Detection of Genetic Alterations in Cancer by fully automated Next Generation Sequencer at Cancer Biology Department, GCRI

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Cancer is now considered as Genetic Disease meaning that cancer is caused by certain changes to genes that control the cells function, especially how they grow and divide. Certain gene changes can cause cells to evade normal growth controls and transform into cancer. Some cancer-causing gene changes increase production of a protein that makes cells grow. The genetic changes are of two types, germline changes and somatic changes. Genetic changes that promote cancer can be inherited from parents to offspring called germline changes. Cancer-causing genetic changes can also be acquired during one's lifetime, as the result of errors that occur as cells divide or from exposure to carcinogenic substances that damage DNA, such as certain chemicals in tobacco smoke and chewing tobacco, and radiation, such as ultraviolet rays from the sun. Genetic changes that occur after conception are called somatic (or acquired) changes.

These gene mutations can be identified in DNA and RNA of an individual by Next Generation Sequencing (NGS). DNA or RNA sequencing tests can "read" DNA or RNA, respectively. By comparing the sequence of DNA or RNA in cancer cells with that in normal cells, identify genetic changes in cancer cells that may be driving the growth of an individual's cancer. This information will help to predict which therapies might work best against a particular tumor.

Currently, cancer treatment decisions are increasingly made on the basis of genomic information, and there are currently large numbers of genomic tests available to oncologists. Genomic tests designed to facilitate decisions about treatment management include those that identify alterations in single genes and multimarker tumor panels. Multigene panels include targeted gene-expression profiling tests that are used to estimate prognosis and/or the likelihood of recurrence. Multimarker panels also include DNA and RNA analysis through NGS technologies, including custom panels that profile multiple actionable driver genes, fusion genes and tumor characteristics that may guide the selection of targeted therapies.

Hence, NGS is of utmost requirement for identification of gene mutations in various cancers as well as in hereditary cancers for identification of family members at risk of cancer.

There are disease specific Multigene-Cancer Panels available that analyze number of genes associated with hereditary and sporadic cancers across major organ systems, including:

- breast and gynecologic (breast, ovarian, uterine)
- gastrointestinal (colorectal, gastric, pancreatic)
- endocrine (thyroid, paraganglioma/ pheochromocytoma, parathyroid, pituitary)
- genitourinary (renal/urinary tract, prostate)
- skin (melanoma, basal cell carcinoma)
- brain/nervous system
- sarcoma
- hematologic(myelodysplastic syndrome/ leukemia)

The Multigene-Cancer Panel is designed to maximize diagnostic yield for individuals with a personal or family history of mixed cancers affecting multiple organ systems.

Genetic testing of these genes may confirm a diagnosis and help guide treatment and management decisions. Identification of a disease-causing variant would also guide testing and diagnosis of at-risk relatives.

Recently, Next Generation Sequencing (NGS) facility: The IontorrentTM Genexus Integrated Sequencer and Ion GeneStudio S5 system (ThermoFisher Scientific) is established at the Cancer Biology Department, GCRI.

The **Genexus Integrated Sequencer - Ion Torrent** is the first turnkey next-generation sequencing (NGS) solution that automates all steps of the targeted NGS workflow starting from Nucleic Acid to results. The Nucleic Acid to Result workflow starts from purified and quantified nucleic acid

samples. Purified nucleic acid samples are pipet into a 96-well sample input plate and then loaded into the Genexus™ Integrated Sequencer for library preparation, templating, and sequencing. With a single touchpoint and five minutes of hands-on time, the Genexus sequencer automates NGS library preparation (including cDNA synthesis), template preparation, sequencing, primary data analysis, and variant reporting for DNA, RNA, and cfDNA applications. Sequencing on the Genexus sequencer is done on a four-lane semiconductor chip: the Ion Torrent GX5 Chip. Each of the four lanes of the GX5 Chip supports the output of 12–15 million reads, and they can be used individually or all at once depending on throughput needs. Ion Torrent Genexus Software streamlines the NGS workflow by integrating the setup-to-report workflow within a single software ecosystem.

The **Ion GeneStudio S5 system** is designed to enable a broad range of targeted next-generation sequencing (NGS) applications with speed and scalability. Five Ion S5 chips (Ion 550 chip, Ion 540 chip, Ion 530 chip, Ion 520 chip and Ion 510 chip) enable a sequencing throughput range of 2M to 130M reads per run.

During standardization on NGS, eight samples of each DNA and RNA of AML patients were sequenced using the Ion Torrent Oncomine Myeloid Assay GX v2 by Molecular Diagnostics & Research Lab-2 (MDRL-2). Moreover, DNA and RNA samples of four lung carcinoma and four brain tumor patients were sequenced and analyzed using the Oncomine Precision Assay (OPA) by MDRL-3 and MDRL-1 of Cancer Biology Department, respectively.

Ion Torrent Oncomine Myeloid Assay GX v2

The Ion Torrent Oncomine Myeloid Assay GX v2 is a comprehensive targeted next-generation sequencing (NGS) assay designed for sensitive detection of myeloid disorder-associated DNA mutations and RNA fusion transcripts in blood and bone marrow samples. This assay is compatible with the Genexus Integrated Sequencer, which performs library preparation, sequencing, analysis, and reporting in an automated sample-to-result workflow. Depending on the workflow, results can be obtained in as little as a single day.

Oncomine Myeloid Assay GX v2 features include:

- Comprehensive coverage of key DNA mutations and >700 fusion transcripts associated with myeloid disorders
- Automated sample-to-report workflow on the Genexus sequencer in less than a day
- Sequencing of up to eight samples (DNA & RNA) per lane on a GX5 Chip in a single run
- Less than 15 minutes hands-on time
- Detection of somatic variants down to 5% allele frequency

With the Oncomine Myeloid Assay GX v2, 45 DNA target genes and 35 RNA fusion driver genes can be interrogated simultaneously, covering the most relevant targets associated with major myeloid disorders, including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia (JMML) (Table 1).

Table 1: Gene content of the Oncomine Myeloid Assay GX v2 panel

Hotspot genes (28)		**Full genes (17)**		**Fusion driver genes (35)**			**Expression genes (5)**	**Expression control genes (5)**
ANKRD2 6 ABL1	KRAS MPL	ASXL1 BCOR	PRPF8 RB1	ABL1 ABL2	HMGA2 JAK2	NUP214 NUP98	BAALC MECOM	EIF2B1 FBXW2
BRAF	MYD88	CALR	RUNX1	BCL2	KAT6A	PAX5	MYC	PSMB2
CBL	NPM1	CEBPA	SH2B3	BRAF	(MOZ)	PDGFR	SMC1A	PUM1
CSF3R	NRAS	ETV6	STAG2	CCND1	KAT6B	PDGFRB	Wt1	TRIM27
DDX41	PPM1D	EZH2	TET2	CREBBP	KMT2A	RARA		
DNMT3 A FLT3	PTPN11	IKZF1	TP53	EGFR	KMT2A-	RUNX1		
(ITD +	SMC1A	NF1	ZRSR2	ETV6	PTDs	TCF3		
TKD)	SMC3	PHF6		FGFR1	MECOM	TFE3		
GATA2	SETBP1			FGFR2	MET	ZNF384		
HRAS	SF3B1			FUS	MLLT10			
IDH1	SRSF2				MRTFA			
IDH2	U2AF1				(MKL1)			
JAK2	Wt1				MYBL1			
KIT					MYH11			
					NTRK2			
					NTRK3			

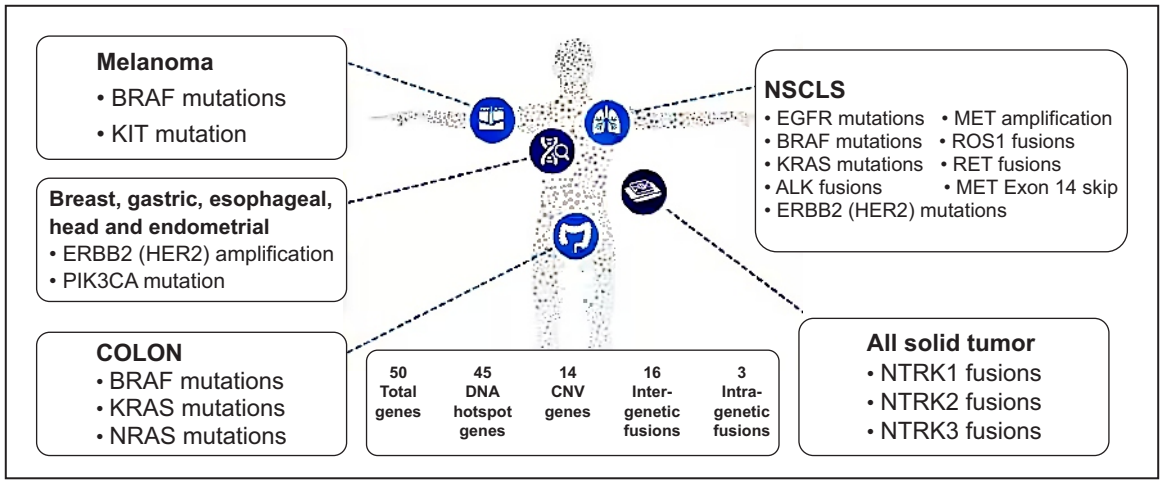

Figure 1: Oncomine Precision Assay content

Table 2: Results of Oncomine Myeloid assay GX v2

Patients	RNA		DNA	
	CNV	Fusion	SNV / Indels	
			Gene	Type of mutation
Patient 1	-	-	WT1	Truncating- Loss of function
Patient 2	-	-	DNMT3A	Hotspot- Loss of function
			PTPN11	Hotspot- Gain of function
Patient 3	-	-	FLT3	Hotspot- Gain of function
			NPM1	Truncating- Loss of function
			WT1	Truncating- Loss of function
Patient 4	-	PML(6)-RARA(3)	DNMT3A	Hotspot- Loss of function
			NPM1	Truncating- Loss of function
Patient 5	-	-	-	-
Patient 6	-	PML(6)- RARA(3)	-	-
Patient 7	-	BCR(13)-ABL1(2)	IDH2	Hotspot- Gain of function
			NPM1	Truncating- Loss of function
Patient 8		BCR(13)-ABL1(2)	IDH2	Hotspot- Gain of function
			NPM1	Truncating- Loss of function

This multiplex primer design leverages Ion AmpliSeq technology to generate results from multiple samples in a single run. Sequencing results are automatically analyzed by Genexus software using an optimized assay-specific analysis workflow.

Oncomine Precision Assay

The Oncomine Precision Assay enables simultaneous detection of biomarkers across 50 genes, including key targets within EGFR, BRAF, KRAS, ALK, ROS1, NTRK, RET, and others, from both solid tissue and liquid biopsy samples. (Figure 1) The kit provides a targeted pan-cancer panel and library reagents sufficient to perform up to 32 sequencing reactions on the Ion Torrent Genexus Integrated Sequencer using the Ion Torrent GX5 Chip.

When used with the Oncomine Precision Assay, the Genexus Integrated Sequencer performs library preparation, sequencing, analysis, and reporting in an automated sample-to-result workflow that delivers results in as little as a single day. Moreover, the Oncomine Precision Assay, based on Ion Torrent™ AmpliSeq™ HD technology, requires only 10 ng of DNA or RNA, resulting in more than 95% of samples producing sequencing results. Minimum sample input and maximum sample success rate Key benefits of the Oncomine Precision Assay on the integrated Genexus System all operated by one Ion

Table 3: Results of Oncomine Precision Assay (OPA)

Patients	Cancer site	DNA			RNA
		CNV	SNV / Indels		Fusion genes
			Gene	Type of mutation	
Patient 1	Brain	-	TP53	Hotspot- Loss of function	-
			FLT3	Hotspot- Gain of function	
			IDH1	Hotspot- Gain of function	
Patient 2	Brain	-	IDH1	Hotspot- Gain of function	ALAS1, ANKRD17, AR, EIF2B1, FGFR3 G6PD, HMBS, MET, TBP
Patient 3	Brain	AR	FLT3	Hotspot- Gain of function	ESR1-CCDC170, BAG4-FGFR1.B1F2,
			IDH2	Hotspot- Gain of function	
			NTRK1	Hotspot- Gain of function	
Patient 4	Brain	EGFR	FLT3	Hotspot- Gain of function	
			IDH1	Hotspot- Gain of function	
Patient 5	Lung	-	EGFR	Hotspot- Gain of function	
Patient 6	Lung	-	HRAS	Hotspot- Gain of function	
			TP53	Hotspot- Loss of function	
Patient 7	Lung	-	EGFR	Hotspot- Gain of function	AR, EGFR, EIF2B1, G6PD, HMBS, MET, TBP, TRIM27
Patient 8	Lung	AR	EGFR	Exon 9 deletion- Gain of Function	
			Tp53	Hotspot- Gain of function	

Table 4: Oncomine Assays and Panels compatible with Genexus Integrated Sequencer and Ion GeneStudio S5 system

Genexus	S5 Sequencer
	Oncomine Precision Assay Plus – 500 genes panel includes TMB, MSI
Oncomine Precision Assay – 50 genes panel	Oncomine Precision Assay – 50 genes panel
	Oncomine Focus Assay – 52 genes panel
Oncomine Comprehensive Assay v3 – 161 genes panel	Oncomine Comprehensive Assay v3 – 161 genes panel
	15-30 genes panel
	Oncomine Bladder Panel
Oncomine BRCA Panel	Oncomine BRCA Extended Panel
	Oncomine CRC and Pancreatic Panel
	Oncomine Gastric & Esophageal Panel
	Oncomine Gynecological Panel
	Oncomine Kidney Panel
	Oncomine Liver Panel
	Oncomine Lymphoma Panel
	Oncomine Melanoma Panel
	Oncomine Prostate Panel
Oncomine Myeloid Assay	Oncomine Myeloid Assay
Oncomine cfDNA Assay	Oncomine cfDNA Assay
	Oncomine Tumor Mutation Load Assay
	HLA Sequencing

Torrent™ Genexus™ software solution.

Oncomine Precision Assay key features include:

- Mutation, CNV, and fusion variant types across 50 key genes such as EGFR, ALK, BRAF, ROS1, RET, KRAS, PIK3CA, and ERBB2, among others
- One-day, hands-free workflow with only two touch points and 10 minutes of hands-on time
- Only 10 ng of DNA/RNA required, allowing for more samples to be tested
- Compatible with FFPE tissue as well as liquid biopsy samples

The Oncomine Precision Assay analyzes 78 variants, including mutations (45), CNVs (14), and fusion variants (19), across 50 key genes. Included are tumor suppressor genes such as TP53, cancer drivers, and resistance mutations. Content has been carefully curated to include all potentially relevant targets of emerging importance for fast genomic profiling in clinical cancer research.

The results of Ion Torrent Oncomine Myeloid Assay GX v2 and the Oncomine Precision Assay (OPA) are shown in Table 2 and Table 3, respectively.

Apart from these, there are multimarker panels for different malignancies that are compatible with Genexus Integrated Sequencer and Ion GeneStudio S5 system (Table 4).

The NGS facility is installed with the help of Corporate Social Responsibility (CSR) of Gujarat Government.

Figure 2 and 3 show the representative images of results for Oncomine Precision Assay (OPA) and Oncomine Myeloid assay GX v2, respectively by Ion Torrent Genexus Software.

Variant Report

Oncomine Precision - GX5 - Solid Tumor - DNA and Fusions - w3.2.0

Cancer Type: **Glioblastoma** Date: 22 Sep 2022

Sample Details		Sample Details	
Sample Name:	**Brain-3**	Collection Date:	**20 SEP 2022**
Application Category:	**Solid Tumor**	Gender:	**Unknown**
Sample Type:	**DNA & RNA**	%Cellularity:	**50**
Cancer Type:	**Glioblastoma**	%Necrosis:	
Cancer Stage:	**Unknown**		

Results for Sequence Variations Detected

SNVs/Indels

Gene	Variant ID	Oncomine Variant Class	Oncomine Gene Class	AA Change	Call	Allele Frequency
FLT3	COSM785	Hotspot	Gain-of-Function	p.D835H	PRESENT (HETEROZYGOUS)	0.311
IDH2	COSM33733	Hotspot	Gain-of-Function	p.R172K	PRESENT (HETEROZYGOUS)	0.423
NTRK1	BT104	Hotspot	Gain-of-Function	p.V573M	PRESENT (HETEROZYGOUS)	0.041

Fusions

Oncomine Driver Gene	Variant ID	Oncomine Variant Class	Oncomine Gene Class	Type	Call	Read Counts
ESR1	ESR1-CCDC170.E2C8.1	Fusion	Gain-of-Function	Fusion	PRESENT	41
FGFR1	BAG4-FGFR1.B1F2	Fusion	Gain-of-Function	Fusion	PRESENT	26

CNVs

Gene	Oncomine Variant Class	Oncomine Gene Class	Call	Copy Number
AR			PRESENT (LOSS)	0.08

Figure 2: Ion Torrent Genexus Software results for Oncomine Precision Assay (OPA)

Variant Report
Oncomine Myeloid v2 - GX5 - DNA and Fusions - w4.2.2

Cancer Type: **Myoepithelial Carcinoma** Date: 17 Sep 2022

Sample Details		Sample Details	
Sample Name:	**AML-10**	Collection Date:	**13 SEP 2022**
Application Category:	**Hematologic Cancer**	Gender:	**Female**
Sample Type:	**DNA & RNA**	%Cellularity:	
Cancer Type:	**Myoepithelial Carcinoma**	%Necrosis:	
Cancer Stage:	**Unknown**		

Results for Sequence Variations Detected

SNVs/Indels

Gene	Variant ID	Oncomine Variant Class	Oncomine Gene Class	AA Change	Call	Allele Frequency
FLT3	.	FLT3ITD	Gain-of-Function	p.Asp586_Arg595dup	PRESENT (HETEROZYGOUS)	0.002
FLT3	.	FLT3ITD	Gain-of-Function	p.Val592_Asp593insAlaMetThrGlySerSerAspAsnGluTyrPheTyrVal	PRESENT (HETEROZYGOUS)	0.035
FLT3	.	FLT3ITD	Gain-of-Function	p.Glu598_Tyr599insSerTyrValAspPheArgGluTyrGlu	PRESENT (HETEROZYGOUS)	0.003
FLT3	.	FLT3ITD	Gain-of-Function	p.Tyr597_Glu598insAspArgValGlnValThrSerSerSerAspAsnGluTyrPheTyrValAspPheArgGluTyr	PRESENT (HETEROZYGOUS)	0.211
FLT3	.	FLT3ITD	Gain-of-Function	p.Glu596_Tyr597insAspProAspPheArgGlu	PRESENT (HETEROZYGOUS)	0.003
FLT3	.	FLT3ITD	Gain-of-Function	p.Glu596_Tyr597insAspProAspPheArgGlu	PRESENT (HETEROZYGOUS)	0.077
FLT3	.	FLT3ITD	Gain-of-Function	p.Glu596_Tyr597insAspProAspPheArgGlu	PRESENT (HETEROZYGOUS)	0.074
FLT3	.	FLT3ITD	Gain-of-Function	p.Leu601_Lys602insGlySerGlnLeuGlnMetValGlnValThrGlySerSerAspAsnGluTyrPheTyrValAspPheArgGluTyrGluTyrAspLeu	PRESENT (HETEROZYGOUS)	0.002
FLT3	.	FLT3ITD	Gain-of-Function	p.Tyr597_Glu598insAspArgValGlnValThrGlySerSerAspAsnGluTyrPheTyrValAspPheArgGluTyr	PRESENT (HETEROZYGOUS)	0.204
FLT3	.	FLT3ITD	Gain-of-Function	p.Arg595_Glu596insLysGluAsnAsnGluTyrPheTyrValAspPheArg	PRESENT (HETEROZYGOUS)	0.009
WT1	.	Truncating	Loss-of-Function	p.Leu383CysfsTer11	PRESENT (HETEROZYGOUS)	0.095
WT1	.	Truncating	Loss-of-Function	p.Arg375AspfsTer8	PRESENT (HETEROZYGOUS)	0.353
WT1	COSM27309	Truncating	Loss-of-Function	p.Ser386LeufsTer71	PRESENT (HETEROZYGOUS)	0.477

Fusions

None Detected

CNVs

None Detected

Figure 3: Ion Torrent Genexus Software results for Oncomine Myeloid assay GX v2