Abstract #618

# Genomic profiling of circulating tumor DNA (ctDNA) from patients with advanced cancers of the GI tract and anus

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## Abstract

**Background**: The treatment of GI carcinomas (CA) is influenced by the presence or absence of prognostic and predictive genomic alterations (GA). Tissue sampling is the historical platform for genomic biomarker assessment, but non-invasive ctDNA assay provides an alternative when tissue is unavailable or cannot be safely obtained.

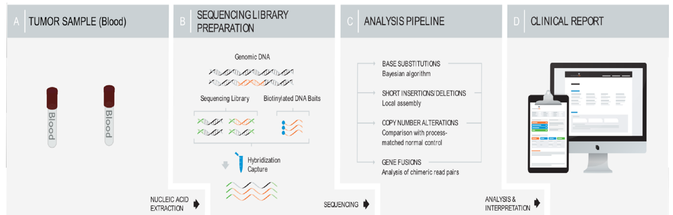
**Methods**: Hybrid-capture based genomic profiling using a ctDNA assay (FoundationACT™) was performed on blood samples from 201 consecutive pts with lower alimentary canal CA.

**Results:** Median age was 60 (range 27-92) and 59% were male. Anatomic breakdown included CRC (n = 143), esophageal (n = 23), gastric (n = 19), gastroesophageal (n = 5) and small bowel adenoCA (SBA, n = 2), anus squamous cell CA (n = 7), and other GI CA (n = 2). At least one GA was reported in 71% of cases. In 59 cases with no GA reported, the average maximum somatic allele frequency was 0.17% (95% CI: 0.10-0.24%) vs. 15.1% (95% CI: 12.0-18.2%) for the 142 cases with GAs (P <0.0001). For the 13 of 43 patients with both blood and tissue testing performed and samples collected within a 60-day interval, 29/32 (91%) GA detected in tissue were also detected in ctDNA.

An average of 1.8 GA/sample were detected in ctDNA. The most commonly altered genes were *TP53* (56%), *KRAS* (26%), *PIK3CA* (11%), and *BRAF* (7%). Comparative analysis using the tissue-based FoundationCORE™ database showed a similar trend with overall slightly higher frequencies of GAs in individual genes. *RAF* and *RAS* short variants (SV) were largely exclusive to lower GI and anal CA. *KRAS* and *RAF1* amplification (amp) occurred only in esophageal CA. *FGFR* SV or amp was identified in 7 cases across the cohort. Of CRC, 5 (4%) had ≥1 *ERBB2* activating SV or amp, 2 had *IDH1/2* hotspot SV, and 2 had *BRCA2* inactivating alterations. Additional potentially actionable alterations identified across the cohort include *ERBB2* amp and SV, *EGFR* amp and extracellular domain SV, *MET* amp, *FGFR* amp and SV, and *PIK3CA* SV. Activating kinase fusions involving *ALK*, *ROS1*, *EGFR*, or *PDGFRA* were identified in 5 cases of CRC and 1 SBA.

**Conclusion:** Our results provide early clinical support and confirm that hybrid-capture based ctDNA testing can reliably detect all 4 classes of GA and provide a molecular profiling option for patients with GI CA.

## Materials and Methods




**Sample requirements**
- 10-20 ml blood
- 5-10 ml plasma
- ≥50 ng extracted ctDNA
- Smear analysis to quantify cfDNA content

**Laboratory process**
- Adaptor-ligation library construction
- Molecular and sample barcodes
- Hybridization capture with biotinylated DNA oligonucleotides
- 2x175 paired-end sequencing on Illumina HiSeq 2500 platform.

**Analysis methods**
- Error correction to <0.05%
- Target depth post correction: ≥5,000x
- Variant calling
  - Subs: down to <0.5% VAF
  - indels and fusions: down to 1% VAF
  - Hi-level amplifications

**Reporting approach**
Interpretation without a matched normal
- Known driver alterations (COSMIC, documented fusions)
- Likely driver alterations (hotspots & truncations)



## Results

**Figure 1. Long tail of alterations detected in ctDNA compared to frequent alterations in the FoundationCORE™ database for cancer of the GI tract and anus**

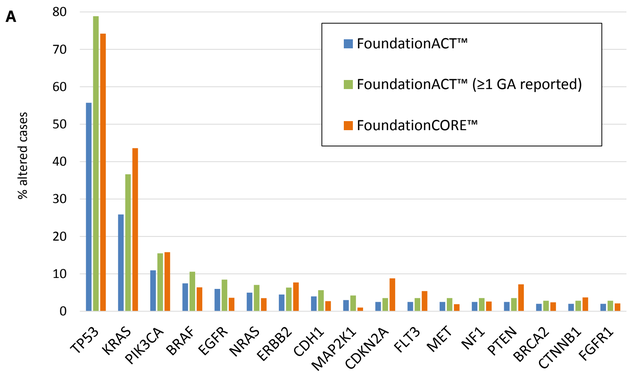



**Figure 1. (A)** Genes altered in >2% of ctDNA cases, compared with frequencies of alterations in these genes in the FoundationCORE™ database for tissue samples from cancers of the GI tract and anus (n =13,522). Frequencies are reported for all ctDNA cases (n = 201) as well as for ctDNA cases with ≥1 genomic alteration (GA) reported (n = 142). **(B)** Maximum somatic allele frequency (MSAF) for ctDNA cases without reported GAs (no variant report: NVR) was significantly lower than for cases with ≥ 1 GA.

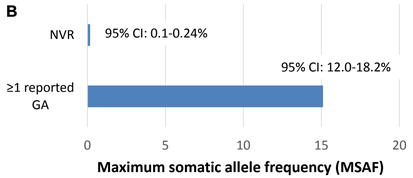



**Figure 2. Comparison of alterations detected in 13 patients with tissue and blood samples collected within a 60-day interval**

| Case: | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | GOPC-ROS1# | NRAS Q61R/K | BRAF V600E | EGFR S464L | EGFR G465R | EGFR amp | CCND1 amp | MYC amp | CTNNB1 splice | TP53 R248W |
| 2 | KRAS Q61H | EGFR S492R | PDGFRB amp | TP53 R282W | TP53 V143A | TP53 C275Y | TP53 splice | | | |
| 3 | SLC34A2-ROS1# | KRAS G12V | FLT3 amp | BRCA2 V2205fs | TP53 R273H | | | | | |
| 4 | NRAS G12D | PIK3CA G12D | NF1 splice | CDH1 S838G | TP53 P27fs | | | | | |
| 5 | KRAS Q61H | KRAS G12V | CDKN2A A102E | TP53 R273S | TP53 C238Y | | | | | |
| 6 | ERBB2 S310F | FLT3 amp | TP53 R175H | TP53 splice | | | | | | |
| 7 | STRN-ALK | CDK6 amp | MYD88 L265P | TP53 R175H | | | | | | |
| 8 | PIK3CA E546K | MYC amp | TP53 R175H | | | | | | | |
| 9 | ERBB2 V777L | ERBB2 amp | TP53 R248W | | | | | | | |
| 10 | KRAS G12D | TP53 R175H | | | | | | | | |
| 11 | KIF5B-PDGFRB | TP53 R273H | | | | | | | | |
| 12 | TP53 P177L | | | | | | | | | |
| 13 | TP53 R196* | | | | | | | | | |

**Figure 2.** 13 patients with GI tract or anal carcinomas had matched tissue and blood-based ctDNA samples collected within a 60 day interval and assayed using similar hybrid-capture based genomic profiling platforms (FoundationOne® and FoundationACT™). Alterations detected in tissue that are not covered by ctDNA testing are not listed. Blue: alteration detected in both ctDNA and tissue; Orange: alteration detected only in ctDNA; Purple: alteration detected only in tissue.

- 29/32 (91%) alterations detected in tissue were also detected in ctDNA (shown in blue)
- 21 alterations were detected in ctDNA that were not detected in tissue (shown in orange), likely reflecting tumor heterogeneity. #This included 2 *ROS1* fusions, which were detected in just 3 and 8 sequence reads, respectively, in ctDNA

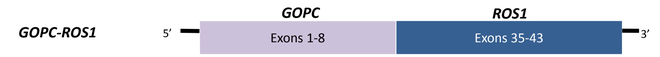
**Figure 3. Overview of potentially actionable alterations identified in ctDNA samples from patients with GI tract and anus carcinomas**

| RAS/RAF/MEK | ERBB2 (HER2) | EGFR | FGFR | MET | PIK3CA | RTK fusion |
|---|---|---|---|---|---|---|
| • *RAS* SV in 40% of CRC, but only 5% of non-CRC<br>• *BRAF* SV in 9% of CRC, but only 3% of non-CRC<br>• *MEK1* (*MAP2K1*) SV exclusive to CRC (4%)<br>• *RAS* amp (3/23, 13%) and *RAF1* amp (1 case) were exclusive to EC | • *ERBB2* amp in 5 cases (2 CRC, 2 EC, 1 GEJ), 2 with SV<br>• *ERBB2* SV in the absence of amp in 3 CRC and 1 SBA<br>• Overall *ERBB2* alts in 4.5% of cases | • 3 cases with *EGFR* amp including 2 (9%) EC<br>• 6 (3%) CRC cases with *EGFR* extracellular domain SV predicting resistance to EGFR antibodies | • *FGFR1/2* amp in 3 CRC, 1 anus SCC, 1 GEJ<br>• Activating *FGFR2* SV in 1 EC and 1 anus SCC | • 4 cases with *MET* amplification including 2 CRC, 1 EC, and 1 GC<br>• 1 CRC case with an activating *MET* kinase SV | • *PIK3CA* SV in 11% of CRC and 13% of EC<br>• Majority (16/22) of SV affected exon 9 (E542K, E545K, Q546K) or exon 20 (H1047L/R) | • Activating RTK fusions in 5 (3.5%) CRC and 1 SBA case<br>• *GOPC-ROS1* (2)<br>• *SLC34A2-ROS1* (1)<br>• *STRN-ALK* (1)<br>• *KIF5B-PDGFRA* (1)<br>• *EGFR-SEPT14* (1) |

CRC: colorectal adenocarcinoma; EC: esophagus carcinoma; GEJ: gastroesophageal junction adenocarcinoma; GC: gastric carcinoma; SCC: squamous cell carcinoma; RTK: receptor tyrosine kinase; SBA: small bowel adenocarcinoma; SV: short variant.

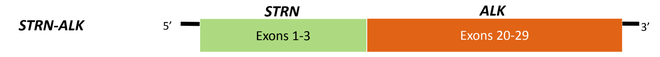
## Case Examples

**Case 1. Small bowel adenocarcinoma with *GOPC-ROS1* fusion detected in tissue and ctDNA with prolonged clinical benefit on crizotinib therapy**




Case #1 is a 54 year-old woman who was diagnosed with stage IV small bowel adenocarcinoma (SBA) with a proximal jejunal lesion and multiple liver lesions. She achieved a near complete response following multiple rounds of FOLFOX, and upon progressive disease was unsuccessfully treated with 6 cycles of FOLFIRI. Both tissue and blood samples were sent for hybrid-capture based genomic profiling, and a *GOPC-ROS1* fusion retaining the GOPC coil-coiled domain and the ROS1 kinase domain was detected in each sample. Mutations in TP53 and APC were also detected, and no *RAF* or *RAS* alterations were detected. The patient was started on the ALK inhibitor crizotinib (250 mg BID) and experienced clinical symptom improvement within days of starting therapy. The patient has remained on crizotinib for almost 11 months, with one interruption due to hospitalization. CT scans performed 2.5 months after starting crizotinib showed a slight interval decrease in the jejunal mass as well as decreased peritoneal metastases and adnexal masses, and increased necrosis of hepatic disease.

**Case 2. Colorectal adenocarcinoma with *STRN-ALK* fusion detected in tissue and ctDNA**




Case #2 is a 62 year-old woman who was diagnosed with stage IV ascending colorectal adenocarcinoma (CRC) with a near obstructive hepatic flexure lesion and metastases to the lymph nodes, liver, and vagina. The vaginal biopsy tissue specimen was submitted for comprehensive genomic profiling, but tissue was insufficient for testing. While a second biopsy was obtained, a blood sample was also submitted for molecular testing. Hybrid-capture based genomic profiling revealed a STRN-ALK fusion retaining the STRN coil-coiled domain and the ALK kinase domain was detected in the blood sample as well as a subsequent tissue sample. No *RAF* or *RAS* alterations were detected. Due in part to restrictive enrollment criteria for available clinical trials of ALK inhibitors, a bevacizumab-based regimen with curative intent was initiated. After 3 months (6 cycles) of FOLFOX with bevacizumab, tumor markers dropped from 203 to 4, and the patient had significant pain relief and normalization of bowels. Currently the patient has no evidence of disease (NED) post synchronous right hemicolectomy and right trisegmentectomy. Based on the results of genomic profiling, targeted therapy with an ALK kinase inhibitor may be pursued at progression.

## Conclusions:

- Hybrid-capture based genomic profiling of ctDNA samples from 201 patients with carcinomas of the GI tract or anus detected one or more genomic alterations (GAs) in 71% of samples for an average of 1.8 GAs/sample. Samples with no reportable GAs had significantly lower MSAF than those with reportable GAs
- The frequency of GAs detected in ctDNA per gene was comparable to that of alterations detected through tissue based testing using a similar hybrid-capture based platform of samples from patients with the same spectrum disease histologies
- In 13 patients with blood and tissue samples collected within a 60-day interval, genomic profiling of ctDNA detected 91% (29/32) alterations that were detected in tissue, and identified 21 alterations not detected in paired tissue, likely reflecting tumor heterogeneity
- Potentially actionable GAs were identified across disease histologies and included amplifications and mutations in *RAS*, *RAF*, *ERBB2* (*HER2*), *EGFR*, *MET*, *FGFRs*, *PIK3CA*, *MAP2K1* (*MEK1*), *BRCA1/2*, and *IDH1*. Many of these alterations have been shown to predict resistance to EGFR antibodies and/or sensitivity to matched targeted therapies
- Activating RTK fusions were identified in 6 cases including 3 *ROS1*, 1 *ALK*, 1 *PDGFRB*, and 1 *EGFR* fusion. One patient with SBA and a *GOPC-ROS1* fusion was treated with crizotinib and has experienced ongoing clinical benefit for 11 months
- These data provide early clinical support for blood-based ctDNA testing and show that this non-invasive methodology can be used to detect diverse actionable genomic alterations in patients with GI cancers