The Newsletter for the Society for Medicines Research

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SMR Webcast and Website: Moving on through technology

The Millennial SMR Case Histories of Drug Discovery and Design conference, reported later in this newsletter (see page 6), was a tremendous success. There was huge interest before the event from people as far away as the Americas and Australia. Even locally, many unfortunately had to be excluded because of over-demand.

We found a solution to this problem — which breaks new ground for the SMR — by collaborating with the publishers Prous to make the proceedings available on the internet in order that those people unable to come could see and hear what was said. Prous have an ongoing initiative to produce webcasts of prominent scientific conferences which are currently made available free. Synchronised audio and visual versions of the symposium, together with written transcripts of the presentations are available.

This particular meeting is an apposite choice for the webcast because case histories of drug discovery are rarely published, and the importance of the meeting as an educational resource is evident.

On a related note, the SMR website has undergone some developments in recent times. First, it has moved to its own server, and the longstanding problem with redirection has finally been sorted out. Second, as reported last newsletter, there is a facility to register electronically for meetings, as well as to receive further information about events sometime in the future and to join the SMR. Meeting reports have for some time been routinely uploaded, but now in addition, this newsletter is also available in portable document format at the site.

In this new age, electronic communication is far cheaper for the SMR, more convenient for the recipient and more efficient in the use of natural resources. If you would like to receive your mailings by this means, simply let us know by e-mail to smr@iob.org, or by any of the more traditional means to our Secretariat.•

Related websites: http://www.socmr.org (SMR home page);http://www.prous.com/smr99 (webcast).

New Chairman for SMR

David Cavalla takes over as Chairman of the SMR. He is a synthetic organic chemist with pharmacological experience who has worked at the American NIMH, Glaxo (where he was involved in the discovery of ondansetron and sumatriptan) and Napp (where he worked on an asthma drug) before starting his own virtual drug development company, Arachnova — named after the web-building activity of the spider.

He follows his father, Dr John Cavalla, as SMR Chairman.•

Genomics: From the Cell to the Clinic

by Ray Jupp

The forthcoming March 2000 SMR meeting on Functional Genomics has been organised in recognition that following the unravelling of the sequence of the human genome, the next big question is: What does it do?

Over the last few years, many people have started to use the word 'genomics' in the same sentence with talk about a revolution sweeping through biology. What, you may ask is genomics? What has happened to create an environment in which a biological revolution can occur? Finally, and probably the most difficult question of all, what does it mean for the pharmaceutical industry?

Unfortunately, the word 'genomics' does not appear in even the most recent dictionary. Therefore, I will attempt to give you my definition: 'genomics is the study of many genes, simultaneously'. There are two aspects here, 'many genes' and 'simultaneously'. Let's take the 'many genes' part first. All Homo sapiens, contain genetic material which is comprised of about 100,000 different genes. It is these genes which make us human beings and not monkeys, sharks, trees or mushrooms. It is also the small differences in this same set of genes that are responsible for our individuality.

A global undertaking

Currently, there is a major global undertaking called the Human Genome Project to sequence or record precisely how each gene is put together. This project has already provided information on over half of our genes and possibly during this year a rough draft of all the genes (the whole human genome), will be complete. Here's where the word 'simultaneously' comes into the second part of the definition.

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Nuclear Receptors as Therapeutic Targets

by Alan Palmer

A large proportion of drug-discovery programmes target receptors localised on the plasma membrane. The Nuclear Receptors meeting, held in London on 16 September 1999, focused on the possibilities of targeting receptors that are present in the cytosol, which, when activated, move to the nucleus and modulate gene expression. A number of these nuclear receptors were considered in this meeting, including receptors for oestrogen, retinoic acid, vitamin D, glucocorticoids and thyroid hormone; the peroxisome proliferator-activated receptor (PPAR) was also considered. The biochemistry, physiology and clinical relevance of these receptors were described and prospects for new therapies considered.

Introduction

This symposium, organised by the Society for Medicines Research and chaired by Drs Mark Giembycz (Imperial College, London), Alan Palmer (Cerebrus Ltd, Winnersh) and Roger Horton (St George's Hospital Medical School, London), was attended by 60 participants from both industry and academia. The meeting focused on nuclear receptors and the new drug development possibilities they represent, together with aspects of mechanism of action of nuclear receptors and their role in both normal function and in certain disease states.

Nuclear receptors are members of a family of ligand-inducible transcription regulators for steroids and steroid hormones, as well as retinoids, vitamin D and certain drugs (such as the fibrate group of lipid-lowering drugs, which bind to the peroxisome proliferator-activated receptor). In addition, there are a large number of 'orphan' nuclear receptors for which the ligands have not yet been established.

The biology of the oestrogen receptor was described by Dr Malcolm Parker (Imperial Cancer Research Fund, London) in order to illustrate the general principles underlying the mechanism of action of nuclear receptors. The classical oestrogen receptor (ERα), like most other nuclear receptors, has both a DNA-binding domain and a ligand-binding domain. The receptor dimerises before binding to target genes and modulating transcription (transactivation). Fine adjustments to gene expression are effected by transcriptional control of the expression of other transcription factors, such as activator protein-1 (AP-1) or nuclear factor-κB (NF-κB). Thus ERα can transactivate AP-1- or NF-κB-responsive genes.

Oestrogen receptors

The role of oestrogen receptor variants in breast cancer was reviewed by Dr Valerie Speirs (University of Hull). A number of variant forms of the classic oestrogen receptor (ERα) have been identified in human breast tumours and breast cancer cell lines. Exon 7 and exon 4 deletion variants are common and over-expression or altered expression of such variants has been correlated with carcinogenesis and tumour progression. A second ER, ERβ, was cloned in 1996 and recent studies have also indicated variant forms of this receptor in normal and malignant breast tissue. Using nested reverse-transcription polymerase chain reaction (RT-PCR) with oligo-nucleotide primers spanning exons 4 to 7, 82 breast tumours were analysed for the presence of wild-type and variant ERβ. Co-expression of ERβ-deletion variants with wild-type ERβ was common; ERβ was detected in over 50% of samples. Sequence analysis of the deletion variants revealed that the deleted portion corresponds to the entire exon 5 of human ERβ. This corresponds to a portion of the ligand-binding domain. No direct associations were observed between expression of this deletion variant and clinical prognostic factors including tumour grade, node status and expression of ERα. It appears that, like ERα variants, ERβ variants are also common in breast tumours, although their functional significance remains unclear.

Retinoid acid receptor biology

The biology of retinoic acid receptors (RARs) was reviewed by Dr Christopher Redfern (University of Newcastle upon

Tyne). Retinoids have been used since the 1960s for the treatment of acne, psoriasis and premalignant skin lesions. Toxicity has limited their use, emphasising the need for new therapies.

Retinoic acid receptors were discovered over 12 years ago as a mechanism-mediating cellular responses to retinoic acid (RA). These receptors are closely related to thyroid hormone receptors and function as heterodimers with related receptors called retinoid-X receptors (RXRs). Three types of RAR (RARα, β and γ), and three of RXR (RXRα, β and γ), each encoded by separate genes, have been identified. The two classes of retinoid receptors (RARs and RXRs) have different ligand-binding properties: RARs bind all-trans and 9-cis RA, whereas RXRs bind only 9-cis RA.

RARs function as ligand-dependent transcription factors in the context of RAR/RXR heterodimers binding to recognition sequences predominantly consisting of two direct repeats (DR) or half-sites separated by five base pairs, and referred to as a DR5 RARE. As RAR heterodimer partners, RXRs play an important role in mediating RA responses at the level of gene expression, but may also affect other hormone-response pathways since they are able to form heterodimers with other nuclear receptors. In addition, RXR homodimer formation may be induced in response to 9-cis RA and, since RXR homodimers are reported to control transcription via DR1 RARE, this is an additional mode of gene regulation by RXRs.

The discovery of different classes of retinoid receptors and different types within each class has prompted a fruitful search for synthetic retinoid receptor type-specific and class-specific retinoids. A range of compounds which bind specifically to different RARs has been identified, and may act as RAR agonists or antagonists. These compounds are useful as tools to elucidate the molecular pathways of retinoid action and may have clinical applications as biological-response modifiers. In addition, compounds which show specifically for RXRs and act as RXR agonists or RXR antagonists have also been developed and show considerable clinical potential for disease therapy. However, as with many synthetic agonists/antagonists, many of these retinoid-receptor specific compounds are active at relatively high concentrations, and may have effects unrelated to their ability to bind RARs or RXRs.

This is illustrated by recent work on a RARβ/γ-specific retinoid fenretinide (McNeil Pharmaceuticals Inc). Fenretinide is an effective inducer of apoptosis in neuroblastoma cells, a property not shown (at least to the same extent) by RA. Although RARβ/γ antagonists block the ability of fenretinide to induce apoptosis, this may result from effects other than inhibition at a receptor level. Clearly, empirical studies on synthetic retinoids are important to elucidate the mechanisms of biological responses.

Retinoic acid and pulmonary alveoli

The role of retinoic acid in the formation of pulmonary alveoli was discussed by Dr Donald Massaro (Georgetown University, Washington DC).

Pulmonary alveoli are formed, in part, by the developmentally regulated subdivision (septation) of the large saccules that constitute the gas-exchange region of the architecturally immature lung. Administration of dexamethasone (DEX), a synthetic glucocorticosteroid to rats in the early post-natal period prevents septation; 'catch-up' septation does not spontaneously occur after treatment with DEX is stopped. The molecular signals responsible for septation are poorly understood.

On the basis that retinoids are key signalling ligands, all-trans retinoic acid (RA) was administered daily to rats from postnatal day three to day 13, the usual period of septation, and a 50% increase in the number of alveoli without an increase in lung volume was observed; treatment with RA also prevented the low number of alveoli and low body mass-specific alveolar surface area caused by treatment with DEX. Instilling elastase into the trachea of adult rats resulted in lowered lung elastic recoil, larger but fewer alveoli,

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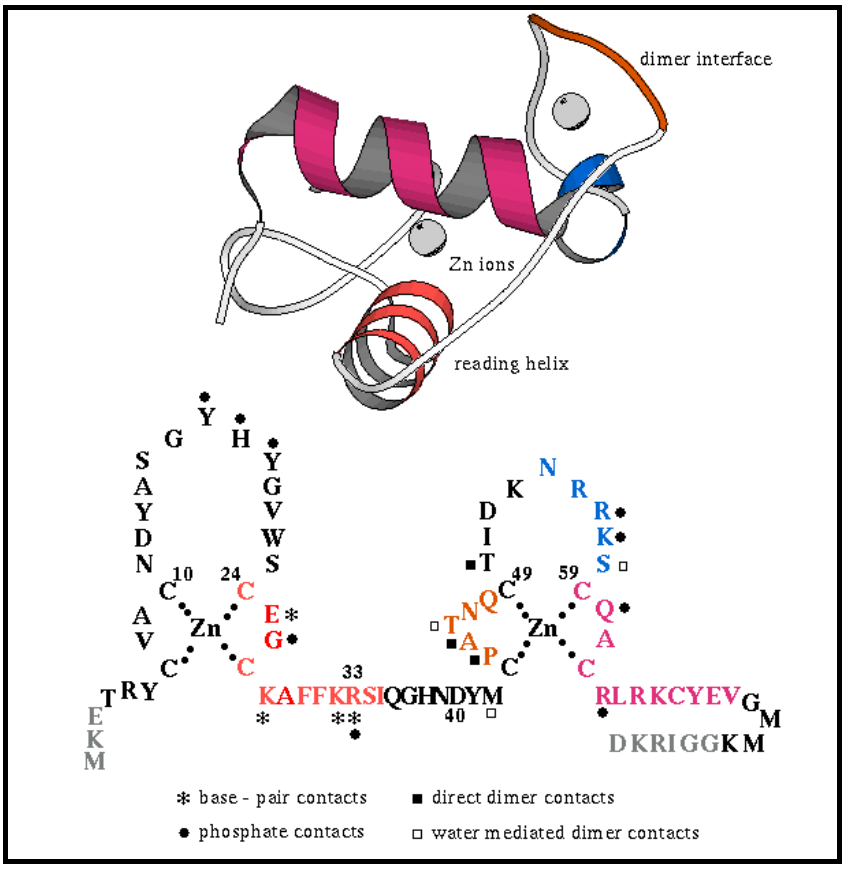

DNA binding domain of the oestrogen receptor (with acknowledgements to the Theoretical Biophysics Group, UIUC)

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and diminished volume-corrected alveolar surface area. Treatment with RA reversed these changes.

These findings support the possibility that, in individuals with too few alveoli for adequate gas-exchange, treatment with a pharmacological agent might provide remedial therapy.

Vitamin-D receptors

The molecular mechanisms of the selective action of vitamin-D analogues together with their therapeutic implications were reviewed by Dr Carsten Carlberg (University of Düsseldorf, Germany).

The nuclear hormone 1α,25-dihydroxyvitamin D_3 (VD), which is the physiologically active form of vitamin D_3, plays a key role in calcium homeostasis and bone formation. However, its ability to induce cellular differentiation and apoptosis and to inhibit cellular proliferation makes VD and its synthetic analogues a potential therapy of hyperproliferative diseases, such as psoriasis and different types of cancer. VD binds with high affinity to the nuclear vitamin D_3 receptor (VDR), which is a member of a super family of structurally related nuclear receptors that can act as ligand-inducible transcription factors. Thus, VD directly modulates transcription of those genes that have a functional building site for the VDR, referred to as a VD response element (VDRE), in their regulatory region.

Present evidence strongly suggests that VDR-RXR heterodimers are the major components in VD signalling, but this single type of heterodimer complex binds to several different VDRE types. These protein-DNA complexes are the molecular switches in VD signalling. The sharp biological profile of the model VD analogues EB-1089 (Leo Pharmaceutical Products Ltd A/S), namely, its high antiproliferative effect combined with low calcemic actions, has been correlated with the selectivity of EB-1089 to activate VDR-RXR heterodimers on VDREs that are formed by an inverted palindromic arrangement of two hexameric core-binding motifs spaced by nine nucleotides (IP9-type VDREs), rather than for VDRE that are formed by direct repeats with three intervening nucleotides (DR3). On each VDRE, two different functional conformations of the VDR can be differentiated and allow a more differential view on DNA-complexed VDR-RXR heterodimers.

The more ligand-sensitive VDR-RXR conformation gains, through EB-1089, a clearly higher affinity for DNA binding and provides a more sensitive activation of an IP9-type VDRE than of a DR3-type VDRE, whereas with the natural hormone VD, no VDRE-type preference is observed. This indicates that a promoter selectivity of VDR ligands is based on their property selectively to increase affinity for VDREs and very sensitively stabilise VDR conformations in VDR-RXR-VDRE complexes. Moreover, the analysis of the conformations of VDR in solution in comparison to those of DNA-complexed VDR-RXR heterodimers allows a differentiation between DNA-dependent and DNA-independent VD-signalling pathways that can be used for the identification of pathway-selective VDR agonists. Therefore, analysing the interaction of VD analogues with the VDR-RXR conformation presently appears to be the most informative method for an *in-vitro* evaluation of these analogues.

An update of the PPAR was provided by Dr Colin Palmer (University of Dundee). The PPARs are well-known targets for lipid-lowering and antidiabetic drugs. However, knowledge of the spectrum of action of these receptors is in its infancy.

Peroxisome proliferator-activated receptor

Initially, it was found that PPARα was the target for the fibrate group of lipid-lowering drugs. The toxicology of these compounds in rodents has been well characterised; however, their action in humans is quite distinct and poorly characterised. Another receptor, PPARγ, has been widely studied, owing to its interaction with the thiazolidinedione class of insulin-sensitising drugs. PPARγ has been described as a fatty-acid sensor in the formation of adipose tissue from skeletal muscle and fibroblasts and it is thought that this is where the insulin-sensitising function resides.

The tightest binding fatty acids to PPARγ are the ω-3 fatty acids such as the fish oils, all-cis-4, 7, 10, 13, 16, 19-docosahexanoic acid (DHA) and all-cis-5, 8, 11, 14, 17-eicosatetraenoic acid (EPA). DHA is a potent activator of PPARγ; however, EPA does not activate PPARγ and will block the action of DHA or rosiglitazone (SmithKline Beecham) in an adipogenesis assay. This is the first description of a natural antagonist of PPARγ and suggests that the characterisation of PPAR antagonists may be important for the formulation of improved pharmaceuticals.

PPARγ has also been shown to be an important regulator of cancer cell growth. Aromatic fatty acids have been found to bind and activate PPARγ at concentrations required to halt the growth of cancer cell lines. In addition, over-expression of PPARγ in cancer cell lines confers a greater sensitivity to aromatic fatty acids, demonstrating that PPARγ is a molecular target for these drugs. Non-steroid anti-inflammatory drugs (NSAIDs) have been studied in the treatment and prevention of colon cancer and it has been shown that several NSAIDs bind weakly to and activate PPARγ; however, the relevance of this to colon cancer remains unclear. It has also been shown that there are certain NSAIDs that have a high affinity for PPARγ and that some of these may have antagonistic activities towards PPARγ.

Glucocorticoid receptors

The mechanism of action of glucocorticoids was reviewed by Dr Ian Adcock (Imperial College School of Medicine, London). Glucocorticoids are the most effective anti-inflammatory drugs used in the treatment of chronic inflammatory diseases, such as asthma. They act by binding to a specific receptor that, upon activation, translocates to the nucleus and either increases (transactivates) or decreases (transrepresses) the expression of responsive genes.

In order to investigate the relative roles for transactivation and transrepression in the control of asthmatic inflammation, the ability of dexamethasone (DEX) to regulate interleukin (IL)-1β-induced gene expression, histone acetyltransferase (HAT) and deacetylase (HDAC)

activity was investigated. Low concentrations of DEX (10^{-10} M) repress IL-1β-stimulated granulocyte macrophage colony-stimulating factor (GM-CSF) expression and fail to stimulate secretory leukocyte proteinase inhibitor (SLPI) expression. DEX (10^{-7} M) and IL-1β stimulated HAT activity, but showed a different pattern of histone H4 acetylation. DEX targeted lysines K5 and K16, whereas IL-1β targeted K8 and K12. Low concentrations of DEX (10^{-10} M), that do not transactivate, repressed IL-1β-stimulated K8 and K12 acetylation. In contrast, RU-486 (Hoechst Marion Roussel AG) repressed IL-1β-stimulated HAT activity without upregulating K5 and K16 acetylation.

The activated GR complex acts both as a direct inhibitor of cyclic AMP response-element binding-protein (CREB)-binding protein (CBP)-associated HAT activity and also by recruiting HDAC2 to the p65/CBP HAT complex. This action does not involve *de-novo* synthesis of HDAC protein or altered expression of p300/CBP-associated factor (PCAF). This mechanism for glucocorticoid repression is novel and establishes that repression of histone acetylation is an additional level of control for inflammatory gene expression. This further suggests that pharmacological manipulation of specific histone acetylation status is a potentially useful approach for the treatment of inflammatory diseases.

Identification of the precise mechanism by which activated GR recruits HDAC2 may reveal new targets for the development of drugs that may dissociate the anti-inflammatory actions of glucocorticoids from their side-effects that are largely due to gene induction.

Dr Björn Vennström (Karolinska Institute, Stockholm) described how gene knock-out studies have helped elucidate the function of thyroid hormone receptor action.

Thyroid hormone receptor

Thyroid hormone (T3) has widespread functions in development and homeostasis, although the receptor pathways by which this diversity arises are unclear. Deletion of the T3 receptors TRαl or TRβ individually reveals only a small proportion of the phenotypes that arise in hypothyroidism. For instance, TRαl-deficient mice have cardiac-function abnormalities and low body temperature, whereas TRβ -/- mice have impaired hearing, a dysfunctional pituitary-thyroid axis of hormone control and abnormal regulation of 7-α-hydroxylase, a key enzyme in lipid metabolism. However, analyses of mice lacking both TRαl and TRβ (TRαl -/-β-/-) identified an array of phenotypes not found in single receptor-deficient mice, including an extremely hyperactive pituitary-thyroid axis, low female fertility, intolerance to cold and retarded growth and bone maturation.

These results establish that major T3 actions are mediated by common pathways in which TRαl and TRβ co-operate with, or substitute for, each other in some tissues. Thus, varying the balance of use of TRαl and TRβ individually or in combination facilitates control of an extended spectrum of T3 actions. Compared to the debilitating symptoms of severe hypothyroidism, the milder overall phenotype of TRαl -/-β-/- mice, lacking all known T3 receptors, suggests that T3-independent actions of T3 receptors, previously demonstrated *in vitro*, have a significant physiological role.

Summary

This meeting illustrated the increased understanding of nuclear receptors that has occurred in the last decade and highlighted a number of disorders that are associated with dysfunction of nuclear receptors. Linking structure to function has been greatly assisted by gene knock-out studies, illustrated here by studies of the thyroid hormone receptor. A clear link between nuclear receptors and human disease was illustrated by studies showing that oestrogen-receptor variants are common in breast tumours.

The increased understanding of the biology of nuclear receptors, together with a more detailed appreciation of the link between nuclear receptors and human disease serves to increase the opportunity for the development of new therapies based on the modulation of nuclear receptors.

Other exciting possibilities for new therapies derive from the number of orphan receptors that have been identified, the oldest of which is RXR. The development of RXR-specific ligands for its various heterodimeric partners shows considerable potential for the development of new medicines.•

Bottles of champagne to winners of SMR writing competition

For those who can still look a bottle of champagne squarely in the face after last month's celebrations, this picture is to celebrate the award of a bottle each to the winners of last newsletter's writing competition. Geoff Stemp presented these bottles to Julie Holder and Dave Nash at SmithKline Beecham's New Frontier Science Park on September 23rd last year. Unfortunately the runners-up were unable to attend.

That piece was the first in our *Viewpoint* series and dealt with the importance of education and training for the pharmaceutical industry. In continuing the *Viewpoint* theme, this edition's article deals specifically with genomics and the use of this new technology for drug discovery (see page 1). Further articles from readers are always welcome; please submit to the SMR Secretariat.•

Case Histories of Drug Discovery and SMR Award Meeting

by David Cavalla

The Society for Medicines Research millennial Case Histories of Drug Discovery and Design meeting took place on 2 December 1999, presented to an audience of 170. One reason for the popularity of the event was the interest in hearing the views of the opening speaker, Dr James Niedel of Glaxo Wellcome on the subject of pharmaceutical R&D in the next millennium.

Three trends are expected to shape the pharmaceutical industry. These are improved performance, value of medicines and social responsibility. The first topic relates to the attrition rate in pharmaceutical development. Only one in 10 drugs that enter preclinical development are successfully registered, and this rate has to date not changed as a result of new technology in drug discovery. There is a great need to improve the selection processes of late discovery in order to limit the number of drugs to fail in development, since these failures are so costly. Candidate selection is a product of the ability to create chemical diversity and to choose molecules with optimal properties. The current standard approach is to use rapid parallel synthesis and combinatorial chemistry, followed by robotic screening. At Glaxo Wellcome, the Combinatorial Lead Optimisation Programme (CLOP) has recently been introduced to measure and predict selectivity, physicochemical properties, absorption, metabolism, tissue penetration an carcinogenicity.

If the analogy of drug discovery with finding needles in haystacks is correct, we require smaller haystacks with more needles. Glaxo Wellcome's approach to this is through intelligent library design, involving an understanding of the properties of drugs and the ability to predict activity. However, it is also important to improve target selection, a process that is increasingly dependent upon knowledge of human genetics.

A good example of the power of genetics is in the study of Single Nucleotide Polymorphisms (SNPs), which represent a small proportion of our DNA but are critically involved in generating our uniqueness. Mapping of the three million SNPs within the three billion base pairs of human genome is being used to identify genetic associations with disease. A consortium of 10 major pharmaceutical companies is financing the work, co-ordinated by the Wellcome Trust, and public availability of the data is guaranteed by publication on the internet. Five academic institutions in the UK and US are generating the data. Susceptibility genes to a certain disease are determined by comparison of the SNP profile of a population without the disease to that of a matched population with the disease. For instance, in a piece of work that took four months to complete, three SNPs within a 10kb region on chromosome 12 show significant association with adult-onset diabetes. The expressed sequence tags (ESTs) and exons in the region have been predicted.

On the second subject, value of medicines, this is defined as perceived benefits less costs. For example, the costs of H_2 antagonists for gastrointestinal ulcers have been compared to the costs of hospitalisation for surgical treatment. This may result in a new approach to paying for drugs, in which patients are paying for quality of life. The pharmaceutical industry as a whole is devoting increasing effort to understanding value from the customer's perspective. Value can also be added with genetics, which can be applied to guide the prescription of the right drug to the right patient. Genetic analysis can permit the correct diagnosis of a disease; and SNPs may also be used where side-effects of a drug are associated with genotype; for instance, with Glaxo Wellcome's lamotrigine for epilepsy, 3% of patients get rash (1% severe rash), thereby limiting overall prescription rates.

Finally, social responsibility may not be a term naturally connected with the pharmaceutical industry, but in Dr Niedel's view, should become so. Glaxo Wellcome is supporting a substantial amount of basic research through partnership, for instance programmes for new vaccines at the Edward Jenner Institute, and into tuberculosis. Less well known is Glaxo Wellcome's donation of malorone for malaria in Africa. This is a useful drug for strains resistant to existing anti-malarials.

Dr Irene François presented the 1999 SMR Award for Drug Design and Discovery for Olanzapine (Zyprexa™) to (from left) Drs David Tupper, Terrence Hotten and Nick Moore of Lilly (UK).

Anti-inflammatory theme

Throughout the day there were three talks about new drugs for arthritis. The first of these concerned the development of **Remicade™ (infliximab)** from Dr Tom Schaible (Centocor). Infliximab is a chimaeric antibody to the pro-inflammatory cytokine tumour necrosis factor (TNF) α, which is produced by macrophages, endothelium and fibroblasts in inflammatory conditions. Significant efforts were made to maintain potency while reducing the immunogenicity of the antibody through humanisation. Infliximab is a 25% mouse (binding site region) and 75% human Ig G_1-type antibody, with very high K_a of $10^{10}\ M^{-1}$.

In the collagen-induced arthritis mouse model, the anti-TNF antibody

stabilised progression of both swelling and histological damage. In cultured human synovial cells, infliximab was demonstrated to reduce IL-1 production.

Clinically, the measure that was used to establish infliximab's efficacy was the ACR 20% response, the number of patients reporting 20% fewer swollen and tender joints, and a 20% improvement in other criteria such as pain and a global assessment. Trials with 1, 3 and 10mg/kg with or without methotrexate (MTX) showed a similar effect to MTX, but the durability of effect after dosing halted was better.

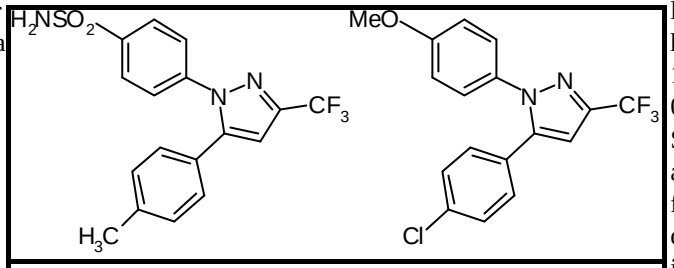
Subsequent trials used a dose of 3- or 10mg/kg with four- or eight-week infusion intervals on MTX-resistant patients. A clinical response was observed after 30 weeks, which could be quantified in terms of joint damage after 54 weeks' treatment. Results from extension of treatment to 102 weeks in terms of physical disability measurements are still due in 2000. Compared with MTX, infliximab at 3mg/kg at eight-week infusion intervals was more effective (20 vs 50% improvement).

Despite the clinical success with infliximab, a major problem is its cost, currently $10,000 per patient per year of dosing. This was one of the reasons for looking at another indication involving even greater patient suffering.

Crohn's Disease is a serious disorder for 500,000 patients in the US, approximately 70% of whom will eventually require surgery; 20–40% will develop fistulae (channels from gut to skin). It is neither medically nor surgically curable.

In a clinical trial of a single infusion of infliximab at 5mg/kg, a 70% decrease in Crohn's Disease Activity Index (CDAI), and a 70–80% response were observed. Clinical remission was observed in 39% of patients at two weeks and 48% at four weeks. Endoscopic examination showed reduced mucosal inflammation of the bowel. A second trial looked at healing of fistulae; infliximab produced complete closure of all fistulae in 40–50% of patients, and closure of at least half in 68% of patients. This represented the first drug to give such an effect in treating this horrible disease.

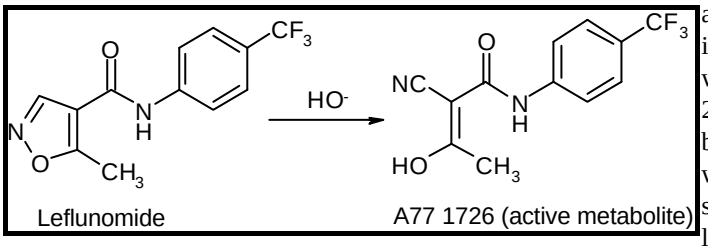
The second anti-arthritic was **Celecoxib (Celebrex™)**, a selective COX-2 inhibitor from Searle (Dr Timothy Maziasz). Non-steroidal anti-inflammatory agents (NSAIDs) have been a mainstay of anti-inflammatory treatment in arthritis for many years, and their mechanism of action has since 1971 been known to derive from inhibition of cyclo-oxygenase (COX), a key enzyme in the production of prostaglandins. However, some prostaglandins have beneficial effects such as supporting renal and platelet function; and in protection of the gastroduodenum.



Celecoxib (left) and its COX-1-selective analogue SC-560, used to prove the COX-2 hypothesis

In 1989, the presence of two types of COX activity was demonstrated. One was constitutive (COX-1), present in the stomach, intestine, kidney and platelets; the other was inducible by inflammatory cytokines, and found at inflammatory sites in macrophages, synoviocytes and endothelial cells. Glucocorticoids block the mRNA expression of COX-2.

In 1991 the second COX gene was cloned and this led in 1992 to a research programme at Searle to find a selective COX-2 inhibitor. The *in-vitro* model that was deployed for the initial screening of new compounds used a human cloned enzyme. Drugs such as naproxen and other standard NSAIDs are non-selective for COX-1/COX-2 enzymes.



Leflunomide A77 1726 (active metabolite)

The synthetic programme produced over 2,500 compounds, of which over 75% were screened *in vivo*. Over 280 compounds had an IC_{50} less than 0.1μM and over 340 were evaluated for oral activity. Seven compounds were taken into multi-species two-week toxicology, pharmacokinetics, process chemistry and formulation.

Celecoxib, the eventual development compound, was identified by random screening (from their agrochemical libraries). The enzyme inhibitory activity (Ki) was 15μM against COX-1 and 0.04μM against COX-2. Structurally, the sulfonamidophenyl group is a key feature, permitting time-dependent pseudo-irreversible block, by binding in a side-pocket of the enzyme's active site formed by conformational change. Interestingly, changing the sulfonamidophenyl group to a methoxy produces a COX-1 selective compound (COX-1: 0.009 μM; COX-2: 6.3 μM). This compound was used to prove the COX-2 hypothesis, since unlike the COX-2 selective compounds, SC-560 is neither anti-inflammatory nor analgesic.

There was an important issue with consistency of different *in-vitro* enzyme assays, which was overcome by using an *in- vivo* model of selectivity following oral dosing. COX-2 enzyme activity was determined from lavage of a carrageenan-induced air pouch, and COX-1 from the gastric mucosa of the same rodent. Using this model, celecoxib exhibited an ED_{50} of 0.2mg/kg for COX-2 vs. over 200mg/kg for COX-1.

Other *in-vivo* models used to determine efficacy included carrageenan-induced paw swelling and adjuvant-induced chronic inflammation. In the latter, the ED_{50} was 0.3mg/kg, whereas acute gastrointestinal toxicity (a COX-1-related effect) was observed only above 2,000mg/kg in the rat. The benefit of COX-2 selectivity was marked in the dog, since this animal is particularly sensitive to conventional NSAIDs. Naproxen or ibuprofen, for instance, are only tolerated in the dog at one-tenth of the human therapeutic dose. Celecoxib, on the other hand was tolerated at six times the human therapeutic dose (of 6mg/kg/day) in the dog.

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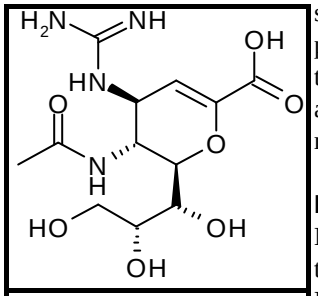
Celecoxib entered development 1994 and progressed rapidly. By May 1996, Phase-II efficacy had been carried out, Phase III was completed by December 1997, and the compound entered clinical use in January 1999. Beyond arthritis, there are a number of additional indications that are currently being targeted by COX-2 inhibitors. Trials are already under way in colon cancer and Alzheimer's Disease. COX-2 also plays physiological roles in female reproduction, renal activity and neuronal plasticity.

The third and final new anti-arthritic of the day was **leflunomide (Arava™)**, presented by Dr Uli Elben of Aventis.



Zanamivir (Relenza™)

The development story of leflunomide is unusual in a number of ways, the first being that it was discovered through an effect in an *in-vivo* rather than an *in-vitro* assay. In the adjuvant arthritis rat model, leflunomide had an ED_{50} on paw volume of 1mg/kg/day; it also reduced skeletal decay (by X-ray analysis). At the time of discovery, the mechanism of action was unknown, and it was not until 1995 that another company (Syntex) showed that leflunomide interfered with *de-novo* pyrimidine nucleotide biosynthesis. The biochemical basis for this effect was tracked to an inhibitory action on a mitochondrial enzyme, dihydro-orotate dehydrogenase (DHODH), which is involved in the conversion of dihydro-orotate to orotate in the uridine biosynthesis pathway. Activated lymphocytes are particularly dependent on pyrimidine synthesis as they lack an alternative pathway, and leflunomide therefore blocks T-cell clonal expression between the G_1 and S phase.

Leflunomide is much more potent in rodents than in humans (for example, IC_{50} to rat DHODH is 16nM, but 657nM to the human form), a complicating factor during development since the levels required to produce human effects were toxic in animals. Another complication was that the compound has an active metabolite (A77 1726).

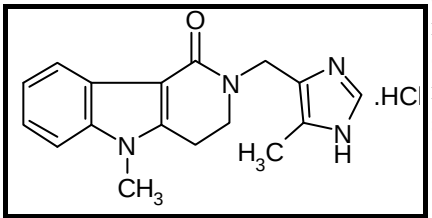
Clinical studies showed a long half-life of 14–16 days (due to enterohepatic recirculation), which was reduced with co-administration of cholestyramine. The long half-life led to a two-part dosing regime in the Phase-III studies of 100mg over three days (loading), then 20mg/day. This was part of a 12-month study in patients with rheumatoid arthritis for at least six months in comparison with methotrexate (MTX). There was little statistical difference in efficacy between the drugs, but quality-of-life studies do show significant improvement with leflunomide over MTX. In a further study in comparison with sulfasalazine, extended to 24 months, leflunomide showed significantly superior physiological function, ACR response rates and patient global assessment.

Influenza

Moving away from arthritis to infective diseases, Dr Rob Fenton (Glaxo Wellcome) presented the story of the discovery of Relenza™ (zanamavir) for influenza infection of the upper respiratory tract.

Influenza virus is transmitted in droplets as people sneeze, cough or talk, particularly in close contact. Sufferers are highly infectious from a few days before to 5–7 days after the appearance of symptoms. After an incubation period of 1–5 days, there is a rapid onset of fever, chills, cough, myalgia, malaise and anorexia. Fever (up to 41°C) can lead to viral pneumonia, and the seriousness of the disease is attested by 50,000–300,000 hospitalisations annually in the US, substantial absenteeism from school and workplace, together with the associated burden on families.

Biologically this is a negative-strand RNA virus, coated with haemagglutinin and neuraminidase. Viral replication occurs internally to the host cell, but disaggregation of viral particles requires the action of the enzyme neuraminidase.



Alosetron (Lotronex™)

Research into neuraminidase inhibitors started in the 1960s with compounds structurally related to sialic acid. Meindl and Tuppy (1969) developed a non-selective analogue but it was rapidly cleared by the kidney and produced no activity in animal models. In 1978 Graeme Laeve prepared crystals of neuraminidase and in 1983 Peter Colman and José Varghese produced 3D images by x-ray. This led in 1993 to the rational design and synthesis by Mark van Itzstein of potent inhibitors. These compounds were licensed by Glaxo Wellcome from Biota, Australia.

Zanamavir binds to neuraminidase three times stronger than the natural substrate sialic acid. The compound inhibits all standard influenza A and B viruses (IC_{50} 0.004–0.014μmol/l on A; 0.005 μmol/l on B). In addition there is a good profile to the development of resistance. A ferret animal model was developed, in which zanamavir suppressed the pyrexic response. Zanamavir is delivered using a dry-powder inhaler which permits good deep-lung distribution and very rapid action. Given at a dose of 10mg b.i.d. for five days, the compound was effective in the clinic in reducing symptoms by approximately 1–2.5 days. There was low systemic exposure and no increase in adverse events compared to a placebo.

Zanamavir is currently the topic of a great deal of debate about reimbursement. In Britain, there is concern about the lack of clinical data in high-risk populations. This group includes those with chronic respiratory or cardiovascular disease, the elderly (>65) and those who are compromised immunologically. There is an improvement in these patients, but because only low numbers have been tested (n = 89) this improvement has not yet reached statistical significance. Zanamavir is also potentially useful prophylactically, for instance in the family of a sufferer. There is a substantial expense associated with zanamavir, but flu is a disease which is, in many ways, costly to society.

Irritable bowel syndrome

The next case history concerns a second use for the $5\text{-}HT_3$ antagonist class of drugs — the first use was for cancer therapy-induced emesis. Professor Pat Humphrey (Glaxo Wellcome), who has pioneered research in the serotonin field and already been integral to the discovery of two new agents in this area (imigran and ondansetron) presented the story of the development of alosetron for irritable bowel disease.

The 5-HT_3 receptor is unusual in the serotonin family of receptors in that it is a cation channel rather than a G-protein-coupled receptor (GPCR). It was thought initially to be important in migraine, but the first clinical use derived from the finding that ondansetron, with a pK_b 8.6 for 5-HT_3 receptors, was anti-emetic in the ferret.

The therapeutic importance of serotonergic agents in gastrointestinal conditions has not been given substantial attention, despite the presence of the 5-HT_3 receptor on sensory nerves in the gut, and the fact that substantial amounts of 5-HT are released from enterochromaffin cells, via 5-HT_3-mediated vagal nerve transmission.

Irritable bowel syndrome is one of most common GI-related disorders, occurring in 15% of the population at one time or another. It is more often seen in females, which account for 70–75% of all cases, and characterised by abdominal pain or discomfort and altered bowel function. There are different kinds of IBS, but in the diarrhoea-predominant type, there is a postprandial increase in plasma 5-HT.

Glaxo Wellcome used a rat model in which a latex balloon was inserted in the colorectum of fasted males and inflated to cause rectal distension, leading to a hypotensive response. This was thought to be nociceptive reflex as it was blocked by opiates. The 5-HT_3 antagonists, such as ondansetron (ID_{50} of 18μg/kg), were also inhibitors.

Clinically, it has been found that alosetron affects not only bowel function, but also the pain severity and urgency in IBS. The pain component is thought to be a visceral allodynia. Alosetron has no effect on normal small intestine transit, but reverses egg albumin-induced increase in transit, a model of the diarrhoea in IBS.

Schizophrenia

Lastly, **olanzapine (Zyprexa™)** is the winner of the 1999 SMR Award for drug discovery, and a presentation was made to the three recipients (see photo). The story was told by Dr David Tupper.

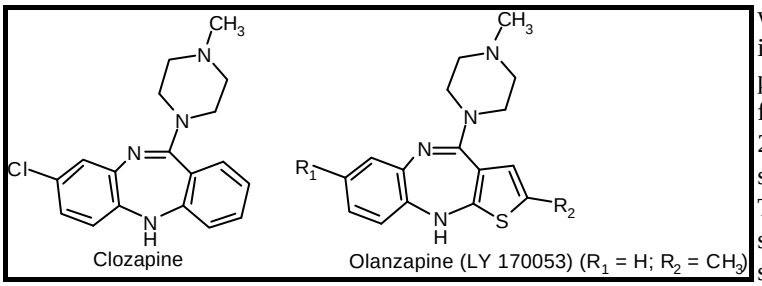
Schizophrenia one of most severe of mental illnesses affecting 1% of population before age of 45 (50 million worldwide) and characterised by three groups of symptoms:

- positive: delusions, hallucinations, bizarre behaviour and impaired communication;
- negative: social withdrawal, slowness of thinking, emotional blunting and lack of drive; and
- cognitive dysfunction: affecting memory, reasoning, etc.

The consequences of schizophrenia can be severe: suicide in sufferers is 10–12 times normal.

Typical antipsychotic agents such as haloperidol and chlorpromazine are dopamine antagonists, effectively treating positive symptoms but ineffective against negative or cognitive dysfunction. All have unpleasant mechanism-related side-effects such as extrapyramidal symptoms (EPS) and tardive dyskinesias (TD).

Market introduction in 1970 of the first atypical antipsychotic, clozapine, produced for the first time effective control of positive symptoms with a low incidence of EPS and TD and an effect on negative symptoms. However, agranulocytosis (depletion of white cells) in some patients led to its withdrawal in 1975. It was re-introduced in 1989 as second-line therapy, but is an expensive option as it requires blood monitoring.



Clozapine

Olanzapine (LY 170053) (R_1 = H; R_2 = CH_3)

Clozapine one of a series of diarylepines. Lilly's chemical approach included investigation of a series of thiophene isosteres for either of the phenyl rings in clozapine. Of the various isomeric possibilities, only the (1, 5) system gave activity in models of antipsychosis, of which the (2, 3-b) isomer had the best overall pharmacological profile.

Four members of the series went into development. Three were terminated: one due to granulocytopenia in dog (LY 120062; R_1 = F, R_2 = Et), another due to hepatotoxicity in man (flumezapine; R_1 = F, R_2 = Me), and a third due to increased cholesterol in the dog (LY 120363; R_1 = H, R_2 = Et). The fourth was olanzapine (LY 170053; R_1 = H, R_2 = CH_3) which progressed without the toxicological problems associated with the others. The structural chemical reasons for these problems remain unexplained.

In-vitro binding studies were unavailable for early studies, so behavioural studies were used instead. These included looking for a block of the conditioned avoidance response as an index of antipsychotic activity, and an induction of catalepsy as an index of EPS liability. The ratio between these two effects needed to be high. Based on this assessment, olanzapine was as se-lective as clozapine (whereas haloperidol was non-selective), but five times as potent. However, when analysed by binding studies alone, olanzapine had mixed effects against a number of dopamine, 5-HT and muscarinic receptors. Had binding studies been a primary method of selecting compounds, it is unlikely olanzapine would have been developed.

Clinical development initially involved five small pilot studies; these were sufficiently encouraging to undertake overlapping pivotal Phase-II trials. In four multinational trials over 2,500 patients were treated, some in excess of one year. The results of these studies showed that olanzapine was superior to haloperidol in treatment of positive symptoms, effective with negative symptoms and produced a low incidence of EPS side-effects, leading to superior long-term compliance. The drug was also effective in clozapine-resistant and intolerant patients. EU and US registrations were submitted simultaneously in September 1995, with approval a year later. By the end of Q3 1999, 3.5 million patients worldwide had received Zyprexa™, and sales had exceeded $3.5 billion). It is approved now in 87 countries and marketed in 78.

The SMR Case Histories Meeting is probably unique in the calendar, focusing in detail on the stories behind the research successes that led to new therapeutics. This year's programme featured talks on a number of very difficult-to-treat diseases, and the value of these case histories is the greater for that. In many ways the symposium is an educational resource, and the opportunity for it to reach a substantial number of people internationally through the Webcast is heartening for many in drug discovery.•

(continued from page 1)

Researchers who can study many genes simultaneously will be at a distinct advantage compared to those who can only study one gene at a time. The Human Genome Project is one programme aimed at acquiring knowledge of all the genes to facilitate such simultaneous studies.

I am sure you have guessed that the Human Genome Project is one factor contributing to this 'biological revolution'. What are the other factors? They include the new technologies that are beginning to allow us to study all 100,000 genes together. As you might imagine, there are several different technologies with interesting names such as genotyping, pharmacogenetics, micro-arrays or biochips, differential display and bioinformatics. Each of these technologies has a valuable contribution to make and most large pharmaceutical companies are actively building all of them into their research programmes. Some of these technologies are being established internally, while others are accessed through external alliances to increase rapidly range and depth of expertise.

DNA chips

DNA chips or micro-arrays, are a particular technology at hand. As with all good inventions they are based on a very simple idea; imagine a small square of thin glass about 1cm X 1cm. In your mind, overlay on this piece of glass a grid of approximately 100 X 100 smaller squares, rather like an extended miniature chess or checkerboard. Next, into the first square in the top left-hand corner of the checkerboard imagine stuck on to the surface a set of probes capable of detecting the presence or absence of one of our 100,000 different genes. The probes are short fragments of single-stranded DNA. In the adjacent square, imagine another set of probes stuck on to the surface capable to recognising a different gene, and so on until the entire checkerboard is full of unique gene probes. Currently, each biochip can detect between 7,000 and 10,000 different genes.

As discussed above, it is our genes that help make us who we are, in addition these same genes, if they become mutated, or turned on or off in the wrong place, cause diseases such as cancer, heart failure, stroke or asthma. Now you know what a biochip is, but what can it do? You may have had a friend or relative, or seen someone on television, undergoing surgery who had to have a biopsy or piece of tissue from a particular organ (prostate, breast, lung, etc.) removed for further analysis. In screening for diseases such as cancer, this analysis can determine if the tissue is benign or malignant, but will not tell the cause of the tumour. This is where the biochip comes in, a sample of the normal as well as tumour tissue is prepared in which all of the active genes in each sample are labelled with a fluorescent dye. Note that each tissue normally only expresses or has active a subset of the total pool of genes which is responsible for that tissue.

Next, the tumour preparation is coated on to the surface of the biochip and each of the active genes is given the opportunity to bind to the detection probes. The biochip is then washed to remove any excess label and scanned using a laser to measure the amount of fluorescent label bound to each set of probes on the checkerboard. The higher the fluorescence detected in each of the squares on the checkerboard, the higher the activity of that gene. The activities of each gene detected in the tumour sample can be compared to the normal sample. In this way abnormally active genes (turned on or off to a greater or lesser extent) in the tumour compared to the normal tissue can be identified. This is one example of the use of biochips, there are many others — including the identification of the genes regulated following drug therapy in an attempt to improve the way existing drugs work. This research is begining to reveal interesting differences in the patterns of gene expression.

The excitement of identifying the genes responsible for causing cancer, stroke or asthma is becoming evident in the pharmaceutical industry. One could imagine a small molecule screening programme to develop a drug that would modulate its activity or even replace it via gene therapy. In this way we can move from the cell to the clinic.

We have now taken the first step and like Aladdin, removed the stopper from the cell and many genes, or if you prefer 'genies', have come out, but which one will grant us three wishes?•

SMR Notes

DIARY

2–7 April 2000. Advanced methods in Pharmacokinetics and Pharmaco-dynamics: a one-week workshop, Sils Maria, Switzerland. Contact: Mrs Irene Sung, School of Pharmacy, University of Manchester, Manchester M13 9PL. Tel: 0161 275 2348; fax: 0161 273 8196; e-mail: irene.sung@man.ac.uk.

5–8 April 2000. 6th International Stockholm/Springfield Symposium on Advances in Alzheimer's Disease, Stockholm, Sweden. Contact: Ms Ann Ogden, Office of Continuing Education, Southern Illinois University, School of Medicine, PO Box 19230, Springfield, IL 62794-1218. Tel: +1 217 782 7711; fax: +1 217 785 4413; e-mail: aogden@wpsmtp.siumed.edu.

NEW MEMBERS

Cambridge Combinatorial Ltd: R.G. Boyle; *Celltech*: S. Brand, D.M. Devine, S.J. Francis, J.L. Fraser, T.J. Norman; *CeNeS Ltd*: I. Collie, P. Daum, M. Huckstep, D. Wu; *Glaxo Wellcome*: S.L. Hind; *Merck Sharp & Dohme*: S.K.F. Cheng, S.J. Lewis; *MSD*: J.G. Neduvelil; *Napp Pharmaceuticals Research*: P. Gharagozloo; *National Heart & Lung Institute, Imperial College*: M. Salmon, E. Renzoni; *Organon Laboratories Ltd*: D.J. Miller, J.M. Worrall; *OSI*: M.J. Procter; *Pfizer Central Research*: M.D. Andrews, J. Huggins, F.S. McIntosh, M. Rahelu; *Rademacher Group Ltd*: K.M. Elased; *Regal Group UK*: W.R. Pitcher; *Rhône-Poulenc Rorer*: I. McLay; *Royal Brompton Harefield Trust Hospital*: D. Garbis; *SmithKline Beecham*: G.F. Atkinson, M. Caltabiano, S. Coulton, P. Jeffrey, C.M. Sabido-David; *St Thomas' Hospital*: M.J. Collins; *University of Bath*: S.E. Abbot; *West LB Panmure Ltd*: E. Palmer.

NEWSLETTER PRODUCTION

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