The effect of a selective CXCR2 antagonist (AZD5069) on human blood neutrophil count and innate immune functions.

Jurcevic, S.

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The effect of a selective CXCR2 antagonist (AZD5069) on human blood neutrophil count and innate immune functions

Supplementary information

**Supplementary figure 1.** Reduction in blood neutrophil count on Day 3 of treatment period. Each point is shown as the arithmetic mean (± SD) neutrophil percent change from naïve (pre-treatment) baseline versus time by treatment, on linear scale.

![Supplementary figure 1](image)

**Supplementary figure 2.** Images of neutrophils from representative subjects treated with either placebo or AZD5069.

Neutrophil phagocytic function was visualised using a multispectral imaging flow cytometry instrument (Imagestream-X, Amnis). Images taken at 0°C are negative controls; phagocytosis was triggered by cell incubation at 37°C. Individual neutrophils are visualised in bright field (BF), green fluorescence (*E. coli* FITC) that shows FITC-labelled *E. coli* engulfed within the cell. After exposure
to propidium iodide (PI), which stains the cell nucleus, the figure shows typical segmented nuclei and a combination/superimposition of BF/E. coli FITC images.

The Phagotest assay used in this study includes the fluorescence quenching step, which neutralises FITC fluorescence in E. coli that are not protected by being inside the neutrophils. Thus, on the placebo 37°C phagocytosis BF/E. coli FTC image, bacteria attached to the surface of the neutrophil are visible but lack green fluorescence label.
**Supplementary figure 3.** Serum levels of high sensitivity C-reactive protein (hsCRP) remained low during both treatment periods. Figure shows mean values (SD).
### Supplementary table 1. Demographics of human volunteer study population.

<table>
<thead>
<tr>
<th>Variable/Category</th>
<th>All subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=30</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>30 (8)</td>
</tr>
<tr>
<td>Median</td>
<td>28</td>
</tr>
<tr>
<td>Minimum/maximum</td>
<td>18/45</td>
</tr>
<tr>
<td>Gender (n [%])</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29 (96.7%)</td>
</tr>
<tr>
<td>Female</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Race (n [%])</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>29 (96.7%)</td>
</tr>
<tr>
<td>Other*</td>
<td>1 (3.3%)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>178 (9)</td>
</tr>
<tr>
<td>Median</td>
<td>179</td>
</tr>
<tr>
<td>Minimum/maximum</td>
<td>158/191</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>75.6 (10.7)</td>
</tr>
<tr>
<td>Median</td>
<td>75.0</td>
</tr>
<tr>
<td>Minimum/maximum</td>
<td>50.2/108.0</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>23.9 (2.5)</td>
</tr>
<tr>
<td>Median</td>
<td>23.9</td>
</tr>
<tr>
<td>Minimum/maximum</td>
<td>19.0/30.0</td>
</tr>
</tbody>
</table>

SD: standard deviation
*Subject E0002003 was Middle Eastern.
**Supplementary table 2.** Circulating neutrophil counts over time in human volunteers

<table>
<thead>
<tr>
<th>Circulating neutrophil count (10^9/L)</th>
<th>Baseline</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 14</th>
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</thead>
<tbody>
<tr>
<td>AZD5069</td>
<td>n</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>3.43</td>
<td>1.10</td>
<td>1.42</td>
<td>1.36</td>
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<tr>
<td></td>
<td>SD</td>
<td>0.89</td>
<td>0.61</td>
<td>0.70</td>
<td>0.65</td>
</tr>
<tr>
<td>Change from baseline</td>
<td>n</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>-1.67</td>
<td>-1.34</td>
<td>-0.40</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.67</td>
<td>0.69</td>
<td>0.60</td>
<td>0.74</td>
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<tr>
<td>Placebo</td>
<td>n</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>3.21</td>
<td>3.00</td>
<td>2.90</td>
<td>2.92</td>
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<tr>
<td></td>
<td>SD</td>
<td>0.80</td>
<td>0.89</td>
<td>0.87</td>
<td>0.94</td>
</tr>
<tr>
<td>Change from baseline</td>
<td>n</td>
<td>29</td>
<td>29</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>0.19</td>
<td>0.09</td>
<td>0.11</td>
<td>0.22</td>
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<tr>
<td></td>
<td>SD</td>
<td>0.78</td>
<td>0.80</td>
<td>0.86</td>
<td>1.23</td>
</tr>
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</table>

**Supplementary table 3.** Human volunteer neutrophil functional assays at the indicated timepoints.

<table>
<thead>
<tr>
<th>Phagocytosis (%)</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD5069 (n=29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean</td>
<td>96.2</td>
<td>96.2</td>
<td>97.5</td>
</tr>
<tr>
<td>CV%</td>
<td>1.4</td>
<td>2.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>96.2</td>
<td>96.2</td>
<td>97.5</td>
</tr>
<tr>
<td>SD</td>
<td>1.2</td>
<td>2.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Median</td>
<td>96.4</td>
<td>96.9</td>
<td>97.8</td>
</tr>
<tr>
<td>95% CI</td>
<td>(97.7, 98.7)</td>
<td>(97.3, 97.1)</td>
<td>(96.9, 98.1)</td>
</tr>
<tr>
<td>Placebo (n=29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean</td>
<td>97.9</td>
<td>97.6</td>
<td>98.0</td>
</tr>
<tr>
<td>CV%</td>
<td>1.8</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>97.9</td>
<td>97.6</td>
<td>98.0</td>
</tr>
<tr>
<td>SD</td>
<td>1.7</td>
<td>1.6</td>
<td>1.1</td>
</tr>
<tr>
<td>Median</td>
<td>98.4</td>
<td>98.2</td>
<td>98.3</td>
</tr>
<tr>
<td>95% CI</td>
<td>(97.2, 98.6)</td>
<td>(97.0, 98.2)</td>
<td>(97.6, 98.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oxidative burst (%)</th>
<th>Day 1</th>
<th>Day 4</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD5069 (n=29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean</td>
<td>99.1</td>
<td>99.0</td>
<td>99.7</td>
</tr>
<tr>
<td>CV%</td>
<td>1.0</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Arithmetic mean</td>
<td>99.1</td>
<td>99.0</td>
<td>98.7</td>
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<tr>
<td>SD</td>
<td>0.9</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Median</td>
<td>99.4</td>
<td>99.3</td>
<td>99.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>(98.8, 99.5)</td>
<td>(98.7, 99.3)</td>
<td>(96.4, 99.1)</td>
</tr>
<tr>
<td>Placebo (n=29)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean</td>
<td>99.2</td>
<td>99.3</td>
<td>98.3</td>
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<tr>
<td>CV%</td>
<td>0.5</td>
<td>0.5</td>
<td>2.9</td>
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<tr>
<td>Arithmetic mean</td>
<td>99.2</td>
<td>99.3</td>
<td>98.3</td>
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<tr>
<td>SD</td>
<td>0.5</td>
<td>0.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Median</td>
<td>99.4</td>
<td>99.3</td>
<td>99.1</td>
</tr>
<tr>
<td>95% CI</td>
<td>(99.0, 99.4)</td>
<td>(99.1, 99.5)</td>
<td>(97.3, 99.3)</td>
</tr>
</tbody>
</table>

CV, geometric coefficient of variation; SD, standard deviation; CI, confidence interval.