Biomarkers and the Changing Landscape of Laboratory Testing for AML

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Disclosures

I have the following financial relationships to disclose.

Consultant for:

Astellas Pharma Canada
Astra Zeneca
Celgene/BMS
Diaceutics
Hoffmann-La ROCHE
Janssen Canada INC.
Jazz Pharma
Novartis Pharma Canada Inc.
Precision RxDx
Seagen Canada

AND

I will not discuss off label use and/or investigational use in my presentation.

Objectives

- Overview of bone marrow triage & the evolving approach to laboratory testing for acute leukemia.
- Describe how selective rapid molecular profiling can help guide early decision-making for treatment.
- Review how comprehensive genomic assessment is increasingly used in pursuit of improved risk-adapted clinical care.
- Discuss quantitative PCR and opportunities for measurable residual disease testing in AML.
- Highlight shifts in the treatment paradigm for acute leukemia and possibilities for maintenance-like therapies.

Case presentation

- 65M presenting with mild pan-cytopenia NYD.

- FMHx of CRC and no secondary causes for cytopenia identified.
  - **Hgb** **110 g/L (MCV 98.2 fL)**
  - **Plt** **88 x 10^9/L**
  - **WBC** **4.8 x 10^9/L**
  - **ANC** **1.8 x 10^9/L**

- Clinical history provided with requisition: *? MDS*

Case presentation

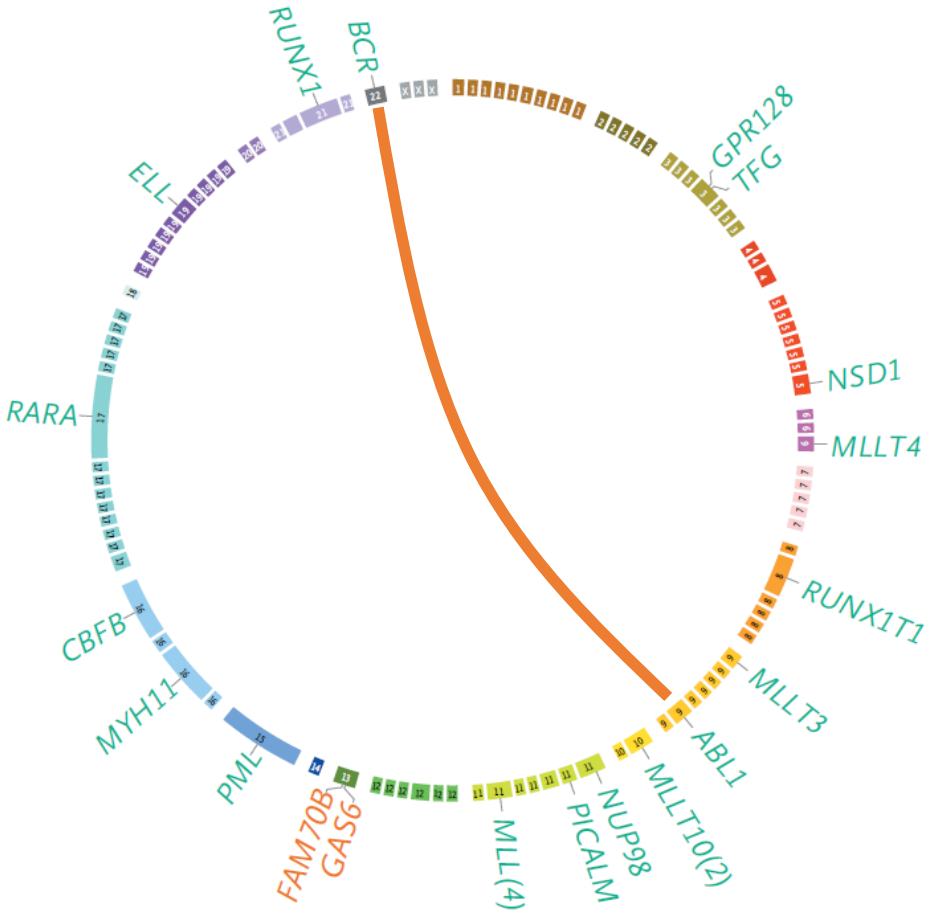
- Unremarkable PB and BMA shows trilineage hematopoiesis, mild dyspoiesis and 4% blasts.
- The HP reviewing the case asks for a 10-colour ‘screening tube’ by flow cytometry.
- Sample is also sent for cytogenetic karyotyping and NGS myeloid sequencing panel (?CCUS).
- PCR-based assessment for mutations in NPM1 and FLT3-ITD are ordered directly by CPOE.

Indications for bone marrow aspiration and biopsy

Unexplained anemia
Macrocytic anemia (to distinguish megaloblastic from normoblastic maturation)
Unexplained leukopenia
Unexplained thrombocytopenia
Pancytopenia
Presence of blasts on peripheral smear (investigation for possible leukemia)
Presence of teardrop red cells on peripheral smear (possible myelofibrosis)
Presence of hairy cells on peripheral smear (possible hairy cell leukemia)
Suspected multiple myeloma
Staging of non-Hodgkin's lymphoma
Unexplained splenomegaly (possible lymphoma)
Suspected storage disease (eg, Gaucher disease, Niemann-Pick)
Fever of unknown origin
Suspected chromosomal disorders in neonates (requiring rapid confirmation)
Confirmation of normal marrow in potential allogeneic donor
Work-up of amyloidosis (to detect clonal plasma cell disorder)

UpToDate®

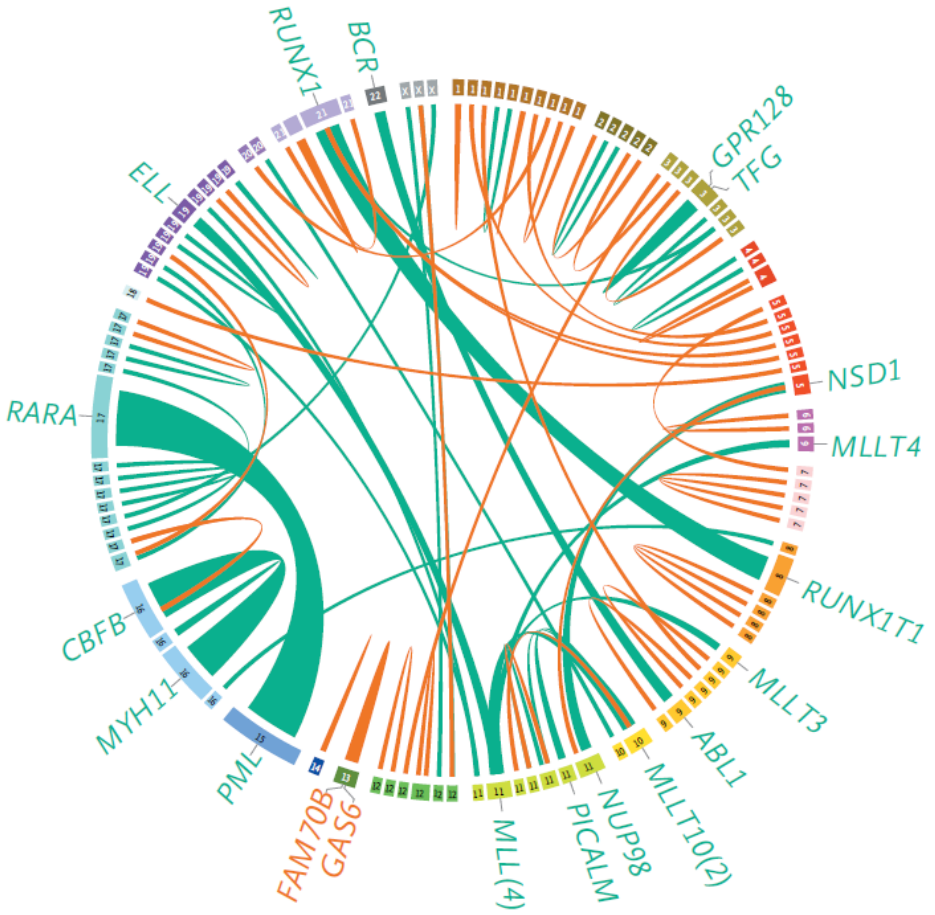
Genomic Landscape of Myeloid Neoplasms



N ENGL J MED 368;22 NEJM.ORG MAY 30, 2013

- Philadelphia chromosome identified as playing a key role in CML pathogenesis in 1960.
- Subsequent development of PCR-based RUO assay to measure BCR-ABL1 published in late 1980's.
- Small-molecule TKI used to treat CML patients dramatically improve outcome (FDA approval 2001).
- Multiple generations of TKI's have culminated in deeper sustained responses, more sensitive testing and trials of TFR.

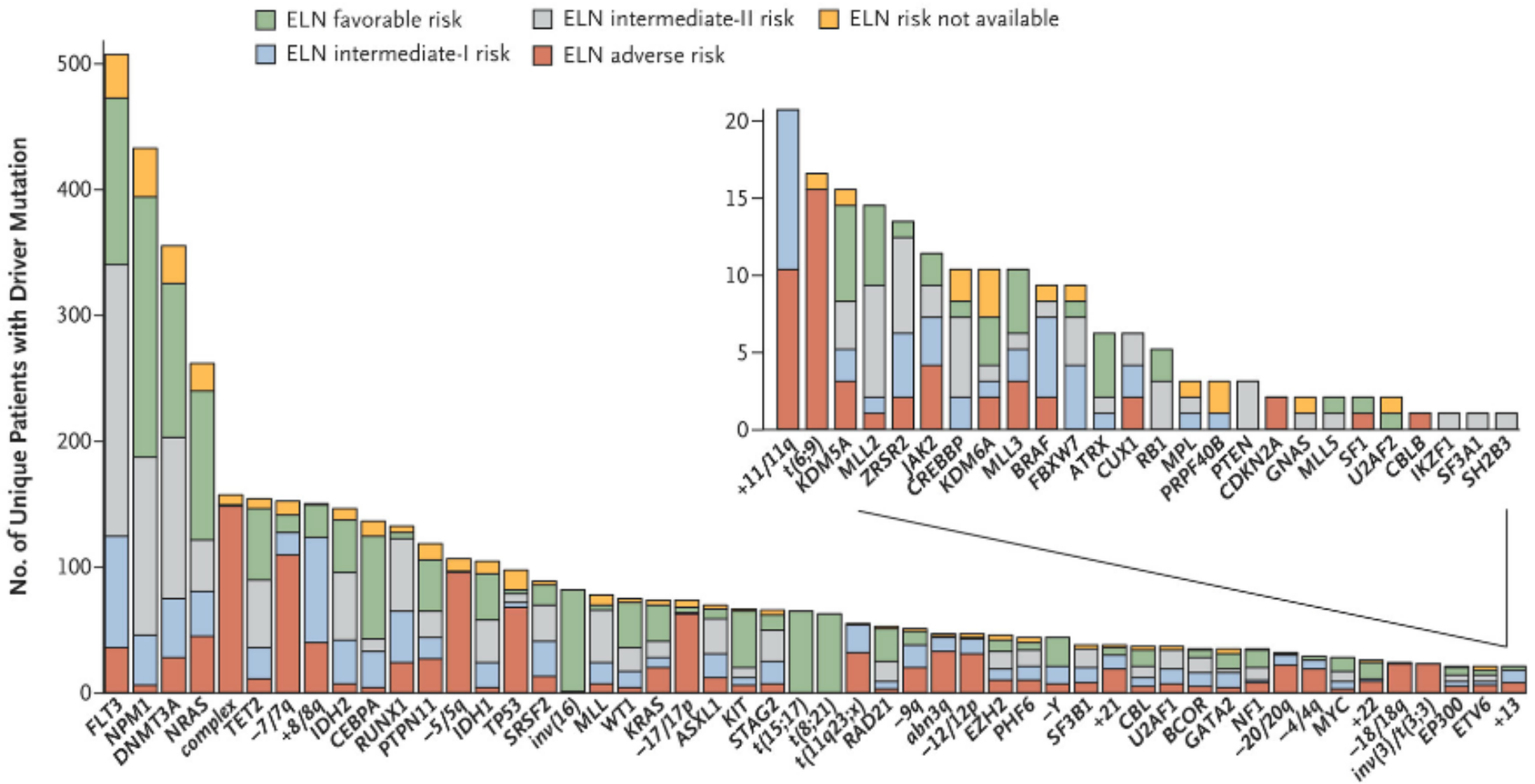
Genomic Landscape of Myeloid Neoplasms



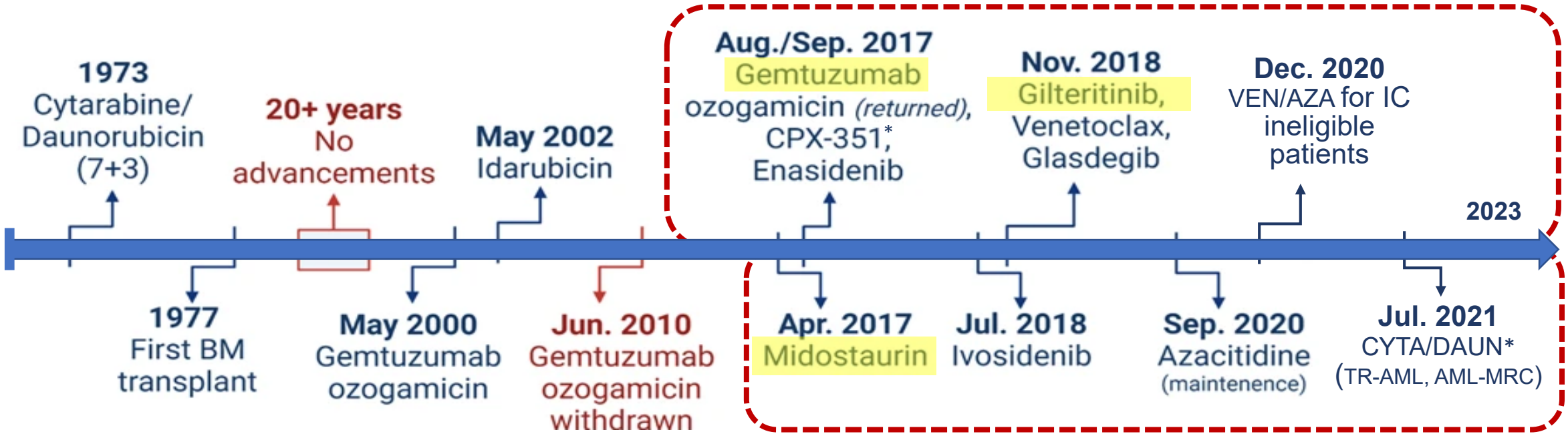
N ENGL J MED 368;22 NEJM.ORG MAY 30, 2013

- Gene fusions are common in AML and are frequently used for post-treatment monitoring.
- Somatic mutations (not shown) are also key drivers of leukemogenesis and are being targeted using novel small molecule inhibitors.
- More options for *quantitative* assessment of disease burden are needed as more treatment options emerge.
- A pathology-informed approach to AML monitoring is needed since the development of an all-purpose method is not likely.

Genomic Landscape of Myeloid Neoplasms



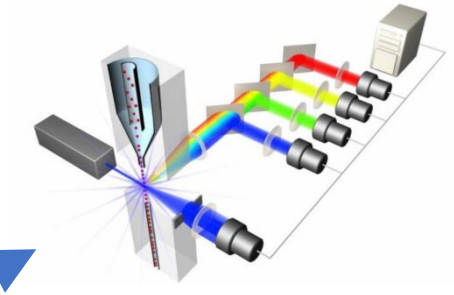
Emerging Therapies for AML

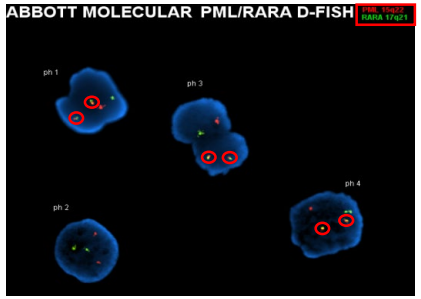


Carter, J.L., Hege, K., Yang, J. *et al. Sig Transduct Target Ther* 5, 288 (2020).

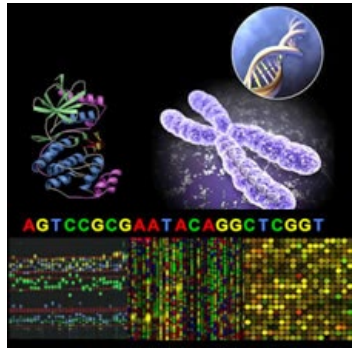
Diagnosis of Myeloid Neoplasms

Triage



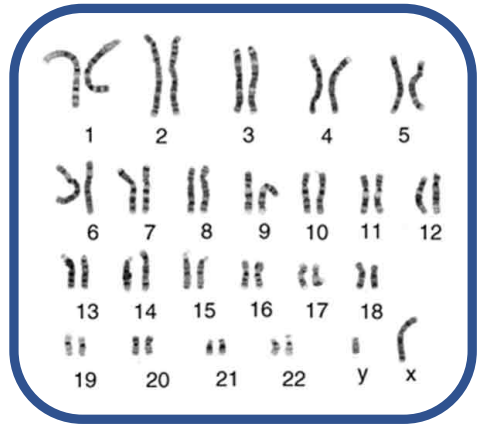


Triage



OGM?

ELN/NCCN

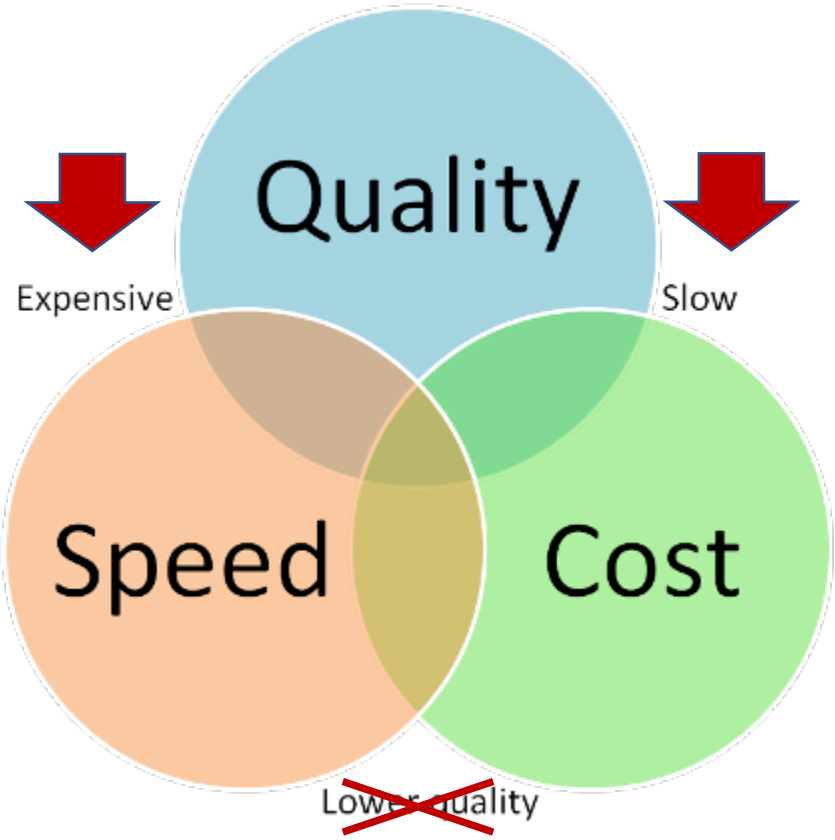


Germline Testing

HOURS **DAYS** **WEEKS**

Rapid Molecular Testing for Acute Leukemia

- Introduction of targeted therapies early in the treatment course demand more rapid molecular testing.
- PCR-based assessment of FLT3, NPM1, inv(16), t(8;21), t(9;22), t(15;17) when appropriate guide treatment decisions.
- Higher cost/test, technical demands and shrinking workforce offers motivation for increased efficiency.



Case presentation

- Bone marrow shows mild dyspoiesis and PCR-based (qualitative) assessment of NPM1 → **MUTATED**.
- Considerations: **MDS, AML, CHIP, CCUS, myeloid neoplasm NYD.**
- **NB:** WHO2022 *AML with defining genetic abnormalities*.

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

Leukemia (2022) 36:1703 – 1719

Test Ordering and Resource Allocation

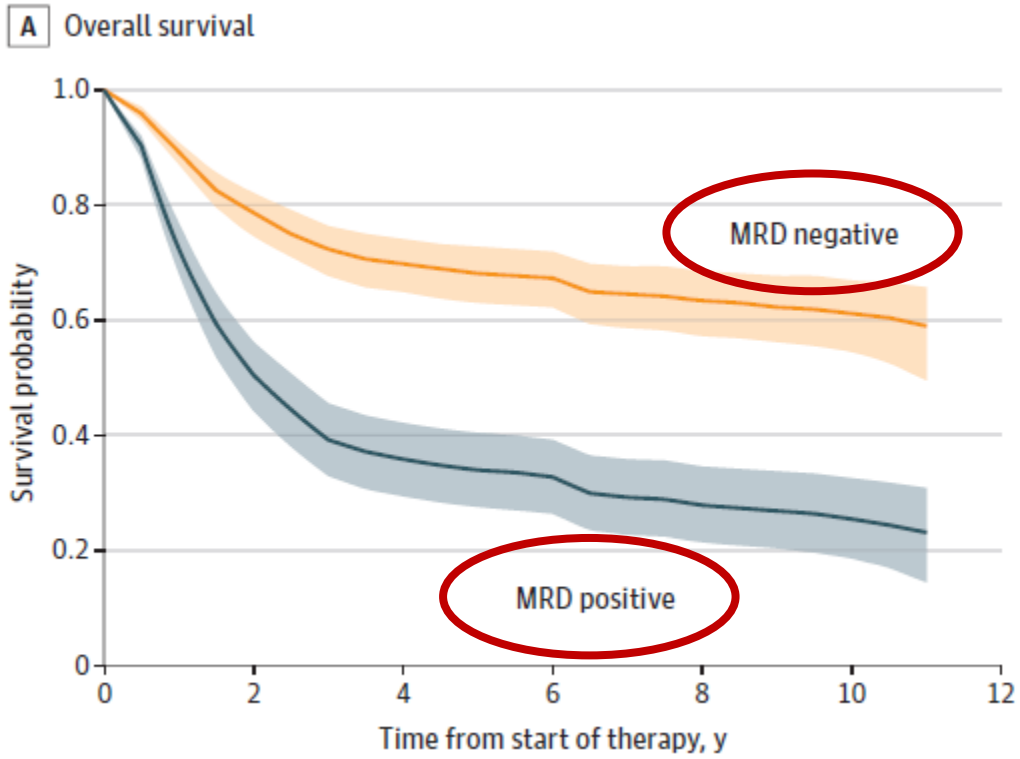
- As laboratory protocols used for AML diagnosis change, adopting best practices for *targeted testing* become more relevant.
- Debate over ‘reflex’ testing on all new cases of acute leukemia versus a more managed and data-driven approach.
- Potential merits of reflex testing include less time required for triage, faster TAT and economy of scale achieved due to higher test volumes.
- The perceived increases in *lab-associated* cost due to ‘unnecessary’ testing make this approach less palatable to administrators/health care funders.

Factors for Treatment Planning

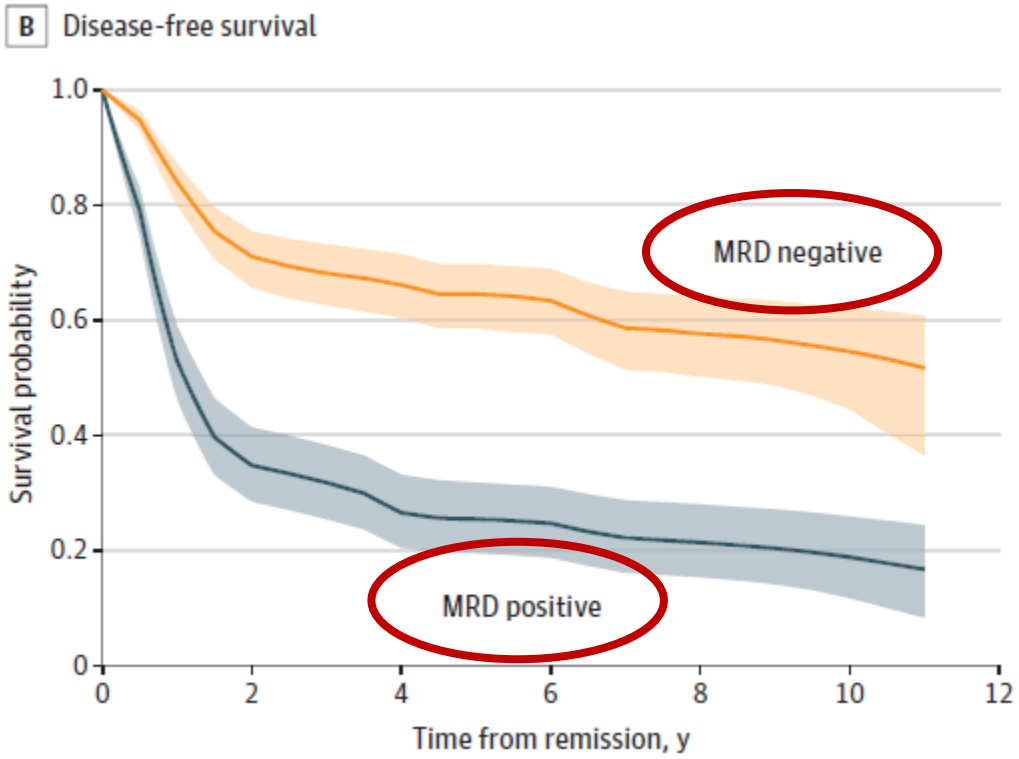
- Most traditional risk-stratification methods, including CK, were based on younger patients (<65) who received IC.
- More recent data shows that these methods do not accurately predict outcomes for treatment naïve AML and older patients.
- An updated risk stratification system is needed in the era of newer targeted therapies and alternatives to IC.
- Measurement of deep early response following IC +/- targeted therapies might be a reliable addition to RS.

Meta-analysis of OS/DFS Stratified by MRD

5yr OS
68% MRD-/34% MRD+



5yr DFS
64% MRD-/25% MRD+

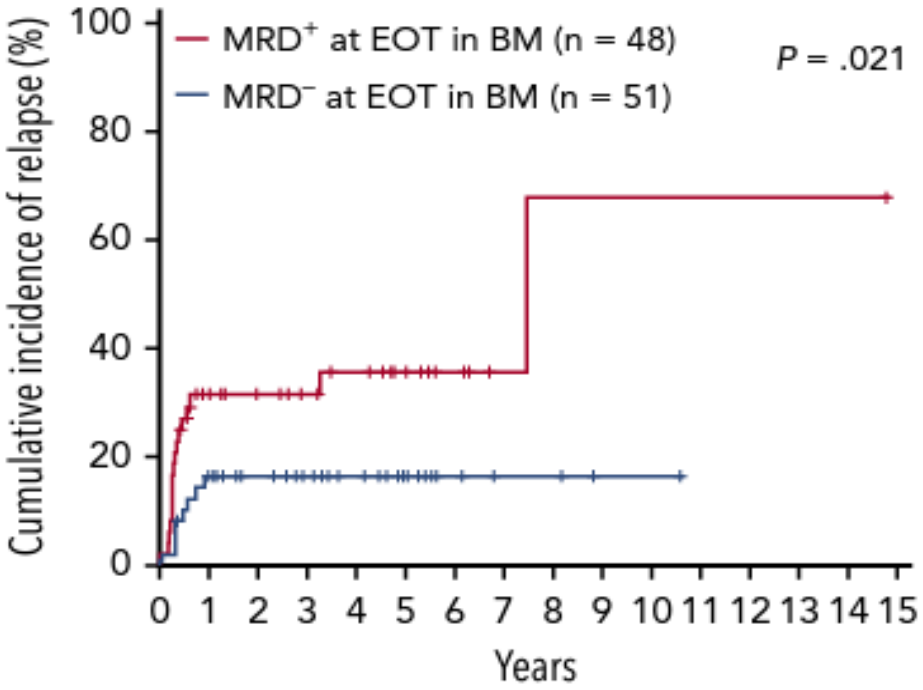


Short NJ et al., *JAMA Oncol* 2020; 6(12):1890-9

Informative for all Risk Categories

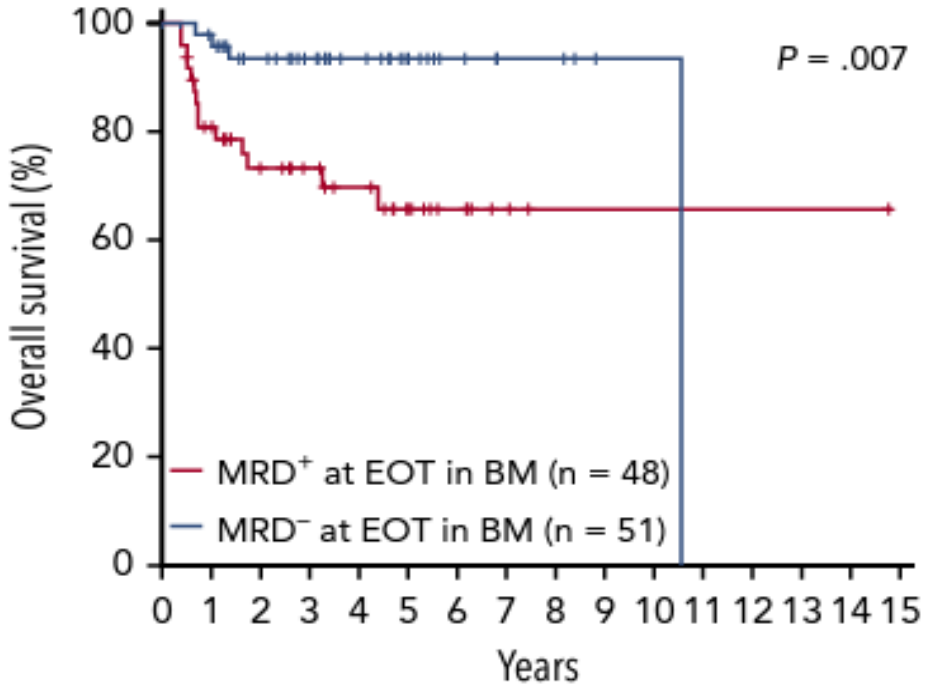
Decreased CIR in MRD- patients.

4-year CIR: 17% vs 36%



Superior OS in MRD- patients.

4-year OS: 93% vs 70%

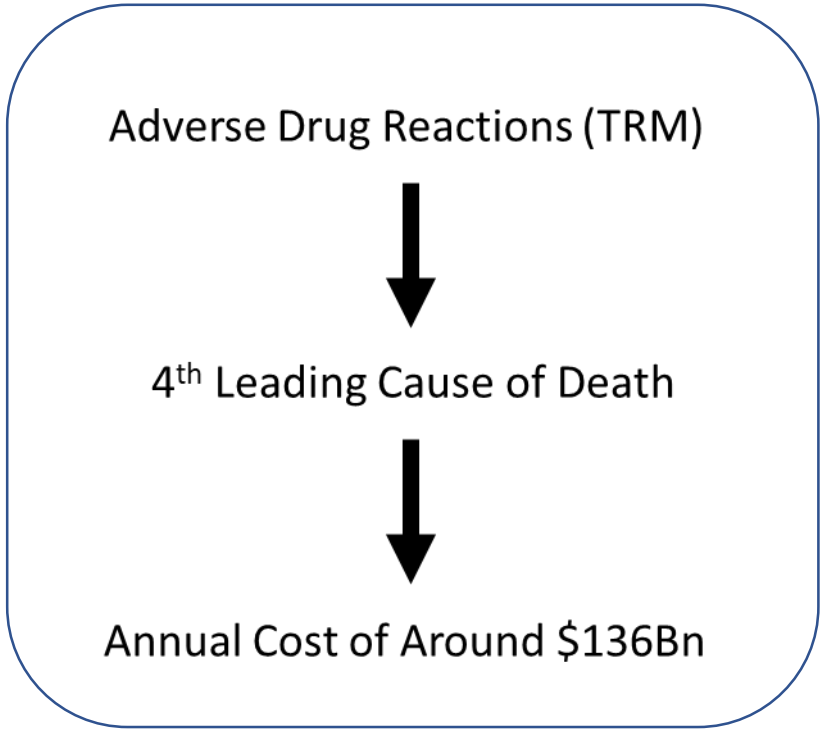


t(8;21)(q22;q22.1)

F. G. Rucker et al. *Blood*. 2019;134(19):1608-1618

Does response-adapted therapy add value?

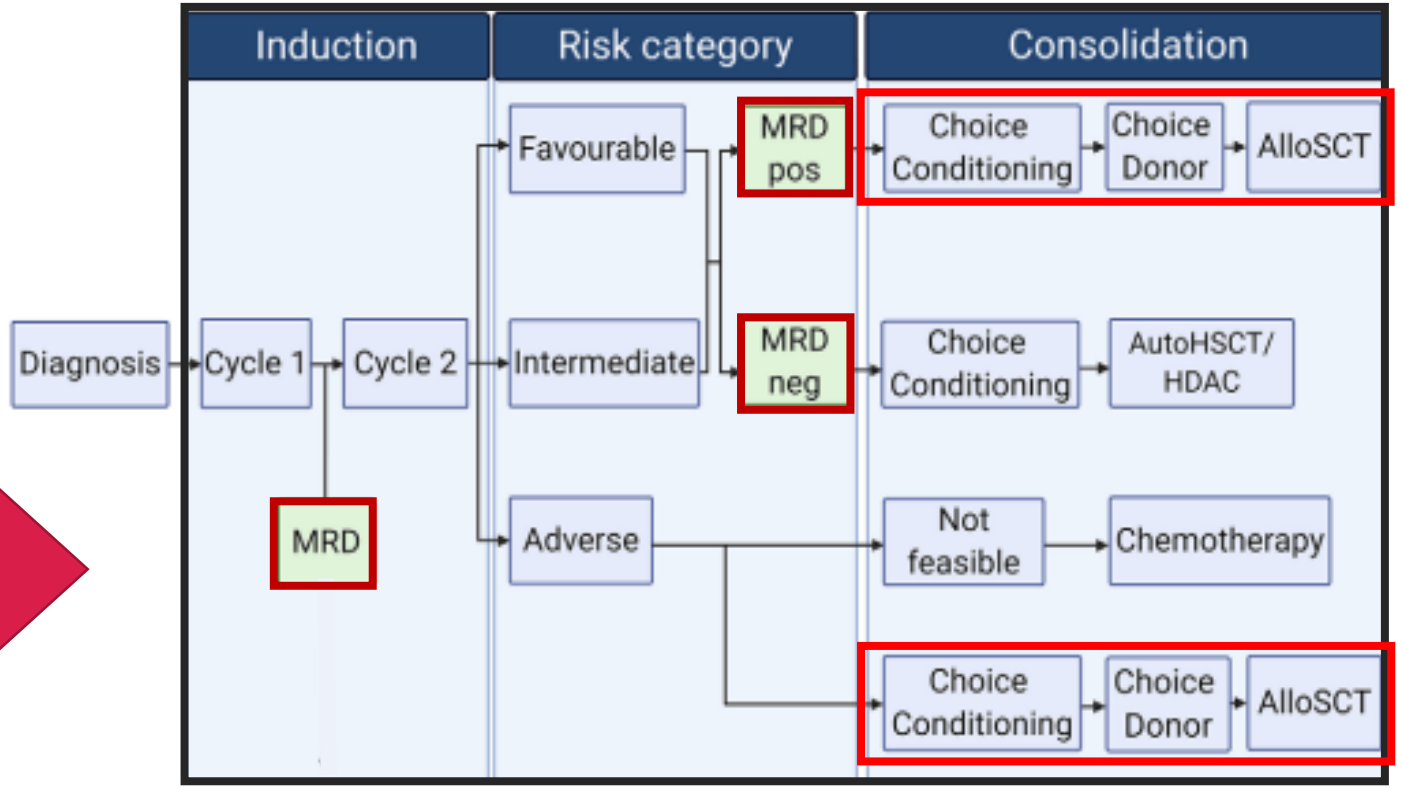
- Consolidation (post-remission therapy) is considered in younger/fit patients and includes options of additional chemo (HiDAC), allogeneic HSCT or autologous SCT.
- The best option of consolidation largely depends on the risk of the leukemia relapsing after treatment.
- Allogeneic HSCT may only benefit patients with a relapse risk of greater than **35%** (Cornelissen et al, BLOOD. 2007).
- Based on the observation that allo-HSCT can have serious complications, including an increased risk of death from treatment (TRM).



Harvard Business Review. 2007. Realizing the Promise of Personalized Medicine; FDA; bioMérieux Internal Database; Cowen & Co.

Frontiers in AML Monitoring/Risk Assessment

Ngai et al. Front Oncol. 2021 Jan 15;10:603636.



For AML, early deep response has an excellent NPV.
There is an unmet clinical need for performing high-sensitivity MRD testing to help inform clinical decision making (i.e. HSCT).

Molecular markers for AML monitoring

- NPM1
- IDH1/2
- DNMT3A
- CEBPA
- MLL-PTD

- t(8;21)/RUNX1-RUNX1T1
- Inv(16)/CBFB-MYH11
- t(15;17)/PML-RARA
- t(7;11)/NUP98-HOXA9
- t(11;v)/MLL-partner gene

	Optimal	Warning	Failure
Baseline	NA	High-risk ACA, high-risk ELTS score	NA
3 months	≤10%	>10%	>10% if confirmed within 1–3 months
6 months	≤1%	>1–10%	>10%
12 months	≤0.1%	>0.1–1%	>1%
Any time	≤0.1%	>0.1% Loss of ≤ 0.1% (MMR) *	>1%, resistance mutations high-risk ACA

* Loss of MMR (BCR-ABL1 > 0.1%) indicates failure after TFR. NA: not applicable; ELTS: EUTOS score for long-term survival; ACAs: additional chromosomal aberrations; MMR: major molecular remission.

MILESTONES

Case presentation

- Cytogenetic karyotyping results show 46XY.
- NGS confirms NPM1 mutation with a **VAF of 9%**.
- Case signed out as:

> ✓ *Trilineage hematopoiesis with mild dyspoiesis (NPM1 mutated).*
>
> ✓ *Close follow up and repeat bone marrow biopsy is recommended.*

Conclusion

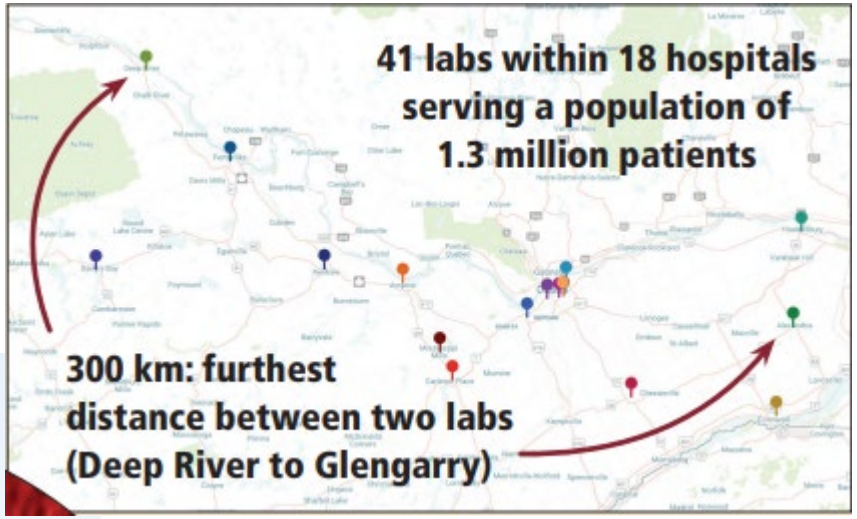
- NPM1 mutation is a durable marker and in the context of AML requires correlation b/w morphologic findings and molecular genetic studies.
- Some argue this approach is more appropriate than assigning an ‘arbitrary’ lower bone marrow blast cut-off (i.e. ICC ≥ 10% blasts).
- Recent data shows that cases previously classified as MDS or MDS/MPN with *NPM1* mutated progress to AML in a short period of time.
- Similar data have emerged from patients with clonal hematopoiesis who acquire *NPM1* mutation.

Conclusion

- Despite improved genetic classification and CR rates close to **~80%**, more than **50%** of adult patients with AML will undergo disease relapse.
- Traditional diagnostic and frontline treatment algorithms continue to improve but morbidity/mortality in AML remains high.
- Standardizing diagnostic/monitoring protocols comes with challenges due to complexity in which clonal heterogeneity prohibits a "**one size fits all**".
- Canadian consensus recommendations aimed at reducing subjectivity and defining best practices are needed.

Thank you


Cancer Centre
Centre de cancérologie


41 labs within 18 hospitals
serving a population of
1.3 million patients
300 km: furthest
distance between two labs
(Deep River to Glengarry)