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MEETING REPORTS

Highlights of the Society for Medicines Research symposium held on September 21, 2006, at the National Heart and Lung Institute, London, U.K.

Therapeutic Approaches Towards the Treatment of Gastroinstestinal Disorders

by Steve Collingwood and Jason Witherington

Summary

The Society for Medicines Research gathered an international panel of speakers and about 60 delegates for their symposium September 21, 2006, on *Therapeutic Approaches Towards the Treatment of Gastroinstestinal Disorders*, at the National Heart and Lung Institute, in London, U.K. The focus of the conference was to discuss therapeutic strategies taken towards the treatment of inflammatory bowel disease, acid-related disorders and irritable bowel syndrome. Key note lectures addressed the development of tegaserod, a 5-HT_4 receptor agonist, for the treatment of constipation dominant irritable bowel syndrome (cIBS), the use of tumor necrosis factor α (TNFα) inhibitors in the treatment of chronic inflammatory diseases, including Crohn's disease, the development of effective inhibitors of gastric acid secretion, the role of $\alpha_4\beta_7$ integrin in the development of Crohn's disease and ulcerative colitis, the parts played by the neuropeptides ghrelin and motilin in the control of gastrointestinal motility, and the role of bacteria in functional gastrointestinal disease.

An international panel of speakers together with around 60 delegates were brought together by the Society for Medicines Research's symposium on *Therapeutic Approaches Towards the Treatment of Gastroinstestinal Disorders*, held on September 21, 2006, at the National Heart and Lung Institute in London, U.K. The focus of the conference was to discuss therapeutic strategies taken towards the treatment of inflammatory bowel disease, acid-related disorders and irritable bowel syndrome.

Dr. Pfannkuche (Novartis) delivered the opening lecture which detailed the research and development of tegaserod (*Zelnorm®*/*Zelmac®*), a 5-HT_4 receptor agonist which is currently being used in the clinic as a gastroprokinetic for the treatment of irritable bowel syndrome with constipation (cIBS) and chronic idiopathic constipation. The hypothesis proposed was that functional gastrointestinal (GI) disorders result from dysfunction in the enteric nervous system and/or the brain–gut axis. The key features that can lead to symptoms are GI hypersensitivity, altered GI motility and imbalanced intestinal secretion. IBS patients represent 4% of all primary care visits and 50% of all gastroenterology visits in the United States. The desire is to identify agents that treat the multiple symptoms of IBS wherein a successful clinical trial would demonstrate ~15% superiority over placebo. Current therapies for diarrhea-dominant IBS (dIBS) range from antidiarrheal agents such as loperamide and the 5-HT_3 receptor antagonist alosetron. Agents such as tegaserod, laxatives and soluble fibers are the current therapies used to treat cIBS. Meanwhile, antidepressants and spasmolytics have been used to treat both GI disorders.

While there are many receptor subtypes for serotonin (5-HT) the four main subtypes in the GI tract are

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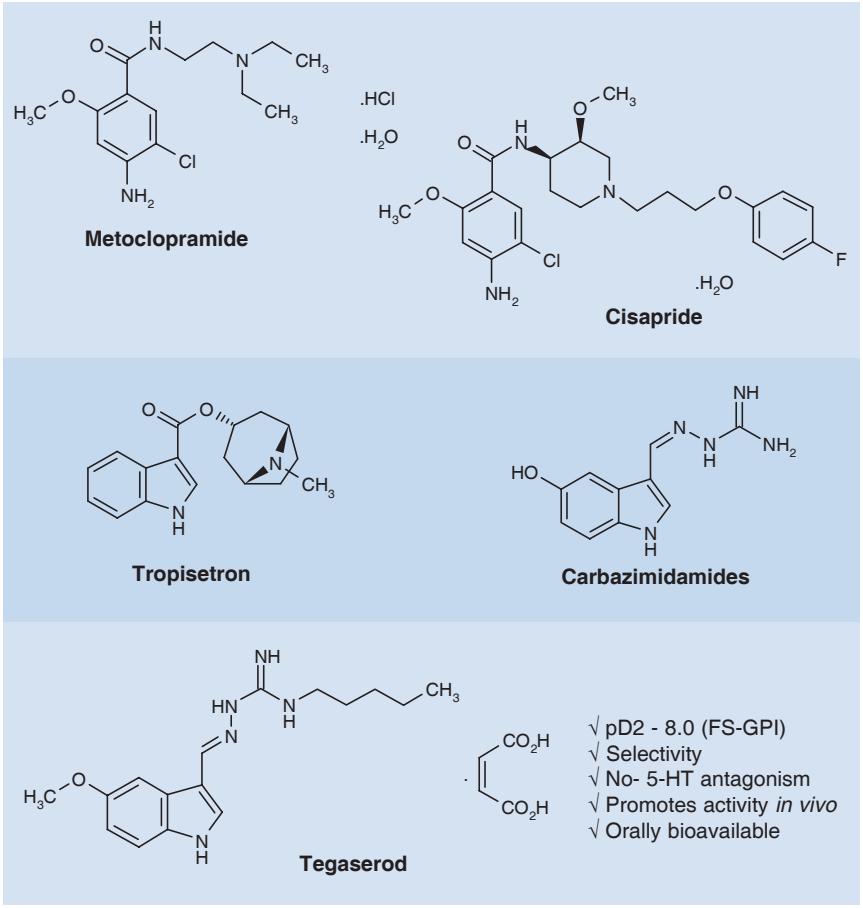
5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4. Furthermore, both 5-HT_3 and 5-HT_4 appear to be crucially involved in the regulation of smooth muscle tone, motility and water/electrolyte secretion. Early pharmacology studies around the 5-HT_4 receptor enabled the discovery of substituted benzamides (**metoclopramide**, **cisapride**) and indole carboxylates (e.g., **tropisetron**) as 5-HT_4 receptor agonists and antagonists, respectively. A new class of 5-HT_4 agonists classified as carbazimidamides displayed excellent potency (pD2 8.8, electrically stimulated guinea pig ileum) and indeed stimulated gastrointestinal motor activity in the dog at 0.01 mg/kg s.c. Unfortunately, this promising series lacked oral bioavailability.



However, chemical optimization of the indole moiety and alkylation of the guanidine group led to the team identifying **tegaserod**, which had excellent potency and selectivity while possessing oral bioavailability. Preclinically, tegaserod increased propulsion in both guinea pigs and dogs, stimulated postprandial GI motility in dogs and stimulated chloride/water secretion in rat colonocytes.

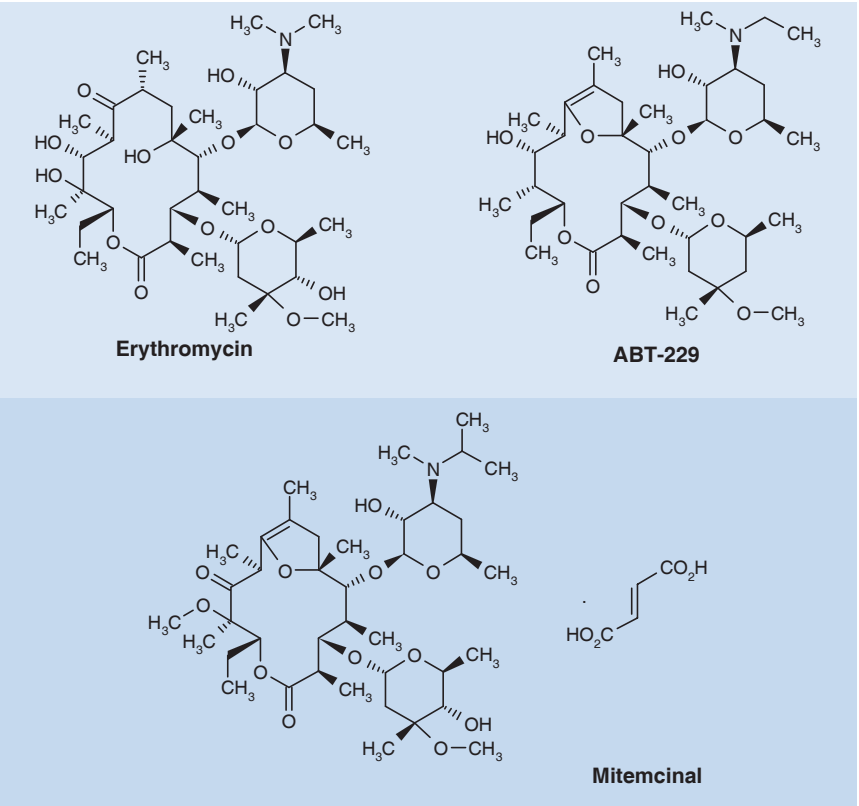
Tegaserod also demonstrated a dose-dependent inhibition of afferent nerve activity in a cat rectal distension visceral hypersensitivity model. Although the discovery of tegaserod started in the 1980s with the first synthesis in 1989, the development in the early 1990s was slowed by the uncertainty over its target indication, clinical endpoints and defining therapeutically relevant efficacy. Nevertheless, in a large IBS clinical program utilizing over 2,500 patients in 15 countries, tegaserod demonstrated a rapid and sustained improvement of IBS symptoms, in particular abdominal pain and discomfort, increased stool frequency and consistency. Importantly, the drug maintained efficacy over a sustained period with low discontinuation due to incidence of diarrhea. In 2002 the U.S. FDA approved tegaserod for cIBS, and in 2004 the FDA also granted approval for its use in chronic idiopathic constipation.

Dr. O'Mahony from University College Cork, Ireland, delivered the second lecture, which focused on the role of bacteria in functional GI disease. The average human being has between 1 and 2 kg of bacteria within the gut, consisting of over 500 commensal species, with the number and diversity of bacteria greatest in the distal GI tract. Preclinical research has demonstrated that germ-free animals have less mucosal cell turnover, digestive enzyme activity, cytokine production, mucosal lymphoid tissue, lamina propria cellularity, vascularity and reduced muscle wall thickness and motility. However, these animals possess more enteroendocrine cell (EC) area, caloric intake and an exaggerated hypothalamic–pituitary–adrenal (HPA) stress response. Interestingly, while incidence of infectious diseases such as tuberculosis, mumps, measles, etc., has declined rapidly since the 1950s, the incidence of immune disorders such as multiple sclerosis and Crohn's disease is on the increase. The rationale for the use of a bifidobacterium in IBS originates from the finding that it can accelerate oro-cecal transit, demonstrates antibacterial and antiviral effects, and has a role in immune modulation. Furthermore, bifidobacterium burden is suppressed in IBS patients. The rationale that IBS represents an inflammatory disorder stems from the finding that IBS patients have increased mast cells in the ileum and colon, display increased colonic mast cell degranulation and also have an increase in rectal ECs, IEL's (CD8), CD3 lymphocytes and IL-1β in the rectal mucosa. The early clinical trials with probiotics were often small, underpowered, poorly controlled in terms of IBS definition and used variable organisms and concentrations. The outcome of these trials was mixed, but in general the trend was

positive wherein improvements in symptoms such as flatulence and bloating were reported. O'Mahoney et al. reported the outcome of a study in 2005 wherein they demonstrated that while no effect on the composite symptom score (CSS) was found using lactobacillus, bifidobacterium (Bif) had a statistically significant effect in lowering the CSS over the course of the treatment period. Furthermore, a 4-week study using 362 females with IBS demonstrated that 1 x 10^8 of *Bifidobacterium infantis* had a significant effect on improving symptoms. Interestingly, increasing the bacteria exposure to 1 x 10^{10} failed to demonstrate efficacy over placebo. In order to understand this finding the researchers undertook a careful analysis of the capsule which is required to protect the bacteria from the acidity of the stomach. They observed that while the capsule containing the 1 x 10^8 bacteria took 10 minutes to rupture, the 1 x 10^{10} capsule took over an hour; and once ruptured its contents appeared to from a solid 'clump,' hence hindering dispersal of the bacterium. The 'bacterial hypothesis' for attenuation of the symptoms of IBS was also discussed. The molecular basis of cross-talk between enteric pathogens and the intestinal epithelium has been extensively characterized. While exposure to salmonella or TNFα leads to an increase in NF-κB activation, exposure to Bif 35624 has no effect on activation, suggesting that epithelial cells act as gut sensors. Furthermore salmonella, but not Bif 35624, stimulates IL-8 secretion and gene expression. However, O'Hara et al. have demonstrated that Bif 35624 co-incubation dose-dependently reduces salmonella-induced IL-8 response. Preclinically, it has also been demonstrated that probiotic feeding has a preventive effect against inflammatory bowel disease in the IL-10 knockout mouse, wherein a statistically significant lowering in interferon-gamma, IL-12 and TNFα was observed following dosing with bifidobacteria. In the T-cell transfer model of colitis (SCID) *Bifidobacterium infantis* also demonstrated a statistically significant effect on clinical score and myeloperoxidase levels.

Dr. Sanger (GSK) opened the afternoon session with a review of the role of neuropeptides in the control of gastrointestinal motility. Two peptides, ghrelin and motilin, have been shown to increase gastric emptying, while ghrelin has also been shown to increase food intake. Motilin, a 22-amino acid peptide found in endocrine cells of the upper small intestine is a powerful gastroprokinetic. It has been reported that motilin (10 $pmol^{-1}$ kg^{-1} min infused for 90 min) reduced the half-emptying time of solids in diabetic gastroparesis patients from 111 to 51 minutes. The peptide has been shown to act as an agonist on the G-protein–coupled receptor GPR38, now known as the motilin receptor. Interestingly, it has been shown that **erythromycin** also activates the motilin receptor, and a study by Quilgley et al. has reported some symptom response to the use of erythromycin (i.v.) in patients with severe gastroparesis. This early work has prompted many companies to explore the structural activity relationship of erythromycin with the aim to identify a gastroprokinetic that is devoid of the antibacterial properties. Abbott discovered **ABT-229**, which has reportedly failed in several clinical studies such as gastroparesis, functional dyspepsia and gastroesophageal reflux disease. However, a close analogue, **mitemcinal** (GM611) has been demonstrated to increase gastric emptying in patients with diabetic/idiopathic gastroparesis. The clinical failure of ABT-229 has prompted many to question the validity of the trial itself. Hypotheses such as tolerance to repeat dosing, incorrect dosage and the long half-life of ABT-229 have all been debated. Interestingly, in contrast to motilin, ABT-229 and erythromycin also display efficacy in rats, and this is particularly noteworthy since the motilin receptor is absent in rodents.

Recently, a related peptide, ghrelin, has also demonstrated a prokinetic activity in both healthy volunteers and patients with diabetic gastroparesis. Importantly, one study has reported improvements in symptom scores for pain and fullness in patients with



idiopathic gastroparesis. Preclinical reports have shown that ghrelin not only facilitates gastric emptying but also has an effect on appetite, inhibits emesis and can stimulate colonic motility and defecation. In 2005 Zhang et al. reported the discovery of obestatin, a peptide derived from the same ghrelin gene. Contrary to the appetite-stimulating effects of ghrelin, treatment of rats with obestatin suppressed food intake, inhibited jejunal contraction and decreased body weight gain. The authors postulated that obestatin binds to the orphan G-protein–coupled receptor GPR39. Several papers are now starting to appear questioning this ligand receptor pairing hypothesis; however, interestingly, it has been shown that GPR39 knockout mice have increased gastric emptying.

Dr. Brown (UCB) reviewed the use of TNFα inhibitors in the treatment of chronic inflammatory diseases, including Crohn's disease. TNF is a multifunctional cytokine produced by macrophages, neutrophils, and lymphocytes, mast cells, fibroblasts, endothelial and epithelial cells. Its normal role appears to be to activate and regulate both innate and acquired immune responses and so protect against infection. It also has a role in the development of splenic and lymph node architecture and germinal center organization. However, its overexpression has been linked to a wide variety of human diseases, including rheumatoid arthritis (RA), Crohn's, multiple sclerosis, diabetes, psoriasis, etc. Anti-TNF antibodies have made a large impact in several of these diseases. TNF mediates its various effects by signaling via the two different receptors, p55TNFR (TNFR1) and p75TNFR, (TNFR2)A. A significant amount of literature has accumulated examining the role of these receptors, and this work continues. There are distinct differences between the two receptors: p55TNFR is constitutively expressed on most cell types and tissues at a low level while p75TNFR is found only on hematopoietic cells, endothelial cells and some neural cell types, and is highly inducible. Both receptors have similar overall affinities (0.3 nM) for TNF and lymphotoxin-α, although p75TNFR is reported to have higher "on" and "off" rates. They can recruit different adapter molecules and signal through different but overlapping pathways. Currently, it is thought that TNF-induced trimerization of the receptors is required for signaling. Possible outcomes of receptor-mediated TNF signaling include apoptotic programmed cell death or necrotic cell death, gene activation by signaling via NF-κB/AP-1, etc., which can lead to proliferation, secretion of cytokines and expression of cell adhesion molecules. These responses may depend on the cell type and context, and it is now becoming clear that the overall outcome *in vivo* is a balance between these different effects and can be either pro- or antiinflammatory. It has been suggested that an inappropriate immune response to luminal bacteria may contribute to the development and maintenance of Crohn's. TNF was known to be a proinflammatory cytokine, and anti-TNFs were effective in animal models of Crohn's disease. Based on this, anti-TNFs were examined in the clinic, and in 1993 infliximab was first shown to induce profound clinical responses in Crohn's disease. Although infliximab is effective in Crohn's disease, its mechanism of action is not clearly understood. It neutralizes both soluble and membrane-bound TNF. It also induces the lysis of membrane TNF-bearing cells by apoptosis, antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). Another anti-TNF agent, adalimumab, has also been shown to be effective in Crohn's disease and can induce apoptosis. In contrast, several clinical trials with etanercept, surprisingly, demonstrated little efficacy in Crohn's, even though it also neutralizes soluble and membrane TNF and works in rheumatoid arthritis. Earlier studies suggested that it did not induce apoptosis, CDC or ADCC of inflammatory cells. These observations have led to the hypothesis that the ability to induce apoptosis is required for efficacy in Crohn's disease. A more recent anti-TNFα agent, certolizumab pegol, has recently shown efficacy in phase III clinical trials. Certolizumab pegol has no Fc fragment and cannot induce CDC or ADCC. In addition, it does not induce apoptosis of membrane TNF-bearing cells. The affinity of these four anti-TNF agents for soluble TNF has also been studied (Table I).

TABLE I.

	Ka (M–1s–1)	Kd (s–1)	KD
Etanercept	4.16 ± 0.29 e 6	1.39 ± 0.37 e –4	33.4 pM
Adalimumab	0.724 ± 0.30 e 6	1.14 ± 0.12 e –4	157.4 pM
Infliximab	1.01 ± 0.06 e 6	2.30 ± 0.34 e –4	227.2 pM
Certolizumab pegol	1.22 ± 0.09 e 6	1.09 ± 0.13 e –4	89.3 pM

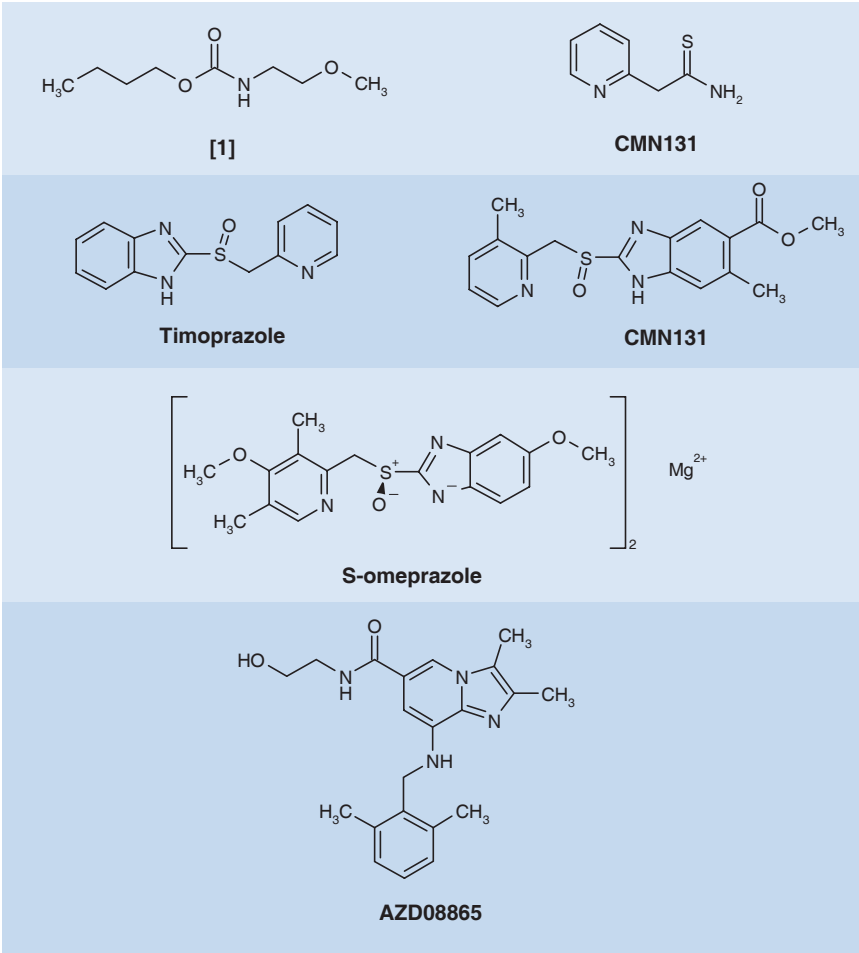
Further studies have been carried out with the four agents exploring their effect on CDC and ADCC of TNF6.5 cells. In contrast with etanercept, adalimumab and infliximab, certolizumab had no effect on CDC or ADCC; furthermore, it also has been demonstrated that it has no effect on apoptosis *in vitro*. However, unlike the other three antibodies, certolizumab displays excellent efficacy for inhibition of cytokine secretion, which is thought to be part of the inflammatory process in Crohn's disease. Interestingly, etanercept displays very little efficacy in this model, and this finding may go some way to suggest why it also performed poorly in the Crohn's clinical trial.

Dr. Andersson gave a highly entertaining lecture focusing on the trials and tribulations of developing effective inhibitors of gastric acid secretion. The introduction of the H_2 antagonists totally changed the treatment of peptic ulcer disease: firstly, cimetidine by SKF followed closely by ranitidine from Glaxo. Work in AstraZeneca started in 1966 with the aim to find a blocker of gastric acid secretion.

Project 1 (1966–1972) led to the identification of **compound [1]**, which

displayed excellent efficacy in rats but unfortunately none in humans. The second project was initiated in 1972 with **CMN131** as the lead.

This resulted in the discovery of **timoprazole** in 1974. Unfortunately, a 3-month rat toxicity study showed findings in the thyroid gland. While the second candidate, **picoprazole**, identified in 1976, also displayed toxicity findings in a 3-month dog study (intestinal vasculitis). However, some careful detective work indicated the problem was due to the strain of dog rather than the drug substance itself, since it could be seen that certain dogs in the placebo arm also developed vasculitis. Importantly, it was shown that the time to healing with a proton pump inhibitor (PPI) was superior to that of a histamine H_2 receptor antagonist. This certainly justified the discovery of the over six clinical candidates! Comparison of omeprazole with its single enantiomers certainly produced some interesting findings.



S-omeprazole produced a significant advantage in terms of inhibition of stimulated acid secretion in the rat compared to either the R-enantiomer or the racemate, although the exposure of the R-enantiomer, following oral dosing, was superior. However, following oral dosing in man, the plasma concentration of the S-enantiomer is higher while the R-enantiomer is lower than the racemate. This also translates to efficacy wherein the S-enantiomer outperforms both the racemate and the R-enantiomer. Interestingly, the clearance pathways have been elucidated and the S-enantiomer is metabolized by 2C19 (73%) and 3A4 (27%) while the R-enantiomer is predominantly cleared by 2C19 (98%). These mechanisms translate into lower clearance for the S-enantiomer (Cl_{int} 14.6 μL/min/mg) than the R-enantiomer (Cl_{int} 42.5 min/mg). Subsequently, the S-enantiomer was launched as *Nexium*™. Research for a K+-competitive inhibitor has been ongoing at the Mölndal research site since the mid 1980s since it had been shown that omeprazole possessed a slow onset of action, building up to steady state over 4 days and with a long duration of action, which may have been a concern given the potential issue with ECL cell carcinoids. Thus it appeared at the time that alternative compounds with attractive properties such as a faster onset but shorter duration may enhance the efficacy while mitigating any safety concerns. Five compounds were selected for clinical studies in man, and this ultimately led to the identification of **AZD0865**.

The mechanism of action of the PPIs is dependent on their weakly basic properties (pKa's ~4); hence they concentrate within the acidic environment of the parietal cell, wherein they are protonated and converted to the active, sulphonamide, form. Likewise, AZD0865 also behaves as a weak base with a measured pKa of 6.1; therefore it is also concentrated within the parietal cell wherein in its protonated form binds to the potassium-competitive proton pump. The reversible nature of the binding has been demonstrated *in vitro*, and it has been shown that AZD0865 also displays selectivity over the sodium–potassium ATPase. Inhibition of acid secretion in the dog was also determined, and AZD0865 had an ED_{50} of 0.25 μmol/kg (0.09 mg/kg). Furthermore a similar repeat dose of AZD0865 in the HP dog resulted in superior level of inhibition of acid secretion relative to omeprazole. AZD0865 was well tolerated in the clinic wherein a 65 mg dose resulted in about 14 hours control of stomach acidity (above pH-4). Noteworthy was the finding that a 45 mg dose of AZD0865 appeared to have better efficacy in terms of lowering intragastric pH when compared to a 40 mg dose of

esomeprazole. Given these promising findings AZD0865 was advanced into a GERD clinical trial. However, surprisingly, no difference relative to S-omeprazole was observed either in time to heal esophagitis or symptom relief; and the clinical advancement of AZD0865 was thus put on hold.

The final lecture of the day was delivered by Robert Egan of Millennium. The lecture focused on the role of $\alpha_4\beta_7$ integrin in the development of Crohn's disease and ulcerative colitis. The $\alpha_4\beta_1$ integrin is broadly expressed, binds to VCAM-1 and fibronectin and directs T-cell trafficking to many tissues including gut, brain and bone marrow. In contrast $\alpha_4\beta_7$ exclusively facilitates T-cell and B-cell trafficking to the gut, while the $\alpha_E\beta_7$ integrin directs T-cell trafficking within the gut. $\alpha_4\beta_7$ selectively binds to MAdCAM-1, which is selectively expressed in mucosal vasculature, highly expressed in gut-associated lymphoid tissue (e.g., Peyer's patches) and is upregulated on vascular endothelium in the gut in inflammatory bowel disease. Furthermore MEdCAM-1 is not expressed significantly on extragastrointestinal sites of inflammation. Anti-$\alpha_4\beta_7$ monoclonal antibodies (mAbs) block adhesion of $\alpha_4\beta_7$+ T cells and B cells to MAdCAM-1, and it has been demonstrated that antibodies to $\alpha_4\beta_7$ inhibit trafficking of lymphocytes to inflamed gastrointestinal endothelium in animal models. The attractiveness of targeting $\alpha_4\beta_7$ over $\alpha_4\beta_1$ or TNFα is that an $\alpha_4\beta_7$-specific mAb should not have the broader effects of a α_4 mAb (immune suppression, effect on cardiac development, and PML) or anti-TNF therapy. MLN0002 is a genetically engineered human IgG1κ monoclonal antibody created by insertion of the complementarity determining regions from the Act-1 mAb into a human IgG1 framework. MLN0002 contains two amino acid substitutions within the Fc receptor binding region that abrogate Fc receptor binding and complement fixation, and these mutations also successfully abrogate *in vitro* ADCC and CDC reactions. Preclinical studies have demonstrated MLN0002 to be a potent inhibitor of $\alpha_4\beta_7$ (IC_{50} 5.4 nM), which displays therapeutic activity with ACT-1 in colitis of cotton-top tamarins. No systemic immunosuppression with ACT-1 in rhesus monkeys was observed with MLN0002, and the toxicological profile in cynomolgus monkeys supported human evaluation. In a phase II ulcerative colitis study a third of patients achieved clinical remission (placebo 14%), and the tolerability profile of MLN0002 was acceptable. The responder rate might have been predicted to be greater; however, 44% of patients receiving MLN0002 developed anti-MLN0002 antibodies (HAHA) by day 57, and by day 180, 72% of treated patients developed HAHA. In a Crohn's disease phase II trial MLN0002 achieved its secondary endpoint for induction of remission in a phase with a 2 mg/kg dose. However, MLN0002 did not achieve its primary endpoint (as defined by reduction of CDAI score by 70 points).

The SMR Committee organizes conferences on behalf of the Society for Medicines Research four times a year, with a focus on different aspects of medicines research. Details about forthcoming meetings can be obtained from: the SMR Secretariat, 840 Melton Road, Thurmaston, Leicester, LE4 8BN, U.K. Tel: +44 (0)116 269 1048; Fax: +44 (0)116 264 0141;E-mail: secretariat@smr.org.uk; URL: http://www.smr.org.uk.