

# CRACKING THE CODE OF CHILDHOOD CANCER

One in 600 children develops cancer. We are searching for genes and gene products which we can use to better diagnose tumour subtypes, and to predict clinical behaviour and response to therapy.



**Chris Jones**

**PhD**

Chris Jones is Team Leader of the Paediatric Molecular Pathology Team in the Section of Paediatric Oncology at The Institute of Cancer Research.

## Unique insights, unique challenges

Childhood cancers are the principal cause of death from disease between infancy and adulthood in developed countries. Although they are only a small contributor to the overall cancer burden in society, the study of paediatric malignancies punches above its weight, both in the clinical and basic science settings. As well as some of the most dramatic rises in treatment success in the last 30 years being achieved in paediatric oncology, a disproportionate degree of insight into the molecular genetics of cancer as a whole has been gleaned from the study of childhood cancer. Such groundbreaking discoveries include:

- The concept of tumour suppressor genes and the 'two-hit' theory in retinoblastoma;
- Oncogenic gene amplification of *NMYC* in neuroblastoma;
- Epigenetic mechanisms of gene expression in Beckwith–Wiedemann syndrome;
- The identification of a 'single-hit' tumour suppressor gene on the X chromosome in Wilms tumour.

The relative rarity of these diseases, however, poses practical obstacles to building on these achievements. In the clinic, large national and international collaborative groups have been instrumental in producing the studies which have seen the cure and long-term survival of children with cancer rise to over 75%. In order to further our understanding of the biology driving the molecular pathogenesis of the disease, it is essential that clinicians and basic science researchers engage with each other in order to perform well-designed molecular studies on large clinical trial-based patient cohorts.

## Biomarker identification through genome-wide analysis

Our approach within the Paediatric Molecular Pathology Team is to utilise the latest techniques for the molecular genetic analysis of childhood cancers treated in national and international clinical trials. The publication of the

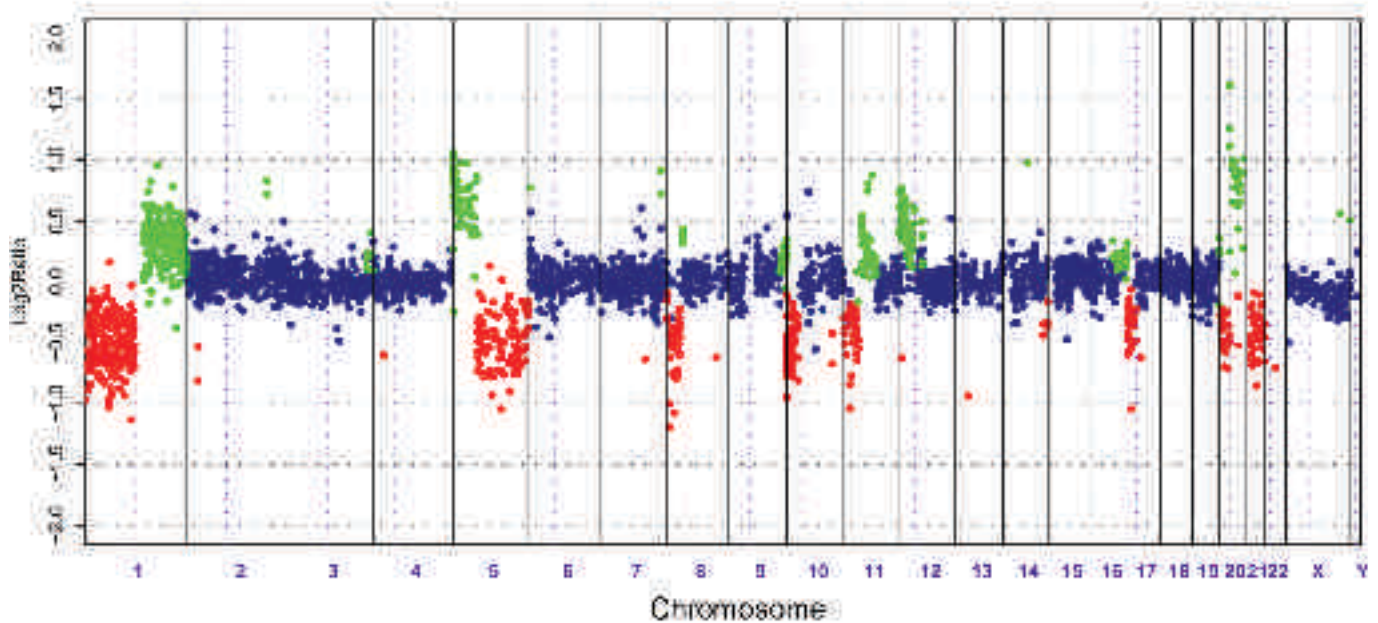


Figure 1: Gene copy profiling of Wilms tumour by array CGH. Probes are aligned along the horizontal axis in genome order, from chromosome 1 through to X and Y. Copy number gains (positive values, highlighted in green), losses (negative values, red) and no change (blue) are plotted on the vertical axis.

human genome sequence, and the recent advances in high-throughput instrumentation, allow us for the first time to carry out these experiments on a genome-wide scale. We are searching for genes and gene products which may be useful biomarkers in paediatric malignancies; that is molecular readouts which we can utilise to diagnose tumour subtypes better, and to predict clinical behaviour and response to therapy.

■ Our work focuses on two relatively under-studied diseases with a high burden of childhood cancer deaths: Wilms tumour (a cancer of the kidney) and malignant gliomas of the brain. ■

We start at the DNA level, using a technique called microarray comparative genomic hybridisation (array CGH) in order to map the changes in gene copy number comprehensively throughout the length of the tumour genomes (see Figure 1). An important aspect to getting the most out of these experiments is in the analysis of these very large datasets. Our group has developed new techniques that enable us to catalogue these changes, and to link the alterations at a genetic level to the clinical outcome of the patient. Applying this approach to a large series of Wilms tumours, we were able to identify a unique increase in DNA copy number at a specific location

on chromosome 15. This was found to be present in around 15% of our Wilms tumour cases, and was strongly associated with treatment failure and tumour relapse. Mapping this region in more detail, we determined the driver of this alteration to be a gene called *IGF1R* (insulin-like growth factor 1 receptor), which encodes a cell surface tyrosine kinase receptor that plays a key role in cell growth, division and survival.

The identification of *IGF1R* as a potential biomarker in Wilms tumour is exciting, not least because it has been known for some time that one of the molecules which binds to and activates the receptor, *IGF2*, can be produced in large amounts by these and other childhood tumours. This led us to speculate that upregulation of this signalling pathway may play an important role in treatment failure in these children (see Figure 2). In order to probe the *IGF1R* network more thoroughly in our tumour specimens, we applied another genome-wide assay to measure comprehensive gene expression at the RNA level. By once more linking to clinical outcome data, we observed a significant upregulation of genes that play a role in *IGF1R*-related signalling in Wilms tumours which went on to relapse. This provided us with evidence that the genomic change we identified was having an important functional effect within the tumours.

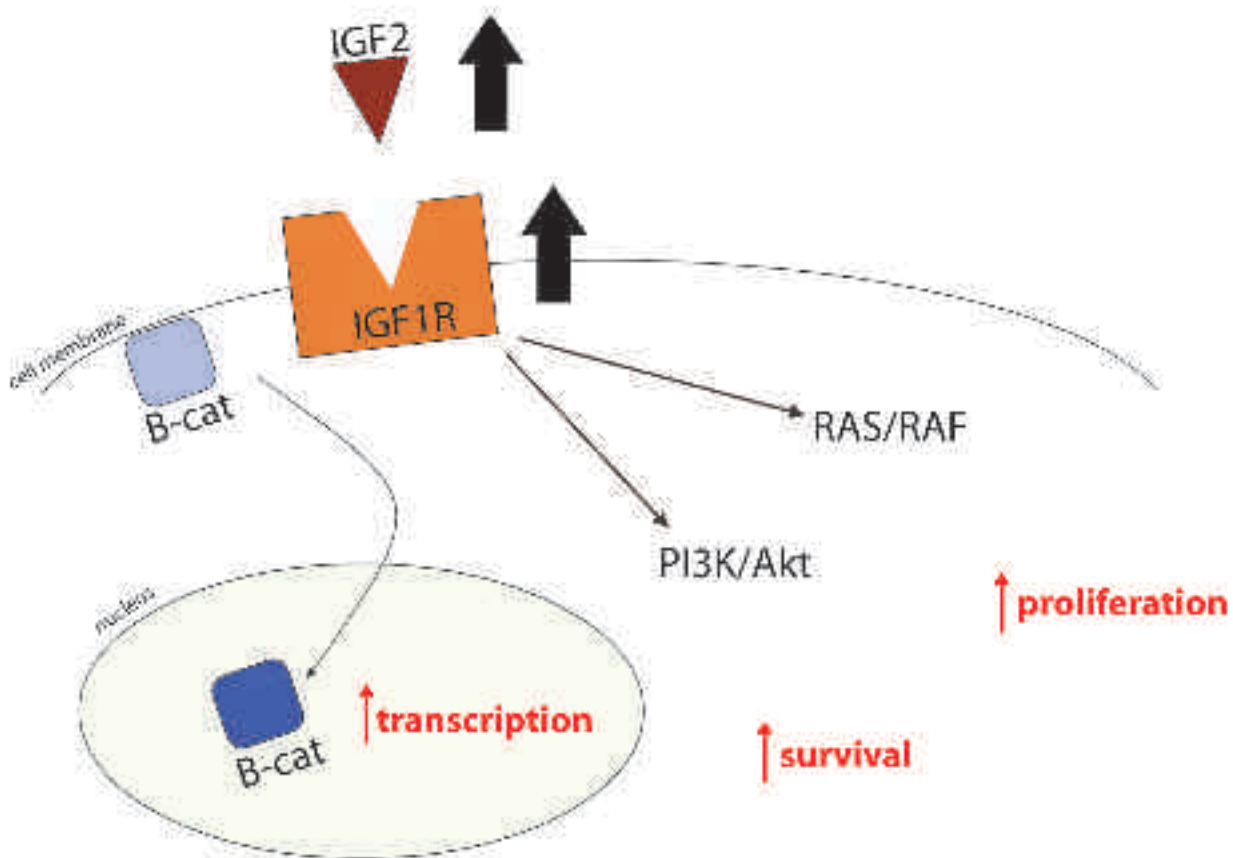


Figure 2: IGF1R signalling in Wilms tumours. An increase in both receptor (IGF1R) and ligand (IGF2) expression may lead to increased cell proliferation and survival, through the RAS/RAF and PI3K/Akt pathways, as well as via downstream targets of  $\beta$ -catenin, translocated to the nucleus in a process mediated by IGF1R.

### Molecular pathology: Discovery, validation and mechanistic clues

The application of modern molecular techniques to pathological tumour specimens performs a vital role in the development of biomarkers from interesting observation to 'prime-time'. By constructing tissue microarrays, comprising hundreds of tiny tumour cores embedded in a single paraffin block, we are able to bring molecular pathology up to the high throughput level of the genomic profiling technologies. By mapping our molecular genetic observations directly back on the pathological tissue specimen, we may also be able to derive more than just validation of our initial findings.

In order to determine whether the IGF1R receptor protein was upregulated at the cell surface of the tumour cells, we used immunohistochemistry on a collection of more than 300 Wilms tumours on a tissue microarray. We discovered that although the epithelial cells ubiquitously expressed the receptor, there was an additional upregulation in around 15% of the tumours in the blastemal cells – undifferentiated embryonic cells from which Wilms tumours are thought to arise. Furthermore, by applying a measure of DNA copy number using chromogenic *in situ* hybridisation (CISH; a technique optimised in collaboration with Dr Jorge Reis-Filho from

the Breakthrough Toby Robins Breast Cancer Research Centre at The Institute), we determined that it was these cases which contained the increased copies of the *IGF1R* gene (see Figure 3). Remarkably, we were able to demonstrate a genomic alteration leading to a cell-type specific upregulation of the receptor protein.

▣ These observations provide us with a first glimpse of a possible novel biological mechanism in Wilms tumours. ▣

Upregulation of the receptor, in conjunction with an over-production of IGF2 by the tumour cells, can lead to uncontrolled cell growth and division, as well as a shutting down of drug sensitivity pathways. Intriguingly, this may also be linked to another Wilms tumour gene – which encodes the transcription factor  $\beta$ -catenin. Activating mutations in this gene lead to the switching on of a raft of cancer-associated genes. A similar effect can be achieved by transport of  $\beta$ -catenin to the nucleus of the cell – a function controlled by IGF1R. We have observed both the expression of  $\beta$ -catenin in the nucleus,

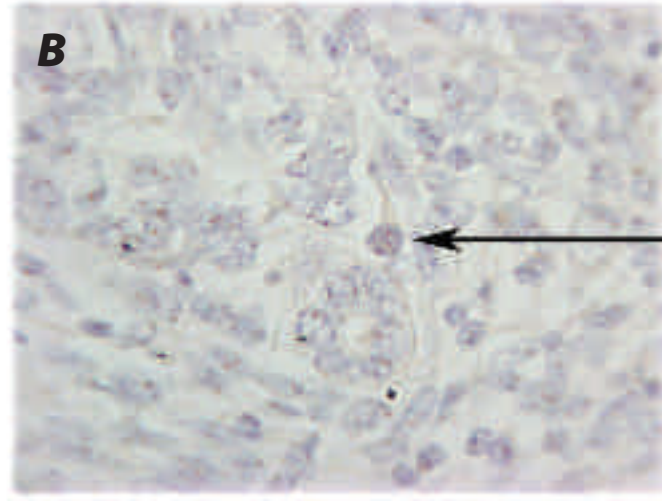
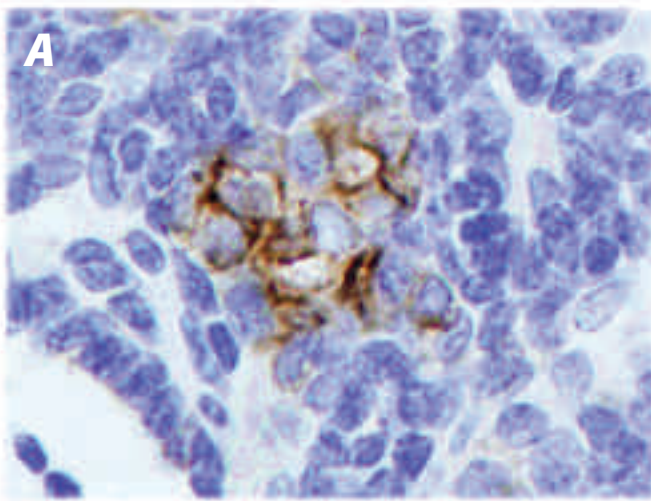


Figure 3: IGF1R expression in the blastemal cells of Wilms tumours is driven by an increase in gene copy number. (A) Immunohistochemistry shows strong receptor protein expression at the cell membrane of the blastemal cells. (B) Chromogenic *in situ* hybridisation (CISH) demonstrates additional copies of the *IGF1R* gene in these blastemal cells (arrow).

and the overexpression of its target genes in our Wilms tumours, and deconvoluting this pathway is one of our immediate research priorities.

### Making rare cancers rarer

Of course the ultimate goal of our work is to translate our basic science findings into practical benefit for the patient. Studying the molecular pathology of the disease and understanding the key pathways involved will enable us to guide the paediatric oncologist towards obtaining agents that will target these pathways. In order for the future clinical development of such compounds, our aim is to demonstrate that the tumour cells have a functional dependence on the activity of the signalling cascade. We are providing evidence that over-reliance of Wilms tumour cells on the IGF-signalling cascade seems an excellent 'Achilles heel' to exploit for children whose tumours have proved resistant to conventional therapies. In fact, there are already monoclonal antibodies and small molecules which specifically target the IGF1R receptor in early clinical trials in adults.

▣ The testing of new, molecularly-targeted drugs in childhood cancer is becoming a hot area of research. ▣

Multinational collaborative groups are setting up screening programmes designed to identify those compounds which may be of most benefit to patients with a number of the most common malignancies. We feel that it is important that relevant cell line model systems are used to demonstrate efficacy and function in the appropriate paediatric tumour types. For the diseases we focus on, Wilms tumour and high grade glioma, there is a lack of available models and such work

has not been extensively carried out. We are addressing this by assembling a panel of cell lines representative of these cancers, for both drug testing and mechanistic studies. It is hoped that our work on the identification and validation of novel genetic targets will go some way to achieving the major goal of the Section of Paediatric Oncology - improving the survival of children with cancer.