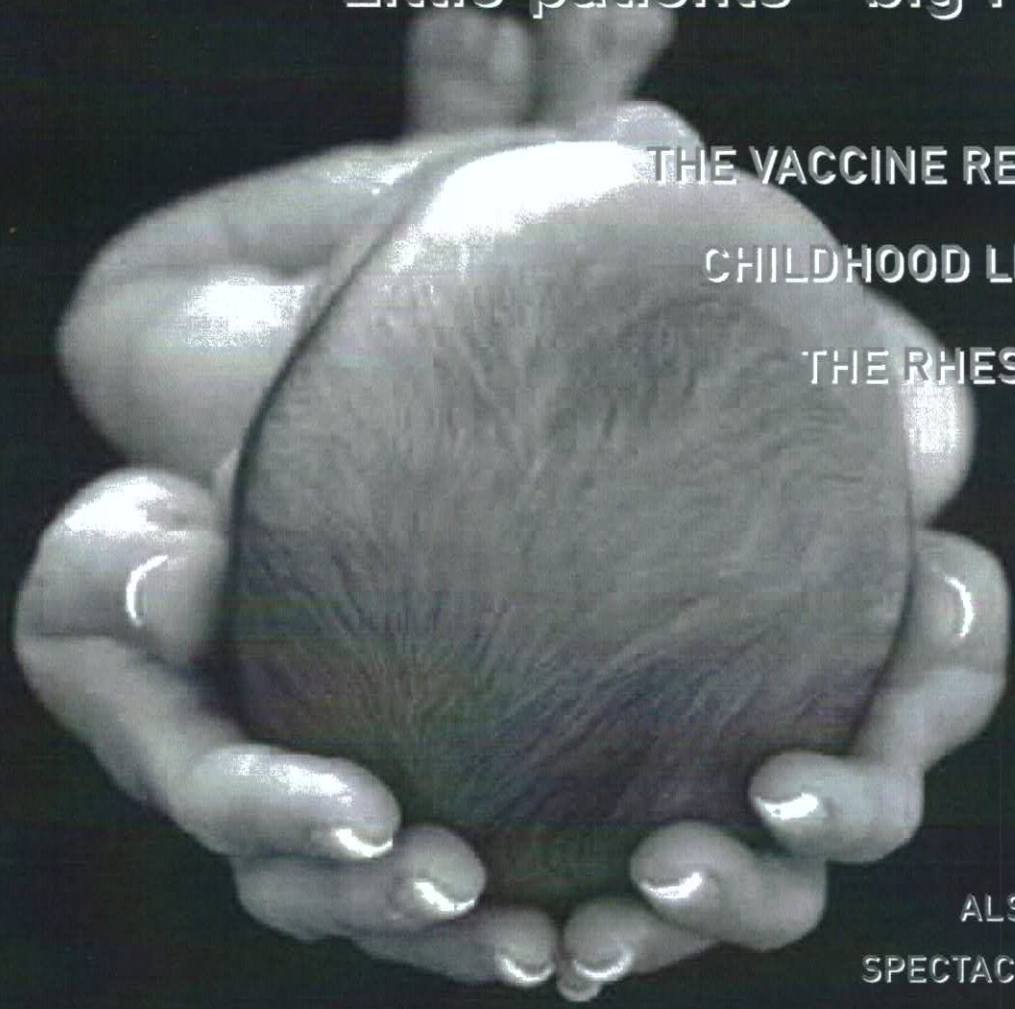
PathWay

THE ROYAL COLLEGE OF PATHOLOGISTS OF AUSTRALASIA

Winter 2008 | Issue #16

Paediatric Pathology

Little patients - big rewards



THE VACCINE REVOLUTION

CHILDHOOD LEUKAEMIA

THE RHESUS STORY

ALSO FEATURING

SPECTACULAR SYDNEY

$7.50 (inc. gst)

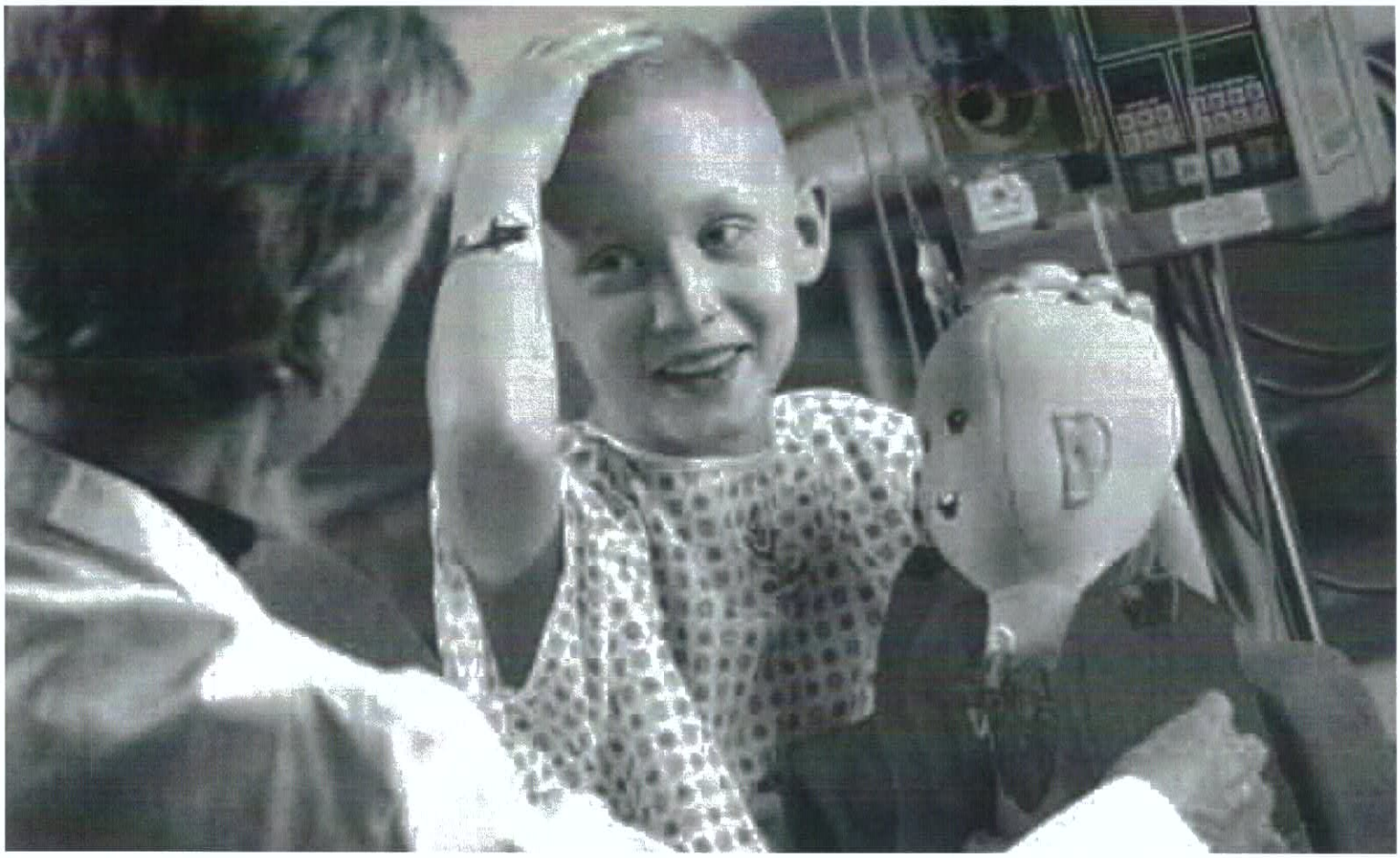
ISSN 1449-793X

9 771449 793082

Childhood leukaemia

– A GENETIC SUCCESS STORY

THE ADVANCES IN UNDERSTANDING, DIAGNOSING AND TREATING ACUTE LYMPHOBLASTIC LEUKAEMIA REPRESENT SOME OF THE BEST MODERN MEDICINE HAS TO OFFER, AS **ALEX WILDE** REPORTS.



Mass murder is not only the stuff of action movies. When the enemy is leukaemia, traditional chemotherapy indiscriminately attacks all newly-dividing cells, whether leukaemic or not – with severe side effects.

But it is now known that not all patients with childhood leukaemia need such aggressive treatment.

With survival rates for acute lymphoblastic leukaemia currently approaching 80% – up from a dismal 5% in the 1940s – doctors have noticed individual differences in the likelihood of remission.

Overall about 20% of young patients with this type of leukaemia fail to respond to therapy and experience a relapse. But researchers have found that this 20% don't always have the classic clinical indicators of poor prognosis, such as a high initial white blood cell count. Thanks to recent advances in DNA technology, pathologists can now analyse features of the cancer and have identified particular pathological markers that are associated with a higher risk of relapse.

The upside of this, is that patients without these markers generally have a better chance of remission and therefore can be given less intensive chemotherapy, with the advantage of lower toxicity and fewer side effects. The most intensive treatment, including bone marrow transplantation, is reserved for patients with highest risk of relapse.

Identifying those at higher risk of relapse

Dr Luciano Dalla-Pozza, paediatric oncologist and senior staff specialist in childhood cancer at the Children's Hospital at Westmead says high risk patients can be identified by various means.

One marker of higher risk is how well that patient initially responds to treatment. Determining the response to treatment involves measuring the concentration of new leukaemic cells, known as blast cells, in the blood in the seven days following treatment with a single agent.

"If the level falls to under 1000 (1.0 x 10^9 per litre) within seven days then that child has good risk disease, if it stays above 1000 that child has high risk disease."

Another means of identifying those at high risk of relapse is determining the presence or absence of one or both of two critical genetic mutations.

Having the Philadelphia chromosome t(9;22) translocation or the t(4;11) translocation in a leukaemia cell is a stronger predictor for high risk of relapse, Dr Dalla-Pozza says.

The Philadelphia chromosome – named after its co-discoverer from Philadelphia – occurs due to a fusion of the ABL gene on chromosome 9 and a gene known as the BCR gene, on chromosome 22. This 'new' gene known as a BCR-ABL produces an abnormal fusion protein, which stimulates tyrosine kinase activity, resulting in leukaemic disease.

Another strong predictor of relapse is the presence of a tiny number of cancer cells that sometimes remain in the patient during treatment or after treatment when the patient is in remission.

>

"There are a myriad of pathways to get to cancer but they all involve key genes, at least 100-200 key genes. So it is very unlikely you will get the precisely same pathway in the development of leukaemia in one child to the next."

"You take two children and give them chemotherapy and let's say one loses her hair and one doesn't. There is something about the child that enables them to be resistant to that.

Known as minimum residual disease, or MRD, laboratory tests, up until a decade ago were not sensitive enough to detect its presence.

But since the development of polymerase chain reaction (PCR) technology, minute levels of cancer cells can be measured in tissue samples - sometimes in concentrations as low as one cancer cell in a million normal cells.

"Having minute amounts of leukaemia carries a poor prognosis, yet the bone marrow of these patients looks normal. So the tests we would do by other means would say that the child is in remission," Dr Dalla-Pozza says

"The technology used [to detect MRD] is based on the fact that leukaemia cells .. arise from one cell, and [each] cell carries a unique signature."

"So .. we take the leukaemia cells, recognising that all those leukaemia cells came from the same parent cell, so they all have exactly the same signature," Dr Dalla-Pozza says.

"We sequence the relevant part of the .. gene to find a signature that is unique for that leukaemia cell for that patient."

Techniques

Dr Ellie Smith, senior staff specialist in cytogenetics at the Children's Hospital, Westmead, says a range of techniques are employed by pathologists to identify high risk markers.

"Techniques can include cytogenetics with G banded chromosomes, molecular cytogenetics (FISH) with probes containing sequences from ABL and BCR and the DNA based techniques of PCR," she says.

"Sensitivity increases from cytogenetics to PCR, but these techniques are complementary. For example FISH can show if another genetic event such as deletion of ABL has happened," Dr Smith explains.

Detecting MRD in patients with acute lymphoblastic leukaemia in remission has profound prognostic importance says clinical haematologist and oncologist Professor Glenn Marshall.

"Survival curves go up by 5-10% every 5-10 yrs and that is because we're conducting continual clinical research into identifying patients who are at high risk of relapse, and giving them stronger treatment earlier," says Professor Marshall, who is also director of the Centre for Children's Cancer and Blood Disorders at Sydney Children's Hospital.

"High risk patients did have a 30-40% cure rate, which reached 60% in our last study. But increasing intensity of treatment increases toxicity... High risk patients now get a bone marrow transplant even in first remission, [which means] there is also a high risk of fatality."

"It is important to continue to identify new kinds of drugs that are directed against particular molecular targets in childhood acute lymphoblastic leukaemia and that's where we see the advances coming in the next decade or so."

Molecular assassins

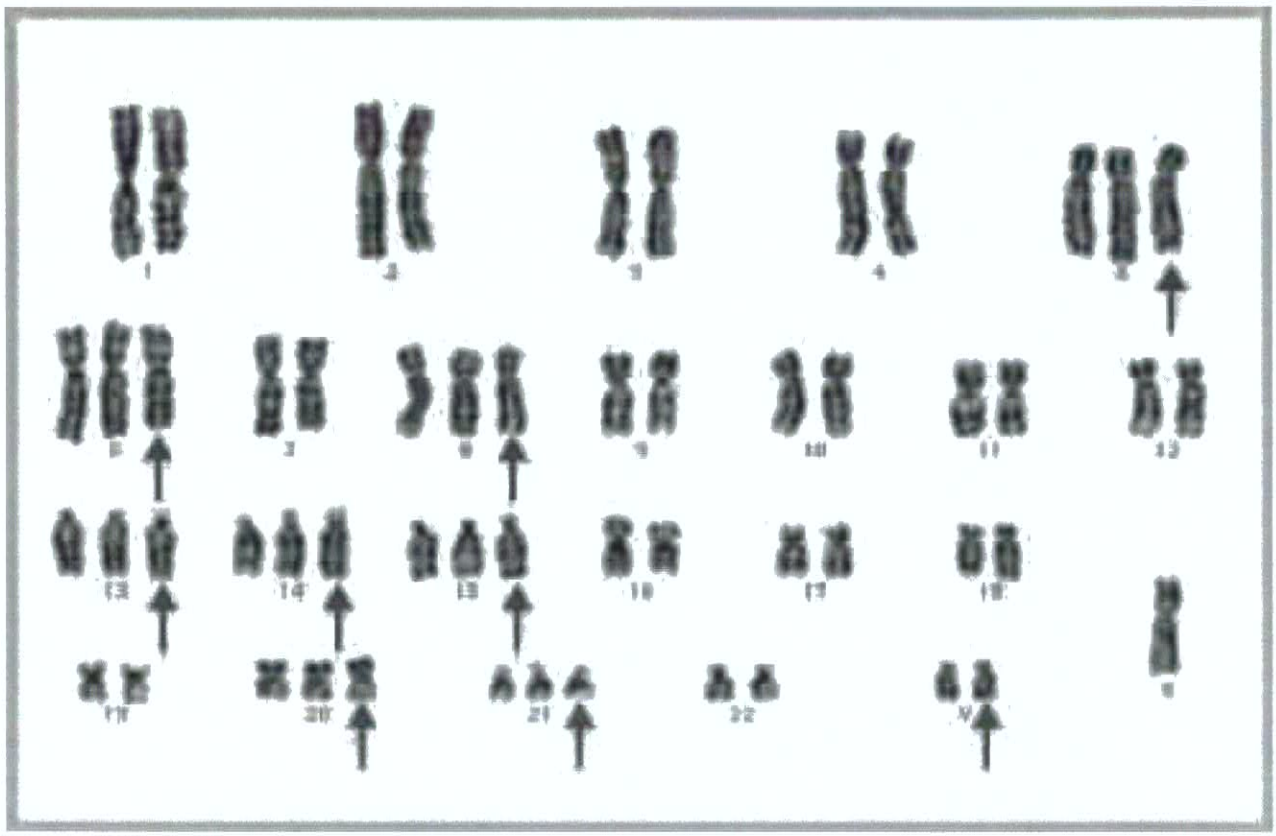
Unselective anti-leukaemic agents bombarding young developing bodies take innocent casualties; typically cells responsible for hair growth and cells that replace the lining of the intestine are destroyed along with the newly dividing cancer cells.

Developing molecular-based targeted therapies that fight specific cancer cells while leaving normal cells unharmed is the ultimate aim of cancer researchers. And it is already a reality in chronic myeloid leukaemia.

Chronic myeloid leukaemia was the first malignant disease to be linked to genetic abnormality. The first targeted therapy was the tyrosine kinase inhibitor, imatinib mesylate, (Glivec) which specifically inhibits the activity of the BCR-ABL protein.

The hunt is on to find the genetic mutations involved in other similar cancers. But while new technology has enabled researchers to look at the activity of thousands of genes at once, the scientists are yet to confirm single genes involved in acute lymphoblastic leukaemia or acute myeloid leukaemia.

Dr David Joske, head of clinical haematology Charles Gairdner Hospital in Perth says researchers are closing in on candidate groups of genes linked to acute lymphoblastic leukaemia, but points out the molecular pathways involved are much more complex than those associated with chronic myeloid leukaemia.



G banded chromosomes in a child with ALL and a hyperdiploid karyotype (count 55, additional chromosomes arrowed), associated with a favourable prognosis.

"In acute lymphoblastic leukaemia, we have found abnormalities in genes of the receptors of the bone marrow cells that affect their growth. We have found abnormalities in genes within the cell, directly involved with cell growth, like tyrosine kinase.

"There are probably genes that affect the blood supply to the tumour cells even within the bone marrow .. and there are even some genes that probably alter the bone marrow micro environment which do or don't permit the malignant cells to grow."

"There are several new classes of chemo agents coming through and there is a lot of work to be done in the coming weeks and months to ascertain which are going to be effective in the acute leukaemias."

The significance of individual variation

Dr Dalla-Pozza predicts that advances in the study of individual differences based on genetic variation will eventually be highly influential in helping pathologists understand more about the behaviours of cancers and patients' likely response to treatment.

"There are a myriad of pathways to get to cancer but they all involve key genes, at least 100-200 key genes. So it is very unlikely you will get the precisely same pathway in the development of leukaemia in one child to the next."

"You take two children and give them chemotherapy and let's say one loses her hair and one doesn't. There is something about the child that enables them to be resistant to that.

Each child has a set of polymorphisms which explain we believe why they either experience little toxicity or why they experience a lot of toxicity; why they might be at risk for heart disease later on as a consequence of treatment; why their body might eliminate the drug from their body so quickly so that the cancer cell isn't exposed for long enough."

"Current single nucleotide polymorphism [SNP] analyses, where you can do all these wonderful precise assessments of sequences of genes, are very important in providing you with clues."

But Dr Dalla-Pozza warns that untangling which particular abnormalities are relevant is going to take some time.

"There will be so many different spots, if you take a gene and you find 20 different alleles or 20 different SNPs on them you might very well find two are highly relevant, three are only relevant 70% of the time. And considering they are usually only relevant in conjunction with differences in other genes, we still need to be able to put them all together into some sort of interpretable collage."

"Ultimately I think proteomics will be the next key area of development. We will be able to define that certain proteins were produced or that they cooperate in pushing the cell into the cancer state," he says.