Paired Tumor Sequencing and Germline Testing in Breast Cancer Management: An Experience of a Single Academic Center

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Methods and Results: 43 breast cancer patients treated at Rush University Medical Center underwent paired germline and somatic variant testing in 2015–2017. A retrospective chart review was conducted with the analysis of demographic, clinical, and genomic data. Three actionable germline variants were found in the CHEK2 (2) and ATM (1) genes. 95% of tumors had somatic mutations. 77% of tumors had genomic
alterations targetable with agents approved for breast cancer and 88% had molecular targets for agents approved for other cancers. Clinical examples of such use are described and potential future directions of tumor and paired testing are discussed.

Conclusions: Germline variants were present in a relatively small patient group not routinely tested for inherited alterations. Potentially targetable somatic alterations were identified in the majority of breast cancers. Paired testing is a feasible and efficient approach that delivers valuable information for the care of breast cancer patients and eliminates serial testing.

Keywords: Breast cancer, genomic profiling, next-generation sequencing, germline testing, cancer predisposition, clinical utility
Paired Tumor Sequencing and Germline Testing in Breast Cancer Management: An Experience of a Single
Academic Center

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Running Title: The use of combined tumor molecular profiling and germline testing in breast cancer

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Introduction:

Recent advances in precision oncology allow for the administration of more effective and less toxic treatments, leading to improved patient outcomes. Molecularly targeted agents (MTAs) such as imatinib in chronic myelogenous leukemia or trastuzumab in Her-2/neu-positive breast cancer have shown significant prolongation of overall survival in the corresponding malignancies and sometimes, cures.[1] A meta-analysis of Phase I trials with more than 13,000 patients showed that biomarker-driven MTAs as compared to non-personalized treatments resulted in a higher response rates (30.6% vs 4.9%) and better progression-free survival (PFS; 5.7 mo vs 2.9 mo).[2] In addition, MTAs usually have fewer side effects (on-target or off-target) than cytotoxic agents. There is an ever-increasing number of clinical trials underway testing new targets and matching therapies.[3] In January of 2018, a PARP-inhibitor olaparib became the first genomically-based treatment approved by the United States FDA (Food and Drug Administration) for patients with metastatic breast cancer (MBC), specifically those who carry germline \textit{BRCA1} or \textit{BRCA2} mutations.[4,5] Subsequently, two more MTAs (talazoparib and alpelisib) have been FDA-approved for metastatic breast cancer with a germline \textit{BRCA} mutation (talazoparib) and somatic \textit{PIK3CA} alteration (alpelisib).[6,7] Advances such as these confirm the promise of molecularly based approaches in breast cancer (BC) management. Currently, therapy in BC is guided by the presence of estrogen and progesterone receptors as well as human epidermal growth factor receptor 2 (HER2) protein overexpression or amplification on pathologic examination.[8-11] The introduction of next-generation DNA sequencing (NGS) and recently, mapping, whole cancer genomes to find cancer “drivers”, allowed to identify promising MTAs. [12, 13] At present, however, alpelisib is the only MTA which requires identification of \textit{PIK3CA} mutation in the tumor to predict its efficacy. Tumor molecular profiling (TMP) is usually employed in advanced BC when all standard treatments have been exhausted.[14] Since the technology of TMP with NGS became available, many experts considered it investigational and appropriate only in a clinical trial setting. Yet in March of 2018 Centers for Medicare & Medicaid Services made the decision that NGS is "reasonable and necessary" in advanced cancer for Medicare beneficiaries, opening the doors for wider use of this laboratory testing.[15] NGS is also employed in germline hereditary predisposition testing, which typically involves the use of multi-gene panels on constitutional DNA isolated from blood cells. Germline testing provides valuable information on cancer surveillance and risk reduction for patients and their
biological relatives. It may also provide information for MTA selection, such as identifying a germline \textit{BRCA1/2} mutation in a patient with metastatic BC who may benefit from treatment with Poly ADP Ribose Polymerase (PARP) inhibitors. Combining germline testing with TMP (paired testing or PT) may provide a time and resource efficient option to reveal molecular targets for therapy and genetic predisposition to cancer.[16]

We performed a pilot study of PT in a series of 43 BC patients to assess the feasibility and clinical impact of this testing approach at a single academic medical center. We discuss the implications of PT results for treatment selection, subsequent cancer surveillance, and screening of family members. We also discuss efficiency of the PT approach.

\textbf{Methods:}

We performed a retrospective chart review of women with the diagnosis of BC who underwent PT when deemed appropriate by their oncologists in the context of routine clinical care at Rush University Medical Center from November 2015 to February 2017. The following data were collected: patient age, ethnic background, clinical stage at diagnosis, histology, ER/PR/HER2 receptor status, BC treatment history, and PT results. All patients received pre-test risk assessment and genetic counseling to inform them about PT. Post-test genetic counseling was provided for patients and/or their families who tested positive for a pathogenic or likely pathogenic germline variant. The study was approved by the Rush University Institutional Review Board (IRB# 17041703-IRB01). Participants were deidentified before group statistical analyses.

\textit{Laboratory analysis}

DNA was extracted from a primary tumor for the majority of subjects while a metastatic lesion was analyzed when the primary tumor was not available. Blood or saliva was also submitted for each patient for germline DNA extraction. Samples were processed as previously described.[16] PT consisted of a custom probe-based NGS tumor panel (Illumina HiSeq) for the detection of single nucleotide variants, small insertions and deletions in 142 genes that frequently harbor somatic and/or germline mutations in cancer (Online Resource Supplemental Table 1). The NGS panel used detects gene fusions and structural variants, such as tandem duplications and inversions, in 15 frequently disrupted oncogenes and tumor suppressors. Tumor tissue and a matched blood specimen were analyzed using a custom bioinformatics pipeline to differentiate between somatic and germline mutations, allowing for precise variant classification. Briefly, paired normal samples were analyzed using
Novoalign V3.02.07 to align FASTQ reads to a reference sequence (hg19) and GATK (V3.2.2) to generate variants and no/low coverage reports. Germline variants were filtered using a Q score of 30, coverage of 10X, het ratio of 10% and filtered out if determined to be a sequencing artifact or common polymorphism utilizing population frequency data from NCBI dbSNP, NHLBI Exome Sequencing Project (ESP), 1000 Genomes, and internal Ambry data. In tumor-normal analysis mode, Varscan2 (v2.3.6), was used to detect somatic variants as low as 3% minor allele frequency. Structural variants were annotated using Oncofuse v1.0.7 and DELLY v.0.6.1 respectively.

Germline genetic variants were assessed using Ambry’s five-tier classification framework based on guidelines published by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (pathogenic; likely pathogenic; variant of uncertain significance; likely benign; benign). [17,18] Tumor specimens were also analyzed using the Affymetrix OncoScan platform, an array technology for high resolution copy number variant detection that can detect single copy amplifications, hemizygous deletions and copy neutral loss of heterozygosiy.

Alterations identified in TMP and associations with MTAs were reported based on peer reviewed studies and other publicly available resources. Germline variants when identified in blood or tumor were reported for the following genes: APC, BRCA1, BRCA2, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, RB1, RET, SDHAF2, SDHB, SDHC, SDHD, STK11, TP53, TSC1, TSC2, and VHL. In some cases, germline testing was performed on additional cancer susceptibility genes as clinically indicated based on genetic risk assessment. Actionable germline alterations were defined as those alterations associated with a currently available option for cancer surveillance, prevention or treatment in either the patient or their close family members.

Results:

Age at diagnosis, ethnicity, stage at the time of testing, histologic type, and tumor markers of breast cancer for 43 patients are reported in Table 1. Our patient population had a greater proportion of stage IV disease at diagnosis and a younger age than average BC population when compared to National Cancer Institute (NCI) epidemiological data which may reflect the referral bias since patients with advanced disease were more likely to get referred for paired testing. [19,20] The primary histological BC subtype of this patient group was infiltrating ductal carcinoma (IDC), which correlates with NCI data, yet there was a somewhat larger proportion of infiltrating lobular
carcinoma, which may be the result of a small sample.\[19\] The majority of our patients had hormone receptor positive, HER2 negative BC, consistent with national data.\[21\]

Somatic genomic alterations in the tumors tested were highly prevalent with 95% (42/43) of tumors having at least one reported mutation with an average of 6.5 (range 0-17) mutations per tumor. Overall, 77% of tumors contained at least one target for BC therapy, and 88% contained an FDA-approved targeted therapy for another type of cancer, potentially available off-label for BC. (Table 2)

A total of 278 alterations in 81 genes were clinically reported in Figure 1, and are broken down by ER receptor status in Supplemental Graph 1. Hemizygous loss of a gene was the most common alteration (n=96) while missense alterations (n=79) and gene amplifications (n=66) were also frequent (Online Resource Supplemental Table 2). Genes known to be frequently mutated in BC (BRCA1, BRCA2, CDH1, PIK3CA, PTEN, and TP53) accounted for 40% of alterations. 22 genes altered in this cohort of patients had an associated MTA for breast cancer while alterations in additional genes added to the number of patients who were potentially eligible for an MTA (Figure 2). Patient characteristics in relation to the alterations can be found in Online Resource Supplemental Table 3.

Three patients were found to have actionable germline variants that were not previously suspected including one pathogenic mutation in CHEK2 (c.1100delC), a likely pathogenic variant in the CHEK2 gene (c.1427C>T), and a likely pathogenic splice site variant in the ATM gene (2466+1G>C). None of these three patients met the then current National Comprehensive Cancer Network (NCCN) germline genetic testing criteria.\[22\] We have performed TMP on breast cancers of the three germline carriers described above (Table 3). Two of these tumors had multiple molecular alterations.

**Clinical Application of PT Results:**

Because 47% of our sample (20 patients) consisted of patients with early-stage breast cancer, real time clinical care was usually not impacted by PT but rather, adjuvant or neoadjuvant treatment was based on stage, pathologic characteristics of the tumor including tumor markers, and in some cases on the results of molecular prognostic assays such as Oncotype DX® and Mammaprint®. In one patient (Patient C), an MTA was considered in adjuvant setting and in two women (Patient A and Patient B), MTAs were administered when BC recurred.
Patient 404122 (Supplemental Table 3) was diagnosed with inflammatory triple-negative left-sided IDC, clinical stage III, grade 3, at age 66. She received four cycles of neoadjuvant dose-dense doxorubicin and cyclophosphamide, followed by four cycles of paclitaxel, and underwent left modified radical mastectomy which demonstrated significant residual BC: ypTis N3a. After radiation to the left chest wall, adjuvant chemotherapy with carboplatin was attempted but not tolerated due to rash and weakness. She was diagnosed with histologically confirmed recurrence of triple-negative BC and metastases to intra-thoracic lymph nodes a year after her surgery. PT was performed on the primary tumor and revealed four somatic genomic alterations including \textit{KRAS} amplification, \textit{BARD1} p.A724V, \textit{TP53} p. K139_P142del, and \textit{TP53} copy neutral LOH. Based on these findings, she was prescribed a suggested MTA off-label, Trametinib, a mitogen-activated protein kinase kinase (MEK) inhibitor which targets \textit{KRAS} amplification. Although it does not directly inhibit MEK, Trametinib has been shown to limit tumor progression via CD8 T-cell mediated factors by altering signaling along the RAS-ERK pathway in cancers with \textit{KRAS} amplification.[23] Unfortunately, trametinib was discontinued one month later due to progression.

Patient 435327 (Supplemental Table 3) was diagnosed with clinical stage III, grade 3, triple-negative right-sided IDC at age 62. She received four cycles of neoadjuvant dose-dense doxorubicin and cyclophosphamide, followed by 12 cycles of weekly paclitaxel. She underwent subsequent right modified radical mastectomy with residual tumor noted, ypT2N0. She then completed radiation and received six cycles of adjuvant chemotherapy with capecitabine. She also was diagnosed with BC recurrence and histologically-confirmed pulmonary metastases one year after surgery. She underwent PT using a tissue sample from her mastectomy, notable for four somatic genomic alterations: \textit{PIK3R1} p.R461_E462delinsQ, \textit{FGF3} amplification, \textit{FGF4} amplification, and \textit{HER2-Neu (ERBB2)} mutation, p.S310F. Given the finding of \textit{HER2-Neu} mutation, she was offered participation in phase II study with neratinib. [24]. Because somatic \textit{HER2-Neu} mutations activate the \textit{HER2-Neu} oncogene without its amplification, Trastuzumab and other commonly used \textit{HER2-Neu} directed agents are usually ineffective in \textit{HER2-Neu}-mutated BC. Neratinib is a small molecule which irreversibly inhibits both \textit{HER2-Neu} amplifications and mutations.[25] The patient enrolled in this trial but it was discontinued 3 months later due to cancer progression.

Patient 487820 (Supplemental Table 3) was diagnosed with clinical stage III, Grade 2, ER-positive, PR-negative, HER2-negative IDC with neuroendocrine features at age 52. She received neoadjuvant doxorubicin and cyclophosphamide, followed by dose-dense paclitaxel. She underwent total left mastectomy with sentinel lymph
node biopsy which showed residual BC, ypT1cN1. PT of her surgical sample revealed somatic mutations in TP53 and a hemizygous loss of NF2, yielding four possible therapies including an mTOR inhibitor everolimus, approved in combination with anti-hormonal therapy for metastatic BC. Given the significant amount of residual disease and this molecular alteration, she was evaluated for the adjuvant trial with everolimus.[26] However, she was found to be ineligible because positive lymph node biopsy was not followed by axillary lymph node dissection based on patient and surgeon preference. She underwent adjuvant radiation therapy and started anti-hormonal therapy with tamoxifen. At the time of this writing, she remains without evidence of disease 3.5 years after surgery.

Discussion:

In our study, 95% of all tumors analyzed demonstrated at least one somatic mutation presumed to be involved in tumorigenesis. The most commonly detected mutation was in TP53 gene, followed by CDH1 and PIK3CA. This correlates with other studies, which have described TP53 as the most commonly mutated gene in human cancers.[27] As seen in Figure 1, the rate of gene alteration in TP53 was less than 12% and that in PIK3CA was less than 6%, which are lower than reported results for breast cancer. Those studies, however, include non-coding regions with high mutation frequencies not all of which can be classified as driver mutations. Mutational signatures extended to genome rearrangements, characterized by tandem duplications or deletions, appear to be associated with defective homologous recombination-based DNA repair.[28] Analysis of all classes of somatic mutation across exons, introns and intergenic regions might generate a higher overall mutation rate, not all of which will be driver mutations. In general, mutations in tumor suppressor genes (loss of function mutations) like TP53 are more difficult to target than mutations in oncogenes (gain of function mutations).[29] PARP-inhibitors is the first class of MTA that targets mutations in tumor suppressor genes (BRCA1 and BRCA2). Preliminary data suggest that these agents can also elicit synthetic lethality in the presence of mutations in other tumor suppressor such as TP53 and PTEN which are mutated in 37% and 3% of all BCs respectively and represented 11.8% and 5.4% of mutations in our sample. [30,31]

Gain-of-function mutations in genes such as PIK3CA which allow unregulated cell proliferation had associated MTAs (mTOR inhibitors: temsirolimus and everolimus; recently approved PIK3CA inhibitor: alpelisib) while others, such as a loss-of-function mutations in the tumor suppressor gene CDH1 were not targetable in our patients (Online Supplemental Table 2). Notably, only one of our tumor samples demonstrated a mutation of ESR1
(estrogen receptor 1). This may be explained by the number of primary, non-metastatic breast tumor tissue in our sample, as the incidence of ESRI mutations rises to 15-20% in metastatic ER positive tumors after prior endocrine treatments, suggesting the development of resistance. [32]

A molecular target for breast cancer treatment was available for a majority (33/43) of patients. However, we did not observe responses in two patients who received MTAs. This is consistent with initial results of clinical trials looking at the use of MTAs in prospective fashion.[33,34] For instance, in SAPHIR01 trial for women with metastatic BC, 13% of patients were able to receive MTAs based on the genomic data and of those patients, only 9% had objective tumor response.[35]

When analyzing the results of MTA clinical trials, it is important to remember that they depend on target identification in tumor tissue and availability of matching MTAs. In the initial phase of the NCI sponsored MATCH (Molecular Analysis for Therapy Choice) trial, only 9% of patients had a matched targeted therapy, when there were only 10 MTAs and 10 corresponding arms in the study. However, the percentage of patients increased to 23% when the number of MTAs and the respective arms in the study went up to 24%.[36]

Previous studies have revealed the prevalence of germline variants discovered in paired testing. A retrospective study of Stanford patients with BRCA1/2 somatic mutations found 55.7% were positive for pathogenic BRCA1/2 germline mutations, confirming “a second hit” hypothesis.[37] In a study of patients with advanced cancer diagnoses, presumed pathogenic germline variants were found in 17 of 269 (6.3%) breast cancer patients (6.3%).[38] In another study of 1040 breast, prostate, renal, pancreatic, and colon cancer patients, 182 (17.5%) had clinically actionable mutations conferring cancer susceptibility. Of these, 101 patients would not have had these mutations detected using clinical guidelines. Germline findings led to predictive testing in the families of 13 individuals (1.3%), including 6 for whom genetic evaluation would not have been initiated by guideline-based testing. [39] In our small sample, we identified three patients (7.0% of those tested) with previously unrecognized actionable germline mutations in CHEK2 and ATM genes which is consistent with these data.

Variants in CHEK2 and ATM can impact cancer surveillance and cancer screening and prevention for blood relatives.[40,41] For our patients, the discovery of these actionable germline mutations led to recommendations for increased breast cancer surveillance with the addition of screening breast MRIs (for both ATM and CHEK2 mutation carriers) and more frequent colonoscopies for colon cancer surveillance (for CHEK2 mutation carriers only). [42]
The same recommendations would apply to their blood relatives if they carry these mutations. Currently, germline genetic testing for hereditary predisposition to breast and other cancers is only offered to individuals who meet specific testing criteria set forth by different societal guidelines, most commonly, the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines. The NCCN guidelines recommend genetic testing for BRCA1/2 mutations based on age at breast cancer diagnosis and burden of breast, ovarian and other related cancers in the family. While these guidelines are useful to identify individuals who should be considered for genetic testing, germline mutations in the BRCA1/2 and other BC susceptibility genes may be missed in individuals who do not meet these criteria as their sensitivity is limited.[43,44] PT may allow for detecting pathogenic germline mutations in such BC patients. Importantly, somatic BRCA 1/2 mutations in the tumors of BRCA 1/2 mutation carriers can cause functional reversal of the germline mutation and restoration of the wild-type BRCA which results in resistance to Carboplatin and PARP-inhibitors in these patients. As opposed to “a second-hit” mutation which affects a normal allele, a “reversing” mutation usually affects the allele with a germline mutation.[45]

Ongoing studies evaluate a potential benefit of PARP-inhibitors in the presence of somatic BRCA1/2 mutations as well as germline and somatic mutations in other genes in homologous recombination (HR) pathway, including ATM and CHEK2. In a Phase 2 Study of Olaparib Monotherapy in Metastatic Breast Cancer Patients With Germline or Somatic Mutations in DNA Repair Genes (Olaparib Expanded) the response to olaparib is studied in patients with metastatic BC and germline or somatic mutations in the following genes: ATM, ATR, BARD1, BRIP1 (FANCJ), CHEK2, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCM, MRE11A, NBN, PALB2, RAD50, RAD51C, RAD51D.[46] In our cohort of women, if their BC recurs, three germline mutation carriers can be candidates for this trial as well as seven patients with somatic mutations in at least one of the above listed genes.

The same genomic alterations can be a target for a chemotherapy agent carboplatin which is known to double the objective tumor response rate in women with metastatic BC and germline BRCA1/2 mutations.[47] HR deficiency in early- stage BC as measured by a HRD (Homologous Recombination Deficiency) molecular assay was associated with a higher chance to benefit from Carboplatin in neoadjuvant setting.[48,49] In a study of triple negative breast cancer, 59% of cases with homologous-recombination-repair deficiency had better outcome on
adjuvant chemotherapy for invasive disease-free survival (hazard ratio 0.42) compared to those without, regardless of whether a genetic/epigenetic cause was identified. [13]

In our study, all three germline mutation carriers had mutations in HR pathway (CHEK2 and ATM genes). In addition, the somatic alterations in two of these three tumors also included many genes in HR pathway (BRCA1 and BRCA2, NF1 and NF2, FANCA) (Table 3). It is logical to suggest that these somatic alterations (frequently, hemizygous losses) in the same pathway played a key role in the tumor development initiated by the germline mutation. Given this constellation of germline and somatic variants, it is likely that these cancers would respond to PARP-inhibitors and carboplatin if they recur.

As discussed earlier, TMP and PT data are usually not incorporated in treatment decisions for early BC. However, this may change with the increased use of platinum agents for adjuvant and neoadjuvant treatment of BC in BRCA1/2 mutation carriers. Also, clinical trials are looking at a potential benefit of PARP-inhibitors in adjuvant and neoadjuvant treatment of high-risk BC patients with these mutations.[50] A recent study showed that patients with BC harboring kinase or helical domain PIK3CA mutations derived significantly greater benefit from letrozole over tamoxifen in BIG 1-98 adjuvant trial.[51] If confirmed prospectively this molecular finding can inform the selection of adjuvant anti-hormonal agents in the future. An integrative analysis of 2,658 whole-cancer genomes and their matching normal tissues across 38 tumor types from the Pan-Cancer Analysis of Whole Genomes (PCAWG) Consortium found cancer genomes contained 4–5 driver mutations, however, in around 5% of cases no drivers were identified, suggesting that cancer driver discovery is not yet complete. [12]

Our study showed that the use of PT is feasible in an actual clinical setting and a single academic institution. Even though TMP can potentially identify germline mutations, their ascertainment as such is difficult without a paired germline testing. [52] PT is more efficient than a germline testing or tumor sequencing alone, providing valuable information for the care of BC patients and eliminating their “testing fatigue” when asked to do serial tests; however, larger prospective studies are needed to assess the clinical impact and cost-effectiveness of PT for women with newly-diagnosed BC.

Conclusions:
With the rising demand for targeted therapies and the rapidly-changing landscape of MTAs, it is obvious that tumor sequencing holds promise for BC. Outcomes of the three patients in this study treated with MTA based on somatic alterations demonstrate the limitations that remain for their current use. In the years to come, cancer treatment may be dictated more by tumor mutations than tumor type. Yet, before BC tumor sequencing and paired testing become routine, active targets must be validated and more MTAs developed, including those that aim at targets currently deemed “not-druggable.” Achieving these goals is likely to improve insurance reimbursement for BC sequencing and PT which at present, is often lacking.

Clinical trials are desperately needed to evaluate the efficacy and safety of MTAs. However, enrollment into these trials can be challenging as they are increasingly conducted in smaller biomarker-enriched patient populations selected by the presence of the target. In addition, since <1% - 20% of tumors harbor individually rare somatic mutations”, collecting and reporting individual responses to MTAs as described in this paper (“N-of-one experiences”) are crucial for future success of MTAs.[53] For this reason, we support the establishment of an up-to-date Web-based open-access database of molecular targets and responses to MTAs in individual patients such as the ASCO initiative CancerLinQ. [54]

Analysis of cancer-related genes in paired germline and tumor DNA samples can lead to increased detection of clinically significant heritable mutations compared to the predicted yield of targeted germline testing based on current clinical guidelines. Identification of germline variants can help guide therapeutic and preventive interventions. Drawing from our experience in this study, we predict expanding indications for paired somatic and germline testing in the future for BC as well as other types of cancer.
Ethical Statement
The study was approved by the Rush University Institutional Review Board (IRB# 17041703-IRB01).

Data Availability Statement
Our data is not currently shared in a repository, but can be made available upon request.

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Conflicts of Interest
At the time research was conducted, Virginia Speare, Holly LaDuca, Carin Espenschied, Amal Yussuf, and Philip Gray were full time employees of Ambry Genetics. Carin Espenschied is a current employee and stock holder at Guardant Health. Otherwise we have no other conflicts of interest to report.

Authors’ Contributions
All authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conceptualization, L.U., C.E., PG; Methodology, E.E., V.S, K.B., P.G., C.E., L.U.; Investigation, E.E., M.C., R.R., K.B., H.L., A.Y., L.U.; Formal Analysis, P.G., A.Y., V.S.; Resources, M.C., R.R., P.G., L.U; Writing – Original Draft, E.E., J.C., J.P., K.B., and L.U.; Writing – Review & Editing, E.E., J.C., J.P., K.B., V.S., C.E., H.L., A.Y., P.G., T.K., L.B., H.L., M.C., R.R, and L.U.; Visualization, C.E., A.Y., V.S, J.C.; Supervision, C.E., L.U., V.S.; Funding Acquisition, L.U., H.L., P.G., V.S.
References:
1. Hochhaus A., Larson R.A., Guilhot F., Radich J.P., Branford S., Hughes T.P., Baccarani M., Deininger M.W., Cervantes F., Fujihara S., Ortmann C.-E., Menssen H.D., Kantarjian H., O’Brien S.G., Druker B.J., for the I.I. (2017) Long-term outcomes of imatinib treatment for chronic myeloid leukemia. The New England Journal of Medicine 376 (10):917-927. doi:10.1056/NEJMoa1609324
2. Schwaederle M., Zhao M., Lee J.J., Eggermont A.M., Schlisky R.L., Mendelsohn J., Lazar V., Kurzrock R. (2015) Impact of precision medicine in diverse cancers: A meta-analysis of phase ii clinical trials. Journal of Clinical Oncology 33 (32):3817-3825. doi:10.1200/JCO.2015.61.5997
3. Tsimberidou A.-M. (2015) Targeted therapy in cancer. Cancer Chemotherapy and Pharmacology 76 (6):1113-1132. doi:10.1007/s00280-015-2861-1
4. Robson M., Im S.-A., Senkus E., Xu B., Domchek S.M., Masuda N., Delaloge S., Li W., Tung N., Armstrong A., Wu W., Goessl C., Runswick S., Conte P. (2017) Olaparib for metastatic breast cancer in patients with a germline brca mutation. New England Journal of Medicine 377 (6):523-533. doi:10.1056/NEJMoa1706450
5. Administration U.S.F.D. (2018) Fda approves olaparib for germline brca-mutated metastatic breast cancer. https://www.fda.gov/drugs/informationondrugs/approveddrugs/ucm592357.htm. Accessed April 16, 2018
6. Talazaparib [package insert]. New York, NY. Pfizer. 2018.
7. Apelisib [package insert]. East Hannover, NJ. Novartis. 2019.
8. Rakha E.A., Starczynski J., Lee A.H.S., Ellis I.O. (2014) The updated asco/cap guideline recommendations for her2 testing in the management of invasive breast cancer: A critical review of their implications for routine practice. Histopathology 64 (5):609-615. doi:10.1111/his.12357
9. Hammond M.E.H., Hayes D.F., Dowsett M., Allred D.C., Hagerty K.L., Badve S., Fitzgibbons P.L., Francis G., Goldstein N.S., Hayes M., Hicks D.G., Lester S., Love R., Mangu P.B., McShane L., Miller K., Osborne C.K., Paik S., Perlmutter J., Rhodes A., Sasano H., Schwartz J.N., Sweep F.C.G., Taube S., Torlakovic E.E. et al (2010) American society of clinical oncology/college of american pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Journal of Clinical Oncology 28 (16):2784-2795. doi:10.1200/JCO.2009.25.6529
10. Krishnamurti U., Silverman J.F. (2014) Her2 in breast cancer: A review and update. Advances in Anatomic Pathology 21 (2):100-107. doi:10.1097/PAP.0000000000000015
11. Desch C.E., McNiff K.K., Schneider E.C., Schrag D., McClure J., Lepisto E., Donaldson M.S., Kahn K.L., Weeks J.C., Ko C.Y., Stewart A.K., Edge S.B. (2008) American society of clinical oncology/national comprehensive cancer network quality measures. Journal of Clinical Oncology 26 (21):3631-3637. doi:10.1200/jco.2008.16.5068
12. Campbell, P.J., Getz, G., Korbel, J.O. et al. Pan-cancer analysis of whole genomes. Nature 578, 82–93 (2020). https://doi.org/10.1038/s41586-020-1969-6
13. Staf, J., Gloedzik, D., Bosch, A. et al. Whole-genome sequencing of triple-negative breast cancers in a population-based clinical study. Nat Med 25, 1526–1533 (2019). https://doi.org/10.1038/s41591-019-0582-4
14. Ian S. Hagemann (2016) Molecular testing in breast cancer: A guide to current practices. Archives of Pathology and Laboratory Medicine 140 (8):815-824. doi:10.5858/arpa.2016-0051-RA
15. Center for Medicare and Medicaid Services (2018) Decision memo for next generation sequencing (ngs) for medicare beneficiaries with advanced cancer (cag-00450n). https://www.cms.gov/medicare-coverage-database/details/nca-decision-memo.aspx?NCAId=290. Accessed July 1, 2018
16. Gray P.N., Vuong H., Tsai P., Lu H.M., Mu W., Hsuan V., Hoo J., Shah S., Uyeda L., Fox S., Patel H., Janicek M., Brown S., Dobrea L., Wagman L., Pлимак Е., Mehra R., Golemis E.A., Bilsus M., Zibelman M., Elliott A. (2016) Tumornext: A comprehensive tumor profiling assay that incorporates high resolution copy number analysis and germline status to improve testing accuracy. Oncotarget 7 (42):68206-68228. doi:10.18632/oncotarget.11910
17. Pesaran T, Karam R, Huether R, et al. Beyond DNA: An Integrated and Functional Approach for Classifying Germline Variants in Breast Cancer Genes. Int J Breast Cancer. 2016;2016:2469523. doi:10.1155/2016/2469523
18. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424
19. National Cancer Institute Surveillance Epidemiology and End Results (SEER) Program (Released April 2017, based on the November 2016 submission). https://www.seer.cancer.gov. Accessed October 24 2017
20. Iqbal J., Ginsburg O., Rochon P.A., Sun P., Narod S.A. (2015) Differences in breast cancer stage at diagnosis and cancer-specific survival by race and ethnicity in the United States. JAMA 313 (2):165-173. doi:10.1001/jama.2014.17322
21. Howlader N., Altekruse S.F., Li C.I., Chen V.W., Clarke C.A., Ries L.A.G., Cronin K.A. (2014) Use incidence of breast cancer subtypes defined by joint hormone receptor and her2 status. JNCI Journal of the National Cancer Institute 106 (5):duj055. doi:10.1093/jnci/duj055
22. NCCN Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for genetic/familial high-risk assessment: Breast and Ovarian v.2.2019. © National Comprehensive Cancer Network, Inc. 2018. All rights reserved. Accessed July 30, 2019. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way. https://www.nccn.org/professionals/physician_gls/pdf/genetics_screening.pdf.
23. Allegrezza M.J., Rutkowski M.R., Stephen T.L., Svoronos N., Perales-Puchalt A., Nguyen J.M., Payne K.K., Singhal S., Erusalov E.B., Tchou J., Conejo-Garcia J.R. (2016) Trametinib drives t cell-dependent control of k-ras-mutated tumors by inhibiting pathological myelopoiesis. Cancer Research 76 (21):6253-6265. doi:10.1158/0008-5472.CAN-16-1308
24. Ma C.X., Bose R., Gao F., Freedman R.A., Pegram M.D., Blackwell K., Bedard P.L., Hayes D.F., Goetz M.P., Niravath P.A., Russell C.A., Tripathy D., Coleleigh M.A., Forero A., Pluard T.J., Anders C.K., Bumb C., Naughton M., Al-Kateh H., Ellis M.J. (2016) Phase ii trial of neratinib for her2 mutated, non-amplified metastatic breast cancer (her2mut mbc). Journal of Clinical Oncology 34 (15_suppl):516-516. doi:10.1200/JCO.2016.34.15_suppl.516
25. Bose R., Kavuri S.M., Searleman A.C., Shen W., Shen D., Koboldt D.C., Monsey J., Goel N., Aronson A.B., Li S., Ma C.X., Ding L., Mardis E.R., Ellis M.J. (2013) Activating her2 mutations in her2 gene amplification negative breast cancer. Cancer Discovery 3 (2):224-237. doi:10.1158/2159-8290.CD-12-0349
26. S1207 hormone therapy with or without everolimus in treating patients with breast cancer (2018) National Library of Medicine. https://ClinicalTrials.gov/show/NCT01674140.
27. Duffy M.J., Synnott N.C., Crown J. (2017) Mutant p53 as a target for cancer treatment. European Journal of Cancer 83:258-265. doi:10.1016/j.ejca.2017.06.023
28. Nik-Zainal S., Davies H., Staaf J., et. al. (2016). Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature 534(7605):47-54. doi: 10.1038/nature17676. Epub 2016 May 2.
29. Guo X.E., Ngo B., Modrek A.S., Lee W.H. (2014) Targeting tumor suppressor networks for cancer therapeutics. Current Drug Targets 15 (1):2-16
30. Mendes-Pereira A.M., Martin S.A., Brough R., McCarthy A., Taylor J.R., Kim J.-S., Waldman T., Lord C.J., Ashworth A. (2009) Synthetic lethal targeting of pten mutant cells with parp inhibitors. EMBO Molecular Medicine 1 (6-7):315-322. doi:10.1002/emmm.200900041
31. The Cancer Genome Atlas N., Koboldt D.C., Fulton R.S., McLellan M.D., Schmidt H., Kalicki-Veizer J., McMichael J.F., Fulton L.L., Dooling D.J., Ding L., Mardis E.R., Wilson R.K., Ally A., Balasundaram M., Butterfield Y.S.N., Carlisen R., Carter C., Chu A., Chuah E., Chun H.-J.E., Coope R.N.J., Dhalla N., Guin R., Hirst C., Hirst M. et al (2012) Comprehensive molecular portraits of human breast tumours. Nature 490:61. doi:10.1038/nature11412
32. Jeselsohn R., De Angelis C., Brown M., Schiff R. (2017) The evolving role of the estrogen receptor mutations in endothelial therapy-resistant breast cancer. Current Oncology Reports 19 (5):35. doi:10.1007/s11912-017-0591-8
33. Astsaturov I. (2017) Future clinical trials: Genetically driven trials. Surgical Oncology Clinics of North America 26 (4):791-797. doi:10.1016/j.soc.2017.05.014
34. Le Tourneau C, Delord J-P, Goncalves A, et al. Molecular Targeted Therapy Based on Tumour Molecular Profiling Versus Conventional Chemotherapy for Advanced Cancer (SHIVA): A Multicentre, Open-Label, Proof-of-Concept, Randomised, Controlled, Phase 2 Trial. Lancet Oncol. 2015;16(13):1324-1334. doi:10.1016/S1470-2045(15)00188-6
35. Andre F., Bachelot T., Commo F., Campone M., Arnedos M., Dieras V., Lacroix-Triki M., Lacroix L., Cohen P., Gentien D., Adelaide J., Dalenc F., Goncalves A., Levy C., Ferrero J.M., Bonneterre J., Lefeuvre C., Jimenez M., Filleron T., Bonnefoi H. (2014) Comparative genomic hybridisation array and DNA sequencing to
direct treatment of metastatic breast cancer: A multicentre, prospective trial (safir01/unicancer). Lancet Oncology 15 (3):267-274. doi:10.1016/S1470-2045(13)70611-9
36. ECOG-ACRIN (2016) Executive summary: Interim analysis of the nci-match trial. Available via ECOG-ACRIN Cancer Research Group. http://ecog-acrin.org/nci-match-eay131/interim-analysis. Accessed August 18 2018
37. Vlessis K, Purington N, Chun N, et al.Germline Testing for Patients With BRCA1/2 Mutations on Somatic Tumor Testing. *JNCI Cancer Spectrum*, Volume 4, Issue 1, February 2020, pkz095
38. Schrader K.A., Cheng D.T., Joseph V., et. al. (2016). Germline variants in targeted tumor sequencing using matched normal DNA. JAMA Oncology 2(1):104-11. doi: 10.1001/jamaoncol.2015.5208
39. Mankelker D., Zhang L., Kemel Y., et. al. (2017). Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs. guideline-based germline testing. JAMA 318(9):825-835. doi:10.1001/jama.2017.11137.
40. Yadav S., Reeves A., Campian S., Sutfka A., Zakalik D. (2017) Preoperative genetic testing impacts surgical decision making in brca mutation carriers with breast cancer: A retrospective cohort analysis. Hereditary Cancer in Clinical Practice 15:11. doi:10.1186/s13053-017-0071-z
41. Jerzak K.J., Mancuso T., Eisen A. (2018) Ataxia–telangiectasia gene (atm) mutation heterozygosity in breast cancer: A narrative review. Current Oncology 25 (2):e176-e180. doi:10.3477/co.25.3707
42. NCCN (2018) Referenced with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for Genetic/Familial High-Risk Assessment: Colorectal v.1.2018 © National Comprehensive Cancer Network, Inc 2018. All rights reserved. Accessed July 12, 2018. To view the most recent and complete version of the guideline, go online to NCCN.org. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.
https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf. Accessed July 12 2018
43. Koumpis C., Dimitrakakis C., Antsaklis A., Royer R., Zhang S., Narod S.A., Kotsopoulos J. (2011) Prevalence of brca1 and brca2 mutations in unselected breast cancer patients from greece. Hereditary Cancer in Clinical Practice 9 (1):10-10. doi:10.1186/1897-4287-9-10
44. Norquist B, Wurz KA, Pennil CC, et al. Secondary somatic mutations restoring BRCA1/2 predict chemotherapy resistance in hereditary ovarian carcinomas. *J Clin Oncol*. 2011;29(22):3008-3015. doi:10.1200/jco.2010.34.2980
45. Olaparib in breast cancer (2018) National Library of Medicine.
https://clinicaltrials.gov/ct2/show/NCT03344965. Accessed September 5, 2018
46. Tutt A., Tovey H., Cheang M.C.U., Kernaghan S., Kilburn L., Gazinska P., Owen J., Abraham J., Barrett S., Barrett-Lee P., Brown R., Chan S., Dowsett M., Flanagan J.M., Fox L., Grigoriadis A., Gutin A., Harper-Wynne C., Hatton M.Q., Hoadley K.A., Parikh J., Parker P., Perou C.M., Roylance R., Shah V. et al (2018) Carboplatin in brca1/2-mutated and triple-negative breast cancer brcaness subgroups: The tnt trial. Nature Medicine 24 (5):628-637. doi:10.1038/s41591-018-0009-7
47. Minckwitz G.V., Timms K., Untch M., Elkin E.P., Fasching P.A., Schneeweiss A., Salat C., Rezai M., Blommer J.U., Zahm D.M., Jackisch C., Gerber B., Klare P., Kümmel S., Eidmann H., Paepeke S., Reid J.E., Nekljudova V., Hartman A.-R., Loibl S. (2015) Prediction of pathological complete response (pCR) by homologous recombination deficiency (hrd) after carboplatin-containing neoadjuvant chemotherapy in patients with tnbc: Results from geparSixto. Journal of Clinical Oncology 33 (15_suppl):1004-1004. doi:10.1200/jco.2015.33.15_suppl.1004
48. Telli M.L., Timms K.M., Reid J., Hennessy B., Mills G.B., Jensen K.C., Szallasi Z., Barry W.T., Winer E.P., Tung N.M., Isakoff S.J., Ryan P.D., Greene-Colozzi A., Gutin A., Sangale Z., Iliev D., Abkevich V., Jones J.T., Lanchbury J.S., Hartman A.-R., Garber J.E., Ford J.M., Silver D.P., Richardson A.L. (2016) Homologous recombination deficiency (hrd) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple-negative breast cancer. Clinical Cancer Research 22 (15):3764-3773. doi:10.1158/1078-0432.ccr-15-2477
49. Litton J.K., Scoggins M., Ramirez D.L., Murthy R.K., Whitman G.J., Hess K.R., Adrada B.E., Moulder S.L., Barcenas C.H., Valero V., Gomez J.S., Mittendorf E.A., Thompson A., Helgason T., Mills G.B., Piwnica-Worms H., Arun B.K. (2017) A feasibility study of neoadjuvant talazoparib for operable breast cancer patients with a germline brca mutation demonstrates marked activity. npj Breast Cancer 3 (1):49. doi:10.1038/s41523-017-0052-4
51. Luen S.J., Asher R., Lee C., et al. (2018) Association of somatic driver alterations with prognosis in postmenopausal, hormone receptor–positive, her2-negative early breast cancer: A secondary analysis of the big 1-98 randomized clinical trial. JAMA Oncology. doi:10.1001/jamaoncol.2018.1778
52. Ngeow, J., Eng, C. Precision medicine in heritable cancer: when somatic tumour testing and germline mutations meet. npj Genomic Med 1, 15006 (2016). https://doi.org/10.1038/npjgenmed.2015.6
53. Santarpia L., Bottai G., Kelly C.M., Győrffy B., Székely B., Pusztai L. (2016) Deciphering and targeting oncogenic mutations and pathways in breast cancer. The Oncologist 21 (9):1063-1078. doi:10.1634/theoncologist.2015-0369
54. Oncology A.S.o.C. (2018) Asco cancerlinq. Available via American Society of Clinical Oncology. https://cancerlinq.org/. Accessed July 1 2018
### Table 1: Demographic and Clinical Characteristics of Breast Cancer Cases

| Patient Characteristics | Number of Patients | Patients with MTA for Breast Cancer (%) |
|-------------------------|--------------------|----------------------------------------|
| Number of patients      | 43                 | 33/43 (76.7)                           |
| Median Age at Diagnosis (IQR) [years] | 66 (53, 72) | -                                      |
| Median Age at Tumor Testing (IQR) [years] | 64 (51, 70.5) | -                                      |
| Age at Diagnosis Group  | N (%)              |                                        |
| 30-39                   | 1 (2.3)            | 1/1 (100.0)                            |
| 40-49                   | 7 (16.3)           | 2/7 (28.6)                             |
| 50-59                   | 10 (23.3)          | 8/10 (80.0)                            |
| 60-69                   | 12 (27.9)          | 9/12 (75.0)                            |
| 70-79                   | 6 (14.0)           | 6/6 (100.0)                            |
| 80 and over            | 7 (16.3)           | 6/7 (85.7)                             |
| Race/Ethnicity (%)      |                    |                                        |
| Caucasian               | 23 (53.4)          | 19/23 (82.6)                           |
| Ashkenazi Jewish        | 0 (0.0)            | -                                      |
| African American        | 12 (27.9)          | 9/12 (75.0)                            |
| Asian                   | 1 (2.3)            | 0/1 (0)                                |
| Hispanic                | 4 (9.3)            | 4/4 (100.0)                            |
| Multiple/Other/Unknown  | 3 (7.0)            | 1/3 (33.3)                             |
| Clinical Stage at Time of Paired Testing (%) |                |                                        |
| I                       | 4 (9.3)            | 3/4 (75)                               |
| II                      | 6 (14.0)           | 5/6 (83.3)                             |
Table 1 depicts demographic and clinical characteristics of the breast cancer cases included in this study.

| III | IV | 2/4 (50.0) |
|-----|----|------------|

| Incomplete early stage b | 6 (14.0) | 3/6 (50.0) |

| Histology |
|-----------|
| IDC | 29 (67.4) | 26/29 (90.0) |
| ILC | 7 (16.3) | 5/7 (71.4) |
| Mixed Histology | 6 (14.0) | 1/6 (16.7) |
| Unknown Histology | 1 (2.3) | 1/1 (100.0) |

| Receptor Status |
|----------------|
| Triple Negative (ER-/PR-/Her2-) | 5 (11.6) | 2/5 (40.0) |
| (ER+/PR+/Her2+) or (ER+/PR-/Her2+) or (ER-/PR+/Her2+) | 6 (14.0) | 3/6 (50.0) |
| (ER+/PR+/Her2-) or (ER+/PR-/Her-2-) or (ER-/PR+/Her2-) | 28 (65.1) | 26/28 (93.0) |
| (ER-/PR-/Her2+) | 4 (9.3) | 4/4 (100.0) |

*a IQR=interquartile range

*b T1 - T2 tumors without nodal assessment
Table 2: Genomic Alterations and Available Therapy and Clinical Trials

| Genomic Alterations (Patients)* | 278 (43) |
|---------------------------------|----------|
| Patients with at least one FDA approved therapy for breast cancer (% of tumors affected) | 33 (76.7) |
| Patients with at least one FDA approved therapy for other tumor type (% of tumors affected) | 38 (88.3) |
| Patients who were eligible for a clinical trial based on genomic alteration(s) (% of tumors affected) | 40 (93.2) |

Table 2 depicts clinical implications of genomic alterations identified. It stratifies results based on percentage of total alterations meeting specific clinical criteria, and percentage of tumors included in the study which meet clinical criteria mentioned.

* Gene alterations found in multiple patients were counted separately.
| Patient | Germline Mutation | Somatic Variants | Selected Demographic and Clinical Characteristics. Disease Status as of July 2020 |
|---------|-------------------|------------------|------------------------------------------------------------------|
| 1       | CHEK2, likely pathogenic c.1427C>T (p.Thr476Met) | 4 somatic single nucleotide variants (SNVS): PIK3CA p. H1047L, AR p.R13W, TP53, p. Y220C, CDH1 p.N297S | Caucasian
Diagnosed in 2011 at age 64 with stage I breast cancer ER and PR positive, Her-2/neu negative. Currently, NED. Also, has a history of clear cell papillary renal cell carcinoma. |
| 2       | ATM, likely pathogenic c.2466+1G>C (splicing site exon 18) | 10 somatic alterations: CDKN2A hemizygous loss, CDKN2B hemizygous loss, PTEN hemizygous loss, NF2 hemizygous loss, BRCA2 hemizygous loss, ATM LOH, FANCA hemizygous loss, FLCN hemizygous loss, T53 (pT155_R156del and T53 LOH) | Caucasian
Diagnosed in 2015 at age 50 with stage II breast cancer, ER positive, PR and Her-2/neu negative. Currently, NED. |
| 3       | CHEK2, pathogenic c.1100del (pT367Mfs*15) | 13 somatic alterations: CDKN2A hemizygous loss, CDKN2B hemizygous loss, PTEN hemizygous loss, NF1 hemizygous loss, BRCA1 hemizygous loss, ATM hemizygous loss, FLCN hemizygous loss, T53 hemizygous loss, CDH1 hemizygous loss, TSC1 hemizygous loss, APC hemizygous loss, CDK4 amplification, FRS2 amplification | African-American
Diagnosed in 2016 at age 79 with stage II breast cancer, ER positive, PR and Her-2/neu negative. Died in 2019 with no evidence of breast cancer recurrence. |

Table 3 captures somatic variants and selected clinical and demographic information for the three patients with germline variants that were not previously suspected.
Figure 1: Frequency of Somatic Alterations by Gene

Figure 1 depicts frequency of specific genomic mutations identified across all tumors studied. 278 somatic alterations (mutations, allelic loss and amplifications) in 81 genes were clinically reported. The single most frequently observed gene was TP53. Genes known to be frequently mutated in BC (BRCA1, BRCA2, CDH1, PIK3CA, PTEN, including TP53) accounted for 40% of alterations.
Figure 2: Genes with Associated FDA Approved Therapy

A.

B.

Figure 2 depicts frequency of genomic mutations with FDA approved therapies (both for breast cancer and for other tumor types) identified across all tumors studied. 22 genes altered in this patient group had an associated MTA for breast cancer (A). Alterations in additional genes had an associated MTA for other cancers (B).
### Supplemental Table 1. TumorNext Solid Tumor Panel

#### Genes with full exon coverage

| Genes | Genes | Genes | Genes | Genes | Genes | Genes | Genes | Genes | Genes |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| ABL1  | BCL2L1| CSF1R | FANCA | GNAQ  | MAP2K2| MYD88 | PDGFRB|
| ABL2* | BCL2L2| CTNNB1| FANCC | GNAS  | MAP2K4| NBN   | PIK3CA|
| AKT1  | BMPR1A| DDR2  | FANCD2| HRAS  | MAX   | NF1   | PIK3CB|
| AKT2  | BRAF  | DDRN1 | FBBX7 | HSP90AA1| MDM2 | NF2   | PIK3CG|
| AKT3  | BRCA1 | EGFR  | FGFR1 | IDH1  | MET   | NOTCH1| PIK3R1|
| ALK   | BRCA2 | EPHA2 | FGFR2 | IDH2  | MITF  | NOTCH2| PIK3R2**|
| APC   | BRIP1 | EPHA3 | FGFR3 | IGF1R | MLH1  | NOTCH3| PIK3R2***|
| AR    | BTK   | EPHA5 | FGFR4 | IL7R  | MLL   | NOTCH4| PTCH1 |
| ARAF  | CB1   | EPHA7 | FGFR4 | IL1R  | MPL   | NRAS  | PTEN  |
| ATM   | CCND1 | EPHB1 | FLCN  | JAK1  | MLL   | NOTCH4| PTCH1 |
| AURKA | CCND3 | ERBB2 | FLT1  | JAK3  | MSH2  | PAK7  | RAD50 |
| AURKB | CDH1  | ERBB3 | FLT3  | KDR   | MSH6  | PALB2 | RAD51C|
| AXL   | CDK4  | ERBB4 | FLT4  | KIT   | NRAS  | PALB2 | RAD51D|
| BARD1 | CDKN2A| ESR1  | FOXL2 | KRAS  | MUTYH | PARP1 | RAFL  |
| BCL2  | CHEK2 | EZH2  | GNA11 | MAP2K1| MYCN  | PDGFR | RARAF |

#### Genes analyzed for structural variants

| Genes | Genes | Genes | Genes | Genes | Genes | Genes | Genes | Genes | Genes |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| ALK   | BRAF  | BRCA2 | FGFR2 | KIT   | NTRK1 | RAF1  | ROS1  |
| BCL2  | BRCA1 | FGFR1 | FGFR3 | NOTCH2| PDGFR | RET   |

*ABL2 exon 2 is not included in the assay
**PIK3R2 exon 13 is not included in the assay
***Only PMS2 exons 1-10 are analyzed
| Genes     | Genes     |
|-----------|-----------|
| RB1       | TET2      |
| RET       | TMEM127   |
| ROS1      | TOP1      |
| RUNX1     | TP53      |
| SDHA      | TSC1      |
| SDHAF2    | TSC2      |
| SDHB      | VHL       |
| SDHC      |           |
| SDHD      |           |
| SMAD4     |           |
| SMARCB1   |           |
| SMO       |           |
| SRC       |           |
| STAT3     |           |
| STK11     |           |

*ABL2 exon 2 is not included in the assay
**PIK3R2 exon 13 is not included in the assay
***Only PMS2 exons 1-10 are analyzed
| Gene       | Mutation          |
|------------|-------------------|
| ABL1       | p.K628del         |
| AKT1       | p.E17K            |
| AKT3       | amplification     |
| APC         | hemizygous loss   |
| ARID1A     | hemizygous loss   |
| AR          | p.R13W            |
| AR          | p.Y858C           |
| ATK1        | p.E17K            |
| ATM         | hemizygous loss   |
| ATM LOH     |                   |
| ATM p.2466+1G>C |               |
| ATM p.R2746fs*12 |          |
| BARD1      | p.A724V           |
| BRCA1       | copy neutral LOH  |
| BRCA1       | hemizygous loss   |
| BRCA1 loss  |                   |
| BRCA1 p.N1018Mfs*8 |         |
| BRCA2       | hemizygous loss   |
| BRIP1       | hemizygous loss   |
| CCND1       | amplification     |
| CCNE1       | amplification     |
| CD274 (PD-L1) | amplification   |
| CDH1        | hemizygous loss   |
| CDH1         | hemizygous deletion |
| CDH1 intron 14 2296-3A>G |     |
| CDH1 LOH    |                   |
| CDH1 p.HI047R |                |
| CDH1 p.K187Qfs*12 |           |
| CDH1 p.N297S |                   |
| CDH1 - KITPDGF |             |
| Mutation                    |
|----------------------------|
| CDH1 p.N405Qfs*14          |
| CDH1 p.T211Rfs*11          |
| CDH1 p.Y827*               |
| CDK4 amplification         |
| CDK6 amplification         |
| CDKN2A hemizygous loss     |
| CDKN2A loss                |
| CDKN2B hemizygous loss     |
| CDKN2B loss                |
| CHEK2 hemizygous loss      |
| CHEK2 LOH                  |
| CHEK2 p.K373E              |
| CHEK2 p.T367Mfs*15         |
| CHEK2 p.T476M              |
| DDR2 amplification         |
| DNMT3A hemizygous loss     |
| EGFR amplification         |
| EPHA3 splice site 2497-2A>T |
| EPHA5 amplification        |
| EPHA5 splice site 2236+1G>T |
| ERBB2 amplification        |
| ERBB2 p.D769Y              |
| ERBB2 p.S310F              |
| ERBB3 p.E928G              |
| ERBB4 amplification        |
| ERBB4 p.H582Y              |
| ERBB4 p.R81*               |
| ESR1 p.D530G               |
| FANCA hemizygous loss      |
| FANCQ2 p.N405S             |
| FBXW7 hemizygous loss      |
| FGFR3 amplification        |
| FGFR4 amplification        |
| FGFR1 amplification        |
| FGFR1 BAG4-FGFR1 fusion    |
Alterations by ER Receptor status
| Gene/Amplification | Characteristics | ER Status |
|-------------------|-----------------|-----------|
| FGFR1 p. E140K    |                 | ER Positive |
| FGFR2 amplification |                | ER Positive |
| FGFR3 amplification |               | ER Positive |
| FLN hemizygous loss |              | ER Negative |
| FH amplification |                | ER Negative |
| FRS2 amplification |               | ER Negative |
| GNAQ P. L391F  |                | ER Negative |
| JAK3 amplification |              | ER Negative |
| KRAS amplification |             | ER Negative |
| MAP2K1 p. K71E  |                | ER Positive |
| MAP3K5 amplification |            | ER Negative |
| MCL1 amplification |            | ER Negative |
| MDM2 amplification |             | ER Positive |
| MDM4 amplification |            | ER Positive |
| MLH1 hemizygous loss |            | ER Negative |
| MLL1 P. G260D |                 | ER Positive |
| MSH6 amplification |             | ER Positive |
| NF1 LOH        |                | ER Negative |
| NF1 loss       |                 | ER Negative |
| NF1 p. W837*   |                | ER Negative |
| NF1 p. Y488C |                 | ER Negative |
| NF2 hemizygous loss |              | ER Negative |
| NOTCH2 P. V1669F |               | ER Negative |
| NOTCH1 P. R568S |                | ER Negative |
| NTRK1 P. R568S |                | ER Negative |
| PAFAH1 amplification |            | ER Negative |
| PAK1 amplification |            | ER Negative |
| PDGK1 amplification |            | ER Negative |
| PKC-CA amplification |          | ER Negative |
| PKC3A p. E545D |                 | ER Negative |
| PKC3A p. E545K |                 | ER Negative |
### Supplement Table 2: Genetic Alterations Found and Available MTA

| Gene     | Alteration                          | Number of times observed | Source       |
|----------|-------------------------------------|--------------------------|--------------|
| ABL1     | p.K628del                           | 1                        | Somatic      |
| AKT1     | p.E17K                              | 2                        | Somatic      |
| AKT3     | amplification                       | 3                        | Somatic      |
| APC      | hemizygous loss                     | 2                        | Somatic      |
| AR       | p.R13W                              | 1                        | Somatic      |
| AR       | p.Y858C                             | 1                        | Somatic      |
| ARID1A   | hemizygous loss                     | 1                        | Somatic      |
| ATM      | p.2466+1G>C                         | 1                        | Germline     |
| ATM      | LOH                                 | 3                        | Somatic      |
| ATM      | p.R2746fs*12                        | 1                        | Somatic      |
| ATM      | hemizygous loss                     | 5                        | Somatic      |
| BARD1    | p.A724V                             | 1                        | Somatic      |
| CHEK2    | p.T367Mfs*15                        | 1                        | Germline     |
| BRCA1    | hemizygous loss                     | 13                       | Somatic      |
| BRCA1    | loss                                | 1                        | Somatic      |
| BRCA1    | copy neutral LOH                    | 1                        | Somatic      |
| BRCA2    | hemizygous loss                     | 12                       | Somatic      |
| BRIP1    | hemizygous loss                     | 2                        | Somatic      |
| CCND1    | amplification                       | 4                        | Somatic      |
| CCNE1    | amplification                       | 1                        | Somatic      |
| CD274 (PD-L1) | amplification               | 1                        | Somatic      |
| CDH1     | p.N297S                             | 1                        | Somatic      |
| CDH1     | p.N405Qfs*14                        | 1                        | Somatic      |
| CDH1     | LOH                                 | 6                        | Somatic      |
| CDH1     | p.Y827*                             | 1                        | Somatic      |
| CDH1     | p.H1047R                            | 1                        | Somatic      |
| CDH1     | p.K187Qfs*12                        | 1                        | Somatic      |
| CDH1     | intron 14 2296- 3A>G                | 1                        | Somatic      |
| CDH1     | hemizygous deletion                 | 1                        | Somatic      |
| CDH1     | hemizygous loss                     | 2                        | Somatic      |
| CDH1     | p.T211Rfs*11                        | 1                        | Somatic      |
| CDK4     | amplification                       | 1                        | Somatic      |
| CDK6     | amplification                       | 1                        | Somatic      |
| CDKN2A   | loss                                | 3                        | Somatic      |
| CDKN2A   | hemizygous loss                     | 4                        | Somatic      |
| CDKN2B   | loss                                | 3                        | Somatic      |
| CDKN2B   | hemizygous loss                     | 3                        | Somatic      |
| CHEK2    | p.T476M                             | 1                        | Germline     |
| CHEK2    | hemizygous loss                     | 1                        | Somatic      |
| CHEK2    | p.K373E                             | 1                        | Somatic      |
| CHEK2    | LOH                                 | 1                        | Somatic      |
| DDR2     | amplification                       | 1                        | Somatic      |
| DNMT3A   | hemizygous loss                     | 1                        | Somatic      |
| EGFR     | amplification                       | 1                        | Somatic      |
| EPHA3    | splice site 2497-2A>T               | 1                        | Somatic      |
| EPHA5    | amplification                       | 1                        | Somatic      |
| EPHA5    | splice site 2236+1G>T               | 1                        | Somatic      |
| Gene       | Mutation Type          | Number | Tumor Type |
|------------|------------------------|--------|------------|
| ERBB2      | amplification          | 6      | Somatic    |
| ERBB2      | p.S310F                | 2      | Somatic    |
| ERBB2      | p.D769Y                | 1      | Somatic    |
| ERBB3      | p.E928G                | 1      | Somatic    |
| ERBB4      | amplification          | 1      | Somatic    |
| ERBB4      | p.H582Y                | 1      | Somatic    |
| ERBB4      | p.R81*                 | 1      | Somatic    |
| ESR1       | p.D538G                | 1      | Somatic    |
| FANCA      | hemizygous loss        | 10     | Somatic    |
| FANCD2     | p.N405S                | 2      | Somatic    |
| FBXW7      | hemizygous loss        | 2      | Somatic    |
| FGFR1      | amplification          | 3      | Somatic    |
| FGFR1      | BAG4-FGFR1 fusion      | 1      | Somatic    |
| FGFR1      | p.K164I                | 1      | Somatic    |
| FGFR2      | amplification          | 1      | Somatic    |
| FGFR4      | p.V550L                | 1      | Somatic    |
| FGFR4      | amplification          | 1      | Somatic    |
| FH         | amplification          | 1      | Somatic    |
| FLCN       | hemizygous loss        | 10     | Somatic    |
| FRS2       | amplification          | 3      | Somatic    |
| GNAQ       | p.Y101*                | 1      | Somatic    |
| JAK2       | amplification          | 1      | Somatic    |
| JAK3       | amplification          | 1      | Somatic    |
| KRAS       | amplification          | 1      | Somatic    |
| MAP2K1     | p.K57E                 | 1      | Somatic    |
| MAP2K1     | amplification          | 1      | Somatic    |
| MCL1       | amplification          | 1      | Somatic    |
| MDM2       | amplification          | 2      | Somatic    |
| MDM4       | amplification          | 1      | Somatic    |
| MLH1       | p.F3L                  | 1      | Somatic    |
| MLH1       | hemizygous loss        | 1      | Somatic    |
| MUTYH      | p.G396D                | 1      | Somatic    |
| MYC        | amplification          | 2      | Somatic    |
| NF1        | hemizygous loss        | 3      | Somatic    |
| NF1        | LOH                    | 2      | Somatic    |
| NF1        | p.W837*                | 1      | Somatic    |
| NF1        | p.Y489C                | 1      | Somatic    |
| NF2        | hemizygous loss        | 5      | Somatic    |
| NOTCH2     | p.V1666F               | 1      | Somatic    |
| NTRK1      | p.R568S                | 1      | Somatic    |
| PAK1       | amplification          | 1      | Somatic    |
| PARP1      | amplification          | 1      | Somatic    |
| PDK1       | amplification          | 1      | Somatic    |
| PIK3       | p.H1047R               | 1      | Somatic    |
| PIK3CA     | p.E542K                | 5      | Somatic    |
| PIK3CA     | p.H1047R               | 3      | Somatic    |
| PIK3CA     | p.H1047L               | 1      | Somatic    |
| PIK3CA     | p.E545K                | 2      | Somatic    |
| PIK3CA     | p.E545D                | 1      | Somatic    |
| Gene      | Alteration                  | Recurrence Count | Type       |
|-----------|-----------------------------|------------------|------------|
| PIK3CA    | p.N345K                     | 1                | Somatic    |
| PIK3CA    | amplification               | 2                | Somatic    |
| PIK3CA    | p.R88Q                      | 1                | Somatic    |
| PIK3CG    | p.A186V                     | 1                | Somatic    |
| PIK3R1    | splice site 1746-2A>G       | 1                | Somatic    |
| PIK3R1    | p.R461_E462delinsQ         | 1                | Somatic    |
| PIK3R2    | amplification               | 1                | Somatic    |
| PTEN      | hemizygous loss             | 3                | Somatic    |
| PTEN      | LOH                         | 3                | Somatic    |
| PTEN      | p.S113Rfs*21                | 1                | Somatic    |
| PTEN      | p.D92Tfs*7                  | 1                | Somatic    |
| PTEN      | loss                        | 1                | Somatic    |
| PTEN      | p.D92H                      | 1                | Somatic    |
| PTEN      | p.G129R                     | 1                | Somatic    |
| PTEN      | p.F347Sfs*5                 | 1                | Somatic    |
| PTEN      | p.R130Q                     | 1                | Somatic    |
| PTEN      | copy netural LOH            | 1                | Somatic    |
| PTEN      | p.H93R                      | 1                | Somatic    |
| RAD51C    | hemizygous loss             | 3                | Somatic    |
| RAF1      | amplification               | 1                | Somatic    |
| RARA      | amplification               | 3                | Somatic    |
| RB1       | hemizygous_deletion         | 1                | Somatic    |
| RPTOR     | amplification               | 1                | Somatic    |
| RUNX1     | p.G408Vfs*193               | 1                | Somatic    |
| RUNX1     | p.S167R                     | 1                | Somatic    |
| SMARCB1   | p.T357*                     | 1                | Somatic    |
| SMARCB1   | Copy neutral LOH            | 1                | Somatic    |
| SMO       | amplification               | 1                | Somatic    |
| STAT3     | amplification               | 1                | Somatic    |
| STK11     | hemizygous loss             | 1                | Somatic    |
| TET2      | p.Q740E                     | 1                | Somatic    |
| TOP2A     | amplification               | 3                | Somatic    |
| TP53      | p.Y220C                     | 2                | Somatic    |
| TP53      | p.G245C                     | 1                | Somatic    |
| TP53      | LOH                         | 9                | Somatic    |
| TP53      | p.E285K                     | 1                | Somatic    |
| TP53      | p.K139_P142del              | 1                | Somatic    |
| TP53      | copy neutral LOH            | 1                | Somatic    |
| TP53      | hemizygous loss             | 8                | Somatic    |
| TP53      | p.R175H                     | 1                | Somatic    |
| TP53      | p.R280T                     | 1                | Somatic    |
| TP53      | p.R248Q                     | 1                | Somatic    |
| TP53      | p.I251Sfs*94                | 1                | Somatic    |
| TP53      | p.T155_R156del              | 1                | Somatic    |
| TP53      | p.R213*                     | 1                | Somatic    |
| TP53      | p.H193Lfs*54                | 1                | Somatic    |
| TP53      | p.R249G                     | 1                | Somatic    |
| TSC1      | hemizygous loss             | 1                | Somatic    |
| TSC2      | p.I61_G62insQ               | 1                | Somatic    |
| VEGFA     | amplification               | 1                | Somatic    |
| VHL       | hemizygous loss             | 1                | Somatic    |

*Some alterations recurred in multiple patients.*
Not every drug was available to each patient and associated therapy is reported based on availability.

LOH = loss of heterozygosity
| FDA Approved Therapies for Breast Cancer | Reference                  |
|------------------------------------------|----------------------------|
| None                                     |                            |
| Everolimus                               | PMID: 23215720             |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| Everolimus                               | PMID: 23215720             |
| Olaparib                                 | PMID: 21424107, 21771338, 18971340, 18649131, 21482494 |
| Olaparib                                 | PMID: 21424107, 21771338, 18971340, 18649131, 21482494 |
| Palbociclib                              | PMID: 25524798, 26030518   |
| Palbociclib                              | PMID: 25524798, 26030519   |
| Palbociclib                              | PMID: 25524798, 26030520   |
| Palbociclib                              | PMID: 25524798, 26030521   |
| Palbociclib                              | PMID: 25524798, 26030522   |
| Palbociclib                              | PMID: 25524798, 26030523   |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| None                                     |                            |
| Drug Combination | PMIDs |
|------------------|-------|
| Trastuzumab, Ado-trastuzumab | 20728210 |
| None | None |
| Pertuzumab | None |
| Lapatinib | 16014882, 22285168, 23816960, 24263064, 19692680 |
| None | None |
| None | None |
| None | None |
| Everolimus | 23215720 |
| None | None |
| None | None |
| None | None |
| Everolimus | 23215720 |
| None | None |
| None | None |
| None | None |
| Everolimus | 23215720 |
| None | None |
| None | None |
| None | None |
| Everolimus, Lapatinib | 23215725 |
| None | None |
| None | None |
| None | None |
| Everolimus | 23215720 |
| Everolimus | 23215721 |
| Everolimus | 23215722 |
| Everolimus | 23215723 |
| Everolimus | 23215724 |
| Everolimus, Lapatinib | 23215725 |
| None | None |
| None | None |
| None | None |
| Everolimus | 23215720 |
| Everolimus | 23215721 |
| Everolimus | 23215722 |
| Everolimus | 23215723 |
| Everolimus | 23215724 |
| Everolimus | 23215725 |
| Everolimus | 23215725 |
| Everolimus | PMID: 23215726 |
|------------|----------------|
| Everolimus | PMID: 23215727 |
| Everolimus | PMID: 23215728 |
| None       |                |
| Everolimus | PMID: 23215720 |
| None       |                |
| None       |                |
| Everolimus | PMID: 23215720 |
| Everolimus | PMID: 23215721 |
| Everolimus | PMID: 23215722 |
| Everolimus | PMID: 23215723 |
| Everolimus | PMID: 23215724 |
| Everolimus | PMID: 23215725 |
| Everolimus | PMID: 23215726 |
| Everolimus | PMID: 23215727 |
| Everolimus | PMID: 23215728 |
| None       |                |
| None       |                |
| None       |                |
| None       |                |
| None       |                |
| None       |                |
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| None       |                |
| None       |                |
| None       |                |
| None       |                |
| None       |                |
| Everolimus | PMID: 23215720 |
| Everolimus | PMID: 23215721 |
| Everolimus | PMID: 23215722 |
| Everolimus | PMID: 23215723 |
| Everolimus | PMID: 23215724 |
| Everolimus | PMID: 23215725 |
| Everolimus | PMID: 23215726 |
| Everolimus | PMID: 23215727 |
| Everolimus | PMID: 23215728 |
bility at the time of testing.
| FDA Approved Therapies for Other Tumor Type | Clinical Trial Available |
|--------------------------------------------|--------------------------|
| None                                       | None                     |
| Everolimus, Temsirolimus                   | Yes                      |
| Everolimus, Temsirolimus                   | Yes                      |
| Celecoxib, Aspirin                         | Yes                      |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | Yes                      |
| Olaparib                                   | yes                      |
| Olaparib, Rucaparib                        | Yes                      |
| None                                       | Yes                      |
| Olaparib                                   | yes                      |
| None                                       | None                     |
| None                                       | None                     |
| Olaparib, Niraparib, Rucaparib             | Yes                      |
| None                                       | Yes                      |
| None                                       | Yes                      |
| Olaparib, Niraparib, Rucaparib             | Yes                      |
| None                                       | None                     |
| Palbociclib                                | Yes                      |
| None                                       | None                     |
| Pembrolizumab, Atezolizumab, Nivolumab     | Yes                      |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| Palbociclib                                | Yes                      |
| None                                       | None                     |
| Pembrolizumab, Atezolizumab, Nivolumab     | Yes                      |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| Imatinib, Nilotinib, Dasatinib             | Yes                      |
| Azacitidine, Decitabine                    | Yes                      |
| Afatinib, Gefitinib, Cetuximab, Erlotinib, Panitumumab, Necitumumab | Yes |
| None                                       | None                     |
| None                                       | None                     |
| None                                       | None                     |
| Drug Combination                                                                 | Compatibility |
|---------------------------------------------------------------------------------|---------------|
| Trastuzumab, Afatinib                                                           | Yes           |
| Trastuzumab, Lapatinib, Afatinib                                                | Yes           |
| Ado-trastuzumab emtansine, Lapatinib, Pertuzumab, Trastuzumab, Afatinib          | Yes           |
| Lapatinib, Afatinib, Gefitinib                                                  | Yes           |
| Gefitinib, Afatinib, Erlotinib                                                  | Yes           |
| None                                                                            | None          |
| None                                                                            | None          |
| None                                                                            | None          |
| Olaparib                                                                        | Yes           |
| None                                                                            | None          |
| Everolimus, Temsirolimus                                                        | Yes           |
| Pazopanib, Ponatinib, Regorafenib, Lenvatinib, Nintedanib                       | Yes           |
| Pazopanib, Ponatinib, Regorafenib, Lenvatinib, Nintedanib                       | Yes           |
| Lenvatinib, Ponatinib, Pazopanib, Nintedanib, Regorafenib                       | Yes           |
| Lenvatinib, Regorafenib, Ponatinib, Pazopanib, Nintedanib                       | Yes           |
| None                                                                            | None          |
| Lenvatinib, Regorafenib, Pazopanib, Ponatinib, Nintedanib                       | Yes           |
| None                                                                            | None          |
| Lenvatinib, Ponatinib                                                           | Yes           |
| None                                                                            | None          |
| Everolimus, Temsirolimus                                                        | Yes           |
| None                                                                            | None          |
| None                                                                            | None          |
| None                                                                            | None          |
| Ruxolitinib                                                                     | Yes           |
| Ruxolitinib, Tofacitinib                                                        | Yes           |
| Trametinib, Cobimetinib                                                         | Yes           |
| Trametinib, Cobimetinib                                                         | Yes           |
| Trametinib, Cobimetinib                                                         | Yes           |
| None                                                                            | None          |
| None                                                                            | None          |
| None                                                                            | Yes           |
| None                                                                            | None          |
| None                                                                            | None          |
| Atezolizumab, Pembrolizumab, Nivolumab                                          | Yes           |
| None                                                                            | None          |
| None                                                                            | None          |
| Everolimus, Temsirolimus, Trametinib, Cobimetinib                               | Yes           |
| Everolimus, Temsirolimus, Trametinib, Cobimetinib                               | Yes           |
| Everolimus, Temsirolimus, Trametinib, Cobimetinib                               | Yes           |
| Everolimus, Temsirolimus, Trametinib, Cobimetinib                               | Yes           |
| Everolimus, Temsirolimus, Trametinib, Cobimetinib                               | Yes           |
| Everolimus, Temsirolimus, Trametinib, Cobimetinib                               | Yes           |
| None                                                                            | None          |
| None                                                                            | None          |
| None                                                                            | None          |
| Olaparib                                                                        | Yes           |
| None                                                                            | None          |
| Everolimus, Temsirolimus                                                        | Yes           |
| Everolimus, Temsirolimus                                                        | Yes           |
| Everolimus, Temsirolimus                                                        | Yes           |
| Everolimus, Temsirolimus                                                        | Yes           |
| Everolimus, Temsirolimus                                                        | Yes           |
| Everolimus, Temsirolimus                                                        | Yes           |
| Drug Molecule                                      | Yes/No |
|---------------------------------------------------|--------|
| Everolimus, Temsirolimus                          | Yes    |
| Everolimus, Temsirolimus                          | Yes    |
| Everolimus, Temsirolimus                          | Yes    |
| None                                              | None   |
| Everolimus, Temsirolimus                          | Yes    |
| Temsirolimus, Everolimus                          | Yes    |
| Everolimus, Temsirolimus                          | Yes    |
| Everolimus, Temsirolimus                          | Yes    |
| Everolimus, Temsirolimus                          | Yes    |
| Everolimus, Temsirolimus                          | Yes    |
| Everolimus, Temsirolimus                          | Yes    |
| Everolimus, Temsirolimus                          | Yes    |
| Everolimus, Temsirolimus                          | Yes    |
| Everolimus, Temsirolimus                          | Yes    |
| Everolimus, Temsirolimus                          | Yes    |
| Olaparib, Niraparib, Rucaparib                    | Yes    |
| Sorafenib, Cobimetinib, Trametinib, Regorafenib   | Yes    |
| Atra, Ato                                         | No     |
| None                                              | None   |
| Everolimus, Temsirolimus                          | Yes    |
| None                                              | None   |
| None                                              | None   |
| None                                              | None   |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| None                                              | Yes    |
| Ramucirumab, Sorafenib, Sunitinib, Bevacizumab, Axitinib, Lenvatinib, Pazopanib, Regorafenib | Yes |
| Everolimus, Bevacizumab, Sunitinib, Temsirolimus, Vandetanib, Pazopanib, Sorafenib, Axitinib | Yes |
| DE-IDENTIFIED ID | Origin of Sample |
|------------------|------------------|
| 381373           | Primary          |
| 416170           | Primary          |
| 375882           | Primary          |
| 381163           | Primary          |
| 384827           | Primary          |
| 408655           | Metastasis       |
| 411460           | Metastasis       |
| 416144           | Primary          |
| 487820           | Primary          |
| 385187           | Primary          |
| 413764           | Primary          |
| 419398           | Primary          |
| 374383           | Primary          |
| 376490           | Metastasis       |
| 381390           | Metastasis       |
| 400589           | Primary          |
| 404122           | Metastasis       |
| 418122           | Primary          |
| 435327           | Primary          |
| 464520           | Primary          |
| 370700           | Metastasis       |
| 373384           | Metastasis       |
| 432004           | Primary          |
| 441866           | Primary          |
| 482444           | Primary          |
| 489490           | Primary          |
| 419641           | Primary          |
| 391993           | Primary          |
| 508422           | Primary          |
| 429264           | Primary          |
| 429968           | Primary          |
| 430972           | Primary          |
| 469972           | Primary          |
|    |     |
|----|-----|
| 480099 | Primary |
| 487331 | Primary |
| 398306 | Primary |
| 411436 | Primary |
| 486602 | Primary |
| 425270 | Primary |
| 410276 | Metastasis |
| 441238 | Primary |
| 466056 | Primary |
| 391849 | Primary |
### Patient Clinical History and Pathologic Diagnosis

| Breast - type | Histology |
|---------------|-----------|
| ILC - grade 2-3 | ILC       |
| IDC - grade unknown | IDC       |
| ILC - grade 2 | ILC       |
| IDC - grade 2 | IDC       |
| DCIS - grade 3 | DCIS ONLY |
| Carcinoma, unspecified; bx of mets | Unknown Histology |
| IDC - grade unknown | IDC       |
| IDC - grade 3; DCIS - grade 2 | Mixed Histology |
| IDC, Neuroendocrine features - grade unknown | IDC       |
| IDC - grade 3 | IDC       |
| IDC - grade 2 | IDC       |
| IDC - grade 3 | IDC       |
| IDC - grade 3 | IDC       |
| IDC - grade 2 | IDC       |
| IDC - grade 3 | IDC       |
| IDC - grade 3; DCIS - grade 3 | Mixed Histology |
| IDC - grade 3 | IDC       |
| IDC - grade 2 | IDC       |
| IDC - grade 3 | IDC       |
| DCIS - grade 2, invasive, ductal and lobular features grade 2 out 3 | Mixed Histology |
| IDC - grade unknown | IDC       |
| ILC - grade unknown | ILC       |
| ILC - grade 2 | ILC       |
| IDC - grade 2 | IDC       |
| IDC - grade 2 | IDC       |
| ILC - grade unknown | ILC       |
| IDC - grade 1; DCIS - grade unknown | Mixed Histology |
| ILC - grade 2 | ILC       |
| ILC - grade 2 | ILC       |
| IDC - grade 2 | IDC       |
| IDC - grade unknown | IDC       |
| Description                          | Case Type       |
|-------------------------------------|-----------------|
| IDC - grade 2; DCIS - grade 2       | Mixed Histology |
| IDC - grade 3                       | IDC             |
| DCIS - grade 3                      | DCIS ONLY       |
| IDC - grade 2                       | IDC             |
| IDC - grade 3                       | IDC             |
| IDC - grade 2; IDC - grade 2        | IDC             |
| IDC - grade 3; DCIS - grade 3       | Mixed Histology |
| IDC - grade 3                       | IDC             |
| IDC - grade 3                       | IDC             |
| IDC - grade unknown                 | IDC             |
| Age at DX Group (%) | Breast Receptor Status | Somatic Alteration | Gene 1 | Alteration 1 | Gene 2 |
|---------------------|------------------------|-------------------|--------|-------------|--------|
| 60-69               | pos/pos/neg            | 0                 |        |             |        |
| 50-59               | pos/pos/pos            | 0                 |        |             |        |
| 50-59               | pos/pos/neg            | 2                 | CDH1   | p.Y827*     | CDH1   |
| 40-49               | pos/pos/neg            | 2                 | FGF3   | amplification | FGF4 |
| 40-49               | neg/neg/neg            | 2                 | TP53   | p.E285K     | TP53   |
| 70-79               | pos/neg/neg            | 2                 | ESR1   | p.D538G     | PIK3CA |
| 60-69               | pos/neg/neg            | 2                 | FGFR1  | amplification | PIK3CA |
| 80 and over         | pos/pos/neg            | 2                 | PIK3CA | p.E542K     | DNMT3A |
| 50-59               | pos/pos/neg            | 2                 | TP53   | hemizygous loss | NF2 |
| 60-69               | neg/neg/neg            | 3                 | ERBB2  | p.D769Y     | NTRK1  |
| 50-59               | pos/neg/neg            | 3                 | PIK3R1 | splice site 1746-2A>G | PTEN |
| 80 and over         | pos/neg/neg            | 3                 | BRCA1  | hemizygous loss | PTEN |
| 60-69               | pos/pos/neg            | 4                 | AR     | p.R13W      | CDH1   |
| 60-69               | pos/pos/neg            | 4                 | ATM    | p.R2746if5*12 | ATM |
| 70-79               | pos/pos/neg            | 4                 | AR     | p.Y858C     | PIK3CG |
| 60-69               | pos/pos/neg            | 4                 | PIK3CA | p.H1047R    | CDH1   |
| 60-69               | neg/neg/neg            | 4                 | BARD1  | p.A724V     | KRAS   |
| 50-59               | neg/neg/neg            | 4                 | ERBB2  | amplification | RARA |
| 60-69               | neg/neg/neg            | 4                 | PIK3R1 | p.R461_E462delinsQ | FGF3 |
| 60-69               | pos/pos/neg            | 4                 | PIK3CA | p.H1047R    | FANCA  |
| 30-39               | pos/pos/neg            | 6                 | AKT1   | p.E17K      | FRS2   |
| 70-79               | pos/pos/neg            | 6                 | PIK3CA | p.E545K     | AKT3   |
| 40-49               | pos/neg/neg            | 6                 | FGFR1  | BAG4-FGFR1 fusion | FGFR1 |
| 40-49               | pos/pos/neg            | 6                 | PIK3CA | p.E542K     | PIK3CA |
| 60-69               | pos/pos/neg            | 6                 | AKT1   | p.E17K      | VHL    |
| 80 and over         | neg/neg/neg            | 6                 | ERBB3  | p.E928G     | ERBB2  |
| 40-49               | neg/neg/neg            | 7                 | BRCA1  | hemizygous loss | CDKN2A |
| 80 and over         | pos/pos/neg            | 8                 | BRCA2  | hemizygous loss | CDH1   |
| 40-49               | pos/pos/pos            | 8                 | BRCA1  | hemizygous loss | BRCA2 |
| 80 and over         | pos/neg/neg            | 9                 | BRCA1  | hemizygous loss | CDH1   |
| 50-59               | pos/pos/neg            | 9                 | AKT3   | amplification | CDH1   |
| 70-79               | pos/pos/neg            | 9                 | ATM    | hemizygous loss | BRCA1 |
| 50-59               | pos/pos/neg            | 9                 | PIK3CA | p.E545K     | AKT3   |
| Age Range | Status/Markers | Case | Genes/Alterations |
|-----------|---------------|------|------------------|
| 50-59     | pos/pos/pos   | 9    | CDKN2A loss      |
| 70-79     | pos/neg/pos   | 9    | ERBB2 amplification |
| 80 and over | neg/pos/pos  | 10   | BRCA1 loss       |
| 50-59     | pos/neg/NOS   | 10   | CDKN2A hemizygous loss |
| 80 and over | pos/pos/neg | 13   | CDK4 amplification |
| 70-79     | pos/neg/neg; pos/pos/neg | 14 | CCND1 amplification |
| 60-69     | neg/neg/pos   | 14   | ATM LOH          |
| 60-69     | pos/pos/neg   | 16   | BRCA1 copy neutral LOH |
| 50-59     | pos/neg/neg   | 16   | PTEN p.G129R     |
| 40-49     | pos/pos/pos   | 17   | ERBB2 amplification |

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| Alteration 2 | Gene 3 | Alteration 3 | Gene 4 | Alteration 4 |
|-------------|--------|-------------|--------|-------------|
| LOH         |        | LOH         |        |             |
| amplification |      | LOH         |        |             |
| p.E545D     |        | p.N345K     |        |             |
| hemizygous loss |    | hemizygous loss |        |             |
| p.R568S     | TET2   | p.Q740E     |        |             |
| p.S113Rfs*21| PTEN   | LOH         |        |             |
| p.D92Tfs*7  | TP53   | p.R175H     |        |             |
| p.N297S     | PIK3CA | p.H1047L    | TP53   | p.Y220C     |
| LOH         | PIK3CA | p.E542K     | RUNX1  | p.S167R     |
| p.A186V     | TP53   | p.G245C     | TP53   | LOH         |
| hemizygous_deletion | RB1 | hemizygous_deletion | FANCD2 | p.N405S |
| amplification | TP53 | p.K139_P142del | TP53 | copy neutral LOH |
| amplification | TOP2A | amplification | TP53 | hemizygous loss |
| amplification | FGF4 | amplification | ERBB2 | p.S310F |
| hemizygous loss | CDH1 | hemizygous loss | NOTCH2 | p.V1666F |
| amplification | MAP2K1 | p.K57E | MDM2 | amplification |
| amplification | FANCA | hemizygous loss | FLCN | hemizygous loss |
| amplification | NF1 | p.W837* | NF1 | LOH |
| hemizygous loss | BRCA1 | hemizygous loss | BRCA2 | hemizygous loss |
| p.S310F | PTEN | p.H93R | PTEN | LOH |
| loss | CDKN2B | loss | ERBB2 | amplification |
| intron 14 2296-3A>G | CDH1 | LOH | CHEK2 | hemizygous loss |
| hemizygous loss | RAD51C | hemizygous loss | TP53 | p.Y220C |
| p.H1047R | CDH1 | LOH | FANCA | hemizygous loss |
| p.K187Qfs*12 | CDH1 | LOH | CDKN2A | hemizygous loss |
| hemizygous loss | CCND1 | amplification | CDKN2A | hemizygous loss |
| amplification | DDR2 | amplification | BRCA1 | hemizygous loss |
| Loss Type          | Gene   | Mutation       | Gene   | Status               |
|--------------------|--------|----------------|--------|----------------------|
| Loss               | PTEN   | p.R130Q        | PTEN   | Copy neutral LOH     |
| Amplification      | PIK3CA | p.E542K        | PTEN   | Hemizygous loss      |
| Loss               | CDKN2B | Loss           | EGFR   | Amplification        |
| Hemizygous Loss    | PTEN   | Hemizygous loss| NF2    | Hemizygous loss      |
| Hemizygous Loss    | CDKN2B | Hemizygous loss| PTEN   | Hemizygous loss      |
| p.R88Q             | PTEN   | p.D92H         | PTEN   | LOH                  |
| Hemizygous Loss    | BRCA2  | Hemizygous loss| CCND1  | Amplification        |
| Hemizygous Loss    | CCNE1  | Amplification  | EPHA5  | Splice site 2236+1G>T|
| p.F347Sfs*5        | FGFR2  | Amplification  | FBXW7  | Hemizygous loss      |
| Amplification      | FGFR4  | Amplification  | JAK2   | Amplification        |
| Gene 5 | Alteration 5 | Gene 6 | Alteration 6 | Gene 7 | Alteration 7 |
|--------|--------------|--------|--------------|--------|--------------|
| MYC    | amplification| RUNX1  | p.G408Vfs*193|        |              |
| CDH1   | p.N405Qfs*14 | CDH1   | LOH          |        |              |
| BRCA2  | hemizygous loss| FLCN  | hemizygous loss|        |              |
| TP53   | p.R248Q      | TP53   | LOH          |        |              |
| CDH1   | p.T211Rfs*11 | CDH1   | LOH          |        |              |
| BRCA2  | hemizygous loss| TP53  | hemizygous loss|        |              |
| PIK3CA | amplification| PTEN   | loss         | TP53   | p.R280T      |
| FANCA  | hemizygous loss| FANCD2| p.N405S      | NF2    | hemizygous loss|
| TP53   | LOH          | FGFR1  | p.K164I      | ABL1   | p.K628del    |
| FLCN   | hemizygous loss| NF1   | hemizygous loss| PIK3CA| p.H1047R     |
| CDKN2B | hemizygous loss| FANCA | hemizygous loss| NF2   | hemizygous loss|
| FANCA  | hemizygous loss| NF1   | hemizygous loss| NF2   | hemizygous loss|
| PARP1  | amplification| MDM4   | amplification| FH    | amplification|
| Gene   | Alteration       | Other Gene | Alteration       | Other Gene | Alteration       |
|--------|------------------|------------|------------------|------------|------------------|
| FBXW7  | hemizygous loss  | NF1        | loss             | BRCA1      | hemizygous loss  |
| FGF4   | amplification    | FLCN       | hemizygous loss  | TP53       | p.R249G          |
| EPHA3  | splice site 2497-2A>T | ERBB2 | amplification    | ERBB4      | amplification    |
| BRCA2  | hemizygous loss  | ATM        | LOH              | FANCA      | hemizygous loss  |
| FRS2   | amplification    | NF1        | hemizygous loss  | BRCA1      | hemizygous loss  |
| FGF3   | amplification    | FGF4       | amplification    | BRCA2      | hemizygous loss  |
| CDK6   | amplification    | CHEK2      | p.K373E          | CHEK2      | LOH              |
| FLCN   | hemizygous loss  | JAK3       | amplification    | MLH1       | p.F3L            |
| NF1    | p.Y489C          | NF1        | LOH              | BRCA1      | hemizygous loss  |
| STAT3  | amplification    | SMARCB1    | p.T357*          | SMARCB1    | Copy neutral LOH |
| Gene 8 | Alteration 8 | Gene 9 | Alteration 9 | Gene 10 | Alteration 10 | Gene 11 |
|----------------|--------------|--------|--------------|---------|---------------|--------|
| PIK3 | p.H1047R |        |              |        |               |        |
| BRIP1 | hemizygous loss |        |              |        |               |        |
| RAD51C | hemizygous loss | TP53 | hemizygous loss |        |               |        |
| PIK3CA | p.E542K | TP53 | hemizygous loss |        |               |        |
| PAK1 | amplification | TP53 | hemizygous loss |        |               |        |
| MCL1 | amplification | BRIP1 | hemizygous loss |        |               |        |
| Gene   | Status     | Gene   | Status     | Gene   | Status     |
|--------|------------|--------|------------|--------|------------|
| BRCA2  | hemizygous loss | APC    | hemizygous loss | RARA   | amplification |
| TP53   | LOH        | MAP2K1 | amplification | TOP2A  | amplification |
| FLCN   | hemizygous loss | TP53   | p.T155_R156del | TP53   | LOH         |
| ATM    | hemizygous loss | APC    | hemizygous loss | FLCN   | hemizygous loss |
| ATM    | hemizygous loss | FANCA  | hemizygous loss | FLCN   | hemizygous loss |
| ERBB2  | amplification | FGF3   | amplification | FGF4   | amplification |
| PIK3R2 | amplification | STK11  | hemizygous loss | TP53   | p.I251Sfs*94 |
| BRCA2  | hemizygous loss | ATM    | hemizygous loss | FANCA  | hemizygous loss |
| FRS2   | amplification | BRCA1  | hemizygous loss | ATM    | hemizygous loss |

**Note:** The table lists various genetic alterations associated with specific genes. For example, BRCA2 has a hemizygous loss, TP53 has a LOH, and RARA has an amplification.
| Alteration 11 | Gene 12 | Alteration 12 | Gene 13 | Alteration 13 | Gene 14 |
|--------------|---------|--------------|---------|--------------|---------|

...
| Event                  | Gene 1 | Mutation 1 | Gene 2 | Mutation 2 | Gene 3 |
|------------------------|--------|------------|--------|------------|--------|
| hemizygous loss        | TP53   | hemizygous loss | CDH1   | hemizygous loss |        |
| amplification          | FGFR4  | p.V550L    | ERBB4  | p.H582Y    | ERBB4  |
| p.Y101*                | RAF1   | amplification | TP53   | p.H193Lfs*54 | TP53   |
| copy neutral LOH       | TSC2   | p.I61_G62insQ | BRCA1  | hemizygous loss | FANCA |
| hemizygous loss        | MLH1   | hemizygous loss | ARID1A | hemizygous loss | FLCN  |
| amplification          | MDM2   | amplification | TP53   | hemizygous loss | MYC   |
| Alteration 14 | Gene 15 | Alteration 15 | Gene 16 | Alteration 16 | Gene 17 |
|--------------|---------|---------------|---------|---------------|---------|
| p.R81* | LOH |
|--------|-----|
| hemizygous loss | **FLCN** | hemizygous loss | **RPTOR** | amplification |
| hemizygous loss | **TP53** | p.R213* | **TP53** | LOH |
| amplification | **RARA** | amplification | **SMO** | amplification | **TOP2A** |
### Germline Alterations

| Alteration 17 | Germline Alteration Count | Gene | Alteration | Germnline Classification |
|---------------|---------------------------|------|------------|--------------------------|
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 1                         | CHEK2 | p.T476M   | VLP                      |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|               | 0                         |      |            |                          |
|   |   |   |   |
|---|---|---|---|
|   |   | ATM p.2466+1G>C VLP |   |
| 1 |   | CHEK2 p.T367Mfs*15 Mutation |   |
| 1 |   | MUTYH p.G396D Mutation |   |
|   | 1 | BRCA1 p.N1018Mfs*8 Mutation |   |
|   | amplification | 0 |   |