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News in brief

Targets and mechanisms

Could this be the end of malaria?

A new drug to help combat malaria has successfully passed the first stage of preclinical testing in rodents[1]. Researchers at Johns Hopkins University (Baltimore, MD, USA) designed the compound, carboxyphenol trioxane, and test results were announced at the *222nd American Chemical Society's National Meeting* (26–30 August 2001, Chicago, IL, USA).

Malaria infects between 300 million and 500 million people each year and causes 1.5–3.0 million fatalities. The strain of the malaria parasite that causes the most deaths is becoming increasingly resistant to current treatments, therefore, making the development of new drugs a pressing issue.

The new drug is water-soluble and so is easy to administer either intravenously or orally. After first-stage testing of the drug in mice and rats, it was found to compare favourably with another water-soluble malaria treatment that is currently under development at the US Walter Reed Army Institute of Research (Washington, DC, USA), which they claim also has a 'very good' therapeutic index. Both drugs are derived from the pharmacologically active component of a plant from the *Artemisia* genus, which has been historically used by the Chinese to treat malaria. A peroxide bond in this active compound reacts with metabolic products from the malaria parasite, generating oxidizing agents and carbon-free radicals that kill the parasite. This knowledge of the mechanism enabled researchers to screen for a derivative of this compound that had improved antimalarial characteristics.

The next step is to produce a large quantity of the carboxyphenol trioxane to start testing in larger animals and humans.

1 Posner, G.H. *et al.* (2001) Antimalarial simplified 3-aryltrioxanes: synthesis and preclinical efficacy/toxicity testing in rodents. *J. Med. Chem.* 44, 3054–3058

Long live chromosome 4

New research suggests that a locus on chromosome 4 is linked with exceptional human longevity[2]. One in 10,000 Americans are centenarians, topping the average life expectancy by 20 years, and centenarians are the fastest growing segment of the US population. The researchers from the Children's Hospital (Boston, MA, USA), Beth Israel Deaconess Medical Center (Boston, MA, USA), Howard Hughes Medical Institute (Boston, MA, USA), Whitehead Institute for Biological Research (Cambridge, MA, USA), Rutgers University (Piscataway, NJ, USA) and Centagenetix (Boston, MA, USA), discovered that many centenarians also have long-lived siblings. This suggests that genetic aetiology might be an important component of exceptionally long life. Scientists have long wondered what enables centenarians to maintain good health for so long. The authors of this study hypothesize that centenarians age slowly and either delay age-related diseases or avoid them altogether.

Previously, it was thought that as many as 1000 genes influence aging, and studies that looked for loci that might predispose people to longevity were limited to association studies of candidate gene polymorphisms. This study, however, involved a genome-wide scan for such predisposing loci using 137 sets of two or more exceptionally long-lived siblings. The results of a sibling-pair linkage analysis, using 400 markers spread along the entire genome, indicate that there is a gene (or genes) that exert a substantial influence on the ability to achieve exceptional old age. The study led them to chromosome 4, which contains between 100 and 500 genes. Now, further research is needed to identify specific longevity-related genes and then to study the biochemical pathways the gene(s) affect with respect to longevity and the aging process.

2 Puca, A. *et al.* (2001) A genome-wide scan for linkage to human exceptional longevity identifies a locus on chromosome 4. *Proc. Natl. Acad. Sci. U. S. A.* 98, 10505–10508

HIV resistance linked to genetic mutation

Individuals who carry one copy of a mutation in a specific gene could be partially resistant to HIV, the virus causing AIDS[3]. The study links the gene encoding the CCR5 receptor, which is found on the surface of T-helper cells, to a decreased risk of infection by HIV.

To enter a cell, the HIV virus must fuse with receptors on the cell surface. The Δ32 mutation in the gene encoding the CCR5 protein results in a defective receptor site that blocks viral entry. Individuals carrying two copies of this mutation, one from each parent (homozygotes), have no functional CCR5 receptors on their T-helper cells and appear to be resistant to HIV infection despite repeated exposure to the virus.

The study focussed on a large group of high-risk individuals who were HIV-negative. The group was followed for 18 months and tested every six months for HIV infection. The results showed that bisexual and homosexual Caucasian men who carried one copy of the mutation (heterozygotes) had a 70% reduced risk of HIV infection compared with individuals who had no copy of the mutation.

In a related study, a mathematical model was used to predict the prevalence of HIV epidemics among heterosexuals[4]. The model correlated CCR5 mutations with the spread of AIDS in entire populations.

The study compared two populations, one that was homozygous for the Δ32 mutation and the other that was a combination of individuals that were heterozygous for the mutation and homozygous for the normal gene (no mutations). The model used demographic data and initial values for infection from UNAIDS surveys conducted in Malawi, Zimbabwe and Botswana.

In the population with none or one mutation, the model showed that HIV prevalence increased logarithmically in the first 35 years of the epidemic, reaching 18% before levelling off. In the two-mutation population, the epidemic spread more slowly in the first 50 years and prevalence reached ~12%. Prevalence then began to decline after 70 years. Because HIV eventually kills its host, the number of individuals with the protective mutation tends to increase over time.

Denise Kirschner, a co-author of the study, suggests that the mutation could be more common in European countries because of widespread epidemics of smallpox and bubonic plague during the Middle Ages. Several studies have implicated the CCR5 receptor in the infection of these viruses.

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Another study highlighted the risks associated with incorrect use of antiretroviral drugs[5]. A theoretical model showed that although the number of drug-resistant cases of HIV has reached epidemic proportions in the gay community of San Francisco, it is mainly caused by inappropriate drug treatment rather than transmission of drug-resistant strains.

The study estimated that the number of drug resistant cases in San Francisco will rise from 3% in 1997 to 42% by 2005.

3 Marmor, M. *et al.* (2001) Homozygous and heterozygous CCR5-Δ32 genotypes are associated with resistance to HIV infection. *J. Acquired Immune Defic. Syndr.* 27, 472–481

4 Sullivan, A.D. *et al.* (2001) The coreceptor mutation CCR5 Δ32 influences the dynamics of HIV epidemics and is selected for by HIV. *Proc. Natl. Acad. Sci. U. S. A.* 98, 10214–10219

5 Blower, S.M. *et al.* (2001) Predicting the unpredictable: transmission of drug-resistant HIV. *Nat. Med.* 7, 1016–1020

Protein engineering and bioelectronic sensors

Researchers in the USA have developed a novel technology that will enable proteins to be engineered as sensitive 'bioelectronic' sensors for many chemicals[6]. The team of biochemists, led by Homme Hellinga from the Duke University Medical Center (Durham, NC, USA), demonstrated the ability of engineered proteins to detect, when attached to electrodes, a specific chemical from a mixture and to produce an electronic signal to indicate the identity and concentration of the chemical. Researchers have already been able to engineer proteins to determine the levels of glucose in blood serum, indicating the specificity of these proteins for compounds contained in a complex mixture.

The team of scientists started their research with bacterial periplasmic binding-proteins from the surface of bacteria, which are sensors for nutrients and toxin avoidance. These proteins thus have a hinge-bending mechanism, which is ligand-inducible, and it is this bending motion that is being exploited here. For example, the maltose-binding protein was engineered and tethered to a metal ruthenium group, so that any conformational change would produce a voltage. Thus, upon the addition of maltose, an electric current was produced that was proportional to the maltose concentration.

Hellinga says, 'For medical applications, you could imagine a multitude of sensors on a tiny chip that physicians could use at a patient's bedside to immediately determine from a drop of blood the concentrations of drugs, or metabolites such as glucose… Thus, with these biosensors… you would no longer need expensive laboratories and time-consuming equipment.'

6 Benson, D.E. *et al.* (2001) Design of bioelectronic interfaces by exploiting hinge-bending motions in proteins. *Science* 293, 1641–1644

Clinical trials

Clinical trials show drug reduces age-related blood vessel stiffness

The drug ALT711, a thiazolium-based compound developed by Alteon (Ramsey, NJ, USA), has been shown in clinical trials to reduce age-associated blood vessel stiffness[12], providing support for its potential use in the treatment of high blood pressure, heart failure and diabetic complications. After 56 days of treatment, arterial pulse pressure was significantly decreased when compared with a placebo group. Meanwhile, arterial flexibility and blood volume capacity was shown to have increased by 15%.

The study, conducted at nine clinical centers, involved 93 people over the age of 50 years who showed evidence of vascular stiffening, either by a systolic blood pressure of >140 mm Hg or by a pulse pressure of >60 mm Hg. No significant side effects were noted in the study. Participants were allowed to continue taking treatments for high blood pressure if they had begun at least four weeks before the study commenced and their regime was kept constant throughout the trial.

'Arterial stiffening is a major factor in many of the vascular diseases associated with advancing age,' said Edward Lakatta, co-author of the study and Chief of the National Institute of Aging's Laboratory of Cardiovascular Sciences (Bethesda, MD, USA). 'The significance of this drug is that it alters the properties of the arterial wall and makes it easier for the heart to eject blood into the vessels.'

Blood vessel stiffness is suspected to be caused by blood glucose reacting with the amino groups of proteins such as collagen and elastin to create restrictive crosslinks[13].

12 Kass, D.A. *et al.* (2001) Improved arterial compliance by a novel advanced glycation end-product crosslink breaker. *Circulation* 104, R8–R14

13 Whelan, J. (2000) Reversing age-related and diabetic cardiovascular disease. *Drug Discov. Today* 5, 272–273

Agouron loses partner in Remune study

The Immune Response Corporation (IMNR; Carlsbad, CA, USA) is to cease its collaboration with Agouron Pharmaceuticals (a Pfizer company; La Jolla, CA, USA) concerning the Study 202 of Agouron's immune-based HIV therapy, Remune. IMNR, who are a former corporate partner of Agouron, said the drug was highly unlikely to achieve its primary objectives: time to virological failure and conditional power (estimation of the likelihood of correctly determining a treatment effect). Although conditional power could be as great as 70%, it could also be as low as 40%.

The primary objective of the randomized, double-blind, adjuvant-controlled study was to determine whether the addition of Remune to a highly-active antiretroviral therapy (HAART) regimen of Viracept (nelfinavir mesylate) and Combivir delayed the time to virologic failure (failure to fall below or relapse above plasma HIV-1 RNA levels of 50 copies per ml).

Improving AD cognition with oestrogen

High doses of oestradiol have been found to enhance attention and memory for postmenopausal women with Alzheimer's disease (AD)[7]. The study conducted at VA Puget Sound Health Care System (Washington, DC, USA) showed significant improvements in attention (Stroop Color Word Interference Test), verbal memory (Buschke Selective Reminding Test), visual memory (Figure Copy/Memory) and semantic memory (Boston Naming Test) in

20 women with mild-to-moderate AD treated for eight weeks with a 0.10 mg day^{-1} 17β-oestradiol skin patch.

Women receiving oestradiol improved their performance in attention tests by 20%, verbal and visual memory by 35 and 30%, respectively, and semantic memory by 10% when compared with a placebo group. Previous studies have shown conflicting evidence for oestrogen having a beneficial effect on patients with AD. However, the researchers of this study suggest that the significant difference they noted here is a result of the use of oestradiol compared with other studies that used a combination of different types of oestrogen. Furthermore, they say that the largest study that showed no effect of oestrogen had only examined patients who had undergone hysterectomies and that this might be a factor in affecting the brain's response to oestrogen. The mechanism of action underlying an effect of oestrogen on the brain is still unknown although it has been suggested that it might increase acetylcholine levels, a substance that is depleted in AD patients.

'These results are hopeful, but they need to be confirmed with larger studies with more participants and longer treatment times,' said Sanjay Asthana, lead author of the study. Further studies will look at whether oestrogen is effective in preventing AD or delaying the onset of the disease.

7 Asthana, S. *et al.* (2001) High-dose estradiol improves cognition for women with AD. *Neurology* 57, 605–612

NMR predicts drug efficacy

Nuclear magnetic resonance (NMR) could help physicians to treat patients at risk of heart disease. Researchers at the Northwestern University Medical School (Chicago, IL, USA), the Centers for Disease Control (Atlanta, GA, USA) and North Carolina State University (Raleigh, NC, USA) are using NMR to identify which patients might benefit most from specific cholesterol-lowering drugs.

NMR uses radio waves to analyze the size and concentration of lipoproteins. Research has shown that the effect of cholesterol-lowering drugs varies in individuals because of lipoprotein variation.

The study, which will be published in a forthcoming issue of the journal *Atherosclerosis*, analyzed the effect of the cholesterol-lowering drug, pravastatin, over a six-month period in a group of 262 patients with high-risk heart disease. Results of the study showed reductions in the number of total and small low-density lipoprotein (LDL) particles and increases in the number of beneficial high-density lipoprotein (HDL) particles.

'By evaluating specific medications with the NMR technology, physicians will be better able to select cholesterol-lowering medications that will have optimal results for the patient depending on his or her lipoprotein size and concentration,' said Robert Rosenson, Director of the Preventive Cardiology Center at Northwestern University Medical School and a cardiologist at Northwestern Memorial Hospital.

Recent projections from new heart health guidelines estimate that 36 million Americans should be taking cholesterol-lowering drugs.

Cancer targets and mechanisms

The fingerprints of a killer

The key to understanding the relationship between gene expression and the future prognosis for men with prostate cancer has been determined. A recent study by scientists at the University of Michigan (UM) Medical School (Ann Arbor, MI, USA) has elucidated the genetic and molecular profile of prostate cancer[8]. Researchers at the UM's Comprehensive Cancer Center analyzed tissue samples from 50 men and found that almost 200 genes (or gene fragments) had expression profiles that varied consistently, depending on the state of the tissue (i.e. normal or malignant). Using microarray technology, the researchers were able to analyze thousands of genes simultaneously; this will become a powerful tool in furthering the molecular profiling of human cancers.

Prostate cancer is the most frequently diagnosed cancer in American men. Previously, screening for elevated levels of prostate-specific antigen (PSA) has been complicated by non-malignant conditions, such as benign prostatic hyperplasia (BPH). Since 1987, when the PSA assay became available, determining the best treatment for patients has not been easy. Now, signature expression profiles of normal adjacent prostate (NAP), BPH, localized prostate cancer and metastatic, hormone-refractory cancer have been determined.

Another important development is the recent work by scientists at the University of California, Berkeley (UCB; CA, USA), where microchip microcantilever technology was developed as a bioassay of PSA[9]. This novel technology involves 'biosensing' upon binding of PSA to the microcantilevers, which were fabricated from silicon nitride and are half the width of a human hair. The cantilever motion results from the free-energy change induced by specific biomolecular binding. The microcantilevers are coated with antibodies, to which the proteins bind, forcing one another apart and inducing the lever to bend downwards. The levers are also concentration sensitive, and the degree of deflection is measured using a laser.

'The technique is sensitive enough to detect levels [of PSA] 20-times lower than the clinically relevant threshold,' says lead author, Arun Majumdar, Professor of Mechanical Engineering at UCB. He continues, 'This could lead to fast screening and molecular profiling… and a possible cancer chip for detecting cancer.' The far reaching applications of this new technique include the possible detection of any disease that is characterized by protein or DNA markers in the blood or urine, such as breast cancer and AIDS.

8 Dhanasekaran, S.M. *et al*. (2001) Delineation of prognostic markers in prostate cancer. *Nature* 412, 822–826

9 Wu, G. *et al*. (2001) Bioassay of prostate-specific antigen (PSA) using microcantilevers. *Nat. Biotechnol.* 19, 856–860

Genetic model shows breast cancer to be a multi-step process

A study has shown for the first time that the genetic abnormalities associated with breast cancer occur not only in the epithelium, but in the surrounding stromal compartment[10]. Loss of heterozygosity (LOH) at various chromosomal arms is a technical indicator of the loss of a tumour suppressor gene and occurs at high frequency in breast cancer. Until now, this had only been studied in the epithelium because it is widely thought that the genetic events that lead to breast cancer occur only in this tissue.

Research led by Charis Eng, Director of the Clinical Cancer Genetics Program at Ohio State University (Columbus, OH, USA), has shown that this is not the case.

Using laser capture microdissection (LCM), Eng's team separated the neoplastic epithelium from the surrounding stroma in 41 sporadic, invasive adenocarcinomas of the breast. They identified frequent LOH in both the neoplastic epithelial and/or stromal compartments. A higher frequency of LOH was found in the neoplastic epithelial cells than in the stromal cells, which, according to the researchers, suggests that LOH in epithelial cells might precede that in stromal cells. Moreover, genetic alterations in chromosomal markers in the epithelium were found to have a statistically significant association with genetic changes in the stroma.

Charis Eng says, 'We have shown for the first time that genetic mutations can occur with some frequency in the stromal cells, too.' She continues, 'Before LCM, the study of cancer genetics entailed… examining a ''mixed bag'' of cells for alterations. We really couldn't attribute genetic changes to any one cell type. LCM allows us to do that.'

10 Kurose, K. *et al.* Genetic model of multi-step breast carcinogenesis involving the epithelium and stroma: clues to tumour–microenvironment interactions. *Hum. Mol. Genet.* (in press)

Daily alcohol consumption increases risk of familial breast cancer

Scientists have discovered a link between alcohol consumption in women and their risk of developing familial breast cancer[11]. A population-based study funded by the National Cancer Institute (Bethesda, MD, USA) was carried out at the Mayo Clinic Cancer Center (Rochester, MN, USA) to examine the relationship between alcohol intake and family history of breast cancer in 426 families with several generations of breast cancer sufferers.

Researchers evaluated whether alcohol was a greater risk factor for blood-relatives of breast cancer probands rather than for female relatives who married into the family. (A proband is defined as the first patient within a family study to whom all relationships are referred.)

Of the 426 families, first-degree relatives (mothers, siblings and children) of the proband daily alcohol drinkers had a significantly increased risk of breast cancer compared with those who never drank alcohol. However, this increase was not as evident either in second-degree relatives or marry-in relatives who drank alcohol daily. Similar results were obtained with an additional subset of 132 families with more than three breast and/or ovarian cancer patients within their family.

'Daily alcohol consumption over a woman's lifetime might be a greater risk factor for breast cancer in women who have a first-degree relative with breast cancer,' says Celine Vachon, principal investigator of the study. 'However,' she continues, 'these data are quite preliminary and we need longer follow-up time to better answer this question.'

11 Vachon, C. *et al.* (2001) Investigation of an interaction of alcohol intake and family history on breast cancer risk in the Minnesota Breast Cancer Family Study. *Cancer* 92, 240–248

Miscellaneous

Brands unprotected online

Few UK pharmaceutical companies are taking steps to safeguard their brand identities on the Internet despite a majority of them knowing it is important to do so, reported Marks & Clerk (London, UK) recently. Of the companies questioned, only 26% monitored and defended against online threats, such as cybersquatting, counterfeiting and copyright infringement, despite 64% knowing this could be done. Forty percent said they searched the Internet to ensure that their brand was not being misappropriated and had registered important trademarks as domain names in the countries in which they trade.

'Despite a range of high-profile cases of cybersquatting involving multinationals like Walmart and McDonalds… pharmaceutical companies are failing to ensure that they are adequately protected,' said Stephanie Loeffler, Partner at Marks & Clerk. 'A record number of cases involving alleged cybersquatting disputes were filed in May 2001 with the World Intellectual Property Organisation (WIPO, Geneva, Switzerland). While the two are often confused, there is no automatic legal correlation between a mark and a domain name.'

SNP Consortium forms alliances to enhance final product

The SNP Consortium will be collaborating with Celera Genomics and Applied Biosystems, and will gain the genotyping services of Motorola Life Sciences (Northbrook, IL, USA) in the final few months before the project to create a genome-wide, SNP-based human linkage map is completed. The data produced by the collaboration will be analyzed by the Laboratory of Computational Genetics, Department of Genetics at Rutgers University (Piscataway, NJ, USA). The linkage map is expected to be published at the end of the year.

'The SNP analyses that Motorola Life Sciences will deliver to the Consortium will provide key information and further our goal of constructing a high quality, highly annotated, and publicly available SNP map,' said Arthur Holden, Chairman and Chief Executive Officer on the SNP Consortium.

Approval of new cancer therapies dire in Europe

The current delay in waiting for new cancer treatments to be reviewed by the European Medicines Evaluation Agency (EMEA) has been suggested to be dire in a recent report by the Cancer Research Campaign (London, UK). Furthermore, cancer patients might have to wait at least two years for new legislation that is aimed at speeding up the approval of anti-cancer drugs.

The legislation, proposed by the EU Commission, could reduce approval times from 18 months towards the six months that it takes in the USA. Gordon McVie, the report's author and Director General of the Cancer Research Campaign says, 'Speeding up approval of anti-cancer treatments in Europe has to be a key priority. We need to look to America, where it takes half the time to approve double the [number of] treatments.'

McVie continued by pointing out that EMEA approval is only the start of the process that makes new treatments available to patients in Europe. 'Each new treatment also has to undergo lengthy bureaucratic national reimbursement procedures before becoming available for prescription… it could take at least two years to get these new proposals through the European parliament,' he commented.

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