

## Liquid Trace Solid Tumor

Patient Name:	<input type="text"/>	Ordering Physician:	<input type="text"/>
Date of Birth:	<input type="text"/>	Physician ID:	<input type="text"/>
Gender (M/F):	<input type="text"/>	Accession #:	<input type="text"/>
Client:	<input type="text"/>	Specimen Type:	CSF
Case #:	<input type="text"/>	Specimen ID:	<input type="text"/>
Body Site:	<input type="text"/>		

MRN:	<input type="text"/>	Indication for Testing:	<input type="text"/>
Collected Date:	<input type="text"/>		
Received Date:	<input type="text"/>	Tumor Type:	<input type="text"/>
Reported Date:	<input type="text"/>	Stage:	<input type="text"/>

Detected Genomic Alterations				
Level 1 (FDA-Approved)	Level 2 (Standard of Care)	Level 3 (Clinical Evidence)	Level 4 (Biological Evidence)	Other
ERBB2 (exon 20 insertion)	Tumor Mutation Burden Low: 3 Mut/Mb	TP53, PPM1D (2 mutations)	BCORL1, MYCN, KMT2C, ACVR1	Autosomal chromosomes show : 1p-, 1q+, +2, 5p+, +7, +8, 9p-(Bi-allelic deletion of CDKN2A/B), 9q+, 10q-, +17, +19, +20, +21.

### Results Summary

- **-Mutations in ERBB2, BCORL1, MYCN, TP53, PPM1D (2 mutations), KMT2C, and ACVR1 genes**
  - **-Tumor Mutation Burden Low: 3 Mut/Mb**
  - **-No evidence of EGFR, KRAS, BRAF, MET, ALK, IDH1/2, H3 K27M, or PTEN mutations**
  - **-EBV viral RNA: Not detected**
  - **-HPV viral RNA: Not detected**
  - **-TTV viral RNA: Not detected**
  - **-HLA Genotyping:**
    - HLA-A: A\*24:02-A\*24:02
    - HLA-B: B\*07:02-B\*27:05
    - HLA-C: C\*01:02-C\*07:02
  - **-Autosomal chromosomes show : 1p-, 1q+, +2, 5p+, +7, +8, 9p-(Bi-allelic deletion of CDKN2A/B), 9q+, 10q-, +17, +19, +20, +21.**
  - **-Increased Keratin mRNA**
  - **-Increased ERBB2 mRNA**
- These findings are consistent with the presence of solid tumor DNA/RNA with markedly high tumor burden.

-ERBB2 exon 20 insertions suggests response to mobocertinib and amivantamab and trastuzumab deruxtecan.

-TP53 mutation suggests possible response to eprenetapopt (APR-246), Aurora kinase A and Wee1 inhibitors.

**See quantitative presentation of mutations at the end of the report.**

### Tumor Heterogeneity

There is a dominant abnormal clone with ERBB2 mutation. The BCORL1 mutation is detected in a subclone. There are abnormal low-level clones with MYCN, TP53, PPM1D (2 mutations), KMT2C, and ACVR1 mutations.

### Expression

Increased Keratin mRNA

Increased ERBB2 mRNA

### Diagnostic Implications

ERBB2, BCORL1, MYCN, TP53, PPM1D (2 mutations), KMT2C, ACVR1

These findings suggest the presence of solid tumor DNA/RNA (see results summary).

### Relevant Alteration Associated with Resistance

TP53 mutation is associated with resistance to therapy.

PPM1D mutations may confer resistance to chemotherapy

### Levels 2, 3 & 4 (Standard of Care and Clinical/Biological Evidence)

ERBB2 - exon 20

HER2-targeting tyrosine kinase inhibitor

TP53

Aurora kinase A inhibitors, Wee1 inhibitors, Chk1 inhibitors, kevetrin, APR-246, nutlins, gene therapy

PPM1D

PPM1D inhibitors

### Relevant Genes with NO Alteration

No evidence of mutation in KRAS, NRAS, EGFR, BRAF, or BRCA 1/2

No specific mutation in DPYD gene, associated with enzymatic deficiency

No evidence of METex14 skipping or EGFRvIII

## Test Description:

This is a comprehensive molecular profile which uses next generation sequencing (NGS) to identify molecular abnormalities, including single nucleotide variants (SNVs), insertions/deletions (indels), copy number variants (CNVs), tumor mutation burden (TMB), fusions, B- and T-cell clonality, and viruses (HPV, EBV, and TTV), in cell-free (cf) DNA of 302 genes and cfrRNA in greater than 1600 genes implicated in solid tumors. Whenever possible, clinical relevance and implications of detected abnormalities are described below.

## Biological relevance of detected Alterations

- **ERBB2.** This gene encodes a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases. This protein has no ligand binding domain of its own and therefore cannot bind growth factors. However, it does bind tightly to other ligand-bound EGF receptor family members to form a heterodimer, stabilizing ligand binding and enhancing kinase-mediated activation of downstream signalling pathways, such as those involving mitogen-activated protein kinase and phosphatidylinositol-3 kinase. Allelic variations at amino acid positions 654 and 655 of isoform a (positions 624 and 625 of isoform b) have been reported, with the most common allele, Ile654/Ile655, shown here. Amplification and/or overexpression of this gene has been reported in numerous cancers, including breast and ovarian tumors. Alternative splicing results in several additional transcript variants, some encoding different isoforms and others that have not been fully characterized. [provided by RefSeq, Jul 2008]
- **BCORL1.** The protein encoded by this gene is a transcriptional corepressor that is found tethered to promoter regions by DNA-binding proteins. The encoded protein can interact with several different class II histone deacetylases to repress transcription. Two transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, May 2010]
- **MYCN.** This gene is a member of the MYC family and encodes a protein with a basic helix-loop-helix (bHLH) domain. This protein is located in the nucleus and must dimerize with another bHLH protein in order to bind DNA. Amplification of this gene is associated with a variety of tumors, most notably neuroblastomas. Multiple alternatively spliced transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jun 2014]
- **TP53.** This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome. Alternative splicing of this gene and the use of alternate promoters result in multiple transcript variants and isoforms. Additional isoforms have also been shown to result from the use of alternate translation initiation codons from identical transcript variants (PMIDs: 12032546, 20937277). [provided by RefSeq, Dec 2016]
- **PPM1D.** The protein encoded by this gene is a member of the PP2C family of Ser/Thr protein phosphatases. PP2C family members are known to be negative regulators of cell stress response pathways. The expression of this gene is induced in a p53-dependent manner in response to various environmental stresses. While being induced by tumor suppressor protein TP53/p53, this phosphatase negatively regulates the activity of p38 MAP kinase, MAPK/p38, through which it reduces the phosphorylation of p53, and in turn suppresses p53-mediated transcription and apoptosis. This phosphatase thus mediates a feedback regulation of p38-p53 signaling that contributes to growth inhibition and the suppression of stress induced apoptosis. This gene is located in a chromosomal region known to be amplified in breast cancer. The amplification of this gene has been detected in both breast cancer cell line and primary breast tumors, which suggests a role of this gene in cancer development. [provided by RefSeq, Jul 2008]
- **KMT2C.** This gene is a member of the myeloid/lymphoid or mixed-lineage leukemia (MLL) family and encodes a nuclear protein with an AT hook DNA-binding domain, a DHHC-type zinc finger, six PHD-type zinc fingers, a SET domain, a post-SET domain and a RING-type zinc finger. This protein is a member of the ASC-2/NCOA6 complex (ASCOM), which possesses histone methylation activity and is involved in transcriptional coactivation. [provided by RefSeq, Jul 2008]
- **ACVR1.** Activins are dimeric growth and differentiation factors which belong to the transforming growth factor-beta (TGF-beta) superfamily of structurally related signaling proteins. Activins signal through a heteromeric complex of receptor serine kinases which include at least two type I (I and IB) and two type II (II and IIB) receptors. These receptors are all transmembrane proteins, composed of a ligand-binding extracellular domain with cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine specificity. Type I receptors are essential for signaling; and type II receptors are required for binding ligands and for expression of type I receptors. Type I and II receptors form a stable complex after ligand binding, resulting in phosphorylation of type I receptors by type II receptors. This gene encodes activin A type I receptor which signals a particular transcriptional response in concert with activin type II receptors. Mutations in this gene are associated with fibrodysplasia ossificans progressive. [provided by RefSeq, Jul 2008]

## Drug Information

## Poziotinib

Poziotinib, previously known as HM781-36B, is a covalent, irreversible tyrosine kinase inhibitor targeting EGFR and HER2 with exon 20 insertion mutations.

## APR-246

APR-246 is a first-in-class agent targeting mutant p53. In vitro and in vivo preclinical models have demonstrated that APR-246 has excellent efficacy in OC (both adenocarcinoma and squamous cell carcinoma) and potently synergises with chemotherapies used in the treatment of OC, restoring sensitivity to chemotherapy-resistant tumours. An initial phase I clinical trial has shown APR-246 to be safe in humans and early results from a currently running phase Ib/II trial of APR-246 with carboplatin and liposomal doxorubicin in ovarian cancer have been promising. Together, these data provide a strong rationale for investigating the efficacy of APR-246 in OC.

APR-246 has been used in trials studying the treatment of Prostatic Neoplasms, Hematologic Neoplasms, and Platinum Sensitive Recurrent High-grade Serous Ovarian Cancer With Mutated p53.

APR-246 is an analogue of PRIMA-1, which modifies the core domain of mutant p53, resulting in restoration of wild-type p53 conformation and reactivation of normal p53 function, leading to increased cell cycle arrest and tumor cell death (PMID: 20498645).

## Potential Clinical Trials

Trial URL	Status	Title	Disease	Drug	Sites
<a href="https://clinicaltrials.gov/study/NCT05546268">https://clinicaltrials.gov/study/NCT05546268</a>	Recruiting	A Phase 1/2 Study of Oral MRT-2359 in Patients With MYC-Driven and Other Selected Solid Tumors Including Lung Cancer	Non-small Cell Lung Cancer	Oral MRT-2359	Sarah Cannon Research Institute, Lake Mary, Florida 32746 Indiana University, Bloomington, Indiana 46202 Dana-Farber Cancer Institute, Boston, Massachusetts 02215
<a href="https://clinicaltrials.gov/study/NCT05650879">https://clinicaltrials.gov/study/NCT05650879</a>	Recruiting	A Phase 1a/1b Study of ELVN-002 for the Treatment of Patients With HER2 Mutant Non-Small Cell Lung Cancer	HER2 Mutant Non-small Cell Lung Cancer	ELVN-002, Fam-Trastuzumab Deruxtecan-Nxki, Trastuzumab emtansine	Advent Health Orlando, Orlando, Florida 32804 BRCR Medical Center Inc, Plantation, Florida 33322 NEXT/Virginia Cancer Specialists, Fairfax, Virginia 22031
<a href="https://clinicaltrials.gov/study/NCT06253871">https://clinicaltrials.gov/study/NCT06253871</a>	Recruiting	A Phase 1/1b Study of IAM1363 in Patients with Advanced Cancers Harboring HER2 Alterations	HER2 Mutant Non-small Cell Lung Cancer	IAM1363	Comprehensive Hematology Oncology, St. Petersburg, Florida 33709 University of Michigan, Ann Arbor, Michigan 48109 SCRI Oncology Partners, Nashville, Tennessee 37203

## Detailed Results

Single Nucleotide Variant (SNV) and Insertions-Deletions (INDELS)								
Gene name	Hgvsp	Hgvsc	Amino acids	Codons	Consequence	Allele frequency	Read depth	Predicted effect on protein
ERBB2	NP_004439.2:p.Tyr772_Ala775dup	NM_004448.2:c.2313_2324dupA TACGTGATGCC	-/AYVM	-/GCATACG TGATG	inframe_insertion	75.33	531	0

BCORL1	NP_068765.3:p. Ser1246ProfsTer14	NM_021946.4:c. 3736_3749delAGCCAGGAAGTCTT	SQEVF/X	AGCCAGGAAGTCTTc/c	frameshift_variant	50.46	329	0
MYCN	NP_005369.2:p. Asp376_Ser377dup	NM_005378.4:c. 1125_1130dupTGACTC	N/NSD	aac/aACTCTGac	inframe_insertion	0.94	638	0
TP53	NP_000537.3:p. Cys238Tyr	NM_000546.5:c. 713G>A	C/Y	tGt/tAt	missense_variant	0.89	449	deleterious (0)
PPM1D	NP_003611.1:p. Cys478TrpfsTer3	NM_003620.3:c. 1433dupG	C/WX	tgc/tGgc	frameshift_variant	0.7	853	0
PPM1D	NP_003611.1:p. Ser468Ter	NM_003620.3:c. 1403C>G	S/*	tCa/tGa	stop_gained	0.49	812	0
KMT2C	NP_733751.2:p. Gly908Asp	NM_170606.2:c. 2723G>A	G/D	gGc/gAc	missense_variant	0.47	850	0
ACVR1 (RNA)	NP_001104537.1:p.Arg206His	NM_001111067.2:c.617G>A	R/H	cGc/cAc	missense_variant	34.86	109	deleterious (0)

## Methodology and Test Background

This is a next generation sequencing (NGS) test that analyzes cfDNA in 302 genes and cfRNA in >1600 genes for abnormalities that are reported to be altered in various types of solid tumors. For cases with detectable circulating solid tumor DNA, tumor mutation burden (TMB) is reported. The assay also detects several viruses that are important in oncology, including EBV, HPV and TTV. TTV (torque teno virus) was first discovered in a patient with non-A-E hepatitis and is now regarded as a part of the human virome. In general, TTV does not cause pathology in immunocompetent individuals. TTV is considered as a marker of immune competence in patients with immunological impairment and inflammatory disorders. High TTV load is associated with increased risk of infection. In patients with organ transplant, low TTV load is associated with an increased risk of rejection.

Nucleic acid is isolated from peripheral blood plasma. Performance of the assays may vary depending on the quantity and quality of nucleic acid, sample preparation and sample age. Testing is performed using massive parallel sequencing of the coding DNA of the listed genes. This includes sequencing of all the exons as well as approximately 50 nucleotides at the 5' and 3' ends of each coding exon to detect splice site abnormalities. The TERT promoter region, including the hotspots at -124 and -146 bp, is also covered. Our cfDNA sequencing method has a sensitivity of 0.1% for detecting hot spot mutations, 0.5% for detecting single nucleotide variants (SNVs) and 1% for small (<60 bp) insertions/ deletions (indels). Known hot spots in specific genes such as IDH1/2, NRAS, and KRAS are reported at levels of 0.01% and higher when both cfRNA and cfDNA results are combined. Significant gene amplification and deletion (copy number variants) are also reported. TMB is calculated and cut-off points were determined based on comparison with tissue samples obtained from the same patient. Using cut-off of 6 mut/Mb, 17% of cases called as negative by cfDNA are false negative (FN). However, cases with  $\leq 3$  show only 6% FN. Intermediate cases (TMB between 6 and 9 mut/Mb) show 51% false positivity. Positive cases (TMB  $\geq 9$  Mut/Mb) show only 7% false positive. Cases without circulating solid tumor DNA are reported as "unable to evaluate" for TMB. Targeted RNA NGS is performed by hybrid capture and duplicates are excluded for levels measurements. The Universal Human Reference (UHR) RNA is used as control. All detected fusion transcripts are reported. While the major focus of the RNA analysis is the detection of fusion mRNA, mutations in the expressed RNA of the analyzed genes, HLA class I genotyping, and Epstein-Barr virus (EBV), human papillomavirus (HPV) and torque teno virus (TTV) viral RNA are also analyzed and reported. B- and T-cell clonality will be reported, if clonal or clinically relevant. The sensitivity of this assay in detecting fusion mRNA is between 5% and 10%. This test specifically covers translocations that lead to the expression of fusion RNA. Translocations that lead to deregulation of expression can be addressed by this test if compared to the expression proper normal control. Since the clinical relevance of the RNA expression level of most of the genes is not characterized at this time, only a few specific genes will be commented on when abnormalities are detected. CD274 (PD-L1) mRNA levels are reported when they are significantly elevated.

Based on our validation study, the following exonic regions of the genes listed below are not covered appropriately <100 X coverage and sequencing by NGS may not be reliable in these regions. The poor coverage is primarily due to the inherent difficulty in obtaining adequate sequencing coverage in regions with high GC content. No well-

characterized hotspots are present in these regions. RAD51 NM\_133487 chr15:40994004-40994124, BRCA1 NM\_007300 chr17:41231351-41231416, FUBP1 NM\_003902 chr1:78435609-78435699, CBLB NM\_170662 chr3:105420938-105421303, TERT NM\_198253 chr5:1295183-1295250, ARID1B NM\_017519 chr6:157098715-157100605, CUX1 NM\_001202543 chr7:101740644-101740781, KMT2C NM\_170606 chr7:151891314-151891346 and 151935792-151935911, GALNT12 NM\_024642 chr9:101569952-101570351, ATM NM\_000051 chr11:108164040-108164204, CDK17 NM\_001170464 chr12:96679880-96679926, RB1 NM\_000321 chr13:48954189-48954220, SETBP1 NM\_015559 chr18:42643044-42643692, KMT2B NM\_014727 chr19:36208921-36209283, AR NM\_000044 chrX:66764889-66766604, STAG2 NM\_001042749 chrX:123200025-123200112.

**The table below may contain a partial list of the tested DNA genes. For a complete list, please go to:**  
<https://genomictestingcooperative.com/genomic-tests/liquid-trace-solid-tumor/> (click the DNA tab)

**For a complete list of tested RNA genes (Fusions/Expression), please go to:**  
<https://genomictestingcooperative.com/genomic-tests/liquid-trace-solid-tumor/> (click the RNA tab)

## Tested genes

Genes Tested for Abnormalities in Coding Sequence																
ABL1	ATRX	BRAF	CDK12	CUX1	EPHA5	FGF4	GNAQ	IL7R	MAP2K1	MSH3	NPM1	PIM1	RAD21	SDHB	SRSF2	TRAF3
ABRAXAS1	AURKA	BRCA1	CDK4	CXCR4	ERBB2	FGF6	GNAS	INHBA	MAP2K2	MSH6	NRAS	PLCG1	RAD50	SDHC	STAG2	TSC1
ACVR1B	AURKB	BRCA2	CDK6	CYLD	ERBB3	FGFR1	GNB1	IRF4	MAP2K4	MTOR	NSD1	PMS1	RAD51	SDHD	STAT3	TSC2
AKT1	AURKC	BRIP1	CDKN1B	DAXX	ERBB4	FGFR2	GREM1	JAK1	MAP3K1	MUTYH	NSD2 (WHSC1)	PMS2	RAD51C	SETBP1	STAT5B	TSHR
AKT2	AXIN1	BTK	CDKN2A	DDR2	ERG	FGFR3	GRIN2A	JAK2	MAP3K14	MYC	NTHL1	POLD1	RAD51D	SETD2	STK11	U2AF1
AKT3	AXIN2	CALR	CDKN2B	DDX41	ESR1	FGFR4	H3-3A (H3F3A)	JAK3	MAPK1	MYCL	NTRK1	POLE	RAF1	SF3B1	SUFU	U2AF2
ALK	B2M	CARD11	CDKN2C	DICER1	ETNK1	FH	H3C2	KAT6A	MCL1	MYCN	NTRK2	POT1	RB1	SMAD2	SUZ12	UBA1
AMER1	BAP1	CBL	CEBPA	DNM2	ETV6	FLCN	HGF	KDM5C	MDM2	MYD88	NTRK3	PPM1D	RET	SMAD4	TAL1	VHL
ANKRD26	BARD1	CBLB	CHEK1	DNMT3A	EXO1	FLT3	HNF1A	KDM6A	MDM4	NBN	PAK3	PPP2R1A	RHEB	SMARCA4	TCF3	WT1
APC	BCL2	CBLC	CHEK2	DOT1L	EZH2	FLT4	HOXB13	KDR	MED12	NF1	PALB2	PRDM1	RHOA	SMARCB1	TENT5C (FAM46C)	XPO1
AR	BCL2L1	CCND1	CIC	EED	FANCA	FOXL2	HRAS	KEAP1	MEF2B	NF2	PAX5	PRKAR1A	RIT1	SMC1A	TERC	XRCC2
ARAF	BCL6	CCND3	CREBBP	EGFR	FANCC	FUBP1	HSP90AA1	KIT	MEN1	NFE2	PBRM1	PRKDC	RNF43	SMC3	TERT	XRCC3
ARID1A	BCOR	CCNE1	CRLF2	EGLN1	FANCD2	GALNT12	ID3	KMT2A	MET	NFE2L2	PDGFRA	PRPF8	ROS1	SMO	TET2	ZNF217
ARID1B	BCORL1	CD274	CSF1R	ELANE	FANCE	GATA1	IDH1	KMT2B	MITF	NFKBIA	PDGFRB	PRSS1	RUNX1	SOCS1	TGFBR2	ZRSR2
ARID2	BCR	CD79A	CSF3R	EP300	FANCF	GATA2	IDH2	KMT2C	MLH1	NKX2-1	PHF6	PTCH1	SAMD9	SOX2	TMEM127	-
ASXL1	BIRC3	CD79B	CTCF	EPAS1	FANCG	GATA3	IGF1R	KMT2D	MPL	NOTCH1	PIK3CA	PTEN	SAMD9L	SOX9	TNFAIP3	-
ATM	BLM	CDC73	CTNNA1	EPCAM	FAS	GEN1	IKZF1	KRAS	MRE11	NOTCH2	PIK3R1	PTPN11	SDHA	SPOP	TNFRSF14	-
ATR	BMPR1A	CDH1	CTNNB1	EPHA3	FBXW7	GNA11	IKZF3	LRP1B	MSH2	NOTCH3	PIK3R2	RAC1	SDHAF2	SRC	TP53	-

## RNA Fusions/Expression

Fusion/Expression													
ABL1	BCL6	CD274 (PD-L1)	EGFR	EWSR1	FLI1	IKZF3	MAP3K1	NRG1	NUP98	PML	RET	SS18	THADA
AKT3	BRAF	CIC	ERG	FGFR1	FOXO1	JAK2	MECOM	NTRK1	PAX8	PPARG	RHOA	STAT6	TMPRSS2
ALK	CAMTA1	CREB1	ETS1	FGFR2	FUS	KIAA1549	MYB	NTRK2	PDGFRA	PRKACA	ROS1	TAL1	YAP1
AR	CBFB	CREBBP	ETV1	FGFR3	GLI1	KMT2A	MYC	NTRK3	PDGFRB	RAF1	RUNX1	TCF3	YWHAE
BCL2	CCND1	ERBB2	ETV6	FIP1L1	HMGA2	MAML2	NOTCH1	NUP214	PICALM	RARA	RUNX1T1	TFG	ZFTA

## Reference

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4. Management of HER2 alterations in non-small cell lung cancer - The past, present, and future. N tzingler J, Bum Lee J, Li Low J, Ling Chia P, Talisa Wijaya S, Chul Cho B, Min Lim S, Soo RA. Lung Cancer. 2023 Dec;186:107385. doi: 10.1016/j.lungcan.2023.107385. Epub 2023 Sep 28. PMID: 37813015.
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## Electronic Signature

## Additional Report Information

