Methodological Overview:
MRD in Acute Myeloid Leukemia

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3rd CDDF Multi-stakeholder Workshop
Minimal Residual Disease (AML/CLL)
4pm Thursday 8th November 2018

NIH National Heart, Lung, and Blood Institute

Disclosures

- **Research Funding:**

Collaborative Research And Development Agreements

- Sellas Life Sciences AG (laboratory funding)
- Merck (laboratory and clinical trial funding)

Research collaborations (incl. pricing discounts): Qiagen, Archer.

- **Advisory Boards:**

Janssen, Novartis (*as official duties*)

Disclaimer

Any views expressed here represent personal opinion and do not necessarily reflect those of the U.S. Department of Health and Human Services, or the United States federal government

My Mandate Today

Evaluation methods (MFC and molecular): Patient selection, method selection, technical requirements, thresholds, compartment (BM vs PB), timepoints for measurement, other aspects. Status of standardization and quality assurance, expected next steps. Areas of certainty and uncertainty.

Outline

1) Why is MRD hard in AML?

2) Tools to estimate AML MRD

3) Where does NGS fit in? Limits to the routine clinical use of AML MRD and where we go next…

Outline

1) **Why is MRD hard in AML?**

2) Tools to estimate AML MRD

3) Where does NGS fit in? Limits to the routine clinical use of AML MRD and where we go next…

“*Oh honey, there ain’t nothing complete about a complete remission*”

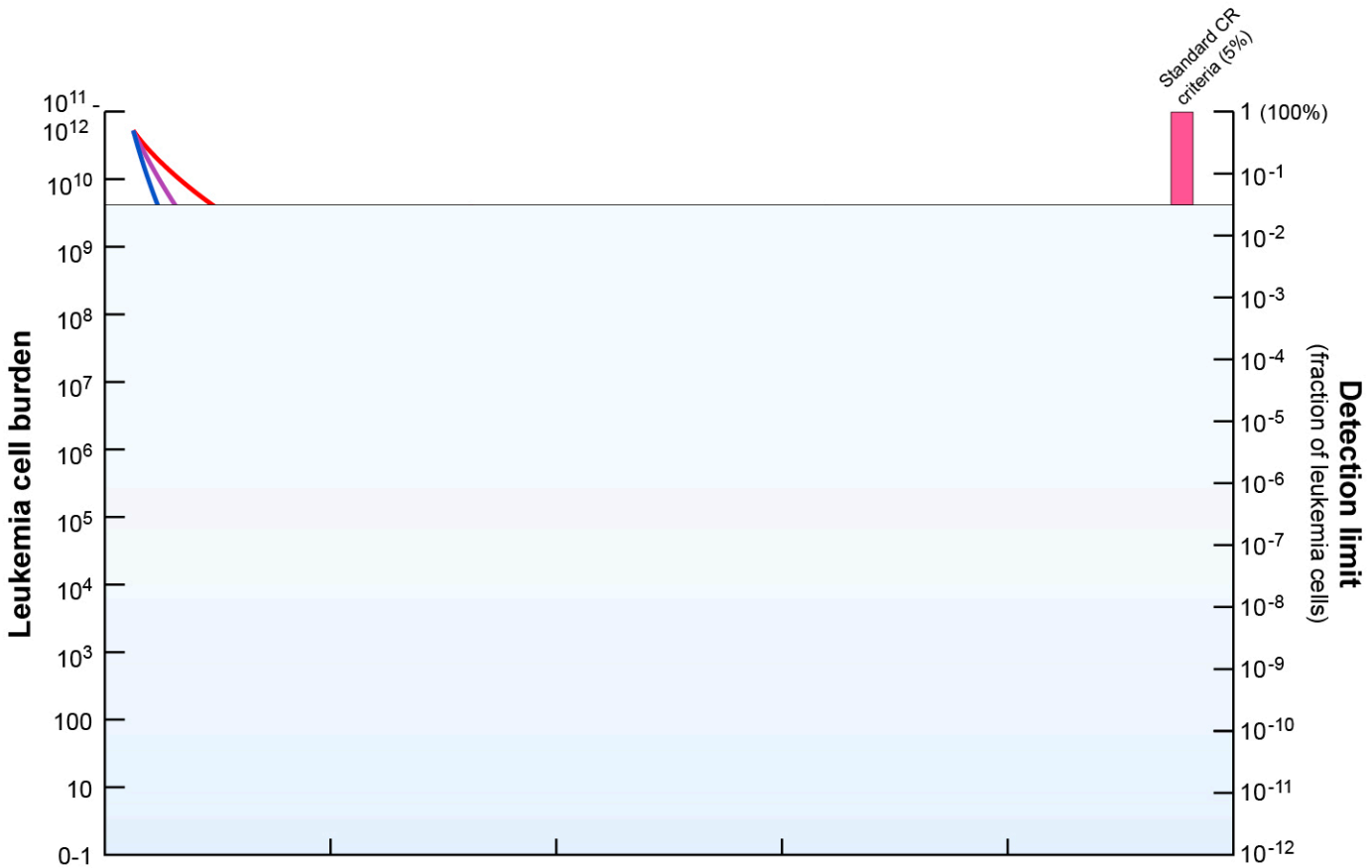
Professor Judy Karp
Head of Leukemia Program, Johns Hopkins
Heard on ward-round, ~2008

Aaron Logan, MD, PhD
@hemedoc

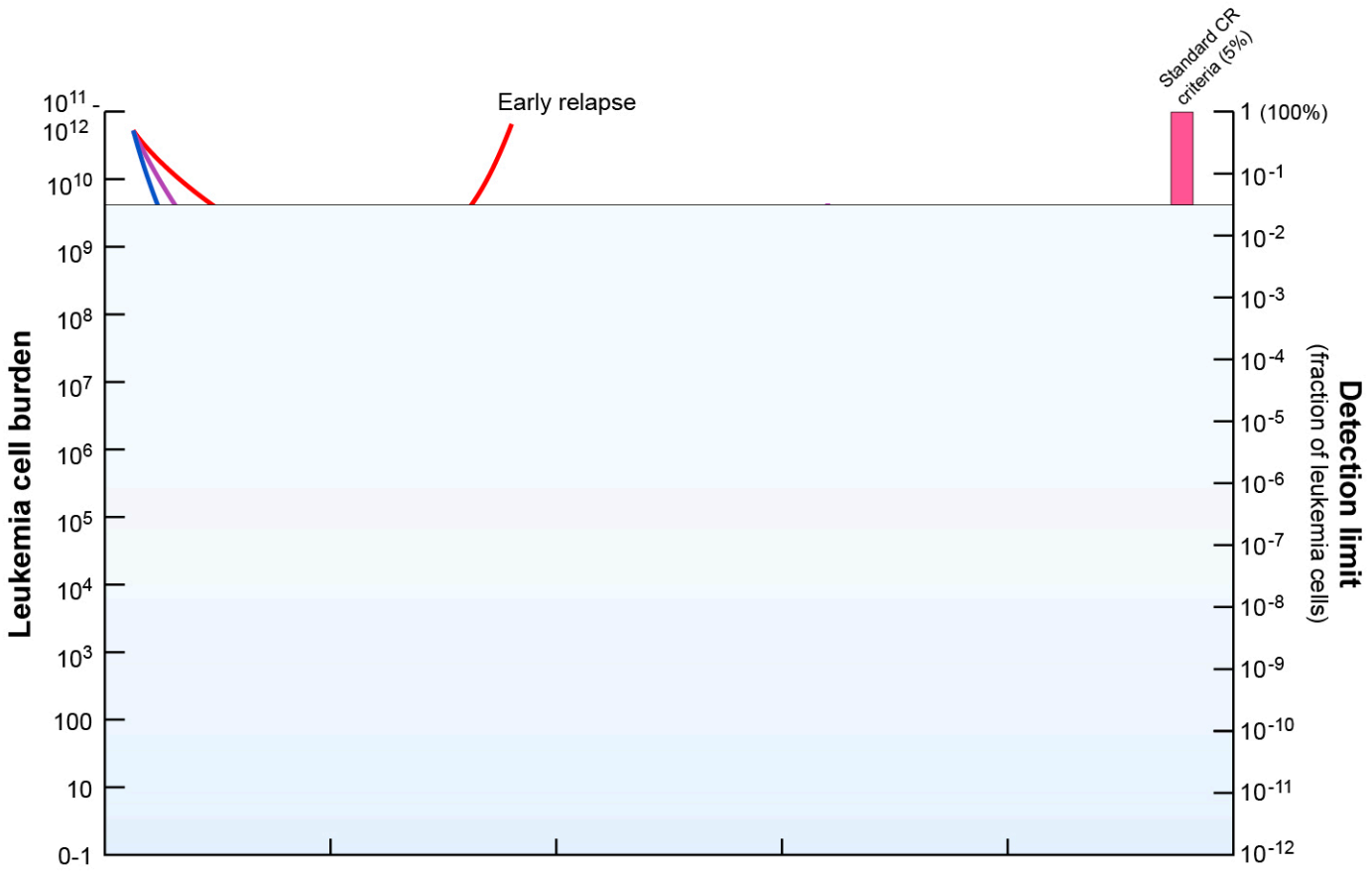
Following

Christopher Hourigan: What is MRD? Not minimal residual disease but MEASURABLE residual disease. #bmttandem14

8:36 AM - 1 Mar 2014



Hourigan and Karp, Nat Rev Clin Onc. 2013



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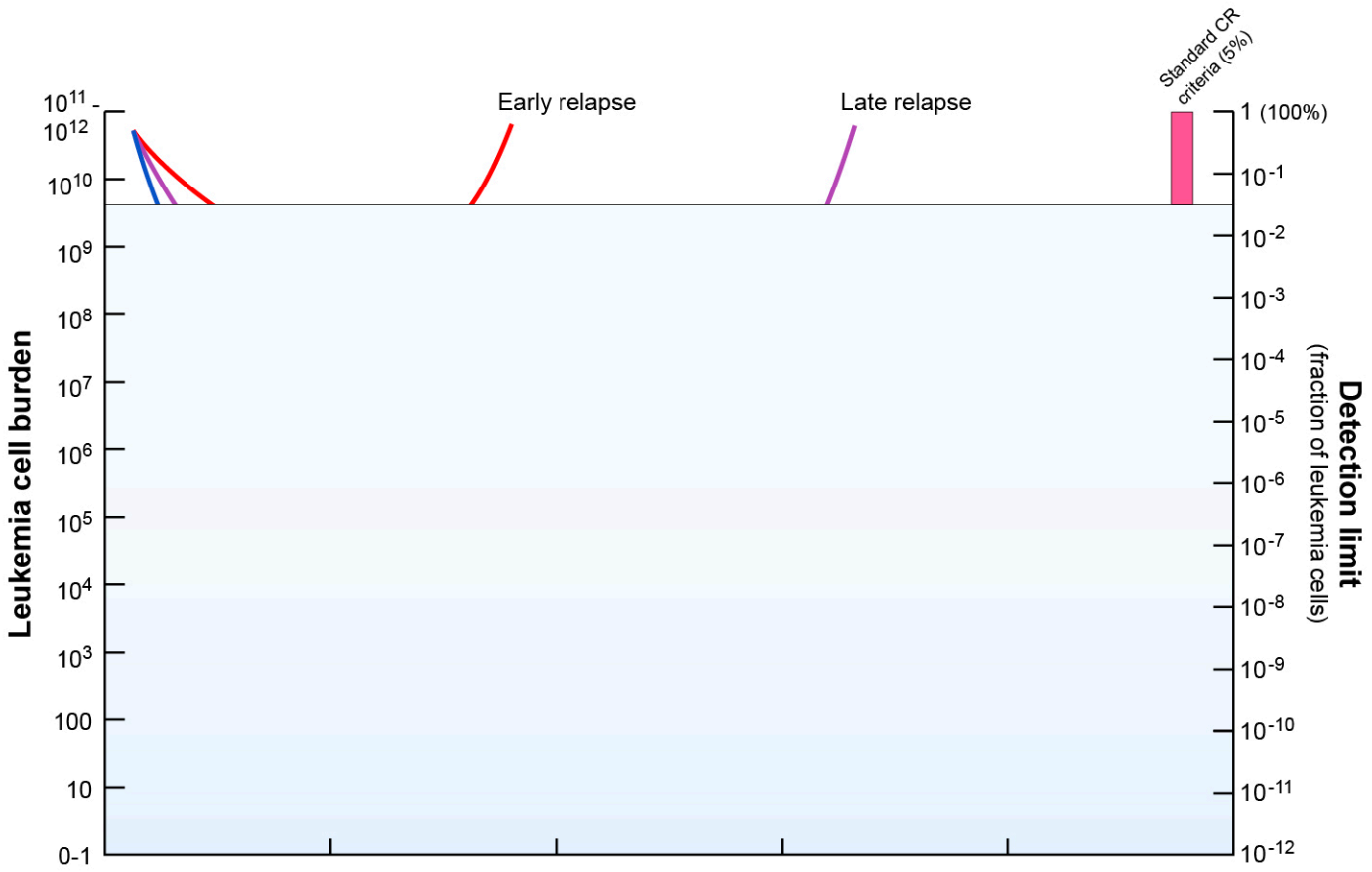

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Letter to the Editor—*Criteria for the Evaluation of Response to Treatment in Acute Leukemia*

The Clinical Studies Panel of the Cancer Chemotherapy National Service Center believes that a precise definition of response to treatment in acute leukemia will allow better comparison of results among patients treated by different drugs and by different physicians. Criteria for evaluation of response are appended. Since they represent an initial attempt at codification, errors of omission or commission may be present. The Panel believes that observation of these criteria in practice and in published reports will provide a basis for more meaningful interpretation of response to drugs currently in use, and to new agents which may come under study. Constructive criticism of these criteria is invited, and if modifications are adopted by the Panel, they will be submitted to this journal.

Blood, July 1956

Table 1 | Evolution of criteria for complete remission in adult patients with AML

Response criteria	1956
Bone marrow	Less than 5% blasts and absence of cells that can be individually identified as leukaemic
Extramedullary disease	Subsidence of all evidence of leukaemic infiltration
Platelets	>100,000
Neutrophil count	>200/mm^3
Haemoglobin	>12 gm/dl for 1 month
Clinical	No symptoms attributable to leukaemia

An additional category of 'cytogenetics normal in those with previously abnormal cytogenetics' is listed, but not required for a morphological complete response in the complete remission category of 2012 National Comprehensive Cancer Network guidelines[10] and is "commended primarily for use in clinical research studies" in the current International Working Group criteria[137] that those guidelines are based on. There is evidence, however, that persistence of cytogenetic abnormalities in patients with AML in complete remission is associated with a worse prognosis.[138] Abbreviation: AML, acute myeloid leukaemia.

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Table 1 | Evolution of criteria for complete remission in adult patients with AML

Response criteria	1956	2016
Bone marrow	Less than 5% blasts and absence of cells that can be individually identified as leukaemic	Less than 5% blasts including no blasts with Auer rods
Extramedullary disease	Subsidence of all evidence of leukaemic infiltration	No residual evidence of extramedullary disease
Platelets	>100,000	>100,000
Neutrophil count	>200/mm^3	>1,000/mcL
Haemoglobin	>12 gm/dl for 1 month	Transfusion-free
Clinical	No symptoms attributable to leukaemia	No criteria

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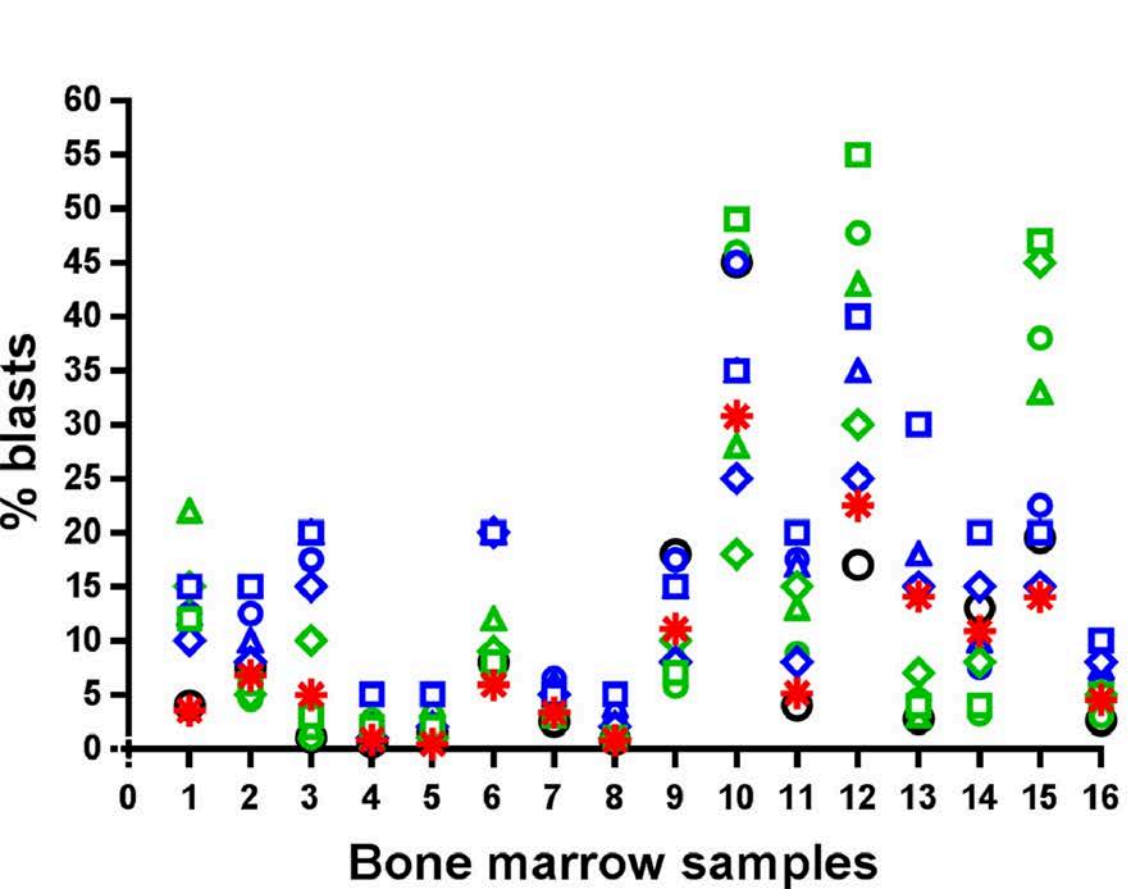
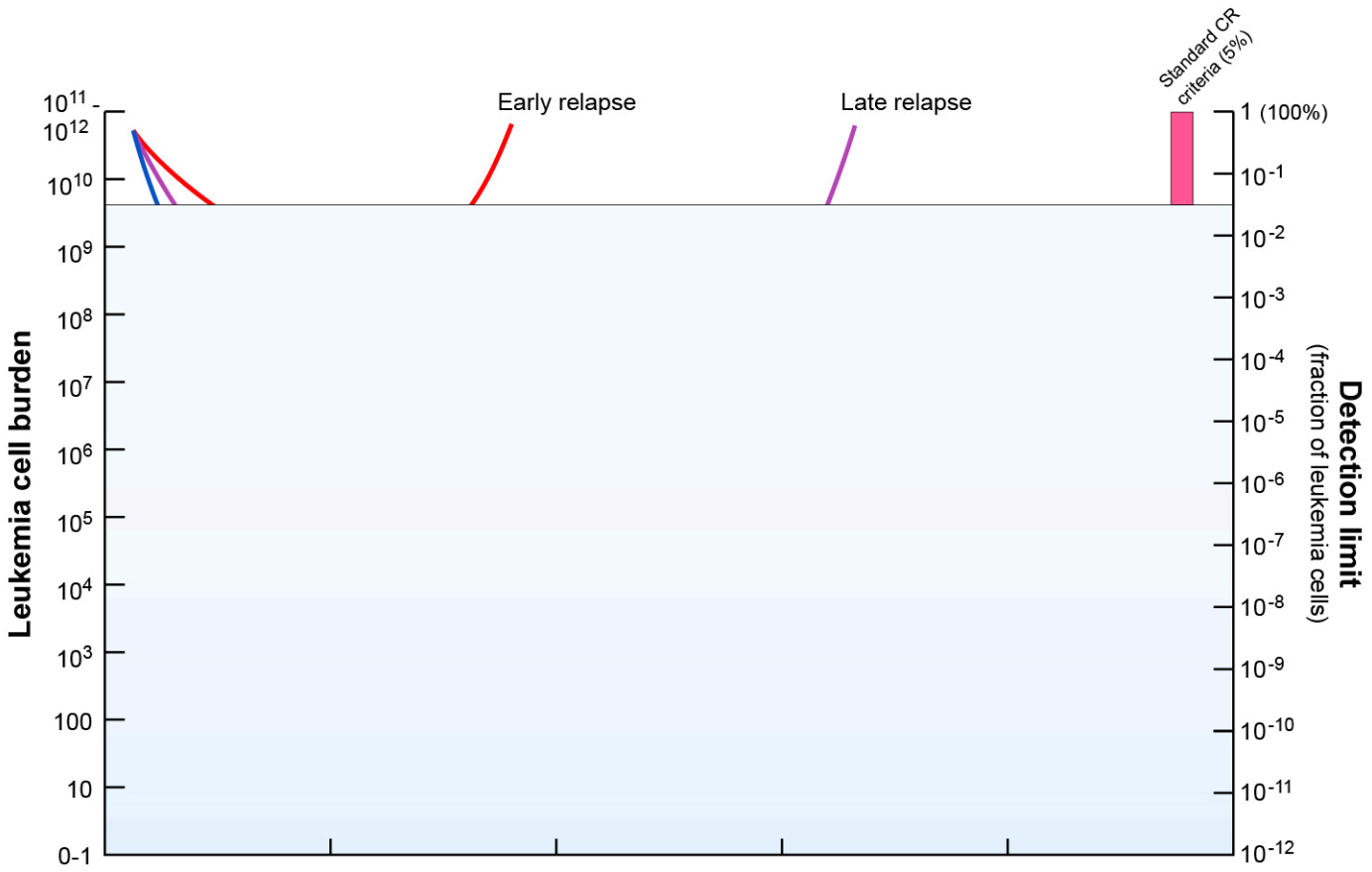


- Mean blast percentage (Digital Reference)
- Hematopathologist A Biopsy
- Hematopathologist A Smear
- Hematopathologist B Biopsy
- Hematopathologist B Smear
- Hematopathologist C Biopsy
- Hematopathologist C Smear
- Hematopathologist D Biopsy
- Hematopathologist D Smear
- Flow Cytometry

Hodes, Seminars in Hematology, 2018



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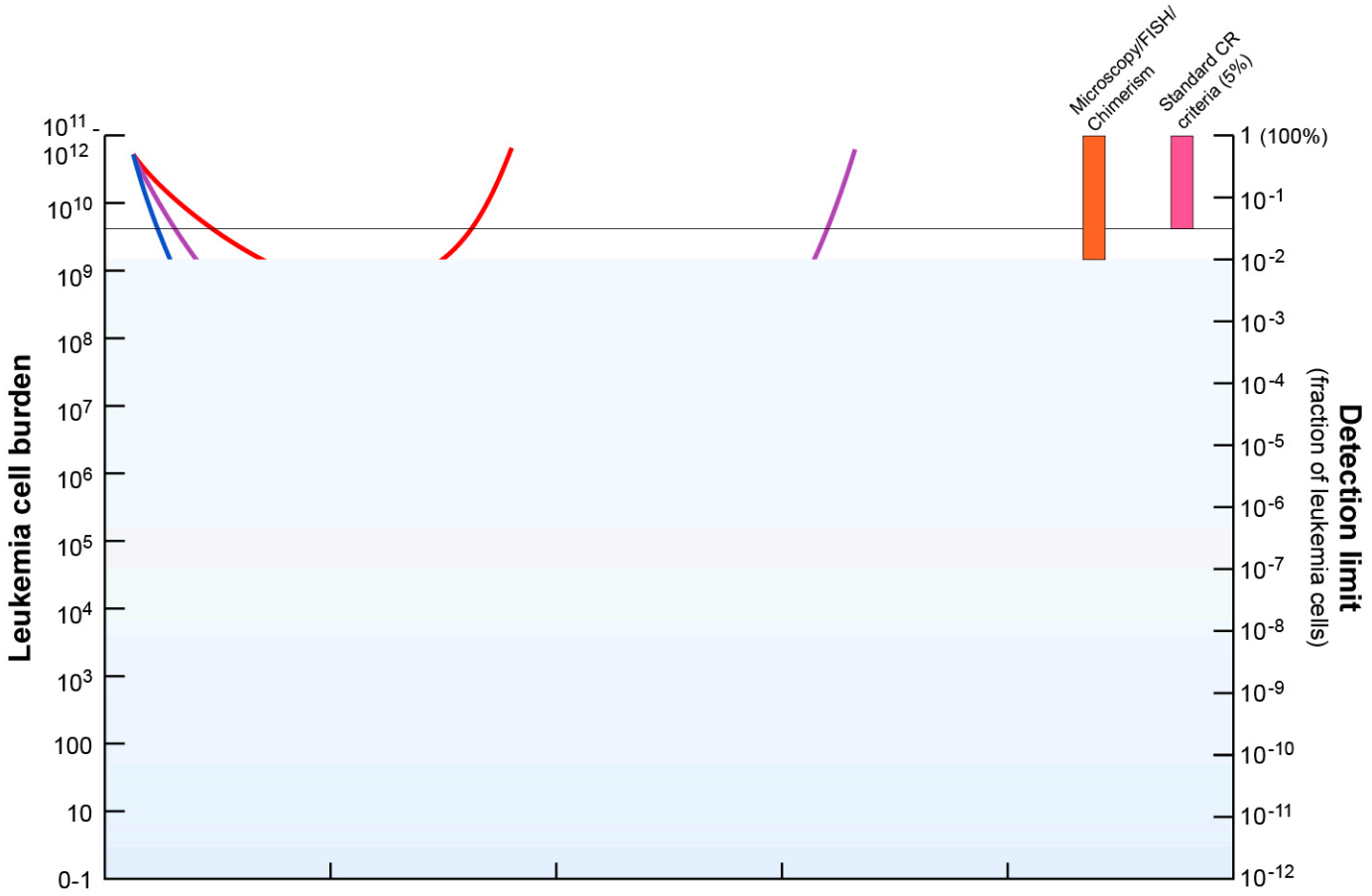

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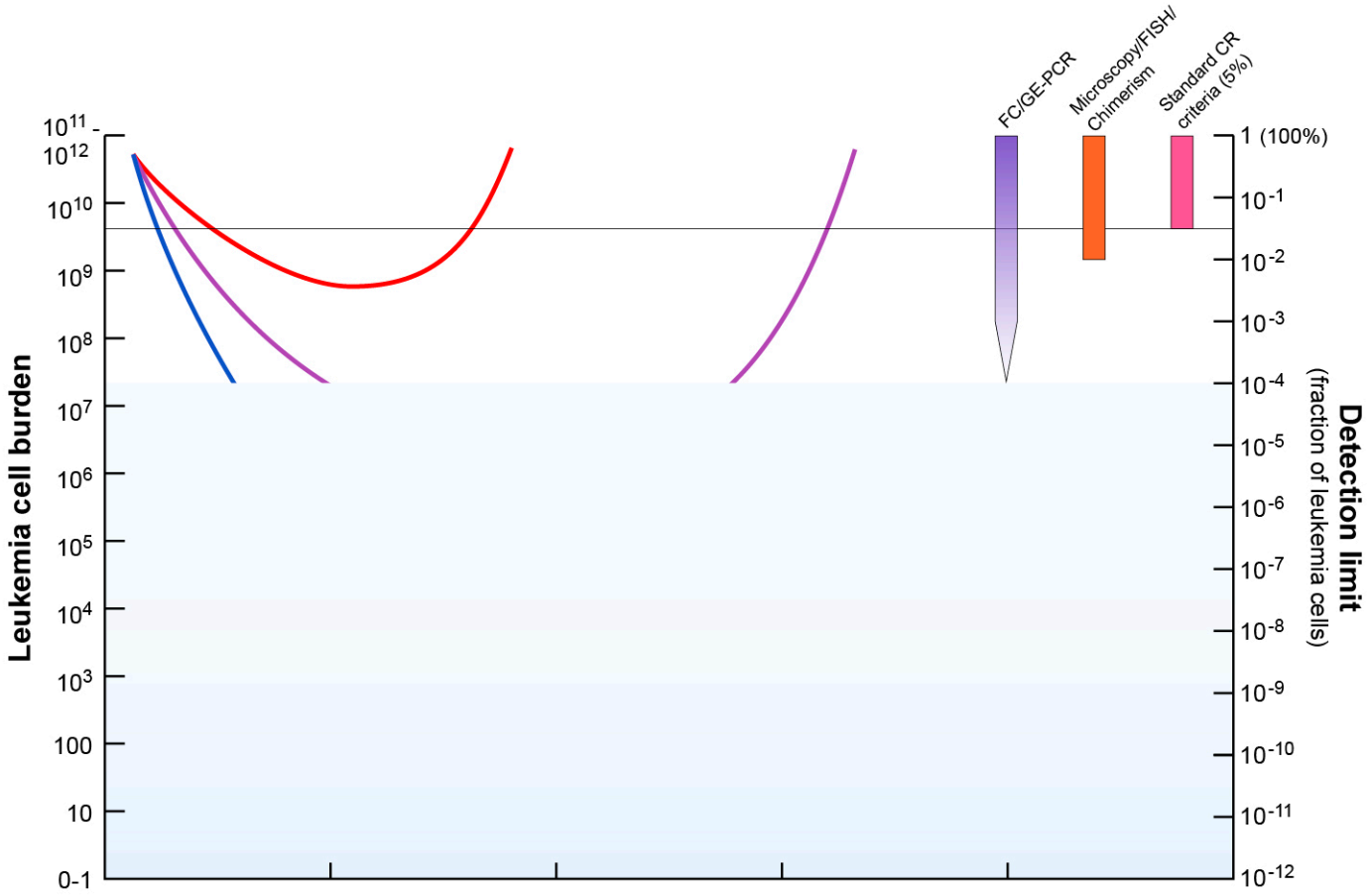

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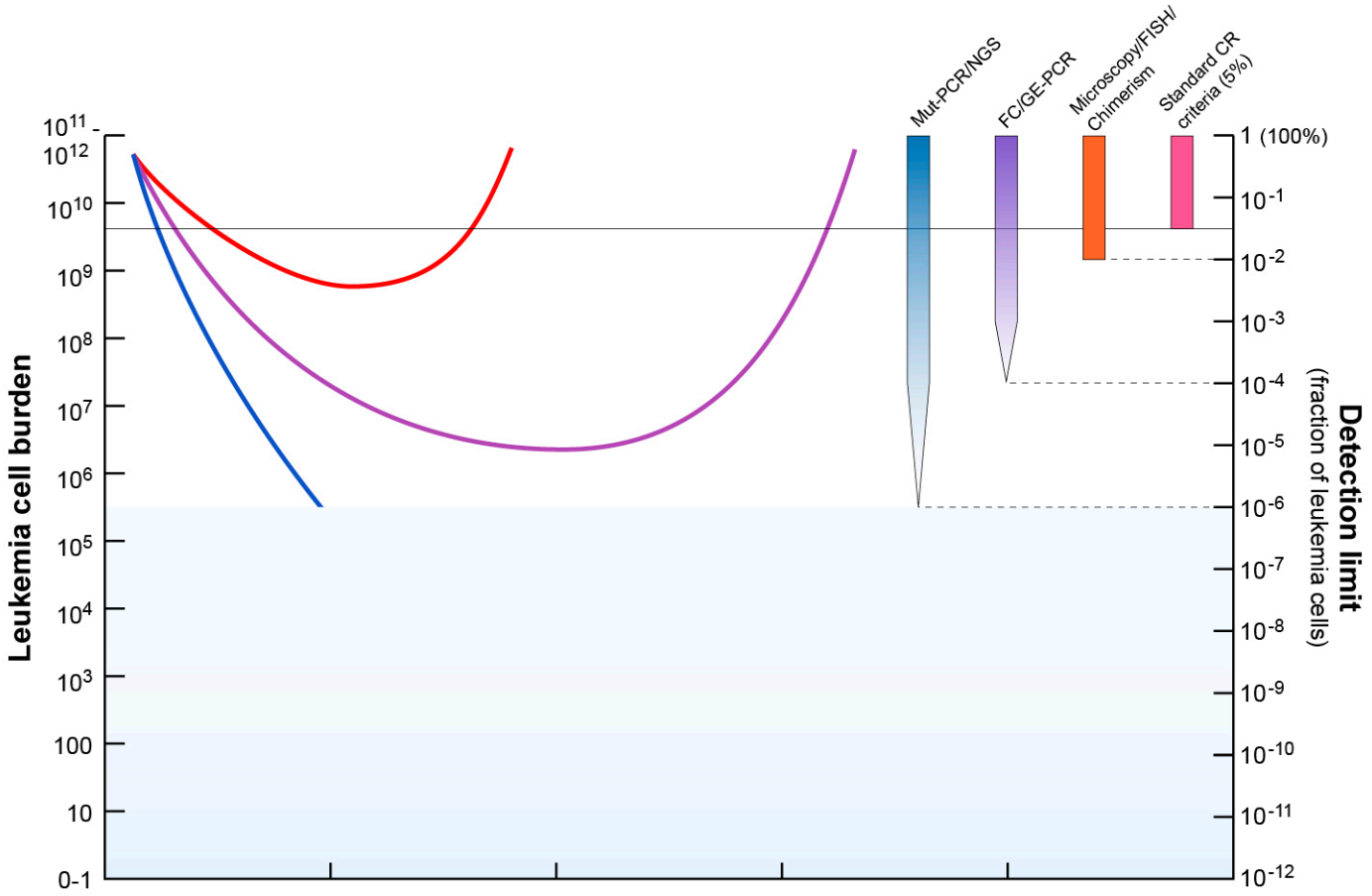

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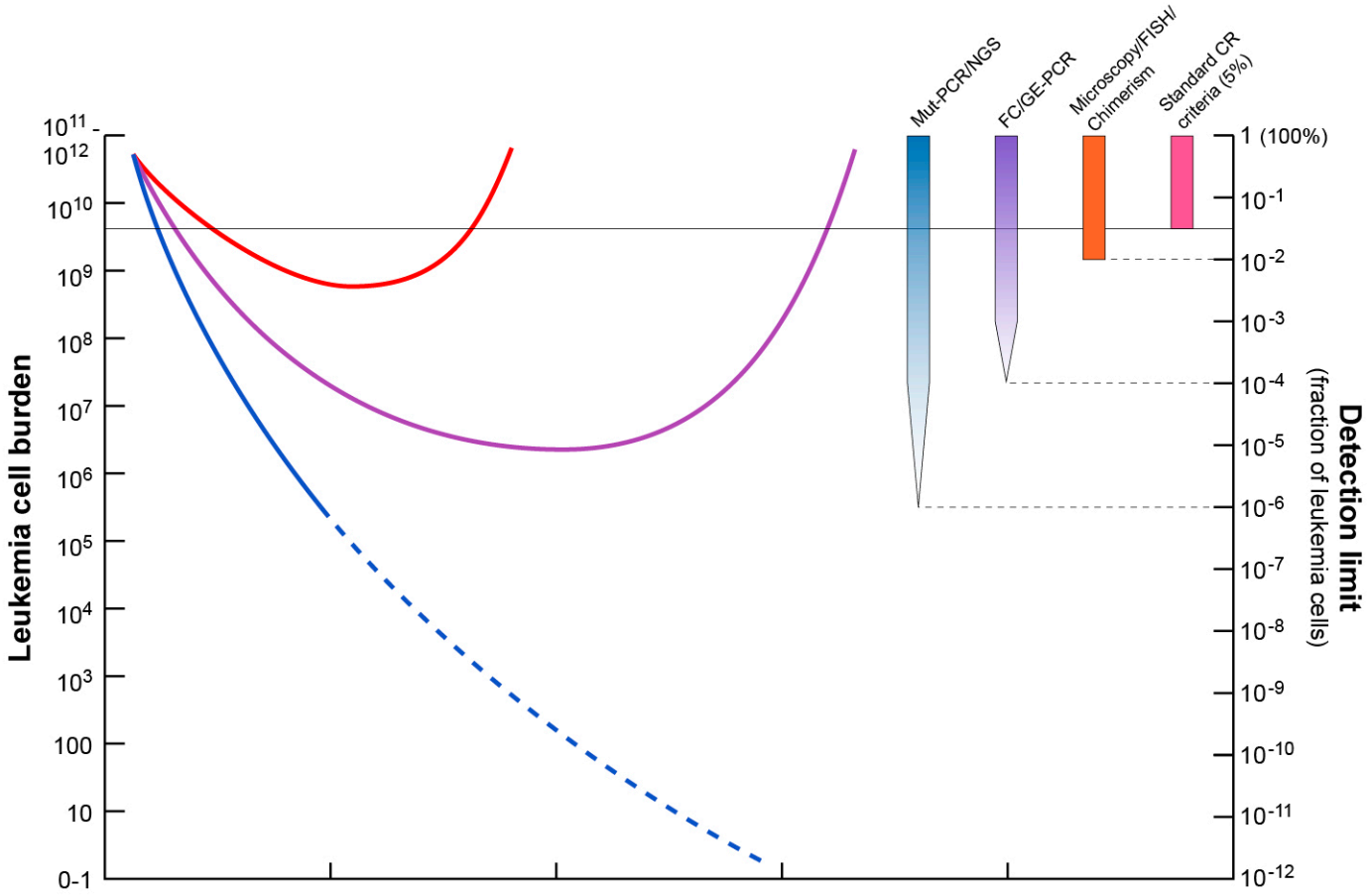

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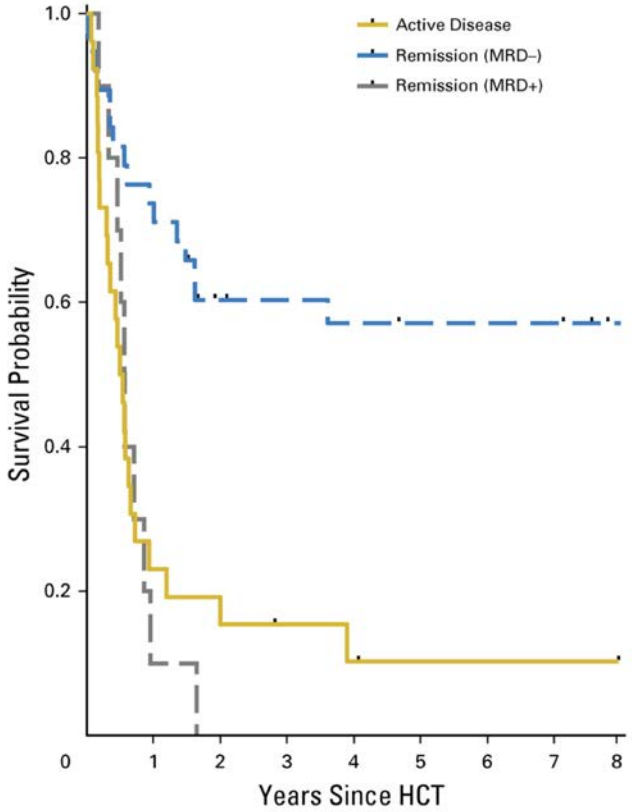
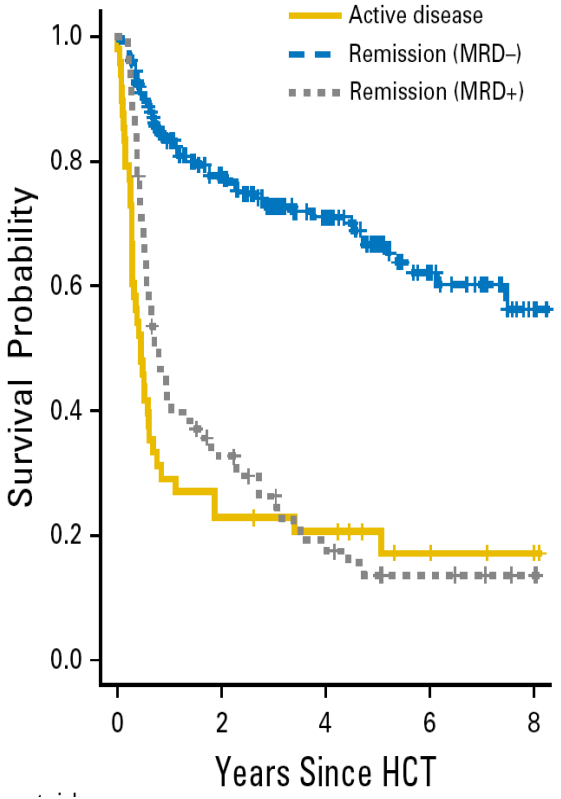
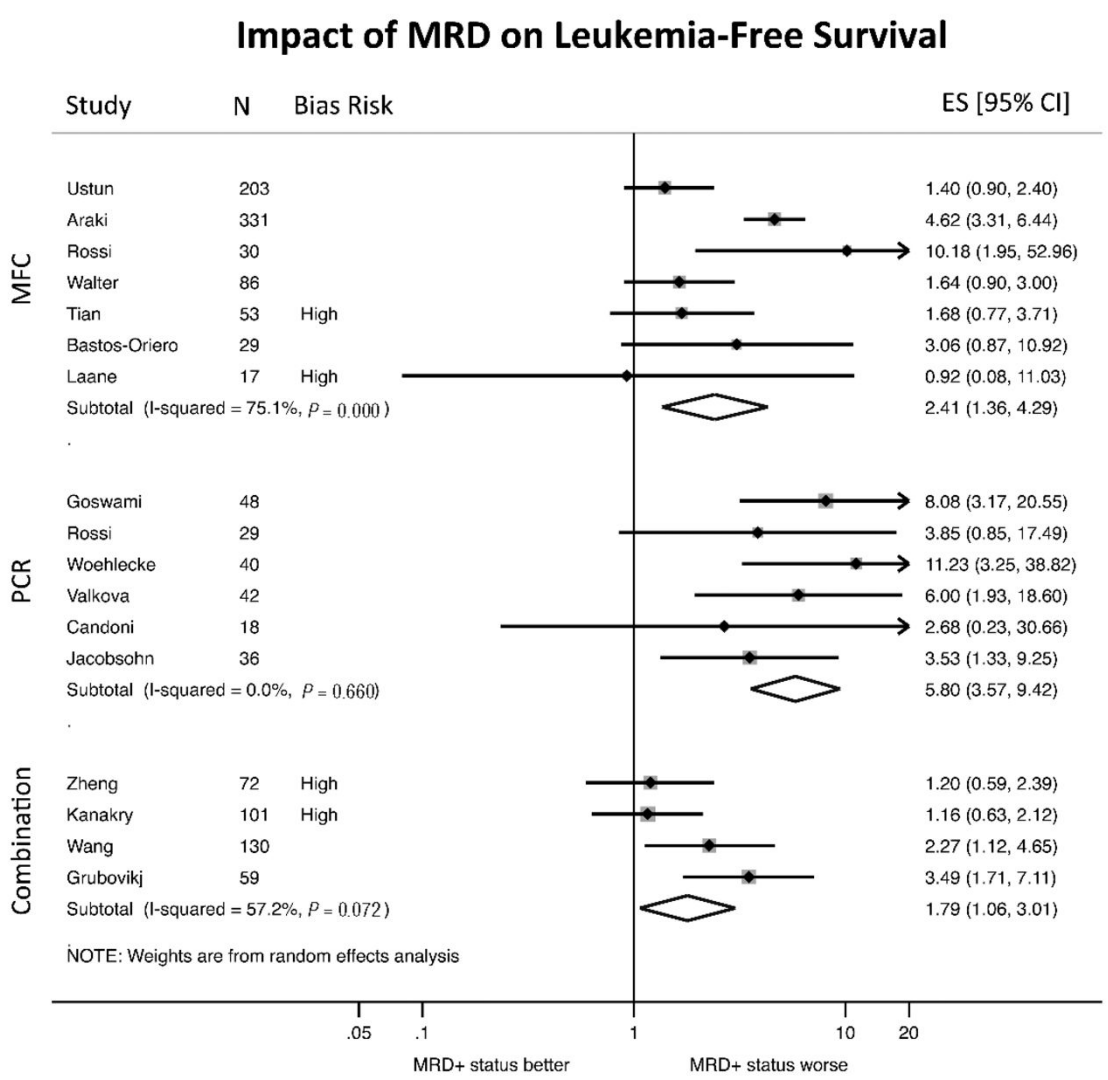
PRESENCE OF AML MRD BEFORE ALLO-SCT IS ASSOCIATED WITH POOR SURVIVAL



Hourigan *et. al.*, JCO 2016



Araki *et. al.*, JCO 2016



Buckley *et. al.*, 2017

Blood. 2017 Jan 26;129(4):424-447. doi: 10.1182/blood-2016-08-733196. Epub 2016 Nov 28.

Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel.

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⊕ Author information

Abstract

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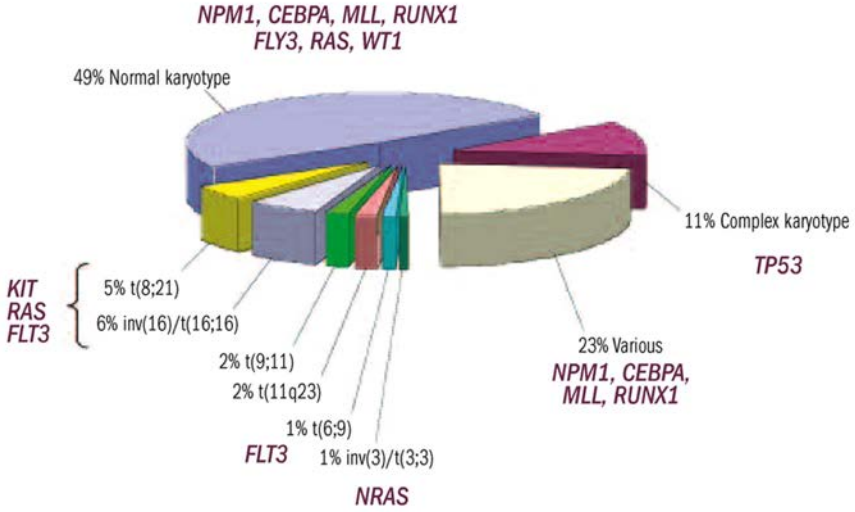
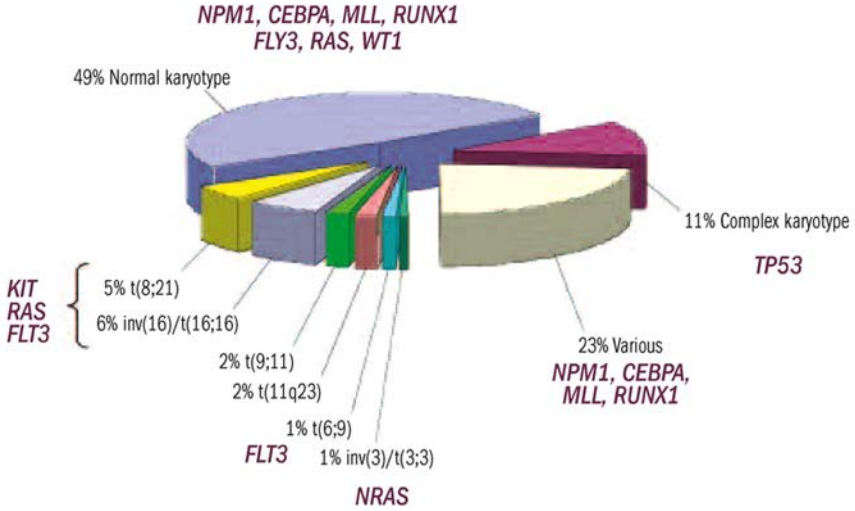
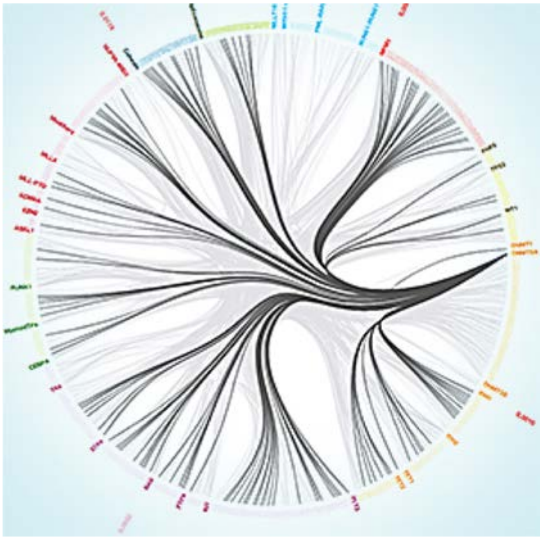
Category	Definition	Comment
Response		
CR without minimal residual disease (CR_{MRD-})	If studied pretreatment, CR with negativity for a genetic marker by RT-qPCR, or CR with negativity by MFC	Sensitivities vary by marker tested, and by method used; therefore, test used and sensitivity of the assay should be reported; analyses should be done in experienced laboratories (centralized diagnostics)
Complete remission (CR)	Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC ≥1.0 × 10^9/L (1000/μL); platelet count ≥100 × 10^9/L (100 000/μL)	MRD^+ or unknown
CR with incomplete hematologic recovery (CR_i)	All CR criteria except for residual neutropenia (<1.0 × 10^9/L [1000/μL]) or thrombocytopenia (<100 × 10^9/L [100 000/μL])	
Morphologic leukemia-free state (MLFS)	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required	Marrow should not merely be "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%
Partial remission (PR)	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%	Especially important in the context of phase 1-2 clinical trials

Why did it take 61 years to update response criteria?



Why MRD is hard in AML…





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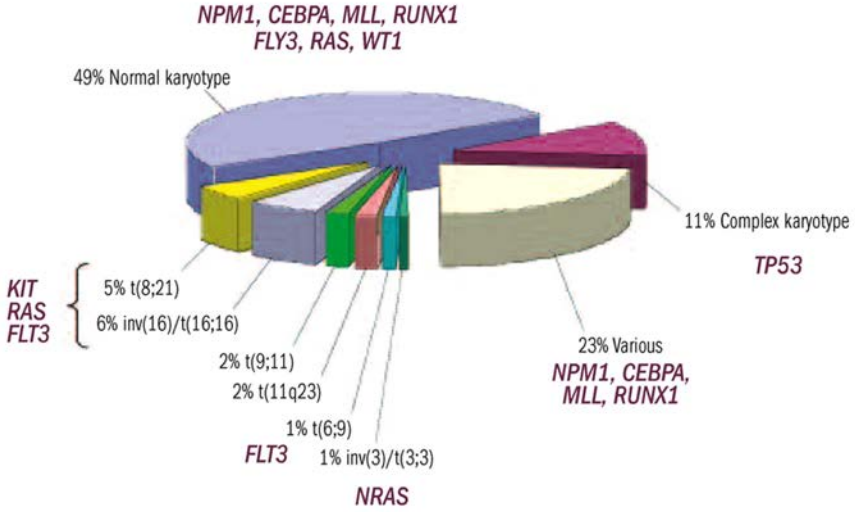

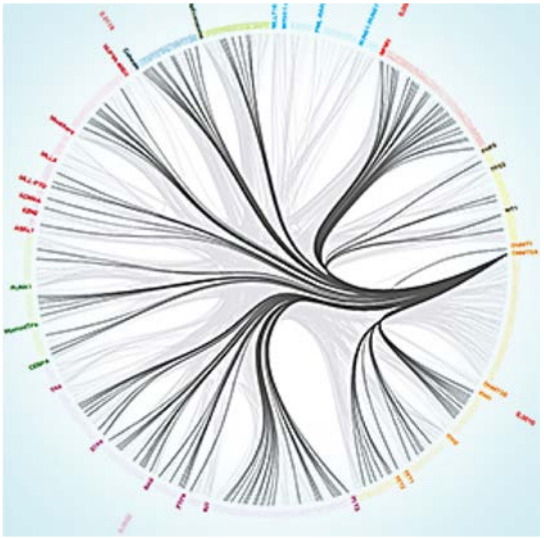

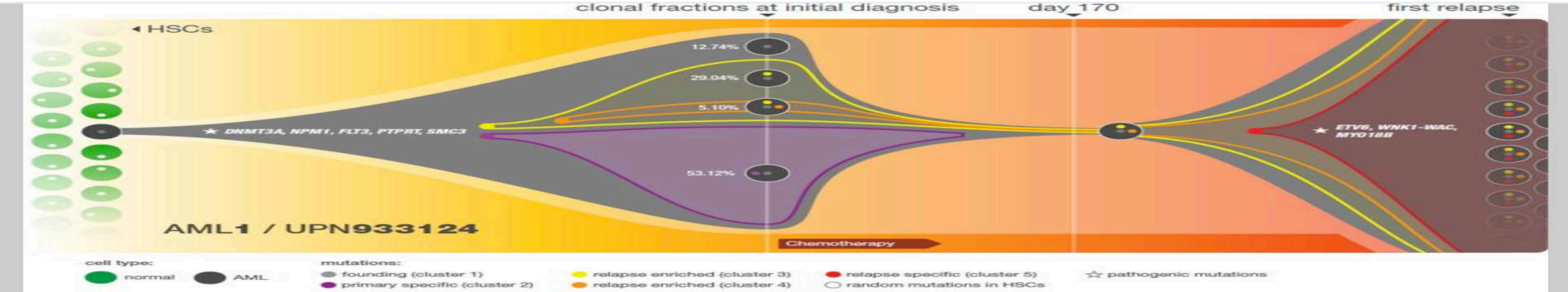

Part 1 - Summary

- “Complete remission” as defined by cytomorphology and “relapse” in AML are artificial constructs – our appreciation of residual disease burden is limited by the sensitivity of the tools we use and the samples that we measure.

- AML MRD tests provide additional information about those in CR (beyond current risk stratification using pre-treatment “surrogates” of disease biology), allowing better estimates of current disease burden and allowing stratification into those with high and low risks of “relapse” following equivalent treatment.

- AML is not one cancer, so no reason why MRD biomarker would be one test.

Outline

1) Why is MRD hard in AML?

2) **Tools to estimate AML MRD**

3) Where does NGS fit in? Limits to the routine clinical use of AML MRD and where we go next…

blood
Special Report

Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party

Gerrit J. Schuurhuis,[1] Michael Heuser,[2,*] Sylvie Freeman,[3,*] Marie-Christine Béné,[4] Francesco Buccisano,[5] Jacqueline Cloos,[1,6] David Grimwade,[7] Torsten Haferlach,[8] Robert K. Hills,[9] Christopher S. Hourigan,[10] Jeffrey L. Jorgensen,[11] Wolfgang Kern,[8] Francis Lacombe,[12] Luca Maurillo,[5] Claude Preudhomme,[13] Bert A. van der Reijden,[14] Christian Thiede,[15] Adriano Venditti,[5] Paresh Vyas,[16] Brent L. Wood,[17,18] Roland B. Walter,[17,19] Konstanze Döhner,[20,†] Gail J. Roboz,[21,†] and Gert J. Ossenkoppele[1]

Blood. 2018 Mar 22;131(12):1275-1291.

Tools	
qPCR (mutations)	~40% of AML cases
Flow	>90%+ of AML cases

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qPCR (mutations)	Highly standardized for AML MRD by EAC. Subtotal coverage of AML (~40%)

Target	Classification	Frequency (%)
NPM1	Insertion mutation	27.2
PML-RARA	Fusion transcript	9.2
CBFB-MYH11	Fusion transcript	5.8
RUNX1-RUNX1T1	Fusion transcript	4
BCR-ABL1	Fusion transcript	1.7

Flow	>90%+ of AML cases

ELN Consensus Summary 2018 - MOLECULAR

- **Real-time qPCR** *…high sensitivity … therefore currently considered the gold standard….limited to … ~40% of AML patients*

- 100ng cDNA/rxt (10K ABL1 copy)
- Run Triplicates
- EAC assays/criteria
- Ref. standards, pos. and no template control

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- **Bone Marrow** (5-10ml, first pull, EDTA or Heparin okay) **AND Blood**

- **Complete molecular remission:** Must be in morphological CR. Two successive MRD negative samples obtained within interval of ≥ 4 weeks at a sensitivity level of at least 1 in 1000.
- **Molecular Relapse:** ↑MRD level of 1 $\log^{10}$ between 2 positive samples (4wk) in a patient who previously tested negative.
- **Molecular Persistence:** *<100-200 copies/10^4 ABL copies corresponding to <1% to 2% of target to reference gene or allele burden.* ***Progression:*** *↑MRD level of 1 $\log^{10}$ any 2 positive samples.*

ELN Consensus Summary 2018 - FLOW

- **“LAIP-based DfN approach”**

LAIPs are DfN abnormalities in the vast majority of cases, and the difference between these 2 approaches is likely to disappear if an adapted, sufficiently large panel of antibodies (preferably ≧8 colors) is used.

- ***≧8 colors***

(CD45, CD34, CD117, CD13, CD33, CD64/CD11b/CD4, CD7, CD15, CD19, CD56, HLA-DR, CD123)
Use of same tubes (same antibody-fluorochrome combinations) at diagnosis and at follow-up. Future: add LSC?

- **Bone Marrow** (5-10ml, first pull, >90% mature neutrophils = significant hemodilution)
- **Acquire 500,000-1 million events** *(excluding CD45neg cells and debris)*
- **Threshold of 0.1% to distinguish MRD^{pos} from MRD^{neg}**

(noted that MRD tests with MRD quantified below 0.1% may still be consistent with residual leukemia, and several studies have shown prognostic significance of MRD levels below 0.1%)

- **Multicenter studies samples may be processed locally but must be analyzed centrally.** *3d at RT undiluted okay.*

ELN Consensus Summary 2018 – CLINICAL

- Patients with **mutant NPM1, RUNX1-RUNX1T1, CBFB-MYH11, or PML-RARA should have molecular assessment** of residual disease.
- AML patients **not included in the molecularly** defined subgroups above, MRD should be assessed using Multiparameter **Flow** Cytometry
- Molecular – assess MRD at **diagnosis, after 2 cycles** of standard chemotherapy, (pre-transplant) and after the **end of treatment** in PB and BM. **Follow-up**: Every 3 mo for 24 mo after the end of treatment in BM and in PB. or PB only every 4-6 wk.
- Failure to achieve an MRD-negative CR, or rising MRD levels during or after therapy are associated with disease relapse and inferior outcomes and should prompt consideration of changes in therapy.
- MRD should be assessed **pre and post transplant**.
- **All clinical trials require molecular and/or MFC assessment of MRD at all times of evaluation of response.**

Tools	CSH says….
qPCR (mutations)	
Flow	

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NGS	Wild-west. Confusion with diagnostic panels. Insufficient rigor in most centers/hands. Meaning of individual variants unclear. DNA >> RNA. A hot mess, but undoubtedly the future of AML MRD
Single cell NGS	V. expensive, low LOD currently. Useful for resolving clonality. RNA >> DNA
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Part 2 Summary

- A set of **five targets** (mutant NPM1, RUNX1-RUNX1T1, CBFB-MYH11, PML-RARA and BCR-ABL1), covering ~40-50% of AML cases, have been approved by **molecular testing** (qPCR) by the international consensus committee on AML MRD

- For **other cases** recommendation for AML MRD monitoring is for **flow** *("LAIP-based DfN approach", ≧8 colors, same tubes at Dx and F/u, Bone Marrow not blood, 500K-1M events, 0.1% threshold, central analysis in multi-site studies*)

- **Minimal Timepoints:** Test MRD at **diagnosis, after 2 cycles** chemotherapy, (**pre- and post-transplant**) and after the **end of treatment** in PB and BM.
  Follow-up: q3 mo x 24 mo after end of treatment in BM and PB. or PB only q4-6 wk.

- **All clinical trials require molecular and/or MFC assessment of MRD at all times of evaluation of response.**

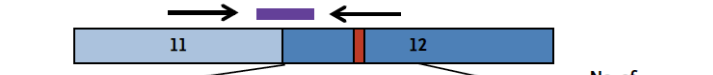
Outline

1) Why is MRD hard in AML?

2) Tools to estimate AML MRD

3) **Where does NGS fit in? Limits to the routine clinical use of AML MRD and where we go next…**

Why NGS in AML MRD is appealing…



Type		No. of Patients	Percent
A	GCTATTCAAGATCTCTG TCTG GCAGTGGAGGAAGTCTCTTTAAGAAAATAG	257	74
B	GCTATTCAAGATCTCTG CATG GCAGTGGAGGAAGTCTCTTTAAGAAAATAG	22	6
D	GCTATTCAAGATCTCTG CCTG GCAGTGGAGGAAGTCTCTTTAAGAAAATAG	31	9
	GCTATTCAAGATCTCTG CTTG GCAGTGGAGGAAGTCTCTTTAAGAAAATAG	5	
	GCTATTCAAGATCTCTG TATG GCAGTGGAGGAAGTCTCTTTAAGAAAATAG	5	
	GCTATTCAAGATCTCTG TCGG GCAGTGGAGGAAGTCTCTTTAAGAAAATAG	3	
	GCTATTCAAGATCTCTG CAGG GCAGTGGAGGAAGTCTCTTTAAGAAAATAG	2	
	GCTATTCAAGATCTCTG TAAG GCAGTGGAGGAAGTCTCTTTAAGAAAATAG	2	
	GCTATTCAAGATCTCTG CGTG GCAGTGGAGGAAGTCTCTTTAAGAAAATAG	1	
	GCTATTCAAGATCTCTG TTTG GCAGTGGAGGAAGTCTCTTTAAGAAAATAG	1	
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	GCTATTCAAGATCTC TCCATGCTCC TGGAGGAAGTCTCTTTAAGAAAATAG	1	
	GCTATTCAAGATCTCTGGCAG CGGA TGGAGGAAGTCTCTTTAAGAAAATAG	1	
	GCTATTCAAGATCTCTGGCAG AGGC TGGAGGAAGTCTCTTTAAGAAAATAG	1	
	GCTATTCAAGATCTCTGGCAGT CTTTCGCTCAC GTCTCTTTAAGAAAATAG	1	
	GCTATTCAAGATCTCTGGCAGTG TTTTGCTC AAGTCTCTTTAAGAAAATAG	1	
	GCTATTCAAGATCTCTGGCAGTG TTTTTCCC AAGTCTCTTTAAGAAAATAG	1	
		346	Total No. of Patients

Ivey *et al.* NEJM. 2016

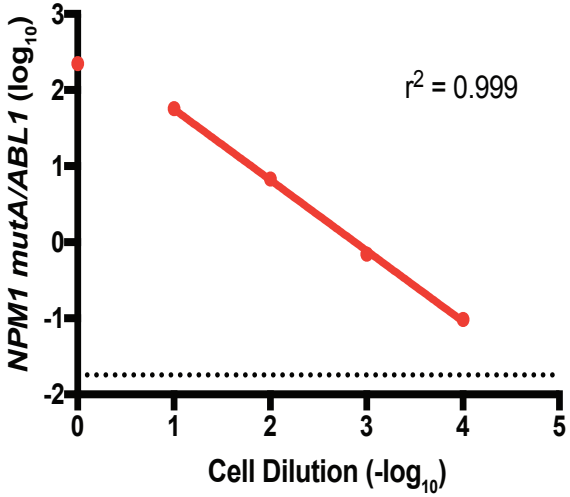
Potential advantages of NGS for AML MRD

- Ability to capture multiple variants within one gene region with a single assay
- Ability to multiplex detection of many different MRD targets with one test
- Allows validation of only one workflow = quicker translation to the clinic
- “Throughput” of NGS (vs. qPCR or ddPCR)

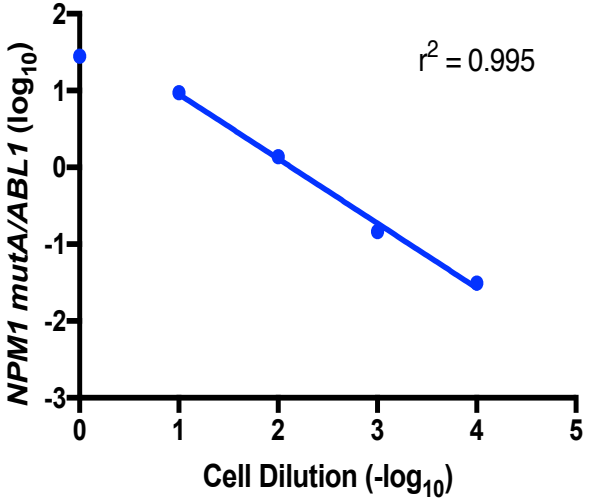
NPM1 Insertion LOD using NIH developed UMI-RNA-seq is Comparable to Gold Standard qPCR Assay

***NPM1* exon12 type A insertion**

Targeted RNA-seq



qPCR

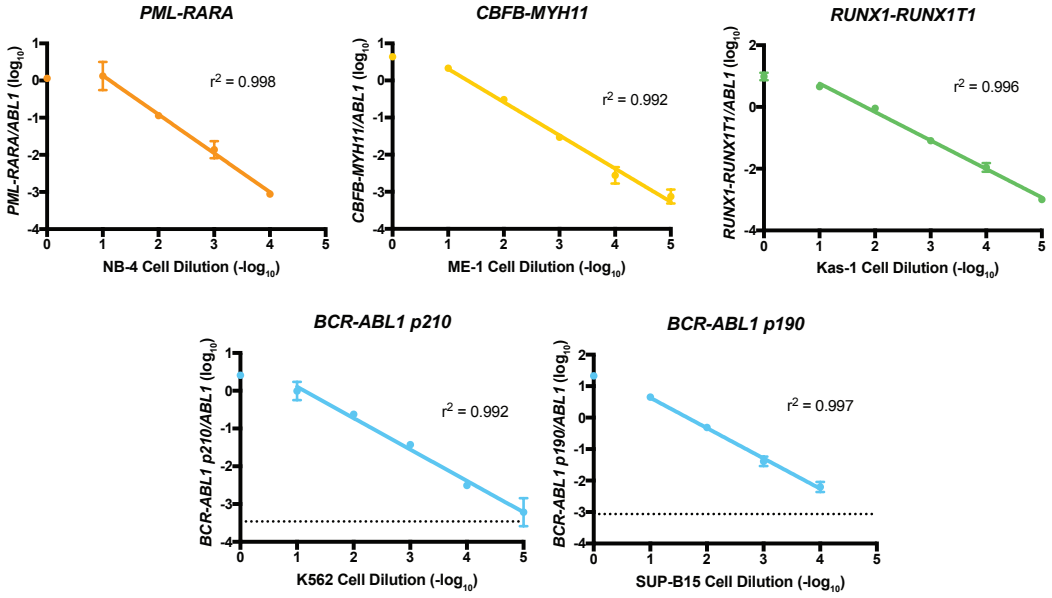


Laura Dillon PhD

Dashed line represents healthy donor background

Fusion Transcript LOD using UMI-RNA-Seq – 1:100,000

Mutation Classification: ***Fusion***



Dashed line represents healthy donor background

Haematologica
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NIH National Heart, Lung, and Blood Institute

DNA NGS for AML MRD

Molecular Minimal Residual Disease in Acute Myeloid Leukemia ***NEJM 2018***

Clearance of Somatic Mutations at Remission and the Risk of Relapse in Acute Myeloid Leukemia. **JCO 2018**

Persistence of pre-leukemic clones during first remission and risk of relapse in acute myeloid leukemia. **Leukemia 2018**

A next-generation sequencing-based assay for minimal residual disease assessment in AML patients with FLT3-ITD mutations. ***Blood Adv 2018***

Residual disease detection using targeted parallel sequencing predicts relapse in cytogenetically normal acute myeloid leukemia. **AJH 2018**

Deep NPM1 Sequencing Following Allogeneic Hematopoietic Cell Transplantation Improves Risk Assessment in Adults with NPM1-Mutated AML. **BBMT 2018**

Multicolor Flow Cytometry and Multigene Next-Generation Sequencing Are Complementary and Highly Predictive for Relapse in Acute Myeloid Leukemia after Allogeneic Transplantation. **BBMT 2017**

Association Between Mutation Clearance After Induction Therapy and Outcomes in Acute Myeloid Leukemia. **JAMA 2015**

IDH1/2 but not DNMT3A mutations are suitable targets for minimal residual disease monitoring in acute myeloid leukemia patients: a study by the Acute Leukemia French Association. **Oncotarget 2015**

Next-generation sequencing of FLT3 internal tandem duplications for minimal residual disease monitoring in acute myeloid leukemia. **Oncotarget 2015**

Next-generation sequencing for minimal residual disease monitoring in acute myeloid leukemia patients with FLT3-ITD or NPM1 mutations. **Genes Chromosomes Cancer 2012**

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Next-generation sequen *al residual disease monitoring in acute myeloid leukemia patients with FLT3-ITD or NPM1 mutations.* **Genes Chromosomes Ca** **2**

Highly publishable, but none ready for primetime clinical use in individual patients yet…

DNA NGS for AML MRD – Known Issues

(1) Stability of Marker/Genetic clonal heterogeneity. Evolution over time and possibly emergence or selection of small sub-clone(s) at relapse *(eg: FLT3-ITD).*

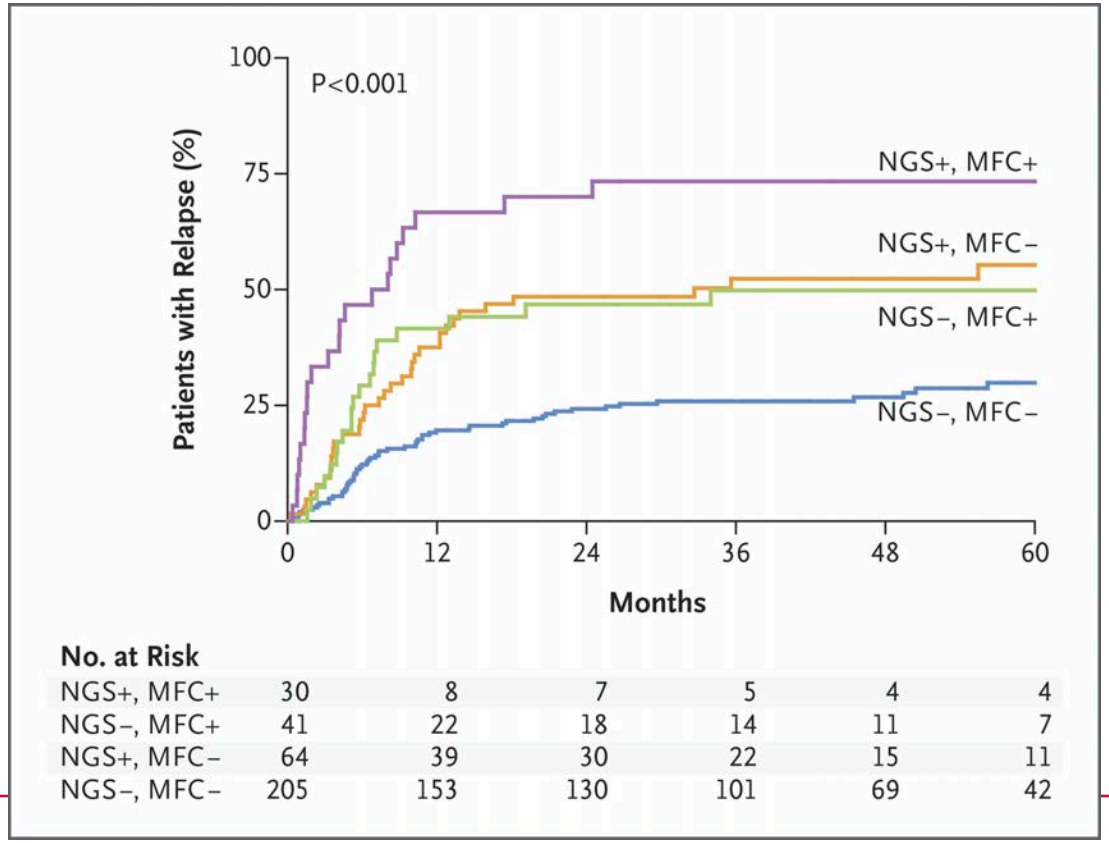
(2) Error rates intrinsic to most conventional NGS techniques allow only for low-sensitivity detection of mutated sequences. Even with ECCS/UMI approaches serious concerns regarding "index-hopping" when multiplexing especially on patterned flow cells.

(3) False positive rates. Regulatory agencies may not accept biomarker strategies that rely on statistical/probabilistic post-test filtering of falsely positive results (rate *influenced by library method, design, sequencing platform used, even other samples on same or prior runs!*).

(4) Understanding clonality in any sample may be limited by amount of genetic material, depth of sequencing and algorithms used for mutation calling. Deep workup at diagnosis helpful.

(5) Somatic mutant ≠ AML! Mutated genes (for example, DNMT3A, TET2 and ASXL1 amongst others) can be detected in healthy people without haematological abnormalities (**CHIP**) **and can persist in persons in long-term remission**.

(6) Uniform reporting standards. How many reads per UMI needed to call a variant? (3, 5)? how many distinct UMI families per variant needed to call MRD? Genomic equivalents input? Standardized filtering, consensus clustering and variant calling needed? What about controls, duplicates, platforms?

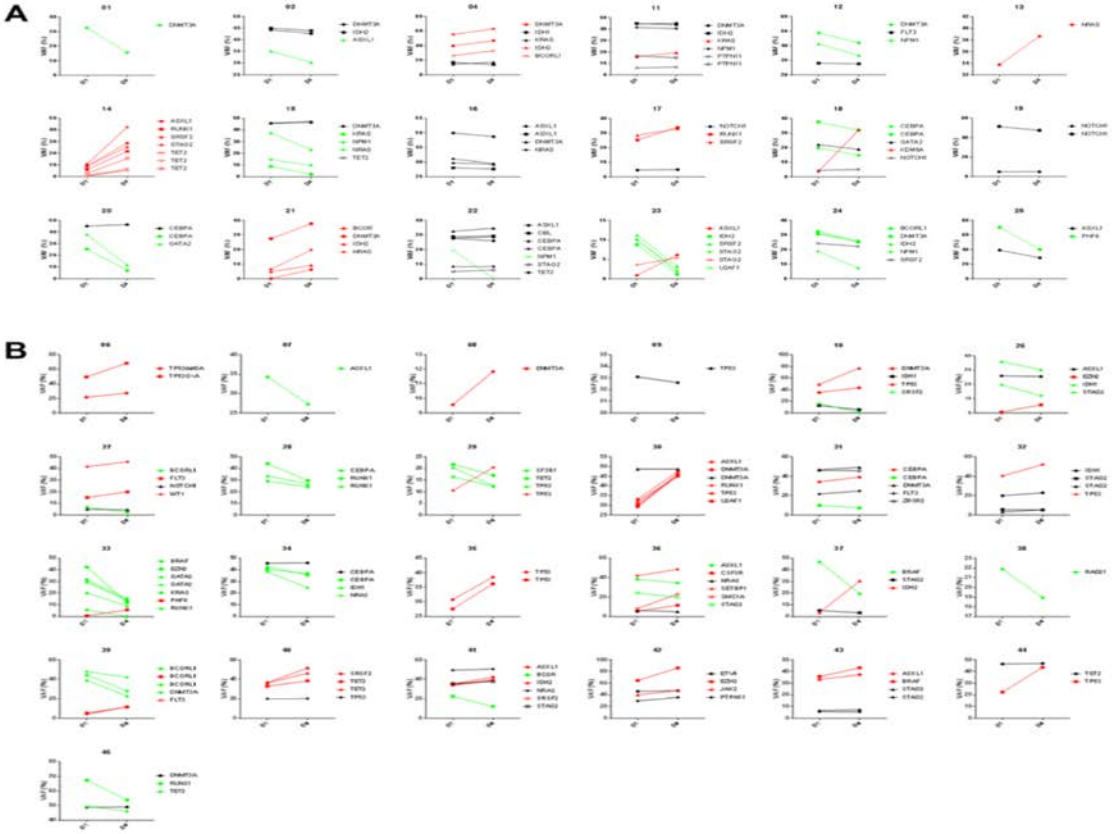


No. at Risk

NGS+, MFC+	30	8	7	5	4	4
NGS–, MFC+	41	22	18	14	11	7
NGS+, MFC–	64	39	30	22	15	11
NGS–, MFC–	205	153	130	101	69	42

Jongen-Lavrencic *et. al.*, 2018

How early is too early?

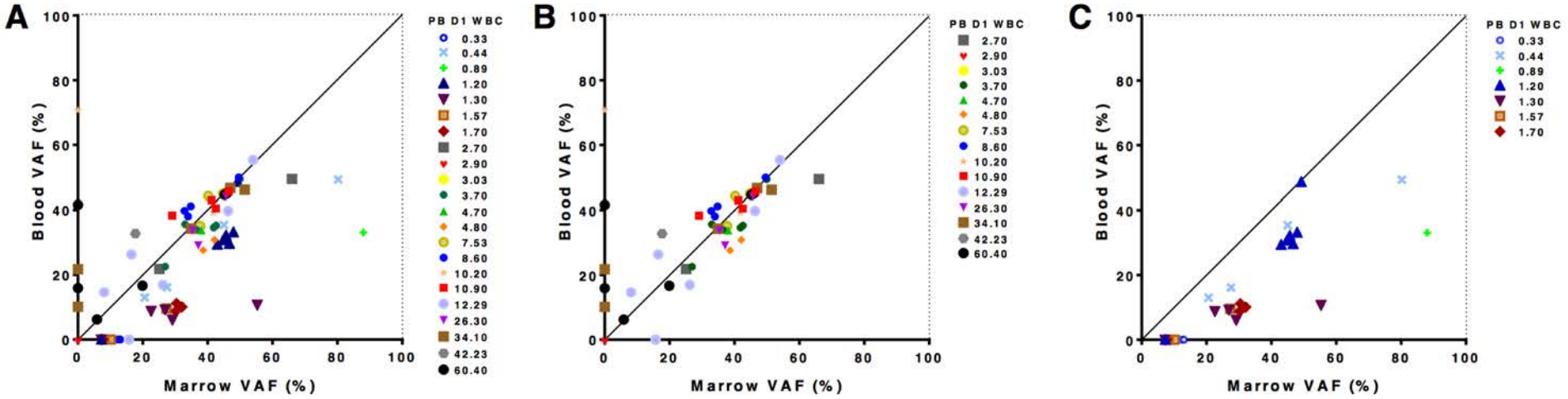


Day4 of induction in PB

Wong et. al., *unpublished*

NIH National Heart, Lung, and Blood Institute

Blood vs. Bone Marrow



Wong et. al.,
unpublished

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No MRD test will have perfect specificity for residual leukemia when measured at a single-time point ***(= expect false positives***)

MRD testing, @ single landmark timepoint, has good ability to risk stratify AML pts in cytomorphological remission **@ a cohort level,** but suboptimal @ individual pt. level.

Some known unknowns

- Will centralized analysis and logistical challenges for MPFC be overcome with single tube approaches? How much sensitivity are we losing in edge cases and is that a systemic bias?
- How do we move away from idea of “thresholds” at “landmarks” to a more sophisticated sequential monitoring with evaluation of kinetics?
- How do techniques complement, or confuse, an assessment? Is orthogonal validation possible, and desirable?
- Which somatic mutations can be used for AML MRD tracking. True for every case? How can analysis of large datasets help?
- **N.B.**: How to overcome challenge of detecting clonal evolution in NGS / requirement for a diagnostic sample? Prospective determination of low-level true positives by NGS in remission samples, with a prior, remains extremely challenging.

Questions?

hourigan@nih.gov

@DrChrisHourigan

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www.nature.com/leu

REVIEW

Measurable residual disease testing in acute myeloid leukaemia

CS Hourigan[1], RP Gale[2], NJ Gormley[3], GJ Ossenkoppele[4] and RB Walter[5,6,7]

1) *BCR-ABL1* test took time and coordinated effort.

2) No AML MRD test has perfect sens. or spec. We still need physicians.

3) Flow is great for AML MRD if you have access to great flow cytometry.
≥ 50% of AML can be monitored by molecular (= qPCR but ?soon NGS)

4) MRD testing @ ≥2 time points as feature of high-quality AML trials

5) MRD test trends likely to be more informative @ indiv. pt. level.

6) **RANDOMIZED TRIALS NEEDED**

7) Potential for MRD as surrogate endpoint in AML drug approvals

Box 1 Suggestions for MRD-testing in AML

1. Considerable efforts were, and are, needed to standardize *BCR/ABL1* testing in CML. Given the diverse genetic aetiology and clonal heterogeneity of AML, even greater standardization efforts are likely needed before MRD-testing is sufficiently accurate and reproducible to be integrated into clinical standard-of-care guidelines.
2. No current AML MRD-test has perfect sensitivity and specificity to accurately predict leukaemia relapse. Physicians need to carefully consider relapse probability and therapy risks before proposing an intervention based on results of MRD-testing.
3. Flow cytometry-based AML MRD-testing is applicable to most cases but has limitations. AML subtypes caused by, or associated with, a canonical genetic abnormality (for example, APL, mutated *NPM1* or core-binding factor translocations) may reasonably be monitored using qPCR. Sequencing-based methods with error correction are likely to become available soon.
4. We believe some MRD-testing, typically at ⩾2 time points, will be a feature of most high-quality clinical trials in AML in the future.
5. MRD-testing at one time point may have insufficient specificity for clinical decision-making whereas trends in MRD-tests over time are likely to be more informative.
6. Randomized trials (for example, intervention based on MRD vs no intervention) are required to determine if MRD-guided therapy is associated with a reduced relapse risk and longer survival.
7. Determination of end point surrogacy requires multiple randomized trials to prove the relationship between effect of treatment on MRD state and the effect of treatment on survival and/or other clinical benefit end point.