Application Note

Customer led pan haematological malignancy NGS solution

A clinical application of the Nonacus Panel Design Tool

Haematological maligancies

The National Cancer Institute (NCI) defines haematological cancer, as any cancer that begins in blood-forming tissue, such as the bone marrow, or in the cells of the immune system. Disruption of normal haematopoietic differentiation can result in three main types of blood cancers; leukaemia, lymphoma, and multiple myeloma.

Cancer genomic testing contributes to Precision medicine by defining cancer types and subtypes based on their genetics. This molecular taxonomy of cancer can provide patients with a more precise diagnosis, and therefore a more personalized treatment strategy.

The abundance of easily accessible fresh cells and high-quality DNA derived from bone marrow and peripheral blood puts haematological malignancies at the forefront of the application of genomics.

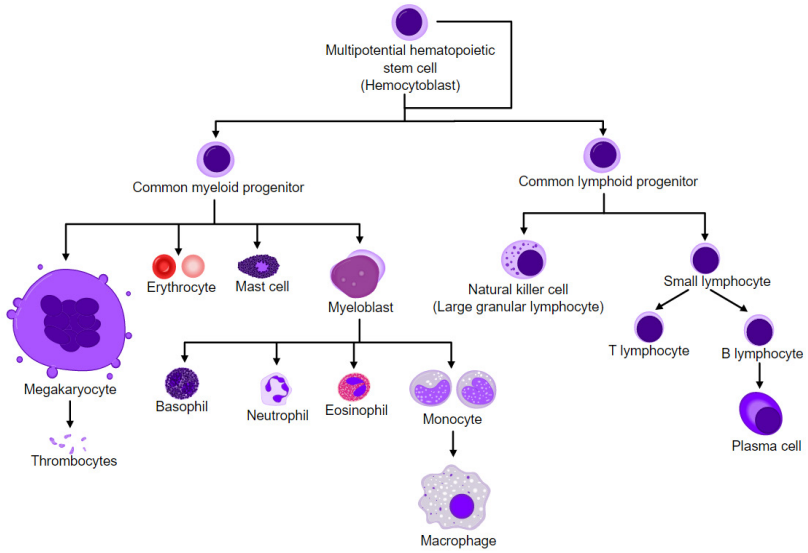

Figure 1: Haematopoiesis Overview

Disruption of normal haematopoietic differentiation can result in three main types of blood cancers; leukaemia, lymphoma and multiple myelomas

Incidence rates

Haematological cancer is the fifth most common cancer in the UK, with over 41,000 people being diagnosed every year. There are approximately 250,000 people living with blood cancer in the UK[1].

One in every 16 men and one in every 22 women will develop a haematological malignancy at some point in their lives. Just 67% of people with blood cancer only have to see their GP once or twice before being diagnosed. This compares to 94% for breast cancer, and 82% for prostate cancer. Successful treatment and improved prognosis is directly impacted by the speed and efficacy of diagnosis[1].

Current testing strategies

Given their inherent complexity, the analysis of haematological malignancies requires an interrogative and iterative testing approach, often across multiple different modalities.

Haematological cancers and in particular acute myeloid leukaemia (AML) currently requires 3 genetic tests; karyotyping, FISH and an NGS panel to cover all genetic variants known to be associated with AML (a 21 day testing approach). Replacing these workflows with one single workflow that can detect SNVs, Indels and CNVs offers huge advantages to genetic testing labs in reducing both turnaround times and cost burdens.

1 Blood Cancer UK. Understanding blood cancer, 2023. https://bloodcancer.org.uk/understanding-blood-cancer/

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Nonacus collaborative pan haemotological malignancy NGS panel

Nonacus has collaborated with a partner trust from the Central and South (CAS) Genomic Laboratory Hub (GLH) based in Birmingham, UK, who currently provide clinical testing services for a population of 6 million patients. Their aim was to design a comprehensive hybridization-capture panel to detect all variant types across all regions identified as clinically relevant in the UK NHSE National Genomic Test Directory, plus additional genes likely to be included in future updates to the test directory (e.g. UBA1:VEXAS). The resulting pan haematological solution will be used to deliver testing across both myeloid and lymphoid neoplasia and will replace multiple existing workflows.

Design Specification

- Detect SNVs with a limit of detection (LOD) of 1% across 132 clinically relevant genes, including those listed on the UK NHSE National Genomic Test Directory
- Inclusion of additional genes expected to be included in future updates to the test directory
- Detect CNVs with a LOD of 20% in target regions
- Inclusion of specific intronic regions in the design of the panel and deployment of variant callers in the bioinformatic pipeline to enable the detection and characterization of FLT3-ITDs, KMT2A(MLL)-PTDs and structural rearrangements for BCR:ABL1
- CNV Backbone comprising 2213 SNPs, for gross CNV calling

Nonacus Panel Design Tool

Targeted DNA sequencing approaches rely on a successful NGS panel design to provide high on-target rates with good uniformity of coverage, to accurately and robustly call genomic variants with high sensitivity and specificity.

The clinical scientists at Central and South (CAS) Genomic Laboratory Hub (GLH) used our online Panel Design Tool which uses proprietary algorithms built in house by experts in assay design. The tool automatically masks highly repetitive regions and includes an exome gap-fill to ensure that the probes designed capture target regions efficiently and with uniform coverage.

We were able to achieve 93.95% coverage of the desired targets.

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Pan haematological NGS panel final design

- 134 genes (including SRY for gender ID only)
- 13 regions for CNV: CNV del(1p), CNV gain(1q), CNV (11q), CNV 13q14; del(17p), CNV del(17p), CN-LOH 6p, CEBPA, GATA2, RUNX1, TERC, TERT, trisomy12
- KMT2A including exons and introns 1-14
- FLT3 with introns 14 & 15
- 38 informative SNPs for genotyping (sample tracking)
- Covering whole coding regions for all genes apart from two –BTK and GNB1 where only hotspots are required
- Overlapping Coverage for exons at least +/-5bp; certain genes +10/-30bp per each exon
- Captures whole gene deletion events for CEBPA, GATA2, RUNX1, TERC, TERT
- All transcripts covered for all genes

ABL1	CBL	ERBB3	IRF4	NOTCH2	SAMD9	TERT
ANKRD26	CBLB	ERCC6L2	JAK2	NPM1	SAMD9L	TET2
ARAF	CBLC	ETNK1	JAK3	NRAS	SAMHD1	THPO
ARID1A	CCND3	ETV6	KDM6A	PAX5	SETBP1	TNFAIP3
ASXL1	CD79B	EZH2	KIT	PHF6	SETDB1	TP53
ATM	CDKN2A	FBXW7	KLF2	PIGA	SF3B1	U2AF1
ATRX	CDKN2C	FLT3	KMT2A	PIK3CA	SH2B3	U2AF2
B2M	CEBPA	FOXO1	KMT2C	PIK3CD	SH3BP1	UBA1
BCL2	CIITA	FYN	KRAS	PLCG1	SMC1A	UBTF
BCOR	CKS1B	GATA1	MAP2K1	PLCG2	SMC3	VAV1
BCORL1	CREBBP	GATA2	MAP3K1	POT1	SRP72	WT1
BCR	CSF3R	GATA3	MBD4	PPM1D	SRSF2	XPO1
BIRC3	CUX1	GNAS	MECOM	PRPF8	SRY	ZNF217
BLL1B	CXCR4	GNB1	MEF2B	PTEN	STAG1	ZRSR2
BRAF	DDX3X	HRAS	MPL	PTPN11	STAG2	
BRCC3	DDX41	ID3	MYD88	RAD21	STAT3	
BTG1	DHX34	IDH1	NF1	RHOA	STAT5B	
BTK	DIS3	IDH2	NFE2	RPS15	TCF3	
CALR	DNMT3A	IKZF1	NFKBIE	RTEL1	TENT5C (FAM46C)	
CARD11	EP300	IL7R	NOTCH1	RUNX1	TERC	

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FLT3-ITD detection significance for AML

The FLT3-internal tandem duplication (FLT3-ITD) is the most common gene alteration found in patients diagnosed with acute myeloid leukaemia (AML) and has been associated with poor prognosis, especially when the ITD is located in the tyrosine kinase domain[2]. Patients with FLT3-ITD have an increased risk of relapse and shorter overall survival rates; therefore, accurate detection of FLT3-ITDs is paramount in standard of care testing.

FLT3-ITDs can be inherently difficult to detect using some NGS techniques due to reliance on the reconstruction of short (<300 base pair) sequences, therefore longer length ITDs or those of poorer quality may be difficult to detect.

Nonacus design strategy to overcome FLT3-ITD variant detection

Hybridisation strategies can capture a wide range of repetitive sequences that occur within internal tandem duplication events, that differ dramatically from typical genomic sequence. The Nonacus design strategy maximises the efficient capture of these aberrations within exons 13-15 (including the intronic sequence) that can be missed by amplicon or sub-optimal placement and density of probes. This design allows detection of large ITDs of several hundred bases.

NGS is becoming more commonly used in monitoring for FLT3 minimal residual disease (MRD) following treatment, therefore strategies to ensure efficient capture of FLT3 aberrations is key in patient care for AML[2].

Customer data analysis and results summary

The team at the Central and South (CAS) Genomic Laboratory Hub (GLH) based in Birmingham, UK, have created a bespoke bioinformatics pipeline using a python script employing Illumina DRAGEN Bio-IT Platform v3.10 software, converting BCL files to ORA compressed FASTQs, before aligning reads to genome build GRCh38 with BWA-MEM. For variant calling, the DRAGEN variant caller tool is used for identifying SNVs while an extended version of Manta is implemented for calling SVs. Each sample concatenated VCF file is uploaded to Alissa Interpret for filtering and annotation.

The pan haematological NGS panel solution coverage was >99% at >500X. A range of SNVs were detected at varying Variant Allele Fractions (VAF) as low as 1%, along with indels in TP53 and CEBPA, FLT3-ITDs and KMT2A-PTDs.

Conclusion

The team at the Central and South (CAS) Genomic Laboratory Hub (GLH) based in Birmingham, UK, used the Nonacus Panel Design Tool to design an NGS panel to detect variants associated with both myeloid and lymphoid disorders. This will allow streamlining of the testing workflow, reducing both turnaround time and cost burden, which will ultimately enable quicker diagnosis and treatment decisions, improving the patient management and prognosis.

2 Bibault JE, et al. Next-generation sequencing of FLT3 internal tandem duplications for minimal residual disease monitoring in acute myeloid leukemia. Oncotarget. 2015 Sep 8;6(26):22812-21. doi: 10.18632/oncotarget.4333. PMID: 26078355; PMCID: PMC4673201.

Nonacus Limited
Quinton Business Park
11 Ridgeway
Birmingham B32 1AF
info@nonacus.com **www.nonacus.com**