**Title**

**Molecular features of tumor-derived genetic alterations in circulating cell-free DNA in virtue of autopsy analysis**

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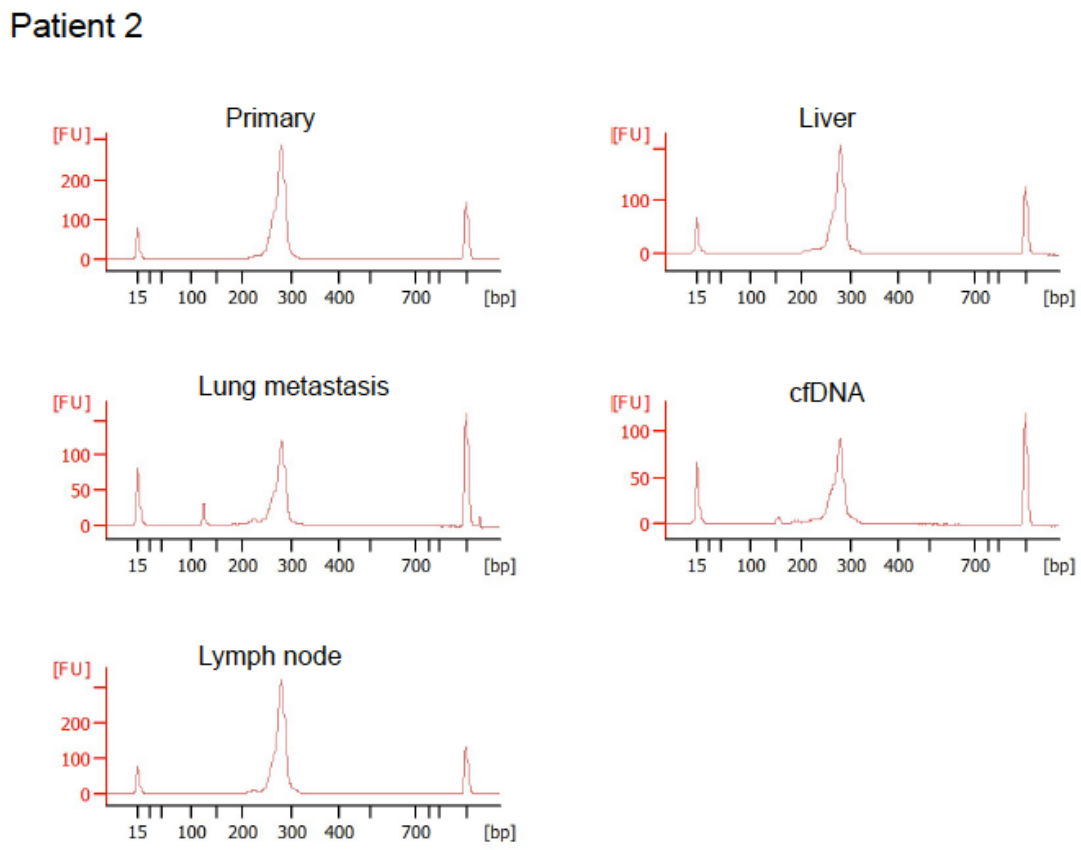
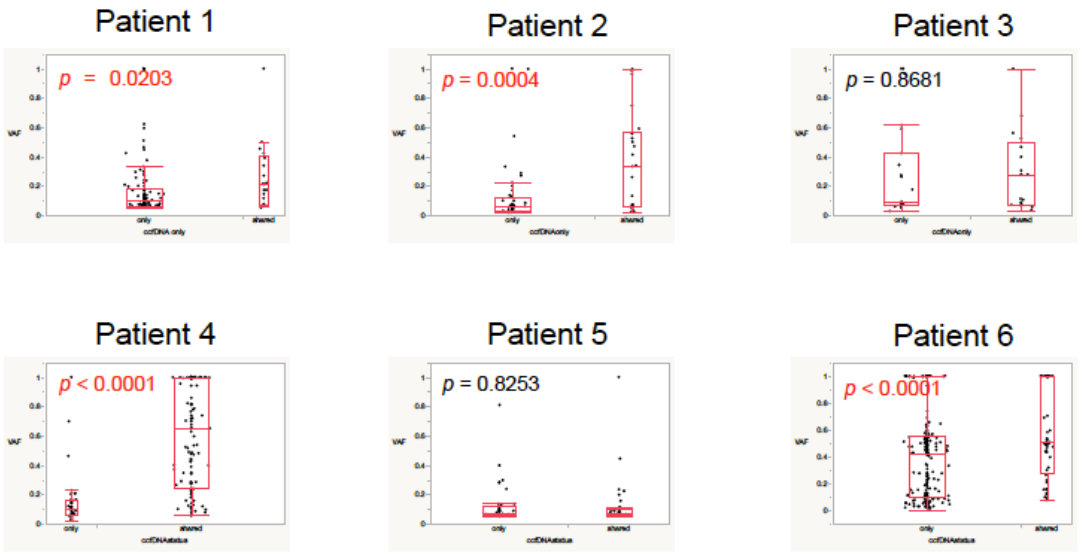
**Supplementary Fig. 1. Bioanalyzer image of library.**




**Bioanalyzer image of library.** Examples of library quality from the NGS analysis conducted in patient #2 using the BIOANALYZER HIGH SENSITIVITY DNA ASSAY KIT (Agilent Technologies). Each library exhibited a single peak around 280 bp and had a sufficient concentration to perform NGS analysis.

**Supplementary Fig 2. Distribution maps showing the variant allele frequencies (VAFs) of the genetic alterations in cfDNA.**




**Distribution maps showing the variant allele frequencies (VAFs) of the genetic alterations in cfDNA according to whether the alterations were detected exclusively in cfDNA (left box plot) or in both cfDNA and tDNA (right box plot).** In four patients (#1, 2, 4 and 6), the VAFs of the alterations detected exclusively in cfDNA were significantly lower than those of the alterations detected in both cfDNA and tDNA. The Mann–Whitney *U* test was used to compare the VAF of a genetic alteration detected in both tDNA and cfDNA with that of an alteration detected in cfDNA only (and not tDNA).

Supplementary Table 1. List of the genes included in the GENEREAD DNASEQ TARGETED PANELS V2 HUMAN COMPREHENSIVE CANCER PANEL (QIAGEN).

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *ABL1* | *BUB1B* | *DDR2* | *FGFR2* | *IDH2* | *MEN1* | *PDGFRA* | *SMARCA4* |
| *AKT1* | *CARD11* | *DICER1* | *FGFR3* | *IKZF1* | *MET* | *PHF6* | *SMARCB1* |
| *AKT2* | *CBL* | *DNMT3A* | *FH* | *IL6ST* | *MLH1* | *PIK3CA* | *SMO* |
| *ALK* | *CBLB* | *ECT2L* | *FLCN* | *IL7R* | *MSH2* | *PIK3R1* | *SPOP* |
| *AMER1* | *CD79A* | *EGFR* | *FLT3* | *JAK1* | *MSH6* | *PMS2* | *SRC* |
| *APC* | *CD79B* | *EP300* | *FUBP1* | *JAK2* | *MTOR* | *PPP2R1A* | *STK11* |
| *AR* | *CDC73* | *EPCAM* | *GATA1* | *JAK3* | *MUTYH* | *PRDM1* | *SUFU* |
| *ARID1A* | *CDH1* | *ERBB2* | *GATA2* | *KDM6A* | *MYC* | *PRKAR1A* | *TERT* |
| *ARID2* | *CDK12* | *ERBB3* | *GATA3* | *KDR* | *MYD88* | *PTCH1* | *TNFAIP3* |
| *ASXL1* | *CDK4* | *ERBB4* | *GNA11* | *KIT* | *NF1* | *PTEN* | *TNFRSF14* |
| *ATM* | *CDKN2A* | *ERCC5* | *GNAQ* | *KLF6* | *NF2* | *PTPN11* | *TP53* |
| *ATRX* | *CHEK2* | *ESR1* | *GNAS* | *KMT2D* | *NFE2L2* | *PAC1* | *TSC1* |
| *BAP1* | *CIC* | *EZH2* | *GPC3* | *KRAS* | *NFKBIA* | *RB1* | *TSC2* |
| *BCL6* | *CREBBP* | *FAM46C* | *GRIN2A* | *MAP2K1* | *NOTCH1* | *RET* | *TSHR* |
| *BCOR* | *CRLF2* | *FANCA* | *H3F3A* | *MAP2K2* | *NOTCH2* | *ROS1* | *U2AF1* |
| *BRAF* | *CSF1R* | *FANCD2* | *HIST1H3B* | *MAP2K4* | *NPM1* | *SDHB* | *VHL* |
| *BRCA1* | *CTNNB1* | *FANCE* | *HNF1A* | *MAP3K1* | *NRAS* | *SETD2* | *WT1* |
| *BRCA2* | *CYLD* | *FAS* | *HRAS* | *MAP4K3* | *PALB2* | *SF3B1* | *XPC* |
| *BRIP1* | *DAXX* | *FBXO11* | *HSPH1* | *MDM2* | *PAX5* | *SLC7A8* | *ZNF2* |
| *BTK* | *DDB2* | *FBXW7* | *IDH1* | *MED12* | *PBRM1* | *SMAD4* | *ZRSR2* |

Supplementary Table 2. Genetic alterations shared between the cfDNA samples of at least two patients

| Gene_Name | Variant_Type | AA_Change | COSMIC_ID | snpEff_Effect | function |
|---|---|---|---|---|---|
| *ALK* | SNP | p.I1461V | | NON_SYNONYMOUS_CODING | Oncogene, Immune response, RTK |
| *ZNF2* | DEL | p.LR156L | | CODON_DELETION | Transcription factor |
| *APC* | SNP | p.V1822D | | NON_SYNONYMOUS_CODING | Tumor Suppressor gene, Adhesion, Apoptosis, Cell cycle, DNA damage & repair |
| *EGFR* | SNP | p.R521K | | NON_SYNONYMOUS_CODING | Oncogene, Adhesion, RTK |
| *ATM* | SNP | p.N1983S | | NON_SYNONYMOUS_CODING | Tumor Suppressor gene, DNA damage & repair |
| *BRCA2* | SNP | p.V2466A | | NON_SYNONYMOUS_CODING | Tumor Suppressor gene, DNA damage & repair |
| *BIVM-ERCC5* | SNP | p.G1507R | | NON_SYNONYMOUS_CODING | Nucletide excision repair |
| *BIVM-ERCC5* | SNP | p.G1534R | | NON_SYNONYMOUS_CODING | |
| *TSHR* | SNP | p.E727D | | NON_SYNONYMOUS_CODING | Oncogene, G-protein coupled receptor signaling |
| *FANCA* | SNP | p.G809D | COSM435949 | NON_SYNONYMOUS_CODING | DNA damage & repair |
| *FANCA* | SNP | p.G501S | | NON_SYNONYMOUS_CODING | |
| *FANCA* | SNP | p.T266A | | NON_SYNONYMOUS_CODING | |
| *TP53* | SNP | p.P72R | COSM250061 | NON_SYNONYMOUS_CODING | Tumor Suppressor gene, Cell cycle, DNA damage & repair, Immune response, Transcription factor |
| *BRIP1* | SNP | p.S919P | | NON_SYNONYMOUS_CODING | DNA damage & repair |
| *ASXL1* | SNP | p.L815P | | NON_SYNONYMOUS_CODING | Tumor Suppressor gene, Epigenetics |

Abbreviations: AA, amino acid; SNP, single nucleotide polymorphism; RTK, receptor tyrosine kinases; DEL, deletion; MNP, manganese peroxidase.

Supplementary Table 3. The differences in mutation detection rates in cfDNA between common mutations (truncal or shared) and individual mutations in each patient.

| Patient 1 | cfDNA positive | cfDNA negative | |
|---|---|---|---|
| Truncal or shared | 21 | 64 | P < 0.0001 |
| Individual | 10 | 2067 | |

| Patient 2 | cfDNA positive | cfDNA negative | |
|---|---|---|---|
| Truncal or shared | 82 | 112 | P < 0.0001 |
| Individual | 21 | 110 | |

| Patient 3 | cfDNA positive | cfDNA negative | |
|---|---|---|---|
| Truncal or shared | 24 | 119 | P = 0.8966 |
| Individual | 10 | 43 | |

| Patient 4 | cfDNA positive | cfDNA negative | |
|---|---|---|---|
| Truncal or shared | 124 | 55 | P < 0.0001 |
| Individual | 16 | 50 | |

| Patient 5 | cfDNA positive | cfDNA negative | |
|---|---|---|---|
| Truncal or shared | 15 | 14 | P = 0.0236 |
| Individual | 18 | 52 | |

| Patient 6 | cfDNA positive | cfDNA negative | |
|---|---|---|---|
| Truncal or shared | 87 | 77 | P = 0.0006 |
| Individual | 29 | 67 | |

P values were calculated by the chi-square test with Yate's correction.

Supplementary Files.

File 1-31 are all genetic alteration profile from all samples. File number corresponds the following:

1. Primary lesion of patient #1
2. Adrenal metastasis of patient #1
3. Soft tissue metastasis of patient #1
4. cfDNA of patient #1
5. Germline DNA of patient #1
6. Primary lesion of patient #2
7. Lung metastasis of patient #2
8. Lymph node metastasis of patient #2
9. Liver metastasis of patient #2
10. cfDNA of patient #2
11. Germline DNA of patient #2
12. Primary lesion of patient #3
13. Liver metastasis of patient #3
14. Renal metastasis of patient #3
15. cfDNA of patient #3
16. Germline DNA of patient #3
17. Primary lesion of patient #4
18. Lymph node metastasis of patient #4
19. cfDNA of patient #4
20. Germline DNA of patient #4
21. Primary lesion of patient #5
22. Lymph node metastasis of patient #5
23. cfDNA of patient #5
24. Germline DNA of patient #5
25. Primary lesion of patient #6
26. Right lung metastasis of patient #6
27. Left lung metastasis of patient #6
28. Diaphragm metastasis of patient #6
29. Liver metastasis of patient #6
30. cfDNA of patient #6
31. Germline DNA of patient #6.

File 32 is an original “terminal app” script.