Results for Sample Patient

Date of birth: MM/DD/YYYY
Sex at birth: Female
Medical record #: MR-000000
Client accession #: CA-0000000
Diagnosis: Lung Cancer

Normal collection date: MM/DD/YYYY
Tumor collection date: MM/DD/YYYY
Specimen type: FFPE Block
Specimen site: Lung
Tumor content: 20%

Ordering provider: Sample Physician
Health care office: Cancer Center
Order ID: WF00008824
Received date: MM/DD/YYYY
Report date: MM/DD/YYYY

Biomarkers with therapy associations

Analytes tested: DNA, RNA, Protein
Clinical trials available: Yes, see page 6 for details

Tumor mutation burden (TMB): Intermediate (8 mut/Mb)
Microsatellite status (MSI): Stable

Biomarker details	Associated therapies	Level of evidence[1]	Analyte
CD74::ROS1 (Fusion)	**Possible benefit:** crizotinib, entrectinib, lorlatinib, repotrectinib, taletrectinib	A	RNA
	ceritinib	C	
	cabozantinib	D	
NF1 (Q369*) VAF: 19%	**Possible benefit:** trametinib	D	DNA
PD-L1 (22C3) (Low) TPS: 1-4	**Possible benefit:** atezolizumab, atezolizumab + bevacizumab, durvalumab, nivolumab + ipilimumab, pembrolizumab	A	Protein
	nivolumab	B	
	avelumab, dostarlimab-gxly, retifanlimab-dlwr, tislelizumab, toripalimab	C	

[1] Level of evidence classification: Level A - FDA-approved or included in guidelines for patient's tumor type; Level B - strong evidence from clinical studies; Level C - FDA-approved for another tumor type or moderate evidence from clinical studies; Level D - case reports.

Tumor-specific biomarkers with no actionable alterations

ALK, BRAF, EGFR, ERBB2, KRAS, MET, NRG1, NTRK1, NTRK2, NTRK3, RET

Notes

*NOTE: The CD74::ROS1 fusion was detected at both the RNA level and as a structural translocation at the DNA level in the sample. The CD74::ROS1 fusion event is reported in the Biomarkers with therapy associations section of the report, and the structural translocation at the DNA level of the same is listed in the Variants of Unknown Significance section to avoid repetition of contents related to therapy and clinical trials.
**NOTE: Contraindications for treatment with certain PD-1/PD-L1 inhibitors may include presence of some oncogenic drivers (i.e., ALK, RET or ROS1 rearrangements, EGFR exon 19 deletion or L858R), which has been shown to lower response rates (PMID: 27225694, 31125062). Targeted therapy for an oncogenic driver should take precedence over treatment with an immune checkpoint inhibitor per clinical guidelines for NSCLC.

IHC result

IHC biomarker (clone)	IHC results	Approved by
ALK (D5F3)	Intensity: 2+ %Cells: 1-4 Negative	Dr. Sample Pathologist MM/DD/YYYY
MSH6 (EP49)	Intensity: 1+ %Cells: 5-9 Positive	Dr. Sample Pathologist MM/DD/YYYY
PMS2 (A16-4)	Intensity: 2+ %Cells: 5-9 Positive	Dr. Sample Pathologist MM/DD/YYYY
PD-L1 (22C3)	%TPS: 1-4 Low	Dr. Sample Pathologist MM/DD/YYYY
PD-L1 (SP142)	%IC: 0 Negative	Dr. Sample Pathologist MM/DD/YYYY
	%TC: 0 Negative	Dr. Sample Pathologist MM/DD/YYYY
MMR Status: Proficient		Dr. Sample Pathologist MM/DD/YYYY

IHC interpretation thresholds

IHC biomarker (clone)	Negative	Equivocal or not significant	Positive
ALK (D5F3)	0 or 1+/2+ and >0%	Not Applicable	3+ and >0%
MSH6 (EP49)	0	Not Applicable	1+/2+/3+ and >0%
PD-L1 (22C3) TPS	0 and <1	Not Applicable	≥1 and ≤49 (Low) or ≥50 (High)
PD-L1 (SP142) TC	0 and <50%	Not Applicable	≥50%
PD-L1 (SP142) IC	0 and <10%	Not Applicable	≥10%
PMS2 (A16-4)	0	Not Applicable	1+/2+/3+ and >0%

Biomarkers with therapy association details

Genomic alteration: CD74::ROS1 (Fusion) VAF: -	
HGVS: N/A	**Coordinate:** chr5:149784243; chr6:117645578
Alteration type: Fused Genes	**Transcript ID:** ENST00000009530.7; ENST00000368508.3
Read depth: N/A	**Location:** E6; E34

The ROS1 gene encodes an orphan receptor tyrosine kinase that may activates multiple pathways involved in cell survival and transformation (PMID: 23719267). This fusion is expected to result in a constitutively active oncogenic chimeric protein (PMID: 23719267, 30262706, 30171048, 22659450, 24828671, 25264305, 26673800, 26917690, 30413378, 32776005). The FDA has approved crizotinib, entrectinib, repotrectinib and taletrectinib in NSCLC patients harboring ROS1 gene fusions.

Drug Evidence for crizotinib:

- In a Phase II study, 37 patients with non-small-cell lung cancer harboring ROS1 translocations were treated with crizotinib. The study reported an objective response rate (ORR) of 47.2% at two cycles, a best ORR of 69.4% during treatment, and a disease control rate of 83.3% at four cycles. The median overall survival and median progression-free survival were 17.2 and 5.5 months, respectively (PMID: 31584608).
- In a Phase I study, 11 patients with non-small cell lung cancer harboring CD74::ROS1 fusion were treated with crizotinib. Ten patients demonstrated significant response with duration of treatment ranging from 5 to 42 months. Three patients experienced 100% tumor regression (PMID: 25264305).

Drug Evidence for repotrectinib:

- In a Phase I/II trial (TRIDENT-1) to assessed the efficacy and safety of repotrectinib in patients with advanced solid tumors, including ROS1 fusion-positive non-small cell lung cancer (NSCLC), an objective response rate of 79% (56 out of 71 patients, comprising 7 complete and 49 partial responses) was observed among TKI-naive ROS1 fusion-positive NSCLC patients. Additionally, the median duration of response and the median progression-free survival were 34.1 months and was 35.7 months, respectively. For those ROS1 fusion-positive NSCLC who had previously received one ROS1 TKI and had never received chemotherapy, the objective response rate was 38% (21 out of 56 patients, with 3 complete and 18 partial responses), with a median duration of response of 14.8 months and a progression-free survival of 9.0 months (PMID: 38197815).

Drug Evidence for lorlatinib:

- In a Phase I study, 12 patients with non-small cell lung cancer harboring ROS1 fusions were treated with lorlatinib. An overall response rate of 50% with a median progression free survival of 7 months were reported in this cohort (PMID: 29074098).
- In a Phase I/II study, 69 patients with ROS1-positive non-small-cell lung cancer were treated with tyrosine kinase inhibitor (TKI) lorlatinib. An objective response rate (ORR) of 41% was reported in this cohort. An ORR of 62%, with a median progression-free survival (mPFS) of 21 months, was reported in 21 TKI-naive patients. An ORR of 35% and a mPFS of 8.5 months was reported in 40 patients previously treated with crizotinib as their only TKI (PMID: 31669155).

Drug Evidence for entrectinib:

- In a combination analysis of two Phase I studies and a Phase II study, thirty-two non-small cell lung cancer patients harboring ROS1 fusions were treated with entrectinib. An overall response rate of 78% and a median progression free survival of 29.6 months were reported in this cohort (PMID: 30050303).

Drug Evidence for taletrectinib:

- The Phase II TRUST I and II trials evaluated the efficacy of taletrectinib in 273 patients with locally advanced or metastatic ROS1+ NSCLC. In TRUST-I, TKI-naïve patients (n=160) had a confirmed objective response rate (cORR) of 88.8% and an intracranial cORR of 76.5%. In TRUST-II, TKI-pretreated patients (n=113) had a cORR of 55.8% and an intracranial cORR of 65.6%. The median duration of response for TKI-naïve patients was 44.2 months, while it was 16.6 months for TKI-pretreated patients. Median progression-free survival was 45.6 months for TKI-naïve patients and 9.7 months for TKI-pretreated patients. Among 32 patients who underwent re-biopsy after failing previous lines of TKIs, 13 patients were found to have the ROS1 G2032R mutation at baseline. Of these patients, eight (61.5%) achieved responses (PMID: 40179330).

Drug Evidence for cabozantinib:

- In a case report, a non-small cell lung cancer patient harboring CD74::ROS1 fusion was treated with cabozantinib, which significantly suppressed tumor growth and relieved respiration symptoms. The patient remained on cabozantinib for 1.5 years, then was treated with crizotinib after tumor progression (PMID: 32103985)

Drug Evidence for ceritinib:

- In a Phase II study, 32 patients with non-small cell lung cancer (disorder) harboring ROS1 rearrangement mutations, were treated with ceritinib. An overall response rate of 62% (1 complete response and 19 partial responses) and a disease control rate of 81%, with a median overall survival of 24 months and a median progression free survival of 9.3 months were reported in this cohort (PMID: 28520527).

Genomic alteration: NF1 (Q369*) VAF: 19%

HGVS: c.1105C>T (p.Gln369Ter)	**Coordinate:** chr17:29528097
Alteration type: Stop Gain	**Transcript ID:** ENST00000358273
Read depth: 576	**Location:** 10|58

The NF1 gene encodes a tumor suppressor protein that activates Ras GTPase activity, thus leading to the inactive Ras conformation and repression of Ras signaling (PMID: 19380279, 30562755). This alteration is predicted to result in the loss of Nf1 protein function (PMID: 19410195, 8628317, 20833335).

Drug Evidence for trametinib:

- One patient with adenocarcinoma of the lung with NF1 mutation had a near-CR on the basis of CR of target disease but non-CR in non-target disease with a PFS of 6.4 months (PMID: 37053535).

Protein biomarker: PD-L1 (22C3) (Low)

Drug Evidence for durvalumab:

- In a Phase III study, 473 patients with non-small cell lung cancer were treated with durvalumab. A median progression-free survival of 17.8 months was reported for patients with TC ≥25% of PD-L1 expression compared to 3.7 months with placebo. Durvalumab improved overall survival versus placebo in the TC ≥1% PD-L1 subgroup (HR, 0.52) but not in the tumor cells <1% subgroup (HR, 1.18) (PMID: 32209338).

Drug Evidence for nivolumab:

- In a Phase III study, patients with nonsquamous non-small-cell lung cancer were treated with nivolumab. A median overall survival of 12.2 months was reported compared to 9.4 months with docetaxel. Nivolumab was associated with greater efficacy than docetaxel across all end points in subgroups defined according to prespecified levels of PD-L1 tumor-membrane expression (≥1%, ≥5%, and ≥10%) of the PD-1 ligand (PMID: 26412456).

Drug Evidence for retifanlimab-dlwr:

- In a Phase II study, 121 patients with selected solid tumors were treated with retifanlimab. An overall response rate of 40.0% was reported in the melanoma cohort (n=35), 34.8% in the NSCLC cohort (n=23, PD-L1 high (TPS≥50%)), 37.9% in the UC cohort (n=29, PD-L1 positive (CPS ≥10%)), and 23.5% in the RCC cohort (n=34). Median duration of response was 11.5 months (95% CI 2.2-not reached) in the UC cohort and was not reached in the other cohorts (PMID: 38401247).

Drug Evidence for avelumab:

- In a Phase Ib study, patients with urothelial carcinoma were treated with avelumab. A median overall survival of 13.7 months was reported along with a median progression-free survival of 11.6 weeks and an overall response rate of 18.2%; a complete response was reported in 5 patients, and partial response was reported in 3 patients. Seven of eight responding patients had PD-L1-positive tumors (PMID: 28375787).
- A multicenter, open-label Phase I study, 22 (12% [95% CI 8-18]) metastatic or recurrent NSCLC patients achieved a confirmed objective response, including one complete response and 21 partial responses when treated with avelumab. Progression free survival was longer in those patients with PD-L1+ tumors compared with PD-L1− tumors (hazard ratio= 0.45 [95% CI, 0·27–0·75]) in an analysis based on a 1% cut-off for tumor cell staining (PMID: 28373005).

Drug Evidence for dostarlimab-gxly:

- In a Phase I, multi-center, open-label, 2-part (dose escalation and cohort expansion) trial, 67 patients with recurrent/advanced non-small cell lung cancer (NSCLC) were treated with dostarlimab. Immune-related objective response rate (irORR) was 26.9%, including 2 complete and 16 partial responses. Responses were observed in 2 of 24 (16.7%) patients with PD-L1 TPS < 1%, 4 of 20 (20.0%) patients with PD-L1 TPS 1%-49% and 2 of 5 (40.0%) patients with PD-L1 TPS ≥ 50%. The median duration of response of 11.6 months (range: 2.8-19.4) (PMID: 35729005).

Drug Evidence for atezolizumab + bevacizumab:

- In the open-label, Phase III IMpower150 study, patients with stage IV, non-squamous NSCLC were randomly assigned to receive atezolizumab plus bevacizumab with carboplatin/paclitaxel (ABCP group, n=356), or bevacizumab with carboplatin/paclitaxel (BCP group, n=336). Among patients without ALK or EGFR alterations, median progression-free survival (PFS) was significantly longer in the ABCP group than in the BCP group (8.3 months vs. 6.8 months, respectively). Median overall survival was also longer in the ABCP group than in the BCP group (19.2 months vs. 14.7 months; hazard ratio for death, 0.78; 95% CI, 0.64 to 0.96; P=0.02). Among patients with high PD-L1 expression (>50% tumor cells or >10% immune cells), PFS was 12.6 months with ABCP vs. 6.8 months with BCP. Improved PFS (8.0 months vs 6.8 months, ABCP vs. BCP) was also seen within a subgroup of patients with low or negative PD-L1 (<50% expression on tumor cells, and <10% on immune cells) (PMID: 29863955).

Drug Evidence for tislelizumab:

- In a Phase III clinical study, 1657 patients with HER2 negative locally advanced unresectable or metastatic gastric or gastro-esophageal junction adenocarcinoma, were randomly assigned (1:1) to receive intravenous tislelizumab (anti PD-1) plus chemotherapy or placebo plus chemotherapy. The primary endpoint was overall survival (OS) both in patients with a PD-L1 positive (tumor area positivity (TAP) score of ≥5%) and in all randomized patients. OS was significantly longer with tislelizumab plus chemotherapy versus placebo plus chemotherapy in patients with PD-L1 positive (median 17.2 months vs 12.6 months; hazard ratio 0.74 (95% CI 0.59 to 0.94); P=0.006) at interim analysis and in all randomized patients (median 15.0 months vs 12.9 months; hazard ratio 0.80 (95% CI 0.70 to 0.92); P=0.001) at final analysis (PMID: 38806195).

Drug Evidence for toripalimab:

- In a Phase III trial, 289 patients with recurrent or metastatic nasopharyngeal carcinoma were treated with toripalimab in combination with gemcitabine-cisplatin chemotherapy. A median progression-free survival of 11.4 months was reported for PD-L1 positive patients (PMID: 34341578).
- In a Phase II study (POLARIS-03), 151 patients with metastatic urothelial carcinoma were treated with toripalimab. PD-L1+ patients had significantly better objective response rate (ORR) and progression-free survival (PFS) than PD-L1- patients (ORR 42% vs 17%, P = 0.002; median PFS 3.7 vs 1.8 months, HR = 0.60). In addition, PD-L1+ patients had numerically better overall survival than PD-L1- patients (median OS 35.6 vs 11.2 months, HR = 0.85), but the difference did not reach significance (PMID: 34740921).

Drug Evidence for atezolizumab:

- In a Phase II randomized controlled trial, 142 patients with NSCLC were treated with atezolizumab. A median overall survival of 12.6 months was reported. Increasing improvement in overall survival was associated with increasing PD-L1 expression (PMID: 26970723).

Drug Evidence for pembrolizumab:

- In a Phase I/Ib study (KEYNOTE-028), patients with solid tumors with PD-L1 expression were treated with pembrolizumab. An overall response rate of 30% was reported with a median duration of response of 15 months in PD-L1-positive (1% or more) advanced esophageal carcinoma cohort (PMID: 29116900).
- In a Phase II/III study, patients with previously treated non-small cell lung cancer were treated with pembrolizumab. Among patients with at least 50% of tumor cells expressing PD-L1, a median overall survival of 14.9 months (2 mg/kg dose) and 17.3 months (10 mg/kg dose) was reported, compared to 8.2 months with docetaxel. A median progression-free survival of 5.0 months (2 mg/kg dose) and 5.2 months (10 mg/kg dose) was observed, compared to 4.1 months with docetaxel (PMID: 26712084).

Drug Evidence for nivolumab + ipilimumab:

- In a Phase I study, patients with advanced NSCLC were treated with nivolumab + ipilimumab (ipilimumab every-6-weeks or 12-weeks). In patients with PD-L1 of 1% or greater, a median progression-free survival of 8.1 months in the ipilimumab every-12-weeks cohort (n=21) and 10.6 months in the ipilimumab every-6-weeks cohort (n=23) was reported along with an overall response rate of 57% in both cohorts. Of the 13 patients with 50% or greater PD-L1 expression, 12 had a confirmed objective response and one had an unconfirmed response (PMID: 27932067).

Clinical trials

Potential trials based on genomic targets identified. The below is ordered by distance from patient.

Biomarker	NCT ID	Study phase	Distance from patient (miles)
NF1 (Q369*)	NCT04892017	Phase 1/Phase 2	4
CD74::ROS1 (Fusion)	NCT02693535	Phase 2	52
CD74::ROS1 (Fusion)	NCT04589845	Phase 2	74
ARID1A (S2249*)	NCT04104776	Phase 1/Phase 2	132
CD74::ROS1 (Fusion)	NCT04919811	Phase 2	162
NF1 (Q369*)	NCT05578092	Phase 1/Phase 2	162
ARID1A (S2249*)	NCT05803382	Phase 1	302
NF1 (Q369*)	NCT05803382	Phase 1	302
ARID1A (S2249*)	NCT04550494	Phase 2	676
ARID1A (S2249*)	NCT05023655	Phase 2	687
NF1 (Q369*)	NCT03340506	Phase 4	704
TP53 (I195T)	NCT04802174	Phase 1/Phase 2	704
CD74::ROS1 (Fusion)	NCT01639508	Phase 2	785
ARID1A (S2249*)	NCT05036226	Phase 1/Phase 2	880

Biomarker	NCT ID	Study phase	Distance from patient (miles)
NF1 (Q369*)	NCT05036226	Phase 1/Phase 2	880
ARID1A (S2249*)	NCT03065062	Phase 1	935
NF1 (Q369*)	NCT03065062	Phase 1	935
ARID1A (S2249*)	NCT04957615	Phase 2	980
NF1 (Q369*)	NCT05039801	Phase 1	980

Variants of unknown significance

Alteration	Alteration Type	VAF %
ACAP2 (L768R)	Missense	7
ADCK5 (L554P)	Missense	6
ADNP2 (S910F)	Missense	38
AKAP13 (V2409A)	Missense	37
ALB (L55V)	Missense	30
ALDOC (R149H)	Missense	40
ANK3 (D2040N)	Missense	37
ATP5SL (N234S)	Missense	45
C18orf25 (c. 1023-1G>A)	Splice Acceptor Variant	35
C9orf78 (G13D)	Missense	10
CAMSAP2 (H381Y)	Missense	30
CCZ1	Deletion	-
CD74_ROS1	Breakpoint: Translocation	-
CNPY3 (R121Q)	Missense	39
COL6A6 (D920N)	Missense	36
CPAMD8 (E787Q)	Missense	6
DAB1 (R124L)	Missense	7
FAT4 (A423S)	Missense	10
FGF2 (M218I)	Missense	33
FZD8 (D576N)	Missense	34

Alteration	Alteration Type	VAF %
NUP50	Breakpoint: Translocation	-
OR5T3 (S165N)	Missense	39
OR9G1 (R169C)	Missense	29
PAFAH1B2 (V122I)	Missense	35
POLR2J3	Deletion	-
PRAMEF1 (E416Q)	Missense	5
PRAMEF13 (E416Q)	Missense	34
PRRC2B (Q1997E)	Missense	5
PRSS12 (G826R)	Missense	17
PSD3	Breakpoint: Translocation	-
RECQL5 (S77C)	Missense	6
RP11-514P8.6	Deletion	-
RP11-514P8.7	Deletion	-
SENP8 (L6F)	Missense	35
SERPINB1 (*380Yext*?)	Stop Lost	34
SERPINB1 (D303N)	Missense	35
SLC2A4RG (R317H)	Missense	11
SPAG11B	Deletion	-
SVEP1 (T3461A)	Missense	32
TOMM40 (S320T)	Missense	43

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Alteration	Alteration Type	VAF %
GNA13 (R200T)	Missense	36
HSP90AB1 (K64fs)	Frameshift	35
HSPB1 (W95*)	Stop Gain	78
KIF27 (Q843H)	Missense	34
MACC1 (S61F)	Missense	61
MROH8 (S456T)	Missense	36
MYLK (E969Q)	Missense	38
NFKBIA (c.834G>A)	Protein Protein Contact	41

Alteration	Alteration Type	VAF %
TTC12 (S549C)	Missense	35
TULP4 (R1338Q)	Missense	25
USP36 (S1120G)	Missense	38
USP37 (L709V)	Missense	35
ZNF260 (E31Q)	Missense	40
ZNF503 (E105K)	Missense	36
ZNF589 (S242L)	Missense	40

Methodology

OncoExTra Test is a Next Generation Sequencing tumor/normal exome and tumor RNA Seq assay that detects substitutions, insertions, deletions, copy number events, and fusions in tumor tissue. MET exon 14 skipping, AR-v7, EGFRvIII and EGFRvIV variants are also detected in RNA. Genomic DNA is extracted from the patient's normal (peripheral white blood cells) and tumor samples. The isolated DNA is then prepared using custom xGen target capture (IDT). This library preparation includes shearing, purification, adaptor ligation and PCR amplification. Total RNA is extracted from the patient's tumor sample. The isolated RNA is then prepared using KAPA HyperPrep with Riboerase (Kapa Biosystems). Libraries are then clustered on a flow cell and sequenced using the Illumina NovaSeq 6000.

Sequence data are analyzed using various validated bioinformatics tools and custom Next Generation Sequencing pipeline NG2-LDT 1.1.2. The reference genome assembly used for alignment is NCBI Not Available. Each tumor's cancer-specific mutations are then queried against a proprietary gene-drug database based on peer reviewed literature to identify potential therapeutic associations. Level of evidence provided for each gene-drug association is based on the AMP/ASCO/CAP standards and guidelines for the interpretation of somatic sequence variants (PMID: 27993330).

Copy number events (amplifications/deletions) reported are focal in nature (<25mb).

Allele fraction is dependent on tumor content. Tumor content is not taken into account when reporting allele fractions.

Tumor Mutation Burden (TMB) is determined by measuring the number of somatic mutations occurring in sequenced genes, counting all mutations expected to change the amino acid sequence of the impacted protein. TMB results are rounded to the nearest integer and are classified as follows: TMB-High: ≥ 20 mutations per megabase (mut/Mb); TMB Intermediate: 6-19 mut/Mb inclusive; TMB-Low: ≤ 5 mut/Mb. "Indeterminate" results may be due to poor sample quality or sequencing coverage. MSI is calculated by scanning certain indels indicative of microsatellite instability. If the number of these, exome wide, is ≥5, then the sample is declared to be "MSI-High". Otherwise, the sample is labelled "MSI-Stable".

Mean target coverage for tumor sample DNA averages 440x (unique reads) and ≥180x reads for the normal sample. Tumor sample RNA averages 121 million reads.

Immunohistochemistry

IHC testing is performed on formalin fixed paraffin-embedded tissue (FFPE) utilizing the detection method of avidin-biotin free polymer and is employed according to an optimized protocol. HER2 testing meets the 2018 ASCO-CAP HER2 testing guidelines in breast cancer and results are reported using the ASCO/CAP scoring criteria as defined in the IHC Thresholds table appearing in the report. For ER and PR, historical cut-offs for all non-breast tissues are followed.

The following Food and Drug Administration (FDA) approved antibody clones are used: PATHWAY anti-HER2 (4B5)/ Roche; PD-L1 22C3 IHC pharmDx/ Agilent; Ventana PD-L1 (SP142)Assay/ Roche; Ventana PD-L1 (SP263) Assay/ Roche; Ventana ALK (D5F3) CDx Assay/ Roche; Ventana MMR RxDx Panel/ Roche.

These assays have not been validated on decalcified specimens.

Results should be interpreted with caution given the possibility of false negative results on decalcified specimens.

External tissue controls are performed and reviewed on all stains for appropriate positive and negative immunoreactivity and found to be acceptable.

If HER2 by FISH is required, it is currently being performed by PhenoPath: 1737 Airport Way S, Ste 201 Seattle, WA 98134. HER2 FISH testing and scoring by PhenoPath is being completed according to the 2018 ASCO-CAP Guidelines, with its methodology listed in their final report. A copy of the final FISH report is stored and can be provided by Exact Sciences/GHI upon request.

Digital images of immunohistochemical (IHC) stained tissues for manual scoring and/or image review are visualized using the Philips Image Management System Version 3.3. Image analysis software is not utilized.

Limitations

Samples with a tumor content of less than 20% may have reduced sensitivity and lead to false negative results. It is also possible that the sample contains a mutation below our established limit of detection (1% allele fraction in hotspots, 5% in other regions), or in a region excluded by our assay.Alterations present in repetitive or high GC content region or non-coding areas may not be detected. Indels larger than 40bp may not be detected. Copy number signal relative to background noise inherent in DNA from FFPE samples may affect sensitivity of reporting amplifications/deletions. Some gene rearrangements like internal tandem duplications (ITD) involving FLT3 and BCOR may not be reliably detected by the test.

The lack of a variant call does not necessarily indicate the absence of a variant since technical limitations to acquire data in some genetic regions may limit assay detection.

Given the nature of RNA isolated from FFPE, sequencing failures may be seen with highly degraded samples, as they may produce sequence reads too short to align informatically.

Previously unspecified fusions cannot be called by the informatics pipeline if the partner genes occur between two closely adjacent genes on the same strand of the same chromosome. In addition, some fusions that are important in hematolymphoid malignancies, including those involving IGH, are difficult to detect with short read sequencing and may be better detected by other modalities.

Disclaimer

This report does not make any promise or guarantee that a particular drug or treatment regimen will be effective or helpful in the treatment of disease in any patient. This report also makes no promise or guarantee that a drug with a potential clinical benefit will in fact provide a clinical benefit or that a drug with potential lack of clinical benefit will in fact provide no clinical benefit. Exact Sciences expressly disclaims and makes no representation or warranties whatsoever relating, directly or indirectly, to this review of evidence or identified scientific literature, the conclusions drawn from it or any of the information set forth in this report that is derived from such review, including information and conclusions relating to therapeutic agents that are included or omitted from this report. This assay has not been validated on decalcified tissues. Results should be interpreted with caution given the possibility of false negative results on decalcified specimens.

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The tests included in this report were developed, and their performance characteristics determined by Exact Sciences. They have not been cleared or approved by the US Food and Drug Administration. The test has been validated as a Laboratory Developed Test per institutional and applicable CLIA regulation (CLIA ID 03D2048606) and College of American Pathology (CAP# 8869063) as qualified to perform high complexity clinical laboratory testing. Data interpretations are based on our current understanding of genes and variants as of the report date. Alterations are listed alphabetically and not in order of strength of evidence or appropriateness for the patient's disease. When the report does identify variants with therapeutic implications, this does not promise or guarantee that a particular drug or treatment regimen will be effective or helpful in the treatment of disease in any patient, and the selection of any drug for patient treatment is done at the discretion of the treating physician.

General genomic alterations should be considered in the context of the patient's history, risk factors and any previous genomic testing. Consideration of Variants of Unknown Significance (VUS) may associate with potential therapies in the future. Exact Sciences does not update reports or send notification regarding reclassification of these alterations.

Electronically signed by

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