Presatovir is a potent, small-molecule respiratory syncytial virus (RSV) fusion inhibitor with activity against both species of RSV A and B clinical isolates. The efficacy of presatovir was evaluated in a randomized, double-blind, placebo-controlled, Phase 2a viral challenge study in healthy adults (aged 18–45 years) experimentally infected with RSV Memphis 37b (NCT01756482). The frequency of resistance to presatovir was lowest for the 5 day 50/25-mg treatment group. No significant adverse events were reported. Competitive fitness assays demonstrated WT RSV recombinants with reduced susceptibility to presatovir remained sensitive to inhibition by palivizumab. Exposure level of presatovir above EC95 for RSV Memphis 37b (NCT01756482) was monitored for RSV in nasal wash samples by qualitative reverse-transcription–polymerase chain reaction (RT-PCR) assay and were randomized into cohorts when RSV-positive or 5 days after inoculation. SD, single dose.

**Methods**

**Healthy subjects RSV M37b Infection (n=140)**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Cohort</th>
<th>Placebo</th>
<th>Presatovir 50/25 mg, 5 days</th>
<th>Presatovir 25/25 mg, 5 days</th>
<th>Presatovir 10/5 mg, 5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>39</td>
<td>100% SO</td>
<td>100% SO</td>
<td>100% SO</td>
<td>100% SO</td>
<td>100% SO</td>
</tr>
<tr>
<td>17</td>
<td>50% 25 mg, 5 days</td>
<td>50% 25 mg, 5 days</td>
<td>50% 25 mg, 5 days</td>
<td>50% 25 mg, 5 days</td>
<td>50% 25 mg, 5 days</td>
</tr>
<tr>
<td>14</td>
<td>100% SO</td>
<td>100% SO</td>
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</tr>
<tr>
<td>15</td>
<td>50% 25 mg, 5 days</td>
<td>50% 25 mg, 5 days</td>
<td>50% 25 mg, 5 days</td>
<td>50% 25 mg, 5 days</td>
<td>50% 25 mg, 5 days</td>
</tr>
</tbody>
</table>

**Competition Fitness Assay**

- **Endpoint**
  - Viral load: 100 %<br>  100 μL of serial dilutions of presatovir Vero E6 cells infected with RSV 100 μL<br>  - HEp-2 cells (3x10^3 cells/well) infected with RSV vs. 100 μL<br>  - Virus replication quantified 4 days postinfection by RSV-specific ELISA using RSV F-protein-specific murine monoclonal antibody (1x10^4.5 tissue infectious doses/mL) in media in presence of serial dilutions of presatovir<br>  - Plasma concentration of presatovir above EC95 for RSV Memphis 37b correlated with antiviral response

**Resistance Frequency**

- **Resistance Sampling**
  - Viral RNA extracted from nasal wash samples using QIAamp RNA Isolation System (QIAGEN, Valencia, CA)  - Nucleotide sequence of RSV F gene determined by Sanger sequencing  - Surveillance of RSV recombinants containing TEMs related to isolated RSV  - wild-type (WT) RSV F protein  - Sequence F gene of several viral isolates from subjects experimentally challenged with RSV and treated with presatovir  - Resistant RSV variants

**Exposure-Driver Antiviral Activity of Presatovir**

- **Methods**
  - **Exposure level of presatovir above EC95 for RSV Memphis 37b correlated with antiviral response**

**Clinical Response to Presatovir in Subjects With TEMs Associated With RSV Resistance**

- **Clinical Parameter**
  - Viral load: 100 %<br>  CT<200 in 5 d<br>  - Symptom score: 50 %<br>  CT<200 in 5 d<br>  - Morbidity: 100 %<br>  CT<200 in 5 d