

Analysis of Mutational Status of Clonal IGVH Gene Rearrangements in Chronic Lymphocytic Leukaemia

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Sample Required:

See Sample requirements page at www.nbt.nhs.uk/genetics for full details

A 3-5ml sample of peripheral blood or bone marrow collected into EDTA.

Samples should be accompanied by a FULLY completed request form (available as download at www.nbt.nhs.uk/genetics or from the laboratory).

Please include details of test, family history, address and POSTCODE, NHS number, referring clinician and unit/hospital.

Consent and DNA/RNA Storage

All genetic testing requires consent. It is the responsibility of the referring clinician to ensure that appropriate consent has been obtained.

Stored material from all referrals may be retained for quality assurance purposes and may be used anonymously for the development of new tests for the disorder in question.

Clinical Background and Genetics

- Chronic lymphocytic leukaemia (CLL) is one of the most common lymphoid malignancies in the Western world and makes up over 40% of all leukaemia in patients over 65 years of age. The overall incidence each year is approx. 3/100,000 where the average age of onset is between 65 and 70 years
- The clinical course of the disease is notable for marked variability. Generally thought to be an indolent disease, a significant proportion of patients do however have a more rapid disease progression requiring immediate therapy. Accurate prognostication is therefore required for this cohort of patients.
- In recent years a number of biological features have been shown to correlate with prognosis.
- One such biological marker includes analysis of IGVH mutation status. Studies by several groups have shown that the median survival for patients with unmutated VH genes was significantly worse as compared to those with mutated VH genes (95 months compared to 295 months – Hamblin *et al.*, 1999).
- The presence of somatic hypermutation of IGVH genes (defined as greater than or equal to 2% divergence from the germline variable gene segments) has subsequently been shown to provide important prognostic information for patients with CLL and small lymphocytic lymphoma.
- Work undertaken by Thorselius *et al.*, 2006 showed that patients with IGH rearrangements that involve the VH3-21 gene region were associated with poor outcome irrespective of mutational status.

Service offered

- BGL is part of the Bristol Haemato-oncology Diagnostic Service (BHODs) and has access to a full range of complementary pathology services.
- Rearrangements of antigen genes occur during ontogeny in B and T lymphocytes. Gene rearrangements that result are unique in length and sequence. PCR can therefore be used to identify lymphocyte populations derived from a single cell by detecting the unique V-D-J gene rearrangements present.
- Multiple consensus primers that target the conserved regions within the gene partners are therefore used as PCR primers to amplify the products of gene rearrangements.
- Analysis of IGVH gene usage and mutation status is carried out by PCR using VH Leader family specific primers in conjunction with downstream JH consensus primers. On occasions when the VH Leader primers fail to produce a product the BIOMED VH FR1 family-specific primers are used.
- Amplicons are evaluated by heteroduplex analysis and clonal rearrangements sequenced.
- V, D and J gene usage is determined by comparison to germline sequence databases (IMGT or IgBlast) and hypermutation analysis is undertaken to determine the divergence from the germline VH region.
- All results are analysed and interpreted in accordance with the original and revised ERIC recommendations for IG gene sequence analysis in CLL (Langerak *et al.*, 2011 & Rosenquist *et al.*, 2017).

Referrals

- Diagnostic Testing**
Immunoglobulin gene sequence analysis is used for prognostication in chronic lymphocytic leukemia (CLL)
- Target reporting Time**

	TAT
IGVH mutation screen	20 days

For up-to-date prices please contact the laboratory.