

Supplemental Figure S1A



Supplemental Figure S1B

Supplemental Fig S1. Confirmation of the fragmentation pattern of plasma-/

serum-PBcfDNA in agarose gel electrophoresis.

(A) Plasma-/serum-PBcfDNA was analyzed with 1% agarose gel electrophoresis. The DNA amount loaded in one lane corresponded to DNA from 45 \textcircled L plasma or serum. DNA appeared in a ladder pattern as indicated by the red arrowheads. Numbers (1 to 32) indicate the MDS patient number. H1 to H14 indicate plasma-PBcfDNA from healthy donors. P; plasma, S; serum, M; 100 bp markers. (B) Representative pattern of PBcfDNA was analyzed with BioAnalyzer (Agilent Technologies, Inc.). The DNA amount in this analysis corresponds to DNA from 0.9 µL plasma. The ladder pattern likely comes from mono-, di-, and tri-chromatin.



Supplemental Figure S2

Supplemental Fig S2. The difference of PBcfDNA concentration in peripheral

blood samples harvested at the different time points.

Peripheral blood obtained from a patient was aliquated into 6 tubes and stored at 4 C for further use. PBcfDNA was harvested at the time points as indicated, and was visualized in agarose gel electrophoresis and the concentration was measured.



BM blast [%]	43
PB blast [%]	44
LDH [U/L]	514
PBcfDNA conc. (Plasma) [ng/mL]	169.8
PBcfDNA conc. (Serum) [ng/mL]	93.2

BM blast [%]	2
PB blast [%]	0
LDH [U/L]	473
PBcfDNA conc. (Plasma) [ng/mL]	32.5
PBcfDNA conc. (Serum) [ng/mL]	7.5

Supplemental Figure S3AB

C UPN #33



BM blast [%]	14.5
PB blast [%]	5
LDH [U/L]	262
PBcfDNA conc. (Plasma) [ng/mL]	58.2
PBcfDNA conc. (Serum) [ng/mL]	79

Supplemental Figure S3C

Supplemental Fig S3. Detection of genetic mutations in PBcfDNA.

Genetic mutations were confirmed by Sanger sequencing of BM-DNA, PBMNC-DNA, plasma-/serum-PBcfDNA, and DNA from germline cells. Genetic mutations detected in UPN #3 (MDS/AML) (**A**), UPN #6 (chronic myelomonocytic leukemia; CMML), (**B**) and UPN #33 (MDS/AML) are shown. BM and PB blast percentage and the serum LDH level at the time of PBcfDNA sample collection and the PBcfDNA concentration are also indicated below the sequencing data. Red arrows; mutated sites, blue arrows; wild-type sequence.

UPN #7



Supplemental Figure S4

Supplemental Fig S4. Detection of *IDH2* mutation using plasma-/serum-PBcfDNA in UPN #7.

IDH2 mutation confirmed in BM-DNA was analyzed in plasma-/serum-PBcfDNA. *IDH2* mutation detected in BM-MNCs was confirmed in three plasma-PBcfDNA samples harvested at different time points of the clinical course, but was not detected in serum-PBcfDNA.