Abstract: S174

Title: MYELOID NGS ANALYSES OF PAIRED SAMPLES FROM BONE MARROW AND PERIPHERAL BLOOD YIELD CONCORDANT RESULTS: A PROSPECTIVE COHORT ANALYSIS OF THE AGMT STUDY GROUP

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Background:

Next generation sequencing (NGS) has become indispensable for diagnosis, risk stratification, prognostication, and monitoring of response in patients with myeloid neoplasias. Guidelines require bone marrow (BM) evaluations for the above, which are only performed in ~50% of patients outside of clinical trials [Dinmohamed A, Leuk Res 2015, 177; Pleyer L, ASH 2022, oral #561], indicating a need for surrogate samples.

Aims:

To compare NGS analyses of non-selected, consecutive, prospectively collected, paired BM and peripheral blood (PB) samples from patients treated at our institute.

Methods:

Inclusion criteria were a signed informed consent, a routine request for a myeloid NGS analysis by the treating physician, the availability of a paired BM/PB sample, and a minimum read depth of ~500.

The commercially available AmpliSeq™ myeloid panel from Illumina® (40 genes) was used. Software tools for read alignment/variant calling included Local Run Manager DNA Amplicon Analysis Module (3.24.1.8+), Burrow-Wheeler Aligner Maximal Exact Match Whole-Genome Aligner (0.7.9a-isis-1.0.2), Pisces Variant Caller (5.2.9.23), Illumina Annotation Engine (2.0.11-.-g7fb24a09), Binary Alignment Map (BAM) Metrics (0.0.22) and Sequence Alignment/Map tools (0.1.19-isis-1.0.3). Illumina VariantStudio v3.0 was used for variant annotation and filtering, setting sensitivity to 1% variant allele frequency (VAF).

After exclusion of single nucleotide polymorphisms present in >1% of the general population, all remaining variants were manually checked (BAM files visualized in IGV) for sequencing artefacts. In discordant cases the 1% VAF filter was removed and mutations found in only one of the samples were manually reviewed.

Correlations between mutations found in the BM vs the PB were assessed with Spearman's correlation. Agreement was assessed acc. to Bland JM, Lancet 1986, 307. Sensitivity analyses were performed using Kendall's Tau. Assign Data Management and Biostatistics GmbH performed statistical analyses with SAS®9.3.

Results:

307 paired BM/PB samples were collected between 18DEC2019 and 14FEB2023. One sample pair was excluded due to low read depths. 285 (92.8%) of sample pairs were drawn on the same day, 8 (2.6%) <1 week, 7 (2.3%) <4 weeks, 6 (2.0%) 4-8 weeks, and 1 (0.3%) >8weeks apart in an untreated patient. Median age at sampling was 70.0 (range 19-90) years; 77% of sample pairs stemmed from patients with myeloid neoplasias. Mean read depth was 9134. After manual correction, 4 of 1842 (0.22%) results remained discordant, 3 of which originated from serial sample pairs of the same patient.

Very strong correlation ($r=0.96220$, $p<0.0001$), high concordance (99.9%), sensitivity (99.7%), specificity (100%), positive predictive value (99.9%), and negative predictive value (99.9%) between NGS analyses of paired BM/PB samples was observed (**Table 1**).

VAFs between BM and PB samples were in good agreement (**Fig 1A**) and very strongly correlated in the total cohort ($r=0.94295$, $p<0.001$) and in subgroups without circulating blasts ($r=0.93189$, $p<0.0001$) or with

neutropenia <1.0 G/L (r=0.88492, p<0.0001) (**Fig 1B-D**). Negligible correlation was found between the VAF of a detected mutation and the blast count in either the BM (r=0.09631) or the PB (r=0.17833) (**Fig 1E-F**), in line with the fact that clonal involvement has been shown for most PB cells in patients with myeloid neoplasias.

Summary/Conclusion:

Our data show for the first time that, even in the absence of circulating blasts or in neutropenic patients, PB samples can be reliably used to molecularly classify and monitor myeloid neoplasms via NGS without loss of sensitivity/specificity

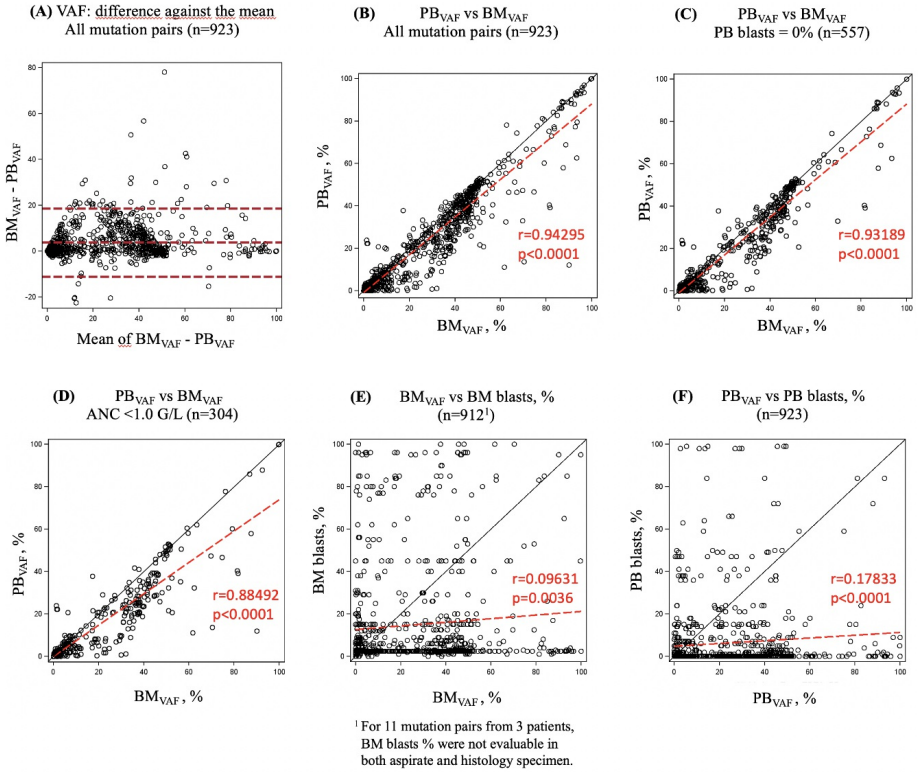
Table 1. NGS sensitivity and specificity.

		PERIPHERAL BLOOD		
		POSITIVE	NEGATIVE	TOTAL
BONE MARROW	POSITIVE	865	3	868
	NEGATIVE	1	2160[1]	2161
	TOTAL	866	2163	3029

[1] The number of negative sample pairs (n=54) multiplied by the number of genes in the panel (n=40)

Concordance = 99.9% (i.e. (865 + 2160)/3029*100).
Positive prediction value (PPV) = 99.9% (i.e. 865/866*100).
Negative prediction value (NPV) = 99.9% (i.e. 2160/2163*100).
Sensitivity = 99.7% (i.e. 865/868*100).
Specificity = 100% (i.e. 2160/2161*100).

Figure 1. Scatterplots of variant allele frequencies (VAF). (A) Assesment of agreement: Difference against the mean. **(B)** BM_{VAF} vs PB_{VAF} for all mutation pairs (n=923). **(C)** BM_{VAF} vs PB_{VAF} for sample pairs with PB blasts = 0% on the day of sampling (n=560). **(D)** BM_{VAF} vs PB_{VAF} for sample pairs with absolute neutrophil count (ANC) <1.0 G/L on the day of sampling (n=296). **(E)** BM_{VAF} vs BM blast percentage for all mutation pairs (n=912). **(F)** PB_{VAF} vs PB blast percentage for all mutation pairs (n=923).



[1] For 11 mutation pairs from 3 patients, BM blasts % were not evaluable in both aspirate and histology specimen.

r indicates Spearman's correlation, whereby values of $0 \le |r| < 0.30$, $0.30 \le |r| < 0.50$, $0.50 \le |r| < 0.70$, $0.70 \le |r| < 0.90$, and $0.90 \le |r| \le 1.00$ indicate negligible, weak, moderate, strong, and very strong correlation [Mukaka MM, Malawi Med J 2012, 69].

Keywords: Bone Marrow, Myeloid malignancies, Peripheral blood, Myelodysplastic syndrome