Utilizing Ultra-Sensitive Techniques (NGS and ddPCR) in MRD-Guided Treatment Strategies for AML Patients

NGS

Next-Generation Sequencing for MRD detection

ddPCR

Droplet Digital PCR for precise MRD quantification

MRD-Guided Treatment

Personalized treatment plans based on MRD results

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Presentation Overview

1

AML & MRD Background

Setting the stage with essential context.

2

Ultra-Sensitive Detection

Exploring advanced methodologies.

3

ASH, EHA, ESMO Updates

Key insights from major conferences.

4

Clinical Case Study

Real-world applications and analysis.

5

Future Directions

Addressing challenges and innovations.

Understanding Minimal Residual Disease (MRD)

Definition

MRD refers to the small number of cancer cells that remain in the body after treatment.

Importance

MRD status can be a significant prognostic factor, potentially more so than genetic subtype, indicating relapse risk in AML.

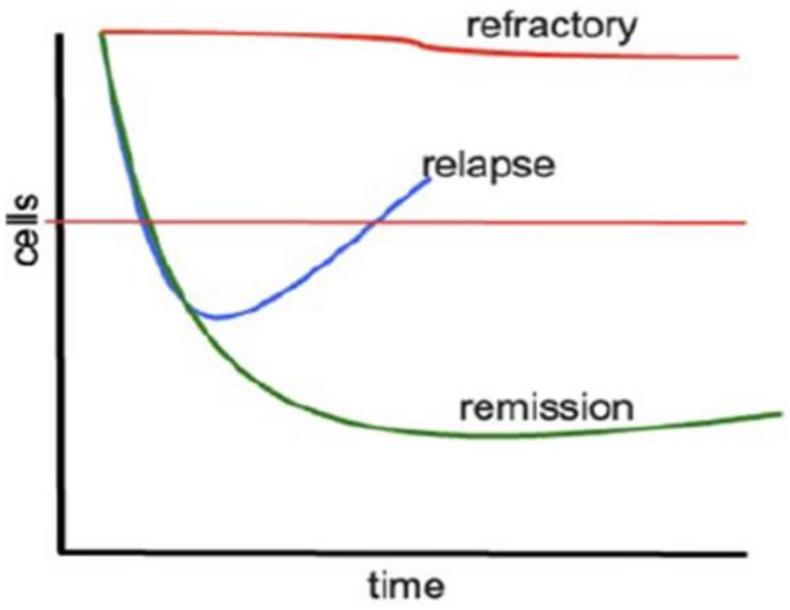
Detection

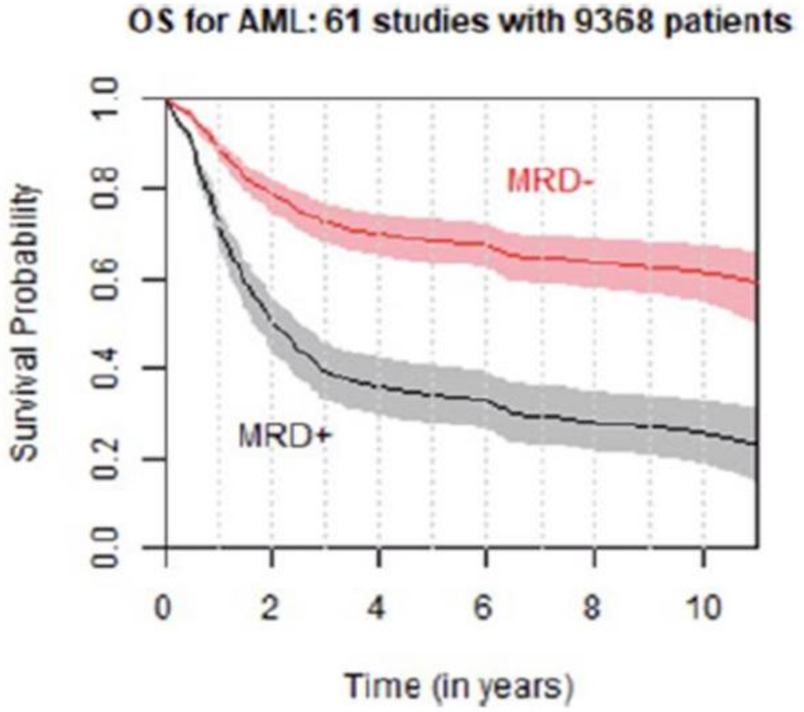
Sensitive methods are essential for identifying these minute cancer cell populations.

Clinical Impact

MRD status influences treatment decisions and prognosis, making post-treatment monitoring crucial.

Resistance, Response, and MRD in AML





A meta-analysis of nearly 10,000 AML cases highlights the impact of MRD status on patient outcomes. However, the analysis also reveals that MRD status is not perfectly predictive: some MRD-negative patients relapse, while some MRD-positive patients do not. Understanding the limitations of MRD status is key to improving treatment strategies.

Importance and Study of Clonal Ontogeny

1 Clonal Evolution and Heterogeneity

Study clonal ontogeny to identify clonal competition/cooperation, using evolutionary rules against virulent clones.

2 Tracking Residual Disease

Characterize/track leukemic clones to predict and potentially abort future relapse.

3 Targeted Therapy

Detect persistent rare clones with sensitive methods for specific targeted therapy.

4 Predicting Treatment Outcomes

Improve clonal detection/characterization as early marker of therapeutic efficacy.

Clonal ontogeny: **acquisition, evolution, and selection of genetic clones**. Understanding clonal ontogeny in the context of AML treatment and response follows several paths:

Techniques to Study Clonal Ontogeny and MRD

1

Next-Generation Sequencing (NGS)

Genetic panel, whole-exome, or whole-genome sequencing

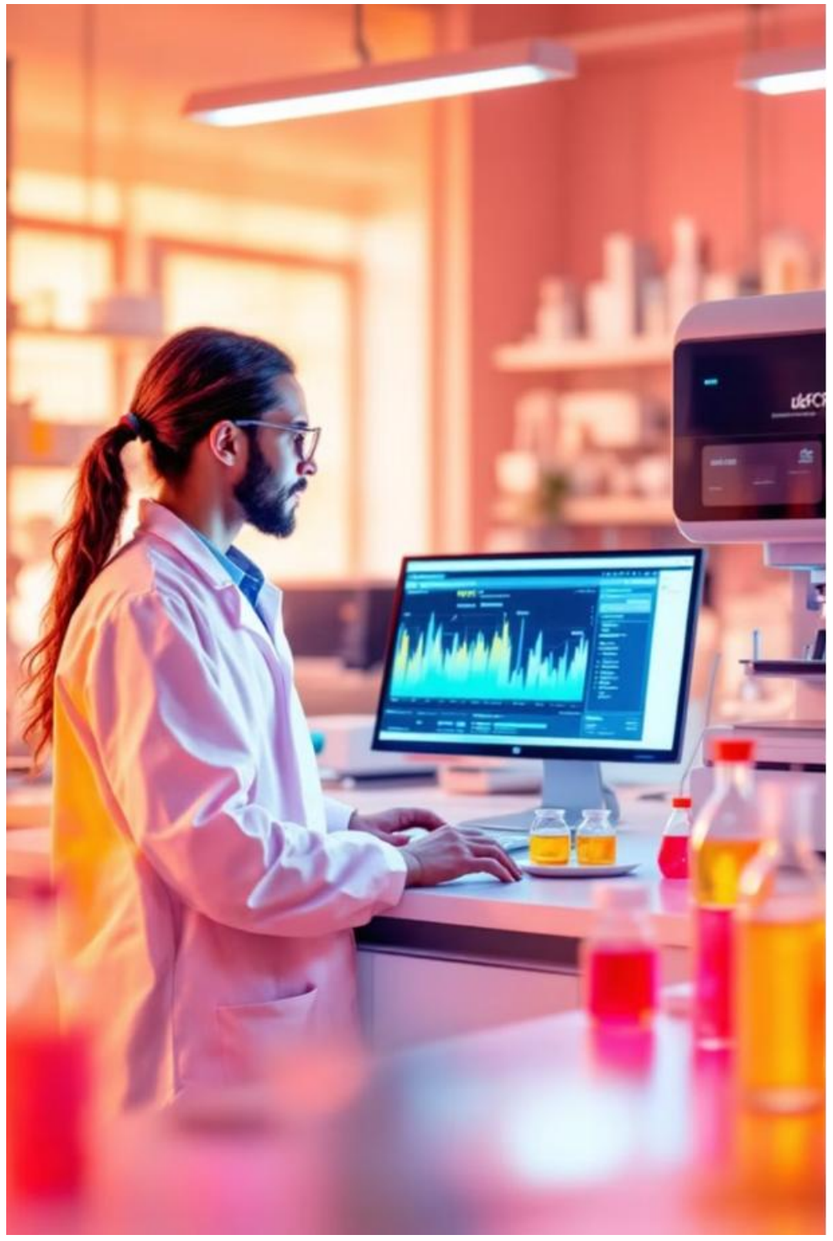
2

Single-Cell Genomics

3

Targeted Gene Molecular Monitoring

Quantitative PCR or digital PCR



Comparing NGS and ddPCR

Aspect	NGS	ddPCR
Sensitivity	High	Very High
Specificity	High	High
Turnaround Time	Longer	Shorter
Cost	Higher	Lower
Sample Requirements	More	Less

By combining MRD with mutation tracking, we can gain a better understanding of how clones evolve over time and predict relapse more accurately.

The Discrepancy in MRD Results

MRD-positive Patients

Some MRD-positive patients remain disease-free for extended periods, challenging the direct correlation between MRD and relapse.

MRD-negative Patients

Some MRD-negative patients later relapse, indicating that current detection methods may not always capture the full picture.

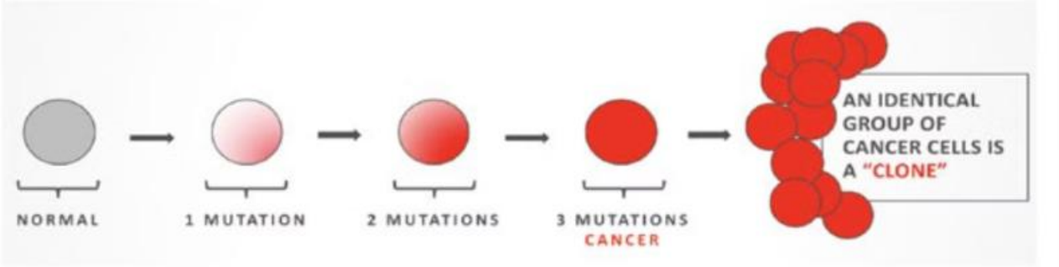
	Relapse status	
MRD status	**Yes**	**No**
Yes	Disease persists Test works!	Mutations not responsible for pre-malignant transformation *and/or* Mutation present in lineage not prone to leukemia *and/or* Active immune suppression *and/or* Other molecular reprogramming that prevents progression
No	Insufficient test sensitivity *and/or* Genetic marker instability *and/or* New clone/disease	No evidence of disease Test works!

MRD is a strong predictor, but it's not without flaws. MRD alone doesn't tell the full story.

Linear vs. Branched Evolution

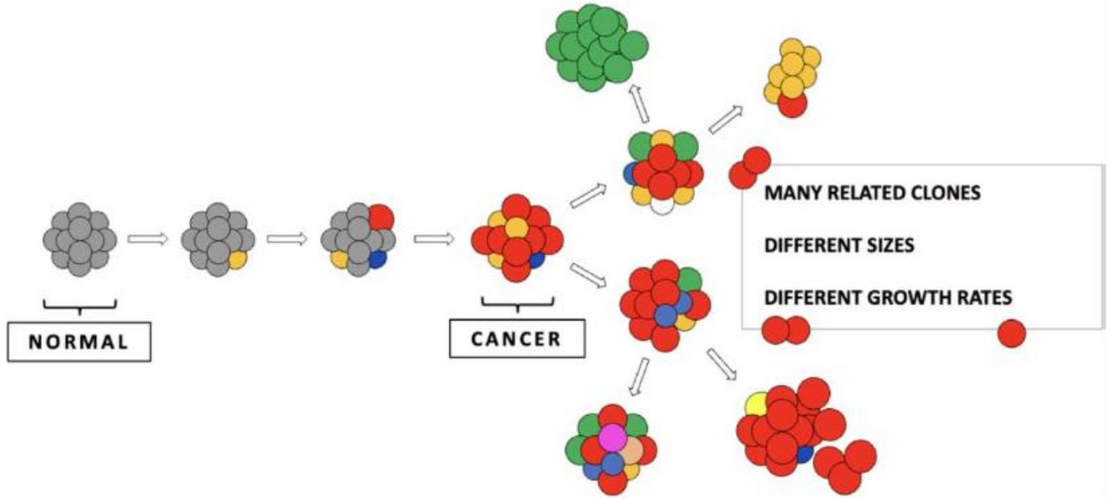
Linear Evolution

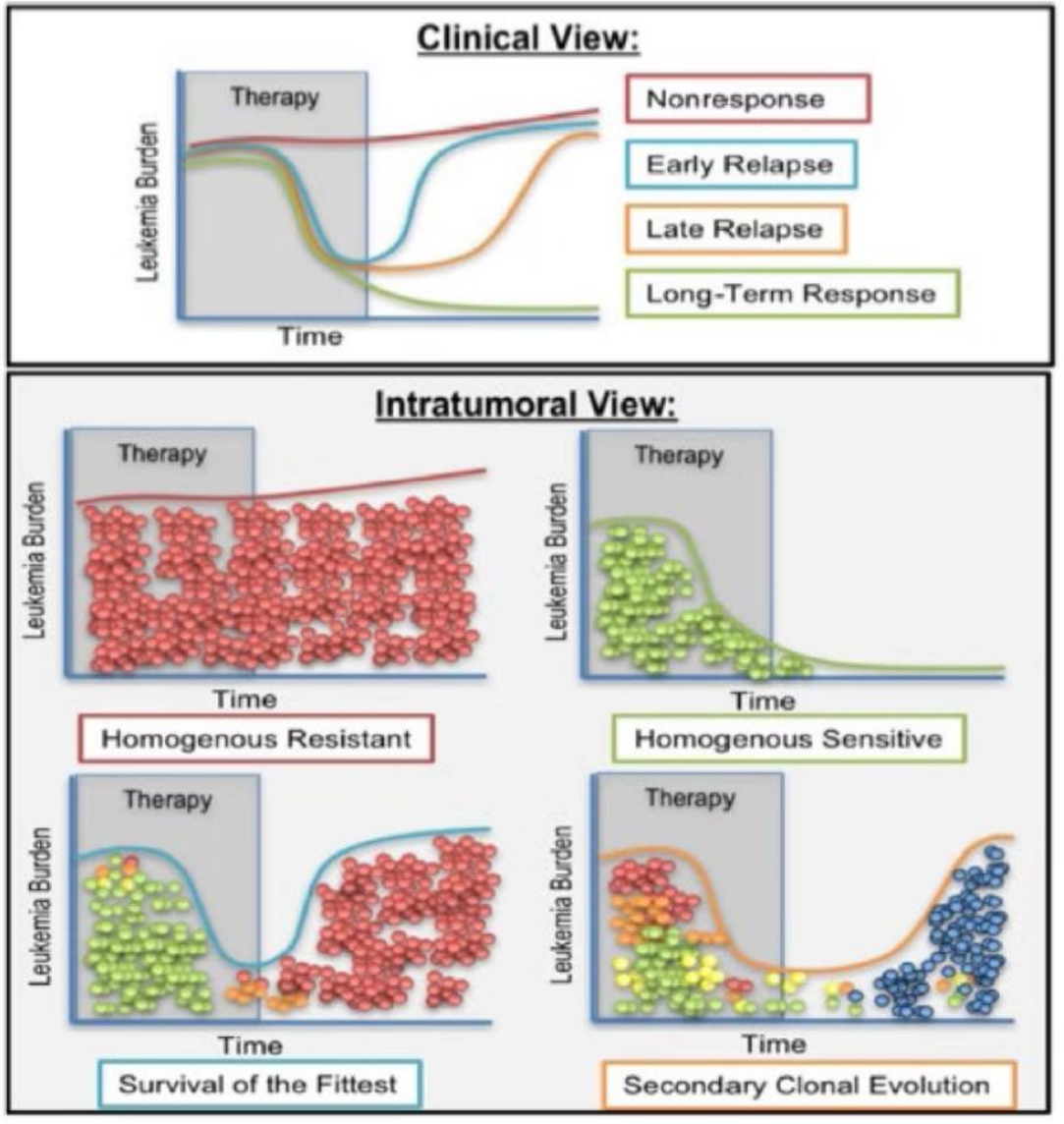
A clone evolves by accumulating sequential, biologically relevant mutations, creating a large cancer clone of a similar phenotype (resistant or sensitive).



Branched Evolution

Related clones with similar or dissimilar biological properties compete, cooperate, and are differentially selected by therapy in a Darwinian landscape.





Patient Profile and MRD Assessment Timeline

1 Initial Patient Profile

45-year-old female with mutations in genes A, B, and C. Treated with induction therapy X and Y.

2 Day 14 Post-Induction

Hypoplastic bone marrow observed after initial induction therapy.

3 Day 30 Post-Induction

Normal bone marrow morphology, but 0.2% abnormal blasts detected by flow cytometry.

4 Post-Treatment NGS Results

No mutations detected by NGS. MRD is positive despite absence of detectable mutations.

5 Clinical Implications

MRD and mutation data provide **insights into relapse risk and guide personalized treatment strategies**. More aggressive treatment may be needed.

The Role of CHIP in MRD Interpretation

What is CHIP?

Clonal Hematopoiesis of Indeterminate Potential (CHIP): Low-level mutations found in normal individuals that may also be present in AML patients.

Why it Matters

Implications: CHIP mutations can affect MRD interpretation and complicate diagnosis, influencing relapse predictions.

CHIP refers to mutations that may exist in normal hematopoietic cells without leading to leukemia. These mutations can sometimes be detected in AML patients, complicating MRD interpretation and relapse predictions.

MRD-Informed Treatment Decisions for AML Patients

Transplant-Eligible

Mutation- and MRD-informed treatment decisions

Transplant-Ineligible

Mutation- and MRD-informed treatments

MRD-Guided Treatment Strategies for AML

1

Select MRD Technology

Choose appropriate technology based on ELN risk groups.

2

Apply Prognostic Implications

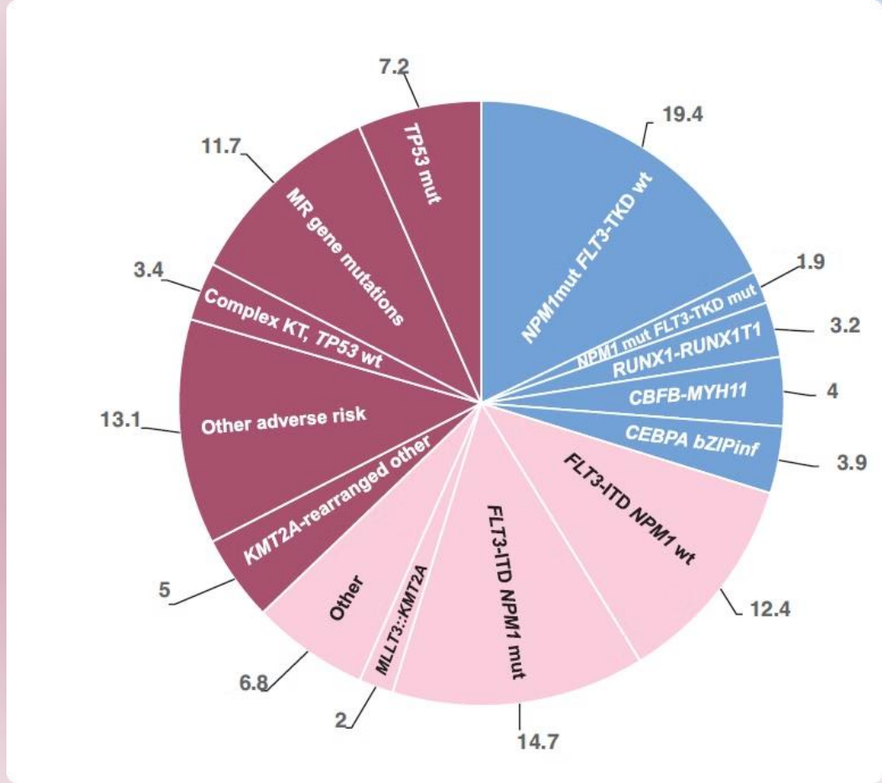
Apply mutations and MRD to risk groups and treatment phases.

3

Compare Treatment Options

Evaluate options for MRD-positive and MRD-negative patients.

ELN 2022 Classification for AML



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Patients

Intensively treated AML cohort

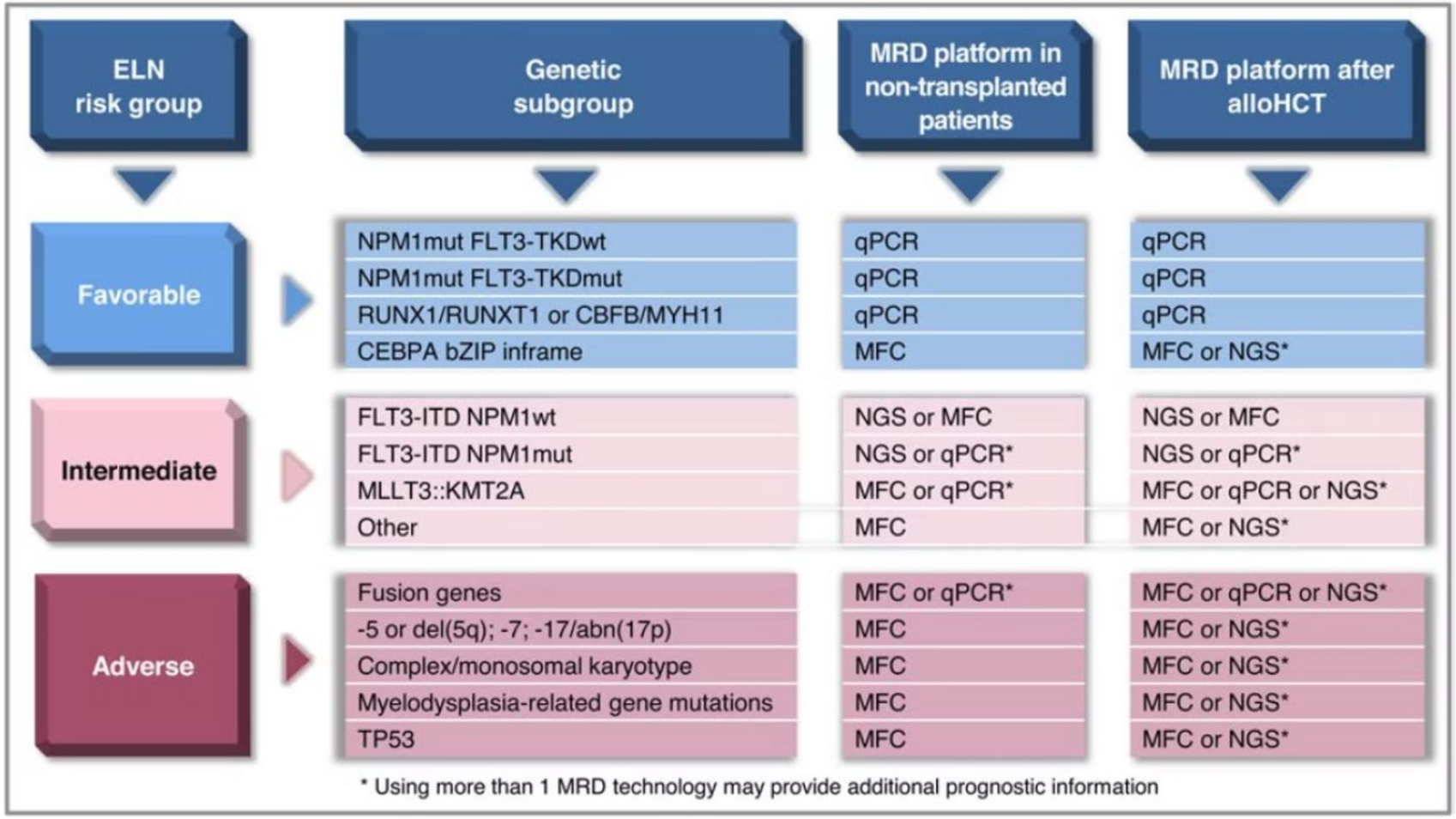
58

Median Age

Range: 18-86 years

Frequency of molecular and therapeutic subgroups based on the ELN 2022 classification modified from Rausch et al. Reported frequencies of ELN 2022 subgroups from an intensively treated AML cohort.

Recommended MRD technology by ELN risk and genetic subgroup for pre-alloHCT and post-alloHCT MRD monitoring



ELN risk group	Genetic subgroup	MRD platform in non-transplanted patients	MRD platform after alloHCT
Favorable	NPM1mut FLT3-TKDwt	qPCR	qPCR
	NPM1mut FLT3-TKDmut	qPCR	qPCR
	RUNX1/RUNXT1 or CBFB/MYH11	qPCR	qPCR
	CEBPA bZIP inframe	MFC	MFC or NGS*
Intermediate	FLT3-ITD NPM1wt	NGS or MFC	NGS or MFC
	FLT3-ITD NPM1mut	NGS or qPCR*	NGS or qPCR*
	MLLT3::KMT2A	MFC or qPCR*	MFC or qPCR or NGS*
	Other	MFC	MFC or NGS*
Adverse	Fusion genes	MFC or qPCR*	MFC or qPCR or NGS*
	-5 or del(5q); -7; -17/abn(17p)	MFC	MFC or NGS*
	Complex/monosomal karyotype	MFC	MFC or NGS*
	Myelodysplasia-related gene mutations	MFC	MFC or NGS*
	TP53	MFC	MFC or NGS*

* Using more than 1 MRD technology may provide additional prognostic information

Therapeutic consequences of MRD after 2 cycles of treatment, at the end of treatment, and during follow-up for thegenetic subgroups defined by

ELN risk group	Genetic subgroup	After two cycles	At end of treatment	During follow-up (q3 months in BM or q4-6 weeks in PB)
Favorable	NPM1 mut FLT3-TKDwt	MRD- Chemo	MRD- -	MRD- -
		MRD+ Chemo, may consider alloHCT	MRD+ alloHCT	MRD+ alloHCT[3], DLI[3], HMA/VEN
	NPM1 mut FLT3-TKDmut	MRD- Chemo+FLT3i	MRD- FLT3i maint. (optional)	MRD- FLT3i maint. (optional)
		MRD+ Chemo+FLT3i, may consider	MRD+ alloHCT+FLT3i maint	MRD+ alloHCT[3]+FLT3i maint, DLI[3], HMA/VEN, FLT3i
	RUNX1/RUNXT1 or CBFB/MYH11	MRD- Chemo	MRD- -	MRD- -
		MRD+ Chemo	MRD+ alloHCT (no allo if MRD at low level)	MRD+ alloHCT[3], DLI[3]
	CEBPA bZIP inframe	MRD- MRD not established	MRD- MRD not established	MRD- MRD not established
		MRD+ MRD not established	MRD+ MRD not established	MRD+ MRD not established
Intermediate	FLT3-ITD NPM1wt	MRD- alloHCT+FLT3i maint	MRD- alloHCT+FLT3i (or oral AZA[2] or FLT3i[2])	MRD- FLT3i maint.
		MRD+ alloHCT+FLT3i maint	MRD+ alloHCT+FLT3i maint.	MRD+ DLI[3] or alloHCT[3] with FLT3i maint
	FLT3-ITD NPM1mut	MRD- Chemo+FLT3i[1] or alloHCT+FLT3i	MRD- Oral AZA or FLT3i maint. or alloHCT	MRD- oral AZA (if no prior allo) or FLT3i maint.
		MRD+ alloHCT+FLT3i maint	MRD+ alloHCT+FLT3i maint.	MRD+ DLI[3] or alloHCT[3] +FLT3i maint, HMA/VEN
	MLLT3::KMT2A	MRD- Chemo or alloHCT	MRD- Oral AZA or alloHCT	MRD- oral AZA (if no prior allo)
		MRD+ alloHCT	MRD+ alloHCT (or oral AZA[2])	MRD+ DLI[3] or alloHCT[3]
	Other	MRD- Chemo or alloHCT	MRD- Oral AZA or alloHCT	MRD- oral AZA (if no prior allo)
		MRD+ alloHCT	MRD+ alloHCT (or oral AZA[2])	MRD+ DLI[3] or alloHCT[3]
Adverse	Fusion genes	MRD- alloHCT	MRD- alloHCT (or oral AZA[2])	MRD- continue observation or maintenance
		MRD+ alloHCT	MRD+ alloHCT (or oral AZA[2])	MRD+ DLI[3] or alloHCT[3]
	-5 or del(5q); -7; -17/abn(17p)	MRD- alloHCT	MRD- alloHCT (or oral AZA[2])	MRD- continue observation or maintenance
		MRD+ alloHCT	MRD+ alloHCT (or oral AZA[2])	MRD+ DLI[3] or alloHCT[3]
	Complex/monosomal karyotype	MRD- alloHCT	MRD- alloHCT (or oral AZA[2])	MRD- continue observation or maintenance
		MRD+ alloHCT	MRD+ alloHCT (or oral AZA[2])	MRD+ DLI[3] or alloHCT[3]
	Myelodysplasia-related gene mutations	MRD- alloHCT	MRD- alloHCT (or oral AZA[2])	MRD- continue observation or maintenance
		MRD+ alloHCT	MRD+ alloHCT (or oral AZA[2])	MRD+ DLI[3] or alloHCT[3]
	TP53	MRD- alloHCT	MRD- alloHCT (or oral AZA[2])	MRD- continue observation or maintenance
		MRD+ alloHCT	MRD+ alloHCT (or oral AZA[2])	MRD+ DLI[3] or alloHCT[3]

Clinical Case 1: FLT3-ITD Mutated AML

1

Diagnosis

53-year-old woman diagnosed with FLT3-ITD and IDH2 mutations.

2

CR Achieved

Achieved complete remission (CR) after induction and consolidation therapy.

3

Pre-AlloHCT

MRD-negative before receiving myeloablative alloHCT from a matched related donor.

4

Post-AlloHCT

FLT3-ITD positive by NGS (VAF: 0.09%) post-transplant.

5

Sorafenib Maintenance

Received maintenance sorafenib from day 6 after alloHCT.

6

1 Year Later

Stopped sorafenib after 1 year due to poor tolerance; NGS-MRD was negative.

FLT3-ITD Mutated AML: Treatment & MRD Timeline

Initial Diagnosis & Induction

7 + 3 chemotherapy with FLT3 inhibitors (midostaurin or quizartinib) for FLT3-ITD mutated AML. ELN 2022 assigns intermediate risk.

1

2

Post-Induction MRD Monitoring

NGS recommended for FLT3-ITD MRD monitoring. Prognostic value after 2 chemotherapy cycles.

MRD-Positive Post-Induction

Proceed to alloHCT. FLT3 inhibitors can convert MRD-positive to MRD-negative.

3

4

Post-alloHCT Maintenance

FLT3 inhibitors (e.g., sorafenib, quizartinib) improve outcomes in MRD-positive patients. Maintenance therapy duration: 5-24 months. Recommended in all FLT3-mutated patients.

AlloHCT converts 73% of MRD-positive patients to MRD negativity, improving OS in converting patients. MRD positivity before or after alloHCT is a critical indicator of a high risk of relapse.

Clinical Case 2: AML with Myelodysplasia-Related (MR) Gene Mutations

1

Initial Diagnosis

65-year-old man with SRSF2 and STAG2 mutations, adverse risk per ELN 2022. Diagnosed with AML with normal cytogenetics; sequencing revealed SRSF2 (47%) and STAG2 (36%) mutations.

2

Treatment & Response

Achieved CR after induction and consolidation therapy.

3

MRD Monitoring Before alloHCT

MRD-negative before alloHCT.

4

MRD Monitoring Post-alloHCT

SRSF2 positive post-transplant. NGS-MRD monitoring post-alloHCT can detect relapse early. ELN 2021 recommends MFC for MRD assessment in patients without an established molecular MRD marker. MFC is recommended for MRD monitoring in patients without molecular markers.

5

Treatment Approach

Standard treatment: 7 + 3 chemotherapy or CPX-351 for MRC-AML. AlloHCT is recommended for eligible patients. Treatment for patients with MR gene mutations is heterogeneous, depending on co-occurring aberrations.

6

MRD-Guided Treatment

MRD monitoring can guide preemptive DLIs or salvage treatment. Longitudinal MRD monitoring post-alloHCT is crucial. **MRD monitoring after alloHCT may guide the tapering of immunosuppression and the use of preemptive DLIs or salvage treatment of MRD relapse.**

Advancements in AML Treatment

Acute Myeloid Leukemia (AML) poses significant challenges, particularly for older patients, due to comorbidities and the limitations of intensive treatments. Recent advances, however, are transforming the prognostic outlook.

Challenges in Treating Older AML Patients

- High treatment-related mortality
- Limited use of intensive chemotherapy (IC) and allogeneic hematopoietic cell transplantation (HCT)
- Persistent measurable residual disease (MRD) leading to relapse

Evolution of AML Treatment

- Traditional treatments: Anthracycline + cytarabine or hypomethylating agent (HMA) monotherapy
- New standard: Azacitidine + Venetoclax (AZA-VEN)
- Targeted therapies (IDH1, IDH2, FLT3, menin inhibitors) transforming outcomes

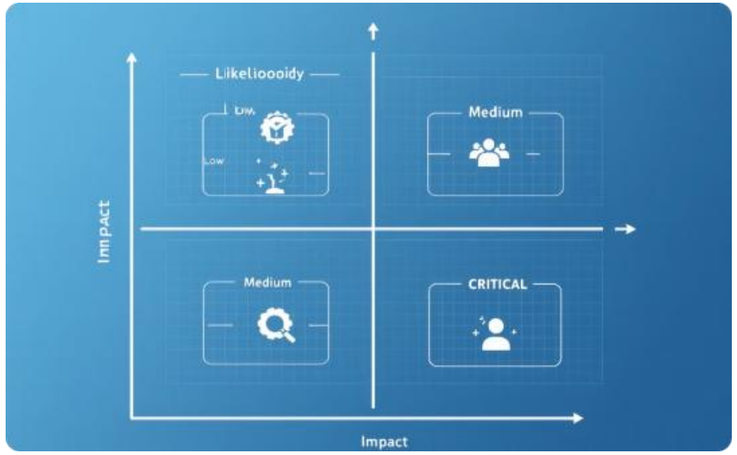
Clinical Case Presentation

Patient Profile

73-year-old man with a history of head and neck cancer, COPD, and CHF presents with anemia and neutropenia. Bone marrow biopsy confirms AML.

Molecular Prognostication

Testing reveals a diploid karyotype and mutations in DNMT3A and IDH1. Not a candidate for induction chemotherapy due to comorbidities.



Risk Stratification

ELN 2022 guidelines are inadequate for HMA-VEN therapy. Consider 4-gene mPRS and Mayo Clinic model.

Prognostic Risk Models in AML

4-Gene Molecular Prognostic Risk Signature (mPRS)

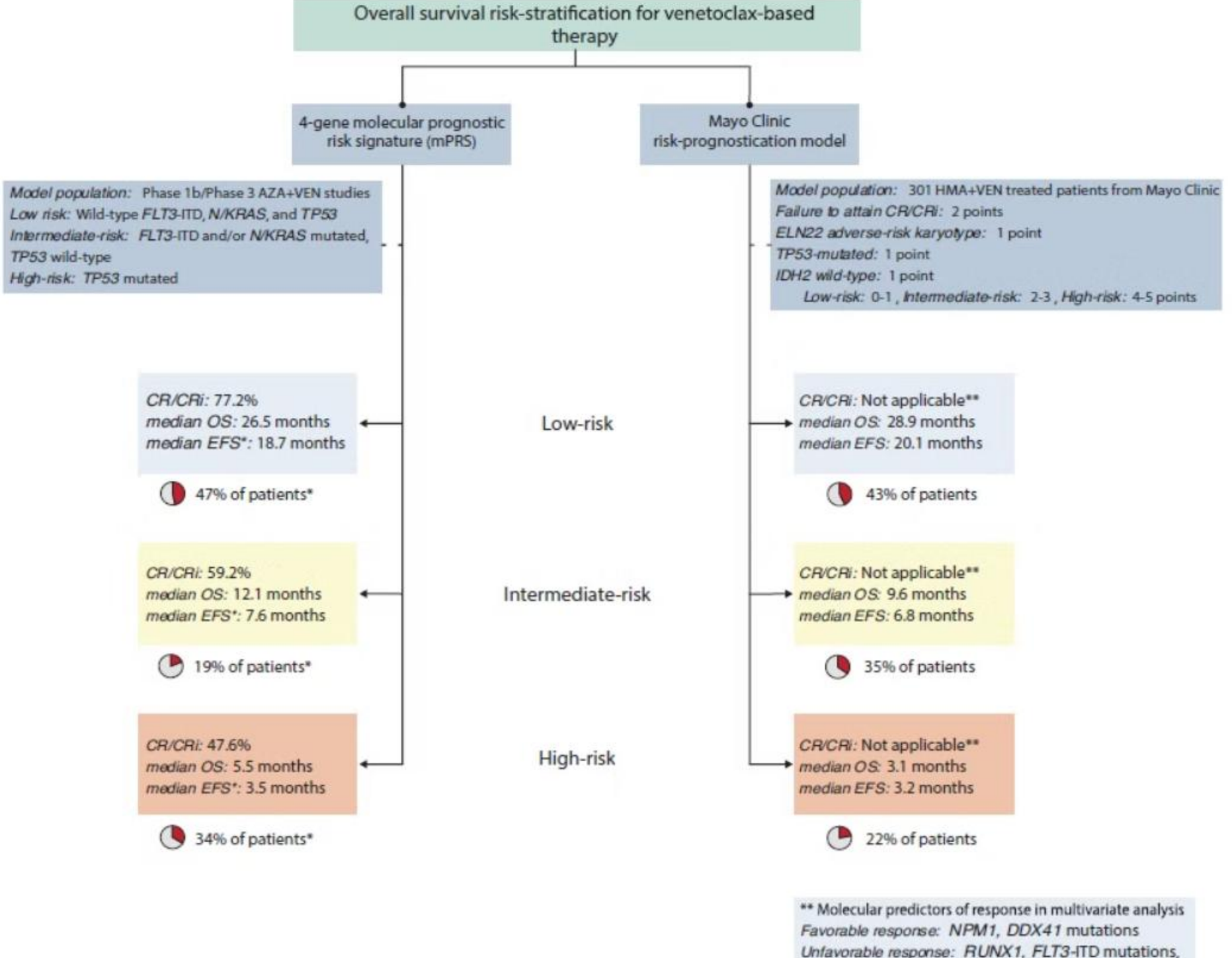
The mPRS, defined by Döhner et al., categorizes AML patients into three groups based on potential treatment benefit:

- **Higher benefit:** Wild-type FLT3-ITD, N/KRAS, TP53
- **Intermediate:** Mutations in FLT3-ITD and/or N/KRAS, wild-type TP53
- **Lower benefit:** TP53 mutation

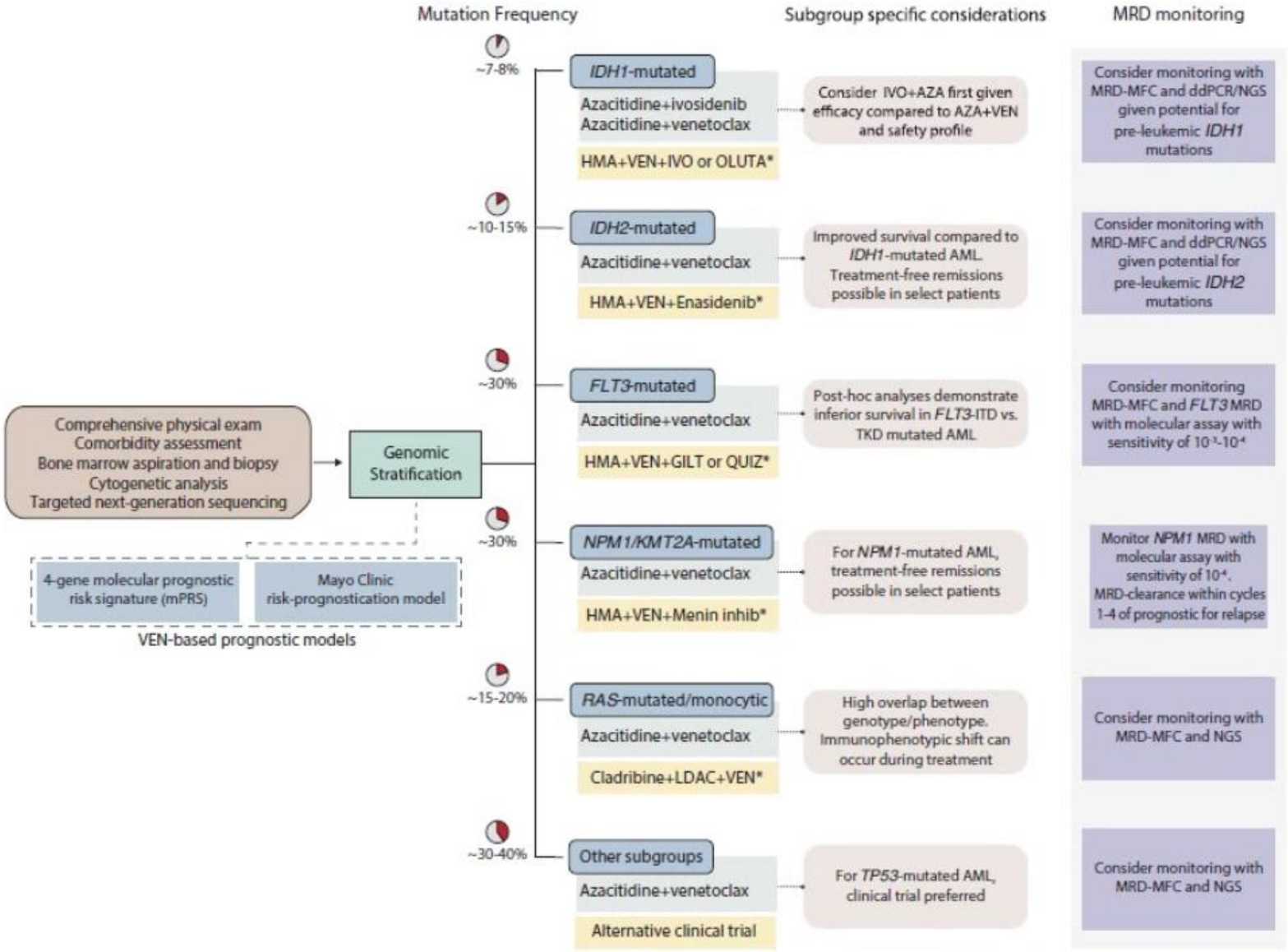
Mayo Clinic Risk-Prognostication Model

This model integrates cytogenetics, mutational status, and clinical response to assess risk:

- **Low-risk:** 0-1 point
- **Intermediate-risk:** 2-3 points
- **High-risk:** 4-5 points


Overall survival risk-stratification for venetoclax-based therapy
4-gene molecular prognostic risk signature (mPRS)
Mayo Clinic risk-prognostication model
Model population: Phase 1b/Phase 3 AZA+VEN studies
Low risk: Wild-type FLT3-ITD, N/KRAS, and TP53
Intermediate-risk: FLT3-ITD and/or N/KRAS mutated, TP53 wild-type
High-risk: TP53 mutated
Model population: 301 HMA+VEN treated patients from Mayo Clinic
Failure to attain CR/CRi: 2 points
ELN22 adverse-risk karyotype: 1 point
TP53-mutated: 1 point
IDH2 wild-type: 1 point
Low-risk: 0-1 , Intermediate-risk: 2-3 , High-risk: 4-5 points
CR/CRi: 77.2%
median OS: 26.5 months
median EFS*: 18.7 months
47% of patients*
Low-risk
CR/CRi: Not applicable**
median OS: 28.9 months
median EFS: 20.1 months
43% of patients
CR/CRi: 59.2%
median OS: 12.1 months
median EFS*: 7.6 months
19% of patients*
Intermediate-risk
CR/CRi: Not applicable**
median OS: 9.6 months
median EFS: 6.8 months
35% of patients
CR/CRi: 47.6%
median OS: 5.5 months
median EFS*: 3.5 months
34% of patients*
High-risk
CR/CRi: Not applicable**
median OS: 3.1 months
median EFS: 3.2 months
22% of patients
** Molecular predictors of response in multivariate analysis
Favorable response: NPM1, DDX41 mutations
Unfavorable response: RUNX1, FLT3-ITD mutations,

lower-intensity treatment perspectives for older, unfit patients with AML and provides a practical



*Currently under clinical investigation. Inhib: inhibitor; HMA: Hypomethylating agent (azacitidine or decitabine); VEN: Venetodax;

MRD-Directed Therapies

Table 1. Outcomes of genetic subgroups with VEN-based therapy

Molecular subgroup	Response (CR/CRi)	MRD-negative CR/CRi (MFC)	OS (median or 2-year)	References
IDH1	66.7% (n = 22/33)	42% (n = 5/12)	10.2 mo	Pollyea et al. CCR 2022;[18] Pratz et al. AJH 2024[38]
IDH2	86% (n = 43/50)	50% (n = 16/32)	27.5 mo	Pollyea et al. CCR 2022;[18] Pratz et al. AJH 2024[38]
NPM1	66.7% (n = 18/27)	88% (n = 15/17)	2-year OS: 71.8%[64]	DiNardo et al. Blood 2020;[11] Pratz et al. AJH 2024[38]
FLT3-ITD	63.3% (n = 19/30)	53% (n = 9/17)	9.9 mo	Konopleva et al. CCR 2022[12]
FLT3-TKD	76.9% (n = 10/13)	50% (n = 2/4)	19.2 mo	Konopleva et al. CCR 2022[12]
N/KRAS	45% (n = 13/29)	-	12 mo	Rivera et al. AJH 2022[51]
TP53	55% (n = 21/38)	30% (n = 6/20)	5.5–7.4 mo	DiNardo et al. Blood 2020;[11] Pollyea et al. CCR 2022[13]

1

Preventing Relapse

MRD monitoring is critical for preventing relapse in AML patients.

2

Rationale for MRD-Directed Therapies

Persistent MRD has an adverse prognostic impact, supporting the development of MRD-directed therapies.

3

Ongoing Studies

Studies like INTERCEPT and MyeloMatch are investigating MRD-directed interventions to improve survival.

Conclusion

1 Evolving Treatment Landscape

AML treatment has evolved significantly with emerging therapies and individualized prognostication.

2 MRD Monitoring

MRD monitoring is improving outcomes in AML patients.

> "AML treatment has evolved tremendously since the binary options of hypomethylating agent monotherapy vs induction chemotherapy. These emerging therapeutic options, along with the preemptive treatment of MRD, rationally designed treatment combinations and sequencing approaches, and increasingly individualized prognostication tools, will assuredly further improve the treatment and survival of older adults with AML."