FOUNDATIONONE®LIQUID CDx

PATIENT
Lin, Chin-Chun

TUMOR TYPE
Liver cholangiocarcinoma
COUNTRY CODE
TW

REPORT DATE
04 October 2022
ORDERED TEST #
ORD-1461672-01

ABOUT THE TEST FoundationOne®Liquid CDx is a next generation sequencing (NGS) assay that identifies clinically relevant genomic alterations in circulating cell-free DNA.

PATIENT
DISEASE Liver cholangiocarcinoma
NAME Lin, Chin-Chun
DATE OF BIRTH 20 June 1961
SEX Male
MEDICAL RECORD # 21384474

PHYSICIAN
ORDERING PHYSICIAN Yeh, Yi-Chen
MEDICAL FACILITY Taipei Veterans General Hospital
ADDITIONAL RECIPIENT None
MEDICAL FACILITY ID 205872
PATHOLOGIST Not Provided

SPECIMEN
SPECIMEN ID CCL 6/20/1961
SPECIMEN TYPE Blood
DATE OF COLLECTION 15 September 2022
SPECIMEN RECEIVED 21 September 2022

Biomarker Findings

Blood Tumor Mutational Burden - 18 Muts/Mb
Microsatellite status - MSI-High Not Detected
Tumor Fraction - Elevated Tumor Fraction

Genomic Findings

For a complete list of the genes assayed, please refer to the Appendix.

CCNE1 amplification
FGFR1 amplification - equivocal†
MDM2 amplification
PTEN splice site 165-1G>C
STK11 F157fs*5
ASXL1 E635fs*15
ESR1 S341L
KDM6A R1351*
NSD3 (WHSC1L1) amplification - equivocal†
RAD21 R450fs*6
RB1 R698S
TP53 R280T
ZNF703 amplification - equivocal†

† See About the Test in appendix for details.

Report Highlights

- Targeted therapies with **NCCN categories of evidence** in this tumor type: **Nivolumab** (p. 15)
- Evidence-matched **clinical trial options** based on this patient's genomic findings: (p. 17)
- Variants that may represent **clonal hematopoiesis** and may originate from non-tumor sources: ***ASXL1* E635fs*15** (p. 9)

BIOMARKER FINDINGS	THERAPIES WITH CLINICAL RELEVANCE (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL RELEVANCE (IN OTHER TUMOR TYPE)
Blood Tumor Mutational Burden - 18 Muts/Mb	Dostarlimab	Nivolumab 2B
	Pembrolizumab	Cemiplimab
		Nivolumab + Ipilimumab
10 Trials see p. 17		
Microsatellite status - MSI-High Not Detected	**MSI-High not detected. No evidence of microsatellite instability in this sample (see Appendix section).**	
Tumor Fraction - Elevated Tumor Fraction	**Tumor fraction is considered elevated when ctDNA levels are high enough that aneuploidy can be detected. There is higher sensitivity for identifying genomic alterations and a lower risk of false negative results in specimens with elevated tumor fraction; the positive percent agreement observed between liquid and tissue for defined short variants is ≥ 90% (Li et al., 2021; AACR Abstract 2231) (see Biomarker Findings section).**	

GENOMIC FINDINGS	VAF%	THERAPIES WITH CLINICAL RELEVANCE (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL RELEVANCE (IN OTHER TUMOR TYPE)
***CCNE1* -** amplification	-	None	None
4 Trials see p. 19			

☐ *NCCN category*

Electronically signed by Douglas A. Mata, MD, MPH | 04 October 2022
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Nimesh R. Patel, M.D., Laboratory Director CLIA: 34D2044309
Foundation Medicine, Inc. | 1.888.988.3639

Sample Preparation: 150 Second St., 1st Floor, Cambridge, MA 02141 · CLIA: 22D2027531
Sample Analysis: 150 Second St., 1st Floor, Cambridge, MA 02141 · CLIA: 22D2027531
Post-Sequencing Analysis: 150 Second St., 1st Floor. Cambridge, MA 02141 · CLIA: 22D2027531

GENOMIC FINDINGS		VAF%	THERAPIES WITH CLINICAL RELEVANCE (IN PATIENT'S TUMOR TYPE)	THERAPIES WITH CLINICAL RELEVANCE (IN OTHER TUMOR TYPE)
FGFR1 -	amplification - equivocal	-	None	None
10 Trials see p. 20				
MDM2 -	amplification	-	None	None
2 Trials see p. 22				
PTEN -	splice site 165-1G>C	17.8%	None	None
10 Trials see p. 23				
STK11 -	F157fs*5	21.4%	None	None
1 Trial see p. 25				

☐ *NCCN category*

VARIANTS THAT MAY REPRESENT CLONAL HEMATOPOIESIS (CH)

Genomic findings below may include nontumor somatic alterations, such as CH. The efficacy of targeting such nontumor somatic alterations is unknown. This content should be interpreted based on clinical context. Refer to appendix for additional information on CH.

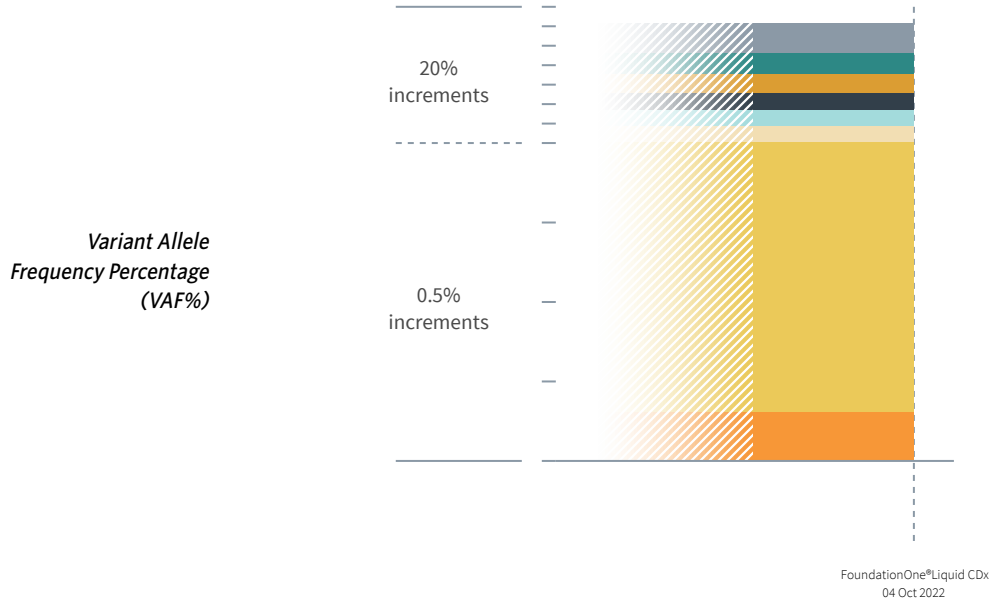
ASXL1 - E635fs*15 ..... p. 9

GENOMIC FINDINGS WITH NO REPORTABLE THERAPEUTIC OR CLINICAL TRIAL OPTIONS

For more information regarding biological and clinical significance, including prognostic, diagnostic, germline, and potential chemosensitivity implications, see the Genomic Findings section.

NOTE Genomic alterations detected may be associated with activity of certain approved therapies; however, the therapies listed in this report may have varied clinical evidence in the patient's tumor type. Therapies and the clinical trials listed in this report may not be complete and/or exhaustive. Neither the therapies nor the trials identified are ranked in order of potential or predicted efficacy for this patient, nor are they ranked in order of level of evidence for this patient's tumor type. This report should be regarded and used as a supplementary source of information and not as the single basis for the making of a therapy decision. All treatment decisions remain the full and final responsibility of the treating physician and physicians should refer to approved prescribing information for all therapies. Therapies contained in this report may have been approved by the US FDA or other national authorities; however, they might not have been approved in your respective country. In the appropriate clinical context, germline testing of *APC, ATM, BAP1, BRCA1, BRCA2, BRIP1, CHEK2, FH, FLCN, MEN1, MLH1, MSH2, MSH6, MUTYH, NF1, NF2, PALB2, PMS2, POLE, PTEN, RAD51C, RAD51D, RB1, RET, SDHA, SDHB, SDHC, SDHD, SMAD4, STK11, TGFBR2, TP53, TSC1, TSC2, VHL,* and *WT1* is recommended.

Variant Allele Frequency is not applicable for copy number alterations.



HISTORIC PATIENT FINDINGS		ORD-1461672-01 VAF%
Blood Tumor Mutational Burden		18 Muts/Mb
Microsatellite status		MSI-High Not Detected
Tumor Fraction		24%
CCNE1	amplification	Detected
FGFR1	amplification	Detected
MDM2	amplification	Detected
PTEN	splice site 165-1G>C	17.8%
STK11	F157fs*5	21.4%
ASXL1	E635fs*15	3.2%
ESR1	S341L	16.7%
KDM6A	R1351*	30.7%
NSD3 (WHSC1L1)	amplification	Detected
RAD21	R450fs*6	0.31%

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HISTORIC PATIENT FINDINGS		ORD-1461672-01 VAF%
RB1	R698S	15.9%
TP53	R280T	19.6%
ZNF703	amplification	Detected

NOTE This comparison table refers only to genes and biomarkers assayed by prior FoundationOne®Liquid CDx or FoundationOne®CDx tests. Up to five previous tests may be shown.

For some genes in FoundationOne Liquid CDx, only select exons are assayed. Therefore, an alteration found by a previous test may not have been confirmed despite overlapping gene lists. Please refer to the Appendix for the complete list of genes and exons assayed. The gene and biomarker list will be updated periodically to reflect new knowledge about cancer biology.

As new scientific information becomes available, alterations that had previously been listed as Variants of Unknown Significance (VUS) may become reportable.

Tissue Tumor Mutational Burden (TMB) and blood TMB (bTMB) are estimated from the number of synonymous and non-synonymous single-nucleotide variants (SNVs) and insertions and deletions (indels) per area of coding genome sampled, after the removal of known and likely oncogenic driver events and germline SNPs. Tissue TMB is calculated based on variants with an allele frequency of ≥5%, and bTMB is calculated based on variants with an allele frequency of ≥0.5%.

Not Tested = not baited, not reported on test, or test preceded addition of biomarker or gene

Not Detected = baited but not detected on test

Detected = present (VAF% is not applicable)

VAF% = variant allele frequency percentage

Cannot Be Determined = Sample is not of sufficient data quality to confidently determine biomarker status

BIOMARKER

Blood Tumor Mutational Burden

RESULT
18 Muts/Mb

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of clinical evidence in solid tumors, increased blood tumor mutational burden (bTMB) may be associated with greater sensitivity to immunotherapeutic agents, including anti-PD-L1[1-3], anti-PD-1[3-4], and anti-PD-1/CTLA4 therapies[5-6] . A Phase 2 multi-solid-tumor trial showed that bTMB ≥16 Muts/Mb (as measured by this assay) was associated with improved survival from treatment with a PD-1 inhibitor alone or in combination with a CTLA-4 inhibitor[5]. In non-small cell lung cancer (NSCLC), multiple clinical trials have shown patients with higher bTMB derive clinical benefit from immune checkpoint inhibitors following single-agent or combination treatments with either CTLA4 inhibitors or chemotherapy, with reported high bTMB cutpoints ranging from 6 Muts/Mb-16 Muts/Mb[1]. In head and neck squamous cell carcinoma (HNSCC), a Phase 3 trial showed that bTMB ≥16 Muts/Mb (approximate equivalency ≥8 Muts/Mb as measured by this assay) was associated with improved survival from treatment with a PD-L1 inhibitor alone or in combination with a CTLA-4 inhibitor[7].

FREQUENCY & PROGNOSIS

Average bTMB levels in solid tumors other than NSCLC have not been evaluated (PubMed, Mar 2022). Published data investigating the prognostic implications of bTMB levels in biliary tract cancer are limited (PubMed, Jul 2022). Although cases with hypermutated biliary tract cancer were enriched in a subgroup with poor prognosis in 1 study[8], TMB-high (≥10 mut/Mb) status in biliary adenocarcinoma not treated with immunotherapy was not significantly associated with OS in another study, in which patients with TMB-high tumors experienced numerically longer OS compared with patients with TMB-low tumors (11.5 vs. 8.4 months, adjusted HR=0.65)[9].

FINDING SUMMARY

Blood tumor mutational burden (bTMB, also known as mutation load) is a measure of the number of somatic protein-coding base substitution and insertion/deletion mutations from circulating tumor DNA in blood. TMB is affected by a variety of causes, including exposure to mutagens such as ultraviolet light in melanoma[10-11] and cigarette smoke in lung cancer[12-13], treatment with temozolomide-based chemotherapy in glioma[14-15], mutations in the proofreading domains of DNA polymerases encoded by the POLE and POLD1 genes[16-20], and microsatellite instability (MSI)[16,19-20]. This sample harbors a bTMB level that may be associated with sensitivity to PD-1- or PD-L1-targeting immune checkpoint inhibitors, alone or in combination with other agents[1-2,4].

BIOMARKER

Tumor Fraction

RESULT
Elevated Tumor Fraction

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Specimens with elevated tumor fraction have high circulating-tumor DNA (ctDNA) content, and thus high sensitivity for identifying genomic alterations. Such specimens are at low risk of false negative results. Tumor fraction levels currently have limited implications for diagnosis, surveillance, or therapy and should not be overinterpreted or compared from one blood draw to another. There are currently no targeted approaches to address specific tumor fraction levels. In the research setting, changes in tumor fraction estimates have been associated with treatment duration and clinical response and may be a useful indicator for future cancer management[21-26].

FREQUENCY & PROGNOSIS

Detectible ctDNA levels have been reported in a variety of tumor types, with higher tumor fraction levels reported for patients with metastatic (Stage 4) tumors compared with patients with localized disease (Stages 1 to 3)[27]. Elevated tumor fraction levels have been reported to be associated with worse prognosis in a variety of cancer types, including pancreatic cancer[28], Ewing sarcoma and osteosarcoma[29], prostate cancer[24], breast cancer[30], leiomyosarcoma[31], esophageal cancer[32], colorectal cancer[33], and gastrointestinal cancer[34].

FINDING SUMMARY

Tumor fraction provides an estimate of the percentage of ctDNA present in a cell-free DNA (cfDNA) sample. The tumor fraction estimate for this sample is based on the observed level of aneuploid instability. The tumor fraction algorithm utilized for FoundationOne Liquid CDx uses the allele frequencies of approximately 1,000 single-nucleotide polymorphism (SNP) sites across the genome. Unlike the maximum somatic allele frequency (MSAF) method of estimating ctDNA content[35], the tumor fraction metric does not take into account the allele frequency of individual variants but rather produces a more holistic estimate of ctDNA content using data from across the genome. The amount of ctDNA detected may correlate with disease burden and response to therapy[36-37].

GENE
CCNE1

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no approved therapies that directly target CCNE1 alterations. Because amplification or overexpression of CCNE1 leads to increased genomic instability though the ATR-CHK1-WEE1 pathway[38-39] and cyclin E1 promotes cell cycle progression in a complex with CDK2[40], clinical and preclinical studies have investigated inhibitors of CHK1, ATR, CDK2, and WEE1 as potential therapeutic approaches for tumors with CCNE1 activation. Clinical benefit has been reported for patients with recurrent high-grade serous ovarian carcinoma (HGSOC) with CCNE1 amplification or expression in response to treatment with the CHK1 inhibitor prexasertib[41]. Studies of the WEE1 inhibitor adavosertib observed PRs in patients with CCNE1-amplified HGSOC and ovarian cancer[42-43]. Similarly, in a Phase 2 study of patients with CCNE1-amplified solid tumors, adavosertib elicited an ORR of 26% with PRs reported for patients with ovarian cancer, urothelial carcinoma, or melanoma[44]. Preclinical studies have demonstrated that cell lines with CCNE1 amplification or overexpression were sensitive to inhibitors of ATR[45-46], CDK2[47], or WEE1[39,48]. However, other studies have shown that sensitivity of various cell lines to CDK2 inhibitors, including SNS-032, dinaciclib, and seliciclib, at clinically achievable doses, is largely independent of CCNE1 copy number or expression[49-52]. One study has reported a reduction in tumor CCNE1 levels in 4/6 lung and esophageal cancer cases following treatment with the HDAC inhibitor vorinostat[53].

FREQUENCY & PROGNOSIS

CCNE1 amplification has been reported in 1-2% of samples in published cholangiocarcinoma datasets[54-55]. Published data investigating the prognostic implications of CCNE1 alterations in biliary tract carcinoma are limited (PubMed, Mar 2022).

FINDING SUMMARY

CCNE1 encodes the protein cyclin E1, which plays a role in the regulated transition from the G1 to S phase by binding to and activating cyclin-dependent protein kinase 2 (CDK2). It also has a direct role in initiation of replication and the maintenance of genomic stability[40]. Amplification of chromosomal region 19q12-q13 has been demonstrated in many types of cancer, and CCNE1 is a well-studied gene within this amplicon[56-57]. Increased copy number of CCNE1 is highly associated with overexpression of the cyclin E1 protein[58-59]. Cyclin E1 overexpression can lead to cell transformation as a result of an increase in cyclin E1 activity[40,60].

GENE
FGFR1

ALTERATION
amplification - equivocal

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Alterations that activate FGFR1 may predict sensitivity to selective FGFR inhibitors including erdafitinib[61-63], pemigatinib[64], infigratinib[65-66], futibatinib[67-69], rogaratinib[70], Debio 1347[71-72], and derazantinib[73], or multikinase inhibitors such as pazopanib[74] and ponatinib[75-77]. The activity and efficacy of selective FGFR inhibitors for FGFR1-amplified tumors has been modest with limited responses reported in FGFR1-amplified lung squamous cell carcinoma (SCC) treated with infigratinib[78] or AZD4547[79] and no responses reported among patients with FGFR1-amplified breast cancer treated with infigratinib[78]. Two case studies reported PRs in patients with FGFR1-amplified breast cancer treated with pazopanib[74].

FREQUENCY & PROGNOSIS

Although FGFR1 amplification and mutation have been reported infrequently in biliary tract tumors and cholangiocarcinoma[8,80-84], recurrent activating FGFR2 fusions have been reported in 8-50% of intrahepatic cholangiocarcinomas[8,82-83,85-88]. Published data investigating the prognostic implications of FGFR1 alterations in biliary tract carcinoma or cholangiocarcinoma are limited (PubMed, Aug 2021).

FINDING SUMMARY

FGFR1 encodes the protein fibroblast growth factor receptor 1, which plays key roles in regulation of the cell cycle and angiogenesis and is an upstream regulator of the RAS, MAPK, and AKT signaling pathways[89]. Amplification of FGFR1 has been correlated with protein expression[90-91] and may predict pathway activation and sensitivity to therapies targeting this pathway[92-93].

GENE
MDM2

ALTERATION
amplification

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

MDM2 antagonists disrupt the MDM2-p53 interaction, thereby stabilizing p53[94]. Preclinical studies have suggested that the amplification of MDM2, in the absence of concurrent TP53 mutations, may increase sensitivity to these agents[95-96]. Preliminary Phase 1 studies of the MDM2-p53 antagonist alrizomadlin (APG-115) reported a PR in a patient with liposarcoma harboring an MDM2 amplification and wildtype for TP53 and SD in 21%–38% (6/28 and 5/13, respectively) of patients in genomically unselected solid tumors[97-98] . A Phase 2 trial of alrizomadlin in combination with pembrolizumab reported a PR in 1 of 3 patients with malignant peripheral nerve sheath tumor that had failed standard therapy, as well as PRs in patients with multiple types of solid tumors that had failed immunotherapy, including 1 out of 14 patients with non-small cell lung cancer; 1 out of 5 patients with urothelial carcinoma; and 2 out of5, 1 out of 5, and 1 out of 11 patients with mucosal, uveal, and cutaneous melanoma, respectively[99]. Phase 1b studies of the MDM2 inhibitor idasanutlin for refractory AML in combination with cytarabine or venetoclax reported anti-leukemic response rates of 33% (25/75) and 37% (11/30), respectively[100-101] ; clinical benefit (58% ORR, 7/12) with idasanutlin monotherapy has been reported for patients with polycythemia vera[102]. The dual MDM2/MDM4 inhibitor ALRN-6924 led to an ORR of 27% (4/15) for patients with TP53 wildtype peripheral T-cell lymphoma in a Phase 2 study[103]; responses have also been observed in TP53 wildtype AML, MDS, Merkel cell carcinoma, colorectal cancer, and liposarcoma[104-105] .

FREQUENCY & PROGNOSIS

MDM2 amplification has been observed in 10.8% of gallbladder carcinoma and 3.2% of cholangiocarcinoma samples[54,106-107]. MDM2 overexpression has been reported to be a common event in biliary tract cancers, occurring in 75% of gallbladder adenocarcinoma and in 38-68% of ICC cases, respectively[108-111]. MDM2 overexpression in pancreatic carcinomas and biliary tract tumors has been associated with advanced tumor stage, metastasis, and poor patient prognosis[109-110,112].

FINDING SUMMARY

MDM2 encodes an E3 ubiquitin protein ligase, which mediates the ubiquitination and subsequent degradation of p53, Rb1, and other proteins[113-115]. MDM2 acts to prevent the activity of the tumor suppressor p53; therefore, overexpression or amplification of MDM2 may be oncogenic[116-117]. Overexpression or amplification of MDM2 is frequent in cancer[118]. Although two retrospective clinical studies suggest that MDM2 amplification may predict a short time-to-treatment failure on anti-PD-1/PD-L1 immune checkpoint inhibitors, with 4/5 patients with MDM2 amplification[119] and 2/3 patients with MDM2 or MDM4 amplification[120] experiencing tumor hyperprogression, amplification of MDM2 or MDM4 was not associated with shorter progression-free survival (PFS) in a retrospective analysis of non-small cell lung cancer (NSCLC) outcomes with immune checkpoint inhibitors (hazard ratio of 1.4, p=0.44)[121]. The latter study reported PFS of >2 months for 5/8 patients with MDM2/MDM4 amplification[121].

GENE

PTEN

ALTERATION
splice site 165-1G>C

TRANSCRIPT ID
NM_000314

CODING SEQUENCE EFFECT
165-1G>C

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

PTEN loss or mutation leads to activation of the PI3K-AKT-mTOR pathway and may predict sensitivity to inhibitors of this pathway[122-125]. A Phase 2 trial examining treatment with the PI3K inhibitor copanlisib in combination with gemcitabine and cisplatin in biliary tract cancer reported longer response rates for patients with low PTEN expression (PFS of 8.5 months, OS of 18 months) compared with patients with high PTEN expression (PFS of 4.6 months, OS of 7.0 months); however, differences were not statistically significant (p=0.19)[126]. Preclinical data indicate that PTEN loss or inactivation may predict sensitivity to PARP inhibitors[127-131], and clinical benefit has been observed for patients with PTEN-altered breast cancer including triple negative breast cancer[132], ovarian cancer[133], uterine leiomyosarcoma[134], and endometrial cancer[131] treated with PARP inhibitors. However, some studies have reported a lack of association between PTEN mutation and PARP inhibitor sensitivity[135-136].

FREQUENCY & PROGNOSIS

PTEN mutations have been reported in 1-11% of cholangiocarcinoma samples[81,83,137]. PTEN homozygous deletion was reported in fewer than 1% of gallbladder cancer cases[106]. PTEN was not observed as a significantly altered gene in an analysis of biliary tract cancers[8]. In a study of 60 cholangiocarcinoma samples, 60% (36/60) showed a loss of PTEN expression[138]. Loss of PTEN expression has been reported in 51.8% of gallbladder adenocarcinomas[139]. Loss of PTEN has been associated with increased invasion, advanced tumor stage, and shorter survival in patients with cholangiocarcinoma[138,140-141], and with poor prognosis in patients with gallbladder adenocarcinoma[139].

FINDING SUMMARY

PTEN encodes an inositol phosphatase that functions as a tumor suppressor by negatively regulating the PI3K-AKT-mTOR pathway; loss of PTEN can lead to uncontrolled cell growth and suppression of apoptosis[123]. Alterations such as seen here may disrupt PTEN function or expression[142-183].

POTENTIAL GERMLINE IMPLICATIONS

One or more of the PTEN variants observed here has been described in the ClinVar database as a likely pathogenic or pathogenic germline mutation (by an expert panel or multiple submitters) associated with hamartoma tumor syndrome (ClinVar, Sep 2022)[184]. Follow-up germline testing would be needed to distinguish whether the finding in this patient is somatic or germline. PTEN mutations underlie several inherited disorders, collectively termed PTEN hamartoma tumor syndrome (PHTS), which include Cowden syndrome (CS) and its variant Lhermitte-Duclos disease (LD), Bannayan-Riley-Ruvalcaba syndrome (BRRS), PTEN-related Proteus syndrome (PS), and Proteus-like syndrome[185-186]. The mutation rate for PTEN in these disorders ranges from 20 to 85% of patients[185,187]. The estimated incidence of Cowden syndrome is 1/200,000, which may be an underestimate due to the high variability of this disorder[185]. Given the association between PTEN and these inherited syndromes, in the appropriate clinical context, germline testing for mutations affecting PTEN is recommended.

GENE
STK11

ALTERATION
F157fs*5

TRANSCRIPT ID
NM_000455

CODING SEQUENCE EFFECT
471_472delCT

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Increased mTOR signaling is present in LKB1-deficient tumors, suggesting therapies targeting mTOR may be relevant for tumors with STK11 alterations[188-192]. Case studies have reported PRs for 2 patients with STK11-mutated pancreatic cancer following treatment with the mTOR inhibitor everolimus[193], with 1 PR observed in a PJS patient for 9 months until progression[193]. However, retrospective analysis of a Phase 2 trial for patients with endometrial carcinoma found LKB1 (STK11) protein levels were not significantly correlated with response to everolimus treatment[194]. In one preclinical study, STK11 loss was associated with sensitivity to combination treatment including an SRC inhibitor[195]; however, the clinical relevance of these findings has not been established.

— Potential Resistance —

STK11 alteration is associated with poorer response to immune checkpoint inhibitors for patients with NSCLC, including those with tumors harboring co-occurring KRAS mutation[196-208] .

FREQUENCY & PROGNOSIS

STK11 mutations have been reported in 3% of biliary tract carcinomas analyzed in the COSMIC database (Mar 2022)[209]. STK11 mutation or loss have also been reported in biliary tract adenocarcinomas in the literature[210-212]. Loss of STK11 protein expression in intrahepatic cholangiocarcinoma has been reported as an independent predictor of shorter OS and time to recurrence[213].

FINDING SUMMARY

The serine/threonine kinase STK11 (also called LKB1) activates AMPK and negatively regulates the mTOR pathway in response to changes in cellular energy levels[188]. LKB1 acts as a tumor suppressor in cancer, as loss of function promotes proliferation and tumorigenesis[195,214]. Alterations such as seen here may disrupt STK11 function or expression[215-227].

POTENTIAL GERMLINE IMPLICATIONS

Germline mutations in STK11 underlie Peutz-Jeghers syndrome (PJS), a rare autosomal dominant disorder associated with a predisposition for tumor formation[228]. This disorder has an estimated frequency between 1:29,000 and 1:120,000, although reported rates in the literature vary greatly[228-230]. Although gastrointestinal tumors are the most common malignancies associated with PJS, patients also exhibit an 18-fold increased risk of developing other epithelial cancers[228-230], and individuals with this syndrome have a 30-50% risk of developing breast cancer[228,230]. Given the association with PJS, in the appropriate clinical context testing for the presence of germline mutations in STK11 is recommended.

GENE
ASXL1

ALTERATION
E635fs*15

TRANSCRIPT ID
NM_015338

CODING SEQUENCE EFFECT
1900_1922del23

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no targeted therapies available to address genomic alterations in ASXL1.

FREQUENCY & PROGNOSIS

ASXL1 alterations occur infrequently across various solid tumor types[231] and are not known to act as drivers in any specific solid cancer type[232]. Published data investigating the prognostic implications of ASXL1 alterations in solid tumors are limited (PubMed, May 2022). In the context of clonal hematopoiesis, ASXL1 mutations are significantly enriched in current or former smokers[233].

FINDING SUMMARY

ASXL1 regulates epigenetic marks and transcription through interaction with polycomb complex proteins and various transcription activators and repressors[234-236]. Alterations such as seen here may disrupt ASXL1 function or expression[237-239].

POTENTIAL CLONAL HEMATOPOIESIS

IMPLICATIONS

Variants seen in this gene have been reported to occur in clonal hematopoiesis (CH), an age-related process in which hematopoietic stem cells acquire somatic mutations that allow for clonal expansion[240-245]. CH in this gene has been associated with increased mortality, risk of coronary heart disease, risk of ischemic stroke, and risk of secondary hematologic malignancy[240-241]. Clinical management of patients with CH in this gene may include monitoring for hematologic changes and reduction of controllable risk factors for cardiovascular disease[246]. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH[244,247-248]. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH.

GENE
ESR1

ALTERATION
S341L

TRANSCRIPT ID
NM_000125

CODING SEQUENCE EFFECT
1022C>T

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

Therapies that directly target ER-alpha, such as selective ER modulators (SERMs) and the selective ER degrader (SERD) fulvestrant, as well as aromatase inhibitors (AIs) that inhibit estrogen production, are approved to treat ER-positive (ER+) and/or hormone receptor-positive (HR+) breast cancer (NCCN Guidelines v4.2022). AI treatment has also been reported to provide clinical benefit in a subset of HR+ gynecologic malignancies[249-253]. Combinations of fulvestrant and CDK4/6 inhibitors such as abemaciclib, palbociclib, and ribociclib, have also demonstrated efficacy for patients with ESR1-mutated breast cancer[254]. It is not known whether these therapeutic approaches would be relevant in the context of alterations that have not been fully characterized, as seen here.

FREQUENCY & PROGNOSIS

ESR1 mutation has not been reported in multiple cholangiocarcinoma datasets[80-82]. Published data investigating the prognostic implications of ESR1 alterations in biliary tract carcinoma are limited (PubMed, Dec 2021).

FINDING SUMMARY

ESR1 encodes estrogen receptor alpha (ER-alpha), one of the major estrogen receptor isoforms in humans. Along with co-activator proteins, the ER complex promotes transcription of genes involved in cell cycle progression and survival[255]. Although alterations such as seen here have not been fully characterized and are of unknown functional significance, similar alterations have been previously reported in the context of cancer, which may indicate biological relevance.

GENE
KDM6A

ALTERATION
R1351*

TRANSCRIPT ID
NM_021140

CODING SEQUENCE EFFECT
4051C>T

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no therapies available to address KDM6A alterations in cancer.

FREQUENCY & PROGNOSIS

KDM6A mutations have been reported in 3.9% of samples analyzed, with the highest incidence in tumors of the urinary tract (31%), liver (7.3%), endometrium (6.7%), salivary gland (6.0%), and pancreas (5.1%) (COSMIC, Jan 2022)[209]. KDM6A mutations or copy number alterations have also been identified in medulloblastoma (8.9%)[256], adenoid cystic carcinoma (6.7%)[257], and metastatic prostate cancer (10%)[258]. KDM6A inactivation has been found as a recurrent tumorigenic event in male T-cell acute lymphoblastic leukemia (T-ALL), and loss of KDM6A increased the sensitivity of T-ALL cells to therapies targeting histone H3 lysine 27 methylation in preclinical assays[259]. However, KDM6A overexpression has been noted in breast cancer and renal cell carcinoma, and correlated with inferior prognosis in patients with breast cancer[260-262].

FINDING SUMMARY

KDM6A encodes a histone H3 lysine 27 demethylase UTX, which functions as a transcriptional regulator[263]. A significant number of inactivating KDM6A mutations have been found across multiple tumor types, suggesting a role as a tumor suppressor[263].

GENE
NSD3 (WHSC1L1)

ALTERATION
amplification - equivocal

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no targeted therapies available to address genomic alterations in NSD3.

FREQUENCY & PROGNOSIS

In TCGA datasets, NSD3 amplification has been most frequently observed in lung squamous cell carcinoma (17%)[264], breast invasive carcinoma (13%)[265], bladder urothelial carcinoma (9%)[266], and head and neck squamous cell carcinoma (9%)[267] samples[268-269]. Amplification of at least one member of the NSD3-CHD8-BRD4 pathway has been associated with worse overall survival in ovarian high-grade serous carcinoma and endometrial cancer[270]. In endometrial cancers, amplification of this pathway was more frequent in endometrial serous and endometrioid serious-like carcinomas compared to low-grade endometrioid endometrial adenocarcinomas[270].

FINDING SUMMARY

NSD3, also known as WHSC1L1, encodes an enzyme that mediates histone methylation[271]. NSD3 has been shown to be amplified in various cancers[272-274].

GENE
RAD21

ALTERATION
R450fs*6

TRANSCRIPT ID
NM_006265

CODING SEQUENCE EFFECT
1348delC

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no therapies to target alterations in this gene.

FREQUENCY & PROGNOSIS

RAD21 amplifications have been reported in solid tumors, including breast cancers (7%), melanoma (5.4%), and prostate (2.4%) cancers[231]. RAD21 overexpression has been correlated with poor prognosis in endometrial cancer[275], breast cancer[276-277], Ewing sarcoma[278], and colorectal cancer (CRC), especially in KRAS-mutant CRC[279].

FINDING SUMMARY

RAD21 encodes a protein involved in DNA double-strand break repair and sister chromatid cohesion as a part of the cohesin complex[280-283]. In preclinical studies, downregulation of RAD21 or other cohesin components leads to loss of expression from amplified genes, as well as amplifications themselves upon cell passaging[284], but also leads to an increase in deletions, insertions, and other rearrangements[285]. High RAD21 expression has also been associated with increased genomic instability[286]. Cohesin complex also organizes chromatin domains and regulates gene expression[287-288]. Both overexpression and reduction of expression of RAD21 has been reported to alter gene expression[289]. RAD21 amplification has been correlated with increased expression in breast[276,286,290] and endometrial[275] cancers. Other RAD21 alterations, including truncating and point mutations, have been reported in the context of cancer, but the majority have not been characterized.

GENE
RB1

ALTERATION
R698S

TRANSCRIPT ID
NM_000321

CODING SEQUENCE EFFECT
2094G>T

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

On the basis of limited clinical data[291] and strong preclinical data[292-295], RB1 inactivation may be associated with sensitivity to inhibitors of Aurora kinase A, particularly in small cell lung cancer (SCLC). A clinical study evaluating the Aurora kinase A inhibitor alisertib for patients with prostate cancer did not find an association between RB1 deletion and clinical benefit[296]. Other approaches to target RB1 inactivation under investigation in preclinical studies include inhibitors of BCL-2 family members[297] and activation of the NOTCH pathway[298]. It is not known whether these therapeutic approaches would be relevant in the context of alterations that have not been fully characterized, as seen here.

FREQUENCY & PROGNOSIS

A study of 18 intrahepatic cholangiocarcinoma patients did not detect loss of heterozygosity at the chromosomal region 13q32 (where RB1 is located) in any cases[299]. However, studies have demonstrated that 12-41% of analyzed cholangiocarcinomas lack expression of Rb protein[300-301]. RB1 mutations have been reported across solid tumors including small cell lung cancer (60%), bladder (13%), uterine sarcoma (10%), non-melanoma skin cancer (9.5%), and gastrointestinal neuroendocrine tumors (9.3%)[302]. One analysis of 50 cholangiocarcinoma cases did not find a correlation between RB1 gene expression and disease metastasis[303].

FINDING SUMMARY

RB1 encodes the retinoblastoma protein (Rb), a tumor suppressor and negative regulator of the cell cycle[304-305]. Although alterations such as seen here have not been fully characterized and are of unknown functional significance, similar alterations have been previously reported in the context of cancer, which may indicate biological relevance.

POTENTIAL GERMLINE IMPLICATIONS

One or more of the RB1 variants observed here has been described in the ClinVar database as a likely pathogenic or pathogenic germline mutation (by an expert panel or multiple submitters) associated with retinoblastoma (ClinVar, Sep 2022)[184]. Follow-up germline testing would be needed to distinguish whether the finding in this patient is somatic or germline. Mutations in RB1 underlie the development of retinoblastoma (RB), a rare tumor that arises at a rate of approximately 1:20,000 live births, with nearly 5,000 new cases worldwide per year[306]. Germline mutations in RB1 account for approximately 40% of RB tumors[307] and are associated with an increased risk of developing secondary malignancies that include soft tissue and bone sarcoma and malignant melanoma[308-309]. In the appropriate clinical context, germline testing of RB1 is recommended.

GENE
TP53

ALTERATION
R280T

TRANSCRIPT ID
NM_000546

CODING SEQUENCE EFFECT
839G>C

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no approved therapies to address TP53 mutation or loss. However, tumors with TP53 loss of function alterations may be sensitive to the WEE1 inhibitor adavosertib[310-313] or p53 gene therapy such as SGT53[314-318]. In a Phase 1 study, adavosertib in combination with gemcitabine, cisplatin, or carboplatin elicited PRs in 9.7% and SDs in 53% of patients with solid tumors; the response rate was 21% (4/19) for patients with TP53 mutations versus 12% (4/33) for patients who were TP53 wildtype[319]. A Phase 2 trial of adavosertib in combination with chemotherapy (gemcitabine, carboplatin, paclitaxel, or doxorubicin) reported a 32% (30/94, 3 CR) ORR and a 73% (69/94) DCR for patients with platinum-refractory TP53-mutated ovarian, Fallopian tube, or peritoneal cancer[320]. A smaller Phase 2 trial of adavosertib in combination with carboplatin achieved a 43% (9/21, 1 CR) ORR and a 76% (16/21) DCR for patients with platinum-refractory TP53-mutated ovarian cancer[321]. The combination of adavosertib with paclitaxel and carboplatin for patients with TP53-mutated ovarian cancer also significantly increased PFS compared with paclitaxel and carboplatin alone[322]. In the Phase 2 VIKTORY trial, patients with TP53-mutated metastatic and/or recurrent gastric cancer experienced a 24% (6/25) ORR with adavosertib combined with paclitaxel[323]. A Phase 1 trial of neoadjuvant adavosertib in combination with cisplatin and docetaxel for head and neck squamous cell carcinoma (HNSCC) elicited a 71% (5/7) response rate for patients with TP53 alterations[324]. The Phase 2 FOCUS4-C trial for patients with TP53- and RAS-mutated colorectal cancer reported improvement in PFS (3.61 vs. 1.87 months, HR=0.35, p=0.0022), but not OS (14.0 vs 12.8 months, p=0.93), following adavosertib treatment compared with active monitoring[325]. In a Phase 1b clinical trial of SGT-53 in combination with docetaxel for patients with solid tumors, 75% (9/12) of evaluable patients experienced clinical benefit, including 2 confirmed and 1 unconfirmed PRs and 2 instances of SD with significant tumor shrinkage[318]. Missense mutations leading to TP53 inactivation may be sensitive to therapies that reactivate mutated p53 such as eprenetapopt. In a Phase 1b trial for patients with p53-positive high-grade serous ovarian cancer, eprenetapopt combined with carboplatin and pegylated liposomal doxorubicin achieved a 52% (11/21) response rate and 100% DCR[326]. A Phase 1 trial of eprenetapopt with pembrolizumab for patients with solid tumors reported an ORR of 10% (3/29)[327].

FREQUENCY & PROGNOSIS

Inactivation of p53, through mutation, deletion, or loss of heterozygosity (LOH), has been observed in 25-63% of gallbladder carcinomas and 10-61% of cholangiocarcinomas[8,80-83,85,328-331]. TP53 mutations occur more frequently in tumors caused by liver fluke (O. viverrini) infection (40%) than in cholangiocarcinoma cases not related to infection (9%)[80]. Aberrant TP53 expression, which is indicative of TP53 dysregulation, has been observed in 20–62% of gallbladder carcinomas and 25% (5/20) of cholangiocarcinomas[332-334]. Data regarding the prognostic significance of TP53 mutation in cholangiocarcinoma are conflicting[107,335-342]. Overexpression of p53 protein has been associated with reduced patient survival in poorly differentiated gallbladder adenocarcinomas and biliary tract cancers[343-344]; however, another study did not find such a correlation[337].

FINDING SUMMARY

Functional loss of the tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers[116]. Alterations such as seen here may disrupt TP53 function or expression[345-349].

POTENTIAL GERMLINE IMPLICATIONS

Germline mutations in TP53 are associated with the very rare autosomal dominant disorder Li-Fraumeni syndrome and the early onset of many cancers[350-352], including sarcomas[353-354]. Estimates for the prevalence of germline TP53 mutations in the general population range from 1:5,000[355] to 1:20,000[354]. For pathogenic TP53 mutations identified during tumor sequencing, the rate of germline mutations was 1% in the overall population and 6% in tumors arising before age 30[356]. In the appropriate clinical context, germline testing of TP53 is recommended.

POTENTIAL CLONAL HEMATOPOIESIS IMPLICATIONS

Variants seen in this gene have been reported to occur in clonal hematopoiesis (CH), an age-related process in which hematopoietic stem cells acquire somatic mutations that allow for clonal expansion[240-245]. CH in this gene has been associated with increased mortality, risk of coronary heart disease, risk of ischemic stroke, and risk of secondary hematologic malignancy[240-241]. Clinical management of patients with CH in this gene may include monitoring for hematologic changes and reduction of controllable risk factors for cardiovascular disease[246]. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH[244,247-248]. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH.

GENE
ZNF703

ALTERATION
amplification - equivocal

POTENTIAL TREATMENT STRATEGIES

— Targeted Therapies —

There are no available targeted therapies to directly address ZNF703 alterations in cancer. One preclinical study suggested that ZNF703 expression in breast cancer cell lines is associated with reduced sensitivity to tamoxifen through AKT-mTOR activation[357], although these findings have not been verified in the clinical setting.

FREQUENCY & PROGNOSIS

Amplification and high expression of ZNF703 has been observed in luminal B breast tumors, a subtype associated with aggressive disease progression and poor patient outcomes[358-360]. ZNF703 expression has also been linked with aggressive tumor characteristics in patients with gastric and colorectal cancers[361-362]. Putative high-level amplification of ZNF703 has been reported with the highest frequency in breast carcinoma, bladder urothelial carcinoma, uterine carcinosarcoma, lung squamous cell carcinoma (SCC), esophageal carcinoma and head and neck SCC (5-13% of samples)(cBioPortal, 2022)[268-269].

FINDING SUMMARY

ZNF703 encodes a transcriptional repressor that plays roles in stem cell proliferation, cell cycle progression, and other key cellular functions[359,363]. Amplification of ZNF703 has been correlated with protein expression[358-359]. ZNF703 was established as a breast cancer oncoprotein by studies showing that ZNF703 expression resulted in transformation and increased proliferation of cultured cells[358-359,364], as well as increased lung metastases in a breast cancer xenograft model[364].

Dostarlimab

Assay findings association

Blood Tumor Mutational Burden
18 Muts/Mb

AREAS OF THERAPEUTIC USE

Dostarlimab is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, reducing inhibition of the antitumor response. It is FDA approved to treat patients with mismatch repair deficient recurrent or advanced endometrial cancer or solid tumors. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical data across solid tumors[5], blood tumor mutational burden (bTMB) ≥16 Muts/Mb (based on this assay) may predict sensitivity to immune checkpoint inhibitors targeting PD-1.

SUPPORTING DATA

In the Phase 1 GARNET trial of dostarlimab as a single agent for patients with mismatch repair-deficient tumors, 1 patient with a gallbladder tumor and 1 patient with a biliary neoplasm each exhibited a CR[365]. Dostarlimab has been studied primarily in recurrent and advanced mismatch repair-deficient (dMMR) endometrial and non-endometrial cancers[365-367] . In the Phase 1 GARNET trial, single-agent dostarlimab elicited an ORR of 39% (41/106) and an immune-related ORR of 46% (50/110) for patients with non-endometrial dMMR solid tumors[365,368] .

Pembrolizumab

Assay findings association

Blood Tumor Mutational Burden
18 Muts/Mb

AREAS OF THERAPEUTIC USE

Pembrolizumab is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with the ligands PD-L1 and PD-L2 to enhance antitumor immune responses. It is FDA approved for patients with tumor mutational burden (TMB)-high (≥10 Muts/Mb), microsatellite instability-high (MSI-H), or mismatch repair-deficient (dMMR) solid tumors; as monotherapy for PD-L1-positive non-small cell lung cancer (NSCLC), head and neck squamous cell cancer (HNSCC), cervical cancer, or esophageal cancer; and in combination with chemotherapy for PD-L1-positive triple-negative breast cancer (TNBC) or cervical cancer. It is also approved in various treatment settings as monotherapy for patients with melanoma, HNSCC, urothelial carcinoma, hepatocellular carcinoma, Merkel cell carcinoma, cutaneous squamous cell carcinoma, endometrial carcinoma that is MSI-H or dMMR, classical Hodgkin lymphoma, or primary mediastinal large B-cell lymphoma; and in combination with chemotherapy or targeted therapy for NSCLC, HNSCC, esophageal or gastroesophageal junction cancer, renal cell carcinoma, TNBC, or endometrial carcinoma that is not MSI-H or dMMR. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical data across solid tumors[5], blood tumor mutational burden (bTMB) ≥16 Muts/Mb (based on this assay) may predict sensitivity to immune checkpoint inhibitors targeting PD-1.

SUPPORTING DATA

A Phase 2 study of single-agent pembrolizumab reported a 5.8% (6/104) ORR for patients with advanced biliary tract cancer; a Phase 1b study for patients with PD-L1-positive advanced biliary tract cancer reported an ORR of 13% (3/23)[369]. Clinical benefit has been observed from pembrolizumab in combination with other therapies. The combination of pembrolizumab or nivolumab with the antiangiogenic multikinase inhibitor lenvatinib achieved an ORR of 21.4% (3/14) and median PFS (mPFS) of 5 months for patients with intrahepatic cholangiocarcinoma[370], as well as an ORR of 10% (3/31) and mPFS of 6.1 months for patients with advanced biliary cancers[371]. Combination of pembrolizumab with granulocyte-macrophage colony-stimulating factor (GM-CSF) achieved an ORR of 19% (5/27) for patients with advanced biliary cancer; a longer mPFS was observed for patients with intermediate or high tumor mutational burden (TMB) compared with low TMB (2.1 vs. 12.8 months, p=0.012)[372]. Pembrolizumab combined with the VEGFR2-targeting antibody ramucirumab elicited an ORR of 4.2% (1/24) for patients with advanced biliary tract carcinoma; an improved median OS (11.3 vs. 6.1 months), but not mPFS (1.5 vs. 1.6 months), was reported for patients with PD-L1-positive tumors compared with those with PD-L1-negative tumors (combined positive score [CPS] ≥1%)[373].

Cemiplimab

Assay findings association

Blood Tumor Mutational Burden
18 Muts/Mb

AREAS OF THERAPEUTIC USE

Cemiplimab is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with the ligands PD-L1 and PD-L2 to enhance antitumor immune responses. It is FDA approved to treat patients with NSCLC with high PD-L1 expression (TPS ≥ 50%), cutaneous squamous cell carcinoma (CSCC), or basal cell carcinoma (BCC). Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical data across solid tumors[5], blood tumor mutational burden (bTMB) ≥16 Muts/Mb (based on this assay) may predict sensitivity to immune checkpoint inhibitors targeting PD-1.

SUPPORTING DATA

Clinical data on the efficacy of cemiplimab for the treatment of biliary tract carcinoma are limited (PubMed, Jun 2022). Cemiplimab has been studied primarily in advanced cutaneous squamous cell carcinoma (CSCC), where it elicited a combined ORR of 48% (41/85) in Phase 1 and 2 studies[374]. A Phase 2 trial of cemiplimab in patients with basal cell carcinoma (BCC) reported ORRs of 31% (5 CRs and 21 PRs) in patients with locally advanced BCC and 21% (6 PRs) in patients with metastatic BCC[375-376] . The Phase 3 EMPOWER-Lung 1 trial for advanced non-small cell lung cancer (NSCLC) with PD-L1 expression ≥50% reported that cemiplimab is associated with improved PFS (8.2 vs. 5.7 months), OS (not reached vs. 14.2 months), and ORR (37% vs. 21%) compared with chemotherapy[377].

Nivolumab

Assay findings association

Blood Tumor Mutational Burden
18 Muts/Mb

AREAS OF THERAPEUTIC USE

Nivolumab is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, reducing inhibition of the antitumor immune response. It is FDA approved as a monotherapy in various treatment settings for patients with melanoma, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), urothelial carcinoma, colorectal cancer (CRC), classical Hodgkin lymphoma (cHL), gastric cancer, gastroesophageal junction cancer, or esophageal adenocarcinoma or squamous cell carcinoma (ESCC). It is also approved in combination with chemotherapy to treat ESCC, in combination with cabozantinib to treat RCC, and in combination with relatlimab to treat melanoma. Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical data across solid tumors[5], blood tumor mutational burden (bTMB) ≥16 Muts/Mb (based on this assay) may predict sensitivity to immune checkpoint inhibitors targeting PD-1.

SUPPORTING DATA

In the Phase 2 CheckMate 848 multi-solid-tumor trial, treatment with the PD-1 inhibitor nivolumab led to improved ORR for patients with a blood tumor mutational burden (bTMB) of 16 Muts/Mb or higher (based on this assay) compared with those with bTMB of 10 Muts/Mb or higher but <16 Muts/MB (22% [5/23] vs. 9.1% [2/22])[5]. A Phase 2 study of nivolumab for patients with advanced biliary tract cancers reported an ORR of 10.9% (5/46) and a DCR of 58.7% (27/46) by independent central review; median PFS and OS were 3.7 and 14.2 months, respectively[378]. This study reported a significant association between PD-L1 tumor cell expression >1% and improved median PFS (10.4 vs. 2.3 months, HR=0.23)[378]. A case report described an ongoing, 1+ year response to nivolumab in a patient with intrahepatic cholangiocarcinoma and a high TMB[379].

Nivolumab + Ipilimumab

Assay findings association

Blood Tumor Mutational Burden
18 Muts/Mb

AREAS OF THERAPEUTIC USE

Nivolumab is a monoclonal antibody that binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, reducing inhibition of the antitumor immune response, and ipilimumab is a cytotoxic T-lymphocyte antigen 4 (CTLA-4)-blocking antibody. The combination is FDA approved in various treatment settings for patients with melanoma, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), hepatocellular carcinoma (HCC), pleural mesothelioma, and esophageal squamous cell carcinoma (ESCC). Furthermore, nivolumab is approved in combination with ipilimumab to treat patients with mismatch repair-deficient (dMMR) or microsatellite instability-high (MSI-H) colorectal cancer (CRC). Please see the drug label for full prescribing information.

GENE ASSOCIATION

On the basis of clinical data across solid tumors[5], blood tumor mutational burden (bTMB) of ≥16 Muts/Mb (based on this assay) may predict sensitivity to combination nivolumab and ipilimumab treatment.

SUPPORTING DATA

In the Phase 2 CheckMate 848 multi-solid tumor trial, combination treatment with nivolumab and ipilimumab led to improved ORR for patients with blood tumor mutational burden (bTMB) ≥ 16 Muts/Mb (based on this assay) compared with those with bTMB ≥10 Muts/Mb but <16 Muts/Mb (34% [13/38] vs 12% [5/42])[5]. A Phase 2 study evaluating nivolumab combined with ipilimumab for patients with biliary cancers, including intrahepatic cholangiocarcinoma and gallbladder carcinoma, reported ORR of 23% (9/39), median PFS of 2.9 months, and median OS of 5.7 months[380].

NOTE Genomic alterations detected may be associated with activity of certain US FDA or other specific country approved therapies; however, the therapies listed in this report may have varied evidence in the patient's tumor type. The listed therapies are not ranked in order of potential or predicted efficacy for this patient or in order of level of evidence for this patient's tumor type. The therapies listed in this report may not be complete and/or exhaustive. Furthermore, the listed therapies are limited to US FDA approved pharmaceutical drug products that are linked to a specific genomic alteration. There may also be US FDA approved pharmaceutical drug products that are not linked to a genomic alteration. Further there may also exist pharmaceutical drug products that are not approved by the US FDA or other national authorities. There may also be other treatment modalities available than pharmaceutical drug products.

IMPORTANT Clinical trials are ordered by gene and prioritized by: age range inclusion criteria for pediatric patients, proximity to ordering medical facility, later trial phase, and verification of trial information within the last two months. While every effort is made to ensure the accuracy of the information contained below, the information available in the public domain is continually updated and should be investigated by the physician or research staff. This is not a comprehensive list of all available clinical trials. There may also be compassionate use or early access programs available, which are not listed in this report. Foundation Medicine displays a subset of trial options and ranks them in this order of descending priority: Qualification for pediatric trial → Geographical proximity → Later trial phase. Clinical trials are not ranked in order of potential or predicted efficacy for this patient or in order of level of evidence for this patient's tumor type. Clinical trials listed here may have additional enrollment criteria that may require medical screening to determine final eligibility. For additional information about listed clinical trials or to conduct a search for additional trials, please see clinicaltrials.gov. However, clinicaltrials.gov does not list all clinical trials that might be available.

BIOMARKER

Blood Tumor Mutational Burden

RESULT

18 Muts/Mb

RATIONALE

Increased tumor mutational burden may predict response to anti-PD-1 (alone or in combination with anti-CTLA-4) or anti-PD-L1 immune checkpoint inhibitors.

NCT04237649

PHASE NULL

KAZ954 Alone and With PDR001, NZV930 and NIR178 in Advanced Solid Tumors

TARGETS
ADORA2A, CD73, PD-1

LOCATIONS: Taipei (Taiwan), Shatin, New Territories (Hong Kong), Sunto Gun (Japan), Singapore (Singapore), Milano (Italy), Barcelona (Spain), California, Illinois, Toronto (Canada), Missouri

NCT05007106

PHASE 2

MK-7684A With or Without Other Anticancer Therapies in Participants With Selected Solid Tumors (MK-7684A-005)

TARGETS
PD-1, KIT, VEGFRs, FGFRs, PDGFRA, RET, TIGIT

LOCATIONS: Taipei (Taiwan), Taoyuan (Taiwan), Tainan (Taiwan), Seoul (Korea, Republic of), Tokyo (Japan), Kashiwa (Japan), Alaska, Adana (Turkey), Ankara (Turkey), Ramat Gan (Israel)

NCT04152018

PHASE 1

Study of PF-06940434 in Patients With Advanced or Metastatic Solid Tumors.

TARGETS
PD-1

LOCATIONS: Taipei (Taiwan), Tainan (Taiwan), Seoul (Korea, Republic of), Wollongong (Australia), Washington, Arizona, Missouri, Texas

NCT03396445

PHASE 1

Safety and Pharmacokinetics Study of MK-5890 as Monotherapy and in Combination With Pembrolizumab (MK-3475) in Adults With Advanced Solid Tumors (MK-5890-001)

TARGETS
PD-1, CD27

LOCATIONS: Taipei (Taiwan), Seoul (Korea, Republic of), Be'er Sheva (Israel), Amsterdam (Netherlands), Rotterdam (Netherlands), Barcelona (Spain), Madrid (Spain), Pozuelo de Alarcon (Spain), Santiago (Chile)

NCT03530397

PHASE 1

A Study to Evaluate MEDI5752 in Subjects With Advanced Solid Tumors

TARGETS
PD-L1, PD-1, CTLA-4

LOCATIONS: Taipei (Taiwan), Taichung (Taiwan), Tainan (Taiwan), Cheongju-si (Korea, Republic of), Incheon (Korea, Republic of), Seoul (Korea, Republic of), Gyeonggi-do (Korea, Republic of), Melbourne (Australia), Amsterdam (Netherlands), Ravenna (Italy)

NCT02628067

PHASE 2

Study of Pembrolizumab (MK-3475) in Participants With Advanced Solid Tumors (MK-3475-158/ KEYNOTE-158)

TARGETS
PD-1

LOCATIONS: Taipei (Taiwan), Makati (Philippines), Seoul (Korea, Republic of), Beijing (China), North Ryde (Australia), Moscow (Russian Federation), Hod Hasharon (Israel), Drammen (Norway), Glostrup (Denmark), Haar (Germany)

NCT03861793

PHASE 1/2

A Dose Escalation and Cohort Expansion Study of Subcutaneously-Administered Cytokine (ALKS 4230) as a Single Agent and in Combination With Anti-PD-1 Antibody (Pembrolizumab) in Subjects With Select Advanced or Metastatic Solid Tumors (ARTISTRY-2)

TARGETS
PD-1

LOCATIONS: Taipei (Taiwan), Tainan (Taiwan), Suwon (Korea, Republic of), Incheon (Korea, Republic of), Seoul (Korea, Republic of), Edmonton (Canada), Badalona (Spain), Rotterdam (Netherlands), Valencia (Spain), Madrid (Spain)

NCT04047862

PHASE 1

Study of BGB-A1217 in Combination With Tislelizumab in Advanced Solid Tumors

TARGETS
PD-1, TIGIT

LOCATIONS: Taipei (Taiwan), Taoyuan (Taiwan), Hualien City (Taiwan), Taichung (Taiwan), Fujian (China), Hangzhou (China), Shanghai (China), Guangdong (China), Changsha (China), Wuhan (China)

NCT04261439

PHASE 1

A Phase I/Ib Study of NIZ985 Alone and in Combination With Spartalizumab

TARGETS
PD-1

LOCATIONS: Taipei (Taiwan), Chuo ku (Japan), Essen (Germany), Napoli (Italy), Leuven (Belgium), Barcelona (Spain), California, Texas

NCT04892498

PHASE 2

Hypofractionated Radiotherapy Combined With PD-1 Inhibitor Sequential GM-CSF and IL-2 for the Treatment of Advanced Refractory Solid Tumors (PRaG2.0)

TARGETS
PD-1

LOCATIONS: Hangzhou (China), Suzhou (China), Wuxi (China), Hefei (China), Xuzhou (China)

GENE
CCNE1

ALTERATION
amplification

RATIONALE
Strong preclinical and clinical data suggest that CCNE1 amplification may predict sensitivity to WEE1 inhibitors.

NCT04768868

PHASE 1

The Safety and Pharmacokinetics Preliminary Efficacy of IMP7068 in Patients With Advanced Solid Tumors

TARGETS
WEE1

LOCATIONS: Taipei (Taiwan), Taoyuan (Taiwan), Taichung (Taiwan), Tainan (Taiwan), Shanghai (China), Wuhan (China), Beijing (China), Chengdu (China), Kansas, Texas

NCT05128825

PHASE 2

A Study of ZN-c3 in Subjects With Malignant Tumors

TARGETS
WEE1

LOCATIONS: Nevada, Colorado, Texas, Ohio, Pennsylvania, Maryland, Virginia

NCT03968653

PHASE 1

Study of Oral Debio 0123 in Combination With Carboplatin in Participants With Advanced Solid Tumors

TARGETS
WEE1

LOCATIONS: Groningen (Netherlands), Nijmegen (Netherlands), Leiden (Netherlands), Barcelona (Spain)

NCT05109975

PHASE 1

A Study to Evaluate Safety and Preliminary Anti-tumor Activity of Debio 0123 as Monotherapy in Adult Participants With Advanced Solid Tumors

TARGETS
WEE1

LOCATIONS: Bellinzona (Switzerland), Zürich (Switzerland), Michigan, Texas

GENE
FGFR1

RATIONALE
FGFR inhibitors may be relevant in tumors with alterations that activate FGFR1.

ALTERATION
amplification - equivocal

NCT05024214

PHASE 1/2

Phase Ib/II Trial of Envafolimab Plus Lenvatinib for Subjects With Solid Tumors

TARGETS
PD-L1, FGFRs, RET, PDGFRA, VEGFRs, KIT, FLT3, CSF1R

LOCATIONS: Hangzhou (China), Shanghai (China), Dongguan (China), Guangzhou (China), Zhuhai (China), Benbu (China), Zhengzhou (China), Jinan (China), Dalian (China), Tianjin (China)

NCT05254847

PHASE 2

Capecitabine Combined With Lenvatinib and Tislelizumab as Adjuvant Treatment After Resection in Patients With BTC

TARGETS
FGFRs, RET, PDGFRA, VEGFRs, KIT, PD-1

LOCATIONS: Shanghai (China)

NCT05156788

PHASE 2

Tislelizumab◈Anti PD-1), Lenvatinib and GEMOX Transformation in the Treatment of Potentially Resectable, Locally Advanced Biliary Tract Cancer

TARGETS
FGFRs, RET, PDGFRA, VEGFRs, KIT, PD-1

LOCATIONS: Shanghai (China)

NCT05010668

PHASE 2

Cryoablation Combined With Sintilimab Plus Lenvatinib in Patients With Advanced Intrahepatic Cholangiocarcinoma

TARGETS
FGFRs, RET, PDGFRA, VEGFRs, KIT, PD-1

LOCATIONS: Shanghai (China)

NCT04169672

PHASE 2

Study of Surufatinib Combined With Toripalimab in Patients With Advanced Solid Tumors

TARGETS
FGFR1, CSF1R, VEGFRs, PD-1

LOCATIONS: Shanghai (China), Beijing (China)

NCT05010681

PHASE 2

Lenvatinib Plus Sintilimab in Patients With Immune Checkpoint Inhibitor Previously Treated Advanced Liver Cancer

TARGETS
PD-1, FGFRs, RET, PDGFRA, VEGFRs, KIT

LOCATIONS: Shanghai (China)

NCT05098847

PHASE 2

Cryoablation Combined With Sintilimab Plus Lenvatinib In Previously Treated Unresectable Liver Metastasis From Solid Tumors

TARGETS
FGFRs, RET, PDGFRA, VEGFRs, KIT, PD-1

LOCATIONS: Shanghai (China)

NCT04550624

PHASE 2

Pembrolizumab in Combination With Lenvatinib in Patients With Advanced Cholangiocarcinoma

TARGETS
PD-1, KIT, VEGFRs, FGFRs, PDGFRA, RET

LOCATIONS: Shanghai (China)

NCT05215665

PHASE NULL

GEMOX Combined With Targeted Therapy and Immunotherapy for Patients With Advanced Cholangiocarcinoma

TARGETS
FGFRs, RET, PDGFRA, VEGFRs, KIT, PD-1

LOCATIONS: Tianjin (China)

NCT04976634

PHASE 2

Pembrolizumab Plus Lenvatinib in Combination With Belzutifan in Solid Tumors (MK-6482-016)

TARGETS
HIF2a, PD-1, KIT, VEGFRs, FGFRs, PDGFRA, RET

LOCATIONS: Seoul (Korea, Republic of), Gosford (Australia), Westmead (Australia), Epping (Australia), Malvern (Australia), Haifa (Israel), Jerusalem (Israel), Ramat Gan (Israel), Tel Aviv (Israel), Utrecht (Netherlands)

GENE
MDM2

ALTERATION
amplification

RATIONALE
Inhibitors of the MDM2-p53 interaction are being tested in clinical trials. Overexpression or amplification of MDM2 may increase sensitivity to these agents, but more data are required.

NCT04785196

PHASE 1/2

APG-115 in Combination With PD-1 Inhibitor in Patients With Advanced Liposarcoma or Advanced Solid Tumors

TARGETS
PD-1, MDM2

LOCATIONS: Shanghai (China), Guangzhou (China)

NCT03611868

PHASE 1/2

A Study of APG-115 in Combination With Pembrolizumab in Patients With Metastatic Melanomas or Advanced Solid Tumors

TARGETS
MDM2, PD-1

LOCATIONS: Brisbane (Australia), South Brisbane (Australia), Bedford Park (Australia), Heidelberg (Australia), California, Arizona, Missouri, Arkansas, Ohio, Pennsylvania

GENE
PTEN

ALTERATION
splice site 165-1G>C

RATIONALE
PTEN loss or inactivating mutations may lead to increased activation of the PI3K-AKT-mTOR pathway and may indicate sensitivity to inhibitors of this pathway. PTEN loss or inactivation may also predict sensitivity to PARP inhibitors.

NCT04644068

PHASE 1/2

Study of AZD5305 as Monotherapy and in Combination With Anti-cancer Agents in Patients With Advanced Solid Malignancies

TARGETS
ERBB2, TROP2, PARP

LOCATIONS: Shanghai (China), Seoul (Korea, Republic of), Chongqing (China), Chuo-ku (Japan), Koto-ku (Japan), Melbourne (Australia), Warszawa (Poland), Gdynia (Poland), Grzepnica (Poland), Budapest (Hungary)

NCT04341259

PHASE 1

A Study Of The Pharmacokinetics And Safety Of Ipatasertib In Chinese Participants With Locally Advanced Or Metastatic Solid Tumors.

TARGETS
AKTs

LOCATIONS: Shanghai City (China)

NCT04337463

PHASE NULL

ATG-008 Combined With Toripalimab in Advanced Solid Tumors

TARGETS
mTORC1, mTORC2, PD-1

LOCATIONS: Chongqing (China), Chengdu (China)

NCT02264678

PHASE 1/2

Ascending Doses of AZD6738 in Combination With Chemotherapy and/or Novel Anti Cancer Agents

TARGETS
ATR, PARP, PD-L1

LOCATIONS: Seongnam-si (Korea, Republic of), Seoul (Korea, Republic of), Goyang-si (Korea, Republic of), Cambridge (United Kingdom), Withington (United Kingdom), Manchester (United Kingdom), London (United Kingdom), Coventry (United Kingdom), Sutton (United Kingdom), Oxford (United Kingdom)

NCT04001569

PHASE 1/2

AZD8186 and Paclitaxel in Advanced Gastric Cancer

TARGETS
PI3K-beta

LOCATIONS: Seongnam-si (Korea, Republic of)

NCT04298021

PHASE 2

DDR-Umbrella Study of DDR Targeting Agents in Advanced Biliary Tract Cancer

TARGETS
PD-L1, ATR, PARP

LOCATIONS: Seoul (Korea, Republic of)

NCT05035745

PHASE 1/2

Selinexor & Talazoparib in Advanced Refractory Solid Tumors; Advanced/Metastatic Triple Negative Breast Cancer (START)

TARGETS
XPO1, PARP

LOCATIONS: Singapore (Singapore)

NCT03772561

PHASE 1

Phase I Study of AZD5363 + Olaparib + Durvalumab in Patients With Advanced or Metastatic Solid Tumor Malignancies

TARGETS
PARP, AKTs, PD-L1

LOCATIONS: Singapore (Singapore)

NCT04801966

PHASE NULL

Safety and Oversight of the Individually Tailored Treatment Approach: A Novel Pilot Study

TARGETS
CDK4, CDK6, PI3K-alpha, PD-L1, MEK, PARP, PD-1, BRAF

LOCATIONS: Melbourne (Australia)

NCT04497116

PHASE 1/2

Study of RP-3500 in Advanced Solid Tumors

TARGETS
ATR, PARP

LOCATIONS: Copenhagen (Denmark), Newcastle Upon Tyne (United Kingdom), Manchester (United Kingdom), London (United Kingdom), Illinois, Toronto (Canada), Massachusetts, Rhode Island, New York, Tennessee

GENE
STK11

ALTERATION
F157fs*5

RATIONALE
Increased mTOR signaling is present in LKB1-deficient tumors, suggesting therapies targeting mTOR may be relevant for tumors with STK11 alterations.

NCT04337463

PHASE NULL

ATG-008 Combined With Toripalimab in Advanced Solid Tumors

TARGETS
mTORC1, mTORC2, PD-1

LOCATIONS: Chongqing (China), Chengdu (China)

NOTE One or more variants of unknown significance (VUS) were detected in this patient's tumor. These variants may not have been adequately characterized in the scientific literature at the time this report was issued, and/or the genomic context of these alterations makes their significance unclear. We choose to include them here in the event that they become clinically meaningful in the future.

ALOX12B
C341R

ATR
I1145T and L196F

CUL4A
E277K

ERBB4
P98L

ERRFI1
V359D

FGFR4
L546P

GRM3
E55K

MLL2
G872R

MST1R
D172N

PTPRO
E722K

RARA
Y2F

SMAD4
H92Y and P320L

ORDERED TEST # ORD-1461672-01

APPENDIX Genes assayed in FoundationOne®Liquid CDx

FoundationOne Liquid CDx interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage (indicated with an *) ; 75 genes (indicated in bold) are captured with increased sensitivity and have complete exonic (coding) coverage unless otherwise noted.

ABL1 **Exons 4-9**	*ACVR1B*	***AKT1*** **Exon 3**	*AKT2*	*AKT3*	***ALK*** **Exons 20-29, Introns 18, 19**	*ALOX12B*	*AMER1* (FAM123B or WTX)	***APC***
AR	***ARAF*** **Exons 4, 5, 7, 11, 13, 15, 16**	*ARFRP1*	*ARID1A*	*ASXL1*	***ATM***	***ATR***	*ATRX*	*AURKA*
AURKB	*AXIN1*	*AXL*	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*
BCOR	*BCORL1*	*BCR** Introns 8, 13, 14	***BRAF*** **Exons 11-18,** Introns 7-10	***BRCA1*** Introns 2, 7, 8, 12, 16, 19, 20	***BRCA2*** Intron 2	*BRD4*	*BRIP1*	*BTG1*
BTG2	***BTK*** **Exons 2, 15**	*CALR*	*CARD11*	*CASP8*	*CBFB*	*CBL*	***CCND1***	*CCND2*
CCND3	*CCNE1*	*CD22*	*CD70*	*CD74** Introns 6-8	*CD79A*	*CD79B*	***CD274*** **(PD-L1)**	*CDC73*
CDH1	***CDK12***	***CDK4***	***CDK6***	*CDK8*	*CDKN1A*	*CDKN1B*	***CDKN2A***	*CDKN2B*
CDKN2C	*CEBPA*	*CHEK1*	***CHEK2***	*CIC*	*CREBBP*	***CRKL***	*CSF1R*	*CSF3R*
CTCF	*CTNNA1*	***CTNNB1*** **Exon 3**	*CUL3*	*CUL4A*	*CXCR4*	*CYP17A1*	*DAXX*	*DDR1*
DDR2 **Exons 5, 17, 18**	*DIS3*	*DNMT3A*	*DOT1L*	*EED*	***EGFR*** **Introns 7,** 15, **24-27**	*EMSY* (C11orf30)	*EP300*	*EPHA3*
EPHB1	*EPHB4*	***ERBB2***	***ERBB3*** **Exons 3, 6, 7, 8, 10, 12, 20, 21, 23, 24, 25**	*ERBB4*	*ERCC4*	*ERG*	***ERRFI1***	***ESR1*** **Exons 4-8**
*ETV4** Intron 8	*ETV5** Introns 6, 7	***ETV6**** **Introns 5, 6**	*EWSR1** Introns 7-13	***EZH2*** **Exons 4, 16, 17, 18**	*EZR** Introns 9-11	*FANCA*	*FANCC*	*FANCG*
FANCL	*FAS*	*FBXW7*	*FGF10*	*FGF12*	*FGF14*	*FGF19*	*FGF23*	*FGF3*
FGF4	*FGF6*	***FGFR1*** Introns 1, 5, **Intron 17**	***FGFR2*** Intron 1, **Intron 17**	***FGFR3*** **Exons 7, 9 (alternative designation exon 10), 14, 18, Intron 17**	*FGFR4*	*FH*	*FLCN*	*FLT1*
FLT3 **Exons 14, 15, 20**	***FOXL2***	*FUBP1*	*GABRA6*	*GATA3*	*GATA4*	*GATA6*	*GID4* (C17orf39)	***GNA11*** **Exons 4, 5**
GNA13	***GNAQ*** **Exons 4, 5**	***GNAS*** **Exons 1, 8**	*GRM3*	*GSK3B*	*H3-3A* (H3F3A)	*HDAC1*	*HGF*	*HNF1A*
HRAS **Exons 2, 3**	*HSD3B1*	*ID3*	***IDH1*** **Exon 4**	***IDH2*** **Exon 4**	*IGF1R*	*IKBKE*	*IKZF1*	*INPP4B*
IRF2	*IRF4*	*IRS2*	*JAK1*	***JAK2*** **Exon 14**	***JAK3*** **Exons 5, 11, 12, 13, 15, 16**	*JUN*	*KDM5A*	*KDM5C*
KDM6A	*KDR*	*KEAP1*	*KEL*	***KIT*** **Exons 8, 9, 11, 12, 13, 17,** Intron 16	*KLHL6*	*KMT2A* (MLL) Introns 6, 8-11, Intron 7	*KMT2D* (MLL2)	***KRAS***

ORDERED TEST # ORD-1461672-01

APPENDIX Genes assayed in FoundationOne®Liquid CDx

FoundationOne Liquid CDx interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage (indicated with an *) ; 75 genes (indicated in bold) are captured with increased sensitivity and have complete exonic (coding) coverage unless otherwise noted.

LTK	*LYN*	*MAF*	***MAP2K1*** **(MEK1) Exons 2, 3**	***MAP2K2*** **(MEK2) Exons 2-4, 6, 7**	*MAP2K4*	*MAP3K1*	*MAP3K13*	*MAPK1*
MCL1	***MDM2***	*MDM4*	*MED12*	*MEF2B*	*MEN1*	*MERTK*	***MET***	*MITF*
MKNK1	*MLH1*	***MPL*** **Exon 10**	*MRE11* (MRE11A)	*MSH2* Intron 5	*MSH3*	*MSH6*	*MST1R*	*MTAP*
MTOR **Exons 19, 30, 39, 40, 43-45, 47, 48, 53, 56**	*MUTYH*	*MYB** Intron 14	***MYC*** **Intron 1**	*MYCL* (MYCL1)	***MYCN***	***MYD88*** **Exon 4**	*NBN*	***NF1***
NF2	*NFE2L2*	*NFKBIA*	*NKX2-1*	*NOTCH1*	*NOTCH2* Intron 26	*NOTCH3*	***NPM1*** **Exons 4-6, 8, 10**	***NRAS*** **Exons 2, 3**
NSD2 (WHSC1 or MMSET)	*NSD3* (WHSC1L1)	*NT5C2*	***NTRK1*** **Exons 14, 15, Introns 8-11**	*NTRK2* Intron 12	***NTRK3*** **Exons 16, 17**	*NUTM1** Intron 1	*P2RY8*	***PALB2***
PARP1	*PARP2*	*PARP3*	*PAX5*	*PBRM1*	*PDCD1* (PD-1)	***PDCD1LG2*** **(PD-L2)**	***PDGFRA*** **Exons 12, 18, Introns 7, 9, 11**	***PDGFRB*** **Exons 12-21, 23**
PDK1	*PIK3C2B*	*PIK3C2G*	***PIK3CA*** **Exons 2, 3, 5-8, 10, 14, 19, 21 (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)**	*PIK3CB*	*PIK3R1*	*PIM1*	*PMS2*	*POLD1*
POLE	*PPARG*	*PPP2R1A*	*PPP2R2A*	*PRDM1*	*PRKAR1A*	*PRKCI*	*PRKN* (PARK2)	*PTCH1*
PTEN	***PTPN11***	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAD51B*	*RAD51C*
RAD51D	*RAD52*	*RAD54L*	***RAF1*** **Exons 3, 4, 6, 7, 10, 14, 15, 17,** Introns 4-8	*RARA* Intron 2	***RB1***	*RBM10*	*REL*	***RET*** Introns 7, 8, **Exons 11, 13-16, Introns 9-11**
RICTOR	*RNF43*	***ROS1*** **Exons 31, 36-38, 40, Introns 31-35**	*RPTOR*	*RSPO2** Intron 1	*SDC4** Intron 2	*SDHA*	*SDHB*	*SDHC*
SDHD	*SETD2*	*SF3B1*	*SGK1*	*SLC34A2** Intron 4	*SMAD2*	*SMAD4*	*SMARCA4*	*SMARCB1*
SMO	*SNCAIP*	*SOCS1*	*SOX2*	*SOX9*	*SPEN*	*SPOP*	*SRC*	*STAG2*
STAT3	***STK11***	*SUFU*	*SYK*	*TBX3*	*TEK*	*TENT5C* (FAM46C)	*TERC** ncRNA	***TERT**** **Promoter**
TET2	*TGFBR2*	*TIPARP*	*TMPRSS2** Introns 1-3	*TNFAIP3*	*TNFRSF14*	***TP53***	*TSC1*	*TSC2*
TYRO3	*U2AF1*	***VEGFA***	*VHL*	*WT1*	*XPO1*	*XRCC2*	*ZNF217*	*ZNF703*

ADDITIONAL ASSAYS: FOR THE DETECTION OF SELECT CANCER BIOMARKERS

Microsatellite (MS) status
Blood Tumor Mutational Burden (bTMB)
Tumor Fraction

Electronically signed by Douglas A. Mata, MD, MPH | 04 October 2022
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Nimesh R. Patel, M.D., Laboratory Director CLIA: 34D2044309
Foundation Medicine, Inc. | 1.888.988.3639

Sample Preparation: 150 Second St., 1st Floor, Cambridge, MA 02141 · CLIA: 22D2027531
Sample Analysis: 150 Second St., 1st Floor, Cambridge, MA 02141 · CLIA: 22D2027531
Post-Sequencing Analysis: 150 Second St., 1st Floor. Cambridge, MA 02141 · CLIA: 22D2027531

FoundationOne Liquid CDx fulfills the requirements of the European Directive 98/79 EC for in vitro diagnostic medical devices and is registered as a CE-IVD product by Foundation Medicine's EU Authorized Representative, Qarad b.v.b.a, Cipalstraat 3, 2440 Geel, Belgium. The CE-IVD regulatory status of FoundationOne Liquid CDx is applicable in countries that accept and/or recognize the CE mark.

CE IVD

ABOUT FOUNDATIONONE LIQUID CDx

FoundationOne Liquid CDx was developed and its performance characteristics determined by Foundation Medicine, Inc. (Foundation Medicine). FoundationOne Liquid CDx may be used for clinical purposes and should not be regarded as purely investigational or for research only. Foundation Medicine's clinical reference laboratories are qualified to perform high-complexity clinical testing.

Please refer to technical information for performance specification details.

INTENDED USE

FoundationOne Liquid CDx is a next generation sequencing based *in vitro* diagnostic device that analyzes 324 genes. Substitutions and insertion and deletion alterations (indels) are reported in 311 genes, copy number alterations (CNAs) are reported in 310 genes, and gene rearrangements are reported in 324 genes. The test also detects the genomic signatures blood tumor mutational burden (bTMB), microsatellite instability (MSI), and tumor fraction. FoundationOne Liquid CDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from the anti-coagulated peripheral whole blood of cancer patients. The test is intended to be used as a companion diagnostic to identify patients who may benefit from treatment with targeted therapies in accordance with the approved therapeutic product labeling. Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with malignant neoplasms.

TEST PRINCIPLES

The FoundationOne Liquid CDx assay is performed exclusively as a laboratory service using circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood from patients with solid malignant neoplasms. The assay employs a single DNA extraction method to obtain cfDNA from plasma from whole blood. Extracted cfDNA undergoes whole-genome shotgun library construction and hybridization-based capture of 324 cancer-related genes including coding exons and select introns of 309 genes, as well as only select intronic regions or non-coding regions of 15 genes. Hybrid-capture selected libraries are sequenced with deep coverage using the NovaSeq® 6000 platform. Sequence data are processed using a customized analysis pipeline designed to accurately detect genomic alterations, including base substitutions, indels, select copy number variants, and select genomic rearrangements. Substitutions and insertion and deletion alterations (indels) are reported in 311 genes, copy number alterations (CNAs) are reported in 310 genes, and gene rearrangements are reported in 324 genes. The assay also reports tumor fraction, and genomic signatures including MSI and bTMB. A subset of targeted regions in 75 genes is baited for increased sensitivity.

THE REPORT

Incorporates analyses of peer-reviewed studies and other publicly available information identified by Foundation Medicine; these analyses and information may include associations between a molecular alteration (or lack of alteration) and one or more drugs with potential clinical benefit (or potential lack of clinical benefit), including drug candidates that are being studied in clinical research. *Note:* A finding of biomarker alteration does not necessarily indicate pharmacologic effectiveness (or lack thereof) of any drug or treatment regimen; a finding of no biomarker alteration does not necessarily indicate lack of pharmacologic effectiveness (or effectiveness) of any drug or treatment regimen.

QUALIFIED ALTERATION CALLS (EQUIVOCAL)

All equivocal calls, regardless of alteration type, imply that there is adequate evidence to call the alteration with confidence. However, the repeatability of equivocal calls may be lower than non-equivocal calls.

RANKING OF THERAPIES AND CLINICAL TRIALS

Ranking of Therapies in Summary Table

Therapies are ranked based on the following criteria: Therapies with clinical benefit (ranked alphabetically within each evidence category), followed by therapies associated with resistance (when applicable).

Ranking of Clinical Trials

Pediatric trial qualification → Geographical proximity → Later trial phase.

LIMITATIONS

1. For *in vitro* diagnostic use.
2. For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
3. A negative result does not rule out the presence of a mutation below the limits of detection of the assay. Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an appropriately validated tumor tissue test, if available.
4. The FoundationOne Liquid CDx assay does not detect heterozygous deletions.
5. The test is not intended to provide information on cancer predisposition.
6. Performance has not been validated for cfDNA input below the specified minimum input.
7. Tissue TMB and blood TMB (bTMB) are estimated from the number of synonymous and nonsynonymous single-nucleotide variants (SNVs) and insertions and deletions (indels) per area of coding genome sampled, after the removal of known and likely oncogenic driver events and germline SNPs. Tissue TMB is calculated based on variants with an allele frequency of ≥5%, and bTMB is calculated based on variants with an allele frequency of ≥0.5%.
8. Tumor fraction is the percentage of circulating tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample. The tumor fraction estimate is computationally derived from the observed level of aneuploidy in the sample. Tumor fraction is considered elevated when ctDNA levels are high enough that aneuploidy can be detected and is significantly distinct from that typically found in non-tumor samples.
9. Microsatellite instability (MSI) is a condition of genetic hypermutability that generates excessive amounts of short insertion/deletion mutations in the tumor genome; it generally occurs at microsatellite DNA sequences and is caused by a deficiency in DNA mismatch repair (MMR) in the tumor. The MSI algorithm is based on genome wide analysis of 1765 microsatellite loci and not based on the 5 or 7 MSI loci described in current clinical practice guidelines for solid tissue testing.
10. Genomic findings from circulating cell-free DNA (cfDNA) may originate from circulating tumor DNA fragments, germline alterations, or non-tumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP). Genes with alterations that may be derived from CHIP include, but are not limited to: *ASXL1*, *ATM*, *CBL*, *CHEK2*, *DNMT3A*, *JAK2*,

KMT2D (*MLL2*), *MPL*, *MYD88*, *SF3B1*, *TET2*, *TP53*, and *U2AF1*.

11. Alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. If a reported alteration is suspected to be germline, confirmatory testing should be considered in the appropriate clinical context.
12. The test is not intended to replace germline testing or to provide information about cancer predisposition.

REPORT HIGHLIGHTS

The Report Highlights includes select genomic and therapeutic information with potential impact on patient care and treatment that is specific to the genomics and tumor type of the sample analyzed. This section may highlight information including targeted therapies with potential sensitivity or resistance; evidence-matched clinical trials; and variants with potential diagnostic, prognostic, nontargeted treatment, germline, or clonal hematopoiesis implications. Information included in the Report Highlights is expected to evolve with advances in scientific and clinical research. Findings included in the Report Highlights should be considered in the context of all other information in this report and other relevant patient information. Decisions on patient care and treatment are the responsibility of the treating physician.

VARIANTS TO CONSIDER FOR FOLLOW-UP GERMLINE TESTING

The variants indicated for consideration of follow-up germline testing are 1) limited to reportable short variants with a protein effect listed in the ClinVar genomic database (Landrum et al., 2018; 29165669) as Pathogenic, Pathogenic/Likely Pathogenic, or Likely Pathogenic (by an expert panel or multiple submitters), 2) associated with hereditary cancer-predisposing disorder(s), 3) detected at an allele frequency of >30%, and 4) in select genes reported by the ESMO Precision Medicine Working Group (Mandelker et al., 2019; 31050713) to have a greater than 10% probability of germline origin if identified during tumor sequencing. The selected genes are *ATM, BAP1, BRCA1, BRCA2, BRIP1, CHEK2, FH, FLCN, MLH1, MSH2, MSH6, MUTYH, PALB2, PMS2, POLE, RAD51C, RAD51D, RET, SDHA, SDHB, SDHC, SDHD, TSC2,* and *VHL*, and are not inclusive of all cancer susceptibility genes. The content in this report should not substitute for genetic counseling or follow-up germline testing, which is needed to distinguish whether a finding in this patient's tumor sequencing is germline or somatic. Interpretation should be based on clinical context.

VARIANTS THAT MAY REPRESENT CLONAL HEMATOPOIESIS

Variants that may represent clonal hematopoiesis (CH) are limited to select reportable short variants in defined genes identified in solid tumors only. Variant selection was determined based on gene tumor-suppressor or oncogene status, known role in solid tumors versus hematological malignancies, and literature prevalence. The defined genes are *ASXL1, ATM, CBL, CHEK2, DNMT3A, IDH2, JAK2, KMT2D (MLL2), MPL, MYD88, SF3B1, TET2,* and *U2AF1* and are not inclusive of all CH genes. The content in this report should not substitute for dedicated hematological workup. Comprehensive genomic profiling of solid tumors detects nontumor alterations that are due to CH. Patient-matched peripheral blood mononuclear cell sequencing is required to conclusively determine if this alteration is present in tumor or is secondary to CH. Interpretation should be based on clinical context.

NATIONAL COMPREHENSIVE CANCER NETWORK® (NCCN®) CATEGORIZATION

Biomarker and genomic findings detected may be associated with certain entries within the NCCN Drugs & Biologics Compendium® (NCCN Compendium®) (www.nccn.org). The NCCN Categories of Evidence and Consensus indicated reflect the highest possible category for a given therapy in association with each biomarker or genomic finding. Please note, however, that the accuracy and applicability of these NCCN categories within a report may be impacted by the patient's clinical history, additional biomarker information, age, and/or co-occurring alterations. For additional information on the NCCN categories, please refer to the NCCN Compendium®. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). © National Comprehensive Cancer Network, Inc. 2022. All rights reserved. To view the most recent and complete version of the guidelines, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.

LEVEL OF EVIDENCE NOT PROVIDED

Drugs with potential clinical benefit (or potential lack of clinical benefit) are not evaluated for source or level of published evidence.

NO GUARANTEE OF CLINICAL BENEFIT

This report makes no promises or guarantees that a particular drug will be effective in the treatment of disease in any patient. This report also makes no promises or guarantees that a drug with potential lack of clinical benefit will in fact provide no clinical benefit.

NO GUARANTEE OF REIMBURSEMENT

Foundation Medicine makes no promises or guarantees that a healthcare provider, insurer or other third party payor, whether private or governmental, will reimburse a patient for the cost of FoundationOne Liquid CDx.

TREATMENT DECISIONS ARE THE RESPONSIBILITY OF PHYSICIAN

Drugs referenced in this Report may not be suitable for a particular patient. The selection of any, all or none of the drugs associated with potential clinical benefit (or potential lack of clinical benefit) resides entirely within the discretion of the treating physician. Indeed, the information in this Report must be considered in conjunction with all other relevant information regarding a particular patient, before the patient's treating physician recommends a course of treatment. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community. A treating physician's decisions should not be based on a single test, such as this test or the information contained in this report.

Certain sample of variant characteristics may result in reduced sensitivity. These include: low sample quality, deletions and insertions >40bp, or repetitive/high homology sequences. FoundationOne Liquid CDx is performed using cell-free DNA, and as such germline events may not be reported.

SELECT ABBREVIATIONS

ABBREVIATION	DEFINITION
CR	Complete response
DCR	Disease control rate
DNMT	DNA methyltransferase
HR	Hazard ratio
ITD	Internal tandem duplication
MMR	Mismatch repair
Muts/Mb	Mutations per megabase
NOS	Not otherwise specified
ORR	Objective response rate
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PR	Partial response
SD	Stable disease
TKI	Tyrosine kinase inhibitor

REFERENCE SEQUENCE INFORMATION

Sequence data is mapped to the human genome, Genome Reference Consortium Human Build 37 (GRCh37), also known as hg19.

MR Suite Version (RG) 7.1.0

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