PDE4 inhibitors in COPD—a more selective approach to treatment

Antonio Maurizio Vignola*

Instituto di Biomedicina e Immunologia Molecolare, Consiglio Nazionale delle Ricerche; Ospedale V. Cervello, IT-90146, Palermo, Italy

Received 4 December 2003; accepted 11 December 2003

Summary Chronic obstructive pulmonary disease (COPD) is a serious and mounting global public health problem. Although its pathogenesis is incompletely understood, chronic inflammation plays an important part and so new therapies with a novel anti-inflammatory mechanism of action may be of benefit in the treatment of COPD. Cilomilast and roflumilast are potent and selective phosphodiesterase (PDE)4 inhibitors, with an improved therapeutic index compared with the weak, non-selective PDE inhibitor, theophylline. Unlike theophylline, which is limited by poor efficacy and an unfavourable safety and tolerability profile, the selective PDE4 inhibitors are generally well tolerated, with demonstrated efficacy in improving lung function, decreasing the rate of exacerbations and improving quality of life, with proven anti-inflammatory effects in patients with COPD. Theophylline is a difficult drug to use clinically, requiring careful titration and routine plasma monitoring due to the risk of toxic side effects, such as cardiovascular and central nervous system adverse events, with dose adjustments required in many patients, including smokers, the elderly and some patients on concomitant medications. In contrast, the selective PDE4 inhibitors are convenient medications for both patient and physician alike. Hence these agents represent a therapeutic advance in the treatment of COPD, due to their novel mechanism of action and potent anti-inflammatory effects, coupled with a good safety and tolerability profile.

Introducing...
that is not fully reversible. Airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases, in particular, tobacco smoke. COPD appears to be a multi-component disease, characterized by airway inflammation, airway obstruction, structural remodelling of the airway wall and mucus hypersecretion, as well as systemic changes, including systemic inflammation, nutritional abnormalities and weight loss, and skeletal muscle dysfunction. Of note, these components may lead to acute exacerbations of COPD, defined by increased symptoms and worsening lung function, which have profound effects on both quality of life and healthcare costs.

Current pharmacological therapies for the treatment of COPD have limitations, particularly in relation to reducing the progression of the disease. Given the multi-component nature of COPD, multiple therapies will probably be required to treat it and one strategy may be to use current treatment options in combination. However, since chronic inflammation is a critical underlying factor in the development of COPD, new therapies with a novel anti-inflammatory mechanism of action may provide additional benefits. One potential candidate is the selective phosphodiesterase (PDE)4 inhibitors, which have proven anti-inflammatory effects, coupled with an improved therapeutic ratio versus existing non-selective PDE inhibitors.

In this article, I describe the significant role that inflammation appears to play in the pathogenesis of COPD, the impact of PDE4 inhibitors in reducing inflammation via increased accumulation of the cyclic nucleotide, adenosine 3',5'-cyclic monophosphate (cAMP), and the potential advantages of second generation, selective PDE4 inhibitors (e.g. cilomilast and roflumilast) over first generation compounds (e.g. rolipram) and non-selective PDE inhibitors (e.g. theophylline) in the treatment of COPD.

**Mechanism of chronic inflammation in COPD**

Although the processes underlying COPD are not yet fully elucidated, chronic inflammation appears to have an important part (Fig. 1). Of note, substantial differences are apparent compared with the inflammatory mechanisms involved in the development of asthma. Inflammation plus structural alterations occurring in the small airways and lung parenchyma, such as fibrosis, smooth muscle hypertrophy, goblet cell metaplasia and luminal occlusion by mucus plugging, are major contributors to the airflow limitation and accelerated decline of forced expiratory volume in one second (FEV1) observed in COPD. Key inflammatory cells implicated in COPD pathophysiology include neutrophils, macrophages and T cells (predominantly, CD8+ cells). In addition, structural cells, e.g. epithelial cells, fibroblasts and smooth muscle cells, participate via the release of a variety of mediators, including cytokines, chemokines and growth factors, such as leukotriene B4 (LTB4), interleukin (IL)-8 and tumour necrosis factor (TNF)-α. It has been proposed that an imbalance between pro- and anti-inflammatory cytokines may be involved in the process of neutrophil accumulation, for example, IL-8 and TNF-α levels are elevated. Together, airway inflammation and remodelling may also lead to degradation of the lung parenchyma, most likely mediated through release of proteolytic enzymes (such as elastase) from inflammatory cells, resulting in emphysema.

Degradation of cAMP by the PDEs (in particular, PDE4) has been hypothesized to be an important target to reduce the chronic inflammation, that is, characteristic of COPD. cAMP is a secondary messenger involved in signal transduction in a range of cellular processes, including cellular growth, sensory signalling, neuroplasty and transcription, as well as inflammation. Increases in cAMP levels result in activation of protein kinase A (PKA) and enhanced protein phosphorylation, which in turn leads to inhibitory effects on many inflammatory and immunomodulatory cells. These effects include relaxation of airway smooth muscle, inhibition of chemotaxis, abnormal release of inflammatory and cytotoxic mediators and inhibition of proliferation (as well as infiltration).
of inflammatory cells. Intracellular cAMP levels are regulated by a number of factors, notably by the PDEs, which catalyse the degradation of cAMP. The PDE superfamily consists of at least 11 isozymes, which vary in their distribution among different tissues/cell types (Fig. 2). The predominant isoenzyme in inflammatory and immunomodulatory cells, PDE4, is expressed in many airway cells involved in the pathogenesis of COPD, including neutrophils, macrophages, T cells and endothelial cells (Table 1). Hence, since PDE4 inhibition has the potential to produce significant anti-inflammatory and disease modifying effects by increasing accumulation of intracellular cAMP, PDE4 represents a promising molecular target for the treatment of COPD.

PDE4 inhibitors—mechanism of action and benefits over theophylline

In spite of their collective effects on PDE inhibition, there are a number of important differences between the mechanisms of action of the non-selective PDE inhibitor, theophylline, and that of the selective PDE4 inhibitors (Table 2). Furthermore, second generation compounds have significant benefits over the first generation PDE4 inhibitors.

Theophylline

Theophylline, a broad spectrum PDE inhibitor, has been used to treat pulmonary diseases for over a century. It has established anti-inflammatory and immunomodulatory effects, possibly mediated by inhibition of the degradation of cAMP, although PDE inhibition may be negligible at therapeutically relevant concentrations of theophylline (see below). However, although the mechanism of action of theophylline remains unclear, it appears to have a range of distinct features compared with the selective PDE4 inhibitors.

Firstly, theophylline is a weak and non-selective PDE inhibitor, which indiscriminately inhibits all PDEs in all tissues and organs of the body, including the gastrointestinal, cardiovascular and central nervous systems (Fig. 2). Hence, since PDE4 inhibition has the potential to produce significant anti-inflammatory and disease modifying effects by increasing accumulation of intracellular cAMP, PDE4 represents a promising molecular target for the treatment of COPD.

---

**Figure 2** Tissue distribution of human PDEs, adapted with permission from Essayan.

**Table 1** cAMP-metabolizing enzymes in human immune and inflammatory cells.

<table>
<thead>
<tr>
<th>Cell</th>
<th>cAMP PDEs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basophils</td>
<td>3 and 4</td>
</tr>
<tr>
<td>B cells</td>
<td>Unknown</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>4</td>
</tr>
<tr>
<td>Macrophages</td>
<td>3 and 4</td>
</tr>
<tr>
<td>Mast cells</td>
<td>3 and 4</td>
</tr>
<tr>
<td>Monocytes</td>
<td>4</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>4</td>
</tr>
<tr>
<td>T cells</td>
<td>3, 4 and 7</td>
</tr>
</tbody>
</table>

Adapted with permission from Torphy.
### Table 2  Comparison of cilomilast and theophylline.

<table>
<thead>
<tr>
<th></th>
<th>Theophylline</th>
<th>Cilomilast</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mechanism of action</strong></td>
<td>Non-selective PDE inhibitor, leading to ↑ cAMP and ↑ cGMP 26</td>
<td>Potent and selective PDE4 inhibitor, leading to ↑ cAMP only</td>
</tr>
<tr>
<td></td>
<td>PDE inhibition may be negligible at therapeutic concentrations 26</td>
<td>PDE4 inhibition at therapeutic concentrations</td>
</tr>
<tr>
<td></td>
<td>Adenosine receptor antagonist 26</td>
<td>No action on adenosine receptors</td>
</tr>
<tr>
<td><strong>Pharmacokinetics</strong></td>
<td>Non-linear pharmacokinetics, with significant inter-subject variability, including effects of smoking, age, concomitant medications—necessitates plasma monitoring 42,43,47,50,56</td>
<td>Linear pharmacokinetics, unaffected by smoking—no requirement for plasma monitoring 1,49,53</td>
</tr>
<tr>
<td><strong>General</strong></td>
<td>Variable, depends on formulation 67</td>
<td>Convenient oral tablet, dosed twice-daily</td>
</tr>
<tr>
<td></td>
<td>~ 7–9 h 47</td>
<td>Rapid (T(_{\text{max}}) ~ 1–2 h), slower with food 1,49,53</td>
</tr>
<tr>
<td></td>
<td>0.5 l/kg body weight; plasma protein binding ~ 56% 50</td>
<td>100%; unaffected by food or co-administration of antacids 3,49,53</td>
</tr>
<tr>
<td></td>
<td>~ 0.4 l/h/kg; affected by genetic factors, environmental agents (including tobacco smoke), pathological conditions and concomitant drug treatments that affect hepatic metabolism 47</td>
<td>~ 7–8 h 3,49,53</td>
</tr>
<tr>
<td></td>
<td>Low; plasma protein binding ~ 99.5% 3,49,53</td>
<td>Low (&lt; 2.1 h) 3,53</td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td>~ 90% metabolized in liver by the CYP pathway, mainly by CYP1A2 (CYP2E1/ CYP3A3/4 also contribute) 47,50</td>
<td>Subject to negligible first pass hepatic metabolism, extensively metabolized (main routes = decyclopentylation, acyl glucuronidation and 3-hydroxylation of the cyclopentyl ring) 3</td>
</tr>
<tr>
<td></td>
<td>Essentially no inhibition of CYP isoenzymes (CYP2C8?) 3</td>
<td>Low potential for drug interactions, and can be taken safely with many other medications that are commonly prescribed to patients with COPD 3</td>
</tr>
<tr>
<td><strong>Drug interactions</strong></td>
<td>Potential drug interactions include propafenone, mexiletine, enoxacin, ciprofloxacin, cimetidine, propanolol, oral contraceptives, erythromycin, rifampicin, phenytoin, carbamazepine, phenobarbital, isoproterenol, tobacco smoke 47,50-52</td>
<td>No dose adjustment required in smokers or the elderly 1,49; use with caution in mild-to-moderate hepatic impairment and severe renal impairment; contraindicated in severe hepatic impairment</td>
</tr>
<tr>
<td><strong>Excretion</strong></td>
<td>Only ~ 10% excreted via the kidneys in unchanged form 47,50</td>
<td>Mainly excreted in urine (~ 90%) and faeces (6–7%), unchanged cilomilast accounts for less than 1% of administered dose 3</td>
</tr>
<tr>
<td><strong>Special populations</strong></td>
<td>Dose adjustment may be required in smokers, the elderly, those with liver disease or patients taking concomitant medications; contraindicated in heart disease, seizure disorders and gastroesophageal reflux 46,47,50,52,54</td>
<td>No dose adjustment required in smokers or the elderly 1,49; use with caution in mild-to-moderate hepatic impairment and severe renal impairment; contraindicated in severe hepatic impairment</td>
</tr>
<tr>
<td><strong>Clinical efficacy</strong></td>
<td>Modest dose-response effect 42</td>
<td>Dose dependent improvements in lung function, with FEV(_1) improvements maintained over 6 months 55,57</td>
</tr>
<tr>
<td></td>
<td>Only effective in subset of patients 42</td>
<td>Effective in smokers and non-smokers 58</td>
</tr>
<tr>
<td></td>
<td>Slow onset of effect 42</td>
<td>Decreases rate of exacerbations and improves quality of life 5,55,56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Demonstrated anti-inflammatory effects on CD8 (\pm) T cells and CD68 (\pm) macrophages from bronchial biopsies 40</td>
</tr>
<tr>
<td><strong>Safety and tolerability</strong></td>
<td>Serious cardiovascular and central nervous system side effects 45</td>
<td>No cardiovascular/central nervous system side effects</td>
</tr>
<tr>
<td></td>
<td>10–15% of patients experience gastrointestinal side effects, insomnia or other minor side effects 42</td>
<td>Generally well tolerated—mild-to-moderate GI effects may occur, but these are self-limiting and easily managed 43</td>
</tr>
</tbody>
</table>
antagonism are likely to be responsible for many or all of the toxic side effects associated with theophylline use.\textsuperscript{26,27,29} Of note, the degree of PDE inhibition may be negligible at concentrations of theophylline that are therapeutically relevant.\textsuperscript{26} For example, in human lung extracts, theophylline at therapeutic concentrations has been shown to inhibit total PDE activity by only 5–10\%.\textsuperscript{26} There is growing evidence that theophylline works via other mechanisms than PDE inhibition and recent findings from asthma studies have indicated that its mode of action involves modification of histone acetylation, leading to changes in gene expression.\textsuperscript{26,30} However, theophylline’s precise mechanism of action in COPD remains unknown.

**First generation PDE4 inhibitors (e.g. rolipram)**

Preclinical studies with first generation compounds such as rolipram that selectively inhibit PDE4 have demonstrated impressive activity in animal models of pulmonary inflammation.\textsuperscript{18,27,31} However, these agents were found to be associated with significant class-associated side effects, such as nausea and vomiting, and gastric acid secretion, caused by inhibition of PDE4 in the central nervous system and parietal glands, respectively.\textsuperscript{14} These findings necessitated the development of PDE4 inhibitors with an improved therapeutic ratio.

**Second generation PDE4 inhibitors (e.g. cilomilast and roflumilast)**

The most clinically advanced selective PDE4 inhibitors (cilomilast and roflumilast) have a superior side effect profile compared with theophylline and first generation compounds.\textsuperscript{14} These compounds were designed with the knowledge that PDE4 exists in two distinct conformations, high affinity rolipram-binding PDE4 (HPDE4; which predominates in the central nervous system and parietal glands) and low affinity rolipram-binding PDE4 (LPDE4; which predominates in immunocompetent cells).\textsuperscript{14} Unlike rolipram, which targets HPDE4, second generation compounds (such as cilomilast) primarily target LPDE4 (Fig. 2), resulting in an improved therapeutic index.\textsuperscript{14}

Selective inhibition of PDE4 increases cAMP content in many inflammatory and immunomodulatory cells, leading to suppression of numerous aspects of the inflammatory response, as well as effects on smooth muscle and pulmonary nerves (Fig. 3). Indeed, second generation PDE4 inhibitors have demonstrated pronounced anti-inflammatory effects in various animal models.\textsuperscript{18,21,22,32–34} Cilomilast/roflumilast inhibit the activity of cells that have been implicated in the pathogenesis of COPD, e.g. neutrophils,\textsuperscript{35,36} monocytes,\textsuperscript{35} macrophages,\textsuperscript{35} CD4\textsuperscript{T} cells,\textsuperscript{35} epithelial cells,\textsuperscript{37} and fibroblasts.\textsuperscript{35,38,39} Additionally, cilomilast has recently been shown to decrease levels of CD8\textsuperscript{T} cells and CD68\textsuperscript{+} macrophages, thus demonstrating its potent anti-inflammatory effects.\textsuperscript{40} Other effects of cilomilast and roflumilast include reduced chemotaxis, activation, degranulation and adherence of inflammatory cells, impact on key mechanisms involved in airway remodelling and modulation of the release of inflammatory mediators, such as TNF-$\alpha$, IL-8, and GM-CSF.\textsuperscript{32,34,37–39,41} Significantly, cilomilast retains the anti-inflammatory actions of rolipram, but is substantially less likely to stimulate gastric acid secretion.\textsuperscript{22}

**PDE4 inhibitors—pharmacokinetics and benefits over theophylline**

The pharmacokinetics of the selective PDE4 inhibitors are markedly different from those of theophylline (for a detailed comparison of cilomilast versus theophylline, see Table 2). This has a number of implications in terms of the relative ease of use of these compounds and the extent of plasma monitoring required.

Theophylline is a difficult drug to use clinically, owing to significant inter- and intra-subject variability in pharmacokinetics.\textsuperscript{42} It has a narrow therapeutic margin and requires careful titration, with routine plasma monitoring, in order to reduce the risk of related adverse events.\textsuperscript{42,43} For example, potentially dangerous complications can
occur with theophylline when plasma levels rise above 20 μg/mL, e.g. cardiovascular events, including arrythmias and central nervous system events, including convulsions. In particular, careful plasma monitoring and dose adjustments are required in smokers and elderly patients. This has important consequences for the cost of therapy. While theophylline is generally considered to be a low price medication, it has been estimated that total annual costs, including hospital admissions for toxic events and plasma monitoring, are higher for patients taking theophylline compared with other bronchodilators. In contrast to asthma, where recent evidence suggests that many of the beneficial effects of theophylline can be observed at low doses, there is evidence of a modest dose–response effect in patients with COPD, which is particularly relevant to the problems associated with its narrow therapeutic margin.

In contrast, the pharmacokinetics of cilomilast are unaffected by smoking. In the elderly, although there may be a small reduction in cilomilast clearance, this is not considered to be of clinical concern and does not necessitate dose adjustment. Use of theophylline is also complicated by the range of potential drug interactions that may occur. Theophylline is metabolized by the cytochrome P450 (CYP) pathway, predominantly by CYP1A2, with a smaller contribution from CYP2E1 and CYP3A4. This leads to numerous potential drug interactions, including B2 agonists, cimetidine, ciprofloxacin, lithium, prednisolone, oral contraceptives, rifampicin, digoxin, warfarin, propafenone, mexiletine, enoxacin, propranolol, erythromycin, phenytoin, carbamazepine, phenobarbital, isoproterenol and tobacco smoke. In contrast, cilomilast has a superior drug interactions profile compared with theophylline. Cilomilast does not inhibit any important CYP isoenzymes and therefore has a low potential for drug interactions. Although CYP2C8 has been implicated in cilomilast metabolism, this enzyme has few other substrates or inhibitors. Importantly, there is no evidence of drug interactions between cilomilast and warfarin, theophylline, digoxin, aluminium/magnesium hydroxide antacid, prednisolone or salbutamol. Furthermore, several pathological conditions affect theophylline metabolism and clearance, including congestive heart failure, cirrhosis, pulmonary oedema, cor pulmonale/ COPD, obesity and systemic viral infection. Theophylline is contraindicated in patients with heart disease, seizure disorders and gastroesophageal reflux.

Clinical data—benefits of PDE4 inhibitors over theophylline

Efficacy

Theophylline is the most frequently prescribed oral bronchodilator for the chronic maintenance treatment of chronic obstructive airway disorders. However, there is some debate as to the role of theophylline in the treatment of COPD compared with newer agents (Table 2). For example, only a modest dose–response effect has been demonstrated, with just small changes in FEV1, forced vital capacity (FVC) and peak expiratory flow rate (PEFR) observed over the range of doses that induce theophylline concentrations of 5–10 μg/mL, 10–15 μg/mL and 15–20 μg/mL. It has been suggested that high doses of theophylline may be needed to induce a significant increase in FEV1, but even when this is achieved, its bronchodilator action is often limited. In fact, the slow onset of action of theophylline coupled with difficulties in achieving stable plasma levels mean that most effects do not occur until 2–6 weeks. Of further concern, its effectiveness may be restricted to a subset of patients with COPD and it may be difficult to predict responders. Additionally, withdrawal of theophylline should be carried out with caution because of possible deterioration in lung function. Given these efficacy concerns, together with its toxicity issues, use of theophylline as monotherapy in COPD should be restricted to the rare cases where patients cannot adequately administer inhalers. Indeed, GOLD guidelines recommend that inhaled bronchodilators are preferable to theophylline, when available.

In contrast, the selective PDE4 inhibitors have demonstrated encouraging efficacy to date. According to published data, the most advanced PDE4 inhibitor in clinical development is cilomilast. This compound has been shown to improve both pulmonary function and symptoms of COPD in 424 patients with moderate-to-severe disease, producing significant improvements in FEV1, FVC and PEFR versus placebo over 6 weeks. In this study, cilomilast use was also associated with improvements in rescue bronchodilator use, exertional dyspnea, and resting and post-exercise arterial oxygen saturation (SaO2). In addition, consistent improvements in patient quality of life approaching that defined as clinically relevant were observed compared with placebo. Furthermore, cilomilast significantly reduces the risk of exacerbations, both self-managed and those requiring physician intervention or hospitalization.
and provides sustained improvements in lung function over 6 months in patients with mild-to-moderate disease. Importantly, the improvements in lung function observed with cilomilast are independent of smoking status. More recently, the anti-inflammatory effects of cilomilast observed in preclinical studies have been confirmed in a small, randomized, placebo-controlled trial of 59 patients with COPD. After 12 weeks of cilomilast therapy, bronchial biopsies taken from treated patients indicated that cilomilast significantly reduces levels of inflammatory markers, i.e. CD8⁺ T cells and CD68⁺ macrophages. These results represent the first demonstration by any agent of a reduction in airway tissue inflammatory cells characteristic of COPD.

Roflumilast has also shown encouraging efficacy in patients with COPD, with significant improvements observed in FEV₁ and PEFR versus baseline, although only limited published data are available at present.

Safety and tolerability

A major benefit of cilomilast and roflumilast is their superior safety and tolerability profile versus theophylline and the first generation PDE4 inhibitors (Table 2).

Theophylline is associated with serious cardiovascular and central nervous system side effects, even at therapeutic doses. These effects are generally attributed to non-selective inhibition of PDEs, as well as concomitant adenosine receptor antagonism, and include tachycardia and serious arrhythmias, focal and generalized seizures, and coma. Acute theophylline overdose is associated with metabolic and electrolyte abnormalities, including hypokalaemia, hyperglycaemia, leukocytosis and elevated serum catecholamine levels. Moreover, approximately 10–15% of patients receiving theophylline will experience gastrointestinal adverse events, insomnia or other minor side effects. Although side effects can be reduced by ensuring that theophylline plasma concentrations do not enter the high therapeutic range, many physicians do not provide adequate monitoring of theophylline levels before or during long-term treatment. Despite the low price of theophylline, the overall cost may be greater than other bronchodilators owing to its toxic effects, including hospital admissions and blood level monitoring.

When the first generation PDE4 inhibitors were developed, it was envisaged that these compounds would have fewer side effects than theophylline, due to their selectivity for PDE4. However, these agents were associated with a number of adverse events, including nausea, vomiting and gastric acid secretion, which limit their clinical use.

In contrast, second generation PDE4 inhibitors have an improved therapeutic ratio. Cilomilast has proven safe and well tolerated at doses of up to 15 mg in both short- and long-term dosing trials, with a low incidence of adverse events. Although cilomilast does cause some gastrointestinal adverse events, most notably, nausea, this is generally mild-to-moderate and self-limiting. Cilomilast can also be taken safely with many other medications that are commonly prescribed to patients with COPD. Similarly, preliminary findings indicate that roflumilast is also safe and well tolerated in patients with COPD, although only limited published data are available at present.

Conclusions

COPD is a serious chronic condition, which is increasingly being recognized as having a significant inflammatory component. New treatments are required that reduce inflammation and delay disease progression, and inhibition of PDE4 represents a promising mechanism to treat COPD, given the resulting effects on inflammation and associated underlying disease processes. Although our preconceptions of theophylline as an agent with PDE inhibitory activity draws us to compare theophylline with specific second generation PDE4 inhibitors, this may not be consistent with the current state of our scientific understanding. Theophylline and first generation PDE4 inhibitors (e.g. rolipram) are limited by poor efficacy and unfavourable safety and tolerability, resulting from their lack of selectivity. In contrast, second generation PDE4 inhibitors (e.g. cilomilast and roflumilast) are more selective and preliminary efficacy and safety/tolerability findings from large clinical trials, and using newer methods of measuring efficacy, are promising. These agents are likely to be of benefit in the treatment of COPD, particularly in combination with other agents, due to their novel mechanism of action and anti-inflammatory effects, and without the hazards and inconvenience of older agents posed to the prescribing physician.

Acknowledgements

This manuscript was supported by an educational grant from Glaxosmithkline.
References
