Review

The Evolving Genetics of AML: Implications for Practice in Albania and Kosovo

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Review

The Evolving Genetics of AML: Implications for Practice in Albania and Kosovo

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Abstract

Acute myeloid leukemia (AML) is a genetically diverse clonal illness marked by the accumulation of somatically acquired genetic abnormalities in hematopoietic progenitors, leading to poor differentiation and unchecked proliferation. Over the last twenty years, the amalgamation of traditional cytogenetics, molecular genetics, and, more recently, next-generation sequencing (NGS) has transformed diagnostic procedures, enhanced risk stratification models, and revealed new therapeutic targets that are altering the treatment landscape. Molecular genetic analysis of *CEBPA*, *NPM1*, and *FLT3* is currently standard of care in AML patients, and mutations in several other genes are becoming more important, including *NPM1, TET2, KIT, DNMT3A, IDH1/2, RUNX1, AXSL1, WT1,* and *RAS*. In this article, the authors review the most relevant literature concerning AML genetics and discuss, based on their own experience and expertise, the perspectives on precision medicine in AML, and addresses practical issues faced in low-resource nations like Albania and Kosovo.

Keywords: acute myeloid leukemia (AML); cytogenetics; European Leukemia Net (ELN); PCR; next generation sequencing (NGS); targeted therapy; precision medicine; Albania; Kosovo; Western Balkans; resource-limited settings

1. Introduction

Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disorder characterized by the accumulation of somatically acquired genetic lesions in hematopoietic progenitors, resulting in impaired differentiation and uncontrolled proliferation. During the past two decades, the integration of conventional cytogenetics, molecular genetics and, more recently, next-generation sequencing (NGS) has revolutionized the diagnostic work-up, refined risk stratification models and uncovered novel therapeutic targets that are reshaping the therapeutic landscape (Table 1) [1–4].

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Table 1. WHO 2022 classification of acute myeloid leukemias.

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

A quantitative view of the mutational burden is essential for resource allocation and for designing therapeutic algorithms. Conventional cytogenetics and FISH still identify the bulk of recurrent structural abnormalities. Based on large cohorts studied between 2010 and 2020, t(8;21)(q22;q22)/ *RUNX1::RUNX1T1* occurred in 6%, inv(16)/t(16;16)/ *CBFB::MYH11* in 5%, t(15;17)/ *PML::RARA* in 5%, *KMT2A* rearrangements (most commonly t(9;11), t(11;19) and t(6;11)) in 2%, complex karyotype in 12%, monosomy 7/del(7q) in 6%, monosomy 5/del(5q) in 5%, t(6;9)/ *DEK::NUP214* in 1% and inv(3)(q21q26)/ *MECOM* activation in 1%. Roughly 45% of adults have a normal karyotype; within this subset, deep sequencing performed within the BEAT-AML program (coverage >500×, 671 genes) revealed *NPM1* mutations in 30%, *FLT3-ITD* in 25%, *DNMT3A* in 22%, *IDH1* in 8%, *IDH2* in 12%, *NRAS*/*KRAS* in 17%, *TET2* in 10%, *CEBPA* (biallelic) in 8%, *RUNX1* in 7%, *ASXL1* in 8% and *TP53* in 5% (Figure 1A).

Pediatric distributions differ significantly. According to most recent data, t(8;21) was present in 12%, inv(16) in 8%, *PML::RARA* in 4%, and *KMT2A* fusions—predominantly t(9;11)(p22;q23) and t(11;19)(q23;p13)—in 15%. Monosomy 7 occurred in 4%, while true complex karyotypes were uncommon (≈2%). Whole-exome sequencing of 684 normal-karyotype samples in the TARGET AML initiative found *FLT3*-ITD in 15%, *NPM1* in only 8%, *WT1* in 12%, *RAS* pathway lesions (*NRAS*/*KRAS*/*PTPN11*) in 16%, *CEBPA* in 6%, *GATA2* in 4%, *SETBP* in 3%, and clonal hematopoiesis–associated genes such as *DNMT3A* or *TET2* in fewer than 2%, underscoring the lower age-related background mutational load in children [5]. Importantly, more than 60% of pediatric patients with a normal karyotype harbor at least one lesion that is currently targetable in an interventional trial, providing a strong rationale for routine upfront DNA- and RNA-based sequencing panels in this age group (Figure 1B).

This review summarizes current knowledge on AML genetics in adult patients, briefly overview pediatric AML, and discusses practical challenges in low-resource countries such as Albania and Kosovo.

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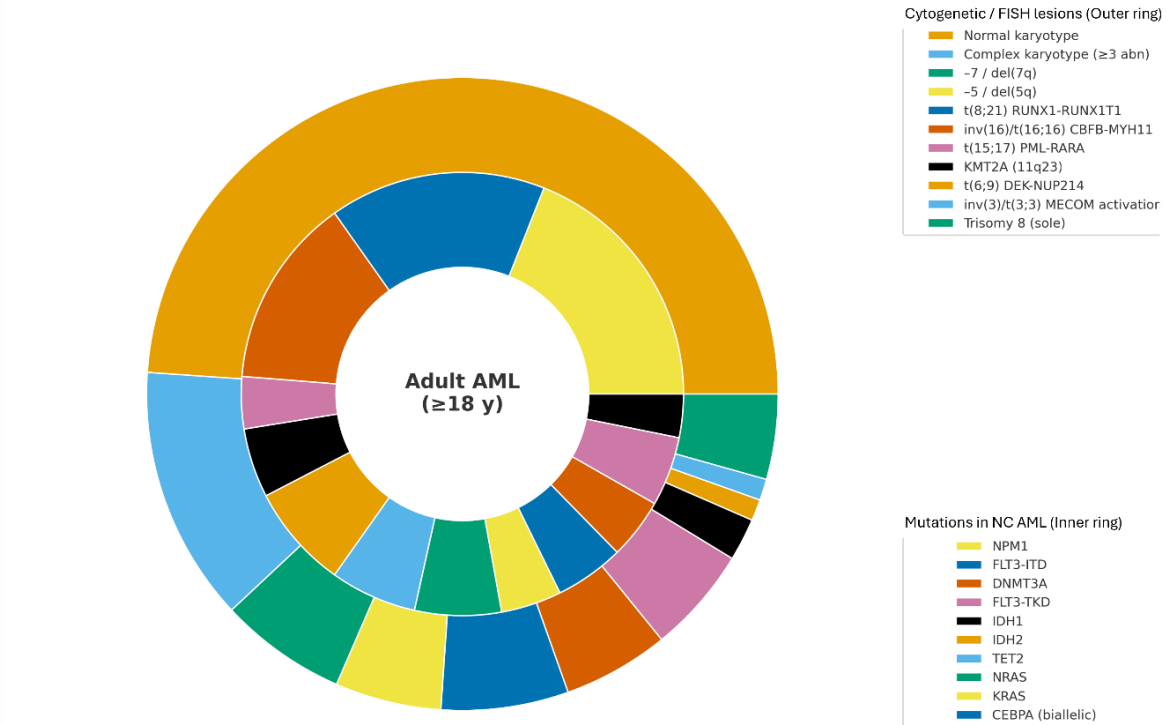
(**A**)



(**B**)

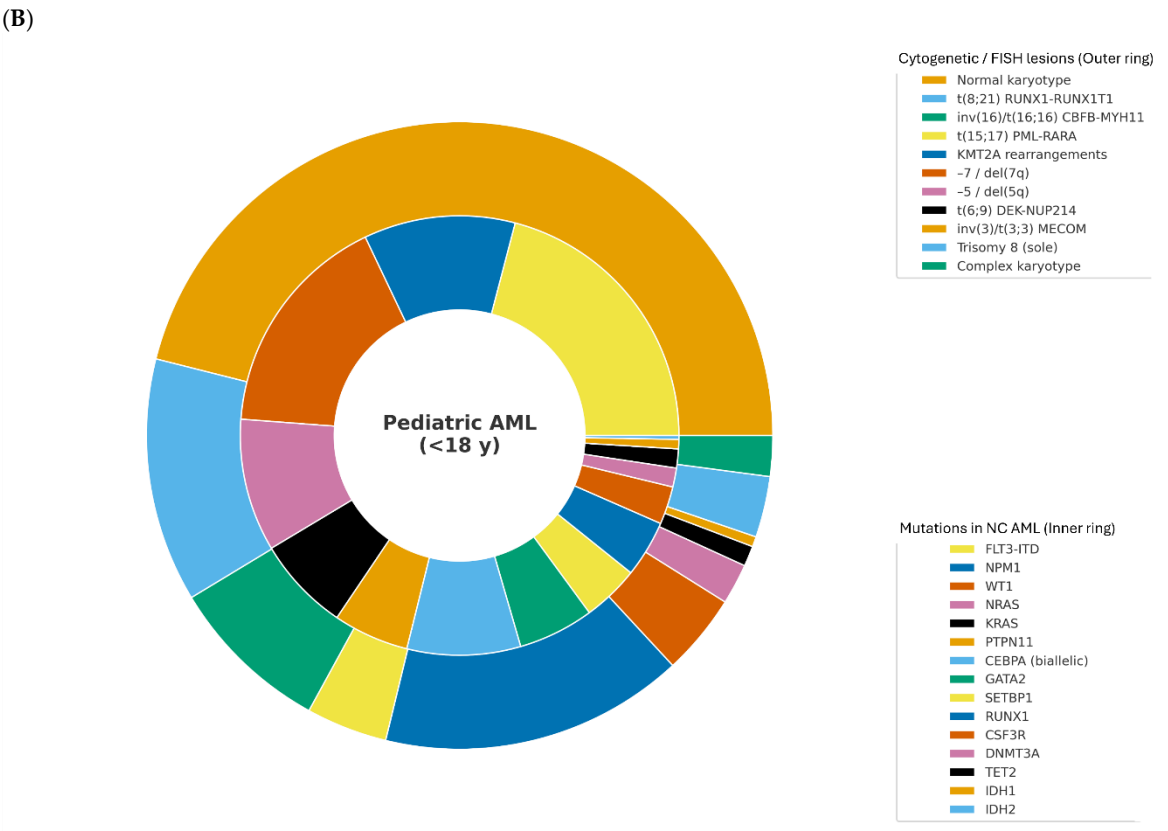


Figure 1. Nested donut chart showing the cytogenetic/FISH lesions and the mutation spectrum within the normal karyotype fraction. The outer ring represents the 'cytogenetic / FISH lesions in unselected adults', and the inner ring shows the 'mutation spectrum inside the 45% CN fraction' (which corresponds to the 'Normal karyotype' segment of the outer ring). A) Adult AML; B) Pediatric AML.

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2. Methods and Techniques to Reveal Genetic Lesions in AML

2.1. Conventional Karyotyping

Conventional G-banded metaphase cytogenetics remains the backbone of initial AML evaluation. Recurrent balanced translocations such as t(8;21)(q22;q22.1)—*RUNX1::RUNX1T1*, inv(16)(p13.1q22)/t(16;16)(p13.1;q22)—*CBFB::MYH11*, and t(15;17)(q24.1;q21.2)—*PML::RARA* define biologically and clinically distinct entities and confer favorable prognosis when treated with appropriate regimens (including the chemo-free combination all-trans retinoic acid and arsenic trioxide, ATRA-ATO for acute promyelocytic leukemia, APL). Unbalanced abnormalities, e.g., monosomy 5/del(5q), monosomy 7/del(7q), i(17q), or complex karyotype (CK, ≥3 clonal abnormalities), are associated with poor outcome [1,2].

Its main advantages are its genome-wide view, the unbiased ability to detect unexpected abnormalities, and the relatively low reagent cost compared with FISH, RT-PCR or NGS. A functioning cytogenetics unit, however, requires a CO_2 incubator, centrifuge, fluorescence microscope with imaging software, and—mostly—skilled technologists able to culture bone-marrow cells and analyze ≥20 metaphases; start-up and maintenance costs are therefore non-trivial for low-income countries. Additional drawbacks include the need for fresh, viable marrow (as blood is often hypocellular). In addition, it is limited to a resolution of ≈5–10 Mb that misses cryptic lesions such as *FLT3*-ITD or *KMT2A* partial tandem duplications (see below). Culture failure rates of 5–15% are higher in resource-limited laboratories owing to delays in transport and lack of antibiotics. Even so, when infrastructure is shared regionally and staff are cross-trained, conventional cytogenetics remains the most cost-effective first-line genomic test in low- and middle-income settings; more targeted assays (FISH, RT-PCR) can then be reserved for cryptic lesions or quality assurance, maximizing clinical impact while containing expense. It should be noted, however, that the need for fresh marrow makes it often difficult to refer samples when referral labs are distant.

2.2. Fluorescence In Situ Hybridization (FISH) and Chromosomal Microarrays

Interphase FISH complements metaphase analysis in samples with low mitotic index or to detect submicroscopic lesions (e.g., cryptic *KMT2A* rearrangements). Array-comparative genomic hybridization (a-CGH) and single nucleotide polymorphism (SNP) arrays detect sub-megabase copy number variants and regions of loss of heterozygosity/uniparental disomy that may escape standard cytogenetics, refining the definition of CK and identifying lesions such as 17p loss involving *TP53*. SNPs arrays as well as optical space genomics have been proposed to surrogate cytogenetics when fresh material is not available. However, those approaches are not standardized and adopted in routine diagnostics.

2.3. Conventional Molecular Methods

Before the NGS era, targeted PCR, RT-PCR and capillary sequencing identified critical gene lesions. The affected genes can be classified based on their function and how the cell functions are impacted. Overall, combined with cytogenetics, the presence of specific mutations is associated with a specific prognostic profile (Table 2).

Table 2. ELN 2022.

Risk category	Genetic abnormality
Favorable	*RUNX1::RUNX1T1* [t(8;21)]
	CBFB::MYH11 [Inv(16)]
	Mutated *NPM1* without *FLT3*-ITD
	bZIP in-frame mutated *CEBPA*
Intermediate	*FLT3*-ITD
	MLLT3::KMT2A [t(9;11)]

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	Cytogenetic or mutations not classified as favorable or adverse
Adverse	*DEK::NUP214* [t(6;9)]
	KMT2A rearranged (other than *MLLT3::KMT2A*)
	BCR::ABL1 [t(9;22)]
	KAT6A::CREBBP [t(8;16)]
	MECOM rearranged [incl. Inv(3)]
	ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2, TP53
	Complex and/or monosomy karyotype
	-5/del(5q); -7; -17/abn(17p)

2.3.1. Class I Mutations (Signal Transduction and Proliferation)

FLT3 internal tandem duplications (ITD; ~25%) and TKD point mutations (~5–10%) augment STAT5/PI3K signaling. High allelic burden ITD confers poor prognosis but is now actionable with multi-kinase inhibitors (midostaurin, gilteritinib, quizartinib). *KIT* mutations (~25% of CBF-AML) increase relapse risk but may be offset by high-dose cytarabine or dasatinib [6–13]. Activating *RAS* mutations have been described in about 10-15% of patients, with some differences across studies [14] Of note, the prognostic impact is not clarified yet [14].

2.3.2. Class II Mutations (Transcription Factors)

RUNX1 point mutations (~5–10%) promote leukemogenesis by impairing core-binding factor function; they are now considered adverse by ELN 2022. *CEBPA* biallelic mutations (~10%) delineate a favorable entity; monoallelic variants have neutral/negative impact [11–13].

2.3.3. Epigenetic Regulators

NPM1 frameshift mutations (~30% of adults) relocate nucleophosmin to the cytoplasm, cooperate with *DNMT3A* (~25%) or *TET2* (~10%), and define a favorable/intermediate group unless accompanied by *FLT3*-ITD-high [15–18]. *IDH1*/2 mutations (~20%) generate oncometabolite D-2-hydroxyglutarate; small-molecule inhibitors ivosidenib and enasidenib are now approved [11–13].

2.3.4. Tumor Suppressors

TP53 mutations (~5–10%, enriched in therapy-related AML and CK) are independently associated with chemoresistance and poor overall survival [11–13].

The timeline of gene discovery and evolution of concepts and management principles in acute myeloid leukemia is summarized in Figure 2.

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Figure 2. Timeline of gene discovery and evolution of concepts and management principles in acute myeloid leukemia. This timeline illustrates the shift in AML understanding from a purely morphological disease to a highly molecularly defined malignancy, driven by technological advancements like **NGS** and **WGS**, leading to more personalized and effective management strategies. NGS: Next Generation Sequencing, CG: cytogenetics; FAB: French, American and British classification; WHO: World Health Organization classification; LSC: leukemia stem cells; WGS: whole genome sequencing; MRD: measurable residual disease.

2.4. Next-Generation Sequencing

In the last two decades, large academic efforts (TCGA, Beat AML, BLUEPRINT) have provided comprehensive catalogs of somatic mutations, structural variants and mutational signatures associated with AML. Whole genome sequencing (WGS) revealed cryptic rearrangements (e.g., *NUP98* fusions) and enhancer hijacking events (e.g., MECOM) [19–21]. However, high cost, turnaround time and bioinformatic complexity limit routine use.

Targeted gene panels covering 40 to about 400 genes (so call myeloid vs. pan cancer panels) capture >95% of clinically relevant variants within days. In newly diagnosed AML, NGS guides ELN classification, detects minimal/measurable residual disease (MRD) markers, and identifies actionable lesions (e.g., *FLT3, IDH1/2, KIT, KRAS/NRAS, CSF3R*) [1,2] (Figure 3). Overall, they appear cost effective; however, they still require the initial investment and a certain expertise. Noteworthy, centralization of samples and procedures is currently recommended for all NGS analyses, and this may favor their spread and standardization.

According to next-generation sequencing, AML is not a monoclonal illness; rather, it results from a hierarchy of genetically unique clones emerging, competing, and being transformed by treatment [22]. Usually, AML clones carry age-related mutations in epigenetic regulators (*DNMT3A, TET2, ASXL1*) or splicing factors, a "founding" dominating clone [22]. However, it usually co-exists with one to several genetically identifiable subclones at diagnosis. By stepwise acquisition of cooperating lesions in signaling genes (*FLT3, RAS*), transcription factors (*CEBPA, RUNX1*), DNA-damage pathways, or chromatin modifiers, these subclones generate a branching rather than linear phylogeny [20–22]. Many times, bulk sequencing underestimates this complexity; single-cell DNA/RNA-seq and error-corrected sequencing, by contrast, have revealed hundreds of low-frequency (<1%) subclones may be present at diagnosis, creating a reservoir for therapy resistance [22].

Pre-leukemic hematopoietic stem cells (pHSCs) with early mutations but maintaining multilineage differentiation potential provide most leukemic founder clones. In long-term survivors, these pHSCs can survive induction chemotherapy and produce clonal hematopoiesis of

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indeterminate potential (CHIP), or gain more hits to seed relapse. Two main evolutionary patterns are shown by comparative sequencing of paired diagnosis/relapse samples: (1) "relapse from subclone,," in which a minor diagnosis subclone acquires resistance-conferring mutations (e.g., *FLT3-TKD, RAS, TP53*) and expands; and (2) "relapse from founder,," in which the ancestral clone persists and evolves along a new trajectory following cytotoxic bottleneck [22–25]. Allogeneic HSCT increases immunological pressure and favors HLA-loss variations or mutations in antigen-processing genes.

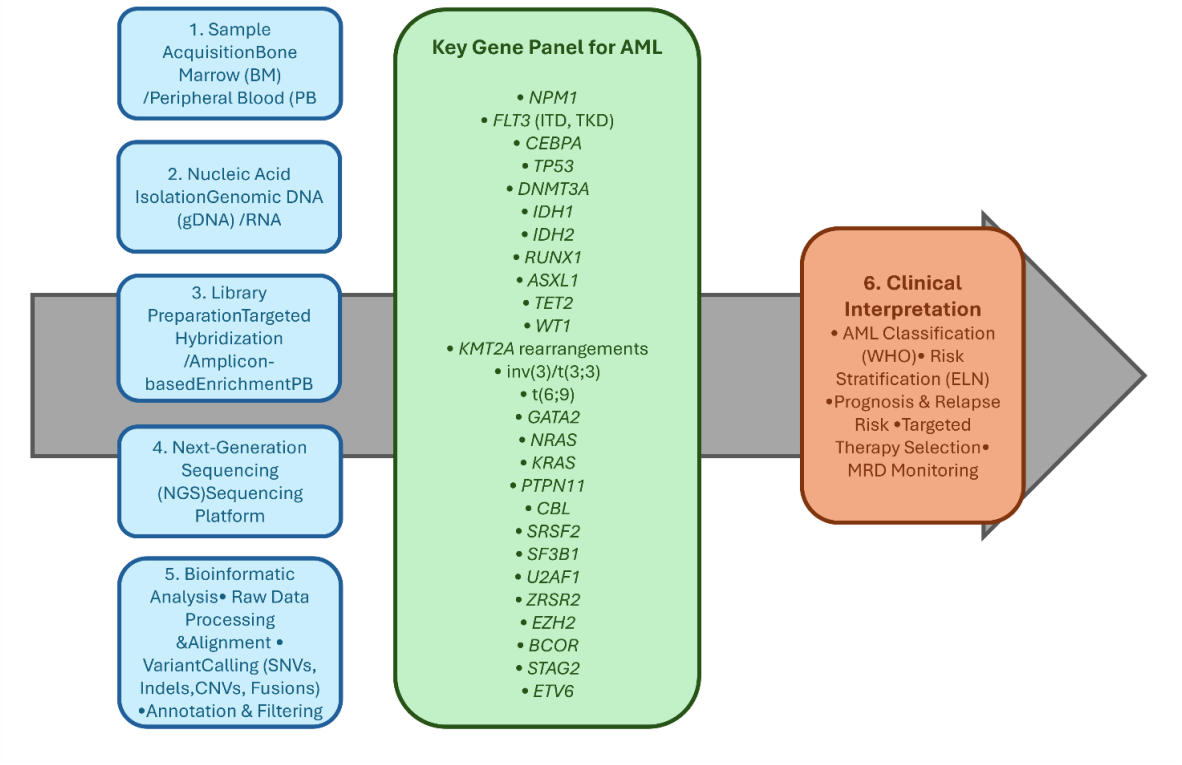

Figure 3. Workflow of myeloid-focused next-generation sequencing panels for AML.

Therapeutically, this clonal variety explains late relapses, MRD kinetics, and primary refractoriness. Minimal panels for high-sensitivity MRD assays are now being informed by integration of variant allele frequency-based clonality with single-cell sequencing [26], which also identifies liabilities such dependence on oxidative phosphorylation in pre-leukemic clones [27]. While early post-remission maintenance (e.g., azacitidine/venetoclax) strives to destroy the founding clone before clonal diversification resumes, rational combination therapy—e.g., FLT3 inhibitors + BCL2 blockade—aims to suppress both dominant and emergent subclones.

2.5. Prognostic Integration

The 2022 European LeukemiaNet (ELN) revision maintains cytogenetic abnormalities as a major risk determinant [1]. For example, t(8;21) and inv(16) remain favorable, whereas CK with or without *TP53* mutation is extremely adverse. Importantly, cytogenetics interacts with molecular genetics; for instance, *FLT3*-ITD high allelic ratio mitigates the favorable impact of core-binding factor (CBF) translocations [1,28].

3. Genetics-Driven Targeted Therapies for AML

The rapid deciphering of the mutational landscape of AML has transformed therapeutic decision-making from a morphology-based discipline into a genetically driven one. Proof of concept was first achieved with all-trans retinoic acid and arsenic trioxide for *PML::RARA*–positive acute promyelocytic leukemia [29], but over the past decade small-molecule inhibitors directed at recurrent driver lesions in non-APL AML have moved from early-phase trials to standard practice. The most

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mature experience involves *FLT3*, *IDH1/2* and, more recently, *NPM1*-mutated or *KMT2A*-rearranged leukemias, with each target illustrating distinct principles of oncogene addiction, clonal evolution and resistance.

FLT3-mutated AML, present in ~25% of newly diagnosed adults and ~15% of children (predominantly internal tandem duplications, ITD), is now treated with first-generation multikinase inhibitors in combination with intensive chemotherapy and with second-generation selective inhibitors at relapse [30–35]. The phase III RATIFY trial showed that adding midostaurin to "7+3" induction and high-dose cytarabine consolidation improved 4-year overall survival to 51% versus 44% with chemotherapy alone, leading to its approval for newly diagnosed *FLT3*-mutated patients irrespective of allelic ratio [31]. At relapse, the highly selective type I inhibitor gilteritinib doubled median overall survival compared with salvage chemotherapy (9.3 vs 5.6 months, ADMIRAL study) and produced composite complete remission (CRc) rates of 34% [36]. Quizartinib, a type II inhibitor active mainly against *FLT3*-ITD, prolonged overall survival over chemotherapy in the phase III QuANTUM-R trial [30]; its recent FDA approval for newly diagnosed *FLT3*-ITD AML in combination with standard chemotherapy (QuANTUM-First) has expanded first-line options [37]. Early-phase data suggest that crenolanib retains activity against many resistance-conferring kinase-domain mutations that emerge after type II inhibitors, supporting sequential use guided by serial NGS [38]. The current paradigm is therefore to use a type I FLT3 inhibitor (midostaurin, gilteritinib or, where available, quizartinib) during induction and consolidation, proceed to allogeneic transplantation in first remission for patients with high allelic-ratio ITD or TKD co-mutations, and maintain with a single-agent inhibitor post-transplant. When relapse occurs after a type I drug, on-target resistance usually involves the "gatekeeper" F691L or activation-loop D835 substitutions; these changes remain sensitive to the type II agent quizartinib, supporting a switch in kinase-inhibitor class. Conversely, quizartinib failures are often rescued by gilteritinib or crenolanib because they retain activity against the Y842 and D835/V mutations that emerge after type II therapy.

Mutations in the IDH enzymes, which block normal α-ketoglutarate–dependent dioxygenase activity and create an oncometabolite (R-2-hydroxyglutarate), occur in 7–10% (*IDH1* R132) and 8–12% (*IDH2* R140/R172) of adults but are rare in children. The oral inhibitors ivosidenib (IDH1) and enasidenib (IDH2) induce differentiation rather than cytotoxicity, with overall response rates of 42% and 40%, respectively, in relapsed/refractory (R/R) cohorts and durable remissions exceeding one year in a subset [39]. Both agents gained regulatory approval for R/R disease and—after single-arm phase II data—for newly diagnosed older adults unfit for intensive chemotherapy [39]. Combination strategies are rapidly moving forward: the randomized phase III AGILE trial showed that ivosidenib plus azacitidine halved the risk of death versus azacitidine alone in newly diagnosed IDH1-mutated AML (hazard ratio 0.44, median OS 24 vs 7.9 months) [40]. Triplet regimens that add venetoclax exploit the dependence of IDH-mutant blasts on BCL-2; early reports reveal CR/CRi rates >70% with acceptable myelosuppression [41]. Relapse is often characterized by either second-site mutations within the allosteric pocket, isoform switching (*IDH1* → *IDH2* or vice versa) or outgrowth of *FLT3*-positive subclones. Serial NGS of blood or bone marrow every one to two cycles has therefore become routine at leading centers and is now endorsed by the 2022 ELN recommendations [1,2].

Unlike *FLT3* and *IDH*, *NPM1* does not encode a druggable enzymatic pocket, but its mutant protein mislocalizes to the cytoplasm, disrupting *HOX* gene regulation and rendering leukemic cells exquisitely sensitive to BCL2 inhibition. In the pivotal VIALE-A trial, azacitidine-venetoclax produced a 66% CR+CRi rate and a median overall survival of 18.9 months in older/unfit AML; exploratory analyses demonstrated even higher response rates and minimal residual disease (MRD) clearance in *NPM1*-mutated cases, leading NCCN and ELN guidelines to recommend venetoclax-based therapy as the default frontline option for this genotype [42]. Additional NPM1-directed approaches include exportin-1 (XPO1) inhibitors (selinexor, eltanexor), which restore nuclear localization; phase I/II trials report CR/CRh rates of 30–40% in heavily pretreated *NPM1*-mutated R/R AML, with deeper responses when combined with FLT3 or BCL2 blockade [43]. Mutant-specific

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degraders that couple a NPM1c-binding peptide to E3 ligase recruiters are progressing through preclinical development and may ultimately provide the first truly allele-selective therapy in AML.

Rearrangements of the *KMT2A* gene on 11q23 comprise ~5% of adult AML but up to 15–20% of pediatric cases and confer a dismal prognosis across age groups. Menin, an essential cofactor that tethers *KMT2A* fusion proteins to chromatin and sustains HOXA/MEIS1 expression, has emerged as a tractable dependency. In 2023 the first-in-human phase I/II study of the oral menin inhibitor revumenib reported a 30% CR/CRh rate and 78% MRD negativity among responding patients with *KMT2A*-rearranged or *NPM1*-mutated AML [44]; differentiation syndrome rather than cytopenia was the dominant toxicity [44]. Additional oral menin antagonists (ziftomenib, JNJ-75276617, BMS-986158) are entering phase II expansion, and early signals suggest that combining these molecules with hypomethylating agents or venetoclax deepens responses and may overcome resistance mediated by RAS pathway mutations—an adaptive escape that appears in roughly one third of patients after single-agent therapy [45].

APR-246 (eprenetapopt) restores WT conformation of mutant *TP53* and, combined with azacitidine, yields ORR 33% in AML but 50–60% in high-risk MDS. Magrolimab (anti-CD47) plus azacitidine induces macrophage-mediated phagocytosis independent of *TP53* status; phase III ENHANCE-2 randomizes against venetoclax/AZA.

H3K27me3 demethylase inhibitor (bomedemstat) and SF3B1 modulators (H3B-8800) target *ASXL1*/*SRSF2*-mutated AML. Early data suggest spliceosome modulation sensitizes to venetoclax via *BCLXL* down-regulation.

In pediatric AML, the impact of targeted drugs is only beginning to be appreciated. Gilteritinib combined with backbone chemotherapy has achieved a composite CR rate of about 70% with minimal additional toxicity [46], and an international phase I study of revumenib in infants with *KMT2A* rearrangements has documented molecular remissions lasting beyond six months, validating menin dependence in this age group [44]. Because *IDH* mutations are vanishingly rare in children, *FLT3* and *KMT2A* remain the dominant actionable lesions, although emerging data indicate that *RAS*-mutated or *KIT*-mutated core-binding-factor AML may respond to MEK or selective KIT inhibition, respectively [14].

Taken together, these data illustrate that the actionable genome of AML is now sufficiently well mapped to justify a genotype-first therapeutic algorithm. In adults, FLT3 mutations account for roughly one quarter of all cases and should prompt inclusion of a type I inhibitor during induction, with class-switching at relapse guided by real-time NGS. *IDH1* and *IDH2* mutations together affect close to one fifth of cytogenetically normal adults and can be addressed with differentiation therapy that is synergistic with hypomethylating agents and venetoclax. *NPM1* mutations, the single most frequent lesion in de novo adult AML, sensitize leukemic cells to BCL2 blockade and to emerging XPO1 or menin inhibition, whereas *KMT2A* rearrangements—especially prevalent in infants—represent the paradigm for transcription-factor addiction that is now being exploited by menin antagonists. As additional dependencies such as mutant *KIT* in core-binding-factor AML, *CSF3R* and *JAK2* in myeloid/lymphoid neoplasms with eosinophilia, or *RAS* pathway lesions responsive to SHP2 or MEK inhibitors move from proof-of-concept into the clinic, the therapeutic armamentarium will continue to diversify, making longitudinal molecular monitoring indispensable.

4. Use of Genetics for Minimal Residual Disease (MRD) Assessment in AML

Minimal residual disease (MRD) has been studied by several methods in AML including flow cytometry (to identify leukemia associated phenotypes), PCR-based methods, Sanger sequencing, and lately NGS [47,48]. NGS achieves sensitivities of 10^{-4} to 10^{-5} when using error-corrected sequencing (duplex molecular barcodes). Standardization is ongoing (EuroMRD consortium), and NGS-MRD may soon complement/replace flow cytometry and quantitative PCR.

One of the first targets evaluated for MRD had been *PML*/*RARA* in APL by quantitative PCR and more recently ddPCR, with a limit of detection (LoD) of 10^{-4} to 10^{-5} [49]. Several data indicate that MRD monitoring may be crucial for detecting relapse in patients treated with all-trans retinoic

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(ATRA) based regimes, specially within one year post-therapy due to the rarity of late molecular recurrence [49]. Specifically, a single positive PCR during consolidation predicted relapse with 90-95% sensitivity and preceded haematologic relapse by a median of 3 months, according to the Italian GIMEMA/AIDA and the PETHEMA trials [50,51]. The occurrence of hematological relapses can be then prevented by properly modulating the treatment [52,53]. In PCR-positive patients, pre-emptive arsenic-based therapy cut overt relapse to less than 5% [52,54].

Based on such evidence, regular qPCR monitoring every three months for two years is a standard of care and enables pre-emptive salvage, aiming to almost eradicate APL mortality.

In non-APL AML, the prognostic significance of MRD assessment has then been increasingly recognized, particularly for core-binding factor AML, *NPM1*-mutated AML, *FLT3*-ITD AML, and *KMT2A*-rearranged AML.

For core-binding factor AML characterized by *RUNX1-RUNX1T1* or *CBFB-MYH11* fusions, MRD is typically monitored using RT-qPCR with a LoD of 10^{-4}. Recent studies elucidated that a three-year relapse rate of 60-70% was associated with transcript levels $\geq 10^{-3}$ post-course 1 or $\geq 10^{-4}$ after consolidation, contrasting sharply with a 10-15% relapse rate when below these thresholds [55]. Rising transcript levels, indicated by a >1-log increase during follow-up, can predict relapses approximately three months in advance, as corroborated by CALGB 10503 and AMLSG 12-09. Consequently, many centers initiate pre-emptive FLAG-Ida ± gemtuzumab ozogamicin when increasing transcripts are detected. The ELN 2022 guidelines recommend intensifying therapy, such as allogeneic hematopoietic stem cell transplantation (allo-HSCT), if the PCR remains $\geq 10^{-3}$ post-induction or fails to decline by ≥3 logs after consolidation [1].

In *NPM1*-mutated AML qRT-PCR of *NPM1* mutant transcripts remains the reference approach, with well-validated thresholds that predict relapse risk [56–58]; droplet-digital PCR achieves comparable or greater analytical sensitivity for low-level clones and is particularly useful when assay standardization is required [59]. Targeted NGS of genomic DNA complements transcript assays by detecting persistent leukemic clones and clonal evolution, informing transplant timing and maintenance strategies [57]. Longitudinal MRD kinetics—more than single timepoints—best stratify relapse risk and influence therapeutic escalation [60].

In *FLT3*-ITD–mutated AML, error-corrected multiplex PCR with capillary electrophoresis (limit of detection ~10^{-4}) remains the standard for MRD assessment. Schroeder et al. demonstrated that a pre-transplant *FLT3*-ITD variant allele frequency >10^{-4} predicted markedly higher post–HSCT relapse risk (two-year CIR 58% vs. 17%) [61,62]. Clinical trials such as RATIFY and QuANTUM-R established that early molecular clearance of *FLT3*-ITD—typically by week four of induction or targeted therapy with midostaurin or gilteritinib—correlates with prolonged event-free survival [30,31]. Consequently, MRD kinetics now inform post-HSCT maintenance strategies, with pre-emptive gilteritinib initiation increasingly employed upon molecular relapse.

In *KMT2A*-rearranged (previously *MLL*) AML, RT-qPCR for patient-specific fusion transcripts (LoD ~10^{-4}) is used to quantify MRD [63]. Adult data parallel findings from the COG AAML0531 trial, where persistence of *KMT2A* fusion transcripts after induction predicted a five-year relapse rate of 65% [64].

5. Implementation Challenges in Low-Resource Settings

In nations lacking in-country cytogenetics/NGS infrastructure, AML classification still relies on morphology and limited immunophenotyping, leading to therapeutic empiricism and suboptimal outcomes. Barriers include: (i) cost (>€500 for 50-gene panel may even exceed *pro capite* monthly income), (ii) scarcity of cytogeneticists/bioinformaticians, (iii) logistic hurdles for sample shipment, and (iv) delayed turn-around times (>3 weeks). Without genomic stratification, transplant allocation, targeted agents and MRD monitoring cannot be rationally deployed, perpetuating inferior OS (<20% at 3 yrs). Advocacy for inclusion of NGS in national essential diagnostics lists and generic procurement of *FLT3*/*IDH* inhibitors is critical.

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A pragmatic approach recently launched in Albania and Kosovo under the Joint Initiative for Hematology Albania Kosovo involve:

- Establishing regional hubs with subsidized courier pipelines and cloud-based data return ≤7 days.
- Adopting low-cost, RT-PCR for *PML::RARA*, *NPM1* and *FLT3*-ITD screening, which covers ~55% of actionable adult AML.
- Participation in international aid programs to access diagnostics and drugs.
- Training local haematopathologists via digital cytogenetics/tele-hematology platforms, bridging the human-resource gap.

6. Conclusions

The genomic dissection of AML has transitioned from descriptive cytogenetics to precision medicine anchored in NGS. Integrated mutational and cytogenetic data inform diagnosis, refine ELN risk stratification, enable MRD quantification and underpin an expanding repertoire of targeted therapies. Genetic-driven directed agents herald the next therapeutic wave, while clonal tracking promises adaptive, response-guided treatment. Yet, translational gains remain uneven globally; implementing cost-effective genomic testing in resource-limited countries is imperative.

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