fusion

THE NEWSLETTER OF THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

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UNIVERSITY OF OXFORD

Interview with Georgina Ferry

A Blue Plaque for Penicillin

Focus on Translational Medicine

Dangerous RNA

SIR WILLIAM DUNN SCHOOL OF PATHOLOGY

Welcome

Penicillin is the gift that keeps on giving, not only to global health but also - albeit a little more parochially - to opportunities for celebrations at the Dunn School. This May we received a belated blue plaque on our building to recognise the site of the epoch-changing research done by Chain, Florey, Heatley and their colleagues. The blue plaque scheme is a national system for marking historically-significant buildings. It is normally reserved for homes of famous people rather than workplaces, but the organisers recognised penicillin as such an important event that they made an exception. The significance was also highlighted by a rare double unveiling, with a second plaque installed at the old Radcliffe Infirmary building on Woodstock Road, marking the site of the first systemic administration of penicillin to Albert Alexander, the famous Abingdon policeman.

The unveiling itself at the Dunn School was performed by Benny Chain, son of Ernst, and Rose Heatley, daughter of Norman, and brought together many alumni, friends and current members of the department. The weather was damply English but did not prevent a great celebratory atmosphere, which was reported by the BBC and local newspapers (see News section).

Jumping forward almost 80 years, those of us who are current members of the Dunn School are very conscious of the spectacular research history of the department; indeed, penicillin is the pinnacle of an impressive portfolio of other discoveries. We eagerly look forward to the celebrations that will mark our current and future successes. Our goal is to be the number one research department in Europe focusing on the cell and molecular biology that underlies human disease, and we are certainly already in the very top league. A few personal successes to recognise this year include the election to the Academy of Medical Sciences of Jordan Raff, the César Milstein Professor of Cancer Cell Biology; the award of the Royal Society's AstraZeneca Award to Ervin Fodor for his work on influenza virus; the award to Monika Gullerova of a prestigious Cancer Research UK Senior Fellowship; and Omer Dushek becoming the first Wellcome Trust Henry Dale Fellow (an early career fellowship for people starting their independent groups) to be awarded their Senior Research Fellowship. Importantly, members of the Dunn School also pick up regular awards that recognise the quality of our teaching as well as research.

There are, of course, many other achievements of Dunn School members and I'd encourage all readers of *Fusion* to look at our website, where news stories describe our successes and other notable events, including a number of important public engagement projects. We have also recently updated our homepage to include an important new initiative that highlights our latest research. It had been pointed out that, paradoxically for such an active research department, our recently redesigned website did not provide much focus on our actual science. I am very pleased that the research digests of some of the top papers that we publish are now being written by a dedicated and talented team of postdocs and students.

This year we have been pleased to welcome two new visiting professors, appointed to broaden the range of experience and expertise associated with the department. Professor Maria Leptin will be known to many of you as the Director of EMBO, the European academy for bioscience. She is also an eminent cell and developmental biologist who continues to run a successful research group. Her new Oxford interactions will primarily be scientific, but she will, of course, be a valuable adviser on broader issues of international science policy, careers and funding. Professor Roger Highfield is the director of external affairs at the Science Museum Group and a distinguished writer and journalist. As a visiting professor of public engagement, there will be many opportunities for him to advise and interact with us all. He points out that there may also be ways for Dunn School initiatives to be showcased through Science Museum channels – an exciting possibility, given their huge audience.

This year I have much enjoyed meeting many alumni and friends of the Dunn School, and we are always very pleased to welcome visits from the readers of *Fusion* who want to know more about what is happening in the department. One of the characteristics that I like most - and which I often boast about being crucial to our success - is the collegiate, almost family, spirit of the Dunn School. And I know that sense of pride of belonging extends to our wider circle of alumni and friends. We do hope that you will enjoy reading this edition of *Fusion* and that it will inspire as many of you as possible to stay in touch.

Matthew Freeman

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Front cover image:
Aftermath of the 'Beast from the East' taken on the original date planned for unveiling of the blue plaque. Photograph by Tatjana Terentjeva.

News

Unveiling a Blue Plaque for Penicillin

Photograph Paul Fairchild

On Tuesday 29th May 2018, some 78 years after the pioneering research that led to the world's first antibiotic, Matthew Freeman, Head of the Sir William Dunn School of Pathology hosted an unveiling ceremony for a blue plaque to honour those who worked in the department on the development of penicillin. The original ceremony planned for March, had been postponed courtesy of the 'Beast from the East', the aftermath of which is captured on the front cover of this edition of *Fusion*. Professor Freeman spoke of the unique importance of the work leading to the advent of the antibiotic era: penicillin alone has been estimated to have saved over 200 million lives since its first systemic use in man. Professor Benny Chain, son of scientist Ernst Chain, and Rose Heatley, daughter of Norman Heatley, unveiled the plaque in front of a large crowd of local dignitaries and current members of the Dunn School as well as reporters from the BBC.

Later the same day a second blue plaque was unveiled on the Woodstock Road end of the building that now houses the University Department of Primary Care. This building used to be home to Briscoe Ward of the Radcliffe Infirmary where the first patients involved in the clinical trial of penicillin in 1941 were treated. Eric Sidebottom, the unofficial penicillin historian, who spent most of his professional life working in the Dunn School, spoke of that first trial. He described the plight of Albert Alexander, the Abingdon policeman, who became the first patient in the trial and who improved dramatically on administration of the wonder drug, starting on 12th February 1941, but sadly succumbed to infection and eventually died when supplies ran out. Most other patients in the trial made dramatic recoveries, as a result of which the world's first antibiotic was well and truly born!

Dunn School Group Leaders Receive Recognition for their Research

Ervin Fodor

Jordan Raff

Two group leaders at the Dunn School have recently received awards in recognition of their outstanding research and its potential impact on society. Ervin Fodor was awarded the 2019 AstraZeneca prize by the Biochemical Society at its spring meeting, which he will receive next year at a ceremony during which he will deliver an invited lecture. The award is made every three years for seminal research that has led to the development of a new reagent or method in the biomedical sciences. In the late 90s, the Fodor laboratory developed a reverse genetics approach to the production of a live attenuated vaccine for influenza virus which has since been adopted by the UK National Childhood Flu Immunization Programme.

Similarly, the outstanding quality of Jordan Raff's research led to the announcement on 10th May of his election to the Academy of Medical Sciences, one of 48 new Fellows this year to join the prestigious body. The Academy's primary aim is to advance biomedical and health research and its translation into benefits for society. Jordan Raff, César Milstein Professor of Cancer Cell Biology, has made important contributions to our understanding of the underlying mechanisms of cell division and, in particular, the role played by centrosomes, yielding important insights into how this complex process can go wrong.

Graduate Student Association Hosts *The Future of Science* Symposium in Celebration of the 90th Anniversary of the Dunn School

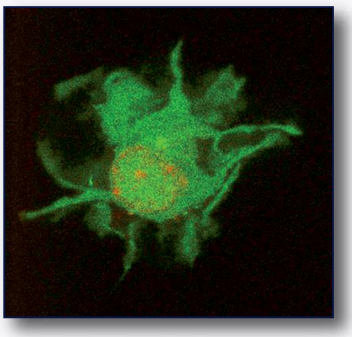
Following the success of the Careers Day in 2016, the Dunn School Graduate Student Association organized The Future of Science Symposium that took place in September 2017. The event, which drew nearly 200 participants, coincided with the 90th anniversary of the opening of the Sir William Dunn School of Pathology and aimed to explore how science will change in the next decade. It was an opportunity for graduate students and postdoctoral fellows across the University to hear new ideas and engage in an open dialogue with the leaders in biotechnology, publishing, public engagement, academia and government. The day was packed with eight talks that ranged over a number of subjects from the accelerating impact of AI to the reproducibility crisis and the role of scientific facts in the post-truth era. The symposium also considered the challenges and opportunities that researchers will face in the next ten years. The spotlight speaker, Sir John Walker, highlighted the importance of mentorship and support that is essential for any fundamental scientific discovery. The talk was followed by a discussion panel that brought together distinguished academics, policy workers and campaigners to discuss the role of public engagement and the future of research training in the era of a competitive academic environment and scarce funding. The speakers remained positive about what the future holds and during the interactive Q&A session shared their advice on how to integrate new technology, creativity and broader collaborations to succeed in science and beyond.

The Symposium was powerful in its examination of scientific progress and the role that scientists will play in shaping the future of the scientific community. The recorded talks are available on Oxford University podcast: http://podcasts.ox.ac.uk/series/future-science-symposium

Mariya Lobanovska

An Important Milestone Reached in Translational Medicine

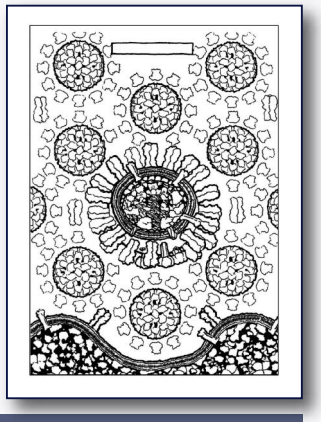
Immunotherapy for the treatment of cancer has recently been revolutionized by the advent of immune checkpoint inhibitors and so-called 'CAR T-cells' but neither has turned out to be the panacea it was once thought to be. In June this year, Asterias Biotherapeutics announced that it had dosed the first patients in a Phase I clinical trial with its proprietary cancer vaccine, AST-VAC2, based on a novel source of dendritic cells differentiated from human embryonic stem (ES) cells, providing an alternative approach to immunotherapy. This achievement marks an important milestone in the development of a cell therapy that originates from work first conducted by Paul Fairchild while a post-doc in Herman Waldmann's laboratory. The patent filed by Paul Fairchild and Herman Waldmann on protocols for the directed differentiation of dendritic cells from ES cells was licensed first by Geron Corporation and subsequently Asterias who will focus on the treatment of non-small cell lung cancer in a clinical trial funded, in part, by Cancer Research UK (CRUK). A number of recent patents that enable the production of more refined 'next generation' dendritic cell vaccines from patient-specific induced pluripotent stem cells (iPSC) will form the IP base of a proposed spin-out company from the Fairchild lab. The Dunn School has, therefore, been responsible for one of only a handful of cell therapies that have ever progressed to clinical trials.



Innovative Outreach: Art Goes Viral

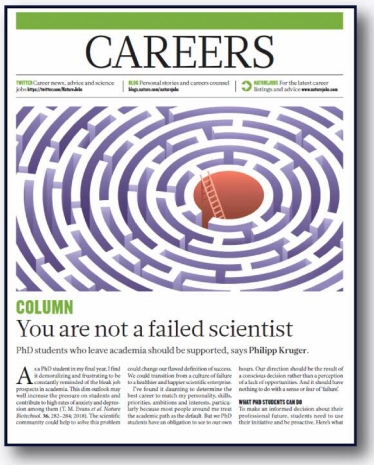
The Dunn School has long been committed to engaging with the public in order to draw awareness to the research carried out within the department and its significance for the treatment of disease. New approaches to outreach are, therefore, always welcome. Ed Hutchinson, a former post-doctoral fellow in Ervin Fodor's laboratory, has contributed to an innovative new colouring book *Art Goes Viral – Exploring the Fascinating World of Viruses through Colour!* which covers the basic concepts of virology in a series of easy-to-colour pictures. It is hoped that the book will help convey the importance of viruses for human health while providing a welcome relief from the stresses of life!

The book can be viewed at the following link: www.gla.ac.uk/media/media_531204_en.pdf.



Fostering Debate about Academic Careers

As an essential part of the life of the Dunn School, the Graduate Student Association has organised numerous events to showcase the variety of alternative career paths open to students who opt not to pursue academic science. Recently, Philipp Kruger, a final year student in Omer Dushek's laboratory, shared insights from his own journey with graduate students worldwide in an article published in *Nature* (*Nature* (2018) **560**:133-134). In addition to providing practical tips on how best to identify the career most appropriate for each person's skills and interests, Philipp argues for an urgent need to end the culture among scientists that views those who take alternative paths to academia as having somehow failed. He concludes that it is simply not possible to complete a PhD without being resilient, hard-working and motivated, to be able to make decisions based on evidence and develop skills necessary to interpret data, communicate complex concepts and prioritize tasks, none of which are the hallmarks of failure in any career!


CAREERS

COLUMN

You are not a failed scientist

PhD students who leave academia should be supported, says Philipp Kruger.

The Dunn School Science and Art Competition 2018

On 21st June the Graduate Student Association (GSA) hosted the first ever Science and Art Competition. For one evening, the Dunn School was transformed into a gallery for displays of the creative artworks made by Dunn School researchers and staff. Members of the department were invited to discover photography images, paintings, sculptures and even jewellery pieces that showcased the work that researchers at the Dunn School do. The panel of Dunn School judges had a difficult task of assessing the scientific content, aesthetics and technique of the artworks presented and the winners were announced at the Graduate Student Symposium. Attendees had an opportunity to vote for the 'Pubic Choice' award and judging by the number of votes, the exhibition received an enthusiastic response. By melding science and art, the event proved there are no boundaries between the two disciplines and in future the GSA is hoping to make the Science and Art Competition an annual celebration of the Dunn School's creative achievements. Congratulations to all our winners who describe their work below!

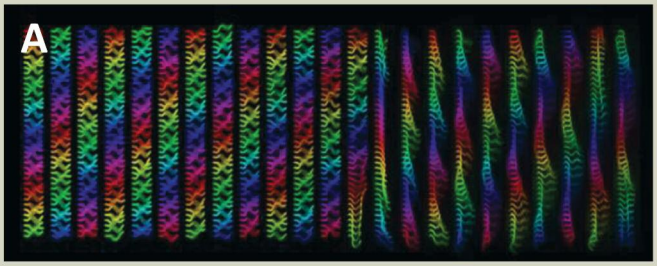
A) Digital Art winner Richard Wheeler 'SWITCH': A representation of how a flagellum, used to drive cell swimming, switches behaviour. It is a digital composite of a microscope image sequence. The shape of the flagellum waveform over time is shown in columns, progressing from top to bottom and from left to right. All the flagellum movement in this image took place in just 4.6 seconds. The design was heavily inspired by the representation of radio signals from the pulsar CP 1919, probably the most famous astronomical data plot after it was used as the cover art for Joy Division's 1979 debut album, *Unknown Pleasures*.

B) 3D Art winner Errin Johnson 'MICROSCOPY - THE LIGHT AND THE DARK': A ceramic 3D sculpture of a lymphocyte. The front of the cell is in cross-section to depict the organelles as seen by both fluorescence light microscopy (left side) and electron microscopy (right side).

C) 2D Art winner Heather Jeffery 'IN THE GROOVE': Regardless of external differences, animals and plants all share the same genetic material: DNA. This is encompassed in the artwork which portrays animals interacting with the structure of DNA, particularly in the major groove (the large gaps between helical rotations of the DNA). We are not as different from animals and plants as first appears.

D) 1st Public Choice winners Cyril Deroy and Cristian Soitu 'THE SCIENCE OF EXODUS - DIVIDING WATERS LIKE MOSES': The principles of fluid mechanics were used to shape and shift liquids on a 60mm petri dish, thereby creating any design imaginable. In the first instance, a fluidic matrix was created which was filled using differently shaded colours to produce mosaic portraits. In the second instance, the fluid was physically 'cut' to create drawings, similarly to one line patterned tattoos.

E and F) 2nd and 3rd Public Choices winner Stephen Cobbold E) 'UNIVERSAL FLOW CYTOMETRY': The way cells are focussed past the lasers of a flow cytometer is similar to how matter spins as it gets sucked into a black hole before being ejected. Fluorescent measurements or images are then acquired – in the case of Flow Imaging this is by a camera that electronically "pans" with the cells as they move past the laser beams to take highly sensitive, multicolour images of each cell. F) 'THE SPIRIT OF THE DUNN SCHOOL': The Dunn School building overshadowed by the historical figures that still influence much of the work we do today: Florey, Chain, Heatley, Abraham, Harris and Medawar. Items associated with the work of each of them are depicted as contributing to the building and its surroundings.


A


B


C


D
Oxford Logo
Dunn Logo
Dunn School
Charles Darwin
Stephen Hawking
Peter Cook
Albert Einstein
Cristian Soitu
Cyril Deroy


E


F

In 2017, the Bodleian Libraries commissioned a series of oral history interviews as part of a project entitled '75 Years of Penicillin in People' funded by the Wellcome Trust. The project involved interviewing scientists, administrators and technicians who work, or formerly worked, at the Dunn School, some with recollections of the penicillin era. The interviews were conducted by Georgina Ferry, an acclaimed science writer, biographer, author and broadcaster who has previously written for* New Scientist, *edited* Oxford Today *and published numerous books, including the biographies of Max Perutz and Dorothy Hodgkin.* Fusion *caught up with her to find out more about the oral histories project and what it has revealed about the Dunn School.

Interview with Georgina Ferry

Tell us a little about the Bodleian's oral history project. How did it first come about and how has it evolved over time?
The oral history project was one strand of a Wellcome Trust-funded curatorial programme called '75 Years of Penicillin in People' which celebrated the Dunn School's history by, among other things, ensuring the long-term preservation of the Dunn School's paper, photo and film archive and upgrading the Dunn School's penicillin exhibition. Susan Thomas, Head of Special Archives and Modern Manuscripts, and project archivist Charlotte McKillop-Mash quickly realised that the oral history strand was not going to centre on penicillin. On the one hand few living witnesses to that period of discovery remained; on the other, they thought it would be more valuable to add to the historical record of the Dunn School by covering its modern history and the challenges faced by modern scientists.

What exactly did the project involve: who was interviewed as a part of the project and how many interviews were conducted in total?
Charlotte worked with Chris Tang to come up with a list of names of people who either had a long-standing association with the Dunn School, or had held significant roles (such as Head of Department). I joined the project at this point, contacting the people on the list to request interviews, and adding a couple of extra names. I was surprised and delighted that almost all immediately agreed.

The numbers were limited by the budget: 21 interviews were completed in total. Not all are PIs or Heads of Department: they also include long-standing facilities manager Pete Stroud and former PA to the Head of Department, Valerie Boasten. The criteria for selection were not very clearly defined, and there were many further names that could have been added to the list if further funding had been available, but those that were interviewed represent the range of research areas and also include some non-research staff. Some had recollections of the Dunn School going back to the 1960s. Some had remained at the School until retirement, while others had moved on to posts elsewhere.

I met each interviewee in person for 1-2 hours, and recorded their accounts using a high-quality digital recorder. I asked about their early education and training, about their first encounter with the Dunn School and their awareness of its reputation, about their work itself and about the context in which the work took place: buildings, funding, collaboration, networking within Oxford and with the wider world. I asked particularly about changes they had seen in their time at the School, in style of management, technology, lab and office space, and external pressures.

What did the project reveal about diversity within the Dunn School? Are there any lessons we should learn in advance of Brexit?
Of those I interviewed, five were women: four of those were PIs and one an administrator. Gillian Griffiths, who came to the Dunn School as a group leader in 1997, says that at the time of her arrival, 'as far as I can recall, there were no female professors and there were very few female group leaders'. Things have changed more recently, says Head of Department Matthew Freeman, who arrived in 2013. 'I have worked very consciously to try and improve our gender balance. We have certainly recruited a much higher ratio of female group leaders than we had in the past.'

Siamon Gordon was born to Jewish Lithuanian immigrant parents in apartheid South Africa, and successfully applied for a readership at the Dunn School in 1976, after beginning his research career in the US. His lab had always been very international and he made a point of recruiting black South African graduate students. 'After 1994, I had several African students' he says. 'I knew they would go back, because they had great opportunities.'

Several interviewees mentioned anxiety about the impact of Brexit on recruitment of research staff. As a well-found institution, access to the best early career researchers, wherever they come from, is more of an issue for the Dunn School than the possible loss of European grants.

The Dunn School's place within the wider University has at times been contentious. Did the project throw any further light of the historical tensions between the two?
Interviewees who date back to Henry Harris's era remember that he ran the Dunn School as a private fiefdom and that there was very little challenge to his authority from the central University or from other departmental heads. His successors have been frank about the Dunn School's unique position among departments in the Science Area, with exclusive rights to the land it stands on and a ready source of funding from the EPA Trusts. For example, Herman Waldmann speaks about his negotiations as Head of Department with the then Vice-Chancellor about building the Medical Sciences Teaching Centre

on the Dunn School site. 'The nice thing about the Dunn School… was, of course, the Trust, the money, and with the heads of the other departments we said, "Look if we can get a good sum of money towards creating space in your departments for you to improve your research…the university wouldn't have to come up with very much." And…when we provided the Vice-Chancellor with that fait accompli,…he accepted…I think all the departments agreed it was a great thing.'

The Dunn School has always been labelled a department of 'Pathology'. Did the interviews provide a sense of how useful the label is today, given the rapidly changing nature of science and a growing trend for interdisciplinary research?
Interviewees tended to identify themselves as immunologists or molecular biologists rather than pathologists, and the techniques they use range from biochemistry to stem cell biology. Matthew Freeman (a developmental biologist) admitted that on his appointment in 2013 he had considered changing the name, but decided it would be an unpopular move. Instead, he came up with a revised definition of pathology as 'the cell biology that underlies human disease'. Most felt that the School's interdisciplinary nature was one of its key strengths.

Were there any surprises or unexpected revelations that came from the interviews you conducted?
Some surprised me but are probably well-known within the Department: for example, Gordon MacPherson remembered that Henry Harris would station his technician Jim Kent outside the door of the lecture room and bar the entry of any student who was late. The myth that Pete Stroud was born on the site proved to be unfounded, although his father ran the workshops: Pete used to come and sit in his office as a boy, and at the age of 17 joined the workshop staff himself.

How will the information provided by the oral history project be collated and disseminated to a wider audience?
Fifteen of the interviews are now available via the University's podcast pages: http://podcasts.ox.ac.uk/series/sir-william-dunn-school-pathology-oral-histories.

All the interviews and their transcripts are held in the Bodleian library, catalogued under the shelfmark MSS. 12467 digital 1-21. Six are currently closed for reasons of personal choice or data protection, but will be made available in the future.

SPOTLIGHT

Tanmay Bharat was recently appointed to a group leader positon at the Dunn School and here discusses with Nina Sulkowski from his laboratory, the nature of the research he intends to pursue and its increasing importance in an era of antibiotic resistance.

Bacterial Biofilms: Multi-cellularity and Antibiotic Tolerance in Microbes

Nina Sulkowski and Tanmay Bharat

Bacteria can exist in liquid culture as single (planktonic) cells; however, they may also adopt an alternative lifestyle and form multi-cellular communities called biofilms. In fact, the majority of the bacterial biomass in nature is found within biofilms. The biofilm environment provides bacteria with protection against external stressors such as desiccation and antibiotics. Biofilms can form on a variety of abiotic and biotic surfaces including human tissue, which can lead to serious infections and disease. Famous examples include biofilms of the bacterium *Pseudomonas aeruginosa*, which cause persistent infection in the lungs of patients with cystic fibrosis.

Biofilm formation consists of several key steps. First, planktonic bacterial cells arrive at a biotic or abiotic surface using tail-like projections known as flagella. Cells can adhere to these surfaces using fimbriae and pili present on their outer surface. The attached cells can then divide and encapsulate themselves in a protective, extracellular matrix rich in polymeric substances. Over time, the biofilm may assume a characteristic spatial architecture involving division of labour and intricate interactions between the constituent cells (Figure 1).

We are interested in studying bacterial biofilm formation at the fundamental molecular level using structural and cell biology techniques. Put simply, we would like to understand how biofilms are 'built', with the cells (the bricks) held together by an extracellular matrix made of polysaccharides, proteins and DNA (the cement). With our research, we also aim to reveal mechanisms by which pathogenic bacteria become tolerant to antibiotic treatment in the biofilm environment. Our end goal is to use knowledge generated in the laboratory to help fight against infectious disease by informing strategies for treatment and prevention.

The central tool in our laboratory is microscopy, more specifically, electron cryo-microscopy (cryo-EM) and electron cryo-tomography (cryo-ET). We combine cryo-EM techniques with additional structural biology methods including X-ray crystallography, mass spectrometry and correlated light

and electron microscopy (CLEM). This allows us to study biofilms at the molecular, cellular and ultrastructural (whole biofilm) level (Figure 1). Recent advancements have revolutionized biological imaging, as reflected in the 2017 Nobel Prize in Chemistry which was awarded to Jacques Dubochet, Joachim Frank and Richard Henderson for development of cryo-EM, a technique that has routinely yielded near atomic resolution structures. In cryo-EM, samples of interest are frozen in a thin layer of vitreous ice and imaged at liquid nitrogen temperature. Next, the resulting cryo-EM images can be processed in a computer to determine three-dimensional structure of the sample and its constituent molecules. Our laboratory therefore features a mixture of expertise, ranging from molecular and structural biology to computational biology and biophysics. Using these methods, the key questions we aim to answer as part of our investigations are:

a) How are bacterial cells held together in biofilms?

b) What is the structure of extracellular matrix surrounding the cells?

c) At what stages of biofilm formation are different components expressed and secreted?

As highlighted in the last edition of *Fusion*, the University of Oxford and the Dunn School have invested significantly in the latest generation of electron microscopes making South Parks Road one of the premier centres for cryo-EM in the UK. We are also joining the ongoing cryo-EM revolution by contributing to the development of novel cryo-EM techniques and software. One of our aims is to develop workflows to perform routine macromolecular structure determination *in situ* (within cells), allowing us to study molecules within their native environment. This approach would provide not only structural insights, but also reveal additional information about cellular localisation of molecules, protein-protein interactions and function. Method development in our laboratory therefore goes hand in hand with the biological questions being addressed. We hope to reveal fundamental insights into the mechanisms of bacterial biofilm formation in the next few years.

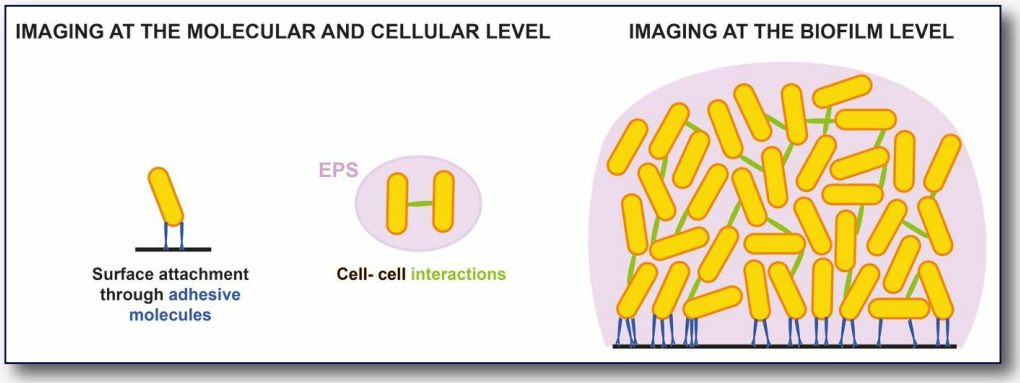


Figure 1. Imaging at multiple scales to reveal biofilm formation in pathogenic bacteria.
Atomic structures of macromolecules regulating biofilm formation are determined using single particle cryo-EM, the cellular function of these molecules is probed using whole cell cryo-ET, and the arrangement of these molecules within mature biofilms is studied using electron tomography.

Celebrating the First 100 Years of the Dunn School and Building for the Future

The Dunn School owes its establishment to a munificent gift given by the group of Trustees set up in the will of Sir William Dunn. The gift of £100,000 was given in June 1922. Around £75,000 was allocated to develop the original Dunn School building on South Parks Road which was subsequently opened in March 1927. Considering the discoveries and developments that have contributed to the Dunn School's reputation as a distinctive, world-class biomedical centre, there are few comparable examples of philanthropy that have had such an influence on biomedical research and teaching.

Those of us in the Dunn School in 2018 recognise that we are only 4 years from the centenary of the legacy gift and only a little further away from the centenary of the opening of the original building. It may seem that we have plenty of time to plan, however we want to ensure all Dunn School alumni are aware of these initiatives and that we get advice, support and input from as many alumni and friends as possible. We want to celebrate all aspects of life in the Dunn School; the many facets of its history and the different people who have worked and trained here. Furthermore, we want to use some of the events organised around the centenary celebrations as a way of facilitating planning and support for future generations of Dunn School researchers.

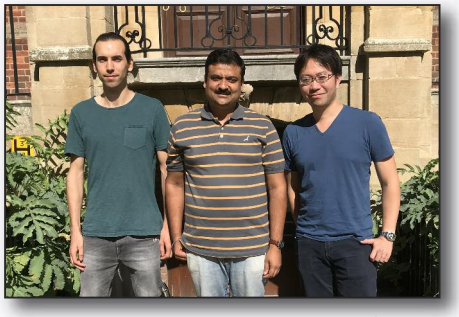
To ensure that we are in touch with as many alumni as possible, please ensure we have your contact details for future *Fusion* editions and event announcements. Also, please encourage others to register their interest for future Dunn School Centenary events by sending their contact details directly to myself (keith.gull@path.ox.ac.uk) or to the Editor of *Fusion* (Paul.Fairchild@path.ox.ac.uk). I will be pleased to receive ideas for events, meetings, actions or gatherings (both scientific and non-scientific) from the Dunn School alumni community. Please email me your ideas with "Dunn Centenary" in the subject line. We look forward to keeping you informed of developments in future editions of *Fusion* as we approach this important milestone in the history of the department!

Keith Gull

RESEARCH INSIGHT

Dangerous RNA

Nick Proudfoot



Michael Tellier, Ashish Dhir and Takayuki Nojima (left to right)

The human genome is beginning to be decoded. The development of DNA sequencing technology started with Fred Sanger and his ingenious technique that relied on incorporation of nucleotide specific DNA chain terminators (dideoxy nucleotides) into DNA followed by a simple gel read out of the sequence. This led to massive parallel sequencing with the derived sequences directly fed into computers. Many technologies from Illumina to Nanopore have opened up the possibility of sequencing whole genomes in hours rather than years. This same revolution directly applies to RNA sequencing. We used to laboriously purify RNAs and map their ends one by one and then derive the RNA sequence by reverse transcription followed by Sanger sequencing of the cDNA. Now transcriptomes can be readily sequenced all in one go, so allowing a snap shot of every transcript being made in a particular population of cells. But what have we learnt so far from these amazing technical advances? I would suggest that one principal outcome is that everything about the human genome is far more complex than any of us could ever have imagined at the beginning of this genomic exploration (that began a mere 50 years ago). What we now appreciate about transcriptomes is that a lot of the RNA produced appears to have little if any specific function. Indeed, nuclear RNase activities work very hard to rid the cell of much of this unwanted transcriptome. In effect, mammalian genomes appear to be subjected to transcriptional anarchy. Surprisingly, most transcripts are degraded either during or soon after their synthesis. Of the around 20,000 protein coding genes synthesised, most of their pre-mRNAs are highly intronic with an average gene looking rather like a bar code where the coding, exon sequence (bars) are interrupted by much longer non-coding (nc), intron sequence (gaps). It is intriguing to note that Henry Harris predicted such transcriptional anarchy in the 1960s. He observed that HeLa cells, pulse-labelled with ^{14}C radioactive adenine produced a labile RNA species that was largely retained in the nucleus rather than forming messenger RNA in the cytoplasm.[1,2]

To appreciate the complexity of human transcriptomes, various experimental tricks must be used to generate nascent RNA libraries, that is analysis of RNA as it is being made but before it is summarily degraded. These include pulse-labelling with nucleotide analogues that allow selection of nascent RNA.[3,4] Alternatively, the direct sequencing of the RNA within the RNA polymerase active site referred to as mammalian NET-seq has been developed in my laboratory[5] and has proved a powerful way to characterise nascent transcription. With the benefit of these nascent transcriptomic libraries, many more ncRNAs have come into focus across the human genome. As I will describe, the inappropriate accumulation of such transcripts leads to severe cellular consequences which are, in effect, RNA pathology. Essentially, ncRNAs are considered to be any transcript that lacks protein coding potential. Of course, several highly abundant ncRNAs have key structural roles in translation such as tRNA and rRNA (made by dedicated RNA polymerases I and III). Also, small ncRNAs such as snoRNA, snRNA, microRNA and piRNA, often excised from larger primary transcripts, facilitate rRNA processing, splicing and mRNA or gene silencing respectively. However, in terms of the greatest genome coverage, it is a new class of ncRNA that has received much recent attention. Notably, most of this RNA derives from the fact that RNA polymerase II (Pol II) which makes pre-mRNA, is an adventitious enzyme, that is, it will initiate transcription wherever there is a hole in nucleosome packaging of the chromatin-enshrouded genome. Regulatory sequences for protein coding genes include multiple separate enhancers as well as the gene's promoter and terminator that together define the extent of transcription across a gene, often referred to as a transcription unit (TU). All of these elements display nucleosome depletion and so allow Pol II to initiate transcription in either direction from these positions. Such opportunistic transcription leads to transcripts that run antisense from the regular protein coding gene promoter (called promoter upstream transcripts or PROMPT), in both directions from enhancers (eRNA) or antisense from gene terminators (asncRNA). These extra transcripts are generally referred to as long noncoding (lnc) RNA. Added to these lncRNAs, some independent TUs are evident that run between more widely-spaced protein coding genes and are referred to as long intergenic noncoding (linc) RNA (Figure 1).

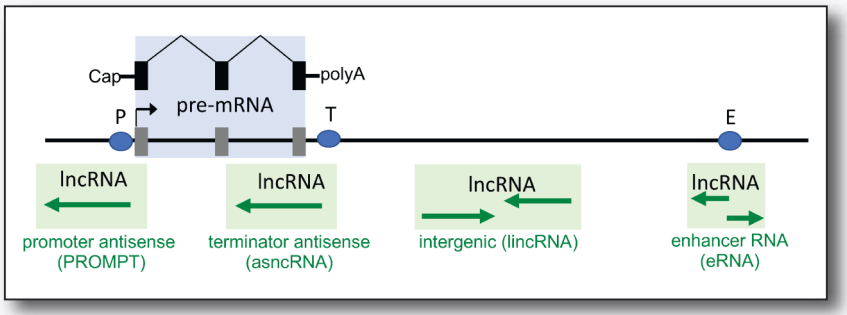


Figure 1. Categories of lncRNA. *Diagram shows protein coding gene (grey shading) with short exons separated by longer introns. P, T and E denote promoter, terminator and enhancer respectively. Pre-mRNA showing 5′ Cap, spliced introns and 3′ polyA. Different types of lncRNA positioned below protein coding gene (green shading).*

We have now shown that lncRNAs are a potential danger to cells if they are not kept under wraps. Notably, we have previously discovered that most lncRNAs (especially lincRNA) are retained and degraded at their site of synthesis on the chromatin template with only a small fraction escaping into the nucleoplasm.[6] In rare cases, lncRNAs may even get all the way to the cytoplasm and potentially associate with ribosomes. They will, however, generally lack protein coding potential beyond the capacity to encode a short peptide. This will normally trigger translation induced degradation. There is also a small subset of

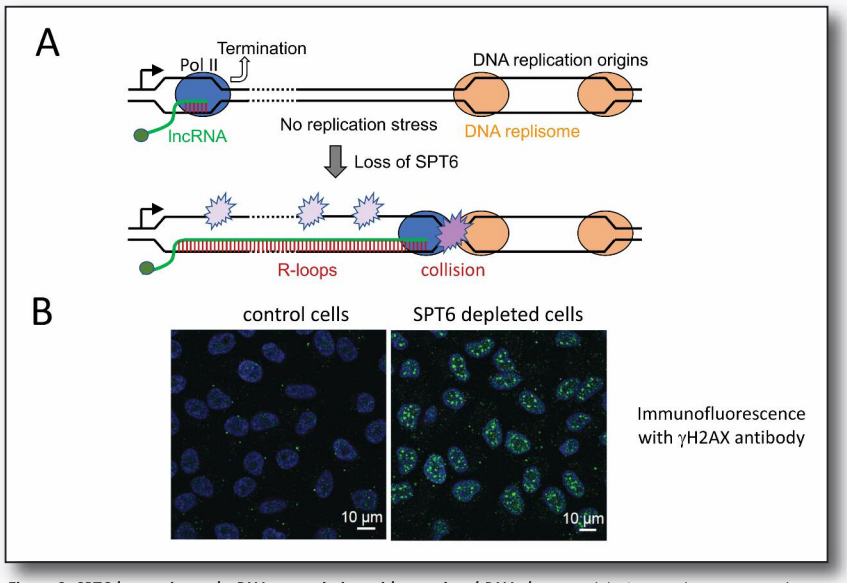


Figure 2. SPT6 loss activates lncRNA transcription with associated DNA damage. *(A) Diagram shows separated, elongating RNA polymerase II (Pol II) and bidirectional DNA replisomes. Following loss of SPT6 Pol II makes extended lncRNA which form R-loops and collide with replisomes in both cases causing DNA damage. (B) Images of cells stained with DAPI (blue) to show nuclei and subjected to anti-γH2AX immunofluorescence. Green nuclear foci indicate DNA damage.*

lncRNAs that do have specific functions such as Xist, a lncRNA required for X chromosome inactivation in female cells[7] or a type of lncRNA called germ line transcripts that are expressed in B cells from antibody gene loci and are involved in the regulated recombination processes associated with this complex locus.[8] However, what we have recently discovered is that a key transcription elongation factor acting with Pol II, called SPT6, plays a pivotal role in promoting efficient transcription of protein coding genes while still keeping lncRNAs at low levels.[9] This study has been a collaboration between Shona Murphy's and my lab at the Dunn School and particularly Takayuki Nojima (NJP lab) performing the molecular biology and genomics and Michael Tellier (SM lab) carrying out extensive bioinformatics. SPT6 was first studied in budding yeast where it has two key features. Firstly, it acts as a nucleosome chaperone to promote the replacement of nucleosomes behind the elongating polymerase as it reads across a gene. Pol II must temporarily displace nucleosomes to allow copying of the DNA template into RNA. The nucleosome chaperone activity of SPT6 was picked up because without it, nucleosome holes appear on transcribed genes which then allow *de novo* Pol II initiation.[10] Secondly, SPT6 helps to recruit the histone modifying methyl transferase Set2 which places methylation marks on the N terminal tails of H3 histone that sticks out from the nucleosome octamer. The H3K36me3 mark is a well-defined feature of active transcription across protein coding TUs.[10]

To look at the role of SPT6 on human transcriptomes, we depleted its levels using standard RNA interference approaches (i.e. transfection of siRNAs into human Hela cell line). Nascent transcript analysis then revealed that, as expected, protein coding gene transcription was reduced especially towards gene ends, entirely consistent with a defect in Pol II transcriptional elongation. However, to our surprise, all of the lncRNA categories mentioned above (PROMPTs, eRNAs and lincRNAs) dramatically increased in levels and extent across the HeLa cell genome. This transcriptional dysregulation appears to have two related deleterious effects. Firstly, the fact that Pol II is allowed to read across wider regions of the human genome will mean that it is more likely to get in the way of the DNA replication process during S phase of the cell cycle. Particularly, head-on collisions between Pol II and the replication fork have been shown to cause gaps in the synthesis of newly replicated DNA which can lead to double-strand breakage and consequent DNA damage. This problem is exacerbated by another consequence of making too much lncRNA. R-loop structures form when the nascent transcript hybridises to the template DNA strand behind the elongating Pol II complex thereby displacing the sense DNA strand. A triple stranded structure is therefore formed comprising a duplex RNA:DNA hybrid and displaced single stranded DNA. These R-loops are especially inclined to form if the nascent RNA dwells on the chromatin template and is neither spliced nor packaged into RNA protein complexes. Such conditions exactly match these elevated and extended lncRNA that are increased in amount following SPT6 depletion and are only inefficiently processed. The consequence of these elevated R-loop levels across the genome is clearly negative and results in extensive DNA damage. Firstly, the single stranded DNA of the R-loop is quite fragile and damage prone. Secondly R-loops associated with elongating Pol II can act to block DNA replication by causing collision between these two nucleic acid copying machines.[11] I illustrate these effects in Figure 2A and show that loss of SPT6 does indeed cause DNA damage, based on the appearance of γH2AX foci (a well-known DNA damage marker) in HeLa cell nuclei (Figure 2B). These DNA damage effects will in turn cause the cell cycle to slow down and even shift out of the regular cycling into a non-dividing or senescent state.[9] We propose that these elevated lncRNAs induced by the loss of SPT6 are highly deleterious to cells. It is critical for lncRNAs to be kept at low levels and preferably degraded before they lead to defects in cell division. In other words, lncRNAs can generally be viewed as extraneous transcripts that have no particular benefit to a cell. Even worse, they are dangerous to the cell since their accumulation is pathogenic.

We have recently uncovered another source of unwanted transcript that, like lncRNA, if allowed to accumulate, can result in RNA pathology. Even though excess nascent transcription is a clear feature of the nucleus, a second organelle, the mitochondrion, is also a source of unwanted RNA. Mitochondria possess multiple copies of a small circular 16.5 kb genome that is transcribed on both strands (L and H) to generate transcripts potentially extending around the entire circular genome. These are broken down into 13 mRNA, 2 rRNA and 22 tRNA sequences. Indeed, tRNA interspersed between the other larger mRNA and rRNA sequences are excised by RNAse P chopping up the longer L and H RNAs. Numerous nuclear proteins (200-300 in total) imported into the mitochondrion combine to generate a functional mitochondrion. In particular a mitochondrial (mt) ribosome is formed to allow translation of the 13 mt mRNA which are translated into key components of the oxidative phosphorylation apparatus, again augmented by many additional nuclear proteins. Major players in RNA metabolism of the L and H mt RNA are two RNA modifying enzymes SUV3 and PNPase encoded by nuclear genes. SUV3 is a helicase that likely unravels L and H transcripts that will otherwise associate to form

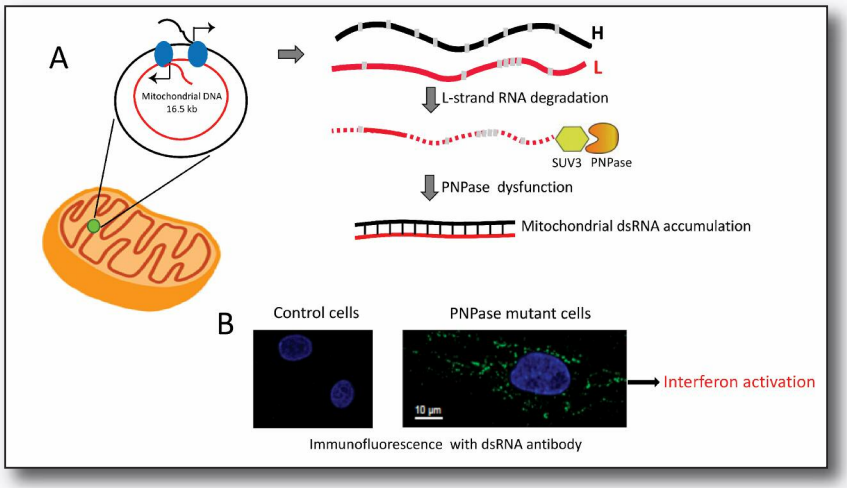


Figure 3. Human mitochondrial double stranded RNA and induction of interferon. (A) Cartoon of mitochondrion showing inner and outer membranes with circular DNA genome in central matrix. Mitochondrial RNA polymerase transcribes L (red) and H (black) strands as indicated. L and H polycistronic transcripts are shown with tRNAs depicted as grey boxes. These are excised to release tRNAs and adjacent mRNAs. Most of L strand RNA is degraded by PNPase exoribonuclease together with SUV3 helicase. Loss of PNPase causes dsRNA accumulation. (B) Images of cells stained with DAPI (blue) to show nuclei and subjected to anti dsRNA immunofluorescence. Green cytoplasmic foci correspond to mitochondrial dsRNA accumulated in the cytoplasm of PNPase mutant cells.

double stranded (ds) RNA. PNPase is an exonuclease that aids in the maturation of the various mt RNA as well as degrading unwanted transcripts.[12] Unexpectedly Ashish Dhir in my lab has now shown that HeLa cells generate a significant amount of mt dsRNA. This is detectible by using a handy dsRNA specific antibody called J2. Immunofluorescence (IF) signals using J2 are only just detectible in HeLa mitochondria but remarkably increase many folds when either SUV3 or PNPase enzymes are depleted by siRNA treatment.[13] A further intriguing twist to this story is that while SUV3 and PNPase act together in the inner matrix of the mitochondria, PNPase has a separate life in the mitochondrial intermembrane space (between the two mitochondrial membranes). Ashish Dhir has now shown that in this separate position PNPase appears to act as a gate keeper to keep mt dsRNA within the mitochondrion[13]. Loss of PNPase not only causes mt dsRNA accumulation in the mitochondrial matrix but also allows its escape into the cytoplasm (Figure 3A illustrates these effects). Here is where the pathology becomes an issue. Notably, cytoplasmic mt dsRNA triggers innate immunity, starting by activation of the dsRNA sensitive interferon response pathway and ending with cell death (apoptosis). It is very apparent that mitochondrial RNAs are potentially dangerous to cellular integrity. Any escape of mt dsRNA into the cytoplasm is catastrophic to the cell. Indeed, we have been able to show that several patients with PNPase gene mutations have increased mt dsRNA J2 signals in primary fibroblast cells isolated from biopsies of their skin. Furthermore, they also show elevated interferon response indicative of severe autoimmunity.[13] Although mutations in PNPase are happily rare in humans, it is a distinct possibility that other conditions might induce the escape of mt dsRNA into the cytoplasm, thereby being of wider relevance to human pathology.

Overall it is clear that the failure to remove unwanted transcripts, whether nuclear lncRNA or mitochondrial dsRNA, through the loss of normal RNA processing activities is a pathogenic condition. On a personal note I have now been working in the Sir William Dunn School of Pathology for almost 40 years. It is very satisfying to finally be able to add a pathological component to my laboratory's otherwise more esoteric basic research into how genes work.

Acknowledgements

I am especially grateful to Taka Nojima and Ashish Dhir for their key discoveries of dangerous lncRNA and double stranded mitochondrial RNA respectively. I thank all members of my lab for their scientific enthusiasm and friendship that makes our science so rewarding. I gratefully acknowledge funding from the ERC (Advanced grant 339270) and the Wellcome Trust (Investigator award: 107928/Z/15/Z).

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Photograph Quentin Sattentau

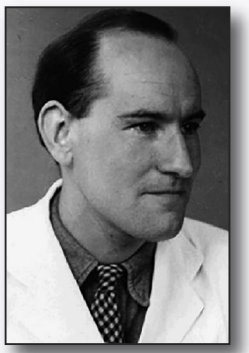
Focus on Translational Medicine

Few departments can boast that the clinical translation of their work has saved hundreds of millions of lives, and yet the story of penicillin is in some ways one of missed opportunities. As a result of Howard Florey's misgivings over the very notion of intellectual property, penicillin was not patented, at least not by the members of the Dunn School who had devised the techniques for its purification: instead patents were filed, unopposed, by their US collaborators who were, therefore, the principle beneficiaries of penicillin's commercial success. By the time that cephalosporin was developed by Abraham and Newton, important lessons had been learned: by protecting their intellectual property, royalty payments on the worldwide sales of the antibiotic led to the establishment of the EPA Trust which continues to provide an important alternative revenue stream for the department. In addition, the Guy Newton Fund, described below by Gillian Helstrom who oversees its administration, provides funding specifically intended to support translational projects and their commercialization. With such resources available and the strong support of Oxford University Innovation, the Dunn School has developed a culture of technology transfer, as a result of which it now holds 30 granted patents and has entered into 48 licensing deals with commercial partners, responsible for returning almost £3m to the University since 2015.

In this edition of *Fusion*, we focus on three current examples of the translation of basic research from the Dunn School towards clinical trials and commercialization by pharmaceutical companies. Former Head of Department, Herman Waldmann, describes the long and sometimes arduous journey that led to the development of Lemtrada (a monoclonal antibody specific for CD52) into a blockbuster drug for the treatment of Multiple Sclerosis, now marketed by Sanofi. Quentin Sattentau and Bass Hassan describe more recent research from their respective laboratories that may lead to the development of a new vaccine for HIV-1 and a novel inhibitor of insulin-like growth factor 2 for the treatment of cancer. Such innovations will help ensure that research from the Dunn School continues to build on the legacy of penicillin and cephalosporin for many years to come.

The Guy Newton Research Fund

Gillian Helstrom



Guy Newton

The Guy Newton Fund is a small trust fund with a value of approximately £12m which supports medical, biological and chemical sciences at the Dunn School. Its history runs parallel to the E. P. Abraham Research Fund as they are both the outcome of the work and generosity of two men, Guy Newton and Edward Abraham, who worked together on the structure and development of Cephalosporin C. Indeed, Newton and Abraham published 50 papers together during their 20 years of collaboration.

Both the Guy Newton Research Fund and the E. P. Abraham Research Fund came into being on 17th March 1967 but the story began at the Dunn School in 1948. Aware that some bacteria were resistant to penicillin, the search was on for the next new antibiotic. Giuseppe Brotzu, an Italian pharmacologist, had, during the war, observed the antibiotic effect of the fungus *Cephalosporium* near a sewage outflow off Sardinia, however his facilities and funding for research were limited and in 1948 a culture of this fungus was sent to the Dunn School. Guy Newton had arrived at the Dunn School in 1947 as a DPhil student of Edward Abraham and, on completion of his studentship, remained within the department supported by the MRC. After several years' work, Newton and Abraham turned Cephalosporin into a marketable drug through Glaxo Laboratories and, unlike penicillin which was not patented, the National Research Development Council (later BTG) obtained patent cover for Cephalosporin C, thereby fulfilling their brief to see that inventions in British universities were protected and commercially developed. The NRDC took half of the income and the rest was agreed to be divided equally between Abraham and Newton. As Guy Newton was funded by the MRC they took part of his share. With this revenue, in 1967 Newton and Abraham established two charitable funds: The Guy Newton Research Fund and the Edward Penley Abraham Research Fund. The history of these funds cannot really be separated but the Guy Newton fund was intended solely to support the Dunn School. It has contributed to the department in many ways over the last 50 years and, more latterly, to the building of OMPI. However, a more recent development has been the advent of Translation Opportunity Grants, small grants to promote commercial translation of research emanating from the Dunn School. Such grants typically support early stage proof-of-concept work that is expected to lead to further funding opportunities and for which there is a clear plan for future development.

It was only two years after setting up the fund, in 1969, that Guy Newton sadly died very suddenly, at the age of 50, of a heart attack but his legacy lives on in the translational focus of research within the department.

For further details of any of the above, please contact the Trust Office: epa.trust@path.ox.ac.uk

30

The number of patents granted

48

Commercial deals negotiated

£2.98m

Returned to the University since 2015

3

Spin-out companies currently in the pipeline

FOCUS ON TRANSLATIONAL MEDICINE

Reprogramming the Immune System

Herman Waldmann

We are all accustomed to the principle that (at least for now) we can abort many infectious diseases with a short pulse of anti-microbial therapy. I came into the field of immunology in 1971 because I felt it offered similar opportunities for 'cure' or (at least) 'near cures' in other disease areas, particularly where the immune system makes unwanted responses. My aim was to 'reprogram' the immune system away from responses to 'self' in so-called autoimmune diseases, such as multiple sclerosis or Type 1 diabetes, to stop rejection of genetically non-identical organ and cell transplants, and to abort allergy to foreign substances. Why would one have imagined this was possible? The answer has evolved over the past 50 years of immunology.

The immune system has the capacity to recognise a vast number of different molecular configurations (antigens) through cell surface receptors that, in each human, are clonally distributed amongst 10 trillion white blood cells known as lymphocytes. Lymphocytes circulate around the body as a surveillance mechanism safeguarding against infections. When lymphocytes first develop from bone marrow stem cells, they randomly acquire expression of these antigen receptors with the potential to recognise any molecular configuration, including many directed to self proteins. Remarkably, such self-recognising lymphocytes are purged once they encounter self, thereby reducing the risk of autoimmune disease in later life. In addition to purging, there are a range of additional fail-safe mechanisms that ensure so-called 'self-tolerance' yet provide the host with an impressive capacity to attack invading microbes. Three major principles guide lymphocyte decisions on whether to become tolerant, or to gain immunity to the antigens they encounter. First, the antigens must be shown to lymphocytes by specialised intermediary antigen-presenting cells that are able to demonstrate 'alarm' features that can indicate if the antigens they have picked up risk danger to the host. If so, then those lymphocytes expand their numbers, and develop a range of immunity mechanisms directed to the invader. In the absence of such alarm signals lymphocytes engaging antigen are directed towards tolerance processes, effectively excluding them from any further involvement in immunity. Second, our pool of lymphocytes needs to be able to recognise antigens in body fluids outside cells, and also antigens that make their way into cells. The job of dealing with extracellular antigens falls on a specialised lymphocyte subset (the so-called bursa equivalent) B-cells, and for intracellular antigens on thymus processed T-cells. All lymphocyte responses require collaborations (T cells with B cells or between different T cell populations). This means that for lymphocytes to participate as collaborators, many of them must have bypassed the anti-self purging mechanisms. This requirement elegantly avoids the likelihood of rogue lymphocytes that survived the thymic purging process autonomously becoming immune to self. Third, from discoveries arising in last 30 years, we have uncovered other important fail-safe mechanisms to ensure self-tolerance. These are embraced by the term 'regulation', a critical player amongst these being a subset of T cells known as regulatory T cells, which constantly police the immune system, and dampen down or suppress anti-self immune responses.

My PhD thesis, guided by my mentor Alan Munro in Cambridge, was devoted to studying mechanisms of lymphocyte cooperation, where we showed a critical role for intercellular mediators (now known as cytokines). The popular view of lymphocyte decision making (tolerance versus immunity) was, at that time, that antigen gave one type of signal to the lymphocyte antigen-receptor (signal 1) which alone would evoke tolerance unless accompanied by signal 2 (cytokines and/or alarm signals) which would authorise immunity. This simple view of the immune system led to the hypothesis that if one could selectively prevent signal 2 events (arising from lymphocyte collaboration), then lymphocytes engaging antigen should be functionally purged from further action; in other words, tolerized. One way of preventing lymphocyte collaboration would be to substantially reduce lymphocyte numbers, but at that time we lacked a safe means of achieving such an outcome.

This all changed when Cesar Milstein and Georg Köhler, at the Laboratory of Molecular Biology (LMB) in Cambridge, discovered a way to generate pure clones of immortalised lymphocytes secreting monoclonal antibodies (mAbs) directed to any antigen one might wish to target (antibodies à la carte). Cesar invited me to attend a seminar at King's College, Cambridge where Georg first presented their work. They had used a technique of cell fusion, first devised by Henry Harris at the Dunn School, to create hybrids of antibody producing cells joined to immortalised B cell lines capable of indefinite and substantive secretion of antibodies. They had generated from immunised rodent lymphocytes a mAb to a standard laboratory antigen, the sheep red blood cell. Sydney Brenner, in the audience, opened the questioning, "Can we make an antibody against anything? Can I make one against my mother-in-law?" That jocular intervention opened up an exciting discussion about the numerous diagnostic, experimental and therapeutic possibilities for mAbs, after which my life was to be changed forever. I asked Cesar if I could spend a short sabbatical with him to learn the technology. My goal was to immunise rats to either mouse or human lymphocytes and, from these, generate mAbs that would prevent lymphocyte collaboration in both mouse and human: the former to test hypotheses, and the latter to put into clinical practice.

An important starting point with this strategy was to establish that our chosen mAb would target lymphocytes and prevent collaboration, but not damage blood stem cells from which new lymphocytes would develop, nor other vital body cell types. This overall goal provided the basis for seven consecutive MRC programme grants, which started in 1980, and saw us through to 2012, when I retired as head of the Dunn School. The first of these programmes was concerned with selecting appropriate candidate antibodies with desired specificity, and

necessitated efforts directed to ensuring avoidance of damage to blood stem cells. This we did through recruitment of Suzanne Watt and Hoang Trang, experts in *in vitro* stem cell assays, and the fortunate acquisition of a Royal Society Guest fellowship for the pioneer in blood stem cell research, Donald Metcalf from the Walter and Eliza Hall Institute. The early immunology and molecular biology underlying this programme was undertaken by Stephen Cobbold, Mike Clark, Geoff Hale and Marianne Bruggemann, who made consistent and invaluable contributions to the collective effort over many years. In the first 5 years, we generated numerous rat antibodies to human and mouse white blood cells, and searched for antibodies that could exploit the body's natural effector mechanisms (for example the complement system), or could block lymphocyte collaborations.

To our delight it wasn't long before we identified a set of mAbs recognising an antigen (known by the code name CD52) present on virtually all lymphocytes, but not on blood stem cells, or other vital body cells. Geoff took on the additional role of establishing a clinical grade manufacturing facility, where selected antibodies could be manufactured, purified and guaranteed safe for clinical testing. The first rat antibody against CD52 was a complement fixing antibody of the IgM class, (CAMbridge PATHology-1M or CAMPATH-1M) which proved useful for *in vitro* purging of potential destructive donor T cells in clinical allogeneic bone marrow transplantation. In this situation donor T-cells in a transplanted bone marrow would otherwise have attacked the recipient causing potentially-fatal graft versus host disease (GvHD). This clinical utility was established through a wonderful collaborative effort with many international haematologists, affectionately known as the CAMPATH users group. To determine whether antibodies to CD52 would be effective when administered to patients, we were able to investigate their utility in patients with drug-refractory lymphocyte malignancies. We soon learned *in vivo* efficacy required a very particular subclass of rat antibodies specific for CD52 (a form called IgG2b, and thus the name CAMPATH-1G). Other rat subclasses were simply unable to recruit the necessary killing mechanisms. My favourite slide summarising these particular findings was entitled '2b or not 2b - that is the question' (Figure 1).

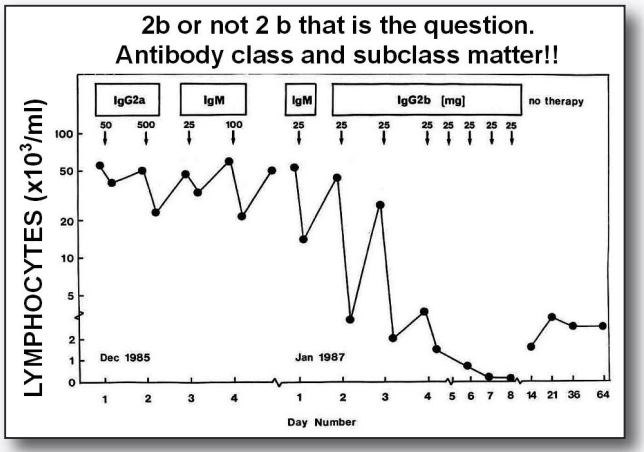


Figure 1. Slide from an early presentation demonstrating the importance of antibody isotype for the purpose of lymphocyte depletion. (Data from Dyer MJS et al, *(1989)* Blood ***73****:1431-1439)*

This finding in the late 1980s that a mAb of a desired subclass could be potently lytic for human lymphocytes in patients inevitably attracted the attention of pharmaceutical companies, one of whom took a license for this rat IgG2b form. While this particular antibody was in commercial development we were finding that these rat antibodies were evoking undesirable anti-rat antibody responses in patients, perhaps not surprising given the foreignness of rat proteins to humans. We and others realised that this problem might be overcome if we were somehow able to develop human mAbs for clinical use. To achieve this Marianne Bruggemann, together with the late Michael Neuberger, took the route to create mice as a source of lymphocytes for cell fusion, in which the murine antibody genes were replaced by the equivalent human genes. This succeeded but took many years to achieve. Fortunately, Greg Winter at the LMB had, at that time, described an ingenious way of genetically engineering rat antibodies in the test tube into the less foreign human form, a process for which he coined the term 'humanization'. Our laboratories collaborated and rapidly humanized our CD52 CAMPATH-1G antibody to generate CAMPATH-1H (Figure 2) which, excitingly, was then the first humanized antibody to enter clinical use. To our delight it retained efficacy but exhibited (desirably) much reduced ability to evoke neutralising antibodies in patients. Greg Winter and I take much pride in this work, as it proved a catalyst for the rapid growth of a multi-billion dollar industry dedicated to clinical benefit. Sadly though, the pharmaceutical company that had initially licensed CAMPATH-1G went through a merger with two other major pharmaceuticals, following which the new enterprise did not see CAMPATH-1H as the blockbuster drug that it needed. Furthermore, our goal throughput this project had been to provide a short-term pulse of antibody treatment to harness tolerance mechanisms, hoping for long-term clinical benefit. We recognised from our previous experience that the pharmaceutical industry was not necessarily convinced by that idea! After all, from their perspective, long-term therapy guaranteed a healthy income.

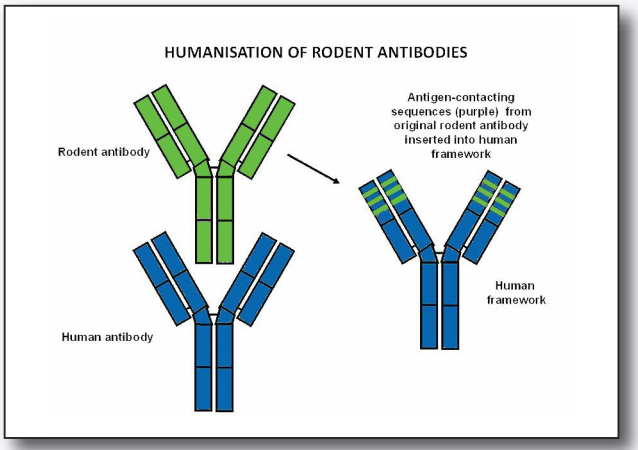


Figure 2. Humanization of rodent antibodies. Schematic showing the principles of 'grafting' the the sequences of rodent antibodies responsible for specificity onto a human antibody framework.

When our team moved to Oxford in 1994, Geoff Hale established a new state-of-the-art antibody manufacturing facility able to produce pharmaceutical-grade material. This enabled us to maintain numerous collaborations in the areas of leukaemia, transplantation and autoimmune disease. Amongst these was an exciting long-term collaboration with two Cambridge neurologists, Alastair Compston and Alasdair Coles, both experts in the management of Multiple Sclerosis. That collaboration (which had begun in 1991) culminated 15 years later with the demonstration that a short pulse of treatment with CAMPATH-1H could induce long-term remissions and substantially slow down the level of disability accumulation in patients. Based on those findings, a USA Biotech company (Genzyme) realised that they could build a business model that might be cost effective to health providers, yet significantly

profitable to them. They licensed the antibody and our patents, and went on to complete the necessary Phase II and Phase III trials required for approval in 2013 by USA and European licensing bodies. And so was born the drug now known as Alemtuzumab or Lemtrada, the first drug for relapsing-remitting Multiple Sclerosis, which is given (as we had always hoped) as a short-term treatment providing remissions lasting many years (Figure 3a), together with a sustained improvement in disability compared to the standard treatment (Figure 3b). Genzyme, with the promise of the success of Lemtrada, was recently acquired by Sanofi.

Looking back over the near 30 years that it took for Alemtuzumab/Lemtrada to achieve its place in the battle against autoimmune disease, I reflect on the many devoted colleagues who were able to turn the idea of harnessing tolerance mechanisms to a commercial reality, despite the pessimism of many 'opinion leaders' within the pharmaceutical sector. After years of battling against such characters and the 'fog' they generated, I am delighted that we persisted in our efforts to create a new treatment paradigm. We are hopeful that Lemtrada and the principle of short-term therapy for long-term benefit, can be more broadly applied to many other areas of unmet medical need (eg autoimmune diseases, transplantation and lymphocyte malignancies), if the pharmaceutical sector and its regulatory bodies, have the will and means to do so.

Closing remarks

Our desire to seek a treatment that would harness tolerance mechanisms was founded on a very simple hypothesis prevalent in the 1970s. Although a useful drug emerged from this effort, we have to acknowledge that the therapeutic benefits of Lemtrada in Multiple Sclerosis, not only result from interfering with lymphocyte collaboration, but also from unwittingly harnessing other failsafe tolerance mechanisms that we and others have uncovered over the past 40 years since this work began. This raises the interesting dilemma of how detailed an understanding of disease pathogenesis or of mechanisms underlying therapeutic agents is needed before embarking on clinical trials. Notwithstanding this, I have little doubt that without our team's and collaborators' relentless efforts in academia, Lemtrada would never have seen the light of day!

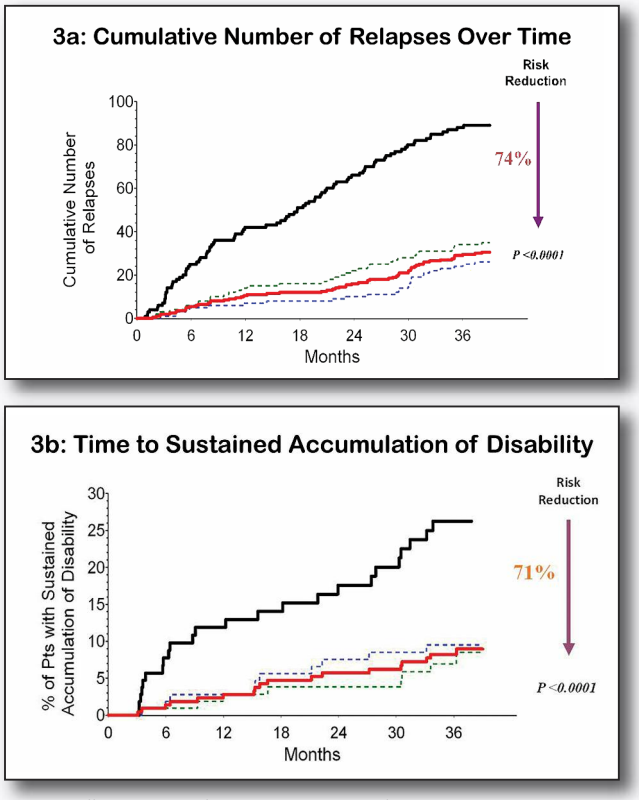


Figure 3. Efficacy outcomes for relapse and disability after short-term treatment in relapsing-remitting Multiple Sclerosis. Key: Black line: Interferon-β-1a, Blue dashed line: Lemtrada at 12 mg/day; Green dashed line: Lemtrada at 24 mg/day; Red line: Lemtrada pooled data. (Data from: CAMMS223 Trial Investigators (2008) N Eng J Med ***359****:1786-1801)*

A fuller account of the emergence of CAMPATH-1 is described by Lara Marks at the following link:
http://www.whatisbiotechnology.org/index.php/exhibitions/campath

Readers who wish to appreciate the impact that Lemtrada has had on the life of an affected individual may like to view the following video:
https://www.youtube.com/watch?v=vQi_834OeQQ

Translational Research Discovery and Basic Mechanistic Science in Rare Cancers

Bass Hassan

Introduction

Whatever we do in science, the basic principles remain the same. Firstly, collection of a detailed set of observations will, one hopes, lead to an interesting discovery. These observations are secondarily used to establish hypothesis, requiring experiments designed to elucidate nature's secret mechanism. We are all aware of the traditional sources of observational discovery in life sciences, such as genetic observations (early lethality) and phenotypic screens (e.g. fly wings) often in wild-type systems. There has, however, been a significant shift in the last decade in terms of the range of sources of biological and pathological 'observations' available to us to draw upon. For example, the exciting enabling genomic scale technologies, such as single cell DNA sequencing, have amplified the depth and breadth of data relating to organism biology and in particular in relation to pathological disease processes in humans. The volume of data arising from such human samples in cancer has rapidly become overwhelming, yet it remains observational. For some reason, observational data derived from human material is also sometimes termed 'translational'. In fact, the definition of translational is now becoming so broad-ranging and mainstream,

extending from basic molecular mechanisms, genetic models, chemical modification to clinical trials, that it is rapidly becoming a somewhat meaningless and redundant term.

I have always been completely integrated and have an agnostic view of observational data. I am constantly stimulated and inspired by the patients I look after through the often complex and prolonged cancer journey, and equally by developments across the sciences. There are fascinating clinical observations that stimulate important mechanistic questions about disease and excellent direct feedback that helps one moderate the potential impacts of the continual and often-hyped claims of basic and early translational science discovery (fake news).

In the last decade, I have been particularly interested and inspired by data from rare cancers, as they frequently display a number of unique pathway drivers and novel mechanisms. Detailed mechanistic understanding underpins drug development but also insights into basic mechanisms. The former remains much-needed and expected in these rare populations. Here, I summarize two recent examples from my group where we have evolved a fluid two-way activity between basic and discovery translational science in rare cancer.

Basic molecular mechanisms to translational development of IGF2-TRAP

Initially supported by Cancer Research UK (CRUK), the first focus has been the need for a selective inhibitor of the growth promoting ligand, Insulin-like growth factor 2. *IGF2* is an imprinted gene, and IGF2 ligand is implicated in both development and tumour growth. We know much about its imprinting regulation, how it activates signaling receptors and its role in cancer, but we have yet to develop a specific IGF2 inhibitor. The natural specific mechanism to limit IGF2 bioavailability, and IGF2 alone, is the Mannose 6-phosphate/IGF2 receptor (*IGF2R*), a non-signaling receptor that selectively binds extra-cellular IGF2 and internalizes it to deliver IGF2 to the lysosomal compartment for degradation. When this mechanism is saturated by excess IGF2, including pro-forms of IGF2 with impaired proteolytic processing (Big-IGF2), IGF2 can activate the constitutive cell surface signaling receptors, Insulin Receptor Isoform A (IR-A) and the IGF1 receptor. Excess IGF2 and its over-supply can occur frequently in cancers, for example due either to loss of imprinting, 11p15 amplification or loss of function of *IGF2R*. When IGF2 levels are very high because of tumours with a large volume, IGF2 can activate systemic IR-A, leading to hypoglycaemia (the IGF2 syndrome) (Figure 1). IGF2 is also experimentally implicated in benign conditions, such as uterine fibroids, and vascular, metabolic and inflammatory conditions.

Attempts at blocking IGF2 have been non-selective, resulting in the additional blockade of IGF1/IGF1R. The consequences of the latter, result in a growth hormone mediated negative feedback resulting in IGF1 production from the liver. Antibodies and small molecules have not been successful as single agents in cancer trials, mainly because of IR-A and the central feedback effects, although there are rare and dramatic responses to IGF1R inhibition in Ewing sarcoma[1] and thyroid associated ophthalmopathy[2]. Given that IGF2 is the key ligand target of IGF signaling pathway activation in most malignant pathological conditions, and blocking its function remains an unmet need, I have continued to actively promote development of IGF2-TRAP.

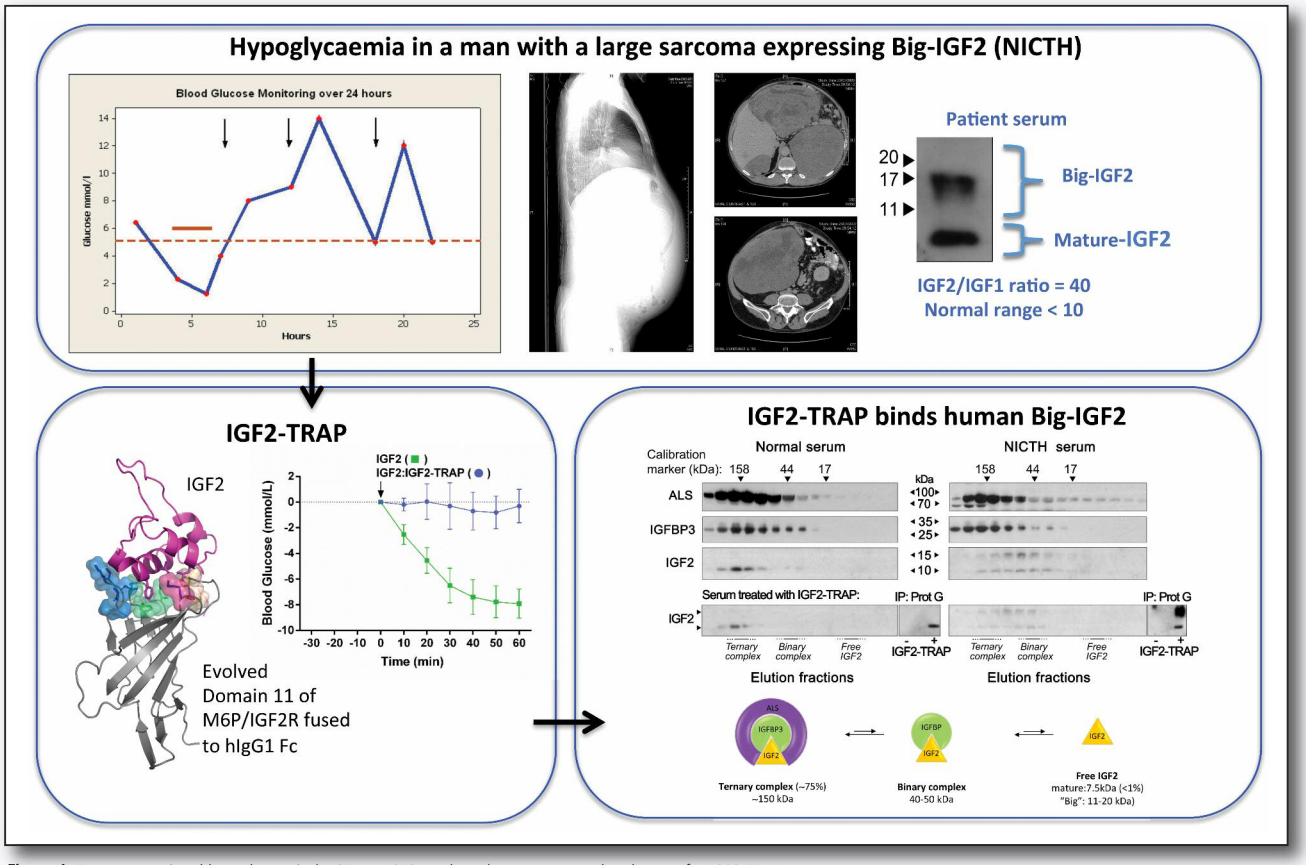


Figure 1. *Tumour associated hypoglycaemia (NICTH or IGF2 syndrome): an unmet need and target for IGF2-TRAP.*

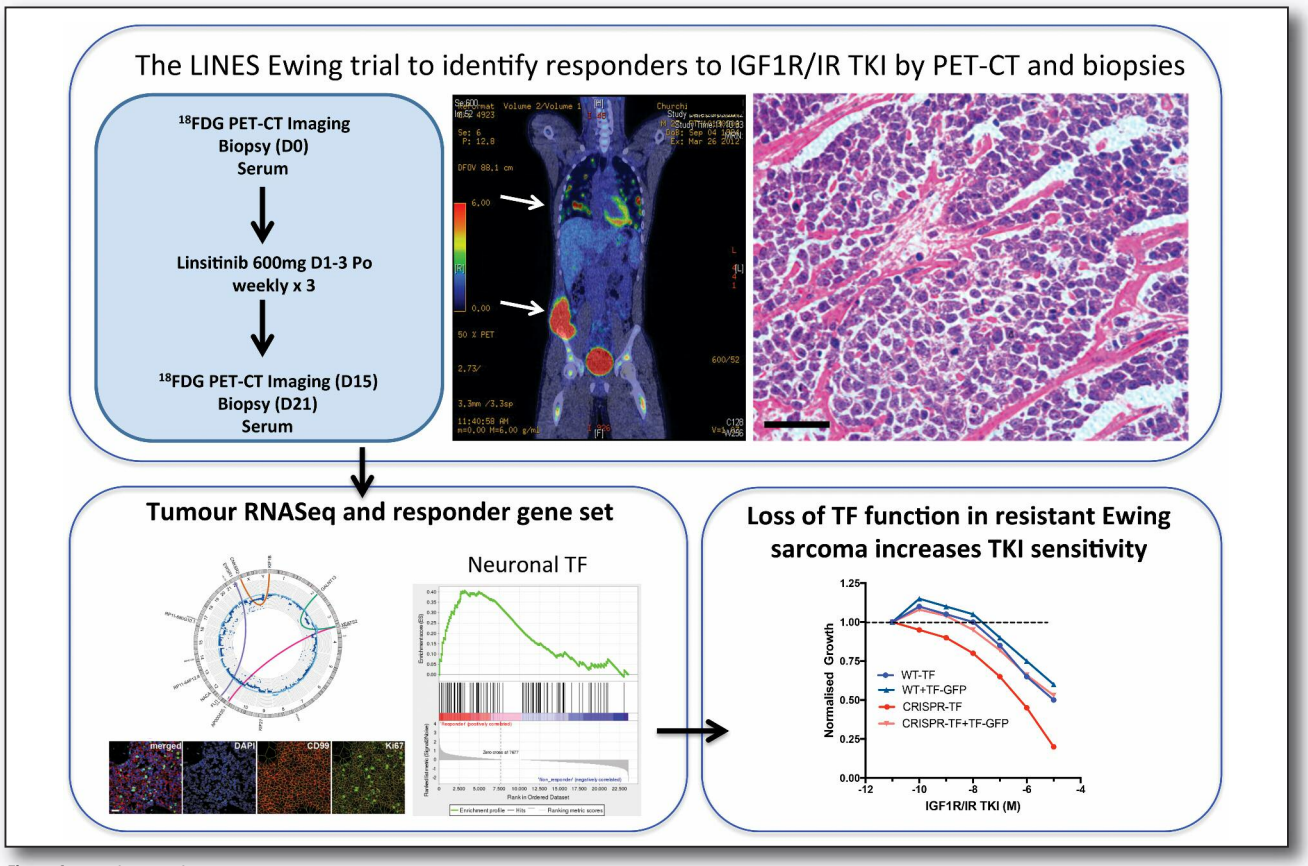


Figure 2. *Identification of a sensitivity mechanism in responders to an IGF1/IR small molecule kinase inhibitor in Ewing Sarcoma.*

The basic experimental data that underpinned the idea of utilizing soluble domain 11 of the M6P/IGF2 receptor as a natural ligand trap was developed when I was a CRUK Senior Clinical Research fellow (1997-2002). The main emphasis of our early CRUK funded work was to understand the structural basis and evolution of the interaction between IGF2 and domain 11, in collaboration with Yvonne Jones (STRUBI-Oxford) and Matt Crump (Chemistry, Bristol). We also extended our knowledge of the functional basis in mouse genetic and cell models. Having identified the key interacting loops of IGF2 with domain 11, and mutated amino acids (forming the basis of a University of Oxford worldwide patent), we performed directed evolution of the interaction using a surface plasmon resonance screen, and identified amino acids that enhanced affinity though 'off' rate modification[3]. We have now expressed these soluble domain 11s fused to human IgG1-Fc, in an Oxford MRC CiC collaboration with UCB Pharma, led by David Humphreys and Richard Taylor, and Matt Crump in Bristol. Once the Fc-fusion proteins (two controls; domain 11 loss of IGF2 binding mutant and wild-type, and two gain of IGF2 affinity mutants, AB3 and AB3P/H) were expressed, along with an Fc-AB5RHH mutant, we established that IGF2-TRAP could reverse the effects of IGF2 induced hypoglycaemia in the mouse. We initiated collaboration with the University of Coimbra for PET labeling half-life and distribution studies in the mouse (led by Antero Abrunhosa). After short infusion and PET imaging, the IGF2 binding IGF2-TRAPs localized to tumour xenografts that expressed human IGF2, indicating that the domain 11 optimization resulted in functional IGF2-TRAP *in vivo*. These pilot studies also identified well-known proteolytic cleavage sites in the linker between the IgG1-Fc and domain 11. Current half-life in the mouse is ~24 hours with evidence through mass spectrometry of a specific cleavage site in the linker. The cleavage site has now been deleted and mutated, and new constructs have been expressed by UCB, and these now require evaluation in order to optimize stability *in vivo*.

The plan is to apply to the MRC Developmental Pathway Funding Scheme (DPFS) with collaborators (UCB, Bristol, Coimbra) to complete linker optimization, which is a key milestone along with establishing, very early, whether the formulation, lyophilization and cGMP process can be achieved and performance is comparable to other antibody and Fc fusion proteins developed by pharma. I will also test functional IGF2-TRAP assays, including IGF2 pathway biomarkers and IGF2-TRAP pharmacokinetic assays in serum, and work with CROs to complete toxicology and cGMP production of IGF2-TRAP in a Phase I/IIa clinical trial. The Phase I/IIa IGF2-TRAP trial is entitled: 'IGF2-TRAP: first-in-man and treatment of IGF2-related non-islet-cell tumour associated hypoglycemia (NICTH), a phase I/IIa dose escalation trial with a proof of concept cohort'. This is a Phase I weekly i.v. dose escalation study in advanced stage cancer, using an acclerated 2 patient per toxicity level design to achieve MTD and optimal biological dose using QconCAT serum mass spectrometry of the (free) IGF2/IGF1-IGFBP3 ratio. For the Phase IIa expansion cohort, this will be entirely in patients with the IGF2 syndrome (NICTH), with primary endpoints being hypogylcaemic events, symptoms and 24 hour duration of hypogylcaemia using continuous real-time glucose monitoring with approved biosensors. The trial will be developed in collaboration of Oxford Unviversity with the European Organisation for the Research and Treatment of Cancer (EORTC), one of the largest academic CROs for cancer clinical trials. Future Phase II efficacy clinical trials,

for which we anticipate non-DPFS clinical trial funding, would be Phase 0 PET-CT localisation linked to theranostics and drug combination in advanced cancer Phase II, and IGF2-TRAP Phase II/ in non-malignant conditions.

Translational discovery and basic molecular mechanisms of Ewing Sarcoma

Although rapid advances in our understanding of rare Ewing sarcoma have been translated towards the treatment of the teenage patients that develop the disease, there have been no dramatic improvements in outcomes such as survival. Learning from these important examples, I have just completed a more in-depth clinical trial in Ewing sarcoma, going beyond what has been performed so far in terms of collecting material for translational discovery. The LINES trial (Figure 2) was of a small molecular treatment that targets the IGF1R/IR receptor kinase pathway and involved 16 patients in the UK and Europe, with the majority treated by my team in the early phase trials Unit of the Oxford Cancer Centre, and supported by EuroSarc EU-FP7 and the Oxford BRC. We know from previous trials of antibody therapy towards the same target that it can rarely lead to exquisite and durable responses in patients with treatment-resistant Ewing sarcoma, in some cases lasting years. The molecular basis of this observation remains a mystery. I aimed to discover the interacting mechanisms that might account for these responses to pathway blockade, by analyzing Ewing sarcoma patient-derived biopsy material from the trial. From these detailed investigations of the rare responders, the extremes of phenotype, we observed that some patients responded, as determined by PET-CT scans. When David Barnes in the lab completed RNASeq analysis of the small biopsies before and after exposure to the drug, we discovered a gene signature associated with a regulator of neuronal differentiation. We then immediately tested this observation using CRISPR/Cas9 targeting of the candidate regulatory transcription factor in a panel of Ewing sarcoma cell lines, and confirmed that depletion of the transcription factor enhanced the sensitivity to the small molecule drug. These data meant that the drug might work best in the non-responding patients if we could simultaneously block the effects of transcriptional and epigenetic regulation, and the drug pathway at the same time (ie synergistic lethality). We have now identified a candidate drug that does this, and we are testing the synergistic function with the view to validating the mechanism prior to a LINES2 trial that combines both agents. One aspect that these results also uncovered was the lack of a basic understanding of the structure and function of transcriptional regulation. I have recently initiated a basic science collaboration with Susan Lea, Oreste Acuto and the Structural Genomics Consortia to develop the structural basis of protein function. This cross-disciplinary approach, often termed 'from bench to bedside and back', will provide a more definitive personalized approach to developing combined therapies that have the potential of getting us closer to a cure for Ewing sarcoma.

Future work

As a result of these experiences, and with EIT health innovation funding, I am setting up one the largest international trials in Rare Cancers, where the design acts as a large-scale molecular discovery program for response to a series of sequential agents. We will systematically investigate the rare responders and controls, across the 200 rare cancer sub-types, which will be evaluated in depth for insights into the mechanistic basis of responsiveness. This trial is called RareCan1, and will be a further collaboration between Oxford and the EORTC. In the meantime, a number of basic science projects continue to inform new ideas for cancer interventions.

References

1. Olmos D *et al*, (2010) *Lancet Oncology* **11**:129-135
2. Smith TJ *et al*, (2017) *N Eng J Med* **376**:1748-1761
3. Frago S *et al*, (2016) *Proc Natl Acad Sci USA* **113**:E2766-E2775

Fixing the Vaccine

Quentin Sattentau

Vaccines are one of the greatest medical interventions, estimated to have saved many millions of lives. Most traditional vaccines can broadly be divided into inactivated, or infectious but weakened (live-attenuated) types. Inactivated vaccines may comprise an infectious agent, or a component of it, treated with a cross-linking chemical that is usually an aldehyde such as formaldehyde. Although some inactivated vaccines have recently been replaced by genetically engineered substitutes, many formaldehyde-inactivated vaccines are in use today. Thus, millions of doses of trivalent inactivated influenza virus, tetanus toxoid and Salk poliovirus vaccines are still administered each year. How does aldehyde inactivation work? Aldehydes contain one or more reactive groups that covalently crosslink proteins, rigidifying component molecules and preventing them from functioning. Cross-linking also leads to overall molecular stabilization and so aldehyde-inactivated vaccines are more stable than their untreated counterparts, which may be useful for vaccine storage and antigenic stability when formulated with adjuvants.

A Vaccine for HIV-1?

The human immunodeficiency virus type-1 (HIV-1) is a retrovirus that causes Acquired Immune Deficiency Syndrome (AIDS), and is responsible for approximately 40 million deaths and 37 million infections worldwide. Although highly effective antiviral therapy is available that has reduced HIV-1 infection to a chronic but treatable disease, it is only available to about 50% of those in need, and does not eliminate an individual's infection. Thus, a vaccine is considered essential as a means to reduce and potentially eliminate the HIV-1 pandemic. Despite 30 years of effort, a prophylactic vaccine remains elusive, mainly because of the virus' highly evolved immune evasion mechanisms. The most promising approach to developing a preventative vaccine is by the induction of neutralizing

antibodies that block virus infection. Neutralizing antibodies target a surface molecule on HIV-1 called the envelope glycoprotein (Env) but Env has evolved a particular neutralizing antibody evasion mechanism based on molecular instability. The Env molecule is composed of a trimer of non-covalently-linked heterodimers, each made up of the surface glycoprotein gp120 and the transmembrane glycoprotein gp41. The gp120-gp41 protomers oscillate naturally between different conformations, resulting in what is described as 'open' and 'closed' forms of the trimer. Since most HIV-1 neutralizing antibodies bind to the 'closed' form of the trimer across molecular surfaces that are conformationally sensitive, this instability is a potent neutralizing antibody evasion strategy. Thus for Env-specific B cells to successfully engage the Env molecule, clonally expand, and undergo affinity maturation to secrete neutralizing antibodies, Env must be conformationally stabilized.

Chemical Cross-linking of HIV-1 Env

Since cross-linking technology has been successfully applied to inactivation and stabilization of many safe and effective vaccines, we applied this approach to HIV-1 Env. We expressed soluble recombinant forms of Env and treated them with glutaraldehyde (GLA), a common dialdehyde that has a more efficient mode of cross-linking than formaldehyde, or EDC, an agent that cross-links primary amine to carboxyl groups. Both types of soluble Env cross-linking resulted in a highly stable molecule that remained trimeric even after reducing denaturing polyacrylamide gel electrophoresis. Cross-linked Env generally bound neutralizing antibodies equivalently to its unmodified counterpart, and had a much-reduced capacity to engage non-neutralizing antibodies that bind to epitopes exposed on the 'open' trimer. We used single particle cryo-electron microscopy to image GLA cross-linked Env in complex with a neutralizing antibody Fab fragment[1], allowing us to view Env at 4.2Å. Comparing the structure of the cross-linked with the unmodified Env allowed us to establish that the differences observed between the GLA modified and unmodified Env were surprisingly subtle, with an RMSD between the trimers of only 1.5Å. Indeed, the only obvious differences were the cross-links themselves, which presented as extra density between lysine (K) and arginine (R) side chains (Figure 1). Cross-links were observed at the trimer apex and the gp120-gp41 interface, both sites of major conformational instability in the unmodified trimer[1]. This is the first time that such cross-links have been observed in a vaccine antigen, and this information will inform future vaccine design allowing us to move from an entirely empirical approach towards more rational structure-based vaccine design strategies.

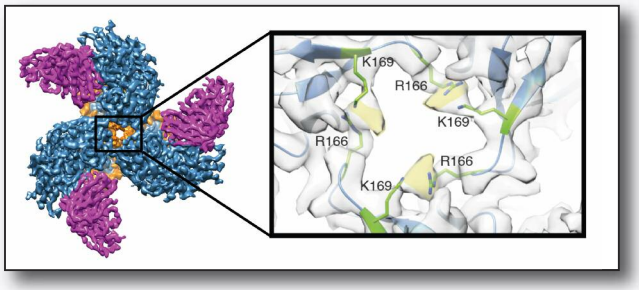


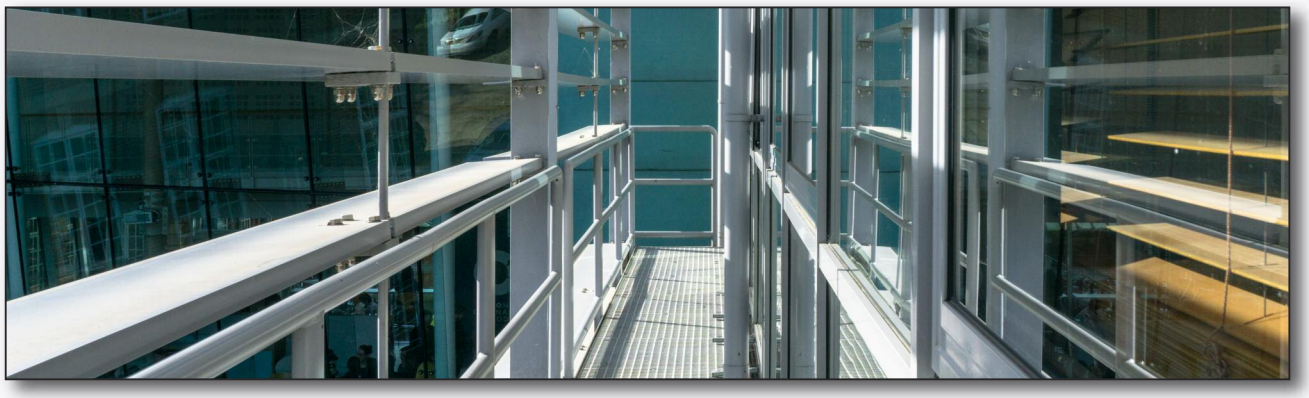
Figure 1. HIV-1 Env cross-linked with GLA and imaged using cryo-electron ***microscopy.*** *The left panel shows a top view of HIV-1 Env with the three gp120 Env subunits (blue) associated with the three gp41 Env subunits (orange), and three bound Fab fragments (lilac). The right panel shows the boxed region magnified to reveal some of the cross-links made by GLA within Env. The cross-links are shown in yellow, and link lysine (K) and arginine (R) amino acid side chains between gp120 subunits at the trimer apex, stabilizing the entire Env trimer.*

Cross-linked Env as an Experimental Vaccine

When used as an experimental vaccine immunogen formulated with a clinically approved adjuvant, cross-linked Env elicited antibodies with broader HIV-1 neutralizing activity than those elicited by its unmodified counterpart[1]. Similar results have more recently been obtained using EDC cross-linked Env, which is even more stable than GLA cross-linked Env and easier to purify using a neutralizing antibody column. As a result of this, EDC cross-linked soluble Env is currently being produced under Good Manufacturing Practice (GMP) and has entered toxicity analysis with the aim of going into a first-in-man experimental medicine clinical trial next year. GMP production, toxicity analysis and the ensuing clinical trial will all be carried out within the Horizon 2020 funded consortium, The European AIDS Vaccine Initiative EAVI2020 (http://www.eavi2020.eu/). This consortium is led by Professor Robin Shattock at Imperial College London and Oxford University is a major participant. Cross-linked Env may be used as a single vaccine antigen, or as part of what is termed a 'heterologous prime-boost' approach, in which combinations of different but related antigens are administered so as to guide rare B cells towards proliferating and producing high-affinity neutralizing antibodies to conserved Env epitopes. Ongoing challenges include raising the titres of neutralizing antibodies elicited by immunization and extending their duration. Future plans to address this include improving cross-linked Env immunogenicity by coupling the trimers to particles, which, we anticipate, will improve B cell activation efficiency. Furthermore, we plan to enhance T cell help by providing additional T cell antigens within the particles.

Reference

1. Schiffner T *et al*, (2018) *PLoS Pathogens* **14**:e1006986



Photograph Tatjana Terentjeva

Dunn School Bioimaging Facility Image Awards 2017

Scientists at the Dunn School spend hours each year using the state-of-the-art microscopes available at the Departmental Facilities, the Central Oxford Structural and Molecular Imaging Centre (COSMIC) and Micron Oxford. Once a year, the Dunn School Bioimaging Facility managers, Dr Errin Johnson (Electron Microscopy) and Dr Alan Wainman (Light Microscopy), give users the opportunity to submit their best images for a chance to win one of the highly-coveted gold microscope awards. The 2017 Bioimaging Facility Image Awards were handed out during the Departmental Symposium on 8th January 2018 and were generously supported by the following sponsoring companies: Thermo Scientific, Photometrics, Gatan, Leica Microsystems, Olympus, Zeiss and Microscope Services Ltd. A judging panel formed by Peter Cook, David Greaves and Melissa Wright chose some stunning images from the following winners, amongst over 30 entries.

Light microscopy

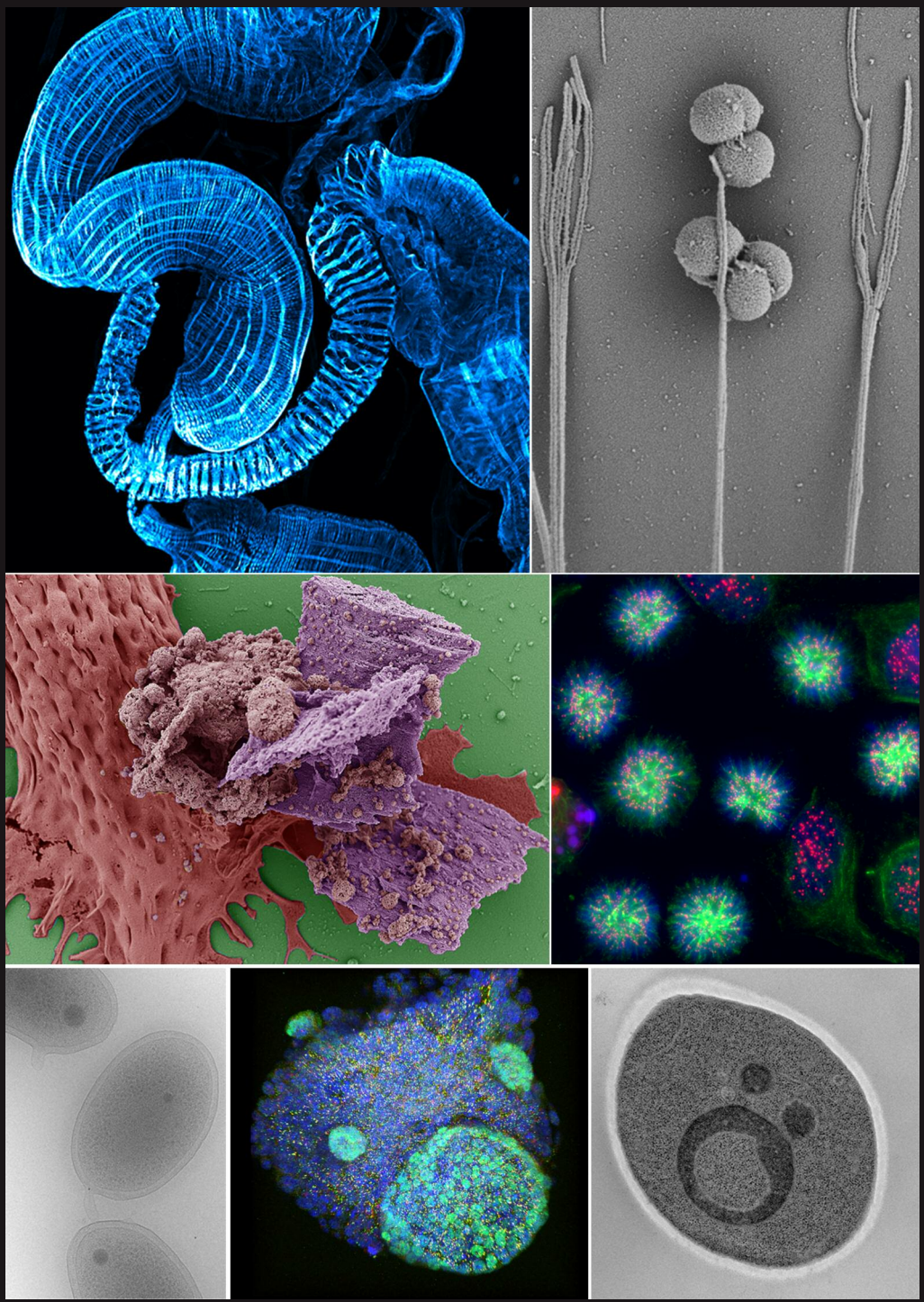
Winner: Sonia Muliyil and Clemence Levet (top left): 'Gut Instinct'. Muscle surrounding the *Drosophila* larva gut stained with phalloidin. Imaged on the Zeiss 880 Airyscan.
Runner-up: James Bancroft (middle right): 'Cellular Fireworks'. HeLa cells treated with kinesin inhibitor monastrol stained with tubulin (green), DNA (blue) and expressing GFP-CENP-A (red) to mark the kinetochores. Wide-field fluorescence image.

Electron microscopy

Winner: Rafael Da Silva Custodio (top right): 'Livin' on the edge'. Image showing a small group of *N. cinerea* "climbing" a filopodia tip during the colonisation of human epithelial cells. Acquired on Zeiss Sigma 300 FEG-SEM.
Runner-up: Kenny Moore (middle left): 'MacroGout'. Induced pluripotent stem cell-derived macrophages (red) treated with deoxyguanosine produce crystals of uric acid (the cause of Gout) as a by-product of purine catabolism (purple), which protrude from the cell alongside cellular debris (brown). Acquired on the Zeiss Sigma 300 FEG-SEM.

Humorous Microscopy

A third category that is gaining popularity each year, is the humorous microscopy – images featuring faces or other curiosities – this year won by Ita Costello (bottom centre) with 'This little piggy'; a 3D projection of a day 6 embryoid body, carrying a Blimp1-venus transgene (green) and counterstained with DAPI (blue) and captured on the Olympus FV1000. The runner-up was Alexis Wang (bottom right) with an 'Emoji yeast', an *S.cerevisiae* cell, surprised by our intrusion of privacy, taken on the Tecnai 12 TEM. An honourable mention goes to Nina Sulkowski for the cryo-EM image of *Asticcacaulis* cells (bottom left), imaged on the Talos Arctica TEM.



TECHNOLOGY FEATURE

Imaging Cytometry

Michal Maj

Flow Cytometry is a powerful analytical technique that allows the measurement of large numbers of cells (approximately 10,000 per second) and the analysis of information on fluorescence and scattered light for every single one of them. A single-cell suspension is processed through the machine sample line and a special quartz cuvette, known as the flow cell. Just before the flow cell, cells are forced into single-file, thanks to the phenomenon of hydrodynamic focusing. Each cell, while passing through the flow cell, interacts with one or more laser beams. As this occurs, it scatters the light of the laser: the characteristics of that scattering depend on shape, size, light refraction index and internal complexity/granularity of each cell. Scattered light alone can give us some information on morphological homogeneity of cells (it is possible, for instance, to differentiate lymphocytes from granulocytes). However, when combined with fluorescence information (for example fluorochromes conjugated with antibodies specific for particular cell-surface or intracellular antigens), we can obtain quite deep phenotype profiles of subpopulations of cells, allowing us to recognise and quantify different subtypes of cells (we can quantify how many T or B lymphocytes there are in the blood sample for example).

The development of this technology is impressive; increasing numbers of available lasers and detectors enable researchers to visualise more antigens at the same time and, with hardware development, more and more fluorochromes have become available. However, the principles by which conventional cytometers work have changed very little over the years. Detection systems always consist of a light path for collected fluorescence (a fibre or in open air), an optical filter (to narrow down fluorescent spectra to particular emission range) and the detector (either a photon multiplier tube (PMT) or an avalanche photo diode (APD) that collects photons emitted at particular energy level). This detection system provides a very useful and clear answer to the question of whether there is fluorescence of particular wavelength on the detected event of interest. What it doesn't answer is where the fluorescence is localised and whether it comes from the actual cell or from fragments of membrane or cellular debris associated with the cell. There is another piece of technology at our finger tips that answers these very questions – the imaging cytometer. Up to the flow cell it works exactly as a regular flow cytometer, but the detection system is different (Figure 1). Optical information from each cell, instead of landing on either a PMT or ADP, goes through a microscopic lens (of 20, 40 or 60x magnification). The detector device itself is different as well; rather than using PMTs nor APDs, CCD cameras are used instead. This allows us to obtain microscopic images of every single cell and the fluorescence intensity in different channels at the same time. With these images, we can not only quantify a fluorescence signal but localise it either on or inside the cell. Dedicated analytical software is available that can be used for multi-parametric analysis of every single obtained image.

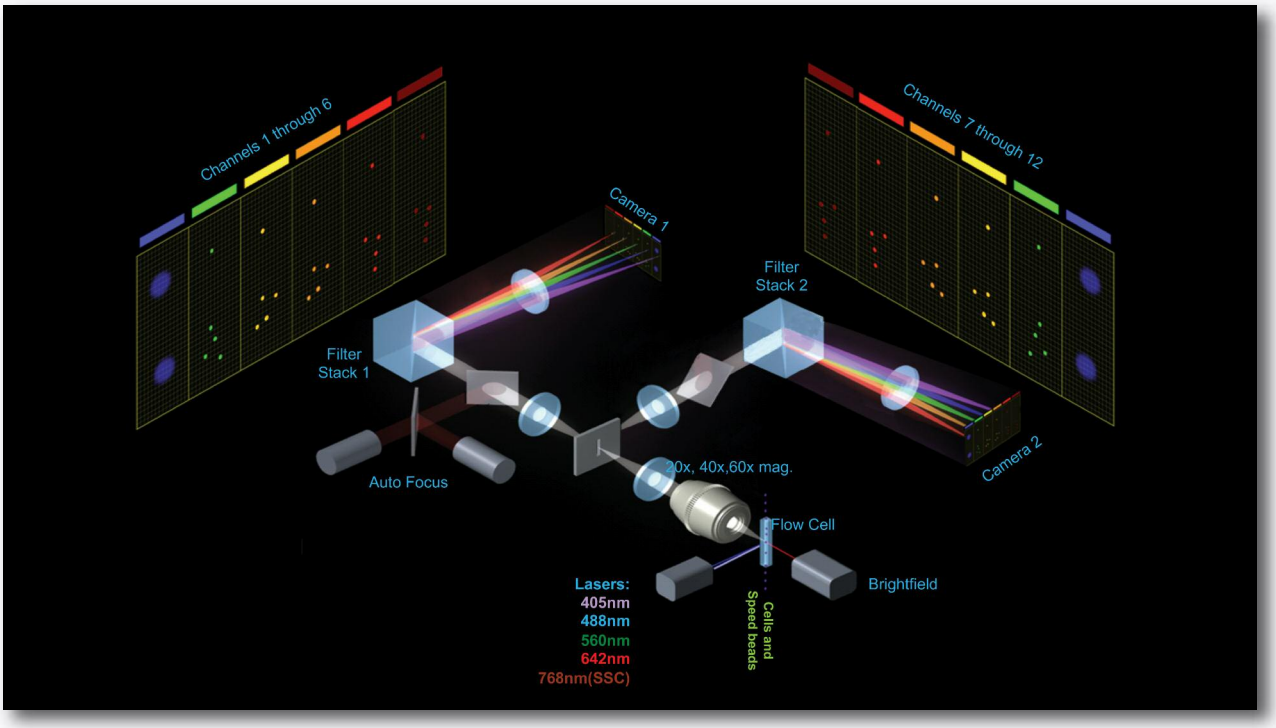


Figure 1. *Arrangement of components of the imaging cytometer.*

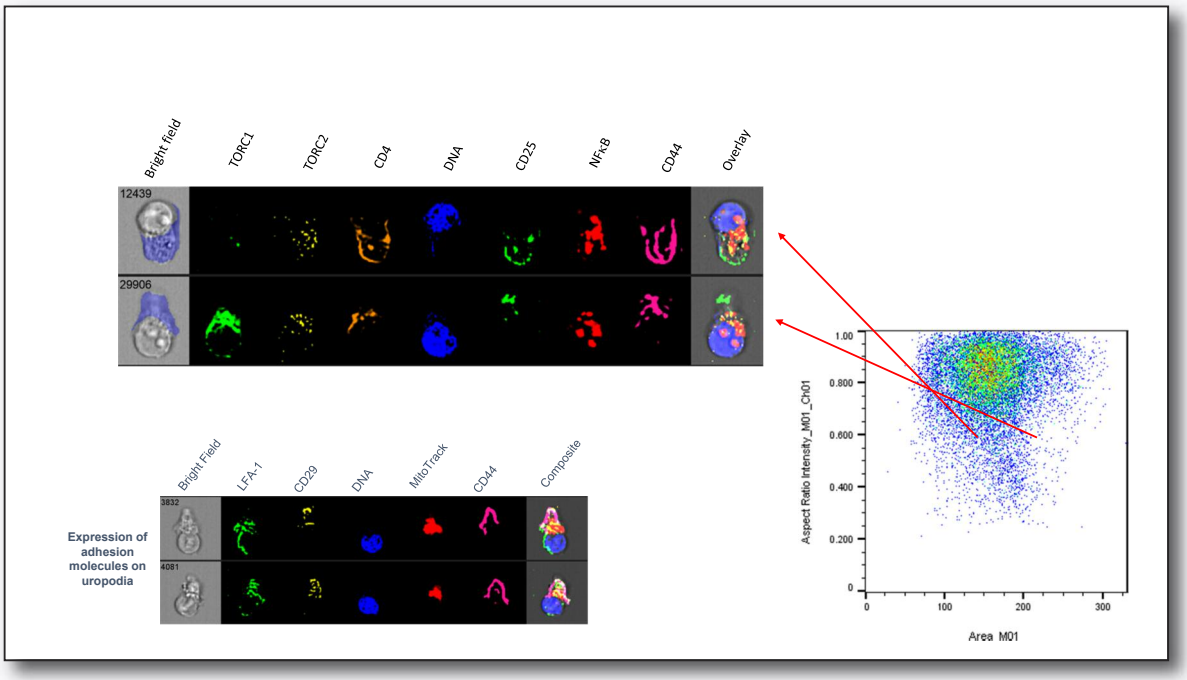


Figure 2. *Use of imaging cytometry to determine the distribution of intracellular and cell surface markers on T cells and their uropodia. Data courtesy of Stephen Cobbold.*

Thanks to high-throughput imaging there are many applications for this particular equipment:

- Co-localisation of fluorescence (for example the trafficking of a marked drug to the lysosomes)
- Internalisation, such as phagocytosis of labelled particles, bacteria or viruses
- Translocation, for example NFκB nuclear translocation in T cells while in contact with antigen presenting cells
- Spot count: quantifying the number of internalised fluorescent particles (beads, extracellular vesicles) by particular subsets of cells
- Morphology analysis: on top of the fluorescence profile of the cell we can search for particular shape properties (for example uropodia structures). This is a great advancement over regular flow cytometer data. Due to much more sophisticated morphology analysis at the population level, researchers can now obtain fluorescent data overlapped with particular structural properties of the cell.

The novel approach to the study of T cells proposed by Stephen Cobbold in the department suggests that the theory of asymmetric T cell division is not correct and that the fate of a dividing T cell is decided before the proliferation process begins. This controversial finding was established thanks to the Imaging Cytometry technique.

Further exploration of this system might open another opportunity, namely label-free phenotyping. Based on machine-learning processes (deep learning), we can now expose computer programs to thousands of images of interest, all presenting a specific experimental feature. Software will run through all of the images and assess any possible combination of recorded features, such as saturation of pixels on the CCD camera, displacement of these pixels, the gradient of saturation between neighbouring pixels and many more. With this information, computers may be able to create their own way of assessing cells and looking for the required feature in multidimensional arrays that are off-limits for conventional human understanding. Engineers have already implemented a simpler protocol for face recognition in smartphone camera systems for example. If we were able to achieve this, we may have the opportunity to quantify different subpopulations of cells and expression levels of different antigens just by looking at the bright-field image from the machine, potentially saving the cost of significant amounts of reagents.

Further reading:

Cobbold SP *et al*, (2018) *Frontiers Immunol* 9:13-81 doi:10.3389/fimmu.2018.01381

Doan M *et al*, (2018) *Trends Biotechnol* **36**:649 – 652

Blasi T *et al*, (2016) *Nat Communications* **7**:10256 doi:10.1038/ncomms10256.

Photograph Paul Fairchild

Laura Hankins, a graduate student in Jordon Raff's laboratory, was recently awarded the runner-up prize in the British Society for Developmental Biology writing competition for her essay describing the seminal experiments that most inspired her to follow a career in development biology. Here we reproduce her winning essay with kind permission of the Society.

Painting the Embryo by Numbers: How Nature Provided the Tools for an Inspirational Experiment

Laura Hankins

Visit a local pond and lie flat on your stomach, allowing the soft mud to seep into your clothes. Be sure to bring a jam jar; it will sparkle in the lazy spring sunlight as you shift it closer to the water's edge. Wait patiently, observing any disturbances to the dappled surface. There! The flat tail of a newt in the breeding season…

Photograph Tim Davies

Pleurodelinae is an unassuming collection of newt species within the Salamander family. As a child, sitting by the pond in our front garden, I was often charmed by the sedate movements of common newts contrasting with the constant hum of traffic whipping past. I think I probably wanted to hunt for new species in undiscovered rainforests, as this seemed a reasonable career move at the time. Little did I know that the humble newt would reappear in a university lecture, starring in an experiment that inspired me to pursue Cell and Developmental Biology.

It is 1924. In Hans Spemann's laboratory, our friends the newts have been the subject of a series of experiments performed by Hilde Mangold as part of her doctoral studies. Spemann was no stranger to amphibians; his work on eye development had made good use of frogs. Now he had turned his attention to how broader embryonic regions are defined.

During gastrulation, the embryo folds in on itself to produce three distinct layers that will ultimately have different fates. This produces the blastopore, an opening that acts like an insatiable mouth as the embryo consumes itself. Spemann, amongst others, had observed that transplanting tissue from the blastopore lip into another embryo resulted in the formation of a second neural tube and its surrounding structures. Many assumed that these features arose exclusively from the donor cells, but Spemann and others hypothesised that these cells could be acting as an 'organiser', signaling to influence their neighbours' fates. But how to test this suggestion?

The breakthrough came with an idea that was beautiful in its simplicity. Mangold repeated the transplantation experiments but moved the tissue between *different* newt species. These newts had distinct pigmentations, so it would be possible to discern host from donor tissue after leaving the embryo to develop following surgery. In 1924, embryos left for sufficient time developed a chimeric conjoined twin with its own neural tube, notochord and somites. After sectioning, Mangold observed that these structures contained both pigmented and unpigmented cells. Remarkably, it seemed Spemann was right: the transplanted tissue had somehow altered the fate of the surrounding host cells, coopting them into forming an artificial twin.

This experiment is inspiring partly due to the minimalism of its approach; it demonstrates that the most influential experiments are designed without unnecessary embellishment. Yet its surgical element made it incredibly *technically* complicated. Thanks to their logical design, and the natural features of newts, Spemann and Mangold changed our perception of cell fate determination. Years later, researchers are still being inspired to use knowledge of the natural world to address questions at the cellular level.

Details of this year's winners of the BSDB writing competition can be found at the following link: http://bsdb.org/2018/04/17/bsdb-writing/

Scarlett Harris, a graduate student in Quentin Sattentau's laboratory, was this year's winner of the Peter Beaconsfield Prize in Physiological Sciences. The Prize rewards young researchers who are capable of escaping from the stereotype of narrow specialisation to engage with translational medicine, and display a wider grasp of the significance and potential applicability of their research. Here we reproduce the winning entry.

Sugar and Protein: A Dangerous Combination

Scarlett Harris

The reaction between a sugar and a free amine group can modify proteins by adding modifications known as advanced glycation end products (AGEs). AGEs are everywhere; they are formed endogenously, with higher levels found in various human diseases, and are also taken into the body through food. It is, therefore, critical that we understand the effects of AGEs on the body. Studies have shown that AGE-modified proteins induce stronger immune responses and I am investigating the mechanism behind this. This work combines both chemistry and immunology and could help us to understand the pathology of AGE-related diseases.

Sugar and Protein: A Dangerous Combination

AGEs are a complex group of non-enzymatic protein modifications formed primarily through the Maillard reaction (Figure 1)[1,2]. Studies in the 1970s and 1980s demonstrated that this reaction occurs *in vivo*. AGEs have since been implicated in the pathology of various diseases including diabetes, atherosclerosis, Alzheimer's disease and cancer[2–5]. AGEs can also enter the body through food. Around 10% of ingested AGEs are absorbed into the circulation and two-thirds of these will remain in the body[6]. Dry heat enhances AGE formation and AGE content is particularly high in fatty and highly processed foods[7]. AGEs are known to induce inflammation through binding to their receptor RAGE[1,4,6], demonstrating a role in innate immune responses. However, less is known about the effect of AGEs on the adaptive immune system; various *in vitro* and *in vivo* studies have shown that AGE-modified proteins induce stronger adaptive immune responses compared to unmodified[8–12], but the mechanism behind this is unclear. I am attempting to determine this mechanism by examining the effects of AGE-modified proteins on dendritic cells (DCs), the immune cell type responsible for initiating adaptive immune responses[13]. Specifically, I'm looking at the role of AGEs in peanut allergy; dry roasting peanuts is reported to add AGEs to peanut proteins[14–16]. Epidemiological data suggest that dry roasted peanuts are more likely to cause allergies and work from our group demonstrated that dry roasted peanuts induced stronger immune responses than raw in three different mouse sensitisation models[9,17]. Peanut allergy is a major medical problem; it tends to be more severe than other allergies, causing the highest number of fatal food allergic reactions, and unlike many other food allergies is not commonly outgrown[18]. It is, therefore, critical that we understand how this disease develops so that we can develop ways to prevent it.

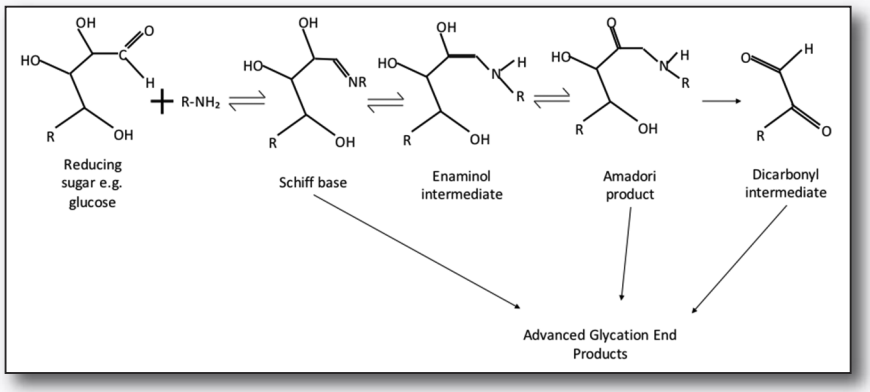


Figure 1. The Maillard reaction[2,14]. The process starts with a condensation reaction between an amine group, most commonly in a lysine side chain, and a reducing sugar[16]. This forms a Schiff base (a carbon-nitrogen double bond in which the nitrogen is not bonded to hydrogen[1]), which is unstable and will rearrange to an Amadori product[2]. The Amadori product can then break down to form intermediate products such as α-dicarbonyls[2]. The α-dicarbonyls are key intermediates which can also react with free amine groups on proteins and are 20,000-fold more reactive with respect to glycation than the original sugar[6]. The end result is a complex set of modifications known as AGEs. These include adducts such as carboxymethyl-lysine (CML) which is frequently used as a marker of AGE modification[6]. Reactive carbonyl groups are also added during this reaction[5,20].

AGEs comprise a huge group of different adducts; each one and different combinations could potentially have vastly different effects on the immune system. Therefore, we need to analyse the extent and type of modification. There are various biochemical tests available for this; for example, the reactive carbonyl content in a sample can be measured using different hydrazine based probes[19]. For other adducts, such as carboxymethyl-lysine (CML), there are antibodies available for detection[20] (Figure 2). However, these tests are limited as they can only measure a single modification at a time and reagents are not available for the detection of every adduct. To more fully analyse the modifications, one would need to use mass spectrometry. This would give a more comprehensive overview of the adducts and could also be used to determine the position of the modified amino acids in a protein[21,22]. Combing the data from the chemical analysis with the immunological data may allow for identification of specific adducts which enhance the development of allergic immune responses. It may be possible to target these adducts or prevent their development, for example by changing food processing techniques.

Having started initial examination of the protein modifications, I then examined the effects of these proteins on DCs. Previous studies examining the effects of AGE-modified proteins on murine DCs have shown that classical markers of DC activation such as costimulatory molecules and pro-inflammatory cytokines are not induced by AGE-modified

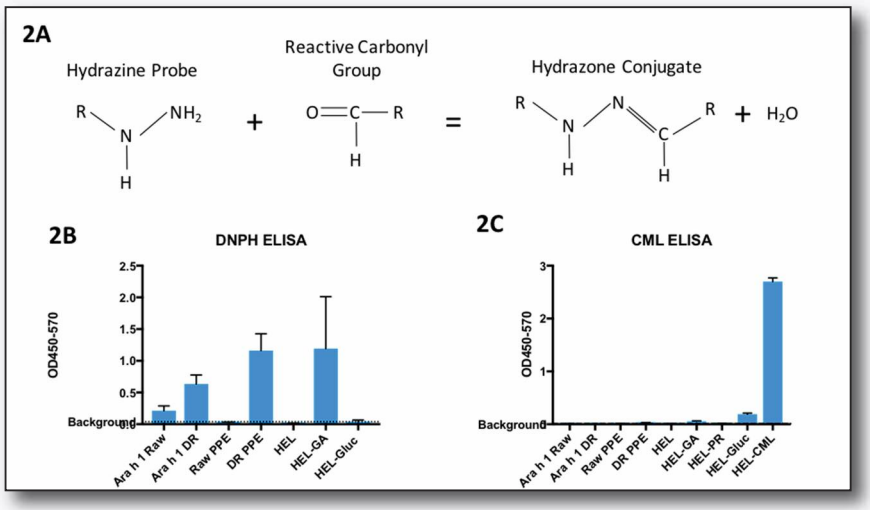


Figure 2. Detection of AGE modifications. (A) shows the chemical reaction between a hydrazine group and a free carbonyl. In (B) the hydrazine probe 2,4-Dinitrophenylhydrazine (DNPH) was used in an ELISA to measure carbonyl content in differently modified samples. Peanut protein extract (PPE) was taken from either raw or dry roasted (DR) peanuts and the peanut allergen Ara h 1 was isolated from this. The egg allergen Hen Egg Lysozyme (HEL) was modified using glucose or the dicarbonyl intermediate glycolaldehyde. The same samples were also assessed for CML content in (C). HEL was also modified with glycoxylic acid to add CML groups specifically.

proteins[9,10]. Therefore, I adopted an approach where I started with large screens examining changes in murine DC biology and then narrowed down my studies, the ultimate aim being to find a few factors which could serve as markers of AGE-induced DC activation. These screens have included RNAseq, which involved collaborating with a team from the Institute of Cancer Research who provided technical and bioinformatics expertise. This was an entirely new field for me and since then I have been developing my own bioinformatics skills with the aim of applying them to future work.

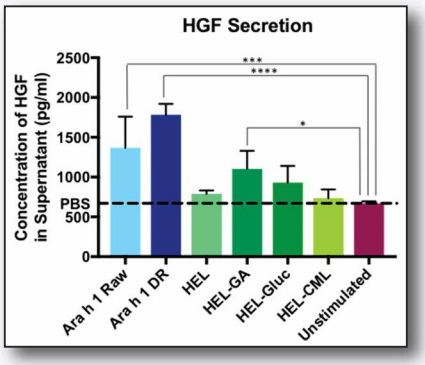


Figure 3. *Murine DCs were pulsed with the samples from Fig 2. After 18 hours the supernatant was collected and the secretion of various cytokine and chemokines was assessed by luminex. The graph shows the results for HGF. The data was analysed using one-way ANOVA, *p≤0.05, ***p≤0.0005 ****p≤0.0001, n=4.*

I have also examined changes to DC secretion of cytokines and chemokines in response to modified proteins. One of the cytokines of particular interest was hepatocyte growth factor (HGF); this factor promotes cell survival, organ regeneration and cell motility[23]. It is linked to several types of cancer where it has been reported to induce cancer cell migration and invasion[24]. HGF is also reported to be critical for DC migration from the skin, with blockade of its receptor, cMET, preventing the development of contact hypersensitivity reactions[25]. This suggests that DC migratory properties may be affected by AGE-modified proteins. This is supported by the RNAseq findings as several of the genes significantly upregulated in response to modified proteins are also involved in cell migration. Based on the protein modification tests, it is unlikely that RAGE signalling is responsible for HGF secretion as HGF is not secreted in response to protein modified specifically to add CML, a modification known to be a RAGE ligand[26]. It is, therefore, possible that another receptor is involved in these responses. This finding highlights the need to combine data from several different disciplines to provide a complete picture.

Moving forward, a key part of future experiments will be to determine if the work in mice holds true for humans. If this is the case, then this study will provide an insight into the development of peanut and other allergies and may in turn allow for allergy prevention strategies to be developed. Moreover, as AGEs are linked to many other human diseases, understanding how the immune system detects and responds to them could provide some idea of how AGEs contribute to the pathology of other illnesses. I think this is particularly important as AGEs have been linked to some of the biggest killers in our society[2–4] which clearly demonstrates the clinical need for a greater understanding of the effects of these modifications on the human body. However, this requirement for understanding is not limited to the clinic as the presence of AGEs in highly processed foods[7], which are an increasingly large part of our diets, shows a need for the whole of society to understand the effect of AGEs on our health.

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HISTORICAL PERSPECTIVE

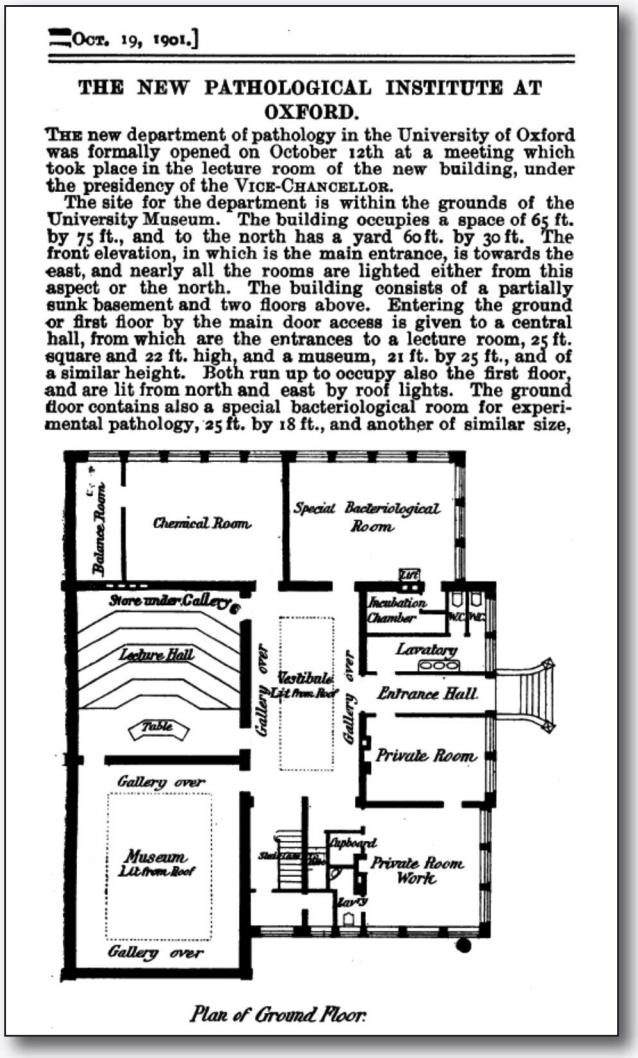
Pathology in Oxford before the Dunn School: Opening of the Dunn School Building in 1927

Eric Sidebottom

As the Dunn School last year passed its 90th anniversary, many scientists who work here do not imagine that pathology in Oxford existed before the founding of this august institution. But the historian in me loves to probe backwards and there are rich pickings…

Matthew Baillie who lived from 1761 to 1823 could be said to be Oxford's earliest pathologist. He was a sometime fellow of Balliol College and published *The Morbid Anatomy of Some of the Most Important Parts of the Human Body* in 1793, possibly England's earliest atlas of Pathology.

Although the first appointment of a 'pathologist' by the University was not made until March 1897 when James Ritchie was appointed to a University Lecturership in Pathology, there was already an established teaching course operating in the Regius Professor of Medicine's Department in the University Museum of Natural History. This was organized by the Professor of Physiology John Burdon Sanderson, ably assisted by James Ritchie. Sanderson, earlier in his career, in 1873, had been the founding Director of the first Research Pathology Laboratory in this country (the Brown Institute in Vauxhall, London) and it is intriguing to note that in his will he left money to the Pathology Department (a fund which still exists) but none to the Department of Physiology, where he was the first Professor.



OCT. 19, 1901.]

THE NEW PATHOLOGICAL INSTITUTE AT OXFORD.

THE new department of pathology in the University of Oxford was formally opened on October 12th at a meeting which took place in the lecture room of the new building, under the presidency of the VICE-CHANCELLOR.

The site for the department is within the grounds of the University Museum. The building occupies a space of 65 ft. by 75 ft., and to the north has a yard 60 ft. by 30 ft. The front elevation, in which is the main entrance, is towards the east, and nearly all the rooms are lighted either from this aspect or the north. The building consists of a partially sunk basement and two floors above. Entering the ground or first floor by the main door access is given to a central hall, from which are the entrances to a lecture room, 25 ft. square and 22 ft. high, and a museum, 21 ft. by 25 ft., and of a similar height. Both run up to occupy also the first floor, and are lit from north and east by roof lights. The ground floor contains also a special bacteriological room for experimental pathology, 25 ft. by 18 ft., and another of similar size,

Plan of Ground Floor.

Opening of the original Pathology Department announced in the BMJ

One of the earliest students to benefit from the pathology course was a rich Australian, Ewan Frazer, who, appalled by the cramped conditions for students, offered £5,000 towards a new laboratory of Pathology providing the university matched his gift. This was eventually agreed and the new laboratory, built behind the University Museum, opened on 12th October 1901, which, coincidentally, was the 80th birthday of Rudolph Virchow, probably the world's most famous pathologist at that time.

Robert Muir, the Professor of Pathology from Glasgow wrote about the new laboratory,

> *"It was carefully and ingeniously planned under Ritchie's direction and was a wonderful example of what could be got for money thoughtfully expended. Situated in quiet and beautiful surroundings and embowered in greenery, it was – I speak from experience (Muir spent a term working in the new department) - an ideal place for research work. Its freedom from dust was a feature that struck one who came from Glasgow".*

This laboratory, directed from 1901 to 1906 by James Ritchie and then until 1927 by Georges Dreyer (when University Pathology moved to the Sir William Dunn School of Pathology), established Pathology teaching and research as subjects central to medical education. The *British Medical Journal* (BMJ) of 19th October 1901 reported at length on the opening ceremony, those attending and on the facilities available for teaching and research in the new building. In 1927 when the Dunn School opened, this building was handed over to the University department of Pharmacology and when that department moved to a new building it became part of the University Chemistry 'empire'. It was eventually demolished to make way for the new 'Earth Sciences' building which opened in 2010.

The BMJ of 19th March 1927 also reported in detail the opening of The Sir William Dunn School of Pathology which had taken place on Friday 11th March. A large gathering of the 'great and the good' attended the ceremonial handing-over of the building, although it was ironic that neither the chancellor of the University, Viscount Cave nor the chairman of the Dunn Trustees, Sir Jeremiah Colman were able to attend due to illness. In their absence Mr Charles Seligman, for the trustees, handed over the building to the University vice-chancellor Dr Pember, Warden of All Souls College.

THE SIR WILLIAM DUNN SCHOOL OF PATHOLOGY
is a department of the University of Oxford
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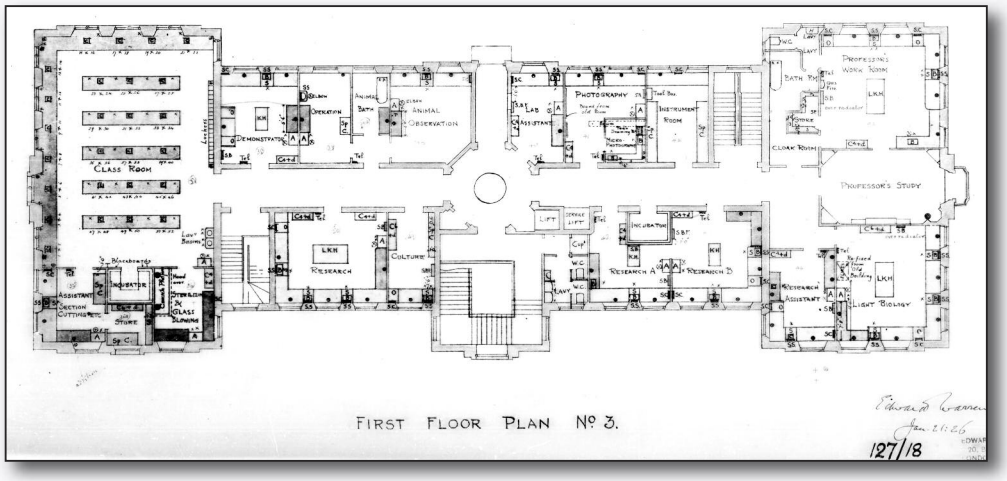
The building of the Dunn School was enabled by the decision of the Dunn Trustees taken in 1922 to award a grant of about £100,000 to Pathology in Oxford. They had earlier, in 1920, agreed to grant £210,000 to Biochemistry in Cambridge. These grants, the largest given by the Trust, were made on the advice of Walter Fletcher, Secretary of the Medical Research Council, and William Hardy, President of the Royal Society that the clause in Sir William's will "to alleviate human suffering" would be better met by supporting medical research than by supporting hospitals directly. It is probably relevant that Georges Dreyer, Professor of Pathology in Oxford, was a member of the Council of the MRC and was also a personal friend of Walter Fletcher.

In complete contrast to the text of the BMJ is an article in the *Architects Journal* published on 23rd March 1927 following the handover of the building on March 11th. The article concludes with the following statement: "Altogether Mr Warren (the architect) is to be congratulated on a notable contribution to the reviving cause of a national architecture. That Oxford should possess it is but one more manifestation of an intelligent conservatism". Moreover, in a further contrast, the MRC published a comprehensive monograph *System of Bacteriology* between 1928 and 1930 which complemented the Dunn School on being an excellent new laboratory for bacteriological work.

For any readers interested in Sir William Dunn, an article on his life and work by Eric Sidebottom and Alison Paul was published in The Journal of Medical Biography *in May 2005*



Original plans of the first floor of the Dunn School building

Making a gift to the Dunn School

The Dunn School owes its existence to a philanthropic gift, from the Trustees of Sir William Dunn, and over the years has been the beneficiary of many acts of philanthropy, not least from those who have worked here. Any gift made to the Dunn School helps to further research here, whether it is made to support a specific initiative such as the ones described in this newsletter, or at the discretion of the Head of Department.

If you would like to make a gift to the Department this year, please use the gift form enclosed with this edition of *Fusion*. Please make sure that you have completed a gift aid form so that we can reclaim tax on your gift, and note that if you are a higher rate tax-payer, you can also set your gift against your tax liability for the year. All gifts made to the Dunn School from the USA are also fully tax-deductible, when made through the University's 'giving vehicle' there, the Americans for Oxford, Inc organization.

Photograph Paul Fairchild

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