Somatic variant detection with Twist Oncology - DNA CGP Panel using QIAGEN® CLC Genomics Workbench and QCI® Interpret One

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Introduction

The Twist Oncology - DNA CGP Panel from Twist Bioscience is designed to enable comprehensive genomic profiling (CGP) of solid tumors for pathogenic variants and scoring of microsatellite instability (MSI) and tumor mutational burden (TMB). QIAGEN CLC Genomics Workbench is a powerful analysis and visualization software with analytical tools and workflows tailored to next-generation sequencing (NGS) data, including data from a wide range of resequencing panel designs. QCI Interpret One is a panel-agnostic interpretation and reporting platform for somatic NGS testing providing comprehensive coverage across all cancer types, dynamic guideline-based variant classification, evidence-rich access to over 800,000 curated variant summaries, and custom reports.

Here, we present highly sensitive somatic detection of commercially available tumor reference samples prepared with the Twist Oncology - DNA CGP Panel and resulting data analyzed and interpreted with QIAGEN CLC Genomics Workbench and QCI Interpret One.

Twist Oncology - DNA CGP Panel features

- The panel covers 2.4 Mb targeting regions of interest from 562 genes of relevance to CGP for detection of variants and scoring of MSI and TMB.
- Additional probes of 57 genes were included to improve the resolution of copy number variant (CNV) detection in these genes. CNV detection is coverage based, so CNVs of other genes in the panel will also be called.
- To detect known fusion events, targets were designed to investigate intronic regions of *ALK, BCR, EFGR, ETV6, EWSR1, FGFR2, FGFR3, NTRK1, NUTM1, PAX8, RET, ROS1, TMPRSS2* and *BRAF*.
- 50 targets were added for the purpose of MSI detection, bringing the total number of MSI loci used by the MSI detection tool to 183.

Methods and materials

Normal controls from Coriell Institute for Medical Research and formalin-fixed, paraffin-embedded (FFPE) samples from LGC Clinical Diagnostics (SeraCare) and Horizon Discovery® were used to evaluate workflow integrity and robustness from extracted gDNA to post-analytical bioinformatics.

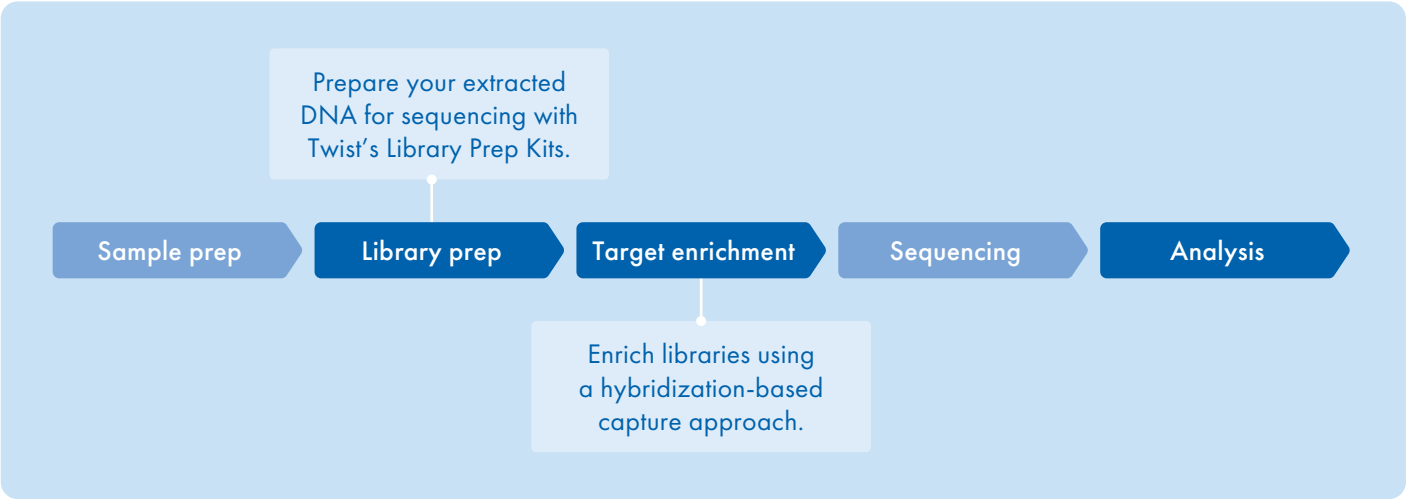
Name	Vendor Cat. No.	Purpose	Reads	Average Coverage
Formalin-Compromised DNA (Moderate) Reference Standard	Horizon Discovery HD799	Demonstrate ability to call 11 SNPs, insertions, and deletions at 0.8% to 24.5% allelic frequency on moder- ately formalin-compromised DNA; demonstrate ability to call MSI-High	28M / 71M / 116M	597x / 754x / 1337x
Formalin-CompromisedDNA (Severe) Reference Standard	Horizon Discovery HD803	Demonstrate ability to call 11 SNPs, insertions, and deletions at0.8% to 24.5% allelic frequency on severely formalin-compromised DNA; demon- strate ability to call MSI-High	29M / 46M / 47M	531x / 438x / 529x
Mimix™ Structural Multiplex, FFPE Reference Standard	Horizon Discovery HD789	Demonstrate ability to call 9 variants with allelic frequencies ranging from 3.5% to 9.7% and CNVs at 4.5x and 8.5x MYC-N and MET focal amplifications as well as RET and ROS1 fusions; demonstrate ability to call MSI-High	41M / 166M / 117M	929x / 2194x / 1774x
Seraseq® FFPE TMB RM Score 26	SeraCare 0710-1307	Demonstrate ability to score TMB-High	142M / 132M	1733x / 1707x
gDNAMSI-High Mix	SeraCare 0710-1670	Demonstrate ability to call MSI-High	86M / 112M	1502x / 1908x
Seraseq MSI Panel Mix AF 5% (Tumor)	SeraCare 0710-1862	Normal control for MSS and CNV baseline creation	33M	802x
Seraseq MSI Matched-WT gDNA (Normal)	SeraCare 0710-1864	Normal control for MSS and CNV baseline creation	33M	786x
HG001	Coriell NA12878	Normal control for MSS and CNV baseline creation	116M	2010x
HG002	Coriell NA24385	Normal control for MSS and CNV baseline creation	110M	1826x
HG004	Coriell NA24143	Normal control for MSS and CNV baseline creation	21M	482x

Table 1. Details of benchmarking control samples, product information, intended function and sequencing metrics in this study.

Library preparation and sequencing

Libraries were prepared with 50 ng to 100 ng of input gDNA using the Twist Library Preparation Enzymatic Fragmentation Kit 2.0 with the Twist Universal Adapter System. Pairs of 10-bp unique dual indices (UDIs) for sample barcoding were added during 6 cycles of pre-capture PCR. Pre-capture libraries of 8 samples at 300 ng each were pooled in a single tube for overnight hybridization with the Twist Oncology - DNA CGP Panel according to Twist Target Enrichment Standard Hybridization v2 workflow. Post-capture libraries were pooled and then sequenced on an MGI DNBSEQ-T7 or Illumina® NextSeq® 2000 platform, targeting at least 32M reads as PE150 (equivalent of 2000X raw coverage for 500X mean coverage).

Figure 1. Schematic of the Twist Oncology - DNA CGP hybrid capture panel laboratory and analysis steps. Shown in dark blue are the topics discussed in this note (library prep, target enrichment and analysis).



Mean target coverage	515x
On-target rate	77%
Fold-80 base penalty	1.32
Duplication rate	20%
Target bases covered ≥ 100x	99.5%

Table 2. Relevant quality control (QC) metrics averaged across samples downsampled to 2000x raw coverage (32M 150PE reads).

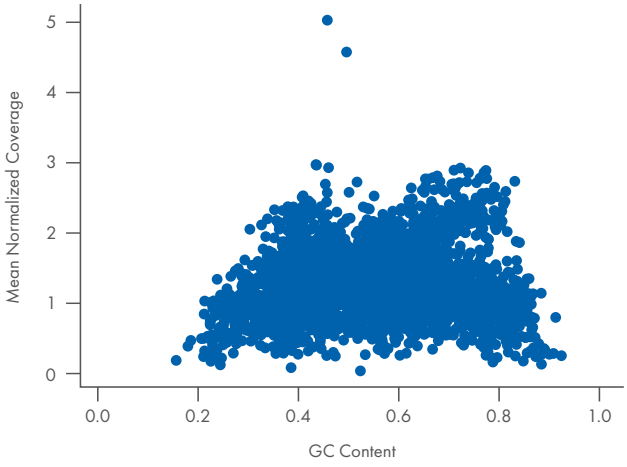


Figure 2. Normalized Twist Oncology DNA CGP panel probe coverage distribution from 2000X downsampled data across varying GC profiles.

Description of QIAGEN CLC Genomics workflow

A QIAGEN CLC Genomics Workbench "Twist Oncology - DNA CGP hybrid capture analysis" workflow was developed as a comprehensive bioinformatic pipeline for the analysis of tumor samples. It is comprised of QIAGEN CLC LightSpeed trimming, read mapping, low allele frequency variant calling with CNV and DNA fusion detection and MSI and TMB estimation steps using hg38 as the reference genome. The analysis is restricted to target regions defined by the panel, and will detect any significant variants within the targets. The workflow has thus no notion of hotspots or whitelists for detection of pre-specified variants; instead, variant detection is limited only by target regions and detection significance thresholds.

The workflow is available as a QIAGEN hosted-based service (QCI® Secondary Analysis) or as a CLC workflow for on-premise deployment.

To assess run time, fastq files of randomly selected case samples were subjected to analysis on a server-grade workstation (2x Intel® Xeon 6238R (28 core, 2.2 GHz base, 4.0 GHz boost) processors with 192 GB RAM). The total run time scaled linearly, varying from 5 minutes for a sample containing 46M reads and 20 minutes for a sample with 164M reads, which are equivalent to 7Gb and 24.6Gb as PE150 reads, respectively.

Description of QCI Interpret One workflow

A QCI Interpret One "Twist Oncology – DNA CGP Panel" workflow was developed as a comprehensive tertiary analysis pipeline for the interpretation and reporting of tumor samples.

- VCFs were uploaded for each sample and relevant case details such as diagnosis and tumor site were entered.
- For each variant, Pathogenicity and Actionability were computed based on the ACMG and AMP/ASCO/CAP guidelines, respectively, and ranked by descending order of clinical significance.
- Variants were matched with relevant treatments, trials and prognostic outcomes accompanied by direct links to supporting evidence from literature, drug labels, databases and professional guidelines.
- Curated, evidence-backed insights and oncologist-reviewed, comprehensive interpretation summaries were retrieved for variants that were reported.
- Customizable reports were generated to include variant specific information, as well as co-occurring variant interactions and overall summary.

Results

Variant detection in reference samples

Detection of mutations (SNPs and Indels)

The CLC LightSpeed module provides an ultra-fast read mapping and variant calling pipeline that forms the basis of the "Twist Oncology - DNA CGP hybrid capture analysis" workflow. Variant types called directly from LightSpeed include SNPs and Indels.

Horizon reference samples HD799 and HD803 were chosen to demonstrate the workflow's ability to call 11 SNPs, insertions, and mutations present in the selected controls at a 0.8% to 24.5% allelic frequency on "moderately" and "severely" formalin-compromised DNA, respectively. Moderately compromised FFPE is defined by the manufacturer as an average fragment length of between 2000 to 4000 bases and DNA integrity Number (DIN) in the range of 2.9 to 3.5. Severely compromised FFPE is defined by the manufacturer as an average fragment length <2000 bases and a DIN in the range of 1.5 to 1.9.

For samples HD799 and HD803, all specified variants were detected at expected variant allele frequencies (VAFs) (Table 3). In addition, 15 parental cell line variants covered by the Twist Oncology - DNA CGP Panel were also detected at expected VAFs (not shown).

Vendor-specified variant specifications				Twist Oncology - DNA CGP variants in triplicate		
Gene	**Prot**	**Expected VAF (%)**	**GRCh38 coordinates**	**Mutation**	**VAF in Moderately compromised FFPE (HD799) (%)**	**VAF in Severely compromised FFPE (HD803) (%)**
NRAS	Q61K	12.50	1:114713909	G>T	10.75 / 7.45 / 11.1	11.27 / 12.47 / 10.68
PIK3CA	E545K	9.00	3:179218303	G>A	6.41 / 4.62 / 6.19	6.69 / 11.46 / 5.98
PIK3CA	H1047R	17.50	3:179234297	A>G	18.34 / 18.44 / 19.74	19.95 / 17.59 / 18.6
cKIT	D816V	10.00	4:54733155	A>T	11.34 / 7.56 / 8.42	7.62 / 8.33 / 9.7
EGFR	G719S	24.50	7:55174014	G>A	23.24 / 24.29 / 23.65	22.86 / 24.96 / 22.04
EGFR	ΔE746 - A750	2.00	7:55174773-55174787		2.03 / 0.75 / 0.77	2.43 / 0.95 / 2.16
EGFR	T790M	1.00	7:55181378	C>T	1.41 / 1.87 / 1.09	1.26 / 1.50 / 1.64
EGFR	L858R	3.00	7:55191822	T>G	4.05 / 2.97 / 3.7	3.04 / 5.04 / 4.51
BRAF	V600E	10.50	7:140753336	A>T	14.06 / 13.22 / 13.05	14.06 / 10.97 / 11.89
KRAS	G13D	15.00	12:25245347	C>T	13.49 / 15.09 / 15.15	19.88 / 10.95 / 12.97
KRAS	G12D	6.00	12:25245350	C>T	5.64 / 4.72 / 3.12	5.29 / 5.07 / 5.54

Table 3. Details of the verified variants spiked into HD799 and HD803. Vendor-specified allele frequencies and Twist Oncology - DNA CGP Panel NGS workflow-determined allele frequencies are indicated together with other relevant variant details.metrics in this study.

For sample HD789, specified variants in *GNA11*, *AKT1*, and *EGFR* were detected at expected VAFs (Table 4). One exception is the mutation leading to the amino acid change, which is vendor-specified at 4.5%, but detected at 12.61%, a deviation seen in only one of three replicates. The sample had fewer reads than the two other replicates (41M vs 166M and 117M), which may be a result of insufficient coverage.

Vendor-specified variant specifications			Twist Oncology - DNA CGP variants in triplicate		
Gene	**Variant (AA)**	**CDS mutation**	**Expected VAF (%)**	**GRCh38 coordinates**	**Measured VAF (%)**
GNA11	Q209L	c.626A>T	4.4	19:3118944	3.82 / 5.01 / 4.67
AKT1	E17K	c.49G>A	3.5	14:104780214	2.92 / 3.27 / 3.44
PIK3CA	E545K	c.1633G>A	4.5	3:179218303	12.61 / 5.16 / 3.58
EGFR	A767_V769dup	c.2300_2308dup	4.4	7:55181317	3.49 / 2.23 / 2.59
EGFR	ΔE746 - A750	c.2235_2249del	4.4	7:55174771	2.67 / 2.97 / 1.97

Table 4. Details of the verified variants spiked into HD789. Vendor-specified VAF and Twist Oncology - DNA CGP workflow measured VAF are summarized with other relevant variant details.

All inspected variants were plotted to assess the ability of the pipeline to call VAFs in line with expectations, yielding a Pearson's correlation coefficient of 0.979 (P-value < 0.01; Figure 3).

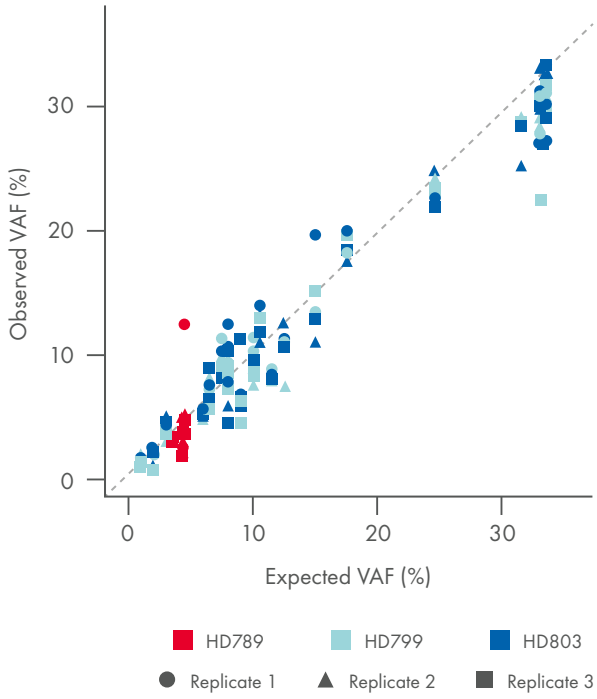


Figure 3. Correlation of VAF in samples. Correlation between observed and expected VAF of the inspected variants in reference samples.

Detection of structural variants

Copy number variants (CNVs)

The Copy Number Variant Detection tool for targeted sequencing in QIAGEN CLC Genomics Workbench detects CNVs (gains and losses) by comparing the coverage levels in a case sample to those exhibited in a set of CNV-free controls. The tool takes a read mapping for the case sample and coverage tables or read mappings for the controls as input. A statistical assessment of the significance of the observed fold changes between the case and the control samples at the target level is made and target-level results are produced.

The Twist Oncology - DNA CGP workflow could detect CNVs at MET (4.5 copies) and *MYC-N* (8.5 copies) in the Horizon Structural Multiplex, FFPE Reference Standard (HD789). DNA from NA24143, MSI Normal, MSI 5% Tumor, NA12878 and NA24385 samples were used as controls.

The CNVs were consistently called at the expected copy numbers (Table 5). For routine testing and especially for sensitive detection of lower-fold changes, we recommend adding control samples (processed in the same way as the test samples) to the normal control pool.

Gene	Vendor-specified copy number	Twist Oncology – DNA CGP detected copy number in triplicate
MET	4.5	5.2; 4.74; 4.86
MYC-N	8.5	9.6; 8.18; 8.34

Table 5. Structural variants. Details of the structural variants identified with the Twist Oncology - DNA CGP workflow on replicates of Horizon Structural Multiplex, FFPE Reference Standard (HD789).

Fusions

The CLC DNA Fusion detection tool takes as input a read mapping and collects evidence from broken pairs and unaligned ends to infer breakpoints and call fusion gene partners.

Twist Oncology DNA CGP has been designed to call fusions in select genes (see section Twist Oncology - DNA CGP Panel Features above). The Horizon Structural Multiplex, FFPE Reference Standard (HD789) DNA carries two fusion events: CCDC6/RET fusion at 4.6% and SLC34A2/ROS1 fusion at 9.7% AF. Both fusions were called in all three replicates of HD789 processed with the Twist Oncology - DNA CGP workflow (Table 6).

Vendor-specified fusions		Twist Oncology - DNA CGP detected fusions in three replicates		
Genes	**Expected AF (%)**	**Replicate 1 AF (%)**	**Replicate 2 AF (%)**	**Replicate 3 AF (%)**
CCDC6/RET	4.6	3.3	3.2	3.4
SLC34A2/ROS1	9.7	12.0	6.0	4.7

Table 6. Fusion allele frequency (AF) estimates. Estimated AFs identified with the Twist Oncology - DNA CGP workflow on replicates of Horizon Structural Multiplex, FFPE Reference Standard (HD789) for the two expected fusions.

MSI detection

The CLC MSI caller evaluates microsatellite instability by comparing repeat lengths at selected loci in a test sample with repeat lengths in a baseline generated with microsatellite stable (MSS) samples.

For MSS baseline creation, we sequenced with the Twist Oncology- DNA CGP Panel the Seraseq MSI Panel Mix AF 5% (Tumor), Seraseq® MSI Matched-WT gDNA (Normal), NA24143, NA24385, and NA12878 samples. Leave-one-out cross-validation confirmed all baseline samples as MSS. The Seraseq MSI Panel Mix AF 5% (Tumor) sample is vendor-engineered by spiking in aberrant repeat lengths for 5 "classic" MSI loci (BAT-25, BAT-26, NR-21, NR-24, and MONO-27) at 5% AF. For the CLC MSI detection algorithm, which assesses loci outlined by the tool MSIsensor2 and which do not include the "classic" loci for MSI testing, this sample represents a normal sample and is therefore useful for baseline creation.

Test Twist Oncology - DNA CGP-processed samples HD799, HD803, HD789, and Seracare gDNA MSI-High Mix were scored in duplicates/triplicates as MSI-High, whereas sample Seraseq FFPE

TMB RM Score 26 (Seracare 0710-1307) was scored in duplicates as MSI-Low (Table 7). Percent loci called instable are highly consistent across replicates. These findings align with vendor sample descriptions or corresponding orthogonal Illumina Trusight® Oncology 500-processed scores of the same samples.

Sample	Twist Oncology - DNA CGP Panel MSI score (% in triplicate)
HD799	High (96 / 95 / 94)
HD803	High (97 / 95 / 95)
HD789	High (93 / 93 / 93)
Seraseq gDNA MSI-High Mix	High (82 / 83)
Seraseq FFPE TMB RM Score 26	Low (4 / 3)

Table 7. MSI scores. MSI scores of samples processed with the Twist Oncology - DNA CGP workflow. Percent loci found instable when compared to stable baseline are indicated in parentheses for replicates.

TMB scores

The CLC TMB score tallies somatic mutations per megabase of panel target region. In order to arrive at a set of somatic mutations, the germline variants have to be subtracted from variants called with the CLC LightSpeed Somatic workflow. We used dbSNP156-common and 1000-Genomes Phase 3 variants for germline filtering. Resulting TMB scores on Seraseq FFPE TMB RM Score 26 (duplicates), NA12878, NA24385, and NA24143 samples were concordant with vendor-specified/ expected scores (Table 8). The use of additional germline filters, such as GnomAD, may help reduce the scores of the Coriell samples further.

Sample	Vendor-specified / expected TMB score	Twist Oncology - DNA CGP Panel TMB score
Seraseq® FFPE TMB RM Score 26	26	26 / 25
NA12878	0-5	3
NA24385	0-5	4
NA24143	0-5	6

Table 8. TMB scores. TMB scores called on samples processed with the Twist Oncology - DNA CGP workflow.

Interpretation and reporting

Following secondary analysis in QIAGEN CLC Genomics Workbench, high-quality VCF files were uploaded to QCI Interpret One for variant classification, curation and reporting. Within minutes, the system automatically annotated variants against QIAGEN's expertly curated knowledge base and computed classifications in line with AMP/ASCO/CAP guidelines for Actionability and ACMG guidelines for Pathogenicity.

QCI Interpret One's automated evidence framework integrated data from more than 800,000 curated variant summaries, professional guidelines, FDA/EMA-approved therapies and global clinical trials.

Detected pathogenic variants (Tier 1A, AMP/ASCO/CAP):

A somatic cancer sample (HorizonDiscovery, HD789) was processed through QCI Interpret One for end-to-end interpretation and reporting. The sample was run with "Cancer" as the diagnosis to showcase relevant drug approvals, clinical practice guidelines, trials, etc., for any cancer type. The platform identified and classified variants as Tier 1A, Pathogenic including, but not limited to:

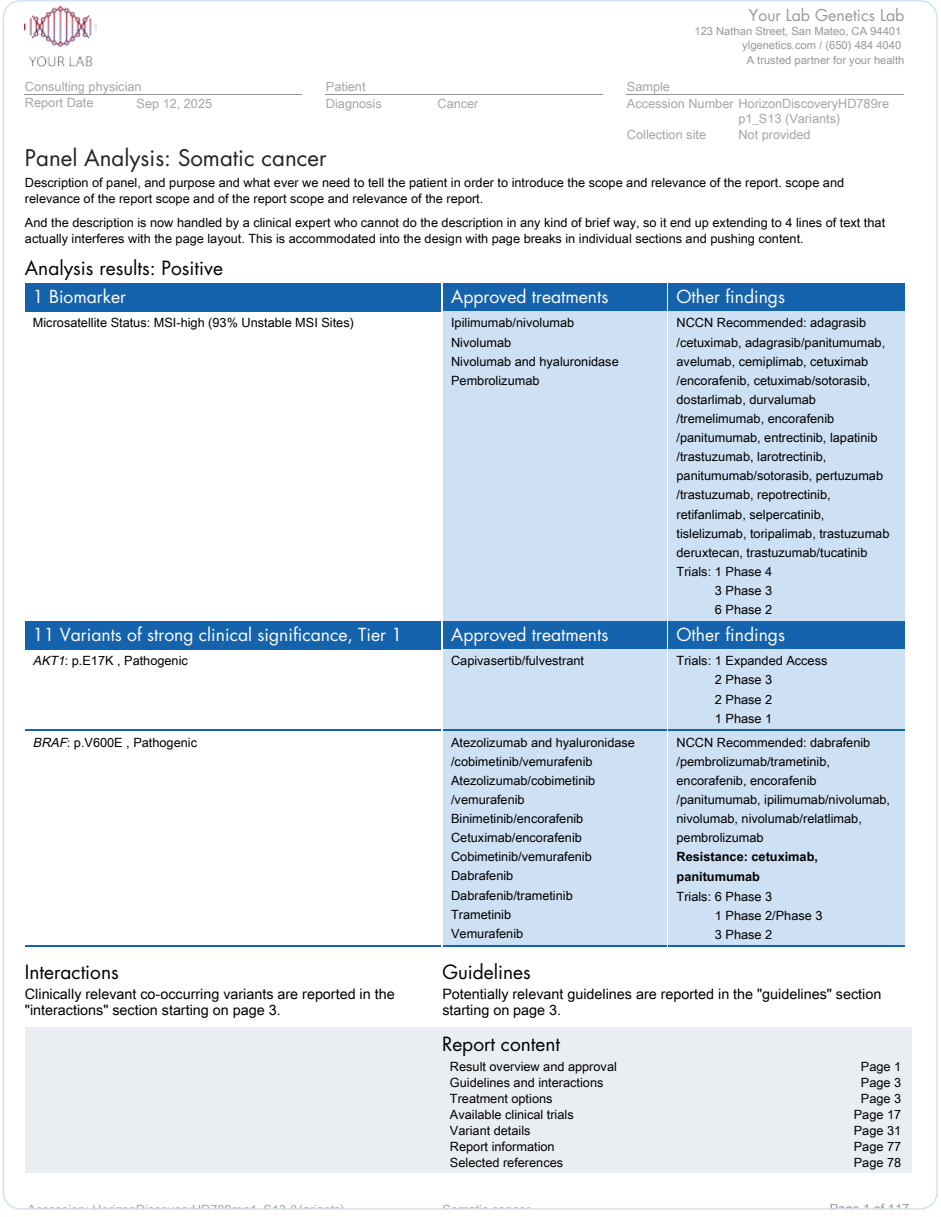
- *BRAF p.V600E*
- *EGFR* p.*E746_A750del* and *p.G719S*
- *RET CCDC6–RET* fusion
- *SLC34A2–ROS1* fusion
- *PIK3CA p.E545K* and *p.H1047R*
- MSI-High

Report output

QCI Interpret One generated a comprehensive, oncologist-ready report summarizing:

- **Variant classifications:** Automatically categorized by tier, significance and therapeutic relevance.
- **Treatment options:** Linked to FDA- and EMA-approved drugs and NCCN guidelines.
- **Clinical trials:** Region-specific trials matched to the patient's genomic profile.
- **Guidelines and interactions:** Contextualized findings with current NCCN guidelines.
- **References:** Selected peer-reviewed literature supporting each variant's evidence base.

The report was completed and approved within hours, demonstrating the efficiency of QCI Interpret One in producing actionable, evidence-backed oncology reports that align with the latest clinical standards. Below are highlights from the report, showing the summary of clinically relevant findings, variant summaries and clinical trial information.



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Consulting physician
Report Date Sep 12, 2025

Patient
Diagnosis Cancer

Sample
Accession Number HorizonDiscoveryHD789re p1_S13 (Variants)
Collection site Not provided

Panel Analysis: Somatic cancer

Description of panel, and purpose and what ever we need to tell the patient in order to introduce the scope and relevance of the report. scope and relevance of the report scope and of the report scope and relevance of the report.

And the description is now handled by a clinical expert who cannot do the description in any kind of brief way, so it end up extending to 4 lines of text that actually interferes with the page layout. This is accommodated into the design with page breaks in individual sections and pushing content.

Analysis results: Positive

1 Biomarker	Approved treatments	Other findings
Microsatellite Status: MSI-high (93% Unstable MSI Sites)	Ipilimumab/nivolumab Nivolumab Nivolumab and hyaluronidase Pembrolizumab	NCCN Recommended: adagrasib /cetuximab, adagrasib/panitumumab, avelumab, cemiplimab, cetuximab /encorafenib, cetuximab/sotorasib, dostarlimab, durvalumab /tremelimumab, encorafenib /panitumumab, entrectinib, lapatinib /trastuzumab, larotrectinib, panitumumab/sotorasib, pertuzumab /trastuzumab, repotrectinib, retifanlimab, selpercatinib, tislelizumab, toripalimab, trastuzumab deruxtecan, trastuzumab/tucatinib Trials: 1 Phase 4 3 Phase 3 6 Phase 2
11 Variants of strong clinical significance, Tier 1	**Approved treatments**	**Other findings**
AKT1: p.E17K , Pathogenic	Capivasertib/fulvestrant	Trials: 1 Expanded Access 2 Phase 3 2 Phase 2 1 Phase 1
BRAF: p.V600E , Pathogenic	Atezolizumab and hyaluronidase /cobimetinib/vemurafenib Atezolizumab/cobimetinib /vemurafenib Binimetinib/encorafenib Cetuximab/encorafenib Cobimetinib/vemurafenib Dabrafenib Dabrafenib/trametinib Trametinib Vemurafenib	NCCN Recommended: dabrafenib /pembrolizumab/trametinib, encorafenib, encorafenib /panitumumab, ipilimumab/nivolumab, nivolumab, nivolumab/relatlimab, pembrolizumab **Resistance: cetuximab, panitumumab** Trials: 6 Phase 3 1 Phase 2/Phase 3 3 Phase 2

Interactions
Clinically relevant co-occurring variants are reported in the "interactions" section starting on page 3.

Guidelines
Potentially relevant guidelines are reported in the "guidelines" section starting on page 3.

Report content

First page of QCI Interpret One report.
The first page provides key sample metadata, a prioritized summary of clinically relevant variants with their diagnostic and therapeutic significance and quick navigation to the detailed evidence that follows.

Variants of strong clinical significance (11)

Phase 1: In a Phase 1 study of the Akt inhibitor capivasertib (AZD5363) in 58 patients with *AKT1* mutations, including 52 with E17K mutations, confirmed partial responses were reported in four ER positive breast carcinomas, two endometrial carcinomas, one cervical carcinoma, one triple-negative breast carcinoma, and one non-small cell lung carcinoma [241]. A Phase 1 study of capivasertib in combination with enzalutamide in 16 metastatic castration-resistant prostate cancer patients who previously failed abiraterone and/or enzalutamide has reported response in 25% (3/12) of evaluable patients, all of whom had either *PTEN* loss or an activating AKT alteration. Common grade 3 or higher adverse events included hypoglycemia and rash [299]. A Phase 1 trial of capivasertib (AZD5363) in 90 solid tumor patients has reported stable disease for more than 6 and 12 weeks in 30% (27/90) and 7% (6/90) of patients, respectively, and one partial response in a cervical cancer patient with a *PIK3CA* mutation. In an expansion cohort of patients with *PIK3CA* mutations, confirmed RECIST responses were observed in 4% (1/28) and 8% (2/26) of breast and gynecologic cancer patients, respectively, resulting in termination of further enrollment [24]. A Phase 1b study of olaparib and capivasertib in 32 evaluable patients with endometrial, triple-negative breast, or ovarian cancer reported that the overall partial response rate was 19%, with partial responses reported in six patients, including 4/9 (44%) of endometrial cancer patients. In addition, stable disease greater than four months was reported in another seven patients [645].

Preclinical: One preclinical study reported that LY294002 (PI3K inhibitor) and A674563 (Akt inhibitor) treatment caused dose dependent decreases in activated Akt, reduced growth, and induced apoptosis and cell cycle arrest in a panel of eight soft tissue sarcoma cell lines; Akt inhibition in a mouse model of soft tissue sarcoma reduced tumor growth [700]. A preclinical study has reported that treatment of melanoma cells with capivasertib (AZD5363) and Wee1 inhibitor adavosertib (MK-1775) synergistically decreased cell viability and inhibited xenograft tumor growth [308].

BRAF V600E

Gene: *BRAF*
Exon: 15
Nucleotide:
NM_004333.6:
g.140753336A>T
c.1799T>A
Amino Acid: p.V600E
Allelic Fraction: 12.0% (of 2402 reads)
Classification: Tier 1A
Assessment: Pathogenic

Treatment options
9 Sensitive
2 Resistance
10 Trials

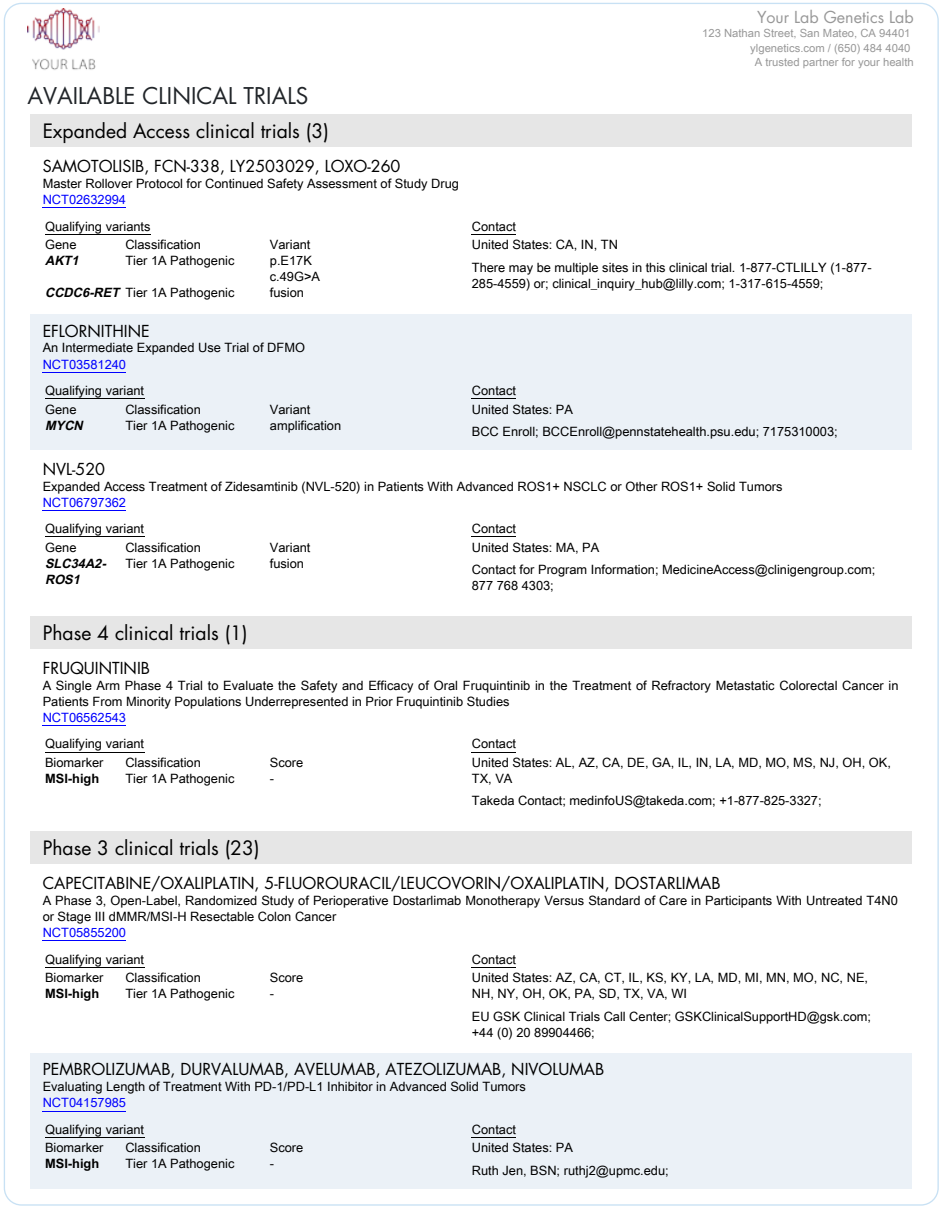
Biomarker summary: *BRAF*-V600E (NM_004333) is an activating mutation.

Clinical relevance: *BRAF* encodes the signaling protein Braf, which is downstream of Ras and activates the MAPK pathway [624]. Activating mutations in *BRAF* may predict sensitivity to Raf or MEK inhibitors, some of which have been approved for certain indications [563, 170, 130, 168, 218, 20, 171, 464]. The *BRAF* V600-specific inhibitors vemurafenib and dabrafenib have been approved for the treatment of *BRAF* V600E-positive melanoma [170, 168]. In addition, the MEK inhibitors trametinib and cobimetinib (in combination with vemurafenib) have been FDA-approved for *BRAF* V600E- and V600K-positive melanoma as has the encorafenib-binimetinib combination [171, 313, 143]. Vemurafenib has additionally been approved for *BRAF* V600-positive Erdheim-Chester disease [127]. Encorafenib in combination with cetuximab (with and without chemotherapy) has been FDA-approved for the treatment of colorectal cancer patients with metastatic disease and harboring a *BRAF* V600E mutation, as detected by an FDA-approved test [301]. Encorafenib in combination with binimetinib has been approved by the FDA for adult patients with metastatic non–small cell lung carcinoma with a *BRAF* V600E mutation [495]. The combination of dabrafenib and trametinib has been FDA-approved for V600E/K-positive melanoma as well as V600E-positive solid tumor (excluding CRC), non-small cell lung carcinoma, anaplastic thyroid carcinoma, and pediatric low-grade glioma [240, 464, 346, 168, 578, 515, 580]. The triple combination of atezolizumab plus cobimetinib and vemurafenib has also been FDA-approved for the treatment of V600E/K-positive melanoma. The pan-Raf inhibitor tovorafenib has been approved by the FDA for the treatment of pediatric patients 6 months of age and older with relapsed or refractory low-grade glioma harboring a *BRAF* fusion or rearrangement, or a *BRAF* V600 mutation [282].

Disease summary: *BRAF* activating mutations or amplification have been reported to result in uncontrolled cell growth and tumorigenesis [114, 624].

Molecular function: *BRAF* V600E is a missense alteration located in the activation domain of the Braf protein [68, 311]. This alteration has been reported as the most frequently occurring *BRAF* mutation in cancer, and shown to lead to constitutive activation of the Braf protein and subsequent activation of the MAPK pathway; *BRAF* V600E has also been shown to be oncogenic and lead to increased survival, proliferation, tumor formation, and invasion, as compared with wild-type *BRAF* [423, 477, 68]. *BRAF* V600E has been reported as a gain-of-function alteration based on gene expression signature changes relative to wild-type expression in a lung cancer cell model [38].

Variant summary.
Variant summaries provide a concise, clinically focused snapshot of each variant's identity, classification, functional impact, cancer relevance, and associated therapeutic, diagnostic, or prognostic significance, with quick access to supporting evidence.



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AVAILABLE CLINICAL TRIALS

Expanded Access clinical trials (3)

SAMOTOLISIB, FCN-338, LY2503029, LOXO-260
Master Rollover Protocol for Continued Safety Assessment of Study Drug
NCT02632994

Qualifying variants

Gene	Classification	Variant
AKT1	Tier 1A Pathogenic	p.E17K c.49G>A
CCDC6-RET	Tier 1A Pathogenic	fusion

Contact
United States: CA, IN, TN
There may be multiple sites in this clinical trial. 1-877-CTLILLY (1-877-285-4559) or; clinical_inquiry_hub@lilly.com; 1-317-615-4559;

EFLORNITHINE
An Intermediate Expanded Use Trial of DFMO
NCT03581240

Qualifying variant

Gene	Classification	Variant
MYCN	Tier 1A Pathogenic	amplification

Contact
United States: PA
BCC Enroll; BCCEnroll@pennstatehealth.psu.edu; 7175310003;

NVL-520
Expanded Access Treatment of Zidesamtinib (NVL-520) in Patients With Advanced ROS1+ NSCLC or Other ROS1+ Solid Tumors
NCT06797362

Qualifying variant

Gene	Classification	Variant
SLC34A2-ROS1	Tier 1A Pathogenic	fusion

Contact
United States: MA, PA
Contact for Program Information; MedicineAccess@clinigengroup.com; 877 768 4303;

Phase 4 clinical trials (1)

FRUQUINTINIB
A Single Arm Phase 4 Trial to Evaluate the Safety and Efficacy of Oral Fruquintinib in the Treatment of Refractory Metastatic Colorectal Cancer in Patients From Minority Populations Underrepresented in Prior Fruquintinib Studies
NCT06562543

Qualifying variant

Biomarker	Classification	Score
MSI-high	Tier 1A Pathogenic	-

Contact
United States: AL, AZ, CA, DE, GA, IL, IN, LA, MD, MO, MS, NJ, OH, OK, TX, VA
Takeda Contact; medinfoUS@takeda.com; +1-877-825-3327;

Phase 3 clinical trials (23)

CAPECITABINE/OXALIPLATIN, 5-FLUOROURACIL/LEUCOVORIN/OXALIPLATIN, DOSTARLIMAB
A Phase 3, Open-Label, Randomized Study of Perioperative Dostarlimab Monotherapy Versus Standard of Care in Participants With Untreated T4N0 or Stage III dMMR/MSI-H Resectable Colon Cancer
NCT05855200

Qualifying variant

Biomarker	Classification	Score
MSI-high	Tier 1A Pathogenic	-

Contact
United States: AZ, CA, CT, IL, KS, KY, LA, MD, MI, MN, MO, NC, NE, NH, NY, OH, OK, PA, SD, TX, VA, WI
EU GSK Clinical Trials Call Center; GSKClinicalSupportHD@gsk.com; +44 (0) 20 89904466;

PEMBROLIZUMAB, DURVALUMAB, AVELUMAB, ATEZOLIZUMAB, NIVOLUMAB
Evaluating Length of Treatment With PD-1/PD-L1 Inhibitor in Advanced Solid Tumors
NCT04157985

Qualifying variant

Biomarker	Classification	Score
MSI-high	Tier 1A Pathogenic	-

Contact
United States: PA
Ruth Jen, BSN; ruthj2@upmc.edu;

Clinical trial information.
The report provides a curated list of relevant, actively enrolling studies linked to the patient's genomic findings, including trial eligibility, associated therapies, variant-specific rationale to support potential enrollment decisions, and geographic and contact information. This is just a preview of potential clinical trials.

Conclusion

The Twist Oncology - DNA CGP workflow has been designed to sensitively detect somatic variants in oncology-relevant genes and estimate MSI and TMB scores. When paired with QIAGEN CLC Genomics Workbench for secondary analysis and QCI Interpret One for interpretation and reporting, this integrated workflow enables accurate detection, classification and contextualization of genomic biomarkers in gDNA from reference samples—delivering end-to-end, evidence-based insights.

Learn more about QIAGEN CLC Genomics Workbench and QCI Interpret at **digitalinsights.qiagen.com**