To the Editor, “Leukemia”:

Male preponderance in chronic lymphocytic leukemia utilizing IGHV 1-69.

Gender plays an important role in the incidence, progression and prognosis of individuals with several forms of hematological malignancy. In the context of B-cell lymphoproliferative disorders, there is a marked preponderance of males (4:1 male to female ratio) in diseases such as hairy cell leukemia (1). For CLL, data derived from both national registries and from prospective clinical trials have shown an increased incidence for males (reviewed in reference 2). One registry study reported a five fold excess of males in patients presenting at about forty years of age (3). Males tend to present with more clinically advanced disease and have a worse prognosis. Randomized clinical trials have shown that even allowing for clinical stage, males have a worse prognosis than females. The biological basis for these differences remains unclear; possible explanations include molecular, genetic and hormonal differences.

Studies on the expression of the immunoglobulin (IG) V region gene segments in CLL have revealed a number of biologically and clinically interesting points. Firstly, there are two subsets of CLL that can be defined by the presence or absence of somatic mutations. Presence of somatic mutations is associated with a better prognosis. In one previous study, there was a 2:1 male excess in CLL patients with unmutated disease, whereas the mutated CLL showed male to female equivalence (4). Secondly, several studies have shown highly restricted IG rearrangements in CLL, both in terms of IGHV gene segment usage and in terms of antigen binding sequences, suggesting a strong role for antigen selection during the development of the disease (5). One of the “stereotypical” IG receptors is that involving the IGHV1-69 gene segments. IGHV1-69 gene
segment usage is found in about 10-15% of all CLL patients. In most instances, these segments remain unmutated. Sequence analysis of both heavy and light chain gene sequences has shown strong conservation of the antigen binding site.

We have studied the frequency and nature of IGHV rearrangements in CLL patients from three separate databases; firstly from the Leukemia Research CLL4 trial (containing only patients requiring therapy), and secondly, from two comprehensive databases from Bournemouth and Leicester in the UK, representing unselected patients seen in both clinics. We report that uniquely, IGHV1-69 gene segment usage occurs predominantly in males. No other significant gender differences were consistently detected in any other common IGHV subgroup.

Blood samples were taken from patients with CLL following informed, written consent and with local ethical committee approval. Genomic DNA and total cellular RNA were isolated using Qiagen columns (Hilden, Germany) from leukemic mononuclear cells prepared by centrifugation on a Ficoll gradient. Amplification and sequence analysis of IGH rearrangements by DNA polymerase chain reaction (PCR) according to BIOMED-2 protocols or reverse transcription-PCR (RT-PCR) was performed, as previously described. Although sequence data were analyzed on at least 2 databases (IMGT, IgBlast, V-BASE), results were reported using the IMGT database (http://imgt.cines.fr). Sequences with a germline homology of 98% or higher were considered as unmutated, and those with a homology less than 98% were considered as mutated. IGVH mutational data were collected from a total of 1239 patients, comprising 540 patients entered into the CLL4 clinical trial, 392 patients attending the Royal Bournemouth Hospital and 307 patients, Leicester Royal Infirmary. The latter two cohorts represent unselected datasets of consecutive patients attending either hospital. The IGHV segment usage in the three different cohorts was broadly similar,
although there was over-representation of \textit{IGHV}1-69 in the CLL4 cohort and possible under-representation of \textit{IGHV}3-21 in the Bournemouth database; \textit{IGHV}4-34 was also under-represented in CLL4. We assessed the male to female ratio in each cohort according to \textit{IGHV} segment usage. Overall, the male to female ratio in all three cohorts was 2.1:1. Data from the eight most commonly utilized \textit{IGHV} gene segments according to gender are shown in Figure 1. In all three cohorts, the \textit{IGHV}1-69 subset contained an excess of males. Overall, there were 133 males and 28 females utilizing \textit{IGHV}1-69 (ratio 4.75:1); comparison of this ratio with the overall ratio within the CLL population showed that this was highly significant (\textit{p} value = 0.000002 using parametric one sample proportion test). Similar gender imbalances were not seen in any other of the other commonly utilized \textit{IGHV} subgroups. Within the \textit{IGHV}1-69 group, there were no significant gender imbalances seen within the described stereotypical subsets of sequences (6).

Eight of the 92 patients with \textit{IGHV}1-69 usage entered into the CLL4 study were mutated. In the Leicester series, there were five out of 28 cases with more than 2\% mutations; none of these were women. All mutated \textit{IGHV}1-69 cases presented with Binet stage A(0) disease and only one has required treatment to date; the lymphocyte doubling time was much slower in \textit{IGHV}1-69 cases as shown in Figure 2. In terms of rate of accumulation of lymphocytes in the peripheral blood, there was a significant difference between mutated and unmutated \textit{IGHV}1-69 (\textit{p}=0.002, Student’s t-test, Figure 2c). Interestingly, whilst most of the \textit{IGHV}1-69 cases required therapy early in the course of their disease, some unmutated \textit{IGHV}1-69 cases exhibited long periods where the disease was only slowly progressive (Figure 2). In one instance, more rapidly progressive disease was associated with a secondary 11q23 chromosomal deletion by FISH (data not shown). Together, these data indicate that the difference between
mutated and unmutated \textit{IGHV}1-69 subgroups may be the rate of acquisition of deleterious secondary genetic events that drive accelerated proliferation.

One other rarer \textit{IGHV} subgroup may also show comparable male imbalance; from the CLL4 study, \textit{IGHV}1-18 was observed in 14 males compared with only one female. However, this gene segment was only utilized in six other patients within the other two cohorts, which did not permit statistical analysis.

The association of \textit{IGHV}1-69 gene segment usage in CLL and male gender is intriguing. The stereotypical nature of the \textit{IGHV\textsubscript{D}J} rearrangements in CLL, and particularly in subsets such as those utilizing \textit{IGHV}1-69, suggests that the same antigen(s) or epitopes are involved in the early stages of the pathogenesis of the disease. \textit{IGHV}1-69 antibodies are poly-reactive but also may bind to apoptotic Jurkat cells (7). The association of male gender with \textit{IGHV}1-69 would indicate that the possible autoantigen would be encoded on the sex chromosomes.

**Renata Walewska*, Aneela Majid*, Zadie Davis\textsuperscript{1}, Palminder Dusanjh, D Ben J Kennedy\textsuperscript{2}, David G Oscier\textsuperscript{1} and Martin JS Dyer.**

*Joint first authors: RW and AM contributed equally to this MS.

From the MRC Toxicology Unit/University of Leicester, Hodgkin Building, Leicester, and \textsuperscript{1}Department of Haematology, Royal Bournemouth Hospital, Castle Lane East, Bournemouth, and \textsuperscript{2}Department of Haematology, University Hospitals, Leicester, UK.

Supported by grants from the Medical Research Council, from the Hope Foundation, and the Leukaemia Research Fund.
Please address correspondence to:-

Professor Martin J.S. Dyer,
MRC Toxicology Unit / Leicester University
Hodgkin Building, PO Box 138, Lancaster Road, Leicester, UK LE1 9HN

Fax: 44-116 252 5616
Tel: 44-116 252 5589
Mobile: 44-7950 859 586
Email: mjsd1@le.ac.uk
References.


Figure 1. *IGHV* gene segment usage in the three different CLL cohorts according to gender. **Figure 1A.** Distribution of the eight most common *IGHV* segments within the different databases as a percentage of all cases. **Figures 1B, C and D.** Distribution of *IGHV* gene segment usage according to gender in the three different databases. All three databases showed a male imbalance in patients utilizing *IGHV*1-69.
Figure 2: Rates of peripheral blood lymphocyte accumulation in mutated and unmutated *IGHV*1-69 gene segment usage. Figures 2A and 2B represent the accumulation of peripheral blood lymphocytes in patients with mutated and unmutated *IGHV*1-69 CLL respectively. **Figure 2C** – rates of accumulation of peripheral blood lymphocytes in 5 mutated and 13 unmutated VH1-69-expressing CLL; recently diagnosed patients with short follow-up have been excluded from this analysis. Changes in peripheral lymphocyte numbers over time up to time of first treatment were calculated for each group with an average follow-up of 1382 days. Counts/day represents the increase in peripheral blood lymphocyte count x 10^9/l per day. The differences in rate of increase between mutated and unmutated *IGHV*1-69 were statistically significant (p= 0.002, Student’s t-test).