Genomic biomarker detection in East Asian clinical practice using circulating tumor DNA (ctDNA) from patients with gastrointestinal (GI) tract cancers

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BACKGROUND

Personalized medicine for patients with advanced-stage GI tract cancers depends on identification of potentially actionable genetic alterations in the tumor. This can be done through sequential testing of individual genes or by a single comprehensive genomic profiling test. Each approach requires either tumor tissue and/or ctDNA.

We summarized the frequency of common and clinically relevant alterations from East Asian patients with common GI cancers whose blood was tested by a commercially available comprehensive next generation sequencing (NGS) assay (Guardant360).

METHODS

- Guardant360 (Guardant Health, Redwood City, CA) is a comprehensive genomic profiling assay of 70 to 74 genes (v2.9 to v2.11). It identifies single nucleotide variants, insertions and deletions, fusions, and amplifications from cell-free plasma DNA. Complete exon sequencing is provided for several genes, including *EGFR* and *KRAS* **(Fig 1)**.
- Guardant360 test results from Japan, Korea, Taiwan, Hong Kong, and Southeast Asia were reviewed (cut-off June 2020).
- We reviewed results from patients with a diagnosis of colorectal adenocarcinoma (CRC), pancreatic adenocarcinoma (PC), gastric and gastroesophageal adenocarcinoma (GEC), and biliary tract carcinoma (BTC).
- Synonymous mutations and variants of unknown significance were excluded. Microsatellite instability-high status was not included in this analysis.
- Samples from patients enrolled in prospective clinical trials were not included.

Figure 1. Guardant360 Gene Panel

Point Mutations, Insertions, Deletions — 74 Genes									
AKT1	*ALK*	***APC***	***AR***	*ARAF*	*ARID1A*	***ATM***	***BRAF***	***BRCA1***	***BRCA2***
CCND1	*CCND2*	*CCNE1*	*CDH1*	***CDK4***	***CDK6***	***CDK12***	*CDKN2A*	*CTNNB1*	*DDR2*
EGFR	***ERBB2 (HER2)***	*ESR1*	*EZH2*	*FBXW7*	*FGFR1*	*FGFR2*	*FGFR3*	*GATA3*	*GNA11*
GNAQ	*GNAS*	*HNF1A*	***HRAS***	*IDH1*	*IDH2*	*JAK2*	*JAK3*	***KIT***	***KRAS***
MAP2K1 (MEK1)	*MAP2K2 (MEK2)*	***MAPK1 (ERK2)***	***MAPK3 (ERK1)***	***MET***	*MLH1*	*MPL*	*MTOR*	***MYC***	*NF1*
NFE2L2	*NOTCH1*	*NPM1*	***NRAS***	*NTRK1*	*NTRK3*	*PDGFRA*	***PIK3CA***	***PTEN***	*PTPN11*
RAF1	*RB1*	*RET*	*RHEB*	*RHOA*	*RIT1*	*ROS1*	*SMAD4*	*SMO*	***STK11***
TERT[†]	***TP53***	*TSC1*	*VHL*						

[†]Includes *TERT* promoter region

Amplifications – 18 Genes								
*AR**	*BRAF**	*CCND1**	*CCND2*	*CCNE1*	*CDK4**	*CDK6**	*EGFR*	*ERBB2**
FGFR1	*FGFR2**	*KIT**	*KRAS**	*MET**	*MYC*	*PDGFRA**	*PIK3CA*	*RAF1**

Fusions – 6 Genes					
ALK	*FGFR2*	*FGFR3*	*RET*	*ROS1*	*NTRK1*

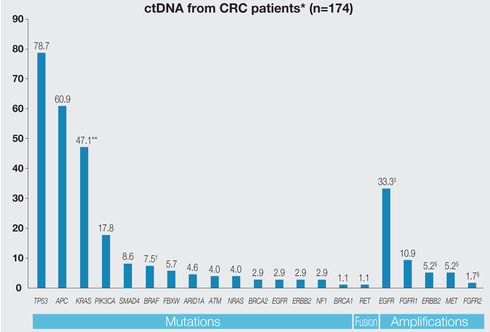
Bold=full exome sequencing
*Focal amplification reported

RESULTS

COLORECTAL CANCER

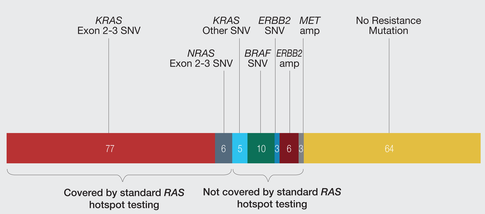
- Includes physician reported diagnoses: colorectal adenocarcinoma, colorectal carcinoma, sigmoid colon adenocarcinoma:
- ctDNA detected in 174 of 191 samples (91%) **(Fig 2)**.
- Samples with ≥1 mutation associated with *EGFR* monoclonal antibody (mAb) resistance[1]: 110 (63%) **(Fig 3)**.

Figure 2: Common alterations (%) in samples with ctDNA from CRC patients* (n=174)



*Samples may contain more than 1 alteration; **1/82 *KRAS* G12C; †11/13 *BRAF V600E*;
‡≥90th percentile: 6/58 *EGFR*, 7/19 *FGFR1*; §Confirmed focal: 8/9 *ERBB2*, 5/9 *MET*, 2/3 *FGFR2*.

Figure 3: *EGFR* mAb resistance mutations detected in 174 plasma samples from patients with metastatic CRC

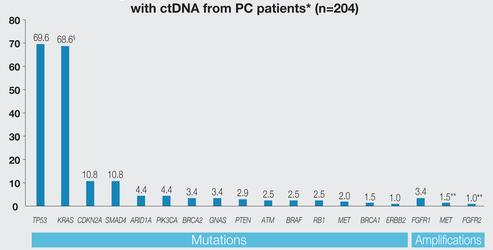


25% of all samples with resistance mutations were detected outside of *RAS* exons 2-3

PANCREATIC ADENOCARCINOMA

- Includes physician reported diagnoses: pancreatic ductal adenocarcinoma, pancreatic mucinous adenocarcinoma, pancreatic carcinoma, pancreatic mixed adenoneuroendocrine carcinoma
- ctDNA detected in 204 of 236 samples (86%) **(Fig 4)**.

Figure 4: Common alterations (%) in samples with ctDNA from PC patients* (n=204)

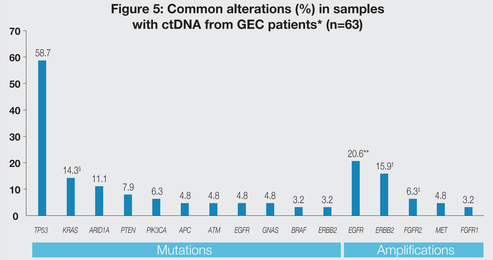


*Samples may contain more than 1 alteration; †6/140 *KRAS* G12C; **All confirmed focal.

GASTROESOPHAGEAL ADENOCARCINOMA

- Includes physician reported diagnoses: gastric adenocarcinoma, gastric carcinoma, gastroesophageal junction adenocarcinoma, gastroesophageal junction carcinoma, esophageal adenocarcinoma
- ctDNA detected in 63 of 74 samples (85%) **(Fig 5)**.

Figure 5: Common alterations (%) in samples with ctDNA from GEC patients* (n=63)

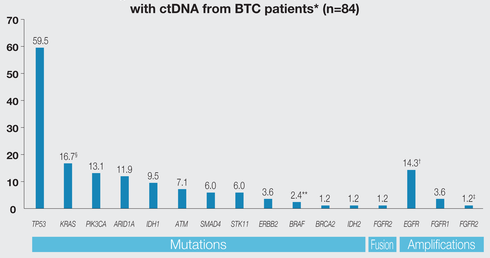


*Samples may contain more than 1 alteration; ‡1/9 *KRAS* G12C; **2/13 ≥90th percentile; †7/10 confirmed focal;
‡All confirmed focal.

BILIARY TRACT CARCINOMA

- Includes physician reported diagnoses: cholangiocarcinoma, gall bladder adenocarcinoma, gall bladder carcinoma, ampullary carcinoma
- ctDNA detected in 84 of 97 samples (87%) **(Fig 6)**.

Figure 6: Common alterations (%) in samples with ctDNA from BTC patients* (n=84)

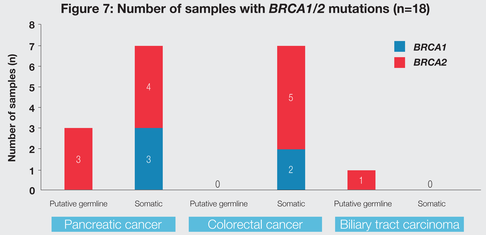


*Samples may contain more than 1 alteration; §2/14 *KRAS* G12C; **1/2 *BRAF* V600E; †4/12 ≥90th percentile;
‡Confirmed focal.

BRCA1/2 MUTATIONS

- Mutations in *BRCA1* or *BRCA2* were present in 4% of samples (10 PC, 7 CRC, 1 BTC, 0 GEC).
- Most samples (14/18; 77.8%) had mutations with allelic frequency <40%, which are likely somatic. Samples with *BRCA* mutation allelic frequency >40% are considered "putative germline" **(Fig 7)**.

Figure 7: Number of samples with *BRCA1/2* mutations (n=18)



Median allelic frequency for *BRCA1/2* mutations
Somatic mutations (n=14): 0.5%
Putative germline mutations (n=4): 53%

LIMITATIONS

This is a retrospective analysis. Treatment history and clinical outcomes prior to and after ctDNA testing were not available. The frequency of specific alterations may be affected by tumor exposure to systemic cancer therapy prior to testing.

CONCLUSIONS

- NGS of ctDNA from clinical samples identified common and clinically relevant genomic alterations in East Asian patients with advanced GI cancers.
- In general, the type and frequency of alterations for each tumor type were similar to those previously reported from primarily Western tumor tissue banks. However, the frequency of *KRAS* mutations in PC, but not in other GI tumor types, was significantly lower than expected. This may be a random finding or may represent a real difference in tumor genomic profiles between East Asian and Western PC patients.
- In the CRC cohort, 25% of all samples with *EGFR* therapy resistance mutations were found outside of *RAS* exons 2-3, suggesting that hotspot testing alone may be insufficient for informing treatment decisions
- Given the ease of blood collection over repeat tissue biopsy, these data support consideration of ctDNA NGS for guiding treatment decisions in patients with advanced GI cancers.

Reference:
1. Zhao B et al. Mechanisms of resistance of anti-EGFR therapy in colorectal cancer. Oncotarget 2017;8:3980-4000.

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Conflict of interest
Sadakatsu Ikeda: No conflicts to report

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