FOUNDATION MEDICINE®

Evidence Dossier
FoundationOne® Liquid CDx

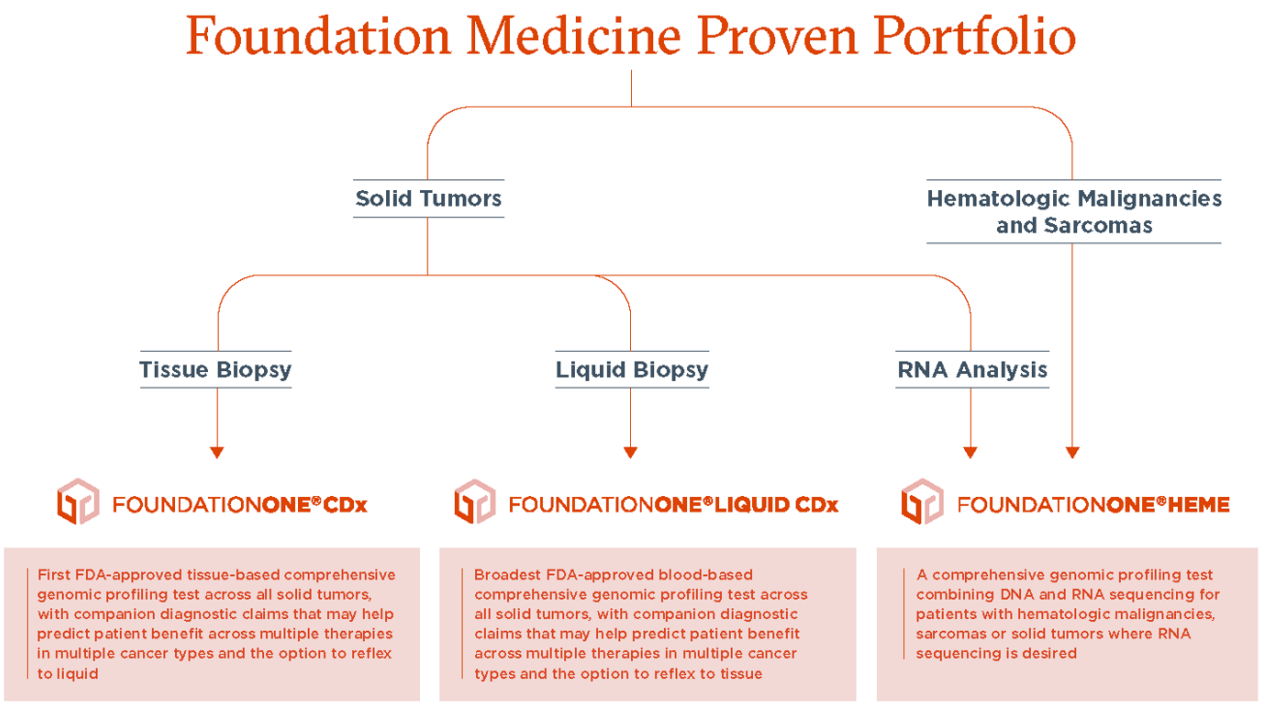
May 18, 2022

US-FLDX-2000011

1 EXECUTIVE SUMMARY

In August 2020, FoundationOne® Liquid CDx became the broadest United States (US) Food and Drug Administration (FDA)-approved circulating tumor deoxyribonucleic acid (ctDNA)-based comprehensive genomic profiling (CGP) assay (liquid biopsy) for use as a companion diagnostic to identify patients who may benefit from treatment with certain targeted therapies in accordance with the approved therapeutic product labeling. FoundationOne Liquid CDx is currently an FDA-approved companion diagnostic for 8 drug therapies in 4 cancer types.[1] Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified healthcare professionals in accordance with professional guidelines in oncology for patients with malignant neoplasms (**FoundationOne Liquid CDx Test Description**). FoundationOne Liquid CDx is the liquid biopsy test that replaces the previously available liquid biopsy laboratory developed test (LDT), FoundationOne Liquid (Table 6-8). FoundationOne Liquid CDx is part of a portfolio of CGP assays that provides a CGP testing option for any advanced cancer patient (Figure 1-1).

Figure 1-1. Foundation Medicine Portfolio



DNA, deoxyribonucleic acid; FDA, Food and Drug Administration; RNA, ribonucleic acid.

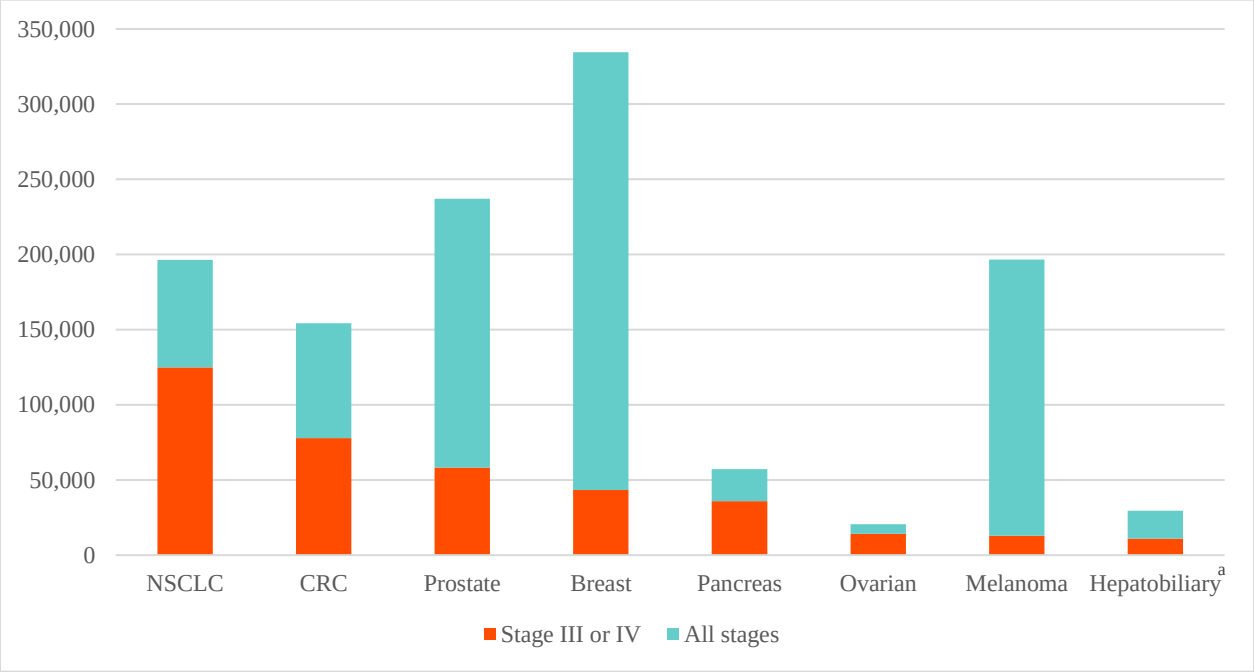
Source: Foundation Medicine, Inc.

Identifying Appropriate Treatment Options in Advanced Cancer Represents Significant Unmet Need

Approximately 545,000 people are diagnosed with advanced cancer annually in the US (Figure 1-2) (**Epidemiology of Advanced Cancer**).[2] Prognosis remains poor for most types of metastatic solid tumors, with relatively low 5-year survival, ranging from 3% in patients with pancreatic cancer or

hepatobiliary cancer to only as high as 40% in patients with head and neck cancer (**Historical Treatment of Advanced Cancer**).[3]

Figure 1-2. Estimated 2021 Distribution of Stage of Disease Within the Select Solid Tumor Cancers



[a] Hepatobiliary only includes HCC for this calculation.

CRC, colorectal cancer; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer.

Source: Kantar Health 2021.[2]

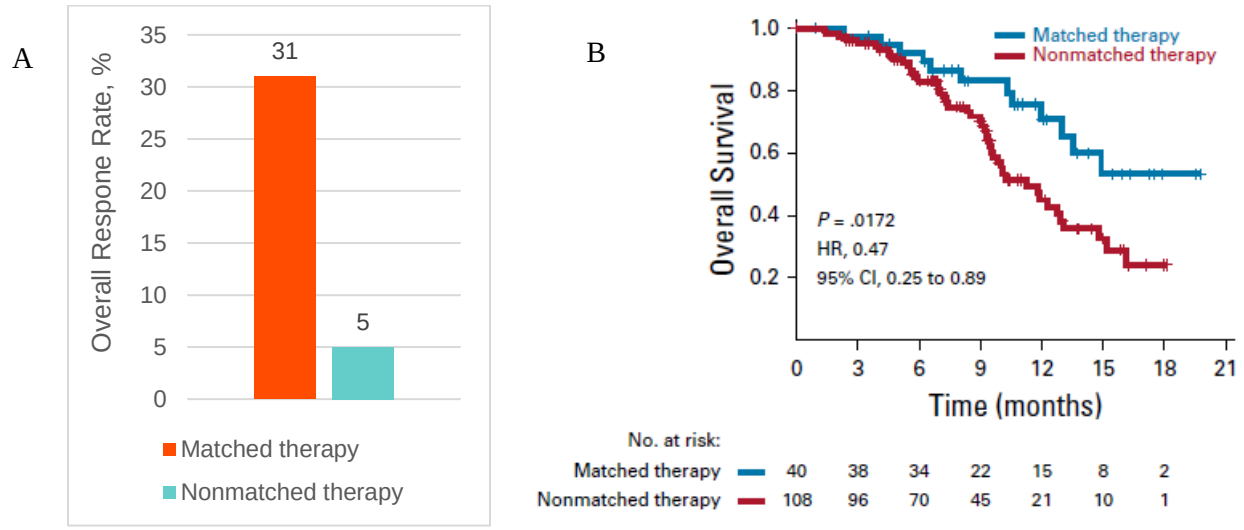
Avoidance of therapies that are unlikely to benefit patients and potentially have serious side effects will maximize patient outcomes and reduce costs (**Historical Treatment of Advanced Cancer**).[4-6]

- Overall response rates (ORR) to chemotherapy, the historical standard of care, are typically poor among patients with advanced cancer, with complete responses (CR) observed in 10% or fewer patients.[7]
- Further, chemotherapy-related adverse events result in hospitalization in up to 20% of advanced cancer patients.[4-6]
- The goal of treatment selection is to provide patients with therapies that have a potential to provide benefit, and although all therapies have toxicities, the benefit:risk ratio is of utmost importance when making therapeutic decisions for patients.[5]

A precision medicine approach can have advantages over cytotoxic regimens.

- The use of biomarker-based targeted therapy has been shown to improve treatment response and survival outcomes in patients with actionable alterations for which there is targeted therapy available (either FDA approved or in clinical trials) as compared with standard of care chemotherapy or best supportive care (BSC) (Figure 1-3) (**Molecularly Matched Therapies Improve Clinical Outcomes**).[8-10]

Figure 1-3. ORR (A) and Survival (B) With Genomically Matched Therapy vs Nonmatched Therapy Across Tumor Types



[a] Meta-analysis comparing patient outcomes from phase 1 studies that used a biomarker-based selection strategy vs those that did not. Source: Schwaederle 2016.[10]

[b] Prospective study comparing patients who received genomically matched therapy vs those who received unmatched therapy. Source: Kopetz 2019.[11]

CI, confidence interval; HR, hazard ratio.

However, the growing complexity of advanced cancer treatment is associated with significant drug spend.

- Costs to treat advanced cancer are generally at least 2-fold higher than for earlier-stage disease.[12-15]
- The recent surge in new treatment options (and the resulting increasing numbers of cancer being treated with available medicines and for longer) is a key driver in spending and growth rates.[16-18]

It is essential to appropriately select patients for treatment with targeted and immunotherapies.

- The majority of cancer types have ≥1 targetable biomarker; despite this, generally fewer than 50% of patients receive molecular testing (**Real-World Molecular Testing Patterns**).[19-24]
- According to NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®), molecular testing is recommended for certain patients with non-small cell lung cancer (NSCLC), prostate cancer, breast cancer, ovarian cancer, bladder cancer, colorectal cancer (CRC), gastric cancer, esophageal and esophagogastric junction cancers, head and neck cancers, hepatobiliary cancers, cutaneous melanoma, pancreatic cancer, uterine cancer, vulvar cancer, central nervous system (CNS) cancers (gliomas, ependymomas, and medulloblastoma), cervical cancer, thyroid cancer, bone cancer, soft tissue sarcoma (STS), and occult primary (cancer of unknown primary); specific recommendations are outlined in Table 6-1.[25-45] Further, the NCCN Guidelines recommend broad molecular testing for certain patients across several solid tumor types; the recommendations regarding broad molecular testing and/or the use of next-generation sequencing (NGS) for select cancer types are also outlined in Table 6-1 (**NCCN Guidelines: Recommendations for Molecular Testing**).[28-31,33,37,40,43-45]
- In addition to the many targeted therapies and immunotherapy agents now available, the NCCN Guidelines state that the best management of any patient with cancer is in a clinical trial[25-45];

approximately 40% of clinical trials utilize the presence of tumor genomic alterations or biomarkers for eligibility and/or stratification.[18]

A CGP Approach to Testing Optimizes Treatment Selection

As the field of molecular profiling is rapidly evolving, there is a shift in focus from a few small, predictive, disease-specific tests to a broader panel testing that can analyze changes in a myriad of genes or gene products (**Evidence of Improved Detection of Genomic Alterations With CGP**).[46] Overall, CGP testing provides valuable information on the presence of actionable biomarkers, which enables healthcare providers to make evidence-based decisions regarding treatments that result in improved outcomes for patients with advanced cancer (**Decision Impact of CGP in Clinical Practice**).[10,47,48]

- CGP utilizes NGS technology to examine entire regions of cancer-relevant genes (in contrast to limited "hotspot" tests) and genes in established cancer pathways for all tumor types, identifying the 4 main classes of genomic alterations (base substitutions, insertions or deletions, copy number alterations [CNAs], gene rearrangements) and reporting complex biomarkers such as tumor mutational burden (TMB) and microsatellite instability (MSI), to inform cancer treatment decisions via a single assay.[46,49-52]
- Results from CGP can provide information about genomic alteration to guide uses of FDA-approved targeted therapies, and potential eligibility for oncology clinical trials, and information that enables physicians to use chemotherapy more effectively (**Decision Impact of CGP in Clinical Practice**).[11,18,53,54]

CGP Allows for Improved Detection of Genomic Alterations

- Of patients with advanced cancer who undergo CGP, 51.7% to 99% will have an actionable alteration that can be matched to either a targeted therapy or to a genomically matched clinical trial.[8,11,53,55-67]
- In patients who previously underwent conventional testing methods (ie, fluorescence *in situ* hybridization [FISH], polymerase chain reaction [PCR], single-gene tests, and hotspot testing), CGP identified at least 1 actionable genomic alteration not previously identified in up to 84% across multiple tumor types (**Evidence of Improved Detection of Genomic Alterations With CGP**).[11,61]
- Further, CGP has been shown to improve detection of genomic alterations within specific tumor types (Table 1-1).[68-71]

Table 1-1. Detection of More Patients With Actionable Genomic Alterations With CGP in Specific Tumor Types

Melanoma (Boussemart 2019)[68]	**CGP can identify** up to 37% more patients with **BRAF alterations compared with traditional PCR-based methods**
CRC (Rankin 2016)[69]	Of the 6.4% of patients who harbor potentially resistant *KRAS* mutations outside of codons 12 and 13, CGP may be able to **identify 88% of those resistance alterations** not assessed by focused PCR-based testing
Breast cancer (Vasan 2019)[70]	CGP can **identify patients who harbor multiple PIK3CA mutations** that are traditionally missed by hotspot testing

NSCLC (Rozenblum 2017)[71]	CGP has been shown to **identify up to 35% more patients with ALK fusions** and **21% more patients with EGFR alterations**[a] compared with traditional methods in NSCLC

[a] 41% of these *EGFR* mutations are common alterations targetable by an FDA-approved therapy in the patient's tumor type.

ALK, anaplastic lymphoma kinase; BRAF, v-raf murine sarcoma viral oncogene homolog B1; CGP, clinical genomic profiling; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; *KRAS*, V-Ki-ras2 Kirsten rat sarcoma; NSCLC, non-small cell lung cancer; PCR, polymerase chain reaction.

CGP Informs Treatment Decisions in Clinical Practice

- A recent national survey using data from the National Survey of Precision Medicine in Cancer Treatment reported that 75.6% of oncologists use multi-marker NGS tumor panels to guide treatment decisions.[72]
- Up to 50% of patients with a treatment plan informed by CGP testing pursue genomically matched therapy, including on-label and off-label FDA-approved therapies and clinical trial enrollment.[53,59-62,64,72,73]
- CGP testing has been associated with a 10% to 20% enrollment rate in clinical trials to date compared with a historical enrollment rate of ≤8%; based on a small cohort analysis from phase 1 clinical trials, this may save payers $25,000 per patient through diversion of drug costs to the study sponsor.[53,59-61,74,75]
- Even for those without or unable to pursue genomically matched options, the personalized treatment plan may confirm chemotherapy as the best option and/or help with discussions about palliative care, thereby avoiding the use of unnecessary therapies.

Patients Have Improved Outcomes Following CGP

- Several pan-tumor and tumor-specific cohort studies have demonstrated substantial improvements in patient outcomes, including ORR, progression-free survival (PFS), and overall survival (OS), associated with CGP testing (**Table 4-1**) (**Evidence of Improved Clinical Outcomes With CGP**).[9,63,66,67,73,74,76-78]
- Improvement in outcomes associated with treatment informed by Foundation Medicine CGP testing as reported in peer-reviewed published studies mirrors the improvement in outcomes associated with the use of FDA-approved targeted therapies as reported in each of the respective drug's FDA labeling.[9,63,66,67,73,74,76-94]

Place of Liquid Biopsy CGP Testing in Treatment

Although tissue-based testing is considered the gold-standard approach to molecular testing, tissue is not always available or feasible to obtain (**Potential Causes of Lack of Tissue-Based CGP Testing**).[95,96]

- Tissue is unavailable in 26.7% to 51% of patients.[95,97-102]
- Liquid biopsy utilizes cell-free DNA (cfDNA); CGP using cfDNA is capable of detecting alterations at high specificity, though false negatives are a limitation and may warrant follow-up using tissue-based testing (**Tissue vs Liquid Biopsy-Based CGP Testing**).

When comparing tissue and liquid biopsy, there are a number of criteria that can factor into clinical decision making; compared with tissue biopsy, liquid biopsies are more convenient and present minimal

procedural risk to the patient, and the collection of these samples is less expensive.[96] For a complete comparison of liquid vs tissue biopsy considerations, please refer to Table 2-5. As noted below, several NCCN Guidelines now specifically recommend plasma testing in certain clinical circumstances.[25,28,30,31,33,35-37,42]

Additionally, clinical guidance on the appropriate use of tumor genomic testing for patients with advanced or metastatic solid tumors was recently published. The opinion strongly recommends genomic testing when there are genomic biomarker-linked therapies approved by regulatory agencies for a specific tumor type and when considering treatment for which there are specific genomic biomarker-based contraindications or exclusions. Multigene panel testing, defined as a next-generation sequencing test which sequences a defined list of genes with at least 50 genes in total, is strongly recommended as part of standard evaluation if more than one biomarker is linked to approved genomic biomarker-linked therapies within the patient's tumor type. Because studies have shown substantial concordance between cfDNA -based testing and tumor testing, in patients without tissue-based genomic test results, treatment may be based on actionable alterations identified in cfDNA.

Oncology Guidelines Recommend Liquid Biopsy Testing

According to NCCN Guidelines, molecular testing is recommended for certain patients with NSCLC, prostate cancer, breast cancer, ovarian cancer, bladder cancer, CRC, gastric cancer, esophageal and esophagogastric junction cancers, head and neck cancers, hepatobiliary cancers, cutaneous melanoma, pancreatic cancer, uterine cancer, vulvar cancer, CNS cancers (gliomas, ependymomas, and medulloblastoma), cervical cancer, thyroid cancer, bone cancer, STS, and occult primary (cancer of unknown primary); specific recommendations are outlined in Table 6-1.[25-45] Additionally, several NCCN Guidelines now specifically recommend liquid biopsy (plasma) testing in certain clinical circumstances, including NSCLC, breast cancer, cervical cancer, colon cancer, esophageal and esophagogastric junction cancers, gastric cancer, pancreatic cancer, prostate cancer, and rectal cancer.[25,28,30,31,33,35-37,42]

Table 1-2 briefly summarizes the NCCN Guidelines that recommend plasma testing in certain clinical circumstances. For additional information concerning the molecular testing recommendations made by NCCN, please refer to **NCCN Guidelines: Recommendations for Molecular Testing** and the individual NCCN Guidelines.

Table 1-2. NCCN Guidelines: Molecular Testing Recommendations in Select Tumor Types

Tumor type/ NCCN Guideline	Category[a] 1 or 2A molecular testing recommendations
Metastatic NSCLC **NCCN Guidelines for NSCLC V.3.2022**[33]	**If there is insufficient tissue to allow testing for all of *EGFR*, *ALK*, *KRAS*, *ROS1*, *BRAF*, *MET* exon 14-skipping, *NTRK1/2/3*, and *RET* in eligible patients with metastatic NSCLC, repeat biopsy and/or plasma testing should be done.**(NSCL-18) **The use of cell-free/circulating tumor DNA can be considered in specific clinical circumstances**, most notably if a patient is medically unfit for invasive tissue sampling; if following pathologic confirmation of a metastatic NSCLC diagnosis, there is insufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow-up tissue-based analysis is planned for all patients in which an oncogenic driver is not identified; or, **in the initial diagnostic setting, if tissue-based testing does not completely assess all recommended biomarkers**

Tumor type/ NCCN Guideline	Category[a] 1 or 2A molecular testing recommendations
	owing to tissue quantity or testing methodologies available, consider repeat biopsy and/or cell-free/circulating tumor DNA testing.(NSCL-18; NSCL-H 7 of 7)
Prostate cancer **NCCN Guidelines for Prostate Cancer V.4.2022**[36]	NCCN strongly recommends a metastatic biopsy for histologic and molecular evaluation. **When unsafe or unfeasible, plasma ctDNA assay is an option, preferably collected during biochemical (PSA) and/or radiographic progression in order to maximize yield**. Caution is needed when interpreting ctDNA-only evaluation due to potential interference from CHIP, which can result in a false-positive biomarker signal.(PROS-B 3 of 3)
Breast cancer **NCCN Guidelines for Breast Cancer V.3.2022**[25]	For stage IV or recurrent unresectable breast cancer, assess for *PIK3CA* mutation with tumor or liquid biopsy if hormone receptor-positive/*HER2*-negative and if considering therapy with alpelisib + fulvestrant. ***PIK3CA* mutation testing can be done on tumor tissue or ctDNA in peripheral blood (liquid biopsy).** If liquid biopsy is negative, tumor tissue testing is recommended. Testing methodology recommendation is molecular panel or PCR (category 1). Fulvestrant + alpelisib for *PIK3CA*-mutated tumors is recommended as a preferred second-line or subsequent treatment (category 1).(BINV-R 1 of 3)
Cervical cancer **NCCN Guidelines for Cervical Cancer V.1.2022**[42]	For persistent or recurrent cervical cancer, consider CGP with a validated and/or FDA-approved assay. **If tissue biopsy of metastatic site is not available, consider CGP via a validated plasma ctDNA assay.**(CERV-10)
CRC **NCCN Guidelines for Colon Cancer V.1.2022**[28] **NCCN Guidelines for Rectal Cancer V.1.2022**[37]	**Methods of testing: The testing can be performed on formalin-fixed paraffin-embedded tissue (preferred) or blood-based assay** (COL-B, 4 of 8; REC-B, 5 of 9) **Determination of tumor gene status for *RAS* and *BRAF* mutation and HER2 amplifications (individually or as part of tissue- or blood-based NGS panel)** for patients with suspected or proven metastatic synchronous adenocarcinoma (any T, any N, M1) or metachronous metastases. If known *RAS/RAF* mutation, HER2 testing is not indicated. **Tissue- or blood-based NGS panels have the ability to pick up rare and actionable mutations and fusions.** Determination of MMR or MSI status recommended (if not previously done).(COL-4, COL-9; REC-7, REC-12)
Gastric, esophageal, and esophagogastric junction cancers **NCCN Guidelines for Gastric Cancer V.2.2022**[31] **NCCN Guidelines for Esophageal and Esophagogastric Junction Cancers V.2.2022**[30]	The genomic alterations of solid cancers may be identified by evaluating ctDNA in the blood, hence a form of "liquid biopsy." **Liquid biopsy is being used more frequently in patients with advanced disease, particularly those who are unable to have a clinical biopsy for disease surveillance and management. The detection of mutations/alterations in DNA shed from gastric, esophageal, and esophagogastric carcinomas can identify targetable alterations or the evolution of clones with altered treatment response profiles. Therefore, for patients who have metastatic or advanced gastric cancer or esophageal/esophagogastric cancer who may be unable to undergo a traditional biopsy, or for disease progression monitoring, testing using a validated NGS-based comprehensive genomic profiling assay performed in a CLIA-approved laboratory may be considered.** A negative result should be interpreted with caution, as this does not exclude the presence of tumor mutations or amplifications.(GAST-B 5 of 6); (ESOPH-B 5 of 6)
Pancreatic cancer	Tumor/somatic gene profiling is recommended for patients with locally advanced/metastatic disease at diagnosis and/or recurrence who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for actionable somatic findings including, but not limited to fusions (*ALK*, *NRG1*, *NTRK*,

Tumor type/ NCCN Guideline	Category[a] 1 or 2A molecular testing recommendations
NCCN Guidelines for Pancreatic Cancer V.1.2022[35]	*ROS1, FGFR2, RET*), mutations (*BRAF, BRCA1/2, KRAS, PALB2*), amplifications (*HER2*), MSI and/or MMR deficiency (detected by tumor IHC, PCR, or NGS). **Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible.**(PANC-1, 1A, PANC-4, PANC-5, 5A, PANC-8, PANC-9 and PANC-10)

[a] **Category 1:** Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate. **Category 2A:** Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate. All NCCN recommendations are category 2A unless otherwise indicated.

ALK, anaplastic lymphoma kinase; BRCA, breast cancer susceptibility gene; CHIP, clonal hematopoiesis of indeterminate potential; CLIA, Clinical Laboratory Improvement Amendments; CRPC, castration-resistant prostate cancer; ctDNA, circulating tumor deoxyribonucleic acid; dMMR, DNA mismatch repair; EGFR, epidermal growth factor receptor; FDA, US Food and Drug Administration; HER2, human epidermal growth factor receptor 2; HRR, homologous recombination repair; IHC, immunohistochemistry; KRAS, V-Ki-ras2 Kirsten rat sarcoma; mCRPC, metastatic castration-resistant prostate cancer; MET, mesenchymal epithelial transition factor receptor; MSI, microsatellite instability; MSI-H, microsatellite instability-high; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NOS, not otherwise specified; NSCLC, non-small cell lung cancer; NTRK, neurotrophic receptor tyrosine kinase; PARP, poly ADP-ribose polymerase; PCR, polymerase chain reaction; PD-L1, programmed death ligand-1; PSA, prostate-specific antigen; TMB, tumor mutational burden; TNBC, triple-negative breast cancer.

Foundation Medicine Testing Results in Better Outcomes for Patients Across Tumor Types

Clinical utility establishes the net clinical benefit to the patient of incorporating CGP to the current standard of care decision making—in effect, answering the question: "Does the intervention (ie, the CGP test) improve patient outcomes?"[103]

Clinical Utility of Foundation Medicine Liquid Biopsy CGP Testing

An increasing body of evidence supports the clinical utility of FoundationOne Liquid CDx to match patients with solid tumors to targeted therapies based on their tumor's genomic alterations and biomarkers. Several tumor-specific cohort studies have demonstrated substantial improvements in outcomes associated with treatment guided by Foundation Medicine liquid biopsy testing (Table 1-3).

Table 1-3. Selected Outcomes[a] in Studies Showing the Clinical Utility of Foundation Medicine Liquid Biopsy CGP

Author/year	Study design	Clinical impact	
		Outcome measure	Outcome[b]
NSCLC			
Madison 2020[104]	A retrospective review of a clinicogenomic database including 6,491 patients with NSCLC and liquid biopsy (n=937 tests) and/or tissue (n=5,582 tests) to evaluate	rwPFS	**rwPFS for patients receiving SOC first-line matched targeted therapy administered following liquid biopsy (N=33) and tissue (N=229) CGP was comparable** ▪ Liquid CGP: 13.8 months

	the clinical outcomes for patients following CGP using liquid biopsy vs tissue biopsy to guide the receipt of matched, targeted therapy in the real-world setting		▪ Tissue CGP: 10.6 months ▪ aHR: 0.68 (95% CI: 0.36, 1.26)
Dziadziuszko 2021[105]	A phase II/III global, multi-center, open-label, prospective clinical trial (BFAST) screened patients (N=2,219) for oncogenic somatic mutations using liquid biopsy with FoundationACT (a prior version of FoundationOne Liquid CDx) for first-line targeted therapies in locally advanced or metastatic NSCLC; a cohort of patients was determined to have ALK-positive disease (n=119), and those patients who met treatment eligibility criteria were treated with alectinib (n=87)	PFS	**ctDNA-based NGS informed clinical decision making in *ALK*-positive NSCLC with significant clinical benefit** ▪ Median PFS: Not reached ▪ 6-month PFS: 90.7% ▪ 12-month PFS: 78.4%
Breast			
Wongchenko 2020[106]	Phase 2, prospective LOTUS trial of patients with metastatic triple-negative breast cancer (N=89) who underwent pre-treatment tissue CGP with FoundationOne and cfDNA analysis with FoundationACT	PFS	**ctDNA successfully selected patients who improved when administered first-line ipatasertib + paclitaxel** First-line ipatasertib + paclitaxel: ▪ Patients with detectable *PIK3CA/AKT1* mutation: HR: 0.15 (95% CI: 0.02, 0.62) ▪ Patients without *PIK3CA/AKT1* mutation: HR: 0.86 (95% CI: 0.48, 1.51)

[a] For more detailed information concerning the clinical validity and utility of Foundation Medicine liquid biopsy CGP, please refer to Table 6-14 and Table 6-15.

[b] All outcomes for PFS and OS outlined above are medians.

CGP, comprehensive genomic profiling; CI, confidence interval; HR, hazard ratio; HRD, homologous recombination deficiency; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; rwPFS, real-world progression-free survival; SOC, standard of care.

Clinical Utility and Validity of FoundationOne Liquid CDx Across Tumor Types

As there is a demonstrable need for an alternative to tissue-based testing in some cases, the FDA has recently evaluated and approved FoundationOne Liquid CDx as a companion diagnostic to identify patients who may benefit from treatment with certain targeted therapies (Table 1-4) or as tumor mutation profiling to be used by qualified healthcare professionals in accordance with professional guidelines in oncology for patients with malignant neoplasms. Please refer to **FoundationOne Liquid CDx Test Description** for the complete intended use statement for FoundationOne Liquid CDx.

Table 1-4. Companion Diagnostic Indications

Tumor type	Biomarker(s) detected	Therapy
NSCLC	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Iressa® (gefitinib) Tagrisso® (osimertinib) Tarceva® (erlotinib)
	ALK rearrangements	Alecensa® (alectinib)
	MET single nucleotide variants and indels that lead to *MET* exon 14 skipping	Tabrecta® (capmatinib)
Prostate cancer	*BRCA1*, *BRCA2* alterations	Rubraca® (rucaparib)
	BRCA1, *BRCA2*, *ATM* alterations	Lynparza® (olaparib)
Breast cancer	*PIK3CA* alterations[a]	Piqray® (alpelisib)
Ovarian cancer	*BRCA1*, *BRCA2* alterations	Rubraca® (rucaparib)

[a] *PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, and H1047Y.

ALK, anaplastic lymphoma kinase; BRCA, breast cancer susceptibility gene; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx.[1]

The sections below highlight the supporting data in patient populations for which FoundationOne Liquid CDx has a companion diagnostic claim. For some of the companion diagnostic claims, the FDA approval was based on concordance data with an approved comparator assay. As such, the clinical benefit associated with such claims is described as a part of the prescribing information for the associated targeted therapy. For an overview of the data supporting the specific companion diagnostic claims, please follow the link associated with each tumor type.

Clinical Utility of FoundationOne Liquid CDx in NSCLC

NSCLC has the greatest number of relevant genomic biomarkers to inform treatment decisions. The landscape of drug therapies with associated biomarkers in NSCLC according to professional guidelines is shown in Table 6-2. Of note, Foundation Medicine testing provides companion diagnostic coverage for 10 out of 22 therapies with a companion diagnostic required per FDA-approved labeling, including liquid biopsy options, for these biomarkers.

FoundationOne Liquid CDx is FDA approved as a companion diagnostic to identify patients who may benefit from treatment with erlotinib, gefitinib, osimertinib, alectinib, and capmatinib.[1]

- The companion diagnostic approvals for *EGFR* mutations (erlotinib, gefitinib, and osimertinib) were based on high concordance to the cobas® *EGFR* Mutation Test v2, as described in Table 1-5.[1]
- As a companion diagnostic for the detection of *ALK* rearrangements, FoundationOne Liquid CDx was approved based on data from a clinical bridging study that showed high concordance to and similar clinical outcomes as the blood-based NGS clinical trial assay (CTA), FoundationACT, from the BFAST trial (Table 1-5 and Table 1-6). [1,105]

- Additionally, FoundationOne Liquid CDx is an FDA-approved companion diagnostic for capmatinib based on data from a clinical bridging study. FoundationOne Liquid CDx showed high concordance to an RNA-based, tissue CTA (reverse transcriptase-polymerase chain reaction [RT-PCR]) (Table 1-5). Additionally, a clinical bridging study reported similar clinical outcomes for FoundationOne Liquid CDx as those whose biomarker status was determined by the RNA-based RT-PCR CTA in the pivotal GEOMETRY mono-1 trial (Table 1-6).[1]

For additional information concerning the clinical utility of FoundationOne Liquid CDx in NSCLC, please refer to **Clinical Utility and Validity of FoundationOne Liquid CDx in NSCLC**.

Table 1-5. Clinical Validity of FoundationOne Liquid CDx for NSCLC Companion Diagnostic Claims

Biomarker	N	PPA, % (95% CI)	NPA, % (95% CI)	Comparator assay
***EGFR* exon 19 deletions** ***EGFR* exon 21 L858R alterations**	177	CCD1: 97.7 CCD2: 97.7	CCD1: 95.6 CCD2: 95.4	cobas® *EGFR* Mutation Test v2
***ALK* rearrangements**	249	84.0 (73.7, 91.4)	100.0 (97.9, 100.0)	CTA (FoundationACT)
***MET* exon 14 skipping mutations**	150	70.5 (59.1, 80.3)	100 (95.0, 100)	RNA-based, tissue CTA (RT-PCR)

CCD1: The first replicate of cobas assay as the reference.

CCD2: The second replicate of cobas assay as the reference.

BICR, blinded independent central review; CI, confidence interval; CTA, clinical trial assay; EGFR, epidermal growth factor receptor; NPA, negative percent agreement; PPA, positive percent agreement.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx.[1]

Table 1-6. Clinical Utility of FoundationOne Liquid CDx for NSCLC Companion Diagnostic Claims

Drug and alteration	Sample description	Clinical endpoint	F1LCDx	CTA[a]
Alecensa® (alectinib) ***ALK* rearrangements**	Pre-treatment samples with ≥30 ng DNA from patients enrolled in BFAST (n=63 for clinical efficacy)	ORR, %[b] (95% CI)	88.9%[c] (78.4, 93.5)	87.4%[c] (78.5, 93.5)
Tabrecta® (capmatinib) ***MET* exon 14 skipping mutations**	Pretreatment samples with ≥30 ng DNA from patients enrolled in GEOMETRY mono-1 (n=39 for F1LCDx and n=69 for CTA for cohort 4 and n=16 for F1LCDx and n=28 for CTA for cohort 5b)	ORR, %[b] (95% CI)	Cohort 4	
			51.3%[c] (34.8, 67.6)	40.6%[c] (28.9, 53.1)
			Cohort 5b	
			81.3%[c]	67.9%[c]

Drug and alteration	Sample description	Clinical endpoint	F1LCDx	CTA[a]
			(54.4, 96.0)	(47.6, 84.1)

[a] The CTA for *ALK* rearrangements was FoundationACT and for *MET* exon 14 skipping mutations was an RNA-based, tissue CTA (RT-PCR).

[b] Investigator-assessed ORR based on confirmed objective response (indicated by 2 objective response assessments) based on RECIST v1.1.

[c] Best ORR per BIRC assessment.

BICR, blinded independent central review; CI, confidence interval; CTA, clinical trial assay; F1LCDx, FoundationOne Liquid CDx; ORR, overall response rate; RECIST, Response Evaluation Criteria in Solid Tumors.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx.[1]

Clinical Utility of FoundationOne Liquid CDx in Prostate Cancer

The landscape of drug therapies with associated biomarkers to inform treatment in prostate cancer is shown below. Of note, Foundation Medicine testing provides companion diagnostic coverage for the 3 therapies that require a CDx per the FDA-approved drug label, including liquid options, for 2 of these biomarkers (Table 6-3).

FoundationOne Liquid CDx is an FDA-approved companion diagnostic to identify patients who may benefit from treatment with both rucaparib and olaparib.[1]

- The approval of FoundationOne Liquid CDx for the detection of *BRCA1/BRCA2* alterations for treatment with rucaparib was based on both high concordance to the CTAs (which included central tissue [Foundation Medicine], tissue and liquid based assays, and local testing [majority tissue-based]) (Table 1-7) and clinical bridging data from the TRITON2 trial that reported clinical outcomes that were similar for patients with biomarker status determined by FoundationOne Liquid CDx or CTAs (Table 1-8).[1,107]
- The companion diagnostic approval for *BRCA1/BRCA2* and *ATM* alterations to determine treatment with olaparib was based on high concordance to the FoundationOne laboratory developed test (LDT) CTA (Table 1-7). Additionally, a clinical bridging study reported similar clinical outcomes when the biomarker was determined by FoundationOne Liquid CDx or the FoundationOne LDT CTA for patients in the PROfound trial (Table 1-8).[1,108,109]

For additional information concerning the clinical utility of FoundationOne Liquid CDx in prostate cancer, please refer to **Clinical Utility and Validity of FoundationOne Liquid CDx in Prostate Cancer**.

Table 1-7. Clinical Validity of FoundationOne Liquid CDx for Prostate Cancer Companion Diagnostic Claims

Biomarker	N	PPA, % (95% CI)	NPA, % (95% CI)	Comparator assay
***BRCA1, BRCA2* alterations**	161	82.4 (73.0, 89.6)	98.6 (92.3, 100)	CTA[a]

***BRCA1, BRCA2, ATM* alterations**	139	79.9 (72.2, 86.2)	91.8 (87.0, 95.2)	CTA (based on FoundationOne CDx)

[a] Clinical bridging via concordance to CTAs, which included central tissue (Foundation Medicine), tissue and liquid based assays, and local testing (majority tissue-based) for some patients.

BRCA, breast cancer susceptibility gene; CI, confidence interval; CTA, clinical trial assay; NPA, negative percent agreement; PPA, positive percent agreement.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1]. Foundation Medicine Inc, PMA P190032.[107] Foundation Medicine Inc, PMA P200016.[108]

Table 1-8. Clinical Utility of FoundationOne Liquid CDx for Prostate Cancer Companion Diagnostic Claims

Drug and alteration	Sample description	Clinical endpoint	F1LCDx	CTA[a]
Rubraca® (rucaparib) ***BRCA1, BRCA2* alterations**	Pretreatment samples with ≥30 ng DNA from patients enrolled in TRITON2 (n=38 for F1LCDx and n=62 for CTA)	ORR, %[b] (95% CI)	47.4% (31.0, 64.2)	43.5% (31.0, 56.7)
Lynparza® (olaparib) ***BRCA1, BRCA2, ATM* alterations**	Pretreatment samples with ≥30 ng DNA from patients enrolled in PROfound (n=139)	rPFS, HR[c] (95% CI)	0.33 (0.21, 0.53)	0.34 (0.25, 0.47)

[a] For rucaparib, FoundationOne Liquid CDx was compared by clinical bridging via concordance to CTAs, which included central tissue (Foundation Medicine), tissue and liquid based assays, and local testing (majority tissue-based) for some patients. For olaparib, FoundationOne Liquid CDx was compared by clinical bridging via concordance to the CTA based on FoundationOne CDx.

[b] ORR per mRECIST v1.1 and /or PCWG-3 criteria by IRR.

[c] rPFS as assessed by BICR per RECIST v1.1 criteria and/or PCWG-3.

BICR, blinded independent central review; BRCA, breast cancer susceptibility gene; CI, confidence interval; CTA, clinical trial assay; F1LCDx, FoundationOne Liquid CDx; IRR, independent radiologic review; mRECIST, modified Response Evaluation Criteria in Solid Tumors; ORR, overall response rate; PCWG-3, Prostate Cancer Working Group-3; RECIST, Response Evaluation Criteria in Solid Tumors; rPFS, radiological progression-free survival.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1]. Abida 2020[109]. de Bono 2020[110]. Foundation Medicine Inc, PMA P190032.[107] Foundation Medicine Inc, PMA P200016.[108]

Clinical Utility of FoundationOne Liquid CDx in Breast Cancer

The landscape of drug therapies with associated biomarkers in breast cancer according to professional guidelines is shown in Table 6-4. Of note, Foundation Medicine testing provides companion diagnostic coverage for 8 out of 10 therapies with a companion diagnostic required per FDA-approved labeling, including liquid options, for some of these biomarkers.

FoundationOne Liquid CDx is FDA approved as a companion diagnostic to identify patients who may benefit from treatment with alpelisib for *PIK3CA*-mutated advanced or metastatic breast cancer.[1]

- The companion diagnostic approval for FoundationOne Liquid CDx for the detection of *PIK3CA* mutations was based on high concordance to the tumor tissue-based PCR CTA, as described in

Table 1-9. Additionally, FoundationOne Liquid CDx has clinical bridging data reporting clinical outcome results similar to those seen when the biomarker status was determined using the tissue-based PCR CTA for treatment with alpelisib + fulvestrant in the pivotal SOLAR-1 clinical trial (Table 1-10).[1,111]

For additional information concerning the clinical utility of FoundationOne Liquid CDx in breast cancer, please refer to **Clinical Utility of FoundationOne Liquid CDx in Breast Cancer**.

Table 1-9. Clinical Validity for FoundationOne Liquid CDx Breast Cancer Companion Diagnostic Claims

Biomarker	N	PPA, % (95% CI)	NPA, % (95% CI)	Comparator assay
***PIK3CA* alterations**[a]	359	71.7 (65.4, 77.5)	100 (97.2, 100)	CTA (based on tumor tissue PCR)

[a] *PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, and H1047Y.

CI, confidence interval; CTA, clinical trial assay; NPA, negative percent agreement; PPA, positive percent agreement.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1].Woodhouse 2020.[111]

Table 1-10. Clinical Utility of FoundationOne Liquid CDx for Breast Cancer Companion Diagnostic Claims

Drug and alteration	Sample description	Clinical endpoint	F1LCDx
Piqray® (alpelisib) + fulvestrant ***PIK3CA* alterations**[a]	Pre-treatment samples from patients enrolled in SOLAR-1 (n=165)	PFS, HR[b,c] (95% CI)	0.46 (0.30, 0.70)

[a] *PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, and H1047Y.

[b] PFS was assessed using RECIST v1.1, based on investigator assessment.

[c] The HR compares alpelisib + fulvestrant to placebo + fulvestrant in patients with *PIK3CA*-mutated advanced breast cancer, with alpelisib + fulvestrant demonstrating an estimated 54% reduction in disease progression or death vs placebo + fulvestrant.

CI, confidence interval; F1LCDx, FoundationOne Liquid CDx; HR, hazard ratio; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumors.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1]. Woodhouse 2020[111].

Clinical Utility of FoundationOne Liquid CDx in Ovarian Cancer

The landscape of drug therapies with associated biomarkers in ovarian cancer according to professional guidelines is shown in Table 6-5. Of note, Foundation Medicine testing provides companion diagnostic coverage for 4 of the 6 therapies with a companion diagnostic required per FDA-approved labeling, including liquid options, for these biomarkers.

FoundationOne Liquid CDx is FDA approved as a companion diagnostic to identify patients who may benefit from treatment with rucaparib in patients with *BRCA1/BRCA2*-mutated ovarian cancer.[1]

- The FDA approval of FoundationOne Liquid CDx for the detection of *BRCA1/BRCA2* alteration for treatment with rucaparib was based on high concordance to the tissue-based CTAs (FoundationFocus™CDxBRCA and FoundationOne CDx), as described in Table 1-11. Further, clinical bridging data for FoundationOne Liquid CDx reported clinical outcome results similar to those seen when the biomarker status was determined using the tissue-based CTA for treatment with rucaparib in the pivotal ARIEL-2 trial (Table 1-12).[1,107,112]

For additional information concerning the clinical utility of FoundationOne Liquid CDx in ovarian cancer, please refer to **Clinical Utility and Validity of FoundationOne Liquid CDx in Ovarian Cancer**.

Table 1-11. Clinical Validity of FoundationOne Liquid CDx for Ovarian Cancer Companion Diagnostic Claims

Biomarker	N	PPA, % (95% CI)	NPA, % (95% CI)	Comparator assay
***BRCA1, BRCA2* alterations**	217	93.8 (84.8, 98.3)	97.4 (93.4, 99.3)	CTAs (based on FoundationFocus™CDxBRCA and FoundationOne CDx)

CI, confidence interval; CTA, clinical trial assay; NPA, negative percent agreement; PPA, positive percent agreement.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1]. Swisher 2017.[112] Foundation Medicine Inc, PMA P190032.[107]

Table 1-12. Clinical Utility of FoundationOne Liquid CDx for Ovarian Cancer Companion Diagnostic Claims

Drug and alteration	Sample description	Clinical endpoint	F1LCDx	CTA[a]
Rubraca® (rucaparib) ***BRCA1, BRCA2* alterations**	Pre-treatment samples from patients enrolled in ARIEL2 (n=26 for F1LCDx and n=61 for CTA for clinical efficacy)	ORR, %[b] (95% CI)	53.8 (33.4, 73.4)	54.1 (40.8, 66.9)

[a] FoundationOne Liquid CDx was compared by clinical bridging to the CTAs based on FoundationFocus™CDxBRCA and FoundationOne CDx.

[b] ORR per RECIST v1.1 per investigator assessment.

CI, confidence interval; CTA, clinical trial assay; ORR, overall response rate; RECIST, Response Evaluation Criteria in Solid Tumors.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1]. Swisher 2017.[112] Foundation Medicine Inc, PMA P190032.[107]

Economic Impact of CGP Is Driven by Drug Costs

As shown by the economic modeling that has been reported to date for CGP, and specifically Foundation Medicine CGP, patients who utilize CGP may have slightly increased total costs in comparison to those who utilize non-CGP molecular testing; the increase primarily occurs because such testing achieves the ultimate goal in oncology—meaningful prolongation of life (**CGP Compared With Conventional Testing**).[113-115]

- The primary cost-drive among patients utilizing CGP is the cost of using effective drugs for a longer period of time.
- Additionally, CGP facilitates identification of patients for clinical trials, thus potentially diverting substantial drug costs to study sponsors (**Economic Value of CGP Increasing Clinical Trial Enrollment**).[59,61,116]
- There may be additional economic benefits that could be derived from a liquid, ctDNA-based CGP approach, including cost avoided for both tissue biopsy procedures and the cost of adverse events related to such procedures.[117,118]

Conclusion

As targeted therapies are efficacious in the right subgroups of patients, it is increasingly important to define these subgroups using an accurate and efficient molecular testing method, such as CGP.[46] A comprehensive approach to testing with Foundation Medicine testing leads to improved outcomes via treatment response and survival, with a manageable budget impact that is driven by longer duration of effective therapy for patients.[95] Not all patients with advanced cancer are able to access tissue-based CGP due to limitations associated with biopsy procedures and/or tissue quality.[95,114] Patients without access to CGP via tissue now have the availability of an FDA-approved blood-based CGP assay to provide the essential information regarding genomically targetable alterations that can inform treatment decisions; this will help better select and stratify cancer patients in order to guide therapy compared with a one-size-fits-all treatment approach.[5]

TABLE OF CONTENTS

2 BACKGROUND: BURDEN / UNMET NEED

- Approximately one-third of patients are diagnosed with their cancer in the advanced stage in the US; survival at 5 years is poor (ranging from 40% down to only 3% depending on cancer site).[2,3,119-121]
- Treatment in advanced tumors has been largely based on tumor site, histology, tumor stage, and prior response to therapy, with the majority of patients receiving chemotherapy; however, overall response rates (ORR) to chemotherapy are typically poor among patients with advanced cancer, with CRs observed in 10% or fewer of patients.[7]
- Both molecularly targeted therapies and immunotherapies have shown improved outcomes of ORR and survival in patients with a corresponding biomarker over standard of care therapy across multiple advanced solid tumors.[8-10,48,122-124]
- Even though guideline recommendations are in place and there is ample evidence supporting molecular testing and biomarker-based therapies, recent evidence shows the majority of advanced cancer patients do not receive molecular testing.[113]
- There are many reasons a patient may not undergo molecular testing via CGP, including clinical factors such as the lack of adequate tissue.

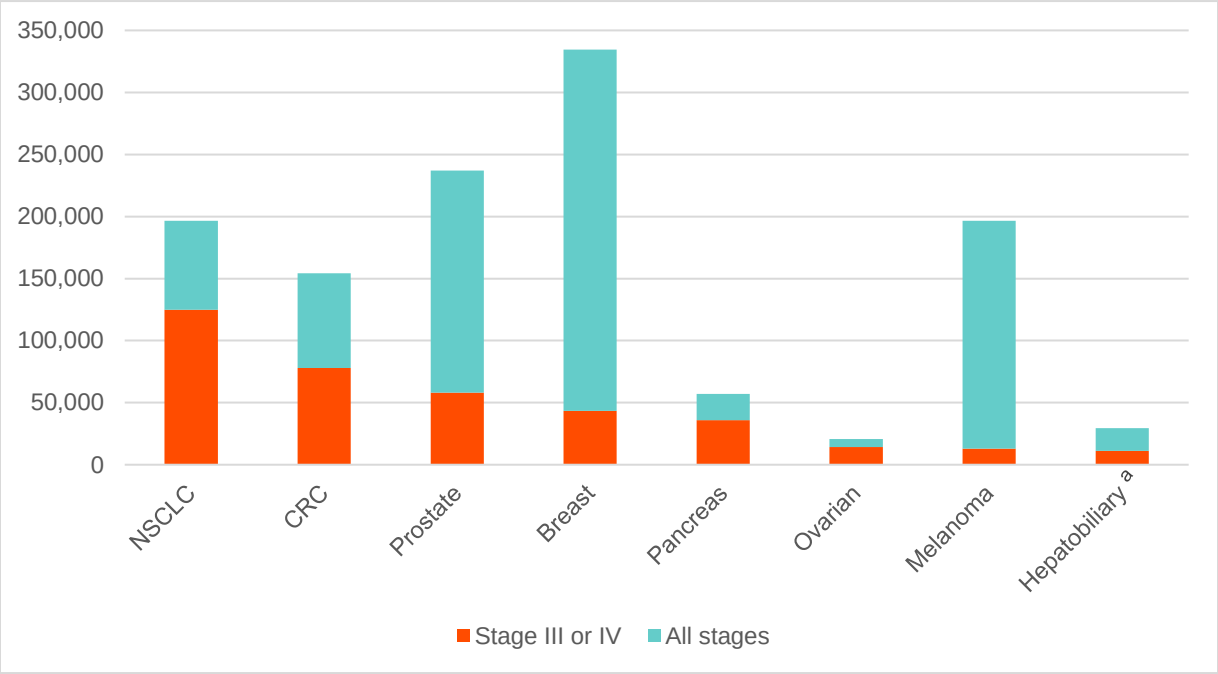
Prevalence of Advanced Cancer and Unmet Treatment Needs

Epidemiology of Advanced Cancer

In the US, approximately 1.9 million people will be diagnosed with cancer and an estimated 608,570 cancer-related deaths will occur in 2021.[119] Approximately one-third of patients diagnosed with a solid tumor will be classified as advanced (defined as stage III or IV cancer).[2,120] Based on this estimation, approximately 545,000 people in the US will be diagnosed with advanced solid tumor cancer per year.[2,119,120]

It is estimated the 10 most common solid tumor cancers in the US, with an incidence of 1,486,738, will comprise approximately 75% of all cancer diagnoses in 2021.[2,119] Among these cancer types, approximately 32% of patients will have stage III or IV disease at diagnosis, equivalent to an incidence of 468,375.[2,120] The distribution of stage of diagnosis within select cancer types is illustrated in Figure 2-1.

Figure 2-1. Estimated 2020 Distribution of Stage of Disease Within the Select Solid Tumor Cancers



[a] Hepatobiliary only includes HCC for this calculation.

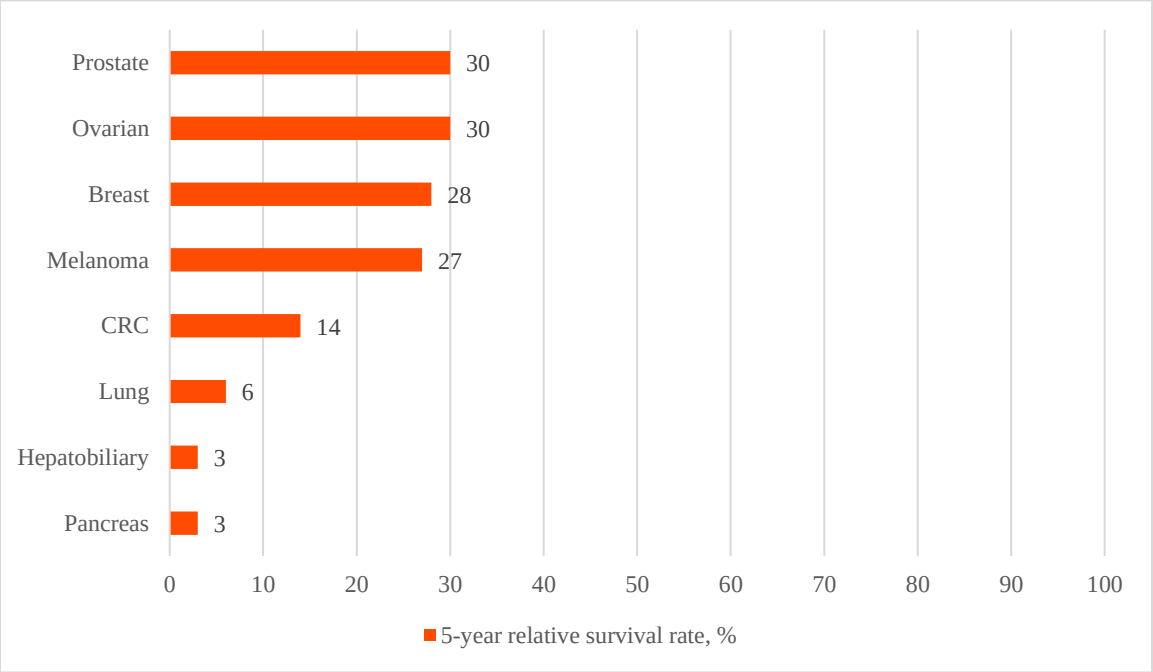
CRC, colorectal cancer; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer.

Source: Kantar Health 2021.[2]

Although many cancers, including prostate cancer and breast cancer, are predominantly diagnosed early in the disease course, even with optimal treatment, up to 30% of patients will relapse and present with metastatic disease.[125,126]

Prognosis remains poor for most types of metastatic cancers, with relatively low 5-year survival when diagnosed as distant. Examples of 5-year relative survival rates in patients diagnosed at distant stage for select tumor types, according to Surveillance, Epidemiology, and End Results (SEER) are shown in Figure 2-2.

Figure 2-2. 5-Year Survival Rates in Select Metastatic Solid Tumors[a]



[a] Data based on the NCI SEER Program in the US using data from 2010–2016.

CRC, colorectal cancer; NCI, National Cancer Institute; SEER, Surveillance, Epidemiology, and End Results; US, United States

Source: Siegel 2021.[3]

Historical Treatment of Advanced Cancer

The diagnosis and treatment selection of all tumor types has historically relied almost exclusively on clinical and pathologic features of the tumor. Prior to the introduction of immunotherapy and targeted therapy, standard of care treatment options for patients with advanced cancer included chemotherapy, radiotherapy, hormone therapy, or surgery.

However, ORR to chemotherapy are typically poor among patients with advanced cancer, with CRs observed in 10% or fewer of patients.[7]

For example, a meta-analysis of 68 chemotherapy trials (2,732 patients) reported a CR rate following cytotoxic chemotherapy treatment of 7.4% (95% confidence interval [CI]: 6.3, 8.4) in patients with late-stage cancer, regardless of tumor type or drug regimen used.[7] The PR rate in this analysis was 27.9%, meaning a total of 35.3% of patients were considered "responders" (ie, CR + PR) to chemotherapy.[7] Conversely, 64.7% of patients were nonresponders to therapy (ie, did not achieve a PR or better with cytotoxic chemotherapy).[7]

Despite significant differences between tumor types (P=0.028), the individual CR rates (defined as disappearance of all cancer as a result of chemotherapy) did not exceed a mean rate of 11% and included:[7]

- 3.2% for patients with pancreatic cancer;
- 5.0% for patients with NSCLC;
- 6.1% for patients with ovarian cancer;

- 6.7% for patients with CRC;
- 8.5% for patients with melanoma;
- 10.1% for patients with breast cancer; and
- 10.9% for patients with prostate cancer.

Biomarker-Based Therapies

Treatment selection in advanced tumors has been largely based on tumor site, histology, tumor stage, and prior response to therapy. However, improvements in our understanding of the biology of cancers have led to the identification of new biomarkers to help guide treatment selection for individual patients. The natural history of solid tumors is a process of multistep carcinogenesis and tumor growth that is driven by changes in the genomic landscape. The genomic status of the tumor, in addition to its location, has substantial implications for effective patient management.[127-131] As such, there has been a shift in the treatment approach of oncology patients toward biomarker-based therapies, the aim of which is to identify interventions likely to be of most benefit to patients based upon features of the individual or their disease.[132] The discovery of multiple new genomic and other biomarkers for use with targeted therapy and immunotherapy has revolutionized the patient journey and treatment paradigm, shifting away from trial-and-error methods and toward biomarker-based selection of the most rational treatment to maximize the likelihood of treatment response and survival.

Molecularly Matched Therapies Improve Clinical Outcomes

Biomarker-based therapy, or targeted therapy, involves treatment that is targeted specifically to the genomic profile of a patient's tumor that may affect certain signaling pathways known to underpin disease progression.[133] Immunotherapy (with immune checkpoint inhibitors such as pembrolizumab and nivolumab) refers to the use of monoclonal antibodies that block key molecules in immune checkpoint pathways, thereby eliciting an immune response that targets and destroys cancer cells.[134] Each therapy type has demonstrated an established clinical benefit in advanced cancers across multiple tumor types.

Targeted Therapy and Immunotherapy

Numerous pan-tumor cohort studies and meta-analyses have demonstrated improvements in outcomes for genomically matched therapy compared with unmatched therapy (Table 2-1).[8-10,48,122,123]

Immunotherapies have also demonstrated significant improvements in outcomes such as ORR, PFS, and OS in patients with advanced cancer. Further, patients having high TMB or microsatellite instability–high/DNA mismatch repair (MSI-H/dMMR) have been shown to have improved outcomes with immunotherapy compared to those without these biomarkers (Table 2-2).[52]

Table 2-1. Improved Clinical Outcomes With Genomically Matched Targeted Therapy

		Clinical impact		
Author/year	**Study design**	**Populations compared**	**Outcome measure[a]**	***P*-value**
Massard 2017[8]	Single-center, single-arm, open-label MOSCATO 01 trial; 199 patients with advanced hard-to-treat solid cancers received therapy matched to a genomic alteration	Intrapatient comparison using PFS2/PFS1[b]	PFS2/PFS1 ratio >1.33: 33% of patients	*P*=NA
Haslem 2017[9]	The Intermountain Healthcare study characterized patients with advanced solid cancers who received genomic testing followed by targeted therapy	Patients with genomic alteration who received targeted therapy (n=36)	PFS (average): 22.9 weeks	*P*=0.002
		Historical control group who received chemotherapy or BSC (n=36)	PFS (average): 12.0 weeks	
Schwaederle 2016[10]	A meta-analysis of 346 phase 1 studies of patients with refractory hematologic and solid tumors (N=13,203)	Genomically matched biomarker-based targeted therapy (n=58 trials)	ORR: 30.6%	*P*<0.001
		Non-matched therapy (n=293 trials)	ORR: 4.9%	
		Genomically matched biomarker-based targeted therapy (n=45 trials)	PFS: 5.7 months	*P*=0.049
		Non-matched therapy (n=45 trials)	PFS: 2.95 months	
Tsimberidou 2019[122]	A prospective study of patients with refractory cancers (N=3,743) who were referred to phase 1 trials	Matched therapy (n=711)	ORR: 16.4%	*P*<0.0001
		Unmatched therapy (n=596)	ORR: 5.4%	
		Matched therapy (n=711)	PFS: 4.0 months	*P*<0.0001
		Unmatched therapy (n=596)	PFS: 2.8 months	
		Matched therapy (n=711)	OS: 9.3 months	*P*<0.0001
		Unmatched therapy (n=596)	OS: 7.3 months	
Jardim 2015[123]	A meta-analysis of 112 registrational trials (57 randomized [32% personalized] and 55 nonrandomized trials [47% personalized]; n=38,104 patients) leading to FDA approval of 58 cancer therapies in patients with hematologic and solid tumor types	Genomically matched targeted therapy (n=44 trials)	ORR: 48%	*P*<0.001
		Unmatched therapy (n=67 trials)	ORR: 23%	
		Genomically matched targeted therapy (n=28 trials)	PFS: 8.3 months	*P*=0.002

Author/year	Study design	Clinical impact		
		Populations compared	**Outcome measure[a]**	***P*-value**
		Unmatched therapy (n=62 trials)	PFS: 5.5 months	
		Genomically matched targeted therapy (n=11 trials)	OS: 19.3 moths	*P*=0.04
		Unmatched therapy (n=49 trials)	OS: 13.5 months	
Schwaederle 2015[48]	A meta-analysis of 570 phase 2 single-agent studies (N=32,149)	Matched therapy	ORR: 31%	*P*<0.001
		Unmatched therapy	ORR: 10.5%	
		Matched therapy	PFS: 5.9 months	*P*<0.001
		Unmatched therapy	PFS: 2.7 months	
		Matched therapy	OS: 13.7 months	*P*<0.001
		Unmatched therapy	OS: 8.9 months	

[a] Unless otherwise indicated, the outcome measures in the table are medians for PFS and OS; for ORR, only complete responses and partial responses were considered (Tsimberidou 2019 used RECIST criteria; Jardim 2015 used RECIST or WHO criteria; Schwaederle 2016 and Schwaederle 2015 did not specify the method of assessment).

[b] The primary objective was to evaluate clinical benefit as measured by the percentage of patients presenting PFS on matched therapy (PFS2) 1.3-fold longer than the PFS on prior therapy (PFS1).

FDA, Food and Drug Administration; NA, not applicable; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

Table 2-2. Improved Clinical Outcomes With Immunotherapy Matched to TMB or MSI-H/dMMR

Author/year	Study design	Clinical impact		
		Populations compared	**Outcome measure[a]**	***P*-value**
Gandara 2018[50]	A retrospective analysis of 2 large randomized trials of atezolizumab in NSCLC as test and validation studies for bTMB measurement as assessed by FoundationACT	bTMB 16 treated with atezolizumab (n=77)	OS: 13.5 months	*P*=0.025
		bTMB 16 treated with docetaxel (n=81)	OS: 6.8 months	
Hellman 2018[52]	A subgroup of high TMB patients (N=299) from the phase 3, randomized, placebo-	Patients with high TMB treated with nivolumab + ipilimumab (n=139)	1-year PFS rate: 42.6%	*P*<0.001

Author/year	Study design	Clinical impact		
		Populations compared	**Outcome measure[a]**	***P*-value**
	controlled CheckMate-227 trial of patients with advanced NSCLC	Patients with high TMB treated with chemotherapy (n=160)	1-year PFS rate: 13.2%	
Goodman 2017[135]	A retrospective pan-tumor study of patients with locally advanced or metastatic cancers who were treated with various immunotherapies with TMB assessed by FoundationOne (N=151)	Patients with high TMB treated with immunotherapy (n=38)	ORR: 58%	*P*=0.0001
		Patients with low TMB treated with immunotherapy (n=113)	ORR: 20%	
		Patients with high TMB treated with immunotherapy (n=38)	PFS: 12.8 months	*P*<0.0001
		Patients with low TMB treated with immunotherapy (n=113)	PFS: 3.3 months	
		Patients with high TMB treated with immunotherapy (n=38)	OS: Not reached	*P*=0.0036
		Patients with low TMB treated with immunotherapy (n=113)	OS: 16.3 months	
Goodman 2019[136]	60 patients seen at UCSD had tumor types analyzed by FoundationOne for MSI and TMB status and response to immunotherapy	Patients with high TMB and MSI-stable treated with immunotherapy (n=15)	PFS: 26.8 months	*P*=0.0173
		Patients with low-intermediate TMB and MSI-stable treated with immunotherapy (n=45)	PFS: 4.3 months	
		Patients with high TMB and MSI-stable treated with immunotherapy (n=15)	OS: NE	*P*=0.0635
		Patients with low-intermediate TMB and MSI-stable treated with immunotherapy (n=45)	OS: 16.3 months	
Le 2015[65]	Phase 2 study of the clinical activity of pembrolizumab in patients with metastatic cancer with or without dMMR (n=41)	Patients with dMMR CRC treated with pembrolizumab (n=10)	ORR: 40%	NR
		Patients with dMMR non-CRC treated with pembrolizumab (n=7)	ORR: 71%	

Author/year	Study design	Clinical impact		
		Populations compared	**Outcome measure[a]**	***P*-value**
		Patients pMMR CRC treated with pembrolizumab (n=18)	ORR: 0%	
		Patients with dMMR CRC treated with pembrolizumab (n=10)	PFS: NE	*P*<0.001
		Patients pMMR CRC treated with pembrolizumab (n=18)	PFS: 2.2 months	
		Patients with dMMR CRC treated with pembrolizumab (n=10)	OS: NE	*P*=0.03
		Patients pMMR CRC treated with pembrolizumab (n=18)	OS: 5.0 months	

[a] Unless otherwise indicated, the outcome measures in the table are medians for PFS and OS; for ORR, only complete responses and partial responses were considered and this was as assessed by RECIST.

bTMB, blood tumor mutational burden; CRC, colorectal cancer; dMMR, DNA mismatch repair; MSI-H, microsatellite instability–high; NE, not evaluable; NR, not reported; NSCLC, non-small cell lung cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; pMMR, proficient DNA mismatch repair; RECIST, Response Evaluation Criteria in Solid Tumors; TMB, tumor mutational burden; USCD, University of California San Diego.

Complexity of the Current Treatment Paradigm

Although therapy targeted to a biomarker has been shown to improve outcomes in patients with advanced cancer, there are many factors physicians must now consider and incorporate into treatment decision making, adding to the complexity of effectively treating these patients.[8-10] Physician understanding of both appropriate molecular testing as well as the results of such testing are vital to ensuring patients receive appropriate treatment. In a recent survey of the 20 top US cancer centers and hospitals, all the respondents indicated genetic test results are reported in patients' electronic medical records in some manner; however, 45% indicated that they don't have decision support tools in place to access relevant genomic information when they need it in patient care.[137] Although clinical guidelines are noted to be effective tools for uniformly and sustainably delivering optimal, quality-focused, patient-centric, safe care, adherence to guideline recommendations are variable.[138] Precision medicine can be very beneficial as it effectively reduces overtreatment by removing more extensive treatment options from consideration if deemed by clinicians to be futile; however, these tests must be ordered, and the results must be understood.[139]

NCCN Guidelines: Recommendations for Molecular Testing

According to NCCN Guidelines, molecular testing is recommended for certain patients with NSCLC, prostate cancer, breast cancer, ovarian cancer, bladder cancer, CRC, gastric cancer, esophageal and esophagogastric junction cancers, head and neck cancers, hepatobiliary cancers, cutaneous melanoma, pancreatic cancer, uterine cancer, vulvar cancer, CNS cancers (gliomas, ependymomas, and medulloblastoma), cervical cancer, thyroid cancer, bone cancer, STS, and occult primary (cancer of unknown primary).[25-45] Additionally, several NCCN Guidelines now specifically recommend plasma testing in certain clinical circumstances, including NSCLC, breast cancer, cervical cancer, esophageal and esophagogastric junction cancers, gastric cancer, pancreatic cancer, and prostate cancer.[25,30,31,33,35,36,42]

Table 2-3 briefly summarizes a selection of applicable guidelines supporting molecular testing, including liquid biopsy recommendations where applicable, of select tumor types of interest. For additional information concerning the molecular testing recommendations made by NCCN, please refer to **NCCN Guidelines: Recommendations for Molecular Testing** and the individual NCCN Guidelines.

Table 2-3. NCCN Guidelines: Recommendations for Molecular Testing in Select Tumor Types

Tumor type/ NCCN Guideline	Category[a] 1 or 2A molecular testing recommendations
Metastatic NSCLC **NCCN Guidelines for NSCLC V.3.2022**[33]	The NCCN NSCLC Guidelines Panel strongly advises broader molecular profiling in eligible patients with metastatic NSCLC with the goal of identifying rare driver mutations for which effective drugs may already be available or to appropriately counsel patients regarding the availability of clinical trials. Broad molecular profiling is a key component of the improvement of care of patients with NSCLC. Establish histologic subtype with adequate tissue for molecular testing (consider re-biopsy or plasma testing if appropriate). Testing for *EGFR* (category 1), *ALK* (category 1), *KRAS*, *ROS1*, *BRAF*, *NTRK1/2/3* gene fusions, *MET* exon 14-skipping mutations, and *RET* is recommended for advanced or metastatic adenocarcinoma, large cell, and NSCLC NOS and should be considered for squamous cell carcinoma. Testing should be conducted as part of broad

Tumor type/ NCCN Guideline	Category[a] 1 or 2A molecular testing recommendations
	molecular profiling which is defined as molecular testing that identifies all of the previously listed biomarkers in either a single assay or a combination of a limited number of assays, and optimally also identifies emerging biomarkers. Emerging biomarkers to identify novel therapies include genetic alteration (ie, driver event), high-level *MET* amplification, and *ERBB2* (HER2) mutations. Tiered approaches based on low prevalence of co-occurring biomarkers are acceptable.(NSCL-18, NSCL-H 2 of 7, NSCL-I) It is recommended at this time that when feasible, testing be performed via a broad, panel-based approach most typically performed by NGS. For patients who in broad panel testing don't have identifiable driver oncogenes (especially in never-smokers), consider RNA-based NGS, if not already performed, to maximize detection of fusion events.(NSCL-H 2 of 7) If there is insufficient tissue to allow testing for all of *EGFR*, *ALK*, *KRAS*, *ROS1*, *BRAF*, *MET* exon 14 skipping, *NTRK1/2/3*, and *RET* in eligible patients with metastatic NSCLC, repeat biopsy and/or plasma testing should be done.(NSCL-18) The use of cell-free/circulating tumor DNA can be considered in specific clinical circumstances, most notably if a patient is medically unfit for invasive tissue sampling; or, if following pathologic confirmation of a metastatic NSCLC diagnosis, there is insufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow-up tissue-based analysis is planned for all patients in which an oncogenic driver is not identified. In the initial diagnostic setting, if tissue-based testing does not completely assess all recommended biomarkers owing to tissue quantity or testing methodologies available, consider repeat biopsy and/or cell-free/circulating tumor DNA testing.(NSCL-18; NSCL-H 7 of 7) Stage IVA, M1a (pleural or pericardial effusion), stage IVA, M1b, and stage IV, MIc: Biomarker testing should include *EGFR* mutations (category 1), *ALK* (category 1), *KRAS*, *ROS1*, *BRAF*, *NTRK 1/2/3*, *MET* exon 14 skipping, and *RET*. Testing should be conducted as part of broad molecular profiling.(NSCL-13, NSCL-14, NSCL-18) There is growing recognition of the molecular mechanisms of resistance to therapy. Plasma or tissue-based testing via broad molecular profiling should be considered at progression, for the T790M mutation and other genomic resistance mechanisms. If plasma-based testing is negative, tissue-based testing with re-biopsy material is strongly recommended. Practitioners may want to consider scheduling the biopsy concurrently with plasma testing referral. Broad genomic profiling may be the most informative approach to examining potential mechanisms of resistance, which may require more than one instance of such profiling over the course of an individual patient's therapy.(NSCL-H 6 of 7, NSCL-22, NSCL-27, NSCL-28, NSCL-30)
Prostate cancer **NCCN Guidelines for Prostate Cancer V.4.2022**[36]	Tumor testing for alterations in homologous combination DNA repair genes, such as *BRCA1*, *BRCA2*, *ATM*, *PALB2*, *FANCA*, *RAD51D*, *CHEK2*, and *CDK12*, is recommended in patients with metastatic prostate cancer. This testing can be considered in patients with regional prostate cancer. Tumor testing for MSI-H or dMMR, is clinically indicated in patients with mCRPC and may be considered in patients with regional or castration-sensitive metastatic prostate cancer. Germline testing for HRRm is recommended for patients with metastatic, regional, very-high-risk, or high-risk prostate cancer

Tumor type/ NCCN Guideline	Category[a] 1 or 2A molecular testing recommendations
	and those with prostate cancer who meet other family or personal cancer history and/or ancestry criteria. TMB testing may be considered in patients with mCRPC.(PROS-B 3 of 3, PROS-1 footnote c- initial diagnosis, PROS-12 footnote uu, PROS-14) At present, tumor molecular and biomarker analysis maybe be used for treatment decision making, including understanding eligibility for biomarker-directed treatments, genetic counseling, early use of platinum chemotherapy, and eligibility for clinical trials. Clinical trials may include established and/or candidate molecular biomarkers for eligibility. Tumor molecular profiles may change with subsequent treatments and re-evaluation may be considered at time of cancer progression for treatment decision making. Patients should be informed that tumor molecular analysis by DNA sequencing has the potential to uncover germline findings. Confirmatory germline testing may be indicated.(PROSB-3 of 3) NCCN strongly recommends a metastatic biopsy for histologic and molecular evaluation. When unsafe or unfeasible, plasma ctDNA assay is an option, preferably collected during biochemical (PSA) and/or radiographic progression in order to maximize yield. Caution is needed when interpreting ctDNA-only evaluation due to potential interference from CHIP, which can result in a false-positive biomarker signal.(PROS-B 3 of 3)
Breast cancer **NCCN Guidelines for Breast Cancer V.3.2022**[25]	Comprehensive germline and somatic profiling is recommended in the workup algorithm for recurrent/stage IV (M1) disease to identify candidates for additional targeted therapies.(BINV-18) For stage IV or recurrent breast cancer, assess for *PIK3CA* mutation with tumor or liquid biopsy if hormone receptor-positive/*HER2*-negative and if considering therapy with alpelisib + fulvestrant. *PIK3CA* mutation testing can be done on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended. Testing methodology recommendation is molecular panel or PCR (category 1). Fulvestrant + alpelisib for *PIK3CA*-mutated tumors is recommended as a preferred second-line or subsequent treatment (category 1).(BINV-R 1 of 3) NGS testing to assess for TMB-H (≥10 muts/Mb) for patients with recurrent or stage IV (M1) disease.(BINV-R 1 of 3) *NTRK* gene fusion testing by NGS, PCR, and FISH for patients with recurrent or stage IV (M1) disease.(BINV-R 1 of 3)
Ovarian cancer **NCCN Guidelines for Ovarian Cancer V.1.2022**[34]	Both somatic and germline *BRCA1/BRCA2* testing is recommended at diagnosis for patients with pathologically confirmed epithelial ovarian cancer/fallopian tube cancer/primary peritoneal cancer. Germline and/or somatic *BRCA1/2* status informs maintenance therapy.(OV-1, OV-2 & OV-3) In the absence of a *BRCA1/2* mutation, homologous recombination status may provide information on the magnitude of benefit of PARP inhibitor therapy.(OV-1 OV-2, OV-3, OV-5) In the up-front setting, choice of somatic testing should, at a minimum, optimize identification of molecular alterations that can inform use of interventions that have demonstrated benefit in this setting, including *BRCA1/2*, loss of heterozygosity (LOH), or homologous recombination (HR) status in the absence of a germline *BRCA* mutation.(OV-B 1 of 3)

Tumor type/ NCCN Guideline	Category[a] 1 or 2A molecular testing recommendations
	Tumor molecular testing is recommended for persistent/recurrent disease, if not previously done. Validated molecular testing should be performed in a CLIA-approved facility using the most recent available tumor tissue. Tumor molecular analysis is recommended to include, at a minimum, tests to identify potential benefit from targeted therapeutics that have tumor-specific or tumor-agnostic benefit including, but not limited to, *BRCA1/2*, homologous recombination status, MSI, TMB, *NTRK* if prior testing did not include these markers. More comprehensive testing may be particularly important in less common histologies (eg, LCOC) with limited approved therapeutic options.(OV-6, OV-7, OV-B, 1 of 3)

[a] **Category 1:** Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate. **Category 2A:** Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate. All NCCN recommendations are category 2A unless otherwise indicated.

ALK, anaplastic lymphoma kinase; BRCA, breast cancer susceptibility gene; CHIP, clonal hematopoiesis of indeterminate potential; CLIA, Clinical Laboratory Improvement Amendments; CRPC, castration-resistant prostate cancer; ctDNA, circulating tumor deoxyribonucleic acid; dMMR, DNA mismatch repair; EGFR, epidermal growth factor receptor; FDA, US Food and Drug Administration; HER2, human epidermal growth factor receptor 2; HRR, homologous recombination repair; IHC, immunohistochemistry; KRAS, V-Ki-ras2 Kirsten rat sarcoma; mCRPC, metastatic castration-resistant prostate cancer; MET, mesenchymal epithelial transition factor receptor; MSI, microsatellite instability; MSI-H, microsatellite instability-high; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NOS, not otherwise specified; NSCLC, non-small cell lung cancer; NTRK, neurotrophic receptor tyrosine kinase; PARP, poly ADP-ribose polymerase; PCR, polymerase chain reaction; PD-L1, programmed death ligand-1; PSA, prostate-specific antigen; TMB, tumor mutational burden; TNBC, triple-negative breast cancer.

Real-World Molecular Testing Patterns

Although both randomized controlled trials and real-world evidence have shown that targeted therapies improve outcomes for patients as compared with standard of care therapy, current molecular testing rates still fall short of guideline recommendations. Recent evidence suggests that many patients with advanced cancer are not undergoing molecular tumor testing.[19]

- A real-world assessment from US labs and claims databases conducted in April 2017 suggest that approximately 6,514 patients monthly and 78,168 annually could be missed from targeted therapy due to suboptimal testing.[23] These were patients whose tests may have led to targeted treatment, but likely did not because the test results were incorrect, too late, or inconclusive due to sample management issues.[23] Importantly, these data are conservative in that they only include known testing issues with molecular testing and do not include patients not tested due to lags in test adoption, which would increase these numbers considerably.[23]
- A recent retrospective claims analysis in the US included a total of 8,193 adults with select metastatic cancers with a diagnosis between January 2010 and March 2015.[19] The observed frequencies of molecular diagnostic tests among all patients were 52% for breast, 42% for NSCLC, 37% for CRC, 34% for head and neck, 41% for ovarian, and 42% for uterine cancer.[19]

In advanced NSCLC, a population representing the vast majority of testing data due to the availability for more than a decade of targeted therapies which have become standard of care, the testing rates remain suboptimal.

- A survey conducted in 2015 of 157 practitioners (n=148 [94%], medical oncologists) responsible

for treating NSCLC reported that in newly diagnosed, metastatic patients with NSCLC, only 72%, 69%, 38%, and 18% tested for *EGFR*, *ALK*, *ROS1*, and *BRAF* gene alterations, respectively.[24]

- A retrospective analysis within a large electronic health record database of patients with advanced NSCLC (n=1,203) receiving treatment within community practices (encompassing 289 oncologists) in 2017 and 2018 found that the testing rate for all biomarkers with an FDA-approved on-label drug (*EGFR*, *ALK*, *ROS1*, and *BRAF*) was 22%.[140] Further, testing for all 7 guideline-recommended genes, excluding PD-L1, for associated therapies was 7%.[140]
- Another retrospective analysis of patients with advanced NSCLC reported that, as of mid-2017, 38% had no record of any biomarker testing.[141]
- A retrospective data analysis comparing biomarker testing rates at academic and community cancer programs and utilizing data input by providers into the Via Oncology clinical pathways software program (Via Portal) was conducted between January 1, 2017 and March 31, 2017.[99] This analysis revealed that testing rates for *ALK*, *EGFR*, and *ROS1* were 94% (n = 285), 95% (n = 288), and 88% (n = 267), respectively, in the overall nonsquamous NSCLC population (N = 304).[99] The testing rate for all 3 biomarkers was 100% in the academic setting; however, in the community setting, the testing rates were lower (*EGFR*: 94%; *ALK*: 92%; *ROS1*: 85%). This study utilized clinical pathways software support that prompted clinicians for biomarker testing results, likely leading to increased compliance with molecular testing in this population.[99]

Within other solid tumor types, there is a paucity of data regarding real-world molecular testing rates. This gap in data may be due to the fact that the therapeutic options for some of targetable genomic alterations in these tumor types are relatively new comparatively to those in NSCLC. However, there are analyses available for metastatic breast cancer and metastatic colon cancer patients that show testing likely remains suboptimal in other tumor types as well.[21]

- A real-world study sought to assess the *BRCA1/2* testing rates in 1,285 HER2-negative adult women with advanced breast cancer in the US.[121] The *BRCA1/2* testing rate observed for the overall sample was 50%, with significantly lower *BRCA1/2* testing seen among HR+/HER2- vs triple-negative breast cancer (TNBC) patients (41% vs 75%; *P*<0.001).[121]
- A retrospective review of the COTA Real-World Data database was performed for 1,497 patients with metastatic colon cancer diagnosed between January 2013 and December 2017 and treated at 23 practiced in the US.[21] Overall guideline-aligned biomarker testing was only completed in 40% of patients in this study; guideline-aligned biomarker testing rates for rat sarcoma (RAS), *BRAF*, and MSI/mismatch repair deficiency over this study period were 41%, 43%, and 51%, respectively.[21]

As studies show that a significant proportion of patients, up to 80%, do not receive guideline-based molecular testing, there is a missed opportunity to ensure patients are receiving optimal treatment.[19-21,23,24,140] Table 2-4 outlines this opportunity in terms of patient numbers.

Table 2-4. Biomarkers and Real-World Testing Patterns in Select Advanced Cancers

	Incidence[a]	Prevalence of biomarkers	Real-world testing rates	Missed opportunity (number of patients)
NSCLC	124,940	30%[b]	33.2%[c]	25,000

Prostate	58,172	15%–30%[d]	41%[e]	5,000–10,000
Breast	43,425	65%[f]	50%[g]	14,000
Ovarian	14,124	50%[h]	41%[i]	4,000
Total estimated number of unidentified patients potentially eligible for targeted treatments				48,000–53,000

[a] Incidence from Kantar Health CancerMPact with estimations for 2021 based on patients with stage III or IV disease at diagnosis.

[b] In eligible patients with metastatic NSCLC, testing is recommended in the NCCN Guidelines for the following biomarkers: *EGFR*, *ALK*, *KRAS*, *ROS1*, *BRAF*, *MET* exon 14-skipping, *RET*, *NTRK1/2/3*, and PD-L1 (NCCN Guidelines for NSCLC V.3.2022[33]). Prevalence from Shepherd et al 2019.

[c] Estimation determined by averaging the studies presented above, which present data on detection of >1 genomic alteration (Mason 2016 and Chawla 2018).

[d] In certain patients with prostate cancer, tumor or somatic testing is recommended or should be considered per the NCCN Guidelines for the following biomarkers: HRR gene mutations (including *BRCA1*, *BRCA2*, *ATM*, *PALB2*, *FANCA*, *RAD51D*, *CHEK2*, *CDK12*), MSI/MMR, and TMB (NCCN Guidelines for Prostate Cancer V.4.2022[36]). Prevalence from Warner et al 2019 and Athie et al 2019.

[e] No specific estimation available for prostate cancer. Estimation determined from Chawla et al 2018 by averaging the molecular testing rate across tumor types.

[f] In patients with recurrent or metastatic breast cancer, testing is recommended in the NCCN Guidelines for the following biomarkers: HER2 amplifications, germline *BRCA1/2* mutations, *PIK3CA* activating mutation, PD-L1 expression, *NTRK* fusion, MSI-H or dMMR, and TMB-H (≥10 mut/Mb) (NCCN Guidelines for Breast Cancer V.3.2022[25]). Prevalence from Pauletti et al 2000, Yaziji et al 2004, and Kratz et al 2018.

[g] Estimation of real-world testing rates taken from Chawla et al 2018 and Lux et al 2020.

[h] In patients with recurrent ovarian cancer, testing is recommended in the NCCN Guidelines for the following biomarkers: *BRCA1/2*, LOH, HR status in the absence of a germline *BRCA* mutation at diagnosis; *BRCA1/2*, HR status, MSI, TMB, *NTRK* if prior testing did not include these markers at persistent/recurrent disease (NCCN Guidelines for Ovarian Cancer V.1.2022[34]). Prevalence from Konstantinopoulos et al 2015, Bonadio et al 2018, and Gee et al 2018.

[i] Estimation of real-world testing rates taken from Chawla et al 2018.

dMMR, DNA mismatch repair; HER2, human epidermal growth factor 2; HRD, homologous recombination deficiency; MSI-H, microsatellite instability-high; NCCN, National Comprehensive Cancer Network; NSCLC, non-small cell lung cancer; TMB, tumor mutational burden.

Source: Kantar Health[2]; Jordan 2017[98]; Warner 2019[142]; Mason 2016[20]; Chawla 2018[19]; Giermann 2019[140]; Kratz 2018[143]; Lux 2020[121]; Konstantinopoulos 2015[144]; Bonadio 2018[145]; Gee 2018[146].

Potential Causes of Guideline Nonadherence for Molecular Testing

Molecular profiling should be considered standard practice for most patients with advanced cancer.[46] However, patients may not undergo molecular testing for a number of reasons, including a lack of knowledge about the benefits of results to help inform treatment decision making, a lack of access to testing, or factors specific to the clinical scenario for a given patient, which make molecular testing utilizing a tissue-based test not feasible.

In patients who do undergo traditional molecular testing methods, such as single-gene tests, hotspot panels, or cancer-specific focused panels (which typically rely on PCR or FISH methodology), there are considerable limitations including:

- Incomplete information as not all relevant genes and/or types of alterations are assessed[71,147-152]
- Inefficiency as these methods may require sequential testing in certain cancer types[147,148,153]
- Risk of re-biopsy as multiple tests exhaust precious tissue[147,148,153]

As tumor molecular profiling is essential to optimizing treatment in clinical practice, options that allow for more complete molecular testing will enable a more informed treatment plan.[46,95]

Comprehensive Genomic Profiling (CGP)

CGP utilizes NGS technology to examine entire regions of cancer-relevant genes (in contrast to limited hotspot tests) and genes in established cancer pathways for all tumor types, identifying the 4 main classes of genomic alterations (base substitutions, insertions or deletions, CNAs, gene rearrangements) and reporting complex biomarkers such as TMB and MSI, to inform cancer treatment decisions via a single assay.[46,49-52]

Evidence from CGP testing has demonstrated the additional value of using a CGP-based approach to match patients to therapy compared with standard genomic tests such as FISH and PCR.

- By increasing the number of targetable genomic alterations identified as compared with single gene or hotspot testing, a tissue-based CGP approach has resulted in improved patient outcomes compared with standard of care (unmatched) therapy through the matching of genomic alterations to effective therapeutic options.[9,63,67,73,154]
- CGP not only allows for identification of genomic alterations but also accurate measurement of complex biomarkers, such as MSI and TMB.[49]
- CGP testing has been associated with a 10% to 20% enrollment rate in clinical trials to date compared with a historical enrollment rate of ≤8%.[53,59-61,74,75]

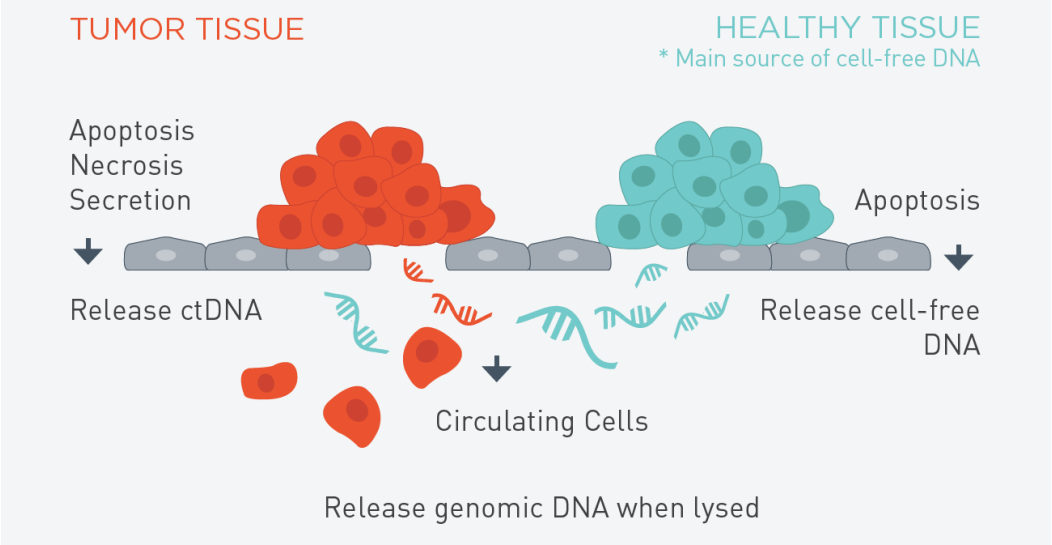
CGP streamlines testing for molecular biomarkers, provides information on genomic signatures that cannot be captured by single-gene tests or smaller panels, and provides context-based test results to allow for evidence-based clinical decision making. With the utilization of broad molecular profiling, such as CGP, treatment options will improve for an increasing number of patients while eventually emerging as a more cost-effective, generally beneficial option compared with the currently accepted trial-and-error treatment model.[46]

Tissue vs Liquid Biopsy-Based CGP Testing

Traditionally for biomarker testing, solid tumor tissue obtained from a biopsy procedure is tested for somatic alterations using a CGP approach. Although tissue-based testing is considered the gold-standard approach to molecular testing, tissue is not always available or feasible to obtain.[95,96] As such, there is a need to provide tumor molecular profiling to patients with advanced cancer in whom tissue-based testing is not available or feasible.[95]

Both normal and tumor cells release small fragments of cfDNA into a patient's bloodstream; a proportion of the cfDNA released contains circulating tumor DNA (ctDNA) (Figure 2-3).[155-160] Using a CGP approach, analysis of blood samples can be used to test for somatic alterations from ctDNA, potentially informing the use of evidence-based therapies when tissue biopsy is not ideal or possible. This approach is commonly referred to as liquid biopsy.

Figure 2-3. ctDNA Release From Tumor Tissue



ctDNA, circulating tumor DNA; DNA, deoxyribonucleic acid.

Source: Foundation Medicine, Inc.

There are advantages and disadvantages to both liquid and tissue CGP, as shown in Table 2-5; some of the advantages associated with liquid biopsy include convenience and minimal procedural risk to the patient.[96]

Table 2-5. General Comparison of Liquid vs Tissue Biopsy-Based CGP

Consideration	Liquid assay	Tissue assay
	Standardization in clinical practice still required for many tumor types	Gold standard in tumor characterization
Sample procurement	Less-invasive blood draw Variable venipuncture risks	Invasive, more challenging to obtain Variable biopsy risks
Clinical and biologic considerations	Comprehensive portrait of the tumor molecular landscape Able to represent tumor heterogeneity of overall disease Usefulness in early detection (minimal residual disease) of relapse and/or metastasis ctDNA release is dependent upon current tumor burden, tumor type, and timing of last therapy Tumor DNA may be <1% of total cfDNA in plasma	Can correlate with histology and cellular phenotype Can assess tumor microenvironment Represents the sampled location only Not feasible in early detection of relapse and/or metastasis Tumor DNA is approximately 20%–40%

Consideration	Liquid assay	Tissue assay
Technical considerations	High sensitivity analysis required Negative results should be confirmed by/reflexed to tissue	Low sensitivity analysis High specificity/sensitivity for genomic alterations

cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; DNA, deoxyribonucleic acid.

Source: Merker 2018.[96]

Evidence of Improved Detection of Genomic Alterations With CGP

Identifying targetable biomarkers may inform therapeutic interventions that are likely to have a greater clinical benefit via a precision medicine approach as opposed to a "one-size-fits-all" approach.[132] Up to 95% of patients with advanced cancer who undergo CGP have an actionable alteration that can be matched either to a targeted therapy or to a genomically matched clinical trial.[8,11,53,55-62] Further, evidence from tissue-based CGP testing has demonstrated the additional value of using a CGP-based approach to match patients to therapy compared with standard genomic tests such as FISH, PCR, single-gene tests, and hotspot testing, as CGP identifies missed genomic alterations from other testing methods in 41% to 84% of previously tested patients (Table 2-6).[11,61]

Table 2-6. Improved Detection of Genomic Alterations With CGP Testing

Author/year	Study description	Percent of patients with ≥1 missed genomic alteration identified with CGP	Percent of patients who received targeted therapy
Kopetz 2019[11]	Prospective study of 521 patients with refractory cancers comparing a 46- or 50-gene NGS assay with a 409-gene whole exome assay	41%	19%
Reitsma 2019[61]	Retrospective analysis of medical records including 96 patients in community oncology practice who received CGP testing Subset of 32 patients who previously received conventional testing	84%	19%

CGP, comprehensive genomic profiling; NGS, next-generation sequencing.

CGP has also been shown to improve detection of actionable genomic alterations within specific tumor types compared with traditional testing methods.[68-71]

- CGP can identify up to 37% more melanoma patients with *BRAF* alterations compared with traditional PCR-based methods.[68]
- Of the 6.4% of CRC patients who harbor potentially resistant *KRAS* mutations outside of codons 12 and 13, CGP may be able to identify 88% of those resistance alterations not assessed by focused PCR-based testing.[69]

- CGP can identify breast cancer patients who harbor multiple *PIK3CA* mutations that are traditionally missed by hotspot testing.[70]
- CGP has been shown to identify up to 35% more patients with *ALK* fusions and 21% more patients with *EGFR* alterations (41% of these *EGFR* mutations are common alterations targetable by an FDA-approved therapy in the patient's tumor type) compared with traditional methods in NSCLC.[71]

Evidence of Detection of Alterations With Liquid Biopsy-Based CGP

Despite decreased tissue requirements for a one-time tissue-based CGP approach compared with sequential approaches via PCR or hotspot testing, some patients may still have too limited or no sample available to pursue a tissue-based CGP approach. In some patients, a tissue sample may be wholly inaccessible, and a presumptive diagnosis can be made based on clinical symptoms and imaging. In other cases, patients may be in a resource-limited setting in which a biopsy procedure is not possible. In all scenarios of unavailable or limited tissue, liquid biopsy may be an alternative method for obtaining genomic information from a patient's ctDNA. A growing amount of evidence is showing that a liquid-based CGP approach is a reliable alternative to tissue-based CGP when tissue testing is not available (Table 2-7).

Table 2-7. Concordance Between Liquid and Tissue Biopsy-Based CGP Testing

Study	N	Patient population	Mutations	Overall concordance	Sensitivity	Specificity	PPV	NPV
Tukachinsky 2021[161]	837	mCRPC	*BRCA1, BRCA2*	97.0%	93.1%	97.4%	NR	NR
Aggarwal 2019[101]	128	Metastatic NSCLC	Therapeutically targetable mutations (*EGFR, ALK, MET, BRCA1, ROS1, RET, ERBB2, BRAF, KRAS*)	81.3%	NR	NR	NR	NR
Leighl 2019[162]	282	Treatment-naïve metastatic NSCLC	Any guideline-recommended biomarker (*EGFR, ALK, ROS1, BRAF* V600E, *RET, MET* amplification, *MET* exon 14 skipping, *ERBB2*)	85.5%	80%	86.9%	62.3%	94.1%
			FDA-approved targets (*EGFR, ALK, ROS1, BRAF*)	>98.2%	NR	100%	100%	NR
Li 2019[163]	96	CRC	*KRAS G12X*	97%	93%	100%	100%	96%
Chowdhury 2018[164]	161	mCRPC	*BRCA1/2*	94.4%	86%	96%	83%	97%

Study	N	Patient population	Mutations	Overall concordance	Sensitivity	Specificity	PPV	NPV
Schrock 2018[75]	33	Advanced NSCLC	*ALK, BRAF, EGFR, ERBB2, FGFR3, KRAS, MET, PDGFRA, RET, ROS1*	NR	64.1%	NR	81.5%	NR
			Professional guideline-recommended (*EGFR, ALK, MET, ERBB2, RET, BRAF, ROS1*)	NR	85.2%	NR	100%	NR
Kato 2018[63]	33	Rare tumors	*TP53*	66.7%	NR	NR	NR	NR
			BRAF	74.1%				
			MYC	88.9%				
			MET	85.2%				

ALK, anaplastic lymphoma kinase; BRCA, breast cancer gene; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; FDA, US Food and Drug Administration; KRAS, V-Ki-ras2 Kirsten rat sarcoma; mCRPC, metastatic castration-resistant prostate cancer; MET, mesenchymal epithelial transition factor receptor; NCCN, National Comprehensive Cancer Network; NPV, negative predictive value; NR, not reported; PPV, positive predictive value.

As not all patients with advanced cancer are able to access tissue-based CGP due to limitations associated with biopsy procedures and/or tissue quality, the availability of CGP via liquid biopsy provides essential information regarding genomically targetable alterations that can inform treatment decisions.[95] This technology has the potential to better select and stratify cancer patients in order to guide therapy compared with a one-size-fits-all treatment approach.[95] For more specific information concerning FoundationOne Liquid CDx, please refer to **FoundationOne Liquid CDx Test Description**.

3 PRODUCT DESCRIPTION

- FoundationOne® Liquid CDx is FDA-approved to report substitutions, insertions, and deletions (indels) in 311 genes, rearrangements in 4 genes, and copy number alterations in 3 genes.[1]
- Comprehensive results across all 324 genes are reported as a laboratory professional service which is not reviewed or approved by the FDA. bTMB, MSI-H status, and tumor fraction are reported as a laboratory professional service which is not reviewed or approved by the FDA.[165]
- FoundationOne Liquid CDx is currently an FDA-approved companion diagnostic for 8 drug therapies in 4 cancer types.[1]

FoundationOne Portfolio and Decision Support

The FoundationOne portfolio facilitates a precision medicine approach for a broad spectrum of patients across both solid tumor and hematologic malignancies.

- In August 2020, FoundationOne Liquid CDx became an FDA-approved ctDNA-based CGP assay (liquid biopsy). It 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. FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified healthcare professionals in accordance with professional guidelines in oncology for patients with malignant neoplasms. FoundationOne Liquid CDx is currently an FDA-approved companion diagnostic for 8 drug therapies in 4 cancer types.[1] For more information concerning FoundationOne Liquid CDx, please refer to the **FoundationOne Liquid CDx label**.
- This assay follows the first FDA-approved tissue-based CGP assay, FoundationOne CDx, which is approved for use as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies in accordance with the approved therapeutic product labeling. Additionally, FoundationOne CDx is intended to provide tumor mutation profiling to be used by qualified healthcare professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. FoundationOne CDx is currently an FDA-approved companion diagnostic for 28 drug therapies in 7 cancer types, which includes 2 therapies indicated for all solid tumor types (pan-tumor). For more information concerning FoundationOne CDx, please refer to the **FoundationOne CDx label**.
- Together, these tests, with FoundationOne Heme and available PD-L1 testing, provide an appropriate genomic testing option to inform precision medicine for any advanced cancer patient.

Foundation Medicine's services go "beyond the test" by providing a clear, in-depth report that supports clinical decision making as well as decision support services and technology solutions to help streamline patient care.

Please refer to **FoundationOne Portfolio Description and Decision Support Services** for more specific information about the FoundationOne Portfolio and the Decision Support Services offered by Foundation Medicine.

FoundationOne Liquid CDx Test Description

Foundation Medicine designed and developed FoundationOne Liquid CDx based on previous versions of the assay, including the FoundationACT and FoundationOne Liquid (a revised version of FoundationACT) laboratory-developed tests (LDTs). The first commercial sample was tested in 2016. The FoundationACT and FoundationOne Liquid LDTs have been used to detect the presence of genomic alterations in blood and plasma specimens. Neither the FoundationACT nor FoundationOne Liquid clinical trial assays were FDA-cleared or -approved.

FoundationOne Liquid CDx is FDA-approved to report substitutions and indels in 311 genes, rearrangements in 4 genes, and copy number alterations in 3 genes. Comprehensive results across all 324 genes are reported as a laboratory professional service which is not reviewed or approved by the FDA. The complete list of genes tested by this assay is described in further detail in the Appendix (Table 6-7). bTMB, MSI-H status, and tumor fraction are reported as a laboratory professional service which is not reviewed or approved by the FDA.[165] FoundationOne Liquid CDx utilizes circulating cfDNA isolated from plasma derived from the anticoagulated 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 the targeted therapies listed in Table 3-1 in accordance with the approved therapeutic product labeling; this information is also available on the **FDA companion diagnostics website**. Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified healthcare professionals in accordance with professional guidelines in oncology for patients with malignant neoplasms.[1]

Table 3-1. Companion Diagnostic Indications

Tumor type	Biomarker(s) detected	Therapy
NSCLC	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Iressa® (gefitinib) Tagrisso® (osimertinib) Tarceva® (erlotinib)
	ALK rearrangements	Alecensa® (alectinib)
	MET single nucleotide variants and indels that lead to *MET* exon 14 skipping	Tabrecta® (capmatinib)
Prostate cancer	*BRCA1*, *BRCA2* alterations	Rubraca® (rucaparib)
	BRCA1, *BRCA2*, *ATM* alterations	Lynparza® (olaparib)
Breast cancer	*PIK3CA* alterations[a]	Piqray® (alpelisib)
Ovarian cancer	*BRCA1*, *BRCA2* alterations	Rubraca® (rucaparib)

[a] *PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, and H1047Y.

ALK, anaplastic lymphoma kinase; BRCA, breast cancer susceptibility gene; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx.[1]

A negative result from a plasma specimen does not mean that the patient's tumor is negative for genomic findings. Patients who are negative for the mutations listed in Table 3-1 should be reflexed to routine biopsy and their tumor mutation status confirmed using an FDA-approved tumor tissue test, if available.

Genomic findings other than those listed in Table 3-1 of the intended use statement (ie, Categories 2, 3 and/or 4) are not prescriptive or conclusive for labeled use of any specific therapeutic product.

FoundationOne Liquid CDx is a single-site assay performed at Foundation Medicine, Inc. in Cambridge, MA.

Assay Description

FoundationOne Liquid CDx received FDA approval as a companion diagnostic for use in lung cancer and prostate cancer on August 26, 2020. Commensurate with the growing field of precision oncology, additional companion diagnostic claims were subsequently approved in NSCLC, breast, prostate, ovarian as outlined in Table 3-1. The test evaluates blood samples from patients with solid tumors for select clinically relevant alterations in 324 commonly altered oncogenes or tumor suppressor genes, of which, FoundationOne Liquid CDx is FDA-approved to report substitutions and indels in 311 genes, rearrangements in 4 genes, and copy number alterations in 3 genes.[1] The test also detects tumor fraction and the genomic signatures bTMB and MSI-H as a professional service which has not been reviewed or approved by the FDA.[165] FoundationOne Liquid CDx features an optimized laboratory process to achieve high sensitivity and specificity with enhanced extraction methodology to generate high-quantity and high-quality ctDNA. The hybrid capture-based NGS test method is combined with proprietary technology to precisely identify accurate variant calls by discriminating sequencing artifacts from bona fide mutations. Specifically, customized software and algorithms are used to determine genomic variants, including substitutions, indels, CNAs, rearrangements, bTMB, and MSI-H.[1,165]

FoundationOne Liquid CDx includes a clinical report that is extensively referenced with up-to-date scientific and clinical publications. The sequencing results are annotated by automated software, merged with patient demographic information, and then combined into an interpretive report that is curated by biomedical informatics scientists and approved by board-certified and licensed pathologists.

An example test report is shown in the Appendix (Figure 6-1).

The robust and comprehensive final clinical report, which is typically obtained within a time frame of <10 working days from receipt of specimen, includes the following:[166]

- Any FDA-approved claims, including companion diagnostic findings, will all be found on page 1 of the report.
- The Professional Services section provides information for all reported biomarker and genomic findings. This section is not reviewed or approved by the FDA.
  Therapies for each associated genomic finding are listed in the therapy table. This table lists therapies within your patient's tumor type and those with proven clinical benefit in other tumor types. Therapy resistance based on the genomic profile will also be indicated.
- The associated NCCN Category that has been assigned to the therapy listed within the tumor type is provided.
- A list of potential clinical trials to consider for identified genomic alterations or genomic signatures.
- Lists variants in select cancer susceptibility genes that have been previously reported as pathogenic or likely pathogenic in the ClinVar genomic database and are identified at an allele frequency that is plausible for potential germline origin for consideration of follow-up germline testing.

The **report guide**, which points out the key features of the FoundationOne Liquid CDx report is show in Figure 3-1.

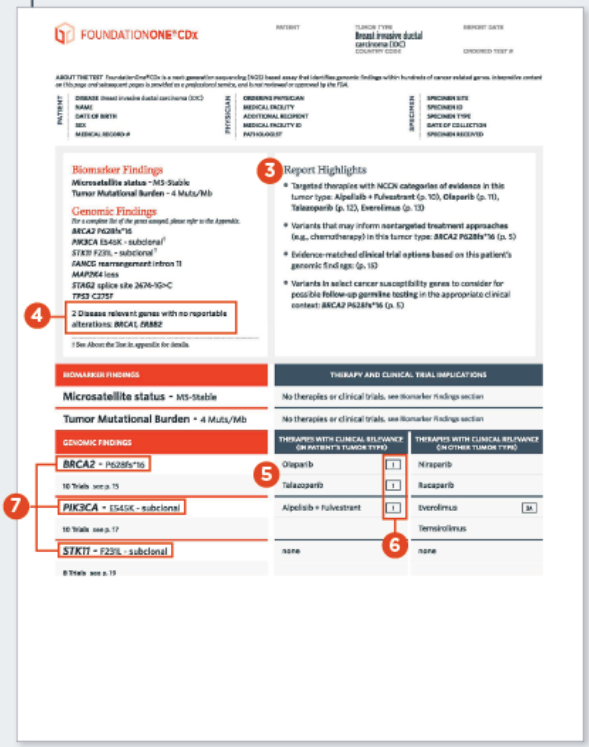
Figure 3-1. FoundationOne Liquid CDx Report Guide

FOUNDATIONONE®CDx FOUNDATIONONE®LIQUID CDx

Guide to FoundationOne®CDx and FoundationOne®Liquid CDx Reports

FDA-Approved Claims
Any FDA-approved claims will always appear at the beginning of the report, starting on page one.

Professional Services
The Professional Services section provides information for all reported biomarker and genomic findings. This section is not reviewed or approved by the FDA.



1 FDA-Approved CDx Claims
List of FDA-approved companion diagnostics associated with your patient's findings.

2 Other Alterations and Biomarkers Identified
For FoundationOne CDx reports, all other genomic and biomarker findings without companion diagnostic claims will appear here. For FoundationOne Liquid CDx reports, alterations shown here are limited to short variants and select rearrangements and copy number alterations. The complete list of genomic and biomarker findings can be found in the Professional Services section.

3 Report Highlights
This feature distills important genomic insights in one easy-to-find place, helping you focus on the key actionable results to inform your patient's treatment plan.

4 Pertinent Negatives
Identifies important negative results that can be used for patient management. Pertinent negatives do not appear for FoundationOne Liquid CDx.

5 Therapies with Clinical Benefit
Therapies for each associated genomic finding are listed in the therapy table. On the left are therapies within your patient's tumor type, and on the right are those with proven clinical benefit in other tumor types. Therapy resistance based on your patient's genomic profile will also be indicated.

6 National Comprehensive Cancer Network® (NCCN®) Categories of Evidence and Consensus[1]
Associated NCCN Category that has been assigned to the therapy listed within your patient's tumor type.

7 Clinical Trials
Identifies number of trials based on your patient's unique genomic profile with page number for quick reference.

Note: The images shown on this piece are of a sample report and do not represent actual test results. This information is intended to educate healthcare providers on the FoundationOneCDx and FoundationOne Liquid CDx reports and should not be used for patient diagnosis or treatment decisions.

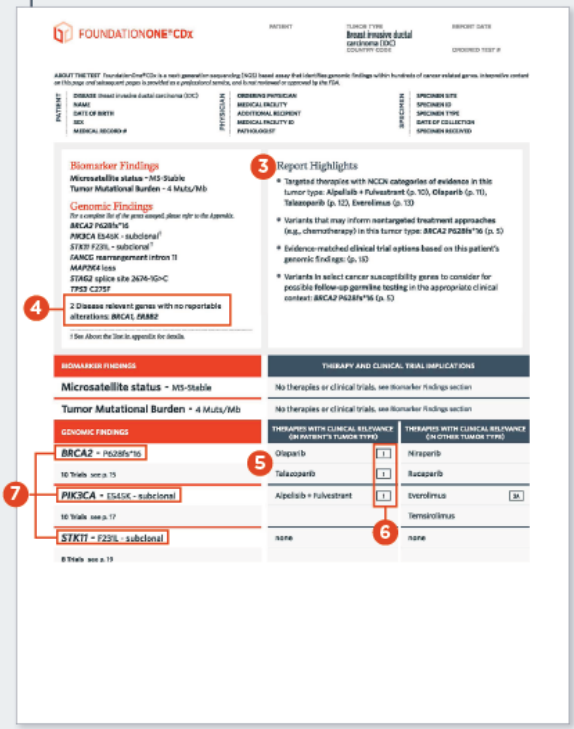
Sample report images last updated December 2021.

FOUNDATIONONE®CDx FOUNDATIONONE®LIQUID CDx

Guide to FoundationOne®CDx and FoundationOne®Liquid CDx Reports

FDA-Approved Claims
Any FDA-approved claims will always appear at the beginning of the report, starting on page one.

Professional Services
The Professional Services section provides information for all reported biomarker and genomic findings. This section is not reviewed or approved by the FDA.



1 FDA-Approved CDx Claims
List of FDA-approved companion diagnostics associated with your patient's findings.

2 Other Alterations and Biomarkers Identified
For FoundationOne CDx reports, all other genomic and biomarker findings without companion diagnostic claims will appear here. For FoundationOne Liquid CDx reports, alterations shown here are limited to short variants and select rearrangements and copy number alterations. The complete list of genomic and biomarker findings can be found in the Professional Services section.

3 Report Highlights
This feature distills important genomic insights in one easy-to-find place, helping you focus on the key actionable results to inform your patient's treatment plan.

4 Pertinent Negatives
Identifies important negative results that can be used for patient management. Pertinent negatives do not appear for FoundationOne Liquid CDx.

5 Therapies with Clinical Benefit
Therapies for each associated genomic finding are listed in the therapy table. On the left are therapies within your patient's tumor type, and on the right are those with proven clinical benefit in other tumor types. Therapy resistance based on your patient's genomic profile will also be indicated.

6 National Comprehensive Cancer Network® (NCCN®) Categories of Evidence and Consensus[1]
Associated NCCN Category that has been assigned to the therapy listed within your patient's tumor type.

7 Clinical Trials
Identifies number of trials based on your patient's unique genomic profile with page number for quick reference.

Note: The images shown on this piece are of a sample report and do not represent actual test results. This information is intended to educate healthcare providers on the FoundationOneCDx and FoundationOne Liquid CDx reports and should not be used for patient diagnosis or treatment decisions.

Sample report images last updated December 2021.

Analytic Validity of FoundationOne Liquid CDx

Sample Validation and Concordance

FoundationOne Liquid CDx was designed to replace Foundation Medicine's previous liquid biopsy tests, FoundationOne Liquid and FoundationACT, with more genes and the addition of bTMB to the previous version, FoundationOne Liquid (for a comparison of FoundationOne Liquid CDx to FoundationOne Liquid, please refer to Table 6-8). As a complement to Foundation Medicine's tissue-based testing, FoundationOne Liquid CDx conveniently provides similar molecular information in the appropriate clinical scenarios, which may include when tissue is unavailable, insufficient, or turnaround time is of the utmost importance. The sample validation for FoundationOne Liquid CDx showed a very low overall false positive rate of 0.01%. Table 3-2 provides the sample validation in terms of limit of detection (LoD) and limit of blank (LoB). Table 3-3 provides the LoD for specific CDx and non-CDx alterations with FoundationOne Liquid CDx.[1]

- The LoD describes the lowest level at which an analyte (genomic variant) can be consistently detected.[111] According to industry standard, consistently detected was defined as the level at which a 95% detection rate is observed.[111] Therefore, LoD indicates the median variant allele frequency (VAF) at which the test has shown 95% probability of detection (sensitivity).[111] The LoD for each variant type was established by processing a total of 1,069 sample replicates across ten contrived (enzymatically fragmented cell-line gDNA) samples representing short variants, rearrangements, copy number amplifications, copy number loss, MSI, and bTMB component variants.[1] The LoD was determined via hit rate and defined as the lowest dilution level tested with at least 95% detection across replicates.[1]
- The LoB describes the highest measurement result that is likely to be observed for a blank sample with a stated probability (α).[111] According to industry standard, an α (type I error rate, false positive rate) of 0.05 was selected.[111] Therefore, LoB evaluates variant calling specificity at 95% in normal blood samples (specificity).[1] The LoB was established by profiling 30 variant-negative DNA samples from heathy donors with 4 replicates per sample.[1] The LoB was estimated via the non-parametric method and was determined to be the ideal value of zero for short variants, rearrangements and copy number alterations.[1]

Table 3-2. Sample Validation of FoundationOne Liquid CDx

Alteration type	Bait set region	Median LoD[a]	LoB
Short variants	Enhanced sensitivity	0.40% VAF	0[b] *Overall false positive rate observed:* *0.01%* *or* *99.99% specificity* *or* *1 in 10,000*
	Standard sensitivity	0.82% VAF	
Rearrangements	Enhanced sensitivity	0.37% VAF	
	Standard sensitivity	0.90% VAF	
Copy number amplification	NA	21.7% TF	
Copy number loss	NA	12.7% TF	
MSI	NA	0.8% unstable loci	
bTMB (component subs)	NA	1.0% VAF	

Alteration type	Bait set region	Median LoD[a]	LoB
bTMB (component indels)	NA	1.0% VAF	

[a] Reported as VAF for short variants, rearrangements; TF for copy number amplifications and copy number loss.

[b] As would be expected in a sampling of human plasma, especially plasma from an aged population, a small number of alterations were detected. Across 30,622 short variants, which include variants classified as VUS/benign, 5 VUSs had a detection rate significantly exceeding 5% on an individual variant basis: TSC1 965T>C, IRF4 1ins87, MSH3 186_187insGCCGCAGCGCCCGCAGCG, IGF1R 568C>T, WHSC1 1582C>A. All other variants were determined to have an LoB of 0 based on the detection rate not significantly exceeding 5%.

bTMB, blood tumor mutational burden; LoB, limit of blank; LoD, limit of detection; MSI-H, microsatellite instability–high; NA, not applicable; TF, tumor fraction; VAF, variant allele frequency; VUS, variant of unknown significance.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx.[1] Woodhouse 2020.[111] Foundation Medicine, Inc, FoundationOne Liquid CDx Technical Specifications.[165]

Table 3-3. LoD for CDx and Non-CDx Alterations With FoundationOne Liquid CDx

Gene	Alteration Subtype	Median LoD
LoD estimation for CDx alterations		
ALK	*ALK-EML4* rearrangement	0.24% VAF
	NPM1-ALK rearrangement	0.94% VAF
ATM	Indels	0.51% VAF
	Rearrangement (*ATM-EXPH5* truncation[a])	1.13% VAF
BRCA1	Indels	0.38% VAF[b]
	Substitutions	0.34% VAF
	Rearrangement[a]	0.87% VAF
BRCA2	Substitutions	0.37% VAF
	Indels	0.36% VAF
	BRCA2- EDA Truncation[a]	0.48% VAF
	Copy Number Loss[a]	48.1% TF
EGFR	Indels (exon 19 deletions)	0.27% VAF
	Substitutions (L858R substitutions)	0.34% VAF
MET	Indels[a]	0.28% VAF
	Substitution[a]	0.40% VAF
PIK3CA	Substitution	0.34% VAF
LoD for highly actionable non-CDx alterations		
BRAF	Substitutions	0.33% VAF[b]
ERBB2	Copy number amplification	19.8% TF[b]
KRAS	Substitutions	0.33% VAF[b]
MET[c]	Indels	0.41% VAF[b]

Gene	Alteration Subtype	Median LoD
NRAS	Substitutions	0.42% VAF[b]
PALB2	Indels	0.37% VAF[b]
	Substitutions	0.51% VAF[b]

The estimated LoDs for *BRCA1* and *BRCA2* subs and indels were confirmed at values higher than the LoDs established in above table.

[a] The LoD for these alterations was determined using clinical specimens.

[b] Quantitative reporting of %VAF/%TF has not been approved by FDA.

[c] This LoD applies to *MET* alterations that do not meet the CDx rules.

cfDNA, cell-free deoxyribonucleic acid; LoD, limit of detection; VAF, variant allele frequency.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1] Woodhouse 2020.[111]

Concordance between FoundationOne Liquid CDx and the respective clinical trial tissue-based assays has been established in NSCLC, prostate cancer, breast cancer, and ovarian cancer for the indications shown in Table 3-4.[1]

Table 3-4. Concordance Results of FoundationOne Liquid CDx to Predicate Assays

Alteration	Drug	Method	PPA (95% CI)	NPA (95% CI)	Comparator assay
NSCLC					
EGFR exon 19 deletions and *EGFR* exon 21 L858R alterations	Iressa® (gefitinib) Tagrisso® (osimertinib) Tarceva® (erlotinib)	Concordance to cobas® *EGFR* Mutation Test v2 (n=177 samples)	CCD1: 97.7% CCD2: 97.7%	CCD1: 95.6% CCD2: 95.4%	cobas® *EGFR* Mutation Test v2
ALK rearrangements	Alecensa® (alectinib)	Pre-treatment samples with ≥30 ng DNA from patients enrolled in BFAST (n=249 for concordance)	84.0% (73.7, 91.4)	100.0% (97.9, 100.0)	CTA (FoundationACT)
MET exon 14 skipping mutations	Tabrecta® (capmatinib)	Pre-treatment samples with ≥30 ng DNA from patients enrolled in GEOMETRY-mono1 (n=150 for concordance)	70.5%[a] (59.1, 80.3)	100% (95.0, 100.0)	RNA-based, tissue CTA (RT-PCR)
Prostate cancer					
BRCA1, *BRCA2* alterations	Rubraca® (rucaparib)	Pre-treatment samples with ≥30 ng DNA from patients enrolled in TRITON2 (n=161 for concordance)	82.4% (73.0, 89.6)	98.6% (92.3, 100)	CTA[b]

Alteration	Drug	Method	PPA (95% CI)	NPA (95% CI)	Comparator assay
BRCA1, *BRCA2*, *ATM* alterations	Lynparza® (olaparib)	Pre-treatment samples with ≥30 ng DNA from patients enrolled in PROfound (n=139 for concordance)	79.9% (72.2, 86.2)	91.8% (87.0, 95.2)	CTA (based on FoundationOne CDx)
Breast cancer					
PIK3CA mutations[c]	Piqray® (alpelisib) + fulvestrant	Pre-treatment samples with ≥30 ng DNA from patients enrolled in SOLAR-1 (n=359 for concordance)	71.7% (65.4, 77.5)	100% (97.2, 100)	CTA (based on tumor tissue PCR)
Ovarian cancer					
BRCA1, *BRCA2* alterations	Rubraca® (rucaparib)	Pre-treatment samples from patients enrolled in ARIEL2 (n=217 for concordance)	93.8% (84.8, 98.3)	97.4% (93.4, 99.3)	CTAs (based on FoundationFocus™ CDxBRCA and FoundationOne®CDx)

CCD1: The first replicate of cobas assay as the reference.

CCD2: The second replicate of cobas assay as the reference.

[a] VAF values down to 0.16% VAF were observed for *MET* short variants.

[b] Clinical bridging via concordance to CTAs, which included central tissue (Foundation Medicine), tissue and liquid based assays, and local testing (majority tissue-based) for some patients.

[c] *PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, and H1047Y.

BRCA, breast cancer susceptibility gene; CI, confidence interval; CTA, clinical trial assay; EGFR, epidermal growth factor receptor; NPA, negative percent agreement; NPV, negative predictive value; PPA, positive percent agreement; PPV, positive predictive value.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx.[1] Foundation Medicine Inc, PMA P190032.[107] Foundation Medicine Inc, PMA P200016.[108] Woodhouse 2020.[111]

Concordance between FoundationOne Liquid CDx and FoundationOne Liquid has also been established (Table 6-9 in Appendix). Further concordance between FoundationOne Liquid and FoundationACT has also previously been established (Table 6-12 in Appendix); therefore, data generated from prior studies that used FoundationACT also support the use of FoundationOne Liquid and FoundationOne Liquid CDx. The extensive validation and concordance data for prior versions of Foundation Medicine's liquid biopsy tests (FoundationOne Liquid and FoundationACT) are provided in the Appendix (**Concordance Between FoundationOne Liquid and FoundationACT** and **Concordance Studies for FoundationACT**).

Tumor Mutational Burden

TMB is a measure of the number of somatic mutations per Mb of sequenced DNA. TMB is a genomic signature biomarker that has emerged as a predictor of immune checkpoint inhibitor treatment outcomes, independent of programmed death ligand-1 (PD-L1) status.[51,167] TMB is optimally calculated by whole exome sequencing (WES), but CGP panels provide TMB estimates in a more time- and cost-effective

manner.[168] However, there is considerable complexity in calculating TMB using a panel and algorithms.[168] A study that compared the results of panel TMB testing vs WES showed that there was variability within and between panel TMB values; despite these findings, this study found that panel TMB values were strongly correlated with WES TMB.[168]

FoundationOne Liquid CDx assesses bTMB through sequencing of all base substitutions with a ≥0.5% allele frequency. A computational model utilizes logic to remove germline polymorphisms and predicted driver mutations to provide a bTMB score that is reported as the number of mutations per Mb. FoundationOne Liquid CDx has a ≥95% probability of detection for bTMB (component indels and component subs) at a frequency of 1.0% variant allele fraction.[111,165]

Microsatellite Instability

FoundationOne Liquid CDx detects MSI-H status which may help inform decisions regarding the use of immunotherapy agents. MSI is a genomic signature biomarker that has been associated with improved responses to certain immunotherapies across a range of tumor types. MSI is a condition of genetic hypermutability that arises from defects in the dMMR system, which then generates excessive amounts of short insertion/deletion mutations in the genome.[169]

FoundationOne Liquid CDx assesses MSI-H by identifying and quantifying “unstable” loci, or loci with lengths that are inconsistent with a reference genome. To determine MSI status, approximately 2,000 repetitive loci (minimum of 5 repeat units of mono-, di-, and trinucleotides) are assessed to determine what repeat lengths are present in the sample. A locus containing a repeat length present in an internal database generated using >3,000 clinical samples is considered to be “unstable.” An MSI indicator is generated by calculating the fraction of unstable loci, considering only those loci that achieve adequate coverage for consideration for the sample. Samples with >0.5% unstable loci are considered to be MSI-High.[111] Instability is assessed for each locus relative to the reference genome after passing through coverage and clinical database germline filters. FoundationOne Liquid CDx has a ≥95% probability of detection for MSI-H with a frequency of 0.8% unstable loci.[111,165]

4 CLINICAL UTILITY OF FOUNDATIONONE LIQUID CDX

Biomarker-based targeted therapies have led to considerable improvements in clinical outcomes including response rates and survival compared with traditional chemotherapy.[8,55-58] CGP testing provides valuable information on the presence of actionable biomarkers, which enables healthcare providers to make evidence-based treatment decisions regarding treatments that result in these improved outcomes for patients with advanced cancer.[10,47,48,170] Of patients with advanced cancer who undergo CGP, 51.7% to 99% will have an actionable alteration that can be matched to either a targeted therapy or to a genomically matched clinical trial.[8,11,53,55-67]

FoundationOne Liquid CDx is an FDA-approved ctDNA-based (liquid biopsy) CGP assay approved for use as a companion diagnostic to identify patients who may benefit from treatment with targeted therapies in accordance with the approved therapeutic product labeling. The following sections review: the clinical utility of CGP overall (**Evidence of Improved Clinical Outcomes With CGP**), liquid biopsy-based CGP overall (**Evidence of Improved Clinical Outcomes With Liquid Biopsy-Based CGP**), and FoundationOne Liquid CDx, specifically in **NSCLC**, **prostate cancer**, **breast cancer**, and **ovarian cancer**.

Evidence of Improved Clinical Outcomes With CGP

Clinical utility establishes the net clinical benefit to the patient of adding CGP to the current standard of care decision making; in effect, does the intervention (ie, the CGP test) improve patient outcomes?[103] As shown in Table 4-1, several pan-tumor and tumor-specific cohort studies have demonstrated substantial improvements in patient outcomes, including RR, PFS, and OS, associated with CGP testing.[22,73]

Table 4-1. Clinical Utility of CGP

Author/year	Study design	Clinical impact		
		Populations compared	Outcome measure[a]	*P*-value
Pan-tumor				
Kato 2018[63]	Prospective study of the utility of tissue and liquid CGP in patients with rare cancers (n=40)	Matched targeted therapy (n=12)	PFS: 19.7 months	*P*=0.008
		Previous unmatched therapy (n=12)	PFS: 3.5 months	
Schwaederle 2016[73]	Retrospective study of the utility of CGP to match patient with advanced solid malignancies to a therapy (n=347)	Matched therapy (n=87)	DCR: 34.5%	*P*≤0.02
		Unmatched therapy (n=93)	DCR: 16.1%	
		Matched therapy (n=87)	PFS: 4.0 months	*P*=0.039
		Unmatched therapy (n=93)	PFS: 3.0 months	
Wheler 2016[67]	Single-arm, nonrandomized study to prospectively investigate the clinical utility of CGP in patients with advanced malignancies (N=500)	Matched therapy (n=122)	DCR: 19%	*P*=0.061
		Unmatched therapy (n=66)	DCR: 8%	
		Matched therapy (n=122)	TTF: 2.8 months	*P*=0.001
		Unmatched therapy (n=66)	TTF: 1.9 months	
		Matched therapy (n=122)	OS: 9.3 months	*P*=0.087
		Unmatched therapy (n=66)	OS: 7.2 months	
Sicklick 2019[66] **I-PREDICT**	Prospective navigation trial at 2 centers using tissue-based CGP to match patients to therapies based on a matching score[b] (n=73)	High matching score (n=28)	PFS: 6.5 months	*P*=0.046
		Low matching score (n=55)	PFS: 3.1 months	
NSCLC				
Madison 2020[104]	A retrospective, real-world study to determine clinical outcomes for NSCLC	Patients with genomic alteration matched to targeted therapy (n=287)	rwPFS: 9.4 months	*P*=0.022

	patients following CGP with liquid biopsy or tissue biopsy (n=6,491)	Patients with genomic alteration not treated with targeted therapy (n=130)	rwPFS: 6.9 months	
		Patients with genomic alteration matched to targeted therapy (n=262)	OS: 26.7 months	*P*=0.035
		Patients with genomic alteration not treated with targeted therapy (n=130)	OS: 17.9 months	
Singal 2019[78]	A retrospective study to determine the clinical utility of a clinico-genomic database (using CGP) in patients with NSCLC (n=4,064)	Patients with driver alteration treated with targeted therapy (n=575)	OS: 18.6 months	*P*<0.001
		Patients with driver alteration not treated with targeted therapy (n=560)	OS: 11.4 months	
Pancreatic				
Pishvaian 2018[74]	Prospective program (Know Your Tumor) using CGP to determine matched therapy in patients with pancreatic cancer (n=640)	Matched therapy (n=17)	PFS: 4.1 months	*P*=0.03
		Unmatched therapy (n=18)	PFS: 1.9 months	
Pishvaian 2020[77]	Prospective program (Know Your Tumor) using CGP to determine matched therapy in patients with pancreatic cancer (n=1,856)	Matched therapy (n=46)	OS: 2.58 years	P=0.0004
		Unmatched therapy (n=143)	OS: 1.51 years	

[a] PFS, TTF, and OS as presented above are all measured as medians (however, for Madison 2020, the type of measurement for rwPFS and OS are not reported). The DCR is the percentage of patients achieving a complete response, partial response, or stable disease for ≥6 months.

[b] A "matching score" score system was then utilized for each patient. Blinded to patient outcomes, the investigators calculated the total number of molecular alterations matched to the drugs administered and divided that number by the total number of characterized genomic aberrations.

CGP, comprehensive genomic profiling; DCR, disease control rate; NSCLC, non-small cell lung cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; rwPFS, real-world progression-free survival; TTF, time to treatment failure.

Decision Impact of CGP in Clinical Practice

CGP most commonly guides the use of genomically matched targeted therapies; in this regard, it can provide information about uses of FDA-approved therapies.[11,53] As NCCN Guidelines state that the best management of any patient with cancer is in a clinical trial[25-45], CGP is also important for determination of eligibility of a substantial proportion of oncology clinical trials (~40%).[18] CGP can also provide genomic information that enables physicians to use chemotherapy more effectively, as in the case of homologous recombination deficiency (HRD) ovarian cancer.[54] Recent data show that the majority of oncologists are using CGP in their current clinical practice.[72]

- A recent national survey using data from the National Survey of Precision Medicine in Cancer Treatment reported that 75.6% of oncologists use CGP to guide treatment decisions.[72] Of these physicians, CGP was used to guide the use of an FDA-approved therapy (33.5%), determine eligibility for clinical trial enrollment (29.1%), and/or make decisions about off-label use of FDA-approved therapies (17.5%).[72] Further, CGP test results informed treatment decisions often and sometimes for 26.8% and 52.4% of respondents, respectfully.[72]

This use of CGP in clinical practice allows patients to receive genomically matched therapy; data show that up to 50% of patients pursue genomically matched therapy, including on-label and off-label FDA approved therapies and clinical trial enrollment.[53,59-62,64,72,73] As approximately 40% of oncology clinical trials require a biomarker for eligibility and/or stratification, CGP-based testing has been associated with a clinical trial enrollment rate between 10% and 20%, compared with a historical enrollment rate of ≤8%.[53,59-61,74,75] Even in those without or unable to pursue genomically matched options, the personalized treatment plan may confirm chemotherapy as the best option and/or help with discussions about palliative care, thereby avoiding the use of unnecessary therapies. By matching more patients to effective therapeutic options, a CGP approach has resulted in improved patient outcomes, including significantly improved response rates and survival, compared with standard of care (unmatched) therapy.[63,66,67,73,74,77,78]

Potential Causes of Lack of Tissue-Based CGP Testing

Even though CGP testing has many advantages over single gene tests or hotspot testing, and tissue-based testing is considered the gold-standard approach to molecular testing, tissue is not always available or feasible to obtain.[95,96]

Tissue Is Insufficient or Inaccessible

Genomic profiling of solid tumors typically requires a sufficient tissue sample for analysis. Most solid tumor diagnoses require a tissue sample; thus, tissue from this initial diagnostic biopsy can, in many cases, be leveraged for genomic profiling. However, the available tissue is inadequate for tissue-based CGP in up to 51% of cases.

- A series of 1,528 solid and hematolymphoid tumors were tested by CGP, of which testing was unsuccessful in 343 specimens (22.5%); the majority of tests were unable to be completed due to insufficient tissue (223/343, 65%) or insufficient DNA (99/343, 28.9%).[171]
- A prospective cohort study of 323 patients with NSCLC included 101 patients who underwent blood-based CGP because tissue-based CGP was not possible (79 of whom had insufficient biopsy tissue).[172]

- In a study of 102 patients with advanced NSCLC, tissue-based CGP could not be completed in 52 (51%) patients due to insufficient quantity (n=24), difficulty accessing tissue (ie, brain or bone metastases; n=12), unavailable tissue (n=9), target gene testing (n=3), and other reasons (n=4).[173]
- In another retrospective study of patients with advanced NSCLC evaluating the genomic alterations in blood-derived ctDNA, 25 of 88 (28.4%) patients did not have any tissue molecular testing completed because of inadequate tissue or biopsy contraindications.[174]
- A prospective study that reported the clinical utility of liquid biopsy using cfDNA-based CGP was undertaken in a cohort of 50 patients at an academic medical center with NSCLC; reasons for liquid biopsy included insufficient tissue (32%), addition to tissue analysis (32%), and alternative to tissue biopsy (13%).[175]
- A report describing the feasibility of tissue-based NGS reported that the assay was able to successfully sequence 55% of samples from 381 patients with NSCLC.[176] The reasons for not performing tissue-based sequencing on the remaining 172 patients included insufficient tissue at intake (at pathologist review, 78 [21%]), study canceled (56 [15%]), inadequate DNA quality or quantity after extraction (32 [8%]), and failed library preparation (6 [2%]).[176]
- A prospective, single-center, single-arm trial enrolled 142 patients with metastatic breast cancer within 10 weeks of starting a new therapy.[177] In this analysis, 21 patients were excluded due to no available tissue, insufficient tissue, or poor DNA quality, meaning 15% of patients considered for tissue testing were unable to be tested.[177]
- A whole genome sequencing study analyzed 570 patients with metastatic breast cancer utilizing tissue biopsied from their metastatic site(s).[178] The metastatic biopsy sites included the liver, lymph node, bone, and soft tissue.[178] Within this analysis, 22% of all metastatic biopsies were non-evaluable, with tissue obtained from bone metastases having the highest failure rated of 33%.[178]

Biopsy or Rebiopsy Poses Unacceptable Risks

Tissue sampling during biopsy can, in many cases, lead to complications that affect patient health. This is particularly notable in the lung where, whether by core needle biopsy or fine-needle approaches, sampling is invasive and associated with high rates of complications.[179]

A meta-analysis of computed tomography-guided transthoracic lung biopsies found that pooled complication rates from core needle biopsy and fine-needle aspiration, respectively, were:

- *Pneumothorax*: 25.3% (95% CI: 22.2–28.6) and 18.8% (95% CI: 14.6–23.9);
- *Pulmonary hemorrhage*: 18.0% (95% CI: 13.4–23.8) and 6.4% (95% CI: 2.5–15.2);
- *Hemoptysis*: 4.1% (95% CI: 2.8–6.1) and 1.7% (95% CI: 0.9–3.1); and
- *Overall complications*: 38.8% (95% CI: 34.3–43.5) and 24.0% (95% CI: 18.2–30.8).

A 15%–25% complication rate for pneumothorax is also associated with transthoracic needle biopsy or transthoracic needle aspiration, of which 4%–6% require chest tube drainage.[180] In general, complication rates following surgical lung biopsy are very high with a 30-day mortality rate of 1%–2%.[181,182]

Time Sensitivity Makes Tissue-Based Testing Impractical

Even in cases where a biopsy or rebiopsy procedure is feasible, patients may still be hindered by a time-sensitive situation where the time required for tissue-based testing will lead to an unacceptable

treatment delay. Logistical burdens associated with sample acquisition and scheduling time involved for tissue biopsy procedures can delay decisions to use time-sensitive treatments, which can negatively affect patient outcomes.[183-185]

Guideline Recommendations for Liquid Biopsy

To date, NSCLC as a disease state has experienced the most advancement with ctDNA technology, and, as such, the guidelines have specific recommendations regarding liquid biopsies in eligible patients with metastatic NSCLC.

- NCCN Guidelines for NSCLC V.3.2022 recommend that plasma-based molecular testing (ie, plasma cell free/ctDNA testing) can be considered in specific clinical circumstances, most notably if a patient is medically unfit for invasive tissue sampling; when there is insufficient material for molecular analysis after pathologic confirmation of a metastatic NSCLC diagnosis, if follow-up tissue-based analysis is planned for all patients for whom an oncogenic driver is not identified; or, in the initial diagnostic setting, if tissue-based testing does not completely assess all recommended biomarkers owing to tissue quantity or testing methodologies available, consider repeat biopsy and/or cell-free/circulating tumor DNA testing.[33] Additionally, if there is insufficient tissue to allow testing for all of *EGFR, ALK, KRAS, ROS1, BRAF, MET, NTRK1/2/3,* and *RET*, repeat biopsy and/or plasma testing should be done.[33]
- Similarly, an updated evidence-based guideline published by the International Association for the Study of Lung Cancer (IASLC), the College of American Pathologists (CAP), and the Association for Molecular Pathology (AMP) recommends blood-based assays to identify *EGFR* mutations in some clinical settings in which tissue is limited and/or insufficient for molecular testing.[186] Specifically, liquid biopsy can be considered at the point of initial diagnosis in all patients who need genomic profiling, and it is particularly recommended when tissue is scarce or unavailable, when a delay in obtaining tissue is expected, or when biopsy poses a risk to the patient's health.[186]
- Since the publication of the first IASLC liquid biopsy statement in 2018, several novel and impressive technological advances have been made; additionally, the growing clinical application of plasma-based NGS and the recent FDA approval of 2 different assays for ctDNA analysis led to the need for an update of this consensus statement from IASLC in 2021.[187,188] In this statement, IASLC states that liquid biopsy represents a practical alternative source to tissue biopsy for investigating tumor-derived somatic alterations.[187] Plasma ctDNA can now be considered a valid tool for genotyping of newly diagnosed patients with advanced NSCLC, and results are often complementary to those of tissue analysis.[187] This consensus statement goes further with the recommendation that in patients with oncogene-addicted NSCLC, liquid biopsy is emerging as not only complementary to tissue-based analysis but also acceptable as the initial approach ("plasma first") for biomarker evaluation at the time of diagnosis and for monitoring the efficacy of targeted therapies.[187] Further, at the time of acquired resistance after tyrosine kinase inhibitor therapy in an oncogene-driven NSCLC, initial use of ctDNA is preferred for evaluation of mechanisms of resistance ("plasma-first") with repeat tissue biopsy if plasma ctDNA is uninformative.[187] As such, liquid biopsy is now the preferred method of molecular testing in some clinical settings and has proven complementary to tumor tissue testing in others.[187] Additionally, the IASLC states that although the data are most robust in NSCLC, patients with other cancer

types may also benefit from this minimally invasive approach to facilitate selection of targeted therapies.[187]

In addition to NSCLC, several NCCN Guidelines now specifically recommend plasma testing in certain clinical circumstances, including breast cancer, cervical cancer, colon cancer, esophageal and esophagogastric junction cancers, gastric cancer, pancreatic cancer, prostate cancer, and rectal cancer; please refer to the **NCCN Guidelines: Recommendations for Molecular Testing** and the individual NCCN Guidelines for more specific information.[25,28,30,31,33,35-37,42]

Evidence of Improved Clinical Outcomes With Liquid Biopsy-Based CGP

The clinical utility of liquid biopsy-based CGP is rapidly evolving, with several studies to date demonstrating substantial improvements in patient outcomes, including RR, PFS, and OS, associated with liquid CGP testing.[47,104,106] Several examples of the improved outcomes associated with CGP testing are briefly discussed in Table 4-2.

Table 4-2. Clinical Utility of Liquid Biopsy-Based CGP

Reference	Study design	Frequent genomic alterations identified	Clinical outcomes
Breast cancer			
Wongchenko et al. (2020)[106] (Manuscript)	Phase 2, prospective LOTUS trial of patients with metastatic triple-negative **breast cancer** (N=89) who underwent pretreatment tissue CGP and cfDNA analysis	≥1 mutation detected by cfDNA: 81 (91%) ▪ PPA with tissue sequencing was 84% for known or likely short variant mutations ▪ 16 alterations detectable in plasma that were not identified using tissue ▪ 18 patients (25% of the 73 patients with evaluable tumor samples), an activating *PIK3CA* (n=12) or *AKT1* (n=6) mutation was detected in ctDNA; concordance with tissue sequencing was 100%	**High- vs low-CTF associated with:** Shorter median PFS: ▪ First-line ipatasertib + paclitaxel (HR: 2.55; 95% CI: 1.14, 5.64) ▪ Placebo + paclitaxel (HR: 2.38; 95% CI: 1.17, 5.05) **Detectable vs non-detectable *PIK3CA/AKT1* mutation associated with:** Improved median PFS: ▪ Patients with detectable *PIK3CA/AKT1* mutation: HR: 0.15 (95% CI: 0.02, 0.62) ▪ Patients without *PIK3CA/AKT1* mutation: HR: 0.86 (95% CI: 0.48, 1.51) ctDNA successfully selected patients who improved when administered first-line ipatasertib + paclitaxel
NSCLC			
Madison et al. (2020)[104]	A retrospective review of a clinicogenomic database including	A targetable GA was detected by liquid	**All patients with a detected targetable GA (liquid biopsy or tissue**

Reference	Study design	Frequent genomic alterations identified	Clinical outcomes
(Manuscript)	6,491 patients with NSCLC and liquid biopsy (n=937 tests) and/or tissue (n=5,582 tests) to evaluate the clinical outcomes for patients following CGP using liquid biopsy and/or tissue biopsy to guide the receipt of matched, targeted therapy in the real-world setting	biopsy in 20.0% tests (188/937) ▪ 95% non-SCC NSCLC ▪ 2% SCC NSCLC ▪ 3% NSCLC-NOS	**CGP) who received first-line matched targeted therapy showed:** Longer rwPFS[a]: ▪ First-line matched targeted therapy (N=287): 9.4 months ▪ Other first-line therapies (N=130): 6.9 months ▪ aHR: 0.72 (95% CI: 0.54, 0.95) Longer OS[a]: ▪ First-line matched targeted therapy (N=287): 26.7 months ▪ Other first-line therapies (N=130): 17.9 months ▪ aHR: 0.70 (95% CI: 0.50, 0.98) **rwPFS was comparable between patients in the liquid biopsy (n=33) and tissue cohorts (n=229) receiving first-line matched targeted therapy after CGP** ▪ Liquid CGP: 13.8 months ▪ Tissue CGP: 10.6 months ▪ aHR: 0.68 (95% CI: 0.36, 1.26)
Dziadziuszko 2021[105]	A phase II/III global, multi-center, open-label, prospective clinical trial (BFAST) screened patients (N=2,219) for oncogenic somatic mutations using liquid biopsy with FoundationACT (a prior version of FoundationOne Liquid CDx) for first-line targeted therapies in locally advanced or metastatic NSCLC; a cohort of patients was determined to have ALK-positive disease (n=119), and those patients who met treatment eligibility criteria were treated with alectinib (n=87)	*ALK*-positive NSCLC was identified in 5.4% ▪ Results were available in 98.6% of cases (1.4% assay failure)	**ctDNA-based NGS informed clinical decision making in *ALK*-positive NSCLC with significant clinical benefit as evidenced by clinical efficacy outcomes[b]** ▪ ORR: 87.4% (95% CI: 78.5, 93.5) ▪ Median DOR: Not reached ▪ 12-month DOR: 75.9% ▪ Median PFS: Not reached ▪ 12-month PFS: 78.4% ▪ 12-month OS: 86.8% Investigators reported that 22/87 (25%) patients did not have a positive tissue test for a BFAST alteration, including *ALK*, yet of those 18/22 (82%) responded to alectinib

[a] In Madison 2020, the outcome measurement of rwPFS and OS were not defined.

[b] Efficacy outcomes are per investigator-assessment.

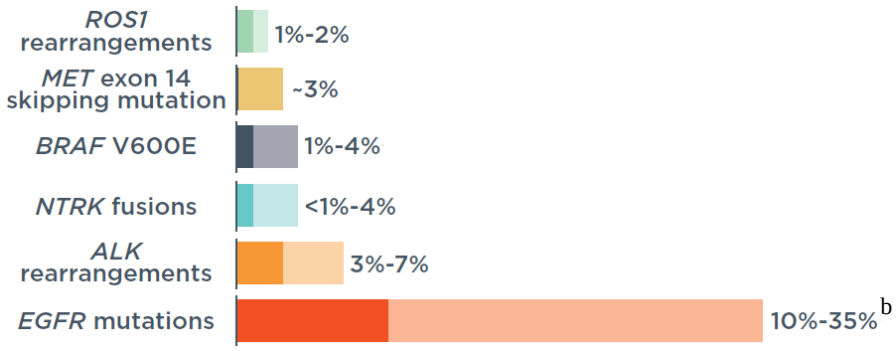
aHR, adjusted hazard ratio; ALK, anaplastic lymphoma kinase; CGP, comprehensive genomic profiling; cfDNA, cell-free DNA; CI, confidence interval; ctDNA, circulating tumor DNA; CTF, circulating tumor DNA fraction; EGFR, epidermal growth factor receptor; GA, genomic alteration; HR, hazard ratio; NSCLC, non-small cell lung cancer; NOS, not otherwise specified; OS, overall survival; ORR, overall response rate; PFS, progression-free survival; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; rwPFS, real-world progression-free survival; SCC, squamous cell carcinoma; STK11, serine/threonine kinase 11.

FoundationOne Liquid CDx is intended to be used as a companion diagnostic to identify patients who may benefit from treatment with specific targeted therapies in NSCLC (**erlotinib**, **gefitinib**, **osimertinib**, **alectinib**, or **capmatinib**), prostate cancer (**rucaparib**, **olaparib**), breast cancer (**alpelisib**), or ovarian cancer (**rucaparib**).

Liquid Biopsy-Based CGP in NSCLC

- Lung cancer accounts for 12.4% of new cancer diagnoses and up to 22% of cancer-related deaths in 2021, with NSCLC accounting for 80% of cases.[3,189,190] Survival for advanced metastatic lung cancer at 5 years is 6.3%, with over half of the patients (56%) diagnosed at this stage.[189]
- Historically, traditional chemotherapy has resulted in poor survival outcomes, with a median survival of less than 1 year in a real-world population with advanced NSCLC.[191]
- Conversely, outcomes of patients with advanced NSCLC with an oncogenic driver given genotype-directed therapy are much improved compared with standard of care chemotherapy.[100]
- Actionable mutations in NSCLC are numerous, with up to 1 in 3 patients having a mutation with an FDA-approved matched therapy (Figure 4-1).[98]

Figure 4-1. Prevalence of Actionable Genomic Alterations in NSCLC Patients[a]



[a] Additionally, PD-L1, an immune biomarker, is found in up to 66% of patients with NSCLC and may be regulated by certain oncogenic drivers.[192]
[b] *EGFR* driver mutation prevalence varies by ethnicity; for example, among East Asians the prevalence is 40%–55% and among Caucasians is 5%–15%.[193]
Sources: Gainor 2013[194]. Bubendorf 2016[195]. Baik 2017[196]. Guo 2019[197]. Chu 2020[198]. Golding 2018[199]. Hofman 2017[200]. Aran 2019[201].

- Pertinent organizations that provide clinical care guidance in metastatic NSCLC are aligned that broad molecular profiling panel tests are preferred over multiple single-gene tests to more broadly capture targeted treatment options. The current recommendations for molecular testing include *EGFR*, *ALK*, *KRAS*, *ROS1*, *BRAF*, *NTRK1/2/3*, *MET* exon 14 skipping, and *RET*.[33,99,186]
- Clinical validity of the FoundationOne Liquid CDx assay was evaluated as a companion diagnostic in identification of patients with advanced NSCLC who may be eligible for treatment with drugs targeting *EGFR* mutations (ie, erlotinib, gefitinib, osimertinib) or *ALK* rearrangements (ie, alectinib).[1] For *EGFR* mutations, noninferiority was demonstrated to a predicate companion diagnostic device, whereas for *ALK* rearrangements and *MET* exon 14 skipping mutations, clinical bridging outcomes from the registrational trials of alectinib and capmatinib, respectively, were used for the companion diagnostic approval.[1]
    - For *EGFR* mutations, the positive percent agreement (PPA) and negative percent agreement (NPA) of the reference test and FoundationOne Liquid CDx were 97.7% and 95.4-95.6%, respectively.[1]
    - For *ALK* rearrangements and *MET* exon 14-skipping mutations, the FoundationOne Liquid CDx assay was shown to be concordant to and have comparable clinical efficacy

results as the clinical trial assay (CTA).[1]

Unmet Need for Molecular Testing in NSCLC

There are numerous mutations in advanced NSCLC that affect treatment decisions; targetable genomic alterations that occur in NSCLC include *EGFR* mutations, *ALK* rearrangements, *KRAS* mutations, *ROS1* rearrangements, *BRAF* mutations, *NTRK1/2/3* gene fusions, *MET* exon 14 skipping mutations, and *RET* rearrangements.[33,98]

Outcomes of patients with advanced NSCLC with an oncogenic driver given genotype-directed therapy are much improved as compared with standard of care chemotherapy; this has been shown in randomized controlled trials as well as in real-world populations of patients with NSCLC.[100]

- In a study that included 14 sites within the US that enrolled patients with metastatic NSCLC (n=1007) and tested their tumors for 10 oncogenic drivers (*EGFR, ALK, KRAS, NRAS, BRAF, ERBB2, PIK3CA, MEK, AKT1*, and *MET*), the median OS was significantly prolonged for patients with an oncogenic driver mutation that received genotype-directed targeted therapy (Table 4-3).[100]

Table 4-3. Overall Survival by Receipt vs No Receipt of Genotype-Directed Therapy in Metastatic NSCLC Patients

Population	n	Median survival (95% CI)
Oncogenic driver and genotype-directed therapy[a]	260	3.5 years (3.0, 4.3)
Oncogenic driver and no genotype-directed therapy[a]	318	2.4 years (1.8, 2.9)
No oncogenic driver detected	360	2.1 years (1.8, 2.5)

[a] Propensity score-adjusted HR (driver mutation detected and genotype-directed therapy given vs driver mutation detected but no genotype-directed therapy given): 0.69 [95% CI: 0.53, 0.9]; *P*=0.006).

CI, confidence interval; HR, hazard ratio; NSCLC, non-small cell lung cancer.

Source: Kris 2014[100]

Due to the improvement in outcomes for patients treated with targeted therapy, the pertinent organizations that provide clinical care guidance for eligible patients with metastatic NSCLC (ie, the NCCN, the American Society of Clinical Oncology [ASCO], CAP, AMP, and IASLC) are all aligned that broad molecular profiling panel tests are recommended over multiple single-gene tests to more broadly capture targeted treatment options. The current recommendations for molecular testing in eligible patients with metastatic NSCLC include *EGFR, ALK, KRAS, ROS1, BRAF, NTRK1/2/3, MET* exon 14 skipping, and *RET*.[33,99,186]

Despite recommendations, and the availability of genotype-directed targeted therapy with improved survival outcomes, not all eligible patients are being tested.

- A retrospective analysis, within a large electronic health record database, of patients with advanced NSCLC (n=1,203) receiving treatment within community practices (encompassing 289 oncologists) found that the testing rate for all biomarkers with an FDA-approved on-label drug (*EGFR, ALK, ROS1,* and *BRAF*) was 22%, and testing for all 7 professional guideline-

recommended genes for associated therapies was 7%, excluding testing for PD-L1 as it is can be measured via immunohistochemistry (IHC) rather than via genomic testing.[140]

A contributing factor to a lack of guideline-recommended testing may be insufficient tissue for CGP. In fact, studies in patients with advanced NSCLC reveal that these patients are at risk of insufficient tissue for complete molecular testing.

- For example, in a single-center prospective study of 229 patients with advanced NSCLC who consented for concurrent testing with both ctDNA and tissue, 34% (n=79) were unable to complete NGS tissue testing because their biopsied tissue was of insufficient quantity or quality; further, an additional 9.6% (n=22) did not have tissue available, and biopsy was not technically possible.[101]
- In another prospective analysis of advanced NSCLC patients (n=264) who consented to undergo molecular testing with both tissue and liquid testing, 32.6% (n=86) did not have successful tissue testing for any genomic alteration due to insufficient tissue quality or quantity.[102]
- In a study of 102 patients with advanced NSCLC, tissue-based CGP could not be completed in 52 (51%) patients due to insufficient quantity (n=24), difficulty accessing tissue (ie, brain or bone metastases; n=12), unavailable tissue (n=9), target gene testing (n=3), and other reasons (n=4).[173]
- A report describing the feasibility of tissue-based NGS reported that the assay was able to successfully sequence 55% of samples from 381 patients with NSCLC.[176] The reasons for not performing tissue-based sequencing on the remaining 172 patients included insufficient tissue at intake (at pathologist review, 78 [21%]), study canceled (56 [15%]), inadequate DNA quality or quantity after extraction (32 [8%]), and failed library preparation (6 [2%]).[176]

Place of Liquid Biopsy-Based CGP in NSCLC

Liquid biopsy can be considered at the point of initial diagnosis in all patients who need genomic profiling, and it is particularly recommended when tissue is scarce or unavailable, when a delay in obtaining tissue is expected, or when biopsy poses a risk to the patient's health.[186]

Liquid biopsies have the potential to overcome potential hurdles associated with accessing CGP. In all scenarios of unavailable or limited tissue, liquid biopsy may be an alternative method for obtaining genomic information from ctDNA.

- A prospective cohort study of 323 patients with NSCLC included 101 patients who underwent blood-based CGP because tissue-based CGP was not possible (79 of whom had insufficient biopsy tissue).[172] Of 113 patients with therapeutically targetable genomic alterations, 35 (31%) had an alteration detected from blood-based CGP, including 27 (26.7%) of the 101 patients who could not undergo tissue-based CGP.[172]
- In a study of 102 patients with advanced NSCLC, tissue-based CGP could not be completed in 52 (51%) patients, whereas ctDNA testing was successful in the entire sample.[173]

Further, per the NCCN Guidelines, plasma-based molecular testing can be considered in specific clinical circumstances in eligible patients with metastatic NSCLC, most notably if a patient is medically unfit for invasive tissue sampling or when there is insufficient material for molecular analysis after pathologic confirmation of an NSCLC diagnosis; if follow-up tissue-based analysis is planned for all patients for whom an oncogenic driver is not identified; or, in the initial diagnostic setting, if tissue-based testing does not completely assess all recommended biomarkers owing to tissue quantity or testing methodologies

available, consider repeat biopsy and/or cell-free/circulating tumor DNA testing.[33] Additionally, if there is insufficient tissue to allow testing for all of *EGFR*, *ALK*, *KRAS*, *ROS1*, *BRAF*, *MET* exon 14 skipping, *NTRK1/2/3*, and *RET*, repeat biopsy and/or plasma testing should be done in eligible patients with metastatic NSCLC.[33]

Similarly, an updated evidence-based guideline published by IASLC/CAP/AMP recommends blood-based assays to identify *EGFR* mutations in some clinical settings in which tissue is limited and/or insufficient for molecular testing.[186]

Since the publication of the first IASLC liquid biopsy statement in 2018, several novel and impressive technological advances have been made; additionally, the growing clinical application of plasma-based NGS and the recent FDA approval of 2 different assays for ctDNA analysis led to the need for an update of this consensus statement from IASLC in 2021.[187,188] In this statement, IASLC states that liquid biopsy represents a practical alternative source to tissue biopsy for investigating tumor-derived somatic alterations.[187] Plasma ctDNA can now be considered a valid tool for genotyping of newly diagnosed patients with advanced NSCLC, and results are often complementary to those of tissue analysis.[187] This consensus statement goes further with the recommendation that in patients with oncogene-addicted NSCLC, liquid biopsy is emerging as not only complementary to tissue-based analysis but also acceptable as the initial approach ("plasma first") for biomarker evaluation at the time of diagnosis and for monitoring the efficacy of targeted therapies.[187] Further, at the time of acquired resistance after tyrosine kinase inhibitor therapy in an oncogene-driven NSCLC, initial use of ctDNA is preferred for evaluation of mechanisms of resistance ("plasma-first") with repeat tissue biopsy if plasma ctDNA is uninformative.[187] As such, liquid biopsy is now the preferred method of molecular testing in some clinical settings and has proven complementary to tumor tissue testing in others.[187] Per the IASLC consensus statement, the literature now supports extension of ctDNA analysis to all guideline-recommended and treatable oncogenic drivers, including *EGFR* mutations, *ALK* rearrangements, *ROS1* rearrangements, *BRAF* mutations, *MET* exon 14 skipping mutations, *RET* rearrangements, and *HER2* mutations.[187]

Table 4-4 summarizes a selection of applicable guidelines supporting molecular profiling for NSCLC, as well as the ability of the FoundationOne Liquid CDx Assay to test for these recommended biomarkers.

Table 4-4. Review of Guideline-Recommended Biomarker Testing and FoundationOne Liquid CDx Assay Capabilities

Applicable guidelines for tumor profiling	Recommended biomarker testing	Biomarker included in FoundationOne Liquid CDx assay
Metastatic NSCLC **NCCN Guidelines for NSCLC[a] (V.3.2022)[33]**	*EGFR* exon 19 deletions or L858R mutation (Category 1 for both)	Yes
	EGFR exon 20 T790M alterations (Category 1)	Yes
	EGFR S768I, L861Q, or G719X mutations	Yes
	EGFR exon 20 insertion mutation	Yes
	ALK rearrangements (Category 1)	Yes
	KRAS G12C mutation	Yes

Applicable guidelines for tumor profiling	Recommended biomarker testing	Biomarker included in FoundationOne Liquid CDx assay
	BRAF V600E	Yes
	ROS1 rearrangements	Yes
	RET rearrangements	Yes
	NTRK1/2/3 gene fusions	Yes
	MET exon 14-skipping mutation	Yes
	PD-L1	No[b]
	High-level *MET* amplification[c]	Yes
	ERBB2 (HER2) mutations[c]	Yes
IASLC/CAP/AMP guideline[186]	*EGFR*	Yes
	ALK	Yes
	ROS1	Yes
	ERBB2[d]	Yes
	MET[d]	Yes
	BRAF[d]	Yes
	KRAS[d]	Yes
	RET[d]	Yes
IASLC statement[187]	*EGFR*	Yes
	ALK	Yes
	ROS1	Yes
	BRAF	Yes
	MET exon 14 skipping	Yes
	RET	Yes
	HER2 mutations	Yes

[a] **Category 1:** Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate. **Category 2A:** Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate. All NCCN recommendations are category 2A unless otherwise indicated.

[b] PD-L1 testing is available from Foundation Medicine.

[c] Defined as an emerging biomarker.

[d] Molecular testing for this gene is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include as part of larger testing panels performed either initially or when routine *EGFR*, *ALK*, and *ROS1* testing are negative.

ALK, anaplastic lymphoma kinase; AMP, Association of Molecular Pathologists; CAP, College of American Pathologists; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; IASLC, International Association for the Study of Lung Cancer; KRAS, V-Ki-ras2 Kirsten rat sarcoma; MET, mesenchymal epithelial transition factor receptor; NCCN, National Comprehensive Cancer Network; NSCLC, non-small cell lung cancer; NTRK, neurotrophic receptor tyrosine kinase; PD-L1, programmed death ligand-1.

Clinical Utility and Validity of FoundationOne Liquid CDx in NSCLC

FoundationOne Liquid CDx is FDA-approved to report substitutions and indels in 311 genes, rearrangements in 4 genes, and copy number alterations in 3 genes. bTMB, MSI-H status, and tumor fraction are reported as a laboratory professional service which is not reviewed or approved by the FDA.[165] Comprehensive results across all 324 genes are reported as a laboratory professional service which is not reviewed or approved by the FDA. FoundationOne Liquid CDx utilizes circulating cfDNA isolated from plasma derived from the anticoagulated peripheral whole blood of cancer patients. The test is intended to be used as a companion diagnostic to identify patients with NSCLC who may benefit from treatment with the targeted therapies listed in Table 4-5 in accordance with the approved therapeutic product labeling. Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified healthcare professionals in accordance with professional guidelines in oncology for patients with malignant neoplasms, including NSCLC.[1]

Table 4-5. Companion Diagnostic Indications Pertinent for Patients With NSCLC

Tumor type	Biomarker(s) detected	Therapy
NSCLC	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Iressa® (gefitinib) Tagrisso® (osimertinib) Tarceva® (erlotinib)
	ALK rearrangements	Alecensa® (alectinib)
	MET exon 14 skipping mutations	Tabrecta® (capmatinib)

EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1]

EGFR to Determine Treatment with Gefitinib, Osimertinib, or Erlotinib

Clinical validity of the FoundationOne Liquid CDx assay was evaluated as a companion diagnostic in identification of patients with advanced NSCLC who may benefit from treatment with drugs targeting *EGFR* mutations (ie, erlotinib, gefitinib, osimertinib).[1] Retrospective samples (n=280) from NSCLC patients were included in this study, which were tested for *EGFR* alterations (specifically, exon 19 deletion and exon 21 L858R) by the FoundationOne Liquid CDx assay and the previously approved cobas® *EGFR* Mutation Test v2 (Roche Molecular Systems, referred to cobas assay).[1] Samples were tested across 2 replicates by the cobas assay (denoted as CCD1 and CCD2) and 1 replicate by FoundationOne Liquid CDx.[1]

- The PPA and NPA between the cobas replicates (CCD1 and CCD2) and FoundationOne Liquid CDx assays were as follows: PPA: 97.7% (CCD1) and 97.7% (CCD2); NPA: 95.6% (CCD1) and 95.4% (CCD2).[1]
- Based on these results, FoundationOne Liquid CDx has been demonstrated to be noninferior to the cobas assay for the detection of *EGFR* exon 19 deletions and *EGFR* exon 21 L858R mutations.[1]

ALK to Determine Treatment with Alectinib

The clinical validity of using FoundationOne Liquid CDx as a companion diagnostic to identify patients

with *ALK* rearrangement-positive NSCLC who may benefit from treatment with alectinib was assessed through a clinical bridging study using screening plasma samples from a cohort within the Blood First Assay Screening Trial (BFAST, NCT03178552).[1,105] The BFAST (Blood First Assay Screening Trial) was a Phase II/III, global, multi-center, open-label, prospective, multi-cohort study designed to evaluate the efficacy and safety of first-line targeted therapies in patients with locally advanced or metastatic NSCLC determined to harbor oncogenic somatic mutations (eg, *ALK*; *RET*) or TMB above the pre-specified cut-points of ≥ 16 mutations per megabase (mut/Mb) and ≥ 10 mut/Mb as identified by two blood-based NGS ctDNA assays.[202] There were multiple cohorts included in this trial: cohort A, *ALK* positive; cohort B, *RET* positive; and cohort C, bTMB.[202] Cohort A of the BFAST trial evaluated the safety and efficacy of alectinib as a treatment for patients with advanced or metastatic NSCLC who tested positive for an *ALK* rearrangement as determined by the blood-based NGS assay FoundationACT, a prior version of FoundationOne Liquid CDx.[1,105] Of the 2,219 patients screened, the CTA (FoundationACT) yielded results in 98.6% of cases; of these, 119 patients were determined to be *ALK*-positive. Of the 119 *ALK*-positive patients, 87 patients were treated with alectinib.[1,105]

- The PPA and NPA for FoundationOne Liquid CDx with FoundationACT as the reference assay were as follows: PPA, 84.0% (95% CI: 73.7, 91.4); NPA, 100.0% (95% CI: 97.9, 100.0). After adjusting for a 5% prevalence of *ALK* rearrangements in the intended use population, the positive predictive values (PPVs) and negative predictive values (NPVs) for FoundationOne Liquid CDx utilizing FoundationACT as the reference assay were as follows: PPV, 100.0% (95% CI: 94.3, 100.0); NPV, 93.5% (95% CI: 89.0%, 96.6%).[1]
- The primary endpoint for the study was investigator-assessed ORR based on confirmed objective response (indicated by two objective response assessments) based on Response Evaluation Criteria in Solid Tumors (RECIST) v1.1.[202] The ORRs were comparable for alectinib-treated patients who were identified by FoundationACT (ORR: 87.4% [95% CI: 78.5, 93.5])[105] and FoundationOne Liquid CDx (ORR: 88.9% [95% CI: 78.4, 95.4]).[1]
    - In the BFAST trial, using the FACT assay, the median DOR was not reached with a 12-month investigator-assessed DOR of 75.9%. Further, the median PFS was also not reached and the 12-month PFS rate per investigator assessment was 78.4%. The 12-month survival rate was 86.8%.[105]

MET to Determine Treatment With Capmatinib

The clinical validity and utility of using FoundationOne Liquid CDx as a companion diagnostic to identify patients with *MET* exon 14 skipping mutation-positive NSCLC who may benefit from treatment with capmatinib was assessed through a clinical bridging study using screening plasma samples from 2 cohorts within the GEOMETRY mono-1 study.[1,203] The GEOMETRY mono-1 study is a prospectively designed, multicenter, open-label, single-arm phase 2 study to evaluate the safety and efficacy of the MET inhibitor capmatinib in adult patients with *EGFR* wild-type and *ALK* rearrangement-negative, locally advanced or metastatic NSCLC harboring *MET* exon 14 skipping alterations.[1,203] There were 6 cohorts included in this trial, of which 2 included patients with *MET* exon 14 skipping mutations (the other 4 included patients with *MET* amplifications): Cohort 4 only enrolled pretreated (second- and third-line) patients with *MET* exon 14 deletions, and Cohort 5b only enrolled treatment-naïve patients with *MET* exon 14 deletions.[1,203] Patients were screened for enrollment into Cohorts 4 and 5b for *MET* exon 14 deletion status using tissue-based MET exon 14 deletion reverse-transcriptase PCR CTA.[1]

- For the 150 patients meeting the ≥30 ng cfDNA input, the PPA and NPA were determined to be 70.5% (95% CI: 59.1, 80.3) and 100% (95% CI: 95.0, 100), respectively. The point estimates of PPA and NPA in Cohort 4 for samples meeting the ≥30 ng cfDNA input sample requirements were 73.6% (95% CI: 59.7, 84.7) and 100% (95% CI: 91.8, 100), respectively, when excluding CDx invalid results. The point estimates of PPA and NPA in Cohort 5b for samples meeting the ≥30 ng cfDNA input sample requirements were 64.0% (95% CI: 42.5, 82.0) and 100% (95% CI: 88.1, 100), respectively, when excluding CDx invalid results.[1]
- The primary endpoint for the study was ORR by BICR assessments in patients with *MET* exon 14-skipping mutation-positive tumors by cohort.[1,203]
    - The ORRs were comparable for capmatinib-treated patients who were identified by the CTA (ORR: 40.6% [95% CI: 28.9, 53.1])[203] and FoundationOne Liquid CDx (ORR: 51.3% [95% CI: 34.8, 67.6]) for cohort 4. Additionally, the DOR was 9.84 months (95% CI: 4.17, 14.06) for FoundationOne Liquid CDx and 9.7 months (95% CI: 5.5, 13.0) [203] for the CTA for cohort 4.[1]
    - The ORRs were also comparable for capmatinib-treated patients identified by the CTA (ORR: 67.9% [47.6, 84.1]) [203] and FoundationOne Liquid CDx (ORR: 81.3% [54.4, 96.0]) for cohort 5b. The DOR was 25.33 months (95% CI: 4.24, 25.33) for FoundationOne Liquid CDx and 12.6 months (95% CI: 5.5, 25.3) [203] for the CTA for cohort 5b. The longer DOR observed with FoundationOne Liquid CDx samples was probably caused by the small number of events in the CTA-positive/FoundationOne Liquid CDx-positive patients (n=7).[1]

bTMB

bTMB is provided as a part of the professional services content for FoundationOne Liquid CDx and it is not part of the FDA-approved intended use for FoundationOne Liquid CDx.[165] The clinical utility of the bTMB FoundationOne® Liquid CDx test was assessed by retrospectively analyzing >1,000 plasma samples from patients with NSCLC receiving second-line therapy with atezolizumab (an anti-PD-L1 antibody) or docetaxel within 2 randomized clinical trials (the POPLAR trial [NCT01903993] and the OAK trial [NCT02008227]).[50] bTMB scoring is defined by counting the total number of variants present at >0.5% mutant allele frequency (MAF) and reported as mut/Mb unit; a threshold of ≥16 mut/Mb was determined to be optimal in terms of predicting treatment effect with atezolizumab.[50]

For patients with a bTMB of ≥16 mut/Mb within the POPLAR trial (n=63), the median PFS was 4.2 months in the atezolizumab arm and 2.9 months in the docetaxel arm (HR: 0.57 [95% CI: 0.33, 0.99]; *P*=0.055). The median OS values were 13.0 and 7.4 months, respectively (HR: 0.56 [95% CI: 0.31, 0.99]).[50] This was confirmed in the OAK trial; for patients with a bTMB ≥16 mut/Mb (n=158), the HRs for PFS and OS associated with atezolizumab vs docetaxel were 0.65 (95% CI: 0.47, 0.92; *P*=0.013) and 0.64 (95% CI: 0.44, 0.92; *P*=0.017), respectively.[50]

Real-World Evidence for FoundationOne Liquid CDx

Retrospective analysis of real-world data has demonstrated that acting on results of liquid biopsy CGP testing (with either FoundationOne Liquid or FoundationACT) is clinically valid, with tumor RRs comparable to results seen with therapy matched to tissue testing results (with either FoundationOne CDx

or FoundationOne).[104] In a study of patients with advanced NSCLC receiving care within the Flatiron database network between January 2011 and June 2019, and who underwent CGP using liquid biopsy CGP (n=934) and/or tissue CGP (n=5,570), a targetable genomic alteration was detected in 20.0% of patients with a liquid biopsy and 21.8% of those with a tissue biopsy.[104] A targetable genomic alteration was defined as either: a) biomarkers for FDA-approved therapies listed in guideline algorithms as follows: *EGFR* mutations, *ALK* rearrangement, *ROS1* rearrangement, and *BRAF* V600E; or b) alterations defined in guidelines as "emerging biomarkers" (*MET* exon 14 mutation/amplification, *RET* rearrangement, and *ERBB2* activating mutations).[104]

Matched targeted therapies were defined as:[104]

1. Erlotinib, gefitinib, afatinib, osimertinib, dacomitinib, and osimertinib for *EGFR* sensitizing mutations
2. Alectinib, brigatinib, ceritinib, crizotinib, and lorlatinib for *ALK* rearrangement
3. Ceritinib, crizotinib, lorlatinib, and entrectinib for *ROS1* rearrangement
4. Dabrafenib, trametinib, and vemurafenib for *BRAF* V600E
5. Ado-trastuzumab, trastuzumab, pertuzumab, afatinib, neratinib, and dacomitinib for *ERBB2* mutation
6. Cabozantinib, vandetanib, alectinib, lenvatinib, and sunitinib for *RET* rearrangement
7. Crizotinib and cabozantinib for *MET* exon 14 mutation or amplification

Among all patients with a targetable genomic alteration detected on either liquid biopsy or tissue CGP, patients who received a matched targeted therapy in the first-line (n=287) had longer rwPFS (9.4 months vs 6.9 months; HR (adjusted for age at onset of therapy, gender, practice type, [aHR]): 0.72 [95% CI: 0.54, 0.95]) and OS (26.7 months vs 17.9 months; aHR: 0.70 [95% CI: 0.50, 0.98]) compared to those who received other first-line therapies (n=130) following testing.[104]

For patients receiving matched targeted therapy based on liquid test (n=33), the rwPFS was comparable with those receiving matched targeted therapy based on tissue testing (n=229) (13.8 vs 10.6 months; aHR: 0.68 [95% CI: 0.36, 1.26]).[104] Further, real-world ORR (defined as patients who achieved PR or CR) to matched targeted therapy was comparable for those tested utilizing liquid vs tissue biopsy (75% vs 66%, respectively; *P*=0.83) and real-world disease control rate (DCR) (defined as patients with CR, PR, or stable disease [SD]) was 88% both for the liquid biopsy and tissue CGP cohorts treated with matched targeted therapies (Table 4-6).[104]

Table 4-6. Tumor Response for Patients With Targetable Genomic Mutations Treated With Targeted Therapy Based on Liquid vs Tissue Biopsy

	Evaluated for response, N		Real-world ORR % (95% CI)		Real-world DCR % (95% CI)	
Targetable GA	**Liquid**	**Tissue**	**Liquid**	**Tissue**	**Liquid**	**Tissue**
***EGFR* exon 19del/L858R**	26[a,b]	166[c]	76.9 (57.8, 89.4)	74.1 (66.9, 80.4)	92.3 (75.4, 98.6)	91.6 (86.2, 95.0)
***EGFR* G719X/S768I/L861Q**	3[b]	22	100 (36.8, 100)	54.5 (33.8, 74.0)	100 (36.8, 100)	90.9 (70.9, 98.4)
***EGFR* T790M**	8[a]	36[c]	50.0	80.6	75.0	91.7

			(19.3, 80.7)	(64.1, 90.7)	(36.5, 95.4)	(78.1, 97.7)
***ALK* rearrangement**	9	73	88.9 (55.7, 99.4)	74.0 (62.3, 83.0)	100 (67.7, 100)	93.2 (85.1, 97.3)
***ROS1* rearrangement**	2	16	100 (22.4, 100)	56.3 (30.5, 79.2)	100 (22.4, 100)	81.3 (56.4, 94.7)
***BRAF* V600**	0	21	N/A	57.1 (35.4, 76.7)	N/A	90.5 (69.5, 89.3)
***MET* exon 14 skipping and/or high-level amplification**	3	28	66.7 (13.5, 98.3)	32.1 (17.5, 51.8)	66.7 (13.5, 98.3)	78.6 (59.1, 90.2)
***RET* rearrangement**	1	3	0.0 (0.0, 95.0)	66.7 (13.5, 98.3)	0.0 (0.0, 95.0)	66.7 (13.5, 98.3)
***ERBB2* (HER2) activating mutation**	1	20	100 (5.0, 100)	20.0 (7.1, 42.4)	100 (5.0, 100)	40.0 (20.9, 62.8)
All targetable genomic alterations	52	385	75.0 (61.6, 85.1)	66.0 (61.0, 70.6)	88.5 (77.1, 94.9)	87.5 (83.8, 90.6)
Standard of care biomarkers	47	334	76.6 (62.5, 86.8)	71.6 (66.5, 76.2)	91.5 (80.0, 97.0)	91.3 (87.8, 94.0)
Emerging biomarkers	5	51	60.0 (18.9, 92.4)	29.4 (18.4, 43.1)	60.0 (18.9, 92.4)	62.7 (48.7, 75.3)

[a] 1 patient with 2 liquid biopsy tests included in *EGFR* exon19del/L858R group and *EGFR* T790M group.

[b] 1 specimen included in *EGFR* exon19del/L858R group and *EGFR* G719X/S768I/L861Q group.

[c] 4 patients with 2 tissue-based tests included in *EGFR* exon19del/L858R group and *EGFR* T790M group.

ALK, anaplastic lymphoma receptor tyrosine kinase; BRAF, v-raf murine sarcoma viral oncogene homolog B1; EGFR, epidermal growth factor receptor; MET, met proto-oncogene (hepatocyte growth factor receptor); NF1, neurofibromin 1; RET, ret proto-oncogene; ROS1, c-ros oncogene 1, receptor tyrosine kinase.

Source: Madison 2020[104].

Liquid Biopsy-Based CGP in Prostate Cancer

- Prostate cancer is the most commonly diagnosed cancer in men, accounting for an estimated 13% of new cancer diagnoses and 5.6% of cancer-related deaths in the US in 2021.[3,204]
- Around 7% of patients present with distant or metastatic disease at diagnosis; additionally, up to 20% of patients with localized disease at diagnosis will progress to castrate-resistant prostate cancer (CRPC), with the majority of these patients having metastatic disease within 5 years of diagnosis.[3]
    - Survival from diagnosis of CRPC is 14 months.[205]
- Germline and somatic mutations of genes involved in DNA damage repair (DDR) pathways occur in 15% to 30% of patients with metastatic prostate cancer; up to 27% may be due to alterations, specifically, in *BRCA*, *ATM*, or *CHEK2*.[142,206]
    - More recent trials of the efficacy of agents targeted against alterations in the DDR pathway vs standard of care in patients with metastatic castration-resistant prostate cancer (mCRPC) have shown improved radiographic PFS, with a favorable trend for OS.[207]
- The NCCN Guidelines for Prostate Cancer recommend tumor testing for HRR gene mutations in all men diagnosed with metastatic prostate cancer, including *BRCA1*, *BRCA2*, *ATM*, *PALB2*, *FANCA*, *RAD51D*, *CHEK2*, and *CDK12*, and consider testing in men diagnosed with regional prostate cancer. Tumor testing for MSI or dMMR is recommended for all men with mCRPC and can be considered for men with castration-naïve metastatic or regional prostate cancer. Additionally, TMB testing may be considered in patients with mCRPC.[36]
- Men with metastatic prostate cancer most frequently have tissue obtained from bone for sequencing as this is the most common site of metastatic disease; this is not optimal, however, as bone has one of the lowest tissue sequencing success rates (42% to 71%).[206,208,209]
- The NCCN strongly recommends a metastatic biopsy for histologic and molecular evaluation. When unsafe or unfeasible, plasma ctDNA assay is an option, preferably collected during biochemical (PSA) and/or radiographic progression in order to maximize yield. Caution is needed when interpreting ctDNA-only evaluation due to potential interference from clonal hematopoiesis of indeterminate potential (CHIP), which can result in a false-positive biomarker signal.[36] In the presence of insufficient tumor content or DNA yield with tissue testing, test success rate utilizing FoundationOne Liquid CDx is 94% in mCRPC patients (with ctDNA fraction of 7.5%).[161]

Unmet Need for Molecular Testing in Prostate Cancer

Despite a decreased trend in mortality seen for patients diagnosed with prostate cancer, mCRPC remains a treatment challenge, with 5-year survival rates of approximately 31% and a median OS of less than 2 years with life-prolonging therapy.[3,210,211] More recently, different DDR pathways have been recognized to be frequently altered in the advanced stages of prostate cancer, with mutations in the DDR genes detected in 15% to 30% of patients with mCRPC (Table 4-7); these can be both inherited (ie, germline) or acquired (ie, somatic).[142,212]

The DDR pathways have different constituent genes that, when mutated, contribute to a deficiency in the DNA repair capability. For example, DNA double-strand breaks are typically repaired by the HRR gene; HRR genes include recombination DNA repair genes such as *BRCA1/2, ATM, CHEK1, CHEK2, WES, BARD1, BRIP1, FAM175A, MRE11A, NBN, PALB2, RAD51C,* and *RAD51D*.[142,213] The MMR pathway is charged with repairing DNA bases that are mispaired during DNA replication; the majority of genes involved in this pathway belong to the *MSH* and *MLH* family, including several sensor molecules such as *MSH2* and *MSH6*.[212]

Table 4-7. DDR Genes Frequently Altered in Prostate Cancer

DDR gene	Common aberration types	Tumor site		References
		Primary	Metastatic	
BRCA2	Deletion, mutation	3%	13.3%	Abeshouse A, et al. *Cell.* 2015;163:1011-1125. Robinson D, et al. *Cell.* 2015;161:1215-1228.
ATM	Deletion, mutation	4%	7.3%	Wu YM, et al. *Cell*. 2018;173:1770-1782.e14. Shelley MD, et al. *Evidence-Based Urol*. 2010:293-303.
CHEK2	Germline mutation	0%	1.87%	Pritchard CC, et al. *N Engl J Med*. 2016;375:443-453.
CDK12	Mutation	1%	6.9%	Wu YM, et al. *Cell*. 2018;173:1770-1782.e14.
BRCA1	Mutation	1%	0.7%	Robinson D, et al. *Cell*. 2015;161:1215-1228.
FANCD2	Copy loss	6%	–	Robinson D, et al. *Cell*. 2015;161:1215-1228.
RAD51C	Copy loss	3%	–	Robinson D, et al. *Cell*. 2015;161:1215-1228.
RAD51D	Germline mutation	–	0.43%	Pritchard CC, et al. *N Engl J Med*. 2016;375: 443-453.
MSH2	Copy loss, mutation, rearrangements	0.3%	2%	Pritchard CC, et al. *Nat Commun.* 2014;5:1-6.
MSH6	Mutation	1.5%	2%	Pritchard CC, et al. *Nat Commun.* 2014;5:1-6. Antonarakis ES, et al. *Eur Urol*. 2018:1-5.
MLH1	Copy loss, epigenetic silencing	0.3%	0.7%	Robinson D, et al. *Cell*. 2015;161:1215-1228.

ATM, ataxia telangiectasia mutated; BRCA, breast cancer susceptibility gene; CHEK2, checkpoint kinase 2; CKD12, cyclin-dependent kinase 12; FANCD2, Fanconi anemia group D2; MLH1, MutL homolog 1; MSH, mismatch repair protein involved in the DNA mismatch repair system; RAD, genes that encode for members of the RAD51 protein family that are known to be involved in homologous recombination and repair of DNA.

Source: Athie 2018[212]; references cited are as cited by this publication.

Inhibition of poly ADP-ribose polymerase (PARP) is currently an active area of investigation in the development of new agents for mCRPC; PARP is involved in the repair of single-stranded DNA breaks, and its inhibition is circumvented by proteins of the HRR pathway.[213] In patients with mutations of the genes involved in the HRR pathway, PARP may effectively block DNA repair, leading to cell death.[213] Three PARP inhibitors, olaparib, rucaparib, and talazoparib, are being evaluated in phase 3 trials in mCRPC, and both olaparib and rucaparib have been granted breakthrough designation by the FDA for expedited review for indication in mCRPC.[212,213]

- For example, in a phase 3 trial evaluating the efficacy of olaparib vs physician choice of a standard of care hormonal agent (pcHA) (ie, enzalutamide or abiraterone) in patients with mCRPC and progression on a previous treatment with enzalutamide or abiraterone and with alterations in any of 15 predefined genes involved in the HRR pathway, olaparib improved radiographic PFS, with a favorable trend for OS despite >80% crossover to olaparib (Table 4-8).[207]

Table 4-8. Efficacy of Olaparib vs Physician Choice Standard of Care Hormonal Agent in mCRPC Patients

	Cohort A (*BRCA1*, *BRCA2*, or *ATM* alterations)		**Cohort A + Cohort B (*BRCA1*, *BRCA2*, or *ATM* alterations plus other HRR alterations, including: *BRIP1*, *BARD1*, *CDK12*, *CHEK-1 or -2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51-B or –C or –D*, or *RAD54L*)**	
Outcome	**Olaparib N=162**	**pcHA N=83**	**Olaparib N=256**	**pcHA N=131**
Median rPFS, months	7.39	3.55	5.82	3.52
HR (95% CI)	0.34 (0.25, 0.47); *P*<0.0001		0.49 (0.38, 0.63); *P*<0.0001	
Median OS, [a] **months**	18.50	15.11	17.51	14.26
HR (95% CI)	0.64 (0.43, 0.97); *P*=0.0173		0.67 (0.49–0.93); *P*=0.0063	

[a] Interim analysis at 38% (Cohort A) and 41% (Cohort A + B) data maturity; of the pcNHA patients whose disease progressed by BICR and were eligible, 80.6% in Cohort A and 84.6% in Cohort B crossed over to olaparib treatment.

HRR, homologous recombination repair; mCRPC, metastatic castrate-resistant prostate cancer; OS, overall survival; pcHA, physician choice of hormonal agent (ie, either enzalutamide or abiraterone); rPFS, radiographic progression-free survival.

Source: Sandhu 2019[207]

In another study evaluating the PARP inhibitor, rucaparib, in patients who have progressed on an androgen receptor (AR)-directed therapy and chemotherapy who harbor an alteration in *BRCA1*, *BRCA2*, *ATM*, or other prespecified DDR gene, revealed an ORR of 43.9% (95% CI: 30.7, 57.6) and a median

time to prostate-specific antigen progression of 6.5 months (95% CI: 5.7, 7.5) in patients with a *BRCA1* or *BRCA2* mutation.[214]

In addition, the MSI-H/dMMR genomic alterations are uncommon yet therapeutically meaningful in patients with prostate cancer; in a small study of mCRPC patients who were MSI-H and treated with immunotherapy, 45% of patients had a durable clinical benefit, which the authors concluded was in line with other neoplasms with this same genomic alteration.[215]

The NCCN Guidelines for Prostate Cancer (V.4.2022) recommend tumor testing for HRR gene mutations in all men diagnosed with metastatic prostate cancer, including *BRCA1, BRCA2, ATM, PALB2, FANCA, RAD51D, CHEK2,* and *CDK12,* and to consider testing for HRR gene mutations in men diagnosed with regional prostate cancer. At present, this information may be used for treatment decision making, including understanding eligibility for biomarker-directed treatments, genetic counseling, early use of platinum chemotherapy, and eligibility for clinical trials. Tumor molecular profiles may change with subsequent treatments, and re-evaluation may be considered at time of cancer progression for treatment decision making. Further, tumor testing for MSI or dMMR is recommended for all men with mCRPC and can be considered for men with castrate-naïve metastatic or regional prostate cancer. TMB testing may be considered in patients with mCRPC.[36]

The rationale for testing is that abnormalities in some of these HRR genes have been specifically associated with poorer outcomes in patients treated based on current (non-biomarker directed) modalities in mCRPC.[216,217] These defects, however, may allow susceptibility to specific targeted therapeutic approaches; as such, the pace and breadth of the clinical development of development of new molecularly targeted agents have recently accelerated in this setting.[212,213] Currently, the NCCN Guidelines for Prostate Cancer recommend:

- olaparib in men with mCRPC who have a pathogenic (germline and/or somatic) HRR mutation and have progressed on prior androgen receptor directed therapy or androgen therapy and a taxane-based chemotherapy;
- pembrolizumab for mCRPC tumors that are MSI-H or dMMR or TMB-H (≥10 mut/Mb) after progression through prior docetaxel and/or a novel hormone therapy; and
- rucaparib in men with mCRPC who have a pathogenic (germline and/or somatic) *BRCA* mutation and have progressed on prior androgen receptor directed therapy or androgen therapy and a taxane-based chemotherapy.[36]

Despite these recommendations, many patients who should be tested pursuant to guidance by the NCCN go untested; and while there is a paucity of data for testing unselected prostate cancer patients, data for germline testing show that guideline adherence is problematic.

- A survey conducted by the Germline Genetics Working Group of the Prostate Cancer Clinical Trials Consortium (PCCTC) that was administered to medical oncologists who see patients with prostate cancer (n=26) from PCCTC affiliate sites revealed that 62% of oncologists surveyed would consider germline genomic testing in all metastatic prostate cancer patients; the remainder would only consider testing for patients with a family history and/or for clinical trial eligibility (27%) or for patients with a family history of genomic testing (12%).[218]

As in other disease states, inability to obtain adequate tissue for sampling may be an issue in prostate cancer, especially for those patients whose biopsies from a metastatic site are utilized. Obtaining

sufficient tumor from metastatic bony lesions is a known challenge in prostate cancer where 85% of mCRPC patients have bone-only disease.

- In a study of 59 patients with metastatic prostate cancer who were biopsied for testing with a tissue-based broad panel molecular test, adequate tissue for testing from a bone biopsy (n=31) was obtained in 71% of patients.[208] Additionally, lymph nodes samples (n=18) resulted in 78% having adequate tissue for testing.[208]
- In another analysis of patients enrolled in the phase 2 TRITON2 and phase 3 TRITON3 studies investigating rucaparib in patients with mCRPC harboring an alteration in an HRR gene, a total of 1,311 tumor tissue samples (from 1,516 patients) were collected to determine patient eligibility for these studies; the test failure rate was 32%, mainly (18%) due to insufficient tumor content or DNA yield.[164]
- An analysis sought to successfully sequence 746 biopsy surgical samples from patients with recurrent or metastatic prostate cancer using the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) sequencing assay.[206] Of the 746 samples, 504 (68%) were successfully sequenced.[206] The highest success rates were for prostate tumor samples that were obtained from diagnostic prostate needle biopsy, radical prostatectomy, or transurethral resection of prostate performed for palliation.[206] For metastatic samples, success rates of >69% were observed for lymph node, liver, and other soft tissue samples, whereas bone and lung samples were more challenging (42% to 52% success rate).[206]
- A retrospective analysis reviewed 59 patients with CRPC who underwent metastatic tissue biopsies between 2012 and 2015 for genotyping with a 37-cancer gene panel.[208] The most frequent sites of biopsy for these patients were bone (53%) and lymph nodes (30%), with liver and soft tissue accounting for approximately 8.5% each.[208] Within the total 59 patients, 46 (78%) had adequate tissue for mutational testing.[208] Of the patients with bone metastasis as the site of biopsy, 71% had adequate tissue for sequencing; 78% of lymph node specimens had adequate tumor for sequencing, and 100% of liver and soft tissue specimens had adequate tumor for sequencing.[208]

Place of Liquid Biopsy-Based CGP in Prostate Cancer

Liquid biopsies have the potential to overcome possible hurdles associated with accessing CGP. In all scenarios of unavailable or limited tissue, liquid biopsy may be an alternative method for obtaining genomic information from ctDNA.

- In a retrospective cohort study of patients with advanced prostate cancer (N=207) who underwent ctDNA analysis with FoundationACT and tissue-based CGP with FoundationOne, the total actionable *BRCA1/2* alterations identified by ctDNA was 15 (7.2%).[219]

The NCCN strongly recommends a metastatic biopsy for histologic and molecular evaluation. When unsafe or unfeasible, plasma ctDNA assay is an option, preferably collected during biochemical (PSA) and/or radiographic progression in order to maximize yield. Caution is needed when interpreting ctDNA-only evaluation due to potential interference from CHIP, which can result in a false-positive biomarker signal..[36] Table 4-9 reviews the recommended biomarker testing to be conducted in prostate cancer, as well as the ability of the FoundationOne Liquid CDx assay to test for these recommended biomarkers.

Table 4-9. Review of Guideline-Recommended Biomarker Testing for Patients With Advanced (Regional and Metastatic) Prostate Cancer and FoundationOne Liquid CDx Assay Capabilities

Applicable guidelines for tumor profiling	Recommended biomarker testing	Biomarker included in FoundationOne CDx assay
NCCN Guidelines for Prostate Cancer V.4.2022[36a,b]	Tumor testing[c]: Homologous recombination gene mutations (eg, *BRCA1*, *BRCA2*, *ATM*, *PALB2*, *FANCA*, *RAD51D*, *CDK12*, *CHEK2*)	Yes
	Tumor testing[d]: dMMR (ie, *MLH1*, *MSH2*, *MSH6*, and *PMS2*) or MSI-H	Yes
	TMB[e]	No[f]

[a] **Category 1:** Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate. **Category 2A:** Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate. All NCCN recommendations are category 2A unless otherwise indicated.

[b] The NCCN Guidelines for Prostate Cancer do not endorse any specific commercially available biomarker assays.

[c] Tumor testing is recommended in patients with metastatic prostate cancer and can be considered in patients with regional prostate cancer.

[d] Tumor testing can be considered in patients with regional or castration-naive metastatic prostate cancer and is recommended in patients with mCRPC.

[e] TMB testing may be considered in patients with mCRPC.

[f] FoundationOne Liquid CDx includes bTMB, which is a distinct biomarker from TMB; bTMB is correlated with TMB.

BRCA, breast cancer susceptibility gene; CHEK2, checkpoint kinase 2; CKD12, cyclin-dependent kinase 12; dMMR, mismatch repair deficient; MLH1, MutL homolog 1; MSH, mismatch repair protein involved in the DNA mismatch repair system; MSI-H, microsatellite instability–high; NCCN, National Comprehensive Cancer Network; RAD, genes that encode for members of the RAD51 protein family that are known to be involved in homologous recombination and repair of DNA.

Clinical Utility and Validity of FoundationOne Liquid CDx in Prostate Cancer

FoundationOne Liquid CDx is FDA-approved to report substitutions and indels in 311 genes, including rearrangements in 4 genes, and copy number alterations in 3 genes. bTMB, MSI-H status, and tumor fraction are reported as a laboratory professional service which is not reviewed or approved by the FDA.[165] Comprehensive results across all 324 genes are reported as a laboratory professional service which is not reviewed or approved by the FDA. FoundationOne Liquid CDx utilizes circulating cfDNA isolated from plasma derived from the anticoagulated peripheral whole blood of cancer patients. The test is intended to be used as a companion diagnostic to identify patients with prostate cancer who may benefit from treatment with the targeted therapies listed in Table 4-10 in accordance with the approved therapeutic product labeling. Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified healthcare professionals in accordance with professional guidelines in oncology for patients with malignant neoplasms, including prostate cancer.[1]

Table 4-10. Companion Diagnostic Indications Pertinent for Patients With Prostate Cancer

Tumor type	Biomarker(s) detected	Therapy
Prostate cancer	*BRCA1*, *BRCA2* alterations	Rubraca® (rucaparib)
	BRCA1, *BRCA2*, *ATM* alterations	Lynparza® (olaparib)

BRCA, breast cancer susceptibility gene.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1]

FoundationOne Liquid CDx provides a means to adhere to guideline recommendations for testing in patients with mCRPC. For patients enrolled in the phase 2 TRITON2 and phase 3 TRITON3 studies investigating rucaparib in patients with mCRPC harboring an alteration in an HRR gene, the test failure rate was 32% utilizing tissue samples, mainly (18%) due to insufficient tumor content or DNA yield; the test success rate utilizing FoundationOne Liquid was 97%.[164]

In addition to a high testing success rate of FoundationOne Liquid, a recent study has shown genomic analysis of ctDNA from patients with mCRPC recapitulates the genomic landscape detected in tissue biopsies, with a high level of agreement in detection of *BRCA1* and *BRCA2* mutations.[161]

- Plasma from 3,334 patients with mCRPC (including 1,674 screening samples from TRITON2/3 and 1,660 samples from routine clinical testing at Foundation Medicine of patients with advanced prostate cancer) were used to assess the landscape of genomic alterations detected in ctDNA and assessed for concordance with tissue-based CGP. The ctDNA assays used in this analysis included FoundationACT (a previous version of FoundationOne Liquid) and FoundationOne Liquid. Of the 3,334 patient samples included, 3,129 patients (94%) had detectable ctDNA. *BRCA1/2* was mutated 8.9% of all patients' plasma samples. For the patient-level *BRCA1* and *BRCA2* mutation status concordance analysis, 837 patient samples were included (liquid biopsy samples with available matched tissue biopsy samples); the PPA was 93.1% and the NPA was 97.4%, with an overall percent agreement of 97.0% between Foundation Medicine liquid biopsy and tissue-based NGS. Additionally, ctDNA harbored some *BRCA1* and *BRCA2* alterations not identified by tissue testing, and ctDNA was enriched in therapy resistance alterations, as well as possible clonal hematopoiesis mutations (eg, in *ATM* and *CHEK2*). Potential androgen receptor resistance alterations were detected in 940 of 2,213 patients (42%), including amplifications, polyclonal and compound mutations, rearrangements, and novel deletions in exon 8.[161]

BRCA1/BRCA2 to Determine Treatment With Rubraca

Clinical validity of the FoundationOne Liquid CDx assay was evaluated as a companion diagnostic in identification of patients with mCRPC harboring *BRCA1* or *BRCA2* alterations who may benefit from treatment with rucaparib using pre-rucaparib treatment blood samples from TRITON2.[1,109] Clinical bridging via concordance to CTAs, which included central tissue (Foundation Medicine), tissue and liquid based assays, and local testing (majority tissue-based) for some patients.[1,107] Pre-rucaparib treatment plasma samples were available for 192 patients, and FoundationOne Liquid CDx data were available for 84% (161/192) of the patients with samples tested.[1]

- The PPA and NPA between the CTA and FoundationOne Liquid CDx assays were as follows: PPA, 82.4% (95% CI: 73.0, 89.6); NPA, 98.6% (95% CI: 92.3, 100.0).[1]
- The primary efficacy endpoint for TRITON2 patients included in this study was confirmed ORR per modified RECIST v1.1/Prostate Cancer Working Group-3 (PCWG-3) criteria by Independent Radiologic Review (IRR) in patients with a *BRCA1* or *BRCA2* alteration and measurable disease at baseline per IRR.[101] *BRCA1/2* alteration status was verified retrospectively by FoundationOne Liquid CDx in 66% (41/62) of the patients in the primary efficacy population. The ORR (95% CI) in the primary efficacy population was 47.4% (31.0, 64.2) in *BRCA1/2* positive patients

determined by FoundationOne Liquid CDx, which is comparable to the ORR of 43.5% (31.0, 56.7) in patients identified by CTA.[1]

BRCA1/BRCA2 and ATM to Determine Treatment with Olaparib

The clinical validity of using FoundationOne Liquid CDx as a companion diagnostic to identify patients with mCRPC harboring *BRCA1, BRCA2* or *ATM* alterations who may benefit from treatment with olaparib was assessed through a clinical bridging study using screening (ie, pre-olaparib treatment) plasma samples from Cohort A of the PROfound trial. The PROfound trial is a phase III, open label, randomized study to assess the efficacy and safety of olaparib vs enzalutamide or abiraterone acetate in men with metastatic castration-resistant prostate cancer who have failed prior treatment with a new hormonal agent and have HRR gene mutations.[220] In total, 387 patients were randomized into the PROfound study by the FoundationOne laboratory developed test (LDT) CTA[1,108,109]; of these, 245 patients were randomized in cohort A and 181 had a plasma sample available for testing. Of these, 139 (76.8%) Cohort A patients had a successful FoundationOne Liquid CDx test result.[1]

- The PPA and NPA between FoundationOne Liquid CDx and the FoundationOne CDx CTA using the CTA as the reference were: PPA, 79.9% (95% CI: 72.2, 86.2); NPA, 91.8% (95% CI: 87.0, 95.2). After adjusting for a 17.1% prevalence of *BRCA1/2* and *ATM* alterations in the intended use population, the PPV and NPV calculated using the CTA as the reference were: PPV, 66.6% (95% CI: 56.0, 77.2); NPV, 95.7% (95% CI: 94.3, 97.1).[1]
- For the primary analyses, clinical efficacy of olaparib vs investigator choice of new hormonal agent in the FoundationOne Liquid CDx *ATM/BRCA1/BRCA2*-positive population was evaluated based on the endpoint radiological progression-free survival (rPFS) as assessed by blinded independent central review per RECIST v1.1 criteria and/or PCWG-3.[220] The estimated rPFS hazard ratio (HR) were 0.331 (95% CI: 0.21, 0.53) for the FoundationOne Liquid CDx population with *BRCA1/2* or *ATM* alterations, which were comparable with the observed rPFS HR of 0.34 (95% CI: 0.25, 0.47) for the FoundationOne CDx CTA population (PROfound Cohort A).[1]

Liquid Biopsy-Based CGP in Breast Cancer

- Breast cancer accounts for 14.8% of new cancer diagnoses and 7.2% of cancer-related deaths in 2021.[3,221]
- Breast cancer is the second-leading cause of cancer-related mortality among women in the US, with an estimated 43,600 deaths in 2021.[3,221]
- Localized and locally advanced breast cancers have high 5-year survival rates at 99% and 86%, respectively; however, the 5-year survival rate for metastatic breast cancer is 29%.[119]
- In advanced breast cancer, the NCCN Guidelines currently recommend patients have access to testing for HER2/*ERBB2* amplification, germline *BRCA1/BRCA2* mutations, *PIK3CA* activating mutation, *NTRK* fusion, PD-L1 expression, MSI-H/dMMR, and TMB-H (≥10 mut/Mb).[25]
- For stage IV or recurrent breast cancer, current NCCN Guidelines recommend assessment of *PIK3CA* mutation with tumor or liquid biopsy if the disease is hormone receptor-positive/HER2-negative and if therapy with alpelisib + fulvestrant is being considered. *PIK3CA* mutation testing can be done on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended. Testing methodology recommendation is molecular panel or PCR (category 1).[25]
- In patients with *PIK3CA*-mutated breast cancer treated with alpelisib + fulvestrant, FoundationOne Liquid CDx was shown to be concordant to and demonstrate similar clinical outcomes as the tumor tissue-based PCR CTA.[1,111]

Unmet Need for Molecular Testing in Breast Cancer

Breast cancer is one of the leading causes of cancer-related mortality in the US, with over 40,000 deaths annually[3,221]; as such, treatment in this setting remains as challenge.[222] Treatment of metastatic breast cancer is largely based on hormone receptor status (estrogen receptor [ER]/progesterone receptor) and HER2 status.[25] Targeting the ER and/or HER2 are the best established targeted treatment approaches in metastatic breast cancer.[223] Still, with currently available therapies, metastatic breast cancer is considered an incurable disease, highlighting the need to define additional actionable targets for the treatment of these patients.[222,224]

Notably, there is a growing number of genomic alterations emerging, which may help facilitate a tailored approach to treatment of metastatic breast cancer.[225] The most common targetable alterations in advanced breast cancer now include[225]:

- Recurrent somatic mutations of *ERBB2* occur in 2% to 4% of patients, most commonly in patients with HER2-negative breast cancer.[143]
- Activation mutations in the phosphoinositide 3 kinase (PI3K) pathway *PIK3CA* gene arise in nearly 40% of tumors (most common mutations in ER+ breast cancer).[225]
- Pathogenic mutations in *BRCA1* and *BRCA2* (*BRCA1/2*) arise in approximately 5% of breast cancers (most cases reflect an underlying germline mutation, although somatic mutations without a predisposing germline mutation can be found).[225]

- Across all breast cancer subtypes, 2% of tumors harbor MSI, reflecting underlying defects in mismatch DNA repair.[225]

Additionally, genomic testing results may also be relevant to determine eligibility for clinical trials with investigational agents.[143] This approach may be particularly relevant for patients with advanced breast cancer, for whom standard treatments are minimally effective.[143] There are several targeted therapies that are actively being studied in metastatic breast cancer, including those targeting *AKT1* mutations or *ESR1* mutations (Table 4-11).[143]

Table 4-11. Genomic Alterations With Targeted Therapies Available or in Clinical Trials

Molecular alteration	Prevalence	Drug class
Targets with FDA-approved therapies		
ER/PR	75%	Aromatase inhibitors/SERMs
HER2 amplification	30%	HER2 mAb or HER2 TKI
BRCA1/BRCA2	5%	PARP inhibitor therapy
***PIK3CA* mutation**	30%–40%; ER+/HER2−, 20%–25%	PI3K inhibitor (α-isoform–specific/selective)
***ERBB2* mutation**	2%–4% HER2-negative	HER2 TKI
***NTRK* fusion**	Enriched in secretory (TNBC)	TRK inhibitor
High tumor mutation burden	1%–5% breast cancers	Immune checkpoint inhibitor
Mismatch repair deficiency signature	<5% breast cancers	Immune checkpoint inhibitor
Targets under investigation in clinical trials		
***AKT1* mutation**	2%–5% breast cancer	AKT inhibitor mTOR inhibitor
***ESR1* mutation**	30%–40% ER+/HER2− after aromatase inhibitor	Oral selective estrogen receptor degrader

AKT1, protein kinase B; BRCA, breast cancer gene; ER, estrogen receptor; ERBB2, Erb-B2 receptor tyrosine kinase 2; ESR1, estrogen receptor 1; HER2, human epidermal growth factor receptor 2; mAb, monoclonal antibody; mTOR, mammalian target of rapamycin; NTRK, neurotrophic tyrosine receptor kinase; PARP, poly (ADP-ribose) polymerase; PR, progesterone receptor; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; SERM, selective estrogen receptor modulator; TKI, tyrosine kinase inhibitor; TNBC, triple negative breast cancer; TRK, tropomyosin receptor kinase.

Source: Kratz 2018[143].

The treatment of breast cancer has been largely determined by HR and HER2 status, as both hormonal and HER2-targeted therapies have greatly improved survival. However, several therapies based on novel (ie, non-HR or HER2) targets are creating opportunities for improved outcomes in women with metastatic breast cancer.[223] These therapies provide potential opportunities, not only for women who are HR- and HER2-negative, but also for women who have exhausted all treatment options. Table 4-12 provides an overview of the improvements in outcomes with the novel targeted therapies for patients with advanced breast cancer, including those targeting *BRCA1/2* mutations, *PI3KCA* mutations, and PD-L1 expression.

Table 4-12. Treatment Outcomes With Targeted Therapies in Advanced Breast Cancer

		Efficacy results	
Regimen	**Patient population**	**Response rate[a]**	**Median PFS**
BRCA1/2 mutations			
Olaparib (Robson 2017)[226]	Patients with a germline *BRCA* mutation and HER2-negative metastatic breast cancer who had received no more than 2 previous chemotherapy regimens for metastatic disease	Olaparib: 59.9% SOC: 28.8%	Olaparib: 7.0 months SOC: 4.2 months HR: 0.58; 95% CI: 0.43, 0.80; *P*<0.001
Talazoparib (Litton 2018)[227]	Patients with advanced breast cancer and a germline *BRCA1/2* mutation	Talazoparib: 62.6% SOC: 27.2%	Talazoparib: 8.6 months SOC: 5.6 months HR: 0.54; 95% CI: 0.41, 0.71; *P*<0.001
PIK3CA mutations			
Alpelisib + fulvestrant (Andre 2019)[228]	Patients with HR-positive, HER2-negative advanced breast cancer who had received endocrine therapy previously	Alpelisib + fulvestrant: 26.6% Placebo + fulvestrant: 12.8%	Alpelisib + fulvestrant: 11.0 months Placebo + fulvestrant: 5.7 months HR: 0.65; 95% CI: 0.50, 0.85; *P*<0.001
PD-L1-positive breast cancer			
Atezolizumab + nab-paclitaxel (Schmid 2018)[229]	Patients with untreated metastatic triple-negative breast cancer	Atezolizumab + nab-paclitaxel: 58.9% Placebo + nab-paclitaxel: 42.6%	In PD-L1-positive patients: Atezolizumab + nab-paclitaxel: 7.5 months Placebo + nab-paclitaxel: 5.0 months HR: 0.62; 95% CI: 0.49, 0.78; *P*<0.001

[a] Response rate defined as ORR per RECIST v1.1 (with the exception of Robson 2017 which used mRECIST v1.1).

BRCA, breast cancer gene; CI, confidence interval; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; HR, hazard ratio; mRECIST, modified Response Evaluation Criteria in Solid Tumors; PD-L1, programmed death-ligand 1; PFS, progression-free survival; RECIST, Response Evaluation Criteria in Solid Tumors; SOC, standard of care; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha.

As targeted therapies have been shown to improve outcomes for women with breast cancer, there is a recognized need for molecular testing for genomic variations, as these have become integral in the management of advanced breast cancer.[230] Although the determination of hormone receptor status and *HER2* status is well known in the treatment paradigm of advanced breast cancer, other genomic alterations and biomarkers (ie, *BRCA1/2* mutations, *PIK3CA* activating mutations, *NTRK* gene fusions, PD-L1 expression status, MSI-H/dMMR, and TMB-H [≥10 mut/Mb]) are actionable either through an FDA-approved therapy or within a clinical trial and are equally important to test for in the management of advanced breast cancer.[25,231]

Although targeted therapies have improved outcomes in women with advanced breast cancer and genomic testing is standard of care per guideline recommendations, there are little data regarding the

status of genomic testing in patients with advanced breast cancer. The data currently available in advanced breast cancer primarily focus on germline cancer genetic testing.[121,230]

- A database registry study reviewed 77,085 women with breast cancer and 6,001 women with ovarian cancer diagnosed between 2013 and 2014 from the Georgia Cancer Registry and the California Cancer registry.[230] The data from these patients were linked with germline genetic testing information from 4 laboratories that performed the majority of clinical testing.[230] Only 24.1% of patients with breast cancer had testing results linked with any laboratory.[230]
- A real-world study sought to assess the somatic and/or germline *BRCA1/2* testing rates in 1,285 HER2-negative adult women with advanced breast cancer in the US (Table 4-13).[121] The *BRCA1/2* testing rate observed for the overall sample was 50%, with significantly lower *BRCA1/2* testing seen among HR+/HER2- vs TNBC patients (41% vs 75%; *P*<0.001).[121] Among HR+/HER2-, lower *BRCA1/2* testing rates were observed among patients known not to have a family history of breast or ovarian cancer.[121]

Table 4-13. *BRCA1/2* Testing by HR Status and Known Family History of Breast or Ovarian Cancer

	HR+/HER2- with FHx (n=141)	HR+/HER2- without FHx (n=713)	TNBC with FHx (n=48)	TNBC without FHx (n=211)
Patients tested, n (%)	97 (69)	289 (41)	36 (75)	146 (69)
***P*-value**	<0.001		0.487	

FHx, family history; HER2, human epidermal growth factor 2; HR, hormone receptor; TNBC, triple-negative breast cancer.
Source: Lux 2020[121].

Many factors are involved in limiting genetic testing in those with clinical indications, including patients' and clinicians' attitudes about the value of genetic testing and the challenges of integrating genetic testing into the cancer treatment workflow.[46,230] Further, issues involving tissue availability also exist in patients with advanced breast cancer, leading to the inability to test.[177,178]

- A prospective, single-center, single-arm trial enrolled 142 patients with metastatic breast cancer within 10 weeks of starting a new therapy, of whom 100 patients had successful FoundationOne CDx NGS testing.[177] In this analysis, 21 patients were excluded due to no available tissue, insufficient tissue, or poor DNA quality (n=21), meaning 15% of patients considered for tissue testing were unable to be tested.[177]
- A whole genome sequencing study analyzed 570 patients with metastatic breast cancer utilizing tissue biopsied from their metastatic site(s).[178] The metastatic biopsy sites included the liver, lymph node, bone, and soft tissue.[178] Within this analysis, 22% of all metastatic biopsies were non-evaluable, with tissue obtained from bone metastases having the highest failure rate of 33%.[178]

Place of Liquid Biopsy-Based CGP in Breast Cancer

Approximately 15% to 20% of all advanced breast cancer patients with testing ordered are unable to get molecular testing results for the following reasons: no available tissue, insufficient tissue, or poor DNA quality; these patients would be optimal candidates to receive liquid-based testing if rebiopsy was not an

option.[177,178] Results from recent analyses show that liquid-based analyses are able to identify genomic alterations in patients with advanced and metastatic breast cancer and match them to appropriate therapies.[232]

- A retrospective study of patients with locally advanced and metastatic breast cancer (N=91) who underwent ctDNA analysis reported that 16 patients (19%) initiated targeted therapy based on ctDNA results.[232] The median PFS and OS of the patients treated with targeted therapy in this analysis were 5.2 months and 21.5 months, respectively.[232]

Molecular testing for genomic variations have become integral in the management of advanced breast cancer.[46,230] For stage IV or recurrent breast cancer, current NCCN Guidelines recommend assessment of *PIK3CA* mutation with tumor or liquid biopsy if the disease is hormone receptor-positive/*HER2*-negative and if therapy with alpelisib + fulvestrant is being considered. *PIK3CA* mutation testing can be done on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended. Testing methodology recommendation is molecular panel or PCR (category 1).[25] Table 4-14 reviews the recommended biomarker testing to be conducted in breast cancer and the ability of the FoundationOne Liquid CDx Assay to test for these recommended biomarkers.

Table 4-14. Review of Guideline-Recommended Biomarker Testing and FoundationOne Liquid CDx Assay Capabilities in Breast Cancer

Applicable guidelines for tumor profiling	Recommended biomarker testing	Biomarker included in FoundationOne Liquid CDx assay
NCCN Guidelines for Breast Cancer[a] (V.3.2022)[25]	*ERBB2* (HER2) amplification	Yes
	BRCA1 mutation (germline)	Yes
	BRCA2 mutation (germline)	Yes
	PIK3CA activating mutation	Yes
	NTRK fusion	Yes
	MSI-H/dMMR	Yes
	TMB-H (≥10 mut/Mb)	No[b]
	PD-L1 expression	No[c]
	Hormone receptor status (ER or progesterone receptor)	No

[a] **Category 1:** Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate. **Category 2A:** Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate. All NCCN recommendations are category 2A unless otherwise indicated.
[b] FoundationOne Liquid CDx includes bTMB, which is a distinct biomarker from TMB; bTMB is correlated with TMB.
[c] PD-L1 testing is available from Foundation Medicine.
dMMR, mismatch repair deficient; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; NCCN, National Comprehensive Cancer Network; NTRK, neurotrophic receptor tyrosine kinase; PD-L1, programmed death ligand-1; TMB, tumor mutational burden.

Clinical Utility and Validity of FoundationOne Liquid CDx in Breast Cancer

FoundationOne Liquid CDx is FDA-approved to report substitutions and indels in 311 genes, rearrangements in 4 genes, and copy number alterations in 3 genes. Comprehensive results across all 324 genes are reported as a laboratory professional service which is not reviewed or approved by the FDA. bTMB, MSI-H status, and tumor fraction are reported as a laboratory professional service which is not reviewed or approved by the FDA.[165] FoundationOne Liquid CDx utilizes circulating cfDNA isolated from plasma derived from the anticoagulated peripheral whole blood of cancer patients. The test is intended to be used as a companion diagnostic to identify patients with breast cancer who may benefit from treatment with the targeted therapy listed in Table 4-15 in accordance with the approved therapeutic product labeling. Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified healthcare professionals in accordance with professional guidelines in oncology for patients with malignant neoplasms, including breast cancer.[1]

Table 4-15. Companion Diagnostic Indications Pertinent for Patients With Breast Cancer

Tumor type	Biomarker(s) detected	Therapy
Breast	*PIK3CA*[a]	Piqray® (alpelisib) (used in combination with fulvestrant)

[a] *PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, and H1047Y.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx.[1]

PIK3CA for Treatment with Alpelisib

The clinical validity of using FoundationOne Liquid CDx to identify breast cancer patients harboring *PIK3CA* alterations who may benefit from treatment with alpelisib was assessed through retrospective testing of plasma samples collected prior to study treatment from advanced or metastatic breast cancer patients enrolled in clinical trial CBYL719C2301 (SOLAR-1). FoundationOne Liquid was compared via a clinical bridging study to the tumor tissue-based PCR CTA.[1,111] SOLAR-1 was a randomized, double-blind, placebo-controlled phase 3 clinical trial to evaluate the safety and efficacy of alpelisib in combination with fulvestrant for men and postmenopausal women with HR-positive, HER2-negative advanced breast cancer that had progressed on or after aromatase inhibitor treatment.[228] In this study, all available plasma samples collected at baseline prior to randomization were tested with FoundationOne Liquid CDx.[1] Of the 572 SOLAR-1 randomized patients, 432 had baseline plasma samples available and were tested using FoundationOne Liquid CDx; of these patients, 359 were available for the primary analysis when excluding those samples with invalid results (DNA input ≥30 ng).

- The point estimates of PPA and NPA between FoundationOne Liquid CDx and the CTA2 assay and the corresponding 95% CIs were: PPA (95% CI): 71.7% (65.4, 77.5); NPA (95% CI): 100% (97.2, 100).[1]
- The primary efficacy analysis in the *PIK3CA* alteration positive population identified by FoundationOne Liquid CDx was based on PFS by local investigator assessment per RECIST v1.1 criteria.[228] Clinical efficacy of alpelisib in combination with fulvestrant for the FoundationOne Liquid CDx-positive population with DNA input ≥30 ng (N=165) was demonstrated with an estimated 54% risk reduction in disease progression or death in the alpelisib plus fulvestrant arm

compared to the placebo plus fulvestrant arm (HR: 0.46; 95% CI: 0.30, 0.70).[1]

- The clinical efficacy of alpelisib + fulvestrant for FoundationOne Liquid CDx-positive patients was very similar to that reported for the tissue-identified *PIK3CA*-mutated patients; the estimated risk reduction for disease progression or death was 35% compared with placebo + fulvestrant (HR: 0.65; 95% CI: 0.50, 0.85; *P*<0.001).[228]

Additionally, the prospective phase 2 LOTUS trial enrolled 124 patients with metastatic TNBC, of whom 88 underwent pre-treatment tissue CGP and liquid ctDNA analysis with FoundationACT.[47,106,233] By analysis utilizing liquid ctDNA, 91% (81/89 patients) had 1 mutation detected; the PPA with tissue sequencing was 84% (106 of 126 alterations detectable in tissue) for known or likely short variant mutations.[106]

- First-line treatment of ipatasertib + paclitaxel was associated with improved PFS in patients with *PIK3CA/AKT1* mutations (HR: 0.15; 95% CI: 0.02, 0.62) vs those without a detectable mutation (HR: 0.86; 95% CI: 0.48, 1.51).[106] Further, ctDNA showed 100% concordance with tumor tissue for variants of interest in this trial (*PIK3CA* and *AKT1*). Importantly, ctDNA successfully selected patients who were treated with ipatasertib + paclitaxel.[106]
- The median OS, with determination of genetic alterations by the tissue-based CTAs (ie, IHC and FoundationOne hybrid capture NGS), favored ipatasertib + paclitaxel vs placebo + paclitaxel in the IHC PTEN-low (n=48; 23.1 vs 15.8 months; HR [95% CI]: 0.83 [0.42, 1.64]) and *PIK3CA/AKT1/PTEN*-altered according to NGS (n=42; 25.8 vs 22.1 months; HR [95% CI]: 1.13 [0.52, 2.47]) subgroups.[106,234] As there was high concordance between the tissue-based CTA and FoundationACT (ctDNA) (as outlined above), similar results may be expected with FoundationOne Liquid CDx as well.[47,106,234]

Liquid Biopsy-Based CGP in Ovarian Cancer

- Although ovarian cancer is a rare cancer type among women (17th most common cancer diagnosis with an estimated 21,410 diagnosed in 2021 in the US), it contributes significantly to cancer-related deaths each year, as it is the fifth most common cause of cancer-related death in women.[3,235]
- Survival for metastatic ovarian cancer at 5 years is 30.3%, with over half of the patients (57%) diagnosed at this state.[235]
- With chemotherapy-based treatment, the vast majority (>70%) of ovarian cancer patients will recur within 2 years.[236-239] As patients with ovarian cancer become platinum-resistant or refractory, the median survival becomes poor, ranging from 9 to 18 months.[240,241]
- In the up-front setting, choice of somatic testing should, at a minimum, optimize the identification of molecular alterations that can inform the use of interventions that have demonstrated benefit in this setting, including *BRCA1/2* mutations, LOH, or HR status in the absence of a germline *BRCA* mutation.[34]
    - Therapies targeting these alterations in patients with ovarian cancer have shown improved outcomes in women with advanced or metastatic ovarian cancer.[242-245,246,247]
- In recurrent ovarian cancer, the NCCN Guidelines currently recommend patients have access to tumor molecular analysis including, but not limited to, testing for *BRCA1/2* mutations, HR status, MSI, TMB, and *NTRK* if prior testing did not include these markers.[34]
- In patients with advanced ovarian cancer, clinical outcomes were comparable between those identified using FoundationOne Liquid CDx or the tissue-based CTAs (FoundationFocus™CDxBRCA and FoundationOne CDx) for detection of *BRCA1/2* mutations.[1,107,112]

Unmet Need for Molecular Testing in Ovarian Cancer

Genomic testing has been an important part of the management of advanced ovarian cancer since *BRCA1* and *BRCA2* were identified in 1994 and 1995, respectively.[230] Approximately 15% to 20% of all patients with ovarian cancer harbor a *BRCA* mutation.[248] Women with *BRCA1* and *BRCA2* mutations have an increased risk of 39% to 46% and 10% to 27%, respectively, of developing ovarian, fallopian tube, or peritoneal cancer by the age of 70 years.[249] Further, HRD tumors, which include *BRCA*-mutated tumors, may serve as a marker for platinum sensitivity; these tumors have a deficiency in the ability to perform high-fidelity repair of double-stranded breaks of DNA through the HRR pathway.[46] Other HRD-related genes, in addition to *BRCA1/2* mutations, include *PALB2, BARD1, BRIP1, RAD51B, RAD51C, RAD51D, ATM, FAAP20, CHEK2, FAN1, FANCE, FANCM,* and *POLQ*.[230,250]

Identifying mutation status may influence treatment recommendations; for example, certain targeted therapies such as PARP inhibitors have been developed specifically for the treatment of ovarian cancer with *BRCA1* or *BRCA2* mutations. Genomic alternations for which targeted therapies are currently available for ovarian cancer are summarized in Table 4-16.

Table 4-16. Genomic Alterations With Targeted Therapies Available for Ovarian Cancer

Molecular Alteration	Prevalence[a]	Drug Class
BRCA1/BRCA2	15%–20%	PARP inhibitor
HRD[b]	40%–50%[c]	PARP inhibitor[d]
NTRK	<1%	TRK inhibitor
MSI-H/dMMR	<1%	PD-1 mAb[e]

[a] The prevalence estimates for molecular alterations are based on data primarily obtained in patients with epithelial ovarian cancer and in particular those with high-grade serous ovarian cancer.

[b] HRD is a deficiency in ability to perform high-fidelity repair of double-strand breaks of DNA through the homologous recombination repair (HRR) pathway. *BRCA1/2* mutations are the most common and well-known aetiology associated with HRD. However, other genomic alterations (ie, *BARD1, BRIP1, PALB2, RB1, NF1, CDKN2A, CHEK1, CHEK2, FAM175A, MRE11A, NBN, RAD51C, RAD51D, CDK12*) are also associated with HRD.

[c] HRD includes *BRCA1/BRCA2* mutations, as these are HRD genetic alterations.

[d] Niraparib is recommended in patients with cancer associated with HRD, defined as either a deleterious or suspected deleterious *BRCA* mutation or genomic instability and progression >6 months after response to last platinum-based chemotherapy.

[e] Pembrolizumab.

BRCA, breast cancer susceptibility gene; dMMR, mismatch repair deficiency; DNA, deoxyribonucleic acid; HR, homologous recombination; HRD, homologous recombination deficiency; mAb, monoclonal antibody; NTRK, neurotrophic tyrosine receptor kinase; PARP, poly (ADP-ribose) polymerase; PD-1, programmed death-1; TRK, tropomyosin receptor kinase.

Source. Konstantinopoulos et al. 2015.[144] Bonadio et al. 2018.[145] Gee et al. 2018.[146]

Tumor genomics for the guidance of treatment decisions in ovarian cancer is recognized by the major clinical guidelines for patients with ovarian cancer, including NCCN and ASCO.[34,103,249,251,252] Clinical efficacy results from FDA-approved therapies for actionable mutations in ovarian cancer are described in Table 4-17.

Table 4-17. Treatment Outcomes With Targeted Therapies in Advanced Ovarian Cancer

Regimen	Patient Population	Efficacy Results
***BRCA1/2* mutations**		
Olaparib		
SOLO-3 (Pujade-Lauraine 2017)[242]	Maintenance therapy for platinum-sensitive, relapsed, *BRCA*-mutant ovarian cancer who received ≥2 prior lines of platinum therapy	Median PFS Olaparib: 19.1 months Placebo: 5.5 months HR: 0.30 (95% CI: 0.22, 0.41)
Study 42 (Domchek 2016)[243]	Deleterious or suspected deleterious *BRCA* mutations who had received ≥3 prior lines of chemotherapy	ORR[a]: 34% (95% CI: 26, 42) Median DOR: 7.9 months (95% CI: 5.6, 9.6)
Niraparib		
NOVA (Mirza 2016)[244]	Recurrent, platinum-sensitive with or without a germline *BRCA* mutation	Median PFS Germline *BRCA* mutation Niraparib: 21.0 months Placebo: 5.5 months HR: 0.27 (95% CI: 0.17, 0.41); *P*<0.0001

		No *BRCA* mutation Niraparib: 9.3 months Placebo: 3.9 months HR: 0.45 (95% CI: 0.34, 0.61); *P*<0.0001
Rucaparib		
ARIEL 3 (Coleman 2017[246])	Maintenance therapy for patients who had received ≥2 platinum-based therapies and achieved response to last treatment, with or without germline *BRCA* mutation	Median PFS Germline *BRCA* mutation Rucaparib: 16.6 months (95% CI: 13.4, 22.9) Placebo: 5.4 months (95% CI: 3.4, 6.7) HR: 0.23 (95% CI: 0.16, 0.34); *P*<0.0001
HRD[b]		
Niraparib		
QUADRA (Moore 2019)[245]	HRD-positive tumors, received ≥3 prior lines of chemotherapy	ORR[a]: 28% (95% CI: 6, 42.6) Median DOR: 9.2 months (95% CI: 5.9, NE)
MSI-H/dMMR		
Pembrolizumab		
KEYNOTE-158 (Marabelle 2020)[247]	MSI-H ovarian cancer	ORR[a]: 33.3% (95% CI: 11.8, 61.6) Median PFS: 2.3 months (95% CI: 1.9, 6.2)

[a] ORR was per RECIST v1.1.

[b] HRD is a deficiency in ability to perform high-fidelity repair of double-strand breaks of DNA through the homologous recombination repair (HRR) pathway. *BRCA1/2* mutations are the most common and well-known aetiology associated with HRD. However, other genomic alterations (ie, *BARD1, BRIP1, PALB2, RB1, NF1, CDKN2A, CHEK1, CHEK2, FAM175A, MRE11A, NBN, RAD51C, RAD51D, CDK12*) are also associated with HRD.

BRCA, breast cancer susceptibility gene; CI, confidence interval; CR, complete response; dMMR, deficient mismatch repair; DOR, duration of response; HR, hazard ratio; MSI-H, microsatellite instability-high; NE, not evaluable; NTRK, neurotrophic tropomyosin receptor kinases; ORR, overall response rate; PFS, progression-free survival; PR, partial response.

As targeted therapies have improved outcomes of patients with advanced ovarian cancer, major medical societies recommend that women with ovarian cancer undergo genetic testing, regardless of family history.

- In women with pathologically confirmed epithelial ovarian cancer, the NCCN Guidelines currently recommend patients have germline and somatic genetic testing.[34] Somatic testing should, at a minimum, optimize the identification of molecular alterations that can inform use of interventions that have demonstrated benefit in this setting, including *BRCA1/2*, LOH, or HR status in the absence of a germline *BRCA* mutation.[34] For patients with recurrent disease, tumor molecular analysis is recommended to include, at a minimum, tests to identify potential benefit from targeted therapeutics that have tumor-specific or tumor-agnostic benefit, including, but not limited to, *BRCA1/2*, HR status, MSI, TMB, and *NTRK* if prior testing did not include these markers. More comprehensive testing may be particularly important in less common histologies

with limited approved therapeutic options.[34]

- The Society of Gynecologic Oncology (SGO) also recommends that all women, regardless of age, with ovarian, tubal, and peritoneal cancer undergo genetic testing even if they do not have a family history of the disease.[249] SGO does not make a recommendation on the use of HRD and dMMR testing.[249]

Both the NCCN and ASCO recommend germline and somatic genetic testing in ovarian cancer.[34,103,252] For the HRD-genes, *BRCA1* and *BRCA2*, a systematic literature review and meta-analysis of 8 studies reported that both germline and somatic mutations in these genes have shown similar response rates to PARP inhibitor therapy.[253] CGP can detect both inherited germline and somatic gene alterations, leading to the potential for targeted therapy or clinical trial enrollment.[254] It should be noted that although both germline and somatic gene alterations can be detected, they cannot necessarily be differentiated using CGP.[254]

Although genetic testing in all women with ovarian cancer is recommended by most guidelines, as previously described, only approximately 10% to 30% of women with ovarian cancer undergo genetic testing.[230,255] Many factors have been cited as contributing to these low rates, including lack of access to testing, lack of physician knowledge, patient lack of knowledge about the value of testing, and/or anxiety around undergoing genetic testing.[256] Moreover, undertesting in ovarian cancer may reflect a relatively low public awareness of and advocacy for ovarian cancer.[230]

Place of Liquid Biopsy-Based CGP in Ovarian Cancer

For patients with ovarian cancer, regardless of stage at diagnosis, surgery is typically recommended as the first treatment option.[34] Based on this treatment pattern, most patients have tumor tissue available or banked for molecular testing. Somatic testing should, at a minimum, optimize the identification of molecular alterations that can inform use of interventions that have demonstrated benefit in this setting, including *BRCA1/2*, LOH, or HR status in the absence of a germline *BRCA* mutation.[34] For patients with recurrent disease, tumor molecular analysis is recommended to include, at a minimum, tests to identify potential benefit from targeted therapeutics that have tumor-specific or tumor-agnostic benefit, including, but not limited to, *BRCA1/2*, HR status, MSI, TMB, and *NTRK* if prior testing did not include these markers. More comprehensive testing may be particularly important in less common histologies with limited approved therapeutic options.[34]Although not currently recommended in the NCCN Guidelines, a reasonable approach in the instance of tumor persistence or recurrence may include a liquid biopsy to identify genomic alterations to inform treatment planning or clinical trial enrollment.

Currently, clinical guidelines from NCCN and ASCO recommend using tumor tissue for molecular testing of patients with ovarian cancer and do not make a recommendation on the use of liquid biopsy for screening, diagnosis, or disease monitoring.[34,252] Table 4-18 reviews the recommended biomarker testing to be conducted in ovarian cancer and the ability of the FoundationOne Liquid CDx Assay to test for these recommended biomarkers.

Table 4-18. Review of Guideline-Recommended Biomarker Testing and FoundationOne Liquid CDx Assay Capabilities in Ovarian Cancer

Applicable guidelines for tumor profiling	Recommended biomarker testing	Biomarker included in FoundationOne Liquid assay

NCCN Guidelines for Ovarian Cancer[a] (V.1.2022)[34]	*BRCA1/2* variants (for histologically confirmed disease)	Yes
	LOH	Yes
	HR status[b]	Yes
	MSI[c]	Yes
	TMB[c]	No[d]
	NTRK gene fusions[c]	Yes
ASCO[252]	BRCA1/2	Yes
	dMMR[c]	Yes

[a] **Category 1:** Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate. **Category 2A:** Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate. All NCCN recommendations are category 2A unless otherwise indicated.

[b] HR status to be determined in the absence of germline *BRCA* mutation.

[c] To be included in tumor molecular analysis only for patients with recurrent disease.

[d] FoundationOne Liquid CDx includes bTMB, which is a distinct biomarker from TMB; bTMB is correlated with TMB.

BRCA, breast cancer gene; dMMR, DNA mismatch repair; HRD, homologous recombination deficiency; MSI-H, microsatellite instability-high; NCCN, National Comprehensive Cancer Network; TMB, tumor mutational burden.

Clinical Utility and Validity of FoundationOne Liquid CDx in Ovarian Cancer

FoundationOne Liquid CDx is FDA-approved to report substitutions and indels in 311 genes, rearrangements in 4 genes, and copy number alterations in 3 genes. Comprehensive results across all 324 genes are reported as a laboratory professional service which is not reviewed or approved by the FDA. bTMB, MSI-H status, and tumor fraction are reported as a laboratory professional service which is not reviewed or approved by the FDA.[165] FoundationOne Liquid CDx utilizes circulating cfDNA isolated from plasma derived from the anticoagulated peripheral whole blood of cancer patients. The test is intended to be used as a companion diagnostic to identify patients with ovarian cancer who may benefit from treatment with the targeted therapy listed in Table 4-19 in accordance with the approved therapeutic product labeling. Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified healthcare professionals in accordance with professional guidelines in oncology for patients with malignant neoplasms, including ovarian cancer.[1]

Table 4-19. FoundationOne Liquid CDx Companion Diagnostic Indications Pertinent for Patients With Ovarian Cancer

Tumor type	Biomarker(s) detected	Therapy
Ovarian	*BRCA1*, *BRCA2* alterations	Rubraca® (rucaparib)

BRCA, breast cancer susceptibility gene.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1]

BRCA1/BRCA2 for Determining Treatment with Rucaparib

Rucaparib is FDA-approved for the treatment adult patients with *BRCA*-associated epithelial ovarian

cancer, fallopian tube, or primary peritoneal cancer who have received ≥2 prior lines of chemotherapy.[257] The clinical validity of using FoundationOne Liquid CDx as a companion diagnostic to identify patients with *BRCA*-mutated ovarian cancer who may benefit from treatment with rucaparib was conducted using pre-rucaparib treatment blood samples from the ARIEL-2 study.[112] The bridging study evaluated (1) the concordance between *BRCA1* and *BRCA2* alternation status by the tissue-based CTA (FoundationFocus™CDxBRCA and FoundationOne CDx) and FoundationOne Liquid CDx and (2) the clinical efficacy of rucaparib treatment in patients who would be eligible for therapy based on *BRCA1* and *BRCA2* alteration status as determined by FoundationOne Liquid CDx.[1,107,112] Plasma samples were available for 55% (271/491) of patients who received rucaparib in the ARIEL2 trial, and FoundationOne Liquid CDx data were obtained for 80% (217/271) of the patients with samples tested. FoundationOne Liquid CDx results were available 41% (26/64) of the patients in the primary efficacy population.[1]

- The PPA and NPA between the FoundationOne Liquid CDx Assay and the CTA for all patients with CTA and FoundationOne Liquid CDx results (n=217) were 93.8% (95% CI: 84.8, 98.3) and 97.4% (95% CI: 93.4, 99.3), respectively.[1]
- The primary efficacy endpoint evaluated for ARIEL2 in this premarket approval was confirmed ORR per RECIST v1.1 by investigator assessment.[112] The ORR in the primary efficacy population was 53.8% (95% CI: 33.4, 73.4) in *BRCA*-positive patients as determined by FoundationOne Liquid CDx, which is comparable to the ORR of 54.1% (95% CI: 40.8, 66.9) in patients identified by the CTA.[1]
- The secondary endpoints of the ARIEL2 trial included duration of confirmed response.[112] The median DOR was 225 days (95% CI: 115, 403) in FoundationOne Liquid CDx *BRCA* positive patients from the primary efficacy population. This is similar to the median DOR of 288 days (95% CI: 170, 403) for the primary efficacy population in *BRCA* positive patients as determined by the CTA.[1]
- Using plasma samples from the ARIEL2 study, the clinical utility of FoundationOne Liquid CDx as a companion diagnostic for identifying patients with *BRCA*-mutated ovarian cancer was demonstrated.[1]

5 ECONOMIC SUMMARY

- Compared to conventional molecular testing, CGP testing shows an incremental cost increase, mostly attributable to drug treatment costs, longer treatment, and longer survival; however, in models to date, the associated budget impact with CGP was $≤0.02 per-member per-month (PMPM).[114,258]
- CGP testing has been associated with a 10% to 20% enrollment rate in clinical trials to date compared with a historical enrollment rate of ≤8%; based on a small cohort analysis of phase 1 clinical trials, this may save payers $25,000 per patient through diversion of drug costs to the study sponsor.[53,59,74,259]

Economic Value of CGP in Advanced Cancer

A budget impact model that describes the effect of increased utilization of tissue- and ctDNA-based CGP testing from the commercial payer perspective is in development and pending publication. This tool is available upon request.

CGP Compared With Conventional Testing

Offering CGP over conventional molecular testing may allow a greater number of patients to benefit from matched therapies without the need for sequential testing for individual genomic alterations. Although it has been suggested that a higher proportion of matched therapy use leads to an increase in cost, this increase has been shown to occur primarily because of improved patient survival, which is the ultimate goal of CGP.[113,114,258] The budget impact of increased CGP use over conventional testing use was estimated in 3 identified economic models.

Budget Impact and Clinical Outcomes of Patients With NSCLC

Of the 3 identified budget impact studies, the first was a study published by Harvey et al in 2021 that assessed the budget impact of increased use of CGP vs conventional testing strategies among patients with advanced NSCLC from a US commercial health plan perspective.[115] A decision analytic model was developed to estimate the incremental benefits and costs across testing methodologies (CGP vs non-CGP), as well as across sample types (tissue-based and liquid-based), for patients with newly diagnosed advanced NSCLC.[115] In a hypothetical 2,000,000-member health plan, 790 members were estimated to have incident advanced NSCLC; 609 underwent molecular diagnostic testing with 122 (20%) tested with CGP (109 tissue-based and 13 liquid) in the base-case (20% CGP testing).[115] Per patient drug costs were estimated to be $237,403, and the total cost of care was estimated to be $325,548 in the base case.[115] With an increase in CGP from 20% to 30%, 183 patients would be tested, and per patient drug costs and total cost of care were estimated to be $237,724 (+$321) and $325,753 (+$205), respectively.[115] An increase in CGP from 20% to 30% (an additional 61 patients tested with CGP) was associated with 3.11 additional life-years gained and a $0.005 in US dollars per member per month budget impact.[115] Approximately 19.6 patients would need to be tested with CGP vs non-CGP to add 1 life-year and 5.9 patients would need to be tested with CGP to treat at least 1 patient with a biomarker-matched therapy.[115]

The second study modeled the incremental increase in a healthcare system budget associated with an increased use of CGP compared with conventional molecular diagnostic testing among patients with advanced NSCLC.[114] An increase in use of Foundation Medicine's tissue-based CGP among 266 patients estimated to be tested with molecular diagnostics from 2% to 10% (the equivalent of 21 additional patients tested with CGP) was associated with an incremental increase of $1,600 in cost (United States dollars [USD]) and 1.9 additional life-years, resulting in a budget impact of $0.02 PMPM.[114] Most of the budget impact was attributable to changes in drug treatment, longer treatment, and longer survival ($0.013 PMPM); the remainder was due to incremental costs of CGP vs conventional molecular diagnostic tests ($0.005 PMPM).[114] With 2% CGP utilization, the total per-patient drug costs were $45,305 and the total costs were $106,119; with 10% CGP utilization, these costs increased only slightly to $45,946 and $107,720, respectively.[114] This analysis also found that, among those tested, approximately 12 patients would need to be tested with CGP compared with conventional molecular diagnostic testing to gain 1 life-year.[114] Overall, this study demonstrated that an 8% absolute increase in the use of CGP in patients with NSCLC led to a modest budget impact. Additional information can be found in the Appendix (Table 6-18).

In a third model, the impact of using a CGP approach instead of single-gene testing for tissue-based molecular assessment of newly diagnosed nonsquamous advanced NSCLC was estimated for a US health plan payer.[258] The study used a Markov model to assess the impact of 100% CGP uptake in a 1-million member plan.[258] Overall, 179 patients were expected to undergo CGP in the first year.[258] It was noted that 32.1% of patients were expected to harbor an actionable alteration; however, only 19.5% of these were expected to have been identified using single-gene testing whereas 30.0% would be identified using CGP.[258] When costs (in USD) of treatment and rebiopsies were included, the total 5-year budget impact for patients who underwent CGP instead of single-gene testing was $432,554 ($0.0072 PMPM).[258]

In sensitivity analyses, the budget impact was most sensitive to the probability of ordering multiple single-gene tests, the cost of a test, and the probability of no result from CGP.[258] Furthermore, budget impact decreased as clinical trial enrollment increased (see **Potential Cost Diversion From Clinical Trial Enrollment** for additional supporting evidence).[258] This model demonstrated that a 100% uptake of CGP led to a modest budget impact on a US health plan while simultaneously improving the detection of actionable alterations in patients with advanced NSCLC.

Matched Compared With Unmatched Therapy

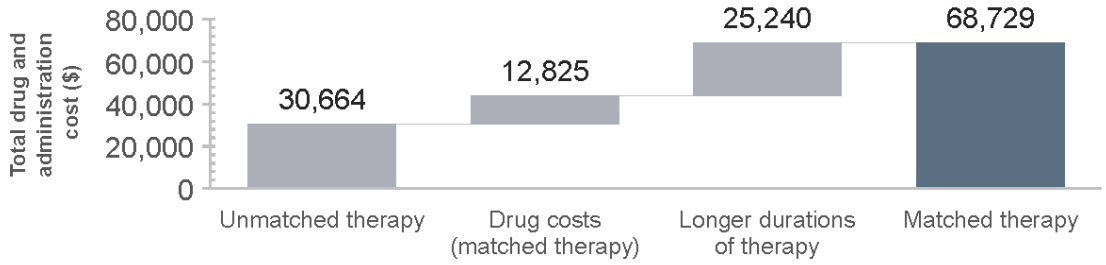
The following studies provide additional clinical evidence that matched therapy may be superior to unmatched therapy and that the additional costs of matched treatment are disproportionally related to improved clinical outcomes rather than to incremental increases in monthly drug costs. Furthermore, the low percentages of patients currently receiving molecular diagnostic testing is highlighted.

Tissue-Based CGP in Patients With Diverse Refractory Cancers

In an analysis that estimated the cost of anticancer therapy directed by CGP, costs were estimated using complete data from a phase 1, nonrandomized, prospective study that investigated patients (N=188) with diverse refractory cancers who underwent Foundation Medicine's tissue-based CGP and were treated with matched (targeted; n=122) or unmatched (n=66) therapy.[67,113] Total drug treatment costs (in USD) were higher among patients treated with matched therapy than among those who were unmatched ($68,729 vs

$30,664; *P*=0.003; Figure 5-1 and Figure 6-2).[113] However, most of the increased costs were attributable to a longer duration of therapy—ie, a longer time to treatment failure (TTF)—rather than higher monthly drug costs (66.3% vs 33.7%, respectively; Figure 5-1 and Figure 6-2).[113] This analysis showed that treatment with matched therapy was associated with longer treatment durations, improved survival, and manageable incremental costs compared with unmatched therapy.

Figure 5-1. Comparison of Total Drug Treatment Costs Between Matched and Unmatched Therapy Among Patients Who Received CGP



Note: Comparison of total drug treatment costs between matched and unmatched therapy in patients with all lines of therapy.

CGP, comprehensive genomic profiling.

Source: Adapted from Chawla et al. (2018).[113]

Real-World, Matched-Cohort Study of Patients With Metastatic Cancers

In a matched-cohort study of 72 patients with metastatic cancers who were treated in the precision cancer medicine program within the Intermountain Healthcare delivery system between 2013 and 2015, outcomes of a cohort of patients who underwent genomic testing and received targeted treatment (n=36) were compared with those of a historical control cohort of patients who received standard chemotherapy (n=29) or BSC (n=7) between 2010 and 2015.[9] Total medical and drug costs (in USD) were both significantly increased among patients receiving targeted therapy treatment compared with historical controls.[9] The difference was somewhat attenuated, however, when the costs were averaged by week of PFS ($4,665 vs $5,000; *P*=0.126).[9] The survival benefit of precision medicine–based treatment over traditional chemotherapy appears to be the driving factor behind increased total costs. Additional information can be found in the Appendix (Table 6-16).

Real-World Utilization of Molecular Diagnostic Testing and Matched Drug Therapies

A US healthcare claims analysis of 8,193 patients with 6 metastatic cancer types (breast, NSCLC, CRC, head and neck, ovarian, and uterine) investigated the rates of utilization and the average costs of molecular diagnostic testing.[113] Genomically matched targeted therapy was used by 6% to 11% of patients with metastatic breast cancer, NSCLC, and CRC; no patients with metastatic head and neck, ovarian, or uterine cancers used this therapy. Unmatched targeted therapy was used by 1% to 21% of patients across the different cancer types.[113]

The cost (in USD) of genomically matched targeted therapy compared with cytotoxic chemotherapy on a per-patient-per-month (PPPM) basis was $349 vs $293 in patients with breast cancer; $255 vs $425 in patients with NSCLC; and $164 vs $701 in patients with CRC.[113] Unmatched targeted therapy was less

costly than cytotoxic chemotherapy for all cancer types with the exception of head and neck cancer ($77 vs $45).[113] Total medical costs, excluding anticancer drug costs, ranged from $6,618 to $9,940 PPPM, driven primarily by outpatient visits and hospitalizations.[113] Overall, this study demonstrated that molecular testing is underutilized despite its relatively low cost of use, and there is no clear trend in higher cost associated with using matched targeted therapy over chemotherapy. Additional information can be found in the Appendix (Table 6-17).

Economic Value of CGP Increasing Clinical Trial Enrollment

Clinical trials provide patients who have advanced cancer and few remaining treatment options with access to investigational agents; often, these are targeted to genomic alterations for which no approved therapies are currently available. In addition to the opportunity to improve outcomes among these patients, enrollment into clinical trials may also lead to an economic benefit to health plans because of the diversion of anticancer drug costs to the study sponsor. Data from 2018 show that an estimated 40% of oncology clinical trials require molecular profiling for enrollment; this number has increased from 2010, at which time only 25% of trials required this information.[18]

Clinical Trial Enrollment Among Patients With Solid Tumors

A prospective, observational study of 120 enrolled patients with incurable, solid tumor malignancies who had progressed on 1 or more prior lines of therapy or who had no standard first-line treatment options available was undertaken to assess the feasibility of CGP in a community practice setting using a centralized molecular tumor board approach.[59] Of 63 patients for whom a treatment was recommended, CGP led to genomically matched therapy in 39 (62%) patients, with 16 of these patients enrolling in a clinical trial. In another study of tumor samples from 156 patients with pancreatic cancer and CGP results from Foundation Medicine's tissue-based CGP, 126 (81%) patients chose 1 of the recommended treatments, with 26 (21%) patients choosing clinical trials compared with a historical rate of 5% among patients with pancreatic cancer.[74,259] In a retrospective sequential cohort analysis of 103 patients with advanced cancers receiving Foundation Medicine testing during the course of clinical care, 18 patients received genotype-matched therapy, with 11 patients enrolling in clinical trials.[53]

Historically, clinical trial enrollment rates have been low (average rate of 8%; 6.3% in the community oncology setting), and identifying trials specific for each patient was burdensome.[75] However, with the advancing knowledge of genomic biomarkers and emerging treatment options as well as the use of CGP testing to increase the number of eligible patients who are identified and ultimately enrolled in clinical trials, trial enrollment remains a potential source of cost diversion back to study sponsors among patients with advanced cancer.

Potential Cost Diversion From Clinical Trial Enrollment

A retrospective analysis of medical records at an oncology practice described 3-year observational results in 86 patients who had clinically relevant genomic alterations (from a total of 96 patients who received CGP).[61] Based on the tissue CGP results, 15 patients were treated with genomically matched therapy and 6 patients enrolled in clinical trials.[61] The potential cost diversion (in USD) from payer to study sponsor in a separate cohort of 20 patients who enrolled in phase 1 clinical trials was explored. Assuming a treatment duration of 3.23 months,[10] it was estimated that the payer may have accrued a total annual cost

benefit of approximately $500,000 ($25,000 per patient) from the diversion of drug costs to the study sponsor.[61] Additional information can be found in the Appendix (Table 6-19).

A retrospective cohort study of patients with metastatic NSCLC (N=70) utilized linked data from electronic medical records to sociodemographic data from a cancer registry and claims data from Medicare and 2 private insurance plans to estimate mean per patient per month (PPPM) total direct medical costs for a second-line clinical trial vs second-line standard of care systemic therapy.[116] Of the 70 eligible patients, 22 (31%) were enrolled in a clinical trial while the remaining patients received second-line standard of care systemic therapy for metastatic NSCLC.[116] For second-line therapy, the mean PPPM total direct medical costs differed substantially, with patients enrolled in clinical trials having significantly lower costs ($4,808; standard deviation [SD]: $3,370 for trial participants vs $12,551; SD: $13,598 for nonparticipants; *P*=0.01).[116] For a mean duration of second-line therapy of 6.8 months, payers saved a mean of $45,308 per patient on a trial.[116]

6 APPENDICES

List of Tables

List of Figures

Terms and Definitions

Actionable alteration	A variation in DNA that is predicted to affect a patient's response to treatment and therefore guides selection of therapies. Also known as actionable mutations.
Advanced cancer	Cancer that is classified as locally advanced or metastatic, which corresponds to the SEER classifications of regional and distant cancer, respectively. Cancer is classified as regional when it has spread beyond the primary site to nearby lymph nodes or organs and tissues. Cancer is classified as distant when it has spread from the primary site to distant organs or distant lymph nodes. Distant cancer is also described as metastatic or stage IV cancer.
Alteration	See *Genomic alteration*.
Base pair	Molecules called nucleotides, on opposite strands of the DNA double helix, form chemical bonds with one another. These chemical bonds act like rungs in a ladder and hold the strands of DNA together. There are 4 nucleotides, or bases, of DNA: adenine (A), cytosine (C), guanine (G), and thymine (T). These bases form specific pairs (A with T, and G with C).
Base pair substitution	An alteration that exchanges 1 single base for another; the result could be a change in DNA sequence that substitutes 1 amino acid for another and may alter the resulting protein, no change in the amino acid sequence and thus no effect on the resulting protein, or a termination of the coding region resulting in a truncated protein.
Cancer of unknown primary (CUP)	A case in which cancer cells are found in the body, but the location where the cells first started growing (the origin or primary site) cannot be determined. Also known as carcinoma of unknown primary.
Cell-free DNA (cfDNA)	DNA that has been released from a cell and is freely circulating within the blood.
Circulating tumor DNA (ctDNA)	A component of cfDNA; DNA that has been shed into the blood specifically from a tumor cell.
Clinico-Genomic Database	A continuously updated database that includes patient data collected routinely as part of health care delivery from Flatiron Health with clinical data linked with CGP results from Foundation Medicine.

Complete response (CR)	The disappearance of all signs of cancer in response to treatment (does not necessarily indicate cure).
Comprehensive genomic profiling (CGP)	A hybrid-capture-based NGS platform that has been optimized to identify all types of molecular alterations (single nucleotide variants, small and large indels, CNAs, and structural variations) in cancer-related genes in a single test using complex and often proprietary bioinformatics. CGP may also include testing for MSI and TMB.
Concordance	Agreement; in the context of FoundationOne Liquid CDx, concordance represents agreement between the results of FoundationOne Liquid CDx and other NGS-based tests or selected FDA-approved non-NGS companion diagnostic assays conducted within the same temporal period.
Confidence interval (CI)	A range provided in conjunction with a point estimate that reflects the true effect on the entire population. For example, a 95% CI indicates a 95% likelihood that the population's result will fall into the range.
Copy number amplification	An alteration that results in a gain of sections of DNA.
Copy number alteration (CNA)	An alteration that results in a gain or loss in copies of sections of DNA.
Distant cancer	Cancer that has spread from the primary site to distant organs or distant lymph nodes. Distant cancer is also described as remote, disseminated, diffuse, metastatic, or stage IV cancer.
DNA sequencing	A laboratory process used to determine the exact sequence (order) of the 4 building blocks, or bases, that make up DNA (identified by the letters A, C, G, and T; see *Base pair* for additional information). DNA sequencing can be used to find genomic alterations.
First-line treatment	The first therapeutic intervention for a disease. When used by itself, first-line therapy represents the accepted best treatment. Also known as induction therapy, primary therapy, and primary treatment.

Fluorescence *in situ* hybridization (FISH)	A laboratory technique used to look at genes or chromosomes in cells and tissues. Pieces of DNA that contain a fluorescent dye are made in the laboratory and added to cells or tissues on a glass slide. When these pieces of DNA bind to specific genes or areas of chromosomes on the slide, they are visible when viewed under a microscope sensitive to fluorescent light. Also known as fluorescent *in situ* hybridization.
Fusion	A hybrid gene formed from 2 previously separate genes. It can occur as a result of translocation, interstitial deletion, or chromosomal inversion.
Gene rearrangement	A large alteration of a chromosome or large chromosomal regions that can take the form of deletions, duplications, insertions, inversions, or translocations.
Genomic alteration	A change in DNA sequence; examples include base pair substitutions, indels, CNAs, and gene rearrangements. Genomic alterations can lead to proteins with abnormal levels of expression and/or function.
Hazard ratio (HR)	A measure of how often a particular event happens in one group compared with how often it happens in another group, over time. In cancer research, HRs are often used in clinical trials to compare survival and other dichotomous outcomes at any point in time between an experimental group of patients who have been assigned a specific treatment and a control group assigned a different treatment or placebo. An HR of 1.0 indicates no difference in outcomes between the groups. An HR >1 or <1 may indicate that the outcome was better in one of the groups. The CI is used to measure the precision of the HR; if the CI includes 1, then the HR is not statistically significant.
Homologous recombination deficiency (HRD)	Deficiency in ability to perform high-fidelity repair of double-strand breaks of DNA through the homologous recombination repair (HRR) pathway. *BRCA1/2* mutations are the most common and well-known etiology associated with HRD. However, other genomic alterations (ie, *BARD1, BRIP1, PALB2, RB1, NF1, CDKN2A, CHEK1, CHEK2, FAM175A, MRE11A, NBN, RAD51C, RAD51D, CDK12*) are also associated with HRD.

Hotspot analysis	In cancer, a hotspot analysis assesses specific alterations in prespecified regions of the gene known to be associated with the diagnosis, prognosis, or treatment of cancer (as opposed to sequencing the entire gene of interest).
Hybrid capture	A signal amplification method where an RNA probe is annealed to target DNA. Subsequently, a captured antibody binds the DNA/RNA hybrid to a solid surface. Also known as hybridization capture.
Immunohistochemistry (IHC)	A laboratory analysis that uses antibodies to test for certain antigens (markers) in a cell or tissue sample. The antibodies are usually linked to an enzyme or a fluorescent dye. When the antibodies bind to the antigen in the tissue sample, the enzyme or dye is activated, and the antigen can be visualized under a microscope. Immunohistochemistry is used to help diagnose diseases, such as cancer.
Immunotherapy	A type of treatment used frequently in patients with cancer that uses substances to stimulate or suppress the immune system. Broadly, this can include cytokines, vaccines, and some monoclonal antibodies; therapies often target certain cells of the immune system although others affect the immune system more broadly. In this dossier, immunotherapy refers more specifically to the use of immune checkpoint inhibitors, including monoclonal antibodies to PD-1, PD-L1, and CTLA-4.
Insertion or deletion alteration (indel)	Alterations in which extra base pairs are inserted into a new place in the DNA (insertions) or in which a section of DNA is removed (deletions).
Liquid biopsy	A test done on a sample of blood to identify cancer cells from a tumor or pieces of DNA from tumor cells that are circulating in the blood.
Matched therapy	Treatment matched to a patient's genomic alteration(s) and/or profile; matched therapy can include both targeted therapy and immunotherapy.
Metastatic cancer	Cancer that has spread from the primary site (where the cancer started) to other places in the body.

Microsatellite instability (MSI)	A change that occurs in the DNA of certain cells (such as tumor cells) in which the number of repeats of microsatellites (short, repeated sequences of DNA) is different than the number of repeats that was in the DNA when it was inherited. The cause of microsatellite instability may be a defect in the ability to repair mistakes made when DNA is copied in the cell.
Mismatch repair (MMR)	Replacement of mismatched DNA base pairs by the enzyme DNA polymerase. Involves the removal of the incorrect base and replacement with the correct base.
Mutant allele frequency (MAF)	The allele frequency at which a specific mutation is detected.
Maximum somatic allele frequency (MSAF)	The maximum allele frequency identified of all alterations measured, which can provide an estimate of ctDNA fraction in the blood.
Next-generation sequencing (NGS)	A high-throughput method used to determine a portion of the nucleotide sequence of an individual's genome. This technique utilizes DNA sequencing technologies that are capable of processing multiple DNA sequences in parallel. Also called massively parallel sequencing and NGS. In the oncology space, this technology is used to interrogate clinically relevant genes to identify 4 classes of actionable alterations: base substitutions, short indels, CNAs, and gene fusions.
Nonsynonymous substitution or mutation	A point mutation (base pair change) in a codon that results in a change in the amino acid produced during translation.
Overall response rate (ORR)	ORR is defined as the proportion of patients with tumor size reduction of a predefined amount and for a minimum time period (ie, until documented tumor progression). ORR equals the sum of confirmed PRs and CRs.

Odds ratio (OR)	A measure of the odds of an event happening in one group exposed to a potential risk factor compared to the odds of the same event happening in another group that has not been exposed to the potential risk factor. In cancer research, ORs are most often used in case-control (backward looking) studies to determine if exposure to a potential risk factor increases the risk of cancer. An OR of 1.0 means that both groups had the same odds of developing cancer regardless of their exposure to the potential risk factor. An OR >1 may indicate that exposure to a risk factor could increase the odds of developing cancer, whereas an OR <1 may indicate that exposure could reduce the risk of cancer. The CI is used to estimate the precision of the OR; a large CI indicates a low level of precision whereas a small CI indicates a higher level of precision. The CI does not report a measure's statistical significance. Also known as relative odds.
Overall survival (OS)	The time from randomization until death from any cause.
Partial response (PR)	A decrease in the size of a tumor, or the extent of cancer in the body, in response to treatment. Also known as partial remission.
Polymerase chain reaction (PCR)	A laboratory method used to make many copies of a specific fragment of DNA from a sample that contains very small amounts of that DNA. The method allows DNA to be amplified sufficiently to detect certain changes in a gene, such as a genomic alteration.
Positive percent agreement (PPA)	The proportion of non-reference standard positive subjects in whom the new test is positive. PPA reflects the frequency of false negatives.
Precision medicine	A form of medicine that uses information about a person's genes, proteins, and environment to prevent, diagnose, and treat disease. In cancer, precision medicine can identify specific information from tumors to help diagnose patients, plan treatment, monitor treatment efficacy, and/or determine disease prognosis. Examples include using targeted therapies to treat specific types of cancer cells, such as HER2-positive breast cancer cells, or using tumor marker testing to help diagnose cancer.
Progression-free survival (PFS)	The time from randomization until objective tumor progression or death. The precise definition of tumor progression is important and should be carefully detailed in the protocol.

Progressive disease (PD)	Cancer that is growing, spreading, or getting worse. According to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, disease progression occurs when there is a 20% increase in the sum of the longest diameter from nadir, 20% increase in the sum of diameters, and at least a 5-millimeter increase from nadir.
Rearrangement	A type of chromosome abnormality involving a change in the structure of the native chromosome. Such changes may involve several different classes of events, including deletions, duplications, inversions, and translocations. Usually, these events are caused by a breakage in the DNA double helices at 2 different locations, followed by a rejoining of the broken ends to produce a new chromosomal arrangement of genes, different from the gene order of the chromosomes before they were broken.
Recurrent cancer	Cancer that has recurred (come back), usually after a period of time during which the cancer could not be detected. The cancer may come back to the same place as the original (primary) tumor or to another place in the body.
Solid tumor	An abnormal mass of tissue that usually does not contain cysts or liquid areas.
Somatic mutation	An alteration in DNA that occurs after conception. Somatic mutations can occur in any of the cells of the body except the germ cells (sperm and egg) and, therefore, are not passed on to children. These alterations can (but do not always) cause cancer or other diseases.
Stable disease (SD)	Cancer that is neither decreasing nor increasing in extent or severity.
Stage IV cancer	Cancer that has spread to distant parts of the body at the time the patient is initially diagnosed with cancer.
Synonymous substitution or mutation	A nucleotide mutation that does not alter the amino acid sequence, involving an insertion or deletion of a single nucleotide during transcription causing a frameshift or point mutation. Also known as a silent mutation or replacement mutation.

Targeted therapy	In cancer, a method of treatment that uses drugs or other substances to identify and attack specific types of cancer cells while causing less harm to non-cancer cells. Some targeted therapies block the action of certain enzymes, proteins, or other molecules involved in the growth and spread of cancer cells. Other types of targeted therapies help the immune system kill cancer cells or deliver toxic substances directly to cancer cells and kill them. Targeted therapy may have fewer adverse events than other types of cancer treatment. Most targeted therapies use either small-molecule drugs or monoclonal antibodies.
Time to treatment failure (TTF)	The time from randomization to treatment discontinuation for any reason, including disease progression, treatment toxicity, patient preference, or death.
Tumor mutational burden (TMB)	Calculated using the number of somatic base substitution or insertion/deletion alterations per megabase of the coding region after filtering to remove known somatic and deleterious mutations and by subsequently extrapolating that value to the exome or genome as a whole.
Unmatched therapy	In cancer, general treatment offered to a patient that is not based on genomic alterations, such as chemotherapy.
Variant	An alteration in the most common DNA nucleotide sequence. The term *variant* can be used to describe an alteration that may be benign, pathogenic, or of unknown significance.
Variant of unknown significance (VUS)	An allele identified through genetic testing whose significance to the function or health of an individual is not known. It is not known whether the allele is a risk factor or a harmless change.

List of Abbreviations

2L	second line
aHR	adjusted hazard ratio
AKT	protein kinase B
ALK	anaplastic lymphoma kinase
AMP	Association for Molecular Pathology
APC	adenomatous, polyposis coli
AR	androgen receptor
ARID1A	AT-rich interaction domain-containing protein 1A
ATM	ataxia-telangiectasia mutated
BRAF	v-raf murine sarcoma viral oncogene homolog B1
BRCA	breast cancer susceptibility gene
BSC	best supportive care
bTMB	blood tumor mutational burden
CAP	College of American Pathologists
CDKN2A	cyclin-dependent kinase inhibitor 2A
cfDNA	cell-free deoxyribonucleic acid
CGDB	clinico-genomic database
CGP	comprehensive genomic profiling
CHEK2	checkpoint kinase 2
CI	confidence interval
CDK12	cyclin-dependent kinase 12
CLIA	Clinical Laboratory Improvement Amendments
CAN	copy number alteration

CR	complete response
CRC	colorectal cancer
CRPC	castration-resistant prostate cancer
CTA	clinical trial assay
ctDNA	circulating tumor deoxyribonucleic acid
CUP	cancer of unknown primary
DCR	disease control rate
ddPCR	droplet digital polymerase chain reaction
DDR	DNA damage repair
dMMR	mismatch repair deficient
DNA	deoxyribonucleic acid
EGFR	epidermal growth factor receptor
ER	estrogen receptor
FANCD2	Fanconi anemia group D2
FDA	U.S. Food and Drug Administration
FISH	fluorescence *in situ* hybridization
GA	genomic alteration
HCC	hepatocellular carcinoma
HER2	human epidermal growth factor receptor 2
HR	hazard ratio
HRD	homologous recombination deficiency
HRR	homologous recombination repair
HRRm	homologous recombination repair gene mutations
IASLC	International Association for the Study of Lung Cancer

IHC	immunohistochemistry
indel	insertion and deletion alteration
IQR	interquartile range
KRAS	V-Ki-ras2 Kirsten rat sarcoma
LDT	laboratory-developed test
LoB	limit of blank
LoD	limit of detection
MAF	mutant allele frequency
Mb	megabase
mCRPC	metastatic castration-resistant prostate cancer
MET	mesenchymal epithelial transition factor receptor
MLH1	MutL homolog 1
MMR	mismatch repair
MSAF	maximum somatic allele frequency
MSH	mismatch repair protein involved in the DNA mismatch repair system
MSI	microsatellite instability
MSI-H	microsatellite instability–high
mut	mutations
NA	not applicable
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NE	not evaluable
NF1	neurofibromin 1
NGS	next-generation sequencing

NNT	number needed to test
NOS	not otherwise specified
NPA	negative percent agreement
NPV	negative predictive value
NR	not reported
NRAS	neuroblastoma rat sarcoma
NSCLC	non-small cell lung cancer
NTRK	neurotrophic receptor tyrosine kinase
OR	odds ratio
ORR	overall response rate
OS	overall survival
PARP	poly ADP-ribose polymerase
pcHA	physician choice of a standard of care hormonal agent
PCCTC	Prostate Cancer Clinical Trials Consortium
PCR	polymerase chain reaction
PD	progressive disease
PDGFRA	platelet-derived growth factor receptor A
PD-1	programmed death-1
PD-L1	programmed death ligand-1
PFS	progression-free survival
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
pMMR	proficient DNA mismatch repair
PMPM	per-member per-month
PPA	positive percent agreement

PPPM	per-patient per-month
PPV	positive predictive value
PR	partial response
PTEN	phosphatase and tensin homolog
RAD	genes that encode for members of the RAD51 protein family that are known to be involved in homologous recombination and repair of DNA
RAS	rat sarcoma
RB1	retinoblastoma-1
RECIST	Response Evaluation Criteria in Solid Tumors
RET	ret proto-oncogene
RNA	ribonucleic acid
ROS-1	c-ros oncogene
rPFS	radiographic progression-free survival
SCC	squamous cell carcinoma
SD	stable disease
SEER	Surveillance, Epidemiology, and End Results
SGO	Society of Gynecologic Oncology
STK11	serine/threonine kinase 11
TCGA	The Tumor Cancer Genome Atlas
TERT	telomerase reverse transcriptase
TKI	tyrosine kinase inhibitor
TMB	tumor mutational burden
TMB-H	tumor mutational burden-high
TP53	tumor protein p53

TTF	time to treatment failure
US	United States
USD	United States dollars
VAF	variant allele frequency
VUS	variant of unknown significance
WES	whole exome sequencing
WT	Wild-type

NCCN Guidelines: Recommendations for Molecular Testing

Table 6-1 reviews the NCCN Guidelines recommendations pertaining to molecular testing across solid tumor types. Table 6-2 (NSCLC), Table 6-3 (prostate cancer), Table 6-4 (breast cancer), and Table 6-5 (ovarian cancer) provide an overview of the NCCN Guidelines recommendations pertaining to biomarker-recommended therapies and which of these therapies require a companion diagnostic in select tumor types; this table also provides alignment for which of these biomarker-directed therapies FoundationOne CDx is the companion diagnostic.

Table 6-1. NCCN Guidelines: Recommendations for Molecular Testing Within Select Cancers and Relevant Foundation Medicine Testing

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
Bladder cancer **NCCN Guidelines for Bladder Cancer V.1.2022**[26]	The panel recommends that molecular/genomic testing be performed for stages IVA and IVB bladder cancer and may be considered for stage IIIB. Testing should be carried out early, ideally at diagnosis of advanced bladder cancer, in order to facilitate treatment decision-making and to prevent delays in administering later lines of therapy. In addition to determining eligibility for FDA-approved therapies, molecular/genomic testing may be used to screen for clinical trial eligibility.(BL-8, BL-9, BL-10, MS-27) Molecular/genomic testing (including testing for *FGFR2* or *FGFR3* alterations*)* is recommended for stage IVA and IVB and should be considered for stage IIIB.(BL-8, BL-9, BL-10, MS-27)	FoundationOne CDx is able to detect alterations in *FGFR2* and *FGFR3.* The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Bone cancer **NCCN Guidelines for Bone Cancer V.2.2022**[43]	For metastatic chondrosarcoma, metastatic/recurrent chordoma, metastatic Ewing sarcoma, and metastatic osteosarcoma, consider CGP with a validated and/or FDA-approved assay to determine targeted therapy opportunities (CHON-4, CHOR-3, EW-3, OSTEO-3). For Ewing sarcoma, consider CGP or other fusion panel to identify translocations if pathologic workup of targeted PCR, FISH, or cytogenetics is negative (EW-1). For metastatic chondrosarcoma, recurrent chordoma, and metastatic osteosarcoma, consider CGP with a validated and/or FDA-approved assay to determine targeted therapy opportunities; consider testing for TMB and MMR/MSI as determined by a validated and/or FDA-approved assay to inform the use of pembrolizumab.(CHON-4, CHOR-3, OSTEO-3)	FoundationOne Heme includes detection of EWS and FUS gene fusions with fusion partner genes described in the discussion section of the guidelines (FLI1, ERG, ETV4, FEV). FoundationOne Heme also includes MSI status. FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb) FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	For metastatic Ewing Sarcoma, consider testing for TMB (category 2B) and MMR/MSI as determined by a validated and/or FDA-approved assay to inform the use of pembrolizumab.(EW-3) Pembrolizumab for TMB-H (≥10 mut/Mb) tumors is listed as a systemic therapy option useful in certain circumstances. TMB-H for patients with unresectable or metastatic tumors who have progressed following prior treatment and who have no satisfactory alternative treatment options. Not for Giant Cell Tumor of Bone.(BONE-B 1 of 5) Pembrolizumab is listed as a preferred therapy option for MSI-H/dMMR tumors. Pembrolizumab is a systemic treatment option for adult and pediatric patients with unresectable or metastatic, MSI-H or dMMR solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options. Not for Giant Cell Tumor of Bone.(BONE-B 1 of 5) For conventional (Grades 1–3) and dedifferentiated chondrosarcoma, testing for *IDH1* mutation can be performed by NGS or targeted exon sequencing to determine treatment with ivosidenib (for susceptible *IDH1* mutations) (BONE-B 1 of 5). Lapatinib is recommended as useful in certain circumstances in patients with *EGFR*-positive chordomas.	treatment with Keytruda (pembrolizumab). The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Breast cancer[b] **NCCN Guidelines for Breast Cancer V.3.2022[25]**	Genetic counseling and testing if patient is at risk for hereditary breast cancer, has TNBC (at any age), or is a candidate for adjuvant olaparib are recommended.(BINV-1) Comprehensive germline and somatic profiling is recommended in the workup algorithm for recurrent/stage IV (M1) disease to identify candidates for additional targeted therapies.(BINV-18) Consider adjuvant olaparib for 1 year for those with germline *BRCA*1/2 mutations and: TNBC, if 1) ≥ pT2 or ≥ pN1 disease after adjuvant chemotherapy, or 2) residual disease after preoperative chemotherapy; HR-positive, HER2-negative tumors, if 1) ≥4 positive lymph nodes after adjuvant chemotherapy, or 2)	FDA-approved to report *ERBB2* (HER2), *PIK3CA* and *BRCA1/2* alterations, and MSI status. FoundationOne CDx is an FDA-approved CDx for Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), Perjeta® (pertuzumab) and Piqray® (alpelisib). FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	residual disease after preoperative therapy and a clinical stage, pathological stage, estrogen receptor status, and tumor grade (CPS+EG) score ≥3. Adjuvant olaparib can be used concurrently with endocrine therapy.(BINV-L 1 of 9) For stage IV or recurrent breast cancer, assess for *PIK3CA* mutation with tumor or liquid biopsy if hormone receptor-positive/HER2-negative and if considering therapy with alpelisib + fulvestrant. *PIK3CA* mutation testing can be done on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended. Testing methodology recommendation is molecular panel or PCR (category 1). Fulvestrant + alpelisib for *PIK3CA*-mutated tumors is recommended as a preferred second-line or subsequent treatment (category 1).(BINV-R 1 of 3) NGS testing to assess for TMB-H (≥10 muts/Mb) for patients with recurrent or stage IV (M1) disease. MSI-H/dMMR testing by IHC or PCR is recommended. Pembrolizumab is indicated for the treatment of patients with unresectable or metastatic MSI-H or dMMR solid tumors, or TMB-H tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options.(BINV-R 1 of 3) *NTRK* gene fusion testing by NGS, PCR, and FISH for patients with recurrent or stage IV (M1) disease. For stage IV or recurrent breast cancer, larotrectinib and entrectinib are FDA-approved therapies useful in certain circumstances for *NTRK* gene fusion-positive patients without a known acquired resistance mutation and have no satisfactory alternative treatments or that have progressed following treatment.(BINV-R 1 of 3) Assess for *BRCA1/2* germline mutations in all patients with recurrent or metastatic breast cancer to identify candidates for PARP inhibitor therapy. While olaparib and talazoparib are FDA-indicated in HER2 negative disease, the panel supports use in any breast cancer subtype	patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). FoundationOne Liquid CDx provides FDA-approved comprehensive genomic profiling from a circulating cell-free DNA (cfDNA) sample. FoundationOne Liquid CDx is an FDA-approved companion diagnostic for Piqray® (alpelisib) in breast cancer. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	associated with a germline *BRCA1* or *BRCA2* mutation (Category 1).(BINV-R 1 of 3) HER2 testing at diagnosis and of a metastatic site at progression.(BINV-A 1 of 2)	
CNS cancers **NCCN Guidelines for CNS Cancers V.2.2021**[27]	Molecular testing of glioblastoma is encouraged because if a driver mutation is detected, it may be reasonable to treat with a targeted therapy on a compassionate use basis and/or the patient may have more treatment options in the context of a clinical trial. Molecular testing also has a valuable role in improving diagnostic accuracy and prognostic stratification that may inform treatment selection.(BRAIN-D 3 of 15) Larotrectinib and entrectinib are options for systemic therapy in both newly diagnosed and recurrent brain metastases for patients with *NTRK* gene fusion-positive tumors. Larotrectinib and entrectinib are useful in certain circumstances for recurrent or progressive *NTRK* gene fusion-positive adult low-grade (WHO Grade 1 or 2) glioma, anaplastic gliomas, and glioblastoma. (BRAIN-D 1-3, 8 of 15) Pilocytic astrocytoma, pleomorphic xanthoastrocytoma, and ganglioglioma: Consider BRAF/MEK inhibitors if *BRAF* V600E-activating mutation as an adjuvant therapy option.(BRAIN-D, 1 of 15, LGG-1) For recurrent or progressive adult low-grade (WHO Grade 1 or 2) glioma, recurrent anaplastic gliomas, or recurrent glioblastoma: Consider BRAF/MEK inhibitors if *BRAF* V600E-activating mutation as an adjuvant therapy option.(BRAIN-D, 1 of 15) *BRAF* fusion and/or mutation testing are clinically indicated in certain patients with low-grade glioma.(MS-6)	FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). FoundationOne CDx is FDA-approved to report MSI status, *NTRK* gene fusions, *BRAF* alterations, and TMB. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Cervical cancer **NCCN Guidelines for Cervical Cancer V.1.2022**[42]	For persistent or recurrent cervical cancer, consider CGP with a validated and/or FDA-approved assay. If tissue biopsy of metastatic site is not available, consider CGP via a validated plasma ctDNA assay.(CERV-10) For patients with stage IVB cervical cancer or distant metastases, consider TMB testing as	FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	determined by a validated and/or FDA-approved assay.(CERV-12) Preferred second-line and subsequent therapy options include pembrolizumab for PD-L1 positive or MSI-H/dMMR tumors.(CERV-F 1 of 3) For second-line and subsequent therapy, pembrolizumab for TMB-H tumors and larotrectinib or entrectinib for NTRK gene fusion-positive tumors (category 2B) are recommended options as useful in certain circumstances.(CERV-F 1 of 3) Pembrolizumab is a treatment option for patients with unresectable or metastatic TMB-H (≥10 mut/Mb) tumors as determined by a validated and/or FDA-approved test, that have progressed following prior treatment and who have no satisfactory alternative treatment options.(CERV-F 1 of 3)	metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). FoundationOne CDx is FDA approved to report MSI status, *NTRK* gene fusions and TMB. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
CRC **NCCN Guidelines for Colon Cancer V.1.2022**[28] **NCCN Guidelines for Rectal Cancer V.1.2022**[37]	Methods of testing: The testing can be performed on formalin-fixed paraffin-embedded tissue (preferred) or blood-based assay (COL-B, 4 of 8; REC-B, 5 of 9) Determination of tumor gene status for *RAS* and *BRAF* mutation and HER2 amplifications (individually or as part of tissue- or blood-based NGS panel) for patients with suspected or proven metastatic synchronous adenocarcinoma (any T, any N, M1) or metachronous metastases. If known *RAS/RAF* mutation, HER2 testing is not indicated. Tissue- or blood-based NGS panels have the ability to pick up rare and actionable mutations and fusions. Determination of MMR or MSI status recommended (if not previously done).(COL-4, COL-9); (REC-7, REC-12) All patients with metastatic CRC should have tumor genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations individually or as part of an NGS panel.(COL-B 4 of 8); (REC-B 5 of 9)	FDA-approved to report *KRAS*, *NRAS*, *BRAF*, *ERBB2* (HER2), *NTRK* alterations, and MSI status. FoundationOne CDx is an FDA-approved companion diagnostic for Erbitux® (cetuximab) and Vectibix® (panitumumab). FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	Universal MMR or MSI testing is recommended in all newly diagnosed patients with colon or rectal cancer.(COL-1,COL-2, COL-B 4 of 8); (REC-1, REC-2, REC-B 5 of 9) While not explicitly recommended, *NTRK* fusions may be actionable if detected. Selection of the appropriate assay for *NTRK* fusion detection depends on tumor type and genes involved, as well as consideration of other factors such as available material, accessibility of various clinical assays, and whether comprehensive genomic testing is needed concurrently. (COL-B 5 of 8; REC-B 6 of 9) HER2 testing is recommended via IHC, FISH, or NGS; if known *RAS/RAF* mutation, HER2 testing is not indicated. Anti-HER2 therapy is only indicated in HER2-amplified tumors that are also *RAS* and *BRAF* wild-type.(COL-B 5 of 8;REC-B 6 of 9)	treatment with Keytruda (pembrolizumab). The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Endometrial cancer/uterine sarcoma[b] **NCCN Guidelines for Uterine Neoplasms V.1.2022[38]**	Recommend genetic evaluation of tumor and evaluation for inherited cancer risk at initial evaluation.(UN-1) Endometrial cancer Molecular analysis of endometrial carcinoma has identified 4 clinically significant molecular subgroups with differing clinical prognoses: *POLE* mutations, MSI-H, copy number low, and copy number high. Consider CGP via a validated and/or FDA-approved assay in the initial evaluation of uterine neoplasms. Ancillary studies for *POLE* mutations, MMR/MSI, and aberrant p53 expression are encouraged to complement morphologic assessment of histologic tumor type.(ENDO-A 2 of 4) Universal testing of endometrial carcinomas for MMR proteins is recommended (MSI testing if results equivocal).(ENDO-A 2 of 4) For recurrent endometrial cancer, MSI-H/dMMR and TMB-H testing is recommended if not previously done. (ENDO-D 3 of 4) Consider TMB testing through a validated and/or FDA-approved assay (reference for TMB validation added [Merino et al 2020]).(ENDO-A 2 and 4 of 4)	FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) for patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is FDA-approved to assess *ERBB2*(HER2), *POLE*, *NTRK* gene fusions, MSI status, and TMB. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	Consider *NTRK* gene fusion testing for metastatic or recurrent endometrial carcinoma.(ENDO-A 2 of 4) Preferred second-line therapy option includes pembrolizumab for TMB-H or MSI-H/dMMR tumors.(ENDO-D 1 of 4) For second-line therapy, nivolumab, dostarlimab-gxly, and avelumab are other recommended therapy options for dMMR/MSI-H tumors. Larotrectinib and entrectinib are other recommended therapy options for *NTRK* gene fusion-positive tumors (category 2B).(ENDO-D 1 of 4) Uterine sarcoma Comprehensive genomic profiling with a validated and/or FDA-approved assay is informative for predicting rare pan-tumor-targeted therapy opportunities and should include at least *NTRK*, MSI, and TMB.(UTSARC-A 1 of 8) Molecular profiling is informative in many mesenchymal malignancies for accurate classification.(UTSARC-A 1 of 8) Pembrolizumab is considered useful in certain circumstances for second-line therapy option for TMB-H tumors. Larotrectinib and entrectinib are considered useful in certain circumstances for *NTRK* gene fusion-positive tumors (category 2B).(UTSARC-C, 1 of 2) Consider PARP inhibitors for *BRCA2*-altered uterine leiomyosarcoma.(UTSARC-C 1 of 2)	NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Gastric, esophageal, and esophagogastric junction cancers **NCCN Guidelines for Gastric Cancer V.2.2022**[31] **NCCN Guidelines for Esophageal and Esophagogastric Junction Cancers V.2.2022**[30]	At initial diagnostic workup universal testing for MSI by PCR/NGS or MMR by IHC is recommended in all newly diagnosed gastric cancer patients; HER2 and PD-L1 testing should be completed if metastatic adenocarcinoma is suspected. NGS may be considered.(GAST-1) Initial diagnostic workup for esophageal cancer should include MSI and PD-L1 testing, if metastatic disease is documented/suspected, and HER2 testing if metastatic adenocarcinoma is documented/suspected. NGS may be considered.(ESOPH-1) For unresectable locally advanced, recurrent, or metastatic gastric cancer: perform HER2, PD-L1, and microsatellite testing (if not done	FDA-approved to report *ERBB2*/HER2, MSI, and *NTRK* gene fusions. FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb).

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	previously) if metastatic cancer is documented or suspected; NGS may be considered via a validated assay.(GAST-9) For unresectable locally advanced, locally recurrent, or metastatic esophageal and esophagogastric junction cancers (SCC histology): perform microsatellite and PD-L1 testing (if not done previously) if metastatic cancer is suspected; perform microsatellite, PD-L1, and HER2 testing (if not done previously) if metastatic cancer is suspected for patients with adenocarcinoma histology. NGS may be considered via validated assay for both SCC and adenocarcinoma histologies.(ESOPH-10, ESOPH-19) NGS: At present, several targeted therapeutic agents, trastuzumab, pembrolizumab/nivolumab, and entrectinib/larotrectinib have been approved by the FDA for use in gastric, esophageal, and esophagogastric junction cancers. Trastuzumab is based on testing for HER2 overexpression. Pembrolizumab/nivolumab are based on testing for MSI by PCR or NGS/MMR by IHC, PD-L1 immunohistochemical expression or high TMB by NGS. The FDA granted approval for the use of select TRK inhibitors for *NTRK* gene fusion-positive solid tumors. When limited tissue is available for testing, or the patient is unable to undergo a traditional biopsy, sequential testing of single biomarkers or use of limited molecular diagnostic panels may quickly exhaust the sample. In these scenarios, comprehensive genomic profiling via a validated NGS assay performed in a CLIA-approved laboratory may be used for the identification of HER2 amplification, MSI status, MMR deficiency, TMB, and *NTRK* gene fusions. The use of IHC/ISH/targeted PCR should be considered first followed by additional NGS testing as appropriate.(GAST-B 5 of 6); (ESOPH-B 5 of 6) Assessment of overexpression or amplification of HER2: NGS offers the opportunity to assess numerous mutations simultaneously, along with other molecular events such as amplification, deletions, TMB, and MSI status. NGS can be considered instead of sequential testing for single biomarkers when limited diagnostic tissue	FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	is available or when the patient is unable to undergo a traditional biopsy. The use of IHC/ISH should be considered first followed by NGS testing as appropriate.(GAST-B 3 of 6); (ESOPH-B 3 of 6)	
	MSI or MMR Testing: Universal testing for MSI by PCR, NGS, or MMR by IHC should be performed for all newly diagnosed gastric cancers. The testing is performed on FFPE tissue and results are interpreted as MSI-H or dMMR in accordance with CAP DNA Mismatch Repair Biomarker Reporting Guidelines. Testing should be performed only in CLIA-approved laboratories.(GAST-B 4 of 6)	
	The genomic alterations of solid cancers may be identified by evaluating ctDNA in the blood, hence a form of "liquid biopsy." Liquid biopsy is being used more frequently in patients with advanced disease, particularly those who are unable to have a clinical biopsy for disease surveillance and management. The detection of mutations/alterations in DNA shed from gastric, esophageal, and esophagogastric carcinomas can identify targetable alterations or the evolution of clones with altered treatment response profiles. Therefore, for patients who have metastatic or advanced gastric cancer or esophageal/esophagogastric cancer who may be unable to undergo a traditional biopsy, or for disease progression monitoring, testing using a validated NGS-based comprehensive genomic profiling assay performed in a CLIA-approved laboratory may be considered. A negative result should be interpreted with caution, as this does not exclude the presence of tumor mutations or amplifications.(GAST-B 5 of 6); (ESOPH-B 5 of 6)	
	For second-line or subsequent therapy for unresectable locally advanced, recurrent, or metastatic gastric, esophageal and esophagogastric junction cancers, dostarlimab-gxly is useful in certain circumstances for MSI-H or dMMR tumors.(GAST-F, 4 of 16; (ESOPH-F 4 of 17)	

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
Head and neck cancer **NCCN Guidelines for Head and Neck Cancer V.2.2022**[45]	For recurrent or persistent very advanced head and neck cancer, consider NGS genomic profiling for biomarker identification.(ADV-3) Salivary gland tumors For salivary ductal carcinomas and adenocarcinomas with recurrent distant metastases, use NGS profiling and other appropriate biomarker testing to check status of androgen reception (AR), HER2, *NTRK*, *HRAS*, *PIK3CA*, and TMB prior to treatment.(SALI-4) Pembrolizumab is recommended as useful in certain circumstances for patients with recurrent, unresectable, or metastatic salivary gland tumors (with no surgery or RT option) that are TMB-H (≥10 mut/Mb).(SALI-B 1 of 2) Larotrectinib or entrectinib are recommended as useful in certain circumstances for patients with recurrent, unresectable, or metastatic salivary gland tumors (with no surgery or RT option) as *NTRK* therapy for *NTRK* gene fusion-positive tumors.(SALI-B, 1 of 2) Nasopharyngeal cancers Pembrolizumab is recommended as useful in certain circumstances for patients with recurrent, unresectable, oligometastatic, or metastatic nasopharyngeal cancers (with no surgery or RT option) that are TMB-H (≥10 mut/Mb) as a subsequent-line therapy. NASO-B 1 of 3) Non-nasopharyngeal cancers NGS genomic profiling may be considered to guide patient treatment options, including clinical trials.(SYST-A 1 of 4) Pembrolizumab is recommended as useful in certain circumstances for MSI-H tumors for first- and subsequent-line therapy for patients with recurrent, unresectable, or metastatic (with no surgery or RT option) non-nasopharyngeal cancer.(SYST-A 2 of 4)	FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Hepatobiliary cancers **NCCN Guidelines for Hepatobiliary Cancers V.1.2022**[32]	Unresectable or metastatic gallbladder cancer or intra- or extra-hepatic cholangiocarcinoma: MMR/MSI and TMB testing are recommended. For patients with dMMR/MSI-H tumors or a family history suggestive of *BRCA1/2* mutations, consider germline testing and/or	FoundationOne CDx is an FDA-approved companion diagnostic in cholangiocarcinoma for Pemazyre® (pemigatinib) or Truseltiq™ (infigratinib). FoundationOne CDx is an FDA-approved companion diagnostic for

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	referral to a genetic counselor.(EXTRA-1; INTRA-1);(GALL-1−GALL-5) Additional molecular testing is recommended. Testing may include *NTRK* gene fusion testing.(EXTRA-1; INTRA-1) ;(GALL-1−GALL-5) For patients with unresectable or metastatic biliary tract cancers the following therapies are useful in certain circumstances systemic therapy options for primary treatment: Entrectinib and larotrectinib for *NTRK* gene fusion-positive tumors; pembrolizumab for MSI-H/dMMR. The following therapies are among the useful in certain circumstances subsequent-line systemic therapy options if disease progression: Entrectinib and larotrectinib for *NTRK* gene fusion-positive tumors; pembrolizumab[c] and dostarlimab-gxly[c,d] for MSI-H/dMMR; pembrolizumab for TMB-H tumors.(BIL-C 2 of 4)	Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). FoundationOne CDx is FDA-approved to report *NTRK* gene fusions, alterations in *IDH1*, and MSI status. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Melanoma: Cutaneous **NCCN Guidelines for Melanoma: Cutaneous V.3.2022**[29]	Stage IIIA (sentinel node-positive): Consider *BRAF* mutation testing.(ME-5) Stage IIIB/C/D (sentinel node-positive): *BRAF* mutation testing is recommended.(ME-5) Adjuvant treatment with dabrafenib/trametinib recommended for patients with *BRAF* V600-activating mutation and sentinel node-positive.(ME-5, ME-6, ME-C 4 of 8) Stage III (clinically node-positive): *BRAF* mutation testing is recommended. Consider broader genomic profiling if the results might guide future treatment decisions or eligibility for participation in a clinical trial. Adjuvant treatment with dabrafenib/ trametinib recommended for patients with *BRAF* V600-	FoundationOne CDx is an FDA-approved companion diagnostic for in melanoma for detection of *BRAF* V600E and V600K mutations for selection of a group of BRAF inhibitors and BRAF/MEK inhibitors in combination. FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic for

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	activating mutation.(ME-6, ME-6A, ME-C 4 of 8) Stage III (clinical satellite/in-transit): *BRAF* mutation testing recommended. Consider broader genomic profiling if the test results might guide further treatment decisions or eligibility for participation in a clinical trial. Adjuvant treatment with dabrafenib/trametinib recommended for patients with *BRAF* V600-activating mutation.(ME-7, ME-7A) Stage IV or clinical recurrence: Obtain tissue to ascertain alterations in *BRAF* and in the appropriate clinical setting, *KIT*, from either biopsy of the metastasis (preferred) or archival material if the patient is being considered for targeted therapy. Broader genomic profiling (eg, larger NGS panels, *BRAF* non-V600 mutations) is recommended if feasible, especially if the test results might guide future treatment decisions or eligibility for participation in a clinical trial.(ME-C 4 of 8) If *BRAF* single-gene testing was the initial test performed and is negative, clinicians should strongly consider larger NGS panels to identify other potential genetic targets (eg, *KIT*, *BRAF* non-V600).(ME-C 4 of 8) The NCCN Guidelines describe the specific implications of testing for *BRAF*, *NRAS*, and *KIT* mutations and less common mutations such as fusions in *NTRK1*, *NTRK2*, *NTRK3*, *ALK*, and *ROS1*, as well as the emerging role of TMB. Fusions in *NTRK1*, *NTRK2*, and *NTRK3* correspond to a high response rate to TRK inhibitors larotrectinib or entrectinib.[260,261] Fusions in *ALK* and *ROS1* may predispose to activity from inhibitors of these genes (eg, crizotinib, entrectinib).(ME-C, 2-5 of 8)[261]	Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is FDA-approved to detect alterations in *BRAF*, *NRAS*, and KIT, as well as rearrangements in *NTRK1*, *NTRK2*, *ALK*, and *ROS1* and to calculate TMB. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Metastatic NSCLC **NCCN Guidelines for NSCLC V.3.2022**[33]	Establish histologic subtype with adequate tissue for molecular testing (consider re-biopsy or plasma testing if appropriate). The NCCN NSCLC Guidelines Panel strongly advises broader molecular profiling in eligible patients with advanced or metastatic NSCLC with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials. Broad	FoundationOne CDx is an FDA-approved companion diagnostic for Gilotrif® (afatinib), Iressa® (gefitinib), Tarceva® (erlotinib), Tagrisso® (osimertinib), Alecensa® (alectinib), Alunbrig® (brigatinib), Xalkori® (crizotinib), Zykadia® (ceritinib), Tafinlar® (dabrafenib) in combination with Mekinist®

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	molecular profiling is a key component of the improvement of care of patients with NSCLC. Testing for *EGFR* mutation (category 1), *ALK* (category 1), *KRAS*, *ROS1*, *BRAF*, *NTRK1/2/3*, *MET* exon 14-skipping, and *RET* is recommended for advanced or metastatic adenocarcinoma, large cell, and NSCLC NOS and should be considered for squamous cell carcinoma. Testing should be conducted as part of broad molecular profiling, which is defined as molecular testing that identifies all of the previously listed biomarkers in either a single assay or a combination of a limited number of assays, and optimally also identifies emerging biomarkers. Emerging biomarkers to identify novel therapies for patients with metastatic NSCLC include the following genetic alterations (ie, driver event): high-level *MET* amplification, and *ERBB2* (HER2) mutations. Tiered approaches based on low prevalence of co-occurring biomarkers are acceptable.(NSCL-18, NSCL-H 2 of 7, NSCL-I) It is recommended at this time that when feasible, testing be performed via a broad, panel-based approach most typically performed by NGS. For patients who in broad panel testing don't have identifiable driver oncogenes (especially in never-smokers), consider RNA-based NGS, if not already performed, to maximize detection of fusion events.(NSCL-H 2 of 7) If there is insufficient tissue to allow testing for all of *EGFR*, *ALK*, *KRAS*, *ROS1*, *BRAF*, *MET*, *NTRK1/2/3*, and *RET* in eligible patients with metastatic NSCLC, repeat biopsy and/or plasma testing should be done.(NSCL-18) The use of cell-free/circulating tumor DNA can be considered in specific clinical circumstances, most notably if a patient is medically unfit for invasive tissue sampling; if following pathologic confirmation of a metastatic NSCLC diagnosis, there is insufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow-up tissue-based analysis is planned for all patients in which an oncogenic driver is not identified; or, in the initial diagnostic setting, if tissue-based testing does not completely assess all recommended	(trametinib), and Tabrecta™ (capmatinib) in NSCLC. FoundationOne Liquid CDx provides FDA-approved comprehensive genomic profiling from a circulating cell-free DNA (cfDNA) sample. FoundationOne Liquid CDx is an FDA-approved companion diagnostic for Alecensa® (alectinib), Iressa® (gefitinib), Tarceva® (erlotinib), Tagrisso® (osimertinib), and Tabrecta® (capmatinib) in NSCLC. FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is FDA-approved to report alterations in *EGFR, ALK, ROS1, BRAF, KRAS, MET, RET, ERBB2, NTRK* gene fusions, *KRAS*, and TMB. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	biomarkers owing to tissue quantity or testing methodologies available, consider repeat biopsy and/or cell-free/circulating tumor DNA testing.(NSCL-18; NSCL-H 7 of 7)	
	Test for *EGFR* mutation (resectable stages IB–IIIA) and PD-L1 status (resectable stages II–IIIA) on surgical tissue or biopsy. (NSCL-3 footnote q)	
	Molecular testing for *EGFR* mutations should be performed when adjuvant TKI therapy is a consideration for resectable NSCLC stage IB–IIIA. While the testing process may be technically easier on a resection specimen, initial diagnostic biopsy specimens are also acceptable for testing for this indication.(NSCL-H 3 of 7)	
	Stage IVA, M1a (pleural or pericardial effusion), stage IVA, M1b, and stage IV, MIc: Biomarker testing should include *EGFR* mutations (category 1), *ALK* (category 1), *KRAS*, *ROS1*, *BRAF*, *NTRK 1/2/3*, *MET* exon 14 skipping, and *RET*. Testing should be conducted as part of broad molecular profiling.(NSCL-13, NSCL-14, NSCL-18)	
	EGFR exon 20 mutations (other than *EGFR* p.T790M) are a heterogeneous group, some of which are responsive to targeted therapy and that require detailed knowledge of the specific alteration. Most *EGFR* exon 20 alterations are a diverse group of in-frame duplication or insertion mutations. These are generally associated with lack of response to first-, second-, and third-generation EGFR TKI therapy, with select exceptions (p.A763_Y764insFQEA is associated with sensitivity to TKI therapy and p.A763_Y764insLQEA may be associated with sensitivity to first- and third-generation TKI therapy). *EGFR* exon 20 insertions/duplications are associated with responsiveness to specific targeted subsequent therapy agents. The most commonly represented *EGFR* exon 20 insertions/duplications in the clinical studies have been insASV, insSVD, and insNPH, although a wide spectrum of other alterations were included. There is currently no evidence that the specific alteration type impacts the probability of responsiveness to the class of	

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	kinase inhibitor. Because some *EGFR* exon 20 mutations are or may be sensitive to first- and third-generation inhibitors, the specific sequence of *EGFR* exon 20 insertion mutations remains important, and some assays will identify the presence of an *EGFR* exon 20 insertion without specifying the sequence. In this scenario, additional testing to further clarify the *EGFR* exon 20 insertion may be indicated for therapy selection. Targeted PCR-based approaches for detection of *EGFR* variants may under-detect *EGFR* exon 20 insertion events; therefore, NGS-based strategies are preferred.(NSCL-H 3 of 7) If p.T790M is identified in the absence of prior *EGFR* TKI therapy, genetic counseling and possible germline genetic testing are-warranted. Identification of germline *EGFR* p.T790M confers a high risk for lung cancer regardless of smoking status.(NSCL-H 3 of 7) A broad range of molecular alterations lead to *MET* exon 14-skipping. NGS-based testing is the primary method for detection of *MET* exon 14-skipping events; RNA-based NGS may have improved detection. IHC is not a method for detection of *MET* exon 14-skipping.(NSCL-H 5 of 7) *RET* common fusion partners are *KIF5B*, *NCOA4*, and *CCDC6*; however, numerous other fusion partners have been identified. FISH break-apart probe methodology can be deployed; however, it may under-detect some fusions. Targeted real-time reverse-transcriptase PCR assays are utilized in some settings, although they are unlikely to detect fusions with novel partners. NGS-based methodology has a high specificity, and RNA-based NGS is preferable to DNA-based NGS for fusion detection.(NSCL-H 5 of 7) There is growing recognition of the molecular mechanisms of resistance to therapy. Plasma or tissue-based testing via broad molecular profiling should be considered at progression, for the T790M mutation and other genomic resistance mechanisms. If plasma-based testing is negative, tissue-based testing with re-biopsy material is strongly recommended. Practitioners may want to consider scheduling the biopsy concurrently with plasma testing referral. Broad	

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	genomic profiling may be the most informative approach to examining potential mechanisms of resistance, which may require more than one instance of such profiling over the course of an individual patient's therapy.(NSCL-H 6 of 7, NSCL-22, NSCL-27, NSCL-28, NSCL-30) Testing in the setting of a limited number of pulmonary nodules can aid in distinguishing separate primary lung carcinoma versus intrapulmonary metastatic disease. Studies to explore tumor relatedness by testing tissue from separately sampled lesions using a broad gene coverage NGS approach suggest it may be superior to histopathologic assessment.(NSCL-H 6 of 7) Contraindications for treatment with PD-1/PD-L1 inhibitors may include active or previously documented autoimmune disease and/or current use of immunosuppressive agents, or presence of an oncogene (ie, *EGFR* exon 19 deletions or L858R, *ALK* rearrangements), which would predict lack of benefit.(NSCL-K 1 and 2 of 5) Suspected multiple lung cancers: Lesions with different cell types (eg, SCC, adenocarcinoma) are usually different primary tumors. Multiple studies suggest that NGS testing with broad gene coverage may allow for unambiguous determination of clonal relatedness among separate lung nodules.(NSCL-10)	
Occult primary/cancer of unknown primary **NCCN Guidelines for Occult Primary V.1.2022**[40]	Consider NGS to identify actionable genomic aberrations in patients with epithelial, not site-specific, tumors. Consider NGS in patients based on clinicopathologic features and where it guides therapeutic decision making.(OCC-2) TMB determination by a validated and/or FDA-approved assay is recommended in the initial workup of a suspected metastatic malignancy (category 2B) (corresponding reference, Merino DM, et al. J Immunother Cancer. 2020;8:e000147). NGS can be considered in the workup of a suspected metastatic malignancy after an initial determination of histology is made.(OCC-1) Pembrolizumab is useful in certain circumstances for occult primary tumors (both adenocarcinoma and squamous cell) who have either dMMR/MSI-H tumors or TMB-H (≥10	FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	mut/Mb) tumors. Dostarlimab-gxly is useful in certain circumstances for dMMR/MSI-H adenocarcinoma tumors.(OCC-B, 2 of 9 and OCC-B, 4 of 9)	NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Ovarian cancer **NCCN Guidelines for Ovarian Cancer V.1.2022**[34]	Both somatic and germline *BRCA1/BRCA2* testing is recommended at diagnosis for patients with pathologically confirmed epithelial ovarian cancer/fallopian tube cancer/primary peritoneal cancer. Germline and/or somatic *BRCA1/2* status informs maintenance therapy.(OV-1, OV-2 & OV-3) In the absence of a *BRCA1/2* mutation, homologous recombination status may provide information on the magnitude of benefit of PARP inhibitor therapy.(OV-1 OV-2, OV-3, OV-5) In the up-front setting, choice of somatic testing should, at a minimum, optimize identification of molecular alterations that can inform use of interventions that have demonstrated benefit in this setting, including *BRCA1/2*, loss of heterozygosity (LOH), or homologous recombination (HR) status in the absence of a germline *BRCA* mutation.(OV-B 1 of 3) Tumor molecular testing is recommended for persistent/recurrent disease, if not previously done. Validated molecular testing should be performed in a CLIA-approved facility using the most recent available tumor tissue. Tumor molecular analysis is recommended to include, at a minimum, tests to identify potential benefit from targeted therapeutics that have tumor-specific or tumor-agnostic benefit including, but not limited to, *BRCA1/2*, homologous recombination status, MSI, TMB, *NTRK* if prior testing did not include these markers. More comprehensive testing may be particularly important in less common histologies (eg, LCOC) with limited approved therapeutic options.(OV-6, OV-7, OV-B, 1 of 3)	FoundationOne CDx is an FDA-approved companion diagnostic in ovarian cancer for Rubraca® (rucaparib) and olaparib (Lynparza®). FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). FoundationOne Liquid CDx provides FDA-approved comprehensive genomic profiling from a circulating cell-free DNA sample. FoundationOne Liquid CDx is an FDA-approved companion diagnostic for Rubraca® (rucaparib) in ovarian cancer. FoundationOne CDx is FDA-approved to assess *BRCA1/2* and other homologous recombination pathway genes (eg, *ATM*, *BRIP1*, *CHEK2*, *FANCA*, *FANCL*, *FANCM*, *NBN*, *RAD51C*, *RAD51D*, and *RAD54L).*

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	Validated molecular testing should be performed in a CLIA-approved facility (OV-B, 1 of 3).	FoundationOne CDx is FDA-approved to report MSI-H status and *NTRK* gene fusions. FoundationOne CDx is FDA-approved to report genomic LOH from FFPE ovarian tumor tissue. Positive HRD status (defined as t*BRCA*-positive and/or LOH high) in ovarian cancer patients is associated with improved PFS from Rubraca (rucaparib) maintenance therapy in accordance with the Rubraca product label. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Pancreatic cancer **NCCN Guidelines for Pancreatic Cancer V.1.2022**[35]	Tumor/somatic gene profiling is recommended for patients with locally advanced/metastatic disease at diagnosis and/or recurrence who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for actionable somatic findings including, but not limited to fusions (*ALK, NRG1, NTRK, ROS1, FGFR2, RET*), mutations (*BRAF, BRCA1/2, KRAS, PALB2*), amplifications (*HER2*), MSI and/or MMR deficiency (detected by tumor IHC, PCR, or NGS). Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible.(PANC-1, 1A, PANC-4, PANC-5, 5A, PANC-8, PANC-9 and PANC-10) Genetic testing for inherited mutations is recommended for any patient with confirmed pancreatic cancer, using comprehensive gene panels for hereditary cancer syndromes. Genetic counseling is recommended for patients who test	FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is FDA-approved to detect *ALK*, *NTRK*, and *ROS1* gene fusions; detect mutations in *BRAF, BRCA1/2, HER2, KRAS, PALB2*; and report MSI status. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	positive for a pathogenic mutation (*ATM, BRCA1, BRCA2, CDKN2A, MLH1, MSH2, MSH6, PALB2, PMS2, STK11*, and *TP53*).(PANC-1, 1A, PANC-2, PANC-3, PANC-4, PANC-6, PANC-7, PANC-8, and PANC-10) Clinical trial enrollment is preferred in the post-operative adjuvant treatment setting for patients with locally advanced disease at surgery who have no prior neoadjuvant therapy and no evidence of recurrent or metastatic disease. Clinical trial enrollment is also preferred as first-line therapy in patients with locally advanced disease and good PS both at diagnosis and disease progression; it is also recommended as an option for those with good PS and no disease progression. Clinical trial enrollment is preferred for those with metastatic disease and good PS.(PANC-5, PANC-7, PANC-8, PANC-9, PANC-10, PANC-11)	NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
<u>Prostate cancer</u> **NCCN Guidelines for Prostate Cancer V.4.2022**[36]	Tumor testing for alterations in homologous combination DNA repair genes, such as *BRCA1, BRCA2, ATM, PALB2, FANCA, RAD51D, CHEK2*, and *CDK12*, is recommended in patients with metastatic prostate cancer. This testing can be considered in patients with regional prostate cancer. Tumor testing for MSI-H or dMMR, if not previously performed, is clinically indicated in patients with mCRPC and may be considered in patients with regional or castration-sensitive metastatic prostate cancer. Germline testing for HRRm is recommended for patients with metastatic, regional, very-high-risk, or high-risk prostate cancer and those with prostate cancer who meet other family or personal cancer history and/or ancestry criteria. TMB testing may be considered in patients with mCRPC.(PROS-B 3 of 3, PROS-1 footnote c-initial diagnosis, PROS-12 footnote uu, PROS-14) At present, tumor molecular and biomarker analysis maybe be used for treatment decision making, including understanding eligibility for biomarker-directed treatments, genetic counseling, early use of platinum chemotherapy, and eligibility for clinical trials. Clinical trials may include established and/or candidate molecular biomarkers for eligibility. Tumor	FoundationOne CDx is an FDA-approved companion diagnostic for Lynparza® (olaparib). FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) in patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne Liquid CDx provides FDA-approved comprehensive genomic profiling from a circulating cell-free DNA sample. FoundationOne Liquid CDx is an FDA-approved companion diagnostic for Lynparza® (olaparib) and

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	molecular profiles may change with subsequent treatments and re-evaluation may be considered at time of cancer progression for treatment decision making. Patients should be informed that tumor molecular analysis by DNA sequencing has the potential to uncover germline findings. Confirmatory germline testing may be indicated.(PROSB-3 of 3) NCCN strongly recommends a metastatic biopsy for histologic and molecular evaluation. When unsafe or unfeasible, plasma ctDNA assay is an option, preferably collected during biochemical (PSA) and/or radiographic progression in order to maximize yield. Caution is needed when interpreting ctDNA-only evaluation due to potential interference from CHIP, which can result in a false-positive biomarker signal.(PROS-B 3 of 3) DNA analysis for MSI and IHC for MMR are different assays measuring different biological effects caused by dMMR function. If MSI is used, testing using an NGS assay validated for prostate cancer is preferred.(PROS-B 3 of 3)	Rubraca® (rucaparib) in prostate cancer. FDA-approved to report MSI status and alterations in *BRCA1*, *BRCA2*, *ATM*, *PALB2*, *FANCA*, *RAD51D*, and *CHEK2*. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Soft tissue sarcoma **NCCN Guidelines for Soft Tissue Sarcoma V.2.2022**[44]	Molecular genetic testing has emerged as an ancillary testing approach since many sarcoma types harbor characteristic genetic aberrations, including single base pair substitutions, deletions and amplifications, and translocations. Most molecular testing utilizes FISH approaches or PCR-based methods and NGS-based methods. NGS, including DNA and RNA sequencing, may be beneficial in selected patients. The timing of when to perform NGS and for which patients must be evaluated individually. NGS findings can help patients qualify for clinical trials and can identify actionable mutations that may not have targeted by prior therapies. Thus, NGS may be appropriate for patients who may qualify for and who are interested in enrolling in a clinical trial or for patients with disease that is refractory who have failed or progressed on standard therapies or in certain histologies where NGS provides clinically actionable information. NGS should not replace expert pathology review, as NGS only rarely results in a diagnosis change following expert review. Technically successful NGS on bone biopsies requires use of	There are multiple sub-types of sarcoma and FoundationOne Heme combined sequencing of DNA and RNA provides sensitive detection of known, novel and complex fusion events to help determine sarcoma sub-types. FoundationOne Heme can also identify MSI-H status and *NTRK* gene fusions to determine use of targeted therapies. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
	decalcification agents, such as EDTA, that do not interfere with genomic testing.(SARC-C 1 of 3) Larotrectinib and entrectinib are recommended as preferred first-line treatment for advanced/metastatic STS subtypes with non-specific histologies for patients with *NTRK* gene fusion-positive sarcomas only.(SARC-F, 1 of 9) Pembrolizumab is recommended as useful in certain circumstances as subsequent lines of therapy for patients with advanced/metastatic myxofibrosarcoma, undifferentiated pleomorphic sarcoma (UPS), cutaneous angiosarcoma, and undifferentiated sarcomas (for the treatment of unresectable/metastatic TMB-H [≥10 mut/Mb] tumors, as determined by an FDA-approved test, that have progressed following prior treatment and who have no satisfactory alternative treatment options).(SARC-F, 1 and 7 of 11) ALK inhibitors (crizotinib, brigatinib, ceritinib, and lorlatinib) are recommended as preferred regimens for patients with inflammatory myofibroblastic tumor with *ALK* translocation.(SARC-F, 5 of 11)	
Thyroid cancer **NCCN Guidelines for Thyroid Cancer V.2.2022**[41]	Papillary, follicular, and Hürthle cell carcinomas For advanced, progressive, or threatening disease, genomic testing to identify actionable mutations (including *ALK*, *NTRK* and *RET* gene fusions), dMMR/MSI, and TMB.(PAP-9, FOLL-8, HÜRT-8) Medullary carcinoma For patients with recurrent or persistent disease (locoregional and metastatic) who are germline wild-type or germline unknown: Genomic testing including TMB or *RET* somatic genotyping (MEDU-6, MEDU-7) Anaplastic carcinoma Molecular testing for actionable mutations at diagnosis should include *BRAF*, *NTRK*, *ALK*, *RET*, MSI, dMMR, and TMB.(ANAP-1) For metastatic disease, stage IVC, molecular testing for actionable mutations (if not previously done) should include *BRAF*, *NTRK*, *ALK*, *RET*, MSI, dMMR, and TMB.(ANAP-3)	FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) for patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). FoundationOne CDx is FDA-approved to report alterations in *RET*, *NTRK* gene fusions, and TMB.

Tumor type and applicable NCCN Guidelines for tumor profiling	NCCN recommendations[a]	FoundationOne CDx alignment
		The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.
Vulvar cancer **NCCN Guidelines for Vulvar Cancer V.1.2022**[39]	Consider TMB testing through a validated and/or FDA-approved assay (reference Merino et al 2020); consider MMR/MSI, PD-L1, and/or *NTRK* gene fusion for patients with recurrent, progressive, or metastatic disease.(VULVA-A 1 of 3) Pembrolizumab is recommended option as useful in certain circumstances for advanced or recurrent/metastatic cervical cancer as second-line therapy for TMB-H, PD-L1 positive, or MSI-H/dMMR tumors.(VULVA-E 1 of 2) Pembrolizumab is recommended option for the treatment of patients with unresectable or metastatic TMB-H (≥10 mut/Mb) tumors as determined by a validated and/or FDA-approved test that have progressed following prior treatment and who have no satisfactory alternative treatment options.(VULVA-E 1 of 2) Larotrectinib or entrectinib are recommended options as useful in certain circumstances for advanced or recurrent/metastatic cervical cancer for *NTRK* gene fusion-positive tumors (category 2B).(VULVA-E 1 of 2)	FoundationOne CDx is an FDA-approved companion diagnostic for Keytruda® (pembrolizumab) for patients with unresectable or metastatic tumors with TMB-H (≥10 mut/Mb). FoundationOne CDx is an FDA-approved companion diagnostic to identify patients with MSI-H solid tumors who may be appropriate for treatment with Keytruda (pembrolizumab). FoundationOne CDx is an FDA-approved companion diagnostic for Vitrakvi® (larotrectinib) across all solid tumors. FoundationOne CDx is FDA-approved to report MSI status, *NTRK* gene fusions, and TMB. The FoundationOne CDx clinical report lists clinical trials for which the patient may be eligible based on the results of testing. When patients genomically match to an arm of the NCI-MATCH trial, physicians at NCI-MATCH sites will receive notification per email, permitting registration. Since 2017, a significant number of the patients participating in this trial have been identified through Foundation Medicine.

[a] All NCCN recommendations are category 2A unless otherwise indicated. Additionally, NCCN states that the best management of any patient with cancer is in a clinical trial.

[b] Data are specific to women.

[c] For patients who have not been previously treated with a checkpoint inhibitor because there is a lack of data for subsequent use of immunotherapy in patients who have previously been treated with a checkpoint inhibitor.

[d] Dostarlimab-gxly is a recommended treatment option for patients with MSI-H, dMMR recurrent or advanced tumors that have progressed on or following prior treatment and who have no satisfactory alternative treatment options.

AR, androgen receptor; AUC, area under the curve; CAP, College of American Pathologists; CGP, comprehensive genomic profiling; CHIP, clonal hematopoiesis of indeterminate potential; CLIA, Clinical Laboratory Improvement Amendments; CNS, central nervous system; CPS, combined positive score; CRC, colorectal cancer; CRPC, castration-resistant prostate cancer; ctDNA, circulating tumor DNA; dMMR, mismatch repair deficient; DNA, deoxyribonucleic acid; EGFR, epidermal growth factor receptor; ER, estrogen receptor; FDA, Food and Drug Administration; FFPE, formalin-fixed, paraffin-embedded; FISH, fluorescence *in situ* hybridization; HBOC, hereditary breast and ovarian cancer; HER2, human epidermal growth factor receptor; HR, hormone receptor; HRD, homologous recombination deficiency; HRRm, homologous recombination repair gene mutation; IHC, immunohistochemistry; LOH, loss of heterozygosity; mCRPC, metastatic castration-resistant prostate cancer; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, microsatellite instability-high; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NOS, not otherwise specified; NSCLC, non-small cell lung cancer; NTRK, neurotrophic receptor tyrosine kinase; PARP, poly ADP ribose polymerase; PCR, polymerase chain reaction; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PS, performance status; PSA, prostate-specific antigen; RAI, radioactive iodine; RT, radiation therapy; SCC, squamous cell carcinoma; tBRCA, tumor BRCA; TKI, tyrosine kinase inhibitor; TMB, tumor mutational burden; TMB, tumor mutational burden-high; TMB-H, tumor mutational burden-high; TNBC, triple-negative breast cancer; WT, wild-type.

Sources: Foundation Medicine, Inc. (2021)[1,262], Hempelman et al. (2017)[263], Swisher et al. (2017)[112].

Table 6-2. Biomarker-Based Targeted Therapies and Immunotherapies Recommended in NCCN Guidelines[a] for Metastatic NSCLC Along With Companion Diagnostics

Genomic alteration[b]	Drug recommended by NCCN	NCCN recommendation[c]	Is a CDx required per FDA approved drug label?	Does Foundation Medicine have a CDx claim for this alteration?
ALK	Alectinib[d]	Category 1; preferred (1L)	Y	Y
	Ceritinib[d]	Category 1 (1L)	Y	Y
	Brigatinib[d]	Category 1; preferred (1L)	Y	Y
	Crizotinib[d]	Category 1 (1L)	Y	Y
	Lorlatinib[d]	Category 1; preferred (1L)	Y	N
	Alectinib[d]	Category 2A (2L)[e,f,g]	Y	Y
	Brigatinib[d]	Category 2A (2L)[e,f,g]	Y	Y
	Ceritinib[d]	Category 2A (2L)[e,f,g]	Y	Y
	Lorlatinib[d]	Category 2A (2L or 3L)[e,f,g,h,i]	Y	N
	Crizotinib	Category 2A (2L)[e,f,j]	Y	Y
BRAF V600E	Dabrafenib + trametinib[d,k]	Category 2A; preferred (1L) Category 2A (2L)	Y/Y	Y

Genomic alteration[b]	Drug recommended by NCCN	NCCN recommendation[c]	Is a CDx required per FDA approved drug label?	Does Foundation Medicine have a CDx claim for this alteration?
	Vemurafenib[d,k]	Category 2A (1L or 2L)	NA[l]	---
	Dabrafenib[d,k]	Category 2A (1L or 2L)	Y	Y
***EGFR* exon 19 deletion or *L858R* mutations**	Osimertinib[d]	Category 1; preferred (1L)	Y	Y
	Erlotinib[d]	Category 1 (1L)	Y	Y
	Afatinib[d]	Category 1 (1L)	Y	Y
	Dacomitinib[d]	Category 1 (1L)	Y	N
	Gefitinib[d]	Category 1 (1L)	Y	Y
	Erlotinib + ramucirumab	Category 2A (1L)	Y/NA	Y/---
	Erlotinib + bevacizumab[m]	Category 2A (1L)	Y/NA	Y/---
	Osimertinib[d,e,n]	Category 2A (2L)	Y	Y
	Erlotinib[e,o]	Category 2A (2L)	Y	Y
	Afatinib[e,o]	Category 2A (2L)	Y	Y
	Dacomitinib[e,o,p]	Category 2A (2L)	Y	N
	Gefitinib[e,o]	Category 2A (2L)	Y	Y
	Erlotinib + ramucirumab[e,o]	Category 2A (2L)	Y/NA	Y/---
	Erlotinib + bevacizumab[e,m,o]	Category 2A (2L)	Y/NA	Y/---
EGFR T790M	Osimertinib[d]	Category 1 (2L)	Y	Y
***EGFR S768I, L861Q,* and/or *G719X* mutations**	Afatinib[d]	Category 2A; preferred (1L)	NA[l]	---
	Osimertinib[d]	Category 2A; preferred (1L)	NA[l]	---
	Erlotinib[d]	Category 2A (1L)	NA[l]	---
	Gefitinib[d]	Category 2A (1L)	NA[l]	---
	Dacomitinib[d]	Category 2A (1L)	NA[l]	---
***EGFR* exon 20 insertion mutation positive**	Amivantamab-vmjw[d]	Category 2A (2L or 3L)	Y	N
	Mobocertinib[d]	Category 2A (2L or 3L)	Y	N
***KRAS* G12C**	Sotorasib[d]	Category 2A (2L)	Y	N
ROS-1	Crizotinib[d]	Category 2A; preferred (1L)	Y	N

Genomic alteration[b]	Drug recommended by NCCN	NCCN recommendation[c]	Is a CDx required per FDA approved drug label?	Does Foundation Medicine have a CDx claim for this alteration?
	Entrectinib[d,q]	Category 2A; preferred (1L)	N[r]	---
	Ceritinib[d]	Category 2A (1L)	NA[l]	---
	Crizotinib[e,f,s]	Category 2A (2L)	Y	N
	Entrectinib[e,f,t]	Category 2A (2L)	Y	N
	Ceritinib[e,f,s]	Category 2A (2L)	N[r]	---
	Lorlatinib[e,f,u]	Category 2A (2L)	N[r]	---
NTRK1/2/3	Larotrectinib[d]	Category 2A; preferred (1L) Category 2A (2L)[v]	Y	Y
	Entrectinib[d]	Category 2A; preferred (1L) Category 2A (2L)[v]	N[r]	---
***MET* exon 14 skipping**	Capmatinib[d,e]	Category 2A; preferred (1L or 2L)[w]	Y	Y
	Tepotinib[d,e]	Category 2A; preferred (1L or 2L)[w]	Y	N
	Crizotinib[d,e]	Category 2A (1L or 2L)[w]	NA[l]	---
RET	Selpercatinib[d,e]	Category 2A; preferred (1L or 2L)[x]	NA[l]	---
	Pralsetinib[d,e]	Category 2A; preferred (1L or 2L)[x]	Y	N
	Cabozantinib[d,e]	Category 2A (1L or 2L)[x]	NA[l]	---
PD-L1 ≥50% and negative for actionable molecular markers[y,z]	Pembrolizumab ± platinum-based chemotherapy	Category 1; preferred (1L)	Y (PD-L1)[aa]	N
	Atezolizumab	Category 1; preferred (1L)	Y (PD-L1)	N
	Cemiplimab-rwlc	Category 1; preferred (1L)	Y (PD-L1)	N
	Atezolizumab + platinum-based chemotherapy + VEGF inhibitor[m]	Category 1[bb] (1L)	N[cc]	---
	Atezolizumab + platinum-based chemotherapy	Category 2A[bb] (1L)	N[cc]	---
	Nivolumab + ipilimumab	Category 1 (1L)	Y (PD-L1)[dd]	N

Genomic alteration[b]	Drug recommended by NCCN	NCCN recommendation[c]	Is a CDx required per FDA approved drug label?	Does Foundation Medicine have a CDx claim for this alteration?
	Nivolumab + ipilimumab + platinum-based chemotherapy	Category 1 (1L)	Y (PD-L1)[dd]	N
PD-L1 ≥1%—49% and negative for actionable molecular markers[y,z]	Pembrolizumab	Category 2B (1L)[ee]	Y (PD-L1)[aa]	N
	Pembrolizumab + platinum-based chemotherapy	Category 1 (1L); preferred	N[aa]	---
	Atezolizumab + platinum-based chemotherapy + VEGF inhibitor[m]	Category 1[bb] (1L)	N[cc]	---
	Atezolizumab + platinum-based chemotherapy	Category 2A[bb] (1L)	N[cc]	---
	Nivolumab + ipilimumab	Category 1 (1L)	Y (PD-L1)[dd]	N
	Nivolumab + ipilimumab + platinum-based chemotherapy	Category 1 (1L)	Y (PD-L1)[dd]	N
PD-L1 <1% and *EGFR, ALK, ROS1, BRAF, MET* exon 14 skipping mutation, *NTRK1/2/3*, and *RET* negative[z,ff]	Pembrolizumab + platinum-based chemotherapy	Category 1 (1L); preferred	N[aa]	---
	Atezolizumab + platinum-based chemotherapy + VEGF inhibitor[m,gg]	Category 1[bb] (1L)	N[cc]	---
	Atezolizumab + platinum-based chemotherapy	Category 2A[bb] (1L)	N[cc]	---
	Nivolumab + ipilimumab	Category 2A (1L)	N[dd]	---
	Nivolumab + ipilimumab + platinum-based chemotherapy	Category 1 (1L)	N[dd]	---
Emerging Biomarkers				
High-level *MET* amplification[hh]	Crizotinib	Category 2A	NA[l]	---
	Capmatinib	Category 2A	NA[l]	---
	Tepotinib	Category 2A	NA[l]	---

Genomic alteration[b]	Drug recommended by NCCN	NCCN recommendation[c]	Is a CDx required per FDA approved drug label?	Does Foundation Medicine have a CDx claim for this alteration?
ERBB2 (HER2) mutations[ii]	Ado-trastuzumab emtansine	Category 2A	NA[l]	---
	Fam-trastuzumab deruxtecan-nxki	Category 2A	NA[l]	---

Green cells outline those therapies that require a companion diagnostic per the FDA label and for which FoundationOne Liquid CDx is an approved companion diagnostic.

[a] Individual guidelines contain differing recommendations for extent of molecular testing; please refer to the individual guidelines at NCCN.org for information on individual cancers by site.

[b] The NCCN Guidelines recommend biomarker testing in eligible patients with metastatic NSCLC and strongly advise broad molecular profiling, most typically performed by NGS, to identify actionable biomarkers, including rare oncogenic driver variants, for which effective therapy may be available. The NCCN Guidelines for NSCLC provide recommendations for individual biomarkers that should be tested and recommend testing techniques but do not endorse any specific commercially available biomarker assays or commercial laboratories.

[c] Category 1: Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2A: Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2B: Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.

Category 3: Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

All NCCN recommendations are category 2A unless otherwise indicated.

[d] For performance status 0–4.

[e] Beware of flare phenomenon in subset of patients who discontinue TKI. If disease flare occurs, restart TKI.

[f] Plasma or tissue-based testing via broad molecular profiling should be considered at progression for genomic resistance mechanisms (eg, *T790M* for *EGFR*-mutated disease, or other genomic resistance mechanisms). If plasma-based testing is negative, tissue-based testing with rebiopsy material is strongly recommended. Practitioners may want to consider scheduling the biopsy concurrently with plasma testing referral.

[g] Ceritinib, alectinib, brigatinib, or lorlatinib are treatment options for patients with *ALK*-positive metastatic NSCLC that has progressed on crizotinib, or for patients who are intolerant to crizotinib.

[h] Recommended for *ALK* G1202R mutation if asymptomatic or if symptomatic and patient has brain metastases or limited systemic metastases.

[i] Lorlatinib is a treatment option for patients with symptomatic systemic disease and multiple lesions after progression on alectinib, brigatinib, or ceritinib if lorlatinib was not previously given.

[j] Crizotinib is only recommended at second-line therapy with progression on crizotinib as continuation in asymptomatic patient or in symptomatic patients with limited systemic metastases.

[k] Single-agent vemurafenib or dabrafenib are treatment options if the combination of dabrafenib + trametinib is not tolerated.

[l] This therapy is not FDA approved for this indication.

[m] Criteria for treatment with bevacizumab: non-squamous NSCLC and no type of hemoptysis; FDA-approved biosimilar is an appropriate susbstitute

[n] Consider osimertinib (regardless of T790M status) for progressive CNS disease or leptomeningeal disease. In the Bloom study, osimertinib was used at 160 mg for patients with leptomeningeal disease.

[o] Erlotinib (ramucirumab or bevacizumab), afatinib, gefitinib, or dacomitinib are only recommended with progression on these agents as continuation in asymptomatic patients or symptomatic patients with brain metastases or limited systemic metastases if T790M-negative.

[p] In the randomized phase III trial of dacomitinib, patients with brain metastases were not eligible for enrollment. In the setting of brain metastases, consider other options.

[q] Entrectinib may be better for patients with brain metastases.

[r] This indication is approved under accelerated approval based on overall response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trial(s).

[s] Entrectinib, crizotnib, or ceritinib are only recommended with progression on these agents as continuation in asymptomatic patients or in symptomatic patients with limited systemic metastases.

[t] Entrectinib is recommended for symptomatic brain metastases if previously treated with crizotinib or ceritinib.

[u] Lorlatinib is only recommended with progression on entrectinib, crizotinib, or ceritinib in asymptomatic patients or in symptomatic patients with systemic metastases.

[v] If *NTRK1/2/3* inhibitors not used 1L.

[w] If *MET* exon 14 skipping mutation inhibitor not used 1L.

[x] If *RET* inhibitor not given 1L.

[y] For performance status 0–2. Best supportive care for PS 3–4.

[z] Contraindications for treatment with PD-1/PD-L1 inhibitors may include active or previously documented autoimmune disease and/or current use of immunosuppressive agents or presence of an oncogene, which would predict lack of benefit.

[aa] Pembrolizumab is approved for the treatment of NSCLC in combination with platinum-based chemotherapy in patients whose tumors do not harbor *EGFR* or *ALK* genomic alterations; no testing is required for PD-L1 for pembrolizumab in combination with platinum-based chemotherapy. Pembrolizumab is also approved as a single agent for tumors expressing PD-L1 (tumor proportion score ≥1%) as determined by an FDA-approved test, with no *EGFR* or *ALK* genomic alterations or disease progression of FDA-approved therapies for these aberrations prior to pembrolizumab.

[bb] Atezolizumab in combination with platinum-based chemotherapy ± VEGF inhibitor is only recommended for patients with adenocarcinoma, large cell, or NSCLC NOS. Carboplatin + paclitaxel + bevacizumab + atezolizumab is Category 1; carboplatin + albumin-bound paclitaxel + atezolizumab is Category 2A.

[cc] Atezolizumab is approved for the treatment of NSCLC in combination with platinum-based chemotherapy in patients whose tumors do not harbor *EGFR* or *ALK* genomic alterations; no testing is required for PD-L1 for atezolizumab in combination with platinum-based chemotherapy. Atezolizumab is also approved as a single agent for tumors with a high PD-L1 expression (PD-L1 stained ≥50% of tumor cells [TC ≥50%] or PD-L1 stained tumor-infiltrating immune cells [IC] covering ≥10% of the tumor area [IC ≥10%]) as determined by an FDA-approved test, with no *EGFR* or *ALK* genomic alterations or disease progression of FDA-approved therapies for these aberrations prior to atezolizumab.

[dd] Nivolumab + ipilimumab is approved regardless of PD-L1 expression in adult patients with metastatic or recurrent NSCLC with no EGFR or ALK genomic tumor aberrations as first-line treatment in combination with 2 cycles of platinum-doublet chemotherapy.

[ee] Pembrolizumab monotherapy can be considered in PD-L1 1%-49% in patients with poor PS or other contraindications to combination chemotherapy.

[ff] For PS 0-1. Various chemotherapy regimens without immunotherapy recommended for PS 2 and best supportive care for PS 3-4.

[gg] Bevacizumab should not be given as a single agent, unless as maintenance if initially used with chemotherapy; bevacizumab should be given until progression. Any regimen with a high risk of thrombocytopenia and the potential risk of bleeding should be used with caution in combination with bevacizumab.

[hh] The definition of high-level *MET* amplification is evolving and may differ according to the assay used for testing. For NGS-based results, a copy number >10 is consistent with high-level *MET* amplification.

[ii] For oncogenic or likely oncogenic *HER2* mutations, refer to definitions at oncokb.org.

1L, first line; 2L, second line; ALK, anaplastic lymphoma kinase; BRAF, v-raf murine sarcoma viral oncogene homolog B1; CDx, companion diagnostic; CNS, central nervous system; EGFR, epidermal growth factor receptor; ERBB2, Erb-B2 receptor tyrosine kinase 2; FDA, Food and Drug Administration; HER2, human epidermal growth factor receptor 2; MET, mesenchymal

epithelial transition factor receptor; NA, not applicable; NCCN, National Comprehensive Cancer Network; NGS, next generation sequencing; N, no; NSCLC, non-small cell lung cancer; NTRK, neurotrophic tyrosine kinase receptor; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PS, performance status; RET, ret proto-oncogene; ROS-1, c-ros oncogene 1; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; Y, yes.

Sources: Alecensa PI 2019[79]; Alunbrig PI 2021[264]; Avastin PI 2021[265]; Cabometyx PI 2020[266]; Caprelsa PI 2018[267]; Cyramza PI 2021[268]; Enhertu PI 2019[269]; Exkivity PI 2021[270]; Gilotrif PI 2019[82]; Iressa PI 2019[84]; Kadcyla PI 2013[271]; Keytruda PI 2021[272]; Libtayo PI 2021[273]; Lorbrena PI 2021[274]; Lumakras PI 2021[275]; Mekinist PI 2020[85]; NCCN NSCLC V.3.2022[33]; Opdivo PI 2020[276]; Retevmo PI 2020[277]; Rozlytrek PI 2019[278]; Rybrevant PI 2021[279]; Tabrecta PI 2020[280]; Tafinlar PI 2020[88]; Tagrisso PI 2019[89]; Tarceva PI 2019[90]; Tecentriq PI 2020[281]; Tepmetko PI 2021[282]; Tykerb PI 2018[283]; Vitrakvi PI 2021[284];Vizimpro PI 2018[285]; Xalkori PI 2019[92]; Yervoy PI 2020[286]; Zelboraf PI 2020[93]; Zykadia PI 2019[94].

Table 6-3. Biomarker-Based Targeted Therapies Recommended in NCCN Guidelines[a] for Prostate Cancer Along With Companion Diagnostics

Genomic alteration	Drug recommended by NCCN	NCCN recommendation[b,c]	Is a CDx required per FDA approved drug label?	Does Foundation Medicine have a CDx claim for this alteration?
***BRCA1/2*m[d]**	Rucaparib[e]	Category 2A (2L+)	Y	Y
HRRm[d]	Olaparib[f]	Category 1 (2L+)[g]	Y	Y
MSI-H/dMMR	Pembrolizumab	Category 2A (2L+)	N[h]	Y
TMB-H (≥10 mut/Mb)	Pembrolizumab	Category 2A (2L+)	Y	Y

Green cells outline those therapies that require a companion diagnostic per the FDA label and for which FoundationOne Liquid CDx is an approved companion diagnostic.

[a] Individual guidelines contain differing recommendations for extent of molecular testing; please refer to the individual guidelines at NCCN.org for information on individual cancers by site.

[b] Category 1: Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2A: Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2B: Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.

Category 3: Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

All NCCN recommendations are category 2A unless otherwise indicated.

[c] All systemic therapies for treatment following docetaxel and a novel hormone therapy are Category 2B if visceral metastases are present.

[d] *BRCA1/2* mutations and HRR mutations refer to germline and/or somatic pathogenic mutations.

[e] Rucaparib is a treatment option for patients with mCRPC and a pathogenic *BRCA1* of *BRCA2* mutation (germline and/or somatic) who have been treated with androgen receptor-directed therapy and a taxane-based chemotherapy. If the patient is not fit for chemotherapy, rucaparib can be considered even if taxane-based therapy has not been given.

[f] Olaparib is a treatment option for patients with mCRPC and a pathogenic mutation (germline and/or somatic) in a homologous recombination repair gene (*BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *RAD51B*, *RAD51C*, *RAD51D*, or *RAD54L*) who have been treated previously with androgen receptor-directed therapy. Patients with *PPP2R2A* mutations in the PROfound trial experienced an unfavorable risk-benefit profile. Therefore, olaparib is not recommended in patients with a *PPP2R2A* mutation. There may be heterogeneity of response to olaparib for non-*BRCA* mutations based on which gene has a mutation.

[g] Olaparib has a Category 1 recommendation for treatment following prior abiraterone or enzalutamide. Olaparib has a Category 1 recommendation for treatment after prior docetaxel and prior novel hormone therapy if visceral metastases are not present.

[h] This indication is approved under accelerated approval based on surrogate endpoint(s) (eg, event-free survival, objective response rate, complete response, PFS or time to progression). Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

1L, first line; 2L, second line; BRCA, breast cancer susceptibility gene; CDx, companion diagnostic; dMMR, DNA mismatch repair; FDA, Food and Drug Administration; HRR, homologous recombination repair; HRRm, homologous recombination repair mutation; MSI-H, microsatellite instability-high; NA, not applicable; NCCN, National Comprehensive Cancer Network; N, no; PFS, progression-free survival; Y, yes.

Sources: Keytruda PI 2021[272]; Lynparza PI 2020[287]; NCCN Prostate Cancer V.4.2022[36]; Rubraca PI 2020[257].

Table 6-4. Biomarker-Based Targeted Therapies and Immunotherapies Recommended in NCCN Guidelines[a] for Recurrent or Metastatic Breast Cancer Along With Companion Diagnostics

Genomic alteration	Drug recommended by NCCN	NCCN recommendation[b]	Is a CDx required per FDA approved drug label?	Does Foundation Medicine have a CDx claim for this alteration?
***ERBB2* (HER2)**	Pertuzumab + trastuzumab + taxane[c]	Category 1 (with docetaxel), preferred; Category 2A (with paclitaxel), preferred (1L+)	Y[d]	Y
	Fam-trastuzumab deruxtecan-nxki[e]	Category 1; preferred (2L+)[f,g]		
	Ado-trastuzumab emtansine (TDM-1)	Category 2A (2L+)[f]	Y	Y
	Tucatinib + trastuzumab + capecitabine[c]	Category 1 (3L+)[h]	N	---
	Trastuzumab + chemotherapy[c,i]	Category 2A (3L+)[j]	Y	Y
	Capecitabine + anti-HER2 therapy[c,k]	Category 2A (3L+)[j]	Y	Y
	Trastuzumab + lapatinib (without cytotoxic therapy)[c]	Category 2A (3L+)[j]	Y[d]	Y
	Neratinib + capecitabine	Category 2A (3L+)[j]	N	---
	Margetuximab-cmkb + chemotherapy[l]	Category 2A (3L+)[j]	N	---
***BRCA1/BRCA2*[m]**	Olaparib	Category 1; preferred (1L+)	Y	Y
	Talazoparib	Category 1; preferred (1L+)	Y	Y

Genomic alteration	Drug recommended by NCCN	NCCN recommendation[b]	Is a CDx required per FDA approved drug label?	Does Foundation Medicine have a CDx claim for this alteration?
PIK3CA **(HR-positive/HER-2 negative)**[n]	Alpelisib + fulvestrant[o]	Category 1; preferred (2L+)	Y	Y
PD-L1 expression (using 22C3 antibody) **Threshold for positivity CPS ≥10**[p]	Pembrolizumab + chemotherapy[q]	Category 1; preferred first-line therapy[r] (1L+)	Y (PD-L1)	N
NTRK[s]	Larotrectinib[t]	Category 2A (2L+)	Y	Y
	Entrectinib[t]	Category 2A (2L+)	N[u]	---
MSI-H/dMMR[v]	Pembrolizumab[w]	Category 2A (2L+)	N[u]	Y
	Dostarlimab-gxly[x]	Category 2A (2L+)	Y	N
TMB-H (≥10 mut/Mb)[y]	Pembrolizumab[w]	Category 2A (2L+)	Y	Y

Green cells outline those therapies that require a companion diagnostic per the FDA label and for which FoundationOne Liquid CDx is an approved companion diagnostic.

[a] Individual guidelines contain differing recommendations for extent of molecular testing; please refer to the individual guidelines at NCCN.org for information on individual cancers by site.

[b] Category 1: Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2A: Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2B: Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.

Category 3: Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

All NCCN recommendations are category 2A unless otherwise indicated.

[c] An FDA-approved biosimilar is an appropriate substitute for trastuzumab.

[d] Within this regimen, trastuzumab is the only therapy that requires an FDA approved companion diagnostic test.

[e] Fam-trastuzumab deruxtecan-nxki is contraindicated for patients with pneumonitis or interstitial lung disease (ILD).

[f] Regimen may also be used as an option for third-line and beyond; the optimal sequence for third-line therapy and beyond is not known.

[g] Fam-trastuzumab deruxtecan-nxki may be considered in the first-line setting as an option for select patients (ie, those with rapid progression within 6 months of neoadjuvant or adjuvant therapy [12 months for pertuzumab-containing regimens]).

[h] Tucatinib + trastuzumab + capecitabine is preferred in patients with both systemic and CNS progression in the third-line setting and beyond; this regimen may be given in the second-line setting.

[i] Chemotherapy to be used in combination with trastuzumab includes paclitaxel ± carboplatin, docetaxel, vinorelbine, capecitabine, and other agents. Trastuzumab given in combination with an anthracycline is associated with significant cardiac

toxicity. Concurrent use of trastuzumab and pertuzumab with an anthracycline should be avoided. Trastuzumab may be safely combined with all non-anthracycline containing preferred and other single agents for recurrent or metastatic breast cancer.

[j] Multiple lines of concurrent chemotherapy with anti-HER2 therapy (trastuzumab or a TKI) offer clinical benefit for recurrent unresectable HER2+ metastatic breast cancer and have been studied in phase 2 or 3 trials. Clinical experience suggests frequent clinical benefit for such treatment. However, there are no meaningful data for use of any of these regimens among patients previously treated with pertuzumab-based chemotherapy, ado-trastuzumab emtansine, fam-trastuzumab deruxtecan-nxki, or trastuzumab/capecitabine/tucatinib regimens. Thus, the optimal sequence or true benefit of therapy is not known.

[k] Trastuzumab or lapatinib.

[l] Chemotherapy options include capecitabine, eribulin, gemcitabine, or vinorelbine.

[m] Assess for germline *BRCA1/2* mutations in all patients with recurrent or metastatic breast cancer to identify candidates for PARP inhibitor therapy. While olaparib and talazoparib are FDA indicated in HER2-negative disease, the panel supports use in any breast cancer subtype associated with a germline *BRCA1* or *BRCA2* mutation.

[n] For HR-positive/HER2-negative breast cancer, assess for *PIK3CA* mutations with tumor or liquid biopsy to identify candidates for alpelisib plus fulvestrant. *PIK3CA* mutation testing can be done on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended.

[o] The safety of alpelisib in patients with Type 1 or uncontrolled Type 2 diabetes has not been established.

[p] Assess for PD-L1 expression in TNBC subtype; detection per IHC.

[q] Chemotherapy options include albumin-bound paclitaxel, paclitaxel, or gemcitabine and carboplatin.

[r] While available data are in the first-line setting, this regimen can be used for second and subsequent lines of therapy if PD-1/PD-L1 inhibitor therapy has not been previously used. If there is disease progression while on a PD-1/PD-L1 inhibitor, there are no data to support an additional line of therapy with another PD-1/PD-L1 inhibitor.

[s] Assess for *NTRK* fusion in all breast cancer subtypes; detection per FISH, NGS, or PCR (tissue block).

[t] Larotrectinib and entrectinib are indicated for the treatment of solid tumors that have an *NTRK* gene fusion without a known acquired resistance mutation and have no satisfactory alternative treatments or that have progressed following treatment.

[u] This indication is approved under accelerated approval based on surrogate endpoint(s) (eg, event-free survival, objective response rate, complete response, PFS or time to progression). Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

[v] Assess for MSI/dMMR in all breast cancer subtypes; detection per IHC or PCR (tissue block).

[w] Pembrolizumab is indicated for the treatment of patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors, or TMB-H tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options.

[x] Dostarlimab-gxly is indicated for adult patients with MSI-H/dMMR unresectable or metastatic tumors that have progressed on or following prior treatment and who have no satisfactory alternative treatment options.

[y] Assess for TMB in all breast cancer subtypes; detection per NGS.

1L, first line; 2L, second line; 3L+, third line and beyond; BRCA, breast cancer susceptibility gene; CDx, companion diagnostic; CNS, central nervous system; ctDNA, circulating tumor DNA; dMMR, DNA mismatch repair; ERBB2, Erb-B2 receptor tyrosine kinase 2; FDA, Food and Drug Administration; FISH, fluorescence in situ hybridization; HER, human epidermal growth factor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IHC, immunohistochemistry; MSI-H, microsatellite instability-high; NA, not applicable; NCCN, National Comprehensive Cancer Network; NGS, next generation sequencing; N, no; NTRK, neurotrophic tyrosine kinase receptor; PARP, poly (ADP-ribose) polymerase; PCR, polymerase chain reaction; PD-L1, programmed death-ligand 1; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; TNBC, triple negative breast cancer; Y, yes.

Sources: Enhertu PI 2019[269]; Herceptin PI 2018[83]; Jemperli PI 2021[288]; Kadcyla PI 2013[271]; Keytruda PI 2021[272]; Lynparza PI 2020[287]; Margenza PI 2021[289]; NCCN Breast Cancer V.3.2022[25]; Nerlynx PI 2020[290]; Perjeta PI 2012[86]; Piqray PI 2019[87]; Rozlytrek PI 2019[278]; Talzenna PI 2018[291]; Tukysa PI 2020[292]; Vitrakvi PI 2021[284].

Table 6-5. Biomarker-Based Targeted Therapies and Immunotherapies Recommended in NCCN Guidelines for Recurrent Ovarian Cancer[a,b] Along With Companion Diagnostics

Genomic alteration	Drug recommended by NCCN	NCCN recommendation[c]	Is a CDx required per FDA approved drug label?	Does Foundation Medicine have a CDx claim for this alteration?
HRD[d]	Niraparib	Category 2A (4L+)	Y	N
Deleterious *BRCA1/2* (germline) mutation	Olaparib	Category 2A (3L+)	Y	Y
Deleterious *BRCA1/2* (germline and/or somatic) mutation	Rucaparib	Category 2A (3L+)	Y	Y
***NTRK* gene fusion positive tumors**	Entrectinib	Category 2A (2L+)	N[e]	---
	Larotrectinib	Category 2A (2L+)	Y	Y
MSI-H/dMMR solid tumors	Pembrolizumab	Category 2A (2L+)	N[e]	Y
	Dostarlimab-gxly	Category 2A (2L+; recurrent or advanced tumors)	Y	N
TMB-H (≥10 mut/Mb)	Pembrolizumab	Category 2A (2L+)	Y	Y

Green cells outline those therapies that require a companion diagnostic per the FDA label and for which FoundationOne Liquid CDx is an approved companion diagnostic.

[a] Individual guidelines contain differing recommendations for extent of molecular testing; please refer to the individual guidelines at NCCN.org for information on individual cancers by site.

[b] This includes both platinum-sensitive recurrent ovarian cancer and platinum-resistant recurrent ovarian cancer. Biomarker-based recommendations in other settings are not included in this table.

[c] Category 1: Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2A: Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Category 2B: Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.

Category 3: Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

All NCCN recommendations are category 2A unless otherwise indicated.

[d] HRD defined by either: 1) a deleterious or suspected deleterious *BRCA* mutation; or 2) genomic instability and progression >6 months after response to the last platinum-based chemotherapy.

[e] This indication is approved under accelerated approval based on surrogate endpoint(s) (eg, event-free survival, objective response rate, complete response, PFS or time to progression). Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

2L+, second-line and beyond; 3L+, third line and beyond; 4L+, fourth line and beyond; BRCA, breast cancer susceptibility gene; CDx, companion diagnostic; dMMR, DNA mismatch repair; FDA, Food and Drug Administration; HRD, homologous recombination deficiency; MSI-H, microsatellite instability-high; NA, not applicable; NCCN, National Comprehensive Cancer Network; N, no; NTRK, neurotrophic tyrosine kinase receptor; PFS, progression-free survival; Y, yes.

Sources: Jemperli 2021 PI[288]; Keytruda PI 2021[272]; Lynparza PI 2020[287]; NCCN Ovarian Cancer V.1.2022[34]; Rozlytrek PI 2019[278]; Rubraca PI 2020[257]; Vitrakvi PI 2021[284]; Zejula PI 2020[293].

FoundationOne Portfolio Description and Decision Support Services

As shown in Table 6-6, Foundation Medicine provides a portfolio of CGP tests and services to ensure patient access to genomic insights regardless of cancer type or specimen type.

Table 6-6. Foundation Medicine Portfolio

	FoundationOne® CDx	FoundationOne® Liquid CDx	FoundationOne® Heme	IHC
FDA-approved CDx claims	FDA-approved CDx for 28 targeted therapies	FDA-approved CDx for 8 targeted therapies	–	FDA-approved CDx for 2 immunotherapies
Target tumor types	All solid tumors	Liquid biopsy (ctDNA): all solid tumors	Hematologic malignancies, sarcomas (soft tissue + bone), or solid tumors where RNA sequencing is desired	Specific solid tumors
Number of genes analyzed	324 (DNA)	324 (DNA)[a]	406 (DNA) 265 (RNA)	-
Genomic signatures/ biomarkers	▪ TMB ▪ MSI	▪ bTMB ▪ MSI-H[b] ▪ Tumor fraction	▪ TMB ▪ MSI	▪ PD-L1
Specimen[c]	FFPE tissue	Peripheral whole blood	▪ FFPE tissue ▪ Bone marrow aspirate ▪ Peripheral whole blood	FFPE tissue
	10 USS or 1 block[d] + 1 H&E slide	2 tubes (8.5mL each) of peripheral whole blood	16 USS + 1 H&E slide or 1 FFPE block or 2.5 mL bone marrow aspirate or 1 filled EDTA tube + 2.5 mL Paxgene tube peripheral whole blood	4 USS
Report features	Point mutations, insertions/deletions, copy number alterations, select rearrangements	Point mutations, insertions/deletions, copy number alterations, select rearrangements	Point mutations, insertions/deletions, copy number alterations, rearrangements	TPS and/or CPS for approved/validated tumor types

	FoundationOne® CDx	FoundationOne® Liquid CDx	FoundationOne® Heme	IHC
Typical turnaround time[e]	<12 days	<10 days	2 weeks	5 days

[a] FoundationOne Liquid CDx is FDA-approved to report substitutions and indels in 311 genes, including rearrangements in *ALK* and *BRCA1/2* and copy number alterations in *BRCA1/2* and *ERBB2* (HER2). Comprehensive results across all 324 genes are reported as a laboratory professional service which is not reviewed or approved by the FDA. bTMB, MSI-H status, and tumor fraction are reported as a laboratory professional service which is not reviewed or approved by the FDA.

[b] MSI status will be reported for samples determined to have high microsatellite instability.

[c] For full details, refer to specimen instructions at www.foundationmedicine.com.

[d] FFPE is preferred.

[e] Based on typical turnaround time from receipt of specimen.

bTMB, blood tumor mutational burden; CDx, companion diagnostic; CPS, combined positive score; ctDNA, circulating tumor DNA; DNA, deoxyribonucleic acid; EDTA, ethylenediaminetetraacetic acid; FFPE, formalin-fixed paraffin embedded; H&E, hematoxylin and eosin; mL, milliliter; MSI, microsatellite instability; MSI-H – microsatellite instability-high; PD-L1, programmed death ligand-1; TMB, tumor mutational burden; TPS, tumor proportion score.

Source: Foundation Medicine, Inc.

Foundation Medicine's Services go "beyond the test" by providing a clear, in-depth report that supports clinical decision making by:

- Providing insights on the patient's genomic profile and associated targeted therapies, immunotherapies, and relevant clinical trials
- Highlighting important disease-relevant genes with no reportable alterations identified
- Highlighting genomic alterations associated with potential resistance to therapy to help rule out potentially ineffective treatment.

Please refer to the sample report (Figure 6-1) for an example of this clinical decision support.

In addition to the in-depth report, Foundation Medicine helps offers decision support services and technology solutions to help streamline patient care, including:

- FoundationINSIGHTS™
- Clinical trial matching
- Digital access
- Electronic medical record (EMR) interfacing
- MD Case Consultant Program
- Molecular tumor boards (>30 nationwide)

FoundationOne Liquid CDx Product Description

As part of its FDA-approved intended use, the FoundationOne Liquid CDx assay interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage; select regions in 75 genes are captured with increased sensitivity (Table 6-7).[1]

Table 6-7. Complete List of Genes Targeted by FoundationOne Liquid CDx

ABL1	*ACVR1B*	***AKT1 [Exon 3]***	*AKT2*	*AKT3*	***ALK***	*ALOX12B*	*AMER1 (FAM123B)*	***APC***	***AR***

[Exons 4-9]					***[Exons 20-29, Introns 18,19]***				
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]*
[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]*	*C11orf30* *(EMSY)*	*C17orf39* *(GID4)*
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]*	*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]*	*FAM46C*	*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*	*GNA11* *[Exons 4, 5]*	*GNA13*	*GNAQ* *[Exons 4, 5]*	*GNAS* *[Exons 1, 8]*	*GRM3*
GSK3B	*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*	*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]***	*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 (TTF-1)*	*NOTCH1*	*NOTCH2 [Intron 26]*	*NOTCH3*	***NPM1 [Exons 4-6, 8, 10]***	***NRAS [Exons 2, 3]***	*NSD3 (WHSC1L1)*	*NT5C2*	***NTRK1 [Exons 14, 15, Introns 8-11]***
NTRK2 [Intron 12]	***NTRK3 [Exons 16, 17]***	*NUTM1* [Intron 1]*	*P2RY8*	***PALB2***	*PARK2*	*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	*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*

								(LKB1)	
SYK	*TBX3*	*TEK*	*TERC* {ncRNA}*	***TERT* {Promoter}***	*TET2*	*TGFBR2*	*TIPARP*	*TMPRSS2* [Introns 1-3]*	*TNFAIP3*
TNFRSF14	***TP53***	*TSC1*	*TSC2*	*TYRO3*	*U2AF1*	***VEGFA***	*VHL*	*WHSC1*	*WTI*
XPO1	*XRCC2*	*ZNF217*	*ZNF703*						

75 genes indicated in bold have regions captured with increased sensitivity (as indicated with brackets).

* 15 genes with only select non-coding coverage.

^ *BRCA1* or *BRCA2* variants as described by FoundationOne Liquid CDx may be either germline or somatic in nature. Follow-up germline testing would be needed to distinguish whether the finding is somatic or germline.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1].

FoundationOne Liquid CDx Sample Report

Figure 6-1. FoundationOne Liquid CDx Sample Report

FOUNDATIONONE®LIQUID CDx

PATIENT

TUMOR TYPE
Lung adenocarcinoma

REPORT DATE

ORDERED TEST #

PATIENT	PHYSICIAN	SPECIMEN
DISEASE Lung adenocarcinoma	ORDERING PHYSICIAN	SPECIMEN ID
NAME	MEDICAL FACILITY	SPECIMEN TYPE
DATE OF BIRTH	ADDITIONAL RECIPIENT	DATE OF COLLECTION
SEX	MEDICAL FACILITY ID	SPECIMEN RECEIVED
MEDICAL RECORD #	PATHOLOGIST	

Companion Diagnostic (CDx) Associated Findings

GENOMIC FINDINGS DETECTED	FDA-APPROVED THERAPEUTIC ONS
ALK EML4-ALK fusion (Variant 1)	ALECENSA® (alectinib)

Other Short Variants Identified

Results reported in this section are not prescriptive or conclusive for labeled use of any cific therapeutic product.
See *professional services* section for information on the alterations listed in this section as has any additional detected copy number alterations, gene rearrangements, or biomarkers.

OTHER BIOMARKERS WITH POTENTIAL CLINICAL SIGNIFIC CE

CTNNB1 N387K

Please refer to appendix for Explanation of Clinical Significance Classification d f riants of unkn ignificance (VUS).

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

Electronically signed by Erik Williams, M.D. |
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Shakti Ramkissoon, M.D., Ph.D., M.M. Sc, 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

Note: The intended use (IU) statement and claims made on this sample report may not be up to date. For the latest version of the FoundationOne Liquid CDx claims and IU, please see the current label: www.foundationmedicine.com/f1lodx

FDA APPROVED CLAIMS - PAGE 1 of 1

FOUNDATIONONE®LIQUID CDx

PATIENT

TUMOR TYPE
Lung adenocarcinoma
COUNTRY CODE
US

REPORT DATE

ORDERED TEST #

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

Interpretive content on this page and subsequent pages is provided as a professional service, and is not reviewed or approved by the FDA.

PATIENT

DISEASE Lung adenocarcinoma
NAME
DATE OF BIRTH
SEX
MEDICAL RECORD #

PHYSICIAN

ORDERING PHYSICIAN
MEDICAL FACILITY
ADDITIONAL RECIPIENT
MEDICAL FACILITY ID
PATHOLOGIST

SPECIMEN

SPECIMEN ID
SPECIMEN TYPE
DATE OF COLLECTION
SPECIMEN RECEIVED

Biomarker Findings

Blood Tumor Mutational Burden - 5 Muts/Mb
Microsatellite status - Cannot Be Determined
Tumor Fraction - 17%

Genomic Findings

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

ALK **EML4-ALK fusion (Variant 1)**
CTNNB1 **N387K**

6 Therapies with Clinical B ft
0 Therapies with Lack of Resp

20 Clinical T

BIOMARKER FINDINGS
Blood Tumor Mutational Burden - 5 Muts/Mb
Microsatellite status - Cannot B Determined
Tumor Fraction - 1

GENOMIC FINDINGS	VAF %
ALK **-** EML4-ALK sion (V iant 1)	%
10 Trials see p. 10	
CTNNB1 **-** 87K	7.5%
10 Trials see p. 12	

THE Y AND CLINICAL TRIAL IMPLICATIONS
o therap or clinical trials. See Biomarker Findings section
Una e to determine Microsatellite status due to insufficient evidence of genomic sta ty.
Tumor fraction is an estimate of the percentage of circulating-tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample based on observed aneuploid instability.

THERAPIES WITH CLINICAL BENEFIT (IN PATIENT'S TUMOR TYPE)		THERAPIES WITH CLINICAL BENEFIT (IN OTHER TUMOR TYPE)
Alectinib	1	None
Brigatinib	1	
Ceritinib	1	
Crizotinib	1	
Lorlatinib	2A	
Entrectinib		
None		None

NCCN category

Electronically signed by Erik Williams, M.D. |
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Shakti Ramkissoon, M.D., Ph.D., M.M. Sc, 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

PATIENT

TUMOR TYPE
Lung adenocarcinoma
COUNTRY CODE
US

REPORT DATE

ORDERED TEST #

IMPORTANT NOTE Genomic alterations detected may be associated with activity of certain FDA-approved drugs, however, the agents listed in this report may have varied clinical evidence in the patient's tumor type. Neither the therapeutic agents nor the clinical 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. In the appropriate clinical context, germline testing of *APC, BRCA1, BRCA2, BRIP1, MEN1, MLH1, MSH2, MSH6, MUTYH, NF2, PALB2, PMS2, 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.

Electronically signed by Erik Williams, M.D. |
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Shakti Ramkissoon, M.D., Ph.D., M.M. Sc, 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

PATIENT

TUMOR TYPE
Lung adenocarcinoma

REPORT DATE

ORDERED TEST #

HISTORIC PATIENT FINDINGS		VAF%
Blood Tumor Mutational Burden		5 Muts/Mb
Microsatellite status		Cannot Be Determined
Tumor Fraction		17%
ALK	EML4-ALK fusion (Variant 1)	8.2%
CTNNB1	● 387K	7.5%

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

For some genes in FoundationOne Liquid CDx, only select exons 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 biomarker list will be updated periodically to reflect new knowledge about cancer biology.

As new 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 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 germline and likely somatic 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 Reported = not baited (not reported on test); may precede addition of biomarker or gene

Not Detected = 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

Electronically signed by Erik Williams, M.D. |
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Shakti Ramkissoon, M.D., Ph.D., M.M. Sc, 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

PATIENT

TUMOR TYPE
Lung adenocarcinoma

REPORT DATE

ORDERED TEST #

BIOMARKER FINDINGS

BIOMARKER
Blood Tumor Mutational Burden

RESULT
5 Muts/Mb

POTENTIAL TREATMENT STRATEGIES
On the basis of clinical evidence in NSCLC and HSNCC, increased bTMB may be associated with greater sensitivity to immunotherapeutic agents, including anti-PD-L1[1,2] and anti-PD-1[3] therapies. In 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 to 16 Muts/Mb[1]. In 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[4].

FREQUENCY & PROGNOSIS
NSCLC harbors a median bTMB of 16.8 Muts/Mb (range 1.9-52.5 Muts/Mb)[3]. Published data investigating the prognostic implications of bTMB levels in lung cancer are limited (PubMed, Jul 2020). A large study of Chinese patients with lung adenocarcinoma reported a shorter median OS for tumors with a higher number of mutations in a limited gene set compared with a lower mut n number (48.4 vs. 61.0 months)[5]. Another study patients with NSCLC correlated elevated TMB with poorer prognosis and significantly associated lower TMB in combination with tive status with longer median su l in patie with lung adenocarcinoma wever, no significant prognostic a ation of TMB an PD-L1 status with su l has been reporte patients with lung SCC

FINDING SUMMARY
Blood tumor mutational b den (bTMB, also known as mutation loa a measure of the number of somatic p n-coding base substitution and i tion/deletion mutations from circulatin mor DNA in bloo TMB is affected by iety of causes, in ing exposure to mutagen h as ultraviol ht in melanoma[8-9] a igarette ke in lung cancer[10-11], treatm wi mozolomide-based chemotherapy in gl [3], mutations in th proofreading domains NA polymeras encoded by the POLE and D1 gen , and microsatellite instability (MS [18] gh bTMB levels were not detected in this le. It is unclear whether the bTMB levels in this sample uld be predicted to be a sociated with s ivity to PD-1- or P 1-targeting immune chec nt inhibitor ne or in combination with o gents[1] epending on the clinical context, T ng of an alternate sample or by another meth logy could be considered.

BIOMARKER
Tumor Fraction

RESULT
17%

POTENTIAL TREATMENT STRATEGIES
There are currently no targeted approaches to address specific tumor fraction els; however, on the basis of emerging clinica id hanges in tumor fraction may correlate h treatm duration and clinical response a may be a us indicator for cancer managemen [4].

FREQUEN & OGNOSIS
Detectible ctD le as been rep in a variety of tumo pes, higher tum ction levels reported in tients metastatic (ge 4) tumors compar with pa with localized ase (Stages 1 to 3 [5]. Elevated or fraction le e been repo d to be ass ated with orse pr is in a v ety of cancer types, ding pan can r[26], Ewing sarcoma and os rcoma[27], pros cer[22], breast cancer[28], leiom coma[29], esophageal cancer[30], colorectal cancer[3], gastrointestinal cancer[32].

INDING SU MARY
ction is an estimate of the percentage of circula tumor DNA (ctDNA) present in a cell-free DNA (cfDNA) sample. Tumor cells in most advanced solid tumor types may shed ctDNA through the process of apoptosis or necrosis[25,33-34]. Tumor fraction has been proposed to be a noninvasive surrogate biomarker of disease burden dynamics. Elevated tumor fraction levels have been associated with inferior prognosis, and therapeutic resistance to treatment in certain tumor types[22,28,31], whereas reduced levels have been correlated with tumor shrinkage and improved clinical outcome in patients with non-small cell lung cancer, urothelial cancer, and melanoma treated with immunotherapy[20,24,35]. The tumor fraction estimate, shown here, is computationally derived from observed aneuploid instability in the sample.

Electronically signed by Erik Williams, M.D. |
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Shakti Ramkissoon, M.D., Ph.D., M.M. Sc, 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

PATIENT

TUMOR TYPE
Lung adenocarcinoma

REPORT DATE

ORDERED TEST #

GENOMIC FINDINGS

GENE
ALK

ALTERATION
EML4-ALK fusion (Variant 1)

POTENTIAL TREATMENT STRATEGIES

ALK mutations or rearrangements may confer sensitivity to ALK TKIs such as crizotinib[36-37], ceritinib[38], brigatinib[39-40], alectinib[41], lorlatinib[42], and entrectinib[43]. An ongoing Phase 2 study of lorlatinib for patients with ALK- positive NSCLC previously treated with second-generation TKIs reported an intracranial ORR of 54% and an extracranial ORR of 37%[44]. Lorlatinib also elicited significant clinical activity for patients with NSCLC and intracranial[45] or intrathecal[46] metastases and against resistance mutations associated with progression on first- and second-generation ALK TKIs such as G1202R[47-48]. Crizotinib[49], ceritinib[50], and lorlatinib[51-52] further displayed antitumor activity against ALK+ inflammatory myofibroblastic tumors (IMTs) in Phase 1/2 trials. Crizotinib has also shown clinical activity in ALK-mutated neuroblastoma[53], and both crizotinib[54-55] and lorlatinib exhibited preclinical activity against activating ALK point mutations[56-57]. Phase 1 studies of the ALK/ROS1/TRK inhibitor entrectinib have reported respon for 4 of 7 (57%) kinase inhibitor-naive patien with ALK-rearranged solid tumors, including patients with NSCLC, renal cell carcinoma, and colorectal cancer as well as for 1 patient with ALK F1245V mutant neuroblastoma, but in 0 of 13 patients with ALK fusion-posi tumors previously treated with an A in and in none of the other patients with LK non alterations[43]. A Phase 1/1B trial of entrectinib for children and adolescents with recurrent or refractory solid tumors reported a CR in a patient with infantile fibrosarcoma (IFS) and an ALK fusion, a CR in a patient with neuroblastoma and an ALK F1174L mutation, and a PR in a patient with an inflammatory myofibroblastic tumor (IMT) and an ALK fusion[58]. A Phase 2 trial of the HSP90 inhibitor ganetespib reported PRs for a small number of patients with ALK-rearranged NSCLC[59]. A Phase 3 study for patients with inhibitor-naive ALK-positive non-small cell lung cancer (NSCLC) reported superior clinical benefit with ensartinib, a second-generation ALK inhibitor, compared with crizotinib treatmen (median PFS 25.8 vs. 12.7 months [HR=0.51], OR 75% vs. 67%, 36-month duration of response [DOR] 59% vs. 27%); intracranial ensartinib for these patients w so imp d compared with crizotinib (O 64% [7/11] 21% [4/19])[60]. A Phase 2 dy for patients w ALK-positive NSCLC progressed on crizotinib reported an ll ORR of 52% median PFS of 9.6 month d an intra ial ORR of 70% /40)[61]. The P 3 IM wer150 study show he addition zolizumab to bevacizumab us motherapy tr ent also had clinical effi cy tients with ated EGFR-mutated ALK anged meta NSCLC[62]; therefo the pa 's clinical co xt should be consider

FR ENCY & PR GNOSIS

LK rear ents a frequently observed in adenocarc [63]. The EML4-ALK gene fus as been obser approximately 3-7% of non ll cell lung carcinoma cases, more frequentl younger patients, non-smokers, females, and ents of Asian heritage[66-72]. ALK protein expression has been associated with poor prognosis in some cancer types, including NSCLC, renal cell carcinoma, an uroblastoma[73-75]. EML4-ALK fusions h been reported to be a significant indicat f poor prognosis in advanced stage CLC[72].

FINDING MARY

ALK encodes ptor ty ne kinase, a member of the insulin rec s family, whose activation induces t wnstream pathway associated with cell sur l angiogenesi d cell proliferation[76]. Different E -ALK nts have been identified in cancer, all i ontain the intracellular tyrosine kinase dom of ALK[77]. The most commonly observed rearrangements consist ALK exon 20 fused to a ariety of breakpoints in L4: exon 13 (varia , 33-54% of cases)[78-81], exon variant 2, 1 % of cases)[78-81], exon 6 (varian b, 26 of cases)[78-79,81-83], exon 15 (variant 4, ses)[67,84-85], exon 18 (variant 5, 1.6-3% of cas [84], exon 2 (variant 5 a/b, 1-2% of cases)[78,85-87], and exon 17 (variant 8 a/b, less than 1%)[80,84,88]. All of these variants have been characterized as, or are predicted to be, activating and sensitive to ALK inhibitors, including crizotinib and ceritinib[79,82,89]; however, variants 3a/b are less sensitive to crizotinib in vitro[79,81]. Although retrospective analyses of crizotinib–treated non-small cell lung cancer (NSCLC) have reported significant differences in outcomes among EML4-ALK variants, specifically longer median progression-free survival (PFS) in patients with variant 1 and improved 2-year PFS and time to progression in patients with variants other than 3a/b[81,90-91], other studies have not found correlation between EML4-ALK variants and response to crizotinib in NSCLC[80,83].

Electronically signed by Erik Williams, M.D. |
Julia Elvin, M.D., Ph.D., Laboratory Director CLIA: 22D2027531
Shakti Ramkissoon, M.D., Ph.D., M.M. Sc, 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

Comparison of FoundationOne Liquid CDx to FoundationOne Liquid

FoundationOne Liquid CDx replaces and expands upon the previous version of the Foundation Medicine liquid test, FoundationOne Liquid, a LDT assay (Table 6-8).

Table 6-8. Comparison of FoundationOne Liquid CDx and FoundationOne Liquid

	FoundationOne Liquid CDx[a]	FoundationOne Liquid
FDA status	FDA approved as companion diagnostic for 8 targeted therapies	LDT; not FDA approved
Target tumor types	All solid tumors	
Number of gene analyzed	324 (DNA)[a]	70 (DNA)
Genomic signatures/biomarkers	bTMB, MSI-H[b], TF[a]	MSI-H
Specimen	Peripheral whole blood	
Report features	Point mutations, insertions/deletions, CNAs (amplifications and select losses), and rearrangements	Point mutations, insertions/deletions, CNAs (select amplifications), and rearrangements
Turnaround time	Typically within 2 weeks from receipt of specimen	

[a] FoundationOne Liquid CDx is FDA-approved to report substitutions and indels in 311 genes, rearrangements in 4 genes, and copy number alterations in 3 genes. Comprehensive results across all 324 genes are reported as a laboratory professional service which is not reviewed or approved by the FDA. bTMB, MSI-H status, and tumor fraction are reported as a laboratory professional service which is not reviewed or approved by the FDA.

[b] MSI status will be reported for samples determined to have high microsatellite instability.

bTMB, blood tumor mutational burden; CNA, copy number alterations; DNA, deoxyribonucleic acid; FDA, Food and Drug Administration; LDT, laboratory derived test; MSI-H, microsatellite instability-high; TF, tumor fraction.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1]. Foundation Medicine Inc, FoundationOne Liquid CDx Technical Specifications[165].

Analytical Validity of FoundationOne Liquid CDx

Concordance of FoundationOne Liquid CDx to FoundationOne Liquid

This study evaluated the concordance of 927 unique samples processed on both the FoundationOne Liquid laboratory developed test (LDT) and FoundationOne Liquid CDx assays. A total of 3,366 alterations, consisting of only those in common between the assays were evaluated. The concordance analysis using FoundationOne Liquid LDT or FoundationOne Liquid CDx as the reference assay is summarized by variant category in Table 6-9.[1]

The overall PPA between FoundationOne Liquid LDT and FoundationOne Liquid CDx assays, with FoundationOne Liquid LDT as the reference assay, was 94.8% with a (95% two-sided CI: 94.0%, 95.5%). The respective short variant, rearrangement, and copy number amplification PPA values were: 95.9% (95% two-sided CI: 95.1%, 96.6%), 88.0% (95% two-sided CI: 82.1%, 92.5%), and 84.4% (95% two-sided CI: 78.7%, 89.1%). These results support the agreement between FoundationOne Liquid LDT and FoundationOne Liquid CDx and the applicability of the tumor comparability analysis performed using historical FoundationOne Liquid data.[1]

Table 6-9. Concordance Between FoundationOne Liquid CDx and FoundationOne Liquid LDT

Variant/ Mutation Type	CDx+ / LDT+	CDx- / LDT+	CDx+ / LDT-	CDx- / LDT-	PPA (95% CI)	NPA (95% CI)	OPA (95% CI)
All Short Variants	2871	123	32	1171180	95.9% (95.1%, 96.6%)	>99.9% (>99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)
Base Substitutions	2415	104	31	999032	95.9% (95.0%, 96.6%)	>99.9% (>99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)
Indels	456	19	1	172148	96.0% (93.8%, 97.6%)	>99.9% (>99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)
Rearrangements	147	20	24	59587	88.0% (82.1%, 92.5%)	>99.9% (>99.9%, 100.0%)	99.9% (99.9%, 99.9%)
Copy number amplifications	173	32	0	59463	84.4% (78.7%, 89.1%)	99.8% (>99.9%,100.0%)	99.8% (>99.9%, 100.0%)
Total	3191	175	166	1290230	94.8% (94.0%, 95.5%)	>99.9% (>99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)

CI, confidence interval; CDx, FoundationOne Liquid CDx; ; LDT, FoundationOne Liquid laboratory derived test; NPA, negative percent agreement; OPA, overall percent agreement; PPA, positive percent agreement; VUS, variants of unknown significance.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1].

Analytical Validity of Previous Versions of Foundation Medicine Liquid Assay

Validation of FoundationOne Liquid

The analytical performance of the previously available FoundationOne Liquid assay is displayed in Table 6-10 for historical purposes and to support the application of previously generated evidence to FoundationOne Liquid CDx.

Table 6-10. Performance Specifications for FoundationOne Liquid

Variable	MAF/tumor fraction, %[a]	Sensitivity, % (95% CI)	PPV, % (95% CI)
Base substitutions	>0.5	99.9 (99.7–99.9)	100 (99.9–100)
	0.25–0.5	95.8 (94.5–96.9)	99.8 (99.3–99.9)
	0.125–0.25	68.4 (65.7–70.9)	96.1 (94.8–97.1)
Indels (1–40 base pairs)	>0.5	99.7 (98.7–99.9)	100 (99.3–100)
	0.25–0.5	87.7 (81.1–92.2)	98.8 (95.4–99.8)

	0.125–0.25	60.5 (52.7–67.7)	96.8 (92.3–98.8)
Rearrangements[b]	>0.5	100 (85.9–100)	100 (85.9–100)
	0.25–0.5	89.4 (65.5–98.2)	100 (77.1–100)
	0.125–0.25	68.4 (43.5–86.4)	100 (71.7–100)
Copy number amplifications[c]	≥20	95.3 (82.9–99.2)	97.6 (85.9–99.9)
	<20	Varies depending on amplitude of copy number amplification and ctDNA fraction	97.6 (85.9–99.9)
MSI-H[d]	>2.0	92.0 (72.5–98.6)	100 (82.2–100)

[a] Copy number amplifications were calculated using tumor fraction.
[b] Performance for gene fusions within targeted introns only. Sensitivity for gene fusions occurring outside targeted introns or in highly repetitive intronic sequence contexts is reduced.
[c] Copy number ≥8.
[d] Reported when MSI is determined to be high.
CI, confidence interval; ctDNA, circulating tumor DNA; indels, insertions and deletions; MAF, mutant allele frequency; MSI-H, microsatellite instability-high; PPV, positive predictive value.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1].

Validation of FoundationACT®

The analytical validation of FoundationACT was performed on 2,666 test alterations from reference samples derived from cell line models, synthetic gene fusions, and clinical samples.[294] The FoundationACT assay achieved more than 99% overall sensitivity (95% CI: 99.1–99.4) for short variants at allele frequency of more than 0.5%, more than 95% sensitivity (95% CI: 94.2–95.7) for allele frequency of 0.25% to 0.5%, and 70% sensitivity (95% CI: 68.2–71.5) for allele frequency of 0.125% to 0.25%.[294] A summary of the analytical validation results are presented in Table 6-11.

Table 6-11. Summary of the Analytical Validation Results of FoundationACT

Variable	MAF, %	Sensitivity, % (95% CI)	PPV, % (95% CI)
Base substitutions	>0.5	99.3 (99.1–99.4)	100 (>99.9–100)
	0.25–0.5	95.7 (94.9–96.4)	100 (99.8–100)
	0.125–0.25	70.0 (68.3–71.6)	99.9 (99.8–100)
Indels	>0.5	98.5 (97.3–99.2)	100 (99.4–100)
	0.25–0.5	86.6 (81.4–90.5)	100 (97.8–100)
	0.125–0.25	68.5 (62.1–74.3)	100 (97.1–100)
Rearrangements	>0.5	100 (77.1–100)	100 (77.1–100)
	0.25–0.5	100 (56.1–100)	100 (56.1–100)
	0.125–0.25	80.0 (29.9–99)	100 (39.6–100)
Copy number amplifications	≥20% ctDNA fraction	95.3 (82.9–99.9)	97.6 (85.9–99.9)

	<20% ctDNA fraction	Varies depending on amplitude of copy number amplifications and ctDNA fraction

CI, confidence interval; ctDNA, circulating tumor DNA; indel, insertions and deletions; MAF, mutant allele frequency; PPV, positive predictive value.

Source: Clark et al. 2018.[294]

Concordance Between FoundationOne Liquid and FoundationACT

Findings from and analysis of the concordance between FoundationOne Liquid and FoundationACT are described in Table 6-12.[1]

Table 6-12. Concordance Between FoundationOne Liquid and FoundationACT

Alteration type	Samples analyzed, n	Observed sensitivity (95% CI)	FoundationACT only	FoundationOne Liquid only	Positive concordance
Short variants and rearrangements, AF ≥0.5%	33	0.984 (0.938–0.997)	2	2	0.969
Short variants and rearrangements, 0.25% ≤AF <0.5%	33	0.909 (0.693–0.984)	2	3	0.800
Copy number calls	65	–	0	2	0.818

AF, allele frequency; CI, confidence interval.

Source: Foundation Medicine Inc, FoundationOne Liquid CDx[1]

Concordance Studies for FoundationACT

Concordance study results for FoundationACT are described in Table 6-13.

Table 6-13. Concordance Results for FoundationACT

Tumor type	Key citation / publication	Sample size	Time between sample collection (days)	Concordance	
				Short variants	Overall (all alteration types)
Breast	Chung et al (2017)[295]	14	<60	89%	67%
	Kim et al (2017)[296]	71	not defined	100% (*PIK3CA*)	_ [a]
NSCLC	Schrock et al (2019)[75]	33	<30	78%	64%
CRC	Li et al (2019)[163]	96	<30	100%	_ [b]

			<90	95%	
GI	Schrock et al (2018)[297]	25	<30	95%	86%
Multiple tumor types	Clark et al (2018)[294]	36	≤60	83%	75%
Breast / lung / ovarian	Zhou et al (2018)[298]	51	not defined	-	85%[c]

[a] Described as 75%; calculation not provided.

[b] Not evaluated for these time periods.

[c] For guideline-recommended molecular targets (except for ERBB2 amplification).

CRC, colorectal cancer; GI, gastrointestinal; NSCLC, non-small cell lung cancer; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha phosphatidylinositol-4,5-bisphosphate 3-kinase.

Clinical Validity of Previous Versions of Foundation Medicine Liquid Assay

The clinical validity study results associated with FoundationACT are presented in and Table 6-14.

Table 6-14. Clinical Validity of FoundationACT in Multiple Tumor Types

Reference	Study design	Frequent genomic alterations identified	Additional results and conclusions
Pan-tumor			
Clark et al. (2018)[294] (Publication)	Validation study of FoundationACT; routine clinical cases with **advanced cancer** (N=860) obtained by FoundationACT	≥1 genomic alteration detected by ctDNA: 70.6% ▪ *TP53* ▪ *KRAS* ▪ *EGFR* ▪ *PIK3CA* ▪ Kinase rearrangements: 22/869 (2.6%)	Median mutant allele frequency of 1.3% detected in 31.6% of patients at low allele frequencies (<0.5%) Novel gene fusion partners detected by ctDNA: *ALK* (*PLEKHA7-ALK*) and *FGFR2* (*FGFR2-NOL1*) The observed frequency of genomic alterations in samples sequenced using FoundationACT and tissue samples from an internal database using FoundationOne were highly correlated, r=0.98 (*P* < .0001)
Zhou et al. (2018)[298] (Publication)	Retrospective study of patients with advanced **solid tumors** (N=81) who underwent ctDNA analysis with FoundationACT and tissue-based CGP with FoundationOne to detect genomic alterations	≥1 mutation detected by ctDNA: 69 (85%)	**Number of professional guideline-recommended molecular targets detected by both assays vs those unique to FoundationACT** NSCLC (n=33): ▪ *EGFR* L858R and exon deletions: 8 vs 0 ▪ *EGFR* T790M: 2 vs 0 ▪ *ALK* rearrangements: 4 vs 0 ▪ *BRAF* V600 mutation: 1 vs 0 ▪ *MET* exon 14 skip site alterations: 2 vs 0 ▪ *ERBB2* mutations: 0 vs 2 Breast (n=7): ▪ *ERBB2* amplification or mutation: 1 vs 1 ▪ *BRCA 1/2* mutation: 2 vs 4 Ovarian (n=2): ▪ *BRCA 1/2* mutation: 2 vs 0
Creelan et al. (2018)[299]	Blood samples from patients with **solid tumors** (N=6,571) underwent ctDNA analysis with FoundationACT, and	Kinase rearrangements in, n/N (%): ▪ Lung cancer: 134/2,709 (4.9) ▪ Non-lung cancer: 93/3,862 (2.4)	**In the non-lung cancer group (n=3,862), kinase rearrangements were detected in, n/N (%):** ▪ Bladder: 3/57 (5.3) ▪ Liver: 5/120 (4.2)

Reference	Study design	Frequent genomic alterations identified	Additional results and conclusions
(Conference abstract)	kinase fusions and rearrangements were evaluated followed by an analysis with a tissue database	Common rearranged kinases: ▪ *ALK*: 45% ▪ *RET*: 15% ▪ *ROS1*: 15% ▪ *FGFR3*: 8% ▪ *FGFR2*: 5% ▪ *EGFR*: 4%	▪ CRC: 18/500 (3.6) ▪ Esophagus: 2/56 (3.6) ▪ Unknown primary: 19/618 (3.1) ▪ Pancreas: 9/332 (2.7) ▪ Prostate: 8/390 (2.1) ▪ Breast: 16/933 (1.7) ▪ Ovarian: 1/181 (0.6) Correlation of kinase rearrangements between temporally matched (≥60 days apart) blood and tissue sample: 46% **Comparison with >70,000 tissue genomic profiles in FoundationCORE™ analyzed frequency of cases with *ALK, RET,* or *ROS* kinase rearrangements over the same time period:** ▪ Lung ctDNA vs tissue: 4.3% vs 4.9% ▪ Non-lung ctDNA vs tissue: 1.3% vs 0.4% (*P*<0.001)
NSCLC			
Schrock et al. (2018)[75] (Publication)	Retrospective study of patients with advanced **NSCLC** (N=1,552) who underwent ctDNA analysis with FoundationACT, followed by an analysis with tissue databases	≥ 1 mutation detected by ctDNA: 1,075 (86)% Detected by ctDNA: ▪ *TP53*: 59% ▪ *EGFR*: 25% ▪ *KRAS*: 17% ▪ *NF1*: 7.1% ▪ *PIK3CA:* 4.3% Kinase fusions (*ALK, ROS1, RET, FGFR3, PDGFRA, PDGFRB*): 5%	Evidence of ctDNA in the blood (MSAF >0): 80% **New mechanisms of acquired resistance detected in patients tested with plasma-based FoundationACT:** ▪ *MET* Y1230C and *EGFR* amplification after treatment with crizotinib ▪ *FGFR3-TACC3* fusion after treatment with *EGFR* inhibitor ▪ Multiple *EGFR*-acquired resistance mutations after treatment with osimertinib **Comparative analysis between ctDNA results and FoundationCORE and TCGA tissue databases (N=21,500):** ▪ *TP53:* 58.3% vs 62% (*P*>0.05) ▪ *NFI:* 6.84% vs 5.87% (*P*>0.05) ▪ *PIK3CA:* 4.02% vs 6.01% (*P*>0.05) ▪ *EGFR:* 23.8% vs 14.8% (*P*<0.0001) ▪ *KRAS:* 16.3% vs 29% (*P*<0.0001) ▪ Gene amplifications were less common in ctDNA

Reference	Study design	Frequent genomic alterations identified	Additional results and conclusions
Schrock et al. (2018)[300] (Publication)	Retrospective case study of patients with *EGFR*-mutated **NSCLC** (N=3,505) who underwent ctDNA analysis with FoundationACT and tissue-based CGP with FoundationOne	*EGFR*+ samples (n=31) with concurrent kinase fusions detected by ctDNA: ▪ *BRAF*: 10 (32%) ▪ *ALK*: 7 (23%) ▪ *RET*: 6 (19%) ▪ *FGFR3*: 6 (19%) ▪ *EGFR*: 1 (3.2%) ▪ *NTRK1*: 1 (3.2%)	12 patients with *EGFR* alterations underwent TKI treatment followed by repeat CGP, which identified an *ALK* fusion that was not detected in the pretreatment period ▪ 5 of these patients underwent initial tissue CGP followed by ctDNA analysis after treatment ▪ 2 patients underwent ctDNA analysis both before and after treatment
Breast cancer			
Chung et al. (2017)[295] (Publication)	Retrospective study of patients with ER+ **breast** cancer (N=254) who had genomic profiling with FoundationACT	≥ 1 reportable alteration detected by ctDNA: 78% ▪ *TP53*: 38% ▪ *ESR1*: 31% ▪ *PIK3CA*: 31% ▪ *CDH1:* 10% ▪ *ERBB2:* 8% Concurrent alterations with *ESR1:* ▪ *PIK3CA*: 35% ▪ *FGFR1*: 16% ▪ *ERBB2*: 8% ▪ *BRCA1/2*: 5% ▪ *AKT1*: 4%	Evidence of ctDNA (MSAF >0): 84% Matched ctDNA and tissue samples were available for 14 patients ▪ 89% of short-variant mutations detected in tissue were also detected in ctDNA; additional *ESRI, TP53, PIK3CA* alterations were identified in ctDNA only ▪ Multiple concurrent *ESR1* genomic alterations were observed in 40% of *ESR1*-altered cases ▪ 27% of amplifications detected in tissue were also detected in ctDNA
Sokol et al. (2018)[301] (Publication)	Prospective cohort study of patients with **invasive lobular and invasive ductal breast cancer** whose tissue (N=336 and 485) and metastatic biopsy specimens (N=180 and 191) were assayed with FoundationOne and liquid ctDNA with FoundationOne Liquid	Most common genomic alterations in metastatic invasive lobular carcinoma: ▪ *CDH1*: 77% ▪ *PIK3CA*: 53% ▪ *TP53*: 24% ▪ Co-amplified 11q13 locus genes *CCND1*: 22% ▪ *FGF19*: 21% ▪ *FGF4*: 19% ▪ *FGF3*: 19% ▪ *ESR1*: 17%	*NF1* alterations are predominantly under loss of heterozygosity (11/14, 79%), are mutually exclusive with *ESR1* mutations (OR: 0.24, $P < .027$), and are frequently polyclonal in ctDNA assays ▪ *NF1* alterations harbored: 33/569 ▪ Strong polyclonality as designated by ≥3 *NF1* alterations: 5/33 (15%) *NF1* alterations arise in the setting of acquired resistance: 3/3 of *NF1* altered samples profiled by FoundationOne Liquid had a co-occurring *CDH1* and *AKT* pathway alteration

Reference	Study design	Frequent genomic alterations identified	Additional results and conclusions
Gornstein et al. (2018)[302] (Publication)	Retrospective case report of a patient with ER+, progesterone receptor-negative, HER2-negative, **metastatic breast cancer** who underwent tissue CGP with FoundationOne and ctDNA analysis with FoundationACT	*ESR1* *BRCA2*	**Case study:** ▪ 55-year-old female with breast cancer underwent tissue CGP, which identified co-occurring *ESR1* and *BRCA2* mutations. The *BRCA2* mutation was targeted with olaparib as 8th-line treatment, which led to a positive response and long-term benefit after progression on 7 prior lines of therapy. At disease progression on olaparib, a *BRCA2* reversion mutation was detected in both tissue and liquid biopsies, providing a molecular explanation for olaparib resistance.
Colon cancer			
Gregg et al. (2018)[303] (Conference abstract)	Substudy of a multicenter prospective study validating FoundationACT in multiple solid tumors; substudy includes patients with **colon cancer** (N=98) who underwent ctDNA analysis with FoundationACT and tissue-based CGP with FoundationOne	Detected by ctDNA[b] ▪ *TP53* ▪ *BRAF* ▪ *KRAS* ▪ *EGFR* ▪ *PIK3CA*	Frequency of genomic alterations detected in ctDNA per gene in the main study was comparable to that of alterations detected through clinical FoundationACT testing and tissue-based testing of samples from the TCGA database
Other tumor types			
Schrock et al. (2018)[297] (Publication)	Prospective study of patients with **advanced gastrointestinal and anus cancers** (N=417) who underwent ctDNA analysis; a subset of patients (N=25) underwent ctDNA analysis with FoundationACT and tissue-based CGP with FoundationOne	≥ 1 reportable alteration detected by ctDNA: 89% ▪ *TP53*: 72% ▪ *KRAS*: 35% ▪ *PIK3CA*: 14% ▪ *BRAF*: 8% ▪ *EGFR:* 7%	Evidence of ctDNA in the blood (MSAF >0): 344 (82%) **Matched ctDNA and tissue samples were available for 25 patients:** ▪ Of 57 alterations detected in tissue samples, 49 (85%) were also detected in ctDNA ▪ 95% of short-variant mutations, 50% of amplifications, and 1/1 rearrangements detected in tissue were also detected in ctDNA ▪ 63% of alterations detected in ctDNA were also detected in tissue

Reference	Study design	Frequent genomic alterations identified	Additional results and conclusions
			Comparative analysis between ctDNA results and FoundationCORE (N=15,948) and TCGA (N=212) tissue databases: ▪ *TP53:* 72.1% vs 73.6% ▪ *KRAS:* 34.0% vs 41.5% ▪ *PIK3CA:* 14.0% vs 15.5% ▪ *BRAF:* 8.1% vs 6.7%
Pal et al. (2018)[304] (Publication)	Retrospective case series in patients with metastatic **papillary renal cell carcinoma** (N=3) with *EML4-ALK* fusions who underwent ctDNA analysis and were treated with alectinib	*EML4-ALK* fusions: 100%	**Case study:** ▪ ctDNA with FoundationACT successfully identified *EML4-ALK* fusion and alterations in *BRCA2* and *TERT-promoter* in a 66-year-old male. Patient started treatment with alectinib and has experienced SD for 9 months.
Wang et al. (2017)[305] (Publication)	Retrospective case study of a patient with **metastatic atypical neuroendocrine** tumor who received ctDNA analysis with FoundationACT when tissue biopsy was unsuccessful	*ALK* translocation	**Case study:** ▪ 52-year-old never-smoking male with neuroendocrine tumor of the atypical carcinoid subtype assuming lung origin could not receive molecular genotyping due to insufficient material. Patient received systemic therapy with temozolomide and capecitabine; restaging scans showed SD but worsening brain metastases. ctDNA with FoundationACT revealed an *ALK* translocation.
Lara et al. (2017)[219] (Conference abstract)	Retrospective cohort study of patients with advanced **prostate cancer** (N=207) who underwent ctDNA analysis with FoundationACT and tissue-based CGP with FoundationOne	Total *BRCA1/2* alterations detected by ctDNA: 15 (7.2%) ▪ *BRCA1*: 4 ▪ *BRCA2*: 12	ctDNA analysis identified an additional 17 cases (8.2%) harboring *BRCA1/2* alterations (categorized as VUS) 118/936 tissue tumor specimens (12.6%) harbored *BRCA1* (n=11) or *BRCA2* (n=107) alterations ctDNA and tissue profiling successfully identified actionable *BRCA1/2* alterations in up to 15% of men with prostate cancer

Reference	Study design	Frequent genomic alterations identified	Additional results and conclusions
McGregor et al. (2018)[304] (Conference abstract)	Retrospective study of patients with metastatic **urothelial carcinoma** (N=80) whose genomic alterations were identified by ctDNA analysis with FoundationACT and then were compared to the Foundation Medicine database of CGP (ctDNA or tissue) in patients with advanced urothelial carcinoma (N=2035)	≥1 mutation detected by ctDNA: 59/80 (74%) ▪ *TP53*: 64% ▪ *TERT-promoter*: 39% ▪ *FGFR3*: 16% ▪ *PIK3CA*: 12% ▪ *KRAS*: 12% ▪ *ERBB2*: 6% ▪ *RAS/RAF/MEK* pathway mutations: 22%	Evidence of ctDNA in the blood (MSAF >0): 67 (84%) Median estimated ctDNA fraction in the blood: 1.9% (IQR: 0.4–5.4) **Genomic alterations identified in ctDNA by FoundationACT assay (n=67) vs independent genomic datasets (ctDNA or tissue; n=80) were consistent:** ▪ *TP53*: 64.2% vs 53.8% ▪ *TERT-promoter*: 38.8% vs 32.5% ▪ *FGFR3*: 16.4% vs 13.8% ▪ *PIK3CA*: 12% vs 10% ▪ *KRAS*: 11.9% vs 10% ▪ *ERBB2*: 6% vs 5% Observed frequencies were consistent with published genomic datasets in both ctDNA and tissue
Bahary et al. (2017)[306] (Conference abstract)	Retrospective cohort study of patients with **pancreatic ductal adenocarcinoma** (N=78) who underwent CGP on samples collected during the course of clinical care with ctDNA (FoundationACT) and tissue-based sequencing	≥1 mutation detected by ctDNA: 77% ≥1 mutation detected by ctDNA when prior tissue testing failed sample preparation: 18/30 (60%)	Other clinically relevant alterations detected by FoundationACT: *ALK*, *BRCA1/2*, *NF1*, *PIK3CA*, *PTEN* Significant differences were demonstrated between the established driver oncogenic alterations for pancreatic ductal adenocarcinoma using ctDNA and tissue-based CGP

[a] Results do not specify which assay (ctDNA or tissue-based) was used to identify genomic alterations.

[b] Not a complete list of all genomic alterations identified

ALK, anaplastic lymphoma kinase; AKT, protein kinase B; BRCA, breast cancer susceptibility gene; CDH1, cadherin-1; CGP, comprehensive genomic profiling; ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; ER, estrogen receptor; ERBB2, erythroblastic oncogene B; FGFR, fibroblast growth factor receptor; IQR, interquartile range; KRAS, Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MSAF, maximum somatic allele frequency; NF1, neurofibromin 1; NSCLC, non-small cell lung cancer; NTRK, neurotrophic receptor tyrosine kinase; OR, odds ratio; PCR, polymerase chain reaction; PDGFRA, platelet-derived growth factor receptor A; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha phosphatidylinositol-4,5-bisphosphate 3-kinase; PTEN, phosphatase and tensin homolog; SD, stable disease; TACC3, transforming acidic coiled-coil-containing protein 3; TCGA, The Tumor Cancer Genome Atlas; TERT, telomerase reverse transcriptase; TP53, tumor protein P53; VUS, variants of unknown significance.

Clinical Utility of Previous Versions of Foundation Medicine Liquid Assay

The clinical utility study results associated with FoundationACT are presented in Table 6-15.

Table 6-15. Clinical Utility of FoundationACT in Multiple Tumor Types

Reference	Study design	Frequent genomic alterations identified	Clinical outcomes
Pan-tumor			
Zhou et al. (2018)[298] (Publication)	Retrospective study of patients with advanced **solid tumors** (N=81) who underwent ctDNA analysis with FoundationACT to detect genomic alterations	≥ 1 mutation detected by ctDNA: 69 (85%)	FoundationACT detected 8 unique and 22 concordant genomic alterations ctDNA genomic data resulted in a change in clinical care with clinical benefit in 7/8 (88%) patients with unique genomic alterations
NSCLC			
Schrock et al. (2018)[300] (Publication)	Retrospective case study of patients with *EGFR*-mutated **NSCLC** (N=3,505) who underwent ctDNA analysis with FoundationACT and tissue-based CGP with FoundationOne	Concurrent kinase fusions detected by ctDNA: ▪ *BRAF*: 10 (32%) ▪ *ALK*: 7 (23%) ▪ *RET*: 6 (19%) ▪ *FGFR3*: 6 (19%) ▪ *EGFR*: 1 (3.2%) ▪ *NTRK1*: 1 (3.2%)	**Case study:** ▪ A 70-year-old female who never smoked with an *EGFR* L858R mutation received erlotinib and achieved PR for 12 months ▪ Upon progression while receiving afatinib for 2 months and osimertinib for 10 months, blood-based ctDNA testing showed the original *EGFR* L858 mutation, lack of T790, and a new *PLEKHA7-ALK* fusion ▪ Alectinib was added to full-dose osimertinib; patient had PR and duration of response of 6 months
Schrock et al. (2018)[75] (Publication)	Retrospective study of patients with advanced **NSCLC** (N=1,552) who underwent ctDNA analysis with FoundationACT	≥ 1 mutation detected by ctDNA: 1,075 (86%)	FoundationACT detected a professional guidelines-recommended genomic alteration in 398 (32%)
Young et al. (2017)[307] (Publication)	Prospective study of patients with **NSCLC** (N=269) who underwent ctDNA analysis with FoundationACT when, for	Kinase fusions: 20 (7.4%) ▪ ALK fusions: 13 (4.5%) ▪ *EML4*: 9	6 patients with actionable kinase fusion detected by ctDNA had insufficient tissue for CGP

Reference	Study design	Frequent genomic alterations identified	Clinical outcomes
	the majority, CGP of biopsied tissue could not be performed	▪ *EML4-PPFIBP1*: 1 ▪ *EML4-CACNB4*: 1 ▪ Unidentified partners: 2 ▪ *KIF5B-RET*: 3 (1%) ▪ *CD74-ROS1*: 3 (1%) ▪ *FGFR3-TACC3*: 1 (<1%)	**Case studies:** ▪ *Patient 1*: *EML4-ALK* fusion was detected in both ctDNA and tissue CGP, 6 days apart ▪ *Patient 2*: *EGFR* L858R + *EGFR* L709K alterations were identified by tissue CGP and patient experienced durable response to afatinib/cetuximab; after progression, ctDNA identified possible acquired resistance mechanism *FGFR-TACC3* and *EGFR* L858R ▪ *Patient 3*: ctDNA confirmed tissue CGP detected *CD74-ROS1* fusion and was subsequently switched to crizotinib; patient had a major radiographic response by the second cycle of crizotinib treatment
Dagogo-Jack et al. (2017)[308] (Publication)	Retrospective case study of a patient with *MET*-amplified **lung adenocarcinoma** who underwent ctDNA analysis with FoundationACT and tissue-based CGP with FoundationOne	*MET* amplification *CDK6* amplification Acquired *EGFR* amplification	**Case study:** ▪ *MET* and *CDK6* amplification was detected using tissue CGP in a 70-year-old female who was a former smoker ▪ After 3 weeks of treatment with crizotinib, patient improved; restaging scans demonstrated marked pulmonary improvement and resolution of mediastinal lymphadenopathy ▪ After 6 months of treatment, patient developed new chest wall pain and crizotinib treatment was discontinued ▪ Repeat tissue biopsy was not feasible ▪ ctDNA analysis revealed persistent *MET* and *CDK6* amplification and acquired *EGFR* amplification ▪ Patient declined further therapy and passed away 1 month later
Ou et al. (2017)[309] (Publication)	Retrospective case study of a patient with stage IV metastatic **NSCLC** who underwent pretreatment tissue CGP with FoundationOne and ctDNA analysis with FoundationACT	*EGFR* L858R T790M	**Case study:** ▪ A 69-year-old female who was a former light smoker with known *EGFR* L858R received erlotinib for 8 months with PR

Reference	Study design	Frequent genomic alterations identified	Clinical outcomes
	during the course of clinical care		▪ At progression, rebiopsy and tissue CGP revealed *EGFR* L858R (MAF: 53.75%) and T790M (MAF: 40.87%) mutations ▪ Osimertinib treatment was initiated with disease shrinkage by 2 months and tumor regrowth by 5 months ▪ ctDNA assay confirmed presence of previously detected *EGFR* L858R (MAF: 17.9%) and T790M (MAF: 18.2%) mutations and also detected solvent front mutations of G796S/R (MAF: 14.4%), hinge pocket mutations of L792F/H (MAF: 0.36%), a minor mutation of unknown significance at V802F (MAF: 0.40%), and mutations at C797S/G (MAF: 2.26%)
Ou et al. (2016)[310] (Publication)	Retrospective case study of a patient with stage IV metastatic lung **adenocarcinoma** who underwent pretreatment tissue CGP as part of phase 2 clinical trial with ctDNA analysis using FoundationACT undertaken at progression	*MET* exon14 skipping alteration	**Case study:** ▪ *MET* exon14 alteration was detected using tissue CGP in a 67-year-old Asian female who had never smoked ▪ Patient achieved a confirmed PR after 2 months of treatment with crizotinib during the trial, maintained PR for nearly 13 months, then developed metastasis ▪ ctDNA assay confirmed presence of previously detected primary *MET* exon14 (D1010H) alteration at 10.9% MAF and also detected the *MET* Y1230C resistance mutation at MAF of 3.5% ▪ Patient declined treatment with alternate MET TKI
CRC			
Lai et al. (2017)[311] (Publication)	Case study following a patient with **CRC** who underwent conventional IHC staining followed by parallel-tissue CGP with FoundationOne and	*STRN-ALK* fusion	**Case study:** ▪ Initial *ALK* IHC staining was negative, but parallel-genomic profiling of both ctDNA and tissue identified an identical *STRN-ALK* fusion ▪ Subsequent *ALK* IHC staining of the same specimen was positive (false-negative)

Reference	Study design	Frequent genomic alterations identified	Clinical outcomes
	ctDNA analysis with FoundationACT		▪ Following CGP, patient received bevacizumab-based regimen and had significant pain relief and normalization of bowels after 3 months ▪ Following surgery, and chemotherapy, patient had no evidence of disease; upon progression, patient may enroll in a clinical trial of an *ALK* inhibitor
Ovarian cancer			
Mayor et al. (2017)[312] (Publication)	Retrospective case study of a patient with **ovarian carcinoma** who underwent pretreatment tissue CGP with FoundationOne and ctDNA analysis with FoundationACT during the course of clinical care	*BRCA1* *TP53*	**Case study:** ▪ *BRCA1* mutation was detected using germline genetic testing during chemotherapy treatment in a 58-year-old female ▪ Patient initiated olaparib and experienced PR; treatment discontinued after 6 months ▪ CGP was performed on the tumor resection sample; assay revealed *BRCA1* and *TP53* mutations; patient started cyclophosphamide and bevacizumab after disease progression ▪ ctDNA assay revealed the previously identified *TP53* and *BRCA1* alteration as well as a second *BRCA1* alteration ▪ Patient continued to develop recurrent ascites and pleural effusions and elected to proceed with comfort measures and passed away
Breast cancer			
Wongchenko et al. (2017)[47] (Conference abstract)	Phase 2, prospective LOTUS trial of patients with metastatic triple-negative **breast cancer** (N=88)[233] who underwent pretreatment tissue CGP with FoundationOne and cfDNA analysis with FoundationACT	≥1 mutation detected by cfDNA: 81 (92%) ▪ *PIK3CA*: 84% ▪ *PIK3CA*: 18% ▪ *PTEN*: 10% ▪ *BRCA1*: 9% ▪ *NF1*: 8% ▪ *AKT1*: 7%	**High- vs low-variant allele fraction associated with:** ▪ Shorter PFS: ▪ First-line ipatasertib + paclitaxel (HR, 2.39; 95% CI: 1.04–5.37) ▪ Placebo + paclitaxel (HR, 2.68; 95% CI: 1.31–5.83) **Detectable vs non-detectable *PIK3CA/AKT1* mutation associated with:**

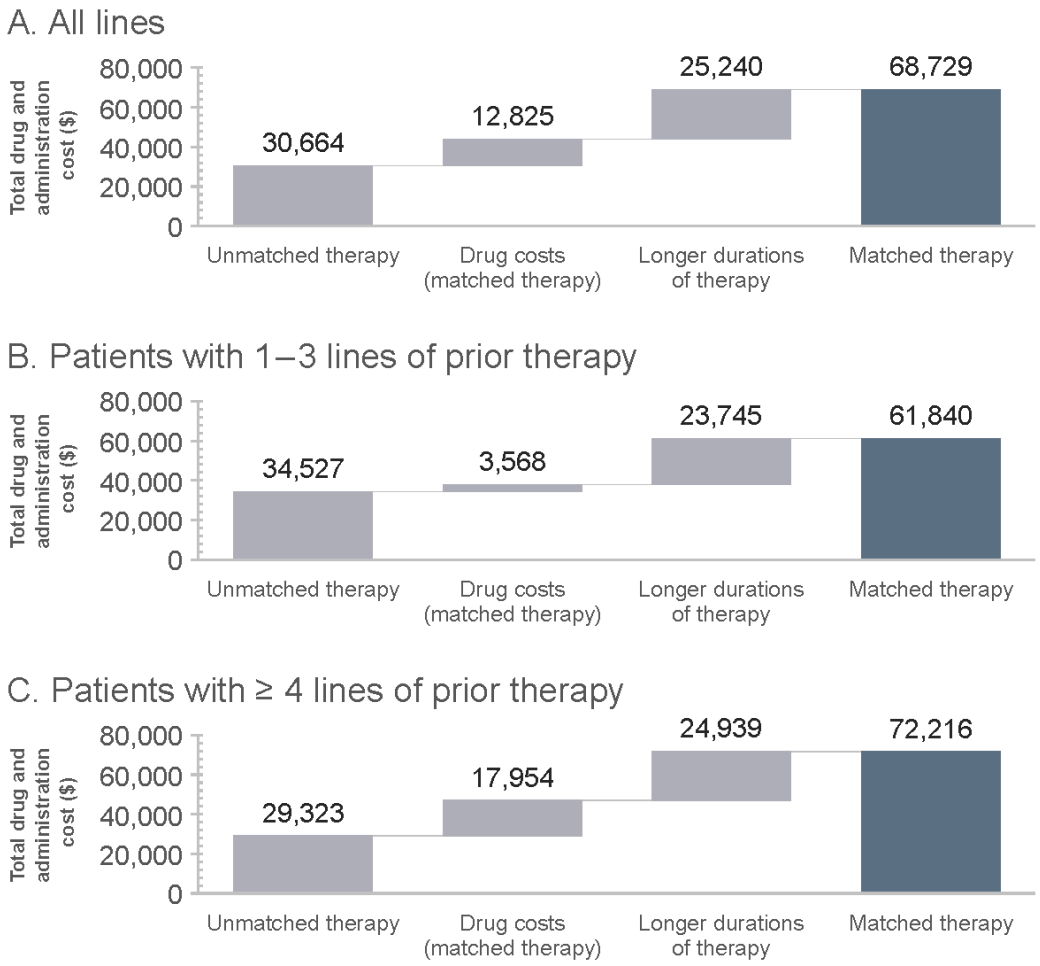
Reference	Study design	Frequent genomic alterations identified	Clinical outcomes
			▪ Improved PFS: ▪ First-line ipatasertib + paclitaxel (HR, 0.15; 95% CI: 0.02–0.62) ▪ Placebo + paclitaxel (HR, 0.82; 95% CI: 0.45–1.44) ▪ 100% concordance with tumor for variants of interest (*PIK3CA*, *AKT1*) ctDNA successfully selected patients who improved when administered first-line ipatasertib + paclitaxel
Other tumor types			
McGregor et al. (2018)[304] (Conference abstract)	Retrospective study of patients with metastatic **urothelial carcinoma** (N=80) whose genomic alterations were identified by ctDNA analysis with FoundationACT and then were compared to the Foundation Medicine database of CGP (ctDNA or tissue) in patients with advanced urothelial carcinoma (N=2,035)	≥ 1 mutation detected by ctDNA: 59/80 (74%) ▪ *TP53*: 64% ▪ *TERT-promoter*: 39% ▪ *FGFR3*: 16% ▪ *PIK3CA*: 12% ▪ *KRAS*: 12% ▪ *ERBB2*: 6% ▪ *RAS/RAF/MEK* pathway mutations: 22%	**Case studies:** ▪ *Patient 1*: *FGFR3* genomic alteration in baseline tumor tissue was no longer detectable and new *TP53* alteration was detected from ctDNA after targeted treatment with FGFR3 inhibitor ▪ *Patient 2*: *ERBB2* and *TP53* genomic alterations in baseline tumor tissue, ctDNA at time of resistance to cisplatin-based therapy showed persistence of *ERBB2* and *TP53* alterations and new *NF1* alteration

[a] Number of alterations detected by several assays: FoundationOne (n=8), FoundationACT (n=3), Guardant360 (n=1)

ALK, anaplastic lymphoma kinase; AKT, protein kinase B; BRCA, breast cancer susceptibility gene; CDK6, cyclin-dependent kinase 6; CGP, comprehensive genomic profiling; CI, confidence interval; CRC, colorectal cancer; ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; ERBB2, erythroblastic oncogene B; FGFR, fibroblast growth factor receptor; HR, hazard ratio; IHC, immunohistochemistry; KRAS, Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MAF, mutant allele frequency; MET, mesenchymal epithelial transition factor; NF1, neurofibromin 1; NSCLC, non-small cell lung cancer; PFS, progression-free survival; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha phosphatidylinositol-4,5-bisphosphate 3-kinase; PR, partial response; PTEN, phosphatase and tensin homolog; TACC3, transforming acidic coiled-coil-containing protein 3; TERT, telomerase reverse transcriptase; TKI, tyrosine kinase inhibitor; TP53, tumor protein P53.

Economic Study Supporting Information

Figure 6-2. Comparison of Total Drug Treatment Costs Between Matched and Unmatched Therapy in Patients Who Received Comprehensive Genomic Profiling



Note: Comparison of total drug treatment costs between matched and unmatched therapy in patients with all lines of therapy (top), patients with 1–3 lines of prior therapy (middle), and patients with ≥4 lines of prior therapy (bottom).

Source: Adapted from Chawla et al. (2018).[113]

Table 6-16. Healthcare-Associated Cost Outcomes With Precision Medicine-Based Treatment Compared With Standard Chemotherapy or BSC

	Precision medicine group (n=22)	Historical controls (n=22)	*P*-value
Total costs per patient	91,790 (85,070)	40,782 (42,267)	0.002
Total drug costs per patient	59,259 (51,425)	20,189 (34,299)	<0.001
Cost per patient per PFS week	4,665 (3,041)	5,000 (6,509)	0.126

Note: Data are presented as mean (standard deviation) in United States dollars unless otherwise stated.

Abbreviation: BSC, best supportive care; PFS, progression-free survival.

Source: Haslem et al. (2017).[9]

Table 6-17. Utilization of Molecular Testing and Healthcare Costs by Cancer-Specific Cohort (Metastatic Cancer)

	Breast (n=3,414)	NSCLC (n=2,231)	CRC (n=1,611)	Head and neck (n=511)	Ovarian (n=275)	Uterine (n=151)
Utilization, %						
Biopsy procedures	23.2	31.1	30.9	33.3	73.1	6.6
Molecular diagnostic tests	52.4	41.9	37.4	34.1	40.7	42.4
Targeted therapy						
Genomically matched	11	9	6	0	0	0
Endocrine	60	0	0	0	14	16
Unmatched	3	7	21	5	9	1
Cytotoxic chemotherapy	41	39	52	26	47	21
Mean (standard deviation) PPPM costs, $						
Biopsy procedures	14 (180)	37 (531)	12 (142)	16 (80)	42 (84)	3 (31)
Molecular diagnostic tests	40 (127)	35 (132)	29 (155)	7 (24)	106 (780)	32 (135)
Targeted therapy						
Genomically matched	349 (1,464)	255 (1,152)	164 (1,005)	NA	NA	NA
Endocrine	60 (238)	NA	NA	NA	4 (31)	3 (14)
Unmatched	84 (697)	240 (1,420)	545 (1,685)	77 (690)	176 (845)	NA
Cytotoxic chemotherapy	293 (823)	425 (1,528)	701 (1,709)	45 (264)	300 (881)	61 (299)
Total medical costs, excluding anticancer drugs	6,667 (11,011)	8,405 (16,642)	8,521 (14,503)	6,618 (9,969)	9,940 (15,043)	7,823 (15,176)
Hospice/palliative care	17 (152)	114 (605)	61 (351)	49 (403)	80 (412)	35 (304)
Emergency department visits	93 (359)	244 (715)	161 (472)	91 (288)	223 (559)	153 (375)
Hospitalizations	1,484 (8,181)	3,582 (12,965)	3,842 (11,847)	1,894 (5,407)	5,306 (13,243)	3,207 (12,930)
Outpatient visits	4,946 (6,642)	4,322 (7,831)	4,172 (6,991)	4,452 (7,113)	3,990 (5,166)	4,258 (7,087)
Other visits	128 (375)	142 (614)	284 (1,322)	132 (430)	341 (1,356)	170 (830)

Note: Utilization is the proportion of patients with any use. Costs are in 2015 USD.

CRC, colorectal cancer; NA, not applicable; NSCLC, non-small cell lung cancer; PPPM, per-patient-per-month; USD, United States dollars.

Source: Chawla et al. (2018).[113]

Table 6-18. Incremental Overall Survival and Budget Impact With Increase in Comprehensive Genomic Profiling

	Base case (CGP testing rate, 2%)	Increase in CGP (CGP testing rate, 10%)	Difference
Testing patterns, n			
Patients with advanced NSCLC	532	532	NA
Patients undergoing molecular diagnostic testing	266	266	NA
Patients undergoing CGP	5	27	21
Costs per patient, $			
Total cost, with any molecular diagnostic testing	106,119	107,720	1,600
Diagnostic testing	1,026	1,415	390
Biopsy	411	396	−15
Medical	59,377	59,963	585
Drug	45,305	45,946	640
Matched	10,679	11,914	1,235
Nonmatched	34,626	34,031	−595
Base case analysis			
Budget impact, $PMPM			0.02
Total life-years	188.5	190.4	1.9
NNT			12

Note: All cost values are reported as United States dollars.

CGP, comprehensive genomic profiling; NA, not applicable; NNT, number needed to test; NSCLC, non-small cell lung cancer; PMPM, per-member per-month.

Source: Signorovitch et al. (2018).[114]

Sensitivity analyses found that the model was most sensitive to the line of therapy at which CGP was used, the CGP test's share of genetic testing, and the proportion of patients using CGP. Adjusting the line of therapy to 1 for all patients increased the budget impact to slightly more than $0.03 PMPM and reduced the NNT to 5.[114]

Table 6-19. Potential Drug Costs Diverted by 20 Patients Who Enrolled in Clinical Trials After CGP

Cancer type	Patient	Per-patient monthly cost of alternate treatment regimen, $	Estimated potential cost diversion per patient,[a] $
Appendiceal adenocarcinoma	1	2,200	7,100
	2	11,800	38,100
Breast invasive ductal carcinoma	3	6,300	20,300
Colon adenocarcinoma	4	10,400	33,600
	5	11,500	37,100
	6	10,400	33,600
Duodenal adenocarcinoma	7	11,500	37,100
Lung adenocarcinoma	8	8,800	28,400
	9	11,400	36,800
Lung adenoid cystic carcinoma	10	7,100	22,900
Lung small cell carcinoma	11	9,600	31,000
	12	100	300
Ovarian adenocarcinoma	13	7,900	25,500
Pancreatic adenocarcinoma	14	2,200	7,100
Rectum adenocarcinoma	15	11,500	37,100
	16	10,400	33,600
Retroperitoneal leiomyosarcoma	17	4,100	13,200
Small bowel adenocarcinoma	18	11,500	37,100
Stomach adenocarcinoma	19	2,600	8,400
Undifferentiated pleomorphic sarcoma	20	5,000	16,200
Total			**504,500**

Note: All cost values are reported as United States dollars and were rounded to the nearest $100.

[a] The monthly cost of each patient's alternate regimen was calculated based on the average sales price plus 6 percent; costs were then multiplied by a PFS duration of 3.23 months, which was the mean PFS reported for patients enrolled in phase 1 clinical trials in a meta-analysis of 346 studies by Schwaederle et al. and then rounded to the nearest $100.[10]

CGP, comprehensive genomic profiling; PFS, progression-free survival.

Source: Reitsma et al. (2018).[61]

References

1. Foundation Medicine Inc. FoundationOne Liquid CDx™ Technical Information. 2022; January 27, 2022. Available at: www.f1lcdxlabel.com.
2. *Kantar Health. CancerMPact Epidemiology, US.* January 26, 2021 2021.
3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. *CA Cancer J Clin.* 2021;71(1):7-33. https://pubmed.ncbi.nlm.nih.gov/33433946/.
4. Wong CI, Zerillo JA, Stuver SO, Siegel J, Jacobson JO, McNiff KK. Adverse events as a cause of unscheduled hospitalizations for solid tumor oncology patients receiving chemotherapy. *Journal of Clinical Oncology.* 2018;36(15_suppl):e18815-e18815. https://ascopubs.org/doi/abs/10.1200/JCO.2018.36.15_suppl.e18815.
5. White Al-Habeeb N, Kulasingam V, Diamandis EP, et al. The Use of Targeted Therapies for Precision Medicine in Oncology. *Clinical chemistry.* 2016;62(12):1556-1564. https://pubmed.ncbi.nlm.nih.gov/27679436/.
6. Hassett MJ, O'Malley AJ, Pakes JR, Newhouse JP, Earle CC. Frequency and cost of chemotherapy-related serious adverse effects in a population sample of women with breast cancer. *Journal of the National Cancer Institute.* 2006;98(16):1108-1117. https://pubmed.ncbi.nlm.nih.gov/16912263/.
7. Ashdown ML, Robinson AP, Yatomi-Clarke SL, et al. Chemotherapy for late-stage cancer patients: Meta-analysis of complete response rates. *F1000Research.* 2015;4:232. https://pubmed.ncbi.nlm.nih.gov/26834979/.
8. Massard C, Michiels S, Ferte C, et al. High-Throughput Genomics and Clinical Outcome in Hard-to-Treat Advanced Cancers: Results of the MOSCATO 01 Trial. *Cancer Discov.* 2017;7(6):586-595. https://pubmed.ncbi.nlm.nih.gov/28365644/.
9. Haslem DS, Van Norman SB, Fulde G, et al. A retrospective analysis of precision medicine outcomes in patients with advanced cancer reveals improved progression-free survival without increased health care costs. *Journal of oncology practice.* 2017;13(2):e108-e119. https://pubmed.ncbi.nlm.nih.gov/27601506/.
10. Schwaederle M, Zhao M, Lee JJ, et al. Association of biomarker-based treatment strategies with response rates and progression-free survival in refractory malignant neoplasms: A meta-analysis. *JAMA oncology.* 2016;2(11):1452-1459. https://pubmed.ncbi.nlm.nih.gov/27273579/.
11. Kopetz S, Shaw KRM, Lee JJ, et al. Use of a Targeted Exome Next-Generation Sequencing Panel Offers Therapeutic Opportunity and Clinical Benefit in a Subset of Patients With Advanced Cancers. *JCO Precision Oncology.* 2019(3):1-14. https://ascopubs.org/doi/abs/10.1200/PO.18.00213.
12. Washington State Health Care Authority. Scott Ramsey Summit Presentation. 2020; April 10, 2020. Available at: https://www.hca.wa.gov/assets/program/scott-ramsey-summit-presentation.pdf.
13. Hutchinson Institute for Cancer Outcomes Research. Community Cancer Care in Washington State: Quality and Cost Report 2019. 2019; April 10, 2020. Available at: https://www.fredhutch.org/content/dam/www/research/institute-networks-ircs/hicor/HICOR-Community-Cancer-Care-Report-2019.pdf.

14. Kaye DR, Min HS, Herrel LA, Dupree JM, Ellimoottil C, Miller DC. Costs of Cancer Care Across the Disease Continuum. *The oncologist.* 2018;23(7):798-805. https://theoncologist.onlinelibrary.wiley.com/doi/full/10.1634/theoncologist.2017-0481.

15. Sun L, Legood R, Dos-Santos-Silva I, Gaiha SM, Sadique Z. Global treatment costs of breast cancer by stage: A systematic review. *PloS one.* 2018;13(11):e0207993. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0207993.

16. Institute of Medicine and National Research Council. Fulfilling the Potential of Cancer Prevention and Early Detection. 2003; April 10, 2020. Available at: https://www.nap.edu/catalog/10263/fulfilling-the-potential-of-cancer-prevention-and-early-detection.

17. Buffery D. Innovation Tops Current Trends in the 2016 Oncology Drug Pipeline. *American health & drug benefits.* 2016;9(4):233-238. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5004820/.

18. IQVIA. Global Oncology Trends 2019. *Institute Report*. 2019; April 21, 2020. Available at: https://www.iqvia.com/insights/the-iqvia-institute/reports/global-oncology-trends-2019.

19. Chawla A, Peeples M, Li N, Anhorn R, Ryan J, Signorovitch J. Real-world utilization of molecular diagnostic testing and matched drug therapies in the treatment of metastatic cancers. *J Med Econ.* 2018;21(6):543-552. https://www.ncbi.nlm.nih.gov/pubmed/29295635.

20. Mason C, Ellis PG, Lokay K, et al. Patterns of Biomarker Testing Rates and Appropriate Use of Targeted Therapy in the First-Line, Metastatic Non-Small Cell Lung Cancer Treatment Setting. *Journal of clinical pathways : the foundation of value-based care.* 2018;4(1):49-54. https://www.journalofclinicalpathways.com/article/patterns-biomarker-testing-rates-and-appropriate-use-targeted-therapy-first-line-metastatic.

21. Gutierrez ME, Price KS, Lanman RB, et al. Genomic Profiling for KRAS, NRAS, BRAF, Microsatellite Instability, and Mismatch Repair Deficiency Among Patients With Metastatic Colon Cancer. *JCO Precision Oncology.* 2019(3):1-9. https://ascopubs.org/doi/abs/10.1200/PO.19.00274.

22. Gutierrez ME, Choi K, Lanman RB, et al. Genomic profiling of advanced non-small cell lung cancer in community settings: Gaps and opportunities. *Clinical lung cancer.* 2017;18(6):651-659. https://www.sciencedirect.com/science/article/pii/S1525730417301092?via%3Dihub.

23. Keeling P. Transforming the pharm business model to prevent lost treatment opportunities and optimize return on investment. *Diaceutics White Paper*. 2017. Available at: https://www.diaceutics.com/docs/ft-2017-white-paper.pdf.

24. Audibert CM SM, Glass DJ, et al. Trends in the Molecular Diagnosis of Lung Cancer: Results from an Online Market Research Survey *Friends of Cancer Research.* 2016:1-19. https://www.focr.org/sites/default/files/pdf/FINAL%202017%20Friends%20NSCLC%20White%20Paper.pdf.

25. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Breast Cancer V.3.2022. © National Comprehensive Cancer Network, Inc.; 2022. All rights reserved. Accessed May 18, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

26. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Bladder Cancer V.1.2022. © National Comprehensive Cancer Network, Inc;

2022. All rights reserved. Accessed March 31, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

27. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Central Nervous System Cancers V.2.2021. © National Comprehensive Cancer Network, Inc; 2021. All rights reserved. Accessed October 7, 2021. To view the most recent and complete version of the guideline, go online to NCCN.org.

28. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Colon Cancer V.1.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed March 31, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

29. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Melanoma: Cutaneous V.3.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed May 18, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

30. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Esophageal and Esophagogastric Junction Cancers V.2.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed March 31, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

31. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Gastric Cancer V.2.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed January 27, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

32. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Hepatobiliary Cancers V.1.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed March 31, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

33. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Non-Small Cell Lung Cancer V.3.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed March 31, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

34. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Ovarian Cancer V.1.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed January 27, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

35. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Pancreatic Adenocarcinoma V.1.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed March 31, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

36. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Prostate Cancer V.4.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed May 18, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

37. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Rectal Cancer V.1.2022. © National Comprehensive Cancer Network, Inc;

2022. All rights reserved. Accessed March 31, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

38. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Uterine Neoplasms V.1.2022. © National Comprehensive Cancer Network, Inc; 2021. All rights reserved. Accessed November 5, 2021. To view the most recent and complete version of the guideline, go online to NCCN.org.

39. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Vulvar Cancer V.1.2022. © National Comprehensive Cancer Network, Inc; 2021. All rights reserved. Accessed October 27, 2021. To view the most recent and complete version of the guideline, go online to NCCN.org.

40. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Occult Primary V.1.2022. © National Comprehensive Cancer Network, Inc; 2021. All rights reserved. Accessed October 7, 2021. To view the most recent and complete version of the guideline, go online to NCCN.org.

41. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Thyroid Carcinoma V.2.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed May 18, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

42. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Cervical Cancer V.1.2022. © National Comprehensive Cancer Network, Inc; 2021. All rights reserved. Accessed November 5, 2021. To view the most recent and complete version of the guideline, go online to NCCN.org.

43. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Bone Cancer V.2.2022. © National Comprehensive Cancer Network, Inc; 2021. All rights reserved. Accessed October 29, 2021. To view the most recent and complete version of the guideline, go online to NCCN.org.

44. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Soft Tissue Sarcoma V.2.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed May 18, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

45. Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Head and Neck Cancers V.2.2022. © National Comprehensive Cancer Network, Inc; 2022. All rights reserved. Accessed May 18, 2022. To view the most recent and complete version of the guideline, go online to NCCN.org.

46. El-Deiry WS, Goldberg RM, Lenz HJ, et al. The current state of molecular testing in the treatment of patients with solid tumors, 2019. *CA Cancer J Clin.* 2019. http://www.ncbi.nlm.nih.gov/pubmed/31116423.

47. Wongchenko M, Dent R, Kim S, et al. Cell-free (CF)DNA analysis identifies PIK3CA/AKT1 mutations associated with greater PFS improvement from the addition of ipatasertib (IPAT) to paclitaxel (P) in triple-negative breast cancer (TNBC). *Annals of oncology : official journal of the European Society for Medical Oncology.* 2017;28(5 Suppl):v22-v42. https://oncologypro.esmo.org/meeting-resources/esmo-2017-congress/Cell-free-cf-DNA-analysis-identifies-PIK3CA-AKT1-mutations-associated-with-greater-PFS-improvement-from-the-addition-of-ipatasertib-IPAT-to-paclitaxel-P-in-triple-negative-breast-cancer-TNBC.

48. Schwaederle M, Zhao M, Lee JJ, et al. Impact of precision medicine in diverse cancers: A meta-analysis of phase II clinical trials. *J Clin Oncol.* 2015;33(32):3817-3825. https://ascopubs.org/doi/10.1200/JCO.2015.61.5997.

49. Trabucco SE, Gowen K, Maund SL, et al. A Novel Next-Generation Sequencing Approach to Detecting Microsatellite Instability and Pan-Tumor Characterization of 1000 Microsatellite Instability-High Cases in 67,000 Patient Samples. *The Journal of molecular diagnostics : JMD.* 2019;21(6):1053-1066. https://www.sciencedirect.com/science/article/pii/S1525157819303587?via%3Dihub.

50. Gandara DR, Paul SM, Kowanetz M, et al. Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab. *Nat Med.* 2018;24(9):1441-1448. https://www.nature.com/articles/s41591-018-0134-3.

51. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome medicine.* 2017;9(1):34. https://genomemedicine.biomedcentral.com/articles/10.1186/s13073-017-0424-2.

52. Hellmann MD, Ciuleanu TE, Pluzanski A, et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. *New Engl J Med.* 2018;378(22):2093-2104. https://www.ncbi.nlm.nih.gov/pubmed/29658845.

53. Johnson DB, Dahlman KH, Knol J, et al. Enabling a genetically informed approach to cancer medicine: a retrospective evaluation of the impact of comprehensive tumor profiling using a targeted next-generation sequencing panel. *The oncologist.* 2014;19(6):616-622. https://theoncologist.onlinelibrary.wiley.com/doi/full/10.1634/theoncologist.2014-0011.

54. Colombo N, Sessa C, du Bois A, et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease†. *Annals of oncology : official journal of the European Society for Medical Oncology.* 2019;30(5):672-705. https://www.sciencedirect.com/science/article/pii/S0923753419311627?via%3Dihub.

55. Marquart J, Chen EY, Prasad V. Estimation of the Percentage of US Patients With Cancer Who Benefit From Genome-Driven Oncology. *JAMA oncology.* 2018;4(8):1093-1098. https://jamanetwork.com/journals/jamaoncology/fullarticle/2678901.

56. Parsons DW, Roy A, Yang Y, et al. Diagnostic Yield of Clinical Tumor and Germline Whole-Exome Sequencing for Children With Solid Tumors. *JAMA oncology.* 2016;2(5):616-624. https://jamanetwork.com/journals/jamaoncology/fullarticle/2484356.

57. Harris MH, DuBois SG, Glade Bender JL, et al. Multicenter Feasibility Study of Tumor Molecular Profiling to Inform Therapeutic Decisions in Advanced Pediatric Solid Tumors: The Individualized Cancer Therapy (iCat) Study. *JAMA oncology.* 2016;2(5):608-615. https://jamanetwork.com/journals/jamaoncology/fullarticle/2484355.

58. Boland GM, Piha-Paul SA, Subbiah V, et al. Clinical next generation sequencing to identify actionable aberrations in a phase I program. *Oncotarget.* 2015;6(24):20099-20110. https://www.oncotarget.com/article/4040/text/.

59. Powell SF, Dib EG, Bleeker JS, et al. Delivering precision oncology in a community cancer program: Results from a prospective observational study. *JCO Precis Oncol.* 2018(2):1-12. http://ascopubs.org/doi/abs/10.1200/PO.17.00220.

60. Sadaps M, Funchain P, Mahdi H, et al. Precision oncology in solid tumors: A longitudinal tertiary care center experience. *JCO Precis Oncol.* 2018;doi:10.1200/PO.18.00186. http://ascopubs.org/doi/abs/10.1200/PO.18.00186.

61. Reitsma M, Fox J, Vanden Borre P, et al. Impact of a collaboration between a health plan, oncology practice, and comprehensive genomic profiling company from the payer perspective. *J Manag Care Spec Pharm.* 2019;doi:10.18553/jmcp.2019.18309. https://www.jmcp.org/doi/10.18553/jmcp.2019.18309.

62. Hirshfield KM, Tolkunov D, Zhong H, et al. Clinical actionability of comprehensive genomic profiling for management of rare or refractory cancers. *The oncologist.* 2016;21(11):1315-1325. https://theoncologist.onlinelibrary.wiley.com/doi/full/10.1634/theoncologist.2016-0049.

63. Kato S, Kurasaki K, Ikeda S, Kurzrock R. Rare Tumor Clinic: The University of California San Diego Moores Cancer Center experience with a precision therapy approach. *The oncologist.* 2018;23(2):171-178. https://theoncologist.onlinelibrary.wiley.com/doi/full/10.1634/theoncologist.2017-0199.

64. Rodriguez-Rodriguez L, Hirshfield KM, Rojas V, et al. Use of comprehensive genomic profiling to direct point-of-care management of patients with gynecologic cancers. *Gynecologic oncology.* 2016;141(1):2-9. https://www.sciencedirect.com/science/article/pii/S0090825816300452?via%3Dihub.

65. Schwaederle M, Daniels GA, Piccioni DE, et al. On the road to precision cancer medicine: Analysis of genomic biomarker actionability in 439 patients. *Molecular cancer therapeutics.* 2015;14(6):1488-1494. https://mct.aacrjournals.org/content/14/6/1488.

66. Sicklick JK, Kato S, Okamura R, et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. *Nat Med.* 2019;25(5):744-750. http://www.ncbi.nlm.nih.gov/pubmed/31011206.

67. Wheler JJ, Janku F, Naing A, et al. Cancer therapy directed by comprehensive genomic profiling: A single center study. *Cancer research.* 2016;76(13):3690-3701. https://cancerres.aacrjournals.org/content/76/13/3690.

68. Boussemart L, Nelson A, Wong M, et al. Hybrid Capture-Based Genomic Profiling Identifies BRAF V600 and Non-V600 Alterations in Melanoma Samples Negative by Prior Testing. *The oncologist.* 2019;24(5):657-663. https://theoncologist.onlinelibrary.wiley.com/doi/full/10.1634/theoncologist.2018-0271

69. Rankin A, Klempner SJ, Erlich R, et al. Broad Detection of Alterations Predicted to Confer Lack of Benefit From EGFR Antibodies or Sensitivity to Targeted Therapy in Advanced Colorectal Cancer. *The oncologist.* 2016;21(11):1306-1314. https://theoncologist.onlinelibrary.wiley.com/doi/full/10.1634/theoncologist.2016-0148.

70. Vasan N, Razavi P, Johnson JL, et al. Double PIK3CA mutations in cis increase oncogenicity and sensitivity to PI3Kα inhibitors. *Science (New York, N.Y.).* 2019;366(6466):714-723. https://science.sciencemag.org/content/366/6466/714.

71. Rozenblum AB, Ilouze M, Dudnik E, et al. Clinical impact of hybrid capture-based next-generation sequencing on changes in treatment decisions in lung cancer. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer.* 2017;12(2):258-268. https://www.sciencedirect.com/science/article/pii/S1556086416312412?via%3Dihub.

72. Freedman AN, Klabunde CN, Wiant K, et al. Use of Next-Generation Sequencing Tests to Guide Cancer Treatment: Results From a Nationally Representative Survey of Oncologists in the United States. *JCO Precision Oncology.* 2018(2):1-13. https://ascopubs.org/doi/abs/10.1200/PO.18.00169.

73. Schwaederle M, Parker BA, Schwab RB, et al. Precision oncology: The UC San Diego Moores Cancer Center PREDICT experience. *Molecular cancer therapeutics.* 2016;15(4):743-752. https://mct.aacrjournals.org/content/15/4/743.

74. Pishvaian MJ, Bender RJ, Halverson D, et al. Molecular profiling of patients with pancreatic cancer: Initial results from the know your tumor initiative. *Clin Cancer Res.* 2018;24(20):5018-5027. https://clincancerres.aacrjournals.org/content/24/20/5018.

75. Schrock AB, Welsh A, Chung JH, et al. Hybrid capture-based genomic profiling of circulating tumor DNA from patients with advanced non-small cell lung cancer. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer.* 2018;doi:10.1016/j.jtho.2018.10.008. https://pubmed.ncbi.nlm.nih.gov/30368012/.

76. Ganesan P, Moulder S, Lee JJ, et al. Triple-negative breast cancer patients treated at MD Anderson Cancer Center in phase I trials: improved outcomes with combination chemotherapy and targeted agents. *Molecular cancer therapeutics.* 2014;13(12):3175-3184. https://pubmed.ncbi.nlm.nih.gov/25253784/.

77. Pishvaian MJ, Blais EM, Brody JR, et al. Overall survival in patients with pancreatic cancer receiving matched therapies following molecular profiling: a retrospective analysis of the Know Your Tumor registry trial. *The Lancet. Oncology.* 2020;21(4):508-518. https://www.sciencedirect.com/science/article/abs/pii/S1470204520300747?via%3Dihub.

78. Singal G, Miller PG, Agarwala V, et al. Association of Patient Characteristics and Tumor Genomics With Clinical Outcomes Among Patients With Non-Small Cell Lung Cancer Using a Clinicogenomic Database. *Jama.* 2019;321(14):1391-1399. https://jamanetwork.com/journals/jama/fullarticle/2730114.

79. Alecensa (alectinib) [prescribing information]. *Genentech, Inc.* June 2018; April 23, 2020. Available at: https://www.gene.com/download/pdf/alecensa_prescribing.pdf.

80. Cotellic (cobimetinib) [prescribing information]. *Genentech, Inc.* January 2018; April 23, 2020. Available at: https://www.gene.com/download/pdf/cotellic_prescribing.pdf.

81. Erbitux (cetuximab) [prescribing information]. *ImClone LLC.* April 2019; April 23, 2020. Available at: https://uspl.lilly.com/erbitux/erbitux.html.

82. Gilotrif (afatinib) [prescribing information]. *Boehringer Ingelheim Pharmaceuticals, Inc.* October 2019; April 23, 2020. Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=fd638e5e-8032-e7ca-0179-95e96ab5d387.

83. Herceptin (trastuzumab) [prescribing information]. *Genentech, Inc.* November 2018; April 23, 2020. Available at: hhttps://www.gene.com/download/pdf/herceptin_prescribing.pdf.

84. Iressa (gefitinib) [prescribing information]. *AstraZeneca Pharmaceuticals LP*. May 2019; April 23, 2020. Available at: https://www.azpicentral.com/iressa/iressa.pdf#page=1.

85. Mekinist (trametinib) [prescribing information]. *Novartis Pharmaceuticals Corporation*. June 2020; August 11, 2020. Available at:

https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=0002ad27-779d-42ab-83b5-bc65453412a1.

86. Perjeta (pertuzumab) [prescribing information]. *Genentech, Inc*. January 2020; April 23, 2020. Available at: https://www.gene.com/download/pdf/perjeta_prescribing.pdf.

87. Piqray (alpelisib) [prescribing information]. *Novartis Pharmaceuticals Corporation*. May 2019; April 23, 2020. Available at: https://www.novartis.us/sites/www.novartis.us/files/piqray.pdf.

88. Tafinlar (dabrafenib) [prescribing information]. *Novartis Pharmaceuticals Corporation*. April 2020; April 23, 2020. Available at: https://www.novartis.us/sites/www.novartis.us/files/tafinlar.pdf.

89. Tagrisso (osimertinib) [prescribing information]. *AstraZeneca Pharmaceuticals LP*. June 2020; August 11, 2020. Available at: https://www.azpicentral.com/tagrisso/tagrisso.pdf#page=1.

90. Tarceva (erlotinib) [prescribing information]. *Genentech, Inc.* October 2016; April 23, 2020. Available at: https://www.gene.com/download/pdf/tarceva_prescribing.pdf.

91. Vectibix (panitumumab) [prescribing information]. *Amgen Inc*. June 2017; April 23, 2020. Available at: https://www.pi.amgen.com/~/media/amgen/repositorysites/pi-amgen-com/vectibix/vectibix_pi.pdf.

92. Xalkori (crizotinib) [prescribing information]. *Pfizer Laboratories Div Pfizer Inc*. June 2019; April 23, 2020. Available at: http://labeling.pfizer.com/showlabeling.aspx?id=676.

93. Zelboraf (vemurafenib) [prescribing information]. *Genentech, Inc.* May 2020; August 11, 2020. Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=38eea320-7e0c-485a-bc30-98c3c45e2763.

94. Zykadia (ceritinib) [prescribing information]. *Novartis Pharmaceuticals Corporation*. March 2019; April 23, 2020. Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=fff5d805-4ffd-4e8e-8e63-6f129697563e.

95. Siravegna G, Mussolin B, Venesio T, et al. How liquid biopsies can change clinical practice in oncology. *Annals of oncology : official journal of the European Society for Medical Oncology*. 2019;30(10):1580-1590. https://www.sciencedirect.com/science/article/pii/S0923753419609725?via%3Dihub.

96. Merker JD, Oxnard GR, Compton C, et al. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. *J Clin Oncol.* 2018;36(16):1631-1641. https://pubmed.ncbi.nlm.nih.gov/29504847/.

97. Geeurickx E, Hendrix A. Targets, pitfalls and reference materials for liquid biopsy tests in cancer diagnostics. *Molecular aspects of medicine.* 2019:100828. https://pubmed.ncbi.nlm.nih.gov/31711714/.

98. Jordan EJ, Kim HR, Arcila ME, et al. Prospective Comprehensive Molecular Characterization of Lung Adenocarcinomas for Efficient Patient Matching to Approved and Emerging Therapies. *Cancer Discov.* 2017;7(6):596-609. https://cancerdiscovery.aacrjournals.org/content/7/6/596.

99. Kalemkerian GP, Narula N, Kennedy EB. Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: American Society of Clinical Oncology Endorsement Summary of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice

Guideline Update. *Journal of oncology practice.* 2018;14(5):323-327. https://ascopubs.org/doi/10.1200/JOP.18.00035.

100. Kris MG, Johnson BE, Berry LD, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. *Jama.* 2014;311(19):1998-2006. https://jamanetwork.com/journals/jama/fullarticle/1872815.

101. Aggarwal C, Thompson JC, Black TA, et al. Clinical Implications of Plasma-Based Genotyping With the Delivery of Personalized Therapy in Metastatic Non–Small Cell Lung Cancer. *JAMA oncology.* 2019;5(2):173-180. https://pubmed.ncbi.nlm.nih.gov/30325992/.

102. Pritchett MA, Camidge DR, Patel M, et al. Prospective Clinical Validation of the InVisionFirst-Lung Circulating Tumor DNA Assay for Molecular Profiling of Patients With Advanced Nonsquamous Non–Small-Cell Lung Cancer. *JCO Precision Oncology.* 2019(3):1-15. https://ascopubs.org/doi/abs/10.1200/PO.18.00299.

103. Deverka P, Messner DA, McCormack R, et al. Generating and evaluating evidence of the clinical utility of molecular diagnostic tests in oncology. *Genetics in medicine : official journal of the American College of Medical Genetics.* 2016;18(8):780-787. https://www.nature.com/articles/gim2015162.

104. Madison R, Schrock AB, Castellanos E, et al. Retrospective analysis of real-world data to determine clinical outcomes of patients with advanced non-small cell lung cancer following cell-free circulating tumor DNA genomic profiling. *Lung cancer (Amsterdam, Netherlands).* 2020;148:69-78. https://www.sciencedirect.com/science/article/pii/S0169500220305572?via%3Dihub.

105. Dziadziuszko R, Mok T, Peters S, et al. Blood First Assay Screening Trial (BFAST) in Treatment-Naive Advanced or Metastatic NSCLC: Initial Results of the Phase 2 ALK-Positive Cohort. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer.* 2021. https://pubmed.ncbi.nlm.nih.gov/34311110/.

106. Wongchenko MJ, Kim S-B, Saura C, et al. Circulating Tumor DNA and Biomarker Analyses From the LOTUS Randomized Trial of First-Line Ipatasertib and Paclitaxel for Metastatic Triple-Negative Breast Cancer. *JCO Precision Oncology.* 2020(4):1012-1024. https://ascopubs.org/doi/abs/10.1200/PO.19.00396.

107. Foundation Medicine Inc. FoundationOne CDx™ Summary of Safety and Effectiveness. PMA P190032. 2021; Decemeber 3, 2021. Available at: https://www.accessdata.fda.gov/cdrh_docs/pdf19/P190032B.pdf.

108. Foundation Medicine Inc. FoundationOne CDx™ Summary of Safety and Effectiveness. PMA P200016. 2021; Decemeber 3, 2021. Available at: https://www.accessdata.fda.gov/cdrh_docs/pdf20/P200016B.pdf.

109. Abida W, Patnaik A, Campbell D, et al. Rucaparib in Men With Metastatic Castration-Resistant Prostate Cancer Harboring a BRCA1 or BRCA2 Gene Alteration. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology.* 2020:Jco2001035. https://pubmed.ncbi.nlm.nih.gov/32795228/.

110. de Bono J, Mateo J, Fizazi K, et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. *N. Engl. J. Med.* 2020;382(22):2091-2102. https://pubmed.ncbi.nlm.nih.gov/32343890/.

111. Woodhouse R, Li M, Hughes J, et al. Clinical and analytical validation of FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of

solid tumor origin. *PLoS One.* 2020;15(9):e0237802. https://pubmed.ncbi.nlm.nih.gov/32976510/.

112. Swisher EM, Lin KK, Oza AM, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. *The Lancet. Oncology.* 2017;18(1):75-87. http://www.ncbi.nlm.nih.gov/pubmed/27908594.

113. Chawla A, Janku F, Wheler JJ, et al. Estimated cost of anticancer therapy directed by comprehensive genomic profiling in a single-center study. *JCO Precis Oncol.* 2018;doi:10.1200/PO.18.00074. http://ascopubs.org/doi/abs/10.1200/PO.18.00074.

114. Signorovitch J, Zhou Z, Ryan J, Chawla A. Budget impact analysis of comprehensive genomic profiling in patients with advanced non-small cell lung cancer. *J Med Econ.* 2018;doi:10.1080/13696998.2018.1549056. https://www.tandfonline.com/doi/abs/10.1080/13696998.2018.1549056?journalCode=ijme20.

115. Harvey MJ, Cunningham R, Sawchyn B, et al. Budget Impact Analysis of Comprehensive Genomic Profiling in Patients With Advanced Non–Small-Cell Lung Cancer. *JCO Precision Oncology.* 2021(5):1611-1624. https://ascopubs.org/doi/abs/10.1200/PO.20.00540.

116. Merkhofer C, Chennupati S, Sun Q, et al. Effect of Clinical Trial Participation on Costs to Payers in Metastatic Non-Small-Cell Lung Cancer. *JCO oncology practice.* 2021;17(8):e1225-e1234. https://pubmed.ncbi.nlm.nih.gov/34375561/.

117. Lokhandwala T, Bittoni MA, Dann RA, et al. Costs of diagnostic assessment for lung cancer: A Medicare claims analysis. *Clinical lung cancer.* 2017;18(1):e27-e34. https://pubmed.ncbi.nlm.nih.gov/27530054/.

118. Arnaud A. Costs and outcomes comparison of tissue and blood based biopsies for the purpose of biomarker testing. *Value Health.* 2016;19:A143. https://www.valueinhealthjournal.com/article/S1098-3015(16)01624-7/fulltext.

119. National Cancer Institute. Cancer Stat Facts. 2021; October 7, 2021. Available at: https://seer.cancer.gov/statfacts/.

120. Kakushadze ZR, Rakesh; Yu, Willie. Estimating Cost Savings from Early Cancer Diagnosis. *Data.* 2017;2(30):1-16. https://www.mdpi.com/2306-5729/2/3/30.

121. Lux MP, Lewis K, Rider A, Niyazov A. Abstract P6-08-09: BRCA1/2 testing in adult women with HER2- advanced breast cancer (ABC): Results from a US real world study. *Cancer research.* 2020;80(4 Supplement):P6-08-09-P06-08-09. https://cancerres.aacrjournals.org/content/80/4_Supplement/P6-08-09.

122. Tsimberidou AM, Hong DS, Wheler JJ, et al. Precision medicine: Clinical outcomes including long-term survival according to the pathway targeted and treatment period–The IMPACT study. *J Clin Oncol.* 2018;36(18 Suppl):LBA2553. https://ascopubs.org/doi/abs/10.1200/JCO.2018.36.18_suppl.LBA2553.

123. Jardim DL, Schwaederle M, Wei C, et al. Impact of a biomarker-based strategy on oncology drug development: A meta-analysis of clinical trials leading to FDA approval. *Journal of the National Cancer Institute.* 2015;107(11):djv253. https://academic.oup.com/jnci/article/107/11/djv253/2457697.

124. Hellmann MD, Ciuleanu TE, Pluzanski A, et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. *The New England journal of medicine.* 2018;doi:10.1056/NEJMoa1801946. https://www.nejm.org/doi/full/10.1056/NEJMoa1801946.

125. Fred Hutchinson Cancer Research Center. Living with Stage 4: The breast cancer no one understands. 2016; April 21, 2020. Available at: https://www.fredhutch.org/en/news/center-news/2014/10/stage-4-metastatic-misunderstood-breast-cancer.html.

126. Pascale M, Azinwi CN, Marongiu B, Pesce G, Stoffel F, Roggero E. The outcome of prostate cancer patients treated with curative intent strongly depends on survival after metastatic progression. *BMC cancer.* 2017;17(1):651. https://bmccancer.biomedcentral.com/articles/10.1186/s12885-017-3617-6.

127. Berger KN, Pu JJ. PD-1 pathway and its clinical application: A 20-year journey after discovery of the complete human PD-1 gene. *Gene.* 2018;638:20-25. https://www.sciencedirect.com/science/article/abs/pii/S0378111917307825?via%3Dihub.

128. Campbell BB, Light N, Fabrizio D, et al. Comprehensive analysis of hypermutation in human cancer. *Cell.* 2017;171(5):1042-1056.e1010. https://www.sciencedirect.com/science/article/pii/S009286741731142X?via%3Dihub.

129. Felsenstein KM, Theodorescu D. Precision medicine for urothelial bladder cancer: update on tumour genomics and immunotherapy. *Nature reviews. Urology.* 2018;15(2):92-111. https://www.nature.com/articles/nrurol.2017.179.

130. Kou T, Kanai M, Matsumoto S, Okuno Y, Muto M. The possibility of clinical sequencing in the management of cancer. *Japanese journal of clinical oncology.* 2016;46(5):399-406. https://academic.oup.com/jjco/article/46/5/399/2218999.

131. Pai SG, Carneiro BA, Chae YK, et al. Correlation of tumor mutational burden and treatment outcomes in patients with colorectal cancer. *Journal of gastrointestinal oncology.* 2017;8(5):858-866. https://jgo.amegroups.com/article/view/14866/13046.

132. Yates LR, Seoane J, Le Tourneau C, et al. The European Society for Medical Oncology (ESMO) Precision Medicine Glossary. *Annals of oncology : official journal of the European Society for Medical Oncology.* 2018;29(1):30-35. https://www.sciencedirect.com/science/article/pii/S0923753419350112?via%3Dihub.

133. Baudino TA. Targeted cancer therapy: The next generation of cancer treatment. *Current drug discovery technologies.* 2015;12(1):3-20. https://www.eurekaselect.com/131876/article.

134. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nature reviews. Cancer.* 2012;12(4):252-264. https://www.nature.com/articles/nrc3239.

135. Goodman AM, Kato S, Bazhenova L, et al. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. *Molecular cancer therapeutics.* 2017;16(11):2598-2608. https://mct.aacrjournals.org/content/16/11/2598.

136. Goodman AM, Sokol ES, Frampton GM, Lippman SM, Kurzrock R. Microsatellite-Stable Tumors with High Mutational Burden Benefit from Immunotherapy. *Cancer Immunol Res.* 2019;7(10):1570-1573. https://cancerimmunolres.aacrjournals.org/content/7/10/1570.

137. Precision Oncoloy News. Survey of Precision Oncology Programs Finds Agreement on Testing Divergence in Care Delivery. 2019; March 29, 2020. Available at: https://www.precisiononcologynews.com/cancer/survey-precision-oncology-programs-finds-agreement-testing-divergence-care-delivery#.XoE9CohKiUl.

138. Hoesing H. Clinical Practice Guidelines: Closing the Gap Betwen Theory and Practice. 2016; March 29, 2020. Available at: https://www.elsevier.com/__data/assets/pdf_file/0007/190177/JCI-Whitepaper_cpgs-closing-the-gap.pdf.

139. Katz SJ, Jagsi R, Morrow M. Reducing Overtreatment of Cancer With Precision Medicine: Just What the Doctor Ordered. *Jama.* 2018;319(11):1091-1092. https://jamanetwork.com/journals/jama/article-abstract/2673741.

140. Gierman HJ, S. G, M. L, et al. Genomic testing and treatment landscape in patients with advanced non-small cell lung cancer (aNSCLC) using real-world data from community oncology practices. *American Society of Clinical Oncology Annual Meeting; June 3, 2019; Chicago, IL.* 2019. https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.15_suppl.1585.

141. Wang L, Page DR, Shewade A, et al. Utilization and timing of Foundation Medicine Inc. (FMI) testing in U.S. advanced non-small cell lung cancer (aNSCLC) patients. *18th IASLC World Conference on Lung Cancer.* 2017. https://www.jto.org/article/S1556-0864(17)31420-X/fulltext.

142. Warner EW, Yip SM, Chi KN, Wyatt AW. DNA repair defects in prostate cancer: impact for screening, prognostication and treatment. *BJU international.* 2019;123(5):769-776. https://bjui-journals.onlinelibrary.wiley.com/doi/abs/10.1111/bju.14576.

143. Kratz J, Burkard M, O'Meara T, Pusztai L, Veitch Z, Bedard PL. Incorporating Genomics Into the Care of Patients With Advanced Breast Cancer. *American Society of Clinical Oncology educational book. American Society of Clinical Oncology. Annual Meeting.* 2018;38:56-64. https://ascopubs.org/doi/10.1200/EDBK_200731.

144. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD. Homologous Recombination Deficiency: Exploiting the Fundamental Vulnerability of Ovarian Cancer. *Cancer Discov.* 2015;5(11):1137-1154. https://cancerdiscovery.aacrjournals.org/content/5/11/1137.

145. da Cunha Colombo Bonadio RR, Fogace RN, Miranda VC, Diz M. Homologous recombination deficiency in ovarian cancer: a review of its epidemiology and management. *Clinics (Sao Paulo, Brazil).* 2018;73(suppl 1):e450s. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1807-59322018000200308&lng=en&nrm=iso&tlng=en.

146. Gee ME, Faraahi Z, McCormick A, Edmondson RJ. DNA damage repair in ovarian cancer: unlocking the heterogeneity. *Journal of ovarian research.* 2018;11(1):50. https://ovarianresearch.biomedcentral.com/articles/10.1186/s13048-018-0424-x.

147. Drilon A, Wang L, Arcila ME, et al. Broad, hybrid capture-based next-generation sequencing identifies actionable genomic alterations in lung adenocarcinomas otherwise negative for such alterations by other genomic testing approaches. *Clin Cancer Res.* 2015;21(16):3631-3639. https://clincancerres.aacrjournals.org/content/21/16/3631.

148. Gray PN, Dunlop CL, Elliott AM. Not All Next Generation Sequencing Diagnostics are Created Equal: Understanding the Nuances of Solid Tumor Assay Design for Somatic Mutation Detection. *Cancers.* 2015;7(3):1313-1332. https://www.mdpi.com/2072-6694/7/3/837.

149. Ali SM, Hensing T, Schrock AB, et al. Comprehensive genomic profiling identifies a subset of crizotinib-responsive alk-rearranged non-small cell lung cancer not detected by fluorescence in situ hybridization. *The oncologist.* 2016;21(6):762-770. https://theoncologist.onlinelibrary.wiley.com/doi/full/10.1634/theoncologist.2015-0497.

150. Chmielecki J, Ross JS, Wang K, et al. Oncogenic alterations in ERBB2/HER2 represent potential therapeutic targets across tumors from diverse anatomic sites of origin. *The oncologist.* 2015;20(1):7-12. https://theoncologist.onlinelibrary.wiley.com/doi/full/10.1634/theoncologist.2014-0234.

151. Ross JS, Gay LM, Wang K, et al. Nonamplification ERBB2 genomic alterations in 5605 cases of recurrent and metastatic breast cancer: An emerging opportunity for anti-HER2 targeted therapies. *Cancer.* 2016;122(17):2654-2662. https://acsjournals.onlinelibrary.wiley.com/doi/full/10.1002/cncr.30102.

152. Suh JH, Johnson A, Albacker L, et al. Comprehensive genomic profiling facilitates implementation of the National Comprehensive Cancer Network guidelines for lung cancer biomarker testing and identifies patients who may benefit from enrollment in mechanism-driven clinical trials. *The oncologist.* 2016;21(6):684-691. https://theoncologist.onlinelibrary.wiley.com/doi/full/10.1634/theoncologist.2016-0030.

153. Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nature biotechnology.* 2013;31(11):1023-1031. https://www.nature.com/articles/nbt.2696.

154. Wheler JJ, Janku F, Naing A, et al. TP53 alterations correlate with response to VEGF/VEGFR inhibitors: implications for targeted therapeutics. *Molecular cancer therapeutics.* 2016;15(10):2475-2485. https://mct.aacrjournals.org/content/15/10/2475.

155. Delgado PO, Alves BC, Gehrke Fde S, et al. Characterization of cell-free circulating DNA in plasma in patients with prostate cancer. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine.* 2013;34(2):983-986. https://pubmed.ncbi.nlm.nih.gov/23269609/.

156. Hashad D, Sorour A, Ghazal A, Talaat I. Free circulating tumor DNA as a diagnostic marker for breast cancer. *Journal of clinical laboratory analysis.* 2012;26(6):467-472. https://pubmed.ncbi.nlm.nih.gov/23143630/.

157. Schwarzenbach H, Stoehlmacher J, Pantel K, Goekkurt E. Detection and monitoring of cell-free DNA in blood of patients with colorectal cancer. *Annals of the New York Academy of Sciences.* 2008;1137:190-196. https://pubmed.ncbi.nlm.nih.gov/18837946/.

158. Park JL, Kim HJ, Choi BY, et al. Quantitative analysis of cell-free DNA in the plasma of gastric cancer patients. *Oncology letters.* 2012;3(4):921-926. https://pubmed.ncbi.nlm.nih.gov/22741019/.

159. Diehl F, Li M, Dressman D, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. *Proceedings of the National Academy of Sciences of the United States of America.* 2005;102(45):16368-16373. https://pubmed.ncbi.nlm.nih.gov/16258065/.

160. Diehl F, Schmidt K, Choti MA, et al. Circulating mutant DNA to assess tumor dynamics. *Nat Med.* 2008;14(9):985-990. https://pubmed.ncbi.nlm.nih.gov/18670422/.

161. Tukachinsky H, Madison RW, Chung JH, et al. Genomic Analysis of Circulating Tumor DNA in 3,334 Patients with Advanced Prostate Cancer Identifies Targetable BRCA Alterations and AR Resistance Mechanisms. *Clin Cancer Res.* 2021;27(11):3094-3105. https://pubmed.ncbi.nlm.nih.gov/33558422/.

162. Leighl NB, Page RD, Raymond VM, et al. Clinical Utility of Comprehensive Cell-free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non-small Cell Lung Cancer. *Clin Cancer Res.* 2019;25(15):4691-4700. https://pubmed.ncbi.nlm.nih.gov/30988079/.

163. Li G, Pavlick D, Chung JH, et al. Genomic profiling of cell-free circulating tumor DNA in patients with colorectal cancer and its fidelity to the genomics of the tumor biopsy. *Journal of gastrointestinal oncology.* 2019;10(5):831-840. https://pubmed.ncbi.nlm.nih.gov/31602320/.

164. Chowdhury S, McDermott R, Piulats JM, et al. Genomic profiling of circulating tumour DNA (ctDNA) and tumour tissue for the evaluation of rucaparib in metastatic castration-resistant prostate cancer (mCRPC). *Annals of oncology : official journal of the European Society for Medical Oncology.* 2018;29(8 Suppl):mdy284.004. http://dx.doi.org/10.1093/annonc/mdy284.004.

165. Foundation Medicine Inc. FoundationOne Liquid CDx™ Technical Specifications. 2021; July 20, 2021. Available at: https://assets.ctfassets.net/w98cd481qyp0/wVEm7VtICYR0sT5C1VbU7/55f0a7f3cbfd30fae686c64c1c3d77ae/F1LCDx_Technical_Specs_072021.pdf.

166. Foundation Medicine Inc. FoundationOne Liquid CDx™ Report Guide. 2021; December 2, 2021. Available at: https://assets.ctfassets.net/w98cd481qyp0/3AHKssP2LZghlHJx4Aj73C/1b5728068e361ee29a2b7315ad494f1d/Portfolio_Report_Guide.pdf.

167. Rizvi H, Sanchez-Vega F, La K, et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell lung cancer profiled with targeted next-generation sequencing. *J Clin Oncol.* 2018;36(7):633-641. https://ascopubs.org/doi/10.1200/JCO.2017.75.3384.

168. Merino DM, McShane LM, Fabrizio D, et al. Establishing guidelines to harmonize tumor mutational burden (TMB): in silico assessment of variation in TMB quantification across diagnostic platforms: phase I of the Friends of Cancer Research TMB Harmonization Project. *J Immunother Cancer.* 2020;8(1). https://jitc.bmj.com/content/8/1/e000147.

169. Kocarnik JM, Shiovitz S, Phipps AI. Molecular phenotypes of colorectal cancer and potential clinical applications. *Gastroenterology report.* 2015;3(4):269-276. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4650976.

170. Gadgeel SM, Mok TSK, Peters S, et al. LBA81_PR - Phase II/III blood first assay screening trial (BFAST) in patients (pts) with treatment-naïve NSCLC: Initial results from the ALK+ cohort. *Annals of Oncology.* 2019;30:v918. http://www.sciencedirect.com/science/article/pii/S0923753419604394.

171. Al-Kateb H, Nguyen TT, Steger-May K, Pfeifer JD. Identification of major factors associated with failed clinical molecular oncology testing performed by next generation sequencing (NGS). *Molecular oncology.* 2015;9(9):1737-1743. https://pubmed.ncbi.nlm.nih.gov/26071350/.

172. Aggarwal C, Thompson JC, Black TA, et al. Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non-small cell lung cancer. *JAMA oncology.* 2018;doi:10.1001/jamaoncol.2018.4305. https://pubmed.ncbi.nlm.nih.gov/30325992/.

173. Thompson JC, Yee SS, Troxel AB, et al. Detection of therapeutically targetable driver and resistance mutations in lung cancer patients by next-generation sequencing of cell-free circulating tumor DNA. *Clin Cancer Res.* 2016;22(23):5772-5782. https://pubmed.ncbi.nlm.nih.gov/27601595/.

174. Schwaederle MC, Patel SP, Husain H, et al. Utility of genomic assessment of blood-derived circulating tumor DNA (ctDNA) in patients with advanced lung adenocarcinoma. *Clin Cancer Res.* 2017;23(17):5101-5111. https://pubmed.ncbi.nlm.nih.gov/28539465/.

175. Tran HT, Elamin Y, Simon GR, et al. Clinical utilization of a CLIA-certified cell-free DNA (cfDNA) blood test for identification of targetable molecular alterations in patients with non-small cell lung cancer (NSCLC). *J Clin Oncol.* 2016;34(15 Suppl):e23064. http://ascopubs.org/doi/abs/10.1200/JCO.2016.34.15_suppl.e23064.

176. Hagemann IS, Devarakonda S, Lockwood CM, et al. Clinical next-generation sequencing in patients with non-small cell lung cancer. *Cancer.* 2015;121(4):631-639. https://pubmed.ncbi.nlm.nih.gov/25345567/.

177. Stover DG, Reinbolt RE, Adams EJ, et al. Prospective Decision Analysis Study of Clinical Genomic Testing in Metastatic Breast Cancer: Impact on Outcomes and Patient Perceptions. *JCO Precision Oncology.* 2019(3):1-11. https://ascopubs.org/doi/abs/10.1200/PO.19.00090.

178. Angus L, Smid M, Wilting SM, et al. The genomic landscape of metastatic breast cancer highlights changes in mutation and signature frequencies. *Nature genetics.* 2019;51(10):1450-1458. https://pubmed.ncbi.nlm.nih.gov/31570896/.

179. Heerink WJ, de Bock GH, de Jonge GJ, Groen HJ, Vliegenthart R, Oudkerk M. Complication rates of CT-guided transthoracic lung biopsy: meta-analysis. *European radiology.* 2017;27(1):138-148. https://pubmed.ncbi.nlm.nih.gov/27108299/.

180. Wiener RS, Wiener DC, Gould MK. Risks of transthoracic needle biopsy: How high? *Clinical pulmonary medicine.* 2013;20(1):29-35. https://pubmed.ncbi.nlm.nih.gov/23525679/.

181. Ost D, Fein AM, Feinsilver SH. Clinical practice. The solitary pulmonary nodule. *The New England journal of medicine.* 2003;348(25):2535-2542. https://pubmed.ncbi.nlm.nih.gov/12815140/.

182. Gould MK, Donington J, Lynch WR, et al. Evaluation of individuals with pulmonary nodules: when is it lung cancer? Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest.* 2013;143(5 Suppl):e93S-e120S. https://pubmed.ncbi.nlm.nih.gov/23649456/.

183. Hansen AR, Bedard PL. Clinical application of high-throughput genomic technologies for treatment selection in breast cancer. *Breast Cancer Res.* 2013;15(5):R97. https://pubmed.ncbi.nlm.nih.gov/24135425/.

184. Prabhakar U, Kelley M. *Validation of Cell-Based Assays in the GLP Setting: A Practical Guide.* Chichester, England: John Wiley & Sons Ltd; 2008.

185. Walker HK, Hall WD, Hurst JW. *Clinical Methods: The History, Physical, and Laboratory Examinations.* Boston: Butterworth Publishers, a division of Reed Publishing; 1990.

186. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: Guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *Archives of pathology & laboratory medicine.* 2018;142(3):321-346. https://meridian.allenpress.com/aplm/article/142/3/321/103064/Updated-Molecular-Testing-Guideline-for-the.

187. Rolfo C, Mack P, Scagliotti GV, et al. Liquid Biopsy for Advanced NSCLC: A Consensus Statement From the International Association for the Study of Lung Cancer. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer.* 2021;16(10):1647-1662. https://pubmed.ncbi.nlm.nih.gov/34246791/.

188. Rolfo C, Mack PC, Scagliotti GV, et al. Liquid biopsy for advanced non-small cell lung cancer (NSCLC): A statement paper from the IASLC. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer.* 2018;13(9):1248-1268. https://www.sciencedirect.com/science/article/pii/S1556086418306804?via%3Dihub.

189. Surveillance, Epidemiology, and End Results (SEER) Program. SEER Cancer Stat Facts: Lung and Bronchus Cancer. National Cancer Institute. Bethesda, MD, . *The National Cancer Institute.* 2021. https://seer.cancer.gov/statfacts/html/lungb.html.

190. Zheng M. Classification and Pathology of Lung Cancer. *Surgical oncology clinics of North America.* 2016;25(3):447-468. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1016%2Fj.soc.2016.02.003.

191. Owonikoko TK, Ragin C, Chen Z, et al. Real-world effectiveness of systemic agents approved for advanced non-small cell lung cancer: a SEER-Medicare analysis. *The oncologist.* 2013;18(5):600-610. https://www.ncbi.nlm.nih.gov/pubmed/?term=The+Oncologist+2013%3B18%3A600%E2%80%93610.

192. Mok TSK, Wu YL, Kudaba I, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. *Lancet.* 2019;393(10183):1819-1830. https://www.ncbi.nlm.nih.gov/pubmed/?term=Lancet.+2019%3B393(10183)%3A1819-1830.

193. Hsu PC, Jablons DM, Yang CT, You L. Epidermal Growth Factor Receptor (EGFR) Pathway, Yes-Associated Protein (YAP) and the Regulation of Programmed Death-Ligand 1 (PD-L1) in Non-Small Cell Lung Cancer (NSCLC). *International journal of molecular sciences.* 2019;20(15). https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fijms20153821.

194. Gainor JF, Shaw AT. Novel targets in non-small cell lung cancer: ROS1 and RET fusions. *The oncologist.* 2013;18(7):865-875. https://www.ncbi.nlm.nih.gov/pubmed/?term=Oncologist.+2013%3B18(7)%3A865-875.

195. Bubendorf L, Buttner R, Al-Dayel F, et al. Testing for ROS1 in non-small cell lung cancer: a review with recommendations. *Virchows Archiv : an international journal of pathology.* 2016;469(5):489-503. https://link.springer.com/article/10.1007%2Fs00428-016-2000-3.

196. Baik CS, Myall NJ, Wakelee HA. Targeting BRAF-Mutant Non-Small Cell Lung Cancer: From Molecular Profiling to Rationally Designed Therapy. *The oncologist.* 2017;22(7):786-796. https://www.ncbi.nlm.nih.gov/pubmed/?term=Oncologist.+2017%3B22(7)%3A786-796.

197. Guo Y, Cao R, Zhang X, et al. Recent Progress in Rare Oncogenic Drivers and Targeted Therapy For Non-Small Cell Lung Cancer. *OncoTargets and therapy.* 2019;12:10343-10360. https://www.dovepress.com/recent-progress-in-rare-oncogenic-drivers-and-targeted-therapy-for-non-peer-reviewed-article-OTT.

198. Chu QS. Targeting non-small cell lung cancer: driver mutation beyond epidermal growth factor mutation and anaplastic lymphoma kinase fusion. *Therapeutic advances in medical oncology.* 2020;12:1758835919895756. https://www.ncbi.nlm.nih.gov/pubmed/?term=10.1177%2F1758835919895756.

199. Golding B, Luu A, Jones R, Viloria-Petit AM. The function and therapeutic targeting of anaplastic lymphoma kinase (ALK) in non-small cell lung cancer (NSCLC). *Molecular cancer.* 2018;17(1):52.

https://www.ncbi.nlm.nih.gov/pubmed/?term=Mol+Cancer.+2018%3B17(1)%3A52.doi%3A10.1186%2Fs12943-018-0810-4.

200. Hofman P. ALK in Non-Small Cell Lung Cancer (NSCLC) Pathobiology, Epidemiology, Detection from Tumor Tissue and Algorithm Diagnosis in a Daily Practice. *Cancers.* 2017;9(8). https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fcancers9080107.

201. Aran V, Omerovic J. Current Approaches in NSCLC Targeting K-RAS and EGFR. *International journal of molecular sciences.* 2019;20(22). https://www.ncbi.nlm.nih.gov/pubmed/?term=10.3390%2Fijms20225701.

202. Mok T SGS, Kim ES, et al. Blood First-Line Ready Screening Trial (B-F1RST) and Blood First Assay Screening Trial (BFAST) Enable Clinical Development of Novel Blood-Based Biomarker Assays for Tumor Mutational Burden (TMB) and Somatic Mutations in 1L Advanced or Metastatic NSCLC. *Presented at the European Society for Medical Oncology 2017 (ESMO 2017); September 8-12; Madrid, Spain.* 2017. https://www.annalsofoncology.org/article/S0923-7534(20)38628-2/fulltext.

203. Wolf J, Seto T, Han JY, et al. Capmatinib in MET Exon 14-Mutated or MET-Amplified Non-Small-Cell Lung Cancer. *The New England journal of medicine.* 2020;383(10):944-957. https://www.nejm.org/doi/10.1056/NEJMoa2002787.

204. Surveillance, Epidemiology, and End Results Program. SEER Cancer Stat Facts: Prostate Cancer. . 2021; October 7, 2021. Available at: https://seer.cancer.gov/statfacts/html/prost.html.

205. Kirby M, Hirst C, Crawford ED. Characterising the castration-resistant prostate cancer population: a systematic review. *International journal of clinical practice.* 2011;65(11):1180-1192. https://www.ncbi.nlm.nih.gov/pubmed/?term=Int+J+Clin+Pract.+2011%3B65(11)%3A1180-1192.

206. Abida W, Armenia J, Gopalan A, et al. Prospective Genomic Profiling of Prostate Cancer Across Disease States Reveals Germline and Somatic Alterations That May Affect Clinical Decision Making. *JCO Precis Oncol.* 2017;2017. https://pubmed.ncbi.nlm.nih.gov/28825054/.

207. Sandhu SK, Hussain M, Mateo J, et al. LBA8 - PROfound: Phase III study of olaparib versus enzalutamide or abiraterone for metastatic castration-resistant prostate cancer (mCRPC) with homologous recombination repair (HRR) gene alterations. *Annals of Oncology.* 2019;30:ix188-ix189. http://www.sciencedirect.com/science/article/pii/S0923753419582126.

208. Tao DL, Bailey S, Beer TM, et al. Molecular Testing in Patients With Castration-Resistant Prostate Cancer and Its Impact on Clinical Decision Making. *JCO Precis Oncol.* 2017;1. https://pubmed.ncbi.nlm.nih.gov/31650098/.

209. Chung JH, Dewal N, Sokol E, et al. Prospective Comprehensive Genomic Profiling of Primary and Metastatic Prostate Tumors. *JCO Precision Oncology.* 2019(3):1-23. https://ascopubs.org/doi/abs/10.1200/PO.18.00283.

210. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin.* 2020;70(1):7-30. https://acsjournals.onlinelibrary.wiley.com/doi/full/10.3322/caac.21590.

211. Higano C, Sternberg CN, Saad F, et al. Treatment patterns and outcomes for metastatic castration-resistant prostate cancer (mCRPC) in a real-world setting: a retrospective study of more than 2500 patients. *Presented at the American Society of Clinical Oncology-Genitourinary*

Cancers Symposium; San Francisco, CA; February 14-16. 2019. https://ascopubs.org/doi/abs/10.1200/JCO.2019.37.7_suppl.256.

212. Athie A, Arce-Gallego S, Gonzalez M, et al. Targeting DNA Repair Defects for Precision Medicine in Prostate Cancer. *Current Oncology Reports.* 2019;21(5):42. https://doi.org/10.1007/s11912-019-0790-6.

213. Saad F, Shore N, Zhang T, Sharma S, Cho HK, Jacobs IA. Emerging therapeutic targets for patients with advanced prostate cancer. *Cancer treatment reviews.* 2019;76:1-9. https://www.sciencedirect.com/science/article/abs/pii/S0305737219300507?via%3Dihub.

214. Wassim Abida, David Campbell, Akash Patnaik, Brieuc Sautois, Jeremy Shapiro, al. E. Preliminary Results from the TRITON2 Study of Rucaparib in Patients with DNA Damage Repair (DDR)-Deficient Metastatic Castration-Resistant Prostate Cancer (mCRPC): Updated Analyses. *ESMO Congress | 27 September–1 October | Barcelona, Spain.* 2019. https://www.clovisoncology.com/media/1160/esmo2019_wabida_poster.pdf.

215. Abida W, Cheng ML, Armenia J, et al. Analysis of the Prevalence of Microsatellite Instability in Prostate Cancer and Response to Immune Checkpoint Blockade. *JAMA oncology.* 2019;5(4):471-478. https://www.ncbi.nlm.nih.gov/pubmed/?term=JAMA+Oncology+5%2C+471%E2%80%93478+(2019).

216. Annala M, Struss WJ, Warner EW, et al. Treatment Outcomes and Tumor Loss of Heterozygosity in Germline DNA Repair-deficient Prostate Cancer. *European urology.* 2017;72(1):34-42. https://pubmed.ncbi.nlm.nih.gov/29367197/.

217. Annala M, Vandekerkhove G, Khalaf D, et al. Circulating Tumor DNA Genomics Correlate with Resistance to Abiraterone and Enzalutamide in Prostate Cancer. *Cancer Discov.* 2018;8(4):444-457. https://pubmed.ncbi.nlm.nih.gov/28259476/.

218. Paller CJ, Antonarakis ES, Beer TM, et al. Germline Genetic Testing in Advanced Prostate Cancer; Practices and Barriers: Survey Results from the Germline Genetics Working Group of the Prostate Cancer Clinical Trials Consortium. *Clinical genitourinary cancer.* 2019;17(4):275-282.e271. https://www.sciencedirect.com/science/article/abs/pii/S1558767319301399?via%3Dihub.

219. Lara P, McPherson J, Heyer W, et al. Comprehensive characterization of BRCA1 and BRCA2 alterations in circulating tumor DNA and tumor tissue in men with prostate cancer: Implications for clinical care. *Annals of oncology : official journal of the European Society for Medical Oncology.* 2017;28(5 Suppl):mdx370.044. http://dx.doi.org/10.1093/annonc/mdx370.044.

220. Hussain M, Mateo J, Fizazi K, et al. Survival with Olaparib in Metastatic Castration-Resistant Prostate Cancer. *The New England journal of medicine.* 2020. https://www.nejm.org/doi/10.1056/NEJMoa2022485.

221. Surveillance, Epidemiology, and End Results Program. SEER Cancer Stat Facts: Breast Cancer. . 2021; October 7, 2021. Available at: https://seer.cancer.gov/statfacts/html/breast.html.

222. Yuan Y, Yost SE, Yim J, et al. Genomic mutation-driven metastatic breast cancer therapy: a single center experience. *Oncotarget.* 2017;8(16):26414-26423. https://www.oncotarget.com/article/14476/text/.

223. Marhold M, Bartsch R, Zielinski C. Recent developments and translational aspects in targeted therapy for metastatic breast cancer. *ESMO open.* 2016;1(3):e000036. https://www.sciencedirect.com/science/article/pii/S2059702920325606?via%3Dihub.

224. Marchio C, Scaltriti M, Ladanyi M, et al. ESMO recommendations on the standard methods to detect NTRK fusions in daily practice and clinical research. *Annals of oncology : official journal of the European Society for Medical Oncology.* 2019;30(9):1417-1427. https://pubmed.ncbi.nlm.nih.gov/31268127/.

225. Litton JK, Burstein HJ, Turner NC. Molecular Testing in Breast Cancer. *American Society of Clinical Oncology educational book. American Society of Clinical Oncology. Annual Meeting.* 2019;39:e1-e7. https://ascopubs.org/doi/10.1200/EDBK_237715.

226. Robson M, Im SA, Senkus E, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. *The New England journal of medicine.* 2017;377(6):523-533. https://www.nejm.org/doi/10.1056/NEJMoa1706450.

227. Litton JK, Rugo HS, Ettl J, et al. Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation. *The New England journal of medicine.* 2018;379(8):753-763. https://www.nejm.org/doi/10.1056/NEJMoa1802905.

228. Andre F, Ciruelos E, Rubovszky G, et al. Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. *The New England journal of medicine.* 2019;380(20):1929-1940. https://www.nejm.org/doi/10.1056/NEJMoa1813904.

229. Schmid P, Adams S, Rugo HS, et al. Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. *The New England journal of medicine.* 2018;379(22):2108-2121. https://www.nejm.org/doi/10.1056/NEJMoa1809615.

230. Kurian AW, Ward KC, Howlader N, et al. Genetic Testing and Results in a Population-Based Cohort of Breast Cancer Patients and Ovarian Cancer Patients. *J Clin Oncol.* 2019;37(15):1305-1315. https://ascopubs.org/doi/10.1200/JCO.18.01854.

231. Hou H, Liu D, Zhou N, et al. Broad, hybrid capture-based next generation sequencing identified actionable genomic alterations in HER2-negative breast cancer. *Journal of Clinical Oncology.* 2016;34(15_suppl):e12544-e12544. https://ascopubs.org/doi/abs/10.1200/JCO.2016.34.15_suppl.e12544.

232. Rossi G, Austin LK, Nagy RJ, et al. Circulating tumor DNA (ctDNA): A real-time application of precision medicine to the management of metastatic breast cancer (MBC). *Cancer research.* 2017;77(4 Suppl):PD1-02. https://cancerres.aacrjournals.org/content/77/4_Supplement/PD1-02.

233. Kim SB, Dent R, Im SA, et al. Ipatasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer (LOTUS): A multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *The Lancet. Oncology.* 2017;18(10):1360-1372. https://pubmed.ncbi.nlm.nih.gov/28800861/.

234. Dent R OA, Isakoff SJ, et al. 139O - Final results of the double-blind placebo (PBO)-controlled randomised phase II LOTUS trial of first-line ipatasertib (IPAT) + paclitaxel (PAC) for inoperable locally advanced/metastatic triple-negative breast cancer (mTNBC). *Presented at the European Society for Medical Oncology 2020 (ESMO 2020); May 23, 2020; Virtual ESMO.* 2020. https://oncologypro.esmo.org/meeting-resources/esmo-breast-cancer-virtual-meeting-2020/final-results-of-the-double-blind-placebo-pbo-controlled-randomised-phase-ii-lotus-trial-of-first-line-ipatasertib-ipat-paclitaxel-pac-for.

235. Surveillance, Epidemiology, and End Results Program. SEER Cancer Stat Facts: Ovarian Cancer. . 2021; October 7, 2021. Available at: https://seer.cancer.gov/statfacts/html/ovary.html.

236. Tsibulak I, Zeimet AG, Marth C. Hopes and failures in front-line ovarian cancer therapy. *Critical reviews in oncology/hematology.* 2019;143:14-19. https://www.sciencedirect.com/science/article/pii/S1040842819301660?via%3Dihub.

237. Arend R, Westin SN, Coleman RL. Decision analysis for secondline maintenance treatment of platinum sensitive recurrent ovarian cancer: a review. *International journal of gynecological cancer : official journal of the International Gynecological Cancer Society.* 2020;30(5):684-694. https://ijgc.bmj.com/content/30/5/684.

238. Pignata S, Pisano C, Di Napoli M, Cecere SC, Tambaro R, Attademo L. Treatment of recurrent epithelial ovarian cancer. *Cancer.* 2019;125 Suppl 24:4609-4615. https://acsjournals.onlinelibrary.wiley.com/doi/full/10.1002/cncr.32500.

239. Giornelli GH. Management of relapsed ovarian cancer: a review. *SpringerPlus.* 2016;5(1):1197. https://springerplus.springeropen.com/articles/10.1186/s40064-016-2660-0.

240. Pujade-Lauraine E, Hilpert F, Weber B, et al. Bevacizumab combined with chemotherapy for platinum-resistant recurrent ovarian cancer: The AURELIA open-label randomized phase III trial. *J Clin Oncol.* 2014;32(13):1302-1308. https://ascopubs.org/doi/10.1200/JCO.2013.51.4489.

241. Lheureux S, Braunstein M, Oza AM. Epithelial ovarian cancer: Evolution of management in the era of precision medicine. *CA Cancer J Clin.* 2019;69(4):280-304. https://acsjournals.onlinelibrary.wiley.com/doi/full/10.3322/caac.21559.

242. Pujade-Lauraine E, Ledermann JA, Selle F, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. *The Lancet. Oncology.* 2017;18(9):1274-1284. https://www.sciencedirect.com/science/article/abs/pii/S1470204517304692?via%3Dihub.

243. Domchek SM, Aghajanian C, Shapira-Frommer R, et al. Efficacy and safety of olaparib monotherapy in germline BRCA1/2 mutation carriers with advanced ovarian cancer and three or more lines of prior therapy. *Gynecologic oncology.* 2016;140(2):199-203. https://www.sciencedirect.com/science/article/abs/pii/S0090825815302237?via%3Dihub.

244. Mirza MR, Monk BJ, Herrstedt J, et al. Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer. *The New England journal of medicine.* 2016;375(22):2154-2164. https://www.nejm.org/doi/10.1056/NEJMoa1611310.

245. Moore KN, Secord AA, Geller MA, et al. Niraparib monotherapy for late-line treatment of ovarian cancer (QUADRA): a multicentre, open-label, single-arm, phase 2 trial. *The Lancet. Oncology.* 2019;20(5):636-648. https://www.sciencedirect.com/science/article/abs/pii/S1470204519300294?via%3Dihub.

246. Coleman RL, Oza AM, Lorusso D, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet.* 2017;390(10106):1949-1961. https://www.sciencedirect.com/science/article/abs/pii/S0140673617324406?via%3Dihub.

247. Marabelle A, Le DT, Ascierto PA, et al. Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From

the Phase II KEYNOTE-158 Study. *J Clin Oncol.* 2020;38(1):1-10. https://ascopubs.org/doi/10.1200/JCO.19.02105.

248. Norquist BM, Harrell MI, Brady MF, et al. Inherited Mutations in Women With Ovarian Carcinoma. *JAMA oncology.* 2016;2(4):482-490. https://jamanetwork.com/journals/jamaoncology/fullarticle/2479125.

249. Lancaster JM, Powell CB, Chen LM, Richardson DL. Society of Gynecologic Oncology statement on risk assessment for inherited gynecologic cancer predispositions. *Gynecologic oncology.* 2015;136(1):3-7. https://www.sciencedirect.com/science/article/abs/pii/S0090825814013122?via%3Dihub.

250. Hoppe MM, Sundar R, Tan DSP, Jeyasekharan AD. Biomarkers for Homologous Recombination Deficiency in Cancer. *Journal of the National Cancer Institute.* 2018;110(7):704-713. https://academic.oup.com/jnci/article/110/7/704/4999673.

251. Previs RA, Sood AK, Mills GB, Westin SN. The rise of genomic profiling in ovarian cancer. *Expert review of molecular diagnostics.* 2016;16(12):1337-1351. https://pubmed.ncbi.nlm.nih.gov/27828713/.

252. Konstantinopoulos PA, Norquist B, Lacchetti C, et al. Germline and Somatic Tumor Testing in Epithelial Ovarian Cancer: ASCO Guideline. *J Clin Oncol.* 2020:Jco1902960. https://ascopubs.org/doi/10.1200/JCO.19.02960.

253. Mohyuddin GR, Aziz M, Britt A, et al. Similar response rates and survival with PARP inhibitors for patients with solid tumors harboring somatic versus Germline BRCA mutations: a Meta-analysis and systematic review. *BMC Cancer.* 2020;20(1):507. https://pubmed.ncbi.nlm.nih.gov/32493233/.

254. Coffee B, Cox HC, Kidd J, et al. Detection of somatic variants in peripheral blood lymphocytes using a next generation sequencing multigene pan cancer panel. *Cancer Genet.* 2017;211:5-8. https://pubmed.ncbi.nlm.nih.gov/28279308/.

255. Childers CP, Childers KK, Maggard-Gibbons M, Macinko J. National Estimates of Genetic Testing in Women With a History of Breast or Ovarian Cancer. *J Clin Oncol.* 2017;35(34):3800-3806. https://ascopubs.org/doi/10.1200/JCO.2017.73.6314.

256. Hoskins PJ, Gotlieb WH. Missed therapeutic and prevention opportunities in women with BRCA-mutated epithelial ovarian cancer and their families due to low referral rates for genetic counseling and BRCA testing: A review of the literature. *CA Cancer J Clin.* 2017;67(6):493-506. https://acsjournals.onlinelibrary.wiley.com/doi/full/10.3322/caac.21408.

257. Rubraca (rucaparib) [prescribing information]. *Clovis Oncology, Inc.* May 2020; August 11, 2020. Available at: https://clovisoncology.com/pdfs/RubracaUSPI.pdf.

258. Yu TM, Morrison C, Gold EJ, Tradonsky A, Arnold RJG. Budget impact of next-generation sequencing for molecular assessment of advanced non–small cell lung cancer. *Value Health.* 2018;21(11):1278-1285. http://www.sciencedirect.com/science/article/pii/S1098301518316759.

259. Hoos WA, James PM, Rahib L, Talley AW, Fleshman JM, Matrisian LM. Pancreatic cancer clinical trials and accrual in the United States. *J Clin Oncol.* 2013;31(27):3432-3438. https://pubmed.ncbi.nlm.nih.gov/23960185/.

260. Drilon A, Laetsch TW, Kummar S, et al. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. *The New England journal of medicine.* 2018;378(8):731-739. http://www.ncbi.nlm.nih.gov/pubmed/29466156.

261. Drilon A, Siena S, Ou SI, et al. Safety and Antitumor Activity of the Multitargeted Pan-TRK, ROS1, and ALK Inhibitor Entrectinib: Combined Results from Two Phase I Trials (ALKA-372-001 and STARTRK-1). *Cancer Discov.* 2017;7(4):400-409. https://www.ncbi.nlm.nih.gov/pubmed/28183697.

262. Foundation Medicine Inc. FoundationOne CDx™ Technical Information. 2022; January 20, 2022. Available at: www.f1cdxlabel.com.

263. Hempelmann JA, Lockwood CM, Konnick EQ, et al. Microsatellite instability in prostate cancer by PCR or next-generation sequencing. *J Immunother Cancer.* 2018;6(1):29. http://www.ncbi.nlm.nih.gov/pubmed/29665853.

264. Alunbrig (brigatinib) [prescribing information]. *Millennium Pharmaceuticals, Inc.* September 2021; October 7, 2021. Available at: https://www.alunbrig.com/assets/pi.pdf.

265. Avastin (bevacizumab) [prescribing information]. *Genentech, Inc.* January 2021; October 7, 2021. Available at: https://www.gene.com/download/pdf/avastin_prescribing.pdf.

266. Cabometyx (cabozantinib) [prescribing information]. *Exelixis, Inc.* July 2020; August 11, 2020. Available at: https://www.cabometyxhcp.com/pi/.

267. Caprelsa (vandetanib) [prescribing information]. *Genzyme Corporation*. June 2020; June 24, 2020. Available at: http://www.caprelsa.com/files/caprelsa-pi.pdf.

268. Cyramza (ramucirumab) [prescribing information]. *Eli Lilly and Company*. June 2021; October 7, 2021. Available at: https://uspl.lilly.com/cyramza/cyramza.html#pi.

269. Enhertu (fam-trastuzumab deruxtecan-nxki) [prescribing information]. *Daiichi Sankyo, Inc.* December 2019; August 11, 2020. Available at: https://dsi.com/prescribing-information-portlet/getPIContent?productName=Enhertu&inline=true.

270. Exkivity (mobocertinib) [prescribing information]. *Takeda Pharmaceuticals America, Inc.* September 2021; October 7, 2021. Available at: https://content.takeda.com/?contenttype=pi&product=exkivity&language=eng&country=usa&documentnumber=1.

271. Kadcyla (ado-trastuzumab emtansine) [prescribing information]. May 2019; Augsut 11, 2020. Available at: https://www.gene.com/download/pdf/kadcyla_prescribing.pdf.

272. Keytruda (pembrolizumab) [prescribing information]. *Merck Sharp & Dohme Corp.* August 2021; October 7, 2021. Available at: https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?setid=9333c79b-d487-4538-a9f0-71b91a02b287.

273. Libtayo (cemiplimab-rwlc) [prescribing information]. *Regeneron Pharmaceuticals, Inc/sanofi-aventis U.S. LLC.* February 2021; October 7, 2021. Available at: https://www.regeneron.com/downloads/libtayo_fpi.pdf.

274. Lorbrena (lorlatinib) [prescribing information]. *Pfizer Laboratories Div Pfizer Inc*. March 2021; October 7, 2021. Available at: http://labeling.pfizer.com/ShowLabeling.aspx?id=11140.

275. Lumakras (sotorasib) [prescribing information]. *Amgen Inc*. May 2021; October 7, 2021. Available at: https://www.pi.amgen.com/~/media/amgen/repositorysites/pi-amgen-com/lumakras/lumakras_pi_hcp_english.ashx.

276. Opdivo (nivolumab) [prescribing information]. *Bristol-Myers Squibb Company*. June 2020; September 15, 2020. Available at: https://packageinserts.bms.com/pi/pi_opdivo.pdf.

277. Retevmo (selpercatinib) [prescribing information]. *Eli Lilly and Company*. May 2020; August 12, 2020. Available at: https://uspl.lilly.com/retevmo/retevmo.html#pi.

278. Rozlytrek (entrectinib) [prescribing information]. *Genentech Inc.* August 2019; April 23, 2020. Available at: https://www.gene.com/download/pdf/rozlytrek_prescribing.pdf.

279. Rybrevant (amivantamab-vmjw) [prescribing information]. *Janssen Biotech, Inc.* July 2021; October 7, 2021. Available at: https://www.janssenlabels.com/package-insert/product-monograph/prescribing-information/RYBREVANT-pi.pdf.

280. Tabrecta (capmatinib) [prescribing information]. *Novartis Pharmaceuticals Corporation*. May 2020; June 24, 2020. Available at: https://www.novartis.us/sites/www.novartis.us/files/tabrecta.pdf.

281. Tecentriq (atezolizumab) [prescribing information]. *Genentech, Inc.* July 2020; August 11, 2020. Available at: https://www.gene.com/download/pdf/tecentriq_prescribing.pdf.

282. Tepmetko (tepotinib) [prescribing information]. *EMD Serono, Inc.* February 2021; October 7, 2021. Available at: https://www.emdserono.com/us-en/pi/tepmetko-pi.pdf.

283. Tykerb (lapatinib) [prescribing information]. *Novartis Pharmaceuticals Corporation*. December 2018; August 11, 2020. Available at: https://www.novartis.us/sites/www.novartis.us/files/tykerb.pdf.

284. Vitrakvi (larotrectinib) [prescribing information]. *Bayer HealthCare Pharmaceuticals Inc.* March 2021; October 7, 2021. Available at: http://labeling.bayerhealthcare.com/html/products/pi/vitrakvi_PI.pdf.

285. Vizimpro (dacomitinib) [prescribing information]. *Pfizer Laboratories Div Pfizer Inc*. September 2018; August 11, 2020. Available at: http://labeling.pfizer.com/ShowLabeling.aspx?id=11019.

286. Yervoy (ipilimumab) [prescribing information]. *Bristol-Myers Squibb Company*. August 2020; September 15, 2020. Available at: https://packageinserts.bms.com/pi/pi_yervoy.pdf.

287. Lynparza (olaparib) [prescribing information]. *AstraZeneca Pharmaceuticals LP*. May 2020; August 11, 2020. Available at: https://www.azpicentral.com/lynparza_tb/lynparza_tb.pdf#page=1.

288. Jemperli (dostarlimab-gxly) [prescribing information]. *GlaxoSmithKline LLC.* August 2021; October 7, 2021. Available at: https://gskpro.com/content/dam/global/hcpportal/en_US/Prescribing_Information/Jemperli/pdf/JEMPERLI-PI-MG.PDF.

289. Margenza (margetuximab-cmkb) [prescribing information]. *MacroGenics, Inc.* December 2020; October 7, 2021. Available at: https://www.margenza.com/pdf/prescribing-information.pdf.

290. Nerlynx (neratinib) [prescribing information]. *Puma Biotechnology, Inc.* July 2020; August 11, 2020. Available at: https://nerlynx.com/pdf/full-prescribing-information.pdf.

291. Talzenna (talazoparib) [prescribing information]. *Pfizer Laboratories Div Pfizer Inc*. March 2020; August 11, 2020. Available at: http://labeling.pfizer.com/ShowLabeling.aspx?id=11046.

292. Tukysa (tucatinib) [prescribing information]. *Seattle Genetics, Inc.* April 2020; August 11, 2020. Available at: https://seagendocs.com/TUKYSA_Full_Ltr_Master.pdf.

293. Zejula (niraparib) [prescribing information]. *GlaxoSmithKline*. April 2020; August 11, 2020. Available at:

https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Zejula/pdf/ZEJULA-PI-PIL.PDF.

294. Clark TA, Chung JH, Kennedy M, et al. Analytical validation of a hybrid capture-based next-generation sequencing clinical assay for genomic profiling of cell-free circulating tumor DNA. *The Journal of molecular diagnostics : JMD.* 2018;20(5):686-702. https://pubmed.ncbi.nlm.nih.gov/29936259/.

295. Chung JH, Pavlick D, Hartmaier R, et al. Hybrid capture-based genomic profiling of circulating tumor DNA from patients with estrogen receptor-positive metastatic breast cancer. *Annals of oncology : official journal of the European Society for Medical Oncology.* 2017;28(11):2866-2873. https://pubmed.ncbi.nlm.nih.gov/28945887/.

296. Kim SB, Dent R, Wongchenko MJ, Singel SM, Baselga J. Concordance between plasma-based and tissue-based next-generation sequencing in LOTUS. *The Lancet. Oncology.* 2017;18(11):e638. https://pubmed.ncbi.nlm.nih.gov/29208392/.

297. Schrock AB, Pavlick D, Klempner SJ, et al. Hybrid capture-based genomic profiling of circulating tumor DNA from patients with advanced cancers of the gastrointestinal tract or anus. *Clin Cancer Res.* 2018;24(8):1881-1890. https://pubmed.ncbi.nlm.nih.gov/29363525/.

298. Zhou C, Yuan Z, Ma W, et al. Clinical utility of tumor genomic profiling in patients with high plasma circulating tumor DNA burden or metabolically active tumors. *J Hematol Oncol.* 2018;11(1):129. https://doi.org/10.1186/s13045-018-0671-8.

299. Creelan BC, Lieber DS, Schrock AB, et al. Landscape of kinase rearrangements (kRE) detected in circulating tumor DNA (ctDNA). *J Clin Oncol.* 2018;36(15 Suppl):12041. http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.15_suppl.12041.

300. Schrock AB, Zhu VW, Hsieh WS, et al. Receptor tyrosine kinase fusions and BRAF kinase fusions are rare but actionable resistance mechanisms to EGFR tyrosine kinase inhibitors. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer.* 2018;13(9):1312-1323. https://pubmed.ncbi.nlm.nih.gov/29883838/.

301. Sokol ES, Feng YX, Jin DX, et al. Loss of function of NF1 is a mechanism of acquired resistance to endocrine therapy in lobular breast cancer. *Annals of oncology : official journal of the European Society for Medical Oncology.* 2019;30(1):115-123. https://pubmed.ncbi.nlm.nih.gov/30423024/.

302. Gornstein EL, Sandefur S, Chung JH, et al. BRCA2 reversion mutation associated with acquired resistance to olaparib in estrogen receptor-positive breast cancer detected by genomic profiling of tissue and liquid biopsy. *Clinical breast cancer.* 2018;18(2):184-188. https://pubmed.ncbi.nlm.nih.gov/29325860/.

303. Gregg JP, Li G, Pavlick D, et al. Comprehensive genomic profiling of ctDNA in patients with colon cancer and its fidelity to the genomics of the tumor biopsy. *J Clin Oncol.* 2018;36(4 Suppl):569. http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.4_suppl.569.

304. McGregor BA, Chung J, Bergerot PG, et al. Correlation of circulating tumor DNA (ctDNA) assessment with tissue-based comprehensive genomic profiling (CGP) in metastatic urothelial cancer (mUC). *J Clin Oncol.* 2018;36(6 Suppl):453. http://ascopubs.org/doi/abs/10.1200/JCO.2018.36.6_suppl.453.

305. Wang VE, Young L, Ali S, et al. A case of metastatic atypical neuroendocrine tumor with ALK translocation and diffuse brain metastases. *The oncologist.* 2017;22(7):768-773. https://pubmed.ncbi.nlm.nih.gov/28507205/.

306. Bahary N, He J, Bailey M, et al. Genomic profiling of circulating tumor DNA (ctDNA) from patients (pts) with pancreatic ductal adenocarcinoma (PDA). *J Clin Oncol.* 2017;35(15 Suppl):4128. http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.15_suppl.4128.

307. Young L, Ali S, Schrock A, et al. Kinase fusions in non-small cell lung carcinoma identified by hybrid capture based ctDNA assay. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer.* 2017;12(1):S524-S525. https://doi.org/10.1016/j.jtho.2016.11.645.

308. Dagogo-Jack I, Fabrizio D, Lennerz J, et al. Circulating tumor DNA identifies EGFR coamplification as a mechanism of resistance to crizotinib in a patient with advanced MET-amplified lung adenocarcinoma. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer.* 2017;12(10):e155-e157. https://pubmed.ncbi.nlm.nih.gov/28499860/.

309. Ou SI, Cui J, Schrock AB, et al. Emergence of novel and dominant acquired EGFR solvent-front mutations at Gly796 (G796S/R) together with C797S/R and L792F/H mutations in one EGFR (L858R/T790M) NSCLC patient who progressed on osimertinib. *Lung cancer (Amsterdam, Netherlands).* 2016;108:228-231. https://pubmed.ncbi.nlm.nih.gov/28625641/.

310. Ou SI, Young L, Schrock AB, et al. Emergence of preexisting MET Y1230C mutation as a resistance mechanism to crizotinib in NSCLC with MET exon 14 skipping. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer.* 2017;12(1):137-140. https://pubmed.ncbi.nlm.nih.gov/27666659/.

311. Lai AZ, Schrock AB, Erlich RL, et al. Detection of an ALK fusion in colorectal carcinoma by hybrid capture-based assay of circulating tumor DNA. *The oncologist.* 2017;22(7):774-779. https://pubmed.ncbi.nlm.nih.gov/28507204/.

312. Mayor P, Gay LM, Lele S, Elvin JA. BRCA1 reversion mutation acquired after treatment identified by liquid biopsy. *Gynecologic oncology reports.* 2017;21:57-60. https://pubmed.ncbi.nlm.nih.gov/28706968/.