Name	: DUMMY Z869	Age/sex	: 55 Y /M
Lab No	: 153672448		
Reporting Centre	: LPL - LPL-ROHINI	Referred by	: UNKNOWN
Receiving Date	: 29/7/2020	Reporting Date	: 4/8/2020

TEST CONDUCTED	CANCER TARGETED GENE PANEL, GASTROINTESTINAL STROMAL TUMOR (GIST) *CD117 *DOG1 *ERBB2 *KIT *BRAF *PDGFRA *TP53

INDICATION & SAMPLE INFORMATION	The patient is a 55 years old male diagnosed with features consistent with Gastrointestinal Stromal Tumor (GIST) from CT guided biopsy from gastro splenic mass lesion with no known treatment history. The patient has a clinical history of ? Gastrointestinal Stromal Tumor (GIST). Sample type for IHC and NGS panel- FFPE (Block No. 191217; Tumour content 90%).

DRUG INFORMATION

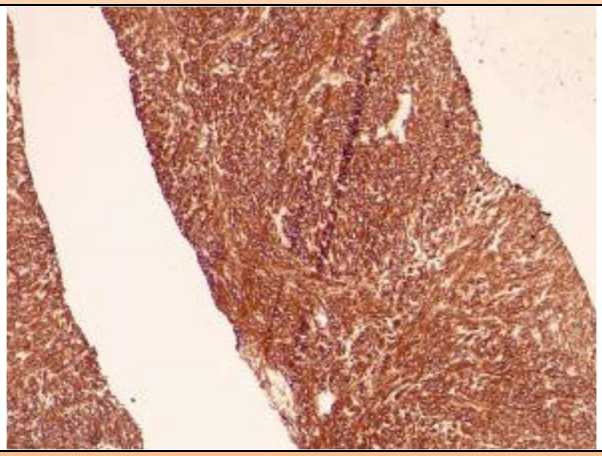
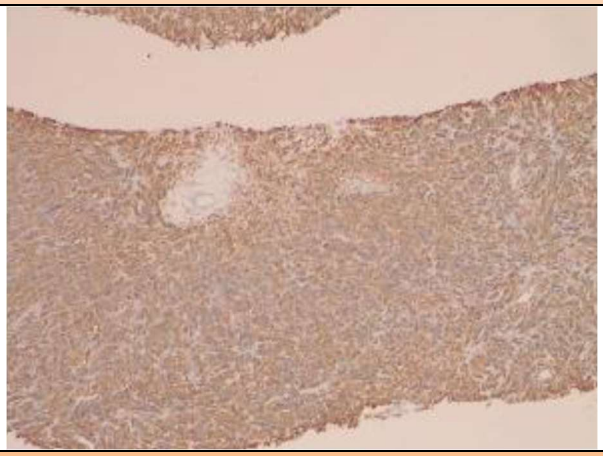
THERAPY	TESTED MARKER	PREDICTED RESPONSE
Imatinib	***KIT, PDGFRA BRAF,*** **TP53**	
Sunitinib	***KIT,*** **PDGFRA, *BRAF,* TP53**	
Sorafenib	**BRAF, KIT, TP53**	**Limited data available**
Regorafenib	***KIT, PDGFRA,*** **BRAF, TP53**	

Predicted Response	Green – Good responders due to mutation Red – Contraindicated due to mutation Yellow – Limited response due to mutation Blue –Therapeutic guidelines not available

RESULTS

CD117 (KIT) Expression	DOG1 Expression	ERBB2 Mutation	KIT Mutation	BRAF Mutation	PDGFRA Mutation	TP53 Mutation
Detected	Detected	Not Detected	Detected	Not Detected	Not Detected	Not Detected

RESULTS OF IHC TESTS

DOG1	CD117
	

RESULT	DOG1: Immunoreactive, score 4+ in neoplastic cells CD117: Immunoreactive, score 3+ in neoplastic cells **** CD117 staining intensity does not correlate with treatment sensitivity or mutational status***

IMPRESSION	Suggestive of Gastrointestinal Stromal Tumour (GIST)

Interpretation	**RESULT**	**SCORE**
	Non immunoreactive	0
	Immunoreactive in 1-25 % cells	1+
	Immunoreactive in 26-50% cells	2+
	Immunoreactive in 51-75% cells	3+
	Immunoreactive in 76-100% cells	4+

Comments	**DOG1:** (**D**iscovered **O**n **G**IST **1**) is a cell surface, calcium regulated chloride channel protein selectively expressed in gastrointestinal stromal tumors (GIST), and rarely expressed in other soft tissue tumors, such as uterine type retroperitoneal leiomyomas, peritoneal leiomyomatosis, and synovial sarcomas. The DOG1 protein shows no homology at the DNA or amino acid level with KIT, and is a sensitive and specific marker for GIST. DOG1 antibody labels the epithelium of the following organs: breast, prostate, salivary gland, liver, stomach, testis, pancreas, and gallbladder. DOG 1 is a useful marker for GISTs, including PDGFRA mutants that fail to express KIT. **CD117 (KIT):** Is a transmembrane tyrosine kinase which serves as a receptor for stem cell factor**.** It is expressed in mastocytosis, melanoma, germ cell tumors and gastrointestinal stromal tumors (GIST). Over 95% of GISTs have CD117 expression. The immunohistochemical positivity for CD117 is independent from the mutational status of KIT and PDGFRA. *References: PMID: 20571340, PMID: 11213830, PMID: 18312355*

Note	1. Type of specimen fixation & processing - formalin fixed paraffin embedded tissue 2. Detection system used is Polymer HRP 3. The impression is based on the material submitted and is not a complete surgical pathology report 4. False negative IHC results due to inadequate fixation of the material sent for evaluation cannot be excluded

Fixation Requirements	The volume of formalin fixative should be at least 10 times the volume of the specimen Decalcification solutions with strong acids should not be used Specimens should be immersed in fixative within 1 hour of the biopsy/resection procedure (time of removal & time of immersion to be mentioned) In all resection (large) specimens, the tumour must be bisected prior to immersion in fixative

RESULTS OF NEXT GENERATION SEQUENCING TESTS

Coverage	**100%**
Depth	**10,510x**

AFFECTED GENES

BRAF (0)	*ERBB2* (0)	***KIT* (1)**	*PDGFRA* (0)	*TP53* (0)

Variants found

Gene	Variant	Exon	Variant Allelic frequency	Pathogenicity	dbSNP ID
KIT	NM_000222.2:c.1669_1674del (p.Trp557_Lys558del)	11	52 %	Likely pathogenic	NA

Variants Interpretation

KIT: NM_000222.2(KIT):c.1669_1674del (p.Trp557_Lys558del)- Likely pathogenic

The human c-KIT gene is a proto-oncogene that belongs to the type III tyrosine kinase receptor family. Binding of endogenous ligand stem cell factor to the extracellular domain causes the c-kit receptor to dimerize resulting in autophosphorylation of the intracellular tyrosine kinase domain. Somatic gain of function mutations of KIT (mutations in exon 9, 11 and 13) have been reported in GIST (PMID: 11719439).

Exon 11 mutations are the most common reported GIST mutations, but are quite heterogenous clustering between codons 556-560 .

Compared to patients with KIT exon 9 mutations and wild type GIST, patients with exon 11 mutations have a worse relapse free survival; however, their tumors have the highest sensitivity to imatinib with a median duration of benefit of approximately 23 months. Patients with exon 11 mutations are less likely to respond to second line sunitinib (PMID: 18955451).

Imatinib is a kinase inhibitor indicated for the treatment of patients with KIT (CD117) positive GIST. This drug is also helpful as an adjuvant treatment of adult patients following resection of KIT positive GIST. However, therapeutic guidelines are not available for present condition.

Imatinib is FDA approved recommended drugs. **However, patient care and treatment decisions should only be made by the physician after taking into account all relevant information available for patient's condition, as well as other clinical details such as the current standards of care.**

References:

Rubin BP, Singer S, Tsao C, Duensing A, Lux ML, Ruiz R, Hibbard MK, Chen CJ, Xiao S, Tuveson DA, Demetri GD, Fletcher CD, Fletcher JA. KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. Cancer Res. 2001 Nov 15;61(22):8118-21. PubMed PMID: 11719439.

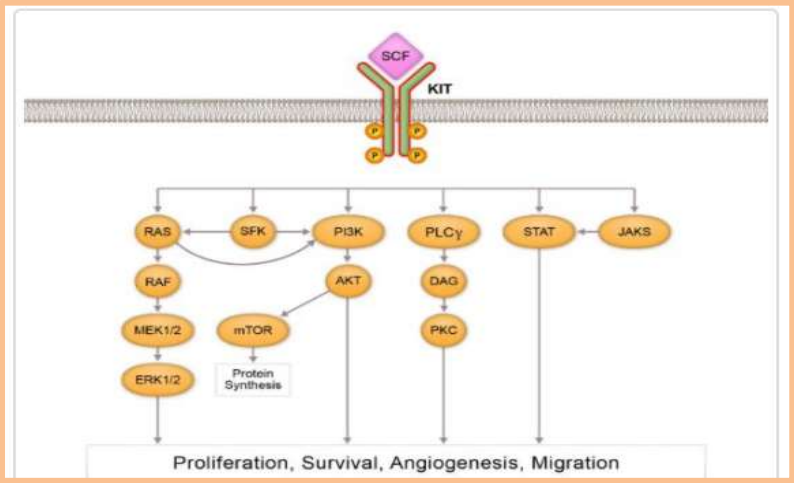
DRUG INFORMATION

Depends on the mutation detected, not on the type of tumor

CLINICAL TRIAL INFORMATION

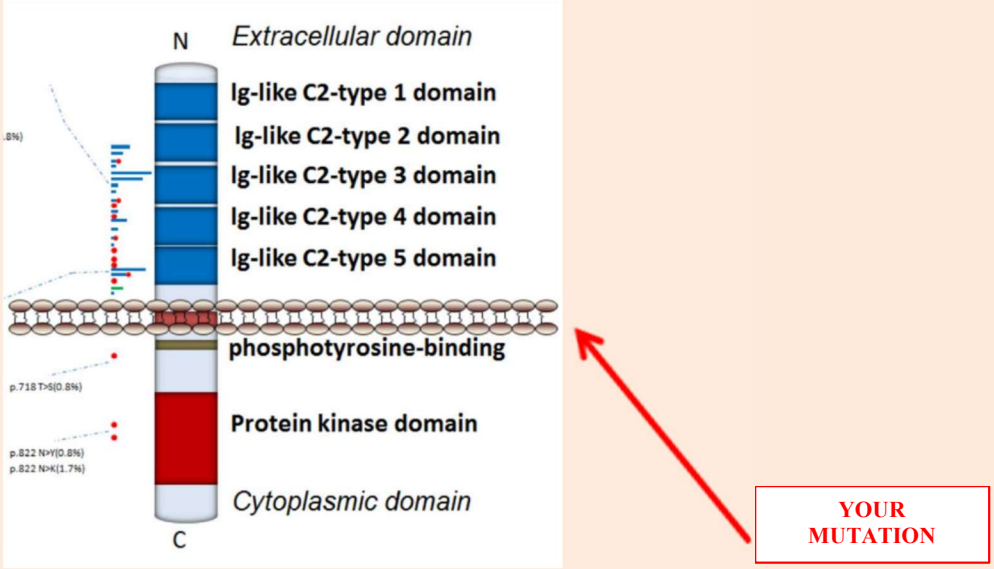
NCT Number	Conditions	Interventions	Start Date	Completion Date
NCT03471468	Pancreatic Cancer|Gastric Cancer	Procedure: kinetics of microparticles under chemotherapy	July 23, 2018	July 2, 2021
NCT03817866	Gastric Neoplasms|Pancreatic Neoplasms|Small Intestinal Neoplasms|Colorectal Neoplasms	Diagnostic Test: BRAHMS CgA II KRYPTOR	January 29, 2019	Dec-21
NCT03787056	Cancer|Breast Cancer|Gastric Cancer|Renal Cancer|Prostate Cancer|Melanoma|Lung Cancer|Hepatocellular Cancer|Colorectal Cancer|Head and Neck Cancer|Pancreatic Cancer|Ovarian Cancer|Glioblastoma|Endometrial Cancer|Bladder Cancer|Esophageal Cancer|B-cell Lymphoma	Other: Blood draws	December 4, 2018	Nov-26

THE KIT PATHWAY



KIT in Gastro-Intestinal Stromal Tumors (GISTs)

The human KIT (c-KIT) gene is a proto-oncogene that belongs to the Type III tyrosine kinase receptor family. Aberrant c-KIT overexpression is reported in multiple tumor types such as gastrointestinal stromal tumors (GISTs), melanoma, acute myeloid leukemia (AML), gliomas and neuroendocrine tumors (15542802, 12780793, 22330882, 16550970). 90% of GIST tumors overexpress the KIT gene product, c-KIT (also referred to as CD117), whereas KIT gene mutations occur in 85-90% of all sporadic GISTs (PMID: 9438854, 18369405). The majority of KIT mutations are deletions or insertions in KIT gene. Though single nucleotide substitution are also commonly reported. In GISTs, oncogenic activation of c-KIT is the most frequent pathogenic mechanism, serving as diagnostic biomarker. The presence of KIT mutations is directly related to increased expression of c-kit in GIST (PMID: 19943934, 12727838).



Mutations in the KIT gene are quite heterogeneous. They are comprised of deletion, insertion, duplication, single nucleotide substitution and other rare complex mutations that cluster within certain regions of exon 8, 9, 11, 13, 17 and 18. Juxtamembrane/JM domain, exon 11 mutations are most commonly reported GIST mutations but are quite heterogeneous clustering between codons 556-560 (PMID: 18265649).

Mutations in exon 17 of KIT are the mutations found in GIST, and confer increased sensitivity to Imatinib. These mutations occur in the activation loop domain, and lead to constitutive activation of tyrosine kinase receptors and downstream signaling pathways, causing uncontrolled cell growth and proliferation.

Imatinib - *CD117 expression, KIT, PDGFRA BRAF, TP53*

KIT
Patients with advanced case of GISTs, 50-90% of patient benefit from Imatinib treatment based on the mutation type.
CD117
CD117 positive cases are eligible for Imatinib therapy
PDGFRA
Mutations in the PDGFRa gene confer favorable response to imatinib except the D842V mutation
BRAF
Wild type BRAF confers decreased sensitivity to Imatinib, and some response to Sunitinib and Sorafenib, and there is evidence of response to Regorafenib.

KIT	**Variant detected** **NM_000222.2:c.1669_1674del (p.Trp557_Lys558del), therapeutic guidelines not available**
PDGFRA	No significant oncogenic variant detected
BRAF	No significant oncogenic variant detected
TP53	No significant oncogenic variant detected
CD117 expression	Immunoreactive, Score 3+ in neoplastic cells

Drug Information

Imatinib is a small molecule kinase inhibitor used to treat certain types of cancer. It is currently marketed by Novartis as Gleevec (USA) or Glivec (Europe/Australia) as its mesylate salt, imatinib mesilate (INN). It is occasionally referred to as CGP57148B or STI571 (especially in older publications). It is used in treating chronic myelogenous leukemia (CML), gastrointestinal stromal tumors (GISTs) and a number of other malignancies.
It is the first member of a new class of agents that act by inhibiting particular tyrosine kinase enzymes, instead of non-specifically inhibiting rapidly dividing cells.

References

- ✓ Vigneri P, Wang JY: Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. Nat Med. 2001 Feb;7(2):228-34. [PubMed:11175855]
- ✓ Droogendijk HJ, Kluin-Nelemans HJ, van Doormaal JJ, Oranje AP, van de Loosdrecht AA, van Daele PL: Imatinib mesylate in the treatment of systemic mastocytosis: a phase II trial. Cancer. 2006 Jul 15;107(2):345-51. [PubMed:16779792]

Sunitinib – *CD117*, *KIT*, PDGFRA, *BRAF*, TP53

***KIT*, PDGFRA**
Useful for the treatment of gastrointestinal stromal tumor after disease progression on or intolerance to Imatinib mesylate
CD117
Sunitinib inhibits KIT (CD117), the RTK that drives the majority of GISTs (PMID: 22439647, 17046465)
BRAF
Non detection of mutations in BRAF may be beneficial as it indicates a favorable response to Sunitinib in some cases.

KIT	**Variant detected.** **NM_000222.2:c.1669_1674del (p.Trp557_Lys558del), therapeutic guidelines not available**
PDGFRA	No significant oncogenic variant detected
BRAF	No significant oncogenic variant detected
TP53	No significant oncogenic variant detected
CD117 expression	**Immunoreactive, Score 3+ in neoplastic cells**

Drug Information

Sunitinib is an oral, small-molecule, multi-targeted receptor tyrosine kinase (RTK) inhibitor that was approved by the FDA for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumor (GIST) on January 26, 2006. Sunitinib inhibits cellular signaling by targeting multiple RTKs. These include all platelet-derived growth factor receptors (PDGF-R) and vascular endothelial growth factor receptors (VEGF-R). Sunitinib also inhibits KIT (CD117), the RTK that drives the majority of GISTs. In addition, sunitinib inhibits other RTKs including RET, CSF-1R, and flt3.

References

1. Demetri GD, van Oosterom AT, Garrett CR, Blackstein ME, Shah MH, Verweij J, McArthur G, Judson IR, Heinrich MC, Morgan JA, Desai J, Fletcher CD, George S, Bello CL, Huang X, Baum CM, Casali PG: Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet. 2006 Oct 14;368(9544):1329-38. [PubMed:17046465]

Sorafenib - BRAF, KIT, TP53

BRAF, KIT
Limited data available for the BRAF and KIT mutated GIST treatment with Sorafenib.
In preclinical models, Sorafenib inhibits KIT activity and cell growth of imatinib-resistant tumors. PMID: 23840364

BRAF	No significant oncogenic variant detected
KIT	**Variant detected** **NM_000222.2:c.1669_1674del (p.Trp557_Lys558del), therapeutic guidelines not available**
TP53	No significant oncogenic variant detected

Drug Information

Sorafenib (rINN), marketed as Nexavar by Bayer, is a drug approved for the treatment of advanced renal cell carcinoma (primary kidney cancer). It has also received "Fast Track" designation by the FDA for the treatment of advanced hepatocellular carcinoma (primary liver cancer), and has since performed well in Phase III trials. Sorafenib is a small molecular inhibitor of Raf kinase, PDGF (platelet-derived growth factor), VEGF receptor 2 & 3 kinases and c Kit the receptor for Stem cell factor.
Sorafenib interacts with multiple intracellular (CRAF, BRAF and mutant BRAF) and cell surface kinases (KIT, FLT-3, VEGFR-2, VEGFR-3, and PDGFR-ß). Several of these kinases are thought to be involved in angiogenesis, thus sorafenib reduces blood flow to the tumor. Sorafenib is unique in targeting the Raf/Mek/Erk pathway. By inhibiting these kinases, genetic transcription involving cell proliferation and angiogenesis is inhibited.

References

✓ Park SH, Ryu MH, Ryoo BY, Im SA, Kwon HC, Lee SS, Park SR, Kang BY, Kang YK. Sorafenib in patients with metastatic gastrointestinal stromal tumors who failed two or more prior tyrosine kinase inhibitors: a phase II study of Korean gastrointestinal stromal tumors study group. Invest New Drugs. 2012 Dec;30(6):2377-83. doi: 10.1007/s10637-012-9795-9. Epub 2012 Jan 25.

Regorafenib - *KIT, PDGFRA, BRAF,* TP53

KIT Mutation
Useful for the treatment of gastrointestinal stromal tumor after disease progression on or intolerance to Imatinib mesylate and Sunitinib.
BRAF
Non detection of activating mutations in genes such as BRAF may indicate a favorable response to Regorafenib, however, few case studies revealed that it can inhibit BRAF V600E (PMID: 25342989, 25213039, PMID: 22568966)

KIT	**Variant detected.** **NM_000222.2:c.1669_1674del (p.Trp557_Lys558del), therapeutic guidelines not available**
PDGFRA	No significant oncogenic variant detected
TP53	No significant oncogenic variant detected
BRAF	No significant oncogenic variant detected

Drug Information

Regorafenib is an orally-administered inhibitor of multiple kinases. It is used for the treatment of metastatic colorectal cancer and advanced gastrointestinal stromal tumours. FDA approved on September 27, 2012 Regorafenib is indicated for the treatment of patients with metastatic colorectal cancer (CRC) who have been previously treated with fluoropyrimidine-, oxaliplatin- and irinotecan-based chemotherapy, an anti-VEGF therapy, and, if KRAS wild type, an anti-EGFR therapy. Regorafenib is also indicated for the treatment of patients with locally advanced, unresectable or metastatic gastrointestinal stromal tumor (GIST) who have been previously treated with imatinib mesylate and sunitinib malate.

References

✓ 1: Demetri GD, Reichardt P, Kang YK, Blay JY, Rutkowski P, Gelderblom H, Hohenberger P, Leahy M, von Mehren M, Joensuu H, Badalamenti G, Blackstein M, Le Cesne A, Schöffski P, Maki RG, Bauer S, Nguyen BB, Xu J, Nishida T, Chung J, Kappeler C, Kuss I, Laurent D, Casali PG; GRID study investigators. Efficacy and safety of regorafenib for advanced gastrointestinal stromal tumours after failure of imatinib and sunitinib (GRID): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet. 2013 Jan 26;381(9863):295-302. doi: 10.1016/S0140-6736(12)61857-1. Epub 2012 Nov 22. PubMed PMID: 23177515; PubMed Central PMCID: PMC3819942.

METHODS

This in-house developed panel targets 5 genes and uses methodologies of IHC, and Next generation sequencing using Ion Ampliseq Cancer Hotspot panel V2. These genes have been selected on the basis of their known impact as actionable targets of existing and emerging anti-cancer therapies, and the prognostic features in specific tumor types.

The sensitivity of the assays depends on the quality of the block, and tumor content. In validation studies using control material and a variety of cell lines, the minimum analytic detection limit for each of the assays is:

Next Generation Sequencing – 5%

IHC – Varies from assay to assay. Refer individual assay

LIMITATION

The accuracy and completeness of this information may vary due to variable information available in different databases Variants with variant allele frequency at nearly 50% or 100% should be considered Germline mutation. To rule out germ line mutations, whole blood sample is recommended to process along with tissue sample. Synonymous mutations were not considered while preparing this report. UDG treatment has not been done. The mutations have not been confirmed using Sanger sequencing and/or alternate technologies and additional testing might be required if clinically indicated. False negative results may be due to sampling error/errors in sample handling as well as clonal density below the limit of detection.

DISCLAIMER

This report provides information about the patient's mutations that may aid the physician's decision making process, but this test should not be the sole source of information for making decisions on patient care and treatment. These tests should be interpreted in the context of standard clinical, laboratory, and pathological findings. Identification of a mutation in one or more of these genes does not guarantee activity of the drug in a given indication.

The information provided in this report was collected from various sources that we believe to be reliable and quality control procedures have been put in place to ensure the information provided is as accurate, comprehensive, and current as possible. The information provided should only be utilized as a guide or aid and the decision to select any therapy option based on the information reported here resides solely with the discretion of the treating physician. Patient care and treatment decisions should only be made by the physician after taking into account all relevant information available including but not limited to the patient's condition, family history, findings upon examination, results of other diagnostic tests, and the current standards of care. This report should only be used as an aid and the physician should employ clinical judgment in arriving at any decision for patient care or treatment.

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