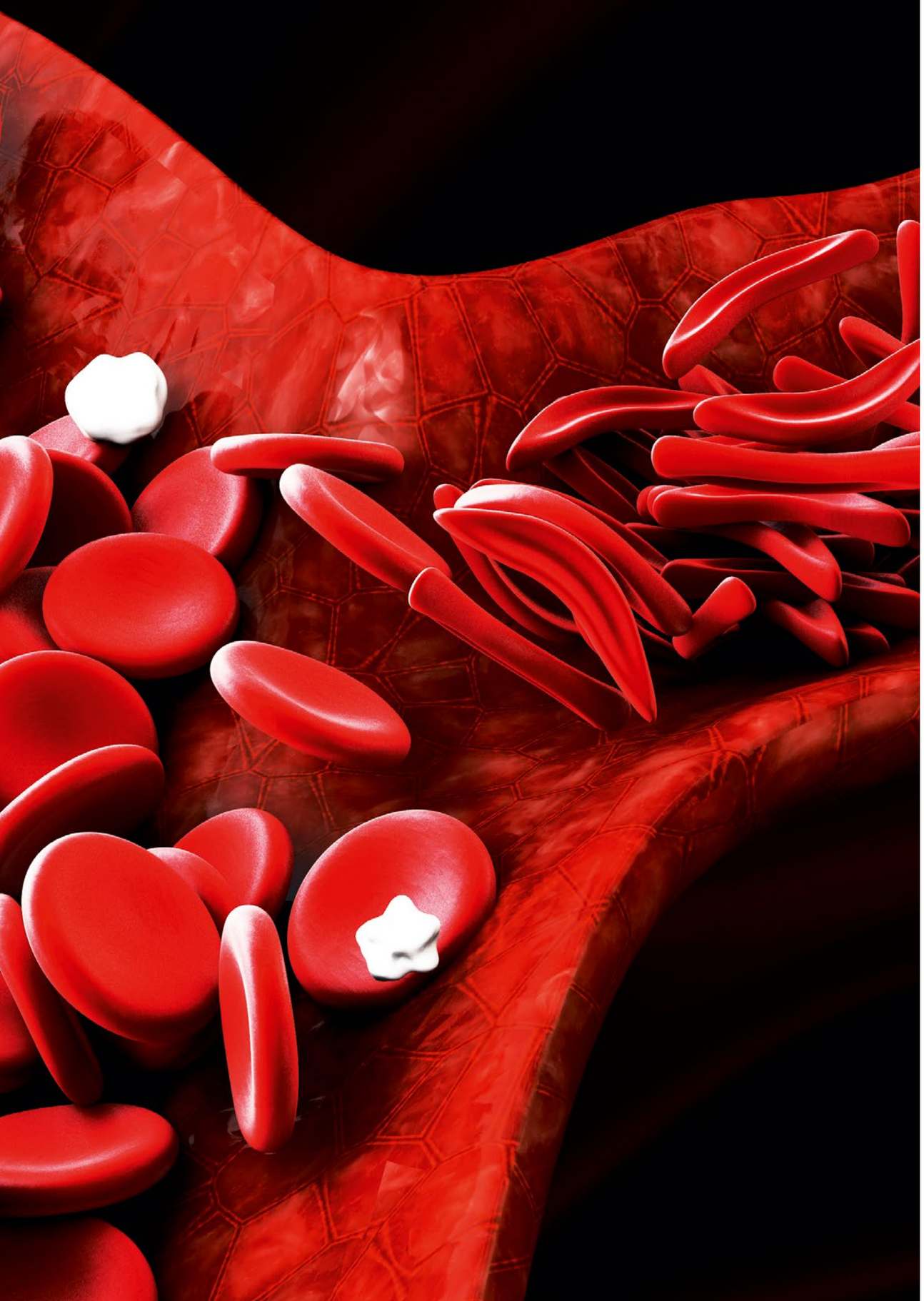

Confronting the challenge of Sickle Cell Anaemia

A distinguished figure in haematology research, **Professor David Nathan** from the Dana-Farber Cancer Institute and the Boston Children's Hospital understands the difficulties posed by disorders of the blood. His latest work focuses on Sickle Cell Disease (SCD) and the drive to find solutions to the debilitating inflammatory crises associated with the condition, always with an eye on its translation to developing economies where the prevalence is greatest.

Sickle cell disease (SCD) is a genetic disorder affecting the haemoglobin genes of red blood cells, a mutation which changes a single amino acid within the haemoglobin protein (the oxygen carrier of the blood) and is responsible for the formation of rigid sickle-shaped red blood cells. These block the fine capillaries of the vascular system leading to acute and chronic episodes of tissue ischemia (oxygen deprivation) and a sustained organ-damaging inflammatory response. The disease is particularly prevalent in developing countries where malaria is endemic, because the heterozygous condition (the largely innocuous state where an individual has both a normal and a mutated version of the gene) confers partial protection from early death from the infection. This in turn confers a survival advantage to the heterozygotes who breed homozygotes (those who inherit two mutated genes and therefore have SCD).

UNDERSTANDING THE DISEASE AND THE CHALLENGES

This produces the first of many challenges. When considering potential treatment options, the ideal therapy would be simple and inexpensive as well as being robust enough to cope with the environment and economic conditions in tropical regions. Another challenge, however, is one of scale; red blood cells account for seventy percent of total cells in an adult, and there may be more than a kilogram of haemoglobin contained within them. As Prof Nathan, former President of the Dana-Farber Institute, makes clear, "Any attempt to apply a drug to haemoglobin must reckon with that awesome fact, treating that quantity of material is no mean feat."

THE PROBLEM WITH SHAPE-SHIFTING

The mutation which causes SCD changes the shape of the haemoglobin molecule, but then haemoglobin changes shape every time it binds or releases oxygen. The trouble with SCD is that as this normal shape change occurs and the haemoglobin S assumes a low oxygen shape, a bulge in the protein caused by the mutation comes to rest in a natural pocket elsewhere in a proximate molecule of the protein. If left long enough (dubbed the delay time), polymerisation of molecules occurs and the haemoglobin polymers become stacks of stiff rods. This causes distortion of red cell membranes and forces open pores within them which disrupt the electrolyte balance. Prof Nathan notes that, "The loss of electrolyte forces loss of water and an obligatory increase in haemoglobin concentration that perversely shortens the delay time", promoting polymerisation of all other mutated molecules.

The transition to the low oxygen conformation is promoted by certain factors in the blood, so individuals with higher levels of these due to other conditions are more likely to suffer SCD crises. Conversely, drugs which can maintain high oxygen conformation may limit the extent of crises. Again, Prof Nathan sees potential, but the same issues arise: "Drugs that bind to haemoglobin may maintain the molecule in the high oxygen affinity state. But such molecules might be expected to be of limited value because very large amounts of drug would be required to deal with [the quantity of] haemoglobin. Such large doses over a protracted period might cause toxicity." Despite this, one such drug is currently undergoing field tests.

LIMITED TREATMENT OPTIONS

The only treatment currently approved for prevention or reduction of SCD symptoms is related to mitigating the polymerisation of haemoglobin. Because mixtures of the constituent haemoglobin proteins within a red cell form hybrids under normal physiological conditions, the presence of certain variants can create hybrids which do not provide a pocket for the abnormal bulge and so inhibit polymerisation. Foetal blood is unaffected by the mutation of SCD because it contains a form of haemoglobin protein which is coded by another gene upstream of the affected SCD gene. The advantage of foetal haemoglobin is its increased affinity for oxygen enabling it to extract it from the mother's blood. It remains in the high oxygen conformation and also does not contain the pocket required for polymerisation, so increased levels of foetal haemoglobin can reduce this damage-causing aspect of SCD. Prof Nathan and colleagues introduced hydroxyurea, first to animal models and then to patients, to successfully increase foetal haemoglobin

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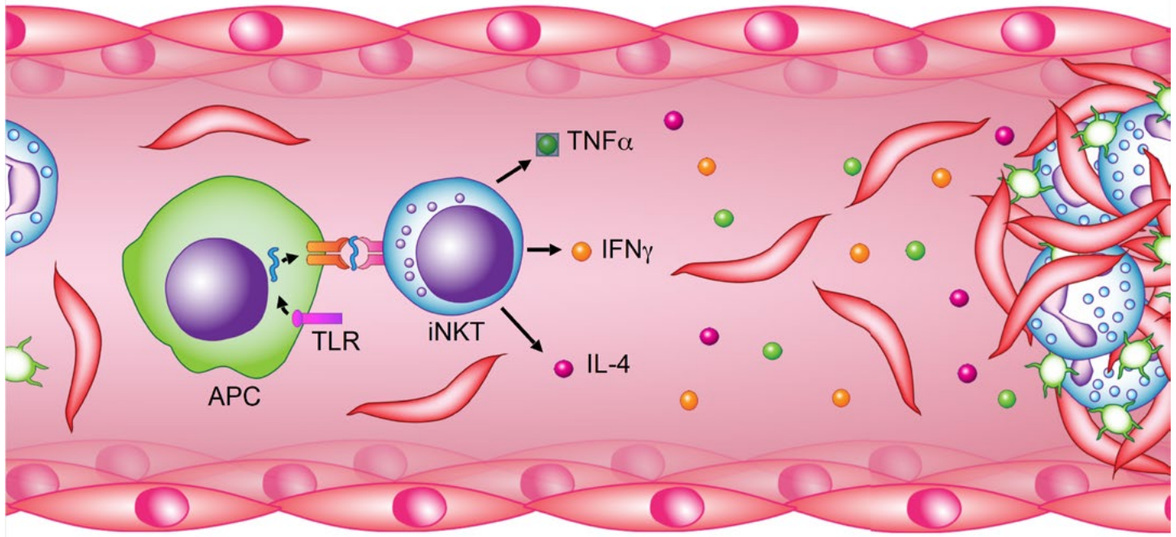

ROLE OF iNKT CELLS IN SICKLE INFLAMMATORY CRISIS Image Courtesy of Dr Joshua Field.
Sickle red blood cells enmeshed in leukocytes, platelets and fibrin stick to endothelial cells and block the capillary as shown on the right. The block causes ischemia and cell death downstream (middle and left). Injured cells (among them antigen presenting cells) release fat fragments that engage the invariant receptor of iNKT cells. These in turn release inflammatory proteins (cytokines) that engage many inflammatory cells to induce severe local inflammation.

and thereby exploit this effect. Other groups are now employing gene therapy approaches to elevating fetal haemoglobin in SCD.

The current focus of Prof Nathan's work is the organ-damaging inflammation which results following multiple short period ischemic events, and which is a major factor in SCD crisis. This inflammatory response is the product of a rush of toxic metabolites into the ischemic region following release of the blockage and resulting in the activation of invariant natural killer T cells (iNKT). These rare cells express a single receptor which responds to fat fragments from damaged cell membranes and causes them to express a massive load of broad inflammatory signals. These engage a panoply of inflammatory cells and the patient experiences this as a 'fire' in the obstructed region which is the SCD crisis. Repeated episodes ultimately damage the organs beyond repair.

POTENTIAL NEW TREATMENTS IDENTIFIED

Biologists previously identified a receptor on immune cells that, when activated by adenosine derived from leaking cancer cells, depresses the anti-cancer cell immune response. Prof Nathan realised the importance of this finding at a Dana-Farber seminar in 2009. He takes up the story: "The seminar focused on the possible use of adenosine receptor blockers to prevent T cell inhibition by adenosine and encourage the anti-cancer immune response. That made me wonder about the use of adenosine analogues to diminish the inflammatory component of sickle cell anaemia."

He got in touch with Dr Joel Linden, La Jolla Institute for Allergy and Immunology, who was happy to share his very successful experience of treating mouse models of SCD with adenosine analogues. Together with Joshua Field, Medical College of Wisconsin and Donna Newburg at Dana-Faber, they embarked on clinical trials of regadenoson, the only sufficiently specific adenosine drug approved for use in patients. Early results were promising, but a larger Phase 2 multi-institutional controlled and double blinded trial was inconclusive. Says Prof Nathan, "There are several potential explanations as to why we did not see a reduction in iNKT cell activation or clinical benefit, with an infusion of low-dose regadenoson during a SCD crisis. One is that the dose of regadenoson used in our trial may have been too low to significantly reduce iNKT cell activation." Other causes of the disparity may be the natural exodus of the iNKT cells from the circulation, the length of time between initiation of the crisis and the patient presenting at the hospital, or the very likely fact that prevention of such crises is much easier to achieve than treating them once they are established.

That made me wonder about the use of such analogues in sickle cell anaemia

NOT THE FINAL WORD

Prof Nathan is undeterred in his search for solutions to the problems presented by SCD. He highlights antibody targeting of iNTK cells (currently in Phase 1 safety trials) and successful therapies which increase foetal blood production, such as suppression of the BCL11A protein which acts as the switch in normal foetal development, as possible avenues of exploration. "All of these trials are somewhat nugatory in the face of the economic and social barriers imposed in Africa and India where sickle cell disease and its complicating *Plasmodium falciparum* malaria are rife." He says, "If we are to be useful in those parts of the world we must provide combinations of inexpensive approaches to disease prevention."

Some of those are uncontroversial, such as the effective treatment of malaria which is a major complicating factor in SCD. Others include prenatal screening to identify homozygotes and encourage termination of the pregnancy which he describes as, "highly controversial, but critically important".

In a career which has spanned decades and seen him hold prestigious positions and collect numerous awards, what is certain is that he will continue to advance the cause of SCD treatment and research, in the hope that effective solutions can be found to these challenging problems.

Q&A

What have been the highlights of your long career in haematology?
The training of scores of clinical and research-oriented haematologists and the opportunity to associate with great haematologists from around the world. The careers of the young trainees give me enormous pleasure.

Why do you have a particular interest in SCD?
It's a vast challenge. It's another disastrous consequence of malaria that potentiates SCD because carriers of SCD are partially resistant to lethal malaria. Hydroxyurea is cheap and it helps to improve the lives of SCD patients. But it must be given properly and compliance is a big problem. Hydroxyurea and anti-malarial are the current standbys in impoverished regions. Gene therapy will eventually work to cure the disease first in wealthier economies that can afford it.

How has the research environment at the Dana-Farber Cancer Institute assisted you in your work?
The atmosphere at DFCI is one of total commitment in the company of superb colleagues who are similarly engaged. The mantra at DFCI is to treat and prevent the best you can today and perform even better tomorrow as a result of research performed today. I don't think there is any biomedical question I can ask that can't be answered at least in part at DFCI and the Harvard/MIT medical research community in which DFCI is embedded.

What are the next steps for SCD research and treatment?
Improvement of basic medical care in tropical regions where SCD is rife, vigorous efforts to protect new-borns with SCD from overwhelming infection, achievement of control of sickling and consequent inflammation with drugs like hydroxyurea, newer anti-sickling agents and agents that inhibit inflammation. If anti-sickling drugs are employed in tropical regions, great care must be devoted to antimalarial prophylaxis.

Ultimately, gene therapy to correct the major inherited disorders of haemoglobin particularly thalassaemia and sickle cell disease will be successful.

What is needed to ensure sufficient emphasis is placed on finding treatments suitable for the health services of developing economies?
Cost of drugs and procedures is an enormous problem, but so are adequate follow up and assurance of compliance with active and preventive therapy. Prevention of early death due to infection by malaria or common bacteria is a major issue. The task of better developed societies is to find therapeutics that can be practically applied in poorer economies. Finally, though controversial, we can prevent the births of SCD patients in the less developed world as well as our own. The cost and invasiveness of foetal genetic testing are rapidly dropping. We could markedly reduce the number of homozygotes if the world community decides to do so.

Detail

RESEARCH OBJECTIVES
Professor David Nathan's research focusses on the pathophysiology and treatment of congenital bone marrow failure and red cell syndromes.

FUNDING
National Heart Lung and Blood Institute (NHLBI)

BIO
Professor Nathan received his MD from Harvard Medical School in 1955. He was trained in medicine at Peter Bent Brigham Hospital and haematology at the National Cancer Institute. Between 1967 and 1984, he was Chief of Haematology at Children's Hospital Boston (CHB), and then Chief of Pediatric Haematology and Oncology at CHB and Dana-Farber Cancer Institute (DFCI). He is currently President Emeritus at DFCI and Physician-in Chief Emeritus at CHB.

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DANA-FARBER CANCER INSTITUTE

Founded in Boston in 1947, the Dana-Farber Cancer Institute is world-renowned for its leadership in adult and paediatric cancer treatment and research. A principal teaching affiliate of Harvard Medical School and a designated Comprehensive Cancer Center, Dana-Farber is a pioneer in cancer care and research. The Boston-based Institute develops and disseminates innovative patient therapies and scientific discoveries throughout the world.

Since 1948, the Jimmy Fund has raised millions of dollars through thousands of community efforts to advance Dana-Farber's lifesaving mission. Dana-Farber cares for adults and children challenged with cancer, blood disorders, and related diseases. Their world-renowned specialists provide comprehensive and personalised care for each patient and support for their families. Their specialised treatment centres are staffed by teams of experts who work closely together to offer patients the latest therapies and strategies, including access to innovative clinical trials. In 2016, 4,826 employees delivered 157,533 infusion treatments via 321,900 patient appointments. Dana-Farber is the only hospital ranked in the top four nationally by US News and World Report in both adult and paediatric cancer care.

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Dana-Farber also remains true to its founder, Sidney Farber, MD, and his vision of a cancer centre that is just as dedicated to discoveries in cancer research as it is to delivering expert, compassionate care. Through strategic investment in research, they support scientific leaders and young investigators, develop new therapies, and ensure a spirit of collaboration and innovation. In the complex fight against cancer, Dana-Farber researchers are advancing the field on every front. They are probing the molecular changes that cause tumours, testing new drug therapies, addressing the needs of cancer survivors, and improving the delivery of care. In 2016, 500 faculty members worked on National Institutes of Health-Sponsored Research and conducted 921 clinical trials. Dana-Farber is a founding member of the Center for AIDS Research at Harvard Medical School.