The Benefits of a Next Generation Sequencing Gene Panel for Lymphoid Malignancies

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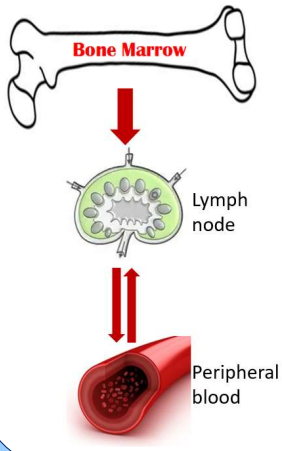
Background Information



Lymphoid malignancies are the most common haematological cancers, with over 115,000 cases of non-Hodgkin lymphoma diagnosed annually in Europe[1]. These malignancies are tumours derived from lymphocytic tissues – a sub-set of white blood cells which arise from the B- and T-cell lineage. The major role of lymphoid tissues is to create and trigger appropriate immune responses within the body to effectively protect against foreign pathogens. As there are limitless foreign pathogens that may invade the human body at any given time, the immune response must be primed to adapt to this. This is achieved via complex processes of genetic alteration to generate various immune responses to give physiological diversity and intricacy.

Initial studies on oncogenesis, describing a few major molecular alterations to be the cause of cancer development, are too simplistic to describe development for many cancers. Evidence supporting this over-simplification is the presence of malignancies which have high mutational heterogeneity and no apparent characteristic mutation[2,3] (i.e. one mutation does not equal to one type of cancer), or poor patient response to targeted therapy based only on molecular aberrations of disease[4]. Diagnosis and classification is a multidisciplinary process encompassing clinical observations, imaging, histopathology, haematology and molecular pathology investigations. Incidence of disease is increasing[1], and new/additional molecular markers have been incorporated into the most recent World Health Organisation (WHO) classification guidelines[5]. Disease entities, such as diffuse large B-cell lymphoma (DLBCL), which were once considered as a single disease are now recognised as molecularly heterogeneous and can be subdivided into at least three distinct diseases with contrasting clinical outcomes[5,6].

Why Employ a Next Generation Sequencing (NGS) panel?

Traditional diagnosis

- Pathological microscope observations can be subjective
- Few molecular markers which are analysed by single gene testing
- Insufficient to allow accurate and reproducible characterisation or subclassification of complex lymphomas.

New Classifications[5]

- Updated guidelines for classification of lymphoid malignancies now recognise the importance of molecular characterisation of this complex and heterogenous group of disorders.

Extended Molecular Repertoire

- Extending the molecular testing repertoire to a 68 gene panel will reduce the subjective nature of parts of the diagnostic process, increasing robustness of lymphoma classification.
- Accurate classification is facilitated, and error reduced, by multimodality testing of diagnostic specimens; convergent results increase the certainty of assigning a specific diagnostic label whilst divergent results flag up alternative possibilities.

Modern lymphoma classification requires identification of a large number of clinically and biologically distinct entities on the basis of shared morphologic, immunophenotypic and genetic characteristics; treatment is dictated by the diagnostic category into which a tumour is placed.

Targeted NGS gene panels allow optimisation of quality, in the way of variant characterisation, read depth, reporting timelines, and cost. These qualities make them a practical option for lymphoma diagnostics within a clinical healthcare setting.

An optimal targeted panel must include markers that give differential or positive diagnosis, prognostic implications and therapeutic requirements for the different lymphoma subtypes. A custom 68 gene lymphoid panel is being clinically validated within NHS Lothian (table 1) with the aim to:

- Assist diagnosis of lymphoid malignancies.
- Provide targeted prognostic information to improve the patient pathway
- Advance therapy stratification ensuring patients obtain optimal treatment options available.
- Enable many clinically relevant molecular markers to be analysed simultaneously.
- Improve the efficiency to diagnose complicated cases.
- Streamline molecular testing by replacing single gene analysis.
- Potentially shorten turnaround times for molecular analysis of lymphoid malignancies due to simultaneous molecular analysis of many different genes.
- Improve the adaptability for the dynamic landscape of lymphoma diagnosis.
- Standardise lymphoid malignancy molecular testing across Scotland.
- Potential cost savings for NHS due to improved therapy stratification for patients to receive treatments more likely to be of benefit.

Table 1: The 68 genes covered by the custom lymphoid panel

ARID1A1	●	*CD28*	●	*FBXW7*	●	***KIT***	●	***NRAS***	●	*SOCS1*	●
ATM	● ●	*CD58*	● ●	*FOXO1*	●	*KLF2*	●	*PIK3CA*	●	*STAT3*	●
B2M	●	*CD79A*	●	*GNA13*	●	*KLHL6*	●	*PIK3CD*	●	*STAT5B*	●
BCL2*	● ● ●	*CD79B*	● ●	*GPR34*	● ●	*KMT2D*	● ●	*PIM1*	●	*STAT6*	●
BIRC3	● ●	*CDKN2A*	● ●	*ID3*	● ●	***KRAS***	●	*PLCG1*	●	*SYK*	●
BRAF	●	*CREBBP*	●	*IDH1*	●	*MAP2K1*	● ●	*PLCG2*	●	*TCF3*	● ●
BTK	●	*CRLF2*	●	*IDH2*	●	***MYC****	●	*POT1*	●	*TET2*	● ● ●
CARD11	● ●	*CXCR4*	●	*IKZF1*	●	***MYD88***	● ●	*PTEN*	●	*TNFAIP3*	●
CCND1*	● ●	*DNMT3A*	● ●	*JAK1*	●	*NF1*	●	*PTPN1*	●	*TNFRSF14*	●
CCND2	● ●	*EP300*	●	***JAK2***	● ● ●	*NOTCH1*	●	*RHOA*	●	***TP53***	● ●
CCND3	● ● ●	***ETV6****	●	*JAK3*	●	*NOTCH2*	●	*SF3B1*	●	*XPO1*	●
CCR6	●	*EZH2*	●	● = Actionable		● = Prognostic		● = Diagnostic			

Genes in bold are those that are currently on the lymphoid malignancy testing repertoire, with the exception for *TP53* the existing genes will only get certain hotspot mutational analysis. The NGS panel is able to detect additional mutational variants out width the hotspot regions.

* Testing carried out by fluorescent *in situ* hybridisation (FISH) which detects fusion genes and therefore misses mutational variants which the NGS panel can detect.

References

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