Clinical trials

In vivo experiments in mice with FLT3–ITD leukaemia showed that a single dose of CEP701 could completely inhibit FLT3 phosphorylation for several hours, and dosing 2–3 times a day improved survival rates; 50% of control mice died by day 16 whereas 50% of the group given 10 mg kg^{-1} CEP701 every 8 hours survived until day 27. Analysis of the result showed that the overall difference in survival was significant ($P<0.001$). The drug was shown to be well tolerated in a previous Phase I trial (Small and colleagues, unpublished data). A Phase II trial for AML patients expressing FLT3 mutations is now under way at the Johns Hopkins Kimmel Cancer Center (http://www.hopkinskimmelcancercenter.org). 'We have just started recruiting patients with relapsed AML into an open-label study to see if they will respond to CEP701 with a reduction in percentage blast cells; we hope that some may even go into remission,' reports Small. If results are promising, the group hopes that the next step will be to combine CEP701 treatment with standard chemotherapy. There is a precedent for this: previously, M3 AML had a low cure rate [3], but after retinoic acid therapy was combined with chemotherapy, the cure rate increased to 70–80%. Small hopes that 'a similar synergistic result may be achieved with CEP701, transforming AML with FLT3 mutations from a worse prognosis to a good prognosis.'

References

1 Levis, M. *et al.* (2002) An FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukaemia cells *in vitro* and *in vivo*. *Blood* 99, 3885–3891

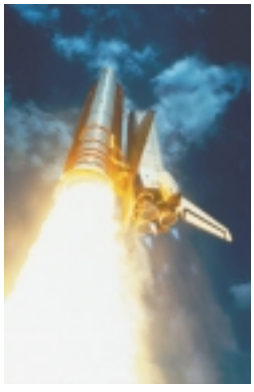
2 Druker, B.J. *et al.* (2001) Activity of a specific inhibitor of BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukaemia and acute lymphoblastic leukaemia with the Philadelphia chromosome. *New Engl. J. Med.* 344, 1038–1042

3 Randolph, T.R. (2000) Acute promyelocytic leukemia (AML-M3) Part 1: Pathophysiology, clinical diagnosis and differentiation therapy. *Clin. Lab. Sci.* 13, 98–105

News in brief

Neurodegenerative diseases

Mouse and computer models of Parkinson's disease



Two separate reports have shed light on how genes misfire in Parkinson's disease (PD), and the origin of the tremors associated with the disorder [1,2]. The first report [1], conducted by researchers at the University of California, Los Angeles (UCLA; http://www.ucla.edu/), produced a method of imaging the misfiring of thousands of genes in a mouse model. This could lead to a research blueprint to pinpoint abnormal regions in the brain linked to autism or schizophrenia.

Desmond Smith, UCLA Assistant Professor of Molecular and Medical Pharmacology, said: 'This approach identifies which genes play a role in abnormal brain function and where they are located. We can use this information to narrow down the brain regions linked to genetic disorders and pinpoint the genes responsible for causing them.'

The study involved the technique of voxelation – involving the analysis of cubes of brain by DNA chip technology – to compare the gene expression in mice brains, half of which were treated with drugs to induce PD. The brains were then 'voxelated' to track the expression of 9000 genes simultaneously. Upon comparing healthy and diseased mice, Smith and co-workers found that mice with PD had an abnormal shift in gene activity and, in particular, the upregulation of genes involved in cell–cell interactions was observed in the PD brains.

The second study, which could explain the origin of the debilitating tremors of PD, was conducted by scientists at the Department of Mathematics, Ohio State University (http://www.osu.edu/), using a computer model of electrochemical activity in a brain affected by PD [2]. The research showed unusual patterns in the way the brain cells fired signals.

David Terman, Professor of Mathematics at Ohio State University, said: 'In a normal brain, every cell is doing its own thing, and the signals create a random pattern. But in our model, we saw cells firing together in lockstep, creating a synchronized pattern that matched the timing of Parkinson's tremors.'

This research could help to elucidate a mystery of the medical community; that is, how the loss of the neurotransmitter dopamine leads to the tremors of PD. In the past, researchers have thought that an increase in the frequency of neural signals was the cause. Although this occurrence of neurons firing almost twice as fast as normal during Parkinson's episodes could explain other symptoms of the disease, such as slowness of movements and stiffness, it could not explain the tremors. 'Our computer model shows that the pattern of signals is important – not just the frequency,' said Terman.

Terman and colleagues hope to extend this research to include other regions of the brain, but these initial results could provide researchers with new directions for therapies for PD.

1 Brown, V.M. *et al.* (2002) Multiplex three-dimensional brain disease expression mapping in a mouse model of Parkinson's disease. *Genome Res.* 12, 868–884

 1359-6446/02/$ – see front matter PII: S1359-6446(02)02397-8

2 Terman, D. *et al.* (2002) Activity patterns in a model for the subthalamopallidal network of the basal ganglia. *J. Neurosci.* 22, 2963–2976

Depression linked to onset of Parkinson's disease

Depressed people are three times more likely to develop Parkinson's disease (PD) than people who are not depressed [3]. In a study conducted by scientists at Maastricht University, The Netherlands, 1358 people diagnosed with depression and 67,570 people born in the same year who had never been diagnosed with depression were compared 25 years later for the incidence of PD. Nineteen (1.4%) of the 1358 people with depression had developed PD compared with 259 (0.4%) out of the 67,570 people who had never been diagnosed with depression.

'This raises the question of whether depression is the first symptom of Parkinson's disease that appears before patients have other symptoms and a diagnosis,' said Agnes Schuurman, author of the study. Both depression and PD are characterised by low levels of serotonin in the brain, and serotonin is known to modulate the release of dopamine, the neurotransmitter that is decreased in PD. It is therefore possible that low levels of serotonin in depression could lead to decreased levels of dopamine in later life, and the onset of PD. 'Because the reduced serotonin activity already exists before any motor neurone symptoms begin, the risk of depression is also increased,' Schuurman said.

3 Schuurman, AG. *et al.* (2002) Increased risk of Parkinson's disease after depression: a retrospective cohort study. *Neurology* 58, 1501–1504

Protease implicated in multiple sclerosis

A new clue has been found in the hunt for an effective multiple sclerosis (MS) therapy. Researchers headed by Isobel Scarisbrick at the Mayo Clinic (http://www.mayo.edu) have found a large increase in levels of the recently discovered myelencephalon-specific protease (MSP) in damaged tissues associated with the disease [4].

MS is an inflammatory CNS disease in which damage is caused to the oligodendroglia tissue of the myelin sheath, which protects the axons of nerve cells. Nerve transmission is therefore disrupted, causing a large variety of symptoms. MS is the most frequent neurological disorder in North America and Europe, affecting 333,000 people in the USA alone. Although the genetics, origin and development of the disease are increasingly well understood, no comprehensively effective treatment yet exists.

The MSP enzyme was discovered in 1997 by Scarisbrick and co-workers [5]. Since then, work has continued to investigate the role of MSP in demyelination. Now, the team have reported the first ever link between MSP and the debilitating effects of MS. They found, in both mouse and human MS tissue, a dramatic increase in the levels of MSP in inflammatory cells associated with the demyelination of MS lesions. MSP facilitates the entry of inflammatory cells into the brain and contributes to tissue destruction when overexpressed. When functioning normally, however, MSP contributes to proper oligodendroglia function. 'If you could control this enzyme, you could possibly decrease the development of disease,' said Scarisbrick. 'We're not reporting this as a cure, but it represents something that could be targeted for therapy. We have a lot more work to do.'

4 Scarisbrick, I.A. *et al.* (2002) Activity of a newly identified serine protease in CNS demyelination. *Brain* 125, 1283–1296
5 Scarisbrick, I.A. *et al.* (1997) Nervous system-specific expression of a novel serine protease: regulation in the adult rat spinal cord by excitotoxic injury. *J. Neurosci.* 17, 8156–8168

Cancer targets and mechanisms

Broccoli: a new weapon against stomach cancer

New research suggests that sulforaphane, found in broccoli and broccoli sprouts, could be used to relieve infection by *Heliobacter pylori*, the bacterium that causes stomach cancers and ulcers. *Helicobacter pylori* was recognized 20 years ago as the cause of stomach ulcers and

stomach cancers, one of the leading causes of cancer-related death worldwide. Although powerful antibiotics can be used to kill the bacterium, it is most common in areas of the world where the use of antibiotics is often economically and logistically difficult.

New research from a team led by Jed Fahey, from the John Hopkins School of Medicine (http://www.hopkinsmedicine.org), has shown that sulforaphane, a compound from broccoli and broccoli sprouts, can kill *H. pylori* under laboratory conditions [6]. Even *H. pylori* strains that are resistant to antibiotics are killed by the compound, which acts on bacteria both inside and outside the cells. This is an important advantage of the compound over antibiotics, because gastric cells can act as reservoirs of bacteria, making it harder to eradicate the infection.

Sulforaphane is thought to act by boosting the production of phase II metabolism enzymes, which can detoxify some carcinogenic agents and damaging free radicals. The antibiotic properties of sulforaphane are, however, still poorly understood.

It is unknown whether dietary sources of sulforaphane will have the same effects as the purified compound. However, if these do prove to be effective against the bacteria, it could lead to native vegetables being used by local populations to reduce infection by *H. pylori,* and thus reduce the incidence of stomach cancer and ulcers.

6 Fahey, J.W. *et al.* (2002) Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of *Heliobacter pylori* and presents benzo[*a*]pyrene-induced stomach tumors. *Proc. Natl. Acad. Sci. U. S. A.* 99, 7610–7615

Homing in on tumour vasculature

A new drug that targets and destroys the blood vessels supplying malignant tumours will hopefully soon enter clinical trials. Scientists at the University of Texas M.D. Anderson Cancer Center (http://www.mdanderson.org) and the University of Texas Southwestern Medical Center (http://www.swmed.edu) have created the drug by linking vascular endothelial growth factor (VEGF) to a toxin. Studies in mice [7] demonstrated that the drug selectively destroys the blood vessels supplying human solid tumours, without harming the vasculature of normal tissue.

VEGF is one of the major factors responsible for angiogenesis – the process by which tumours develop new blood vessels, enabling continued tumour growth and metastasis. Receptors for VEGF are thought to be promising targets for cancer therapies as they are overexpressed on the endothelium of tumour vasculature compared with their expression in normal tissue. A toxin attached to VEGF will therefore target and destroy tumour vasculature in preference to normal tissue. In this study, a gelonin toxin (rGel), genetically engineered and shown to be non-antigenic and non-damaging to normal human blood vessels, was attached to the 'carrier' VEGF.

To test the idea, mice were injected with human melanoma and prostate cancer cells. Tumour growth in the mice that were given the drug was reduced to 16% of that in the untreated mice. Destruction of the tumour vasculature was observed 48 h after administration of the drug, and there was no visible damage to any normal organs. Philip Thorpe, Professor of Pharmacology at Southwestern, described these results as 'impressive in magnitude and prolonged'. He added, 'These studies suggest that VEGF/rGel has potential as an anti-tumour agent for treating cancer patients.'

Although the drug is selective for tumour cells over normal cells, it is less selective for any particular tumour type. 'The significance of this fusion toxin is that it's not specific to one kind of tumour – it has impressive anti-tumour effects in various kinds of tumours – including melanoma and prostate cancers,' said Michael Rosenblum, of M.D. Anderson. It is hoped that this targeted therapy will overcome a major problem with chemotherapy: the ability of tumour cells to mutate and develop resistance to the drug. A clinical trial to test the technique in humans is expected to begin within the year at M.D. Anderson.

7 Veenendaal, L.M. *et al.* (2002) *In vitro* and *in vivo* studies of a VEGF121/rGelonin chimeric fusion toxin targeting the neovasculature of solid tumors. *Proc. Natl Acad. Sci. U. S. A.* 99, 7866–7871

The grim Reaper revealed

New research by scientists at Rockefeller University (http://www.rockefeller.edu) has shown how the protein Reaper triggers apoptosis in *Drosophila*, highlighting a novel possible strategy for fighting cancer cells [8].

In *Drosophila*, the Reaper protein regulates a 'guard' protein called inhibitor of apoptosis protein 1 (DIAP1), which, when eliminated, leads to the self-destruction of the cell. In human cancer cells this mechanism is overcome, and the number of inhibitor of apoptosis proteins (IAPs) is increased, thus preventing apoptosis. However, until now it was not known how Reaper actually worked.

The researchers showed that Reaper causes DIAP1 to undergo auto-ubiquitination. Ubiquitination occurs in all cells at all times and is a way in which protein waste is eliminated. Proteins that are no longer needed by the cell are tagged with ubiquitin ligases, such as DIAP1, which identify the unwanted protein to proteasomes, which then remove it. Auto-ubiquitination is the process whereby proteins tag themselves for destruction, rather than relying on ubiquitin ligases. This is the first time that it has been shown that Reaper acts by stimulating DIAP1 to ubiquitinate itself; this causes caspases, the proteins that eventually kill a cell, to be released, thus ending in cell death.

It is hoped that further research could lead to small-molecule drugs that imitate the ability of Reaper to cause IAPs to self-ubiquitinate, and which could thus be targeted to cancerous cells, leaving healthy cells unharmed. 'This kind of therapy should result in fewer side-effects', says Herman Steller, principal author of the paper.

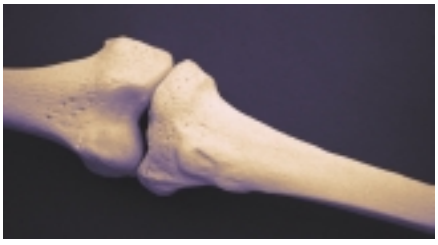
8 Ryoo, H.D. *et al.* (2002) Regulation of *Drosophila* IAP2 degradation and apoptosis by *reaper* and *ubdD2*. *Nat. Cell Biol.* 4, 432–438

Miscellanous

Common drugs could prevent bone healing

Researchers have found that drugs commonly prescribed for arthritis pain and musculoskeletal injuries could impair the healing of bone fractures. The study, published in the *Journal of Bone and Mineral Research* [9] showed that some anti-inflammatory drugs, known as the cyclo-oxygenase 2 (COX-2) inhibitors or coxibs, are often prescribed to people with bone fractures or are already being taken for other ailments, such as headache and menstruation.

The drugs in question are Celebrex™ (celecoxib) and Vioxx™ (rofecoxib), which were developed to treat pain and inflammation without the risks associated with the traditional non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen.



J. Patrick O'Connor, Assistant Professor in the Department of Orthopaedics, University of Medicine and Dentistry of New Jersey (http://www.umdnj.edu/), led a study of the role of prostaglandins in fracture healing. Prostaglandins stimulate both the breakdown and formation of bone and can be synthesized upon induction by COX-2.

The study involved four groups of rats, with hind-leg bone fracture, which were subsequently treated with either the COX-2-selective NSAIDs, celecoxib or rofecoxib, a traditional NSAID

(indomethacin) or a control. Animals were tested weekly at 2–8 weeks when the researchers assessed healing of the fractures using mechanical, radiographic and microscopic tests. The control group demonstrated regular bone healing throughout (new bone tissue had almost bridged the fracture site by 4 weeks), whereas the indomethacin-treated rats exhibited a delay in bone healing by 1 week. Treatment with COX-2 inhibitors showed dramatic results: bone healing was normal at 2 and 4 weeks, however, the fracture was still evident at 8 weeks. Further studies showed that mice lacking a functional *COX-2* gene failed to heal properly, proving that COX-2 is the first gene to be identified as being essential for bone healing, but it is not essential for normal foetal development.

9 Simon, A.M. *et al.* (2002) Cyclo-oxygenase 2 function is essential for bone fracture healing. *J. Bone Min. Res.* 17, 963–976

Tumour transcriptome technique

Scientists have developed a technique that makes it possible to obtain a picture of all the expressed genes in an individual cell at the time of analysis [10]. The team, at the Institute of Immunology, Ludwig-Maximilians-Universitat München (http://www.uni-muenchen.de), in collaboration with Micromet (http://www.micromet.de), showed a technique where genetic aberrations can be identified, as can the functional state of a tumour cell.

This technique could be particularly important in the stage of human cancer where residual individual tumour cells have left their primary sites and invaded foreign tissues, which can lead to metastatic relapse at any time. Because these cells are so scarce, it has been difficult to analyze them in the past, but clinical trials show that their presence is important in diagnosing recurrent cancer.

The researchers investigated micrometastatic cells from the bone marrow of cancer patients, by using transcriptome and genome analysis of individual tumour cells. Genomic abberations were identified upon comparative genome hybridization of the same cells. The technique is PCR-based and the use of this approach for the development of new therapeutics targets was demonstrated by high expression levels of a cell surface antigen (EMMPRIN, also known as CD147), which is involved in tumour invasion.

Patrick A. Baeuerle, CSO of Micromet, said: 'These findings highlight the potential of the method for the identification of novel therapeutic targets on micro-metastatic cells.'

10 Klein, C.A. *et al.* (2002) Combined transcriptome and genome analysis of single micrometastatic cells. *Nat. Biotechnol.* 20, 387–392

Labs lag behind data revolution

The large amount of electronic data now being generated by drug discovery processes has left traditional scientists behind, reveals a survey conducted by NuGenesis Technologies Corporation (http://www.nugenesis.com) at its seminar *Managing the Drug Discovery and Data Explosion* earlier this year.

Of 137 scientists polled, 58% said they did not have a formal method to electronically organize the data they collect and another 22% said they kept records by cutting and pasting into notebooks.

FDA legislation now requires laboratories to convert to a electronic based environment. However, John Helfrich, development manager for the drug discovery and development applications group at Nugenesis was not surprised by the results.

'The drug discovery arena is seeing an explosion of data today unlike anything that has been seen before,' he said. Scientists are catching up but he estimated it would be 'five years, maybe even ten' before 90% of the research population has converted from a paper environment to an electronic one.

Cash and tissue donation aid autism program

Psychiatric Genomics (http://www.psychiatricgenomics.com) has received a Phase 1 Small Business Innovation Research grant of US$100,000 from the National Institute of Child Health and Human Development, National Institutes of Health (http://www.nih.gov). The grant will support a research project to investigate whether small-molecule non-peptide receptor agonists at the human secretin receptor can be used to treat autism.

In a separate donation, the Autism Tissue Program (http://www.autism-society.org) has provided Psychiatric Genomics with postmortem brain tissue from individuals diagnosed with autism and from appropiately matched individuals without the disease. Studying the differences in gene expression between the samples will hopefully lead to the identification of novel gene pathways involved in the disease.

Speaking of the valuable tissue contribution from the NIH brain bank, Jane Pickett, Director of the Autism Tissue Program, said, 'We select recipients based on both the scientific merit of the proposed research and the potential of the research to contribute to the further understanding of autism.'

Competition hots up in systems biology

The market for technologies that can simulate biochemical reactions is expected to rise from US$84.3 million in 2001 to over US$6 billion by 2008 as pharmaceutical companies look to new ways to reduce the costs incurred by lengthy clinical trials.

These large revenues will cause new IT companies to join the market looking for a share of profits, and might also encourage firms selling basic computer hardware to expand from information management to information analyzers.

Although clinical trials are in no danger of being replaced by *in silico* technologies, pharmaceutical companies are on the lookout for new tools that can shorten drug development times. Larger pharmaceutical companies are expected develop their own IT infrastructure as they increasingly look to *in silico* technologies as a way of accelerating the drug discovery process while keeping costs down. 'Considering the potential profits in selling a blockbuster drug, pharmaceutical companies are understandably fearful of losing even a small portion of revenues to partners in the drug discovery process,' says Brad Peters, analyst for Frost & Sullivan (http://www.frost.com), which produced the data.

News in brief was written by *Matt Brown*, *Joanne Clough*, *Ben Ramster* and *Linsey Stapley*