**Institution:** University of Southampton

**Unit of Assessment:** 01 Clinical Medicine

**Title of case study:** 01-21 A genetic predictor of progression in a common chronic leukaemia

## 1. Summary of the impact

Research by the University of Southampton has helped transform the understanding and treatment of chronic lymphocytic leukaemia (CLL), the most common leukaemia, affecting around 2,400 patients each year in the UK and 17,000 in the USA. Southampton's widely cited studies revealing the existence of two subsets of CLL have been crucial in giving clinicians and patients in the UK and overseas a much clearer indication of the likely disease course. The predictive information is now included in all clinical trials and in international guidelines, delivering greatly improved care. The research has also inspired the development of a new drug given "breakthrough" status by the Food and Drug Administration in the United States.

## 2. Underpinning research

In the late 1990s Southampton researchers uncovered a major prognostic marker in chronic lymphocytic leukaemia (CLL), the Western world's most common form of leukaemia. Before the 1999 publication of their findings **[3.1]** CLL was considered a single disease of variable clinical course. The research showed there are two subsets of CLL, with significantly different mean survival rates and treatment requirements.

This finding arose from research employing sequencing techniques developed by Freda Stevenson (joined Southampton 1981, Professor of Immunology from 1997). This involved the analysis of human immunoglobulin variable region (V) genes in B-cell malignancies and in normal B cells by sequencing of the amplified DNA segments. Working with Southampton haematologist Terry Hamblin (Professor of Immunohaematology from 1987, died January 2012) and Dr Caroline Chapman (1992-99), Stevenson began to sequence a range of human B-cell tumours, leading to major insights into a range of haematological malignancies **[3.2, 3.3, 3.4]**.

Once the sequences were established, correlations with disease behaviour were assessed, revealing a connection between Ig V gene mutational status and the subsequent clinical course. Two subsets of CLL were identified: one derived from B cells prior to entry to the germinal centre and carrying no somatic mutations in the Ig variable region genes (unmutated CLL or U-CLL), the other from cells that have traversed this site and accumulated mutations (mutated CLL or M-CLL). The mutational pattern remained fixed and a constant marker of the tumour cells.

Importantly, it was found the ~40% of patients with U-CLL had a more aggressive disease, for which the mean survival rate was eight years – compared to 25 years for the ~60% of patients with M-CLL. This is reflected in U-CLL patients' greater need for chemotherapy.

This subdivision had previously been missed because CLL cells from the two subsets are similar in appearance and phenotype. A USA research group (Damle, R., *Blood*, 1999) concurrently observed the two subsets, confirming the work's international applicability. The findings have since been confirmed by multiple studies and widely accepted. Southampton's 1999 publication in *Blood* has been cited around 1500 times.

The reason for the difference in tumour behaviour is now known to reflect a differential response to antigen engagement. Further research at Southampton on the biology behind disease behaviour has focused on critical signaling pathways mediated by the surface Ig **[3.5, 3.6]**. The U-CLL subset signals more, paving the way for the development of new therapeutic strategies specifically targeting the signalling pathways.

The discovery of this prognostic marker led to an explosion of interest in CLL among biologists. Researchers have sought surrogate markers associated with the Ig V-gene mutational status, which would provide an easier means of measuring the gene analysis. While some have been

found, so far none is sufficiently close to allow this.

Dr David Oscier, a haematologist at Bournemouth Hospital, also made a contribution to this research, as did various post-doctoral fellows and clinician scientists, including Dr M Spellerberg (1983-2001).

## 3. References to the research

**3.1** Hamblin TJ, Davis Z, Oscier DG & Stevenson FK. Unmutated immunoglobulin $V_H$ genes are associated with a more aggressive form of chronic lymphocyte leukemia. *Blood* 1999; 94(6):1848-1854. *(>1500 citations)*

**3.2** Stevenson F, Sahota S, Zhu D, Ottensmeier C, Chapman C, Oscier D, Hamblin T. Insight into the origin and clonal history of B-cell tumors as revealed by analysis of immunoglobulin variable region genes. *Immunol Rev* 1998; 162:247-59. *This review summarizes the work leading up to the discovery reported in the impact paper* **3.1***.*

**3.3** Oscier DG, Thompsett A, Zhu D, Stevenson FK. Differential rates of somatic hypermutation in V(H) genes among subsets of chronic lymphocytic leukemia defined by chromosomal abnormalities. *Blood 1997;* 89(11):4153-60. *This paper describes the beginning of the genetic analysis of CLL cases.*

**3.4** Hamblin TJ, Orchard JA, Davis Z, Gardiner A, Oscier D, & Stevenson FK. Immunoglobulin V gene and CD38 expression in CLL. *Blood 2000;* 95(7):2455-2457. *This is one of several follow up papers.*

**3.5** Lanham S, Hamblin TJ, Oscier DG, Stevenson FK & Packham G. Differential signaling via surface IgM is associated with VH gene mutational status and CD38 expression in chronic lymphocytic leukemia. *Blood* 2003; 101:1087-1093. *This describes the next stage of analysis of the critical signaling pathways mediated by the surface Ig and set the scene for development of inhibitory drugs.*

**3.6** Krysov S, Potter KN, Mockridge CI, Coelho V, Wheatley I, Packham G, Stevenson FK. Surface IgM of CLL cells displays unusual glycans indicative of engagement of antigen in vivo. *Blood* 2010; 15(21):4198-205. *This paper describes further observations on the biology of the surface Ig which again is likely to open new therapeutic strategies.*

**Grants**

All of the work over these years has been funded by external grants to Stevenson from the following national cancer charities:

Tenovus, Cardiff Programme Grants: “Chronic lymphocytic leukaemia: linking clinical behaviour to B-cell biology” (2001-2006 £1,297,500; 2006-2009 £549,429) and

Leukaemia and Lymphoma Research Project Grant: “Differential signaling via the BCR in subsets of CLL” (2003-2006 £130,076).

## 4. Details of the impact

CLL is the most prevalent form of leukaemia in the West. It tends to occur in later life and is more common in men than women. Every year around 17,000 people in the USA and 2,400 in the UK are diagnosed with the disease. As a result of the University of Southampton’s research, patients can now be told the likely course of their disease and their prognosis, while clinicians, by following current international guidelines based on the findings, are able to offer vastly improved care.

The finding that patients can be divided into those with U-CLL (~40% of cases) or M-CLL (~60% of cases) led to a clear subdivision of the disease. This distinction has had far-reaching implications for patients, their families and clinicians since U-CLL has a mean survival period of ~99 months and M-CLL ~293 months.

This widely confirmed correlation between the Ig V-gene mutational status and the progression of CLL discovered by researchers at Southampton has had a dramatic effect on both the understanding of pathogenesis and on clinical management. It is now mandatory for clinical trials of CLL to include this prognostic factor and have Ig V-gene analysis **[5.1]**.

Patients with U-CLL have a significantly lower mean survival rate than those with M-CLL, as well as a greater need for chemotherapy. Southampton's research has helped clinicians predict the time to first treatment and the time to the chemorefractory state (thus alerting clinicians to poor outcome), as well as the likelihood of transformation to the more aggressive Richter's syndrome, which has a mean survival of five to eight months.

A better understanding of appropriate treatment means chemotherapy can be avoided or at least delayed for some patients. This brings both economic savings, given the cost of treatments, and, due to the considerable potential side-effects of many forms of treatment, benefits to patients' well-being. These factors were the driving force behind the publication of international guidelines **[5.1]** in 2008 for the diagnosis and treatment of CLL, which directly referenced Southampton's research and updated previous guidelines issued by the US National Cancer Institute Working Group. The 2008 guidelines prepared by clinicians from Europe and the USA state: "The leukemia cells express immunoglobulin that may or may not have incurred somatic mutations in the immunoglobulin heavy chain variable region genes ($IgV_H$ genes). The outcome of patients with leukemia cells that use an unmutated $IgV_H$ gene is inferior to those patients with leukemia cells that use a mutated $IgV_H$ gene." These are still current.

An important aspect for prognosis is that the $IgV_H$ mutational status is a biomarker applicable to all patients. This contrasts with chromosomal markers e.g. the 17p deletion which is found in ~7% of patients.

An update on the clinical usefulness of Ig V-genes has been published recently by the Swedish CLL group **[5.2]**.

The Southampton findings also directly influenced the formation of practice guidelines from the Italian Society of Haematology, the Italian Society of Experimental Haematology and the Italian Group for Bone Marrow Transplantation. These are still current **[5.3, 5.4]**.

A more recent outcome of the research has been the development of drugs specifically targeting the Ig signaling pathways **[5.5]**. A 2012 paper published in *Leukemia & Lymphoma* **[5.6]** referred to the new drugs as "exciting". Not only is the VH gene status important, it is a target for novel drugs, such as ibrutinib, which promises to have a dramatic effect on CLL treatment. The Food and Drug Administration in the US has already awarded ibrutinib three 'breakthrough' designations, a reflection of its publicised commitment to fast track the delivery of the experimental drug to patients **[5.7]**.

For patients, the National Cancer Institute (US) website for CLL puts Ig gene mutational status first in the list of prognostic factors **[5.8a]**. Consequently, there is evidence that patients are learning of this important prognostic factor and requesting the test: in the US, commercial companies (e.g. Molecular Diagnostic Labs, Barnes-Jewish Hospital, St Louis) offer the assay. Websites set up to support and empower patients with CLL provide some evidence of patients using IGHV mutational status as their own index of prognosis in CLL **[5.8b]**.

The Ig V-gene status is also useful in different clinical settings. For instance, it is recognised that unmutated immunoglobulin variable heavy-chain gene status remains an adverse prognostic factor after autologous stem cell transplantation for chronic lymphocytic leukaemia showing continued relevance after treatment **[5.9, 5.10]**.

## 5. Sources to corroborate the impact

**5.1** Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia, updating the National Cancer Institute-Working Group 1996 guidelines; Michael Hallek et al. *Blood* 2008; 111(12): 5446-5456. *These guidelines are compiled from representatives in Europe and the USA, including the USA CLL Consortium (TJ Kipps).*

**5.2** Rosenquist R et al Prognostic markers and their clinical applicability in CLL:where do we stand? *Leuk and Lymphoma* 2013; 54: 2351-2364.

Two further general references from the large European clinical trial groups:

**5.3** Brugiatelli M et al Management of CLL: practice guidelines from the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation. *Haematologica* 2006; 91(12):1662-1673

**5.4** Hallek M et al. Addition of rituximab to fludaribine and cyclophosphamide in patients with CLL-a randomized open-label phase 3 trial. *Lancet* 2010; 376: 1164-1174

*Clinicians in the USA are also in line with the above, with one example from the MD Anderson Cancer Centre, a major centre for the treatment of CLL, in Houston [5.5] and another from Harvard Medical School [5.6]:*

**5.5** Lin KI, et al. Relevance of the immunoglobulin VH somatic mutation status in patients with chronic lymphocytic leukemia treated with fludarabine, cyclophosphamide, and rituximab (FCR) or related chemoimmunotherapy regimens. *Blood* 2009; 113:3168-3171

**5.6** Davids MS, Brown JR. Targeting the B-cell receptor pathway in CLL. *Leuk and Lymphoma* 2012; 53(12):2362-2370

**5.7** http://www.fiercebiotech.com/story/breakthrough-ibrutinib-nda-makes-rapid-arrival-fda/2013-07-10

**5.8** a) National Cancer Institute [USA] CLL prognostic factors: http://www.cancer.gov/cancertopics/pdq/treatment/CLL/healthprofessional/Page2#Section_179

b) Cancer Research UK: http://www.cancerresearchuk.org/cancer-help/type/cll/treatment/statistics-and-outlook-for-chronic-lymphocytic-leukaemia

**5.9** Ritgen M, Lange A, Stilgenbauer S, Dohner H, Bretscher C, Bosse H, Stuhr A, Kneba M, Dreger P. Unmutated immunoglobulin variable heavy-chain gene status remains an adverse prognostic factor after autologous stem cell transplantation for chronic lymphocytic leukemia. *Blood* 2003; 101(5):2049-53.

**5.10** Our claims can be verified by the President of the European Research Initiative in CLL (ERIC http://www.ericll.org/ ), a subgroup of the European Leukemia Net. Corroborative statement held in online evidence repository.