Supplementary figures and tables
Molecular diagnosis of diffuse glioma using a chip-based digital PCR system to analyze IDH, TERT, and H3 mutations in the cerebrospinal fluid

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## Supplementary table 1

### Assay data

| mutation                  | Assay ID       | product length(bp) | Forward Primer                              | Reverse Primer                              | Wild type probe                  | Mutation probe                  |
|---------------------------|----------------|--------------------|---------------------------------------------|---------------------------------------------|----------------------------------|---------------------------------|
| IDH R132H                 | ANDJ4XD        | 88                 | GCAAAATCACATTATTGCAACA                      | CTTGTGAGTGATGGGTAAAACC                      | AAGCATGACGACCTATT                | TAAGCATGATGACCTATG              |
| H3K27M                    | ANFVT27        | 107                | CTTTGCCCATTTTTTTTCTGTATT                    | GAAGCAACTGGCTACAAAGCC                       | CCGACTTGCGAGCT                   | CGCACTCATCGCGAGC                |
| TERT promoter mutation C228T | Hs000000092_rm | NA                 | NA                                          | NA                                          | NA                               | NA                             |
| TERT promoter mutation C250T | Hs000000093_rm | NA                 | NA                                          | NA                                          | NA                               | NA                             |

NA: not available
Supplementary table 2

**IDH1R132H mutation and H3K27M mutation**

| Stage 1 | Stage 2 | Stage 3 |
|---------|---------|---------|
| 96°C    | 56.0°C  | 98.0°C  | 60.0°C   | 10.0°C   |
| 10 min  | 2 min   | 30 s    | 2 min    | ∞        |
| 1x (Hold) | 39x (Cycles) |   | 1x (Hold) |

**TERT promoter mutation C228T and C250T**

| Stage 1 | Stage 2 | Stage 3 |
|---------|---------|---------|
| 96°C    | 55.0°C  | 98.0°C  | 55.0°C   | 10.0°C   |
| 10 min  | 2 min   | 30 s    | 2 min    | ∞        |
| 1x (Hold) | 54x (Cycles) |   | 1x (Hold) |
### Supplementary table 3

|                          | 1 reaction µL | Final concentration ng/µL |
|--------------------------|---------------|---------------------------|
| Digital PCR master mix v2 | 7.25          | 1x                        |
| Taqman Assay 20x (primer + probe) | 0.75          | 1x                        |
| Nuclease free water      | 6.5-X         |                           |
| Template DNA             | X             | X/14.5                    |
| Total                    | 14.5          |                           |

X: Up to 6.5 µL
### Supplementary table 4

| Taqman Assay                           | X   | Y   |
|----------------------------------------|-----|-----|
| IDH1 R132H mutation                    | 1200| 4000|
| H3F3A K27M mutation                    | 1400| 4000|
| TERT promoter mutation C228T           | 350 | 1200|
| TERT promoter mutation C250T           | 400 | 2000|

VIC threshold = mode + X  
FAM threshold = mode + Y
| Molecular diagnosis | WHO grading | Tumor DNA | N | matched | sensitivity |
|---------------------|-------------|-----------|---|---------|-------------|
| GBM,IDH mut         | IV          | mut       | 4 | 4       | 100%        |
| GBM,IDH wt          | IV          | wt        | 3 | 3       | 100%        |
| DMG,H3K27M          | IV          | wt        | 5 | 4       | 80%         |
| AO,IDH mut          | III         | mut       | 8 | 8       | 100%        |
| AA,IDH mut          | III         | mut       | 2 | 1       | 50%         |
| AA,IDH wt           | III         | wt        | 2 | 0       | 0%          |
| OD,IDH mut          | II          | mut       | 1 | 0       | 0%          |
| DA,IDH mut          | II          | mut       | 1 | 0       | 0%          |
| DA,IDH wt           | II          | wt        | 2 | 0       | 0%          |
| GBMorAAorDA         | -           | wt        | 6 | 6       | 100%        |

mut: mutant  wt: wildtype  GBM: glioblastoma  DMG: diffuse midline glioma  AO: anaplastic oligodendroglioma  AA: anaplastic astrocytoma  OD: oligodendroglioma  DA: diffuse astrocytoma
Supplementary Fig 1a

Scatter plot

UL: Upper left
UR: Upper right
LL: Lower left
LR: Lower right

FAM fluorescence
VIC fluorescence

Histogram
FAM

X

mode

VIC threshold = mode + X

Y

mode

FAM threshold = mode + Y

Fluorescence

no amplification
wildtype DNA

no amplification
& wildtype DNA
Supplementary Fig 1b

FC: 0.01 ng/μL

UL: 0  UR: 0  LR: 25
LL: 18277

FC: 0.1 ng/μL

UL: 0  UR: 5  LR: 476
LL: 17905

VIC fluorescence

FAM fluorescence

Well counts

Fluorescence

VIC

Mode: 500

Threshold: 500 + 1200 = 1700

Mode: 600

Threshold: 600 + 1200 = 1800

Mode: 1100

Threshold: 1100 + 4000 = 5100

Mode: 900

Threshold: 900 + 4000 = 4900
**Supplementary Fig 1c**

IDH1 R132H mutation assay

**FAM10**

**VIC10**

**Well counts**

**Fluorescence**

**Mode:** 400  
**VIC threshold:**  
**Mode + 1200 = 1600**

**Mode:** 600  
**FAM threshold:**  
**Mode + 4000 = 4600**

**FAM fluorescence**

**VIC fluorescence**

**Mutant**

**VIC threshold:** 1600

**FAM threshold:** 4600
Supplementary Fig 2

- Mutant
- Wild type

Axes:
- VIC fluorescence
- FAM fluorescence
- VIC threshold
- FAM threshold
Supplementary Fig 3a: MRI imaging

Supplementary Fig 3b
Pathological finding

Supplementary Fig 3c
Supplementary Fig 3d: Tumor DNA

Digital PCR: positive

Supplementary Fig 3e: cfDNA in CSF

Intracranial CSF

Sanger sequence: wild type

Lumbar puncture CSF