The company also announced a second deal, this time with Eli Lilly and Co. (http://www.lilly.com). Lilly recognized that many of the more potent and selective leads emerging from discovery are also increasingly insoluble and difficult to turn into drugs. To address this problem, TransForm is performing hundreds of experiments within 2–3 week cycles on very small amounts of drug compound, to enable Lilly to select more 'developable' lead candidates. According to TransForm, both deals are ahead of schedule. Although other partnerships are being pursued, the strategy is to keep the number of deals small to ensure close collaboration.

Transform believes it has several advantages over other companies investigating form and formulation issues. According to Colin Gardner, TransForm's Chief Scientific Officer, the partnerships are only half the story. 'We're not just developing technology to sell it, ' he said, 'We're using it both to generate our own product opportunities as well as to make TransForm a partner of choice to pharmaceutical companies that are looking for an effective way to capture more of the intrinsic value of their proprietary pipeline.' In addition, Gardner believes that this two-pronged approach provides TransForm with more stability than start-up companies based solely on technology platforms, or whose early discovery efforts may not yield fruit for many years.

However, Ray Rowe (AstraZeneca UK), cautioned that this science is nothing new and that many companies have in-house high-throughput form and formulation systems, albeit on a smaller scale. Quoting American microscopist Walter McCrone [3], he said, '…every compound has different polymorphic forms and that, in general, the number of forms known for a given compound is proportional to the time and money spent in research on the compound.'

References

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2 Buller, R. *et al.* (2002) Quinolone binding site on malaria pigment crystal: a rational pathway for antimalarial drug design. *Cryst. Growth Des.* DOI: 10.1021/cg02550I (available online at: http://pubs.acs.org/journals/cgdefu/index.html)

3 McCrone, W.C. (1965) Polymorphism. In *Physics and Chemistry of the Organic Solid State.* (Vol. 2) (Fox, D. *et al.*, eds), pp. 726–767, Interscience

News in brief

Targets and mechanisms

Magnesium and calcium lower blood pressure independently

Scientists have revealed how magnesium can regulate blood pressure by activating ion channels in the cell membrane [1]. This new research, by scientists at Case Western Reserve University (CWRU: http://www.cwru.edu) can be used to help understand how magnesium decreases blood pressure, and also heart failure and stroke.

Jianmin Cui, lead researcher and Assistant Professor in the Department of Biomedical Engineering at CWRU, said: '…we have discovered that when magnesium is applied to calcium-activated potassium channels, these channels will open.'

Calcium-activated potassium channels are pathways in the cell membrane that relax smooth muscle in blood vessels, and also modify electrical impulses through nerve cells to the brain. This study used cloned ion channel DNA to express the channels in frog eggs. These large-conductance (BK type) channels are activated by membrane depolarization and intracellular levels of calcium and magnesium.

Various site-directed mutations were made and any functional changes were recorded; it was found that mutations that abolish the sensitivity to magnesium do not affect calcium sensitivity, and vice versa. This indicates that there are two distinct pathways for Mg^{2+}- and Ca^{2+}-dependent activation of BK-type channels.

Cui said, 'Our research is basic science, however, we hope that the results can help to explain why some treatments would work and provide rationale for development of new drugs for hypertension.'

1 Shi, J. *et al.* (2002) Mechanism of magnesium activation of calcium-activated potassium channels. *Nature* 418, 876–880

Caveolae: role in muscular dystrophy

Advances in understanding the tangled web of cell signalling might have implications for sufferers of a form of muscular dystrophy. Scientists at the University of Texas Southwestern Medical Center (http://www3.utsouthwestern.edu/) have published details of the composition of filaments found in caveolae – organelles that form a membrane system which contains cell-signalling molecules – and described how problems with formation of these filaments can lead to the muscle-wasting disease [2].

The researchers, headed by Richard G.W. Anderson, Chairman of Cell Biology at UT Southwestern, have uncovered the mechanism whereby a protein called caveolin, in conjunction with cholesterol, forms filaments on the inside surface of caveolae. Caveolae are important organelles that ensure signalling molecules are in the correct location for both intra- and inter-cell signalling. 'We believe that signal transduction is not an interaction that can take place anywhere in the cell,' said Anderson. 'Caveolae contain a whole array of signalling molecules, and their job is to spatially organize signal transduction at the cell surface.'

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Anderson and co-workers separated caveolin filaments by using cholesterol-attracting drugs to disrupt the cholesterol sheath that surrounds caveolin. This caused the filaments to degrade into individual subunits. Using a combination of electron microscopy, circular dichroism and analytical ultracentrifugation, they found that each subunit was composed of seven caveolin molecules. This research was then applied to limb-girdle muscular dystrophy, in which patients have mutations in their muscle-cell caveolin. It appears that the mutations prevent caveolin subunits from forming filaments, leading to the characteristic wasting of muscle.

2 Fernandez, I. *et al.* (2002) Mechanism of caveolin filament assembly. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11193–11198

Making cancer cells vulnerable

A gene found in a virus responsible for the common cold can render tumour cells vulnerable and hence susceptible to destruction [3]. Researchers, at the University of Illinois at Chicago (UIC) College of Medicine (http://www.uic.edu/com/cancer), have shown that this gene, known as *E1A*, can be used to weaken cancer cells, thus enabling them to be destroyed.

James Cook, lead author of the paper and Chief of Infectious Diseases, and member, of the UIC Cancer Center, said: 'By explaining how *E1A* works, we hope to develop novel strategies to make human immunological defenses against tumours, as well as chemotherapy and radiation therapy, more effective in combating cancer.'

Cook and co-workers tested the *E1A* gene in four animal models and found that it renders malignant cells susceptible to defence cells of the immune system. 'We believe that these observations may reveal a common Achilles heel of many types of cancer cells,' said Cook. In this study, the team used tumour necrosis factor-α (TNFα), which attaches to cell-surface receptors on tumour cells preparing for attack, an assault usually blocked by the cell. However, when the *E1A* gene is inserted in these malignant cells, the tumour defence mechanism is shut down. This is achieved by repression of the nuclear factor-κB (NFκB), which defends against TNFα.

The goal is to find ways of making current cancer treatments more effective: cancer cells become resistant to conventional treatment and, although the reasons for this are unclear, it is thought that mutations occur during the growth of the tumour. Using this novel approach, researchers could one day alter this malignant tissue, making the cancer vulnerable to therapy. Cook said, '*E1A* is helping us identify the set of cellular switches that need to be turned on or off to render cancer cells more sensitive to therapeutic injury.' He added, 'Further definition of these mechanisms will help us develop new concepts that may be useful in treating cancer, in part by enlisting the body to become a more active partner in fighting the disease.'

3 Cook, J.L. *et al.* (2002) Role of the E1A Rb-binding domain in repression of the NFκB-dependent defense against tumour necrosis factor-α. *Proc. Natl. Acad. Sci. U. S. A.* 99, 9966–9971

From LXRs to elixirs?

Another important discovery has been made in the search for therapeutics against cardiovascular disease. Researchers from X-Ceptor Therapeutics (http://www.x-ceptor.com/), the Howard Hughes Medical Institute (http://www.hhmi.org/) and the University of Texas-Southwestern Medical Center (http://www3.utsouthwestern.edu/) have established a direct protective role between the liver X receptors (LXRs) and the progression of cardiovascular disease [4]. The findings increase the interest in developing LXR-based therapeutics to target cardiovascular disease, the leading cause of death in the Western world.

LXRs are receptors that act as sensors for cholesterol and regulate the expression of genes involved in cholesterol metabolism. Elevated cholesterol levels can lead to atherosclerosis, a disease in which macrophages embed in the inner artery wall, forming a plaque and thus causing circulatory problems. Any molecules involved in cholesterol regulation are therefore potential targets for therapeutic intervention to alleviate cardiovascular diseases caused by cholesterol. In this study, normal macrophages were replaced with LXR-deficient macrophages by bone-marrow transplant in mice. The researchers demonstrated that selective loss of macrophage LXR activity significantly increased the development of atherosclerotic lesions. The increase in lesions indicates that LXRs function as endogenous inhibitors of atherosclerosis.

'This demonstrates that the LXRs are protective or anti-atherogenic and thereby represent a novel molecular target for the treatment of cardiovascular disease,' said Richard Heyman, Chief Scientific Officer of X-Ceptor. The researchers believe that LXR-based drugs could be co-administered with statin therapies, or given to patients who do not respond to statin monotherapy.

4 Tangirala, R.K. *et al.* (2002) Identification of macrophage liver X receptors as inhibitors of atherosclerosis. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.182199799 (http://www.pnas.org)

Miscellaneous

Almonds can reduce 'bad' cholesterol

Researchers have discovered that almonds can lower low density lipoprotein (LDL) blood levels in a randomized, controlled, crossover study of people with high cholesterol [5]. Previous research indicates that nut consumption could improve levels of blood lipids and, therefore, reduce the risk of coronary heart disease. The current study, led by David Jenkins, Director at the Clinical Nutrition Risk factor Modification Center at St Michael's hospital, Toronto (http://www.stmichaelshospital.com/) assessed the benefits of almonds at different dosage levels.

The high fat content of nuts has prevented their general recommendation for the diets of people with hyperlipidemia. Therefore, study participants were counselled on how to replace other foods with nuts in their diet. They reduced blood LDL by an average of 9.4% with a daily full portion of almonds (74 g) and 4.4% with a daily half-portion, compared with controls. 'This study suggests that replacing carbohydrates with monounsaturated fat – within the context of a diet that is low in saturated, *trans* fat and cholesterol –

HIV research

Possible multi-strain HIV vaccine emerges

US researchers have developed a new candidate vaccine strategy that has the ability to elicit an antibody response that can prevent infection with multiple HIV strains [10].

HIV, like influenza, exists as multiple strains and the identification of HIV envelope structures that generate neutralizing antibodies has been a major challenge in the development of an effective HIV vaccine.

The surface of HIV is coated with the glycoprotein 120 (gp120), which has chemical features that vary between strains. But all gp120 molecules have a shared characteristic that allows them to bind to CD4.

The researchers found that covalently crosslinked complexes of soluble human CD4 (shCD4) and the envelope glycoproteins (gp120 and gp140) generated antibodies that neutralized a wide range of primary HIV-1 isolates (regardless of the genetic subtype or co-receptor usage) in rhesus macaques. Anthony DeVico from the Institute of Human Virology at the University of Maryland Biotechnology Institute (http://www.umm.edu/medicine/ihv.html) said, 'The preliminary findings indicate the gp120–CD4 complex might serve as a useful model for HIV vaccine development.'

Robert Gallo, a co-discoverer of HIV, added that, although designed as a preventative vaccine, the complex might be useful as a therapeutic vaccine. The gp12–CD4 complex is currently undergoing safety testing but it is hoped that it will proceed within the next two years to Phase I clinical trials.

This finding is very exciting because the development of a single HIV vaccine for multiple viruses could become a reality and could help stop the AIDS epidemic. HIV and AIDS have killed 25 million people worldwide and another 40 million people are infected.

10 Fouts, T. *et al.* (2002) Crosslinked HIV-1 envelope-CD4 receptor complexes elicit broadly cross-reactive neutralizing antibodies in rhesus macaques. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.182412199 (http://www.pnas.org)

HIV favours integration in active genes

HIV-1 selectively integrates itself into active areas of a host cells genome [11], researchers have discovered.

HIV reproduces by infecting a cell, making a DNA copy of the RNA genome and integrating that DNA copy into a chromosome of the host, so that when the host genome transcribed, the viral genome is too.

Human cells infected with HIV were broken open and the DNA was sequenced to identify the location of the viral DNA. By matching DNA segments with the recently published human genome sequence, researchers discovered that the viral DNA was present in areas of the chromosomes where there are human genes. By using gene chips to screen for proteins encoded by active genes, the team discovered that it was mainly the active genes that were targeted.

'HIV seems to be targeting not just genes, but active genes. That makes a lot of biological sense if the targeting has evolved to promote efficient expression of the viral genome once it integrates into the cell,' said Frederic Bushman, a researcher at the Salk Institute (http://www.salk.edu/) and senior author of the study. He went on to add that the genes targeted are ones that are turned on by infection with HIV itself.

When HIV infects a cell it triggers a response in the cell that includes making new proteins. In this way, HIV wields a double-edged sword by creating a weakness and then taking advantage of it. Also because most HIV-infected cells die within a day or two, it is to the virus' advantage to reproduce quickly.

The findings in this study could have implications for the future development of effective gene therapies.

11 Schröder, A.R.W. *et al.* (2002) HIV-1 integration in the human genome favours active genes and local hotspots. *Cell* 110, 521–529

Serendipitous discovery of potential HIV immobiliser

A researcher at Sandia National Laboratories (http://www.sandia.gov/) has developed a compound that could potentially immobilise the AIDS virus [12]. May Nyman found the optimum conditions to synthesise the first niobium heteropolyanion (HPA), altering them slightly to produce an assortment of HPAs. Preliminary work indicates that the new compounds can have a strong binding effect on viruses.

Researchers have known about the HPAs in the form of oxides of tungsten, vanadium and molybdenum since the late-19th century. Their ability to bind viruses and large metal atoms, such as some radionuclides, has long intrigued researchers. Although HPAs are made cheaply and easily at room temperatures and pressures, they are stable only in acidic environments, making the use of HPAs as antivirals difficult because blood is of neutral pH.

Nyman's discovery is the first group of niobium HPAs ever reported. By contrast to other HPAs, nobium HPAs are base-stable and are inexpensively and easily formed. Being base-stable means they can survive longer and could possibly even thrive in neutral environments, such as blood. Once these compounds bind to an AIDS virus, the virus is no longer capable of entering a cell.

This finding came about by accident when Sandia was called in to find the cause of a clogging problem during the Savannah River site's attempts to extract a dangerously radioactive isotope of caesium. Nyman's curiosity led her to investigate the clogging impurity formed during manufacturing, which turned out to be the niobium HPA. 'One man's trash is another's treasure', says Nyman of her experience.

12 Nyman, M. *et al.* (2002) A general synthetic procedure for heteropolyniobates. *Science* 297, 996–998

favourably affects the cholesterol levels and cardiovascular risk' says Alice Lichtenstein, vice-chair of the American Heart Association's (AHA) nutrition committee.

Although this study cofirms that nuts are a good source of protein with no cholesterol, their potential benefits can be negated if they are simply added to the diet without removing other foods. An overall balanced diet, high in fruits, vegetables and whole grains, and including low-fat dairy products,

fish and lean meats is recommended by the AHA.

5 Jenkins, D.J.A. *et al.* (2002) Dose response of almonds on coronary heart disease risk factors: blood lipids, oxidized low-density lipoproteins, lipoprotein(a), homocysteine, and pulmonary nitric oxide: a randomized, controlled, crossover trial. *Circulation* 10.1161/01.CIR.0000028421.91733.20

The most complete protein map so far

Scientists at the Department of Energy's Pacific Northwest National Laboratory (http://www.pnl.gov) have obtained the most complete protein coverage of any organism, to date, with the study of a radiation-resistant microbe known to survive extreme environments. The scientists have identified more than 1900 proteins in *Deinococcus radiodurans* [6]. Potentially, this research could open up new opportunities to harness the microorganism for bioremediation.

Deinococcus radiodurans is interesting because of its potential to degrade radioactive materials, its ability to withstand high levels of radiation and its impressive DNA repair capabilities. Proteins with different functions were identified by exposure to several stresses and environments: heat shock; cold shock; exposure to chemicals that damage DNA, such as trichloroethylene; exposure to ionizing radiation; and starvation. The scientists confirmed the existence of many proteins. In addition, new proteins were found that only become active under specific conditions, as were proteins that appear to exist all the time.

'Because our coverage is unprecedented, we're now able to provide biologists with protein-level information they never had access to before', said Mary Lipton, PNNL senior research scientist.

A new high-throughput mass spectrometer based on Fourier-transform ion cyclotron resonance developed at PNNL was used in the study. This instrumentation enables scientists to identify thousands of proteins within hours. The system relies on a two-step process that first uses tandem MS to identify biomarkers for each protein. 'Once we've identified the protein biomarkers, then we never have to repeat the identification step, thereby speeding up our experiments. As a result we not only have a much more complete view of the proteome than existed previously, but we also can follow changes to it much faster', said Richard Smith, PNNL investigator.

6 Lipton, M.S. *et al.* (2002) Global analysis of the *Deinococcus radiodurans* proteome by using accurate mass tags. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11049–11054

Coffee cancer cure

Researchers have found that common ingredients in many foods and drinks could help defeat cancer and heart disease [7]. A team at University College London (UCL; http://www.ucl.ac.uk) believe that caffeine and theophylline might block a key enzyme implicated in a range of cell functions.

The enzyme involved, phosphoinositide (PI) 3-kinase, is believed to have a key role in signalling mechanisms that determine cell growth and death; it triggers phosphate attachment to cell membrane lipids, which in turn triggers signalling pathways. The isoform p110Δ of the enzyme, which is targeted by caffeine, is also involved in inflammatory bowel disease and is part of the body's response to infection.

The scientists generated 'knockin' mice that expressed a mutated p110Δ: the lipid kinase in this mutant was completely inactive. Also, antigen receptor signalling in B and T cells was defective and immune responses in these mutant mice were decreased.

Peter Shepherd, a Professor at UCL's Department of Biochemistry and Molecular Biology said, 'We've shown that caffeine-like compounds play a novel role in blocking enzymes known to play a critical role in a range of cellular functions in the body. Alongside possible advances in cancer treatment, this research suggests that caffeine-type drugs could be used to treat heart disease and inflammatory illnesses.' He continued, 'But the message to the general public is not to overdose on chocolate or coffee. The study relied on using high concentrations of caffeine that would normally be unhealthy for human use. The next stage of our research will be to develop compounds which mimic the structure of caffeine but without its negative effects.'

7 Okkenhaug, K. *et al.* (2002) Impaired B and T cell antigen receptor signaling in p110Δ PI 3-kinase mutant mice. *Science* 297, 1031–1034

VEGF Trap: a potent angiogenesis blocker

Regeneron Pharmaceuticals (http://www.regeneron.com) has developed a potent vascular endothelial growth factor (VEGF) blocker, the VEGF Trap. The VEGF Trap acts as a decoy receptor and effectively inhibits cancer tumour growth by blocking the signals that promote blood vessel growth in tumours.

Blocking tumour-associated angiogenesis prevents tumour growth and might result in tumour shrinkage. Independent preclinical studies at Columbia University (http://www.columbia.edu/) [8] and Regeneron Research Laboratories [9] have compared the VEGF Trap with three current approaches to angiogenesis inhibition: a monoclonal antibody that binds and blocks VEGF, a monoclonal antibody that binds and blocks the VEGF receptor, and an RNA-based fluoropyrimidine aptamer that binds and blocks VEGF. The studies examined multiple tumour types, including melanoma, glioma, neuroblastoma and rhabdomyosarcoma.

The 'VEGF Trap appears to be one of the most potent inhibitors of angiogenesis, causing regression of a tumour's initial blood vessels as well as blockage of new blood vessel formation', noted Darrell Yamashiro, Assistant Professor of Paediatrics, Pathology and Surgery at Columbia University. Importantly, the results from the study at Columbia University indicate that the more complete blockage achieved by Regeneron's VEGF Trap not only inhibits growth of new blood vessels, but also eventually leads to regression of existing vessels. These results highlight an exciting step towards more complete treatment for diverse tumour types.

8 Kim, E.S. *et al.* (2002) Potent VEGF blockade causes regression of co-opted vessels in a model of neuroblastoma. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11399–11404

9 Holash, J. *et al.* (2002) VEGF Trap: a blocker with potent antitumor effects. *Proc. Natl. Acad. Sci. U. S. A.* 99, 11393–13398

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