


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

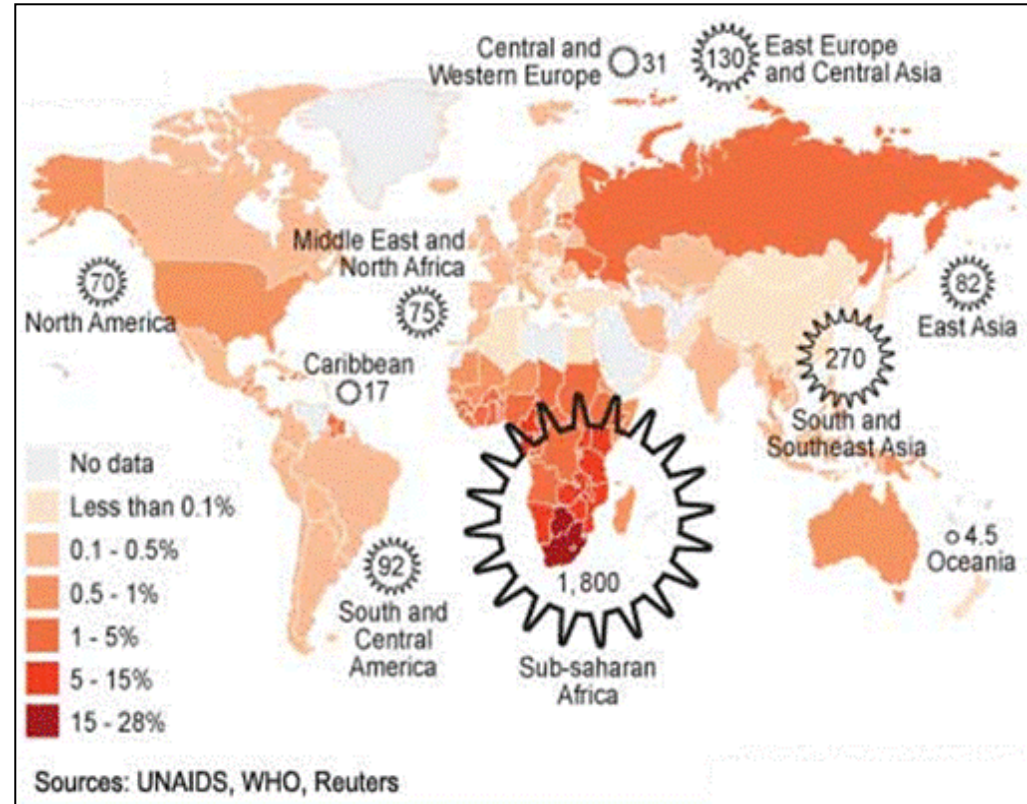


Emerging Researcher Symposium

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10 October 2012

Global view of HIV infection : 2010

- 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 () were reported (in thousands) among adults and children



A lot of progress has been made to manage HIV But....

Condoms

Monogamy

Partner reduction

ARV

Treatment of STI

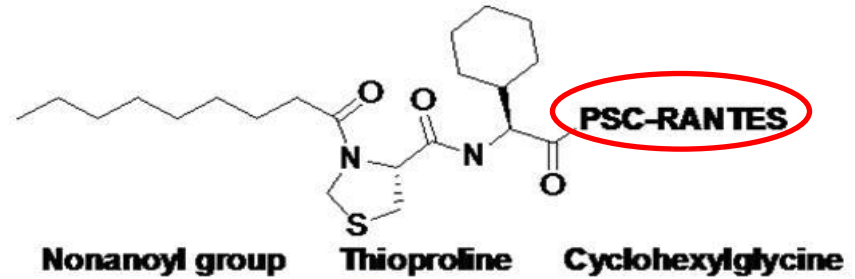
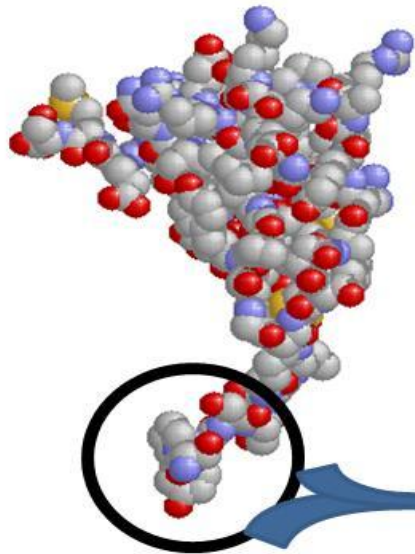
?

More solutions are required

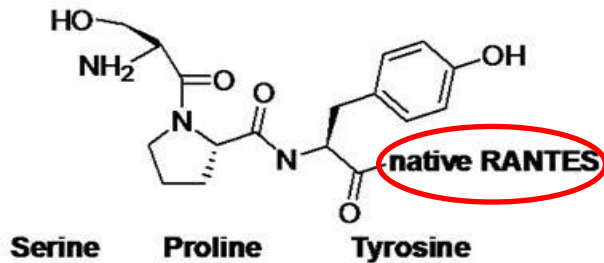


- Globally, 60% of women are affected
- Available protection measures are biased towards male partners
- Most women have little negotiating power on sexual matters
- Microbicides may offer a viable option that women can initiate
 - **Microbicides are molecules that eliminate or reduce the spread of viruses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

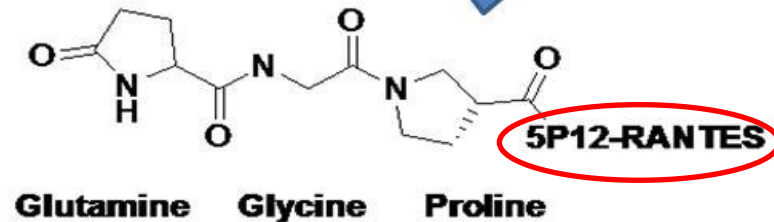
Discovery of 5P12-RANTES



1. Total chemical synthesis



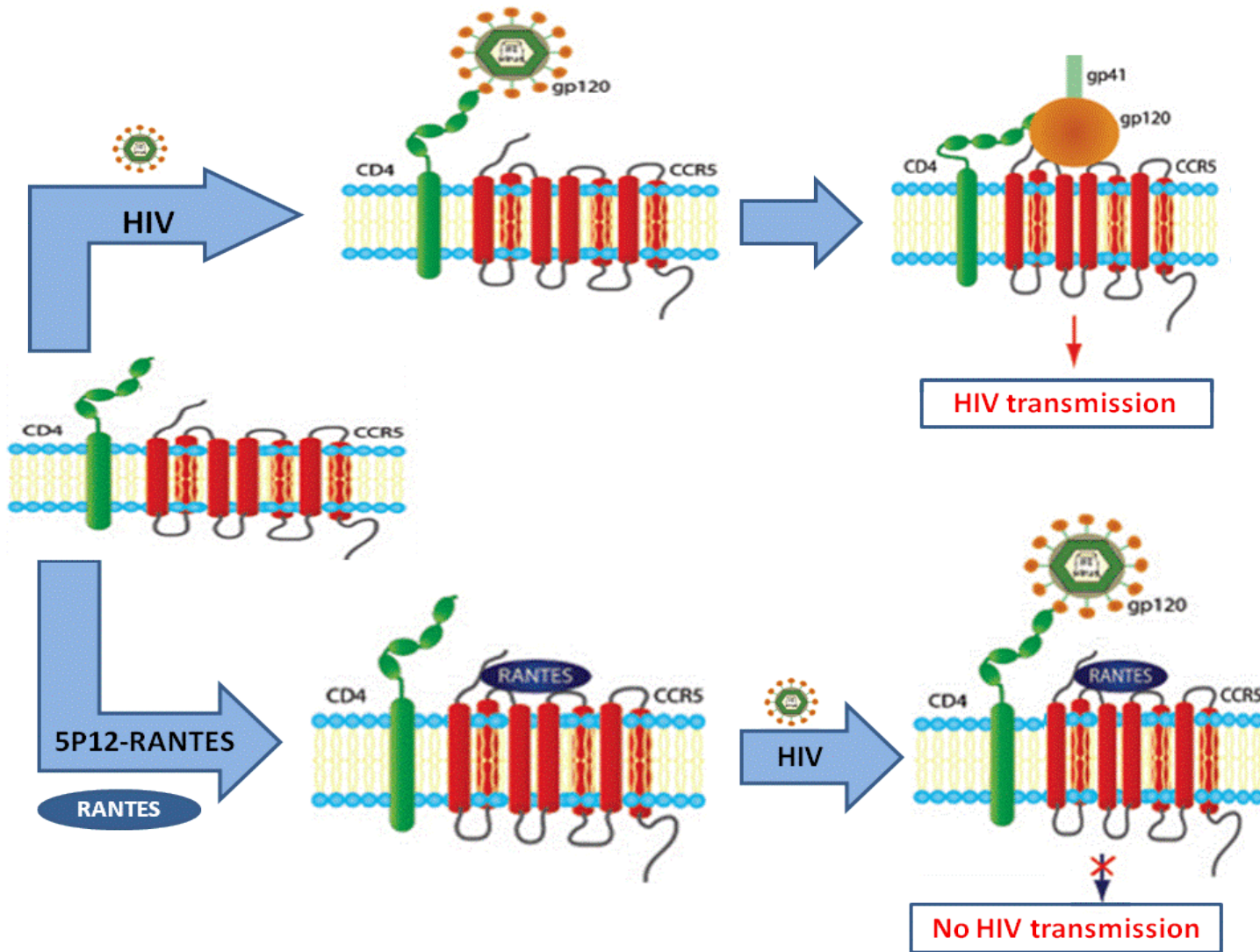
2. Phage display system



RANTES : Regulated upon activation, normal T-cell expressed and secreted

- Small basic chemokine (7.9 kDa)

5P12-RANTES mechanism of action



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



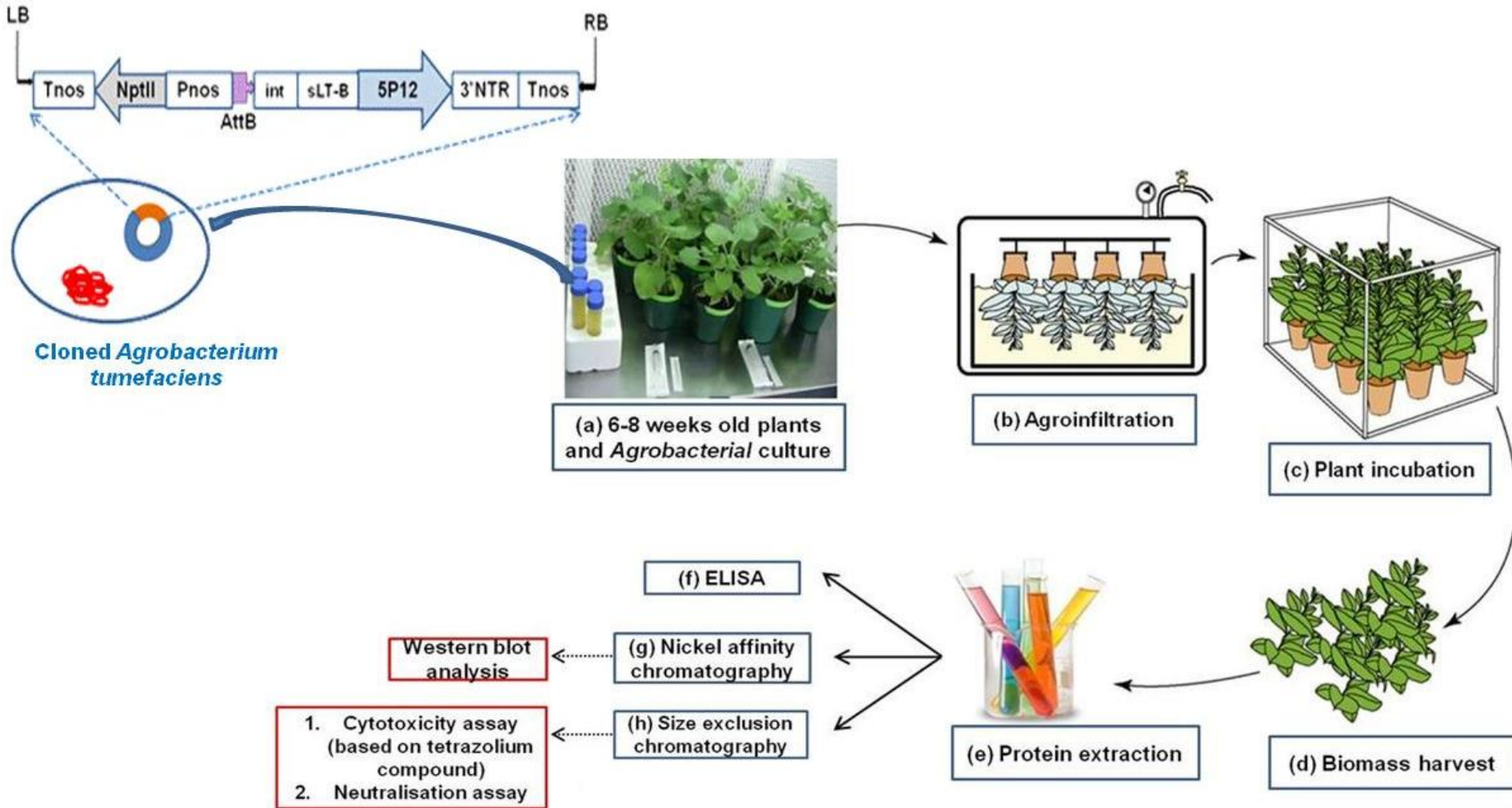
Can functional 5P12-RANTES be expressed in tobacco via transient approaches?

Objectives

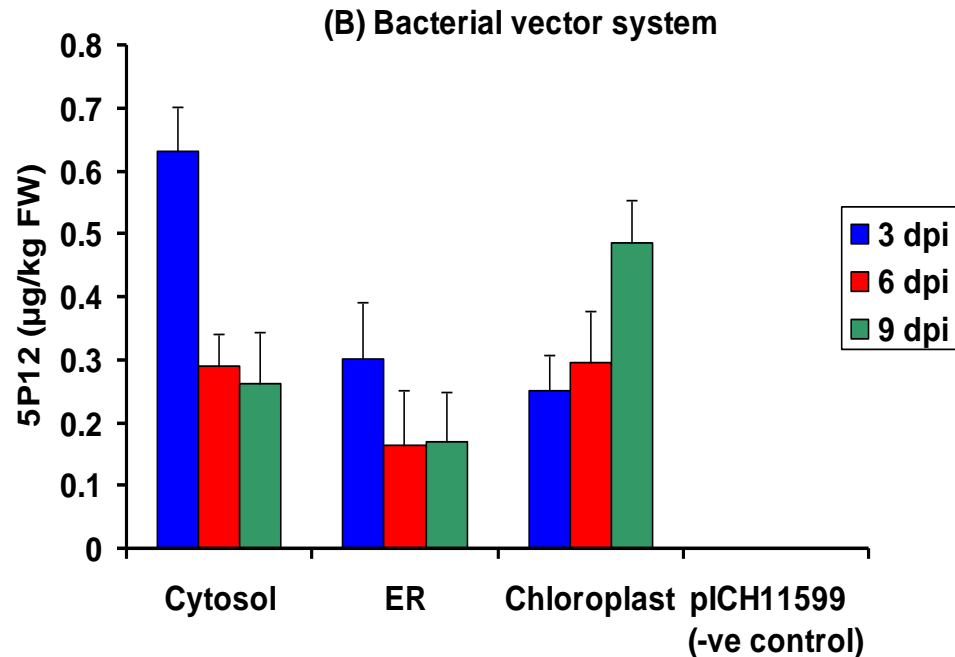
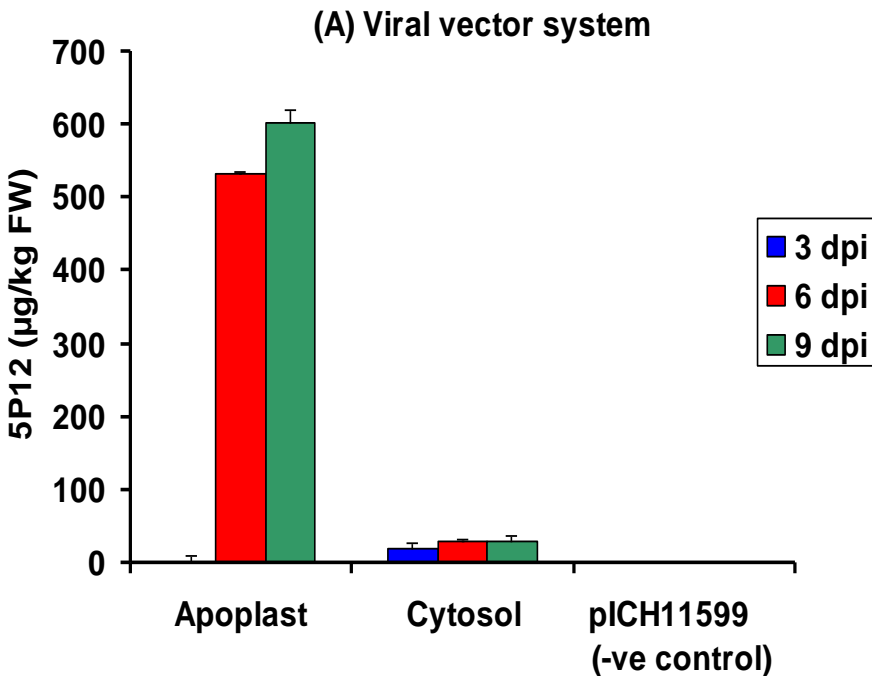


- a) To clone 5P12-RANTES analogue into a transient expression vector (viral and bacterial)
- b) Agroinfiltration of 5P12-RANTES constructs into tobacco leaves
- c) Evaluate protein expression by ELISA and western blot
- d) Optimise protein expression level by subcellular targeting
- e) To develop protein purification process

Materials and methods



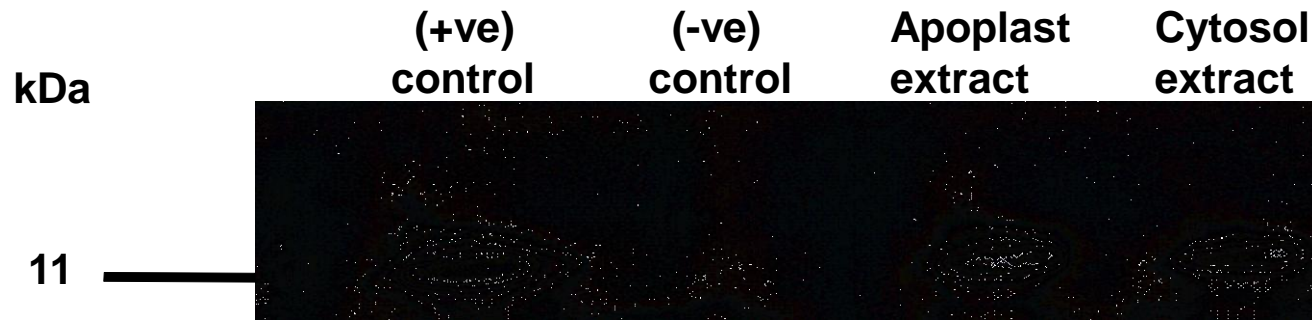
Results - ELISA



dpi – day post infiltration

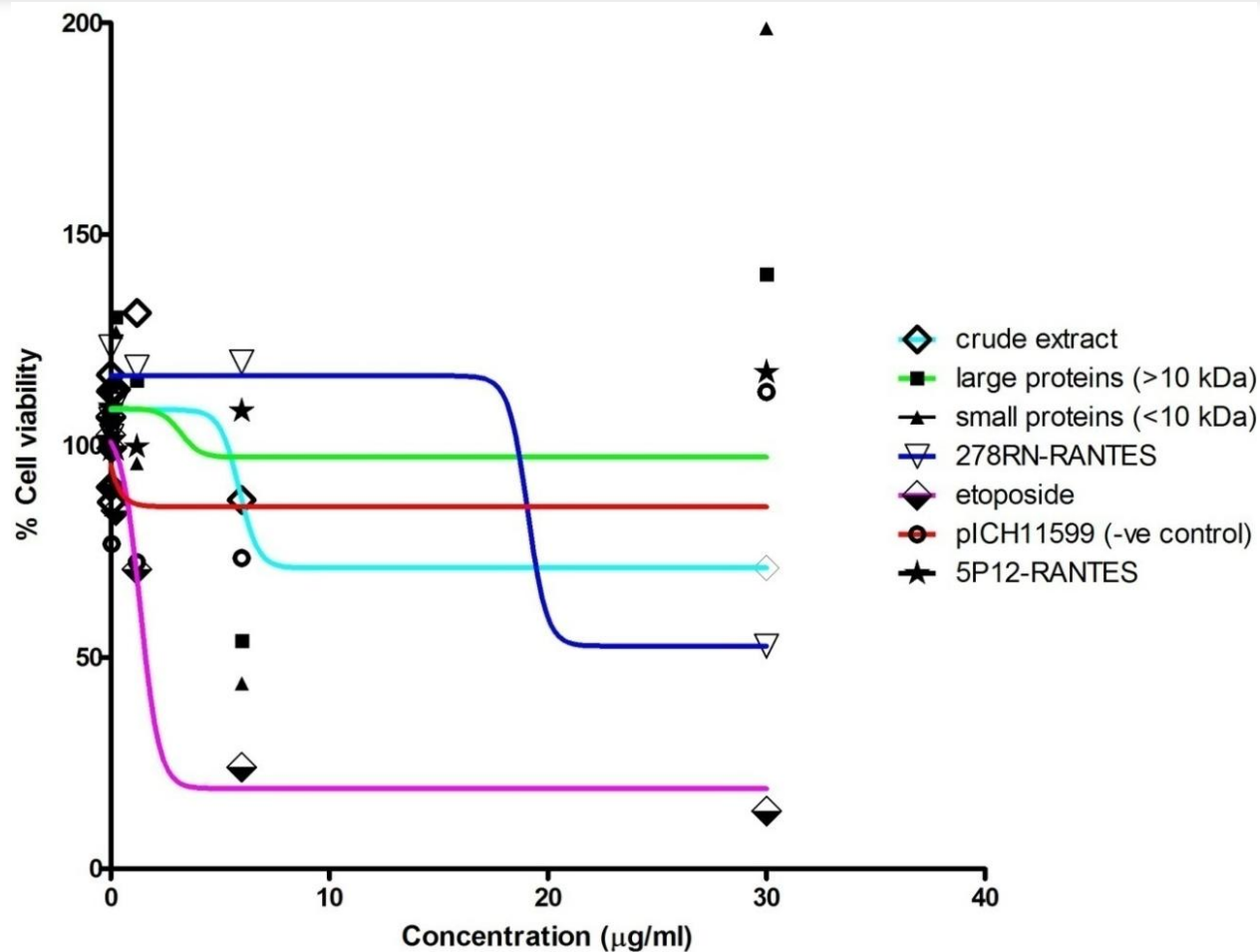
- **ELISA** (enzyme linked immunosorbent assay) was used to evaluate expression with anti-RANTES antibodies

Western blot analysis



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

Cytotoxicity – MTS based



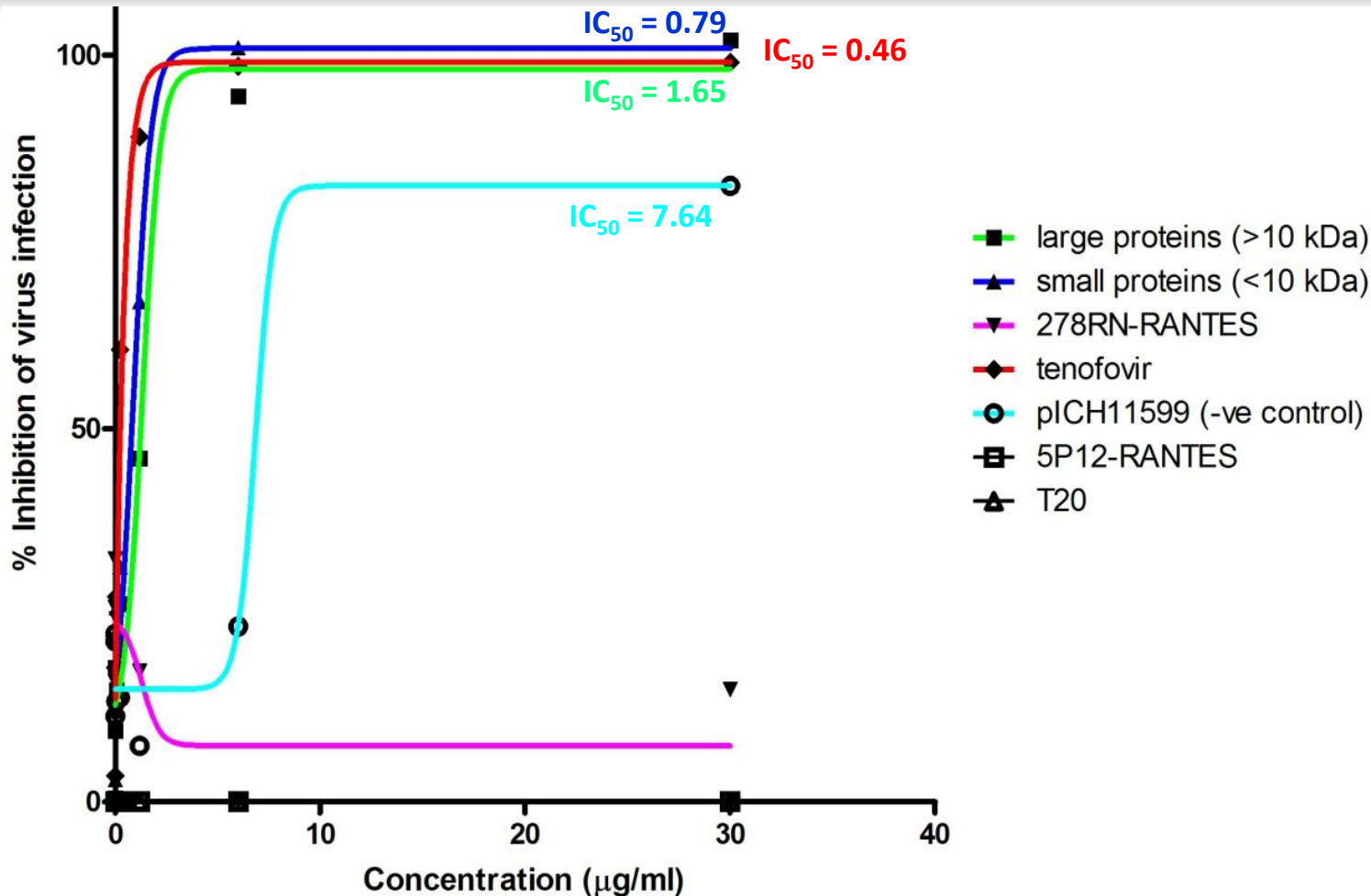
MTS tetrazolium compound, {3-(4,5-dimethyl-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium

Efficacy testing

Table 1 Summary of IC₅₀ values of the HIV-1 pseudovirions screened.

Sample identification	ZM53 (targets CCR5) IC ₅₀ (µg/ml)	HXB2 (targets CXCR4) IC ₅₀ (µg/ml)
Crude extract	0.59	2.05
Large proteins (>10 kDa)	0.09	0.02
Small proteins (<10 kDa)	0.06	0.02
5P12-RANTES (CCR5 inhibitor)	<0.0006	NT
pICH11599 (-ve control)	1.66	0.56
278RN-RANTES (<i>E. coli</i> based)	20-100	>100
T20 (fusion inhibitor)	0.081	0.04459

Non-specific antiviral activity



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

Conclusion



- Proof of concept was obtained
- Viral vector system gave better expression levels (i.e., 603 $\mu\text{g}/\text{kg}$)
- 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

Acknowledgements



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Thank you

