FoundationOne® Liquid CDx

Technical Information

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1 Intended Use
FoundationOne Liquid CDx is a qualitative next generation sequencing based *in vitro* diagnostic test that uses targeted high throughput hybridization-based capture technology to detect and report substitutions, insertions and deletions (indels) in 311 genes, including rearrangements in eight (8) genes and copy number alterations in three (3) genes. FoundationOne Liquid CDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood of cancer patients collected in FoundationOne Liquid CDx cfDNA blood collection tubes included in the FoundationOne Liquid CDx Blood Sample Collection Kit. 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 1 in accordance with the approved therapeutic product labeling.

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Biomarker(s) Detected</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-small cell lung cancer (NSCLC)</td>
<td>ALK rearrangements</td>
<td>ALECENSA® (alectinib)</td>
</tr>
<tr>
<td></td>
<td>EGFR exon 19 deletions and EGFR exon 21 L858R substitution</td>
<td>EGFR tyrosine kinase inhibitors approved by FDA*</td>
</tr>
<tr>
<td></td>
<td>EGFR exon 20 insertions</td>
<td>EXKIVITY® (mobocertinib)</td>
</tr>
<tr>
<td></td>
<td>MET single nucleotide variants (SNVs) and indels that lead to MET exon 14 skipping</td>
<td>TABRECTA® (capmatinib)</td>
</tr>
<tr>
<td></td>
<td>ROS1 fusions**</td>
<td>ROZLYTREK® (entrectinib)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>BRCA1, BRCA2, ATM alterations</td>
<td>LYNPARZA® (olaparib)</td>
</tr>
<tr>
<td></td>
<td>BRCA1, BRCA2 alterations</td>
<td>RUBRACA® (rucaparib)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>PIK3CA mutations C420R, E542K, E545A, E545D [1635G&gt;T only], E545G, E545K, Q546E, Q546R; and H1047L, H1047R, and H1047Y</td>
<td>PIQRAY® (alpelisib)</td>
</tr>
<tr>
<td>Solid Tumors</td>
<td>NTRK1/2/3 fusions**</td>
<td>ROZLYTREK® (entrectinib)</td>
</tr>
<tr>
<td>Colorectal Cancer (CRC)</td>
<td>BRAF V600E alteration</td>
<td>BRAFTOVI® (encorafenib) in combination with cetuximab</td>
</tr>
</tbody>
</table>


Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms.

A negative result from a plasma specimen does not mean that the patient's tumor is negative for genomic findings. Patients with the tumor types above who are negative for the mutations listed in Table 1 (see **Note for NTRK1/2/3 and ROS1 fusions) should be reflexed to routine biopsy and their tumor mutation status confirmed using an FDA-approved tumor tissue test, if feasible.
**Note: When considering eligibility for ROZLYTREK® based on the detection of NTRK1/2/3 and ROS1 fusions, testing using plasma specimens is only appropriate for patients for whom tumor tissue is not available for testing.**

Genomic findings other than those listed in **Table 1** 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.

2 **Contraindication**

There are no known contraindications.

3 **Warnings and Precautions**

- Alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. If a reported alteration is suspected to be germline, confirmatory testing should be considered in the appropriate clinical context.

- The test is not intended to replace germline testing or to provide information about cancer predisposition.

- Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an FDA-approved tumor tissue test, if possible.

4 **Limitations**

1. For in vitro diagnostic use only.

2. For prescription use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.

3. Genomic findings other than those listed in **Table 1** of the intended use are not prescriptive or conclusive for labeled use of any specific therapeutic product.

4. A negative result does not rule out the presence of an alteration in the patient’s tumor.

5. Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient’s condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.

6. The test is intended to be performed on specific serial number-controlled instruments by Foundation Medicine, Inc.

7. Genomic findings from cfDNA may originate from circulating tumor DNA fragments, germline alterations, or nontumor somatic alterations, such as clonal hematopoiesis of indeterminate potential (CHIP). Genes with alterations that may be derived from CHIP include, but are not limited to, the following: **ASXL1, ATM, CBL, CHEK2, DNMT3A, JAK2, KMT2D (MLL2), MPL, MYD88, SF3B1, TET2, TP53**, and **U2AF1**. The efficacy of targeting such nontumor somatic alterations (e.g., CH) is unknown.

8. The false positive rate of this test was evaluated in healthy donors. The detection rate for unique short variants in apparently healthy patients is 0.82%. Across 30,622 short variants, 58 variants had a detection rate of greater than 5%.

9. The analytical accuracy for the FoundationOne Liquid CDx assay has not been demonstrated in all genes.

10. The analytical accuracy for the FoundationOne Liquid CDx assay for the detection of SNVs and indels that lead to **MET** exon 14 skipping has not been demonstrated for samples with variant allele frequencies (VAF) below 0.34% for base substitutions and 0.73% VAF for small insertions and small deletions.
11. The analytical accuracy for the FoundationOne Liquid CDx assay for detection of **EGFR** exon 20 insertions has not been demonstrated for samples with <0.18% VAF.

12. The analytical accuracy for the FoundationOne Liquid CDx assay for detection of **BRAF** V600E alteration has not been demonstrated for samples with <1.02% VAF.

13. TABRECTA® efficacy has not been established in patients with **MET** SNVs <0.21% VAF and in patients with **MET** indels <0.16% VAF tested with FoundationOne Liquid CDx.

14. ALECENA® efficacy has not been established in patients with ALK rearrangements <0.06% VAF tested with FoundationOne Liquid CDx.

15. LYNPARZA® efficacy has not been established in prostate cancer patients with BRCA1/2 or ATM rearrangements with <0.25% VAF or with short variants in BRCA1/2 or ATM <0.11% VAF tested with FoundationOne Liquid CDx.

16. RUBRACA® efficacy has not been established in prostate cancer patients with BRCA1/2 rearrangements with <0.85% VAF or with short variants in BRCA1/2 <0.15% VAF tested with FoundationOne Liquid CDx.

17. PIQRAY® efficacy has not been established in patients with PIK3CA SNVs with <0.14% VAF tested with FoundationOne Liquid CDx.

18. EXKIVITY® efficacy has not been established in patients with **EGFR** exon 20 insertions <0.20% VAF tested with FoundationOne Liquid CDx.

19. BRAFTOVI® (encorafenib) in combination with cetuximab efficacy has not been established in patients with **BRAF** V600E alteration <0.11% VAF tested with FoundationOne Liquid CDx.

20. The precision of FoundationOne Liquid CDx was only confirmed for select variants at the limit of detection (LoD).

21. The FoundationOne Liquid CDx assay does not detect heterozygous deletions.

22. The FoundationOne Liquid CDx assay does not detect copy number losses/homozygous deletions in **ATM**.

23. A complete assessment of the impact of cfDNA blood collection tube lot-to-lot variability on the performance of the test has not been evaluated.

24. The test is not intended to provide information on cancer predisposition.

25. **BRCA1/BRCA2** homozygous deletions and rearrangements were not adequately represented in all analytical studies.

26. Representation of **ALK** rearrangements were limited in the analytical validation studies.

27. The representation of **ATM** short variants and rearrangements was limited in the analytical validation studies.

28. Performance has not been validated for cfDNA input below the specified minimum input.

29. Representation of SNV and indels that lead to **MET** exon 14 skipping that represent biomarker rule category 1 and 2 (refer to Section 11.6 for CDx biomarker definition), were limited in the analytical validation studies.
For optimal ctDNA shed, it is recommended that blood be drawn prior to therapy or at a time of disease progression. The sensitivity of liquid biopsy is related to adequate levels of ctDNA shed. Therefore, assay performance will be dependent upon level of ctDNA shed at time of testing.

Due to the low prevalence of ROS1 fusions and NTRK1/2/3 fusions, the positive predictive value (PPV) of the test (FoundationOne Liquid CDx positive, tissue negative) may be lower than reported in test labeling.

FoundationOne Liquid CDx may miss a subset of patients with NTRK1/2/3 fusion and ROS1 fusion positive solid tumors who may derive benefit from ROZLYTREK®. In a retrospective-prospective clinical study assessing concordance between FoundationOne Liquid CDx test results in plasma and patients whose tumor tissue tested positive and was the basis for enrollment into a clinical trial, the data demonstrated that the FoundationOne Liquid CDx test did not detect approximately 46% of potential responders with NTRK1/2/3 fusions and 49% of responders with ROS1 fusions.

ROZLYTREK® efficacy has not been established in patients with NTRK2 fusions tested with FoundationOne Liquid CDx, given the low prevalence of the biomarker.

In a retrospective-prospective clinical study assessing concordance between FoundationOne Liquid CDx test results in plasma and patients whose tumor tissue tested positive and was the basis for enrollment into a clinical trial, FoundationOne Liquid CDx detected 1 of 7 different NTRK3 fusion partners. Due to the rarity of these fusions, the accuracy of FoundationOne Liquid CDx for NTRK3 fusions has not been adequately determined.

NTRK2 fusions per the FoundationOne Liquid CDx biomarker rules for NTRK1/2/3 fusions were not represented in analytical validation studies.

A study evaluating the concordance to a second method demonstrated that the agreement between FoundationOne Liquid CDx positive results and a comparator method for NTRK1/3, and ROS1 was ≤ 50% (i.e., whether these are potential FoundationOne Liquid CDx false positives or false negatives by the comparator is unknown).

5 Test Principle
The FoundationOne Liquid CDx (F1LCDx) assay is performed exclusively as a laboratory service using circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood from patients with solid malignant neoplasms. The assay employs a single DNA extraction method to obtain cfDNA from plasma from whole blood. Extracted cfDNA undergoes whole-genome shotgun library construction and hybridization-based capture of 324 cancer-related genes. All coding exons of 309 genes are targeted; select intronic or non-coding regions are targeted in fifteen of these genes (refer to Table 2 for the complete list of genes reported by FoundationOne Liquid CDx). Hybrid-capture selected libraries are sequenced with deep coverage using the NovaSeq® 6000 platform. Sequence data are processed using a custom analysis pipeline designed to detect genomic alterations, including base substitutions and indels in 311 genes, copy number variants in three genes, and genomic rearrangements in eight genes. A subset of targeted regions in 75 genes is baited for enhanced sensitivity.

Table 2. 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 (indicated with an *).
Select regions in 75 genes (indicated in bold) are captured with increased sensitivity. Genes are captured for increased sensitivity with complete exonic (coding) coverage unless otherwise noted.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Exons</th>
<th>Intron</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>Exons 11-18, Intron 7-10</td>
<td></td>
</tr>
<tr>
<td>BRCAL</td>
<td>Intron 2, 7, 8, 12, 16, 19, 20</td>
<td></td>
</tr>
<tr>
<td>BRCA2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRD4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRIP1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTG1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTG2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BTK</td>
<td>Exons 2, 15</td>
<td></td>
</tr>
<tr>
<td>CALR</td>
<td>CARD11</td>
<td>CASP8</td>
</tr>
<tr>
<td>CD70</td>
<td>CD74* [Introns 6-8]</td>
<td>CD79A</td>
</tr>
<tr>
<td>CDK8</td>
<td>CDKN1A</td>
<td>CDKN1B</td>
</tr>
<tr>
<td>CREBBP</td>
<td>CRKL</td>
<td>CSF1R</td>
</tr>
<tr>
<td>CYP17A1</td>
<td>DAXX</td>
<td>DDR1</td>
</tr>
<tr>
<td>EPHA3</td>
<td>EPHB1</td>
<td>EPHB4</td>
</tr>
<tr>
<td>FANCL</td>
<td>FAS</td>
<td>FBXW7</td>
</tr>
<tr>
<td>IGF1R</td>
<td>IKBKE</td>
<td>IKZF1</td>
</tr>
<tr>
<td>JUN</td>
<td>KDM5A</td>
<td>KDM5C</td>
</tr>
<tr>
<td>KMT2D (MLL2)</td>
<td>KRAS</td>
<td>LTK</td>
</tr>
<tr>
<td>MAPK1</td>
<td>MCL1</td>
<td>MDM2</td>
</tr>
<tr>
<td>MKNK1</td>
<td>MLH1</td>
<td>MPL [Exon 10]</td>
</tr>
<tr>
<td>MUTYH</td>
<td>MYB* [Intron 14]</td>
<td>MYC1 [Intron 1]</td>
</tr>
</tbody>
</table>
The classification criteria for all CDx variants are outlined at the end of this document. The output of the test includes:

**Category 1:** Companion Diagnostic (CDx) claims noted in Table 1 of the Intended Use

**Category 2:** cfDNA Biomarkers with Strong Evidence of Clinical Significance in cfDNA

**Category 3:** Biomarkers with Evidence of Clinical Significance in tissue supported by:

- 3A: strong analytical validation using cfDNA
- 3B: analytical validation using cfDNA

**Category 4:** Other Biomarkers with Potential Clinical Significance

As part of its FDA-approved intended use, copy number alterations and rearrangements are reported in the genes listed in Table 3.

### Table 3. Genes for which copy number alterations and rearrangements are reported for tumor profiling by FoundationOne Liquid CDx

<table>
<thead>
<tr>
<th>Alteration Type</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copy Number Alterations</td>
<td>BRCA1, BRCA2, ERBB2</td>
</tr>
<tr>
<td>Rearrangements</td>
<td>ALK, BRCA1, BRCA2, NTRK1, NTRK2, NTRK3</td>
</tr>
</tbody>
</table>
6 FoundationOne Liquid CDx cfDNA Blood Specimen Collection Kit Contents

Test Kit Contents
The test includes a sample shipping kit, which is sent to ordering laboratories and physicians. The shipping kit contains the following components:

- Specimen preparation and shipping instructions
- Two FoundationOne Liquid CDx cfDNA blood collection tubes (8.5 mL nominal fill volume per tube)
- Return shipping label

All other reagents, materials and equipment needed to perform the assay are used exclusively in the Foundation Medicine laboratory. The FoundationOne Liquid CDx assay is intended to be performed with serial number-controlled instruments.

7 FoundationOne Liquid CDx Test Ordering
To order FoundationOne Liquid CDx, the test order form in the test kit must be fully completed and signed by the ordering physician or other authorized medical professional. Please refer to Specimen Preparation Instructions and Shipping Instructions included in the test kit.

For more detailed information, including Performance Characteristics, please find the FDA Summary of Safety and Effectiveness Data at: https://www.accessdata.fda.gov/cdrh_docs/pdf19/P190032B.pdf

8 Instruments
The FoundationOne Liquid CDx device is intended to be performed with the following instruments, as identified by specific serial numbers:
- Illumina NovaSeq 6000
- Thermo Scientific Kingfisher Flex DW 96
- Hamilton STARTlet-STAR Liquid Handling Workstation

9 Performance Characteristics
Performance characteristics were established using contrived and clinical circulating cfDNA derived from blood specimens extracted from a wide range of tumor types. Table 4 below provides a summary of the number of tumor types and variants included in each study. As summarized in this table, each study included a broad range of representative alteration types (substitutions, insertion-deletions, copy number alterations, rearrangements) in various genomic contexts across a number of genes. The validation studies included >7,000 sample replicates, >31,000 unique variants [includes variants classified as variants of unknown significance (VUS) and/or benign], >30 tumor types, representing all 324 genes targeted by the assay.

Table 4. Representation of tumor types and variants\(^1\) across validation studies

<table>
<thead>
<tr>
<th>Study Title</th>
<th>Cancer Types Represented</th>
<th># Unique Samples</th>
<th># of Sample Replicates</th>
<th># of Unique Genes</th>
<th># of Unique</th>
<th>Copy Number Amplif.</th>
<th>Copy Number Losses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subs</td>
<td>Indels</td>
<td>Rearrang.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contrived Sample Functional Characterization (CSFC) Study</td>
<td>Breast cancer</td>
<td>13</td>
<td>1843</td>
<td>228</td>
<td>563</td>
<td>81</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Colorectal cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Contrived samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FoundationOne Liquid CDx to Validated NGS Tumor Tissue Test Concordance: BRCA1 and BRCA2 Variants</td>
<td>Prostate cancer</td>
<td>279</td>
<td>N/A</td>
<td>2</td>
<td>100</td>
<td>87</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Ovarian cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Includes variants classified as variants of unknown significance (VUS) and/or benign.
<table>
<thead>
<tr>
<th>Study Title</th>
<th>Cancer Types Represented</th>
<th># Unique Samples</th>
<th># of Sample Replicates</th>
<th># of Unique Genes</th>
<th># of Unique Subs</th>
<th>Indels</th>
<th>Rearrang.</th>
<th>Copy Number Amplif.</th>
<th>Copy Number Losses</th>
</tr>
</thead>
<tbody>
<tr>
<td>FoundationOne Liquid CDx to Validated NGS cfDNA Assay</td>
<td>Breast cancer</td>
<td>412</td>
<td>N/A</td>
<td>1</td>
<td>32</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Concordance: PIK3CA mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Orthogonal Concordance</td>
<td>23 cancer types</td>
<td>278</td>
<td>N/A</td>
<td>64</td>
<td>541</td>
<td>12</td>
<td>11</td>
<td>3</td>
<td>0</td>
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<tr>
<td>Contrived samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LoD Estimation</td>
<td>Prostate Contrived samples</td>
<td>10</td>
<td>877</td>
<td>286</td>
<td>1490</td>
<td>247</td>
<td>32</td>
<td>13</td>
<td>3</td>
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<tr>
<td>LoB Study 1</td>
<td>Healthy Donors</td>
<td>28</td>
<td>79</td>
<td>322</td>
<td>26134</td>
<td>4482</td>
<td>911</td>
<td>222</td>
<td>42</td>
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<tr>
<td>LoB Study 2</td>
<td>Healthy Donors</td>
<td>44</td>
<td>131</td>
<td>532</td>
<td>29507</td>
<td>4438</td>
<td>2752</td>
<td>222</td>
<td>42</td>
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<tr>
<td>Potentially Interfering Substances</td>
<td>Contrived samples</td>
<td>9</td>
<td>336</td>
<td>18</td>
<td>16</td>
<td>11</td>
<td>11</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hybrid Capture Bait Specificity</td>
<td>25 cancer types</td>
<td>3546</td>
<td>N/A</td>
<td>324</td>
<td>N/A</td>
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<td>Platform Precision study 1</td>
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<td>1121</td>
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<td>166</td>
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<td># of Sample Replicates</td>
<td># of Unique Genes</td>
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<td># of Unique Rearrang.</td>
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<td>Copy Number Losses</td>
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<td>Whole Blood Sample Stability</td>
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<td>74</td>
<td>148</td>
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<td>20</td>
<td>17</td>
<td>12</td>
<td>12</td>
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<td>Guard Banding with updated LC input$^3$</td>
<td>Contrived samples</td>
<td>7</td>
<td>105</td>
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<td>Clinical validation for detection of EGFR exon 19 deletions and L858R alterations: non-inferiority study$^2$</td>
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<td>177</td>
<td>N/A</td>
<td>1</td>
<td>5</td>
<td>7</td>
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<td>Clinical validation study for detection of SNVs and indels that lead to MET exon 14 skipping$^2$</td>
<td>Lung Cancer</td>
<td>171$^2$</td>
<td>N/A</td>
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<td>Clinical validation study for detection of rearrangements that lead to NTRK fusions$^2$</td>
<td>Solid Tumor</td>
<td>203</td>
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<td>Lung Cancer</td>
<td>203</td>
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<td># of Unique Genes</td>
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<td>Pan-tumor performance (includes historical analysis)</td>
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<td>324</td>
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<td>N/A</td>
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<td>927</td>
<td>N/A</td>
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<td>1815</td>
<td>376</td>
<td>109</td>
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<td>N/A</td>
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<td>FoundationOne Liquid CDx to Validated cfDNA NGS Assay Concordance: $MET$ exon 14 (Primary Analysis)</td>
<td>Lung Cancer</td>
<td>172</td>
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<td>11</td>
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<tr>
<td>FoundationOne Liquid CDx to Validated cfDNA NGS Assay Concordance: $NTRK$ fusions$^4$</td>
<td>Solid Tumor</td>
<td>116</td>
<td>N/A</td>
<td>5</td>
<td>N/A</td>
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<td>4</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Precision and LoD Confirmation of $NTRK$ Gene Fusions in a Pan-tumor Setting$^4$</td>
<td>Solid Tumor</td>
<td>4</td>
<td>93</td>
<td>6</td>
<td>N/A</td>
<td>N/A</td>
<td>4</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Study Title</td>
<td>Cancer Types Represented</td>
<td># Unique Samples</td>
<td># of Sample Replicates</td>
<td># of Unique Genes</td>
<td># of Unique Subs</td>
<td>Indels</td>
<td>Rearrang.</td>
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</tr>
<tr>
<td>FoundationOne Liquid CDx to Validated cfDNA NGS Assay Concordance: EGFR exon 20 insertions</td>
<td>Lung Cancer</td>
<td>151</td>
<td>N/A</td>
<td>1</td>
<td>N/A</td>
<td>25</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Precision and LoD Confirmation of EGFR exon 20 insertions</td>
<td>Lung Cancer</td>
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<td>72</td>
<td>1</td>
<td>N/A</td>
<td>3</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>FoundationOne Liquid CDx to Validated cfDNA NGS Assay Concordance: BRAF V600E alteration</td>
<td>Colorectal Cancer</td>
<td>189</td>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Precision and Confirmation of LoD of BRAF V600E alteration</td>
<td>Colorectal Cancer</td>
<td>1</td>
<td>24</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</table>

1Variants detected may include variants classified as VUS and benign.
2Clinical validation study was conducted using the original LC input range for F1LCDx (30ng-80ng, with conditional processing of samples between 20-30ng)
3Study was conducted to validate the new LC input range for F1LCDx (20ng-60ng).

### 9.1 Concordance – Comparison to an Orthogonal cfDNA NGS Method #1

The detection of short variants and rearrangements by the FoundationOne Liquid CDx assay was compared to that of an externally validated cfDNA next generation sequencing (NGS) assay in 74 genes common to both assays across 278 samples that represented an array of tumor types (>50 unique disease ontologies across 23 cancer types). The cancer types (#samples) included lung [NSCLC (75) and other (3)]; breast (54); prostate (32); colorectal [colon (27) and rectal (6)]; liver (11); ovarian (6); pancreas (9); gastrointestinal (7); bile duct (2); esophageal (5); skin (6); cervical (1); anal (1); bladder (1); gallbladder (1); salivary gland (2); thymus (1); thyroid (3); uterine (2); fallopian tube (1); head and neck (1); soft tissue (1); and unknown primary (19). The study included samples selected from clinical FoundationOne Liquid testing (n=268) and contrived samples consisting of fragmented gDNA diluted in clinical cfDNA to represent rare alterations (n=10).

Using the externally validated NGS assay as the comparator, the analysis demonstrated a short variant positive percent agreement (PPA) of 96.2% with a 95% two-sided confidence interval (CI) of [94.8%-97.4%]. The short variant negative percent agreement (NPA) was >99.9% with a 95% two-sided CI of [99.9%-100.0%]. The respective PPA of base substitutions and indels with a 95% two-sided CI was 96.1% [94.6%-97.3%] and 100.0% [85.2%-100.0%]. The respective NPA and 95% two-sided CI of base substitutions and indels was >99.9% [99.9%-100.0%] and 100.0% [99.89%-100.0%] (Table 5).

#### Table 5. Concordance of short variants called in FoundationOne Liquid CDx and the cfDNA comparator assay (n= 902 positive variants, n= 152,832 negative variants* by the comparator assay)

<table>
<thead>
<tr>
<th>Variant Type</th>
<th>FoundationOne Liquid CDx(+) Comparator(+)</th>
<th>FoundationOne Liquid CDx(+) Comparator(-)</th>
<th>FoundationOne Liquid CDx(-) Comparator(+)</th>
<th>FoundationOne Liquid CDx(-) Comparator(-)</th>
<th>PPA [95% CI]</th>
<th>NPA [95% CI]</th>
<th>OPA [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Short Variants</td>
<td>868</td>
<td>34</td>
<td>8</td>
<td>152824</td>
<td>96.2% [94.8%-97.4%]</td>
<td>&gt;99.9% [99.9%-100.0%]</td>
<td>&gt;99.9% [99.9%-100.0%]</td>
</tr>
</tbody>
</table>
These data demonstrate that the FoundationOne Liquid CDx assay and an externally-validated NGS assay are highly concordant across the 76 genes common between the two panels.
9.2 Concordance – FoundationOne Liquid CDx to validated NGS tumor tissue assay (BRCA1 and BRCA2 alterations)

Samples from a total of 279 prostate and ovarian cancer patients were tested and the concordance evaluated between FoundationOne Liquid CDx and the validated NGS tumor tissue assay for the detection of deleterious alterations in BRCA1 or BRCA2. As summarized below, a PPA of 88.03% and an NPA of 95.68% were observed on a sample level (Table 8). As summarized in Table 9 an overall PPA of 87.28% and an NPA of 99.83% were observed at the variant level. Some discordance is expected based on biological differences and sampling times between tumor tissue and plasma samples. Considering the impact of biological differences between analytes, these data demonstrate a high concordance between FoundationOne Liquid CDx and the validated NGS tumor tissue assay for the detection of deleterious alterations in BRCA1 or BRCA2.

Table 8. Concordance (by sample) of FoundationOne Liquid CDx and validated NGS tumor tissue assay in prostate and ovarian cancer patients for the detection of alterations in BRCA1 or BRCA2

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<th>NGS Tumor Tissue Assay</th>
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<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>FoundationOne Liquid CDx</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>103</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>PPA: 88.03%</strong></td>
<td><strong>NPA: 95.68%</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>[80.91%-92.74%]</td>
<td>[91.35%-97.89%]</td>
<td></td>
</tr>
</tbody>
</table>

Table 9. Concordance (by variant) of FoundationOne Liquid CDx and validated NGS tumor tissue assay in prostate and ovarian cancer patients for the detection of alterations in BRCA1 or BRCA2

<table>
<thead>
<tr>
<th></th>
<th>F1LCDx+/Tissue+</th>
<th>F1LCDx-/Tissue-</th>
<th>F1LCDx+/Tissue+</th>
<th>F1LCDx-/Tissue-</th>
<th>PPA (95% CI)</th>
<th>NPA (95% CI)</th>
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<td>Substitutions</td>
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<td>29</td>
<td>20255</td>
<td><strong>92.77%</strong></td>
<td><strong>99.86%</strong></td>
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<td>Indels</td>
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<td>16362</td>
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<td>7</td>
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<td><strong>57.14%</strong></td>
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<td>1</td>
<td>263</td>
<td><strong>33.33%</strong></td>
<td><strong>99.62%</strong></td>
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<td>22</td>
<td>68</td>
<td>38819</td>
<td><strong>87.28%</strong></td>
<td><strong>99.83%</strong></td>
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</table>

9.3 Concordance – Comparison to an Orthogonal cfDNA NGS Method #2

The accuracy of using FoundationOne Liquid CDx as a companion diagnostic to identify breast cancer patients harboring PIK3CA alterations was assessed with residual plasma samples from the SOLAR-1 clinical trial. Of the remaining plasma samples, 542 were evaluable by the externally-validated NGS method and produced valid results. 418 were evaluable by FoundationOne Liquid CDx, of which 192 positive variants were detected across 188 patients, with four patients possessing two positive variants each. The distribution of counts per positive variant is listed in Table 10.

Table 10. Distribution of variants detected with FoundationOne Liquid CDx evaluable samples.

<table>
<thead>
<tr>
<th>Protein Effect in PIK3CA</th>
<th># Variant Calls (188 Positive Samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C420R</td>
<td>3</td>
</tr>
<tr>
<td>E542K</td>
<td>25</td>
</tr>
<tr>
<td>E545A</td>
<td>1</td>
</tr>
<tr>
<td>E545G</td>
<td>2</td>
</tr>
<tr>
<td>Protein Effect</td>
<td>PIK3CA</td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
</tr>
<tr>
<td>E545K</td>
<td></td>
</tr>
<tr>
<td>H1047L</td>
<td></td>
</tr>
<tr>
<td>H1047R</td>
<td></td>
</tr>
<tr>
<td>H1047Y</td>
<td></td>
</tr>
<tr>
<td>Q546R</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
</tr>
</tbody>
</table>

A total of 412 valid samples generated valid results with both assays. The primary analysis using NGS Method #2 as the reference assay achieved a PPA [95% CI] of 97.06% [93.27%, 99.04%], and an NPA [95% CI] of 91.74% [87.52%, 94.88%]. The contingency table for this comparison is provided in Table 11, with counts representing number of samples (versus number of variant calls).

The sample counts in the core 2x2 white boxes total to 412 samples. There were seven samples evaluable with FoundationOne Liquid CDx but failed (italicized in Table 11), as well as three samples missing from reference assay data. There were five samples unevaluable by the reference assay; three of these aligned with the 418 evaluable FoundationOne Liquid CDx samples, while two were among the 130 samples not evaluable due to insufficient plasma.

Table 11. Contingency table comparing FoundationOne Liquid CDx with the reference assay, primary analysis with 412 cases.

<table>
<thead>
<tr>
<th>FoundationOne LiquidCDx</th>
<th>Reference Assay</th>
<th>Positive</th>
<th>Negative</th>
<th>Not Evaluable</th>
<th>Missing</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>165</td>
<td>20</td>
<td>2</td>
<td>1</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>5</td>
<td>222</td>
<td>1</td>
<td>2</td>
<td>230</td>
</tr>
<tr>
<td></td>
<td>Evaluable but Failed</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Not Evaluable</td>
<td>35</td>
<td>93</td>
<td>2</td>
<td>0</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>205</td>
<td>342</td>
<td>5</td>
<td>3</td>
<td>555</td>
</tr>
</tbody>
</table>

PPA<sub>ONC</sub>: 97.06% [93.27%, 99.04%]  
NPA<sub>ONC</sub>: 91.74% [87.52%, 94.88%]  
OPA: 93.93% [91.17%, 96.04%]

9.4 Concordance – FoundationOne Liquid CDx to an externally validated cfDNA NGS assay (SNVs and indels that lead to MET exon 14 skipping)

An analytical accuracy study was performed to demonstrate the concordance between FoundationOne Liquid CDx (F1LCDx) and an externally validated cfDNA NGS comparator (evNGS) assay for the detection of SNVs and indels that lead to MET exon 14 skipping. Overall, there were 74 overlapping genes targeted by the two assays and the comparator assay bait set covered the same regions as the FoundationOne Liquid CDx bait set.

The analytical accuracy study was conducted with 45 samples from the clinical bridging study with 41 samples from patients enrolled in the GEOMETRY-mono 1 trial (refer to Section 10.7 below). An additional 100 NSCLC samples were sourced from FMI’s clinical archives, 38 samples from NSCLC patients previously evaluated in the accuracy study to support the original PMA P190032 (refer to section 9.1 above) and 31 externally sourced plasma samples from NSCLC cases whose tissue specimens tested positive for MET exon 14 skipping alterations and were subsequently tested with F1LCDx to determine their MET exon 14 skipping associated alteration status prior to conducting the accuracy study statistical analysis. Samples selected from FMI’s clinical archives that were positive for MET exon 14 skipping alterations had to have a variant allele frequency (VAF) greater than or equal 0.40%.
Of the 214 samples, 179 samples had DNA yield that allowed processing with F1LCDx at the specified LC DNA input of 30ng-80ng. Thirty-five (35) samples were tested with F1LCDx at a lower LC DNA input of out of specification of 20ng<30ng LC DNA input. Of the 179 samples that had sufficient DNA yield for testing with F1LCDx, 3 samples had a F1LCDx sequence analysis QC failure, while 4 had an evNGS QC failure.

The primary analytical concordance analysis, using the evNGS assay results as the reference, included 172 samples that passed QC with both assays. Forty-eight (48) of the 172 samples were identified as positive for MET exon 14 skipping alterations by FoundationOne Liquid CDx. The statistical analysis using the evNGS assay results as the reference showed a PPA of 94.87% with 95% CI (83.11%-98.58%), a NPA of 91.83% with 95% CI (85.80%, 95.32%), a PPV of 77.08% with 95% CI (63.46%, 86.69%) and a negative predictive value (NPV) of 98.39% with 95% CI (94.31%, 99.56%) as shown in Table 12. Since the samples were selected from different sources based on different assays, the unadjusted PPA/NPA and unadjusted PPV/NPV in Table 12 may be subject to potential bias.

Table 12. Primary Concordance Analysis Comparing Sample-level Biomarker Detection between FoundationOne Liquid CDx and Comparator Assay

<table>
<thead>
<tr>
<th>F1LCDx</th>
<th>evNGS</th>
<th>MET ex14 positive</th>
<th>MET ex14 negative</th>
<th>Total</th>
<th>PPV/NPV(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MET ex14 positive</td>
<td>37</td>
<td>11</td>
<td>48</td>
<td>PPV: 77.08% (63.46%, 86.69%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>133</td>
<td>172</td>
<td>NPV: 98.39% (94.31%, 99.56%)</td>
<td></td>
</tr>
</tbody>
</table>

Ten (10) of the eleven (11) samples that were F1LCDx-positive/evNGS-negative [F1LCDx(+)/evNGS(-)] were discordant due to differences in variant reporting by assays. Of the 11 samples, 10 samples harbored MET exon 14 deletions ≥6bp detectable by the evNGS variant caller, which calls variants in the evNGS’s loci of interest (LOI) and indels ≥6bp in MET exon 14. Since MET exon 14 indels ≥6bp are not part of the evNGS’s LOI, this variant type is filtered out and not reported by the evNGS’s analysis software in the default setting, and thus are considered negatives by the evNGS comparator assay. Further the remaining one (1) sample from the 11 samples that were F1LCDx (+)/evNGS(-), contained a MET exon 14 deletion <6bp which cannot be called with the evNGS variant because the variant caller can only output MET exon 14 deletions ≥6bp. The evNGS reporting rules only correspond to biomarker rule category 3, so all 37 samples that were F1LCDx(+)/evNGS(+) had MET exon 14 skipping alterations that correspond to biomarker rule category 3, i.e., these samples had base substitutions and indels affecting positions 0, +1, +2, or +3 at the splice donor site of the 3’ boundary of MET exon 14. The evNGS assay does not call category 1 and 2 biomarkers as they are not included in their LOI. In the two (2) discordant samples that were F1LCDx negative(-)/evNGS(+), base substitutions reported by the evNGS were not detected in the variant analysis pipeline of F1LCDx.

Four (4) of the eleven (11) discordant samples that were F1LCDx(+)/evNGS(-) were from patients evaluated in the clinical therapeutic study for whom efficacy data was available. Of these 4 patients, 3 had partial response to TABRECTA, while one had progressive disease. Although these patients had discordant results, these results appear to suggest that these patient with F1LCDx(+)/evNGS(-) were MET exon 14 deletion positive.

9.5 Concordance – FoundationOne Liquid CDx to an externally validated cfDNA NGS assay (NTRK1/2/3 Fusions)

An analytical accuracy study was performed to demonstrate the concordance between FoundationOne Liquid CDx and an externally validated cfDNA NGS comparator assay for the detection of NTRK fusions. For this study, seven (7) residual cfDNA samples were selected from patients enrolled in the STARTRK-2 trial used to support the effectiveness of the device, seven (7) residual cfDNA clinical samples were externally sourced, and 102 residual cfDNA samples were sourced from FMI’s clinical archives. Overall, a total of 116 sample replicates were processed using F1LCDx in this study. Of the 116 samples, 113 were processed with the evNGS. Of the 113
samples run by both assays for this study, one (1) sample had an F1LCDx post-sequencing QC failure, while 10 had an evNGS post-sequencing QC failure.

Measures of analytical concordance for the 102 samples that passed QC with both assays were determined. Since specimens were selected based on F1LCDx and confirmed by the evNGS agreement, PPV and NPV are estimated conditional on F1LCDx. PPV was estimated as 40% (4/10) with two-sided 95% CI (16.8%, 68.7%), and NPV as 100% (92/92) with two-sided 95% CI (95.99%, 100.00%), as shown in Table 13, below. For informational purposes, unadjusted positive percent agreement (PPA) and negative percent agreement (NPA) are also displayed.

Table 13. Concordance Analysis Comparing Sample-level Biomarker Detection between F1LCDx and evNGS

<table>
<thead>
<tr>
<th>F1LCDx</th>
<th>evNGS</th>
<th>NTRK1/2/3 fusion positive</th>
<th>NTRK1/2/3 fusion negative</th>
<th>Total</th>
<th>PPV/NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTRK1/2/3 fusion positive¹</td>
<td>4</td>
<td>6²</td>
<td>10</td>
<td>PPV: 40.0% (16.8%, 68.7%)</td>
<td></td>
</tr>
<tr>
<td>NTRK1/2/3 fusion negative</td>
<td>0</td>
<td>92</td>
<td>92</td>
<td>NPV: 100% (95.99%, 100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>98</td>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPA/NPA (Unadjusted) (95% CI)</td>
<td>PPA: 100% (51.01%, 100%)</td>
<td>NPA: 93.9% (87.3%, 97.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹No NTRK2 fusion positive samples were evaluated in this study.
²These six samples were discordant due to the fusion breakpoints falling in regions that the evNGS assay does not bait for.

The six (6) samples that were NTRK1/2/3 fusion positive by F1LCDx and NTRK1/2/3 fusion negative by the evNGS were discordant due to the fusion breakpoints falling in regions that the evNGS assay does not bait for. Specifically, the evNGS assay did not claim to generate coverage in certain regions of interest (e.g., intron 8 of NTRK1 and intron 5 of ETV6), and thus were negative by the evNGS comparator assay.

9.6 Concordance – FoundationOne Liquid CDx to an externally validated cfDNA NGS assay (EGFR exon 20 insertions)

An analytical accuracy study was performed to demonstrate the concordance between FoundationOne Liquid CDx and an externally validated cfDNA NGS comparator assay for the detection of EGFR exon 20 insertions. For this study, 101 frozen plasma samples were identified from patients enrolled in the AP32788-15-101 trial and 125 residual cfDNA samples were sourced from FMI’s clinical archives. Of the 125 residual cfDNA samples, four (4) were excluded due to diluted DNA concentration being out of acceptable range or evNGS post-sequencing QC failure. Of the 101 frozen plasma samples, 71 were excluded from the analysis due to insufficient cfDNA yield, diluted DNA concentration being out of acceptable range, or evNGS post-sequencing QC failure. Overall, a total of 151 samples from NSCLC patients were processed using both F1LCDx and an externally validated cfDNA NGS assay in this study.

Analytical concordance was determined for the 151 samples that passed QC with both assays. Since specimens were selected based on F1LCDx and confirmed by the evNGS assay, positive predictive value (PPV) and negative predictive value (NPV) are estimated conditional on F1LCDx. Forty-nine (49) of the 151 samples were identified as positive for EGFR exon 20 insertions by both F1LCDx and evNGS. The statistical analysis showed a PPV of 100% with two-sided 95% CI [92.70%-100%] and a NPV of 99.02% with two-sided 95% CI [94.65%-99.83%], as shown in Table 14 below.

Table 14. Concordance Analysis Comparing Sample-Level Biomarker Detection Between F1LCDx and evNGS

<table>
<thead>
<tr>
<th>evNGS</th>
<th>EGFR exon 20 insertion positive</th>
<th>EGFR exon 20 insertion negative</th>
<th>Total</th>
<th>PPV/NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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In the one (1) discordant sample that was F1LCDx-negative/evNGS-positive, a 3 bp *EGFR* exon 20 insertion reported by the evNGS was not detected in the variant analysis pipeline of F1LCDx.

### 9.7 Concordance – FoundationeOne Liquid CDx to an externally validated ctDNA NGS assay (*BRAF* V600E)

An analytical accuracy study was performed to demonstrate the concordance between F1LCDx and an externally validated ctDNA NGS (evNGS) comparator assay for the detection of *BRAF* V600E alterations. Overall, a total of 203 samples from CRC patients were processed using both F1LCDx and an externally validated ctDNA NGS assay in this study. 189 samples passed QC.

Analytical concordance using the evNGS assay results as the reference for the 189 samples that passed QC with both assays was determined. Since archived specimens were selected based on previous F1LCDx or F1L results and tested again by the evNGS assay and F1LCDx, calculation of percent agreement (PPA) and negative percent agreement (NPA) is presented adjusted for the enrichment of *BRAF* V600E positives in the concordance evaluation sample cohort. Adjusted PPA has a point estimate of 92.31% with a 95% two-sided CI of (78.45%, 100.00%). Adjusted NPA has a point estimate of 100.00% with a 95% two-sided CI of (96.53%, 100.00%). For informational purposes, unadjusted PPA, NPA, PPV and NPV are also displayed, as shown in Table 15, below.

<table>
<thead>
<tr>
<th></th>
<th>F1LCDx</th>
<th>evNGS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>BRAF</em> V600E alteration positive</td>
<td><em>BRAF</em> V600E alteration negative</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>81</td>
<td>0</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>1*</td>
<td>107</td>
<td>108</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>82</td>
<td>107</td>
<td>189</td>
</tr>
<tr>
<td><strong>PPA/NPA (Unadjusted) (95% CI)</strong></td>
<td><strong>PPA: 98.78% (93.41%, 99.78%)</strong></td>
<td><strong>NPA: 100% (96.53%, 100%)</strong></td>
<td></td>
</tr>
</tbody>
</table>

*This discordant sample had very low supporting reads and variant allele fraction in F1LCDx, which did not pass F1LCDx calling threshold.*

### 9.8 Limit of Detection (Analytical Sensitivity)

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, and copy number alterations. The LoD was determined using the conservative hit rate approach for the majority of variants. A probit model was used when appropriate (when ≥3 dilution levels with hit rates between 10% and 90% were observed). LoD by hit rate was defined as the mean variant allele frequency (VAF) value (for short variants and rearrangements) or mean tumor fraction (TF) value (for copy number alterations) at the lowest dilution level tested with at least 95% detection across replicates. The hit rate was computed as the number of replicates with positive variant calls per the total number of replicates tested at each level of the targeted VAF (short variants and rearrangements) or tumor fraction (copy number alterations). Short variants with hit rates of at least 95% at all dilution levels or hit rates below 95% for all dilution levels were excluded from analysis as LoD could not be reliably estimated.
Confirmed LoDs for CDx alterations are presented below in Table 16 and are taken from the confirmation of LoD studies as presented in Section 9.13. The confirmation of LoD studies utilized clinical samples assessed near the established LoD (targeting 1x-1.5x LoD). The confirmed LoD for targeted short variants, rearrangements, and copy number alterations demonstrate at least a 95% hit rate at a level near the established LoD (Table 17).

### Table 16. Established and Confirmed LoD for CDx alterations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration Subtype</th>
<th>Established LoD</th>
<th>Confirmed LoD (Fold LoD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK</td>
<td>Rearrangement</td>
<td>0.24% VAF</td>
<td>0.68% VAF (2.84x)</td>
</tr>
<tr>
<td>ATMs</td>
<td>Substitutions</td>
<td>0.51% VAF</td>
<td>0.56% VAF (1.09x)</td>
</tr>
<tr>
<td></td>
<td>Indels</td>
<td>0.51% VAF</td>
<td>0.86% VAF (1.68x)</td>
</tr>
<tr>
<td></td>
<td>Rearrangement</td>
<td>Not Determined</td>
<td>1.13% VAF (N/A)</td>
</tr>
<tr>
<td>BRAF</td>
<td>Substitutions</td>
<td>0.33% VAF</td>
<td>0.70% VAF (2.12x)</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Substitutions</td>
<td>0.34% VAF</td>
<td>0.51% VAF (1.49x)</td>
</tr>
<tr>
<td></td>
<td>Indels</td>
<td>0.38% VAF</td>
<td>0.55% VAF (1.44x)</td>
</tr>
<tr>
<td></td>
<td>Rearrangement</td>
<td>Not Determined</td>
<td>0.87% VAF (N/A)</td>
</tr>
<tr>
<td>BRCA2</td>
<td>Substitutions</td>
<td>Not Determined</td>
<td>0.71% VAF (N/A)</td>
</tr>
<tr>
<td></td>
<td>Indels</td>
<td>0.36% VAF</td>
<td>0.63% VAF (1.74x)</td>
</tr>
<tr>
<td></td>
<td>Rearrangement</td>
<td>Not Determined</td>
<td>0.48% VAF (N/A)</td>
</tr>
<tr>
<td></td>
<td>Copy Number Loss</td>
<td>48.1% TF^4</td>
<td>N/A</td>
</tr>
<tr>
<td>EGFR</td>
<td>Substitutions (L858R)</td>
<td>0.34% VAF</td>
<td>0.64% VAF (1.90x)</td>
</tr>
<tr>
<td></td>
<td>Indels (exon 19 deletions)</td>
<td>0.27% VAF</td>
<td>0.45% VAF (1.65x)</td>
</tr>
<tr>
<td></td>
<td>Indels (exon 20 insertions)</td>
<td>Not Determined</td>
<td>0.65% VAF (N/A)</td>
</tr>
<tr>
<td>MET</td>
<td>Indels (exon 14)</td>
<td>0.41% VAF</td>
<td>0.28% VAF (0.67x)</td>
</tr>
<tr>
<td></td>
<td>Substitutions (exon 14)</td>
<td>Not Determined</td>
<td>0.40% VAF (N/A)</td>
</tr>
<tr>
<td>NTRK1</td>
<td>Rearrangement</td>
<td>0.44% VAF</td>
<td>0.75% VAF (1.70x)</td>
</tr>
<tr>
<td>NTRK3</td>
<td>Rearrangement</td>
<td>0.27% VAF</td>
<td>0.68% VAF (2.52x)</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>Substitutions</td>
<td>0.34% VAF</td>
<td>0.39% VAF (1.14x)</td>
</tr>
<tr>
<td>ROS1</td>
<td>Rearrangement</td>
<td>0.52% VAF</td>
<td>1.30% VAF (2.51x)</td>
</tr>
</tbody>
</table>

1 Confirmation of LoD was performed without direct LoD establishment data. Platform LoD was used for the targeted dilution level.
2 Confirmed LoD for BRCA1 RE was using the DIBv1 primer set. LoD was also confirmed using the DIBv2 primer set at 1.27% VAF.
3 Confirmed LoD for BRCA2 RE was using the DIBv1 primer set. LoD was also confirmed using the DIBv2 primer set at 1.49% VAF.
4 LoD was established in a clinical sample and therefore confirmation of LoD was not applicable.

The platform LoD for short variants, rearrangements, and copy number losses are presented in Table 17. A total of 864 short variants were included in the platform LoD analysis. The enhanced sensitivity region of the bait set contains 269 of the short variants analyzed and the standard sensitivity region of the bait set contains 595 of the short variants analyzed. The estimated LoD for short variants is 0.40% for the enhanced sensitivity region and 0.82% of the standard sensitivity region. The median LoD is 30.4% tumor fraction for copy number losses.

Because a major component driving the detectability of a variant is genomic context (repetitiveness of the reference genomic region), the LoD analysis by alteration subtype was also evaluated within categories based on genomic context as summarized in Table 17.

### Table 17. LoD by variant subtype based on genomic context

<table>
<thead>
<tr>
<th>Region</th>
<th>Alteration Subtype</th>
<th>LoD Unit</th>
<th>N</th>
<th>Minimum LoD</th>
<th>1st Quantile LoD</th>
<th>Median LoD</th>
<th>3rd Quantile LoD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhanced Sensitivity Region Total</td>
<td>Short Variants: Enhanced Sensitivity Region Total</td>
<td>VAF</td>
<td>269</td>
<td>0.20%</td>
<td>0.33%</td>
<td>0.40%</td>
<td>0.50%</td>
</tr>
</tbody>
</table>
The median LoD for highly-actionable, non-CDx alterations evaluated for LoD are presented in Table 18. The median LoD for these targeted short variants are consistent with the platform LoD presented in Table 16.

### Table 18. LoD for non-CDx alterations

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration Subtype</th>
<th>Number of Samples Evaluated</th>
<th>Median LoD¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>Substitutions</td>
<td>1</td>
<td>0.33% VAF</td>
</tr>
<tr>
<td>KRAS</td>
<td>Substitutions</td>
<td>2</td>
<td>0.33% VAF</td>
</tr>
<tr>
<td>MET²</td>
<td>Indels</td>
<td>1</td>
<td>0.41% VAF</td>
</tr>
<tr>
<td>NRAS</td>
<td>Substitutions</td>
<td>2</td>
<td>0.42% VAF</td>
</tr>
<tr>
<td>PALB2</td>
<td>Indels, Substitutions</td>
<td>2</td>
<td>0.37% VAF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0.51% VAF</td>
</tr>
<tr>
<td>ERBB2</td>
<td>Copy Number Amplification</td>
<td>1</td>
<td>19.8% TF</td>
</tr>
</tbody>
</table>

VAF = variant allele frequency  
TF = tumor fraction  
¹Quantitative reporting of %VAF/%TF has not been approved by FDA.  
²This LoD applies to MET alterations that do not meet the CDx rules.
9.9 Limit of Blank (LoB)
Per CLSI EP17-A2, the limit of blank (LoB) was established by profiling plasma samples from 30 asymptomatic donors with no diagnosis of cancer with 4 replicates per sample. All donors were over the age of 60 with a median age of 68 and included 15 smokers and 15 non-smokers.

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, five variants of unknown significance 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%. Each cancer-related alteration detected in this study was detected in replicates from a single donor, indicating that these are likely true variants present in the sample. On a per unique variant basis (number of unique variants detected at least once across all replicates divided by the total number of unique variants included in the analysis), the overall detection rate for short variants in this study was 0.82%. On a per total variant basis (number of variants detected across all replicates divided by the total number of variants included in the analysis across all replicates), the overall detection rate for short variants in this study was 0.027% (Table 19).

### Table 19. Detection rate for each reporting category in LoB study

| Category | Unique Variant Detection Rate (Unique variants detected) / (total unique variants analyzed) | Total Variant Detection Rate (Total variants detected) / (total variants analyzed)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>0% (0 of 292)</td>
<td>0% (0 of 23,068)</td>
</tr>
<tr>
<td>Level 2</td>
<td>0% (0 of 10)</td>
<td>0% (0 of 790)</td>
</tr>
<tr>
<td>Level 3</td>
<td>0% (0 of 18)</td>
<td>0% (0 of 1,422)</td>
</tr>
<tr>
<td>Level 4</td>
<td>0.82% (47 of 5,760)</td>
<td>0.024% (107 of 455,040)</td>
</tr>
<tr>
<td>VUS</td>
<td>0.83% (203 of 24,542)</td>
<td>0.029% (555 of 1,938,818)</td>
</tr>
<tr>
<td>All categories</td>
<td>0.82% (250 of 30,622)</td>
<td>0.027% (662 of 2,419,138)</td>
</tr>
</tbody>
</table>

1 total variants analyzed = unique variants * 79 replicates

Across 264 copy number alterations and 894 rearrangements, zero variants were detected. These results demonstrate the high specificity of FoundationOne Liquid CDx.

A supplemental LoB study was performed for F1LCDx to support the updated LC input range (20ng-60ng) and evaluate variants observed in gDNA. Whole blood samples from 44 healthy donors were collected to prepare two plasma cfDNA replicates per donor for a total of 88 cfDNA sample replicates. A total of 87 cfDNA replicates were run between 20-60ng DNA input, with 1 cfDNA replicate failure at the DNA extraction step. Additionally, one matched gDNA replicate per donor was isolated from buffy coat and mechanically fragmented by sonication for F1LCDx testing to obtain non-tumor variant (e.g., germline) information and support LoB analysis. A total of 44 gDNA replicates passed the QC steps.

All variants were determined to have an LoB of 0, based on the detection rate not significantly exceeding 5%. On a per unique variant basis, the overall detection rate in this study was 0.24%. On a total variant basis, the overall detection rate was 0.0038%. Table 20 provides the unique variant detection rate and overall variant LoB for variants at each variant level/category using the same definitions of unique variant detection rate and total variant detection rate as in Table 19. The results in Table 20 are based on variants detected in cfDNA replicates only (variant detected in the matching gDNA replicate were subtracted) for each sample.

### Table 20. Detection rate for each reporting category in LoB study

| Category | Unique Variant Detection Rate (Unique variants detected) / (total unique variants analyzed) | Total Variant Detection Rate (Total variants detected) / (total variants analyzed)
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>0.22% (2 of 898)</td>
<td>0.0026% (2 of 78,126)</td>
</tr>
</tbody>
</table>
## Category Unique Variant Detection Rate

(Unique variants detected) / (total unique variants analyzed)

<table>
<thead>
<tr>
<th>Category</th>
<th>Level 2</th>
<th>Level 3a</th>
<th>Level 3b</th>
<th>Level 4</th>
<th>VUS</th>
<th>All categories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% (0 of 1)</td>
<td>N/A (0 of 0)</td>
<td>0.66% (2 of 302)</td>
<td>0.25% (18 of 7,154)</td>
<td>0.23% (65 of 28,606)</td>
<td>0.24% (87 of 36,961)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Category</th>
<th>Level 3b</th>
<th>Level 4</th>
<th>VUS</th>
<th>All categories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.66% (2 of 302)</td>
<td>0.25% (18 of 7,154)</td>
<td>0.23% (65 of 28,606)</td>
<td>0.24% (87 of 36,961)</td>
</tr>
</tbody>
</table>

### Total Variant Detection Rate

(Total variants detected) / (total variants analyzed)

<table>
<thead>
<tr>
<th>Category</th>
<th>Level 2</th>
<th>Level 3a</th>
<th>Level 3b</th>
<th>Level 4</th>
<th>VUS</th>
<th>All categories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% (0 of 87)</td>
<td>N/A (0 of 0)</td>
<td>0.0114% (3 of 26,274)</td>
<td>0.0035% (22 of 622,398)</td>
<td>0.0038% (94 of 2,488,722)</td>
<td>0.0038% (121 of 3,215,607)</td>
</tr>
</tbody>
</table>

1 total variants analyzed = unique variants * 44 replicates

### Across 264 copy number alterations and 2752 rearrangements, one rearrangement variant was detected. These results demonstrate the high specificity of FoundationOne Liquid CDx.

### 9.10 Potentially Interfering Substances

To evaluate the robustness of the FoundationOne Liquid CDx results in the presence of potentially interfering exogenous and endogenous substances, a total of 11 potential interferents were evaluated. These potential interferents included six endogenous substances (albumin, conjugated bilirubin, unconjugated bilirubin, cholesterol, hemoglobin and triglycerides) and five exogenous substances (DNA from another source [the microorganism Staphylococcus epidermidis], excess anticoagulant, proteinase K, ethanol and molecular index barcodes).

A total of 340 samples were tested to evaluate the potential interference of these substances. An assessment of the cfDNA yield obtained during the DNA isolation, purification, and quantification steps, as well as at library construction QC (LCQC) and hybrid capture QC (HCQC) was performed. The process success rates for each step are listed in Table 21.

### Table 21. Process success rates with interfering substances

<table>
<thead>
<tr>
<th>Process</th>
<th># Failed</th>
<th># Pass</th>
<th>Total</th>
<th>Success Rate (%)</th>
<th>95% CI LB (%)</th>
<th>95% CI UB (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Extraction</td>
<td>0</td>
<td>180</td>
<td>180</td>
<td>100.00</td>
<td>97.97</td>
<td>100.00</td>
</tr>
<tr>
<td>LC</td>
<td>1</td>
<td>339</td>
<td>340</td>
<td>99.71</td>
<td>98.37</td>
<td>99.99</td>
</tr>
<tr>
<td>HC</td>
<td>3</td>
<td>336</td>
<td>339</td>
<td>99.12</td>
<td>97.44</td>
<td>99.82</td>
</tr>
<tr>
<td>Sequencing</td>
<td>0</td>
<td>336</td>
<td>336</td>
<td>100.00</td>
<td>98.91</td>
<td>100.00</td>
</tr>
</tbody>
</table>

For each potential interferent, concordance of alteration calls was calculated relative to a control sample without interferent. The pre-defined variants included 27 short variants, 17 rearrangements, and 3 copy number variants. Of the 11 potential interferents tested across 16 conditions, concordance for all variant calls was 100% for 8 conditions and ≥97% for all conditions (Table 22).

### Table 22. Concordance per substance for variants ≥1x LoD

<table>
<thead>
<tr>
<th>Substance</th>
<th>Detected Reps</th>
<th>Total Reps</th>
<th>Concordance</th>
<th>95% two-sided exact CI_lower</th>
<th>95% two-sided exact CI_upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides, 37 mmol/L (or 33 g/L)</td>
<td>80</td>
<td>80</td>
<td>100.00%</td>
<td>95.49%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Hemoglobin, 2.0 g/L</td>
<td>78</td>
<td>78</td>
<td>100.00%</td>
<td>95.38%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Albumin, 60 g/L</td>
<td>80</td>
<td>82</td>
<td>97.56%</td>
<td>91.47%</td>
<td>99.7%</td>
</tr>
<tr>
<td>Bilirubin (conjugated), 0.2 g/L</td>
<td>84</td>
<td>84</td>
<td>100.00%</td>
<td>95.7%</td>
<td>100.00%</td>
</tr>
<tr>
<td>Bilirubin (unconjugated), 0.2 g/L</td>
<td>76</td>
<td>78</td>
<td>97.44%</td>
<td>91.04%</td>
<td>99.69%</td>
</tr>
<tr>
<td>Cholesterol Level 2, 3.88 mmol (150 mg/dL)</td>
<td>80</td>
<td>82</td>
<td>97.56%</td>
<td>91.47%</td>
<td>99.7%</td>
</tr>
<tr>
<td>Cholesterol Level 1, 6.47mmol (250 mg/dL)</td>
<td>74</td>
<td>76</td>
<td>97.37%</td>
<td>90.82%</td>
<td>99.68%</td>
</tr>
</tbody>
</table>
Taken together, these data indicate that the FoundationOne Liquid CDx assay is robust to potential specimen-related endogenous substances and exogenous contaminants or interferents.

9.11 Hybrid Capture Bait Specificity

Bait specificity was addressed through an assessment of coverage of targeted regions in FoundationOne Liquid CDx using 3,546 validation study samples. Results show that targeted genomic regions have consistently high, uniform coverage. For each genomic region associated with a predefined subset of highly-actionable alterations, between 94% to 100% of samples possessed the expected level of coverage. An in-depth, platform-wide examination of the FoundationOne Liquid CDx baitset through the analysis of HapMap process control samples revealed that, on average, 98.8% and 94.1% of platform-wide baited coding and non-coding regions, respectively, met their expected coverage levels. Samples assessed in this study consistently demonstrated high quality uniform and deep coverage across the entire genomic region targeted by the assay.

9.12 Carryover/Cross-Contamination

The study demonstrated that the risk of cross contamination (intra-plate), and carry-over contamination (inter-plate) of samples during the processing of the FoundationOne Liquid CDx assay is low. A total of 376 wells were examined for intra- and inter-plate contamination by processing and sequencing of contrived samples derived from cell lines at high input concentrations with known genomic backgrounds. Unique variants of each cell line were characterized by independent control sequencing runs. The samples were arrayed in a checkerboard fashion across four 96-well PCR plates to detect cross-contamination events. A cross-contamination rate of 0.53% (2/376) was observed in this study. These data demonstrate a low probability of cross contamination during the FoundationOne Liquid CDx process.

9.13 Precision: Reproducibility and Confirmation of LoD

Multiple Precision and Confirmation of LoD studies were performed, using both clinical and contrived samples to evaluate precision and only clinical samples for confirmation of LoD. Precision was evaluated for alterations associated with both CDx claims and tumor profiling. Target alterations were assessed at two target levels each (near LoD and 2-3x LoD) for the contrived samples, and at one level (targeting 1-1.5x LoD) for clinical cfDNA samples.

In all studies, each sample was divided into 24 aliquots, with 12 duplicates being processed on the same plate under the same conditions. Each sample was tested across 24 replicates. Reproducibility was assessed and compared across three lots, two sequences, and two processing runs. Samples were processed near the assay’s minimum DNA input mass.

The studies evaluate the precision of FoundationOne Liquid CDx for detecting a set of highly actionable variants. **Table 23** and **Table 23** summarize the Disease Ontology (if applicable), Variant Subtype, Targeted Variant, Reproducibility, Average Measurand, and LoD for each sample with CDx variants and non-CDx variants, respectively.
<table>
<thead>
<tr>
<th>Targeted Variant</th>
<th>Variant Subtype</th>
<th>Cancer Type</th>
<th>Reproducibility (%) (95% Two-sided CI)</th>
<th>Average Measurand</th>
<th>LoD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALK_EML4_fusion</td>
<td>RE</td>
<td>Lung cancer</td>
<td>100 (86.2, 100)</td>
<td>0.68% VAF</td>
<td>0.24% VAF</td>
</tr>
<tr>
<td>ALK_EML4 fusion</td>
<td>RE</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>1.39% VAF</td>
<td>0.24% VAF</td>
</tr>
<tr>
<td>ALK-EML4 fusion</td>
<td>RE</td>
<td>Prostate cancer</td>
<td>100 (85.75, 100)</td>
<td>0.64% VAF</td>
<td>0.24% VAF</td>
</tr>
<tr>
<td>ALK-EML4 fusion</td>
<td>RE</td>
<td>Contrived</td>
<td>100 (85.18, 100)</td>
<td>0.89% VAF</td>
<td>0.24% VAF</td>
</tr>
<tr>
<td>ALK-NPM1 fusion</td>
<td>RE</td>
<td>Lung cancer</td>
<td>78.26 (56.3, 92.54)</td>
<td>0.4% VAF</td>
<td>0.94% VAF</td>
</tr>
<tr>
<td>ALK-NPM1 fusion</td>
<td>RE</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.64% VAF</td>
<td>0.94% VAF</td>
</tr>
<tr>
<td>ATM I2012fs*4</td>
<td>Indel</td>
<td>Prostate cancer</td>
<td>100 (85.18, 100)</td>
<td>0.86% VAF</td>
<td>0.51% VAF</td>
</tr>
<tr>
<td>ATM K1773fs*3</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.77% VAF</td>
<td>0.51% VAF</td>
</tr>
<tr>
<td>ATM K1773fs*3</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.18, 100)</td>
<td>1.04% VAF</td>
<td>0.51% VAF</td>
</tr>
<tr>
<td>ATM splicing site 8850+1G&gt;A</td>
<td>Sub</td>
<td>Prostate cancer</td>
<td>100 (85.75, 100)</td>
<td>0.56% VAF</td>
<td>0.51% VAF</td>
</tr>
<tr>
<td>ATM-EXPH5 truncation</td>
<td>RE</td>
<td>CRC</td>
<td>100 (85.75, 100)</td>
<td>1.13% VAF</td>
<td>Not Determined</td>
</tr>
<tr>
<td>BRAF 1999T&gt;A</td>
<td>Sub</td>
<td>Lung cancer</td>
<td>100 (86.2, 100)</td>
<td>0.70 VAF</td>
<td>0.33% VAF</td>
</tr>
<tr>
<td>BRCA N1784fs*3</td>
<td>Indel</td>
<td>Stomach cancer</td>
<td>87.5 (69, 95.7)</td>
<td>0.34% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA1 D825fs*21</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.61% VAF</td>
<td>0.38% VAF</td>
</tr>
<tr>
<td>BRCA1 D825fs*21</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.93% VAF</td>
<td>0.38% VAF</td>
</tr>
<tr>
<td>BRCA1 E23fs*17</td>
<td>Indel</td>
<td>Ovary cancer</td>
<td>100 (85.75, 100)</td>
<td>0.66% VAF</td>
<td>0.38% VAF</td>
</tr>
<tr>
<td>BRCA1 P871fs*32</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.18, 100)</td>
<td>0.51% VAF</td>
<td>0.38% VAF</td>
</tr>
<tr>
<td>BRCA1 P871fs*32</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>1.08% VAF</td>
<td>0.38% VAF</td>
</tr>
<tr>
<td>BRCA1 Q780*</td>
<td>Sub</td>
<td>Ovary cancer</td>
<td>100 (85.75, 100)</td>
<td>1.11% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>BRCA1 Y465*</td>
<td>Sub</td>
<td>Prostate cancer</td>
<td>100 (86.2, 100)</td>
<td>0.51% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>BRCA1_D1840fs*32</td>
<td>del</td>
<td>Prostate cancer</td>
<td>95.83 (79.76, 99.26)</td>
<td>0.55% VAF</td>
<td>0.38% VAF</td>
</tr>
<tr>
<td>BRCA1_N/A_truncation</td>
<td>RE</td>
<td>CRC</td>
<td>100 (86.2, 100)</td>
<td>1.27% VAF</td>
<td>Not Determined</td>
</tr>
<tr>
<td>BRCA1_S646fs*5</td>
<td>del</td>
<td>Prostate cancer</td>
<td>100 (85.69, 100)</td>
<td>0.54% VAF</td>
<td>0.38% VAF</td>
</tr>
<tr>
<td>BRCA1_Y1563*</td>
<td>Sub</td>
<td>Ovary cancer</td>
<td>100 (86.2, 100)</td>
<td>1.66% VAF</td>
<td>0.51% VAF</td>
</tr>
<tr>
<td>BRCA1-BRCA1 deletion</td>
<td>RE</td>
<td>Ovary cancer</td>
<td>100 (85.75, 100)</td>
<td>0.87% VAF</td>
<td>0.28% VAF</td>
</tr>
<tr>
<td>BRCA2 C1200fs*1</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.58% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 C1200fs*1</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.92% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 G267*</td>
<td>Sub</td>
<td>Ovary cancer</td>
<td>91.67 (73, 98.97)</td>
<td>0.5% VAF</td>
<td>Not Determined</td>
</tr>
<tr>
<td>BRCA2 N1784fs*7</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>1.22% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 N1784fs*7</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>1.85% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 N1784fs*7</td>
<td>Indel</td>
<td>Ovary cancer</td>
<td>100 (85.18, 100)</td>
<td>1.07% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 N1784fs*7</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>2.24% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 N1822fs*2</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.92% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 N1822fs*2</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.18, 100)</td>
<td>1.19% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 Q1429fs*9</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.94% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 Q1429fs*9</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.18, 100)</td>
<td>1.26% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 S2988fs*12</td>
<td>Indel</td>
<td>Ovary cancer</td>
<td>100 (85.75, 100)</td>
<td>1.07% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 T3033fs*11</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.71% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2 T3033fs*11</td>
<td>Indel</td>
<td>Contrived</td>
<td>91.67 (73, 98.97)</td>
<td>1.03% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2_CDH17_truncation</td>
<td>RE</td>
<td>Prostate cancer</td>
<td>100 (86.2, 100)</td>
<td>1.49% VAF</td>
<td>Not Determined</td>
</tr>
<tr>
<td>BRCA2_E2198fs*4</td>
<td>del</td>
<td>Ovarian cancer</td>
<td>100 (86.2, 100)</td>
<td>0.65% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2_G995fs*4</td>
<td>del</td>
<td>Prostate cancer</td>
<td>95.83 (79.76, 99.26)</td>
<td>0.63% VAF</td>
<td>0.36% VAF</td>
</tr>
<tr>
<td>BRCA2_loss</td>
<td>CN</td>
<td>Prostate cancer</td>
<td>100 (86.2, 100)</td>
<td>53.11% TF</td>
<td>48.1% TF</td>
</tr>
<tr>
<td>BRCA2_loss</td>
<td>CN</td>
<td>Prostate cancer</td>
<td>87.5 (67.64, 97.34)</td>
<td>39.43% TF</td>
<td>48.1% TF</td>
</tr>
<tr>
<td>BRCA2_N/A_truncation</td>
<td>RE</td>
<td>Prostate cancer</td>
<td>70.83 (50.83, 85.09)</td>
<td>1.32% VAF</td>
<td>0.48% VAF</td>
</tr>
<tr>
<td>Targeted Variant</td>
<td>Variant Subtype</td>
<td>Cancer Type</td>
<td>Reproducibility (%) (95% Two-sided CI)</td>
<td>Average Measurand</td>
<td>LoD</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------------</td>
<td>------------------------------</td>
<td>----------------------------------------</td>
<td>-------------------</td>
<td>--------</td>
</tr>
<tr>
<td>BRCA2_N3124l</td>
<td>Sub</td>
<td>Ovarian cancer</td>
<td>100 (86.2, 100)</td>
<td>0.74% VAF¹</td>
<td>0.49% VAF</td>
</tr>
<tr>
<td>BRCA2_Q1361*</td>
<td>sub</td>
<td>Prostate cancer</td>
<td>100 (85.69, 100)</td>
<td>0.71% VAF¹</td>
<td>0.49% VAF</td>
</tr>
<tr>
<td>BRCA2-EDA truncation</td>
<td>RE</td>
<td></td>
<td>100 (85.18, 100)</td>
<td>0.48% VAF¹</td>
<td>0.47% VAF²</td>
</tr>
<tr>
<td>EGFR E746_A750del</td>
<td>Indel</td>
<td>Lung cancer</td>
<td>95.7 (79.99, 2)</td>
<td>0.45% VAF¹</td>
<td>0.27% VAF</td>
</tr>
<tr>
<td>EGFR E746_A750del</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.51% VAF¹</td>
<td>0.27% VAF</td>
</tr>
<tr>
<td>EGFR E746_A750del</td>
<td>Indel</td>
<td></td>
<td>100 (85.75, 100)</td>
<td>0.74% VAF²</td>
<td>0.27% VAF</td>
</tr>
<tr>
<td>EGFR E746_A750del</td>
<td>Indel</td>
<td></td>
<td>100 (85.75, 100)</td>
<td>0.93% VAF²</td>
<td>0.27% VAF</td>
</tr>
<tr>
<td>EGFR E746_A750del</td>
<td>Indel</td>
<td></td>
<td>100 (85.18, 100)</td>
<td>1.2% VAF</td>
<td>0.27% VAF</td>
</tr>
<tr>
<td>EGFR E746_A750del</td>
<td>Indel</td>
<td></td>
<td>100 (85.18, 100)</td>
<td>0.51% VAF²</td>
<td>0.27% VAF</td>
</tr>
<tr>
<td>EGFR E746_A750del</td>
<td>Indel</td>
<td>Lung cancer</td>
<td>100 (85.75, 100)</td>
<td>1.01% VAF¹</td>
<td>0.27% VAF</td>
</tr>
<tr>
<td>EGFR L858R</td>
<td>Sub</td>
<td></td>
<td>100 (85.75, 100)</td>
<td>0.64% VAF¹</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>EGFR L858R</td>
<td>Sub</td>
<td></td>
<td>100 (85.75, 100)</td>
<td>1.64% VAF¹</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>EGFR L858R</td>
<td>Sub</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.46% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>EGFR L858R</td>
<td>Sub</td>
<td></td>
<td>100 (85.75, 100)</td>
<td>0.68% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>EGFR L858R</td>
<td>Sub</td>
<td></td>
<td>100 (85.18, 100)</td>
<td>0.95% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>EGFR ex20 insertion</td>
<td>Indel</td>
<td>Lung Cancer</td>
<td>100 (86.2, 100)</td>
<td>0.98% VAF¹</td>
<td>Not Determined</td>
</tr>
<tr>
<td>ETV6-NTRK3 fusion</td>
<td>RE</td>
<td>Thyroid cancer</td>
<td>100 (86.20, 100)</td>
<td>0.82% VAF¹</td>
<td>0.27% VAF</td>
</tr>
<tr>
<td>ETV6-NTRK3 fusion</td>
<td>RE</td>
<td>Contrived</td>
<td>95.83 (78.88, 99.89)</td>
<td>0.32% VAF</td>
<td>0.47% VAF²</td>
</tr>
<tr>
<td>ETV6-NTRK3 fusion</td>
<td>RE</td>
<td>Lung cancer</td>
<td>95.83 (78.88, 99.89)</td>
<td>0.59% VAF¹</td>
<td>0.47% VAF²</td>
</tr>
<tr>
<td>ETV6-NTRK3 fusion</td>
<td>RE</td>
<td>Salivary gland cancer</td>
<td>100 (85.69, 100)</td>
<td>0.68% VAF¹</td>
<td>0.27% VAF</td>
</tr>
<tr>
<td>GOPC-ROS1 fusion</td>
<td>RE</td>
<td>Contrived</td>
<td>86.96 (66.41, 97.22)</td>
<td>0.35% VAF</td>
<td>0.47% VAF²</td>
</tr>
<tr>
<td>MET exon14 splice site</td>
<td>Indel</td>
<td>Lung cancer</td>
<td>95.8 (79.8, 99.3)</td>
<td>0.28% VAF¹</td>
<td>0.41% VAF</td>
</tr>
<tr>
<td>MET exon14 splice site</td>
<td>Sub</td>
<td></td>
<td>95.8 (79.8, 99.3)</td>
<td>0.45% VAF¹</td>
<td>Not Determined</td>
</tr>
<tr>
<td>MET exon14 splice site</td>
<td>Sub</td>
<td>Lung cancer</td>
<td>95.7 (79.0, 99.2)</td>
<td>0.35% VAF¹</td>
<td>Not Determined</td>
</tr>
<tr>
<td>MET exon14 splice site</td>
<td>Sub</td>
<td></td>
<td>100 (85.7, 100)</td>
<td>0.85% VAF</td>
<td>Not Determined</td>
</tr>
<tr>
<td>MET exon14 splice site</td>
<td>Sub</td>
<td></td>
<td>100 (85.75, 100)</td>
<td>0.76% VAF</td>
<td>Not Determined</td>
</tr>
<tr>
<td>MET splice site 3029-1G&gt;T</td>
<td>Sub</td>
<td>Contrived</td>
<td>62.5 (40.59, 81.2)</td>
<td>0.21% VAF</td>
<td>Not Determined</td>
</tr>
<tr>
<td>MET splice site 3029-1G&gt;T</td>
<td>Sub</td>
<td></td>
<td>91.3 (71.96, 98.93)</td>
<td>0.3% VAF</td>
<td>Not Determined</td>
</tr>
<tr>
<td>MET splice site 2888-17_2888-3del15</td>
<td>Indel</td>
<td>Lung cancer</td>
<td>100 (85.75, 100)</td>
<td>1.17% VAF¹</td>
<td>0.41% VAF</td>
</tr>
<tr>
<td>MET splice site 3005_3028+3+C</td>
<td>Indel</td>
<td></td>
<td>100 (85.75, 100)</td>
<td>1.67% VAF¹</td>
<td>0.41% VAF</td>
</tr>
<tr>
<td>MPRIP-NTRK1 fusion</td>
<td>RE</td>
<td>Contrived</td>
<td>69.57 (47.08, 86.79)</td>
<td>0.49% VAF</td>
<td>0.44% VAF</td>
</tr>
<tr>
<td>MPRIP-NTRK1 fusion</td>
<td>RE</td>
<td></td>
<td>87.5 (67.64, 97.34)</td>
<td>0.69% VAF</td>
<td>0.44% VAF</td>
</tr>
<tr>
<td>PIK3CA E542K</td>
<td>Sub</td>
<td>Breast cancer</td>
<td>100 (85.75, 100)</td>
<td>0.89% VAF¹</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>PIK3CA E545A</td>
<td>Sub</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.52% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>PIK3CA E545A</td>
<td>Sub</td>
<td></td>
<td>100 (85.18, 100)</td>
<td>0.7% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>Targeted Variant</td>
<td>Variant Subtype</td>
<td>Cancer Type</td>
<td>Reproducibility (%) (95% Two-sided CI)</td>
<td>Average Measurand</td>
<td>LoD</td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------</td>
<td>---------------</td>
<td>----------------------------------------</td>
<td>-------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PIK3CA E545K</td>
<td>Sub</td>
<td>Breast cancer</td>
<td>100 (85.75, 100)</td>
<td>0.5% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>PIK3CA E545K</td>
<td>Sub</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.45% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>PIK3CA E545K</td>
<td>Sub</td>
<td>Breast cancer</td>
<td>100 (85.75, 100)</td>
<td>0.66% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>PIK3CA H1047R</td>
<td>Sub</td>
<td>Breast cancer</td>
<td>100 (85.75, 100)</td>
<td>1.04% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>PIK3CA H1047R</td>
<td>Sub</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.41% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>PIK3CA Q546R</td>
<td>Sub</td>
<td>Breast cancer</td>
<td>91.7 (74.2, 97.7)</td>
<td>0.44% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>PIK3CA Q546R</td>
<td>Sub</td>
<td>Contrived</td>
<td>95.65 (78.05, 99.89)</td>
<td>0.49% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>PIK3CA Q546R</td>
<td>Sub</td>
<td>Breast cancer</td>
<td>100 (85.75, 100)</td>
<td>0.92% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>PIK3CA Q546R</td>
<td>Sub</td>
<td>Contrived</td>
<td>95.65 (79.01, 99.23)</td>
<td>0.39% VAF</td>
<td>0.34% VAF</td>
</tr>
<tr>
<td>PTEN_loss</td>
<td>CN</td>
<td></td>
<td>100 (85.75, 100)</td>
<td>46.89% TF</td>
<td>12.7% TF</td>
</tr>
<tr>
<td>ROS1-CD74 fusion</td>
<td>RE</td>
<td>Lung cancer</td>
<td>100 (85.75, 100)</td>
<td>1.32% VAF</td>
<td>0.52% VAF</td>
</tr>
<tr>
<td>ROS1-EZR fusion</td>
<td>RE</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.03% VAF</td>
<td>0.28% VAF</td>
</tr>
<tr>
<td>SLC34A2-ROS1 fusion</td>
<td>RE</td>
<td>Breast cancer</td>
<td>100 (85.75, 100)</td>
<td>1.36% VAF</td>
<td>0.28% VAF</td>
</tr>
<tr>
<td>SLC34A2-ROS1 fusion</td>
<td>RE</td>
<td>Contrived</td>
<td>91.67 (73, 98.97)</td>
<td>8.48% VAF</td>
<td>0.44% VAF</td>
</tr>
<tr>
<td>TPM3-NTRK1 fusion</td>
<td>RE</td>
<td>Lung cancer</td>
<td>100 (85.75, 100)</td>
<td>0.3% VAF</td>
<td>0.44% VAF</td>
</tr>
<tr>
<td>TPM3-NTRK1 fusion</td>
<td>RE</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.4% VAF</td>
<td>0.44% VAF</td>
</tr>
<tr>
<td>TPM3-NTRK1 fusion</td>
<td>RE</td>
<td>Colon cancer</td>
<td>100 (85.69, 100)</td>
<td>0.83% VAF</td>
<td>0.44% VAF</td>
</tr>
<tr>
<td>TPR-NTRK1 fusion</td>
<td>RE</td>
<td>Thyroid cancer</td>
<td>100 (85.69, 100)</td>
<td>0.75% VAF</td>
<td>0.44% VAF</td>
</tr>
</tbody>
</table>

1 LoD was confirmed for these variants with hit rate (same as the reproducibility) which met the acceptance criteria defined in respective study.
2 LoD was not determined for these specific variants; platform LoD for the variant type is listed.

Table 24. Precision and Confirmation of LoD by Targeted Non-CDx Variant
<table>
<thead>
<tr>
<th>Targeted Variant</th>
<th>Variant Subtype</th>
<th>Cancer Type</th>
<th>Reproducibility (%) (95% Two-sided CI)</th>
<th>Average Measurand</th>
<th>LoD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERBB2_amplification</td>
<td>CN</td>
<td>Soft tissue cancer</td>
<td>0 (0, 13.8)</td>
<td>54.53% TF</td>
<td>19.8% TF</td>
</tr>
<tr>
<td>ERBB2_amplification</td>
<td>CN</td>
<td>Lung cancer</td>
<td>0 (0, 14.31)</td>
<td>54.8% TF</td>
<td>19.8% TF</td>
</tr>
<tr>
<td>KRAS G12D</td>
<td>Sub</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.89% VAF</td>
<td>0.49% VAF</td>
</tr>
<tr>
<td>KRAS G12D</td>
<td>Sub</td>
<td>Colon cancer</td>
<td>100 (85.75, 100)</td>
<td>0.94% VAF</td>
<td>0.51% VAF</td>
</tr>
<tr>
<td>KRAS G13D</td>
<td>Sub</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.55% VAF</td>
<td>0.49% VAF</td>
</tr>
<tr>
<td>KRAS G13D</td>
<td>Sub</td>
<td>Colon cancer</td>
<td>100 (85.75, 100)</td>
<td>0.92% VAF</td>
<td>0.49% VAF</td>
</tr>
<tr>
<td>KRAS Q61R</td>
<td>Sub</td>
<td>Colon cancer</td>
<td>100 (85.75, 100)</td>
<td>0.53% VAF</td>
<td>0.33% VAF</td>
</tr>
<tr>
<td>MET L1312fs*4</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.69% VAF</td>
<td>0.56% VAF</td>
</tr>
<tr>
<td>MET L1312fs*4</td>
<td>Indel</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>0.96% VAF</td>
<td>0.56% VAF</td>
</tr>
<tr>
<td>NRAS G12C</td>
<td>Sub</td>
<td>Lung cancer</td>
<td>82.61 (61.22, 95.05)</td>
<td>0.48% VAF</td>
<td>0.49% VAF</td>
</tr>
<tr>
<td>NRAS G12C</td>
<td>Sub</td>
<td>Lung cancer</td>
<td>91.3 (73.2, 97.6)</td>
<td>0.55% VAF</td>
<td>0.42% VAF</td>
</tr>
<tr>
<td>NTRK2-N/A rearrangement</td>
<td>RE</td>
<td>Contrived</td>
<td>95.83 (78.88, 99.89)</td>
<td>1.85% VAF</td>
<td>0.897% VAF</td>
</tr>
<tr>
<td>NTRK2-N/A rearrangement</td>
<td>RE</td>
<td>Contrived</td>
<td>95.83 (78.88, 99.89)</td>
<td>2.03% VAF</td>
<td>0.897% VAF</td>
</tr>
<tr>
<td>PALB2 G808*</td>
<td>Sub</td>
<td>Colon cancer</td>
<td>100 (85.18, 100)</td>
<td>0.48% VAF</td>
<td>0.49% VAF</td>
</tr>
<tr>
<td>PALB2 G808*</td>
<td>Sub</td>
<td>Colon cancer</td>
<td>100 (85.75, 100)</td>
<td>0.92% VAF</td>
<td>0.49% VAF</td>
</tr>
<tr>
<td>PALB2 K908fs*15</td>
<td>Indel</td>
<td>Colon cancer</td>
<td>100 (85.75, 100)</td>
<td>0.52% VAF</td>
<td>0.56% VAF</td>
</tr>
<tr>
<td>PALB2 K908fs*15</td>
<td>Indel</td>
<td>Colon cancer</td>
<td>100 (85.75, 100)</td>
<td>0.74% VAF</td>
<td>0.56% VAF</td>
</tr>
<tr>
<td>PALB2 N280fs*8</td>
<td>Indel</td>
<td>Colon cancer</td>
<td>100 (56.6, 100)</td>
<td>0.48% VAF</td>
<td>0.37% VAF</td>
</tr>
<tr>
<td>PIK3CA D549N</td>
<td>Sub</td>
<td>Colon cancer</td>
<td>100 (85.75, 100)</td>
<td>0.48% VAF</td>
<td>0.49% VAF</td>
</tr>
<tr>
<td>PIK3CA D549N</td>
<td>Sub</td>
<td>Colon cancer</td>
<td>100 (85.75, 100)</td>
<td>0.73% VAF</td>
<td>0.49% VAF</td>
</tr>
<tr>
<td>PTEN_loss</td>
<td>CN</td>
<td>Contrived</td>
<td>75 (53.29, 90.23)</td>
<td>44.04% TF</td>
<td>12.7% TF</td>
</tr>
<tr>
<td>PTEN_loss</td>
<td>CN</td>
<td>Contrived</td>
<td>100 (85.75, 100)</td>
<td>59.26% TF</td>
<td>12.7% TF</td>
</tr>
<tr>
<td>RET-CCDC6 fusion</td>
<td>RE</td>
<td></td>
<td>95.83 (78.88, 99.89)</td>
<td>0.22% VAF</td>
<td>0.474% VAF</td>
</tr>
<tr>
<td>RET-CCDC6 fusion</td>
<td>RE</td>
<td></td>
<td>100 (85.75, 100)</td>
<td>0.39% VAF</td>
<td>0.474% VAF</td>
</tr>
</tbody>
</table>

1 LoD was confirmed for these variants with hit rate (same as the reproducibility) which met the acceptance criteria defined in respective study.

2 LoD was not determined for these specific variants; platform LoD for the variant type is listed.

**Assessment of Tumor Profiling Variants**

Across 39 unique samples, including 8 contrived samples, and 31 clinical samples, a total of 1,240 variants were evaluated for reproducibility and repeatability of tumor profiling variants, with variant types including substitutions, indels, rearrangements, and copy number alterations. The number of variants in each variant bin are summarized in Table 25. The overall repeatability for all variants were 99.47% with 95% 2-sided exact CIs [99.45%, 99.48%]. The overall reproducibility results were 99.59% with the 95% 2-sided exact CIs [99.58%, 99.60%]. The repeatability and reproducibility results for each variant type are summarized in Table 25.
Table 25. Number of each variant type

<table>
<thead>
<tr>
<th>Variant Category</th>
<th>N</th>
<th># of Pairs Agree/ # of Total Pairs</th>
<th>Repeatability (%) [95% Two-Sided Exact CIs (%)]</th>
<th># of Replicates Agree/ # of Total Replicates</th>
<th>Reproducibility (%) [95% Two-Sided Exact CIs (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substitutions</td>
<td>898</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substitution in a non-repetitive region or a repetitive region of &lt;=7 base pairs</td>
<td>882</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substitution in a repetitive region of &gt;7 base pairs</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insertion/Deletion in non-repetitive region or a repetitive region of &lt;=3 base pairs</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insertion/Deletion in a repetitive region of 4 to 6 base pairs</td>
<td>118</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insertion/Deletion in a repetitive region of &gt;=7 base pairs</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rearrangements</td>
<td>60</td>
<td>33105 / 33480</td>
<td>98.88 [98.76, 98.99]</td>
<td>66723 / 67260</td>
<td>99.20 [99.13, 99.27]</td>
</tr>
<tr>
<td>Copy Number Amplification</td>
<td>49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copy Number Loss</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1240</strong></td>
<td><strong>688225 / 691920</strong></td>
<td><strong>99.47 [99.45, 99.48]</strong></td>
<td><strong>1384328 / 1390040</strong></td>
<td><strong>99.59 [99.58, 99.60]</strong></td>
</tr>
</tbody>
</table>

9.14 Reagent Lot Interchangeability

The interchangeability of critical reagent lots for library construction (LC), hybrid capture (HC) and sequencing within the FoundationOne Liquid CDx assay was evaluated by testing eight (8) contrived samples from either enzymatically fragmented cell line genomic DNA containing alterations of interest or enzymatically fragmented plasmid DNA. Each of the contrived samples was tested in triplicate using two different lots each of LC, HC, and sequencing reagents. Eight reagent pairings were assessed. A total of eight analyses for each specimen were completed. A total of 192 tests were included in this study. Four Master Pool Libraries (MPLs) were evaluated on each of two flowcells on a NovaSeq 6000 sequencer, using two different Sequencing reagent lots. Of the 49 alterations assessed in the sample set, 43 had a percent agreement greater than 90% (39 alterations had percentage agreement equal to 100%, one had percent agreement equal to 95.83%, one had percent agreement equal to 95.65%, and two had percent agreement equal to 91.67%), exceeding the pre-specified acceptance criteria. For the remaining six alterations the observed detection rates for these variants were similar to the predicted detection rate based on the LoD analysis. These results demonstrate the interchangeability of critical reagent lots in the FoundationOne Liquid CDx assay.

9.15 Variant Curator Precision

This study was performed to evaluate the precision of genomic variant call curation, following analysis by the FoundationOne Liquid CDx analysis pipeline. This was established by analyzing targeted alterations, including CDx alterations, and platform-wide alterations within samples used in the FoundationOne Liquid CDx Precision and LoD and Precision Confirmation Study. The study design reflected the intermediate precision design and evaluated curator precision in reporting of targeted and platform alterations. A total of 19 samples were selected for this study. Three curators were chosen randomly amongst all qualified curators to curate variant calls in a set of randomly chosen replicates from each of the 19 samples. The variant calls were generated from each sample
per curator. The overall average percent agreement for targeted alterations was 93.3% (95% CI; 83.80%, 98.15%), and for platform genomic alterations was 99.14% (95% CI; 98.47%, 99.57%).

9.16 Stability
9.15.1 Reagent Stability
The reagent stability of FoundationOne Liquid CDx was assessed by analyzing data from each of eight samples in triplicate, per each of three different lots of LC, HC, and sequencing reagents. A total of nine analyses for each specimen were completed for each of six time points assessed. A total of 72 tests were assessed per time period; a total of 432 samples and six time points (one baseline timepoint and 5 subsequent experimental timepoints) were included in this study overall. Each of the three sample Master Library Pools (MPLs), representing three LC and HC reagent lots was evaluated per time point on a NovaSeq 6000 sequencer, using three different sequencing reagent lots. The analysis of baseline timepoint zero (T0) identified the baseline variant calls for each sample.

All five experimental time points have been processed and analyzed for Lot #1, Lot #2, and Lot #3. Concordance was assessed among 127,642 data points for tumor profiling variants across the five experimental timepoints. The three reagent lots achieved ≥90% concordance with the baseline variant calls for all the experimental timepoints (including the last two timepoints T4 and T5 at 12 and 13 months respectively) except for a middle timepoint T3 (9 months) which is present in Table 26. The reason for the failure of T3 (9 months) was a technical error which resulted in lower than planned DNA being transferred for LC and therefore this was not a reagent failure. Reagent stability can be claimed as 12 months.

<table>
<thead>
<tr>
<th>Reagent Lot</th>
<th>Timepoint ( ^1 )</th>
<th># Concordant</th>
<th># Total</th>
<th>Concordance (%)</th>
<th>95% 2-sided score CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOT#1</td>
<td>3 months</td>
<td>1921</td>
<td>1966</td>
<td>97.71%</td>
<td>[96.95%, 98.28%]</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>2082</td>
<td>2151</td>
<td>96.79%</td>
<td>[95.96%, 97.46%]</td>
</tr>
<tr>
<td></td>
<td>9 months</td>
<td>1916</td>
<td>2151</td>
<td>89.07%</td>
<td>[87.69%, 90.32%]</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>1609</td>
<td>1656</td>
<td>97.16%</td>
<td>[96.25%, 97.86%]</td>
</tr>
<tr>
<td></td>
<td>13 months</td>
<td>1918</td>
<td>1973</td>
<td>97.21%</td>
<td>[96.39%, 97.85%]</td>
</tr>
<tr>
<td>LOT#2</td>
<td>3 months</td>
<td>2083</td>
<td>2148</td>
<td>96.97%</td>
<td>[96.16%, 97.62%]</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>2091</td>
<td>2160</td>
<td>96.81%</td>
<td>[95.98%, 97.47%]</td>
</tr>
<tr>
<td></td>
<td>9 months</td>
<td>1851</td>
<td>2160</td>
<td>85.69%</td>
<td>[84.15%, 87.11%]</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>2087</td>
<td>2160</td>
<td>96.62%</td>
<td>[95.77%, 97.3%]</td>
</tr>
<tr>
<td></td>
<td>13 months</td>
<td>2089</td>
<td>2160</td>
<td>96.71%</td>
<td>[95.87%, 97.39%]</td>
</tr>
<tr>
<td>LOT#3</td>
<td>3 months</td>
<td>2086</td>
<td>2139</td>
<td>97.52%</td>
<td>[96.77%, 98.10%]</td>
</tr>
<tr>
<td></td>
<td>6 months</td>
<td>2098</td>
<td>2154</td>
<td>97.4%</td>
<td>[96.64%, 97.99%]</td>
</tr>
<tr>
<td></td>
<td>9 months</td>
<td>1855</td>
<td>2154</td>
<td>86.12%</td>
<td>[84.59%, 87.51%]</td>
</tr>
<tr>
<td></td>
<td>12 months</td>
<td>2097</td>
<td>2154</td>
<td>97.35%</td>
<td>[95.96%, 97.95%]</td>
</tr>
<tr>
<td></td>
<td>13 months</td>
<td>1924</td>
<td>1977</td>
<td>97.32%</td>
<td>[96.51%, 97.94%]</td>
</tr>
</tbody>
</table>

A supplemental study is being conducted to evaluate the stability of updated LC reagents. The study will confirm that reagent stability can be claimed as 12 months for the F1LCDx assay with the changed reagents.

9.15.2 Whole Blood Specimen Stability
The recommended storage temperature is 18°C - 25°C. In this study, stress conditions were simulated through extended storage at elevated (35°C ± 2°C) and reduced (4°C ± 2°C) temperatures.

In this interim analysis, 22 samples (11 sample pairs) were tested, including baseline (within 24 hours of collection) and experimental time points (after 10, 14, or 15 days of storage).

Overall, 100% of samples yielded a cfDNA input ≥30ng. The success rate for DNAx yield, and LC yield was 100% and the success rate of the HC yield was 96.3%. The variant analysis was conducted for variants at ≥2x LoD. For the aggregate 11 pairs of samples processed and reported, 100% agreement was observed between
the baseline and experimental timepoint for short variants and rearrangements for each experimental time point. The percent agreement per sample also resulted in 100% agreement between the baseline and experimental timepoint for short variants and rearrangements. The data is summarized in Table 27.

**Table 27. Aggregate percent agreement per temperature and experimental timepoint**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Experimental Timepoint</th>
<th>N</th>
<th>Short Variants [95% two-sided CI]</th>
<th>Rearrangements</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C</td>
<td>7 Days</td>
<td>4</td>
<td>100.00 [89.72, 100.00]</td>
<td>100.00 [39.76, 100.00]</td>
</tr>
<tr>
<td></td>
<td>14 Days</td>
<td>3</td>
<td>100.00 [91.40, 100.00]</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>15 Days</td>
<td>3</td>
<td>100.00 [83.89, 100.00]</td>
<td>N/A</td>
</tr>
<tr>
<td>35°C</td>
<td>14 Days</td>
<td>1</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

The impact of potential interferents originating from the FoundationOne Liquid cfDNA blood collection tube (BCT) stopper on the performance of the FoundationOne Liquid CDx assay was assessed by evaluating stability of whole blood in tubes stored in an upright or inverted position at 4°C± 2°C, 25°C± 2°C, and 35°C± 2°C for various durations (10, 14, and 15 days).

First, the success rate of the FoundationOne Liquid CDx assay for processing samples was assessed at the DNA extraction (DNax), LC, HC and Sequencing step, based on product in- process quality control (QC) criteria. Samples stratified by the upright and the inverted condition exhibited comparable success rates above 94% at DNax, LC, HC and Seq (Table 28). Thus, the stopper of the FoundationOne Liquid cfDNA BCT does not impact FoundationOne Liquid CDx test performance when stored between 4 and 35°C for up to 15 days.

**Table 28. Process success rate by tube position**

<table>
<thead>
<tr>
<th>Process</th>
<th>Tube Position</th>
<th># Passing Samples</th>
<th># Total Samples</th>
<th>Success Rate (%)</th>
<th>95% 2-sided CIs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Extraction</td>
<td>Upright</td>
<td>139</td>
<td>147</td>
<td>94.6%</td>
<td>[89.6%, 97.2%]</td>
</tr>
<tr>
<td></td>
<td>Inverted</td>
<td>147</td>
<td>150</td>
<td>98%</td>
<td>[94.3%, 99.3%]</td>
</tr>
<tr>
<td>LC</td>
<td>Upright</td>
<td>135</td>
<td>136</td>
<td>99.3%</td>
<td>[96%, 99.9%]</td>
</tr>
<tr>
<td></td>
<td>Inverted</td>
<td>146</td>
<td>146</td>
<td>100%</td>
<td>[97.4%, 100%]</td>
</tr>
<tr>
<td>HC</td>
<td>Upright</td>
<td>134</td>
<td>135</td>
<td>99.3%</td>
<td>[95.9%, 99.9%]</td>
</tr>
<tr>
<td></td>
<td>Inverted</td>
<td>143</td>
<td>146</td>
<td>97.9%</td>
<td>[94.1%, 99.3%]</td>
</tr>
<tr>
<td>Sequencing</td>
<td>Upright</td>
<td>134</td>
<td>134</td>
<td>100%</td>
<td>[97.2%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Inverted</td>
<td>143</td>
<td>143</td>
<td>100%</td>
<td>[97.4%, 100%]</td>
</tr>
</tbody>
</table>

Stability was also evaluated by comparing concordance between baseline and experimental samples. Positive percent agreement (PPA) and negative percent agreement (NPA) for alteration calls at ≥ 2x LoD were computed along with the corresponding two-sided 95% score confidence interval (CI) across all replicates by variant category using the baseline detection as reference. Note that NPA is under-estimated as variants not detected at any of the treatment conditions were not used in the analysis set and hence counted against the NPA calculation.

Concordance between baseline and experimental results from all samples in the upright and inverted position combined demonstrated > 99% PPA and NPA for the detection of short variants and rearrangements. Copy number alterations were only detected in samples treated in the inverted tube position and therefore, not included in this analysis. Furthermore, stratification by the treatment condition (2 tube positions × 3 temperatures × 3 durations) revealed >99.0% PPA and NPA for short variants and rearrangements across the combinations of tube positions, temperatures and durations tested. The data also demonstrate that the detection of copy number alterations is not impacted by the storage of blood in the inverted position at 35°C for up to 14 days. The concordance results by variant type for each of the experimental conditions are provided in Table 29.
Variant calls included in the concordance analysis were identified based on the majority call across all 12 Fisher replicates. The success rate of the DNAx yield for three reagent lots range from 95.8% to 100.0%.

Reproducibility of the FoundationOne Liquid CDx DNA extraction process across KingFisher instruments and extraction reagent lots were analyzed utilizing a factorial design (3 reagent lots x 2 KingFisher instruments x 2 replicates). The success rate of the DNAx yield for three reagent lots range from 95.8% to 100.0% and two King Fisher instruments range from 97.2% to 100.0%.

Table 29. Concordance of detected alterations between baseline sample and experimental conditions for inverted tube stability study

<table>
<thead>
<tr>
<th>Variant Type</th>
<th>Temp.</th>
<th>Tube Position</th>
<th>Exp. Time Point</th>
<th>N Variants Detected at Baseline TimePoint</th>
<th>N Variants Detected at Exp. Time Point</th>
<th>N Variants Agree</th>
<th>PPA</th>
<th>PPA [95% CI]</th>
<th>N Variants Not Detected at Baseline TimePoint</th>
<th>N Variants Not Detected at Exp. Time Point</th>
<th>NPA</th>
<th>NPA [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short variants</td>
<td>04°C</td>
<td>Inverted</td>
<td>Day 10</td>
<td>50</td>
<td>49</td>
<td>98%</td>
<td></td>
<td>[89.5%, 99.6%]</td>
<td>612</td>
<td>612</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>04°C</td>
<td>Upright</td>
<td>Day 10</td>
<td>50</td>
<td>50</td>
<td>100%</td>
<td></td>
<td>[92.9%, 100%]</td>
<td>613</td>
<td>612</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>04°C</td>
<td>Inverted</td>
<td>Day 14</td>
<td>59</td>
<td>58</td>
<td>98.3%</td>
<td></td>
<td>[90.9%, 99.7%]</td>
<td>610</td>
<td>611</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>04°C</td>
<td>Upright</td>
<td>Day 14</td>
<td>44</td>
<td>44</td>
<td>100%</td>
<td></td>
<td>[92.0%, 100%]</td>
<td>611</td>
<td>611</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>04°C</td>
<td>Inverted</td>
<td>Day 15</td>
<td>37</td>
<td>37</td>
<td>100%</td>
<td></td>
<td>[90.6%, 100%]</td>
<td>611</td>
<td>611</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>04°C</td>
<td>Upright</td>
<td>Day 15</td>
<td>52</td>
<td>52</td>
<td>100%</td>
<td></td>
<td>[93%, 100%]</td>
<td>611</td>
<td>611</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>25°C</td>
<td>Inverted</td>
<td>Day 10</td>
<td>78</td>
<td>77</td>
<td>97.1%</td>
<td></td>
<td>[91.1%, 99.2%]</td>
<td>627</td>
<td>628</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>25°C</td>
<td>Upright</td>
<td>Day 10</td>
<td>44</td>
<td>44</td>
<td>100%</td>
<td></td>
<td>[92.0%, 100%]</td>
<td>613</td>
<td>613</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>25°C</td>
<td>Inverted</td>
<td>Day 14</td>
<td>46</td>
<td>46</td>
<td>100%</td>
<td></td>
<td>[92.3%, 100%]</td>
<td>611</td>
<td>609</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>25°C</td>
<td>Upright</td>
<td>Day 14</td>
<td>42</td>
<td>41</td>
<td>97.6%</td>
<td></td>
<td>[87.7%, 99.6%]</td>
<td>610</td>
<td>611</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>25°C</td>
<td>Inverted</td>
<td>Day 15</td>
<td>44</td>
<td>44</td>
<td>100%</td>
<td></td>
<td>[92.0%, 100%]</td>
<td>613</td>
<td>613</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>25°C</td>
<td>Upright</td>
<td>Day 15</td>
<td>49</td>
<td>48</td>
<td>97.8%</td>
<td></td>
<td>[89.3%, 99.6%]</td>
<td>616</td>
<td>617</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>35°C</td>
<td>Inverted</td>
<td>Day 10</td>
<td>15</td>
<td>15</td>
<td>100%</td>
<td></td>
<td>[79.6%, 100%]</td>
<td>609</td>
<td>609</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>35°C</td>
<td>Upright</td>
<td>Day 10</td>
<td>35</td>
<td>35</td>
<td>100%</td>
<td></td>
<td>[90.1%, 100%]</td>
<td>609</td>
<td>609</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>35°C</td>
<td>Inverted</td>
<td>Day 14</td>
<td>55</td>
<td>55</td>
<td>100%</td>
<td></td>
<td>[93.4%, 100%]</td>
<td>611</td>
<td>611</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>35°C</td>
<td>Upright</td>
<td>Day 14</td>
<td>48</td>
<td>47</td>
<td>95.7%</td>
<td></td>
<td>[86.0%, 98.8%]</td>
<td>609</td>
<td>610</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>35°C</td>
<td>Inverted</td>
<td>Day 15</td>
<td>39</td>
<td>39</td>
<td>97.4%</td>
<td></td>
<td>[86.8%, 99.5%]</td>
<td>610</td>
<td>610</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
<tr>
<td>Short variants</td>
<td>35°C</td>
<td>Upright</td>
<td>Day 15</td>
<td>28</td>
<td>29</td>
<td>100%</td>
<td></td>
<td>[87.9%, 100%]</td>
<td>613</td>
<td>612</td>
<td>100%</td>
<td>[100%, 100%]</td>
</tr>
</tbody>
</table>

These results demonstrate that blood is stable in the FoundationOne Liquid CDx cfDNA BCT when stored between 4°C and 35°C for up to 15 days, in an upright or inverted position. Additional data will be generated to further evaluate whole blood stability and potential interference of the blood collection tube cap.

9.17 DNA Extraction

DNA extraction evaluated 72 samples across five cancer types: lung cancer (including NSCLC), colorectal cancer (CRC), prostate cancer, breast cancer, and skin cancer (melanoma, sarcoma), using three reagent lots and two KingFisher Magnetic Particle processors.

Reproducibility of the FoundationOne Liquid CDx DNA extraction process across KingFisher instruments and extraction reagent lots were analyzed utilizing a factorial design (3 reagent lots x 2 KingFisher instruments x 2 replicates). The success rate of the DNAx yield for three reagent lots range from 95.8% to 100.0% and two King Fisher instruments range from 97.2% to 100.0%.

Variant calls included in the concordance analysis were identified based on the majority call across all 12 replicates for a given disease ontology. Positive Percent Agreement (PPA) and Negative Percent Agreement
(NPA) were computed across the replicates for each somatic alteration for each sample, and aggregated by variant type (deletion, insertion, rearrangement, and substitution) for variants at ≥1x LoD. The percent agreement results by disease ontologies are: 90.3% - 99.8% for PPA, and 99.1% - 100.0% for NPA (Table 30) The percent agreement results across all variant types (deletion, insertion, rearrangement and substitution) evaluated at ≥1x LoD are: 90.6% - 96.8% for PPA and 98.9% - 100.0% for NPA (Table 31).

Table 30. Concordance summary by disease ontology at 1x LoD for DNA extraction study

<table>
<thead>
<tr>
<th>Disease Ontology</th>
<th>Positive Detected/ Positive Total</th>
<th>PPA [95% two-sided CI]</th>
<th>Negative Detected/ Negative Total¹</th>
<th>NPA [95% two-sided CI]</th>
<th>Overall Detected/ Total*</th>
<th>OPA [95% two-sided CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast Cancer</td>
<td>347/348</td>
<td>99.7% [98.4%,100.0%]</td>
<td>3144/3144</td>
<td>100.0% [99.9%,100.0%]</td>
<td>3491/3492</td>
<td>100.0% [99.8%,100.0%]</td>
</tr>
<tr>
<td>Colorectal Cancer (CRC)</td>
<td>1122/1188</td>
<td>94.4% [93.0%,95.7%]</td>
<td>2284/2304</td>
<td>99.1% [98.7%,99.5%]</td>
<td>3406/3492</td>
<td>97.5% [97.0%,98.0%]</td>
</tr>
<tr>
<td>Lung Cancer</td>
<td>431/432</td>
<td>99.8% [98.7%,100.0%]</td>
<td>3053/3060</td>
<td>99.8% [99.5%,99.9%]</td>
<td>3484/3492</td>
<td>99.8% [99.5%,99.9%]</td>
</tr>
<tr>
<td>Non-SmallCell Lung Cancer (NSCLC)</td>
<td>600/612</td>
<td>98.0% [96.6%,99.0%]</td>
<td>2878/2880</td>
<td>99.9% [99.7%,100.0%]</td>
<td>3478/3492</td>
<td>99.6% [99.3%,99.8%]</td>
</tr>
<tr>
<td>ProstateCancer</td>
<td>486/492</td>
<td>98.8% [97.4%,99.6%]</td>
<td>2987/3000</td>
<td>99.6% [99.3%,99.8%]</td>
<td>3473/3492</td>
<td>99.5% [99.2%,99.7%]</td>
</tr>
<tr>
<td>Skin Cancer</td>
<td>455/504</td>
<td>90.3% [87.4%,92.7%]</td>
<td>2987/2988</td>
<td>100.0% [99.8%,100.0%]</td>
<td>3442/3492</td>
<td>98.6% [98.1%,98.9%]</td>
</tr>
</tbody>
</table>

¹Variants detected include variants classified as VUS and benign

Table 31. Concordance summary by variant type at 1x LoD for DNA extraction study

<table>
<thead>
<tr>
<th>Variant Type</th>
<th>Positive Detected/ Positive Total</th>
<th>PPA [95% two-sided CI]</th>
<th>Negative Detected/ Negative Total¹</th>
<th>NPA [95% two-sided CI]</th>
<th>Overall Detected/ Total*</th>
<th>OPA [95% two-sided CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deletions</td>
<td>386/408</td>
<td>94.6% [91.9%, 96.6%]</td>
<td>2036/2040</td>
<td>99.8% [99.5%, 99.9%]</td>
<td>2422/2448</td>
<td>98.9% [98.4%, 99.3%]</td>
</tr>
<tr>
<td>Insertions</td>
<td>163/180</td>
<td>90.6% [85.3%, 94.4%]</td>
<td>819/828</td>
<td>98.9% [97.9%, 99.5%]</td>
<td>982/1008</td>
<td>97.4% [96.2%, 98.3%]</td>
</tr>
<tr>
<td>Rearrangements</td>
<td>23/24</td>
<td>95.8% [78.9%, 99.9%]</td>
<td>120/120</td>
<td>100.0% [97.0%, 100.0%]</td>
<td>143/144</td>
<td>99.3% [96.2%, 100.0%]</td>
</tr>
<tr>
<td>Substitutions</td>
<td>2869/2964</td>
<td>96.8% [96.1%, 97.4%]</td>
<td>14358/1438</td>
<td>99.8% [99.7%, 99.9%]</td>
<td>17227/17352</td>
<td>99.3% [99.1%, 99.4%]</td>
</tr>
</tbody>
</table>

¹Variants detected include variants classified as VUS and benign

These results demonstrate robustness of the FoundationOne Liquid CDx DNA extraction process across KingFisher instruments, extraction reagent lots, and cancer types.

9.18 Guard Banding/Robustness
This validation study evaluated the impact on FoundationOne Liquid CDx test performance due to potential process variation with regard to uncertainty in the measurement of DNA concentration. This guard banding evaluation assessed the DNA input into each of the main process steps of the FoundationOne Liquid CDx assay (LC, HC, and sequencing).

Guard bands were evaluated relative to calculated process variability for LC, HC, and sequencing. The assessment of multiple DNA input levels into LC demonstrated robust performance and tolerance of various DNA input levels. The observed results of HC guard banding showed that the HC process is robust within the predefined specifications 1000ng to 2000ng of DNA input into HC. For sequencing, the observed distribution of coverage indicated robust performance within the predefined specifications of 1.0nM of DNA input concentration into sequencing (as summarized in Table 32).
Table 32. Summary of process pass and failure rate at each guard banding DNA input level

<table>
<thead>
<tr>
<th>Process(^1)</th>
<th>Input Level</th>
<th># of Pass</th>
<th>Pass Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-50%</td>
<td>500ng</td>
<td>18/20 90</td>
</tr>
<tr>
<td></td>
<td>-20%</td>
<td>800ng</td>
<td>20/20 100</td>
</tr>
<tr>
<td></td>
<td>Lower limit</td>
<td>1000ng</td>
<td>20/20 100</td>
</tr>
<tr>
<td></td>
<td>Upper limit</td>
<td>2000ng</td>
<td>20/20 100</td>
</tr>
<tr>
<td></td>
<td>+20%</td>
<td>2400ng</td>
<td>20/20 100</td>
</tr>
<tr>
<td></td>
<td>+50%</td>
<td>3000ng</td>
<td>18/20 90</td>
</tr>
</tbody>
</table>

|               | -50%        | 0.5nM     | 20/20 100     |
|               | -20%        | 0.8nM     | 20/20 100     |
|               | Normal input| 1.0nM     | 20/20 100     |
|               | +20%        | 1.2nM     | 20/20 100     |
|               | +50%        | 1.5nM     | 20/20 100     |

\(^1\) Results for guardbanding of LC input levels can be found in Table 33 below.

A second guard banding study was conducted to evaluate the impact of a range of cfDNA input masses (50% below the lower limit and 33% above the upper limit) for F1LCDx using an updated LC input range (20-60ng). Results from this second study are described in Table 33 and Table 34. All 105 sample replicates tested in this study passed processing and post-sequencing metric specifications as shown in Table 33 below. The results demonstrate robust performance across the intended DNA input range.

Table 33. Processing Success Rates by cfDNA Input Level for F1LCDx

<table>
<thead>
<tr>
<th>Process QC</th>
<th>cfDNA Input Level</th>
<th>cfDNA Input (ng)</th>
<th># Total</th>
<th># Pass</th>
<th># Fail</th>
<th>Success Rate</th>
<th>95% Two-sided Score Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC</td>
<td>-50%</td>
<td>10</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Lower limit</td>
<td>20</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Mid-point</td>
<td>40</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Upper limit</td>
<td>60</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>+33%</td>
<td>80</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td>HC</td>
<td>-50%</td>
<td>10</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Lower limit</td>
<td>20</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Mid-point</td>
<td>40</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Upper limit</td>
<td>60</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>+33%</td>
<td>80</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td>Sequencing</td>
<td>-50%</td>
<td>10</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Lower limit</td>
<td>20</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Mid-point</td>
<td>40</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Upper limit</td>
<td>60</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>+33%</td>
<td>80</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td>Post-sequencing QC</td>
<td>-50%</td>
<td>10</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Lower limit</td>
<td>20</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Mid-point</td>
<td>40</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>Upper limit</td>
<td>60</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
<tr>
<td></td>
<td>+33%</td>
<td>80</td>
<td>21</td>
<td>21</td>
<td>0</td>
<td>100%</td>
<td>[84.54%, 100%]</td>
</tr>
</tbody>
</table>
Table 34. Aggregate Percent Agreement Across All Targeted Variants per cfDNA Input Level for F1LCdx

<table>
<thead>
<tr>
<th>cfDNA Input Level</th>
<th>cfDNA Input (ng)</th>
<th>Agreement (# Variants Detected / Total # Variants) [95% Two-sided Score CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>-50%</td>
<td>10</td>
<td>92.86% (117/126) [86.98%, 96.2%]</td>
</tr>
<tr>
<td>Lower limit</td>
<td>20</td>
<td>99.21% (125/126) [95.64%, 99.86%]</td>
</tr>
<tr>
<td>Mid-point</td>
<td>40</td>
<td>100% (126/126) [97.04%, 100%]</td>
</tr>
<tr>
<td>Upper limit</td>
<td>60</td>
<td>100% (126/126) [97.04%, 100%]</td>
</tr>
<tr>
<td>+33%</td>
<td>80</td>
<td>100% (126/126) [97.04%, 100%]</td>
</tr>
</tbody>
</table>

9.19 Pan-Tumor Performance

A large-scale retrospective analysis was performed to demonstrate consistent test performance of FoundationOne Liquid CDx across samples derived from patients with different tumor types. This was evaluated by comparing in-process QC metrics across tumor types using historical data from samples processed in Foundation Medicine’s clinical laboratory using two prior versions of the FoundationOne Liquid CDx assay. The FoundationOne Liquid CDx assay was developed based on two versions of the FoundationOne Liquid LDT assay, each of which targeted a subset of the genomic regions targeted by FoundationOne Liquid CDx. FoundationACT (FACT) targeted 62 genes and FoundationOne Liquid targeted 70 genes. The workflow is substantially similar between the assays. In order to support the use of historical data in this study, the regions commonly baited by the two previous assay versions and by FoundationOne Liquid CDx were evaluated for comparability of test performance (Section 2.15). The sample set for this analysis included 19,868 distinct samples from 25 tumor type categories that had previously been tested using the Foundation Medicine FoundationOne Liquid and FoundationACT assays, previous versions of FoundationOne Liquid CDx. **Table 35** below includes a summary of the tissue types included in the study. Overall, 98.1% of samples yielded ≥25ng DNA, which corresponds to a DNA input mass of 20ng for LC. A total of 89.1% of samples yielded ≥36ng of DNA which corresponds to a DNA input mass of 30ng for LC. The proportion of samples with an LC yield greater than the minimum mass of 500ng and lower than the maximum mass of 2700ng was 99.9%, with one sided 95% confidence interval of [99.8%, 99.9%]. The proportion of samples with an HC yield greater than the minimum mass of 20ng and lower than the maximum mass of 2250ng was 100%, with one sided 95% confidence interval of [99.99%, 100%]. The proportion of samples which met coverage requirements was 96.1%, with one sided 95% confidence interval of [95.9%, 96.3%]. The proportion of samples which met post-sequencing requirements was 95.6%, with one sided 95% confidence interval of [95.3%, 95.8%]. The proportion of samples that generated a passing or qualified (overall pass as results are reported) result after sequencing was 91.7%, with one sided 95% confidence interval of [91.4%, 92.1%].

Table 35. F1L/FACT samples per tumor type and pass rates

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Sample Size</th>
<th>DNA Extraction Pass Rate (≥25 ng²)</th>
<th>DNA Extraction Pass Rate (≥36 ng³)</th>
<th>LC Yield Pass Rate</th>
<th>HC Yield Pass Rate</th>
<th>Median Coverage Pass Rate</th>
<th>Post-sequencing Pass Rate</th>
<th>Overall Pass Rate (≥36 ng³)</th>
<th>Overall Pass Rate (≥25 ng³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare Tumors</td>
<td>1164</td>
<td>97.0%</td>
<td>86.4%</td>
<td>99.9%</td>
<td>100.0%</td>
<td>93.8%</td>
<td>94.3%</td>
<td>93.4%</td>
<td>88.4%</td>
</tr>
<tr>
<td>Biliary Cancer</td>
<td>171</td>
<td>99.4%</td>
<td>95.3%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>98.8%</td>
<td>97%</td>
<td>97.5%</td>
<td>95.9%</td>
</tr>
<tr>
<td>Bladder Cancer</td>
<td>166</td>
<td>97.6%</td>
<td>85.5%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>93.2%</td>
<td>98.7%</td>
<td>95.8%</td>
<td>92%</td>
</tr>
<tr>
<td>Breast Cancer</td>
<td>2775</td>
<td>97.6%</td>
<td>87.7%</td>
<td>99.9%</td>
<td>100.0%</td>
<td>96.4%</td>
<td>95.5%</td>
<td>95.8%</td>
<td>91.9%</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>377</td>
<td>98.9%</td>
<td>96.0%</td>
<td>99.7%</td>
<td>100.0%</td>
<td>98.7%</td>
<td>97.3%</td>
<td>97%</td>
<td>95.7%</td>
</tr>
<tr>
<td>Colorectal Cancer (CRC)</td>
<td>1640</td>
<td>98.5%</td>
<td>92.4%</td>
<td>99.9%</td>
<td>100.0%</td>
<td>97.5%</td>
<td>96.9%</td>
<td>96.1%</td>
<td>94.3%</td>
</tr>
<tr>
<td>Endocrine-Neuro Cancer</td>
<td>75</td>
<td>100.0%</td>
<td>85.3%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>93.3%</td>
<td>96.9%</td>
<td>93.3%</td>
<td></td>
</tr>
<tr>
<td>Endometrial Cancer</td>
<td>231</td>
<td>98.3%</td>
<td>88.3%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>96.5%</td>
<td>95.9%</td>
<td>95.1%</td>
<td>92.5%</td>
</tr>
<tr>
<td>Esophageus Cancer</td>
<td>291</td>
<td>99.7%</td>
<td>92.4%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>97.6%</td>
<td>96.5%</td>
<td>96.3%</td>
<td>94.1%</td>
</tr>
<tr>
<td>Glioma Cancer</td>
<td>59</td>
<td>94.9%</td>
<td>72.9%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>76.8%</td>
<td>86%</td>
<td>76.8%</td>
<td></td>
</tr>
<tr>
<td>Tumor Type</td>
<td>Sample Size</td>
<td>DNA Extraction Pass Rate (≥25 ng)$^1$</td>
<td>DNA Extraction Pass Rate (≥36 ng)$^2$</td>
<td>LC Yield Pass Rate</td>
<td>HC Yield Pass Rate</td>
<td>Median Coverage Pass Rate</td>
<td>Post-sequencing Pass Rate</td>
<td>Overall Pass Rate (≥36 ng)$^1$</td>
<td>Overall Pass Rate (≥25 ng)$^2$</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-------------</td>
<td>--------------------------------------</td>
<td>--------------------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>--------------------------</td>
<td>---------------------------</td>
<td>-------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Head and Neck Cancer</td>
<td>154</td>
<td>96.1%</td>
<td>81.8%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>89.2%</td>
<td>96.2%</td>
<td>95.2%</td>
<td>85.8%</td>
</tr>
<tr>
<td>Kidney Cancer</td>
<td>203</td>
<td>99.0%</td>
<td>87.7%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>95.0%</td>
<td>95.3%</td>
<td>94.9%</td>
<td>90.5%</td>
</tr>
<tr>
<td>Liver Cancer</td>
<td>109</td>
<td>98.2%</td>
<td>95.4%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>95.3%</td>
<td>95.2%</td>
<td>95.3%</td>
</tr>
<tr>
<td>Lung Non-Small Cell Lung Carcinoma (NSCLC)</td>
<td>5919</td>
<td>98.2%</td>
<td>88.8%</td>
<td>99.8%</td>
<td>100.0%</td>
<td>95.5%</td>
<td>95.6%</td>
<td>94.7%</td>
<td>91.1%</td>
</tr>
<tr>
<td>Melanoma</td>
<td>257</td>
<td>96.5%</td>
<td>79.8%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>92.7%</td>
<td>93.5%</td>
<td>93.7%</td>
<td>86.7%</td>
</tr>
<tr>
<td>Ovary Cancer</td>
<td>496</td>
<td>97.8%</td>
<td>88.5%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>95.9%</td>
<td>94.6%</td>
<td>94.5%</td>
<td>90.7%</td>
</tr>
<tr>
<td>Pancreas Cancer</td>
<td>1359</td>
<td>98.8%</td>
<td>94.0%</td>
<td>99.9%</td>
<td>100.0%</td>
<td>97.8%</td>
<td>95.8%</td>
<td>95%</td>
<td>93.6%</td>
</tr>
<tr>
<td>Peripheral Nervous System (PNS)</td>
<td>44</td>
<td>100.0%</td>
<td>90.9%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>93.2%</td>
<td>95%</td>
<td>93.2%</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>1778</td>
<td>97.3%</td>
<td>87.7%</td>
<td>99.9%</td>
<td>100.0%</td>
<td>96.9%</td>
<td>95.1%</td>
<td>95.8%</td>
<td>92.1%</td>
</tr>
<tr>
<td>Small Cell Cancer</td>
<td>135</td>
<td>98.5%</td>
<td>93.3%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>99.2%</td>
<td>99.2%</td>
<td>98.4%</td>
<td>98.5%</td>
</tr>
<tr>
<td>Soft Tissue Sarcoma</td>
<td>130</td>
<td>97.7%</td>
<td>83.1%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>95.3%</td>
<td>91.7%</td>
<td>94.4%</td>
<td>87.4%</td>
</tr>
<tr>
<td>Stomach Cancer</td>
<td>267</td>
<td>98.9%</td>
<td>89.1%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>98.1%</td>
<td>93.8%</td>
<td>95.8%</td>
<td>92%</td>
</tr>
<tr>
<td>Thyroid Cancer</td>
<td>50</td>
<td>98.0%</td>
<td>86.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>81.6%</td>
<td>90.7%</td>
<td>81.6%</td>
</tr>
<tr>
<td>Unspecified</td>
<td>856</td>
<td>98.5%</td>
<td>89.1%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>95.5%</td>
<td>96.6%</td>
<td>96.3%</td>
<td>92.3%</td>
</tr>
<tr>
<td>Unknown Primary Carcinoma (CUP)</td>
<td>1162</td>
<td>98.1%</td>
<td>89.7%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>95.2%</td>
<td>95.9%</td>
<td>94.8%</td>
<td>91.3%</td>
</tr>
</tbody>
</table>

$^1$ 36 ng of extracted cfDNA allows for sufficient cfDNA to process 30 ng of cfDNA

$^2$ 25 ng of extracted cfDNA allows for sufficient cfDNA to process 20 ng of cfDNA

Table 36 summarizes the overall sample pass rate across tumor types as well as performance metrics from key QC points in the process. These results demonstrate comparable test performance across tumor types.

Table 36. Summary of F1L/FACT sample data

<table>
<thead>
<tr>
<th>QC Metric</th>
<th>QC Pass Rate Across Tumor Types$^1$</th>
<th>Tumor Types with ≥ 90% QC Pass Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall report Pass/Qualified rate</td>
<td>76.8%~98.5%</td>
<td>24/25 (96%)$^2$</td>
</tr>
<tr>
<td>Library Construction</td>
<td>99.7%~100%</td>
<td>25/25 (100%)$^1$</td>
</tr>
<tr>
<td>Hybridization Capture</td>
<td>100%</td>
<td>25/25 (100%)$^1$</td>
</tr>
<tr>
<td>Median exon coverage</td>
<td>89.2%~100%</td>
<td>24/25 (96%)$^1$</td>
</tr>
<tr>
<td>Post-sequencing</td>
<td>76.8%~99.2%</td>
<td>23/25 (92%)$^1$</td>
</tr>
</tbody>
</table>

$^1$ Summarized based on 25ng of Extracted cfDNA

$^2$ Summarized based on 36ng of Extracted cfDNA

9.20 Concordance – FoundationOne Liquid Laboratory Developed Test (LDT) to FoundationOne Liquid CDx

In order to support the use of historical data from the FoundationOne Liquid LDT to evaluate performance across cancer types, a study was performed to evaluate concordance between FoundationOne Liquid CDx and the FoundationOne Liquid LDT across the genomic regions targeted by both assays. 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 37.

**Table 37. Concordance between FoundationOne Liquid LDT (F1L LDT) and FoundationOne Liquid CDx (F1LCDx)**

<table>
<thead>
<tr>
<th>Variant/Mutation Type</th>
<th>F1LCDx+ F1L LDT+</th>
<th>F1LCDx- F1L LDT+</th>
<th>F1LCDx+ F1L LDT-</th>
<th>F1LCDx- F1L LDT -</th>
<th>PPA [95% CI]</th>
<th>NPA [95% CI]</th>
<th>OPA [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Short Variants</td>
<td>2871</td>
<td>123</td>
<td>32</td>
<td>1171180</td>
<td>95.9% [95.1%-96.6%]</td>
<td>&gt;99.9% [&gt;99.9%-100.0%]</td>
<td>&gt;99.9% [&gt;99.9%-100.0%]</td>
</tr>
<tr>
<td>Base Substitutions</td>
<td>2415</td>
<td>104</td>
<td>31</td>
<td>999032</td>
<td>95.9% [95.0%-96.6%]</td>
<td>&gt;99.9% [&gt;99.9%-100.0%]</td>
<td>&gt;99.9% [&gt;99.9%-100.0%]</td>
</tr>
<tr>
<td>Indels</td>
<td>456</td>
<td>19</td>
<td>1</td>
<td>172148</td>
<td>96.0% [93.8%-97.6%]</td>
<td>&gt;99.9% [&gt;99.9%-100.0%]</td>
<td>&gt;99.9% [&gt;99.9%-100.0%]</td>
</tr>
<tr>
<td>Rearrangements</td>
<td>147</td>
<td>20</td>
<td>24</td>
<td>59587</td>
<td>88.0% [82.1%-92.5%]</td>
<td>&gt;99.9% [&gt;99.9%-100.0%]</td>
<td>99.9% [99.9%-99.9%]</td>
</tr>
<tr>
<td>Copy Number Amplifications</td>
<td>173</td>
<td>32</td>
<td>0</td>
<td>59463</td>
<td>84.4% [78.7%-89.1%]</td>
<td>99.8% [&gt;99.9%-100.0%]</td>
<td>99.8% [&gt;99.9%-100.0%]</td>
</tr>
<tr>
<td>Total</td>
<td>3191</td>
<td>175</td>
<td>166</td>
<td>1290230</td>
<td>94.8% [94.0%-95.5%]</td>
<td>&gt;99.9% [&gt;99.9%-100.0%]</td>
<td>&gt;99.9% [&gt;99.9%-100.0%]</td>
</tr>
</tbody>
</table>

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 of [94.0%-95.5%]. The respective short variant, rearrangement, and copy number amplification PPA values, with 95% two-sided CI, were: 95.9% [95.1%-96.6%], 88.0% [82.1%-92.5%], and 84.4% [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.

### 9.21 Molecular Index Barcode Performance

To evaluate the molecular index barcode performance, a total of 7,641 sequenced samples from FoundationOne Liquid CDx validation studies were analyzed with the FoundationOne Liquid CDx assay.

The overall coefficient of variation (% CV) of sequencing coverage across all barcodes was 8.95% for the enhanced sensitivity regions and 7.64% for the standard sensitivity regions. This observed small % CV includes both sample variability and barcode variability as these two components were confounded and inseparable. Results demonstrated that all 480 barcodes analyzed are detectable with low differences in sample coverage variance between barcodes, indicating comparable performance of the barcodes.

### 9.22 Automation Line Equivalence

An intermediate precision study was performed to establish equivalence between the Hamilton instrumentation and the Biomek/Bravo instrumentation. The study consisted of eight contrived samples run in triplicate across four runs and both instrumentation platforms resulting in a total of 192 sample replicates included in the study overall. The analysis evaluated the negative call rate (NCR) and positive call rate (PCR) for 1,309 variants from eight contrived samples. The PCR and NCR were also evaluated by the seven variant categories.

The Mann-Whitney test was used for the comparison of PCR and NCR across liquid handling platforms for each sample, all samples in aggregate, and for each variant type. The NCR across platforms for each analysis set (per sample, all samples in aggregate, per variant type) were not statistically significant (p > 0.05) by sample and by variant type. The PCR across platforms were not statistically significant (p > 0.05) with the exception of contrived sample #3, the aggregate of all samples, and substitutions in a non-repetitive region or a repetitive region of ≤7 base pairs. The PCRs for the Hamilton liquid handling platform were slightly higher than the PCRs for the Biomek/Bravo platform (92.08% versus 90.15% for sample #3, 90.75% versus 89.67% for all samples, and 91.14% versus 90.10% for substitutions in a non-repetitive region or repetitive region of ≤7 base pairs). The statistical significance observed was due to large sample sizes allowing for the detection of slight differences that are likely not meaningful in practice; therefore, the Hamilton and Biomek/Bravo liquid handling platforms are considered to be interchangeable in the FoundationOne Liquid CDx assay.
9.23 Updated LC Method Comparison Study
A method comparison study was conducted to demonstrate comparable performance between F1LCDx assay using original and updated LC input ranges. Eighty-one (81) clinical cfDNA samples from 10 unique disease ontologies were processed in triplicate to create 243 sample replicates. Samples were processed at the lower range for cfDNA input, 30ng for the original recommended minimum for LC input and 20ng for the updated minimum for LC input. 1815 unique targeted variants were analyzed, including CDx variants and variants from all alteration sub-types.

For each of the 81 samples, two of the three replicates were processed with F1LCDx around a 30ng input level, using the previous LC method, (referred to as CCD\textsubscript{1} and CCD\textsubscript{2}) and the third replicate was processed with F1LCDx around a 20ng input level, using the updated LC method, (referred to as UCD\textsubscript{ALL}). Two hundred and forty-three (243) sample replicates tested in this study passed all QC metrics. A non-inferiority analysis was performed. Aggregated PPA and NPA across all 1815 targeted variants were calculated for pairwise comparisons between CCD\textsubscript{1} and CCD\textsubscript{2}. PPAs and NPAs for all targeted variants were also calculated for either CCD\textsubscript{1} or CCD\textsubscript{2} versus UCD\textsubscript{ALL}. Agreement differences were calculated with corresponding 95\% upper 1-sided bounds. The upper bounds of the 1-sided 95\% CIs for agreement differences \( \zeta_{PPA1} \), \( \zeta_{PPA2} \), \( \zeta_{NPNA1} \) and \( \zeta_{NPNA2} \) were all <1\% for UCD\textsubscript{ALL}. Therefore, the F1LCDx assay using the updated LC input range was demonstrated to be non-inferior to F1LCDx using the original LC input range for the detection of CDx and non-CDx variants.

10 Clinical Validation Studies

10.1 Clinical Bridging Study: Detection of ALK Rearrangements to Determine Eligibility for Treatment with Alectinib
The clinical validity of using FoundationOne Liquid CDx as a companion diagnostic to identify patients with non-small cell lung cancer (NSCLC) harboring ALK rearrangements for treatment with alectinib was assessed through acinal bridging study using screening (i.e., pre-alectinib treatment) plasma samples from Cohort A of the Blood First Assay Screening Trial (BFAST, BO29554).

The BFAST trial is a Phase II/III multicenter study, in which Cohort A 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 a blood-based NGS assay (CTA).

The concordance between FoundationOne Liquid CDx and the CTA was evaluated as summarized in Table 38.

<table>
<thead>
<tr>
<th></th>
<th>CTA Pos</th>
<th>CTA Neg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FoundationOne Liquid CDx Positive(^1)</td>
<td>63</td>
<td>0</td>
<td>63</td>
</tr>
<tr>
<td>FoundationOne Liquid CDx Negative</td>
<td>12</td>
<td>174</td>
<td>186</td>
</tr>
<tr>
<td>Missing</td>
<td>4</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>183</td>
<td>262</td>
</tr>
</tbody>
</table>

\(^1\) VAF values down to 0.06\% VAF were observed for ALK rearrangements.

The PPA and NPA between FoundationOne Liquid CDx and the CTA using the CTA as the reference for the primary analysis set and the corresponding 95\% confidence intervals were:

- PPA [95\% CI]: 84.0\% [73.7\%, 91.4\%]
- NPA [95\% CI]: 100\% [ 97.9\%, 100.0\%]

After adjusting for a 5\% prevalence of ALK rearrangements in the intended use population, the PPV and NPV calculated using the CTA as the reference and the corresponding 95\% confidence intervals were:

- PPV [95\% CI]: 100.0\% [94.3\%, 200.0\%]
- NPV [95\% CI]: 93.5\% [89.0\%, 96.6\%]
The estimated Overall Response Rate (ORR) and the corresponding 95% confidence intervals was 88.9% [78.4%, 95.4%] for the FoundationOne Liquid CDx ALK-positive population which is comparable with the observed ORR and the corresponding 95% confidence intervals of 87.4% [78.5%, 93.5%] for the CTA ALK- positive population (BFAST Cohort A).

A sensitivity analysis was performed to estimate the clinical efficacy of treating patients with alectinib when considering missing FoundationOne Liquid CDx results. The estimated ORR and the corresponding 95% confidence intervals were 90.4% [90.1%, 90.6%] for the patient population that are both CTA ALK+ and FoundationOne Liquid CDx ALK+, demonstrating the robustness of the clinical efficacy analysis to missing FoundationOne Liquid CDx results.

10.2 FoundationOne Liquid CDx Concordance Study for EGFR exon 19 deletion and EGFR exon 21 L858R Alteration

Clinical validity of FoundationOne Liquid CDx assay was established as a companion diagnostic to identify patients with advanced NSCLC who may be eligible for treatment with TARCEVA® (erlotinib), IRESSA® (gefitinib), or TAGRISSO® (osimertinib). Two hundred and eighty retrospective samples from NSCLC patients were included in this study, which were tested for EGFR exon 19 deletion and exon 21 L858R alterations (EGFR alterations) by the FoundationOne Liquid CDx assay and the previously approved cobas® EGFR Mutation Test v2 (Roche Molecular Systems, referred to as cobas assay). Both EGFR alteration-positive and EGFR alteration-negative samples (based on CTA results) were selected from the screen failed population of an unrelated clinical trial in NSCLC. To avoid selection bias, the samples were selected starting with a specific testing date until the predefined number of 150 EGFR alteration-positive and 100 EGFR alteration-negative samples were fulfilled. Samples were tested across two replicates by the cobas assay (denoted as CCD1 and CCD2) and one replicate by FoundationOne Liquid CDx. The tested samples, from NSCLC patients, were compared against the intended use (IU) population with respect to gender to ensure the screening population is representative of the IU population. The variant calls were evaluated based on the agreement between both the FoundationOne Liquid CDx and the cobas assay results and between the two cobas assay replicates. For any samples in which there was insufficient plasma to process both CCD1 and CCD2, processing was not performed. In total there were 177 samples with complete test results available for analysis. The agreement analysis results between FoundationOne Liquid CDx and the cobas assay for the detection of EGFR exon 19 deletions and L858R alterations are presented in Table 39.

Table 39. Agreement analysis results for EGFR exon 19 deletion and L858R separately.

<table>
<thead>
<tr>
<th>Exon 19 deletion</th>
<th>PPAC1F</th>
<th>95.5%</th>
<th>NPAC1F</th>
<th>95.6%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAC1C2</td>
<td>97.7%</td>
<td>NPAC1C2</td>
<td>98.9%</td>
<td></td>
</tr>
<tr>
<td>PPAC2F</td>
<td>95.5%</td>
<td>NPAC2F</td>
<td>96.0%</td>
<td></td>
</tr>
<tr>
<td>PPAC2C1</td>
<td>96.2%</td>
<td>NPAC2C1</td>
<td>99.4%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>L858R</th>
<th>PPAC1F</th>
<th>100.0%</th>
<th>NPAC1F</th>
<th>95.6%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAC1C2</td>
<td>92.9%</td>
<td>NPAC1C2</td>
<td>98.9%</td>
<td></td>
</tr>
<tr>
<td>PPAC2F</td>
<td>100.0%</td>
<td>NPAC2F</td>
<td>94.7%</td>
<td></td>
</tr>
<tr>
<td>PPAC2C1</td>
<td>96.0%</td>
<td>NPAC2C1</td>
<td>98.0%</td>
<td></td>
</tr>
</tbody>
</table>

The concordance of EGFR mutations as detected by FoundationOne Liquid CDx and the cobas assay were assessed and the data are summarized in Table 40.

Table 40. Concordance among CCD1, CCD2 and FoundationOne Liquid CDx results with eligible samples (n=177)

<table>
<thead>
<tr>
<th></th>
<th>CCD1+</th>
<th></th>
<th></th>
<th>CCD1-</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCD2+</td>
<td>CCD2-</td>
<td>Total</td>
<td>CCD2+</td>
<td>CCD2-</td>
<td>Total</td>
</tr>
<tr>
<td>FoundationOne Liquid CDx+</td>
<td>80</td>
<td>4</td>
<td>84</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>FoundationOne Liquid CDx-</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>87</td>
<td>87</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>4</td>
<td>86</td>
<td>1</td>
<td>90</td>
<td>91</td>
</tr>
</tbody>
</table>
The agreement analysis results between FoundationOne Liquid CDx and the cobas assay are presented in Table 41.

<table>
<thead>
<tr>
<th></th>
<th>PPA</th>
<th>NPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCD2</td>
<td>CCD1(^1)</td>
<td>95.3%</td>
</tr>
<tr>
<td>CCD1</td>
<td>CCD2(^2)</td>
<td>96.1%</td>
</tr>
<tr>
<td>FoundationOne Liquid CDx</td>
<td>CCD1(^*)</td>
<td>97.7%</td>
</tr>
<tr>
<td>FoundationOne Liquid CDx</td>
<td>CCD2(^*)</td>
<td>97.7%</td>
</tr>
</tbody>
</table>

\(^1\) CCD1: the 1st replicate of cobas assay as the reference  
\(^2\) CCD2: the 2nd replicate of cobas assay as the reference

Table 42. Agreement analysis results

The estimates of ζPPA1, ζPPA2, ζNPA1 and ζNPA2 and the corresponding one-sided 95% upper bounds confidence limit computed using the bootstrap method are presented in Table 42.

<table>
<thead>
<tr>
<th></th>
<th>Point Estimate</th>
<th>Mean one-sided 95% upper confidence limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>ζPPA1</td>
<td>-2.3%</td>
<td>2.3%</td>
</tr>
<tr>
<td>ζNPA1</td>
<td>3.3%</td>
<td>6.6%</td>
</tr>
<tr>
<td>ζPPA2</td>
<td>-1.6%</td>
<td>4.7%</td>
</tr>
<tr>
<td>ζNPA2</td>
<td>3.3%</td>
<td>6.6%</td>
</tr>
</tbody>
</table>

Based on these results, FoundationOne Liquid CDx has been demonstrated to be non-inferior to the cobas assay for the detection of EGFR exon 19 deletions and EGFR exon 21 L858R mutations. This study establishes the clinical validity of the FoundationOne Liquid CDx assay for identifying patients eligible for treatment with erlotinib, gefitinib, and osimertinib.

10.3 Clinical Bridging Study: Detection of BRCA1/BRCA2/ATM Alterations to Determine Eligibility for Treatment with Olaparib

The clinical validity of using FoundationOne Liquid CDx as a companion diagnostic to identify patients with metastatic castrate-resistant prostate cancer (mCRPC) harboring BRCA1, BRCA2 or ATM alterations for treatment with olaparib was assessed through a clinical bridging study using screening (i.e., 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 (Lynparza™) versus enzalutamide or abiraterone acetate in men with metastatic castration-resistant prostate cancer who have failed prior treatment with a new hormonal agent and have homologous recombination repair gene mutations. Only Cohort A patients with either BRCA1, BRCA2 or ATM mutations were tested with the FoundationOne Liquid CDx assay.

In total, 4425 patients were screened and 387 (9.6%) were randomized into the PROfound study by the CTA. Of these 387 patients, 245 patients were randomized in cohort A. In cohort A, 181 out of the 245 randomized patients both consented to the use of their sample for ctDNA CDx development and had a plasma sample available for testing. In total, 181/245 (73.9%) of the Cohort A patients were tested using the FoundationOne Liquid CDx assay. Of these, 139 (76.8%) Cohort A patients had a successful FoundationOne Liquid CDx test result and 42 Cohort A patients had a failed FoundationOne Liquid CDx test result. This represents 56.7% (139/245) of total Cohort A patients with a FoundationOne Liquid CDx result. In addition, 250 non-HRRm patient samples were randomly selected for ctDNA testing from the screen-failed population to determine the NPA/NPV of the FoundationOne Liquid CDx assay. A total of 194/250 (77.6%) screen failed non-HRRm patients were successfully tested using the FoundationOne Liquid CDx assay.
Of the 139 successfully tested Cohort A patients, 111 patients were reported as \textit{BRCA1/BRCA2/ATM} mutation positive and 28 randomized patients were reported as biomarker negative by FoundationOne Liquid CDx.

Therefore, the FoundationOne Liquid CDx ctDNA biomarker positive subgroup comprises 111 patients with \textit{BRCA1, BRCA2, and/or ATM} mutations.

Sample accountability for this clinical bridging study is summarized in Table 43.

**Table 43. Sample accountability for olaparib clinical bridging study**

<table>
<thead>
<tr>
<th>Description</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients randomized into PROfound</td>
<td>387</td>
</tr>
<tr>
<td>Patients with qualifying \textit{BRCA1, BRCA2, or ATM} alterations (Cohort A)</td>
<td>245</td>
</tr>
<tr>
<td>Cohort A patients with samples tested by FoundationOne Liquid CDx</td>
<td>181</td>
</tr>
<tr>
<td>FoundationOne Liquid CDx results available</td>
<td>139</td>
</tr>
<tr>
<td>Cohort A patients, biomarker positive by FoundationOne Liquid CDx</td>
<td>111</td>
</tr>
</tbody>
</table>

**Table 44** shows the agreement analysis between CLIA CTA (tissue test) and the FoundationOne Liquid CDx results for PROfound patients, including Invalid and Not Tested results.

**Table 44. Summary of agreement analyses for FoundationOne Liquid CDx compared against CTA tissue test**

<table>
<thead>
<tr>
<th>FoundationOne Liquid CDx assay</th>
<th>CTA Results (n=495)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biomarker positive</td>
</tr>
<tr>
<td>Biomarker positive(^1)</td>
<td>111</td>
</tr>
<tr>
<td>Biomarker(^2) negative</td>
<td>28</td>
</tr>
<tr>
<td>Biomarker(^3) Invalid</td>
<td>42</td>
</tr>
<tr>
<td>Not Tested</td>
<td>64</td>
</tr>
</tbody>
</table>

**Agreement analyses (only Valid results included)**

<table>
<thead>
<tr>
<th></th>
<th>CTA Results (n=495)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA (95% CI(^9))</td>
<td>79.9 (72.2, 86.2) [111/139]</td>
</tr>
<tr>
<td>NPA (95% CI(^9))</td>
<td>91.8 (87.0, 95.2) [178/194]</td>
</tr>
<tr>
<td>OPA (95% CI(^9))</td>
<td>86.8 (82.7, 90.2) [289/333]</td>
</tr>
<tr>
<td>PPV (95% CI(^9))</td>
<td>66.6 (56.0, 77.2)</td>
</tr>
<tr>
<td>NPV (95% CI(^9))</td>
<td>95.7 (94.3, 97.1)</td>
</tr>
</tbody>
</table>

\(^1\) VAF values down to 0.11% VAF were observed for short variants and 0.25% VAF for rearrangements in \textit{BRCA1, BRCA2, or ATM}.

\(^2\) Biomarker refers to patients with eligible \textit{BRCA/ATM} mutations

\(^3\) Confidence intervals calculated using Clopper-Pearson method

The PPA and NPA between FoundationOne Liquid CDx and the CTA using the CTA as the reference for the primary analysis set and the corresponding 95% confidence intervals were:

- PPA [95% CI]: 79.9% [72.2%, 86.2%]
- NPA [95% CI]: 91.8% [87.0%, 95.2%]

After adjusting for a 17.1% prevalence of \textit{BRCA1/2} and \textit{ATM} alterations in the intended use population, the PPV and NPV calculated using the CTA as the reference and the corresponding 95% confidence intervals were:

- PPV [95% CI]: 66.6% [56.0%, 77.2%]
- NPV [95% CI]: 95.7% [94.3%, 97.1%]

The estimated radiological progression-free survival (rPFS) hazard ratio (HR) and the corresponding 95% confidence intervals were 0.331 [0.21, 0.53] for the FoundationOne Liquid CDx biomarker positive population,
which were comparable with the observed rPFS HR and the corresponding 95% confidence intervals of 0.34 [0.25, 0.47] for the CTA biomarker positive population (PROfound Cohort A).

Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the unknown FoundationOne Liquid CDx results was performed using the multiple imputation method in All Patients. After imputing the missing FoundationOne Liquid CDx results, the median rPFS HR and corresponding [95% CI] across the imputed datasets was 0.44 [0.32, 0.59], demonstrating robustness of the analysis to missing FoundationOne Liquid CDx results.

10.4 Clinical Bridging Study: Detection of BRCA1 and BRCA2 Alterations to Determine Eligibility of mCRPC Patients for Treatment with Rucaparib

The clinical performance of FoundationOne Liquid CDx as a companion diagnostic to identify patients with metastatic castration-resistant prostate cancer (mCRPC) harboring breast cancer gene 1 or 2 (BRCA1 or BRCA2) alterations for treatment with rucaparib was demonstrated using pre-rucaparib treatment blood samples from clinical trial NCT0952534 (TRITON2). The clinical data supporting the use of rucaparib in the proposed indication was submitted as New Drug Application (NDA) 209115/S-004.

A bridging study was conducted to evaluate: 1) the concordance between BRCA1 and BRCA2 alteration status by the clinical trial assay (CTA) and FoundationOne Liquid CDx, and 2) the clinical efficacy of rucaparib treatment in patients that would be eligible for therapy based on BRCA1 and BRCA2 alteration status as determined by FoundationOne Liquid CDx.

A total of 209 patients (All Patients) from TRITON2 were included in NDA 209115/S-004. Genomic status was determined using the FoundationOne laboratory developed test (LDT) (F1 LDT), the FoundationOne Liquid LDT (F1L LDT), or a local test, as summarized in Figure 1.

**Figure 1: TRITON2 Patient Enrollment**

<table>
<thead>
<tr>
<th>TRITON2</th>
<th>Enrolled based on F1 LDT</th>
<th>Enrolled based on F1L LDT</th>
<th>Enrolled based on Local Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Patients N=209</td>
<td>N=73</td>
<td>N=72</td>
<td>N=64</td>
</tr>
<tr>
<td>All Patients with Measurable Disease at Baseline N=109</td>
<td>N=40</td>
<td>N=31</td>
<td>Tissue N=40 Non-Tissue N=14</td>
</tr>
<tr>
<td>Primary Efficacy N=62</td>
<td>N=20</td>
<td>N=16</td>
<td>Tissue N=31 Non-Tissue N=7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tissue N=19 Non-Tissue N=7</td>
</tr>
</tbody>
</table>

Pre-rucaparib treatment plasma samples were available for 92% (192/209) of the patients. FoundationOne Liquid CDx data were available for 93% (178/192) of the patients with samples tested; inadequate input material resulted in FoundationOne Liquid CDx test data being unavailable for 14 patients. In total, FoundationOne Liquid CDx data were available for 85% (178/209) of All Patients.

Of the 62 patients in the Primary Efficacy Population (those patients with measurable visceral and/or nodal disease at baseline), FoundationOne Liquid CDx test data were obtained for 84% (52/62) and used for concordance and efficacy analyses. The sample accountability for this clinical bridging study is summarized in Table 45.
Table 45. Sample accountability for rucaparib prostate clinical bridging study

<table>
<thead>
<tr>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Patients in TRITON2</td>
<td>209</td>
</tr>
<tr>
<td>Total samples available for retesting by FoundationOne Liquid CDx</td>
<td>192</td>
</tr>
<tr>
<td>Patients with evaluable FoundationOne Liquid CDx data and cfDNA input ≥ 30ng (All Patients)</td>
<td>161</td>
</tr>
<tr>
<td>Patients with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 20ng (All Patients)</td>
<td>178</td>
</tr>
<tr>
<td>Primary efficacy population in TRITON2</td>
<td>62</td>
</tr>
<tr>
<td>Patients with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 30ng (Primary Efficacy Population)</td>
<td>48</td>
</tr>
<tr>
<td>Patients with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 20ng (Primary Efficacy Population)</td>
<td>52</td>
</tr>
</tbody>
</table>

Concordance between FoundationOne Liquid CDx and the CTAs
The concordance of BRCA status between FoundationOne Liquid CDx and CTA test results were evaluated in all patients as summarized in Table 46 and Table 47.

Table 46. Concordance between FoundationOne Liquid CDx BRCA Status and the CTA BRCA Status in All Patients with FoundationOne Liquid CDx cfDNA input ≥30ng

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>CTA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FoundationOne Liquid CDx</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA Positive</td>
<td>75</td>
<td>1</td>
<td>76</td>
</tr>
<tr>
<td>BRCA Negative</td>
<td>16</td>
<td>69</td>
<td>85</td>
</tr>
<tr>
<td>BRCA Unknown</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>71</td>
<td>164</td>
</tr>
</tbody>
</table>

1 VAF values down to 0.15%VAF were observed for short variants and 0.85%VAF for rearrangements in BRCA1 or BRCA2.

The PPA, NPA between FoundationOne Liquid CDx and the CTA, based on a cfDNA input ≥30ng, were determined using the CTA as the reference for all patients.

- PPA (95% CI): 82.4% (73.0%, 89.6%)
- NPA (95% CI): 98.6% (92.3%, 100.0%)

Table 47. Concordance between FoundationOne Liquid CDx BRCA Status and the CTA BRCA Status in All Patients with FoundationOne Liquid CDx cfDNA input ≥20ng

<table>
<thead>
<tr>
<th></th>
<th>All Patients</th>
<th>CTA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FoundationOne Liquid CDx</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA Positive</td>
<td>82</td>
<td>1</td>
<td>83</td>
</tr>
<tr>
<td>BRCA Negative</td>
<td>18</td>
<td>77</td>
<td>95</td>
</tr>
<tr>
<td>BRCA Unknown</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>80</td>
<td>183</td>
</tr>
</tbody>
</table>

1 VAF values down to 0.15%VAF were observed for short variants and 0.85%VAF for rearrangements in BRCA1 or BRCA2.

The PPA, NPA between FoundationOne Liquid CDx and the CTA, based on a cfDNA input ≥20ng, were determined using the CTA as the reference for all patients.

- PPA (95% CI): 82.0% (73.1%, 89.0%)
- NPA (95% CI): 98.7% (93.1%, 100%)
Efficacy Based on FoundationOne Liquid CDx Results

BRCA1 and BRCA2 alteration status were 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 46.3% [30.7%-62.6%] in BRCA 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 (Table 48).

<table>
<thead>
<tr>
<th>Primary Efficacy Population</th>
<th>FoundationOne Liquid CDx</th>
<th>CTA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRCA Positive N=38</td>
<td>BRCA Positive N= 41</td>
</tr>
<tr>
<td></td>
<td>(≥ 30 ng cfDNA input)</td>
<td>(≥ 20 ng cfDNA input)</td>
</tr>
<tr>
<td>Confirmed ORR (CR +PR), n (%)</td>
<td>18 (47.4)</td>
<td>19 (46.3)</td>
</tr>
<tr>
<td>95% CI (%)</td>
<td>31.0 – 64.2</td>
<td>30.7 - 62.6</td>
</tr>
</tbody>
</table>

Abbreviations: BRCA = breast cancer gene, includes BRCA1 and BRCA2; CI = confidence interval; CTA = clinical trial assay; ORR = objective response rate; CR = complete response; PR = partial response.

Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the unknown FoundationOne Liquid CDx results was performed using the multiple imputation method and demonstrated that the drug efficacy in the FoundationOne Liquid CDx positive population was robust to missing FoundationOne Liquid CDx results.

10.5 Clinical Bridging Study: Detection of PIK3CA Alterations to Determine Eligibility for Treatment with Alpelisib

Clinical validity of using FoundationOne Liquid CDx to identify breast cancer patients harboring PIK3CA alterations eligible for 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). A total of 395 patients were enrolled based on CTA1 results and 177 patients were enrolled based on CTA2 results. All 395 patients enrolled based on CTA1 results were retrospectively tested by CTA2. This clinical bridging study was performed based on CTA2 results.

Samples with ≥30 ng from 375 patients were tested by FoundationOne Liquid CDx. Excluding those with invalid results for either CTA2 or CDx (4 and 12, respectively), the primary efficacy analyses were conducted using data from the 359 subjects who were CTA2- evaluable and CDx- evaluable Table 49.

Table 49. Concordance between FoundationOne Liquid CDx and CTA2

<table>
<thead>
<tr>
<th>CDx</th>
<th>CTA2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>165</td>
</tr>
<tr>
<td>Negative</td>
<td>65</td>
</tr>
<tr>
<td>Invalid</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>237</td>
</tr>
</tbody>
</table>

1 VAF values down to 0.14% VAF were observed for short variants in PIK3CA.

Samples not tested are excluded from the analysis.

Samples tested with cfDNA input < 30 ng are excluded from the analysis.

The point estimates of PPA and NPA between FoundationOne Liquid CDx and the CTA2 assay and the corresponding 95% confidence intervals were:

- PPA [95% CI]: 71.7% [65.4%, 77.5%]
- NPA [95% CI]: 100% [97.2%, 100%]

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 1.1 criteria. Clinical efficacy of alpelisib in combination with fulvestrant for the FoundationOne Liquid CDx-positive population with cfDNA 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).
As summarized in Table 50, the PFS hazard ratio for the 165 tissue CTA2-positive, FoundationOne Liquid CDx-positive patients was 0.46 (95% CI: 0.30, 0.70). Median PFS was 11.0 months for the alpelisib plus fulvestrant arm versus 3.6 months for the placebo plus fulvestrant arm.

Table 50. Progression-free survival in the CTA2-positive, FoundationOne Liquid CDx-positive patients (primary analysis set)

<table>
<thead>
<tr>
<th>Progression free survival (months)</th>
<th>Alpelisib 300mg qd + Fulvestrant N=84</th>
<th>Placebo qd + Fulvestrant N=81</th>
<th>HR (95% CI) Alpelisib 300mg qd + Fulv /Placeboqd + Fulv(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of events (%)</td>
<td>54 (64.3)</td>
<td>67 (82.7)</td>
<td>0.46 (0.30, 0.70)</td>
</tr>
<tr>
<td>PD (%)</td>
<td>52 (61.9)</td>
<td>61 (75.3)</td>
<td></td>
</tr>
<tr>
<td>Death (%)</td>
<td>2 (2.4)</td>
<td>6 (7.4)</td>
<td></td>
</tr>
<tr>
<td>No of censored (%)</td>
<td>30 (35.7)</td>
<td>14 (17.3)</td>
<td></td>
</tr>
<tr>
<td>Median (95% CI)(^2)</td>
<td>11.0 (7.3, 15.9)</td>
<td>3.6 (2.4, 5.8)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Hazard ratio (HR) estimated using Cox regression model stratified by the two stratification factors: presence of lung and/or liver metastases, previous treatment with any CDK4/6 inhibitor, and adjusted for clinically relevant covariates, as well as the imbalanced covariates.

\(^2\) The 95% CI calculated from PROC LIFETEST output using the method of Brookmeyer and Crowley (1982). CDx results from samples tested with cfDNA input < 30 ng are treated as missing.

PD = progressive disease

Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the missing FoundationOne Liquid CDx results was performed using the multivariate imputation by chained equations (MICE0 method. After imputing the missing FoundationOne Liquid CDx results, the hazard ratio was estimated to be 0.63 (95% CI: 0.45, 0.87), demonstrating robustness of the clinical efficacy analysis to missing FoundationOne Liquid CDx results.

10.6 Clinical Bridging Study: Detection of MET single nucleotide variants (SNVs) and indels that lead to MET exon 14 skipping to Determine Eligibility for Treatment with capmatinib

The clinical performance of FoundationOne Liquid CDx for detecting SNVs and indels that lead to MET exon 14 skipping in NSCLC patients who may benefit from treatment with capmatinib (Table 1) was established with clinical data generated from a clinical bridging study using samples from patients enrolled in the GEOMETRY mono-1 study. The study demonstrates concordance between the enrollment assay, i.e., the clinical trial assay (CTA), and the FoundationOne Liquid CDx assay and establish the effectiveness of the FoundationOne Liquid CDx assay.

GEOMETRY mono-1 was a prospectively designed, multi-center, open-label, single arm Phase II study of oral cMET inhibitor, TABRECTA (capmatinib), in adult patients with EGFR wild-type (wt), and anaplastic lymphoma kinase (ALK) negative advanced NSCLC. Patients were enrolled into multiple cohorts of the study, but the bridging study was focused on the fully-enrolled MET exon 14 skipping positive Cohorts 4 and 5b. Cohort 4 only enrolled pretreated (second and third line) patients with MET exon 14 skipping, and Cohort 5b only enrolled treatment-naive patients with MET exon 14 skipping. Patients were screened for enrollment into Cohorts 4 and 5b for MET exon 14 skipping status using a MET exon 14 skipping reverse-transcriptase PCR (RT-PCR) CTA that was detected MET exon 14 skipping in a patient’s tissue. Plasma samples were collected and stored prior to study treatment for retrospective testing. Patients enrolled in Cohorts 4 and 5b received 400mg of capmatinib orally twice daily in tablet form. Efficacy was evaluated every six weeks from the first day of treatment until RECIST 1.1 disease progression.

A clinical bridging study was conducted to evaluate: 1) the concordance between MET single nucleotide variants (SNVs) and indels that lead to MET exon 14 skipping status by the clinical trial assay (CTA) and FoundationOne Liquid CDx, and 2) the clinical efficacy of capmatinib treatment in patients that would be eligible for therapy based on MET biomarker positive status as determined by FoundationOne Liquid CDx.

The primary endpoint of GEOMETRY mono-1 was the overall response rate (ORR) by Blinded Independent Review Committee (BIRC) assessment by cohort to determine whether treatment with capmatinib is effective. Duration of response (DOR) as assessed by BIRC was the key secondary endpoint.
The primary concordance analysis of the status of MET SNVs and indels that lead to MET exon 14 skipping between FoundationOne Liquid CDx and the tissue CTA test results were evaluated in both analysis sets that met ≥30 ng cfDNA input and ≥20 ng cfDNA input. The analysis on the ≥30 ng cfDNA input population evaluated 150 patients (78 MET exon 14 skipping positive patients, and 72 MET exon 14 skipping negative patients), excluding invalid CDx results. The analysis on the ≥20 ng cfDNA input population evaluated 171 patients (83 MET exon 14 skipping positive patients, and 88 MET exon 14 skipping negative patients), excluding invalid CDx results.

Agreement (PPA, NPA and OPA) for combined Cohort 4 and 5b by ≥30 ng cfDNA input and ≥20 ng cfDNA input CDx are shown in Table S1 and Table S2, below. For the 150 patients meeting the ≥30 ng cfDNA input, the PPA, NPA and OPA and respective confidence intervals were determined to be 70.5% (59.1%, 80.3%), 100% (95.0%, 100%) and 84.7% (77.9%, 90.0%). For the 171 patients meeting the ≥20 ng cfDNA input, the PPA, NPA and OPA and respective confidence intervals were determined to be 68.7% (57.6%, 78.4%), 100% (95.9, 100%) and 84.8% (78.5, 89.8%).

Table S1. Agreement between CDx and CTA based on CTA results in combined cohorts by cfDNA input ≥30 ng

<table>
<thead>
<tr>
<th>Measure of agreement</th>
<th>Percent agreement % (n/N)</th>
<th>95% CI (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA¹</td>
<td>70.5 (55/78)</td>
<td>(59.1, 80.3)</td>
</tr>
<tr>
<td>NPA</td>
<td>100 (72/72)</td>
<td>(95.0, 100)</td>
</tr>
<tr>
<td>OPA</td>
<td>84.7 (127/150)</td>
<td>(77.9, 90.0)</td>
</tr>
</tbody>
</table>

¹ VAF values down to 0.16% VAF were observed for MET short variants.
N: The total number of patients. It is the denominator for percentage (%) calculation: Number of patients with agreement between CTA and CDx.
(1) The 95% CI calculated using Clopper-Pearson method

Table S2. Agreement between CDx and CTA based on CTA results in combined cohorts by cfDNA input ≥20 ng

<table>
<thead>
<tr>
<th>Measure of agreement</th>
<th>Percent agreement % (n/N)</th>
<th>95% CI (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA¹</td>
<td>68.7 (57/83)</td>
<td>(57.6, 78.4)</td>
</tr>
<tr>
<td>NPA</td>
<td>100 (88/88)</td>
<td>(95.9, 100)</td>
</tr>
<tr>
<td>OPA</td>
<td>84.8 (145/171)</td>
<td>(78.5, 89.8)</td>
</tr>
</tbody>
</table>

¹ VAF values down to 0.16% VAF were observed for MET short variants.
N: The total number of patients. It is the denominator for percentage (%) calculation: Number of patients with agreement between CTA and CDx.
(1) The 95% CI calculated using Clopper-Pearson method

Based on the PPA of 70.5% (59.1%, 80.3%) between FoundationOne Liquid CDx (F1LCDx) and the tissue CTA, reflex testing using tissue specimens to an FDA approved tissue test is recommended, if feasible, if the plasma test is negative.

Clinical effectiveness of FoundationOne Liquid CDx was evaluated by estimation of clinical efficacy in the CTA-enrolled MET exon 14 deletion positive patient population, as assessed by the primary objective of ORR by BIRC. The GEOMETRY mono-1 clinical trial met its primary objective demonstrating a statistically significant improvement in ORR by BIRC assessments in patients with MET exon 14 deletion positive tumors in each cohort.

Table S3 and Table S4 present the clinical efficacy of TABRECTA analyzed in CTA-positive patients who were tested as CDx-positive (“double positive” patients) in each cohort that met the ≥30 ng cfDNA input and ≥20 ng cfDNA input CDx sample requirements, respectively. In Cohort 4 there were 39 patients with ≥30 ng cfDNA input and 41 with ≥200ng cfDNA input with valid results for analysis of ORR. In Cohort 5b there were 16 patients, all of whom met the ≥30ng cfDNA input.

Patients in Cohort 4 that met the ≥30 ng cfDNA input demonstrated an ORR of 51.3% (34.8%, 67.6%). Patients from Cohort 4 that met the ≥20 ng cfDNA input requirements demonstrated an ORR of 48.8% (32.9%, 64.9%). For
patients in Cohort 5b, all patients met the ≥30 ng cfDNA input and demonstrated an ORR of 81.3% (54.4%, 96.0%).

Table 53. Overall response per BIRC assessment in (CTA-positive, CDx-positive) and CTA-positive patients by cohort and CDx sample requirements (Cohort 4).

<table>
<thead>
<tr>
<th>(CTA+, CDx+)</th>
<th>(CTA+, CDx+)</th>
<th>(CTA+, CDx+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFDA input ≥ 30 ng</td>
<td>CFDA input ≥ 20 ng</td>
<td>CFDA input ≥ 30 ng</td>
</tr>
<tr>
<td>N=39</td>
<td>N=41</td>
<td>N=69</td>
</tr>
<tr>
<td>n (%)</td>
<td>95% CI (1)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Overall Response Rate (ORR: CR + PR)</td>
<td>20 (51.3) (34.8, 67.6)</td>
<td>20 (48.8) (32.9, 64.9)</td>
</tr>
</tbody>
</table>

(1) The 95% CI calculated with the Clopper-Pearson Exact method.

Table 54. Overall response per BIRC assessment in (CTA-positive, CDx-positive) and CTA-positive patients by cohort and CDx sample requirements (Cohort 5b).

<table>
<thead>
<tr>
<th>(CTA+, CDx+)</th>
<th>(CTA+, CDx+)</th>
<th>(CTA+, CDx+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFDA input ≥ 30 ng</td>
<td>CFDA input ≥ 20 ng</td>
<td>CFDA input ≥ 30 ng</td>
</tr>
<tr>
<td>N=16</td>
<td>N=16</td>
<td>N=28</td>
</tr>
<tr>
<td>n (%)</td>
<td>95% CI (1)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Overall Response Rate (ORR: CR + PR)</td>
<td>13 (81.3) (54.4, 96.0)</td>
<td>13 (81.3) (54.4, 96.0)</td>
</tr>
</tbody>
</table>

(1) The 95% CI calculated with the Clopper-Pearson Exact method.

Estimated drug efficacy in FoundationOne Liquid CDx Positive (F1LCDx(+)) patients

The ORR by BIRC assessment in F1LCDx(+) patients was calculated for Cohort 4 and Cohort 5b, separately. Because all CTA(-) patients are tested as negative by CDx (i.e. NPA=100%) and thus PPV is estimated as 100%, the results do not vary with Pr(CTA+) values and the ORR in F1LCDx(+) population is estimated as the same as the ORR in F1LCDx [CTA+(+)CDx+] population. For F1LCDx(+) patients meeting "Recommended" CDx sample requirement (cfDNA input ≥ 30 ng), the ORR (95% CI) is 51.3% (34.8, 67.6%) in Cohort 4 and 81.3% (54.4%, 96.0%) in Cohort 5b, respectively. For CDx(+) patients meeting "Minimum" CDx sample requirement (cfDNA input ≥ 20 ng), the ORR (95% CI) is 48.8% (32.9, 64.9%) in Cohort 4 and 81.3% (54.4%, 96.0%) in Cohort 5b, respectively.

Sensitivity analysis on missing FoundationOne Liquid CDx results

The impact of missing F1LCDx results on the concordance between CTA and F1LCDx and final drug efficacy in F1LCDx(+) patients was evaluated by imputing the missing F1LCDx results using multiple imputation method. For Cohort 4, the imputed ORR (95% CI) by BIRC were estimated to be 46.5% (32.6, 60.9%) given "Recommended" sample requirement and 47.2% (33.3, 61.5%) given "Minimum" sample requirement. For Cohort 5b, the imputed ORRs and two-sided 95% CIs by BIRC were estimated to be 75.3% (53.3, 94.4%) given "Recommended" samplerequirement and 78.1% (55.6, 95.5%) given "Minimum" sample requirement. The sensitivity analysis results demonstrated that the concordance between CTA and F1LCDx and final drug efficacy in F1LCDx(+) population are robust to missing F1LCDx results.

10.7 Clinical Bridging Study: Detection of ROS1 Fusions to Determine Eligibility for Treatment with Entrectinib

The clinical performance of using FoundationOne Liquid CDx as a companion diagnostic to identify NSCLC patients harboring ROS1 fusions eligible for treatment with entrectinib (Table 1) was assessed in this clinical bridging study. All available pre-entrectinib treatment plasma samples from patients enrolled in ALKA, STARTRK-1, and STARTRK-2 clinical trials were tested by FoundationOne Liquid CDx as part of this clinical bridging study. Only samples from STARTRK-2 were available for testing by F1LCDx.

A clinical bridging study was conducted to evaluate the 1) the concordance between the F1LCDx assay and the CTAs used for clinical trial enrollment for the detection of ROS1 fusions and 2) the clinical efficacy of entrectinib treatment in patients who would be eligible for therapy based on ROS1 fusions positive as determined by F1LCDx.
A total of 255 patients were included in the clinical bridging study. Of these 255 patients, 161 were determined as ROS1 fusion positive based on testing by the CTAs. Initially, the clinical bridging study included 51 ROS1 fusion positive NSCLC patients from the new drug application (NDA) efficacy population, 41 additional ROS1 fusion positive, ROS1 inhibitor-naive patients with NSCLC with measurable disease who had insufficient follow-up (<12 months) at the time of the NDA submission, 67 ROS1 fusion positive patients with NSCLC who were enrolled prior to October 31, 2018, and two (2) patients with prior ROS1 inhibitor treatment and used only for the concordance evaluation. In total, clinical outcome data from 161 ROS1 fusion positive patients (as determined by the CTAs) enrolled before October 31, 2018 (based on the May 1, 2019 clinical data cutoff date) were planned for use in the bridging analysis. Of the 94 ROS1 fusion negative samples (as determined by the CTAs), 73 were patients enrolled in the clinical trial by the CTAs as NTRK1/2/3 fusion positive. The remaining 21 ROS1 fusion negative samples were FFPE tissue-matched plasma samples procured from a commercial source, with tissue testing by one of the CTAs used for clinical trial enrollment. Only samples from STARTRK-2 were available for testing by F1LCDx and, thus, 218 of the 255 samples were evaluated by retrospective F1LCDx testing. Among them, 203 samples met the F1LCDx quality control metrics, and 175 samples met the recommended sample input of cfDNA ≥ 30ng. An additional 28 samples met the minimum F1LCDx sample input criteria of cfDNA ≥ 20ng. Sample accountability for this clinical bridging study is summarized in Table 55.

### Table 55. Sample Accountability for the ROS1 Clinical Bridging Study

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>Total # of samples (n=255)</th>
<th>Sample fail/unavailable (n=52)</th>
<th>F1LCDx evaluable (n=203)</th>
<th>DNA ≥ 30 ng (n=175)</th>
<th>DNA ≥ 20 ng and &lt; 30 ng (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procured ROS1 Negative samples</td>
<td>21</td>
<td>2</td>
<td>19</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>ROS1 Negative by CTA test*</td>
<td>73</td>
<td>14</td>
<td>59</td>
<td>51</td>
<td>8</td>
</tr>
<tr>
<td>ROS1 Positive by CTA test</td>
<td>161</td>
<td>36</td>
<td>125</td>
<td>107</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>255</td>
<td>52 (20.4%)</td>
<td>203 (79.6%)</td>
<td>175 (68.6%)</td>
<td>28 (11.0%)</td>
</tr>
</tbody>
</table>

*The CTA ROS1-fusion negative samples were enrolled in the clinical trials as CTA NTRK-fusion positive.

The primary analyses were conducted for the 175 patients with evaluable FoundationOne Liquid CDx results that also had a DNA input of ≥ 30 ng. The concordance between FoundationOne Liquid CDx and the CTAs is summarized in Table 56. Over 20 different types of CTAs with a mix of technologies (RT-PCR, FISH, NGS) and analytes (RNA and DNA) were used to enroll the patients in the clinical trials.

### Table 56. Concordance result between F1LCDx and CTA for the detection of ROS1-fusions for samples with DNA content ≥30 ng (n=175).

<table>
<thead>
<tr>
<th>F1LCDx</th>
<th>Detected</th>
<th>Not Detected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detected</td>
<td>55</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>Not Detected</td>
<td>52</td>
<td>68</td>
<td>120</td>
</tr>
<tr>
<td>Unevaluable</td>
<td>54</td>
<td>26</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>161</td>
<td>94</td>
<td>255</td>
</tr>
</tbody>
</table>

**Agreement Statistics Excluding CDx-Unevaluable Results**

- **PPA** [95% CI]: 51.4% [42.05%, 60.66%]
- **NPA** [95% CI]: 100.0% [94.65%, 100%]

**Percent Unevaluable**

- **PPA** [95% CI]: 33.5% [26.7%, 41.1%]
- **NPA** [95% CI]: 27.7% [19.6%, 37.4%]

*Calculated with Wilson 2-sided 95% CI

The following concordance statistics were calculated for this sample set using the CTA as the reference:

- **PPA** [95% CI]: 51.4% [42.05%, 60.66%]
- **NPA** [95% CI]: 100.0% [94.65%, 100%]
After adjusting for a 1% prevalence of ROS1 rearrangements in the intended use population PPV and NPV were calculated using the CTA as the reference:

- **PPV [95% CI]:** 100% [93.47%, 100%]
- **NPV [95% CI]:** 99.51% [99.41%, 99.61%]

The discordances between the CTAs and F1LCDx among ROS1 fusion positive patients was evaluated by stratifying the PPA into two subgroups, DNA-based NGS CTAs and RNA-based NGS CTAs. The PPA between F1LCDx and DNA-based NGS CTAs was 55.6% (10/18) with 95% two-sided CI (33.7%, 75.4%). The PPA between F1LCDx and RNA-based NGS CTAs was 50.6% (40/79) with 95% two-sided CI (39.8%, 61.4%). Of the 52 CTA positive patients who were F1LCDx negative, 92.3% (48/52) did not have detectable tumor fraction as determined by F1LCDx, suggesting that the ctDNA content in these samples was low.

The clinical efficacy of entrectinib in the clinical trials was measured in ORR with either confirmed complete response (CR) or partial response (PR) based on blinded independent centralized review (BICR). Only clinical samples with clinical outcome data were used in this part of the study analysis.

The ORR in the CTA-positive population was 67.3% (107/159) with 95% two-sided CI (59.7%, 74.1%). Fifty-four (54) patients were CTA positive and had F1LCDx ROS1 fusion-positive results. The ORR for this population was 66.7% (36/54) with 95% two-sided CI (53.4%, 77.8%). Fifty-one (51) patients were CTA positive but had F1LCDx ROS1 negative results. The ORR for this population was 66.7% (34/51) with 95% two-sided CI (53.0%, 78.0%). Fifty-four (54) patients were CTA positive but were unevaluable by F1LCDx. The ORR for this population was 68.5% (37/54) with 95% two-sided CI (55.3%, 79.3%).

### Table 57. ORR in CTA-positive, FoundationOne Liquid CDx-positive patients

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>Total CTA positive population (N=159)</th>
<th>CTA positive and F1LCDx positive (N=54)</th>
<th>CTA positive and F1LCDx negative (N=51)</th>
<th>CTA positive and F1LCDx unevaluable (N=54)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ORR% [95% CI]</strong></td>
<td>67.3% [59.7%, 74.1%]</td>
<td>66.7% [53.4%, 77.8%]</td>
<td>66.7% [53.0%, 78.0%]</td>
<td>68.5% [55.3%, 79.3%]</td>
</tr>
<tr>
<td>Complete response</td>
<td>14 (8.8%)</td>
<td>5 (9.3%)</td>
<td>6 (11.8%)</td>
<td>3 (5.6%)</td>
</tr>
<tr>
<td>Partial response</td>
<td>93 (58.5%)</td>
<td>31 (57.4%)</td>
<td>28 (54.9%)</td>
<td>34 (63.0%)</td>
</tr>
<tr>
<td>Number of responders</td>
<td><strong>N=107</strong></td>
<td><strong>N=36</strong></td>
<td><strong>N=34</strong></td>
<td><strong>N=37</strong></td>
</tr>
<tr>
<td><strong>Duration of response</strong></td>
<td><strong>Median± in months (range)</strong></td>
<td><strong>9.5 (1.8, 42.3)</strong></td>
<td><strong>6.4 (1.8, 20.5)</strong></td>
<td><strong>13.4 (1.9, 27.6)</strong></td>
</tr>
<tr>
<td>% with duration ≥9 months</td>
<td>61.7%</td>
<td>38.9%</td>
<td>70.6%</td>
<td>75.7%</td>
</tr>
<tr>
<td>% with duration ≥12 months</td>
<td>41.1%</td>
<td>19.4%</td>
<td>55.9%</td>
<td>48.6%</td>
</tr>
<tr>
<td>% with duration ≥18 months</td>
<td>19.6%</td>
<td>5.6%</td>
<td>26.5%</td>
<td>27.0%</td>
</tr>
</tbody>
</table>

**Two-sided 95% CI for each subgroup was based on the Wilson-score method**

±Arithmetic median used (not Kaplan-Meier methods) since censoring data was not available

Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the missing FoundationOne Liquid CDx results was performed using the multiple imputation method. Based on the 100 bootstrap samples with 50 times imputation estimated ORR of the FoundationOne Liquid CDx ROS1-positive population was 67.1% [50.7%, 78.9%].

There were 70 ROS1 positive patients by the CTAs with partial or complete response to entrectinib, who also had an F1LCDx result. Among them, only 51.4% (36/70) were positive by F1LCDx (95% CI: 39.9, 62.8). There were 35 ROS1-positive patients by the CTAs who did not respond to entrectinib, who also had an F1LCDx result (54-36=18 and 51-34=17). Among them, 51.4% (18/35) were positive by F1LCDx (95% CI: 35.6, 67.0).
Clinical Bridging Study: Detection of NTRK 1/2/3 Fusions to Determine Eligibility for Treatment with Entrectinib

The clinical performance of using FoundationOne Liquid CDx as a companion diagnostic to identify patients with solid tumors harboring NTRK1, NTRK2, or NTRK3 fusions eligible for treatment with entrectinib (Table 1) was assessed in this clinical bridging study. All patients with available plasma samples from the NDA population from ALKA, STARTRK-1, and STARTRK-2 clinical trials were tested by FoundationOne Liquid CDx as part of this clinical bridging study. Only samples from STARTRK-2 were available for testing by F1LCDx.

A clinical bridging study was conducted to evaluate the 1) the concordance between the F1LCDx assay and the CTAs used for clinical trial enrollment for the detection of NTRK fusions and 2) the clinical efficacy of entrectinib treatment in patients who would be eligible for therapy based on NTRK fusions positive as determined by F1LCDx.

A total of 256 patients were included in the clinical bridging study. Of these 256 patients, 74 were determined as NTRK fusion-positive based on testing by the CTAs. Initially, the clinical bridging study included 54 NTRK fusion-positive patients from the NDA efficacy population, as well as 20 NTRK fusion-positive patients who were enrolled after the data cutoff. Of the 182 NTRK fusion-negative samples, 161 were patients enrolled in the clinical trial by the CTAs as ROS1 fusion-positive. The remaining 21 NTRK fusion-negative samples were FFPE tissue-matched plasma samples procured from a commercial source, with tissue testing by one of the CTAs used for clinical trial enrollment. Only samples from STARTRK-2 were available for testing by F1LCDx and, thus, 218 of the 256 samples were included for retrospective F1LCDx testing. Among them, 203 samples met the F1LCDx quality control metrics, and 175 samples met the recommended sample input of cfDNA ≥ 30ng. An additional 28 samples met the minimum F1LCDx sample input criteria of cfDNA ≥ 20ng. Sample accountability for this clinical bridging study is summarized in Table 58.

Table 58. Sample Accountability for the NTRK Clinical Bridging Study

<table>
<thead>
<tr>
<th>Source of samples</th>
<th>Total # of samples (n=256)</th>
<th>Sample fail/unavailable (n=53)</th>
<th>F1LCDx evaluable (n=203)</th>
<th>DNA ≥30 ng (n=175)</th>
<th>DNA ≥20 ng and &lt;30 ng (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procured NTRK Negative samples</td>
<td>21</td>
<td>2</td>
<td>19</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>NTRK Negative by CTA test*</td>
<td>161</td>
<td>36</td>
<td>125</td>
<td>107</td>
<td>18</td>
</tr>
<tr>
<td>NTRK Positive by CTA test</td>
<td>74</td>
<td>15</td>
<td>59</td>
<td>51</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>256</td>
<td>53 (20.7%)</td>
<td>203 (79.3%)</td>
<td>175 (68.4%)</td>
<td>28 (10.9%)</td>
</tr>
</tbody>
</table>

*The CTA NTRK-fusion negative samples were enrolled in the clinical trial as CTA ROS1-fusion positive.

The primary analyses were conducted for the 175 patients with evaluable FoundationOne Liquid CDx results that also had a DNA input of ≥ 30 ng. A comparison of the clinical outcomes and baseline characteristics demonstrated that the FoundationOne Liquid CDx-eligible population was representative of the FoundationOne Liquid CDx-unevaluable population in this bridging study. The concordance between FoundationOne Liquid CDx and the CTAs is summarized in Table 59. Over 20 different types of CTAs with a mix of technologies (RT-PCR, FISH, NGS) and analytes (RNA and DNA) were used to enroll the patients in the clinical trials.

Table 59. Concordance between FoundationOne Liquid CDx and CTAs for the detection of NTRK1, NTRK2, and NTRK3 fusions

<table>
<thead>
<tr>
<th>CTAs</th>
<th>Detected</th>
<th>Not Detected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1LCDx</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detected</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Not Detected</td>
<td>26</td>
<td>124</td>
<td>150</td>
</tr>
<tr>
<td>Unevaluable</td>
<td>23</td>
<td>58</td>
<td>81</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>182</td>
<td>256</td>
</tr>
</tbody>
</table>
The following concordance statistics were calculated for this sample set:

- **PPA [95% CI]: 49.0% [35.9%, 62.3%]**
- **NPA [95% CI]: 100.0% [97.0%, 100%]**

After adjusting for a 0.32% prevalence of **NTRK** fusions in the intended use population PPV and NPV were calculated using the CTA as the reference:

- **PPV [95% CI]: 100% [86.7%, 100%]**
- **NPV [95% CI]: 99.8% [99.79%, 99.88%]**

The discordances between the CTAs and F1LCDx among **NTRK1/2/3** fusion-positive patients was evaluated by stratifying the PPA into two subgroups, DNA-based NGS CTAs and RNA-based NGS CTAs. The PPA between F1LCDx and DNA-based NGS CTAs was 65.0% (13/20) with 95% two-sided CI (43.3%, 81.9%). The PPA between F1LCDx and RNA-based NGS CTAs was 38.7% (12/31) with 95% two-sided CI (23.7%, 56.2%).

The clinical efficacy of entrectinib in the clinical trials was measured in overall response rate (ORR) with either confirmed complete response (CR) or partial response (PR) based on blinded independent centralized review (BICR). Only clinical samples with clinical outcome data were used in this part of the study analysis.

The ORR in the CTA positive population was 63.5% (47/74) with 95% two-sided CI (52.1%, 73.6%). Twenty-five (25) patients were CTA positive and had F1LCDx **NTRK** positive results. The ORR for this population was 72.0% (18/25) with 95% two-sided CI (52.4%, 85.7%). Twenty-six (26) patients were CTA positive but had F1LCDx **NTRK** negative results. The ORR for this population was 57.7% (15/26) with 95% two-sided CI (38.9%, 74.5%).

Twenty-three (23) patients were CTA positive but were F1LCDx-unevaluable. The ORR for this population was 60.9% (14/23) with 95% two-sided CI (40.8%, 77.8%) (**Table 60**).

**Table 60. ORR in CTA-positive, FoundationOne Liquid CDx-positive patients**

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>Total CTA positive population (N=74)</th>
<th>CTA positive and F1LCDx positive (N=25)</th>
<th>CTA positive and F1LCDx negative (N=26)</th>
<th>CTA positive and F1LCDx unevaluable (N=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR% [95% CI**]</td>
<td>63.5%</td>
<td>72.0%</td>
<td>57.7%</td>
<td>60.9%</td>
</tr>
<tr>
<td></td>
<td>[52.1,73.6]</td>
<td>[52.4, 85.7]</td>
<td>[38.9, 74.5]</td>
<td>[40.8, 77.8]</td>
</tr>
<tr>
<td>Complete response</td>
<td>5 (6.8%)</td>
<td>0 (0.0%)</td>
<td>1 (3.8%)</td>
<td>4 (17.4%)</td>
</tr>
<tr>
<td>Partial response</td>
<td>42 (56.8%)</td>
<td>18 (72.0%)</td>
<td>14 (53.8%)</td>
<td>10 (43.5%)</td>
</tr>
<tr>
<td>Number of responders</td>
<td>N=47</td>
<td>N=18</td>
<td>N=15</td>
<td>N=14</td>
</tr>
<tr>
<td>Duration of response</td>
<td>Median± in months (range)</td>
<td>7.5 (1.4, 26.0)</td>
<td>5.9 (1.9, 16.6)</td>
<td>7.9 (1.4, 26.0)</td>
</tr>
<tr>
<td>% with duration ≥9 months</td>
<td>44.7%</td>
<td>38.9%</td>
<td>46.7%</td>
<td>50.0%</td>
</tr>
<tr>
<td>% with duration ≥12 months</td>
<td>29.8%</td>
<td>22.2%</td>
<td>40.0%</td>
<td>28.6%</td>
</tr>
<tr>
<td>% with duration ≥18 months</td>
<td>10.6%</td>
<td>0.0%</td>
<td>13.3%</td>
<td>21.4%</td>
</tr>
</tbody>
</table>

**Two-sided 95% CI for each subgroup was based on the Wilson-score method**

±Arithmetic median used (not Kaplan-Meier methods) since censoring data was not available
Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the missing FoundationOne Liquid CDx results was performed using the multiple imputation method. Based on the 100 bootstrap samples with 50 times imputation, the estimated ORR of the FoundationOne Liquid CDx NTRK-positive population was 67.5% [52.4%, 87.1%].

There were 33 NTRK1/2/3-positive patients by the CTAs with partial or complete response to entrectinib, who also had an F1LCDx result. Among them, only 54.5% (18/33) were positive by F1LCDx (95% CI: 38.0, 70.2). There were 18 CTA-positive patients who did not respond to entrectinib, who also had an F1LCDx result (25-18=7 and 26-15=11). Among them, 38.9% (7/18) were positive by F1LCDx (95% CI: 20.3, 61.4).

There were 25 patients positive for an NTRK3 fusion in the entrectinib clinical studies. Among them, 68.0% (17/25) were negative for NTRK3 fusions by F1LCDx. Among the 17 patients who were negative for NTRK3 fusions by F1LCDx, 64.7% (11/17) had response to entrectinib. Further, F1LCDx detected one (1) of seven (7) different NTRK3 fusions that were detected by the CTAs.

10.9 Clinical Bridging Study: Detection of EGFR exon 20 Insertions to Determine Eligibility for Treatment with Mobocertinib

The clinical performance of FoundationOne Liquid CDx as a companion diagnostic to identify NSCLC patients harboring EGFR exon 20 insertions eligible for treatment with mobocertinib was assessed in a clinical bridging study. All available plasma samples from patients enrolled in the NDA population from the AP32788-15-101 (Study 101) clinical trial were tested by F1LCDx as part of this clinical bridging study. To further support the clinical validation of F1LCDx for the detection of EGFR exon 20 insertions, additional CTA-positive and CTA-negative patients from the non-NDA population (i.e., patient population that were included as part of the dose-escalation cohort or did not receive prior platinum treatment) of the AP32788-15-101 trial were included in the concordance analysis. Additionally, CTA-negative tissue samples (with matched plasma for F1LCDx testing) procured from commercial sources, and residual plasma samples (not tissue-matched) from the FMI clinical archive and processed in previous studies, were also included in the clinical bridging study.

The clinical bridging study evaluated 1) the concordance between the F1LCDx assay and the CTAs used for clinical trial enrollment for the detection of EGFR exon 20 insertions 2) the clinical efficacy of mobocertinib treatment in patients who would be eligible for therapy based on EGFR exon 20 insertions-positive status as determined by F1LCDx and 3) a sensitivity analysis to assess the robustness of the concordance and efficacy results subject to the missing F1LCDx results.

A total of 342 patients were identified for the clinical bridging study analysis. Among 230 EGFR exon 20 insertion-positive patients by CTA, 46 did not have a plasma sample available for F1LCDx testing and 25 patient samples failed the F1LCDx QC metrics, resulting in a total of 159 EGFR exon 20 insertion-positive samples that had F1LCDx-evaluable results. Among the 159 EGFR exon 20 insertion-positive evaluable samples, 132 had cfDNA ≥30 ng for input to LC and were used for the primary analysis. Twenty-seven (27) EGFR exon 20 insertion-positive samples had cfDNA <30 ng and ≥20 ng for input to LC, and these samples were included in the exploratory analysis.

Among the 112 samples that were EGFR exon 20 insertion-negative by CTA, 3 samples failed F1LCDx QC metrics resulting in a total of 109 EGFR exon 20 insertion-negative samples that had F1LCDx-evaluable results. Among the 109 EGFR exon 20 insertion-negative samples evaluable by F1LCDx, 100 had cfDNA ≥30 ng for input to LC and were used for the primary analysis. The remaining 9 EGFR exon 20 insertion-negative samples had cfDNA <30 ng and ≥20 ng for input to LC, and these samples were included in the exploratory analysis. Sample accountability for this clinical bridging study is summarized in Table 61.
Table 61. F1LCDx Sample Accountability for EGFR exon 20 Insertions

<table>
<thead>
<tr>
<th>CTA Status</th>
<th>Sample Source</th>
<th>Study 101 Population</th>
<th># of Patients</th>
<th># of Failed or Unavailable Samples</th>
<th>F1LCDx Evaluable</th>
<th># of F1LCDx Evaluable Samples</th>
<th># of F1LCDx Samples ≥30 ng</th>
<th># of F1LCDx Samples ≥20 ng and &lt;30 ng</th>
</tr>
</thead>
<tbody>
<tr>
<td>POSITIVE</td>
<td>Study 101</td>
<td>NDA</td>
<td>114</td>
<td>34</td>
<td>80</td>
<td>71</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Study 101</td>
<td>Non-NDA</td>
<td>116</td>
<td>37</td>
<td>79</td>
<td>61</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Positive Subtotal</td>
<td></td>
<td>230</td>
<td>71</td>
<td>159</td>
<td>132</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>Study 101</td>
<td>Non-NDA</td>
<td>43</td>
<td>3</td>
<td>40</td>
<td>34</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Procured</td>
<td>N/A</td>
<td>46</td>
<td>0</td>
<td>46</td>
<td>43</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Retrospective</td>
<td>N/A</td>
<td>23</td>
<td>0</td>
<td>23</td>
<td>23</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative Subtotal</td>
<td></td>
<td>112</td>
<td>3</td>
<td>109</td>
<td>100</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>342</td>
<td>74 (21.6%)</td>
<td>268 (78.4%)</td>
<td>232 (67.8%)</td>
<td>36 (10.5%)</td>
<td></td>
</tr>
</tbody>
</table>

16 additional samples (15 from Study 101 and 1 procured patient sample) failed CTA testing QC.

Results for the primary concordance analysis (total n=232) is summarized in Table 62.

Table 62. Contingency Table Comparing EGFR exon 20 Insertions Status Between the CTAs and F1LCDx

<table>
<thead>
<tr>
<th>F1LCDx</th>
<th>Detected</th>
<th>Not Detected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detected</td>
<td>95</td>
<td>0</td>
<td>95</td>
</tr>
<tr>
<td>Not Detected</td>
<td>37</td>
<td>100</td>
<td>137</td>
</tr>
<tr>
<td>Unevaluable</td>
<td>98</td>
<td>12</td>
<td>110</td>
</tr>
<tr>
<td>Total</td>
<td>230</td>
<td>112</td>
<td>342</td>
</tr>
</tbody>
</table>

Agreement Statistics Excluding CDx-Unevaluable Results

PPA: 72.0% (95/132) 95% CI: (63.8%, 78.9%)
NPA: 100% (100/100) 95% CI: (96.3%, 100%)

Percent Unevaluable

42.6% (98/230) 10.7% (12/112)

16 additional samples (15 from Study 101 and 1 procured patient sample) failed CTA testing QC.
2Calculated with Wilson 2-sided 95% CI.

The following concordance statistics were calculated for this sample set using the CTA as the reference:
- PPA [95% CI]: 72.0% [63.8%, 78.9%]
- NPA [95% CI]: 100% [96.3%, 100%]

Since patients were enrolled and initially tested by local CTAs, the PPV and NPV were calculated using the PPA and NPA, after adjusting for the prevalence of EGFR exon 20 insertions among the intention-to-treat (ITT) population. The prevalence estimate used in the adjusted agreement was 1.8%. In this analysis, F1LCDx demonstrated an adjusted PPV of 100% with 95% two-sided CI [96.1%, 100%]) and NPV of 99.5% with 95% two-sided CI [99.3%, 99.6%].

The primary clinical efficacy of mobocertinib was estimated with NDA patients from Study 101 that had samples with DNA input ≥ 30 ng. The ORR in the CTA-positive population was 28.1% (32/114) with 95% two-sided CI [20.6%, 36.9%]. Fifty-three (53) patients were CTA-positive and had F1LCDx EGFR exon 20 insertion-positive results. The ORR for this population (CTA+/F1LCDx+) was 32.1% (17/53) with 95% two-sided CI [21.1%, 45.5%]. Eighteen (18) patients were CTA-positive but had F1LCDx EGFR exon 20 insertion-negative results. The ORR for this population (CTA+/F1LCDx-) was 16.7% (3/18) with 95% two-sided CI [5.8%, 39.2%]. Forty-three (43)
patients were CTA-positive but were unevaluable by F1LCDx. The ORR for this population (CTA+/F1LCDx unevaluable) was 27.9% (12/43) with 95% two-sided CI [16.7%, 42.7%] (Table 63).

Table 63. Primary Efficacy Analysis Results

<table>
<thead>
<tr>
<th>Clinical outcome</th>
<th>Total CTA+ population (N=114)</th>
<th>CTA+/F1LCDx+ (N=53)</th>
<th>CTA+/F1LCDx– (N=18)</th>
<th>CTA+/F1LCDx unevaluable (N=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORR% [95% CI]</td>
<td>28.1% [20.6%, 36.9%]</td>
<td>32.1% [21.1%, 45.5%]</td>
<td>16.7% [5.8%, 39.2%]</td>
<td>27.9% [16.7%, 42.7%]</td>
</tr>
<tr>
<td>Number of responders²</td>
<td>32</td>
<td>17</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Median³ duration of response in months [95% CI]</td>
<td>17.5 [7.4, 20.3]</td>
<td>7.4 [3.7, N/A⁴]</td>
<td>N/A⁵</td>
<td>20.3 [8.3, N/A⁴]</td>
</tr>
<tr>
<td>% with duration ≥6 months</td>
<td>59.4%</td>
<td>41.2%</td>
<td>66.7%</td>
<td>83.3%</td>
</tr>
</tbody>
</table>

¹ CI for ORR calculated with Wilson 2-sided 95% CI except in the F1LCDx+ population which was calculated using normal approximation CI using the variance.
² All responses were partial response.
³ Median was determined using Kaplan-Meier estimate.
⁴ The upper bound of the 95% CI was not estimable.
⁵ The median could not be calculated for the CTA+/F1LCDx- subgroup due to the small sample size (the survival probability did not reach 50%).

The median DOR in the CTA-positive population that responded to mobocertinib (N=32) was 17.5 months with 95% two-sided CI [7.4, 20.3]. Seventeen (17) patients that were CTA-positive and responded to mobocertinib also had F1LCDx EGFR exon 20 insertion positive results. The median DOR for this population (F1LCDx+CTA+) was 7.4 months with 95% two-sided CI [3.7, N/A]. Twelve (12) patients that were CTA-positive and responded to mobocertinib were not evaluable by F1LCDx. The median DOR for this population (F1LCDx-unevaluable|CTA+) was 20.3 months with 95% two-sided CI [8.3, N/A].

A sensitivity analysis was performed to assess the robustness of the concordance and efficacy results subject to the missing F1LCDx results. F1LCDx results were predicted for the F1LCDx-unevaluable patients (patients with missing or invalid F1LCDx test results), and the PPA and PPV estimates were updated with the complete set of F1LCDx results.

In the sensitivity analysis, the average PPA was 69.7% (95% CI [59.4% 80.6%]). The prevalence-adjusted PPV was still 100%. The ORR estimated for the F1LCDx-positive population was 32.6% (95% CI [17.0% 48.2%]). The sensitivity analysis results demonstrated that the concordance between CTA and F1LCDx and drug efficacy estimated in the F1LCDx-positive population were robust as calculated with the F1LCDx-eligible patients.

10.10 Clinical Bridging Study: Detection of BRAF V600E Alterations to Determine Eligibility for Treatment with encorafenib in combination with cetuximab

The clinical performance of F1LCDx for the detection of BRAF V600E alterations in plasma samples from patients with metastatic CRC for treatment with BRAF TOVI® (encorafenib) in combination with cetuximab was established through a clinical bridging study that assessed clinical efficacy of encorafenib and cetuximab in patients selected based on F1LCDx results. Baseline plasma samples for patients enrolled in the BEACON (ARRAY-818-302) clinical trial were retrospectively tested by F1LCDx in the bridging study. The study results demonstrate concordance between the clinical trial assay (CTA) and the F1LCDx assay, and establishes the clinical effectiveness of the F1LCDx assay in identifying metastatic CRC patients with BRAF V600E alterations for treatment with encorafenib in combination with cetuximab.

The BEACON trial was a randomized, open-label, multi-center, parallel group, three-arm Phase 3 study in patients with BRAF V600E alteration in CRC whose disease had progressed after 1 or 2 prior regimes in the metastatic setting. The study compared the efficacy and safety of binimetinib + encorafenib + cetuximab (Triplet Arm), and encorafenib + cetuximab (Doublet Arm) to irinotecan/cetuximab or FOLFIRI/cetuximab (Control Arm).

The supplemental new drug application population included enrolled patients from the Control and Doublet arms from BEACON. The clinical trial tested the efficacy of therapy with these drugs by screening for and selecting
metastatic CRC patients that harbor the BRAF V600E alteration using the CTA which uses FFPE tissue DNA as the sample input. Overall survival (OS) and objective response rate (ORR) by Response Evaluation Criteria in Solid Tumors (RECIST) V1.1 were the primary efficacy endpoints.

This study evaluated the clinical validity of F1LCDx as a CDx to identify BRAF V600E positive patients from the BEACON clinical trial. F1LCDx testing was performed on patients with available plasma samples from the BEACON clinical trial that tested positive for BRAF V600E by clinical trial assay (CTA+). Additionally, commercially procured BRAF V600E negative CRC patient tissue samples with matched plasma were tested.

The concordance between the CTA and F1LCDx was evaluated by the positive percent agreement (PPA) and negative percent agreement (NPA) (Table 64). The prevalence-adjusted positive predictive value (PPV) and negative predictive value (NPV) were also calculated by adjusting for the prevalence of BRAF V600E mutations among the intention-to-treat (ITT) population, with 10% and 15% as the estimated prevalence. The PPA, NPA, PPV, NPV, and their two-sided 95% CIs are provided in Table 65.

### Table 64. Concordance for BRAF V600E mutation between F1LCDx and the CTA

<table>
<thead>
<tr>
<th></th>
<th>Detected</th>
<th>Not Detected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1LCDx Detected</td>
<td>286</td>
<td>3</td>
<td>289</td>
</tr>
<tr>
<td>F1LCDx Not Detected</td>
<td>42</td>
<td>102</td>
<td>144</td>
</tr>
<tr>
<td>F1LCDx Unevaluable</td>
<td>74</td>
<td>16</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td>402</td>
<td>121</td>
<td>523</td>
</tr>
</tbody>
</table>

Agreement Statistics Excluding CDx-Unevaluable Results

**PPA:** 87.2% (286/328) 95% CI\(^\text{1}\): (83.1%, 90.4%)  
**NPA:** 97.1% (102/105) 95% CI\(^\text{1}\): (91.9%, 99.0%)

Percent Unevaluable

18.4% (74/402) 13.2% (16/121)

\(^{1}\)Calculated with Wilson 2-sided 95% CI.

### Table 65. Concordance Analysis Results

<table>
<thead>
<tr>
<th></th>
<th>Prevalence</th>
<th>Numerator</th>
<th>Denominator</th>
<th>Point Estimate (%)</th>
<th>95% Two-Sided CI(^*) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA</td>
<td>N/A</td>
<td>286</td>
<td>328</td>
<td>87.20</td>
<td>[83.14, 90.39]</td>
</tr>
<tr>
<td>NPA</td>
<td>N/A</td>
<td>102</td>
<td>105</td>
<td>97.14</td>
<td>[91.93, 99.02]</td>
</tr>
<tr>
<td>Adjusted PPV</td>
<td>10%</td>
<td>N/A</td>
<td>N/A</td>
<td>77.23</td>
<td>[59.41, 100.00]</td>
</tr>
<tr>
<td>Adjusted NPV</td>
<td>10%</td>
<td>N/A</td>
<td>N/A</td>
<td>98.56</td>
<td>[98.17, 98.94]</td>
</tr>
<tr>
<td>Adjusted PPV</td>
<td>15%</td>
<td>N/A</td>
<td>N/A</td>
<td>84.34</td>
<td>[69.92, 100.00]</td>
</tr>
<tr>
<td>Adjusted NPV</td>
<td>15%</td>
<td>N/A</td>
<td>N/A</td>
<td>97.73</td>
<td>[97.12, 98.33]</td>
</tr>
</tbody>
</table>

\(^*\)CI was calculated using the Wilson-score method for PPA and NPA, while using the bootstrap method for the adjusted PPV and NPV.

The clinical validity of F1LCDx was demonstrated by assessing clinical efficacy in the F1LCDx BRAF V600E positive population based on the ORR difference between the Doublet Arm and Control Arm, as well as the log hazard ratio (log(HR)) between the two arms from the Cox regression model. The ORR is defined as the proportion of patients with objective response of either confirmed complete response (CR) or partial response (PR) based on RECIST V1.1. The ORR for the Doublet Arm and Control Arm as well as the ORR difference are reported in Table 66 for the following subpopulations: CTA+, F1LCDx+|CTA+, F1LCDx-|CTA+, and F1LCDx unevaluable|CTA+. Table 66 also summarizes the median OS by the Kaplan-Meier method for each arm as well as the log(HR) with 95% two-sided CI for each of the aforementioned subpopulations.
The clinical validity of F1LCDx was demonstrated by estimating the ORR difference and log(HR) between the Doublet Arm and Control Arm. The estimated efficacy results for the F1LCDx-positive (F1LCDx+) population are shown in Table 67 Estimated Efficacy for the F1LCDx+ Population, which were comparable to that in the CTA+ population as shown in Table 66 above.

Table 66 Primary Efficacy in the Bridging Study Subpopulations

<table>
<thead>
<tr>
<th></th>
<th>CTA+</th>
<th>F1LCDx+</th>
<th>F1LCDx-</th>
<th>F1LCDx unevaluable</th>
</tr>
</thead>
<tbody>
<tr>
<td># Total</td>
<td>402</td>
<td>286</td>
<td>42</td>
<td>74</td>
</tr>
<tr>
<td>ORR for Doublet Arm</td>
<td>19.90%</td>
<td>18.49%</td>
<td>17.39%</td>
<td>28.13%</td>
</tr>
<tr>
<td>ORR for Control Arm</td>
<td>1.49%</td>
<td>1.43%</td>
<td>0.00%</td>
<td>2.38%</td>
</tr>
<tr>
<td>ORR Difference (95% two-sided CI)*</td>
<td>18.41% [12.74%, 24.55%]</td>
<td>17.06% [10.51%, 24.22%]</td>
<td>17.39% [-2.39%, 37.14%]</td>
<td>25.74% [9.73%, 43.10%]</td>
</tr>
<tr>
<td>Median OS (months) for Doublet Arm</td>
<td>9.49</td>
<td>7.62</td>
<td>NA§</td>
<td>18.89</td>
</tr>
<tr>
<td>Median OS (months) for Control Arm</td>
<td>5.88</td>
<td>5.38</td>
<td>12.16</td>
<td>7.16</td>
</tr>
<tr>
<td>log(HR) (95% two-sided CI)</td>
<td>-0.51 [-0.76, -0.26]</td>
<td>-0.47 [-0.75, -0.19]</td>
<td>-2.72 [-4.71, -0.74]</td>
<td>-0.44 [-1.23, 0.34]</td>
</tr>
</tbody>
</table>

*CI was calculated using the Newcombe method.
§The estimated median OS is NA due to the small number of events in this group (3 events).

A sensitivity analysis was performed to assess the robustness of the concordance and efficacy results subject to the missing F1LCDx test results. F1LCDx BRAF V600E status were predicted for the F1LCDx unevaluable patients (patients with missing or invalid F1LCDx test results). The concordance analysis and the clinical efficacy were updated by accounting for the imputed data.

The PPA and prevalence adjusted PPV estimates were computed for each of the 50 imputed complete data sets and the summary statistics are shown in Table 68.

Table 68 Summary Statistics of PPA and PPV on Imputed Complete Data

<table>
<thead>
<tr>
<th></th>
<th>Prev</th>
<th>Min</th>
<th>Q1</th>
<th>Median</th>
<th>Mean</th>
<th>Q3</th>
<th>Max</th>
<th>2.5%</th>
<th>97.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPA (%)</td>
<td>N/A</td>
<td>84.29</td>
<td>85.04</td>
<td>85.54</td>
<td>85.46</td>
<td>85.79</td>
<td>86.78</td>
<td>84.54</td>
<td>86.53</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>10%</td>
<td>76.62</td>
<td>76.78</td>
<td>76.89</td>
<td>76.87</td>
<td>76.94</td>
<td>77.14</td>
<td>76.68</td>
<td>77.09</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>15%</td>
<td>83.89</td>
<td>84.01</td>
<td>84.08</td>
<td>84.07</td>
<td>84.12</td>
<td>84.28</td>
<td>83.93</td>
<td>84.24</td>
</tr>
</tbody>
</table>
In addition, the drug efficacy for the F1LCDx+|CTA+ population with the imputed complete data set was shown in Table 69. The estimated efficacy results for the F1LCDx+ population in the sensitivity analysis are shown in Table 70. The sensitivity analysis demonstrated the robustness of the concordance between CTA and F1LCDx and drug efficacy estimated in the F1LCDx+ population by accounting for the missingness of F1LCDx status. This study demonstrated the clinical validity of using F1LCDx as a CDx device to select metastatic CRC patients with BRAF V600E mutations for the treatment with encorafenib in combination with cetuximab.

Table 69 Summary Statistics of Estimated log(HR) and ORR Difference for the F1LCDx+|CTA+ Population (δ,1) on Imputed Complete Data

<table>
<thead>
<tr>
<th>F1LCDx+</th>
<th>CTA+</th>
<th>Min</th>
<th>Q1</th>
<th>Median</th>
<th>Mean</th>
<th>Q3</th>
<th>Max</th>
<th>2.5%</th>
<th>97.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>log (HR)</td>
<td>-0.59</td>
<td>-0.55</td>
<td>-0.53</td>
<td>-0.53</td>
<td>-0.51</td>
<td>-0.47</td>
<td>-0.59</td>
<td>-0.59</td>
<td>-0.48</td>
</tr>
<tr>
<td>ORR (%) Difference</td>
<td>17.88</td>
<td>18.61</td>
<td>18.96</td>
<td>18.91</td>
<td>19.28</td>
<td>19.82</td>
<td>17.92</td>
<td>17.92</td>
<td>17.72</td>
</tr>
</tbody>
</table>

Table 70 Estimated Efficacy for the F1LCDx+ Population in the Sensitivity Analysis

<table>
<thead>
<tr>
<th>Estimated F1LCDx+ Efficacy with 95% CI (log (HR))</th>
<th>Estimated F1LCDx+ Efficacy with 95% CI (ORR difference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>prev = 10%</td>
<td></td>
</tr>
<tr>
<td>c=0%</td>
<td></td>
</tr>
<tr>
<td>-0.41 [-0.66, -0.15]</td>
<td>14.54 [8.27, 20.81]</td>
</tr>
<tr>
<td>c=30%</td>
<td></td>
</tr>
<tr>
<td>-0.44 [-0.71, -0.18]</td>
<td>15.85 [9.81, 21.89]</td>
</tr>
<tr>
<td>c=50%</td>
<td></td>
</tr>
<tr>
<td>-0.47 [-0.74, -0.20]</td>
<td>16.73 [10.71, 22.74]</td>
</tr>
<tr>
<td>c=70%</td>
<td></td>
</tr>
<tr>
<td>-0.49 [-0.77, -0.22]</td>
<td>17.60 [11.50, 23.70]</td>
</tr>
<tr>
<td>c=100%</td>
<td></td>
</tr>
<tr>
<td>-0.53 [-0.82, -0.24]</td>
<td>18.91 [12.49, 25.34]</td>
</tr>
<tr>
<td>prev = 15%</td>
<td></td>
</tr>
<tr>
<td>c=0%</td>
<td></td>
</tr>
<tr>
<td>-0.45 [-0.71, -0.18]</td>
<td>15.90 [9.78, 22.02]</td>
</tr>
<tr>
<td>c=30%</td>
<td></td>
</tr>
<tr>
<td>-0.47 [-0.74, -0.20]</td>
<td>16.81 [10.75, 22.86]</td>
</tr>
<tr>
<td>c=50%</td>
<td></td>
</tr>
<tr>
<td>-0.49 [-0.76, -0.21]</td>
<td>17.41 [11.32, 23.50]</td>
</tr>
<tr>
<td>c=70%</td>
<td></td>
</tr>
<tr>
<td>-0.50 [-0.79, -0.22]</td>
<td>18.01 [11.83, 24.19]</td>
</tr>
<tr>
<td>c=100%</td>
<td></td>
</tr>
<tr>
<td>-0.53 [-0.82, -0.24]</td>
<td>18.91 [12.49, 25.34]</td>
</tr>
</tbody>
</table>

*c is the ratio of efficacy between F1LCDx+|CTA- and F1LCDx+|CTA+ populations.

11 CDx Classification Criteria

11.1 CDx classification criteria for ALK rearrangements, qualifying NSCLC patients for therapy with ALECENSA® (alectinib):
- The ALK rearrangement must have pathogenic driver status (FMI driver status of "known" or "likely")
- AND the disease type must be NSCLC
- AND one of the following two conditions must hold:
  1. The partner gene is EML4, or
  2. The ALK breakpoint occurs within ALK intron 19

11.2 CDx classification criteria for EGFR alterations, qualifying NSCLC patients for therapy with EGFR Tyrosine Kinase Inhibitors (TKI) approved by FDA:
- Base substitutions resulting in EGFR L858R
- In-frame deletions occurring within EGFR exon 19

11.3 CDx classification criteria for BRCA1, BRCA2, and ATM alterations, qualifying prostate cancer patients for therapy with LYNPARZA® (olaparib):
Table 71, Table 72, and Table 73 describe the criteria for classifying BRCA1, BRCA2, or ATM alterations known to be deleterious to protein function
Table 71. Classification Criteria for BRCA1, BRCA2, and ATM

<table>
<thead>
<tr>
<th>Deleterious Variant Criteria</th>
<th>Sequence Classification</th>
<th>CDx Classifier Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>A gene alteration that includes any of the sequence classifications</td>
<td>Protein truncating mutations</td>
<td>Sequence analysis identifies premature stop codons or frame shift indels anywhere in the gene coding region, except: 3' of and including BRCA2 K3326*</td>
</tr>
<tr>
<td></td>
<td>Splice site mutations</td>
<td>Sequence analysis identifies variant splice sequences at intron/exon junctions: within ± 2bp of exon starts/ends, or callable splice variants in Table 73</td>
</tr>
<tr>
<td></td>
<td>Homozygous deletions</td>
<td>Sequence analysis identifies deletions in both gene alleles of ≥ 1 exon in size. Only reported for BRCA1 and BRCA2. Not reported for ATM.</td>
</tr>
<tr>
<td></td>
<td>Large protein truncating rearrangements</td>
<td>Sequence analysis identifies protein-truncating rearrangements</td>
</tr>
<tr>
<td></td>
<td>Deleterious missense mutations</td>
<td>Curated list (Table 72)</td>
</tr>
</tbody>
</table>

Table 72. Deleterious Missense Alterations

<table>
<thead>
<tr>
<th>BRCA1 Protein Effect (PE)</th>
<th>BRCA2 Protein Effect (PE)</th>
<th>ATM Protein Effect (PE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1V</td>
<td>M1R</td>
<td>M1T</td>
</tr>
<tr>
<td>M1I</td>
<td>M1I</td>
<td>R2032K</td>
</tr>
<tr>
<td>C61G</td>
<td>V159M</td>
<td>R2227C</td>
</tr>
<tr>
<td>C64Y</td>
<td>V211L</td>
<td>R2547_S2549del</td>
</tr>
<tr>
<td>R71G</td>
<td>V211I</td>
<td>G2765S</td>
</tr>
<tr>
<td>R71K</td>
<td>R2336P</td>
<td>R2832C</td>
</tr>
<tr>
<td>R1495M</td>
<td>R2336H</td>
<td>S2855_V2856delinsRI</td>
</tr>
<tr>
<td>E1559K</td>
<td></td>
<td>R3008C</td>
</tr>
<tr>
<td>D1692N</td>
<td></td>
<td>R3008H</td>
</tr>
<tr>
<td>D1692H</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1699W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1708E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1788V</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 73. Intronic Variants

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Position</th>
<th>Ref</th>
<th>Alt</th>
<th>dbSNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>chr11</td>
<td>108128198</td>
<td>T</td>
<td>G</td>
<td>rs730881346</td>
</tr>
<tr>
<td>ATM</td>
<td>chr11</td>
<td>108214102</td>
<td>AGTGA</td>
<td>A</td>
<td>rs730881295</td>
</tr>
</tbody>
</table>

11.4 CDx classification criteria for BRCA1 and BRCA2 alterations, qualifying prostate cancer patients for therapy with RUBRACA® (rucaparib):

Table 74 and Table 75 describe the criteria for classifying BRCA1 or BRCA2 alterations known to be deleterious to BRCA protein function rendering the sample BRCA+.

Table 74. Classification Criteria for Deleterious Tumor BRCA Variants

<table>
<thead>
<tr>
<th>Qualification Criteria</th>
<th>Sequence Classification</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>A BRCA1/2</td>
<td>Protein truncating mutations</td>
<td>Sequence analysis identifies premature stop codons anywhere in the gene coding region, except: 3’ of and including BRCA2 K3326*</td>
</tr>
<tr>
<td>Qualification Criteria</td>
<td>Sequence Classification</td>
<td>Methodology</td>
</tr>
<tr>
<td>------------------------------------------------------------</td>
<td>-----------------------------------</td>
<td>----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>alteration that includes any of the sequence classifications</td>
<td>Splice site mutations</td>
<td>Sequence analysis identifies variant splice sequences at intron/exon junctions +/- 2bp of exon starts/ends</td>
</tr>
<tr>
<td></td>
<td>Homozygous deletions</td>
<td>Sequence analysis identifies deletions in both gene alleles of ≥ 1 exon in size</td>
</tr>
<tr>
<td></td>
<td>Large protein truncating rearrangements</td>
<td>Sequence analysis identifies protein truncating rearrangements</td>
</tr>
<tr>
<td></td>
<td>Deleterious missense mutations</td>
<td>Curated list (Table 75)</td>
</tr>
</tbody>
</table>

**Table 75. Deleterious BRCA Missense Alterations**

<table>
<thead>
<tr>
<th>BRCA1 Alterations (Protein Change)</th>
<th>BRCA2 Alterations (Protein Change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1V</td>
<td>M1V</td>
</tr>
<tr>
<td>C61G</td>
<td>R2659T</td>
</tr>
<tr>
<td>D1692H</td>
<td></td>
</tr>
<tr>
<td>G1788V</td>
<td></td>
</tr>
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11.5 CDx classification criteria for PIK3CA alterations, qualifying breast cancer patients for therapy with PIQRAY® (alpelisib):

Presence of PIK3CA mutation(s): H1047R; E545K; E542K; C420R; E545A; E545D [1635G>T only]; E545G; Q546E; Q546R; H1047L; or H1047Y

11.6 CDx classification criteria for SNVs and Indels that lead to MET exon 14 skipping:

A SNV or indel in MET shall be considered to result in skipping of exon 14 if one or more of the following criteria are met:

1. Deletions greater than or equal to 5 bp that affect positions -3 to -30 in the intronic region immediately adjacent to the splice acceptor site at the 5' boundary of MET exon 14.
2. Indels affecting positions -1 or -2 at the splice acceptor site of the 5' boundary of MET exon 14.
3. Base substitutions and indels affecting positions 0, +1, +2, or +3 at the splice donor site of the 3' boundary of MET exon 14.
11.7 CDx classification criteria for NTRK fusions:
Rearrangements in NTRK1, NTRK2, or NTRK3 shall be considered CDx biomarker positive, that is, to lead to a NTRK1, NTRK2, or NTRK3 RNA fusion, if the following criterion is met:

- In-strand rearrangement events that may lead to an NTRK1, NTRK2 or NTRK3 RNA fusion with a previously reported or novel partner gene in which the kinase domain is not disrupted. This also includes rearrangement events that result in reciprocal fusions (NTRK may be on either the 5' or the 3' end of the detected fusion).

In this regard out-of-strand events are considered as non-fusion rearrangements and are classified as CDx biomarker negative. Intragenic fusions in which genomic rearrangement events are wholly internal to the NTRK1, NTRK2, or NTRK3 genes (i.e., NTRK1-NTRK1, NTRK2-NTRK2, NTRK3-NTRK3 events) are also considered biomarker negative. Unidentified partners (encoded as N/A) or LINC non-coding partners are also considered CDx biomarker negative.

11.8 CDx classification criteria for ROS1 fusions
Rearrangements in ROS1 shall be considered CDx biomarker positive, i.e., to lead to ROS1 RNA fusion, if the following condition is met:

- In-strand rearrangement events that may lead to a ROS1 RNA fusion with another protein coding gene in which the ROS1 kinase domain is not disrupted. ROS1 must be on the 3' end of the detected fusion.

In this regard, out-of-strand events are considered as non-fusion rearrangements and are classified as CDx biomarker negative. Intragenic fusions in which genomic rearrangement events are wholly internal to the ROS1 (i.e., ROS1-ROS1 events) are also considered biomarker negative. Unidentified partners (encoded as N/A) or LINC non-coding partners are also considered CDx biomarker negative. ROS1 fusions with novel partners are required to be in frame.

11.9 CDx classification criteria for EGFR exon 20 insertions
CDx positivity for EGFR exon 20 insertions is determined if the following criterion is met:

- Any in-frame insertions affecting amino acids 762 – 775 in exon 20

11.10 CDx classification criteria for BRAF V600E alteration
- Base alterations resulting in BRAF V600E