CellMax LBx
Liquid Biopsy

Test Report

CellMax Life

Test Report

CellMax LBx
Liquid Biopsy

Order Information

Requisition Number	SG001292
Patient Name	[redacted]
ID	[redacted]
Date of Birth	1949/07/11
Gender	M ☐ F ☑
Patient Phone Number	
Patient E-mail	
Name of Hospital / Clinic	
Hospital / Clinic Phone Number	
Name of Physician	[redacted]
Specimen Site	Peripheral blood
Disease	Breast carcinoma (ER positive)
SNOMED Concept ID	417181009
Date of Collection	2018/08/13
Date of Report	2018/09/14

Test Report

CellMax LBx Liquid Biopsy

Patient Test Results

Clinically Relevant Genomic Alterations Detected

TIER 2: VARIANTS OF POTENTIAL CLINICAL SIGNIFICANCE

Predictive Variants

Marker	Alteration	Therapies approved in this indication	Therapies approved in other indications	May indicate resistance to therapies	Trials
ERBB2	L755S	None	Neratinib (C), Afatinib (C)	Lapatinib (C)	Yes

MSI Result

MSI-H	Not detected

Comments

ERBB2 L755S has been identified as a secondary resistance mutation which predicts resistance to the FDA-approved therapy lapatinib in preclinical studies, but confers sensitivity to irreversible dual EGFR/Her2 inhibitors, such as neratinib or afatinib (Kancha et al., 2011; 22046346, Bose et al., 2013; 23220880, Kloth et al., 2016; 26001389, Ben-Baruch et al., 2015; 26358790).

Electronic Signatures

Laboratory Manager

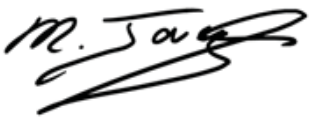
Leon Chen

Date

2018/09/14

Consulting Pathologist

Manana Kvezereli-Javey, MD, PhD



Date

2018/09/14

LBx-Liquid Biopsy Test Report

Report outline:

CellMaxLife

Patient ID: SG001292
Report Date: Sep 13, 2018
Gender: female

Report ID: SG001292
Disease: Breast carcinoma (ER positive)

1. Summary

CLINICALLY RELEVANT ALTERATIONS

TIER 1: VARIANTS OF STRONG CLINICAL SIGNIFICANCE: NONE

TIER 2: VARIANTS OF POTENTIAL CLINICAL SIGNIFICANCE

Predictive Variants

Marker	Alteration	Therapies approved in this indication	Therapies approved in other indications	May indicate resistance to therapies	Trials
ERBB2	L755S	None	Neratinib (C), Afatinib (C)	Lapatinib (C)	Yes

Prognostic and Diagnostic Variants: None

GUIDELINES

Marker-Alteration	Summary
BRCA1	Although NCCN guidelines are defined for BRCA1 in Breast carcinoma (ER positive), the BRCA1 variant in this case is a Variant of Uncertain Clinical Significance, and the relevance of the NCCN guidelines is therefore unknown.

INTERACTIONS: NONE

OTHER ALTERATIONS

TIER 3: VARIANTS OF UNCERTAIN CLINICAL SIGNIFICANCE

Marker	Alteration
EZH2	p.Tyr741Cys (Y741C)
SMAD4	p.Leu529TyrfsTer8 (L529fs*8)

The functional or therapeutic consequences of Variants of Uncertain Clinical Significance are unknown.

TIER 4: BENIGN OR LIKELY BENIGN VARIANTS

Marker	Alteration
BRCA1	p.Asp1739= (D1739D)

The functional or therapeutic consequences of Benign or Likely Benign Variants are unknown.

LABORATORY TECHNICAL DATA

Marker	Alteration	Map Location	Variant Allele Frequency	Coding Sequence Change	Transcript ID
ERBB2	L755S	chr17:37880220	17.96%	c.2264T>C	NM_004448
EZH2	Y741C	chr7:148504772	0.44%	c.2222A>G	NM_004456
SMAD4	L529fs*8	chr18:48604762	9.28%	c.1586delT	NM_005359
BRCA1	D1739D	chr17:41209129	0.449%	c.5217T>C	NM_007294

The data in this table was generated by the laboratory in the course of molecular testing. It has not been altered in any way by CellMax.

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2. Detailed Biomarker Information

2.1. ERBB2-L755S (p.Leu755Ser)

TIER 2: Variant of Potential Clinical Significance

2.1.1 BIOMARKER RESULTS SUMMARY

Marker	Result	Summary
ERBB2	- MUTN (seq): p.Leu755Ser (L755S)	ERBB2-L755S is an activating mutation.
	Clinical relevance	ERBB2 (also known as HER2/neu) encodes the receptor tyrosine kinase Her2, in the same family as Egfr (Higgins and Baselga, 2011; 21965336). Activation of Her2 as a result of mutation or amplification of ERBB2 can lead to excessive proliferation and tumor formation (Higgins and Baselga, 2011; 21965336). ERBB2 gene amplification or mutation, or Her2 overexpression may predict sensitivity to Her2 inhibitors (Minami et al., 2007; 17311002, Tomizawa et al., 2011; 21353324). However, ERBB2 L755S has been identified as a secondary resistance mutation which predicts resistance to lapatinib in preclinical studies, but confers sensitivity to irreversible dual Egfr/Her2 inhibitors, such as neratinib or afatinib (Kancha et al., 2011; 22046346, Bose et al., 2013; 23220880, Kloth et al., 2016; 26001389, Ben-Baruch et al., 2015; 26358790). In addition, separate studies have reported that a colorectal cancer cell line harboring ERBB2 L755S and a colorectal cancer patient with this alteration, along with BRAF N581S and APC Q1429fs mutations, did not respond to trastuzumab (Aung et al., 2016; 27626067, Kloth et al., 2016; 26001389).

2.1.2 BIOLOGICAL RELEVANCE of ERBB2-L755S (p.Leu755Ser)

ERBB2 alterations in Breast carcinoma (ER positive)	
Molecular function	ERBB2 L755S is a missense mutation located within the kinase domain of the Her2 protein; this mutation has been shown to lead to activation of Her2 and have transforming activity (Lee et al., 2006; 16397024, Kancha et al., 2011; 22046346). ERBB2 L755S has been identified as a secondary resistance mutation which predicts resistance to lapatinib in preclinical studies but may predict sensitivity to irreversible dual Egfr/Her2 inhibitors, such as neratinib or afatinib (Kancha et al., 2011; 22046346, Bose et al., 2013; 23220880, Kloth et al., 2016; 26001389, Xu et al., 2017; 28487443). Indeed, one study reported a partial response to neratinib lasting 11 months in a Her2-positive breast cancer patient harboring the ERBB2 L755S mutation (Ben-Baruch et al., 2015; 26358790). A colorectal cancer patient harboring ERBB2 L755S, in addition to BRAF N581S and APC Q1429fs mutations, has been reported to not respond to trastuzumab combined with 5-fluorouracil and leucovorin; in addition, a colorectal cancer cell line harboring ERBB2 L755S was not sensitive to trastuzumab in a preclinical study (Aung et al., 2016; 27626067, Kloth et al., 2016; 26001389).
Incidence in disease	ERBB2 mutations have been reported in 3.3% (26/779) of Breast carcinoma (ER positive) samples analyzed in COSMIC (Jun 2018). ERBB2 mutations have been reported in 2.2% (28/1248) and 2.5% (138/5605) of breast cancer samples (Zuo et al., 2016; 27697991, Ross et al., 2016; 27284958).

2.1.3 CLINICAL RELEVANCE of ERBB2-L755S (p.Leu755Ser)

ERBB2 alterations in Breast carcinoma (ER positive)	
Role in disease	Activation of ERBB2 by amplification or mutation has been reported to play a role in several types of cancer (Herter-Sprie et al., 2013; 23630663). Her2 expression has been associated with increased tumor aggressiveness and risk of recurrence in breast cancer (Kim et al., 2014; 24783266, Pradeep et al., 2012; 22139081, Slamon et al., 1987; 3798106). Her2 positivity has been significantly associated with ER/PR-negative status, invasive ductal subtype, younger age, higher histologic grade, as well as increased tumor size and nodal status in large-scale breast carcinoma studies (Dodson et al., 2018; 30066480, Rüschoff et al., 2017; 27767099).

ERBB2 alterations in Breast carcinoma (ER positive)	
Effect on drug sensitivity	Activating ERBB2 alterations may predict sensitivity to Her-targeted drug therapies. A number of therapies, including antibodies, small molecule inhibitors, and tyrosine kinase inhibitors, have been FDA-approved in various indications; these and other agents are under investigation (Bang et al., 2010; 20728210, Jones and Buzdar, 2009; 19959074, Baselga et al., 2012; 22149875, Verma et al., 2012; 23020162, Gelmon et al., 2015; 25779558, Sequist et al., 2013; 23816960, Soria et al., 2015; 26156651). However, ERBB2 L755S has been associated with resistance to some Her-targeted therapies (Kancha et al., 2011; 22046346, Bose et al., 2013; 23220880, Kloth et al., 2016; 26001389, Ben-Baruch et al., 2015; 26358790, Aung et al., 2016; 27626067). Her2-directed chimeric antigen receptor (CAR) T-cell therapies are additionally being investigated in glioblastoma and other diseases expressing ERBB2/Her2 (Liu et al., 2017; 28977984, Ahmed et al., 2017; 28426845).
Effect on drug resistance	ERBB2 L755S has been identified as a secondary resistance mutation which predicts resistance to the FDA-approved therapy lapatinib in preclinical studies, but confers sensitivity to irreversible dual Egfr/Her2 inhibitors, such as neratinib or afatinib (Kancha et al., 2011; 22046346, Bose et al., 2013; 23220880, Kloth et al., 2016; 26001389, Ben-Baruch et al., 2015; 26358790). In addition, a colorectal cancer cell line harboring ERBB2 L755S, or colorectal cancer patient with this alteration and BRAF N581S and APC Q1429fs mutations, did not respond to trastuzumab (Aung et al., 2016; 27626067, Kloth et al., 2016; 26001389). Her2 overexpression in ER-positive breast cancer has been associated with resistance to endocrine therapy (Wright et al., 1992; 1346366, Houston et al., 1999; 10098763, Dowsett et al., 2008; 18227529, Arpino et al., 2008; 18216219). High ERBB2/CEP17 amplification ratio has been significantly associated with decreased disease-free survival in patients treated with standard trastuzumab-based chemotherapy in a study of 332 patients with Her2-positive breast cancer (Stocker et al., 2016; 27463363).

2.1.4 CLINICAL EVIDENCE in Breast carcinoma (ER positive)

ERBB2 alterations in Breast carcinoma (ER positive)	
FDA Approved	None.
Phase III Data	A Phase 3 randomized, double-blind, placebo-controlled trial of neratinib for 12 months after standard chemotherapy and trastuzumab in 2840 women with Her2-positive early breast cancer reported significantly fewer invasive disease-free survival events in the neratinib than in the placebo group, and a five-year invasive disease-free survival rate of 90.2% for neratinib and 87.7% for placebo, without significant differences in long-term toxicity in the two arms (Martin et al., 2017; 29146401).
Phase II Data	In a Phase 2 trial of neratinib monotherapy compared with lapatinib in combination with capecitabine in Her2-positive advanced breast cancer patients, neratinib showed single-agent activity, with an objective response rate (ORR) of 29% (34/117) and a clinical benefit rate (CBR) of 44% (52/117), but the neratinib arm had a lower ORR and CBR, and non-inferiority of neratinib compared with lapatinib in combination with capecitabine was not demonstrated (Martin et al., 2013; 23953056). In a Phase 2 study of neratinib plus vinorelbine in 79 patients with metastatic breast cancer with ERBB2 amplification and prior trastuzumab treatment, 41% (23/56) of evaluable patients with no prior lapatinib and 8% (1/12) of patients with prior lapatinib exhibited a partial response (Awada et al., 2013; 22967996). A Phase 2 study of 68 evaluable Her2-positive trastuzumab-pretreated metastatic breast cancer patients receiving neratinib in combination with capecitabine has reported complete and partial responses in 12% (8/68) and 51% (35/68) of patients, respectively. Approximately 60% of patients experienced a grade 3/4 adverse event, with the most common being diarrhea (26%) (Saura et al., 2014; 25287822). A Phase 2 clinical trial of afatinib in Her2-negative metastatic breast carcinoma reported no objective responses (Schuler et al., 2012; 22763464). A Phase 2 clinical trial of afatinib in Her2-positive metastatic breast carcinoma reported a 46% clinical benefit rate (Lin et al., 2012; 22418700). A Phase 2 study of afatinib plus letrozole in 28 ER-positive hormone-refractory metastatic breast cancer patients resistant to letrozole monotherapy reported that 14% (4/28) of patients remained progression-free at 16 weeks, including two patients known to be Her2-negative; a best response of stable disease was reported in 54% (15/29) of patients (Gunzer et al., 2016; 26835225). A Phase 2 trial of afatinib in Her2-positive inflammatory breast carcinoma reported clinical benefit in 35% (9/26) of cases. Combination treatment with afatinib and vinorelbine following disease progression resulted in clinical benefit in 2/10 of patients (Goh et al., 2016; 27923043).
Phase I Data	A Phase 1 clinical trial of the pan-ErbB inhibitor dacomitinib in advanced, solid tumor patients reported that 1/13 evaluable patients had a partial response (a lung adenocarcinoma patient) and 9/13 had stable disease for at least six weeks (Takahashi et al., 2012; 22249430).

ERBB2 alterations in Breast carcinoma (ER positive)	
Preclinical	The pan-ErbB inhibitor dacomitinib was reported to inhibit cell proliferation in breast cancer cell lines with ERBB2 amplification, including cell lines with resistance to trastuzumab or lapatinib (Kalous et al., 2012; 22761403). A preclinical study has reported that abemaciclib, a CDK4/6 inhibitor, can overcome lapatinib or trastuzumab resistance in Her2-positive breast cancer cell and mouse models (Goel et al., 2016; 26977878).

2.1.5 SAMPLE RELEVANT THERAPIES

Therapies targeting ERBB2

Drug	Trade Name	Level of Evidence	Target/Rationale	Highest Level of Clinical Development
Neratinib	Nerlynx	C	Egfr/Her2/ErbB4 inhibitor.	FDA Approved (Breast carcinoma) Phase 2 (Glioblastoma, Glioma, Lymphoma, Solid Tumor, Breast carcinoma (triple negative))
Afatinib	Gilotrif	C	Irreversible pan-ErbB kinase inhibitor.	Phase 3 (Breast carcinoma) FDA Approved (Lung squamous cell carcinoma, EGFR-mutant NSCLC)
Dacomitinib		D	Pan-ErbB family tyrosine kinase inhibitor.	Phase 1 (Solid Tumor) Phase 3 (Non-small cell lung carcinoma (NSCLC), Lung cancer)
Pyrotinib		None found	Egfr/Her2 kinase inhibitor.	Phase 3 (Breast carcinoma) Phase 2 (Non-small cell lung carcinoma (NSCLC))
Tesevatinib		None found	Egfr/Her2/VEGFR/EphB4 small molecule kinase inhibitor.	Phase 3 (Non-small cell lung carcinoma (NSCLC))
Poziotinib		None found	Egfr/Her2/ErbB4 kinase inhibitor.	Phase 2 (Breast carcinoma) Phase 2 (Gastric carcinoma, Non-small cell lung carcinoma (NSCLC))
Tucatinib		None found	Her2 kinase inhibitor.	Phase 2 (Breast carcinoma) Phase 2 (Colorectal carcinoma (CRC))
AP32788		None found	EGFR/ERBB2 mutant-specific inhibitor.	Phase 2 (Non-small cell lung carcinoma (NSCLC))
Varlitinib		None found	Egfr/Her2 inhibitor.	Phase 2 (Gastric carcinoma, Pancreatic carcinoma)
ARX788		None found	Anti-HER2 monoclonal antibody conjugated to monomethyl auristatin F.	Phase 1 (Breast carcinoma) Phase 1 (Solid Tumor)
PF-06804103		None found	Anti-Her2 monoclonal antibody conjugated to auristatin-0101.	Phase 1 (Breast carcinoma) Phase 1 (Gastric carcinoma, Non-small cell lung carcinoma (NSCLC))
ADXS31-164		None found	Her2 vaccine.	Phase 1 (Solid Tumor)
DS-8201a		None found	Anti-Her2 antibody conjugated with a TopoI inhibitor.	Phase 1 (Solid Tumor)
Margetuximab		None found	Anti-Her2 monoclonal antibody.	Phase 1 (Solid Tumor)
Pirotinib		None found	ErbB family inhibitor.	Phase 1 (Solid Tumor)

2.1.6 BIOMARKER-MATCHED CLINICAL TRIALS

Trials Prioritized By Clinical Specificity*

	Markers	Trial ID	Title	Phase	Targets	Locations/contact
1	ERBB2	NCT01670877	Neratinib +/- Fulvestrant in Metastatic HER2 Non-amplified But HER2 Mutant Breast Cancer	Phase 2	EGFR, ERBB2, ESR1	•Overall contact: Cynthia Ma, M.D., Ph.D., cynthiaxma@wustl.edu, 314-362-9383 •AL (1), CA (2), FL (1), IL (2), MA (1), MN (1), MO (2), NC (2), SD (1), TX (1)
2	ERBB2	NCT02673398	Neratinib in Treating Older Patients With Metastatic HER2-Positive Breast Cancer	Phase 2	EGFR, ERBB2	•CA (7)
3	ERBB2	NCT02500199	Phase I Study of Pyrotinib in Patients With HER2-positive Solid Tumors	Phase 1	EGFR, ERBB2	•Overall contact: Ewa Matczak, MD, ewa.matczak@hengruitherapeutics.com, (609)423-2155 x215 •FL (1), MA (2), MI (1), MO (1), NY (1), TN (1)
4	ERBB2	NCT02544997	A Phase II, Single-Arm Trial of Poziotinib as Salvage Treatment in Patients With Metastatic Breast Cancer Who Has HER2 or EGFR Mutation or Activated AR or EGFR Pathway	Phase 2	EGFR, ERBB2, ERBB4	•Overall contact: Yeon-hee Park, MD,Ph.D., yeonh.park@samsung.com, 2-3410-3459 x82 •Samsung Medical Center: Seoul, Korea, Republic of, Yeon Hee Park, MD, Ph.D
5	ERBB2	NCT03412383	Pyrotinib in Metastatic HER2 Non-amplified But HER2 Mutant Breast Cancer	Phase 2	EGFR, ERBB2	•Overall contact: Fei Ma, MD, mafei@126.com, +86-10-87787652 •Fei Ma: Beijing, China, Fei Ma, MD, mafei@126.com, (Beijing)

*Note: the trials listed above were matched to the biomarkers identified in this tumor; the list may not be comprehensive.

Trials Prioritized By Region*

	Markers	Trial ID	Title	Phase	Targets	Locations/contact
1	ERBB2	NCT01670877	Neratinib +/- Fulvestrant in Metastatic HER2 Non-amplified But HER2 Mutant Breast Cancer	Phase 2	EGFR, ERBB2, ESR1	•Overall contact: Cynthia Ma, M.D., Ph.D., cynthiaxma@wustl.edu, 314-362-9383 •AL (1), CA (2), FL (1), IL (2), MA (1), MN (1), MO (2), NC (2), SD (1), TX (1)
2	ERBB2	NCT02673398	Neratinib in Treating Older Patients With Metastatic HER2-Positive Breast Cancer	Phase 2	EGFR, ERBB2	•CA (7)
3	ERBB2	NCT02500199	Phase I Study of Pyrotinib in Patients With HER2-positive Solid Tumors	Phase 1	EGFR, ERBB2	•Overall contact: Ewa Matczak, MD, ewa.matczak@hengruitherapeutics.com, (609)423-2155 x215 •FL (1), MA (2), MI (1), MO (1), NY (1), TN (1)
4	ERBB2	NCT02544997	A Phase II, Single-Arm Trial of Poziotinib as Salvage Treatment in Patients With Metastatic Breast Cancer Who Has HER2 or EGFR Mutation or Activated AR or EGFR Pathway	Phase 2	EGFR, ERBB2, ERBB4	•Overall contact: Yeon-hee Park, MD,Ph.D., yeonh.park@samsung.com, 2-3410-3459 x82 •Samsung Medical Center: Seoul, Korea, Republic of, Yeon Hee Park, MD, Ph.D
5	ERBB2	NCT03412383	Pyrotinib in Metastatic HER2 Non-amplified But HER2 Mutant Breast Cancer	Phase 2	EGFR, ERBB2	•Overall contact: Fei Ma, MD, mafei@126.com, +86-10-87787652 •Fei Ma: Beijing, China, Fei Ma, MD, mafei@126.com, (Beijing)

*Note: the trials listed above were matched to the biomarkers identified in this tumor; the list may not be comprehensive.

2.2. EZH2-Y741C (p.Tyr741Cys)

TIER 3: Variant of Uncertain Clinical Significance

2.2.1 BIOMARKER RESULTS SUMMARY

Marker	Result	Summary
EZH2	- MUTN (seq): p.Tyr741Cys (Y741C)	The functional consequences of EZH2-Y741C are unknown.
	Clinical relevance	EZH2 encodes the Ezh2 protein. Activating mutations in EZH2 result in transcriptional repression of some target genes, including many tumor suppressors, and overexpression of EZH2 results in transcriptional activation of other target genes, including MYC and CCND1 (Tan et al., 2014; 24362326). However, the role of EZH2 in cancer is complex, as both activating and inactivating alterations are observed in cancer, suggesting a possible dual-role for the gene (Xu and Li, 2012; 22475286, Chase and Cross, 2011; 21367748). At present there are no approved targeted therapies for EZH2 mutations. However, small molecule inhibitors of Ezh2, such as tazemetostat, are being investigated (Copeland, 2013; 23958745, Knutson et al., 2014; 24563539). Other therapeutic approaches targeting EZH2 alteration are also under investigation, including DNA demethylation agents and histone deacetylation inhibitors (Xu and Li, 2012; 22475286, Chase and Cross, 2011; 21367748, Nielsen et al., 2012; 22575654). As the alteration reported here has not been functionally characterized, the relevance of any available therapeutic approaches is unknown.

2.2.2 BIOLOGICAL RELEVANCE of EZH2-Y741C (p.Tyr741Cys)

EZH2 alterations in Breast carcinoma (ER positive)	
Molecular function	EZH2 Y741C is a missense alteration that occurs outside of several characterized domains of the Ezh2 protein (UniProt) (Ryan et al., 2011; 22194861, Wigle et al., 2011; 21856302). This alteration has not been reported (COSMIC, Sep 2018) or functionally characterized (PubMed, Sep 2018), and its effect on protein function is unknown.
Incidence in disease	EZH2 mutations have been reported in 1.6% (6/376) of Breast carcinoma (ER positive) samples analyzed in COSMIC (Jun 2018).

2.3. SMAD4-L529fs*8 (p.Leu529TyrfsTer8)

TIER 3: Variant of Uncertain Clinical Significance

2.3.1 BIOMARKER RESULTS SUMMARY

Marker	Result	Summary
SMAD4	- MUTN (seq): p.Leu529TyrfsTer8 (L529fs*8)	The functional consequences of SMAD4-L529fs*8 are unknown.
	Clinical relevance	SMAD4 (DPC4) is a tumor suppressor that encodes the Smad4 protein, a transcription factor downstream of TGF-beta (Schutte, 1999; 10436786). Loss or mutation of tumor suppressors may result in excessive cell proliferation. At present, there are no therapies available to address SMAD4 loss or mutation in cancer. In addition, as the alteration reported here has not been functionally characterized, the relevance of any available therapeutic approaches is unknown.

2.3.2 BIOLOGICAL RELEVANCE of SMAD4-L529fs*8 (p.Leu529TyrfsTer8)

SMAD4 alterations in Breast carcinoma (ER positive)	
Molecular function	SMAD4 L529fs*8 is expected to effectively truncate the Smad4 protein at amino acid 529 of 552, resulting in the loss of a small portion of the MH2 domain (UniProt). This alteration has been reported (COSMIC, Sep 2018), but it has not been functionally characterized (PubMed, Sep 2018), and its effect on protein function is unknown.
Incidence in disease	SMAD4 mutations have been reported in 2.6% (14/535) of Breast carcinoma (ER positive) samples analyzed in COSMIC (Jun 2018).

2.4. BRCA1-D1739D (p.Asp1739=)

TIER 4: Benign or Likely Benign Variant

2.4.1 BIOMARKER RESULTS SUMMARY

Marker	Result	Summary
BRCA1	- MUTN (seq): p.Asp1739= (D1739D)	BRCA1-D1739D is predicted to have no effect on the protein function.
	Clinical relevance	BRCA1 inactivation may impair the DNA damage repair process and result in a loss of cell cycle checkpoint control leading to tumorigenesis (Savage et al., 2014; 24638981, Wu et al., 2010; 21203981, Kolinjivadi et al., 2017; 28079255). Inactivating BRCA1 alterations have been reported to predict sensitivity to platinum-based chemotherapy and PARP inhibitors, including olaparib, rucaparib, and niraparib, which are FDA-approved in specific indications (Hollis et al., 2017; 28546758, Kim et al., 2015; 26187614, Swisher et al., 2017; 27908594, Scott, 2017; 28474297). However, as the alteration reported here has been shown to have no effect on Brca1 protein function, PARP inhibitors are not likely to be relevant.

2.4.2 BIOLOGICAL RELEVANCE of BRCA1-D1739D (p.Asp1739=)

BRCA1 alterations in Breast carcinoma (ER positive)	
Molecular function	BRCA1 D1739D is a synonymous alteration between the first and second BRCT domains of the Brca1 protein (UniProt). This alteration has been reported as likely benign in ClinVar. This alteration has not been reported in COSMIC (Sep 2018) or functionally characterized (PubMed, Sep 2018). As it does not result in a protein sequence change, it is predicted to have no effect on Brca1 protein function.
Incidence in disease	BRCA1 mutations have been reported in 1.1% (6/553) of Breast carcinoma (ER positive) samples analyzed in COSMIC (Jun 2018). BRCA1 mutations have been reported in approximately 4-7% of breast cancer patients screened for familial mutations (Ghadirian et al., 2014; 23621881, Huzarski et al., 2013; 23940229, Abugattas et al., 2015; 25256238, Kraus et al., 2017; 27616075).

3. Glossary of Biomarkers

Marker	Description
BRCA1	BRCA1 encodes the protein Brca1, which is involved in the maintenance of genomic stability, including DNA repair, cell cycle checkpoint, and chromosome segregation.
ERBB2	ERBB2 (also known as HER2/neu) encodes the receptor tyrosine kinase Her2, in the same family as Egfr. Amplification or overexpression of ERBB2 can lead to excessive proliferation and tumor formation.
EZH2	EZH2 encodes the protein Enhancer of zeste homolog 2 (Ezh2), a histone lysine methyltransferase that is the catalytic subunit of Polycomb repressive complexes (PRC2/3), which methylate lysine 9 and 27 of histone H3 and lysine 26 of histone H1 and cause transcriptional repression of target genes. Target genes repressed by this complex include HOXC8, HOXA9, MYT1, CDKN2A and retinoic acid target genes. In cancer cells, an Ezh2-containing complex has been suggested to result in de novo DNA methylation to target genes for repression.
SMAD4	SMAD4 encodes the protein Smad4, a transcription factor downstream of TGF-beta. Also known as DPC4 or MADH4, SMAD4 is a homolog of the Drosophila gene MAD (mothers against decapentaplegic), and was first identified in a screen for tumor suppressors in pancreatic cancer.

4. References

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Prostate Cancer V.2.2016, and Uterine Neoplasms V.2.2016.

5. Definitions of Variant Tiers and Levels of Evidence

Tier 1: Variants of Strong Clinical Significance

Level A	**Predictive of response:** Therapy is FDA-approved in this disease, based on the presence of this biomarker. **Predictive of resistance:** Biomarker is included in professional guidelines as providing resistance to therapy. **Diagnostic:** Biomarker is included in professional guidelines as pathognomonic (required for diagnosis; characteristic of a particular disease). **Prognostic:** Biomarker is included in professional guidelines for clinical decision-making; specifically, the molecular criteria is included in an accepted, clinically relevant prognostic scoring system.
Level B	**Predictive of response:** Strong evidence (well-powered studies, consensus from experts) that biomarker predicts sensitivity to therapy. **Predictive of resistance:** Well-powered studies with expert consensus or smaller studies repeatedly confirmed or reproduced by different groups that variant predicts resistance to therapy. **Diagnostic:** Well-powered studies with expert consensus or repeatedly reported in smaller studies with consistent results or reproduced by different groups indicating diagnostic relevance. These markers may be mentioned in professional guidelines, but are suggestive of, rather than conclusive for, a specific diagnosis. **Prognostic:** Well-powered studies with expert consensus or smaller studies repeatedly with consistent results or reproduced by different groups indicating prognostic relevance.
Level B/C	**Predictive of response:** Consensus from experts, but lacking well-powered studies that biomarker predicts sensitivity to therapy. Not applicable for drug resistance, prognostic, or diagnostic levels of evidence.

Tier 2: Variants of Potential Clinical Significance

Level C	**Predictive of response:** Therapy is FDA-approved for a different disease, based on the presence of this biomarker; or, criteria for a clinical trial. **Predictive of resistance:** Preclinical data strongly suggests resistance; reported in clinical cases. **Diagnostic:** Small studies, diagnostic for a group of related cancers or variants that are supportive of a diagnosis along with other genomic variants. **Prognostic:** Multiple small studies providing prognostic relevance.
Level C/D	**Predictive of response:** Case reports or small case series including exceptional responders that indicate sensitivity to therapy. Not applicable for drug resistance, prognostic, or diagnostic levels of evidence.
Level D	**Predictive of response:** Plausible sensitivity to therapy based on preclinical studies, which do not need to be disease specific. **Predictive of resistance:** Limited preclinical data suggesting resistance; no clinical reports. **Diagnostic:** Small studies or a few case reports support this variant alone or in combination with other biomarkers as assisting diagnosis of this disease. **Prognostic:** : Small studies or a few case reports support this variant alone or in combination with other biomarkers as assisting with prognostic assessment in this disease.
Level E	**Predictive of response:** Poor evidence that biomarker predicts sensitivity to an approved therapy. Not applicable for drug resistance, prognostic, or diagnostic levels of evidence.

Tier 3: Variants of Uncertain Clinical Significance

Tier 4: Benign or Likely Benign Variants

Disclaimer

DNA studies do not constitute a definitive test for any disease conditions in any tested individual. This test is developed and its performance characteristics determined by CellMax Life laboratory. This assay is validated on peripheral blood and provides information on somatic alterations, and microsatellite high instability (MSI-H). No further comments are made on low microsatellite instability (MSI-L), or microsatellite stable (MSS) status. Clinical decisions regarding care and treatment of customers should not be solely based on this test. How this information is used to guide customer care is the responsibility of the physician.

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