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MEETING REPORT

Highlights from the Society for Medicines Research symposium* Challenges Facing Drug Discovery in Vascular Disease*, held September 30, 2005, at Organon Research, Newhouse, Scotland.

Challenges Facing Drug Discovery in Vascular Disease

by Phillip Cowley and Jason Witherington

Summary

The Society for Medicines Research symposium *Challenges Facing Drug Discovery in Vascular Disease* was held September 30, 2005, at Organon Research, Newhouse, Scotland. The conference brought together an international panel of speakers representing academia and the pharmaceutical industry to review approaches to the treatment of diseases affecting the vasculature. The focus of the meeting was on atherosclerosis and one of its clinical manifestations, stroke. The meeting reviewed current and emerging therapeutic approaches and improving technologies to monitor risk and disease progression in patients. © 2005 Prous Science. All rights reserved.

An international panel of speakers was brought together by The Society for Medicines Research's symposium on *Challenges Facing Drug Discovery in Vascular Disease*, held on September 30, 2005, at Newhouse, Scotland. The focus of the conference was to discuss approa-ches to treat diseases affecting the vasculature, and on atherosclerosis and stroke, in particular. Current and emerging therapies and the improvement of technologies to monitor risk and disease progression were highlighted.

Professor Chris Packard (University of Glasgow, Scotland) opened the meeting with an excellent presentation that covered present and future approaches in the prevention and treatment of vascular disease. Coronary heart disease (CHD) remains the major cause of morbidity and mortality in developed countries and will continue to be so as long as the population ages. For the many developing nations it is predicted that, in the near future, CHD will replace infection as the principal health problem.

The role of low-density lipoprotein (LDL) in the pathogenesis of atherosclerosis was discussed. It is now accepted that LDL levels need to be lowered in those at risk for CHD. The remarkable success of statins in clinical trials looking at primary and secondary CHD events prevention was used to indicate that the lower LDL levels fall the greater the benefit. Professor Packard suggested that it was safe to assume that statin therapy would be the universal first step in any treatment algorithm. In addition, the dawn of the 'statin plus' era was recognized with combination therapies with ezetimibe, fibrate and HDL-raising drugs specifically mentioned.

It is increasingly recognized that raising HDL levels is the next step, since this lipoprotein is established as a cardioprotective agent. This can be done in a number of ways, including by fibrates, by inhibitors of cholesterol ester transfer protein or by inducing cellular export of cholesterol. HDL structure, function and metabolism are more complex than that of LDL and the outcome of interventions cannot be predicted readily. Highlighting recent publications on antioxidant and antiinflammatory properties, it was suggested that HDL should be considered as 'molecular flypaper.'

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The final section of the presentation addressed C-reactive protein (CRP). Low CRP levels have been linked to a reduced risk of myocardial infarction and a reduced rate of progression of atherosclerosis. In addition, raised CRP levels have been shown to be linked to an increased risk of type 2 diabetes.

A new paradigm for lipid lowering therapy was discussed, in which atherosclerosis imaging played a key role in CHD prevention. Patients with an acute coronary event, diabetes or high global risk score (>3% per year) should be prescribed aggressive statin or statin 'plus' treatment with targets of achieving LDL <70 mg/dl (2.0 mmol/l), HDL >40 mg/dl (1.1 mmol/l) and CRP <1.0 mg/l. Low risk (<1% per year), asymptomatic patients should be advised on changes to diet and lifestyle. For patients in the medium-risk (1–3% per year) group imaging could be used to assess for subclinical atherosclerosis, prior to treatment as for the high or low risk groups as appropriate.

Dr. John Davies (Addenbrooke's Hospital and Cambridge University, UK) outlined the case to support the development of *in vivo* techniques that can identify and quantify plaque inflammation. Such techniques could potentially provide many benefits, namely identification of patients at risk of plaque progression and rupture, monitoring the effects of clinical treatments and the evaluation of novel therapies in preclinical and clinical studies. The researchers at Addenbrooke's have been developing 18-fluorodeoxyglucose (FDG) positron emission tomography (PET) to image and quantify atherosclerotic plaques. Initial studies with human tissue confirmed that FDG is taken up nearly exclusively by macrophages. This has further been substantiated by studies in New Zealand White rabbits where a statistically significant correlation has been established between plaque FDG and macrophage density. Furthermore, *ex vivo* studies using the same experimental model have clearly shown that FDG distinguishes between plaques that display features of instability and those of a more stable histological phenotype. However, the technique has so far been unable to make these distinctions *in vivo*, despite the use of a dedicated animal PET scanner. Some thoughts on why this has proved unsuccessful were discussed, and the three main hypotheses focused on inadequate special resolution leading to partial volume, signal-to-noise ratios and lack of anatomical detail leading to misplacement of ROI. In contrast, *in vivo* FDG-PET imaging of inflamed plaque has proved possible in humans. From studies where stroke had recently been diagnosed, it was reported that determination of uptake of FDG into carotid lesions was deemed possible. More recently, a study combining the power of PET and magnetic resonance imaging (MRI) has also been carried out. From this study it was demonstrated that in some patients, lesions that do not impede blood flow accumulate more FDG than lesions selected for surgical excision based on the associated severity of blood flow obstruction seen on x-ray angiography. These findings raise concerns over the current strategies regarding the selection of lesions for interventional therapy. Further studies have clearly demonstrated the power of FDG-PET in the identification of inflamed plaques in the aorta and coronary arteries.

Dr. Philippe Gervois (Institute Pasteur, France) presented an overview of the role of nuclear receptors in atherosclerosis. Activation of various nuclear receptors can lead to dyslipidemia and vascular inflammation which can ultimately lead to atherosclerosis. This activation, for instance of the peroxisomal proliferator activated receptors (PPARs), is a result of various ligands (fatty acids, fibrates, thiazolidinediones) binding to the receptor to induce the formation of a heterodimer with the retinoid X receptor (RXR). The second stage of this activation is the PPAR-RXR complex recognizes genes carrying its peroxisome proliferator activated receptor response element (PPRE). Activation of PPARα leads to diminished apo C-III production by inducing Reverbα expression. Activation of PPARα also increases LPL gene expression which leads to an increased degradation of triglyceride-rich lipoproteins. Activation of PPARα also leads to an increase in HDL, which has been shown to reduce cardiovascular risk. PPARα also increases ApoAI and ApoAII gene expression, and it has been shown from both the human ApoAI transgenic mouse and rabbit models that ApoAI protects against atherosclerosis. PPARα activation also induces ApoAV gene expression in human primary hepatocytes and, interestingly, data from an ApoAV knockout mouse suggest triglyceride levels are elevated. In adition, data from a human ApoAV transgenic mouse show that triglyceride levels are statistically lower. PPARα activators also induce cholesterol efflux from human macrophages and reverse cholesterol transport. Furthermore, it was reported that activation of PPARα has further effects on fibrinogen concentration, adhesion molecule production and prevents IL-6–induced acute phase response gene expression. Finally, it was reported that fenofibrate lowers C reactive protein in both dyslipidemic patients and dyslipidemic patients with coronary artery disease (CAD), while it also lowers IL-6 levels in dyslipidemic patients with coronary artery disease.

Dr. Bill Cairns (GlaxoSmithKline, Harlow, UK) presented on the challenges and emerging opportunities in the field of nuclear receptors. Following the finding that researchers were able to find the balance between desired pharmacology and potential liabilities in the area of selective estrogen receptor modulators (SERMs), workers at GSK sought to extend this finding to other members of the nuclear receptor family. The liver X receptor (LXR) α and LXRβ are nuclear receptors that bind to oxysterols and regulate the expression of target genes involved in cholesterol metabolism and transport (ABCA1),

inflammation (COX2, iNOS) and gluconeogenesis (PEPCK, G6P).

A first-generation LXR agonist **GW-3965** (Fig. 1) demonstrated antiatherosclerotic activity in ApoE-/- mice (10 mpk/day) as well as antidiabetic activity in ZDF rats comparable to known PPARγ ligands. Furthermore, GW-3965 demonstrated anti-inflammatory activity in antigen-challenged rats. Perhaps the most impressive finding is the GW-3965 displays efficacy in the DSS induced colitis model, where effects were seen in histopathological score and on the disease activity index.

While the efficacy of the first generated LXR agonists is impressive, they do possess a major liability through their ability to induce hepatic lipogenesis and increase LDLc. Indeed, *in vivo* profiling of GW-3965 (30 mpk, b.i.d., 7 days, normal diet) in hamster confirmed the hypothesis, with statistically significant increases observed in SREBP-1c mRNA, triglycerides, vLDLc and LDLc. In order to dissect the desirable pharmacology from the potential liabilities, researchers at GlaxoSmithKline sought to design LXR modulators employing structure-based drug

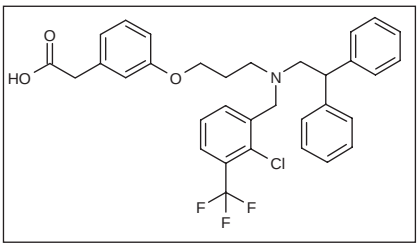


Fig. 1. First-generation LXR agonist **GW-3965**.

design. Analysis of several ligand-bound crystal structures revealed a mobile binding pocket (Fig. 2). Following the observation that the fairly rigid sulfonamide moiety of T1317 could be replaced by a benzyl group without significant loss in potency, researchers set about optimization of the new chemotype with the hypothesis that the LXR receptor pharmacology could be dissected through changes to the benzyl moiety.

Profiling compounds in an antiinflammatory activity efficacy assay (IL-6 secretion) and a liability assay (triglyceride accumulation) in parallel, researchers were quickly able to identify compounds that displayed excellent functional activity with minimal potential to increase triglyceride levels (Table I).

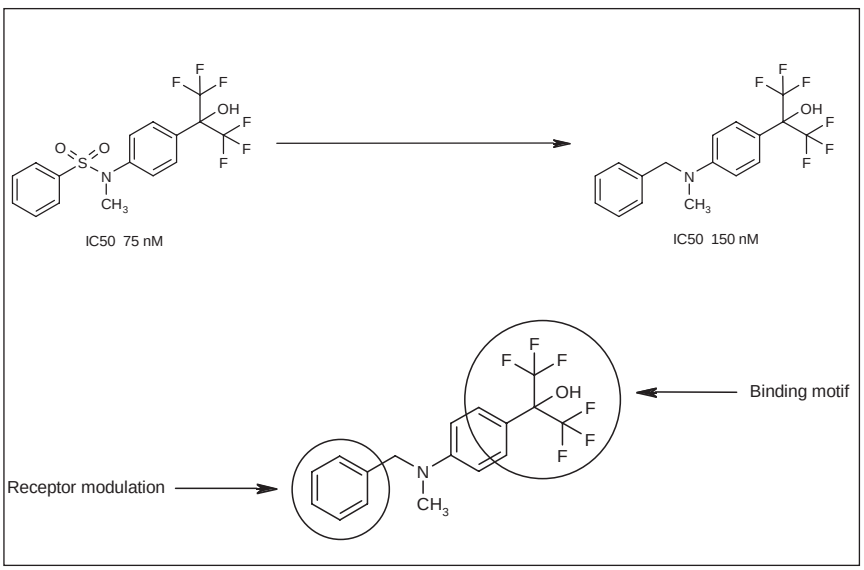
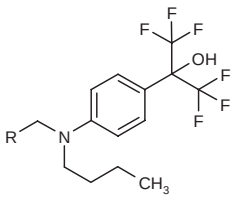


Fig. 2. Ligand-bound crystal structures revealing a mobile binding pocket.

Gratifyingly, the researchers were also able to obtain a ligand-bound co-crystal structure of the most potent selective compound disclosed (GSK 2186, 2.4 Å), which confirmed their hypothesis that while the compound retained the important interaction with H435, in contrast to the first-generation agonists, the benzyl group interacts with S278 in a new binding pocket.

Dr. Colin MacPhee (GlaxoSmith Kline, USA) reported on a novel approach towards the treatment of atherosclerosis. The current main therapy for atherosclerosis is the use of statins. While these are well-established agents they only reduce the risk of cardiovascular events by 20–40%, with the residual risk for high-risk patients considerable. Workers at GSK decided to target a secreted enzyme, lipoprotein-associated phospholipase A_2 (Lp-PLA_2), as a novel approach towards the treatment of atherosclerosis. Lp-PLA_2 is a calcium-independent serine-dependent PLA_2 that displays a preference for polar fatty acid substituents. Lp-PLA_2 is involved in the deposition and modification of ApoB-containing lipoproteins, rapidly cleaving oxidized phospholipids within oxLDL to generate proinflammatory mediators. Many of these mediators such as Lyso-PC have been shown to promote endothelial dysfunction through the inducement of adhesion molecules and chemokines. Furthermore, Lyso-Pc also attract monocytes, macrophages, T lymphocytes and has negative effects on smooth muscle cells. It has also been reported that inhibition of Lp-PLA_2 abolishes Lyso-PC generation without altering the kinetics of LDL oxidation. Upregulation of Lp-PLA_2 has also been demonstrated in atherosclerotic lesions and through microarray analysis of carotid plaques, Lp-PLA_2 was the only phospholipase A_2 upregulated. Epidemiology studies also suggest plasma Lp-PLA_2 also appears to be a strong and independent predictor of cardiovascular risk (stroke, myocardial infarction and CHD). Interestingly, medical genetics have also identified a polymorphism

TABLE I.

	BINDING		FUNCTIONAL	
	R	LXRα	LXRβ	IL-6	triglyceride
	2-HO-3-F-Ph	615 nM	120 nM	100 nM	++++
	2-HO-3,5-diCl-Ph	890 nM	290 nM	>500 nM	+++
	3-Cl-4,5-diMeO-Ph	165 nM	25 nM	10 nM	+

in the Caucasian population (5% homozygous) that appear to have a lower CV risk (Ala379Val). Initial studies appear to suggest the lower cardiovascular risk maybe due to the reduced enzyme kinetics of this polymorph. Work at GSK has identified two classes of Lp-PLA_2 inhibitors typified by **SB-222657** and **SB-480848** (Fig. 3). SB-480848 was selected for clinical evaluation and in patients undergoing carotid endarterectomy, presurgical dosing with SB-480848 dose dependently inhibited Lp-PLA_2 in the plasma and more importantly in the carotid plaque.

Professor Mat Daemen (Cardiovascular Research Institute Maastricht, the Netherlands) presented on the attractiveness of cathepsin K (CatK) as a drug target. CatK, a lysosomal cysteine protease, was identified in a gene-profiling experiment that compared human early plaques, advanced stable plaques and advanced atherosclerotic plaques containing a thrombus, where it was highly upregulated in advanced stable plaques.

In order to assess the function of cathepsin K in atherosclerosis, CatK-/- x ApoE-/- mice were generated. At 26 weeks of age, plaque area in the mice was reduced (41.8%) due to a decrease in the number of advanced lesions as well as a decrease in individual advanced plaque area. These results suggested an important role for cathepsin K in atherosclerosis progression.

Advanced plaques of CatK-/- x ApoE-/- mice showed an increase in collagen content and fibrous cap thickness, giving a more stable plaque phenotype. Medial elastin fibers were less prone to rupture than in ApoE-/- mice. Although the relative macro-phage content did not differ, individual macrophage size increased. *In vitro* studies using bone marrow-derived macrophages confirmed this observation. Scavenger receptor mediated uptake (particularly by CD36) of modified LDL increased in the absence of cathepsin K, resulting in an increased macrophage size due to an increased cellular storage of cholesterol esters, giving enlarged lysosomes.

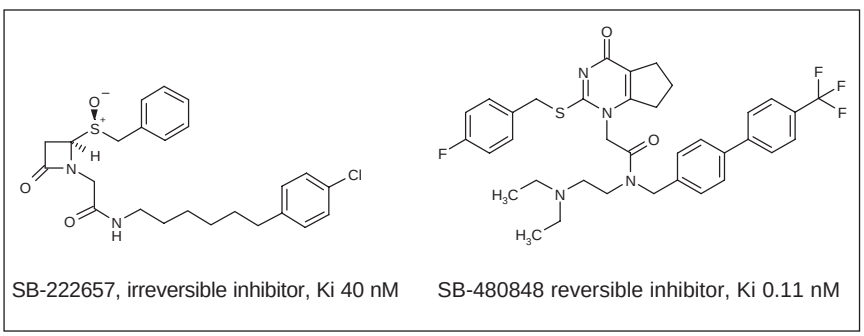


Fig. 3. Two classes of Lp-PLA_2 inhibitors.

Professor Daemen indicated that the cathepsins are not plaque-specific targets, being also present in bone, lung and bone marrow endothelial progenitor cells. Cathepsin K was noted to be an osteoclast gene, suggesting potential as an approach to the treatment of osteoporosis.

Dr. Hugh Marston (Organon Research, Scotland) introduced stroke as a theme for the remainder of the meeting, stating, "The history of the development of an effective, acute treatment for the neurological damage associated with stroke is characterized by preclinical promise followed by clinical disappointment." He then went on to look at this problem from a preclinical perspective, citing research carried out during his time at the Fujisawa Institute of Neuroscience at the University of Edinburgh.

"Stroke" covers a wide range of conditions that result from the interruption of effective blood supply to the brain. The consequent ischemia, if not reversed, leads to cell dysfunction and, ultimately, cell death. Following cardiac arrest, the whole brain is vulnerable to ischemic injury, whereas more limited damage is caused by interruption of flow within blood vessels in the brain. This can occur through rupture of a cerebral blood vessel (hemorrhagic stroke) or via occlusion of a blood vessel in the brain (ischemic stroke). Ischemic stroke is the most prevalent form of stroke and usually results from occlusion of the middle cerebral artery (MCA). It is because of this that most animal models of stroke involve occlusion of the MCA.

There are now many techniques available to occlude vessels in a range of experimental species. Occlusion leads to a pattern of damage that appears similar to the core and penumbra of human stroke. The time course of the vascular dynamics and development of the lesion can be manipulated. Functional deficits associated with different patterns of damage mirror those seen in man. Taken together this would appear to represent excellent validation for the animal models. As a consequence a wide selection of pharmacological approaches, with good mechanistic rationale, has been advanced for clinical development. These include: excitatory amino acid receptor antagonists, interleukins, immunophylins, nitric oxide-releasing molecules and neutrophins, to name but a few. Validation has included convincing evidence as to their intervention in the cascade of cellular events following an ischemic insult, efficacy when administered even several hours post insult, and effective functional protection. Yet, to date, all have failed when taken to the clinic. Instead, clinical progress has been through the development of preventative approaches, clot busting and improvements in rehabilitative care.

A case study was presented looking at both middle cerebral artery occlusion (MCAo) and anterior cerebral artery occlusion (ACAo) in anesthetized rats induced by stereotaxic application of 120 pmol endothelin-1 proximal to the respective arteries. The neuroprotectant properties of FK506 (1.0 mg/kg i.v.), administered after induction of the stroke, were assessed both by histological analysis and by behavioral testing in skilled motor and cognitive tasks.

In rats trained to perform the 'staircase test,' MCAo induction was followed by treatment with FK506 or vehicle. The animals were then reassessed in this test of lateralized skilled motor function 6–15 days after surgery. MCAo produced a profound and lasting impairment in performance in this task that was bilateral but skewed to the contralateral side. Acquisition of the task was also impaired. These impairments correlated with damage to cortical and corticipetal regions rather than the striatum. FK506 treatment attenuated the skilled motor deficit resulting from MCAo by 60%.

In a second model, the effects of ACAo in 'delayed non-match to place' (DNMTP) were assessed. Both acquisition and performance were impaired, with the acquisition deficit being attenuated by FK506 treatment 1 min after surgery. This cognitive deficit was seen to be the result of damage to midline structures. ACAo did not impair the staircase task and MCAo produced no impairment of DNMTP performance.

Dr. Marston summarized that our understanding of the pathophysiology of stroke has advanced solidly over the last decade. The importance of this being: reperfusion is now better understood; the interrelationship between neuronal, glial and other cell types is recognized as possibly key, as is the differential response in white and gray matter. On the clinical side better infrastructure and a realization of the importance of early referral are more wide spread. Animal studies are rarely able to indicate that neuroprotection will be possible with interventions more than 4 hours post infarct, yet the clinical reality is that referral times are rarely inside 4 hours. Perhaps just as importantly, preclinical work is now being performed in model systems that take into account the fact that stroke very rarely occurs in fit young adults. It is hoped that the ongoing reassessment of the complexity of the factors involved both for the preclinical scientist and the clinician will aid the rational development and realistic trial of effective treatments in the future.

Dr. Keith Muir (University of Glasgow, Scotland) looked at the results of stroke trials over the last decade, with a view to learning lessons for the future. He noted that the majority of drug treatments developed for acute stroke over this time frame have failed, with the notable exception of alteplase for the treatment of acute ischemic stroke.

Reasons for the failure were said to include poor understanding of animal model systems by clinical trialists, inadequate preclinical testing, drugs lacking in efficacy, dose-limiting drug toxicity and incomplete characterization of pharmacokinetics. In concurrence with Dr. Marston, the importance of rapid onset of treatment was emphasized. Failure to initiate treatment within 3–4 hours correlates with a markedly reduced chance of a good outcome, yet many trials have failed to provide treatment within this window.

A failure to appreciate the heterogeneity of stroke pathophysiology and mechanisms has also contributed to the failure of trials. This was highlighted as the probable cause of failure for MATCH and other trials of antithrombotic therapy for secondary prevention. Homogenization of populations by the use of brain imaging and the incorporation of imaging biomarkers as outcome surrogates in "proof of concept" trials were noted as factors in successful acute trials.

Recent phase II trials that showed potential for the future were recombinant activated factor VII for primary intracerebral hemorrhage, desmoteplase for ischemic stroke and the nitron free radical spin trap agent NXY-059, which looks like it could well be the first neuroprotectant to show clear efficacy in clinical trials for stroke. An analysis of aspects of these trials and their outcomes suggested that stroke trials are improving. The importance of imaging in entry criterion, classification and as surrogate markers of outcome was emphasized. Changes in the design of trials with shorter time windows,

more realistic sample sizes and better designed scales to define outcome were also noted for their positive contributions.

The webcast of this meeting can be viewed at: http://webcasts.prous.com/SMR_SEP_2005/index.asp.

Dr. Phillip Cowley is a Section Head in the Chemistry Department at Organon Research, Newhouse, Scotland, and Dr. Jason Witherington is a Senior Team leader within the GSK Medicinal Chemistry department of the Neurology and Gastrointestinal CEDD based at Harlow, UK. The SMR Committee organizes conferences on behalf of the Society for Medicines Research four times a year. These one-day conferences are multidisciplinary in nature and focus on various aspects of medicines research. Details of forthcoming meetings can be found at: http://www.smr.org.uk.

FDA ADVISORY COMMITTEE RECOMMENDS MORE DATA FOR EVEROLIMUS APPROVAL

The U.S. FDA's Cardiovascular and Renal Drugs Advisory Committee has recommended that more data be provided to substantiate the efficacy and safety of Novartis' *Certican*® (everolimus) for use as prophylaxis against acute rejection in heart transplant recipients. Data submitted to the FDA from a phase III, multicenter, international, randomized study demonstrated that patients treated with everolimus in combination with ciclosporin experienced a greater than 25% reduction in the incidence of treated acute rejection compared to those receiving conventional therapy. In a substudy, patients demonstrated a highly significant reduction in the incidence and severity of cardiac allograft vasculopathy (CAV). Everolimus 1.5 mg reduced progression of internal coronary artery wall thickening by 60%. This large prospective trial is the first successful superiority trial in heart transplant recipients, validating the significant impact of everolimus on progression of CAV in these high-risk patients. A majority of the Committee members agreed with the FDA and Novartis that everolimus has demonstrated superior efficacy to a comparator in the prevention of acute rejection. Several members of the Committee also recognized the potential benefits everolimus has demonstrated on the progression of cardiac allograft vasculopathy. However, the majority suggested that more prospective data on the everolimus/ciclosporin combination regimen was needed to determine the optimal dosage regimen in order to further enhance renal safety. The Committee also suggested that therapeutic drug monitoring would be a useful approach to address these issues. Novartis has initiated further clinical studies in heart transplantation using everolimus in combination with reduced-dose ciclosporin and therapeutic drug monitoring to supplement the existing clinical registration database from over 3,000 heart and kidney transplant recipients worldwide. Data from one of these studies, an ongoing European post-marketing study in heart transplantation which may be available for review in 2007, might address the committee's concerns. Certican is an orally administered investigational immunosuppressant described as an mTOR inhibitor. It is currently being evaluated for coadministration with *Neoral*® (ciclosporin) Modified, and appears to target many of the underlying causes of chronic allograft dysfunction or late graft loss. An NDA originally submitted to the FDA for indications in both kidney and heart transplantation in 2002 resulted in an approvable letter from the FDA in October 2003. Further analysis, and additional data submitted by Novartis in February 2004 led to a second approvable letter from the FDA in August of that year. In compliance with this approvable letter, two new prospective studies (one in each indication) using everolimus in combination with reduced doses of ciclosporin were initiated this year. A global, multicenter trial, aimed at further refining the use of everolimus in heart transplantation, began enrolling patients this month and was discussed during the Advisory Committee meeting. Another study, a phase IV commitment in Europe comparing everolimus with MMF in heart transplant recipients, was initiated in December 2004.