Evolution in leukemia research: from identification of the *BCR/ABL* fusion to carbohydrate-mediated protection by the bone marrow microenvironment

As a biology student in Groningen, the Netherlands, John Groffen was given the opportunity to learn molecular cloning and Southern blotting in Richard Flavell's lab of Gene Structure and Function in Mill Hill, UK. After graduation, we were recruited to work in John Stephenson's lab at the Laboratory of Viral Carcinogenesis at the NCI Frederick, MD in the USA. Our projects included the molecular cloning of viral-transduced oncogenes. John Stephenson was studying type C RNA transforming viruses, and the lab had obtained a mouse v-Abl clone from David Baltimore. We made probes from it to investigate if human DNA contained homologous sequences. When we discovered that well-conserved sequences appeared to exist, John returned to Mill Hill to make a human cosmid library from which we then cloned out large segments of the human c-*ABL* gene. In collaboration with Nigel Spurr in Walter Bodmer's lab in the UK we were able to utilize a human DNA probe to localize the c-*ABL* gene to human chromosome 9.

Serendipity was responsible for the next development: when Frank Grosveld from Mill Hill visited us, he mentioned that his brother Gerard had just joined Dick Bootsma's lab in Rotterdam and was leading the effort to clone the breakpoint of the Ph-chromosome. Anne Hagemeijer and colleagues had generated somatic cell hybrids containing the Ph-chromosome and when Annelies de Klein used our c-*ABL* probe on a Southern blot, we all discovered that the part of c-*ABL* represented by the probe had been translocated to the Ph-chromosome. This significantly increased the chances that c-*ABL* was positioned near the translocation breakpoint, as the segment of DNA that had moved to chromosome 22 was too small to be visualized by cytogenetic techniques available at the time.

John and I subsequently started molecularly cloning DNA segments 5' to that contained in the cosmids which we had already isolated, and that encompassed what later was shown to be exons 2-11 of c-*ABL.* By combining cloning with Southern blotting of CML patient DNA, we discovered the first translocation breakpoint 5' in the c-*ABL* gene, cloned out a chimeric DNA fragment and by doing so obtained DNA probes from chromosome 22 which we then used to clone a large stretch of chromosome 22 DNA that contained this region. When we joined forces with Gerard to examine a large number of CML DNAs, we found that all contained breakpoints in a relatively small region of DNA, which we named the breakpoint cluster region (*BCR*) by lack of any idea regarding the function of this locus. We later isolated cDNA clones and showed it contains a gene which encodes a protein, and that transiently -in one publication- carried the name "Phl". Using Northern blotting we also showed that CML patients express chimeric *BCR/ABL* mRNAs.

In later studies we identified breakpoints in intron 1 of the *BCR* gene that, when fused to *ABL*, give rise to the P190 fusion protein found in Ph-positive ALL. I learned how to generate transgenic mice in Mill Hill, and our lab generated P190 Bcr/Abl transgenic mice to directly demonstrate that Bcr/Abl causes leukemias. Gene targeting in mice was used to further examine the function of the *BCR* gene. We also identified Crkl as a direct substrate of the Bcr/Abl tyrosine kinase.

Without the advances in basic research and the technologies that emanated from them, such as Southern blotting, molecular cloning and transgenic mouse technology, our studies could not have happened. When we started our research in the early 1980s, chromosome 22 in terms of genes was almost entirely a terra incognita. The techniques we used were considered state-of-the-art at the time, but only 30 years later they have been entirely replaced by faster and more accurate methods which allow the sequencing of complete genomes. Yet, although genomics, transcriptomics and proteomics are able to provide us with a detailed inventory of the content of CML cells, it remains a challenge to relate these components to the way CML cells function and survive in the complex microenvironment of the bone marrow niche.

Our lab still focuses on leukemia but we have directed some of our effort to study it from the viewpoint of glycobiology, a path that is less well-travelled. We know that leukemia cells are protected when they reside in their microenvironmental niche in the bone marrow, and that contact is mediated by cell surface structures of which the carbohydrates are key components. In recent studies, we found that bone marrow stromal cells secrete a lectin that binds to poly-N-lactosamine modified glycoproteins, called Galectin-3, and which is endocytosed by B-cell precursor acute lymphoblastic leukemia (BCP ALL) cells. Stromal cells that can not produce Galectin-3 are defective in their ability to protect BCP ALL cells against chemotherapy. Thus our studies in BCP ALL, and those of Yamamoto-Sugitani et al in CML, suggest that there are still many possible approaches to eradicate leukemia cells other than to directly target them. Given the huge technological and conceptual advances in the past 30 years in CML research, it seems safe to predict that it will not take an additional 30 years to translate this type of approach into a therapy.

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