be effective against the patient's virus, thus raising the chances of a good outcome for patients,' explains Mellors.

Mechanism of search

The first step is to determine the genetic sequence of the protease and reverse transcriptase genes of the HIV samples taken from patients. Mutations in these genes are identified and compared with mutations that have already been linked to resistance or cross-resistance in the database, which Mellors estimates is growing at a rate of approximately 2000 samples each month. Currently, >100 mutations are known to cause HIV drug resistance.

Each search elicits matching genotypes but, importantly, also provides the known rate of replication of strains with that genotype in the presence of all the current antiretroviral drugs (the virtual phenotype). 'If actual phenotypes are compared with virtual phenotypes, a concordance rate of over 90% is achieved. False-positive and false-negative results are very rare and most miscalls occur with strains with intermediate resistance,' says Mellors. He adds: 'This is because our knowledge of HIV mutations is incomplete, but it will improve with time.'

It takes approximately two weeks to produce an accurate virtual phenotype to enable doctors to predict which combination of the three classes of antiretroviral drugs (nucleoside and non-nucleoside reverse transcriptase inhibitors and the protease inhibitors) is likely to be most effective for each patient at the point of diagnosis. This will have the advantage of avoiding the need to try out various drug cocktails until the most effective one is found.

Future uses

'Currently, AIDS and HIV advisory bodies recommend resistance testing for newly diagnosed patients prior to starting therapy,' says Mellors and he predicts that the use of resistance testing will rise in the future. Meanwhile, the company will be working on extending the technique to other diseases, including cancer and hepatitis.

Sharon Dorrell

News in brief

Vagus nerve stimulation for Alzheimer's disease

A pilot clinical study has been approved for the examination of the effects of vagus nerve stimulation (VNS) for the treatment of Alzheimer's disease (AD). Researchers at Cyberonics (Houston, TX, USA) have initiated the study because of VNS treatment-related improvements previously seen in memory in both animal studies[1] and in patients with epilepsy[2]. The first study showed enhanced memory storage while the second study (by the same group) showed enhanced memory by verbal learning in epilepsy patients of 35%.

The three-month pilot study will be carried out at the Sahlgrenska University Hospital in Gothenburg (Sweden) and will implant up to ten patients with the NCP System to stimulate the left cervical vagus nerve. The patients will then be followed up long-term.

As well as examining changes in cognitive performance such as in memory over time, the team hope to examine the effects of VNS on disturbances in attention, mood and executive functions, which are often among the earliest symptoms of AD. Magnus Sjogren, the principal investigator of the pilot study said, 'In a separate study of patients with depression[3]…VNS has been shown to potentially have mood elevating effects. In moderate to severe AD, many patients also develop depressive symptoms, so the potential of VNS to not only enhance memory, but also improve depression is of great interest to us.'

1 Clark, K.B. *et al.* (1998) Posttraining electrical stimulation of vagal afferents with concomitant vagal efferent inactivation enhances memory storage processes in the rat. *Neurobiol. Learn. Mem.* 70, 364–373

2 Clark, K.B. *et al.* (1999) Enhanced recognition memory following vagus nerve stimulation in human subjects. *Nat. Neurosci.* 2, 94–98

3 Rush, A.J. (2000) Vagus nerve stimulation (VNS) for treatment-resistant depressions: a multicenter study. *Biol. Psychiatry* 47, 276–286

Competition increases in the cardiovascular market

The global cardiovascular (CV) disease therapeutic market will continue to be a high risk forum in which to launch new drugs and perform marketing, despite increases in its overall size, reports a recent Decision Resources (Waltham, MA, USA) study entitled *The Marketing Environment for Cardiovascular Agents*. The market, principally comprising the US, France, Germany, Italy, Spain and the UK, is highly competitive because of the large potential profits that are available and the overlapping nature of CV disease pathophysiologies and treatment practices.

Intense R&D effort by commercial companies has resulted in the presence of too many similar drugs which now crowd the market, making the production of a market 'blockbuster' less likely. There is now a realization that the market requires diversification to fill the niches that do exist and develop truly novel agents. Other factors that

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commercial companies will have to address include: the increasingly stringent criteria used to assess reimbursement of novel drugs; the interaction of novel agents with existing medication, which is becoming increasingly important as the population ages; and the increasing availability of health information to the public.

Simultaneous signature sequencing for rapid cDNA expression profile identification

Researchers at Lynx Therapeutics (Hayward, CA, USA) have reported a novel sequencing approach for the characterization of gene expression[4]. This technique, named Massively Parallel Signature Sequencing (MPSS), combines non-gel-based signature sequencing with *in vitro* cloning of millions of templates on separate microbeads and can simultaneously identify hundreds of thousands of cDNA molecules in one run.

A microbead library of DNA templates was created and a planar array of a million template-containing microbeads assembled in a flow cell. Sequences of the free ends of the cloned templates were then analyzed and unique 16–20 base signature sequences obtained and interrogated by encoded hybridization probes. As the technology quantifies the expression of each cDNA by a direct count of the number of times a molecule's signature appears, the company say it can detect cDNA molecules that are expressed in only a few copies per million. This technique was validated by sequencing >269 000 signatures from two cDNA libraries constructed from a full sequenced strain of *Saccharomyces cerevisiae*, and by measuring gene expression levels in the human cell line THP-1.

Norrie Russell, CEO of Lynx Therapeutics said, 'In addition to pursuing applications for its technologies in selected pharma and agbio collaborations, [the company] is now in discussions with others interested in working with Lynx to rapidly construct complete expression databases that could be made broadly available to researchers by subscription.'

4 Brenner, S. *et al.* (2000) Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. *Nat. Biotechnol.* 18, 630–634

New clinical trial designs fuel ischaemic stroke research

The success of the new ischaemic stroke (IS) drug candidate clomethiazole (Zendra) is a tribute to the innovative design of its clinical trial, reported the recent Decision Resources (Waltham, MA, USA) study *Ischemic Stroke*. The trial divided patients into neurologically defined subgroups, enabling results to be more specifically analyzed. Clomethiazole was found to be most effective in treating patients with large infarct strokes, suggesting a possible future application as a specific therapeutic agent.

Other interest centres around IS therapeutics that open potassium channels (e.g. Bristol-Myers Squibb's BMS204352), trap free radicals (e.g. Daiichi's Ebselen), reduce cerebral inflammation (e.g. Pfizer's/Corvas's UK279276), or inhibit apoptosis. There is also enthusiasm regarding the potential use of diffusion-weighted imaging/perfusion imaging (DWI/PI) magnetic resonance imaging (MRI) to improve the design of clinical trials for neuroprotective agents.

The combined sales of acute and secondary preventative IS therapies are predicted to reach $1.5 billion in 2008 by Decision Resources.

Low-frequency ultrasound: possible new therapy for cancer?

Low-frequency focused ultrasound beams have been shown to stimulate apoptosis through the process of cavitation without associated biologically significant temperature increases or free radical generation[5]. High-frequency ultrasound (5–7 mHz) is already commonly used to destroy selected tissues but is always associated with significant rises in temperature.

Researchers from Angiosonics (Morrisville, NC, USA) used a frequency (20–100 kHz) normally used for diagnostics, which caused irreversible cell shrinkage, membrane blebbing, chromatin condensation, nuclear fragmentation and apoptotic body formation, but showed minimal DNA damage when applied to HL-60, K562, U937 and M1/2 leukaemic cell line cultures. The technique uses cavitation, a method of concentrating and controlling the diffuse ultrasound waves. This method works on the principle that sufficiently high levels of ultrasound creates waves in liquids that lead to the formation of cavities (or micro-bubbles), which then implode sending shock waves towards nearby cells.

Uri Rosenschein, Chief Medical Officer at Angiosonics said, 'Modulation of this pathway [apoptosis] is likely to form the basis for a wide range of medical applications including cancer treatment and anti-restenosis therapy.'

5 Ashush, H. *et al.* (2000) Apoptosis induction of human myeloid leukemic cells by ultrasound exposure. *Cancer Res.* 60, 1014–1020

Nitric oxide enhances anti-ulcer drugs

A nitric oxide (NO)-enhanced histamine H_2-receptor antagonist (NMI672) and a NO-enhanced proton pump inhibitor (NMI826) have been reported to be more efficacious in rats than their respective parent molecules, cimetidine and lansoprazole, respectively. These results were presented at the *Experimental Therapeutics/American Society of Biochemistry and Molecular Biology* meeting in Boston (MA, USA).

The research, carried out at NitroMed (Bedford, MA, USA), showed that over a seven-day period, NMI826 healed 90% of acid-induced gastric ulcers in rats compared with lansoprazole. Both compounds were said to accelerate tissue healing and prevent ulcer formation, thought to be caused by the ability of

NO to increase gastric mucosal blood flow and increase mucus secretion and gel thickness, as well as to prevent neutrophil endothelial adhesion.

McGill patent folate metabolism gene

The United States Patent and Trademark Office has assigned a patent for the Human methylenetetrahydrofolate reductase (*MTHFR*) gene[6] to McGill University (Montreal, Canada). MTHFR influences the levels of homocysteine and folates in the blood, two factors that affect the risk of developing cardiovascular and CNS diseases, and cancer.

The patent covers methods for determining MTHFR levels based on the genotype and methods for associating MTHFR deficiency with the risk of developing a variety of disorders. Rima Rozen at McGill University, who cloned the gene, has subsequently identified variant forms of the gene that affect folate metabolism.

It is hoped that such an association might lead to the development of specific genetic diagnostic tests. To help them, the university will receive financial support from Variagenics (Cambridge, MA, USA), with whom they signed an exclusive licensing agreement last year.

6 (McGill University) cDNA for human methylenetetrahydrofolate reductase. US6074821

Positive results with antisense therapy for non-Hodgkin's lymphoma

The antisense drug, Gentasense (G3139), when used together with cyclophosphamide, has been shown to eliminate normally fatal non-Hodgkin's lymphoma in SCID mice[7]. The team from Genta (Lexington, MA, USA) showed that the effects of the two drugs were synergistic, improving survival and eradicating the disease in SCID mice bearing Bcl-2 overexpressing systemic human B-cell lymphoma (DoHH2), as shown by histological and molecular examination. Furthermore, the study showed that reverse-matched or mismatched control antisense compounds had no effect on the disease.

Howard Fingert, Vice-President for Clinical and Regulatory Affairs at Genta said, 'It is especially noteworthy that the synergistic effects in this study were observed using less than half of the effective cyclophosphamide dose… confirming other *in vivo* studies in diseases such as melanoma, breast cancer and prostate cancer.'

7 Klasa, R.J. *et al.* (2000) Eradication of human non-Hodgkin's lymphoma in SCID mice by Bcl-2 antisense oligonucleotides combined with low-dose cyclophosphamide. *Clin. Cancer Res.* 6, 2492–2500

New prostate cancer susceptibility gene discovered

A novel prostate cancer susceptibility gene has been discovered by Myriad Genetics (Salt Lake City, UT, USA). The discovery, made using a proprietary form of linkage analysis, has highlighted inherited gene mutations that confer a significantly higher risk of developing prostate cancer. Scientists hope that this breakthrough will enable other genes in the prostate cancer developmental pathway to be investigated, and eventually lead to the development of a susceptibility test for at risk individuals.

Electronic microarray analysis technology patented

Nanogen (San Diego, CA, USA) have received two United States patents for an electronic microarray technology for use in molecular biological analysis[8] and separation of bioparticles by dielectrophoresis[9].

The first patent describes the use of certain cost-effective substrates and interconnect processes for performing electronically controlled biological operations. Meanwhile, the second patent covers the separation of a mixture of cellular materials in a sample using dielectrophoresis through a channel-less flow chamber. The separated cells can then be moved electronically to a bioelectronic chip where the cells can be lysed and the genetic contents analyzed.

8 (Nanogen) multicomponent devices for molecular biological analysis and diagnostics. US6068818

9 (Nanogen) channel-less separation of bioparticles on a bioelectronic chip by dielectrophoresis. US6071394

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