Poster #3902

Higher Mutation Rate in Patients with Aplastic Anemia Using Peripheral Blood cfDNA as Compared with Bone Marrow Cells

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BACKGROUND

Numerous studies have demonstrated that in patients with neoplastic disorders, peripheral blood may contain tumor-specific DNA, RNA, and protein, and these products can be used for diagnosis or monitoring of various types of cancers. However, in hematologic neoplasms, the neoplastic cells present along with normal cells in blood as a mixed population in suspension. Multiple papers have demonstrated that cell-free (cf) DNA/RNA in peripheral blood might be more representative than DNA from bone aspirates or biopsy, reflective of the entire bone marrow and less influenced by the patchiness frequently seen in bone marrow neoplasms. However, determining the level of sensitivity of cfDNA is difficult due to the difficulty in determining the level of disease in bone marrow.

Aplastic anemia (AA) is believed to be the result of immune-mediated destruction of hematopoietic stem cells. Recent studies have suggested that this immune-mediated destruction of stem cells allows for the emergence of abnormal hematopoietic clones carrying mutations, most of which are detected in patients with myelodysplastic syndrome (MDS). Most of these clones are detected at very low frequency with low variant allele frequency (VAF).

OBJECTIVES

- To determine the sensitivity of cfDNA compared with DNA from bone marrow cells
- To establish that testing cfDNA in patients with aplastic anemia can replace testing using bone marrow cells

METHODS AND SAMPLES

- **Samples**
  120 paired samples (bone marrow and peripheral blood) from 96 patients.
- **DNA from bone marrow aspirate**
  QIAamp DNA Mini Kit (Qiagen; Venlo, Netherlands) in automated (QIAcube) extractions according to manufacturer's instruction used for DNA extraction. Extracted DNA was quantified using a Nanodrop 2000 (Thermo Fisher Scientific; Waltham, MA, U.S.A.) instrument and adjusted to approximately 50 ng/µL with H_2O.
- **DNA from peripheral blood plasma**
  Total nucleic acid was extracted via the NucliSenS EasyMAG automated platform (BioMerieux; Marcy-l'Étoile, France). DNA was quantified using Qubit 2.0 Fluorometer (Thermo Fisher Scientific; Waltham, MA, U.S.A.) and adjusted accordingly.
- **Next-generation DNA sequencing**
- All paired BM and plasma samples (N=120 paired) were tested using the commercially available TruSight Myeloid Sequencing Panel (Illumina; San Diego, CA) according to manufacturer's instructions. Libraries were then sequenced with paired end reads (2x150bp) on either the miSeq or NextSeq instruments (Illumina; San Diego, CA).
- **Bioinformatics**
  Sequences were aligned against the human hg19 reference genome. Variants were called using the Illumina-developed Somatic Variant Caller. RefSeq (NCBI; Bethesda, MD) annotations were applied. Molecular abnormalities were called in Illumina Variant Studio and then individually verified with the Integrated Genome Viewer (Broad Institute; Cambridge, MA).

RESULTS

Detected mutations (Table 1)

- 33 of 96 patients (34%) or 48 of 120 samples (40%) had one or more mutations.
- 54 mutations were detected; 45 of which were unique (Table 1). 40/54 mutations had allele frequency ≤20% in BM cells, while 45 samples had allele frequency ≤20 in cfDNA.

cfDNA versus BM allele frequencies (Figures 1-2)

- No significant difference (P=0.71, Sign test) was found between cfDNA and BM allele frequencies (Figure 1).
- The median mutant allele frequency was 10.9% in cfDNA and 12.6% in BM cells.
- Significant correlations existed between BM and cf allele frequencies for all cases (Figure 2, r=0.77; P-value <0.0001).
- In fact, cfDNA was more sensitive than BM. Mutations were detected in significantly more peripheral blood cfDNA samples than in bone marrow cellular DNA (P-value=0.002).
- ASXL1 and TET2 were the most frequently-detected mutations.

Cases with mutations in plasma cfDNA but not in BM (Table 2)

- Six of the 33 (18%) patients had mutations in plasma but not in BM (Table 2).
- Mutations included RUNX, STAG2, PTEN, ZRSR2, BCOR, NPM1 found in 1/3, 1/1, 1/1, 1/2, 2/5, and 1/1 patients respectively.
- The greatest difference in allele frequency was found in the EZH2 gene, though not enough mutations were found in each gene to make significance statements about allele frequency differences on a gene-by-gene basis.
- Overall concordance between BM cells and cfDNA in the 120 samples was 92%, and there was no statistically significant difference between the two sample types (P=0.6).

Cases with mutations in BM but not plasma cfDNA (Table 3)

- ASXL1 was mutated in 3 BM cases but not plasma.
- Two patients (6%) showed mutations in BM cells and not in cfDNA.
- One patients had a mutation in ASXL1 and a subsequent sample showed the same ASXL1 mutation in BM cells and not in cfDNA. A second clone with a different ASXL1 mutation was detected in both BM cells and cfDNA (Table 3).

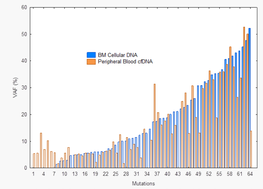
CONCLUSIONS

- Peripheral blood cfDNA can be used as a replacement for bone marrow cells for the purpose of detecting mutations in patients with AA.
- cfDNA is a reliable sample type for testing for the presence of mutations in bone marrow even when the VAF is very low.
- cfDNA can be used to monitor patients with AA.
- The demonstration that cfDNA is as reliable as BM cells in detecting very low level abnormalities suggests that cfDNA is reliable in the diagnosis of hematologic neoplasms and in monitoring residual disease.

Table 1: List of the detected unique mutations

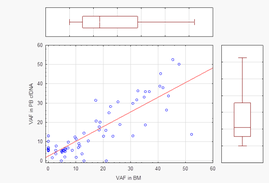
Gene	Hgvsc	Hgvsp
ASXL1	NM_015338.5:c.1771_1772insA	NP_056153.2:p.Tyr591Ter
ASXL1	NM_015338.5:c.1926_1927insG	NP_056153.2:p.Gly646TrpfsTer12
ASXL1	NM_015338.5:c.2197C>T	NP_056153.2:p.Gln733Ter
ASXL1	NM_015338.5:c.2222A>T	NP_056153.2:p.Asp741Val
ASXL1	NM_015338.5:c.2276_2280delGCCAG	NP_056153.2:p.Gln760LeufsTer12
ASXL1	NM_015338.5:c.2287delC	NP_056153.2:p.Leu764TyrfsTer8
ASXL1	NM_015338.5:c.3110G>A	NP_056153.2:p.Trp1037Ter
BCOR	NM_001123385.1:c.3809G>A	NP_001116857.1:p.Trp1270Ter
BCOR	NM_001123385.1:c.4973_4974delAG	NP_001116857.1:p.Gln1658ArgfsTer13
BCOR	NM_001123385.1:c.4988_4989delGG	NP_001116857.1:p.Trp1663SerfsTer8
BCOR	NM_001123385.1:c.756C>A	NP_001116857.1:p.Tyr252Ter
BCORL1	NM_021946.4:c.1942_1943insC	NP_068765.3:p.Val650ArgfsTer15
CBLC	NM_012116.3:c.1303C>T	NP_036248.3:p.Pro435Ser
CSF3R	NM_156039.3:c.2326C>T	NP_724781.1:p.Gln776Ter
DNMT3A	NM_022552.4:c.1634delA	NP_072046.2:p.Glu545GlyfsTer106
DNMT3A	NM_022552.4:c.1913C>A	NP_072046.2:p.Ser638Tyr
DNMT3A	NM_022552.4:c.2470delA	NP_072046.2:p.Ile824Ter
DNMT3A	NM_022552.4:c.2578T>C	NP_072046.2:p.Trp860Arg
DNMT3A	NM_022552.4:c.976C>T	NP_072046.2:p.Arg326Cys
EZH2	NM_004456.4:c.2109delA	NP_004447.2:p.Val704LeufsTer2
EZH2	NM_004456.4:c.630dupA	NP_004447.2:p.Glu211ArgfsTer11
IDH1	NM_005896.2:c.394C>T	NP_005887.2:p.Arg132Cys
JAK2	NM_004972.3:c.1849G>T	NP_004963.1:p.Val617Phe
NPM1	NM_002520.6:c.863_864insCCGC	NP_002511.1:p.Trp288CysfsTer12
NRAS	NM_002524.4:c.35G>C	NP_002515.1:p.Gly12Ala
NRAS	NM_002524.4:c.37G>C	NP_002515.1:p.Gly13Arg
PTEN	NM_000314.4:c.674A>G	NP_000305.3:p.Tyr225Cys
PTPN11	NM_002834.3:c.178G>C	NP_002825.3:p.Gly60Arg
PTPN11	NM_002834.3:c.226G>C	NP_002825.3:p.Glu76Gln
RUNX1	NM_001754.4:c.1440C>A	NP_001745.2:p.Tyr480Ter
RUNX1	NM_001754.4:c.276dupC	NP_001745.2:p.Asp93ArgfsTer45
RUNX1	NM_001754.4:c.965C>G	NP_001745.2:p.Ser322Ter
SETBP1	NM_015559.2:c.2602G>A	NP_056374.2:p.Asp868Asn
SF3B1	NM_012433.2:c.1973G>C	NP_036565.2:p.Trp658Ser
SF3B1	NM_012433.2:c.1998G>T	NP_036565.2:p.Lys666Asn
SRSF2	NM_001195427.1:c.284C>G	NP_001182356.1:p.Pro95Arg
STAG2	NM_001042749.1:c.1027G>T	NP_001036214.1:p.Val343Leu
TET2	NM_001127208.2:c.1118_1122delAAAAT	NP_001120680.1:p.Gln373ArgfsTer15
TET2	NM_001127208.2:c.1147C>T	NP_001120680.1:p.Gln383Ter
TET2	NM_001127208.2:c.1648C>T	NP_001120680.1:p.Arg550Ter
TET2	NM_001127208.2:c.2715_2716insA	NP_001120680.1:p.Met906AsnfsTer18
TET2	NM_001127208.2:c.3763_3764insA	NP_001120680.1:p.Tyr1255Ter
TET2	NM_001127208.2:c.575_576insAAT	NP_001120680.1:p.Tyr192delinsTer
U2AF1	NM_001025203.1:c.101C>T	NP_001020374.1:p.Ser34Phe
ZRSR2	NM_005089.3:c.1346_1360delGGAGCCGCCGCAGCC	NP_005080.1:p.Ser450_Arg454del

Figure 1: Comparison of cfDNA and BM mutation allele frequencies



Only 96 patients were included in the study, but 120 samples of these patients were tested. Mutations were detected in significantly more cfDNA samples than bone marrow samples (P=0.002). The VAF were compared between bone marrow cellular DNA and PB cfDNA. There was no statistical significance between cfDNA and BM in VAF (P=0.071). The levels of the VAF detected in all tested samples are shown in the graph above.

Figure 2: Correlation between cfDNA and BM mutation allele frequencies



r=0.77; P-Value <0.0001

Table 2: Cases with mutations in plasma cfDNA but not in BM

Detected in cfDNA Only

Gene	VAF (%)
BCOR*	5.4
BCOR*	5.58
NPM1	13.08
PTEN	6.96
RUNX1	10.25
STAG2	6.22
ZRSR2	5.71

Table 3: Cases with mutations in BM but not in cfDNA

Detected in BM Cells Only

Gene	VAF (%)
ASXL1	4.64
ASXL1+	13.04
ASXL1+	21.14

*, The same patient; +, the same patient

Two patients had mutations that were detected in BM but not in plasma.