A new class of bronchodilator improves lung function in COPD: A trial with GSK961081

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ABSTRACT: GSK961081 is a bi-functional molecule demonstrating both muscarinic antagonist (MA) and beta agonist (BA) activities (MABA).

This was a 4-week, multicentre, randomised, double-blind, double-dummy, placebo and salmeterol controlled parallel group study. Doses ranging across three twice-daily (BD) doses and three once-daily (OD) doses were assessed in moderate and severe COPD patients. Trough FEV₁ at day 29 was the primary endpoint. At days 1 and 28, 12h FEV₁ spirometry was performed in all patients. A subset of patients had complete 24h spirometry at day 28.

The study recruited 436 patients. GSK961081 showed statistically and clinically significant differences from placebo in all doses and regimens for trough FEV₁ on day 29 (155–277 mL). The optimal total daily dose was 400 µg, either as 400 µg OD or as 200 µg BD, with an improvement in day 29 trough FEV₁ of 215 mL and 249 mL respectively. Other efficacy endpoints also showed improvement. No effects were observed on glucose, potassium, heart rate, blood pressure and no dose response effect on QTc elongation.

This study showed that GSK961081 is an effective bronchodilator in COPD and appeared safe and well tolerated.

KEYWORDS: Beta agonist, bronchodilation, chronic obstructive pulmonary disease, Diskus, bi-functional, muscarinic antagonist
INTRODUCTION

Pharmacological management of chronic, stable COPD is primarily aimed at improving symptoms and quality of life, optimizing lung function, reducing exacerbations and improving exercise tolerance [1, 2]. Inhaled bronchodilators, including β2-agonists and anti-muscarinics are the mainstays of therapy in patients diagnosed with COPD [1].

Whilst the mechanism of dual bronchodilators is not fully understood, addition of a β2-agonist to an anti-muscarinic results in greater bronchodilation in the airways than either component alone. Mechanistically it is thought the addition of a β2-agonist decreases the release of acetyl choline (ACh), through the modulation of cholinergic neurotransmission by pre-junctional β2-adrenergic receptors (β2-AR's), amplifying the bronchial smooth muscle relaxation induced by the muscarinic antagonist. Secondly, the addition of a muscarinic antagonist reduces bronchoconstrictor effects of ACh, whose release has been modified by the β2-agonist, and thereby amplify the bronchodilation elicited by the β2-agonist through the direct stimulation of smooth muscle β2-ARs [3].

Clinical research confirms that addition of β2-agonist to a muscarinic antagonist is more effective at improving lung function and patient centred outcomes than either of the components alone and that there are no untoward safety issues [4-9]. In studies evaluating the combined use of tiotropium (Tio) with formoterol or salmeterol, the number and type of reported adverse events (AEs) were similar when comparing co-administration of monotherapies with individual treatments for up to 1 year [6–10].

As of 2012 there is currently no licensed combination of LABA/LAMA, either as two separate drugs in the same device or as a single molecule. Compounds with both MA and BA activity (MABAs) offer a single pharmacokinetic profile for both pharmacological activities, potential for maximizing the synergy between the two mechanisms, and a simpler technical and clinical development pathway compared to co-formulation of two compounds [11]. GSK961081 is a bi-functional molecule and has
muscarinic antagonist activity at one end of the molecule, separated from β2-agonist activity by an inert linker portion. The bi-functional nature of GSK961081 has been demonstrated in vitro [12] and in vivo in a guinea pig broncho-protection model [13]. In a study in healthy volunteers with and without propranolol (beta-2 adrenergic receptor blockade) GSK961081 showed activity at both receptors with the β2-agonist being longer lasting than the anti-muscarinic activity [14]. In a small, 14-day, crossover study in 50 moderate COPD patients, GSK961081 was found to be safe and well tolerated and showed bronchodilation versus placebo that was comparable to Tio plus salmeterol [15].

This study was designed to determine the bronchodilator effects of GSK961081, the dose and dosing interval (using trough FEV₁ at day 29 as the primary outcome), as well as safety and tolerability in moderate and severe COPD patients. The study evaluated three once-daily (OD) doses and three twice-daily (BD) doses, a placebo arm and an active comparator salmeterol.

METHODS

Study design

This was a 4-week, phase IIb, multicentre, randomised, double-blind, double-dummy, placebo and active controlled, parallel group, dose interval and dose ranging study. After the screening visit and a 7-day run-in period, eligible patients were randomised and entered a 28-day treatment period. Clinic visits were on days 1, 2, 14, 28, 29 plus two telephone contacts on day 7 and seven days after the last clinic visit.

Patients enrolled at centres with overnight accommodation had 24 hour serial spirometry assessed on day 28.

Sample size calculations
Sample size calculations were based on the primary efficacy endpoint and the assumptions are shown in the online supplement 1. Eligible patients were randomised to one of 8 arms, with OD doses of GSK961081 of 100 µg, 400 µg, 800 µg or BD doses of 100 µg, 200 µg, 400 µg, 50 µg salmeterol BD or placebo (as shown in table 1), in a ratio of 2:2:2:2:2:2:2:3. Patients were provided with two Diskus inhalers – one for morning use and one for evening use. For the OD regimen the evening inhaler was a placebo. The study was stratified by reversibility to salbutamol and inhaled corticosteroid (ICS) use.

**Study patients**

This study included both current and former smokers aged 40 years and older, who had a smoking history of ≥10 pack-years. Patients had a clinical diagnosis of moderate-to-severe stable COPD (post-bronchodilator forced expiratory volume in 1 second (FEV₁)/forced vital capacity (FVC) <70% and FEV₁ ≥30% and ≤70% predicted) according to NHANES III [16]. Patients on a stable dose of ICS were allowed into the study.

A current diagnosis of asthma was exclusionary. Due to the presence of vocal fold erosions in dogs given high doses of GSK961081, patients who were symptomatic (or had a documented history of) laryngopharyngeal reflux (LPR), extraesophageal reflux (EER), posterior laryngitis or laryngopharyngeal ulcerations and erosions were also excluded. This was to ensure that patients with pre-existing throat problems would not confound the potential to identify any new instances of throat irritation during the study. Further characteristics of the COPD population are shown in the online supplement 2.

The study was approved by the Medical Ethical Committees of the participating centres, and all patients gave their written informed consent. The study was conducted according to the declaration of Helsinki using Good Clinical Practice.

**STUDY ASSESSMENTS**
**Efficacy**

Spirometry was carried out using the Vitalograph from Biomedical Systems (BMS) (see the online supplement 3). Over-reading of traces was also carried out by BMS. A subject was reversible if they increased their pre-salbutamol FEV₁ by ≥200 mL and ≥12%, 15 minutes after the administration of 400 µg of salbutamol at the screening visit.

**Safety assessments**

The incidence and severity of all AEs was recorded in the electronic case report form (eCRF). Details for electrocardiograms (ECGs), blood chemistry and vital signs are in the online supplement 4. Data were collected for any patients who reported throat symptoms for 7 days or longer, including: hoarseness of voice, sore throat, lump in throat, difficulty in swallowing or any abnormal sensations in the throat. These patients also underwent a protocol-defined flexible laryngoscopy examination by a specialist.

**Endpoints**

The primary endpoint for the study was the change from baseline in trough FEV₁ at day 29. Trough was defined as the mean of 11 and 12h measurement after the evening dosing on day 28.

Secondary endpoints included the weighted mean for 0–24h serial FEV₁ measurements in the subset of patients with overnight spirometry, serial FEV₁ on day 28 at each time point up to 24h post-dose in the subgroup of patients performing overnight spirometry, and serial morning post dose FEV₁ 0-12h on day 1 and day 28 in the whole cohort.

**Statistical analysis**

The primary endpoint was analysed using a repeated measures model, with fixed effects for baseline FEV₁, reversibility, concurrent ICS use, sex, age, smoking status (at screening), treatment, study day, treatment by study day interaction and whether or not the patient participated in 24-hour
spirometry assessments. In order to preserve an overall alpha level of 5% inference versus placebo a closed sequential testing procedure was used within each dosing regimen at a significance level of 2.5%, initially comparing the highest dose with placebo. Subsequent comparisons at lower doses continued in a step-down manner only if the preceding comparison was significant. Inferences for secondary endpoints were not adjusted for multiplicity. A post-hoc analysis was carried out to provide inferences between all groups and salmeterol.

RESULTS

Cohort characteristics

Four-hundred-and-thirty-seven patients were randomised into the study. One patient received no investigational product and was withdrawn, giving a modified intent-to-treat population of 436 patients from nine countries and forty nine sites, with 46% of patients in the overnight cohort. Demographic characteristics of the study population can be found in table 1 and the disposition of patients in figure 1.

For the primary endpoint (morning trough FEV₁ at day 29), all doses of GSK961081 were significantly different from placebo (p<0.001) (table 2). Differences for the OD doses ranged from 155 mL (100 µg) to 277 mL (800 µg) and differences for the BD doses ranged from 173 mL (100 µg) to 258 mL (400 µg). When looking at treatment effects versus placebo within the predefined strata, FEV₁ improvements were generally greater for patients who were reversible to salbutamol and greater for patients who were not concurrent ICS users (see the online supplement table 1). During the study the trough FEV1 increased over the first 14 days and remained constant to 28 days (see online supplement table 2).

The active comparator salmeterol was compared against all treatments in a post-hoc analysis. In this analysis, there was a nominal statistical difference in favour of salmeterol compared to placebo (77 mL difference, p=0.046). Differences between GSK961081 doses and salmeterol ranged from
78 mL (100 µg OD) to 200 mL (800 µg OD), with statistically significant differences in favour of GSK961081 for all doses except 100 µg OD (table 2).

Secondary endpoints

For the subset of overnight patients (n=18–23), the 24h weighted mean FEV₁ differences from baseline at day 28 were statistically significant compared to placebo (p<0.001) for all doses and regimens over the 24h time period, ranging from 226 mL (100 µg BD) to 335 mL (800 µg OD; table 3). Salmeterol had a weighted mean improvement of 85 mL but the difference from placebo was not statistically significant.

The 0–24h FEV₁ profile on day 28 (n=18–23) indicated that patients on placebo showed a reduction in FEV₁ following their evening dose from 11h onwards (fig. 2). Salmeterol also mirrored the evening drop in FEV₁ as did the GSK961081 OD doses with 100 µg and 400 µg, dropping the mean change from baseline below 200 mL by the following morning. The BD doses of 200 µg and 400 µg induced the extra peak at 12–14h, which ensured that the patients remained above 200 mL for mean change from baseline overnight until the following morning.

On day 1, all GSK961081 treatments gave differences over placebo which exceeded 100 mL at all timepoints from 0-12h post-morning dose, with the exception of 100 µg BD and 100 µg OD at 11h and 12h post-dose (fig. 3a). On day 28, the 200 µg BD, 400 µg BD, 400 µg OD and 800 µg OD GSK961081 treatments had differences over placebo which exceeded 200 mL at all timepoints from 0-12h post-morning dose (Fig. 3b).

Other endpoints

The proportion of patients showing an improvement of 100 mL within 15 min on day 1 was between 60% and 81% for GSK961081 doses (table 4) compared to 43% of patients who were treated with salmeterol. The peak FEV₁ response increased from day 1 to day 28 for all doses of GSK961081 except the 100 µg OD, as shown in table 4.
Trough FVC measurements for all doses of GSK961081 showed nominal statistical differences compared to placebo (p=0.014 for 100 µg OD, p<0.001 for all other GSK961081 doses) and varied from 153 mL for GSK961081 100 µg OD to 381 mL for the 800 µg OD dose (table 3). Salmeterol (120mL difference from placebo on day 29) was not statistically different from placebo.

The mean number of occasions per day of salbutamol use prior to treatment was 1.49. During the study, there was a nominal statistical differences (p<0.01)for the reduction in the number of occasions per day for the GSK961081 doses, ranging from 0.45 occasions for the 100 µg OD dose to 0.74 for the 400 µg BD dose (table 3). Salmeterol showed a nominal statistical difference (p=0.026) with a reduction of 0.39 occasions per day over the study period.

**Safety**

One non-fatal serious AE was reported during treatment and required hospitalization. This was an incidence of biliary colic which was reported in a patient, with a suspected past history of gallstones, in the 400 µg OD GSK961081 treatment group and was not considered to be related to the study drug. The incidence of AEs is shown in table 5. GSK961081 was well tolerated with headache, cough, dysgeusia (bad taste) and nasopharyngitis being the most common AEs. Drug related AEs were reported more frequently in the GSK961081 groups than in the placebo or salmeterol arms. The most frequently reported events were cough and dysgeusia. Six COPD exacerbations occurred during the study, four in the placebo group, and one each in the 100 µg OD/BD GSK961081 groups, and none required hospitalisation. There were four post-dose ECG abnormalities. One was a tachycardia in the 100 µg BD group which was judged to be unrelated to study drug by the investigator and did not lead to withdrawal. The other three were deemed treatment related and led to withdrawal of GSK961081. A left bundle branch block and a Wolf Parkinson-White Syndrome were diagnosed on day 1, post-dose ECGs, although review of ECGs indicated both these abnormalities were present on ECGs obtained before dosing. In addition, a patient was withdrawn for first degree atrioventricular
block (PR=244 msec) and had a normal ECG at screening (PR=177 msec) but had a day 1, pre-dose PR interval of 215 msec.

Heart rate, systolic and diastolic blood pressure showed little response to GSK961081. Changes in glucose and potassium were also minimal when on treatment with GSK961081. There was a pharmacological effect with GSK961081 on QTc(F) with a 3–4 msec increase compared to placebo on day 28. However, no dose-response relationship was apparent.

**DISCUSSION**

This study was designed to assess the efficacy and safety of the novel dual bronchodilator GSK961081, in moderate and severe COPD patients. The study showed robust clinically and statistically significant improvements in trough FEV₁ after 28 days of treatment and reduced the use of rescue medication. A comparison of the dosing intervals with the same total daily dose of GSK961081 at 400 µg and 800 µg demonstrated that there were no significant differences between OD and BD dosing with respect to trough FEV₁, FVC trough, rescue medication usage and safety parameters. There was a small increase in trough FEV₁ as the total daily dose increased from 400 µg to 800 µg. Increases in trough FEV₁ greater than the widely accepted minimal clinically important difference (MCID) of 100 mL [17] for a single bronchodilator were observed for doses of 100 µg OD and 100 µg BD; however, increases in trough FEV₁ greater than 200 mL (as expected for a dual bronchodilator) were not observed for these doses. Therefore, compared to a total daily dose of 400 µg the lower daily doses would be considered as suboptimal. Using the safety data there was no clear increase in safety parameters of concern as the dose increased. Therefore we conclude that the optimum total daily dose would be 400 µg, either as a 200 µg BD dose or a 400 µg OD dose in moderate-to-severe COPD patients.
Over the 28 days of the study, a post-hoc analysis showed GSK961081 was consistently better in improving lung function than the active comparator salmeterol. GSK961081 produced improvements of trough FEV₁ by day 29, with mean differences (compared to placebo) which exceeded 150 mL for all doses, and specifically exceeded 200 mL for total daily doses at or above total daily doses of 400 µg.

The 24h spirometric profiles at day 28 in the subset of patients at overnight sites showed in the placebo treated patients a diurnal variation with an FEV₁ decrease of approximately 100 mL overnight. Despite the fluctuations due to diurnal effect, all active treatment arms at least tracked the changes and maintained the differences achieved in lung function compared to placebo. There is a suggestion that BD doses≥200 µg or OD doses ≥400 µg of GSK961081 had a reduced diurnal variation, resulting in greater differences in mean lung function compared to placebo during the evening period. Patients showed sustained bronchodilation over the 24h period with all doses of GSK961081 although less so with the 100 µg OD or the 100 µg BD doses.

The 12h spirometric profiles of all patients showed that there was an increase in improvement of trough FEV₁ versus placebo from day 1 to day 28. The trough FEV₁ increased up to day 14 and then remained constant for the remainder of the study (online supplement table1).

GSK961081 onset was rapid, with at least 60% of patients across all GSK961081 doses reaching 100 mL improvement in FEV₁ by the first post-dose assessment (15 min), with the majority of patients reaching peak bronchodilation between 1–2h. GSK961081 also showed nominal statistical improvements compared to placebo for trough FVC and rescue medication use.

In general, GSK961081 was well tolerated. The most common AEs were headache, cough, dysgeusia and nasopharyngitis. Treatment with GSK 961081 was associated with prolongation of various QTc intervals ranging from 3–5 msec more compared to placebo or salmeterol. However, there was no apparent dose response, and in a previous study [15], where a dose of 1200 µg OD was used, there
was no prolongation of the QT interval seen. In preclinical dog studies, high doses of GSK961081 showed the development of vocal fold erosions in the larynx. Two patients were excluded at screening due to pre-existing laryngopharyngeal reflux or oesophageal reflux. Throat symptoms were monitored throughout the study. On treatment there were two reported incidents of throat symptoms lasting longer than 7 days, but laryngoscopy showed no evidence of vocal fold erosions.

The main limitation of this phase IIb study was that it used a selected population of moderate and severe COPD patients. Whether the effect sizes seen in this study would be maintained for a longer period of time in a broader COPD population will need to be addressed in future studies.

COPD treatment guidelines recommend the use of a single bronchodilator initially. Single bronchodilators like salmeterol, in moderate or severe COPD patients provide approximately 80ml improvement in trough FEV1 versus placebo whereas Tio and indacaterol shows approximately 100ml [18, 19, 21]. If patients remain symptomatic, guidelines suggest an additional bronchodilator with a different mechanism could be added [1]. Clinical studies provide the evidence for this [3-10]. When Tio was added to indacaterol in a moderate to severe COPD population in two separate studies an improvement of 60-90mls vs Tio alone was seen [20]. In a double-blind 26 week study with moderate-to-severe COPD patients taking the fixed dose dual bronchodilator QVA149, there was a 200ml (p <0.001) improvement versus placebo [21]. Therefore the expectation was that a bronchodilator with two mechanisms of action would provide a trough improvement of approximately 200ml. The improvement in trough FEV1 of 200mls or greater was achieved by the 800 µg OD, 400 µg OD 200 µg BD and 400 µg BD GSK961081 versus placebo. The 100 OD or 100 BD whilst improved over single bronchodilators are suboptimal in terms of the 200ml trough FEV1 expectation for a bronchodilator with two mechanisms. Although the improvements in lung function at total daily doses ≥400 µg for GSK961081 appear similar to current combinations, direct comparisons need to be carried out in randomised controlled trials.
In conclusion, GSK961081 is bi-functional, having both muscarinic antagonist and β₂-agonist activities in the same molecule. This study showed that a total daily dose of 400 µg GSK961081 was optimal given either as 400µg OD or 200µg BD. GSK961081 had a rapid onset of action, was a potent bronchodilator in moderate and severe COPD patients, and appeared safe and well tolerated.

CLINICAL TRIAL

The study was registered on the Clinical Trials Register NCT01319019, used the study code MAB115032 and was funded by GlaxoSmithKline.

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**FIGURE LEGENDS**

**FIGURE 1.** Subject disposition consort diagram.

[Diagram showing subject disposition with details provided in the text box.]

**FIGURE 2.** Serial FEV₁ profile over 0-24h on day 28 in the subset of overnight subjects.
FIGURE 3a. Serial FEV₁ profile over 0-12h on day 1 in all subjects.
FIGURE 3b. Serial FEV₁ profile over 0-12h on day 28 in all subjects.
TABLE 1 Patient demographics and clinical characteristics at screening

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>SAL</th>
<th>100 BD</th>
<th>200 BD</th>
<th>400 BD</th>
<th>100 OD</th>
<th>400 OD</th>
<th>800 OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITT Subjects, n</td>
<td>81</td>
<td>47</td>
<td>52</td>
<td>50</td>
<td>54</td>
<td>50</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63 ± 7</td>
<td>61 ± 7</td>
<td>62 ± 9</td>
<td>61 ± 9</td>
<td>63 ± 8</td>
<td>63 ± 9</td>
<td>62 ± 8</td>
<td>61 ± 9</td>
</tr>
<tr>
<td>Male</td>
<td>70%</td>
<td>62%</td>
<td>71%</td>
<td>64%</td>
<td>70%</td>
<td>64%</td>
<td>52%</td>
<td>67%</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26 ± 4</td>
<td>26 ± 4</td>
<td>26 ± 4</td>
<td>26 ± 4</td>
<td>26 ± 4</td>
<td>26 ± 4</td>
<td>27 ± 4</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Current smoker</td>
<td>44%</td>
<td>62%</td>
<td>54%</td>
<td>66%</td>
<td>40%</td>
<td>54%</td>
<td>48%</td>
<td></td>
</tr>
<tr>
<td>Concurrent ICS use</td>
<td>59%</td>
<td>55%</td>
<td>58%</td>
<td>60%</td>
<td>56%</td>
<td>60%</td>
<td>54%</td>
<td>56%</td>
</tr>
<tr>
<td>Reversible to salbutamol</td>
<td>33%</td>
<td>36%</td>
<td>33%</td>
<td>34%</td>
<td>37%</td>
<td>32%</td>
<td>26%</td>
<td>31%</td>
</tr>
<tr>
<td>††FEV₁ (L)</td>
<td>1.56 ±</td>
<td>1.48 ±</td>
<td>1.55 ±</td>
<td>1.59 ±</td>
<td>1.62 ±</td>
<td>1.63 ±</td>
<td>1.53 ±</td>
<td>1.56 ±</td>
</tr>
<tr>
<td>††FEV₁ %predicted</td>
<td>0.53</td>
<td>0.47</td>
<td>0.50</td>
<td>0.46</td>
<td>0.47</td>
<td>0.49</td>
<td>0.45</td>
<td>0.42</td>
</tr>
<tr>
<td>FEV₁ %reversibility</td>
<td>49 ± 11</td>
<td>48 ± 10</td>
<td>50 ± 10</td>
<td>51 ± 10</td>
<td>51 ± 10</td>
<td>53 ± 10</td>
<td>52 ± 10</td>
<td>51 ± 10</td>
</tr>
<tr>
<td>††FEV₁/FVC ratio</td>
<td>0.49 ±</td>
<td>0.50 ±</td>
<td>0.51 ±</td>
<td>0.50 ±</td>
<td>0.50 ±</td>
<td>0.50 ±</td>
<td>0.52 ±</td>
<td>0.51 ±</td>
</tr>
</tbody>
</table>

Summary values are mean ± standard deviation or number of patients (percentage).

† - Reversibility to salbutamol defined as FEV₁ increase of 200mL and 12% following salbutamol administration.

†† - Post-salbutamol measurements.

BD: twice daily; BMI: body mass index; FEV₁: forced expiratory volume in 1 sec; FVC: forced vital capacity; ICS: inhaled corticosteroid; ITT: intent-to-treat; OD: once daily; SAL: salmeterol.
TABLE 2 Results for LS mean change from baseline trough FEV₁ on day 29

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>LS mean change from baseline (mL)</th>
<th>LS mean (95% CI) difference from placebo (mL)</th>
<th>p-value v placebo</th>
<th>†LS Mean (95% CI) difference from salmeterol (mL)</th>
<th>†p-value v salmeterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>71</td>
<td>-7</td>
<td>77 (1, 153)</td>
<td>0.046†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAL</td>
<td>43</td>
<td>71</td>
<td>167 (100, 247)</td>
<td>&lt;0.001</td>
<td>96 (14, 179)</td>
<td>0.023</td>
</tr>
<tr>
<td>100 BD</td>
<td>47</td>
<td>167</td>
<td>173 (100, 247)</td>
<td>&lt;0.001</td>
<td>172 (89, 255)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>200 BD</td>
<td>46</td>
<td>243</td>
<td>249 (175, 323)</td>
<td>&lt;0.001</td>
<td>181 (98, 263)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>400 BD</td>
<td>49</td>
<td>251</td>
<td>258 (185, 330)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 OD</td>
<td>45</td>
<td>148</td>
<td>155 (80, 229)</td>
<td>&lt;0.001</td>
<td>78 (-7, 162)</td>
<td>0.071</td>
</tr>
<tr>
<td>400 OD</td>
<td>41</td>
<td>209</td>
<td>215 (139, 291)</td>
<td>&lt;0.001</td>
<td>138 (53, 223)</td>
<td>0.002</td>
</tr>
<tr>
<td>800 OD</td>
<td>48</td>
<td>270</td>
<td>277 (204, 350)</td>
<td>&lt;0.001</td>
<td>200 (117, 282)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

For the primary endpoint, p-values for GSK961081 doses were compared to placebo at α=0.025 due to separate closed step-down procedures.

† - Inferences involving salmeterol were post-hoc analyses

BD: twice daily; CI: confidence interval; LS: least squares; OD: once daily; SAL: salmeterol.

LS means adjusted for age, sex, smoking status, reversibility stratum, overnight site stratum, concurrent ICS use, baseline and treatment.
TABLE 3 Weighted mean 0–24h on day 28, trough FVC on day 29 and salbutamol use during the study

<table>
<thead>
<tr>
<th></th>
<th>Weighted mean FEV₁ (0–24h) on day 28</th>
<th>Trough FVC, day 29</th>
<th>Salbutamol use</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>LS mean difference (95% CI)</td>
<td>n</td>
</tr>
<tr>
<td>Placebo</td>
<td>34</td>
<td></td>
<td>71</td>
</tr>
<tr>
<td>SAL</td>
<td>19</td>
<td>85 (-21, 191)ns</td>
<td>43</td>
</tr>
<tr>
<td>100 BD</td>
<td>22</td>
<td>126 (125, 327)*</td>
<td>47</td>
</tr>
<tr>
<td>200 BD</td>
<td>21</td>
<td>325 (222, 428)*</td>
<td>46</td>
</tr>
<tr>
<td>400 BD</td>
<td>24</td>
<td>307 (209, 405)*</td>
<td>49</td>
</tr>
<tr>
<td>100 OD</td>
<td>18</td>
<td>246 (139, 353)*</td>
<td>45</td>
</tr>
<tr>
<td>400 OD</td>
<td>18</td>
<td>300 (192, 407)*</td>
<td>41</td>
</tr>
<tr>
<td>800 OD</td>
<td>23</td>
<td>335 (236, 434)*</td>
<td>48</td>
</tr>
</tbody>
</table>

ns: not significant † - p<0.05; ‡ - p<0.01; * - p<0.001.

Inference between salmeterol and placebo was part of a post hoc analysis.

CI: confidence interval; FEV₁: forced expiratory volume in 1 sec; FVC: forced vital capacity; LS: least squares;
SAL: salmeterol; BD: twice daily; OD: once daily.

LS Means adjusted for age, sex, smoking status, reversibility stratum, overnight site stratum, concurrent ICS use, baseline and treatment.
### TABLE 4 Summary of onset of effect and peak FEV₁

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>15 minute onset</th>
<th>Day 1 peak FEV₁</th>
<th>Day 28 peak FEV₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>81</td>
<td>11 (14)</td>
<td>117 ± 117</td>
<td>73 ± 200</td>
</tr>
<tr>
<td>SAL</td>
<td>47</td>
<td>20 (43)</td>
<td>229 ± 134</td>
<td>170 ± 170</td>
</tr>
<tr>
<td>100 BD</td>
<td>52</td>
<td>34 (65)</td>
<td>281 ± 159</td>
<td>317 ± 221</td>
</tr>
<tr>
<td>200 BD</td>
<td>50</td>
<td>34 (68)</td>
<td>339 ± 188</td>
<td>399 ± 239</td>
</tr>
<tr>
<td>400 BD</td>
<td>54</td>
<td>44 (81)</td>
<td>344 ± 144</td>
<td>384 ± 205</td>
</tr>
<tr>
<td>100 OD</td>
<td>50</td>
<td>30 (60)</td>
<td>293 ± 176</td>
<td>279 ± 229</td>
</tr>
<tr>
<td>400 OD</td>
<td>50</td>
<td>34 (68)</td>
<td>295 ± 161</td>
<td>368 ± 201</td>
</tr>
<tr>
<td>800 OD</td>
<td>52</td>
<td>34 (65)</td>
<td>392 ± 250</td>
<td>436 ± 300</td>
</tr>
</tbody>
</table>

Summary values are mean ± standard deviation or number of patients (percentage).

Onset defined as achieving a 100mL improvement from pre-dose trough to the first post-dose measurement.

Peak FEV₁ is defined as the highest FEV₁ value from 0-6 hours post dose.

BD: twice daily; FEV₁: forced expiratory volume in one second; OD: once daily; SAL: salmeterol.
### TABLE 5 Most common on-treatment adverse events (AEs) (≥3% incidence in any treatment group)

<table>
<thead>
<tr>
<th>Preferred term</th>
<th>Placebo</th>
<th>SAL</th>
<th>100 BD</th>
<th>200 BD</th>
<th>400 BD</th>
<th>100 OD</th>
<th>400 OD</th>
<th>800 OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>81</td>
<td>47</td>
<td>52</td>
<td>50</td>
<td>54</td>
<td>50</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>Headache</td>
<td>5 (6)</td>
<td>2 (4)</td>
<td>2 (4)</td>
<td>0</td>
<td>5 (9)</td>
<td>5 (10)</td>
<td>5 (10)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Cough</td>
<td>2 (2)</td>
<td>0</td>
<td>2 (4)</td>
<td>4 (8)</td>
<td>1 (2)</td>
<td>5 (10)</td>
<td>5 (10)</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Dysgeusia</td>
<td>0</td>
<td>0</td>
<td>2 (4)</td>
<td>3 (6)</td>
<td>3 (6)</td>
<td>2 (4)</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>3 (4)</td>
<td>0</td>
<td>1 (2)</td>
<td>3 (6)</td>
<td>0</td>
<td>3 (6)</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Back pain</td>
<td>2 (2)</td>
<td>0</td>
<td>1 (2)</td>
<td>0</td>
<td>0</td>
<td>2 (4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dysphonia</td>
<td>2 (2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Muscle spasms</td>
<td>0</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>0</td>
<td>2 (4)</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Nausea</td>
<td>2 (2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Myalgia</td>
<td>1 (1)</td>
<td>1 (2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Palpitations</td>
<td>0</td>
<td>1 (2)</td>
<td>0</td>
<td>2 (4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Summary values are number of patients (percentage).

BD: twice daily; OD: once daily; SAL: salmeterol.