Identification of Potential Genomic Alterations Using Pan-Cancer Cell-Free DNA Next-Generation Sequencing in Patients with Gastric Cancer

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Supplemental Data Table S1. QC metrics of cfDNA analysis using the Ion Torrent S5 XL system

Oncomine Pan-Cancer Cell-Free Assay		
Category		**Metric**
Sample quality	cfDNA recovered from sample	3.2 (2.37–6.23)
Sample quality	cfDNA input	32.0 (24.10–49.10)
Sample quality	Library (pmol)	2714 (1788.0–3520.5)
Sequencing	ISP loading	94% (94.0%–95.0%)
Sequencing	Key signal	101 (95.0–104.0)
Sequencing	Usable reads	63% (60.5%–65.0%)
Mapping	Percent on-target reads	97% (96.2%–97.0%)
Mapping	Uniformity of base coverage	99% (98.4%–99.4%)
Mapping	Overall mapped reads	19,995,625 (18,176,716.5–22,257,952.5)
Mapping	Median read coverage	60,380 (52,183.5–66,919.5)
Mapping	Median molecular coverage	6,201 (4,980.5–7,796.8)
Mapping	Amplicons reading end-to-end	99% (98.5%–98.9%)

Data are presented as median and 25th and 75th interquartile range.

Abbreviations: cfDNA, cell-free DNA; ISP, ion sphere particle.

Supplemental Data Table S2. QC metrics of cfDNA analysis using the Illumina NextSeq-550 system

AlphaLiquid 100 kit **Category**	**Metric**	
Sample quality	cfDNA recovered from sample	2.8 (1.44–5.60)
Sample quality	cfDNA input	30.9 (30.00–35.00)
Sample quality	Library input	2,000 (2,000.0–2,000.0)
Sequencing	% ≥ Q30 read 1	88% (86.4%–89.8%)
Sequencing	% ≥ Q30 read 4	86% (84.7%–89.1%)
Sequencing	% Clusters passing filter	91% (88.3%–92.8%)
Mapping	Raw reads	High: 89,951,894 (77,083,725.5–105,539,922.0) Mid: 136,254,381 (129,247,796.5–147,541,133.5)
Mapping	Mapped reads	High: 84,960,287 (73,137,845.3–100,027,107.3) Mid: 128,087,414 (120,881,686.5–137,515,767.5)
Mapping	On-target read ratio	High: 64.5 (59.81–69.43) Mid: 73.5 (69.58–76.24)
Mapping	On-target mean depth	High: 13,021 (10,492.9–16,361.0) Mid: 20,875 (19,795.4–22,197.2)
Mapping	Fragment mean depth	2,181 (1,709.1–2,376.7)
Mapping	Fragment uniformity (%)	High: 97% (90.5%–97.8%) Mid: 97% (96.9%–97.8%)

Two flow-cell configurations were used: high-output and mid-output.

Data are presented as median and 25th and 75th interquartile range.

Abbreviation: cfDNA, cell-free DNA.

Supplemental Data Table S4. Exclusion of germline variants using Sanger sequencing

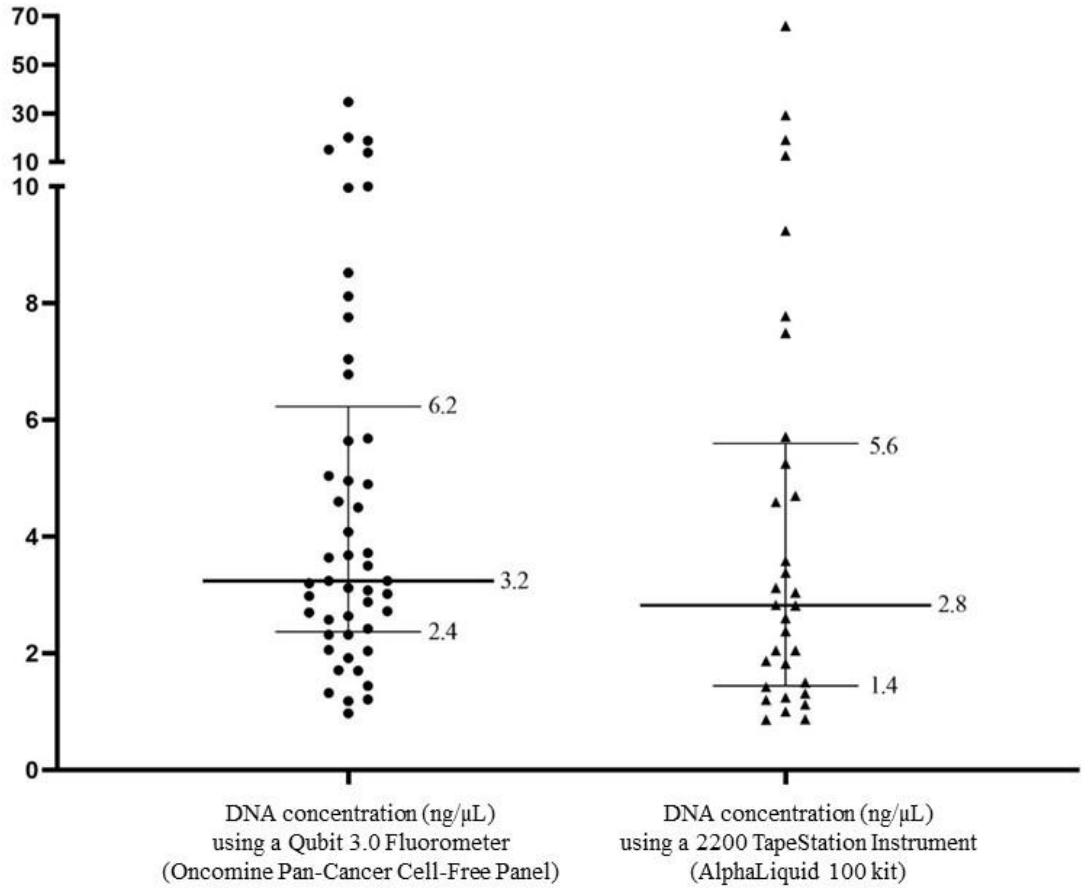
No. case	Tier	SNV gene	Transcript	Nucleotide change	Amino acid change	Allele frequency (%)
60	Tier II	*TP53*	NM_000546.6	c.659A>G	p.Tyr220Cys	57.2*
63	Tier II	*ARID1A*	NM_006015.6	c.2296del	p.Gln766SerfsTer67	41.4*
68	Tier II	*RET*	NM_020975.6	c.2269G>A	p.Val757Met	47.3*
79	Tier II	*APC*	NM_000038.6	c.4547_4562del	p.Ile1516AsnfsTer2	56.5*

*The variant was confirmed to be a somatic mutation via Sanger sequencing.

Supplemental Data Table S5. Comparison of VIKTORY trial data and our data according to the biomarkers

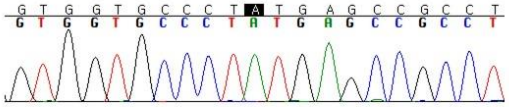
Biomarker	**The present study (N patients/total N patients)**	**VIKTORY trial***
FGFR2 amplification	6.2% (5/81)	4.2%
FGFR1 amplification	1.2% (1/81)	1.4%
EGFR amplification	3.7% (3/81)	2.4%
CCNE1 amplification	9.4% (3/32)	2.0%
RAS mutation or amplification	Mutation: 11.1% (9/81) Amplification: 9.4% (3/32)	12.2%
TP53 mutation	38.3% (31/81)	44.9%
PIK3CA mutation or amplification	2.5% (2/81)	7.6%
MET amplification	3.7% (3/81)	3.5%

*The VICTORY trial included 715 tissue samples.

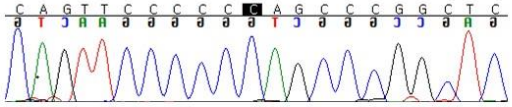


Supplemental Data Figure S1. DNA concentrations of the samples used in the two assays in this study.

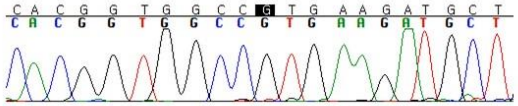
Patient 60 NM_000546.6(TP53):c.659A>G,
p.Tyr220Cys, AF 57.2%



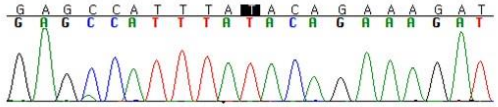
Patient 63 NM_006015.6(ARID1A):c.2296del,
p.Gln766SerfsTer67, AF 41.4%



Patient 68 NM_020975.6(RET):c.2269G>A,
p.Val757Met, AF 47.3%



Patient 79 NM_000038.6(APC):c.4547_4562del,
p.Ile1516AsnfsTer2, AF 56.5%



Supplemental Data Figure S2. Sanger sequencing chromatograms of tier I or II variants with an allele frequency of 40%–60%.