

Table S1 Mutation information of ZJ

Number	Mutation	Initial frequency	Company	Catalog number	Final frequency
1	BRAF:p.V600E	50%	Horizon	HD251 HD253 HD254	10.00%
2	PIK3CA:p.H1047R	50%	Horizon Cobioer	HD251 HD253 HD254 HD258 HD664 CBP60034	10.00%
3	KRAS:p.G12S	100%	Cobioer	CBP60084	5.10%
4	NRAS:p.Q61R	100%	Cobioer	CBP60074	5.10%
5	BRAF:p.G469A	99%	Cobioer	CBP60163	5.05%
6	KRAS:p.G13D	50%	Horizon	HD664	2.55%
7	ALK-EML4:fusion	50%	Horizon	HD664	2.55%
8	EGFR:p.T790M	50%	Horizon	HD258	2.55%
9	EGFR:p.G719S	50%	Horizon	HD253	1.02%
10	KRAS:G12D PIK3CA:1047R	45%	Cobioer	CBP60034	0.92%
11	EGFR:p.L858R	50%	Horizon	HD254	0.51%
12	EGFR:p.746_750del	50%	Horizon	HD251	0.51%
13	PIK3CA:p.E545K	46%	Cobioer	CBP60142	0.23%
14	PTEN:p.R233*	50%	Cobioer	CBP60707	0.26%
15	KRAS:p.G12A	61%	Cobioer	CBP60748	0.31%

Note: 1) The cell line background of HD251, HD253, HD254 and HD258 is RKO, which carries both BRAF:p.V600E and PIK3CA:p.H1047R (https://cancer.sanger.ac.uk/cell_lines/sample/overview?id=909698). So, HD251, HD253, HD254 and HD258 was used to generate lower allelic frequencies of BRAF:p.V600E and PIK3CA:p.H1047R in our study. 2) The cell line background of HD664 is HCT-116, which carries both PIK3CA:p.H1047R and KRAS:p.G13D (https://cancer.sanger.ac.uk/cell_lines/sample/overview?id=905936). So, HD664 was used to generate lower allelic frequencies of PIK3CA:p.H1047R and KRAS:p.G13D here. 3) The cell line background of CBP60034 is LS-180, which carries both PIK3CA:p.H1047R and KRAS:G12D (https://cancer.sanger.ac.uk/cell_lines/sample/overview?id=998189). So, CBP60034 was also used to generate lower allelic frequencies of PIK3CA:p.H1047R and KRAS:G12D. 4) The cell line background of CBP60084 is A549, which carries KRAS:p.G12S (https://cancer.sanger.ac.uk/cell_lines/sample/overview?id=905949). So, CBP60084 was used to generate a lower allelic frequency of KRAS:p.G12S. 5) The cell line background of CBP60074 is NCI-H2347, which carries NRAS:p.Q61R (https://cancer.sanger.ac.uk/cell_lines/sample/overview?id=687820). So, CBP60074 was used to generate a lower allelic frequency of NRAS:p.Q61R. 6) The cell line background of CBP60163 is NCI-H1395, which carries BRAF:p.G469A ([https://www.jto.org/article/S1556-0864\(18\)31510-7/pdf](https://www.jto.org/article/S1556-0864(18)31510-7/pdf)). So, CBP60163 was used to generate a lower allelic frequency of BRAF:p.G469A. 7) The cell line background of CBP60142 is NCI-H596, which carries PIK3CA:p.E545K (https://cancer.sanger.ac.uk/cell_lines/sample/overview?id=908459). So, CBP60142 was used to generate a lower allelic frequency of PIK3CA:p.E545K. 8) The cell line background of CBP60707 is C-33A, which carries PTEN:p.R233* (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3976291/>). So, CBP60707 was used to generate a lower allelic frequency of PTEN:p.R233*. 9) The cell line background of CBP60748 is SW1116, which carries KRAS:p.G12A (https://cancer.sanger.ac.uk/cell_lines/sample/overview?id=909746). So, CBP60748 was used to generate a lower allelic frequency of KRAS:p.G12A.

Table S2 Gene list

SAMD9L
ABCB1
ABCA13
PIK3CG
RAMP3
AMPH
UBE3C
FLNC
HNRNPA2B1
CPA2
TRRAP
OPN1SW
RHBDD2
PTPN12
BRAF
HGF
EGFR
HDAC9
PCLO
ABCB4
MLL3
SMO
NOS3
DDC
SLC25A13
IL6
MET
CARD11
URGCP
SAMD9
ELMO1
FOXA2
GNAS
SRC
SAMHD1
RAC2

*Table S2 (continued)***Table S2** (*continued*)

NF2
MICAL3
EP300
MAPK1
HIF1A
SLC10A1
AKT1
SYNE2
FOXA1
BRF1
ATAD2
MTDH
CHD7
RSPO2
TPD52
DLC1
ZFPM2
PCMTD1
SULF1
WRN
WWP1
PPAPDC1B
UNC5D
PKHD1L1
CLU
RECQL4
FZD6
CSMD3
FGFR1
MYC
NSMCE2
CCNE1
XRCC1
GNA11
OR4F17

Table S2 (continued)

Table S2 (continued)

ZNF737
KEAP1
PEG3
RZR1
TGFB1
SPC24
ZNF226
MAP2K7
JAK3
MLL4
SMARCA4
COMP
STK11
PDE4DIP
ARID4B
FAM5C
NRAS
DDR2
OR4F3
LEPR
MTOR
RPL22
CNTN2
FCRL1
CACNA1E
TMEM51
ASPM
SETDB1
COL11A1
GPATCH3
MPL
MUC1
ISG15
CRP
MCL1
PLXNA2

Table S2 (continued)

Table S2 (continued)

RZR2
IGSF3
EPS15
PRMT6
PARP1
JAK1
TCHHL1
PTPRC
ABCA4
SPAG17
ARID1A
ATAD3B
ERRF1
SPRTN
MYSM1
VEGFA
HLA-DRA
SYNE1
DST
DSE
EYS
ROS1
UBD
HIST1H4B
HIST1H2AL
TLL2
PKHD1
CDKN1A
SLC22A1
HIST1H2BD
IGF2R
MEP1A
LAMA2
CCND3
ITPR3
NEDD9

Table S2 (continued)

Table S2 (continued)

HLA-DQA1
NOTCH4
CEP85L
ARID1B
GJA1
TNF
MEN1
FGF4
SIPA1
MLL
FAT3
CFL1
FGF3
TTC36
IGF2
CCND1
FGF19
HRAS
ATM
SIRT3
CD3D
MAP2K3
ACE
NME1
HN1
SUPT6H
RAD51C
USP6
KRT19
NLRP1
CBX4
SDK2
STAT3
G6PC
BPTF
NCOR1

Table S2 (continued)

Table S2 (continued)

TP53
TMEM99
BRIP1
MAP2K4
FLCN
ERBB2
NF1
CLDN14
USP25
RUNX1
CDH1
PRKCB
BRD7
CREBBP
RBL2
C16orf62
SRCAP
TSC2
AXIN1
TMEM170A
SERPINB3
SMAD2
C18orf34
ATP8B1
ASXL3
SMAD4
EPHB1
RAF1
KAT2B
IGSF10
ZNF717
VHL
CTNNB1
BAP1
FAM157A
LRTM1

Table S2 (continued)

Table S2 (continued)

GLB1
SLC15A2
ATR
COL6A5
PIK3CA
RASSF1
MECOM
SETD2
ADIPOQ
MLL2
MDM2
CDK4
CDKN1B
RAN
CACNA2D4
ARID2
KRAS
CCND2
GXYLT1
SELPLG
MARS
TNFRSF1A
MDM1
NUP107
BAZ2A
RARG
LRP1
PTGES3
NAV3
PTPN11
ERBB3
HNF1A
PTPRB
RYR3
MAP2K1
IL16

Table S2 (continued)

Table S2 (continued)

PML
IGF1R
MAN2C1
NTRK3
CHD2
FBN1
SMAD3
IDH2
VCX
RPS6KA3
FLNA
ZIC3
GPC3
ATRX
DMD
GPR143
TAF1
AR
HUWE1
KDM6A
FGA
KIT
FGFR3
FAT4
NFKB1
EGF
ALB
AFP
PDGFRA
ADH1B
IRF2
SPP1
OTOP1
PLK4
KDR
CCNA2

Table S2 (continued)

Table S2 (continued)

FRAS1
GAB1
TLR3
PROM1
IL8
LRP2
TTN
SCN7A
ERBB4
CYP1B1
UNC80
UBR3
IRS1
BAZ2B
DNMT3A
LRP1B
EPHA4
STAT4
BRE
GLI2
ABCB11
ALK
NFE2L2
MXD1
APOB
ACVR2A
EIF2AK3
IDH1
XRCC5
HOXD13
GALNT14
CDKN2A
TMC1
PTCH1
JAK2
NOTCH1

Table S2 (continued)

Table S2 (continued)

PLIN2
LCN1
GOLM1
GNAQ
TSC1
ABL1
PTPRD
CEL
C9orf3
KLF4
TAF1L
CDKN2B
RB1
DCLK1
SACS
PARP4
BRCA2
FLT3
FLT1
FAM123A
NRG3
DKK1
CYP2E1
SMC3
PTEN
KLF6
RET
FGFR2
RPS24
FLT4
FGFR4
IL6ST
FBN2
LIFR
TAF9
DMXL1

Table S2 (continued)

Table S2 (*continued*)

THBS4
DOCK2
AHRR
MAP1B
ATP10B
ADCY2
CSF1R
CXCL14
TERT
BRD9
GOLPH3
CTNND2
PDGFRB
CHD1
BRD8
APC
HMGCS1
PRLR
NPM1

Table S3 Results of the 3 virtual plasma samples

Sample Name	Kit	Total (ng)	Pre-PCR (ng/uL)	Post-PCR (ng/uL)	Average sequencing depth
QIAGEN-1	QIAGEN	221.60	40.60	19.9	6,389.25
QIAGEN-2	QIAGEN	192.00	46.00		5,456.45
QIAGEN-3	QIAGEN	233.60	52.00		8,454.60

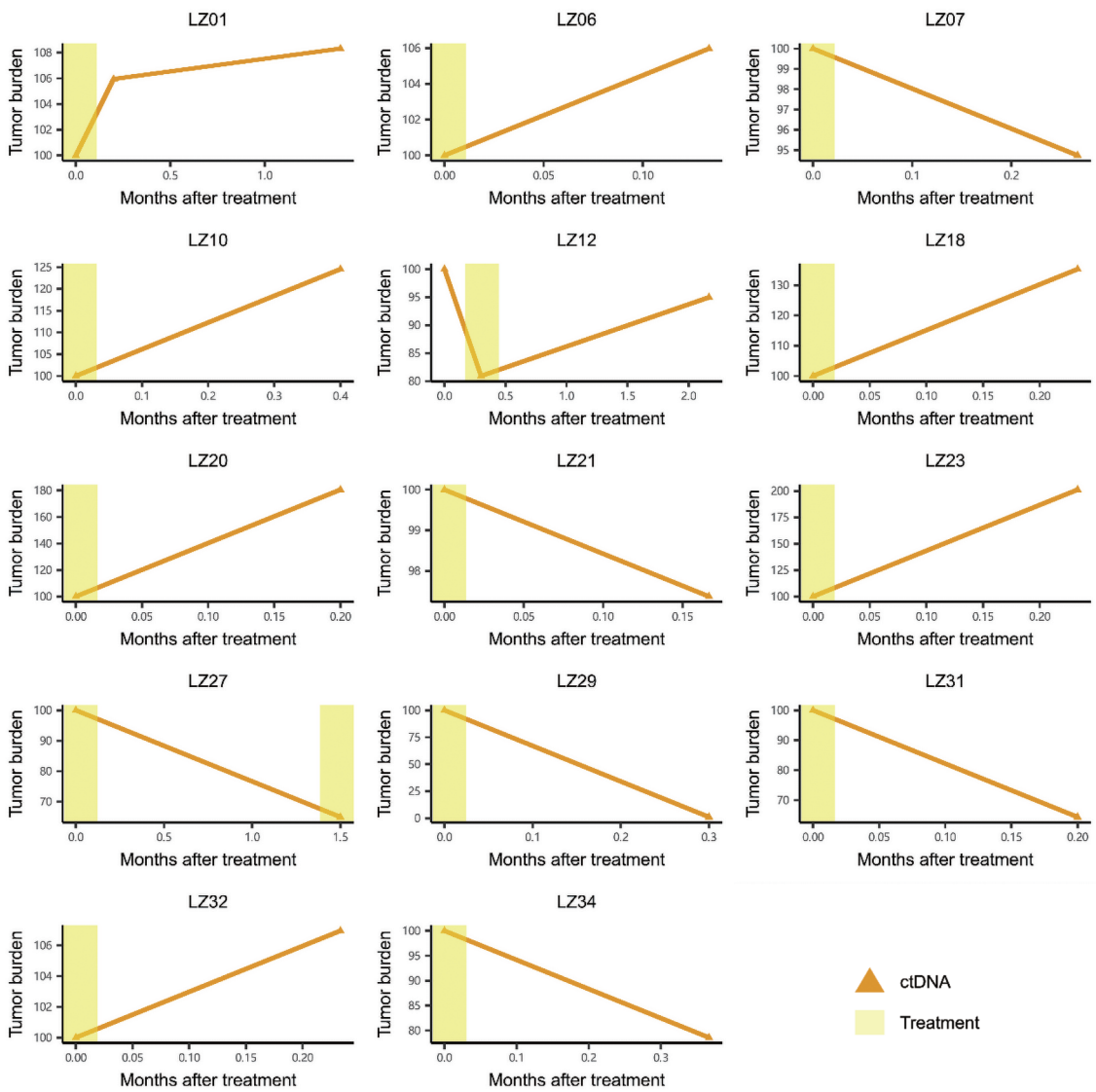


Figure S1 ctDNA levels in patients with normal AFP concentration. AFP, alpha-fetoprotein.