Activity of the novel and selective pan-Janus kinase (JAK) inhibitor VR588 in a murine polyinosinic:polycytidilic acid (Poly(I:C)) model of viral lung inflammation.

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Rationale
VR588 is a selective pan-JAK inhibitor that demonstrates potent activity in a series of in-vitro models (see associated poster). Viral infection is an important trigger of airway disease exacerbation. The JAKSTAT pathway is activated by viral infection in asthmatic and COPD patients and is associated with the related inflammatory process. This study was designed to compare the effects of inhaled and oral VR588 in a murine model of viral lung inflammation.

Methods
The viral RNA mimic Poly(I:C) (1mg/ml) was administered intranasally to male BALB/c mice 1 hour after VR588 administration and again 16 hours later. VR588 was given at intranasal doses of 1.5, 7.5 and 15mg/kg and at an oral dose of 15mg/kg (n=6 per group). Lung tissue and bronchoalveolar lavage (BAL) fluid was harvested 40 hours after the initial Poly(I:C) challenge. Inflammatory cell count from BAL fluid was determined using FACS analysis; JAK-STAT activation (pSTAT1, 3 & 5) was measured by ELISA and BAL inflammatory cytokines by Luminex immunoassay. Fluticasone propionate (intranasal dose of 1.5 mg/kg) and tofacitinib (oral dose of 15 mg/kg) were employed as relevant comparators.

Results I
Poly(I:C) increased total BAL cell count which was reduced in a dose-related manner by intranasal VR588. In contrast, oral administration of VR588 was ineffective.

Table 1. List of inflammatory mediators increased by Poly(I:C) treatment in murine lung.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Saline</th>
<th>Poly(I:C) i.n.</th>
<th>Poly(I:C) p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>100</td>
<td>150</td>
<td>200</td>
</tr>
<tr>
<td>TNF-α</td>
<td>500</td>
<td>750</td>
<td>1000</td>
</tr>
<tr>
<td>MCP-1</td>
<td>10</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>RANTES</td>
<td>20</td>
<td>30</td>
<td>40</td>
</tr>
</tbody>
</table>

Figure 1. The effect of Poly(I:C) and intranasal (i.n.) and oral (p.o.) VR588 on total BAL cell count. Fluticasone propionate (FP) and Tofacitinib (ToF) were used as relevant comparators. **p<0.01 vs. saline group, #p<0.05, ##p<0.01 vs. Poly(I:C) group, Mann Whitney test.

Results II
Intranasal VR588 attenuated the expression of poly(I:C)-induced BAL eotaxin, MIP-1α & β, IP-10, KC, TNF-α and MCP-1 in a dose-related manner to a similar extent as FP and ToF. VR588 did not significantly attenuate VEGF and RANTES.

Figure 2. The effect of poly(I:C) and intranasal (i.n.) and oral (p.o.) VR588 on BAL mediator expression. Fluticasone propionate (FP) and Tofacitinib (ToF) were used as a relevant comparators. **p<0.01 vs. saline group, #p<0.05, ##p<0.01 vs. Poly(I:C) group, Mann Whitney test.

Results III
pSTAT1 and pSTAT5 (Fig 3A & C), but not pSTAT3 (Fig 3B), were increased by Poly(I:C) treatment and attenuated by VR588.

Figure 3. The effect of Poly(I:C) and intranasal (i.n.) and oral (p.o.) RV588 on (A) pSTAT1, (B) pSTAT3 and (C) pSTAT5 activation in murine lung. Fluticasone propionate (FP) was used as a relevant comparator. **p<0.01 vs. saline group, #p<0.05, ##p<0.01 vs. Poly(I:C) group, Mann Whitney test.

Conclusions
VR588 attenuated Poly(I:C)-induced inflammatory cell accumulation, cytokine production and activation of STAT signalling pathways and was more effective by the inhaled versus the oral route.

This supports the concept that JAK inhibition may be a useful strategy for the treatment viral exacerbation in airway disease.

Further studies evaluating the VR588 via the inhaled route in-vivo profile post repeat dose exposure are justified.

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