Axaitech

Chemo Data Matters

Liquid Biopsy Chemotherapy Resistance Screen Report

Sample Number: Axcfdna-Sample

Patient Name: John Milnery

Diagnosis: Colorectal Cancer

975 Clifton Avenue,
Lyttelton Manor X3,
Centurion, Gauteng,
0157
Tel: +27 79 596 6030

Axaitech
Chemo Data Matters

Sample Number:	AxcfdnaSample	**Patient:**	John Milnery
Date of Birth:	18/02/1972	**Gender**	Female
Diagnosis:	Colorectal Cancer	**Ordering Physician/Oncologist**	Dear Angel

Sample Report

Potentially Actionable Variants

Genes	HGVS	Coding Impact	Cosmic Primary Site	Variant Allele Fraction (VAF)
CTNNB1	p.Ser33Tyr	missense	Central Nervous System	**0.37**
PIK3CA	p.Gly914Arg	missense	Large Intestine	**0.35**
EGFR	p.Gly719Ser	missense	Lung	**0.25**
PIK3CA	p.His1047Arg	missense	Breast	**0.18**
KIT	p.Asp816Val	missense	Haematopoietic and Lymphoid Tissue	**0.13**
NRAS	p.Gln61Lys	missense	Skin	**0.13**
CTNNB1	p.Ser45del	in frame	Kidney	**0.11**

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

Variant and Therapy Related Data

CTNNB1

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

Beta catenin is a transcriptional co-regulator and an adapter protein for cellular adhesion; it comprises part of the Wnt signaling pathway and intracellular levels of beta-catenin are regulated by its phosphorylation, ubiquitination and proteosomal degradation. Accumulation of nuclear beta catenin can lead to a tumoral phenotype and oncogenic transformation in a variety of solid tumors. Various oncogenic mutants of beta catenin have been found in different tumor types which alter its degradation, leading to its accumulation and promoting tumor growth. Some of these mutations are located at the N-terminus of the protein at the sites of phosphorylation which normally regulate its degradation.

Tumor	Tissue
Myelodysplastic Syndrome, Acute Myeloid Leukemia	Blood, Bone Marrow

CTNNB Linked Therapies:

Mutations in beta catenin (CTNNB1) are seen in about 90% of adamantinomatous craniopharyngiomas and mutations in BRAF (V600E) in papillary craniopharyngiomas. Accumulation of nuclear beta catenin can lead to a tumoral phenotype and oncogenic transformation in a variety of solid tumors.

Therapy	Type	Tissue	SAHPRA Related Drugs
Vantictumab *FDA Approved: No*	*Approval Status: Preclinical - Pdx*	Colon Cancer	No Registered Drugs. Pre-clinical Phase.

Therapy	Combination	Tissue	SAHPRA Related Drugs
Triciribine *FDA Approved: No*	*Combination Therapy: NVP-TNKS656 + Triciribine* *Approval Status: Preclinical - Pdx*	Colon Cancer	No Registered Drugs. Pre-clinical Phase.

2. PIK3CA

PIK3CA E542K, PIK3CA E545K, PIK3CA H1047R, PIK3CA codon(s) 542, 545, 1047

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 hotspot: 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.

Tumor	Tissue
Adenocarcinoma, Lobular Carcinoma	Breast

PIK3CA exon(s) 10, 20, 21

Somatic mutations in PIK3CA have been found in 10–30% of colorectal cancers. 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 is 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.

Tumor	Tissue
Adenocarcinoma	Colon, Rectum

Combination Therapy Related PIK3CA Data:

The following list of combined therapies may contain experimental therapies. The following data may require research and confirmation regarding efficacy and trial results by the physician by exploring published trial data and relevant mutation data. Scientific references in regards to these therapies may be requested by the physician. Note that the following list is not exhaustive, but a more detailed list of potential therapies could be requested by the physician.

Therapy	Combination	Tissue	SAHPRA Related Drugs
Irinotecan ++	*Combination Therapy:*	Colon Cancer, Colorectal	Irinotecan: Irocan from Innovata Pharmaceuticals

FDA Approved: Yes	*Cetuximab + Irinotecan* Combination Therapy: Cetuximab + Fluorouracil + Irinotecan + Floxuridine	Cancer	(Several Suppliers) + Cetuximab: Erbitux from Merck Fluorouracil: Fluroplex from Cipla Medpro (Several Suppliers) Floxuridine: Not Registered by SAHPRA

Therapy	Combination	Tissue	SAHPRA Related Drugs
Sorafenib ++ *FDA Approved: Yes*	*Combination Therapy: Bevacizumab + Sorafenib* *Combination Therapy: Cisplatin + Gemcitabine + Sorafenib*	Colorectal Cancer, Invasive Bladder Transitional Cell Carcinoma, Non-Small Cell Lung Carcinoma	Sorafenib: Nexavar from Bayer + Bevacizumab: Avastin from Roche Products + Gemcitabine: Cytigem from Eorolab (Several Suppliers) + Cisplatin: Kemoplat from Cipla Medpro (Several Suppliers)

3. EGFR Variant

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.

Tumor	Tissue
Adenocarcinoma	Lung

EGFR G719S, G719D

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

Tumor	Tissue
Non-Small Cell Lung Carcinoma	Lung

EGFR Linked Therapies:

Therapy	Type	Tissue	SAHPRA Related Drugs
Erlotinib	*Drug Family: EGFR*	Advanced Solid Tumor,	Tarceva from Roche

Interaction type: inhibitor, antagonist *FDA Approved: Yes*	*inhibitor 1st gen*	Esophagus Squamous Cell Carcinoma, Glioblastoma Multiforme, Head And Neck Squamous Cell Carcinoma, Lung Adenocarcinoma, Malignant Glioma, Neuroblastoma, Non-Small Cell Lung Carcinoma, Triple-Receptor Negative Breast Cancer	Products

Therapy	Type	Tissue	SAHPRA Related Drugs
Gefitinib *Interaction type: inhibitor, antagonist* *FDA Approved: Yes*	*Drug Family: EGFR inhibitor 1st gen*	Advanced Solid Tumor, Colon Cancer, Colorectal Cancer, Glioblastoma Multiforme, Head And Neck Squamous Cell Carcinoma, Lung, Lung Adenocarcinoma, Non-Small Cell Lung Carcinoma, Triple-Receptor Negative Breast Cancer	Iressa from AstraZeneca Pharmaceuticals

Therapy	Type	Tissue	SAHPRA Related Drugs
Afatinib *Interaction type: inhibitor* *FDA Approved: Yes*	*Drug Family: EGFR inihibitor 2nd gen*	Advanced Solid Tumor, Colorectal Cancer, Esophagus Squamous Cell Carcinoma, Head And Neck Squamous Cell Carcinoma, Lung Adenocarcinoma, Lung Cancer, Lung, Prostate, Non-Small Cell Lung Carcinoma, Urinary Bladder Cancer	No Registered Drugs

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 of Exon 18 G719. If this indication is important, copy number gain tests may need to be considered.

Therapy	Type	Tissue	SAHPRA Related Drugs
Cetuximab *Interaction type: inhibitor, antibody, antagonist* *FDA Approved: Yes*	*Drug Family: EGFR mAb inhibitor*	Advanced Solid Tumor, Colorectal Cancer, Esophagus Squamous Cell Carcinoma, Gastrointestinal System Cancer, Head And Neck Squamous Cell Carcinoma,	Erbitux from Merck

		Lung Adenocarcinoma, Lung Cancer, Olfactory Neuroblastoma	

Therapy	Type	Tissue	SAHPRA Related Drugs
Panitumumab *Interaction type: inhibitor, antibody, suppressor, agonist* *FDA Approved: Yes*	*Drug Family: EGFR mAb Inhibitor*	Colorectal Cancer	Vectibix from Amgen South Africa

Combination Therapy Related EGFR Data

The following list of combined therapies may contain experimental therapies. The following data may require research and confirmation regarding efficacy and trial results by the physician by exploring published trial data and relevant mutation data. Scientific references in regards to these therapies may be requested by the physician. Note that the following list is not exhaustive, but a more detailed list of potential therapies could be requested by the physician.

Therapy	Combination	Tissue	SAHPRA Related Drugs
Erlotinib + Bevacizumab *FDA Approved: Yes*	*Combination Therapy: Bevacizumab + Erlotinib*	Lung Adenocarcinoma	Erlotinib: Tarceva from Roche Products + Bevacizumab: Avastin from Roche Products

Therapy	Combination	Tissue	SAHPRA Related Drugs
Cetuximab + Erlotinib *FDA Approved: Yes*	*Combination Therapy: Erlotinib + Cetuximab*	Advanced Solid Tumor, Colorectal Cancer, Esophagus Squamous Cell Carcinoma, Gastrointestinal System Cancer, Head And Neck Squamous Cell Carcinoma, Lung Adenocarcinoma, Lung Cancer, Olfactory Neuroblastoma	Erlotinib: Tarceva from Roche Products + Cetuximab: Erbitux from Merck

Therapy	Combination	Tissue	SAHPRA Related Drugs
Decitabine ++ *FDA Approved: Yes*	*Combination Therapy: Decitabine + Gefitinib*	Colon Cancer	Gefitinib: Iressa from AstraZeneca Pharmaceuticals + Decitabine: Dacogen from

Therapy	Combination	Tissue	SAHPRA Related Drugs

Trametinib ++ *FDA Approved: Yes*	*Combination Therapy: Trametinib + Dabrafenib*	Collecting Duct Carcinoma, Colorectal Cancer, Non-Small Cell Lung Carcinoma	Trametinib: Mekinist from Novartis + Dabrafenib: Tafinlar from Novartis

4. KIT

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

KIT(cKIT) mutations are present in approximately 8-25% of cases of acute myeloid leukemia and have a higher prevalence in the favorable 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 a 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.

Tumor	Tissue
Acute Myeloid Leukemia, Mast Cell Neoplasm, Acute Leukemia of Unspecified Cell Type, Anemia, Unspecified, Atypical Chronic Myeloid Leukemia, B Lymphoblastic Leukemia/Lymphoma, Chronic Myeloid Leukemia, Chronic Myelomonocytic Leukemia, Chronic Neutrophilic Leukemia, Cytopenia, Eosinophilia, Essential Thrombocythemia, Histiocytic and Dendritic Cell Neoplasms, Langerhans Cell Histiocytosis, Leukocytosis, Leukopenia, MDS with Ring Sideroblasts, Monocytosis, Myelodysplastic Syndrome, Myelodysplastic/Myeloproliferative Neoplasm, Myeloproliferative Neoplasm, Myeloid Neoplasm, Other Acute Leukemia, Polycythemia Vera, Polycythemia, Primary Myelofibrosis, T Lymphoblastic Leukemia/Lymphoma, Thrombocytopenia, Thrombocytosis	Blood, Bone Marrow

KIT D816F, KIT D816V, KIT D816Y

Dasatinib and Nilotinib Sensitivity linked to these specific variants.

Tumor
Gastrointestinal Stromal Tumor

KIT Linked Therapies:

Some studies have shown high frequency of c-Kit overexpression in stage II colon cancer patients (59.3%) with 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. Role of c-Kit continues to be studied in colon cancers.

Therapy	Combination	Tissue	SAHPRA Related Drugs
Dasatinib ++ *Interaction type: inhibitor, antagonist* *FDA Approved: Yes*	*Mechanism Of Interaction: Stem cell growth factor receptor inhibitor* *Drug Family: BCR-ABL inhibitor 2nd gen* *Alteration: KIT:D816Y,D816F,D816V*	Acute Myeloid Leukemia, Advanced Solid Tumor, Gastrointestinal Stromal Tumor, Mast-Cell Leukemia, Melanoma	Dasatinib: Sprycel from Bristol-Myers Squibb + Cytarabine: Cytosar from Pfizer Laboratories (Several Suppliers)

	Combination Therapy: Dasatinib + Cytarabine		

Therapy	Combination	Tissue	SAHPRA Related Drugs
Nilotinib Interaction type: inhibitor, antagonist FDA Approved: Yes	*Drug Family: BCR-ABL inhibitor 2nd gen*	Advanced Solid Tumor, Gastrointestinal Stromal Tumor, Mast-Cell Leukemia, Melanoma	Nilotinib: Tasigna from Novartis South Africa

5. NRAS

NRAS Q61H, NRAS Q61L, NRAS Q61K, NRAS 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 tumors are less likely to respond to cetuximab or panitumumab, but this may not have an effect on Progression Free Survival or Overall Survival.

Tumor	Tissue
Adenocarcinoma	Colon, Rectum

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

Somatic mutations in NRAS have been found in approximately 13–25% of all malignant melanomas. The result of these mutations is constitutive activation of NRAS signaling pathways. NRAS mutations are found in all melanoma subtypes, but may be slightly more common in melanomas derived from chronic sun-damaged (CSD) skin. Currently, there are no direct (inhibitor) anti-NRAS therapies available.

Tumor	Tissue
Melanoma	Skin

NRAS Linked Therapy:

Therapy	Combination	Tissue	SAHPRA Related Drugs
Trametinib ++ *FDA Approved: Yes*	*Combination Therapy: Cetuximab + Trametinib*	Colorectal Cancer	Trametinib: Mekinist from Novartis South Africa + Cetuximab: Erbitux from Merck

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 (SNVs) that are contained within protein coding 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 not reported. The human genome reference version 19 (GRch37) 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.