# fusion

THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

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## Editorial

**The theme of this issue of *fusion* is cancer. Through the study of pathology has come the identification of many of the visible critical changes that distinguish normal and malignant cells. Departments such as ours are, therefore, well suited to extend the pathological description of disease to its molecular origins and to enable its reversal at the molecular level.**

Indeed the Dunn School has made some important contributions to our understanding of cancer and towards creating novel therapies. In this edition, we celebrate the historic work on cell fusion and tumour suppression genes led by Henry Harris at the Dunn School in the 1960s, and look at some more recent successes. Over the past decade, the Dunn School has pioneered the application of humanized monoclonal antibodies in the therapy of leukaemia and lymphoma at the Therapeutic Antibody Centre, and current research in T-cells that regulate the immune system offers scope to enhance therapeutic vaccines against cancer.

We would now like to build on our strengths in cell and molecular biology to expand cancer research within the school. Cancer cells undergo three characteristic general changes that require a molecular understanding. The first is the acquisition of growth independence from nutrients and factors. The second is the development of resistance to signals or drugs that normally would cause them to die. The third is the progressive accumulation of further genetic abnormalities. Such an understanding is essential to evolve new therapeutics for cancer. The key issues now are to identify molecules that cause cancer and to determine how they exert their effect. Such knowledge will allow better drug design to alter the pathways so as either to normalize cancerous cells or alternatively to destroy the cancer and spare the normal. It should also lead to strategies to enhance efficacy of current cancer drugs and avoid the problems of drug resistance. Through our interdisciplinary approach to molecular and cell biology and immunology we are poised to make further significant contributions to cancer research. Our activity in this area has been enhanced by the arrival of Chris Norbury and his group from ICRF, where they have developed new insights in cell-growth and drug sensitivity through use of yeast systems.

In order to provide the additional leadership that would enable us to integrate high-level cancer research into the Dunn School and Oxford we are seeking to endow a Chair in Cancer Cell Biology. If you would like to know more about our plans in this field, or feel able to help us, I would be delighted to discuss them with you.

**Herman Waldmann**

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*Sydney Brenner and Sir Henry Harris at the 11th Heatley Lecture (see page 3)*

# Yeast – our long-lost cousin

**Chris Norbury was appointed Lecturer in Cell Biology at the Dunn School in 2002; prior to that he was at the Weatherall Institute of Molecular Medicine, also in Oxford. His research uses fission yeast as a model to illuminate key aspects of cancer cell biology.**

Modern molecular biology has brought with it the humbling lesson that some of our most important genes have remained largely unchanged since nucleated cells first evolved. This is particularly clear for genes that control the cell cycle processes of DNA replication and chromosome segregation – events that are fundamental to all cell proliferation. It seems the common ancestor we share with simple single-celled organisms such as yeasts had already perfected most aspects of cell cycle regulation a billion years ago, before the proto-yeasts and proto-humans went their separate ways. Recent thinking about cell cycle regulation in human cells has been guided in many key respects by genetic analysis of the corresponding processes in yeast cells. Some human gene products, equivalent to cell cycle regulators first identified in yeast, have been found to be dysfunctional in cancer cells, while others represent attractive new targets for anti-cancer therapies.

Yeast cells offer a number of important advantages for studies of this sort. Classical genetics makes it possible to identify mutants that are conditionally defective in the process of interest, and subsequent isolation of the corresponding genes is generally very straightforward. DNA recombination-based techniques can then be used to modify the yeast genome in very precise ways, for example to place a gene of interest under the control of a regulatable promoter or to alter the protein coding sequence of a gene in its normal chromosomal context. The net result is a level of control over the genetic make-up of the experimental system that is currently beyond the reach of those working with human cells.

My research group has adopted this strategy of identification of cell cycle regulatory genes in the fission yeast *Schizosaccharomyces pombe*, followed by examination of the corresponding human genes and proteins. In recent years we have begun to explore the idea that the same general approach might be applied to another fundamental aspect of cell biology – the acquisition of resistance to diverse drugs, which frequently limits the effectiveness of existing cancer therapies. Here too we are finding that conserved genes perform remarkably similar functions in the two organisms.

Our current research is focused on cell cycle and drug resistance proteins that influence different aspects of gene expression. This is one of the reasons for our excitement at relocating to the Dunn School, where there is a high concentration of directly related research. Another is the growing circle of local groups using fission yeast, including those of Nick Proudfoot in the Dunn School and Stephen Kearsey and my former post-doc Shao-Win Wang in the Zoology Department. Together, we share the view that yeast cells still have a great deal to tell us about human cells, their long-lost cousins.

**Chris Norbury**

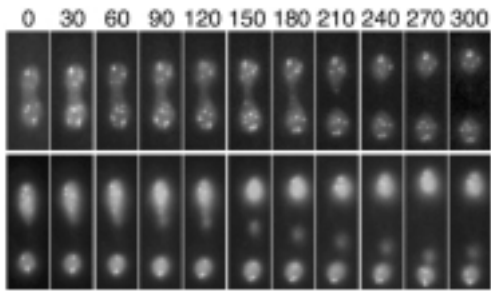



*Figure 1. Mitotic chromosome segregation in living fission yeast cells expressing a GFP-tagged chromatin protein. Individual cells were observed by fluorescence microscopy over a five-minute period, with images collected every 30 seconds. Note the lagging chromosome in the lower panels. Scale bar: 10 microns.*

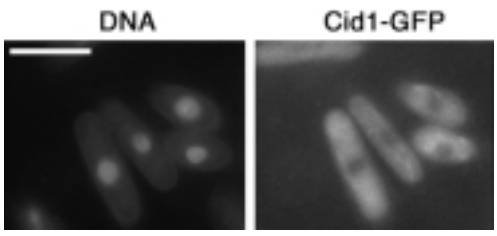



*Figure 2. Our genetic studies identified Cid1 as a new cell cycle regulator in fission yeast. Surprisingly the Cid1 protein, which is conserved in human cells, acts as a poly(A)polymerase yet is localized to the cytoplasm. See Read, R.L.* et al. *(2002).* Proc. Natl Acad. Sci. USA, *99, 12097-84.*

# Sydney Brenner gives the 11th Norman Heatley Lecture

**Sydney Brenner, Nobel laureate and doyen of the molecular biology revolution, gave a memorable lecture on 27 March to a packed house. After completing his medical studies with an intercalated science degree at Witwatersrand in South Africa and an MSc on mammalian heterochromatin, Oxford was lucky enough to attract him to do a D.Phil here, though we failed to keep him.**

A contemporary of Leslie Orgel, in Cyril Hinschelwood's Physical Chemistry Laboratory, he was already convinced that the key to understanding biology was the study of DNA and its mutations. In Cambridge, with Francis Crick and others at the Cavendish, he found colleagues with the same enthusiasm. He predicted that the genetic code would be non-overlapping and discovered two of the STOP codons using elegant, classical genetics. His discovery of mRNA, together with Jacob and Meselson would have been enough to crown the research career of most Nobel laureates, but Sydney was just getting started! He decided by the early 60s that it was time to do something really challenging and proposed to the MRC, in October 1963 as follows. "To start with we propose to identify every cell in the worm and trace lineages. We shall also investigate the constancy of development and study its control by looking for mutants". Almost four decades later, the fulfilment of this prophetic statement by Brenner and his co-workers was recognized with the Award of the 2001 Nobel Prize for Medicine and Physiology.

He is still confident that there is a place for the individual to make a difference in modern biology – and he has an idea he'd like to pursue himself. As he puts it "Many people have forgotten that the basic units of living organisms are not genes but cells. They are the appropriate level of abstraction. The programme for modern biology is to make a map of how cells interact with each other in higher organisms." This is, as he says irrepressibly "an unfunded, *Gedanken* project, but I'm looking for a bank to rob!"

Dr Brenner has agreed to become an Honorary Fellow of the Dunn School.

# People

The Dunn School is pleased to welcome three new University Lecturers: Dr David Greaves is the new Lecturer in Experimental Pathology, Dr Chris Norbury (see p. 2) has come to the Dunn School as Lecturer in Cell Biology, and Dr Quentin Sattentau moves from Imperial College in April to take up the Lectureship in Microbiology. We extend a warm welcome to them all.

Dr Aron Chakera has been awarded the prestigious Bristol-Myers Squibb Prize Fellowship Award 2003, one of only two recipients. He will study novel anti-chemokine strategies in atherosclerosis, supervised by David Greaves.

Siamon Gordon, Glaxo Wellcome Professor of Cellular Pathology, has been elected a Fellow of the Academy of Medical Sciences.

The University's Medical Sciences Board has conferred the title of University Research Lecturer on Dr Marion Brown and Dr Luisa Martinez-Pomares – congratulations to both!

The SARS virus has been much in the news lately. Two former members of the Dunn School have played a key role in unravelling the nature of the virus causing SARS. Malik Peiris (who did his D. Phil in 1981) and Leo Poon who completed his doctorate with George Brownlee in 1999 are based in Queen Mary Hospital, Hong Kong and their paper in *The Lancet* (19 April 2003) indicates that a novel coronavirus is likely to be the primary agent associated with the disease.

Jeff Errington, Professor of Molecular Microbiology, gave the Nordstrom Lecture at the University of Uppsala in November 2002, and a Plenary Lecture at the German Society for General and Applied Microbiology in Berlin in March 2003.

# News

The Bill and Melinda Gates Foundation have awarded Siamon Gordon and his collaborators at Cold Spring Harbor Press and Queen Mary's College, London, $330,000 to continue the Staying Alive: Fighting HIV/AIDS project for South African Children (see *Fusion* issue 1). This will enable the team to revise the content and design of the book in the light of feedback from the community, and to develop a more extensive distribution network.

Good news from the Therapeutic Antibody Centre, which was recently visited by Medicines Control Agency inspectors who found it to be in compliance with the new EU Good Manufacturing Practice guidelines.

Siamon Gordon and David Greaves organized the Medical Sciences Division's third science open day, on the topic of vascular biology. The symposium was held on 19 March in the new Medical Sciences Teaching Centre, which is developing a secondary role as a venue for medical conferences.

# The Discovery of Tumour-Suppressor Genes

*Peter Cook*

**Sir Henry Harris was Head of Department at the Dunn School from 1963-1994. Among his most influential work is the technique of cell fusion and the discovery of tumour-suppressor genes. Peter Cook, Professor of Cell Biology at the Dunn School explains.**

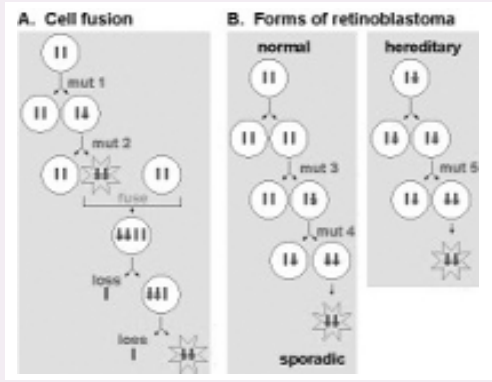
Cancer is mainly a genetic disease. Genetic mutation enables a cell to escape from the normal growth controls into an endless proliferative cycle that eventually generates the tumour. Some mutations occur in genes – known as tumour-suppressor genes or anti-oncogenes – that encode products that normally block proliferation, while mutations in others generate active oncogenes that encode altered proteins that drive unregulated growth. Using a driving analogy, the tumour-suppressor genes act as brakes and the active oncogenes as accelerators; mutation – by inactivating a brake or jamming on the accelerator – can then lead to cancer.

Tumour-suppressor genes were discovered in The Dunn School using the then pioneering technique of cell fusion, also developed at the Dunn School by Henry Harris. Normally only eggs and sperm are able to fuse, and then only if they are of the same species. In 1965, John F. Watkins and Henry Harris made the first inter-species hybrid by fusing together human and mouse cells; the resulting 'humouse' cells grew and divided. In 1969, Harris and his collaborators went on to fuse a malignant mouse cell with a normal mouse cell, and tested the resulting hybrid to see if it was malignant when injected into mice. It proved to be non-malignant, suggesting that something in the normal cell was able to suppress the malignancy of the cancer cell. But when the hybrid was grown in tissue culture, chromosomes were lost spontaneously from the cell and malignancy reappeared. A simple interpretation of these results was that introducing normal genes rectified a genetic defect in the malignant cell, and malignancy returned when these rectifying genes were lost (as shown in Figure 2). This result reverberates to this day: it brings hope that malignancy can be suppressed, but the despair that it will inevitably return as a rare mutant outgrows the suppressed population.

Support for this explanation soon came from an entirely different area. A.G. Knudson was working in Sweden on the epidemiology of retinoblastoma, a rare cancer of children that exists in two forms. One is non-hereditary, occurring sporadically as a single tumour in one eye; the other is hereditary, autosomal dominant, and usually gives multiple tumours in both eyes. Like Harris, Knudson explained both patterns by mutation of two (recessive) tumour-suppressor genes, the Rb genes (see Figure). Many other cancers – including breast cancer – have now been shown to exist in two forms, one of which is non-hereditary and sporadic while the other is hereditary. Many of the underlying tumour-suppressor genes have been identified over the past three decades, and screens to detect mutant ones have been introduced into the clinic. As a result, unfortunate girls born into families with familial breast cancer may already carry one mutant gene, and – because as they age they are so likely to acquire a second mutation and develop cancer – it can make sense to have their breasts removed prophylactically.

Active oncogenes in human tumours were only identified in 1980, and since then we have discovered that regulated growth results from a complex balance between the products of many genes, including the tumour-suppressor genes and oncogenes. For example, more than half the Western population will develop a colorectal tumour by the age of 70, and in 1 in 10 of these individuals that tumour will go on to become malignant as it accumulates around seven independent mutations. The challenge for the future is to build on the hope provided

by Harris' work. That is why we as individuals can help ourselves by reducing the causative mutations, for example by not smoking, while as scientists we can search for ways to brake the active oncogenes and stimulate the action of the tumour-suppressor genes.




*Tumour-suppressor genes.*
*A. Identification by cell fusion. A cell (shown carrying only one maternal and one paternal chromosome) divides many times, but only becomes malignant by accumulating two mutations (mut 1,2) that inactivate both alleles of a tumour-suppressor gene. When this malignant cell is fused with a normal cell (which has two wild-type alleles), the resulting tetrapoloid hybrid contains two wild-type genes and malignancy is suppressed. However, chromosomes can be lost by chance as the hybrid grows, and – if this leads to the loss of both wild-type alleles – malignancy returns as the cell with only mutant alleles outgrows the others. Here, malignancy behaves as a recessive marker.*
*B. Genetic changes occurring during the development of retinoblastoma. Most cells in healthy individuals contain two wild-type Rb alleles, although some occasionally acquire a recessive mutation (mut 3) that goes phenotypically unrecognized. About 1 in 30,000 people acquire a second mutation (mut 4), and – as the lack of any RB protein leads to unregulated retinal growth – tumours develop. Individuals with this sporadic form of the disease contain cells of three types (with 2, 1 and 0 normal alleles). In the hereditary form of the disease, the individual is born with one wild-type and one mutant allele in every diploid cell, and – as there are sufficient cells in the retina that the wild-type allele in one of those cells is likely to mutate during development – tumours almost inevitably develop.*

**References:**

Harris, H. and Watkins, J.F. (1965). Hybrid cells from mouse and man; artificial heterokaryons of mammalian cells of different species. *Nature* 205, 640-646.

Harris, H., Miller, O.J., Klein, G., Worst, P. and Tachibana, T. (1969). Suppression of malignancy by cell fusion. *Nature* 223, 363-368.

*Henry Harris fusing cells at the Dunn School*

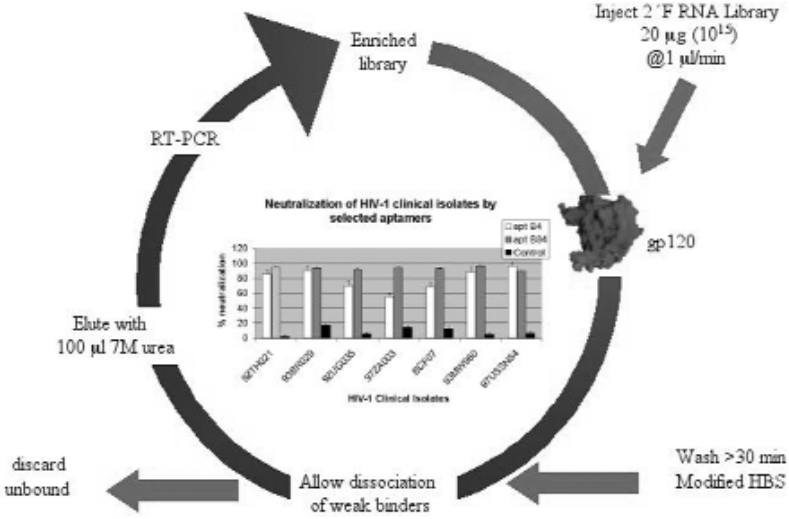
# Simple science on a high impact collision course with the AIDS virus

**Makobetsa Khati is a Wellcome Trust Postdoctoral Fellow from South Africa. He came to the Dunn School of Pathology in October 1998 as a D.Phil. student, after completing a Masters and DIC in Molecular Medicine at the Imperial College School of Medicine, Hammersmith Hospital in London.**

**My mission in the Dunn School has thus been to probe the interaction between HIV-1 and its natural host cells, macrophages.**

I have a special interest in HIV-1, a virus that has killed 28 million people and currently infects more than 42 million worldwide, making it one of the major global causes of mortality. My mission in the Dunn School has thus been to probe the interaction between HIV-1 and its natural host cells, macrophages. Macrophages, centrally involved in both the innate and adaptive arms of the immune system, are not only the chief targets of HIV-1, but also its main reservoir and vehicle of transmission. Macrophage-tropic strains of HIV-1, also called R5 as defined by use of the CCR5 coreceptorin establishing the primary infection, predominate in the asymptomatic phase, and in 50% of the individuals persist throughout infection until the onset of AIDS. Efforts directed at understanding the cellular and molecular mechanisms underlying HIV-macrophage interactions remain the basis for devising novel and efficacious therapeutic strategies against HIV-1 and the AIDS epidemic.

*Figure. Isolation of anti-gp 120 aptamers using the SELEX (systematic evolution of ligands by exponential enrichment) protocol and neutralization of R5 HIV-1 clinical isolates by aptamers (insert).*

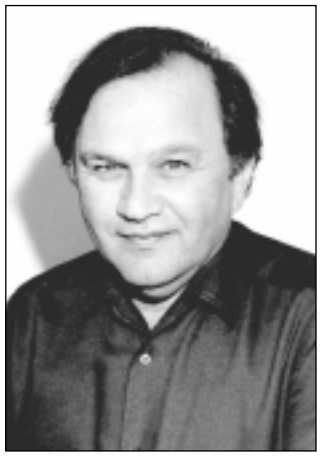



The entry of HIV-1 into target cells, its cellular tropism and the pathogenesis of AIDS are largely determined by the virion surface glycoprotein, gp120. The heroic elucidation of the three dimensional crystal structure of the gp120, and site directed mutagenesis studies that mapped regions of gp120 critical to its function, shed light on how to devise new strategies against HIV-1. Using simple yet ingenious science I isolated novel nucleic acid ligands called aptamers against the HIV-1 gp120. These aptamers not only bound gp120 with high affinities but also neutralized HIV-1 infectivity in human peripheral blood mononuclear cells by up to 100,000–fold, truly unprecedented when compared with natural ligands such as antibodies. Furthermore, these aptamers were able to cross-neutralize the infectivity of clinical isolates of HIV-1 from diverse genetic clades and geographic locations.

The challenge now is to characterize the neutralization sites identified by these aptamers at a functional and structural level in order to gain a deeper understanding of the molecular interactions between HIV-1 and its host cells and to provide detailed structural leads for drug development. These novel ligands or their structural mimetic would provide hope for salvage therapy for patients failing currently used anti-retroviral drugs, as well as alternatives for initial therapy for newly infected individuals.

Through ongoing collaborative research we set up in Oxford and elsewhere, and the Wellcome Trust grant for my post-doctoral fellowship, we have the means to move a step closer in our contribution towards conquering the AIDS virus and providing a lifeline to millions. The Wellcome Trust grant tenure extends beyond my stay in Oxford and allows me to return to South Africa to continue the work on HIV-1 and transfer the skills acquired in the Dunn School.

# Introducing Mark Greene



**Mark Greene, John Eckman Professor of Pathology and Laboratory Medicine at the University of Pennsylvania, has spent 2002/3 as the Newton-Abraham Visiting Professor. *Fusion*'s reporter David Greaves asks the questions.**

**Mark, you have a very impressive list of accomplishments in medical research. What was it that originally attracted you to study science at school when you growing up in Canada?**
I initially studied Fine Arts and Mathematics. It was only after I left school that I switched to Medical School and then later studied for a PhD in Chemistry at the University of Manitoba in Canada.

**Looking back on your time as a PhD student what do you think was the most important thing you learned as a graduate student?**
Patience. I am a very patient person because I studied Chemistry, it's the most humbling of all the sciences.

**After completing your medical training you left Canada and spent the next 10 years in Boston. What factors influenced that decision?**
I had a scholarship from the Canadian MRC, so I could go pretty much anywhere I wanted. I went to Harvard Medical School, initially for one year, and then I was supposed to be going to Cambridge UK but I stayed in Boston until I got a great offer to move to the University of Pennsylvania in Philadelphia.

**You moved to Penn in 1986, where you are now Chairman of the Department of Pathology. What changes have you seen in your University over the last 16 years?**
I was recruited to develop a programme in immunology, molecular biology and receptor biology. Penn now has great strengths in these areas.

**If you were graduating with a PhD in Molecular & Cellular Biology today which areas of scientific research would you be attracted to study and why?**
I was lucky; I studied Chemistry and then moved on to study crystallography. I think the best advice I could give to graduate students is find yourself a niche in an area of science that is fundable.

**Looking back at your 275 published papers which set of experiments gives you the most pleasure and why?**
Back in the 1980s I teamed up with Robert Weinberg to look for new cell surface receptors on the surface of transformed cell lines. These studies identified the Neu oncogene but very soon afterwards we showed that adding anti-Neu antibodies to these cells reversed the transformed phenotype. That result changed the way we thought about immune therapy for cancer. (*Cell, 41:* 695-706, (1985))

**What do you find the most striking difference between the University of Oxford and the University of Pennsylvania?**
Penn and Oxford are very similar in many ways. The great difference is the Oxford College system. The Colleges provide an enduring intellectual education and a great 'civilising' influence on the people who pass through them.

**Outside of science what are your hobbies and interests?**
I still appreciate art and I enjoy drawing. You have some of the greatest museums in the world here such as the British Museum and the National Gallery.

**Mark, many thanks for taking the time to talk to *fusion*. Enjoy the rest of your stay in Oxford!**

# Old Members

We were saddened by the recent deaths of two eminent old members of the Dunn School. Desmond Kay came to the Dunn School with Paul Fildes to work on the biology of bacteriophages and was the acknowledged authority on electron microscopy.

Ann Wakefield did her D.Phil and post-doctoral work with Des Kay, later holding a Royal Society position in the Department of Paediatrics, where she became a world leader on the opportunistic pathogen, Pneumocystis carinii.

# History of the Dunn School

Dr Eric Sidebottom, a former Lecturer at the Dunn School, is compiling a history of the Dunn School. He would be delighted to hear from any readers of *fusion* who would like to share their reminiscences of their time here. You can either write to him at the Dunn School or email eric.sidebottom@path.ox.ac.uk.

**CAMPATH is a humanized antibody discovered by Herman Waldmann and his group and now widely used in the treatment of leukarmia**

# CAMPATH: a fusion of science and therapy

**Steve Cobbold, Reader in Cellular Immunology, describes how Campath came of age.**

December 2000 was the day CAMPATH came of age and was licensed in both the UK and USA to treat a form of cancer in the blood called B Chronic Lymphocylic Leukaemia. When Herman Waldmann was showing me how to do a cell fusion to make monoclonal antibodies, exactly 21 years earlier in the Pathology Department in Cambridge, we had set out to find a way to treat bone marrow transplants, to stop them attacking the patient in what is known as graft versus host disease. The antibody made in that fusion, now called CAMPATH, works by killing lymphocytes and so could also be used to treat autoimmune diseases such as multiple sclerosis and vasculitis. The Therapeutic Antibody Centre was set up with Dr Geoff Hale to help doctors treat these groups of patients that had run out of all conventional therapies, and so that we could learn more about these diseases and how to use monoclonal antibodies.

One such patient is Nicola Cole, who was treated for vasculitis with a severe arthritis-like disease and who recently wrote of her experience with CAMPATH treatment.

'Before being given CAMPATH for the first time, approximately eleven years ago, my joints were extremely stiff, and if I attempted to move I was punished with excruciating pain. I became so ill that my internal organs were shutting down, and my parents were told that I would be lucky to last the weekend. This was when CAMPATH was infused as a last hope.

'Within days of it being given I had begun to show signs of improvement, and thus here I am today! After repeated treatments, my disease has changed and I have been able to undergo various operations, including replacement hips and knees. I am now able to walk a little WITHOUT aids, and I am eternally grateful!

'At present I am at home, but I'm finding that my pain levels are increasing. Unfortunately I have developed an antibody against CAMPATH and cannot have any more infusions, but I remain forever hopeful that something like it will come up which will give me the same feeling of well-being and freedom that CAMPATH once did.'

*Nicola opening the Therapeutic Antibody Centre, with Prof. Geoff Hale (1995).*

When the Therapeutic Antibody Centre moved to Oxford in 1995, we were pleased that Nicola could perform the opening ceremony. Since then, the TAC has continued to be involved in helping doctors throughout the world set up clinical trials with therapeutic antibodies in the management of organ and bone marrow transplantation, multiple sclerosis, diabetes and leukaemia. We are still making improvements to antibodies, and hope that, one day, we may find a way to help Nicola, and those like her, again.

Many thanks to those of you who wrote in following the first edition of *fusion* with your news and comments. In future editions we will include a section for old member's news, so do please continue to send in news items. We would also like to invite you to visit the Dunn School to see the new buildings when you are next in Oxford. Please contact Susan Harrison (susan.harrison@path.ox.ac.uk) to arrange a visit.

**THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY**
is a department of the University of Oxford
**website:**
**www.path.ox.ac.uk**

Contacts:
**Professor Herman Waldmann**, FRS
*Head of Department*
Sir William Dunn School of Pathology,
South Parks Road
Oxford OX1 3RE
email: herman.waldmann@path.ox.ac.uk

**Susan Harrison**
*Development Executive*
Sir William Dunn School of Pathology,
South Parks Road
Oxford OX1 3RE
Tel: (44) (0)1865 285751
email: susan.harrison@path.ox.ac.uk