

# Journal club - Concordance of Peripheral Blood and Bone Marrow Next-Generation Sequencing in Hematologic Neoplasms

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*Research Article*

# **Concordance of Peripheral Blood and Bone Marrow Next-Generation Sequencing in Hematologic Neoplasms**

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A circular inset on the left side of the slide shows a microscopic view of several red blood cells. The cells are spherical and have a characteristic biconcave shape, with a darker red center and a lighter red outer rim. They are set against a light, slightly blurred background.

# Introduction

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- Diagnosis of hematolymphoid neoplasms requires the integration of multiple factors such as:
  1. Clinical presentation
  2. Pathologic features such as morphology and immunophenotype from biopsy material
  3. Cytogenetics
  4. Molecular characteristics
- Molecular characteristics provide prognostic and therapeutic information .



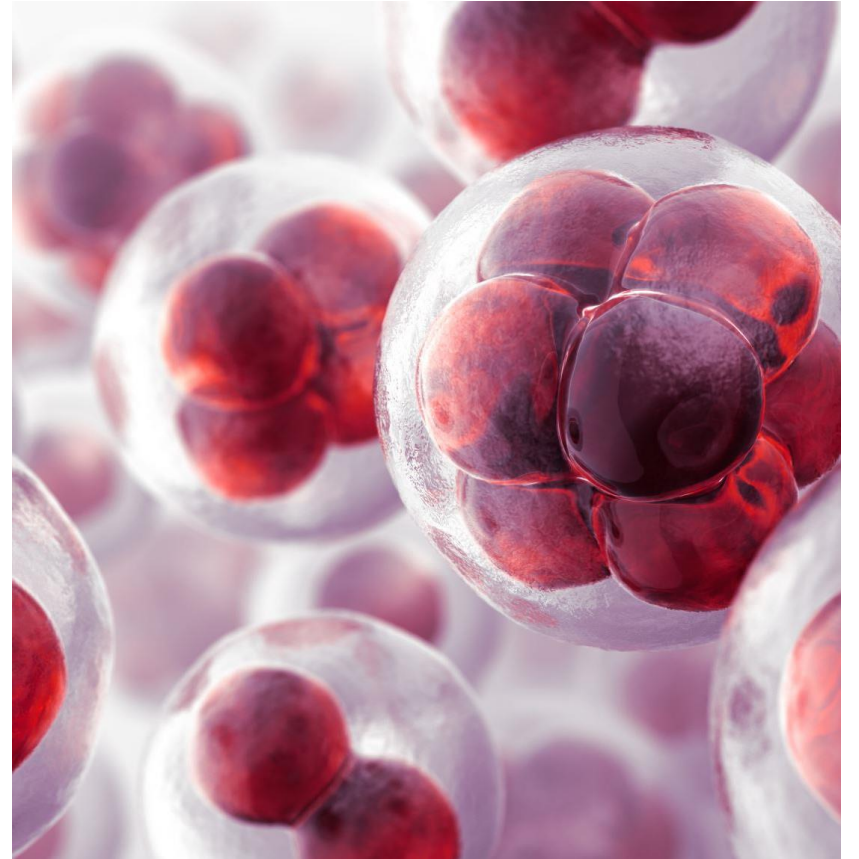
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- Mutational analysis by next-generation sequencing (NGS) is used in clinical practice when evaluating hematologic diseases .
  - Bone marrow aspiration and biopsy remain necessary for the initial diagnosis of neoplastic processes involving bone marrow .
  - Mutational analysis obtained by peripheral blood NGS has been of clinical interest to use as a screening tool due to the less invasive nature of this test



# Introduction

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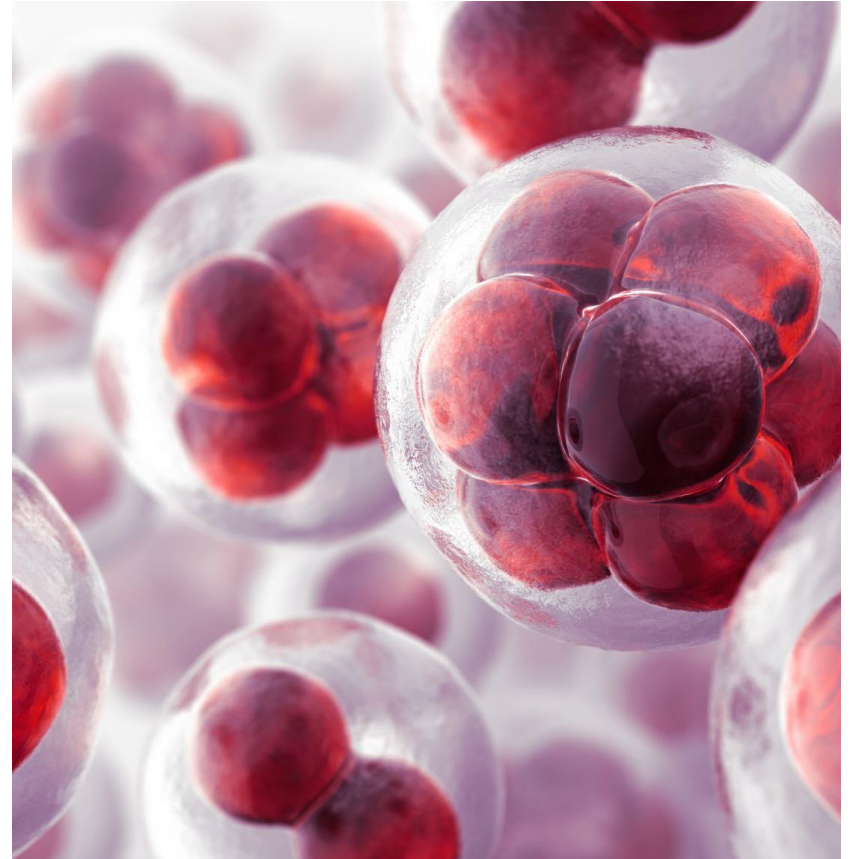
- Peripheral blood NGS has been a reliable tool in **screening for myeloid neoplasms** in patients presenting with cytopenia with negative predictive value of 95%
- The presence of a pathogenic mutation predicted the presence of a myeloid neoplasm, confirmed by subsequent bone marrow biopsy (positive predictive value of 58%)



# Introduction

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- The concordance between NGS performed on peripheral blood (PB) and bone marrow (BM) in the evaluation of hematolymphoid malignancy overall has not been well studied.
- To evaluate the relationship between NGS performed on PB and BM specimens in the setting of the diagnosis of hematolymphoid disease.

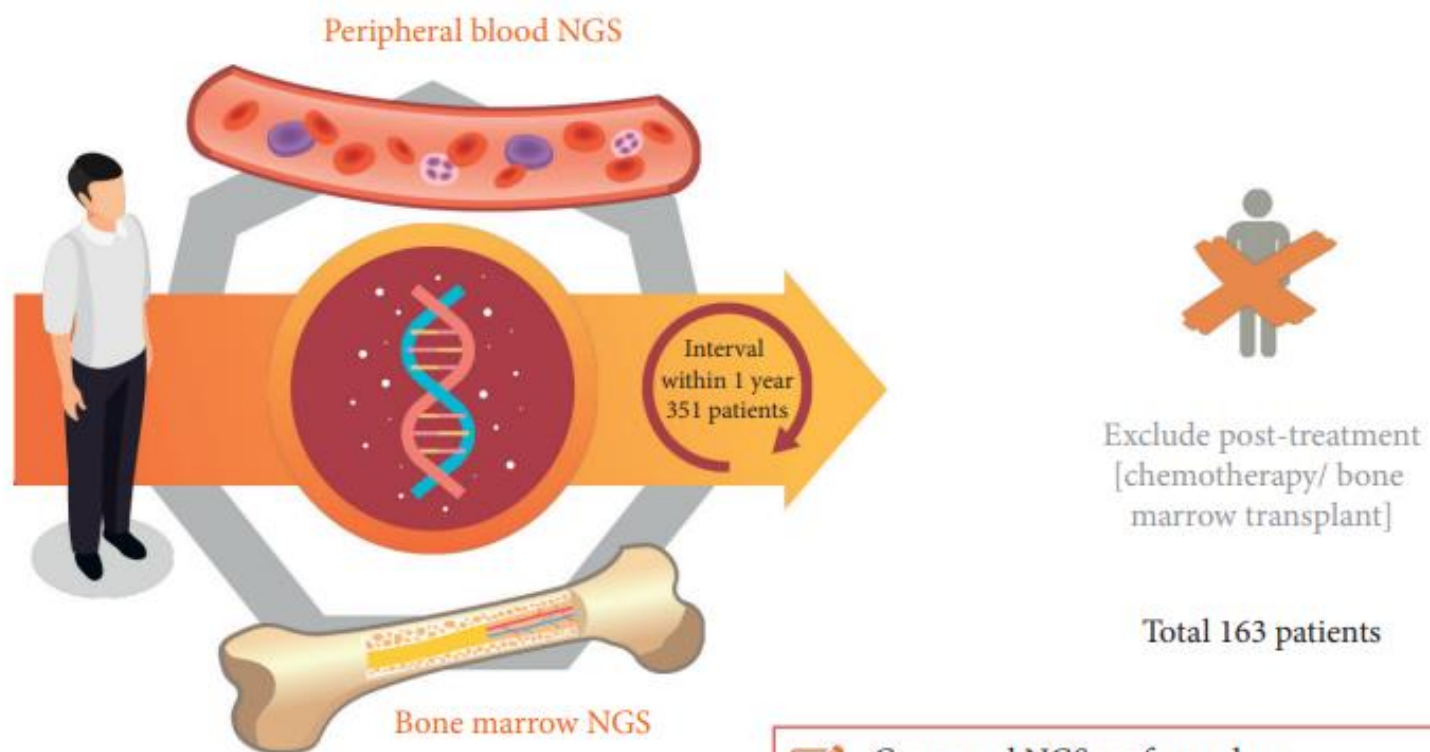


# Methods

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- Patients with peripheral blood NGS performed from January 1st, 2017 till December 31st, 2020 :
- Total 2403 patients were identified
- 368 patients underwent NGS evaluation of a bone marrow specimen
- Excluded patients with interval between bone marrow and peripheral blood NGS was longer than 1 year
- Study population to 351 patients
- Patients who underwent chemotherapy or bone marrow transplant were also excluded
- Study population to **163 patients**





NGS performed on concurrent peripheral blood and bone marrow samples without any treatment in between the two samples

January 2017 - December  
2020



Compared NGS performed on peripheral blood and bone marrow

Classified as

1. Complete concordance
2. Partial concordance
3. Discordance

# Methods

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- We recorded NGS results and compared NGS performed on PB and BM using the same gene panel.
- Cases were classified as
  - 1. Complete concordance**
  - 2. Partial concordance**
  - 3. Discordance**
- Complete concordance: BM and PB NGS show an identical set of abnormal genes or genes.
- Partial concordance : some abnormal genes were identified
- Discordance : there was no overlap between the genes detected in BM and PB

# Methods

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- Statistical analysis was performed with descriptive statistics for demographic data.
- Concordance across peripheral blood and bone marrow NGS was assessed by the kappa coefficient.
- kappa coefficient ( $\kappa$ ) values indicated the strength of agreement based on Altman (1991) as follows:
  1. Poor:  $\kappa \leq 0.20$ ,
  2. Fair:  $\kappa$  0.21–0.40,
  3. Moderate:  $\kappa$  0.41–0.60
  4. Good:  $\kappa$  0.61–0.80
  5. Very good:  $\kappa$  0.81–1.00



# Methods

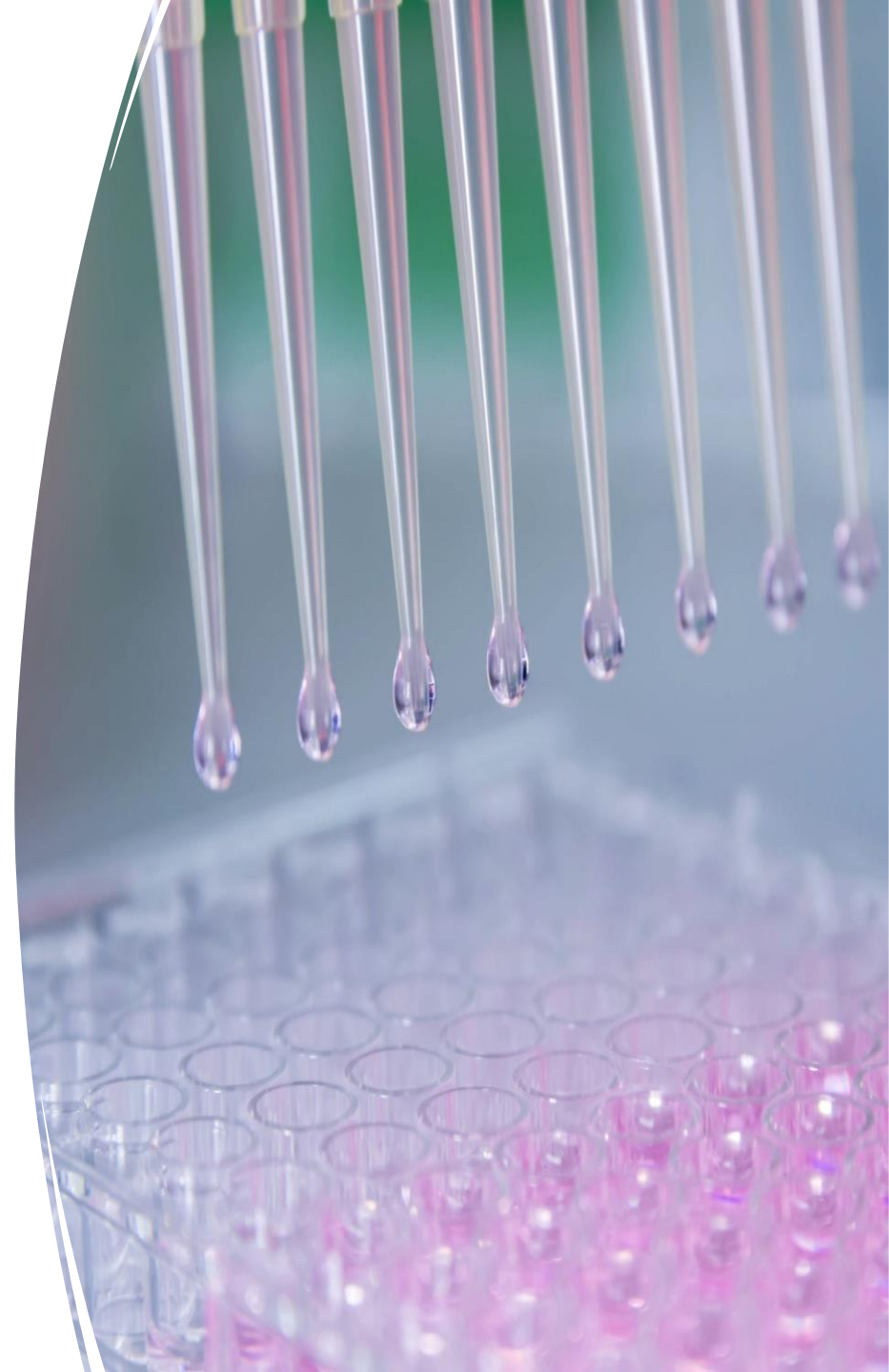
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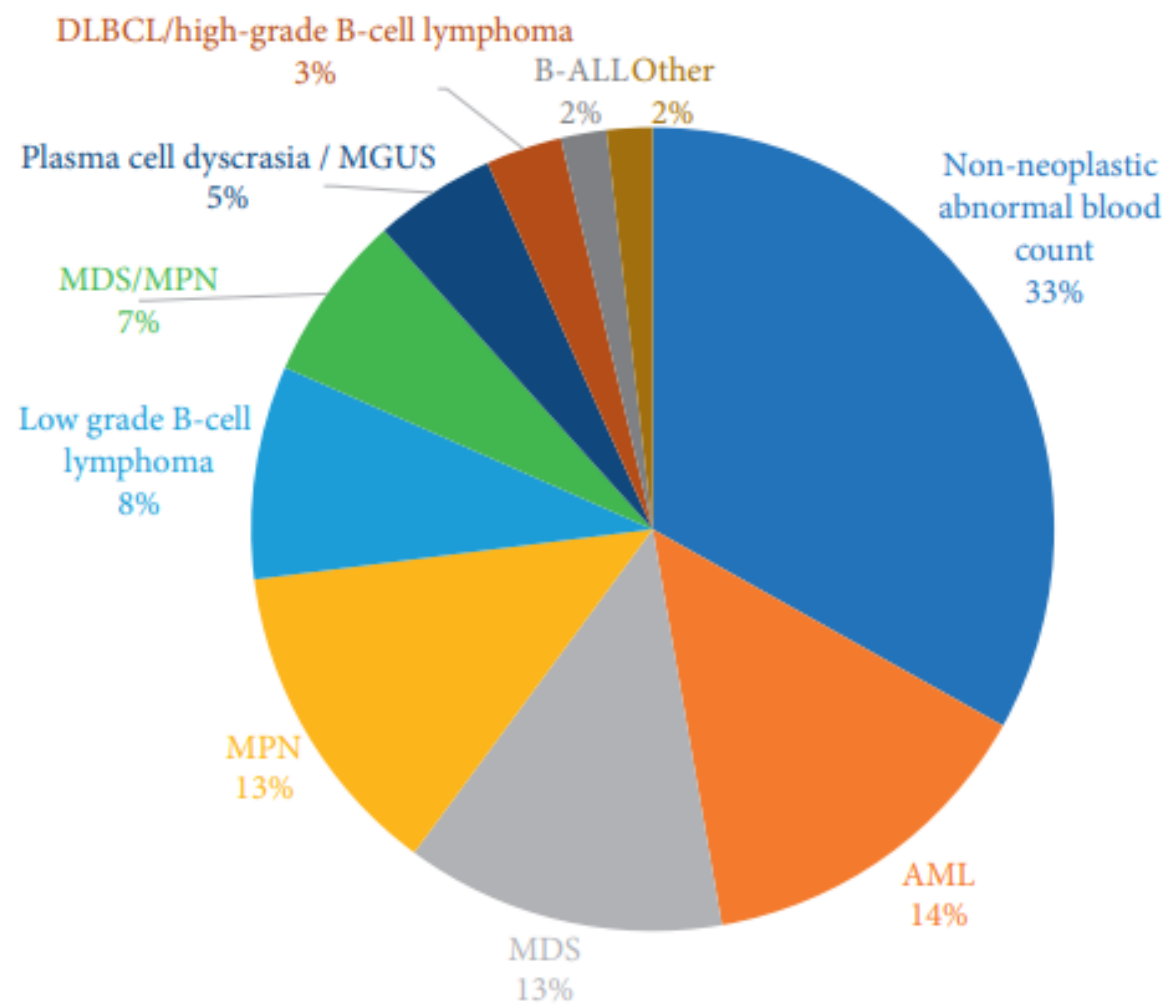
- NGS testing was performed using the **Illumina TruSight sequencing** panel using a **54-gene** comprehensive panel
- Included ABL1, ASXL1, ATRX, BCOR, BCORL1, BRAF, CALR, CBL, CBLB, CBLC, CDKN2A, CEBPA, CSF3R, CUX1, DNMT3A, ETV6, EZH2, FBXW7, FLT3, GATA1, GATA2, GNAS, HRAS, IDH1, IDH2, IKZF1, JAK2, JAK3, KDM6A, KIT, KMT2A, KRAS, MPL, MYD88, NOTCH1, NPM1, NRAS, PDGFRA, PHF6, PTEN, PTPN11, RAD21, RUNX1, SETBP1, SF3B1, SMC1A, SMC3, SRSF2, STAG2, TET2, TP53, U2AF1, WT1, and ZRSR2.

# Methods

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- For lymphoid malignancies - **18 gene panel**
- Includes BCOR, BRAF, CDKN2A, DNMT3A, EZH2, FBXW7, IDH1, IDH2, JAK2, JAK3, KIT, KRAS, MYD88, NOTCH1, NRAS, SF3B1, TET2, and TP53







# Results

- The patients within the study population were those who presented with an abnormal complete blood count (CBC) but whose subsequent bone marrow evaluation was not diagnostic for hematolymphoid disease. This group included 54 cases (33.1%).
- CBC abnormalities seen included cytopenia(s), polycythemia, leukocytosis, thrombocytosis, and eosinophilia.
- Associated conditions included iron deficiency anemia, immune thrombocytopenia, reactive thrombocytosis and idiopathic eosinophilia

# Results

- Myeloid neoplasms consist of
  1. acute myeloid leukemia (AML) (23 cases, 14.1%),
  2. myelodysplastic syndrome (MDS) (21 cases, 12.9%)
  3. myeloproliferative neoplasm (MPN) (21 cases, 12.9%)
  4. myelodysplastic/myeloproliferative neoplasm (MDS/MPN) (11 cases, 6.7%)
  5. mastocytosis (1 case, 0.6%).

# Results

- 31 cases (19%) were lymphoid neoplasms, including
  1. B-lymphoblastic leukemia (BALL) (3 cases, 2%),
  2. chronic lymphocytic leukemia (CLL) (5 cases, 3.1%),
  3. hairy cell leukemia (2 cases, 1.2%)
  4. marginal zone lymphoma (2 cases, 1.2%)
  5. follicular lymphoma (1 case, 0.6%)
  6. mantle cell lymphoma (1 case, 0.6%)
  7. low-grade B-cell lymphoma, unclassified (3 cases, 1.8%)



# Results

- plasma cell myeloma (3 cases, 1.8%)
- monoclonal gammopathy of undetermined significance (MGUS) (5 cases, 3.1%)
- diffuse large B-cell lymphoma (4 cases, 2.4%)
- high-grade B-cell lymphoma (1 case, 0.6%)
- T-cell lymphoma (angioimmunoblastic T-cell lymphoma involved bone marrow) (1 cases, 0.6%).
- One case (0.6%) is mixed-phenotype acute leukemia (MPAL)

## Interval between Peripheral Blood and Bone Marrow NGS.

- Ranged from 0 to 334 days with an average of 63 days.
- 80 cases had an interval of less than 30 days and 83 cases had an interval of more than 30 days

# Correlation between Peripheral Blood and Bone Marrow Mutational Analysis by NGS

- Complete or partial concordance - 150 out of 163 cases (92.0%)
- Complete concordance - 124 cases (76.1%)
- Partial concordance - 26 cases (15.9%).
- Discordance - 13 cases (8.0%).
- Correlation between PB and BM NGS showed good concordance with a kappa coefficient of 0.794 (kappa standard error 0.054) and P value for testing kappa <0.0001



Correlation of peripheral blood NGS and bone marrow NGS(less than 30 days )

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TABLE 1: Correlation of peripheral blood NGS and bone marrow NGS.

Total cases	Count (N= 163)	Percentage (%)	Kappa	Standard error of kappa	P value
Concordance	150	92.0	0.794	0.054	<0.0001
Complete concordance	124	76.1			
Partial concordance	26	15.9			
Discordance	13	8.0			

Correlation of  
PB and BM  
NGS ( more  
than 30 days  
interval )

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TABLE 2: Correlation of peripheral blood NGS and bone marrow NGS.

Cases interval 30 days or less	Count (N= 80)	Percentage	Kappa	Standard error of kappa	P value
Concordance	72	90.0%	0.750	0.083	<0.0001
Complete concordance	59	73.7%			
Partial concordance	13	16.3%			
Discordance	8	10.0%			
Cases more than 30 days interval	Count (N= 83)	Percentage	Kappa	Standard error of kappa	P value
Concordance	78	94.0%	0.839	0.069	<0.0001
Complete concordance	65	78.3%			
Partial concordance	13	15.7%			
Discordance	5	6.0%			

TABLE 3: Correlation of NGS result between peripheral blood and bone marrow NGS grouped by diagnostic groups.

Diagnoses	Count	Percentage (%)	Kappa	Standard error of kappa	<i>P</i> value
<b>Nonneoplastic abnormal blood count (<i>n</i> = 54)</b>					
<b>Concordance</b>	47	87.0	0.743	0.089	<0.0001
Complete concordance	44	81.4			
Partial concordance	3	5.6			
<b>Discordance</b>	7	13.0			
<b>Myeloid neoplasms (<i>n</i> = 78)</b>					
<b>Concordance</b>	77	98.7	0.916	0.083	<0.0001
Complete concordance	59	75.6			
Partial concordance	18	23.1			
<b>Discordance</b>	1	1.3			
<b>Lymphoid neoplasms (<i>n</i> = 31)</b>					
<b>Concordance</b>	26	83.9	0.599	0.157	0.00014
Complete concordance	21	67.7			
Partial concordance	5	16.1			
<b>Discordance</b>	5	16.1			

# Discussion

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- Our study demonstrated a **high degree of concordance** between mutational analysis by NGS on peripheral blood and bone marrow.
- Concordance rate that we observed was similar to a previous study limited to MDS patients performed by Mohamedali et al.
- We found that the concordance of peripheral blood and bone marrow NGS showed no significant differences by the time interval in between the acquisition of the samples



# Discussion

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- In nonneoplastic abnormal blood count patients concordant - 87.0%
- Discordant results were seen in 7 patients
- Discordant genes were
  1. DNMT3A (3 patients)
  2. ASXL1 (2 patients)
  3. CEBPA (1 patient)
  4. BCORL1 (1 patient)
  5. TP53 (1 patient)
- One possible explanation is that DNMT3A and ASXL1 known to represent upto two-thirds of the clonal hematopoiesis genes were **found in low allele frequency in the bone marrow** but not in the peripheral blood

# Discussion

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- Myeloid neoplasms - MDS, MPN and MDS/MPN patients
- total of 53 patients showed a 100% correlation of NGS results obtained from peripheral blood and bone marrow
- In acute leukemia patients concordance - 92.59%
- In myeloid and biphenotypic leukemia concordance - 95.83%

# Discussion

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- lower concordance was seen in B-ALL cases patients because B-ALL occasionally presented with no peripheral blasts.
- Lymphoid neoplasms showed concordance in 83.9% of cases.
- This finding is expected due to the lack of circulating neoplastic cells in many lymphoid neoplasms.
- CLL and hairy cell leukemia- 100% concordance (due to circulating neoplastic cells)

# Discussion

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- Discordant results were observed in a “bidirectional” fashion as demonstrated in a previous study .
- Majority of discordant cases **detect abnormal genes in the bone marrow specimen not in peripheral blood**
- 2 out of 13 discordant cases, genes were only **detected in PB** NGS, while NGS obtained from BM showed no mutations.
- This discordance may be due to **suboptimal bone marrow sampling**, e.g., **subcortical marrow biopsy, a particulate bone marrow aspiration, and marrow fibrosis**.
- PB NGS can provide additional diagnostic value in cases that have limited diagnostic bone marrow material.

# Conclusion

- Mutational analysis by PB NGS showed **significant concordance** with BM NGS.
- **Myeloid neoplasms** showed a **very high concordance**
- **Slightly lower levels of concordance** was seen in **lymphoid neoplasms** and nonneoplastic abnormal blood counts.
- PB NGS is a reliable tool for mutational analysis
- PB NGS can provide a less invasive method for screening and monitoring molecular profile in hematolymphoid conditions.



# Conclusion

- PB NGS immensely helpful in clinical practice, especially when the diagnostic material for bone marrow studies is a particulate
- Periodic PB NGS assays for screening patients with cytopenias as a guide to the overall bone marrow status - clonal cytopenia of undetermined significance (CCUS) or low grade MDS.

# Data Availability

- data are available from the corresponding author on request.

Critical appraisal of the article

# Is the study question relevant?

- Concordance of Peripheral Blood and Bone Marrow Next-Generation Sequencing in Hematologic Neoplasms
- Yes, need of the hour as **less invasive, outpatient based diagnostic tests** are the demand from patients

# Does the study add anything new?

- Mutational analysis by PB NGS showed significant concordance with bone marrow NGS especially in myeloid neoplasms.
- Since it's a less invasive procedure it **validates and justifies** the efforts to get **NGS testing done from PB** in cases where the **general condition of the patient doesn't allow for BM procedure** or the **yield of BM is poor** such as marrow fibrosis or hypocellular marrow.



**Was the study performed according to the original protocol?**

- Yes

**Were the statistical analyses performed correctly?**

- Yes

**Do the data justify the conclusions?**

- Sample size is small in selective subgroups such as lymphoid neoplasms and MDS.
- Requires further studies in a larger cohort
- This limitation has been highlighted by the authors

**Are there any conflicts of interest?**

- None mentioned

# Limitations

- Concordance between the two samples greatly depends on the distribution of the cells of interest in both the specimens.
- PB NGS may not be a good tool to study the clonal architecture in clinical entities that are marrow-centric diseases.
- ALL and high grade MDS with increased blasts, where the blast % in the PB may not be representative of the bone marrow disease burden.

# One possible solution







- Perform flowcytometry on the PB before PB sequencing to increase the reliability of the peripheral blood NGS studies.

Supporting evidence from other  
articles



Article

# Myeloid NGS Analyses of Paired Samples from Bone Marrow and Peripheral Blood Yield Concordant Results: A Prospective Cohort Analysis of the AGMT Study Group

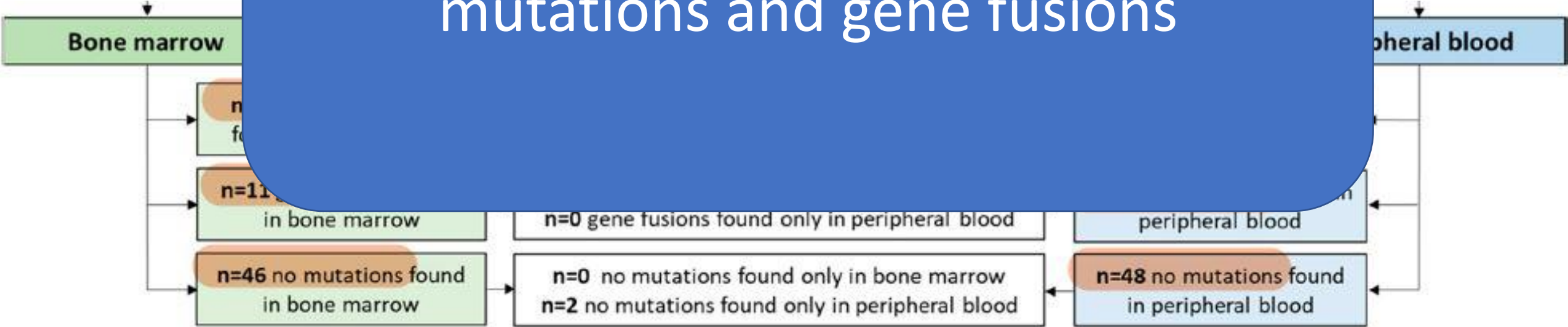
Bettina Jansko-Gadermeir <sup>1,2,3,4,5,6</sup>, Michael Leisch <sup>1,2,3,7</sup>, Franz J. Gassner <sup>1,2,3,4</sup> , Nadja Zaborsky <sup>1,2,3,4</sup> ,  
Thomas Dillinger <sup>2,5</sup>, Sonja Hutter <sup>2,5</sup>, Angela Risch <sup>3,6</sup> , Thomas Melchardt <sup>1,2,3,7</sup>, Alexander Egle <sup>1,2,3,4,7</sup> ,  
Manuel Drost <sup>8</sup>, Julian Larcher-Senn <sup>8</sup> , Richard Greil <sup>1,2,3,4,5,7</sup>  and Lisa Pleyer <sup>1,2,3,4,5,7,\*</sup>

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Patients and samples

n=187 patients  
(bone marrow and peripheral blood sample pairs)

Shows a good correlation in both gene mutations and gene fusions



Jansko-Gadermeir B et al. Myeloid NGS Analyses of Paired Samples from Bone Marrow and Peripheral Blood Yield Concordant Results: A Prospective Cohort Analysis of the AGMT Study Group. *Cancers*. 2023; 15(8):2305. <https://doi.org/10.3390/cancers15082305>



# Their conclusion

- **Sequential molecular analyses** of PB specimens can be reliably used to molecularly classify and monitor myeloid neoplasms without loss of sensitivity or specificity
- BM evaluation solely for the purpose of monitoring of mutations is not necessary
- Myeloid NGS analyses from PB can be used as an **alternative to BM** to identify and monitor gene mutations and to guide treatment decisions.
- Less frequent follow-up of BM evaluations [perhaps be entirely omitted in the future]
- PB Samples can be drawn easily, nearly painlessly, and at multiple time points.
- PB NGS - particular clinical interest
  - **Minimally invasive** screening tool for diagnostic and therapy monitoring
  - Especially in special situations such as a **fibrotic or hypocellular marrow**

# Targeted Next-Generation Sequencing of Circulating Tumor DNA, Bone Marrow, and Peripheral Blood Mononuclear Cells in Pediatric AML

*Min Ruan<sup>1†</sup>, Lipeng Liu<sup>1†</sup>, Benquan Qi<sup>1</sup>, Xiaoyan Chen<sup>1</sup>, Lixian Chang<sup>1</sup>, Aoli Zhang<sup>1</sup>, Fang Liu<sup>1</sup>, Shuchun Wang<sup>1</sup>, Xiaoming Liu<sup>1</sup>, Xiaojuan Chen<sup>1</sup>, Li Zhang<sup>1</sup>, Ye Guo<sup>1</sup>, Yao Zou<sup>1</sup>, Yingchi Zhang<sup>1</sup>, Yumei Chen<sup>1</sup>, LiXia Liu<sup>3</sup>, Shanbo Cao<sup>2</sup>, Feng Lou<sup>2</sup>, Chengcheng Wang<sup>3</sup> and Xiaofan Zhu<sup>1\*</sup>*

**Ruan M, Liu L, Qi B, et al (2021) Targeted Next-Generation Sequencing of Circulating Tumor DNA, Bone Marrow, and Peripheral Blood Mononuclear Cells in Pediatric AML. *Front. Oncol.* 11:666470. doi: 10.3389/fonc.2021.666470**

# GIST of this article

- BM and PB from 20 AML children at the time of initial diagnosis
- ctDNA sample was isolated from PB.
- Detection of mutation was performed on ctDNA, BM, and peripheral blood mononuclear cell (PBMC) by NGS based on a 185-gene panel

## **Conclusion**

- This study demonstrates that ctDNA was a reliable sample in pediatric AML.
- Can be used for mutation detection.
- Consistency analysis showed that ctDNA can mirror the genomic information from BM

# Final verdict

- This study is definitely the need of the hour as **less invasive, outpatient based diagnostic tests** are the demand from patients
- Especially useful to monitor patients after chemotherapy
- Several **newer studies support the above findings** of PB vs BM NGS
- Other studies have gone further to compare **circulating tumour DNA vs PB vs BM NGS testing.**