Evaluation of 5P12-RANTES analogue expression in *Nicotiana benthamiana*

Emerging Researcher Symposium

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Nearly 34 million people were living with HIV

Sub-Saharan Africa shows high proportion (%) of infected adults (people aged 15 to 49 years)

New infections (瑙) were reported (in thousands) among adults and children

Sources: UNAIDS, WHO, Reuters
A lot of progress has been made to manage HIV
But…..

- Condoms
- Monogamy
- Partner reduction
- ARV
- Treatment of STI

Arntzen, 2005
More solutions are required

- Globally, 60% of women are affected
- Available protection measures are biased towards male partners
- Most women have little negotiating power on sexual matters
- Microbicides may offer a viable option that women can initiate
  - Microbicides are molecules that eliminate or reduce the spread of viruses or bacteria
Discovery of 5P12-RANTES

**RANTES**: Regulated upon activation, normal T-cell expressed and secreted
- Small basic chemokine (7.9 kDa)
5P12-RANTES mechanism of action

HIV

5P12-RANTES

RANTES

HIV transmission

No HIV transmission
RANTES is difficult to manufacture

Current bioreactors

- Yeast (e.g. *Pichia*) – linear and cyclic (active) forms
- *E. coli* – insoluble inclusion bodies formation
- Mammalian cells – low expression yield
- Unaffordable

Low-cost bioreactors

Plants - *Nicotiana benthamiana*

- Capacity to carry out post-translational modification
- Used for more than 20 years as an expression host – susceptible to various pathogens, e.g. viruses, bacteria and fungi
- Safe & cheap
Aim

Can functional 5P12-RANTES be expressed in tobacco via transient approaches?
Objectives

a) To clone 5P12-RANTES analogue into a transient expression vector (viral and bacterial)

b) Agroinfiltration of 5P12-RANTES constructs into tobacco leaves

c) Evaluate protein expression by ELISA and western blot

d) Optimise protein expression level by subcellular targeting

e) To develop protein purification process
Materials and methods

1. Cytotoxicity assay (based on tetrazolium compound)
2. Neutralisation assay

(f) ELISA
(g) Nickel affinity chromatography
(h) Size exclusion chromatography

(a) 6-8 weeks old plants and Agrobacterial culture
(b) Agroinfiltration
(c) Plant incubation
(d) Biomass harvest
(e) Protein extraction
Results - ELISA

- ELISA (enzyme linked immunosorbent assay) was used to evaluate expression with anti-RANTES antibodies.

**dpi** – day post infiltration
Western blot analysis

Western blot confirmed expression and size of plant-based 5P12-RANTES
Cytotoxicity – MTS based

MTS tetrazolium compound, \{(3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium\}
## Efficacy testing

**Table 1** Summary of IC$_{50}$ values of the HIV-1 pseudovirions screened.

<table>
<thead>
<tr>
<th>Sample identification</th>
<th>ZM53 (targets CCR5) IC$_{50}$ (µg/ml)</th>
<th>HXB2 (targets CXCR4) IC$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>0.59</td>
<td>2.05</td>
</tr>
<tr>
<td>Large proteins (&gt;10 kDa)</td>
<td>0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>Small proteins (&lt;10 kDa)</td>
<td>0.06</td>
<td>0.02</td>
</tr>
<tr>
<td>5P12-RANTES (CCR5 inhibitor)</td>
<td>&lt;0.0006</td>
<td>NT</td>
</tr>
<tr>
<td>pHICH11599 (-ve control)</td>
<td>1.66</td>
<td>0.56</td>
</tr>
<tr>
<td>278RN-RANTES (<em>E. coli</em> based)</td>
<td>20-100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>T20 (fusion inhibitor)</td>
<td>0.081</td>
<td>0.04459</td>
</tr>
</tbody>
</table>
Non-specific antiviral activity

Plant-based 5P12-RANTES did not inhibit infection downstream of viral entry, hence it is specific to CCR5 inhibition

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Conclusion

• Proof of concept was obtained

• Viral vector system gave better expression levels (i.e., 603 µg/kg)

• Apoplast was the best compartment for heterologous expression of 5P12-RANTES

• Recombinant 5P12-RANTES inhibited binding of ZM53 and HXB2 pseudovirions to CCR5 receptors

• 5P12-RANTES was specific to CCR5 inhibition as it was unable to inhibit the endocytosed VSV-G pseudovirions

• Future work, optimise conditions for better expression of 5P12-RANTES in the apoplast and chloroplast
• Prof JN Eloff, Dr E Chakauya and Dr R Chikwamba

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Thank you