Breaking through low immunogenicity of reverse transcriptase in therapeutic vaccines against drug resistance in HIV infection

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BACKGROUND

- Highly Active Antiretroviral Therapy (HAART) dramatic change Death from AIDS-related diseases reduced significantly

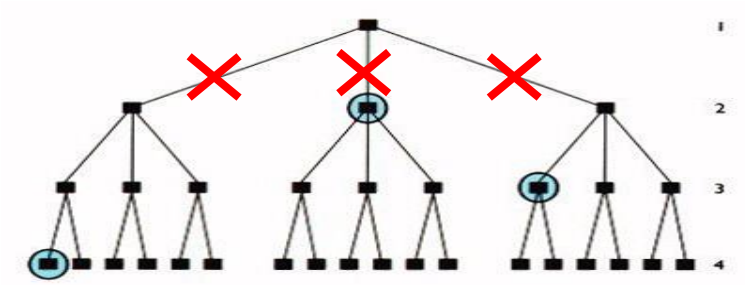
- The emergence of multiple drug-resistant viral strains (drHIV), primary infections with drHIV , failures on HAART regimens

- The immune-mediated control of HIV replication in the absence of ART (also called “functional cure”) are needed

- Eradication strategy aims at the induction of viral replication in latently-infected cells and at the elimination of these reactivated cells by either direct cytolytic targeting or by immunotherapeutic intervention

AIM

Prevent or hinder development of drug resistance in HIV-infection by therapeutic vaccination preceding or parallel to HAART



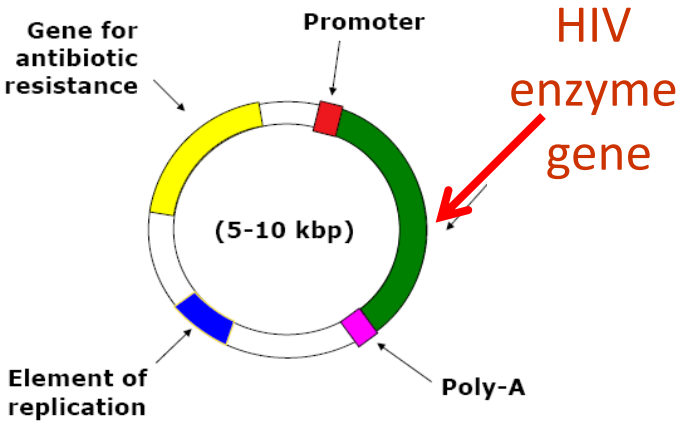
Develop a complex approach for immunotherapy of HIV including generation of synthetic antigens, methods for in vitro, ex vivo and in vivo testing of immunogens, with the emphasis on HIV enzymes.

REVERSE TRANSCRIPTASE

Choice of vaccine vehicle - naked DNA

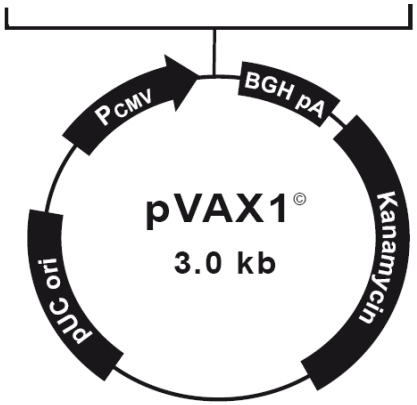
- Genetic vaccine based on plasmid DNA
- Plasmid encodes viral antigen of choice
- Antigen is expressed in the vaccine recipient; correctly processed and folded. No need in expression/purification
- Elicites B and T cell response as attenuated viral vaccine, but totally safe /not virulent
- Standard plasmid manufacture from bacteria
- Highly stable over long period of time
- No cold chain in storage /distribution

Effective methods of vaccination



DESIGN

RT GENE



PLASMID BACKBONE

pVax 1 (Invitrogen)

HIV ENZYME GENES

- Encoding - wild type or with mutation of drug-resistance

wt / 1.14

- Introduce mutations abrogated enzymatic activity

in

- Codon-optimized genes to increase expression

vir / opt

Expression of RT variants in the cells

Plasmids encoding RT variants were generated.
HeLa cells transfected with plasmids and protein synthesis was proved by Western blot

1 - pVax RTwt

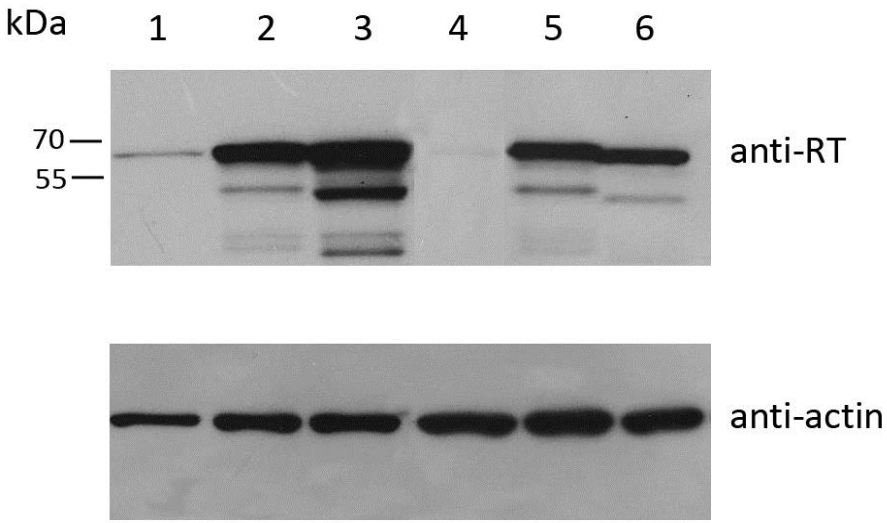
2 - pVax RTwt opt

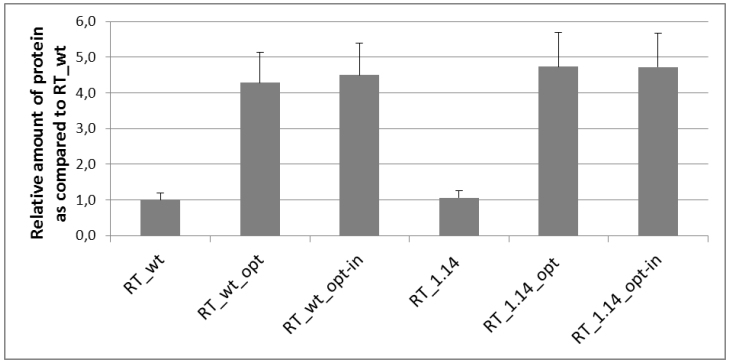
3 - pVax RTwt opt-in

4 - pVax RT 1.14

5 - pVax RT 1.14 opt

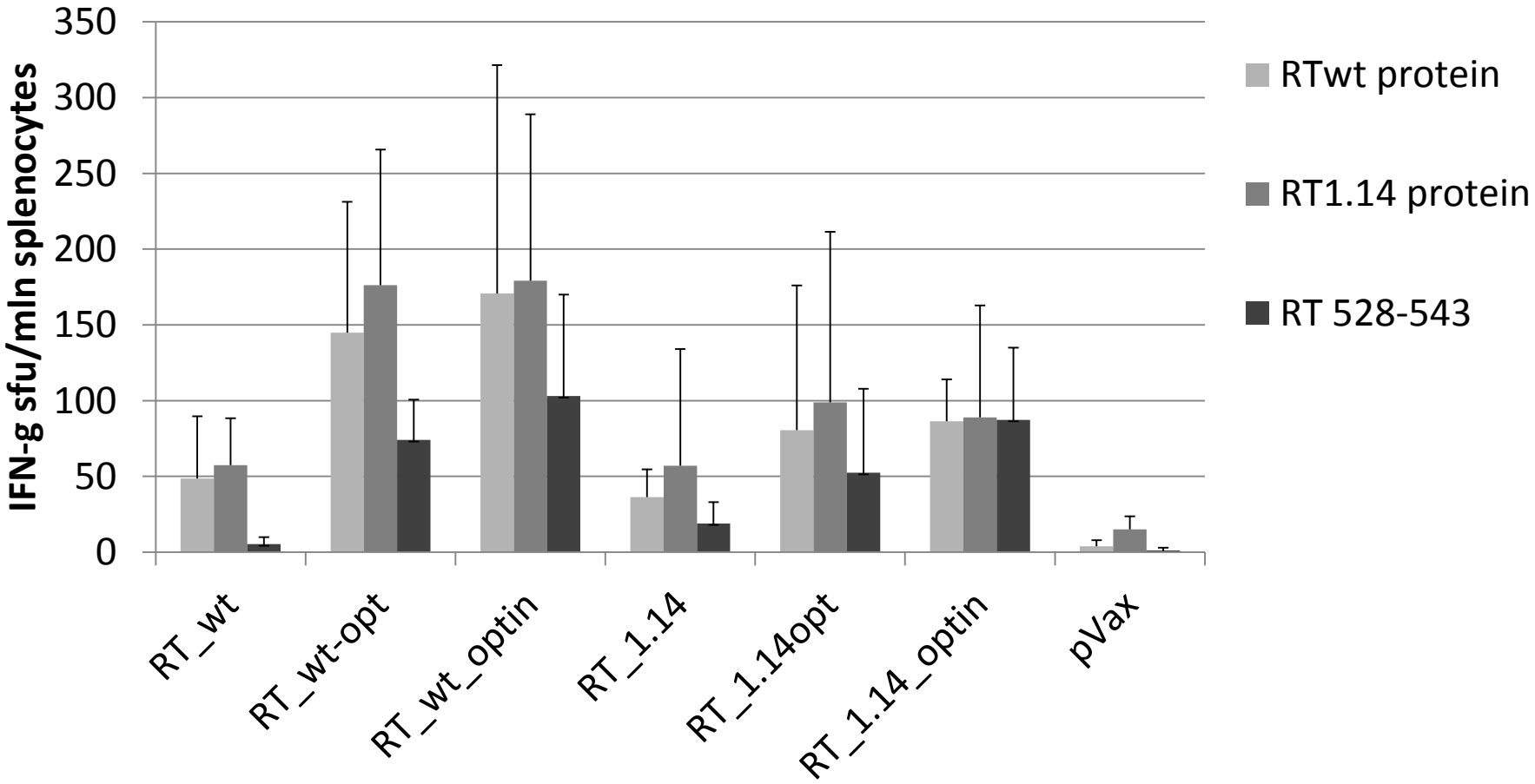
6 - pVax RT1.14 opt-in





Immunogenicity: GENE / PLASMID

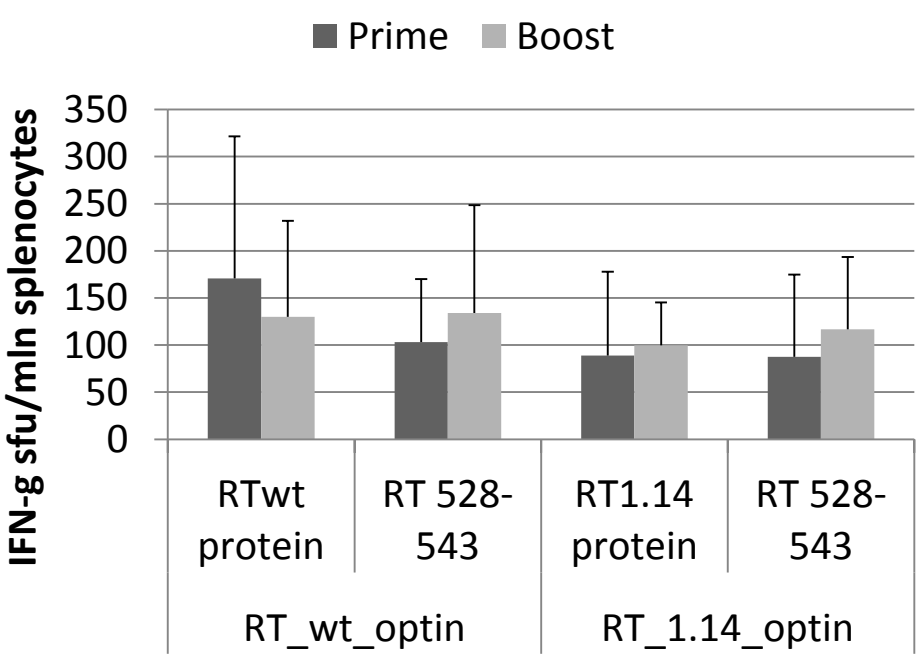
Mice (BALB/c) were immunized – plasmid injection followed by EP
21 days after FluoroSpot was performed

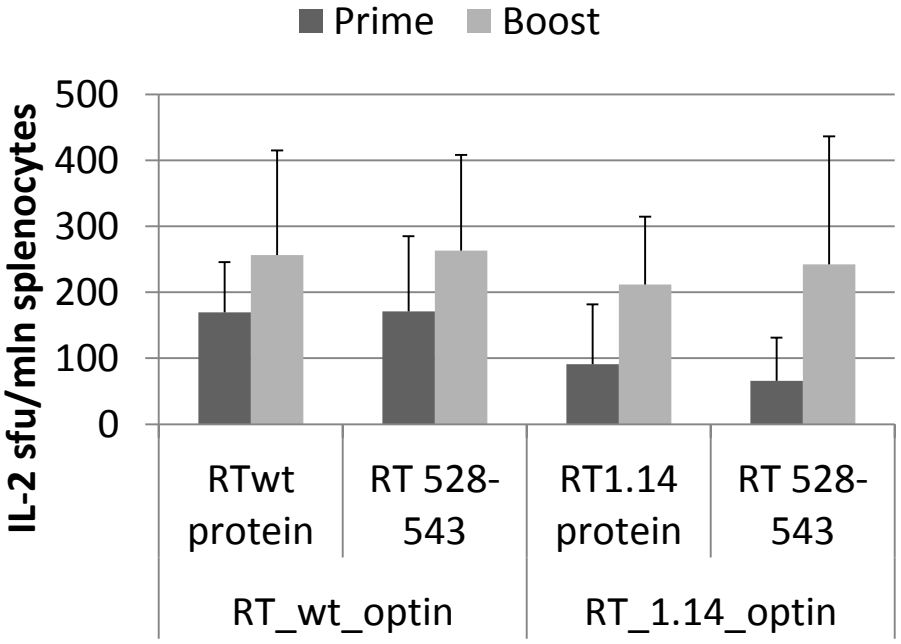


Immunogenicity: PRIME/BOOST REGIMEN

BALB/c mice were primed and one month after boosted with RT DNA. Cytokine assays after prime and boost

Effect of boosting: IL-2 boosted; IFN-g no boost

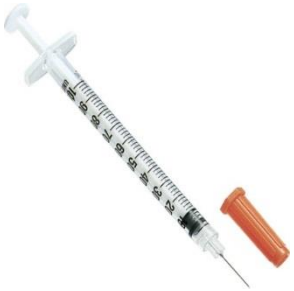




Immunogenicity: DELIVERY REGIMEN

Injection regime

29G needles OR



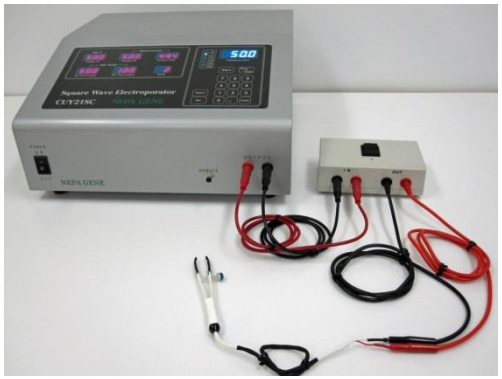
Microneedles

Micronjet (Nanopass Technologies)

Biojector (delivery by gas pressure)

Electroporation regime

Dermavax, Cellectis

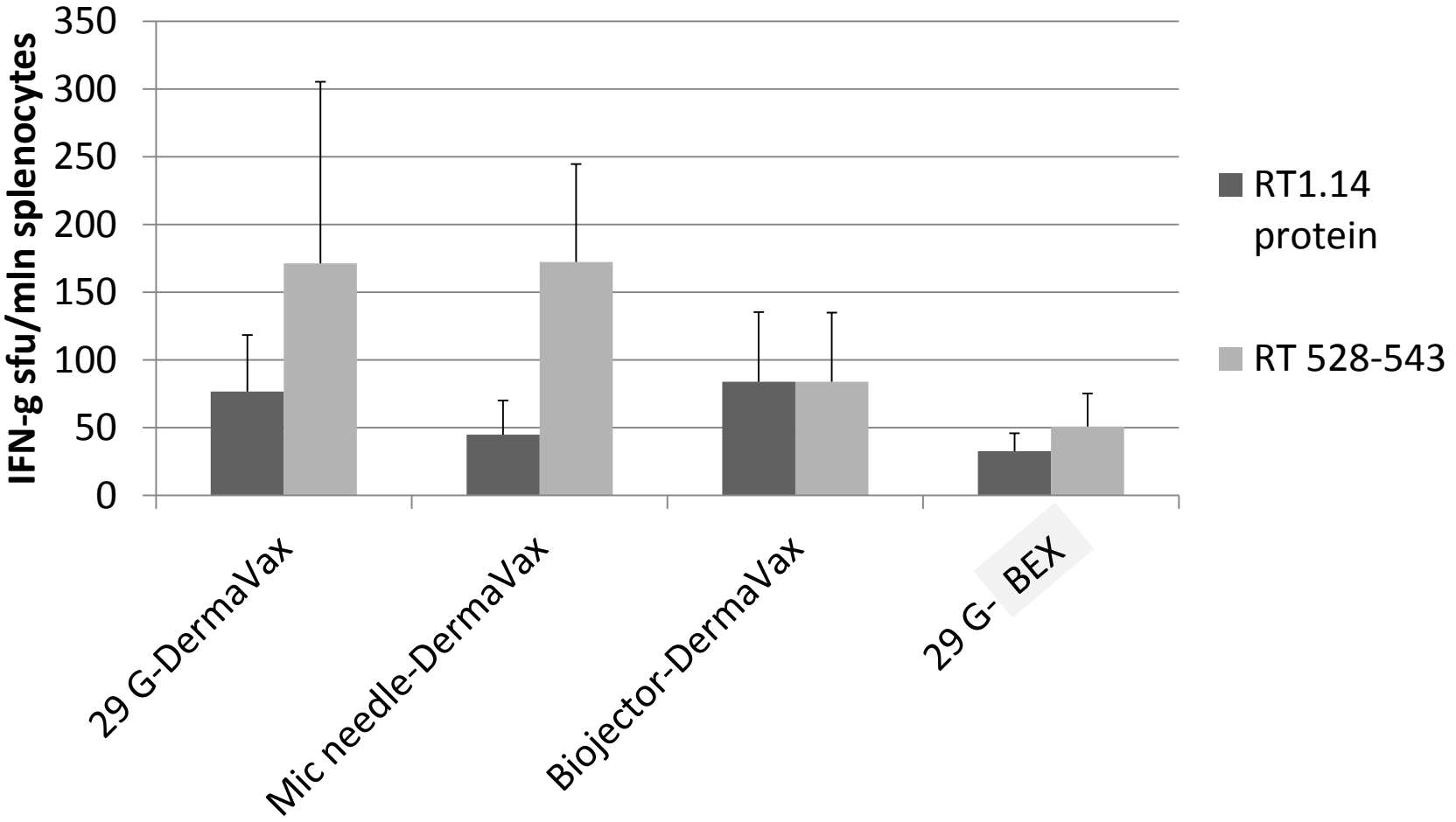


CUY21EDIT, BEX

Immunogenicity: DELIVERY INJECTION/ELECTROPORATION

Mice (BALB/c) were immunized by RT DNA followed by EP IFN-g assay after 3 weeks.

Best: delivery by insulin needles and microneedles.



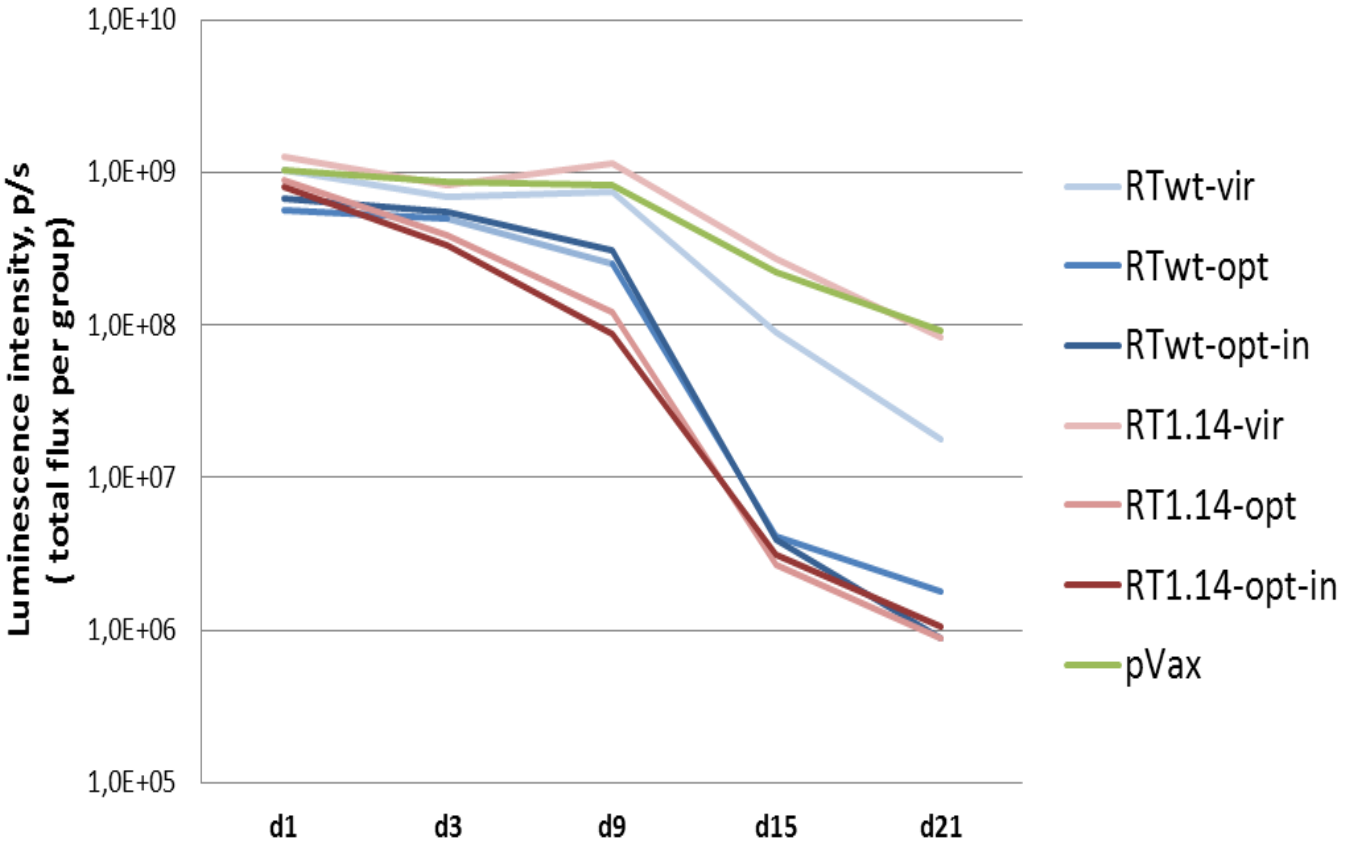
Immunogenicity: FOLLOW-UP OF DELIVERY/IMMUNE RESPONSE *in vivo*

In vivo monitoring of immune response by elimination of antigen-expressing cells

Day 1 – prime
RT DNA + Luc DNA

In vivo monitoring
Day 1, 3, 9, 15, 21

Optical In Vivo Imaging System (IVIS)



Conclusions

Potency of HIV RT as a DNA immunogen can be greatly increased by:

- **Codon optimization of the gene**
- **Delivery by optimal injections**
- **Electroporation**
- **Prime-boost regimen**

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