I was a bit of a sceptic about TCL1 when it was first brought to my attention but now a real story about TCL1 and CLL seems to be emerging.

TCL1 is an oncogene activated by recurrent reciprocal translocations at chromosome segment 14q32.1 in the most common of the mature T-cell malignancies, T-cell prolymphocytic leukemia. It was first recognized in Carlo Croce's lab in a patient with Ataxia Telangectasia who developed T-PLL (Proc Natl Acad Sci U S A. 1989; 86:602-6). In T-PLL it acts to transport the protein Akt1 to the nucleus and enhance Akt1's enzyme activity.

It was also found that TCL1 is expressed in B-cell chronic lymphocytic leukemia (CLL) and the similar disorder of mature B cells, splenic lymphoma with villous lymphocytes, though not in normal mature B-cells (Yuile et al, Genes Chromosomes Cancer. 2001;30:336-41).

The following year in Croce's lab (Bichi et al, PNAS 2002; 99:6955-60) they produced a transgenic mouse in which the TCL1 gene was placed under the control of the immunoglobulin heavy chain promoter so that more of the protein was produced. These mice began to develop a proliferation of CD5+ B cells, starting in the peritoneal cavity at 2 months (where classical B1 cells live in mice, though not in humans). These cells became evident in the spleen at 3-5 months and in the bone marrow at 5-8 months. Cells in the spleen were located in the marginal zone but did not have marginal zone cell markers. Oligo- or monoclonality began appearing at 8 months, first in peritoneal cells and later in other populations. At the age of 13-18 months the mice became visibly ill with enlarged spleens, marked lymphadenopathy and high white cell counts, the blood films showed smudge cells but the predominant cells was a large CD5+/IgM+ lymphocyte; the picture being more characteristic of prolymphocytic transformation of CLL (CLL/PLL) than of CLL itself. The authors suggested that these transgenic mice might serve as an animal model for CLL.

Workers at MD Anderson Cancer Center studied the expression of TCL1 in 213 patients with CLL (Herling et al, Leukemia 2006; 20:280-5). 90% of samples expressed TCL1 whether detected by flow cytometry, immunohistochemistry or Western blotting. The highest levels were less than is seen in T-PLL but these relatively high levels correlated with the presence of unmutated *IGHV* genes, ZAP-70 positivity and the presence of deletions of the long arm of chromosome 11. Stimulation of CLL cells with interleukin-4 caused the expression of TCL1 to irreversibly diminish in association with proliferation.

The question then arises as to whether the TCL1 B-cell lymphoma of mice is not a model of human CLL as a whole, but rather of the more aggressive form with unmutated *IGHV* genes. Confirmation of this idea seemed to come from a study by Yan et al (PNAS 2006; 103:11713-11718). They found that like the more aggressive form of human CLL, the leukaemia of TCL1 mice uses unmutated *IGHV* genes, shows a biased use of these genes and stereotypy between cases of the B-cell receptor (BCR) structure. They also showed that

these stereotypic BCRs reacted with autoantigens and antigens found in bacterial cell membranes.

More recently, workers from Austria (Holler et al. Blood 2009; 113:2791-4) have found evidence that BCR signalling plays an important part in the development of the leukaemia from the expanded polyclonal CD5+ B cell population. Transgenic TCL1 mice in which the protein kinase C beta gene (*PKCbeta*) was knocked out failed to develop the CLL like disease despite developing an expanded CD5+ B-cell population. PKCbeta is an important component of the BCR signalling mechanism.

Micro RNAs (miR) are a large family of highly-conserved, non-coding genes involved in the regulation of what genes are expressed in different tissue and at different times in the cell cycle. They range in size from 19-25 nucleotides and are typically excised from a hairpin RNA structure of 60-110 nucleotides that is transcribed from a larger primary transcript. They are able to bind to other RNAs to reduce the level of their target transcripts as well as the protein coded by the transcript. The first evidence that they were involved in malignancy was the finding by Calin and his colleagues that *miR-15a* and *miR-16-1* were absent or down-regulated in patients with CLL who had chromosomal deletions at 13q14 – the commonest chromosomal abnormality in CLL (PNAS 2002; 99:15524-9).

The same group identified a micro RNA signature associated with prognosis and progression in CLL. Among the miRs associated with a short period between diagnosis and treatment were members of the *miR-29* family (NEJM 2005; 353:1793-1800). Another miR characteristically identified with B-cell lineage tumor cell lines is *miR-181* (Ramkissoon et al. Leuk Res 2006; 30:643-7). In a study of micro RNA expression profiling Pekarsky et al found that the levels of expression of these two micro RNAs were inversely related to the expression of TCL1 (Cancer Res 2006; 66:11590-3). Patients with CLL were divided into three groups: indolent, aggressive and aggressive with an 11q23 chromosomal deletion. TCL1 expression was low in 65% and high in 4% of indolent cases, low in 44% and high in 56% of aggressive cases and low in 3% and high in 75% of aggressive cases with 11q deletions. Levels of expression of *miR-29b* and *miR-181* are inversely correlated with TCL1 protein expression in patients with CLL.

Pekarsky et al also demonstrated that these two micro RNAs could individually inhibit the expression of TCL1 messenger RNA by transfected cell lines and that by transfecting TCL1 together with *miR-29b* and *miR-181* into a mammalian expression vector they demonstrated that the presence of the micro RNAs significantly decreased TCL1 protein expression. Furthermore, both *miR-29b* and *miR-181b* show reciprocal sequence homology with the 3 prime untranslated region (3' UTR) of *TCL1*. Taken together, these results suggest that TCL1 expression is, at least in part, regulated by these two micro RNAs.

Exactly how TCL1 influences the behaviour of CLL cells was made clearer by another paper by Pekarsky et al (PNAS 2008; 105:19643-8). In contrast to T-PLL, the AKT oncoprotein does not seem to be involved. The cAmp Response Element Binding (CREB) protein, p300, is a transcription activator involved in transactivation mediated by several signalling pathways including NF-kappaB. Co-precipitation experiments showed that TCL1 interacts with p300. However, sequence analysis demonstrated two mutant sequences or *TCL1* among the CLL patients studied. These mutant forms of TCL1 did not upregulate the NF-kappaB pathway to the same extent as wild type TCL1, leading the investigators to expect that another pathway might also be involved. One of the best known pathways involving p300 is activator protein 1 (AP-1) –dependent transcription. This complex contains c-Jun and c-Fos. AP-1 induces apoptosis by transactivating proapoptotic genes. Wild type TCL1 inhibits AP-1 dependent transactivation ~ 2.5 fold, however the mutant forms inhibit it ~ 100 fold. Co-precipitation and co-transfection experiments demonstrated that TCL1 interacts physically with AP-1 components c-Fos, c-Jun and JunB and inhibits their pro-apoptotic function, but that the mutant forms did this more effectively.

It is reasonable to conclude that the upregulation of TCL1 in certain CLL cells has multiple consequences including the suppression of apoptosis and increase of NF-kappaB dependent transcription. The fact that mutant forms with increased anti-apoptotic activity may have implications for certain types of transformation of CLL. One of the mutants was present in germ-line DNA, suggesting that it may be implicated in some cases of familial CLL.

The TCL1 mouse seems to have established itself as an animal model for the more aggressive forms of CLL and has been adopted by several groups in order to study putative new therapeutic agents (Lucas et al. Blood. 2009; 113:4656-66), the effect of that CLL has on T-cell function (Gorgun et al. Proc Natl Acad Sci U S A. 2009;106:6250-5) and the importance of macrophages in a novel immunotherapeutic approach (Wu et al. J Immunol 2009; 182:6771-8). It remains to be seen whether the results obtained in the animal model are duplicated in patients with CLL