PATIENT NAME	Ms. A. B. C.		48		Female
DIAGNOSIS	C/O Invasive ductal carcinoma of breast				
RECOMMENDATION NO	V 1.0	RECOMMENDATION DATE	24	02	2021

Therapy Recommendations:

V 1.0	The integrative molecular analysis in the sample provided indicates therapy benefit from the combination of Lenvatinib + Everolimus + Enzalutamide as OFF LABEL THERAPY or Olaparib as STANDARD OF CARE THERAPY.

Targeted and chemotherapy drugs[1]:

OFF LABEL THERAPY

No	Drug	Indication	Dose/Schedule/Notes[2]
1	Lenvatinib + Everolimus + Enzalutamide	VEGFR1/FLT1 and VEGFR2/KDR ICC positive, mTOR ICC positive, AR IHC positive	Lenvatinib + Everolimus - Standard dose Enzalutamide - Standard dose
Combination of Lenvatinib + Everolimus is used in Renal cancer.			

STANDARD OF CARE THERAPY IN PATIENT'S CANCER TYPE

No	Drug	Indication	Dose/Schedule/Notes[2]
1	Olaparib	Germline BRCA2 p.T3033Nfs*11	Standard dose

Other Potentiating Drugs[3]:

No	Drug	Indication	Dose/Schedule/Notes
Please refer to Page 6 of the report. To be used as per clinician's judgement.			

1. Recommendations for cytotoxic and targeted therapy options, based on observed cellular and molecular indications, are as per existing information and literature in public domain.
2. The Treating Oncologist may consider appropriate dosage, as well as suitable dose reduction, based on a real-time clinical evaluation of the patient, as well as the reported and/or expected toxicity profile(s) of the drug(s).
3. Potentiating drugs are to be administered simultaneously with Targeted and Chemotherapy drugs.

Follow-up:

1. Blood collection every 4 weeks for liquid biopsy investigation after initiation of recommended therapy.
2. Follow-up PET-CT, 4 weeks after initiation of recommended therapy.

P.T.O.

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Disclaimer:

1. This interpretation is not a prescription and must be acted upon by independent application of mind and evaluation as to its suitability for the relevant patient by a Registered Medical Practitioner/Board Certified Medical Oncologist/any other medical professional authorized by law to practice medicine for cancer patients in the relevant jurisdiction. This interpretation is a part of the overall molecular and circulating tumor cell analysis and must always be read and understood conjointly. The analysis performed is based upon the bonafide reliance by Datar Cancer Genetics that the clinical history has been disclosed completely and accurately in the Test Request Form (TRF) and no information has been withheld and further that the sample has been obtained from the patient above named and is free from any deliberate and inadvertent contamination.
2. This interpretation of molecular analysis and circulating tumor cell data is based upon best judgment assessment for optimising the drugs and drug combinations which can be suggested to the treating oncologists. The patient is emphatically advised to seek appropriate guidance and independent interpretation of the data provided along with this interpretation and this interpretation should not be used as the sole decision-making tool for the therapy selection.
3. The interpretation provided herein is the subjective approach suggested and is based upon information available in public domain and scientific data accumulated in the laboratories of Datar Cancer Genetic Ltd at the time of the release of the report. It is not the claim of Datar Cancer Genetics that the interpretation provided is the only possible interpretation or that the interpretation is guaranteed to give any outcomes.
4. Cancer therapy selection, dosing, administration, and the management of related adverse events can be a complex process that should be handled by an experienced healthcare team. Clinicians must choose and verify treatment options based on the individual patient; drug dose modifications and supportive care interventions should be administered accordingly. The suggested treatment regimens may include both USFDA-approved and unapproved indications/regimens. Therapy Recommendations are a work in progress that may be refined as often as new significant data becomes available. DCGL 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.
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PATIENT AND SPECIMEN DETAILS

PATIENT NAME	:	Ms. A. B. C.	TUMOR TYPE	:	Invasive ductal carcinoma of breast
AGE	:	48 Years	SPECIMEN TYPE	:	Blood, FFPE Tumor Block
GENDER	:	Female	DATE OF COLLECTION	:	03.02.2021
ADDRESS	:	--	DATE OF ACCESSION	:	08.02.2021
REFERRED BY	:	Dr. X. Y. Z.	DATE OF REPORT	:	15.03.2021

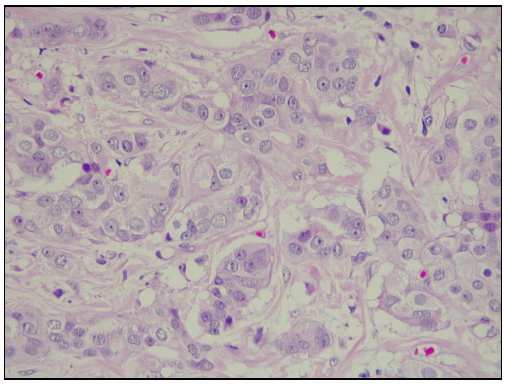


Figure 1: Light microscopic image of Hematoxylin & Eosin stained section of FFPE block (40X).

Microscopy:

Hematoxylin and eosin stained sections from representative areas reveal clusters and tubules of tumor cells. Cells are round to oval with variable amount of eosinophilic cytoplasm and vesicular nuclei with prominent nucleoli. Moderate pleomorphism is seen. Extensive areas of solid DCIS as well as occasional areas of comedo-necrosis are also present. Surrounding stroma is desmoplastic.

Impression:

FFPE block: Histological features are consistent with invasive ductal carcinoma of breast.

SAMPLE PROCESSING SUMMARY

The tumor content determined by microscopic examination of the submitted FFPE block (21/423/2) was **>50%.**

Tumor tissue sample was used for comprehensive gene expression analysis of 20802 genes.

452 genes were analyzed on tumor tissue sample for detection of DNA alterations such as single nucleotide variations (SNVs), insertion-deletions (INDELS), copy number variations (CNVs), BRCA1/2 gene alterations, Tumor mutation burden (TMB) estimation and RNA based fusion analysis.

Tumor tissue was used for Fluorescence In Situ Hybridization (FISH), Immunohistochemistry and microsatellite instability markers analysis described in the report.

Cell free nucleic acids were isolated from plasma separated from patient's blood and used in analysis of 52 genes for detection of DNA alterations such as single nucleotide variations (SNVs), insertion-deletions (INDELS), copy number variations (CNVs) and RNA based fusion analysis.

Pharmacogenetics analysis was performed on genomic DNA isolated from the peripheral blood mononuclear cells (PBMCs).

Peripheral blood was used for circulating tumor cell (CTC) enumeration. Immunocytochemistry (ICC) and chemosensitivity analysis was performed on the isolated CTC.

REPORT HIGHLIGHTS

DRUGS WITH CLINICAL BENEFIT

TARGETED/ HORMONAL/ IMMUNOTHERAPY DRUGS		
INDICATIONS	**USFDA APPROVED* / STANDARD OF CARE* (Breast Cancer)**	**OFF LABEL THERAPY***
Germline BRCA2 p.T3033Nfs*11	Olaparib Talazoparib	Rucaparib Niraparib
ER IHC positive (8/8) ESR1 overexpression (+3.37 FC) Absence of ESR1 mutations CYP2D6 normal metabolizer status (Tamoxifen)	Tamoxifen Letrozole Anastrozole Exemestane Fulvestrant	---
PR IHC positive (8/8)	Megestrol	Medroxy-progesterone
AR IHC positive	---	Enzalutamide Bicalutamide Nilutamide Apalutamide Darolutamide Leuprolide Flutamide Abiraterone
VEGFA overexpression (+2.64 FC)	Bevacizumab	Ziv-Aflibercept
mTOR ICC positive	Everolimus	Temsirolimus
VEGFR1/FLT1 ICC positive VEGFR2/KDR ICC positive	---	Axitinib Lenvatinib Cabozantinib Pazopanib Sorafenib Sunitinib Regorafenib Ponatinib
VEGFR2/KDR ICC positive	---	Vandetanib Ramucirumab
EGFR ICC positive	---	Cetuximab Panitumumab Necitumumab

ICC: Immunocytochemistry; IHC: Immunohistochemistry; FC: Fold change

* The USFDA approval or SOC recommendation may not be for the detected biomarker or alteration. The association of the detected biomarker or alteration and the drug may be based only on the literature evidence.

DRUGS WITH CLINICAL BENEFIT

CYTOTOXIC DRUGS			
USFDA APPROVED / STANDARD OF CARE DRUGS			
IN BREAST CANCER	INDICATION Chemosensitivity – % Cell Death (CD) ± Molecular biomarker	IN OTHER CANCERS	INDICATION Chemosensitivity – % Cell Death (CD) ± Molecular biomarker
Vinblastine	77% CD	Irinotecan	77% CD
Epirubicin	63% CD	Vincristine	68% CD
Carboplatin	52% CD; Germline BRCA2 mutation	Dactinomycin	53% CD
Cyclophosphamide	49% CD	Etoposide	51% CD
Docetaxel	37% CD	Melphalan	49% CD
5FU/Capecitabine	34% CD	Dacarbazine	47% CD
Eribulin	31% CD	Temozolomide	38% CD
Vinorelbine	26% CD	Mitomycin	32% CD
		Cabazitaxel	28% CD

DRUGS WITHOUT CLINICAL BENEFIT/WITH POTENTIAL RESISTANCE

TARGETED/ HORMONAL/ IMMUNOTHERAPY DRUGS		
INDICATIONS	**USFDA APPROVED / STANDARD OF CARE (Breast Cancer)**	**OFF LABEL THERAPY**
HER2 FISH negative	Ado-Trastuzumab emtansine (TDM1) Fam-Trastuzumab deruxtecan Lapatinib Neratinib Tucatinib Pertuzumab Trastuzumab	---

FISH: Fluorescence in situ hybridization

CYTOTOXIC DRUGS			
USFDA APPROVED / STANDARD OF CARE DRUGS			
IN BREAST CANCER	**INDICATION** Chemosensitivity – % Cell Death (CD) ± Molecular biomarker	**IN OTHER CANCERS**	**INDICATION** Chemosensitivity – % Cell Death (CD) ± Molecular biomarker
Cisplatin	<25% CD	Bleomycin	<25% CD
Doxorubicin	<25% CD	Ifosfamide	<25% CD
Gemcitabine	<25% CD	Mitoxantrone	<25% CD
Methotrexate	<25% CD	Oxaliplatin	<25% CD
Paclitaxel	<25% CD	Pemetrexed	<25% CD
		Topotecan	<25% CD
		Trabectedin	<25% CD

ADDITIONAL REPORT HIGHLIGHTS

INDICATIONS FOR NON-CONVENTIONAL DRUGS

DRUG	INDICATION
Diflunisal	Chemosensitivity - 54% CD
Artesunate	Chemosensitivity - 53% CD; MMP11 (+4.36 FC) overexpression
Cannabidiol	Chemosensitivity - 49% CD; MMP11 (+4.36 FC) overexpression
Hypericin	Chemosensitivity - 49% CD
Apigenin	Chemosensitivity - 47% CD
Epigallocatechin-gallate	Chemosensitivity - 42% CD; MMP11 (+4.36 FC) overexpression
Dichloroacetate	Chemosensitivity - 37% CD
Atorvastatin	Chemosensitivity - 33% CD
Glutathione	Chemosensitivity - 29% CD
Celecoxib	Chemosensitivity - 26% CD
Vitamin C	Chemosensitivity - 25% CD
Aspirin	Chemosensitivity - 22% CD
Glibenclamide	Chemosensitivity - 22% CD
Bromelain	Chemosensitivity - 21% CD
Melatonin	Chemosensitivity - 19% CD
Resveratrol	Chemosensitivity - 13% CD; MMP11 (+4.36 FC) overexpression
Propranolol	Chemosensitivity - 13% CD
Berberine	MMP11 (+4.36 FC) overexpression
Mebendazole	MMP11 (+4.36 FC) overexpression
6-Shogaol	MMP11 (+4.36 FC) overexpression

PHARMACOGENETICS - DRUGS WITH INCREASED RISK OF TOXICITY

DRUG	INDICATION	DRUG	INDICATION
Cisplatin	ERCC1, XPC	Gemcitabine	NT5C2
Irinotecan	UGT1A1	Sacituzumab govitecan	UGT1A1

PHARMACOGENETICS - DRUGS WITH LABELED RISK OF TOXICITY

DRUG	INDICATION	DRUG	INDICATION
Belinostat	UGT1A1	Carboplatin	ERCC1, MTHFR
Cyclophosphamide	GSTP1	Dabrafenib	G6PD
Epirubicin	GSTP1	Erdafitinib	CYP2C9
Erlotinib	UGT1A1	Fluoropyrimidines	DPYD
Gefitinib	CYP2D6	Mercaptopurine	TPMT, NUDT15
Methotrexate	ABCB1, MTHFR	Nilotinib	UGT1A1
Oxaliplatin	ERCC1	Pazopanib	UGT1A1
Rasburicase	G6PD	Regorafenib	UGT1A1
Thioguanine	TPMT, NUDT15	Trametinib	G6PD
Vincristine	CEP72		

LONGITUDINAL MONITORING BIOMARKERS

0%	Highest Mutant Allele Frequency (HMAF)
4 CTCs /ml	Number of CTCs detected

GERMLINE MUTATION

BRCA2 p.T3033Nfs*11	Hereditary breast and ovarian cancer (HBOC)

PROGNOSTIC IMPLICATIONS

PROGNOSIS	INDICATION
Adverse	None detected
Favorable	None detected

BIOMARKERS FOR IMMUNE CHECKPOINT INHIBITORS

0.84 Mutations/Mb	Tumor mutation burden (TMB)
MS-Stable	Intact nuclear expression of MLH1, MSH2, MSH6, PMS2
TPS- 0%	PD-L1 22C3 IHC
TPS- 0%	PD-L1 28-8 IHC
IC <1%	PD-L1 SP142 IHC

DISEASE RELEVANT FINDINGS

BIOMARKER	RESULT
BRCA1	No alterations detected
PIK3CA	No mutations detected
ERBB2/ HER2	No amplification detected
NTRK1/2/3	No fusions detected

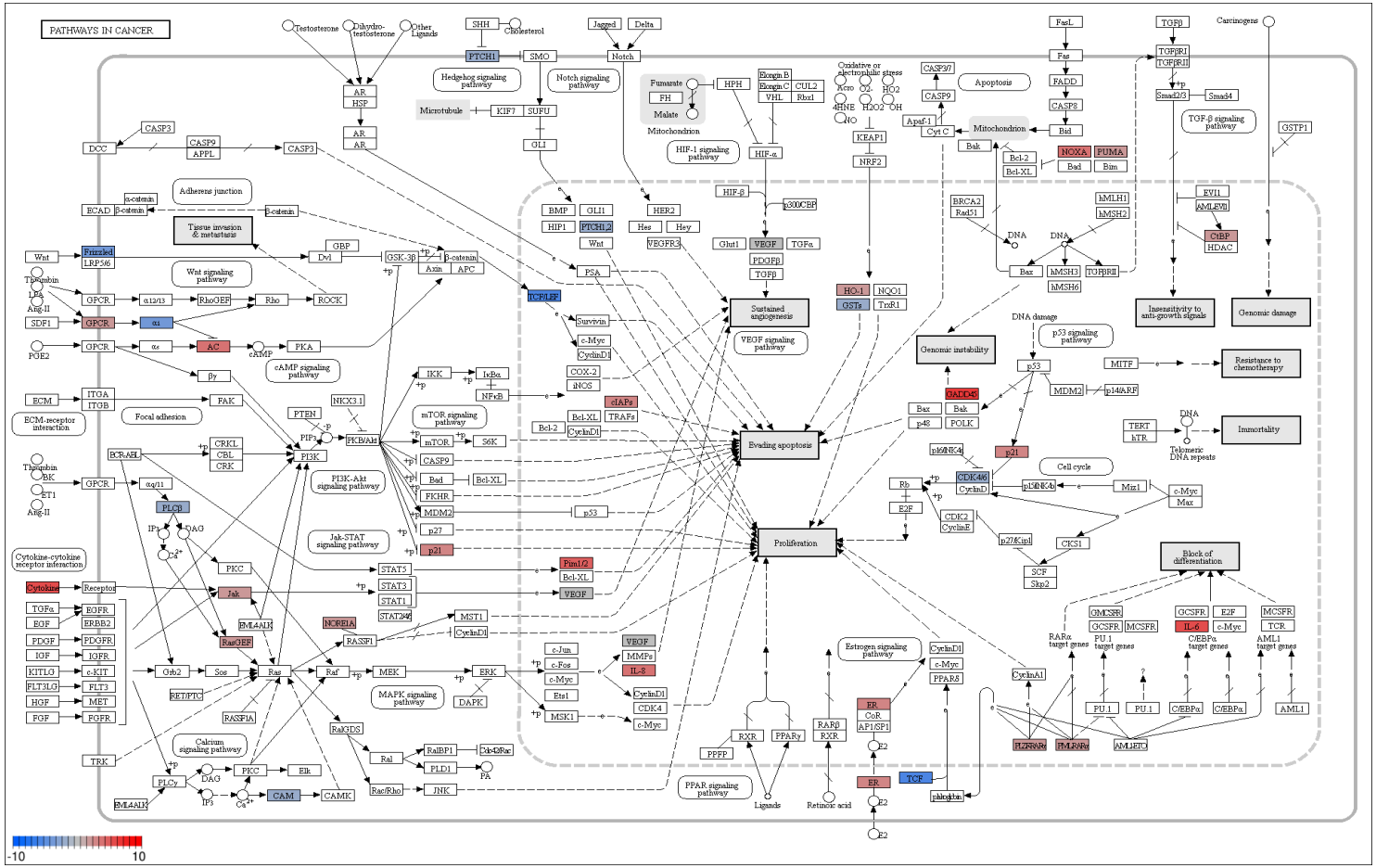
SUMMARY OF OTHER GENOMIC ALTERATIONS

GENE	SNV / INDEL	THERAPEUTIC SIGNIFICANCE
NOTCH1	p.G1301R (Tissue MAF 5.07% at 670X)	---
RECQL4	p.Q471P (Tissue MAF 7.5% at 715X)	---
MBD1	p.G205R (Tissue MAF 5.3% at 2008X)	---
NF1	p.R711C (Tissue MAF 4.62% at 238X)	---

GENE	CNV	THERAPEUTIC SIGNIFICANCE
PIK3C2B, MDM4, IKBKE	Gain (3 copies)	---

GENE	FUSION	THERAPEUTIC SIGNIFICANCE
FGFR1	WHSC1L1-FGFR1 (W1F2)	---

COMPREHENSIVE PATHWAY PERTURBATION IN PRIMARY TUMOR



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GLOBAL GENE EXPRESSION HIGHLIGHTS

Out of 20802 protein coding genes analyzed in the tumor tissue, **7501** genes were expressed in the analyzed tumor tissue.

554 genes were found to be differentially regulated in the tumor tissue.

THERAPEUTIC IMPLICATIONS (TUMOR TISSUE mRNA)

Therapeutic implications section of the report briefly describes the genetic alterations with potential implications to the patient's treatment and cancer management.

Table 1: List of drugs approved for cancers with potential benefit based on tumor tissue gene expression analysis.

Markers	Result (Fold Change)	Drugs With Benefit
ESR1	▲ **+3.37 FC**	**Tamoxifen, Letrozole, Anastrozole, Exemestane, Fulvestrant**

Interpretation: Upregulation of ESR1 is suggestive of potential benefit from Tamoxifen, Letrozole, Anastrozole, Exemestane and Fulvestrant (Mokbel, 2003; Barnadas et al., 2009; Kim et al., 2011; Diaz-Cruz et al., 2013; Dabydeen et al., 2015; Li et al., 2019).

Tamoxifen, Letrozole and Anastrozole are USFDA approved for the treatment of hormone receptor positive early breast cancer and hormone receptor positive locally advanced or metastatic breast cancer.
Exemestane is USFDA approved for the treatment of estrogen receptor-positive early breast cancer and in advanced breast cancer whose disease has progressed following tamoxifen therapy.
Fulvestrant is USFDA approved for the treatment of hormone receptor-positive (HR+) and HER2-negative advanced breast cancer or with Palbociclib in women with HR+ and HER2-negative advanced or metastatic cancer that got worse after treatment with hormone therapy.
All these drugs are also recommended as standard of care drugs for breast cancer as per NCCN guidelines (NCCN guidelines, 2021).

VEGFA	▲ **+2.64 FC**	**Bevacizumab, Ziv-Aflibercept**

Interpretation: Upregulation of VEGFA is suggestive of potential benefit from Bevacizumab and Ziv-Aflibercept (Ranieri et al., 2006; Otrock et al., 2011; Alidzanovic et al., 2016; Zhang et al., 2017).

Bevacizumab is USFDA approved for the treatment of cervical cancer, glioblastoma, colorectal cancer, non-small cell lung cancer, ovarian epithelial, fallopian tube, or primary peritoneal cancer and renal cell carcinoma.
Combination of Paclitaxel and Bevacizumab is a SOC therapy for breast cancer as per NCCN guidelines (NCCN guidelines, 2021).

Ziv-Aflibercept is USFDA approved for the treatment of metastatic colorectal cancer.
In a phase I trial, treatment of Capecitabine with Aflibercept in patients with breast cancer showed manageable safety profile with objective response rate of 15.4% in arm A (continuous Capecitabine dosing) and 7.7% in arm B (intermittent Capecitabine dosing) among 26 assessable patients (Aflibercept was administered at a flat dose of 6 mg/kg every 3 weeks in both arms) (Camera et al., 2020).

Table 2: List of non-conventional agents that may provide therapeutic benefit based on tumor tissue gene expression analysis.

Markers	Result (Fold Change)	Drugs
MMP11	▲ +4.36 FC	Doxycycline, Berberine, Mebendazole, Metformin, Artesunate, Cannabidiol, Resveratrol, Curcumin, Epigallocatechin-gallate (EGCG), 6-Shogaol

Interpretation: The antibiotic agent, doxycycline, non-selectively inhibits MMP activation and expression, and has been shown to suppress MMP activities in human cancer cells (Tang et al., 2013; Cathcart et al., 2015).

Numerous studies have shown that berberine and its derivatives demonstrate important anti-tumor effects. Berberine appears to exert its anticancer properties by inducing ROS production and prevention of cell migration via inhibition of the gene expression of MMP in various cancers (McCubrey et al, 2017; Li et al, 2018; Hu et al, 2019; Zhang et al, 2020).

Mebendazole is found to inhibit invasion and migration of cancer cells by supressing MMP activity (Pinto et al., 2015).

Metformin has been reported to block migration and invasion of tumor cells by inhibition of matrix metalloproteinase-9 (Hwang and Jeong, 2010).

Artesunate inhibits invasion and metastasis in cancer cells through downregulating expression of MMPs (Rasheed et al., 2010; Wang et al., 2016; Ma et al., 2019).

Cannabidiol showed anti-migratory and anti-invasive effects by inhibiting MMPs which in turn degraded the extra-cellular matrix (ECM), thus affecting metastasis of cancer to the distant organs (Chakravarti et al., 2014; Elbaz et al., 2015; Sharafi et al., 2019).
Multiple studies have shown that Resveratrol supresses invasion and growth of cancer cells by inhibiting expression of MMPs (Yu et al., 2008; Weng et al., 2010; Ko et al., 2017).

Curcumin exerts antitumor activity in cancer cells through downregulating MMP activity (Hong et al., 2006; Kumar et al., 2012; Hassan and Daghestani, 2012; Cao et al., 2014; Bachmeier et al., 2018).

Epigallocatechin-gallate (EGCG) is found to inhibit epithelial-mesenchimal transition (EMT) as well as cellular invasion in cancer cells by directly binding and downregulating collagenase activity of MMPs (Negri et al., 2018).

6-Shogaol is reported to inhibit cancer cell invasion by reducing MMP9 expression (Ling et al., 2010; Weng et al., 2010).

HMGB1	▲ +2.85 FC	Chloroquine

Interpretation: In pre-clinical studies, Chloroquine is reported to inhibit HMGB1-induced Iκ-B degradation and NF-κB activation and thereby preventing cytokine-like activities of HMGB1 (Andersson and Tracey, 2011; Zhang et al., 2012; Fiuza et al., 2013). Chloroquine demonstrated anticancer activity by inducing apoptosis in several cancer types (Yang et al., 2013; Wu et al., 2015; Verbaanderd et al., 2017).

Table 3: List of approved drugs that may not provide therapeutic benefit based on tumor tissue gene expression analysis.

Markers	Result	Drugs Without Benefit
None detected		

SOMATIC GENOME ALTERATIONS (TUMOR TISSUE)

Table 4: Genomic alterations identified with gene analyses carried out on the submitted sample.

	Total genomic alterations identified	**07**
A.	**Somatic mutations identified**	**04**
	1. Number of driver mutations identified	03
	2. Number of passenger mutations identified	01
	3. Number of uncategorized mutations	00
	4. Number of mutations with therapy response	00
	5. Number of mutations with therapy resistance	00
B.	**Copy Number Variation (CNV) identified**	**03**
	1. CNV with therapy response	00
	2. CNV with therapy resistance	00

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SINGLE NUCLEOTIDE VARIATIONS/INDELS

Table 5: List of mutations detected in tumor tissue analysis with clinical significance in invasive ductal carcinoma of breast.

Markers (Transcript ID)	Variant	Category
No variants detected		

Table 6: List of mutations detected in tumor tissue analysis with unknown significance in invasive ductal carcinoma of breast.

Markers (Transcript ID)	Variant	Category
NOTCH1 (NM_017617.4)	c.3901G>A, p.G1301R; [p.(Gly1301Arg)]	Tier III

Interpretation: Mutations in NOTCH1 gene are reported in breast cancer (Wang et al., 2015; Zhong et al., 2016). In silico analysis predicts NOTCH1 p.G1301R to be a loss-of-function mutation. It is reported in tumors of skin, stomach and large intestine. The clinical significance of this mutation in invasive ductal carcinoma of breast is not yet known.

The NOTCH1 gene provides instructions for making a protein called Notch1, a member of the Notch family of receptors. The encoded preproprotein is proteolytically processed in the trans-Golgi network to generate two polypeptide chains that heterodimerize to form the mature cell-surface receptor. This receptor plays a role in the development of numerous cell and tissue types. The NOTCH1 protein has such diverse functions that the gene is considered both an oncogene and a tumor suppressor.

RECQL4 (NM_004260.4)	c.1412A>C, p.Q471P; [p.(Gln471Pro)]	Tier III

Interpretation: Mutations in RECQL4 gene are reported in breast cancer (Schrijver et al., 2018). The clinical significance of RECQL4 p.Q471P mutation in invasive ductal carcinoma of breast is not yet known.

The protein encoded by this gene is a DNA helicase that belongs to the RecQ helicase family. DNA helicases unwind double-stranded DNA into single-stranded DNAs and may modulate chromosome segregation.

MBD1 (NM_001204136.1)	c.613G>A, p.G205R; [p.(Gly205Arg)]	Tier III

Interpretation: In silico analysis predicts MBD1 p.G205R to be a loss-of-function mutation. The clinical significance of this mutation in invasive ductal carcinoma of breast is not yet known.

The protein encoded by this gene is a member of a family of nuclear proteins related by the presence of a methyl-CpG binding domain (MBD). These proteins are capable of binding specifically to methylated DNA, and some members can also repress transcription from methylated gene promoters.

NF1 (NM_001042492.2)	c.2131C>T, p.R711C; [p.(Arg711Cys)]	Tier III

Interpretation: Mutations in NF1 gene are reported in breast cancer (Frayling et al., 2019; Pearson et al., 2020). It is reported that loss of NF1 function is suggestive of potential benefit from mTOR inhibitors (Cheaib et al., 2015). In silico analysis predicts activity of NF1 p.R711C to be a normal. Therefore, the clinical significance of this mutation in invasive ductal carcinoma of breast is not yet known.

NF1 p.R711C is reported in tumors of skin and central nervous system.

The NF1 gene provides instructions for making a protein called neurofibromin. This protein is produced in many types of cells, including nerve cells and specialized cells called oligodendrocytes and Schwann cells that surround nerves. This gene product appears to function as a negative regulator of the ras signal transduction pathway. Neurofibromin acts as a tumor suppressor protein.

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TUMOR MUTATION BURDEN (TMB)

Markers	Result	Interpretation
Tumor Mutation Burden (TMB)	**0.84 Mutations/Mb**	**Low TMB**

Patient's tumor mutation burden assessment based on targeted genomic profiling of 409 genes was found to be 0.84 Mutations/Mb.

Tumor mutation burden (TMB), the total number of somatic coding mutations in a tumor, is emerging as a promising predictive biomarker for immunotherapy response in cancer patients (Chan et al., 2018; Fancello et al., 2019). The somatic mutations in tumor DNA can give rise to neoantigens, mutation-derived antigens that are recognized and targeted by the immune system, especially after treatment with agents that activate T cells. Therefore, more somatic mutations a tumor has, the more neoantigens it is likely to form, and TMB can represent a useful estimation of tumor neoantigenic load (Chan et al., 2018; Fancello et al., 2019). Tumor mutation burden (TMB) is, thus, an informative biomarker for predicting response to immune checkpoint inhibitors like Pembrolizumab, Nivolumab, Atezolizumab, Avelumab, Durvalumab and Ipilimumab.
Clinical studies have shown associations between elevated TMB and efficacy of immune checkpoint inhibitors, alone or in combination with other agents, in multiple solid tumors including, lung cancer, urothelial carcinoma, melanoma, colorectal cancer, head and neck squamous cell carcinoma and other cancer types (Johnson et al., 2016; Goodman et al., 2017; Carbone et al., 2017; Hellmann et al., 2018; Eroglu et al., 2018; Miao et al., 2018; Rizvi et al., 2018; Powles et al., 2018; Socinski et al., 2018; Legrand et al., 2018; Chae et al., 2019; Ott et al., 2019).

Analysis of tumor mutation burden (TMB) across more than 100,000 multiple solid cancer specimens suggests that patients with TMB <20 mutations/Mb may not derive benefit from immune checkpoint inhibitors (Chalmers et al., 2017; Samstein et al., 2019).
However, a clinical study of stage IV or recurrent NSCLC patients with >10 mutations/Mb showed that the combination of Nivolumab plus ipilimumab resulted in 1- year progression-free survival rate of 42.6% versus 13.2% with chemotherapy and the median progression-free survival of 7.2 months versus 5.5 months. The objective response rate was 45.3% with Nivolumab plus Ipilimumab and 26.9% with chemotherapy (Hellman et al., 2018). In a phase II trial (CheckMate 568) of 288 chemotherapy-naive stage IV NSCLC patients with tumor available for testing, 98 patients (34%) were evaluable for TMB (49% with TMB ≥10 mutations per megabase, Mut/Mb). These patients when treated with Nivolumab plus Ipilimumab, showed response rate of 30% and median progression-free survival (PFS) of 6.8 months (Remon et al., 2019; Ready et al., 2019). The combination of Nivolumab (Opdivo) plus Ipilimumab (Yervoy) and single agent Nivolumab are NCCN recommended for the treatment of patients with advanced non–small cell lung cancer (NSCLC) based on tumor mutation burden (TMB) (NCCN guidelines, 2021).
In TAPUR study, Pembrolizumab demonstrated anti-tumor activity (disease control rate: 37%, in heavily pre-treated patients with metastatic breast cancer and high tumor mutation burden (hTMB) (greater than or equal to 9 mutations/Mb) (Alva et al., 2019).

Recently, Pembrolizumab has been USFDA approved for the treatment of patients with tumor mutation burden-high (TMB-H) [≥10 mutations/megabase (mut/Mb)] solid tumors.
In a clinical trial of NSCLC patients with blood TMB (bTMB) of 6 or higher, anti-programmed cell death 1 (anti-PD-1) and anti-programmed cell death ligand 1 (anti-PD-L1) therapy showed objective response rate of 39.3% (Wang et al., 2019). Also, it is reported that, TMB measured from the blood is a predictive biomarker for PFS in patients receiving Atezolizumab monotherapy in NSCLC. Analyses of POPLAR and OAK trials demonstrate that, bTMB≥16 is a clinically meaningful and technically robust cut-point to determine clinical benefit from immune checkpoint inhibitors (Gandara et al., 2018).

The median tumor mutation burden (TMB) (n=4297) for breast invasive ductal carcinoma is reported to be 3.6 mutations/Mb, while the maximum TMB is 261.3 mutations/Mb (95% Confidence Interval, 1-1.7) (Chalmers et al., 2017).

High TMB (TMB-H) is indicative of potential benefit from immune checkpoint inhibitors. Tumor mutation burden (TMB) detected in the submitted sample is 0.84 mutations/Mb. Therefore in this case, there is no indication of immune checkpoint inhibitor therapy based on TMB.

COPY NUMBER VARIATIONS

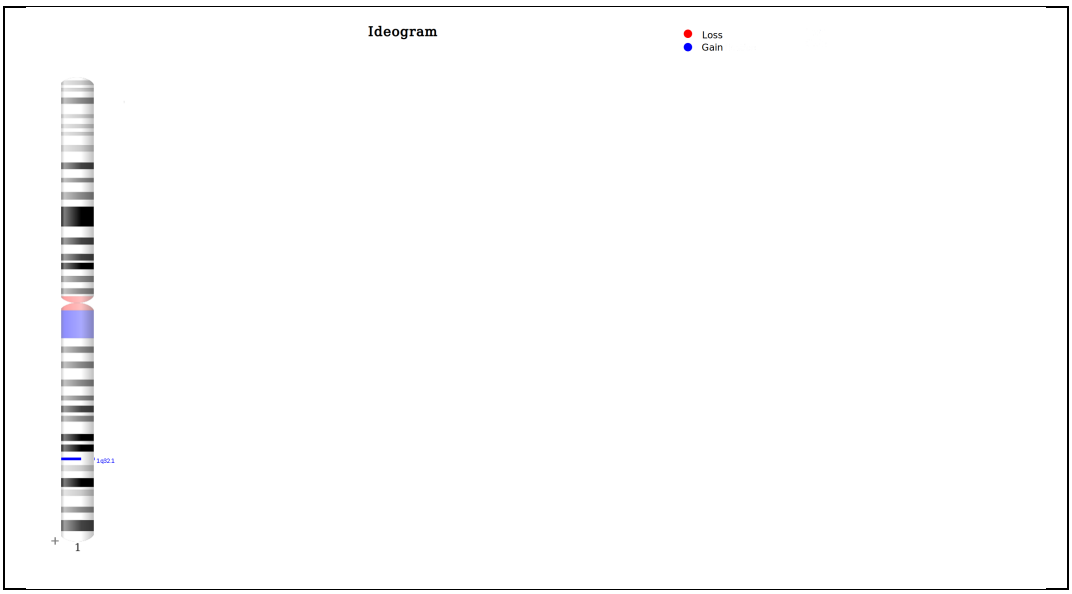


Figure 2: Ideogram of chromosomes showing copy number variations detected in the submitted sample.

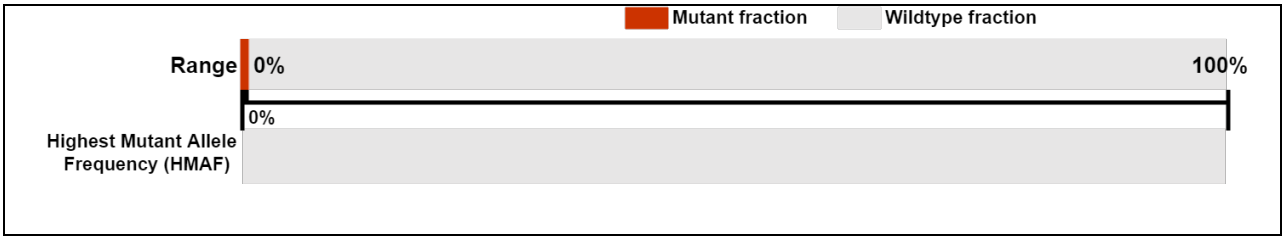
Table 7: List of copy number variations detected in tumor tissue analysis in the submitted sample.

Markers (Cytoband)	Result	Category
PIK3C2B, MDM4, IKBKE (1q32.1)	Gain (3 copies)	Tier III

Interpretation: Copy number gain of chromosome 1q is observed in breast cancer (Orsetti et al., 2006). IKBKE, a breast cancer oncogene, is amplified and overexpressed in approximately 30% of breast carcinomas, in which it induces survival signaling associated with NF-kB pathway activation (Boehm et al., 2007). However, clinical significance of copy number gain of these alterations in invasive ductal carcinoma of breast is not yet known.

SOMATIC GENOME ALTERATIONS (CELL FREE NUCLEIC ACIDS)

Figure 3: Highest Mutant Allele Frequency



1. No mutations were detected in the cell free nucleic acids isolated from patient's plasma.

SINGLE NUCLEOTIDE VARIATIONS/INDELS

Table 8: List of mutations detected in cell-free nucleic acids analysis with clinical significance in invasive ductal carcinoma of breast.

Markers (Transcript ID)	Variant	Category
No variants detected		

Table 9: List of mutations detected in cell-free nucleic acids analysis with unknown significance in invasive ductal carcinoma of breast.

Markers (Transcript ID)	Variant	Category
No variants detected		

BRCA1/2 MUTATION ANALYSIS

Sample is positive for germline pathogenic mutation, p.T3033Nfs*11 [c.9097dupA, p.(Thr3033AsnfsTer11)], in BRCA2 gene, as evaluated by Next Generation Sequencing (NGS).

Patient may derive potential benefit from PARP inhibitors, Olaparib, Rucaparib, Niraparib, Talazoparib as well as platinum therapies, Carboplatin, Cisplatin and Oxaliplatin.

No large genomic rearrangements (LGRs) (large deletions and duplications) detected in the BRCA1 /BRCA2 genes as evaluated by Multiplex Ligation-dependent Probe Amplification (MLPA).

Gene Transcript	Variant	Zygosity	Classification	Disease	Inheritance
BRCA2 (NM_000059.3)	**c.9097dupA, p.T3033Nfs*11; [p.(Thr3033AsnfsTer11)] rs397507419**	**Heterozygous**	**Pathogenic**	Hereditary breast and ovarian cancer (HBOC)	Autosomal dominant

BRCA2: The germline c.9097dupA p.(Thr3033AsnfsTer11), also known as 9325insA and 9317insA, a pathogenic variant in BRCA2 gene, is reported previously in patients with hereditary breast and ovarian cancer (HBOC) (Kauff et al., 2002; Machackova et al., 2008; Kwong et al., 2012; Holter et al., 2015; Kwong et al., 2016; Banda et al., 2018; Apessos et al., 2018; Uyisenga et al., 2020). The c.9097dupA p.(Thr3033AsnfsTer11) mutation, located in coding exon 23 of the BRCA2 gene, results from a duplication of one nucleotide at position 9097, causing a translational frameshift with a predicted alternate stop codon (p.T3033Nfs*11). Loss-of-function mutations in BRCA2 gene are known to be pathogenic (Borg et al., 2010). The allele frequency of this frameshift variant is 4.0 X 10^{-6} in general population (Karczewski et al., 2020). In summary, this collective evidence supports c.9097dupA p.(Thr3033AsnfsTer11) in the BRCA2 gene as a pathogenic variant for HBOC.

Studies indicate that women with pathogenic mutations in BRCA2 have a risk of breast cancer of 69% by age of 80 years. Women with pathogenic BRCA2 mutations have a high risk of developing a new primary cancer in the contralateral breast in the years following a breast cancer diagnosis. Around 38-84% of women with BRCA2 mutation can develop breast cancer. The risk of developing second primary breast cancer is reported to be 10% within next 10 years in BRCA2 carriers. This risk increases to approximately 62% by age of 70 years. The risk of ovarian cancer is 16.5 to 27% in women with BRCA2 mutation. In male carriers, the risk of breast cancer is up to 8% and the risk of prostate cancer is up to 15% by age of 65 years with 20% lifetime risk. There may be an increase in risk of pancreatic cancer and melanoma in BRCA2 carriers. Due to the autosomal dominant inheritance, each first degree relative of this individual has a one-in two chance of having this mutation (Kuchenbaecker et al., 2017). Family members can be tested for this specific mutation.

The presence of loss-of-function, pathogenic BRCA2 mutation, is suggestive of potential therapeutic benefit from PARP inhibitors Olaparib, Rucaparib, Niraparib, Talazoparib as well as platinum based chemotherapy drugs, Carboplatin, Cisplatin and Oxaliplatin (Hennessy et al., 2010; Dann et al., 2012; Alsop et al., 2012; Pennington et al., 2014; Chao et al., 2016; Koczkowska et al., 2016).

Olaparib is USFDA approved for breast, pancreatic, ovarian epithelial, fallopian tube, or primary peritoneal cancer patients with germline BRCA mutations. It is also USFDA approved for prostate cancer patients with germline or somatic mutations in the genes involved in the HRR pathway.

Talazoparib is USFDA approved for patients with germline BRCA-mutated HER2-negative metastatic breast cancer.
Olaparib and Talazoparib are standard of care drugs for breast cancer as per NCCN guidelines (NCCN guidelines, 2021).

Rucaparib is USFDA approved for prostate cancer and advanced ovarian epithelial, fallopian tube, or primary peritoneal cancer patients with BRCA mutations.
In a phase II study (RUBY), Rucaparib demonstrated antitumor activity in a subset of germline BRCA wildtype metastatic breast cancer patients whose tumor had high homologous recombination deficiency (HRD) (assessed by Loss of Heterozygosity (LOH) score). Among 37 evaluable patients, 5 (13.5%) demonstrated clinical benefit (1 CR [LOH high], 3 partial response [2 LOH high, 1 somatic BRCA2] and 1 SD>31 weeks [somatic BRCA1]) (Patsouris et al., 2019).

Niraparib is USFDA approved for ovarian epithelial, fallopian tube or primary peritoneal cancer with BRCA mutations.
In an open-label trial, treatment of Niraparib with Pembrolizumab demonstrated promising antitumor activity (objective response rate of 21% and disease control rate of 49%) in 47 evaluable patients with advanced or metastatic breast cancer, with numerically higher response rates (objective response rate of 47%, disease control rate of 80% and median progression free survival of 8.3 months) in 15 evaluable patients with BRCA mutations (Vinayak et al., 2019).

Carboplatin is USFDA approved for the treatment of ovarian cancer.
Cisplatin is USFDA approved for bladder cancer, ovarian cancer and testicular cancer.
Carboplatin and Cisplatin are standard of care therapies for breast cancer as per NCCN guidelines (NCCN guidelines, 2021).

Oxaliplatin is USFDA approved for the treatment of colorectal cancer and stage III colon cancer.
In a clinical study, combination of Oxaliplatin and Capecitabine in anthracyclines and taxanes pretreated breast cancer patients (n=28) showed moderate activity (objective responses in 32%, median overall survival of 10 months) and was well tolerated (Polyzos et al., 2009).

RECOMMENDATION

- Consultation with a healthcare professional who has training and experience in cancer genetics is strongly recommended for this patient in order to discuss cancer risks and other disease risks associated with this genetic test result. The type and frequency of cancer surveillance, cancer prevention options and strategies and the impact of this result on the cancer risks for members of the patient's family are also recommended topics of discussion with a health care professional.
- Genetic testing for BRCA2 mutation of other family members like siblings (sisters as well as brothers) and children (daughters as well as sons) is recommended after counselling.

FUSION ANALYSIS

Table 10: Fusion analysis

Marker (Transcript ID)	Alteration	Result	Category
FGFR1 (NM_001174067.1)	WHSC1L1-FGFR1 (W1F2)	Detected	Tier III

Interpretation: Fusion of FGFR1 gene with WHSC1L1 is reported in solid tumors, including breast cancer (Kirchner et al., 2019; Williams et al., 2020; Loddo et al., 2021). WHSC1L1-FGFR1 fusion is reported to be a druggable target which may lead to activation of multiple signalling pathways such as RAS/RAF/MEK/ERK, PI3K/AKT/MTOR, JAK/STAT and PLCy/PKC pathways (Williams et al., 2020; Loddo et al., 2021). However, the therapeutic significance of this fusion in breast carcinoma is not well evaluated.

Table 11: Analysis of MSI Markers

Marker	Staining pattern
MLH1	**Intact nuclear expression**
MSH2	**Intact nuclear expression**
MSH6	**Intact nuclear expression**
PMS2	**Intact nuclear expression**

Interpretation:

Immunohistochemistry (IHC) for four mismatch repair (MMR) proteins (MLH1, PMS2, MSH2 and MSH6) was performed on formalin-fixed, paraffin-embedded tissue taken from representative sections of the resection specimens. IHC for MMR proteins is used to identify MMR status: being diffusely positive (intact/retained nuclear staining) or showing loss of nuclear staining (MMR protein deficient) (Kanopiene et al, 2014; McCarthy et al, 2019). Loss of expression of MMR proteins may occur due to germline MMR gene mutations, somatic MMR gene inactivation or epigenetic silencing via promoter hypermethylation.

PD-1/PD-L1 checkpoints have important function in maintaining immune-tolerance and preventing effective antitumor immunity. Various clinical trials have demonstrated that mismatch repair deficiency (dMMR) or microsatellite instability-high (MSI-H) is significantly associated with long-term immunotherapy-related response and better prognosis in various tumors treated with immune checkpoint inhibitors. Tumors with dMMR or MSI-H are sensitive to immune checkpoint blockade (ICB), particularly to PD-1 and PD-L1 inhibitors. It is worth emphasizing that dMMR or MSI-H status could identify responders regardless of tumor location and tumor type, that is, they have the ability to guide different tumor immunotherapies in the same manner. Subsequently, USFDA approved Pembrolizumab for all dMMR/MSI-H solid tumors (Lemery et al., 2017; Zhao et al., 2019; Luchini et al., 2019).

HISTOPATHOLOGICAL ANALYSIS

Sample Received:

Received single FFPE block labeled as 21/423/2 for histopathological and immunohistochemical analysis.

Microscopy:

Hematoxylin and eosin stained sections from representative areas reveal clusters and tubules of tumor cells. Cells are round to oval with variable amount of eosinophilic cytoplasm and vesicular nuclei with prominent nucleoli. Moderate pleomorphism is seen. Extensive areas of solid DCIS as well as occasional areas of comedo-necrosis are also present. Surrounding stroma is desmoplastic.

Impression:

FFPE block: Histological features are consistent with invasive ductal carcinoma of breast.

IMMUNOHISTOCHEMISTRY (IHC) ANALYSIS

Markers	Result
ER	**Positive (8/8)**
Interpretation: Strong nuclear staining for ER in 90% of neoplastic cells indicates potential benefit from Tamoxifen, Letrozole, Anastrozole, Exemestane and Fulvestrant. Kindly refer to USFDA label and/or studies for Tamoxifen, Letrozole, Anastrozole, Exemestane and Fulvestrant mentioned earlier.	
PR	**Positive (8/8)**
Interpretation: Strong nuclear staining for PR in 90% of neoplastic cells indicates potential benefit from Megestrol and Medroxyprogesterone. Megestrol acetate is USFDA approved for the treatment of breast and endometrial cancer. Megestrol acetate is standard of care drug for breast cancer as per NCCN guidelines (NCCN guidelines, 2021).	
AR	**Positive**
Interpretation: Nuclear staining of AR in 90% of neoplastic cells indicates potential benefit from Enzalutamide, Bicalutamide, Nilutamide, Apalutamide, Darolutamide, Leuprolide, Flutamide and Abiraterone. Enzalutamide, Nilutamide, Apalutamide, Bicalutamide, Darolutamide, Leuprolide, Flutamide and Abiraterone are USFDA approved for the treatment of metastatic prostate cancer.	
PD-L1 (antibody clone 22C3)	**Negative** **(TPS- 0%)**
Interpretation: PD-L1 (antibody clone 22C3) is non-immunoreactive in neoplastic cells. Pembrolizumab is USFDA approved for the treatment of melanoma, classical Hodgkin lymphoma, gastric or gastroesophageal junction adenocarcinoma, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) solid tumors, primary mediastinal large B-cell lymphoma, merkel cell carcinoma, cervical, renal cell, endometrial, hepatocellular, urothelial, lung, esophageal, head and neck cancers. Pembrolizumab in combination of chemotherapy is recommended as a standard of care regime for the treatment of breast cancer as per NCCN guidelines (NCCN guidelines, 2021). Cemiplimab-rwlc is USFDA approved for treatment of non-small cell lung cancer, cutaneous squamous cell carcinoma and basal cell carcinoma.	
PD-L1 (antibody clone 28-8)	**Negative** **(TPS- 0%)**
Interpretation: PD-L1 (antibody clone 28-8) is non-immunoreactive in neoplastic cells. Nivolumab is USFDA approved for melanoma, classical Hodgkin lymphoma, lung, renal cell, hepatocellular, colorectal, urothelial and head and neck cancers.	

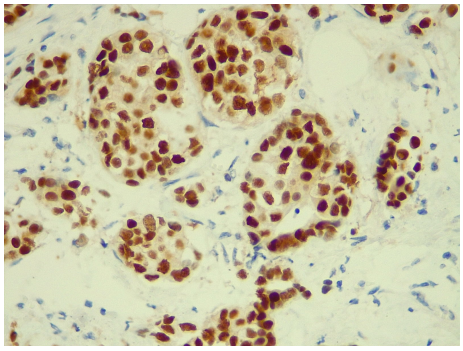
PD-L1 (antibody clone SP142)	**Negative (IC <1%)**

Interpretation: PD-L1 (antibody clone SP142) showed staining in <1% of tumor infiltrating immune cells (IC).

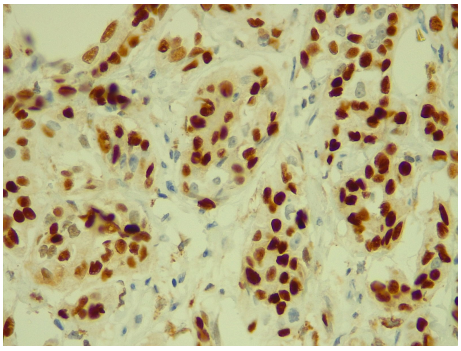
Atezolizumab is USFDA approved for the treatment of multiple tumor types, including triple negative breast cancer. Atezolizumab is recommended as a standard of care drug for the treatment of breast cancer as per NCCN guidelines (NCCN guidelines, 2021).

HER2	**Equivocal (2+)**

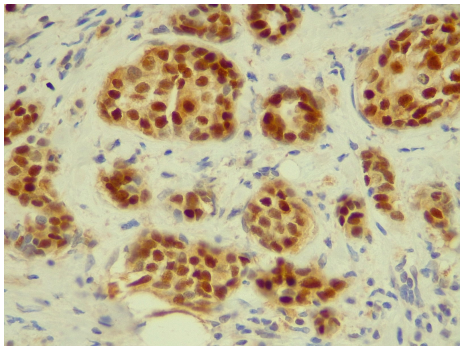
Interpretation: Weak to moderate complete membrane staining of HER2 in >10% of tumor cells.



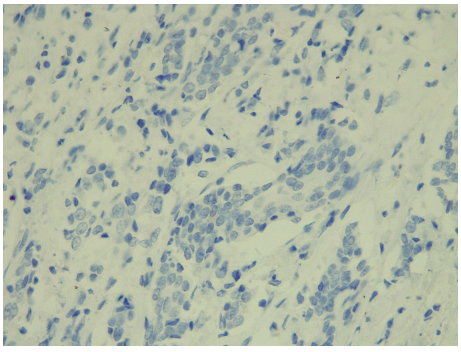
ER IHC positive



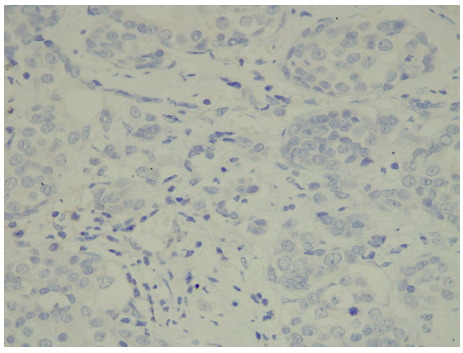
PR IHC positive



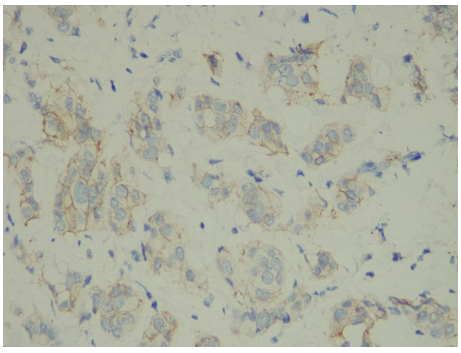
AR IHC positive



PD-L1 (antibody clone 22C3) IHC negative



PD-L1 (antibody clone 28-8) IHC negative



HER2 IHC Equivocal

FLUORESCENCE IN SITU HYBRIDIZATION (FISH) ANALYSIS

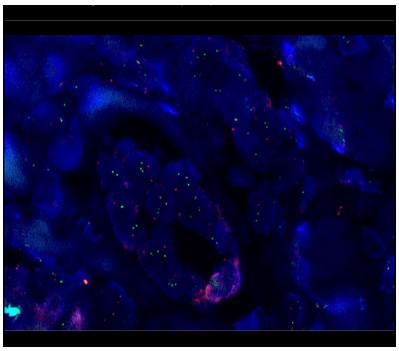
Marker	Result
HER2	Negative

Interpretation: Negative (Non-amplified) status of HER2 is indicative of lack of benefit from Ado-Trastuzumab emtansine (TDM1), Fam-Trastuzumab deruxtecan, Lapatinib, Neratinib, Tucatinib, Pertuzumab and Trastuzumab.

Ado-trastuzumab emtansine (T-DM1) is USFDA approved for treatment of HER2 positive breast cancer patients who have already been treated with trastuzumab and a taxane. It is also used in these patients if the cancer recurs after adjuvant therapy.

Fam-Trastuzumab deruxtecan is USFDA approved for treatment of adult patients with unresectable or metastatic HER2-positive breast cancer who have received two or more prior anti-HER2-based regimens in the metastatic setting.

Lapatinib, Neratinib, Tucatinib and Pertuzumab are USFDA approved for treatment of HER2 positive breast cancer. Trastuzumab is USFDA approved for HER2 positive breast cancer and HER2 overexpressing metastatic gastric or gastroesophageal junction (GEJ) adenocarcinoma.
These are also standard of care drugs for breast cancer as per NCCN guidelines (NCCN guidelines, 2021).



HER2 FISH negative

PHARMACOGENETIC REPORT FOR ONCOLOGY DRUGS - SNAPSHOT

Drug with Contraindication

None

Drug with Increased Risk of Toxicity

Cisplatin
Gemcitabine
Irinotecan
Sacituzumab govitecan

Drug with Labelled Toxicity

Belinostat
Carboplatin
Cyclophosphamide
Dabrafenib
Epirubicin
Erdafitinib
Erlotinib
Fluoropyrimidines
Gefitinib
Mercaptopurine
Methotrexate
Nilotinib
Oxaliplatin
Pazopanib
Rasburicase
Regorafenib
Thioguanine
Trametinib
Vincristine

Table 12: Analysis of pharmacogenetics markers for oncology drugs.

Gene Analysis Results	Interpretation
Cisplatin ERCC1; rs11615 GG XPC; rs2228001 GT	Evidence level : Level 1B,2B The patient has an unfavorable genotype in the analysed XPC gene variant. Patients with such genotype may have an increased risk of hearing loss, neutropenia and decreased but not non-existent risk of nephrotoxicity when treated with Cisplatin (Sakano et al., 2010; Khrunin et al., 2010; Tzvetkov et al., 2011).
Gemcitabine NT5C2; rs11598702 TT	Evidence level : Level 2B The patient has an unfavorable genotype in the analysed variant of NT5C2 gene. Patients with such genotype may have a decreased clearance of Gemcitabine and an increased risk of toxicity (Mitra et al., 2012).
Irinotecan UGT1A1; *1/*28	Evidence level : Level 1A The patient has an intermediate metabolizer status for UGT1A1. Patients with such genotype, who are treated with Irinotecan -based regimens may have an increased risk of neutropenia, diarrhea, or asthenia (Irinotecan FDA Label).
Sacituzumab govitecan UGT1A1; *1/*28	Evidence level : Level 1A The patient has an intermediate metabolizer status for UGT1A1 gene leading to reduced UGT1A1 activity. Patients with such genotype who are treated with Sacituzumab govitecan may have an increased risk of neutropenia and other adverse reactions (Sacituzumab govitecan FDA Label).
Trastuzumab FCGR2A; rs1801274 AA FCGR3A; rs396991 AA	Evidence level : Level 2B The patient has an unfavorable genotype in the investigated FCGR3A gene variant. Breast cancer patient with such genotypes may have reduced response to Trastuzumab and shorter progression-free survival in people with breast cancer (Musolino et al., 2008; Tamura et al., 2010).
5-Fluorouracil DPYD; *1/*1	Evidence level : Level 1A The patient has a normal metabolizer status for DPYD gene leading to normal DPYD activity. Labelled risk for 5-Fluorouracil toxicity. Use as directed (Fluorouracil FDA Label).
Belinostat UGT1A1; *1/*28	Evidence level : Level 1A The patient has an intermediate metabolizer status for UGT1A1 gene leading to reduced UGT1A1 activity. Such genotype does not affect the clearance of Belinostat significantly. Use as directed (Belinostat FDA Label).
Capecitabine DPYD; *1/*1	Evidence level : Level 1A The patient has a normal metabolizer status for DPYD gene leading to normal DPYD activity. Labelled risk for Capecitabine toxicity. Use as directed (Capecitabine FDA Label).
Carboplatin ERCC1; rs11615 GG MTHFR; rs1801133 AG	Evidence level : Level 2A,2B The patient has favorable genotypes in the analysed MTHFR and ERCC1 gene variants. Patients with this genotype may have an decreased risk of drug toxicity including nephrotoxicity, when treated with Carboplatin (Patiño-García et al., 2009; Khrunin et al., 2010; Tzvetkov et al., 2011).
Cyclophosphamide GSTP1; rs1695 AA	Evidence level : Level 2A The patient has a favorable genotype in the analysed variant of GSTP1 gene. Breast cancer patient with such genotype may have an increased drug response and decreased severity of toxicity when treated with Cyclophosphamide (Zhang et al., 2011).

Dabrafenib G6PD; wildtype/wildtype	Evidence level : Level 1A The patient is not a carrier of G6PD deficient genotype. Patients with such genotype who are treated with Dabrafenib may have a reduced risk of hemolysis (Dabrafenib FDA Label).
Epirubicin GSTP1; rs1695 AA	Evidence level : Level 2A The patient has a favorable genotype in the analysed variant of GSTP1 gene. Breast cancer patient with such genotype may have an increased drug response and decreased severity of toxicity when treated with Epirubicin (Zhang et al., 2011).
Erdafitinib CYP2C9; *1/*1	Evidence level : Level 1A The patient has a normal metabolizer status for CYP2C9 leading to an optimal enzyme activity. Patients with such genotype may have an optimal plasma concentration of Erdafitinib. Use as directed (Erdafitinib FDA Label).
Erlotinib UGT1A1; *1/*28	Evidence level : Level 1A The patient has an intermediate metabolizer status for UGT1A1. Patients with such genotype, who are treated with Erlotinib may have an average risk of hyperbilirubinemia. Use as directed (Erlotinib EMA Label).
Gefitinib CYP2D6; *35/*41	Evidence level : Level 1A The patient has a normal metabolizer status for CYP2D6. Patients with such genotype who are treated with Gefitinib may have normal metabolism of Gefitinib. Use as directed (Gefitinib FDA Label).
Mercaptopurine NUDT15; *1/*1 TPMT; *1/*1	Evidence level : Level 1A The patient is a normal metabolizer for TPMT and NUDT 15 genes. Patients with such metabolizer status who are treated with Mercaptopurine may have an increased inactivation of Mercaptopurine and a decreased risk of developing severe, life-threatening myelotoxicity. Use as directed. Start with normal starting dose and adjust doses of Mercaptopurine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment (Mercaptopurine FDA Label).
Methotrexate ABCB1; rs1045642 AG MTHFR; rs1801133 AG	Evidence level : Level 2A The patient has favorable genotypes in the analysed variants of ABCB1 and MTHFR genes. Patients with such genotypes when treated with Methotrexate, may have a decreased risk of toxicity (Suthandiram et al., 2014).
Nilotinib UGT1A1; *1/*28	Evidence level : Level 1A The patient has an intermediate metabolizer status for UGT1A1. Patients with such genotype, who are treated with Nilotinib may have an average risk of hyperbilirubinemia. Use as directed (Nilotinib FDA Label).
Oxaliplatin ERCC1; rs11615 GG	Evidence level : Level 2B The patient has a favorable genotype in analysed variant of ERCC1 gene. Patients with this genotype when treated with Oxaliplatin may have decreased but not non-existent risk for nephrotoxicity (Khrunin et al., 2010; Tzvetkov et al., 2011).
Pazopanib UGT1A1; *1/*28	Evidence level : Level 1A The patient has an intermediate metabolizer status for UGT1A1. Patients with such genotype, who are treated with Pazopanib may have an average risk of hyperbilirubinemia. Use as directed (Pazopanib FDA Label).

Rasburicase G6PD; wildtype/wildtype	Evidence level : Level 1A The patient is not a carrier of G6PD deficient genotype. Patients with such genotype who are treated with Rasburicase may have a reduced risk of hemolysis (Rasburicase FDA Label).
Regorafenib UGT1A1; *1/*28	Evidence level : Level 1A The patient has an intermediate metabolizer status for UGT1A1. Patients with such genotype, who are treated with Regorafenib may have an average risk of hyperbilirubinemia. Use as directed (Regorafenib EMA Label).
Tamoxifen CYP2D6; *35/*41	Evidence level : Level 1A The patient is a normal metabolizer for CYP2D6. Breast cancer patient with this metabolizer status and breast cancer show optimal metabolism of Tamoxifen resulting in optimal endoxifen concentrations, decreased likelihood of recurrence, increased event-free and recurrence-free survival, when treated with Tamoxifen in an adjuvant setting. Use as directed (CPIC Guideline for CYP2D6 and Tamoxifen Therapy).
Tegafur DPYD; *1/*1	Evidence level : Level 1A The patient has a normal metabolizer status for DPYD gene leading to normal DPYD activity. Labelled risk for Tegafur toxicity. Use as directed (Fluorouracil FDA Label).
Thioguanine NUDT15; *1/*1 TPMT; *1/*1	Evidence level : Level 1A The patient is a normal metabolizer for TPMT and NUDT 15 genes. Patients with such metabolizer status who are treated with Thioguanine may have an increased inactivation of Thioguanine and a decreased risk of developing severe, life-threatening myelotoxicity. Use as directed. Start with normal starting dose and adjust doses of Thioguanine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment (Thioguanine FDA Label).
Trametinib G6PD; wildtype/wildtype	Evidence level : Level 1A The patient is not a carrier of G6PD deficient genotype. Patients with such genotype who are treated with Trametinib may have a reduced risk of hemolysis (Trametinib FDA Label).
Vincristine CEP72; rs924607 CT	Evidence level : Level 2B The patient has a favorable genotype in the analysed variant of CEP72 gene. Patients with such genotypes who are treated with Vincristine may have a decreased, but not absent, risk of peripheral nervous system diseases (Diouf et al., 2015).

CIRCULATING TUMOR CELLS ENUMERATION

Circulating Tumor Cells (CTCs): **DETECTED**

Number of CTCs: **4 CTCs** /ml peripheral blood

CTCs are defined as EPCAM+ve, CK+ve, CD45-ve cells

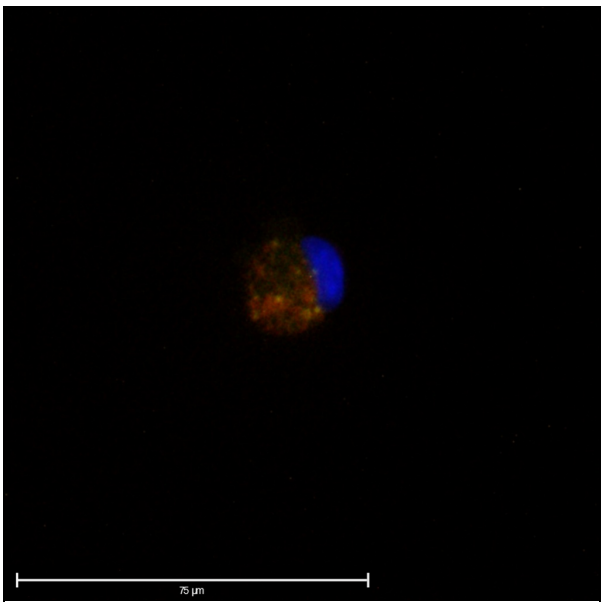


Figure 4: Fluorescent microscopic image of CTC

INTERPRETATION

4 CTCs/ ml peripheral blood detected in the submitted sample.

RECOMMENDATION

Circulating tumor cells enumeration may be done every month to monitor disease status.

IMMUNOCYTOCHEMISTRY (ICC) ANALYSIS

Markers	Result
mTOR	**Positive**

Interpretation: Positive staining of mTOR is indicative of potential benefit from Everolimus and Temsirolimus (Li et al., 2014; Rodriguez-Moreno et al., 2017; Du et al., 2018; Kuo et al., 2019).

Everolimus is USFDA approved for treatment of multiple tumor types, including hormone receptor positive, HER2 negative breast cancer.
Everolimus is also standard of care drug for breast cancer as per NCCN guidelines (NCCN guidelines, 2021).

Temsirolimus is USFDA approved for the treatment of patients with advanced renal cell carcinoma.
In a phase II study, Temsirolimus showed objective response rate of 9.2%, median time to progression of 12 weeks and tolerable safety profile in heavily pre-treated patients with locally advanced or metastatic breast cancer (n=109) (Chan et al., 2005).
In a phase II randomized 3-arm study, combination of Temsirolimus and Letrozole demonstrated a clinical benefit rate of 82% (Letrozole +10 mg daily Temsirolimus), 83% (Letrozole + 30 mg daily Temsirolimus for 5 days every 2 weeks) and 79% (Letrozole alone) in postmenopausal women with locally advanced or metastatic breast cancer (n=92). Progression free survival at one year was higher for the combination arms (69% and 62%), than for the Letrozole alone arm (48%) (Carpenter et al., 2005).

VEGFR2/KDR	**Positive**

Interpretation: Positive staining of VEGFR2/KDR is indicative of potential benefit from Axitinib, Cabozantinib, Lenvatinib, Pazopanib, Sorafenib, Sunitinib, Vandetanib, Regorafenib, Ponatinib and Ramucirumab (Paule et al., 2010; Chiang et al., 2012; Chu et al., 2013; Yamamoto et al., 2014; Daudigeos-Dubus et al., 2015; Kim et al., 2015; Tannir et al., 2017; Ortega et al., 2017; Schmidinger and Danesi, 2018; Morse et al., 2019).

Axitinib is USFDA approved for the treatment of advanced renal cell carcinoma.
In a randomized double-blind phase II study, the combination of Axitinib (AG) with Docetaxel (DOC) demonstrated an acceptable safety profile and promising anti-tumor activity as compared to DOC plus placebo (PL) in metastatic breast cancer (n=168) patients (overall response rate of 40% for AG+DOC arm and 23% for DOC+PL arm) (Rugo et al., 2007).

Cabozantinib is USFDA approved for the treatment of hepatocellular carcinoma, advanced renal cell carcinoma and thyroid cancer.
In a phase II placebo-controlled randomized discontinuation study, Cabozantinib demonstrated clinical activity with objective response rate of 13.6% and disease control rate of 46.7% at week 12 in heavily pretreated metastatic breast cancer patients (n=45) (Tolaney et al., 2016).

Lenvatinib is USFDA approved for the treatment of endometrial, hepatocellular carcinoma, advanced renal cell carcinoma and thyroid cancer.
In a phase Ib/II trial, combination of Lenvatinib and Letrozole showed significant anti-tumor activity with overall disease control rate of 93.8% and stable disease rate of 43.8% in postmenopausal women with hormone receptor positive, locally advanced/metastatic breast cancer (n=16) (NCT02562118, Lim et al., 2019).

Pazopanib is USFDA approved for the treatment of advanced renal cell carcinoma and soft tissue sarcoma.
In a phase II study, Pazopanib was well tolerated and showed a clinical benefit rate of 26% in 19 evaluable patients with recurrent or metastatic invasive breast carcinoma (Taylor et al., 2009).

Sorafenib is USFDA approved for the treatment of advanced renal cell, hepatocellular and thyroid cancer.
In a phase I study, combination of Sorafenib and Pemetrexed showed a stable disease of 45% and overall response rate of 15% in 37 evaluable patients with breast cancer (Poklepovic et al., 2016).

Sunitinib is USFDA approved for the treatment of advanced renal cell carcinoma, gastrointestinal stromal tumor and pancreatic neuroendocrine tumors.
In a phase II study, Sunitinib malate was found to be active with overall response rate of 11%, median time to progression of 10 weeks and overall survival of 38 weeks in patients with metastatic breast cancer (MBC) previously treated with an anthracycline and a taxane (n=64) (Burstein et al., 2008).
Sunitinib in combination with Trastuzumab demonstrated antitumor activity with objective response rate of 37% and clinical benefit rate of 56% in a phase II study on advanced breast cancer patients (n=57). Among these, the patients who were treatment-naive or had only received prior adjuvant treatment showed an objective response rate of 44% and clinical benefit rate of 59% (Bachelot et al., 2014).

Vandetanib is USFDA approved for the treatment of medullary thyroid cancer.
In a phase I study, the combination of Vandetanib and continuous oral metronomic Cyclophosphamide and Methotrexate in 20 response-evaluable metastatic breast cancer patients demonstrated modest clinical activity with partial response in 10%, stable disease in 65%, of which 15% showed a stable disease for ≥ 6 months (Mayer et al., 2012).

Ramucirumab is USFDA approved for the treatment of non-small cell lung cancer, stomach adenocarcinoma or gastroesophageal junction adenocarcinoma and colorectal cancer.
In a multicenter phase Ib study, the combination of Ramucirumab and Docetaxel was tolerable in breast cancer patients with 4 out of 7 showing partial response (Masuda et al., 2016).

Regorafenib is USFDA approved for the treatment of colorectal, hepatocellular cancers and gastrointestinal stromal tumors.
In a pre-clinical study, Regorafenib reduced cell proliferation and enhanced radiosensitivity in breast cancer cells (Mehta et al., 2020).

Ponatinib is USFDA approved for the treatment of acute lymphoblastic leukemia and chronic myelogenous leukemia.
In a pre-clinical study, Ponatinib significantly inhibited the migration and mammosphere formation of breast cancer cells in vitro and blocked breast cancer lung metastasis in multiple in vivo models (Gozgit et al., 2012; Shao et al., 2019).

VEGFR1/FLT1	Positive

Interpretation: Positive staining of VEGFR1/FLT1 is indicative of potential benefit from Axitinib, Lenvatinib, Cabozantinib, Pazopanib, Sorafenib, Sunitinib, Regorafenib and Ponatinib (Paule et al., 2010; Chiang et al., 2012; Chu et al., 2013; Yamamoto et al., 2014; Daudigeos-Dubus et al., 2015; Kim et al., 2015; Tannir et al., 2017; Ortega et al., 2017; Schmidinger and Danesi, 2018; Morse et al., 2019).

Kindly refer to USFDA label and/or studies for Axitinib, Lenvatinib, Cabozantinib, Pazopanib, Sorafenib, Sunitinib, Regorafenib and Ponatinib mentioned earlier.

EGFR	Positive

Interpretation: Positive staining of EGFR is indicative of potential benefit from Cetuximab, Panitumumab and Necitumumab (Douillard et al., 2014; Trivedi et al., 2016; Thakur and Wozniak, 2017; Caratelli et al., 2020).

Cetuximab is USFDA approved for the treatment of head and neck and colorectal cancer.
In a phase I study, Cetuximab / Paclitaxel in patients with advanced stage breast cancer, the combination showed stable disease in 2 among 10 evaluable patients (Modi et al., 2006).

Panitumumab is USFDA approved for treatment of colorectal cancer.
In a clinical study, Panitumumab plus neoadjuvant chemotherapy in patients with primary HER2-negative inflammatory breast cancer (IBC) (n=40) showed an overall pathologic complete response (pCR) rate of 28% (A pCR rate of 42% in triple-negative IBC and 14% in hormone receptor-positive/HER2-negative IBC patients) (Matsuda et al., 2018).

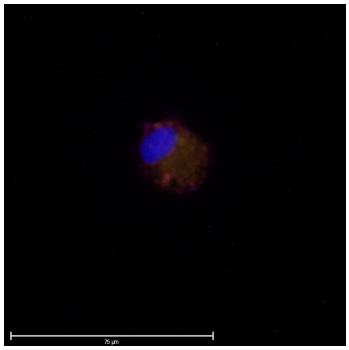
Necitumumab is USFDA approved for the treatment of squamous non-small cell lung cancer.
The efficacy of Necitumumab in breast cancer is not well evaluated.

VEGFA	**Negative**

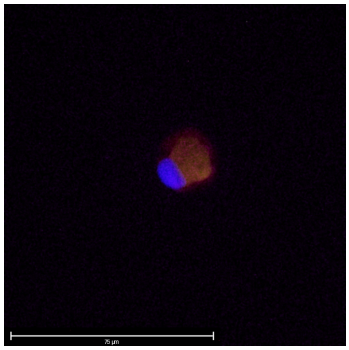
Interpretation: No staining of VEGFA is indicative of potential lack of benefit from Bevacizumab and Ziv-Aflibercept (Weickhardt et al., 2011; Tsai et al., 2015).

However, simultaneous overexpression of VEGFA is indicative of potential benefit from Bevacizumab and Ziv-Aflibercept.

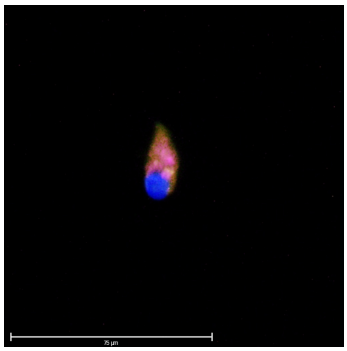
Kindly refer to USFDA label and/or studies for Bevacizumab and Ziv Aflibercept mentioned earlier.



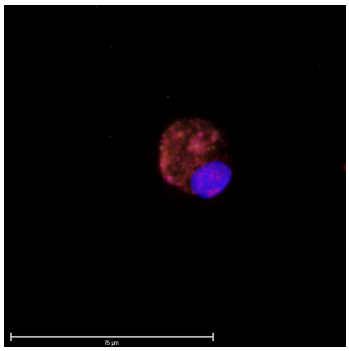
mTOR ICC positive



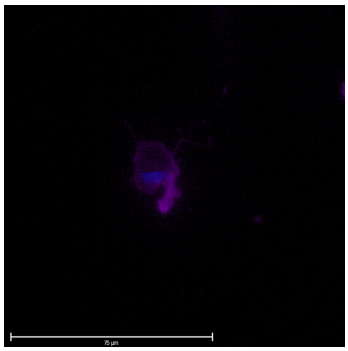
VEGFR2/KDR ICC positive



VEGFR1/FLT1 ICC positive



EGFR ICC positive

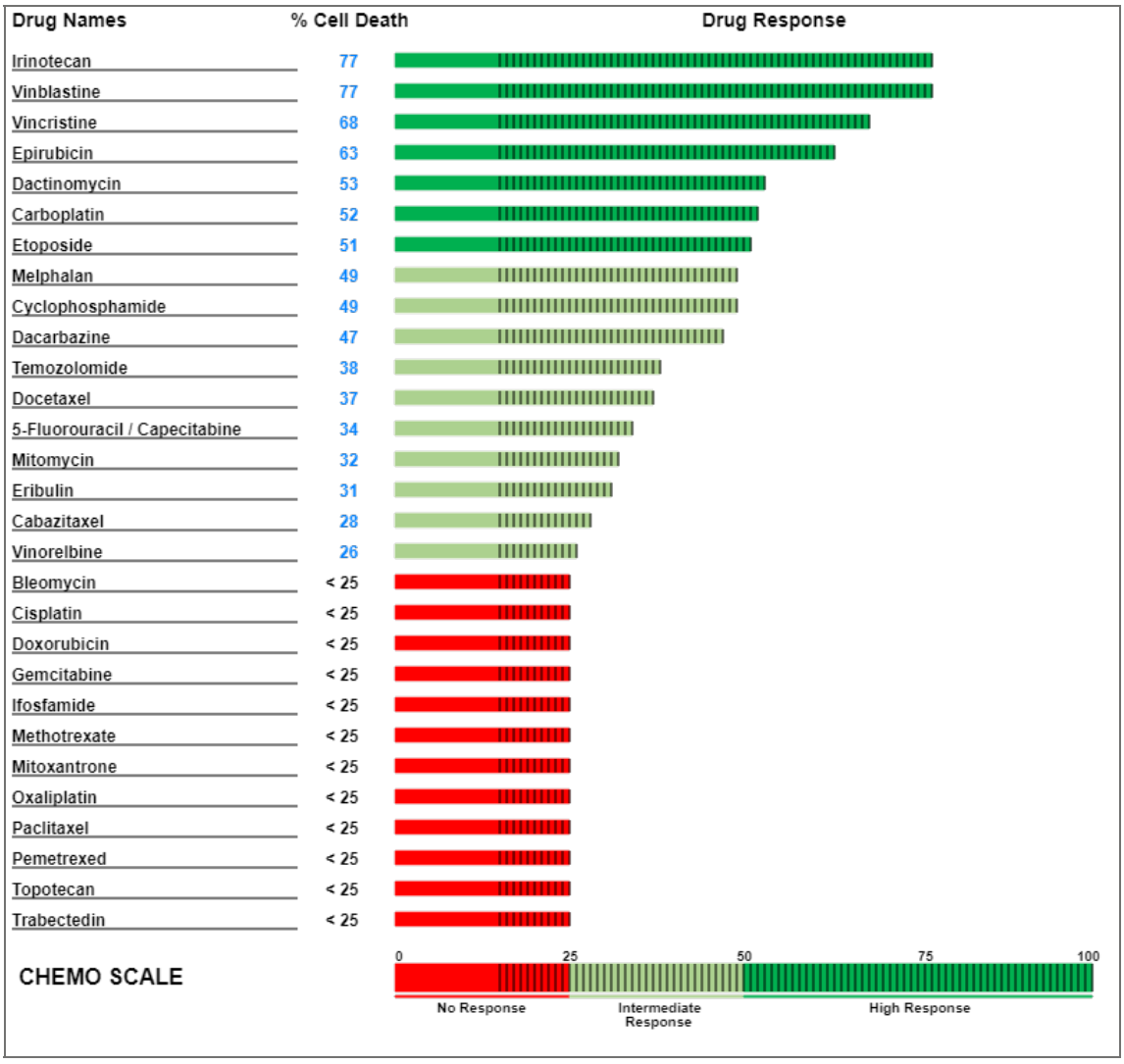


VEGFA ICC negative

CHEMOSENSITIVITY ANALYSIS

Chemosensitivity assay performed on cultured circulating tumor and its associated cells indicates the effectiveness of chemotherapeutic drugs in descending order of efficacy. Depending on viable tumor cell availability, single drugs mentioned below were tested.

% Cell Death (%CD)	> 50%	25 - 50%	< 25%
Response Level	High Response	Intermediate Response	No response



RESPONSE TO REPURPOSED DRUGS

Sr.No.	Drug/Drugs Combination	% Cell Death
1	Diflunisal	54
2	Artesunate	53
3	Cannabidiol	49
4	Hypericin	49
5	Apigenin	47
6	Epigallocatechingallate	42
7	Dichloroacetate	37
8	Atorvastatin	33
9	Glutathione	29
10	Celecoxib	26
11	Vitamin C	25
12	Aspirin	22
13	Glibenclamide	22
14	Bromelain	21
15	Melatonin	19
16	Resveratrol	13
17	Propranolol	13
18	Doxycycline	10
19	Pantoprazole	10
20	Metformin	1
21	Amygdalin	0
22	Curcumin	0
23	Chloroquine	0

VARIANT ALLELE FRACTION AND COVERAGE (TUMOR TISSUE)

Variant (Transcript ID)	Genomic co-ordinates	Allele fraction	Coverage (X)
MBD1 (NM_001204136.1) c.613G>A, p.G205R	chr18: 47802255C>T	5.3	2008
RECQL4 (NM_004260.4) c.1412A>C, p.Q471P	chr8: 145740605T>G	7.5	715
NOTCH1 (NM_017617.4) c.3901G>A, p.G1301R	chr9: 139401168C>T	5.07	670
NF1 (NM_001042492.2) c.2131C>T, p.R711C	chr17: 29553582C>T	4.62	238

Due to minimum coverage or no sequence, the presence or absence of variants contained within the target regions listed below could not be meaningfully assessed.

PIK3CA [NM_006218], Exon 5, Codons 336-353; CTNNB1 [NM_001904], Exon 3, Codons 5-46; MAP2K2 [NM_030662], Exon 6, Codons 194-221; ARID1A [NM_006015], Exon 1, Codons 151-192ATM [NM_000051], Exon 51, Codons 2541-2543ATR [NM_001184], Exon 15, Codons 1052-1057CDK12 [NM_016507], Exon 1,Codons 43-79PALB2 [NM_024675], Exon 4, Codons 71-79PTCH1 [NM_000264], Exon 1, Codons 1-30TERT [NM_198253], Exon 1, Codons 14-58

VARIANT ALLELE FRACTION AND COVERAGE (CELL FREE NUCLEIC ACIDS)

Variant (Transcript ID)	Genomic co-ordinates	Allele fraction	Coverage (X)
None			

FUSION DETAILS

Gene	Variant ID	Molecular Counts
FGFR1	WHSC1L1-FGFR1 (W1F2)	220

CRITERIA FOR CLASSIFICATION OF SOMATIC VARIANTS

The criteria/guidance used in this report is in accordance with the guidelines provided by the American College of Medical Genetics and Genomics (ACMG) for the interpretation and reporting of sequence variants in cancer. Somatic sequence variations are categorized into four tiers based on their clinical significance (Li et al., 2017).

- **Tier I:** Variants/biomarkers with strong clinical significance (therapeutic, prognostic and/or diagnostic)
  - **Level A evidence:** FDA approved therapies or standard guidelines for a specific tumor type.
  - **Level B evidence:** Statistically significant studies with consensus for specific tumor type.
- **Tier II:** Biomarkers with potential clinical significance (therapeutic, prognostic and/or diagnostic)
  - **Level C evidence:** FDA approved therapies or standard guidelines for a different tumor type (off-label use of the drug). An inclusion criteria for clinical trials.
  - **Level D evidence:** No consensus among different studies.
- **Tier III:** Biomarker whose association with cancer is not evident from available literature and is not frequently present in general population.
- **Tier IV:** Biomarker whose association with cancer has not been reported till date and is frequently present in general population. This category of variants is not included in this report as per guidelines.

CRITERIA OF CLASSIFICATION FOR PHARMACOGENETIC ANALYSIS

Each variant-drug combination can be graded based on the measure of confidence in the association and the strength of prescribing recommendation.

- **Level 1:** Evidence based on pharmacogenetics guidelines or well-established association studies
- **Level 2:** Evidence of moderate variant-drug association from studies.
- **Level 3:** Evidence suggests no consensus among different studies.

DRUG METABOLIZER STATUS CATEGORIES

Based on the different combination of haplotypes an individual inherits in each drug metabolizing gene, a drug metabolizer status can be predicted. There are 4 different drug metabolizer status types:

- **Poor Metabolizers (also called "PM"),** Poor metabolizers have two non-functional alleles and therefore have little to no enzyme activity.
- **Intermediate Metabolizers (also called "IM"),** Intermediate metabolizers have one non-functional allele and one normally functioning allele, and therefore have decreased enzyme activity.
- **Normal Metabolizers (also called "NM")** Normal metabolizers have 2 normally functioning alleles and therefore have normal enzyme activity.
- **Ultra-Rapid Metabolizers (also called "UM").** Ultra-rapid metabolizers have one or more alleles which result in increased enzyme activity compared to extensive metabolizers.

The impact of each metabolizer type on medication response depends on the role of the enzyme in the metabolism of the specific drug in question. For example, for a drug that is inactivated by the enzyme, an ultra-rapid metabolizer may need a higher dose of the drug to reach a therapeutic range while for another drug, that is activated by the enzyme; ultra-rapid metabolizer status may be associated with increased exposure to the drug and therefore an increased risk of adverse drug reactions.

CRITERIA FOR CLASSIFICATION OF GERMLINE VARIANTS

The American College of Medical Genetics and Genomics (ACMG) developed guidance for the interpretation of sequence variants and recommended the use of following specific standard terminology to describe variants identified in genes that cause Mendelian disorders (Richards et al.,2015).

- **Pathogenic:** Functional or expression evidence suggests deleterious effect on gene function.
- **Likely Pathogenic/Probably Deleterious:** Limited or no functional evidence available, but overall biological expectations suggestive of deleterious effect.
- **Variants of unknown significance (VUS):** Little or nothing has been reported on this variant or its effects.
- **Likely Benign:** The variant has been seen in cases, but also in controls. Variant may be present in a high percentage of the population, and may be present in a non-conserved region.
- **Benign:** Established in the literature as a variant that is not associated with Mendelian (single-gene inherited) disease, or known to have an allele frequency that is far too high to be compatible with the prevalence of disease, mode of inheritance and penetrance patterns known for that condition.

GENES ANALYZED IN TUMOR TISSUE ANALYSIS

Single Nucleotide Variations (SNVs) and InDels:

ARAF, BRCA1, BRCA2, CDKN1B, FANCI, H3F3A, HIST1H3B, KNSTRN, MAGOH, MAX, MED12, NOTCH3, POLE, RAC1, RAD51, RAD51B, RAD51C, RAD51D, RHEB, RHOA, RNF43, SLX4, SPOP, STAT3, U2AF1

SNVs, InDels and Copy Number Variations (CNVs):

ABL1, ABL2, ACVR2A, ADAMTS20, AFF1, AFF3, AKAP9, AKT1, AKT2, AKT3, ALK, APC, AR, ARID1A, ARID2, ARNT, ASXL1, ATF1, ATM, ATR, ATRX, AURKA, AURKB, AURKC, AXL, BAI3, BAP1, BCL10, BCL11A, BCL11B, BCL2, BCL2L1, BCL2L2, BCL3, BCL6, BCL9, BCR, BIRC2, BIRC3, BIRC5, BLM, BLNK, BMPR1A, BRAF, BRD3, BRIP1, BTK, BUB1B, CARD11, CASC5, CBL, CCND1, CCND2, CCND3, CCNE1, CD79A, CD79B, CDC73, CDH1, CDH11, CDH2, CDH20, CDH5, CDK12, CDK2, CDK4, CDK6, CDK8, CDKN2A, CDKN2B, CDKN2C, CEBPA, CHEK1, CHEK2, CIC, CKS1B, CMPK1, COL1A1, CRBN, CREB1, CREBBP, CRKL, CRTC1, CSF1R, CSMD3, CTNNA1, CTNNB1, CYLD, CYP2C19, CYP2D6, DAXX, DCC, DDB2, DDIT3, DDR2, DEK, DICER1, DNMT3A, DPYD, DST, EGFR, EML4, EP300, EP400, EPHA3, EPHA7, EPHB1, EPHB4, EPHB6, ERBB2, ERBB3, ERBB4, ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERG, ESR1, ETS1, ETV1, ETV4, EXT1, EXT2, EZH2, FAM123B, FANCA, FANCC, FANCD2, FANCF, FANCG, FAS, FBXW7, FGF19, FGF3, FGFR1, FGFR2, FGFR3, FGFR4, FH, FLCN, FLI1, FLT1, FLT3, FLT4, FN1, FOXL2, FOXO1, FOXO3, FOXP1, FOXP4, FZR1, G6PD, GATA1, GATA2, GATA3, GDNF, GNA11, GNAQ, GNAS, GPR124, GRM8, GUCY1A2, HCAR1, HIF1A, HLF, HNF1A, HOOK3, HRAS, HSP90AA1, HSP90AB1, ICK, IDH1, IDH2, IGF1R, IGF2, IGF2R, IKBKB, IKBKE, IKZF1, IL2, IL21R, IL6ST, IL7R, ING4, IRF4, IRS2, ITGA10, ITGA9, ITGB2, ITGB3, JAK1, JAK2, JAK3, JUN, KAT6A, KAT6B, KDM5C, KDM6A, KDR, KEAP1, KIT, KLF6, KRAS, LAMP1, LCK, LIFR, LPHN3, LPP, LRP1B, LTF, LTK, MAF, MAFB, MAGEA1, MAGI1, MALT1, MAML2, MAP2K1, MAP2K2, MAP2K4, MAP3K7, MAPK1, MAPK8, MARK1, MARK4, MBD1, MCL1, MDM2, MDM4, MEN1, MET, MITF, MLH1, MLL, MLL2, MLL3, MLLT10, MMP2, MN1, MPL, MRE11A, MSH2, MSH6, MTOR, MTR, MTRR, MUC1, MUTYH, MYB, MYC, MYCL, MYCL1, MYCN, MYD88, MYH11, MYH9, NBN, NCOA1, NCOA2, NCOA4, NF1, NF2, NFE2L2, NFKB1, NFKB2, NIN, NKX2-1, NLRP1, NOTCH1, NOTCH2, NOTCH4, NPM1, NRAS, NSD1, NTRK1, NTRK2, NTRK3, NUMA1, NUP214, NUP98, PAK3, PALB2, PARP1, PAX3, PAX5, PAX7, PAX8, PBRM1, PBX1, PDE4DIP, PDGFB, PDGFRA, PDGFRB, PER1, PGAP3, PHOX2B, PIK3C2B, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PIK3R1, PIK3R2, PIM1, PKHD1, PLAG1, PLCG1, PLEKHG5, PML, PMS1, PMS2, POT1, POU5F1, PPARG, PPP2R1A, PRDM1, PRKAR1A, PRKDC, PSIP1, PTCH1, PTEN, PTGS2, PTPN11, PTPRD, PTPRT, RAD50, RAF1, RALGDS, RARA, RB1, RECQL4, REL, RET, RHOH, RICTOR, RNASEL, RNF2, RNF213, ROS1, RPS6KA2, RRM1, RUNX1, RUNX1T1, SAMD9, SBDS, SDHA, SDHB, SDHC, SDHD, SEPT9, SETD2, SF3B1, SGK1, SH2D1A, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SMUG1, SOCS1, SOX11, SOX2, SRC, SSX1, STK11, STK36, SUFU, SYK, SYNE1, TAF1, TAF1L, TAL1, TBX22, TCF12, TCF3, TCF7L1, TCF7L2, TCL1A, TERT, TET1, TET2, TFE3, TGFBR2, TGM7, THBS1, TIMP3, TLR4, TLX1, TNFAIP3, TNFRSF14, TNK2, TOP1, TP53, TPR, TRIM24, TRIM33, TRIP11, TRRAP, TSC1, TSC2, TSHR, UBR5, UGT1A1, USP9X, VHL, WAS, WHSC1, WRN, WT1, XPA, XPC, XPO1, XRCC2, ZNF384, ZNF521

Gene Fusions (Inter- and Intragenic):

AKT2, ALK, AR, AXL, BRAF, BRCA1, BRCA2, CDKN2A, EGFR, ERBB2, ERBB4, ERG, ESR1, ETV1, ETV4, ETV5, FGFR1, FGFR2, FGFR3, FGR, FLT3, JAK2, KRAS, MDM4, MET, MYB, MYBL1, NF1, NOTCH1, NOTCH4, NRG1, NTRK1, NTRK2, NTRK3, NUTM1, PDGFRA, PDGFRB, PIK3CA, PPARG, PRKACA, PRKACB, PTEN, RAD51B, RAF1, RB1, RELA, RET, ROS1, RSPO2, RSPO3, TERT.

GENES ANALYZED IN CELL FREE NUCLEIC ACIDS ANALYSIS

SNV Genes: AKT1, ALK, APC, AR, ARAF, BRAF, CHEK2, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ESR1, FBXW7, FGFR1, FGFR2, FGFR3, FGFR4, FLT3, GNA11, GNAQ, GNAS, HRAS, IDH1, IDH2, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, NTRK1, NTRK3, PDGFRA, PIK3CA, PTEN, RAF1, RET, ROS1, SF3B1, SMAD4, SMO, TP53.
Fusion Genes: ALK, BRAF, ERG, ETV1, FGFR1, FGFR2, FGFR3, MET, NTRK1, NTRK3, RET, ROS1.
CNV Genes: CCND1, CCND2, CCND3, CDK4, CDK6, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, MET, MYC.

TUMOR TISSUE GENE EXPRESSION ANALYSIS

Tumor tissue RNA: 20802 mRNA

BIOMARKERS ANALYZED FOR MICROSATELLITE INSTABILITY (MSI)

MLH1, MSH2, MSH6, PMS2

BRCA1/2 MUTATION ANALYSIS

BRCA1 and BRCA2 genes sequencing; deletion & duplication (MLPA)

GENES ANALYZED FOR PHARMACOGENETICS

GENES	VARIANTS ANALYZED
ABCB1	c.3435T>C
CEP72	n.366+1469G>A
CYP2C9	*1, *2, *3, *4, *5, *6, *7, *8, *9, *10, *11, *12, *13, *14, *15, *16, *18, *35
CYP2D6	*1, *2, *3, *4, *6, *7, *8, *9, *10, *11, *12, *15, *17, *19, *20, *29, *35, *38, *41, *42, *44, *56 and *5, XN
DPYD	*1, *10, *11, *12, *13, *2A, *3, *4, *5, *6, *7, *8, *9A, *9B, c.1024G>A, c.1057C>T, c.1314T>G, c.1896T>C, c.2279C>T, c.2639G>T, c.2846A>T, c.2872A>G, c.2933A>G, c.496A>G, c.557A>G, c.61C>T, c.62G>A, c.1129-5923C>G (HapB3), c.1236G>A (HapB3)
ERCC1	c.354T>C
FCGR2A	c.497A>G
FCGR3A	c.526T>G

G6PD	Gaohe; Sunderland; Orissa; Murcia Oristano; Ube Konan; Vancouver; Santa Maria; G6PD A-680T_376G; Mt Sinai; Sierra Leone; G6PD A-968C_376G; Ananindeua; Taipei Chinese-3; Malaga; Mediterranean Haplotype; Mediterranean_Dallas_Panama_Sassari_Cagliari_Birmingham; Coimbra Shunde; Sibari; Cincinnati; Minnesota_Marion_Gastonia_LeJeune; Nanning; Chinese-5; Ierapetra; Serres; Iowa_Walter Reed_Springfield; Guadalajara; Riverside; Asahi; Ludhiana; Pawnee; Surabaya; Japan_Shinagawa; Puerto Limon; Alhambra; Nashville_Anaheim_Portici; Beverly Hills_Genova_Iwate_Niigata_Yamaguchi; Tomah; Montpellier; Loma Linda; Mira d'Aire; Chatham; Rehevot; Kalyan-Kerala_Jamnaga_Rohini; Viangchan_Jammu; Seattle_Lodi_Modena_Ferrara II_Athens-like; Aveiro; Nilgiri; Nankang; Ilesha; Crispim; Sao Borja; Lagosanto; Namouru; A-202A_376G; Hechi; Metaponto; Aures; Acrokorinthos; A; Vanua Lava; Mediterranean_Dallas_Panama_Sassari_Cagliari_Birmingham; wildtype; 202G>A_376A>G_1264C>G
GSTP1	c.313A>G
MTHFR	c.665C>T
NT5C2	c.175+1178A>G
NUDT15	*1, *2, *3, *4, *5, *6
TPMT	*1, *2, *3A, *3B, *3C, *4, *5, *6, *7, *8, *9, *10, *11, *12, *13, *14, *15, *16, *20, *21, *23, *24, *25, *26, *29, *31, *32, *33, *34, *37
UGT1A1	*1, *28
XPC	c.2815C>A

DRUGS TESTED IN CHEMOSENSITIVITY ANALYSIS

5-Fluorouracil, Amygdalin, Apigenin, Artesunate, Aspirin, Atorvastatin, Bleomycin, Bromelain, Cabazitaxel, Cannabidiol, Carboplatin, Celecoxib, Chloroquine, Cisplatin, Curcumin, Cyclophosphamide, Dacarbazine, Dactinomycin, Dichloroacetate, Diflunisal, Docetaxel, Doxorubicin, Doxycycline, Epigallocatechin-gallate, Epirubicin , Eribulin, Etoposide, Gemcitabine, Glibenclamide, Glutathione, Hypericin, Ifosfamide, Irinotecan, Melatonin, Melphalan, Metformin, Methotrexate, Mitomycin, Mitoxantrone, Oxaliplatin, Paclitaxel, Pantoprazole, Pemetrexed, Propranolol, Resveratrol, Temozolomide, Topotecan, Trabectedin, Vinblastine, Vincristine, Vinorelbine, Vitamin C

ANTIBODY DETAILS - IMMUNOCYTOCHEMISTRY (ICC) ANALYSIS

Marker	Clone
EPCAM	REA764
CK	REA831
CD45	REA747
mTOR	Polyclonal
VEGFR1	REA569
VEGFR2	REA1116
VEGFA	JH121
EGFR	EP22

ANTIBODY DETAILS - IMMUNOHISTOCHEMISTRY (IHC) ANALYSIS

Marker	Clone	Vendor	Visualization System
ER	Clone EP1	Dako	Polymer Detection System
PR	PgR 636	Dako	
AR	AR441	Dako	
PD-L1	28-8	Dako	
PD-L1	22C3	Dako	
Her 2	NA	Dako	
MLH1	ES05	Dako	
MSH2	FE11	Dako	
MSH6	EP49	Dako	
PMS2	EP51	Dako	
PD-L1	SP142	Ventana	Optiview Universal DAB Detection Kit and Optiview Amplification Kit (on Ventana Benchmark XT platform)

Scoring:

ER/PR: Allred scoring

Proportion score (PS)	% of cells positive for ER
0	0
1	< 1%
2	1 - 10%
3	11 - 33%
4	34 - 66%
5	67 - 100%

Intensity score	Staining intensity (SI)
0	Negative
1	Weak
2	Moderate
3	Strong

Allred score (PS+SI)	Interpretation
0 - 2	Negative
3 - 8	Positive

HER2:

PD-L1 INTERPRETATION:

PD-L1 (Clone: 22C3): PD-L1 protein expression is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining. PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (Pembrolizumab). According to PD-L1 IHC 22C3 pharmDx literature, specimen should be considered PD-L1 positive if TPS ≥ 50% of the viable tumor cells exhibit membrane staining at any intensity. However, an open-label, phase 3 KEYNOTE-042 study proved Pembrolizumab to be superior over platinum-based chemotherapy in patients with previously untreated advanced/metastatic NSCLC without sensitizing EGFR or ALK alterations and a PD-L1 TPS ≥1%.

Phase 3 trial of Cemiplimab versus platinum-based chemotherapies showed that Cemiplimab is indicated for the first-line treatment of patients with NSCLC whose tumors have high PD-L1 expression [Tumor Proportion Score (TPS) ≥ 50%] as determined by an FDA-approved test, with no EGFR, ALK or ROS1 aberrations (NCT03088540).

#PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using monoclonal Mouse Anti-PD-L1, Clone 22C3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) tissue.

PD-L1 (Clone: 28-8): PD-L1 protein expression is defined as the percentage of tumor cells exhibiting positive complete circumferential or partial linear plasma membrane staining at any intensity. Cytoplasmic staining, if present, is not considered positive for scoring purposes. Non-malignant cells and immune cells (e.g. such as infiltrating lymphocytes or macrophages) may also stain with PD-L1; however, these are not included in the scoring for the determination of PD-L1 positivity.

PD-L1 expression cut off for non-squamous non-small cell lung carcinoma is ≥1%. PD-L1 expression as detected by PD-L1 28-8 pharmDx in non-squamous NSCLC may be associated with enhanced survival from OPDIVO® (Nivolumab).

PD-L1 expression cut off is ≥1% for squamous cell carcinoma of the head and neck (SCCHN), Urothelial carcinoma (UC) and melanoma. Detection of PD-L1 expressing tumor cells in SCCHN and UC patient specimens may indicate an enhanced survival benefit to OPDIVO® (Nivolumab) treatment for the patients. Clinical study CHECKMATE-067 investigated the clinical validity of PD-L1 IHC 28-8 pharmDx for the assessment of PD-L1 expression in melanoma patients treated with OPDIVO®, OPDIVO® in combination with YERVOY® (Ipilimumab), and YERVOY® alone.

#PD-L1 IHC 28-8 pharmDx is a qualitative immunohistochemical assay using monoclonal Rabbit Anti-PD-L1, Clone 28-8 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-squamous non-small cell lung cancer (NSCLC), squamous cell carcinoma of the head and neck (SCCHN), urothelial carcinoma (UC), and melanoma tissues.

PD-L1 (Clone: SP142): Ventana PD-L1 (SP142) assay is a qualitative immunohistochemical assay using rabbit monoclonal anti-PD-L1 antibody (clone SP142), intended for use in the assessment of the programmed death-ligand 1 (PD-L1) protein in tumor cells and tumor infiltrating immune cells in the formalin-fixed, paraffin-embedded (FFPE) tissues with Optiview DAB Detection Kit and Optiview Amplification Kit on a BenchMark XT platform.

Determination of PD-L1 status is indication-specific and evaluation is based on either the proportion of tumor area occupied by PD-L1 expressing tumor-infiltrating immune cells (% IC) of any intensity or the percentage of PD-L1 expressing tumor cells (% TC) of any intensity.

VENTANA PD-L1 (SP142) Assay is indicated as an aid in identifying patients for treatment with Tecentriq (Atezolizumab). Cut-off of PD-L1 (SP142) in breast carcinoma is ≥ 1% IC.

PROBE DETAILS - FLUORESCENCE IN SITU HYBRIDIZATION (FISH) ANALYSIS

DETAILS OF PROBE USED:

- **ZytoLight ® SPEC ERBB2/CEN 17 Dual Color Probe:** The SPEC **ERBB2/CEN 17** Dual Color Probe is a mixture of an **orange** fluorochrome direct labeled CEN 17 probe specific for the alpha satellite centromeric region of **chromosome 17** (D17Z1) and a **green** fluorochrome direct labeled SPEC ERBB2 probe specific for the chromosomal region 17q12-q21.1 harboring the **ERBB2 gene**.

SCORING:

ASCO-CAP 2018 HER2 Testing Guidelines:

Interpretation	HER2/CEN17 Ratio	HER2 Copy Number Signals Per Cell	HER2 Immunohistochemistry
Amplification	≥ 2.0	≥ 4.0	Any Score
	≥ 2.0	< 4.0	3+
	< 2.0	≥ 6.0	2+ to 3+
	< 2.0	≥ 4.0 and < 60	3+
Non-amplification	< 2.0	< 4.0	Any Score
	≥ 2.0	< 4.0	0 to 2+
	< 2.0	≥ 6.0	0 to 1+
	< 2.0	≥ 4.0 and < 60	0 to 2+

METHODS AND LIMITATIONS

Tumor tissue analysis:

Tumor tissue was analyzed for mutation detection using semiconductor based Next Generation Sequencing technology. High quality tumor tissue DNA and RNA extracted from the submitted specimen was subjected to target enrichment by multiplex PCR amplification using panel targeting 452 Oncogenes and Tumor suppressor genes. (see gene list in the 'Genes analyzed section'). Enriched DNA sequences were ligated with platform specific adaptor molecules and was sequenced on using semiconductor chip. Sequenced data was aligned with the human genome (hg19), analyzed at 500x minimum average depth using a customized in-house pipeline DCGL NGS Bioinformatics Pipeline v7.9, DCGL NGS Bioinformatics Pipeline v9.7 and DCGL NGS Bioinformatics Pipeline v10.1 designed to accurately detect the rare somatic variants. Analytical Validation of this assay shown sensitivity of 99.99% and specificity 99.99%.

Cell free nucleic acids analysis:

Cell free nucleic acids were analyzed for mutation and fusion detection using semiconductor based Next Generation Sequencing technology. Cell free nucleic acids extracted from the plasma of submitted specimen was subjected to target enrichment by multiplex PCR amplification using panel of genes (see gene list in the 'Genes analyzed section'). Enriched DNA sequences were ligated with platform specific adaptor molecules and were sequenced on semiconductor P1 chip. Sequenced data was aligned with the human genome (hg19), analyzed at 17000x minimum average depth using a customized in-house pipeline DCGL NGS Bioinformatics Pipeline v11.7, designed to accurately detect the rare somatic variants. Lower limit of detection of the mutations targeted is 0.1% and variants present below 0.1% may not be detectable with this assay, whereas analytical sensitivity is 97.14% and specificity is 93.75% for SNV, CNV and Fusion.

A negative test result does not exclude the possibility of mutations being present in the test sample probably due to the reads representing minor allele fraction is below the detectable limit of the assay or other limiting technical/analytical factors.

The clinical sensitivity of most assays for detection of alterations in cell free nucleic acids is limited as compared with tumor tissue-based testing. This may result from a high ratio of normal to tumor DNA or excess degradation of cell free nucleic acids or may simply reflect the biologic heterogeneity of solid tumors, some of which may shed abundant nucleic acid into the circulation and others that may not. Tumor type, size, disease stage, sites of metastasis, histologic grade, or other features may also affect levels, however, much remains to be elucidated.

Tumor tissue mRNA analysis:

Tumor tissue was analyzed for mRNA expression detection using semiconductor based Next Generation Sequencing method. High quality RNA was extracted from the submitted specimens along with healthy tissue sample and subjected to mRNA library preparation using a targeted panel. RNA sequencing was performed to achieve at least 4 million mappable high-quality reads for the paired analysis. Sequence reads were aligned to the hg19 transcriptome reference sequence in Torrent Suite Software using the Ion Torrent Mapping Alignment Program. Differential Gene Expression analysis was performed using a customized in-house pipeline DCGL NGS Bioinformatics Pipeline v 5.7 designed to detect the Significantly expressed genes.

Multiplex Ligation-dependent Probe Amplification (MLPA) assay:

The simultaneous analysis was performed by the Multiplex Ligation-dependent Probe Amplification (MLPA) for BRCA1 and BRCA2 to rule out deletions and duplications. Genomic DNA was isolated from sample submitted. Using MLPA reagents from MRC-Holland B.V. (Amsterdam, the Netherlands) and the MLPA procedure was performed as recommended by the manufacturer.

Analytical Validation of this assay shown sensitivity of 100% and specificity 100%.

Pharmacogenetic analysis:

Blood was analyzed for genotyping using semiconductor based Next Generation Sequencing technology. High quality genomic DNA was extracted from the submitted specimen and subjected to target enrichment by high multiplex PCR amplification using panel targeting variants of genes. Enriched DNA sequences were ligated with platform specific adaptor molecules and was sequenced on using semiconductor P1 chip. The minimum average depth was 500x for panel of genes analyzed. High quality sequencing data (proportion of Q20 bases ≥75%) was analyzed using DCGL NGS Bioinformatics Pipeline v14.4. This test does not detect polymorphisms other than those listed. Drug metabolism may be affected by non-genetic factors. DNA testing does not replace the need for clinical and therapeutic drug monitoring. Analytical Validation of this assay shown sensitivity of 100% and specificity 98.55%.

IHC analysis:

FFPE tissue was analyzed for immunohistochemistry. The test results relate specifically to the sample received in the lab. The pre-analytical variables like cold ischemia time, fixative and duration of fixation, which are beyond the control of DCGL laboratory, may affect the test results.

FISH analysis:

FFPE blocks prepared from fresh tissue were used for FISH analysis. The probe used was ZytoLight ® SPEC ERBB2/CEN 17 Dual Color Probe: The SPEC ERBB2/CEN 17 Dual Color Probe is a mixture of an orange fluorochrome direct labeled CEN 17 probe specific for the alpha satellite centromeric region of chromosome 17 (D17Z1) and a green fluorochrome direct labeled SPEC ERBB2 probe specific for the chromosomal region 17q12-q21.1 harboring the ERBB2 gene.

CTC Enumeration and ICC analysis:

Enriched CTCs from the submitted peripheral blood were labelled with EPCAM, Cytokeratin and CD45 antibodies and analyzed by High content imaging platform. Analytical Validation of this assay shown sensitivity of 99.99% and specificity 99.99%.

Circulating Tumor and associated cells from the submitted peripheral blood were analyzed through Cell stabilization protocol using Cell Wizard™ System. Cells were labelled with mTOR, VEGFR1, VEGFR2, VEGF-A and EGFR antibodies and analyzed by Fluorescent microscopy for Immunocytochemistry (ICC).

Blood based Chemosensitivity analysis:

Circulating tumor and its associated cells were isolated from the submitted peripheral blood sample. The live cancer cells were tested against multiple chemotherapy agents. The number of drugs selected for testing depend on the number of circulating tumor associated cells isolated from the submitted sample.

A defined number of cells were incubated with different drugs with respective drug concentrations, mean peak plasma concentration and cell death events were measured. The extent of cell death was determined either using Varioskan LUX platform or by fluorescence-based staining of live/dead cells. Percent cell death was calculated to evaluate the response level of the drug. Appropriate positive and negative controls were tested and evaluated in a similar manner simultaneously with the test sample.

Analytical Validation of this assay shown sensitivity of 85.71% and specificity 99.99%.

The performance of the assay specific reagents used in this assay has been established and its performance characteristics defined by Datar Cancer Genetics. This test may not detect all variants in non-coding regions that could affect copy number changes encompassing all or a large portion of the gene. Tumor mutation analysis panel testing is limited in detecting the following types of mutations (this might not be exhaustive): large rearrangements and deletion/ duplications, epigenetic factors, mutations in repetitive or high GC rich regions and mutations in gene with corresponding pseudo genes or other highly homologous sequences. Presence of PCR inhibitors in the sample may prevent DNA amplification for mutation analysis. Rare and novel mutations may be clinically uncharacterized.

Also note that the current knowledge on the genetic of the disease or pathogenic disorder or on the inheritance of the genes may be incomplete. If the test identifies the genetic cause of the disorder, it is possible that this knowledge may or may not help with the prognosis and management of the disease.

This test was developed, and its performance characteristics determined by Datar Cancer Genetics. It has not been cleared or approved by the U.S. Food and Drug Administration.

This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA)-USA as qualified to perform high complexity clinical laboratory testing.

The Patient Analysis raw data may be shared on written request by the individual patient.

DISCLAIMER

This report documents the genetic alterations detected in the submitted sample material. Information in this report is provided for information purpose only and should only be considered in conjunction with all other relevant information regarding a particular patient, before the patient's treating physician recommends a course of treatment.

Decisions on patient care and treatment must be based on the independent medical judgment of the treating physicians, taking into consideration all applicable information concerning the patient's condition, such as personal and family history, physician's examination, information from other diagnostic test and patient references, in accordance with the standard of care in a given community. A treating physician's decisions should not be based on a single test or on the information contained in this report.

This information in this report does not constitute a treatment recommendation by Datar Cancer Genetics, either to use or not to use any specific therapeutic agent, and should not be interpreted as treatment advice. Decisions on patient care and treatment rest solely within the discretion of the patient's treating physician.

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****End of Report****

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