19th Annual INDY HEMATOLOGY REVIEW®
MRD Monitoring in Myeloma, Lymphoma and Leukemia

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Minimal Residual Disease; Myeloma, Lymphoma and Leukemia

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NGS monitoring in Myeloma ?

60 years old with ISS stage II Myeloma, status post 4 cycles of daratumumab + VRD in CR

NCCN/Revised IMWG Response Criteria

IMWG criteria for response assessment including criteria for minimal residual disease (MRD)	
Response Category[a]	Response Criteria
IMWG MRD criteria (requires a complete response as defined below)	
Sustained MRD-negative	MRD negativity in the marrow (next-generation flow [NGF], next-generation sequencing [NGS], or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years).[b]
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF[c] on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher.
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using a validated equivalent method with a minimum sensitivity of 1 in 10^5 nucleated cells[d] or higher.
Imaging plus MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding FDG PET/CT or decrease to less mediastinal blood pool standardized uptake value (SUV) or decrease to less than that of surrounding normal tissue.[e]
Standard IMWG response criteria[f]	
Stringent complete response	Complete response as defined below plus normal FLC ratio[g] and absence of clonal cells in bone marrow biopsy by immunohistochemistry (κ/λ ratio ≤4:1 or ≥1:2 for κ and λ patients, respectively, after counting ≥100 plasma cells).[h]
Complete response[i]	Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <5% plasma cells in bone marrow aspirates.
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis or ≥90% reduction in serum M-protein plus urine M-protein level <100 mg per 24 h.
Partial response	≥50% reduction of serum M-protein plus reduction in 24-h urinary M-protein by ≥90% or to <200 mg per 24 h. If the serum and urine M-protein are unmeasurable, a ≥50% decrease in the difference between involved and uninvolved FLC levels is required in place of the M-protein criteria. If serum and urine M-protein are unmeasurable, and serum-free light assay is also unmeasurable, ≥50% reduction in plasma cells is required in place of M-protein, provided baseline bone marrow plasma-cell percentage was ≥30%. In addition to these criteria, if present at baseline, a ≥50% reduction in the size (sum of the products of the maximal perpendicular diameters [SPD] of measured lesions)[j] of soft tissue plasmacytomas is also required.
Minimal response	≥25% but ≤49% reduction of serum M-protein and reduction in 24-h urine M-protein by 50%–89%. In addition to the above listed criteria, if present at baseline, a 25%–49% reduction in SPD[j] of soft tissue plasmacytomas is also required.

NGS monitoring in Acute Myloigenous Leukemia ?

- 55 years old with IDH-2 Mutant AML:
- Day 14 BM: No morphologic evidence of residual leukemia, positive IDH-2 NGS

NCCN GUIDELINES 2022: Acute Myelogenous Leukemia - MRD MONITORING

MEASURABLE (MINIMAL) RESIDUAL DISEASE ASSESSMENT

- The role of MRD in prognosis and treatment is evolving. Participation in clinical trials is encouraged.
- MRD in AML refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods. MRD is a component of patient evaluation over the course of sequential therapy. If the patient is not treated in an academic center, there are commercially available tests available that can be used for MRD assessment. Patients who achieved a CR by morphologic assessment alone can still harbor a large number of leukemic cells in the BM.[1] The points discussed below are relevant to intensive approaches (induction chemotherapy) but have not been validated for other modalities of treatment.
- The most frequently employed methods for MRD assessment include real-time quantitative polymerase chain reaction (RQ-PCR) assays (ie, *NPM1*,[2] *CBFB-MYH11*, *RUNX1-RUNX1T1*[3]) and multicolor flow cytometry (MFC) assays specifically designed to detect abnormal MRD immunophenotypes.[1] The threshold to define MRD+ and MRD- samples depends on the technique and subgroup of AML. NGS–based assays to detect mutated genes (targeted sequencing, 20–50 genes per panel)[4,5] is not routinely used, as the sensitivity of PCR-based assays and flow cytometry is superior to what is achieved by conventional NGS. Mutations associated with clonal hematopoiesis of indeterminate potential (CHIP) and aging (ie, *DNMT3A*, *TET2*, potentially *ASXL1*) are also not considered reliable markers for MRD.[4-6]
  - There are distinct differences between diagnostic threshold assessments and MRD assessments. If using flow cytometry to assess MRD, it is recommended that a specific MRD assay is utilized, but, most importantly, that it is interpreted by an experienced hematopathologist.
- Based on the techniques, the optimal sample for MRD assessment is either peripheral blood (*NPM1* PCR-based techniques) or an early, dedicated pull of the BM aspirate (ie, other PCR, flow cytometry, NGS). The quality of the sample is of paramount importance to have reliable evaluation.
- Studies in both children and adults with AML have demonstrated the correlation between MRD and risks for relapse, as well as the prognostic significance of MRD measurements after initial induction therapy.[7]
  - MRD positivity is not proof of relapse. However, a persistently positive MRD result after induction, which depends on the technique used and the study, is associated with an increased risk of relapse.
  - For favorable-risk patients, if MRD is persistently positive after induction and/or consolidation, consider a clinical trial or alternative therapies, including allogeneic transplantation.
  - Some evidence suggests MRD testing may be more prognostic than *KIT* mutation status in CBF AML, but this determination depends on the method used to assess MRD and the trend of detectable MRD.
  - After completion of therapy, "Molecular relapses" can predict hematologic relapses within a 3- to 6-month timeframe.
- Timing of MRD assessment:
  - Upon completion of initial induction.[4-6]
  - Before allogeneic transplantation.[8]
  - Additional time points should be guided by the regimen used.[2,3]

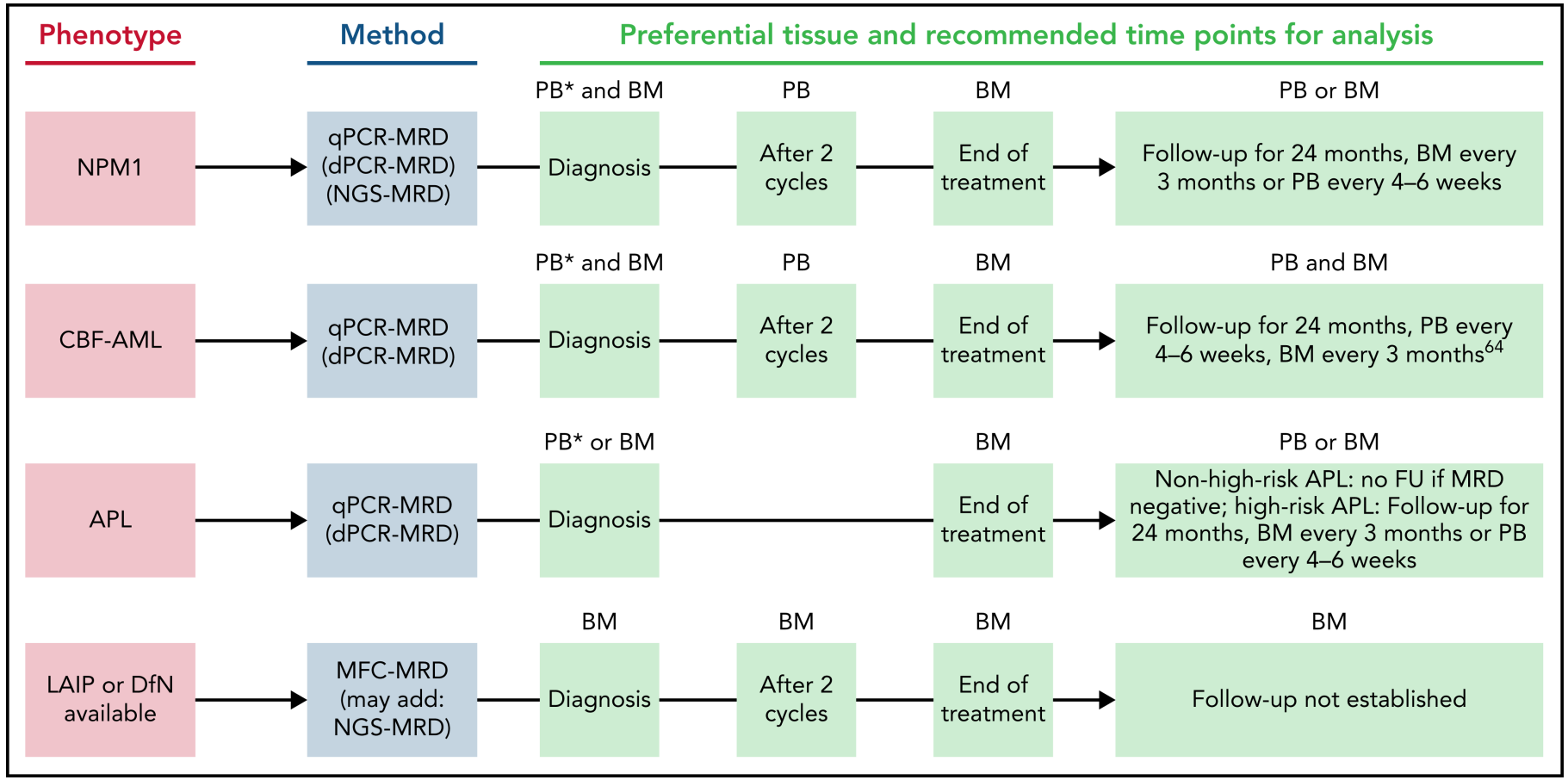
[1] Schuurhuis GJ, Heuser M, Freeman S, et al. Minimal/measurable residual disease in AML: a consensus document from ELN MRD Working Party. Blood

[5] Klco JM, Miller CA, Griffith M, et al. Association between mutation clearance after induction therapy and outcomes in acute myeloid leukemia. JAMA 2015;314:811-822.

[6] Morita K, Kantarjian H, Wang F, et al. Clearance of somatic mutations at

2021 Update on MRD in acute myeloid leukemia: A consensus document from the European LeukemiaNet MRD Working Party

Time points at which MRD is considered a clinically relevant biomarker.



Heuser, M et al2, 021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party, Blood, 2021,

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NCCN MRD MONITORING: Acute Lymphoblastic Leukemia 2022

MINIMAL/MEASURABLE RESIDUAL DISEASE ASSESSMENT

- MRD refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods or standard immunophenotyping.
- MRD quantification is an essential component of patient evaluation over the course of sequential ALL therapy.
- Studies in children and adults with ALL have demonstrated a strong correlation between the presence of MRD during remission and risk for relapse, as well as the prognostic significance of MRD measurements after induction and consolidation therapy.[1]
- The preferred sample for MRD assessment is the first small volume (of up to 3 mL) pull of the bone marrow aspirate, if feasible.
- If validated MRD assessment technology with appropriate sensitivity (at least 10^{-4}) is not available locally, there are commercially available tests.
- The most frequently used methods for MRD quantification include flow cytometry assays[2,3] specifically designed to detect abnormal MRD immunophenotypes at low frequency, real-time quantitative polymerase chain reaction (RQ-PCR) assays (eg, clonally rearranged immunoglobulin [Ig], T-cell receptor [TCR] genes, reverse transcriptase quantitative PCR (RT-qPCR) assays (eg, BCR/ABL1), and NGS-based assays to detect fusion genes or clonal rearrangements in Ig and TCR loci (does not require patient-specific primers).
- High-sensitivity flow cytometry with validated analysis algorithms or PCR methods can quantify leukemic cells at a sensitivity threshold of 1×10^{-4} (0.01%) bone marrow mononuclear cells (MNCs).[2,3] NGS and some PCR methods can detect leukemic cells at a sensitivity threshold of 1×10^{-6} (0.0001%) MNCs.[4,5] The concordance rate between these methods for quantifying MRD >1×10^{-4} is generally high. Methods not achieving sensitivity threshold of 1×10^{-4} or lower are not recommended.
- For flow cytometric quantification of MRD, notify laboratory performing the assay if the patient has received immunotherapy (such as monoclonal antibodies, bispecific antibodies, or CAR-T cells) or HCT as these treatments can affect interpretation. Such testing should be performed in a laboratory with experience performing MRD testing in this clinical setting.
- Timing of MRD assessment:
  - Upon completion of initial induction.
  - End of consolidation
  - Additional time points should be guided by the regimen used.
  - Serial monitoring frequency may be increased in patients with molecular relapse or persistent low-level disease burden.
  - For some techniques, a baseline sample (ie, prior to treatment) is needed to characterize the leukemic clone for subsequent MRD assessment.

NGS monitoring in CLL ?

- 60 years old with status completing time limited therapy with venetoclax and obinutuzumab x 12 cycles:
- NGS positive for RESIDUAL disease

NCCN GUIDELINES: CLL MRD MONITORING

National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2023
Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

NCCN Guidelines Index
Table of Contents
Discussion

RESPONSE DEFINITION AFTER TREATMENT FOR CLL/SLL[a]

Minimal Residual Disease (MRD) Assessment:

- **Evidence from clinical trials suggests that undetectable MRD in the peripheral blood after the end of treatment is an important predictor of treatment efficacy.[e,f,g]**
- **Allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) and six-color flow cytometry (MRD flow) are the two validated methods used for the detection of MRD at the level of 10^{-4} to 10^{-5}.[h,i] Next-generation DNA sequencing (NGS)-based assays have been shown to be more sensitive, thus allowing for the detection of MRD at the level of 10^{-6}.[j,k,l]**
- **MRD evaluation should be performed using an assay with a sensitivity of 10^{-4} according to the standardized European Research Initiative on CLL (ERIC) method or standardized NGS method.**

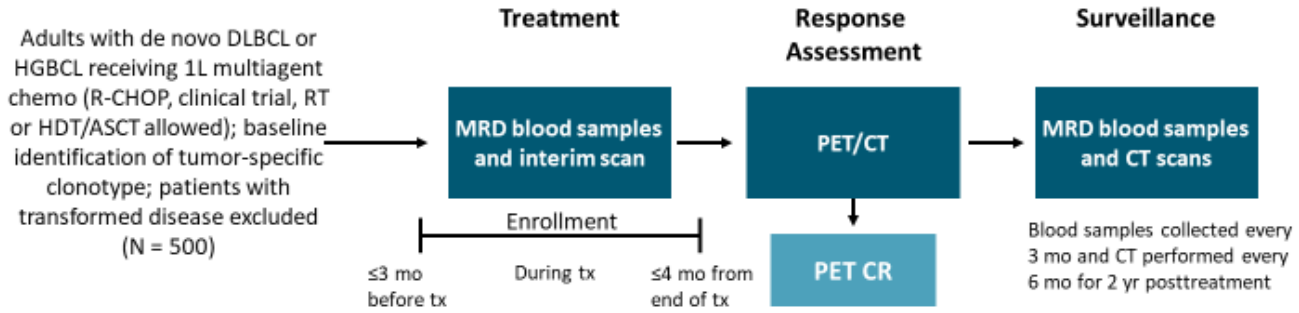
NGS monitoring in Lymphoma ?

- 40-year-old in CR after Pola-R-CHOP for stage IVB DLBCL:
- How to monitor: CT, NGS or both or clinical symptoms only ?

MRD Monitoring in Lymphoma

MRD Surveillance in DLBCL: Study Design

- Prospective, multicenter, study



- Primary endpoint: characteristics of immunosequencing MRD assay (sensitivity, specificity, NPV, PPV)
  - MRD uses multiplex PCR followed by NGS to identify and track rearrangements of IgH-VDJ, IgH-DJ, Igκ, and Igλ, as well as translocations in Bcl1/2-IgH
  - MRD positivity was defined as any detectable rearrangement assay

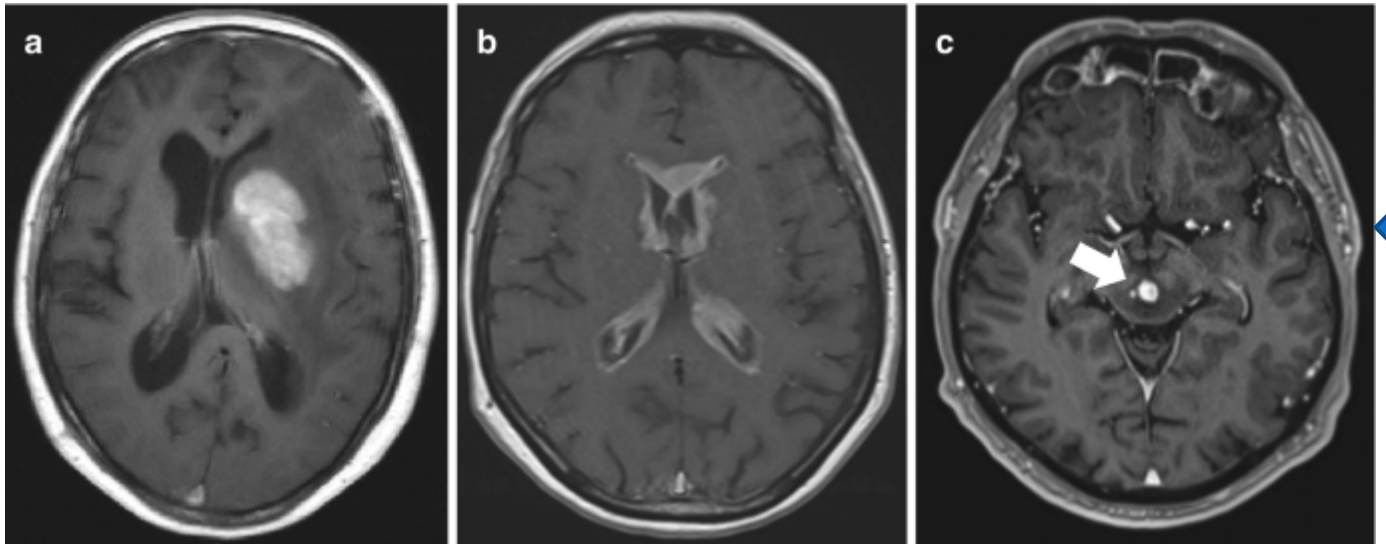
Kumar. ASH 2021. Abstr 52.

Slide credit: clinicaloptions.com

- Study to evaluate MRD in early detection of relapse in DLBCL.
- 45% of clinical relapses detected radiographically in asymptomatic pts.
- MRD assay suboptimal in sensitivity and specificity in the post-treatment surveillance of DLBCL.

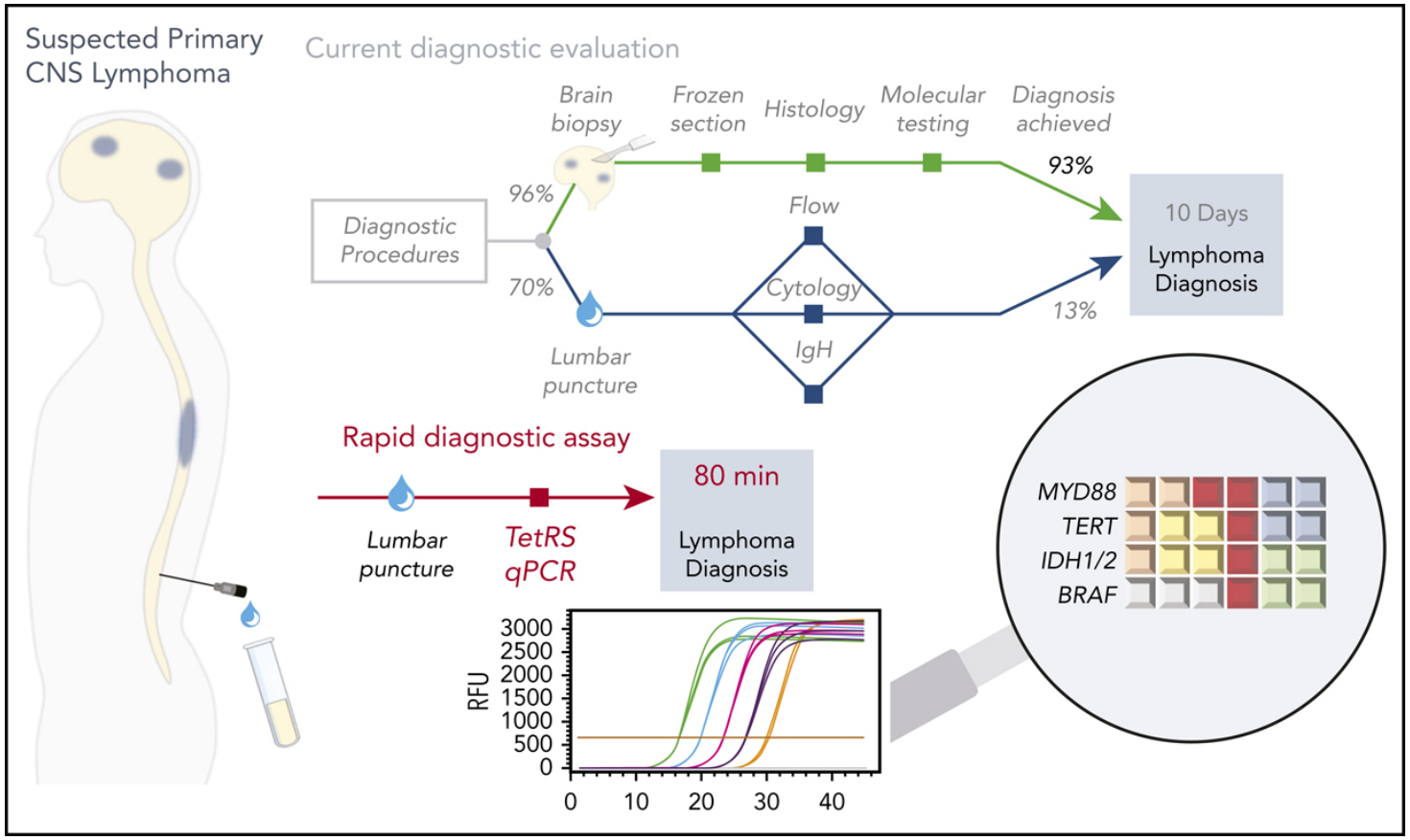
NGS Diagnosis of Lymphoma?

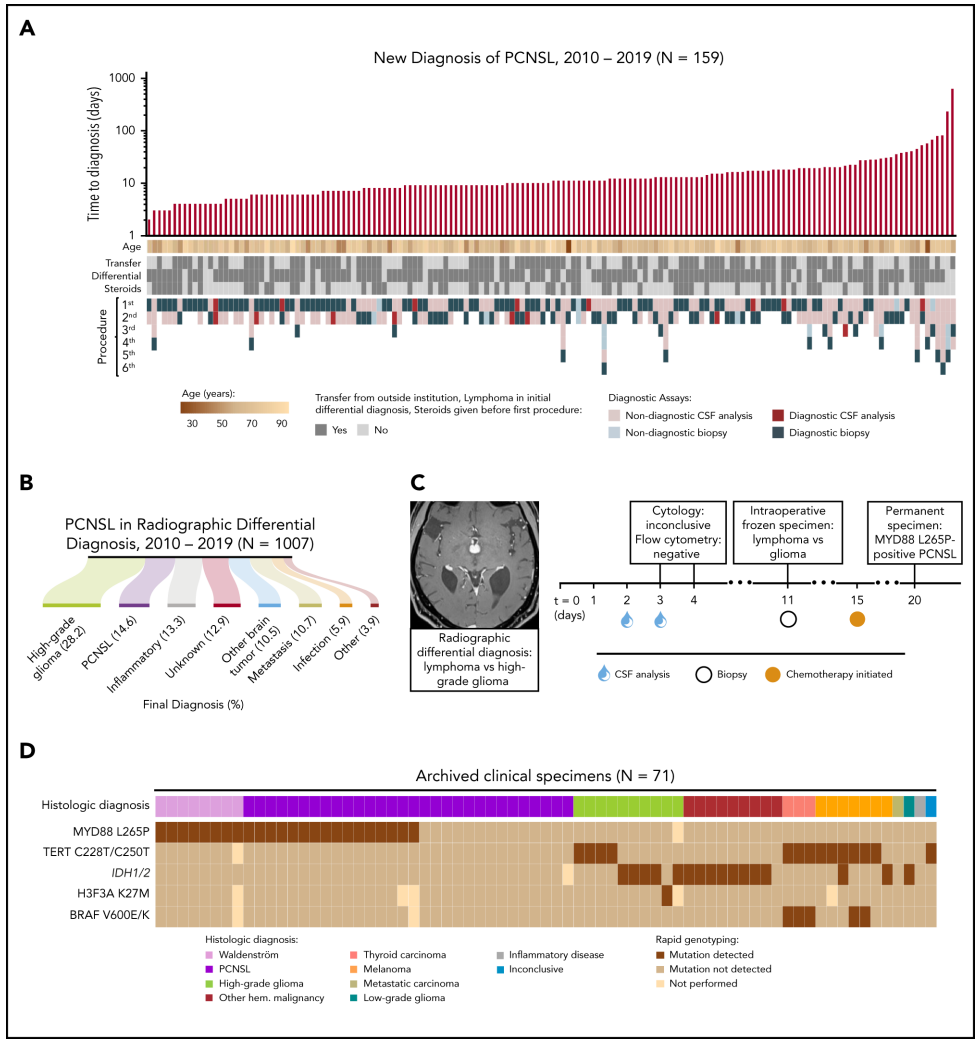
- 67 years old with deep brain enhancing lesion, not amenable to biopsy but suspicious for lymphoma
- Treat empirically or ctDNA diagnosis



Our patient

A Rapid Genotyping Panel for Detection of Primary Central Nervous System Lymphoma





Gupta, M et al. A rapid genotyping panel for detection of primary central nervous system lymphoma, Blood (2021) 138 (5): 382–386.

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