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Axaitech
Chemo Data Matters

Sample Number:	AxcfdnaSample	**Patient:**	Test
Date of Birth:		**Gender**	
Diagnosis:		**Ordering Physician/Oncologist**	

cfDNA Variant Report

Potentially Actionable Variants

Genes	HGVS	Coding Impact	Cosmic Primary Site	Variant Allele Fraction (VAF)
CTNNB1	c.98C>A(p.Ser33Tyr)	missense	Central Nervous System	**0.35**
EGFR	c.2155G>A(p.Gly719Ser)	missense	Lung	**0.25**
PIK3CA	c.3140A>G(p.His1047Arg)	missense	Breast	**0.20**
NRAS	c.181C>A(p.Gln61Lys)	missense	Skin	**0.12**
KIT	c.2447A>T(p.Asp816Val)	missense	Haematopoietic and Lymphoid Tissue	**0.12**
PIK3CA	c.1633G>A(p.Glu545Lys)	missense	Breast	**0.07**
EGFR	c.2573T>G(p.Leu858Arg)	missense	Lung	**0.05**
EGFR	c.2369C>T(p.Thr790Met)	missense	Lung	**0.02**

Detailed information regarding these variants are outlined in the variant and therapy related data section

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Variant and Therapy Related Data

CTNNB1 Gene

The CTNNB1 gene provides instructions for making a protein called beta-catenin. This protein is present in many types of cells and tissues, where it is primarily found at junctions that connect neighbouring cells (adherens junctions). Beta-catenin plays an important role in sticking cells together (cell adhesion) and in communication between cells.

The beta-catenin protein is also involved in cell signalling as an essential part of the Wnt signalling pathway. Certain proteins in this pathway attach (bind) to beta-catenin, which triggers a multistep process that allows the protein to move into the cell nucleus. Once in the nucleus, beta-catenin interacts with other proteins to control the activity (expression) of particular genes. The Wnt signalling pathway promotes the growth and division (proliferation) of cells and helps determine the specialized functions a cell will have (differentiation). Wnt signalling is known to be involved in many aspects of development before birth. In adult tissues, this pathway plays a role in the maintenance and renewal of stem cells, which are cells that help repair tissue damage and can give rise to other types of cells.

CTNNB1 codon(s) 32, 33, 34, 35, 36, 37, 41 and 45

Accumulation of nuclear beta catenin can lead to a tumoral phenotype and oncogenic transformation in a variety of solid tumours. Various oncogenic mutants of beta catenin have been found in different tumour types which alter its degradation, leading to its accumulation and promoting tumour growth. Some of these mutations are located at the N-terminus of the protein at the sites of phosphorylation which normally regulate its degradation.

Inhibition of beta catenin using small molecule inhibitors is currently being investigated in various tumour types. Recent studies suggest that targeting of the Wnt pathway and beta catenin may be promising targets in the therapy of acute myeloid leukemia.

chr3:41266101 C => A (p.Ser33Tyr) (Pathogenic – ACMG, Pathogenic - ClinVar)

This variant is at position 85 of the 3rd exon of transcript NM_001904.4, located on the positive strand of chromosome 3p22.1. It is a missense substitution, as it changes the 33rd amino acid (serine) to tyrosine.

Mutation	Gene(s)	Exon	Variant Type	Variant Allele Frequency (VAF)
HGVS Protein: Ser33Tyr	**CTNNB1**	exon 3 of 15 position 85 of 228	SNV	**0.35**

CTNNB Linked Therapies:

The following list of therapies may contain experimental therapies. Note that the following list is not necessarily exhaustive, but a more detailed list of potential therapies could be requested by the physician, including clinical trial data for experimental therapies.

Therapies	Combination Type	Tissue	SAHPRA Related Drugs
Everolimus Interaction Type: mTOR Inhibitor FDA Approved: Yes **Vantictumab** *FDA Approved: No*	*Combination Therapy*: Everolimus +Letrozole	Breast Cancer Colon Cancer	**Everolimus**: Certican from Novartis SA (Several Licensed) **Letrozole**: Femara from Novartis South Africa (Several Licensed) **Vantictumab**: Not Registered

PIK3CA Gene

The PIK3CA gene provides instructions for making the p110 alpha (p110α) protein, which is one piece (subunit) of an enzyme called phosphatidylinositol 3-kinase (PI3K). The p110α protein is called the catalytic subunit because it performs the action of PI3K, while the other subunit (produced by a different gene) regulates the enzyme's activity.

Like other kinases, PI3K adds a cluster of oxygen and phosphorus atoms (a phosphate group) to other proteins through a process called phosphorylation. PI3K phosphorylates certain signalling molecules, which triggers a series of additional reactions that transmit chemical signals within cells. PI3K signalling is important for many cell activities, including cell growth and division (proliferation), movement (migration) of cells, production of new proteins, transport of materials within cells, and cell survival. Studies suggest that PI3K signalling may be involved in the regulation of several hormones and may play a role in the maturation of fat cells (adipocytes).

PIK3CA Gene in Colorectal Cancer

Somatic mutations in PIK3CA have been found in 10-30% of colorectal cancers. KRAS, NRAS, BRAF and PIK3CA and non-functional PTEN predict resistance to anti-EGFR therapies in metastatic colorectal cancer. According to some reports, co-occurrence of both exon 9 and exon 20 PIK3CA mutations, when present, may be associated with a poor prognosis. Recent 'molecular pathological epidemiology' (MPE) research has shown that aspirin use may be associated with better prognosis and clinical outcome in PIK3CA-mutated colorectal carcinoma, suggesting somatic PIK3CA mutation may be a molecular biomarker that predicts response to aspirin therapy. PIK3CA may also be a target of directed therapy in some clinical settings.

PIK3CA Glu542Lys, Glu545Lys and His1047Arg Mutations

PIK3CA mutations activate the PI3K-PTEN-AKT pathway which is downstream from both the EGFR and the RAS-RAF-MAPK pathways. The somatic mutations found thus far in PIK3CA are oncogenic, and the majority of them are clustered within exon 9 and 20 (helical and kinase domains), with 80% of the identified mutations found within three hotspots: E542K, E545K, and H1047R. PIK3CA mutations are often found in hormone receptor-positive breast cancer and have been associated with resistance to anti-EGFR therapy in some studies but not in others.

chr3:178952085 A => G (p.His1047Arg) (Pathogenic – ACMG, Pathogenic - ClinVar)

This variant is at position 204 of the 21st exon of transcript NM_006218.4, located on the positive strand of chromosome 3q26.32. It is a missense substitution, as it changes the 1047th amino acid (histidine) to arginine.

Mutation	Gene(s)	Exon	Variant Type	Variant Allele Frequency (VAF)
HGVS Protein: His1047Arg	**PIK3CA**	exon 10 of 21 position 94 of 125	SNV	**0.20**

chr3:179218303 G => A (p.Glu545Lys) (Pathogenic – ACMG, Pathogenic - ClinVar)

This variant is at position 94 of the 10th exon of transcript NM_006218.4, located on the positive strand of chromosome 3q26.32. It is a missense substitution, as it changes the 545th amino acid (glutamic acid) to lysine.

Mutation	Gene(s)	Exon	Variant Type	Variant Allele Frequency (VAF)
HGVS Protein: Glu545Lys	**PIK3CA**	exon 10 of 21 position 94 of 125	SNV	**0.07**

PIK3CA Linked Therapies

The following list of therapies may contain experimental therapies. Note that the following list is not necessarily exhaustive, but a more detailed list of potential therapies could be requested by the physician, including clinical trial data for experimental therapies.

Therapies	Combination Type	Tissue	SAHPRA Related Drugs
Cetuximab Inhibitor Type: EGFR Inhibitor FDA Approved: Yes **Alpelisib:** Inhibitor Type: PI3-Kinase p110-α Inhibitor FDA Approved: Yes	*Combination Therapy*: Cetuximab + Irinotecan (+ Fluorouracil) (**Possible Resistance indicated by PIK3CA Variants**) *Combination Therapy*: Alpelisib + Fulvestrant (**Binds downstream PI3-Kinase p110-α**)	Colorectal Cancer, Metastatic Breast Cancer	**Cetuximab**: Erbitux from Merck **Irinotecan**: Irocan from Innovata Pharmaceuticals (Several Licensed) **Alpelisib**: None Registered **Fulvestrant**: Faslodex from AstraZeneca Pharmaceuticals

EGFR Gene

The EGFR gene provides instructions for making a receptor protein called the epidermal growth factor receptor, which spans the cell membrane so that one end of the protein remains inside the cell and the other end projects from the outer surface of the cell. This positioning allows the receptor to attach to other proteins, called ligands, outside the cell and to receive signals that help the cell respond to its environment. Epidermal growth factor receptor binds to at least seven different ligands. The binding of a ligand to epidermal growth factor receptor allows the receptor to attach to another nearby epidermal growth factor receptor protein (dimerize), activating the receptor complex. As a result, signalling pathways within the cell are triggered that promote cell growth, division and cell survival.

EGFR L858R, EGFR exon(s) 18, 19, 20, 21

Somatic mutations in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR) gene are present in approximately 80% of the lung adenocarcinomas that respond to first and second-generation EGFR inhibitors (eg, gefitinib, erlotinib and afatinib). Two types of mutations account for approximately 80-90% of all EGFR mutations: short in-frame deletions in Exon 19 and a point mutation in exon 21 at codon 858 (L858R). Other less common mutations in exons 18, 20, and 21 are found in 10-20% of EGFR-mutated cases. EGFR Exon 19 deletions , EGFR Exon 21 L858R and EGFR Exon 18 G719 mutations correlate strongly with sensitivity to specific EGFR inhibitors and the response rate to therapy with TKIs has been reported to be up to 80% in such cases. The T790M mutation in exon 20 is associated with resistance to some EGFR inhibitors. However, third-generation TKI (eg, osimertinib) can specifically target T790M.

EGFR G719S, G719D

Afatinib, Erlotinib and Gefitinib sensitivity indicated by these specific variants.

In colorectal cancer, EGFR gene amplification is associated with sensitivity EGFR-targeted therapies, such as Erbitux (Cetuximab) and Vectibix (Panitumumab). Note that the variant listed in this test does not represent copy number gain, but only mutation(s). If this indication is important, copy number gain tests may need to be considered.

chr7:55241707 G => A (p.Gly719Ser) (Likely pathogenic – ACMG, Drug Response - ClinVar)

This variant is at position 94 of the 18th exon of transcript NM_005228.5, located on the positive strand of chromosome 7p11.2. It is a missense substitution, as it changes the 719th amino acid (glycine) to serine.

Mutation	Gene(s)	Exon	Variant Type	Variant Allele Frequency (VAF)
HGVS Protein: Gly719Ser	**EGFR**	exon 18 of 28 position 94 of 123	SNV	**0.25**

chr7:55259515 T => G (p.Leu858Arg) (Pathogenic – ACMG, Drug Response - ClinVar)

This variant is at position 104 of the 21st exon of transcript NM_005228.5, located on the positive strand of chromosome 7p11.2. It is a missense substitution, as it changes the 858th amino acid (leucine) to arginine.

Mutation	Gene(s)	Exon	Variant Type	Variant Allele Frequency (VAF)
HGVS Protein: Leu858Arg	**EGFR**	exon 21 of 28 position 104 of 156	SNV	**0.05**

chr7:55249071 C => T (p.Thr790Met) (Pathogenic – ACMG, Drug Response - ClinVar)

This variant is at position 86 of the 20th exon of transcript NM_005228.5, located on the positive strand of chromosome 7p11.2. It is a missense substitution, as it changes the 790th amino acid (threonine) to methionine.

Mutation	Gene(s)	Exon	Variant Type	Variant Allele Frequency (VAF)
HGVS Protein: Thr790Met	**EGFR**	exon 20 of 28 position 86 of 186	SNV	**0.02**

EGFR Linked Therapies:

The following list of therapies may contain experimental therapies. Note that the following list is not necessarily exhaustive, but a more detailed list of potential therapies could be requested by the physician, including clinical trial data for experimental therapies.

Therapies	Combination Type	Tissue	SAHPRA Related Drugs
Erlotinib: Interaction Type: EGFR Inhibitor, 1st Generation FDA Approved: Yes **Gefitinib:** Interaction Type: EGFR Inhibitor, 1st Generation FDA Approved: Yes **Panitumumab:** Interaction Type: EGFR Inhibitor FDA Approved: Yes	*Combination Therapy*: Erlotinib + Cetuximab (**Erlotinib Sensitivity Indicated by EGFR Variants**) *Combination Therapy*: Gefitinib + Decitabine (**Gefitinib Sensitivity Indicated by EGFR Variants**)	Advanced Solid tumour, Non-small Cell Lung Cancer, Colon Cancer	**Erlotinib**: Tarceva from Roche Products **Cetuximab**: Erbitux from Merck **Gefitinib**: Iressa from AstraZeneca **Decitabine**: Dacogen from Janssen Pharmaceuticals **Panitumumab**: Vectibix from Amgen South Africa

KIT Gene

The KIT gene provides instructions for making a member of a protein family called receptor tyrosine kinases. Receptor tyrosine kinases transmit signals from the cell surface into the cell through a process called signal transduction. The KIT protein is found in the cell membrane of certain cell types where a specific protein, called stem cell factor, attaches (binds) to it. This binding turns on (activates) the KIT protein, which then activates other proteins inside the cell by adding a cluster of oxygen and phosphorus atoms (a phosphate group) at specific positions. This process, called phosphorylation, leads to the activation of a series of proteins in multiple signalling pathways.

KIT D816V, KIT exon(s) 11, 17, 8 missense, 9 missense and 10 missense

KIT(cKIT) mutations are present in approximately 8-25% of cases of acute myeloid leukemia and have a higher prevalence in the favourable cytogenetic risk group including core-binding factor (CBF) AMLs (ie, (t(8;21)(q22;q22)(RUNX1-RUNX1T1), inv(16)(p13q22)(CBFB-MYH11)) or normal karyotype AML. Mutations of KIT in AML are most common in KIT exon 17 (within the activation loop of the tyrosine kinase domain) but may also occur in KIT exons 8 (extracellular portion of the receptor implicated in dimerization), 9-11 (juxtamembrane/transmembrane domains). The presence of KIT mutations has been reported to be associated with poorer survival and/or higher risk of relapse than expected for patients with the t(8;21)(q22;q22)(RUNX1-RUNX1T1), and to a lesser extent, in inv(16) AML. The KIT D816V mutation has been shown to be resistant to imatinib; other KIT mutations may show variable responses to imatinib. The KIT D816V mutant has been reported to be sensitive to other tyrosine kinase inhibitors.

KIT D816F, D816V, D816Y

Dasatinib and Nilotinib Sensitivity linked to these specific variants.

Some studies have shown a high frequency of c-Kit overexpression in stage II colon cancer patients (59.3%) with a significant correlation between c-Kit overexpression and reduced disease-free survival. However, other studies failed to demonstrate c-kit expression in a significant number of colorectal cancers suggesting that c-kit kinase activation is not a prominent pathogenetic feature of colorectal cancers. The role of c-Kit continues to be studied in colon cancers.

Chr4:55599321 A => T (p.Asp816Val) (Pathogenic – ACMG)

This variant is at position 86 of the 17th exon of transcript NM_000222.3, located on the positive strand of chromosome 4q12. It is a missense substitution, as it changes the 816th amino acid (aspartic acid) to valine.

Mutation	Gene(s)	Exon	Variant Type	Variant Allele Frequency (VAF)
HGVS Protein: Asp816Val	**KIT**	exon 17 of 21 position 86 of 123	SNV	**0.12**

KIT Linked Therapies

The following list of therapies may contain experimental therapies. Note that the following list is not necessarily exhaustive, but a more detailed list of potential therapies could be requested by the physician, including clinical trial data for experimental therapies.

Therapies	Combination Type	Tissue	SAHPRA Related Drugs
Imatinib: Interaction Type: BCR-ABL Inhibitor 1st Generation, FDA Approved: Yes (**Resistance Indicated by KIT Variant**)		Gastrointestinal Stromal Tumour (GIST),	**Imatinib**: Gleevec from Novartis South Africa (Several Licensed)
Dasatinib: Interaction Type: BCR-ABL Inhibitor, 2nd Generation FDA Approved: Yes	*Combination Therapy*: Dasatinib + Cytarabine (**Sensitivity Indicated by KIT Variant**)	Acute Myeloid Leukemia (AML)	**Dasatinib**: Sprycel from Bristol-Myers Squibb **Cytarabine**: Cytosar from Pfizer Laboratories (Several Licensed)
Nilotinib: Interaction Type: BCR-ABL Inhibitor, 2nd Generation FDA Approved: Yes (**Sensitivity Indicated by KIT Variant**)		Acute Myeloid Leukemia (AML), Advanced Solid Tumour, Gastrointestinal Stromal Tumour (GIST)	**Nilotinib**: Tasigna from Novartis South Africa

NRAS Gene

The NRAS gene provides instructions for making a protein called N-Ras that is involved primarily in regulating cell division. Through a process known as signal transduction, the protein relays signals from outside the cell to the cell's nucleus. These signals instruct the cell to grow and proliferate or to mature and take on specialized functions (differentiate). The N-Ras protein is a GTPase, which means it converts a molecule called GTP into another molecule called GDP. The N-Ras protein acts like a switch, and it is turned on and off by the GTP and GDP molecules. To transmit signals, the N-Ras protein must be turned on by attaching (binding) to a molecule of GTP. The N-Ras protein is inactivated when it converts the GTP to GDP. When the protein is bound to GDP, it does not relay signals to the cell's nucleus.

NRAS Q61H, Q61L, Q61K, Q61R, or NRAS codon(s) 12, 13, 61, 146

NRAS mutations occur in approximately 1--6% of colorectal cancers. Several studies have shown that patients with NRAS-mutated tumours are less likely to respond to cetuximab or panitumumab, but this may not affect Progression-Free Survival or Overall Survival.

Chr1:115256530 G => T (p.Gln61Lys) (Pathogenic - ACMG)

This variant is at position 70 of the 3rd exon of transcript NM_002524.5, located on the negative strand of chromosome 1p13.2. It is a missense substitution, as it changes the 61st amino acid (glutamine) to lysine.

Mutation	Gene(s)	Exon	Variant Type	Variant Allele Frequency (VAF)
HGVS Protein: Gln61Lys	**NRAS**	exon 3 of 7 position 70 of 179	SNV	**0.12**

NRAS Linked Therapies

The following list of therapies may contain experimental therapies. Note that the following list is not necessarily exhaustive, but a more detailed list of potential therapies could be requested by the physician, including clinical trial data for experimental therapies.

Therapies	Combination Type	Tissue	SAHPRA Related Drugs
Cetuximab Inhibitor Type: EGFR Inhibitor FDA Approved: Yes **Panitumumab:** Inhibitor Type: EGFR Inhibitor FDA Approved: Yes (**Resistance Indicated by NRAS Variant**)	*Combination Therapy: Cetuximab + Trametinib* (**Resistance Indicated by NRAS Variant**)	Colorectal Cancer, Melanoma	**Cetuximab**: Erbitux from Merck **Trametinib**: Mekinist from Novartis South Africa **Panitumumab**: Vectibix from Amgen South Africa

Biologically Relevant Variants

MSH6 Gene

The MSH6 gene provides instructions for making a protein that plays an essential role in repairing DNA. This protein helps fix errors that are made when DNA is copied (DNA replication) in preparation for cell division. The MSH6 protein joins with another protein called MSH2 (produced from the MSH2 gene) to form a two-protein complex called a dimer. This complex identifies locations on the DNA where errors have been made during DNA replication. Additional proteins, including another dimer called the MLH1-PMS2 dimer, then repair the errors by removing the mismatched DNA and replicating a new segment. The MSH6 gene is a member of a set of genes known as the mismatch repair (MMR) genes.

A decrease in functional MSH6 protein leads to an increase in unrepaired DNA errors during cell division. The errors accumulate as the cells continue to divide, which may cause the cells to function abnormally, increasing the risk of tumour formation in the colon or another part of the body.

In a small number of people, mutations in the MSH6 gene cause a variant of Lynch syndrome called Muir-Torre syndrome. In addition to colorectal cancer, people with this condition have an increased risk of developing several uncommon skin tumours. These rare skin tumours include sebaceous adenomas and carcinomas, which occur in glands that produce an oily substance called sebum (sebaceous glands). Multiple rapidly growing tumours called keratoacanthomas may also occur, usually on sun-exposed areas of skin.

chr2:47803501 Deletion C (p.Phe1088SerfsTer2) (Pathogenic – ACMG, Pathogenic - ClinVar)

This variant is at position 89 of the 5th exon of transcript NM_000179.3, located on the positive strand of chromosome 2p16.3. It is a frame-shift deletion of one nucleotide at the 1087th amino acid.

Mutation	Gene(s)	Exon	Variant Type	Variant Allele Frequency (VAF)
HGVS Protein: Phe1088SerfsTer2	**MSH6**	exon 5 of 10 position 89 of 266	Deletion (1)	**0.01**

Axaitech cfDNA Screen and Disclaimer

cfDNA Screen Description:

The Axaitech Liquid Biopsy Chemotherapy Resistance Screen (57 Gene) is a next-generation sequencing (NGS) cell-free DNA (cfDNA) profiling assay for identifying genomic alterations. The Screen includes single nucleotide variants (SNVs), insertions and deletions (indels) and copy number variant (ERBB2) detected by amplicon-based panels. The technical sensitivity of this panel has a limit of detection of 1% for SNVs.

Potentially actionable variants are variants that are contained within protein-coding or protein-altering regions with associated therapies from medical literature. Other variants that could potentially be relevant or variants with unknown significance could be requested, but the effects of these variants may be unclear or without sufficient evidence for actionable insight. **Benign Variants** are generally not reported. The human genome reference version 38 (GRCh38) is used for aligning the patient's DNA variants.

Axaitech Disclaimer:

Using next-generation sequencing (NGS) as a platform to analyse DNA, the sensitivity and quality of data produced may be affected by multiple factors including DNA quality, haemolysis of blood samples and low amounts of cfDNA.

Any decisions related to the patient and their care should be based on the independent judgement of the treating physician. All information regarding the patient's diagnosis, family history, physical examinations or other tests and indications should be taken into account by the physician. Axaitech is not liable for medical judgement following the test results.