DNA vaccination against drug resistance in chronic viral infections, example of HIV-1

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BACKGROUND

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

- **How long clinical benefit will last?**
  the emergence of multiple drug-resistant viral strains (drHIV)
  primary infections with drHIV
  failures on HAART regimens

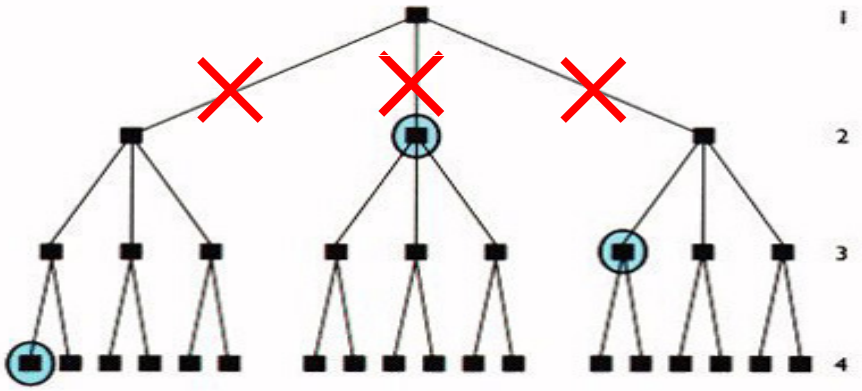
- **Immune response limits HIV-1 replication. Elite controllers!**
  Under HAART – no antigen stimulation - loss of anti-HIV immune response

- **Urgent task – to exert an additonal non-drug pressure on HIV-1**
  to reduce its capacity to develop resistance
  CREATE A ”BOTTLE NECK” EFFECT

CONCEPT - complement HAART with an immune pressure!

AIM

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



Immune prevention of primary DR mutations to

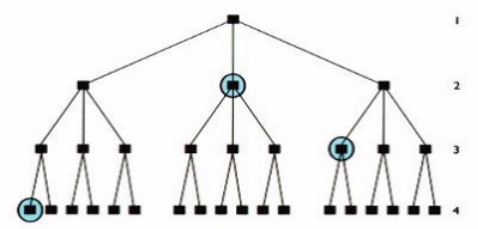
hamper the whole process of drug resistance development

TASKS:

- Selection of antigens responsible for resistance – HIV enzymes, gp41.
- Selection of genetic background – HIV1 FSU-A
- Consensus approach to design of antigens
- Genetic engineering of plasmid vectors
- Modification of antigens by introduction of primary drug resistance mutations
- Efficient protocols for DNA delivery for preclinical trials
- Preclinical trials, also on the background of treatment with antiretroviral drugs

DESIGN OF IMMUNOTHERAPEUTICALS

Choice of components
Antigens involved in HIV resistance – primarily HIV ENZYMES



Design principles:

- **consensus sequence** → HIV-1 subtype A prevalent in Russia and FSU
- **primary DR mutations** → Block appearance of early mutations – hamper the whole process
- **enzyme inactivation for safety** → Introduce mutations abrogated enzymatic activity

Choice of vaccine vehicle - naked DNA

PLASMID BACKBONE

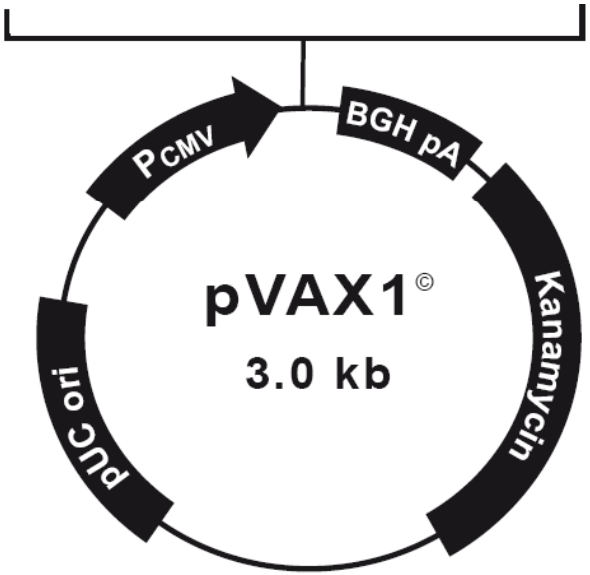
pVax 1 (Invitrogen)

HIV ENZYME GENES

- Consensus HIV-1 subtype A
- Codon-optimized to increase expression

MULTI-GENE SET GENERATED: RTPRIN-A

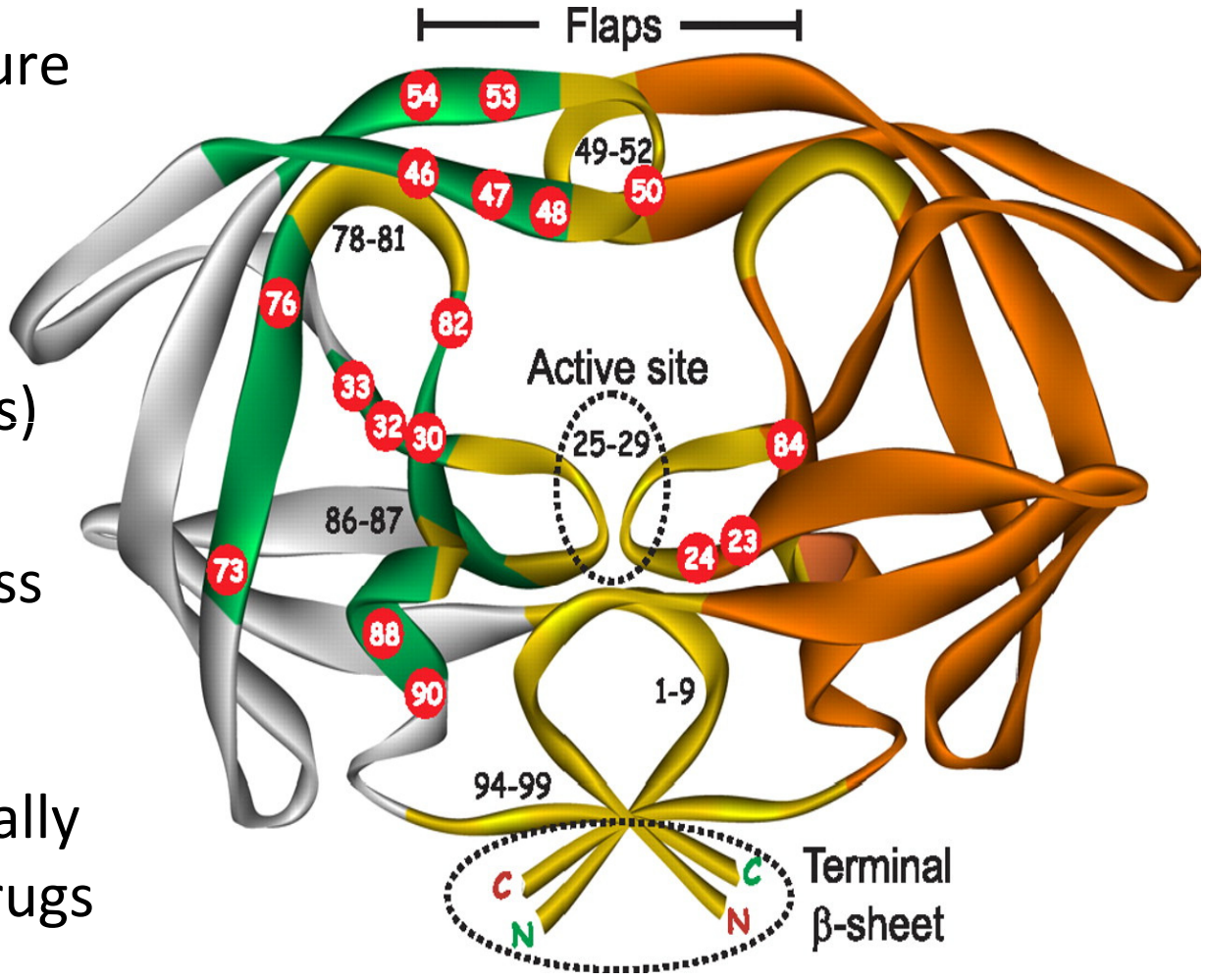
- Reverse Transcriptase;
- Protease;
- Integrase HIV subtype A



DESIGN OF IMMUNOTHERAPEUTICALS: example of protease

- Cleaves Gag and Pol polyproteins into mature forms
- Major target of antiretroviral therapy
- Protease inhibitors (PIs) bind active site
- Long-term effectiveness limited due rapid viral evolution, leading to proteases with drastically lower affinity to the drugs

HIV-1 Protease



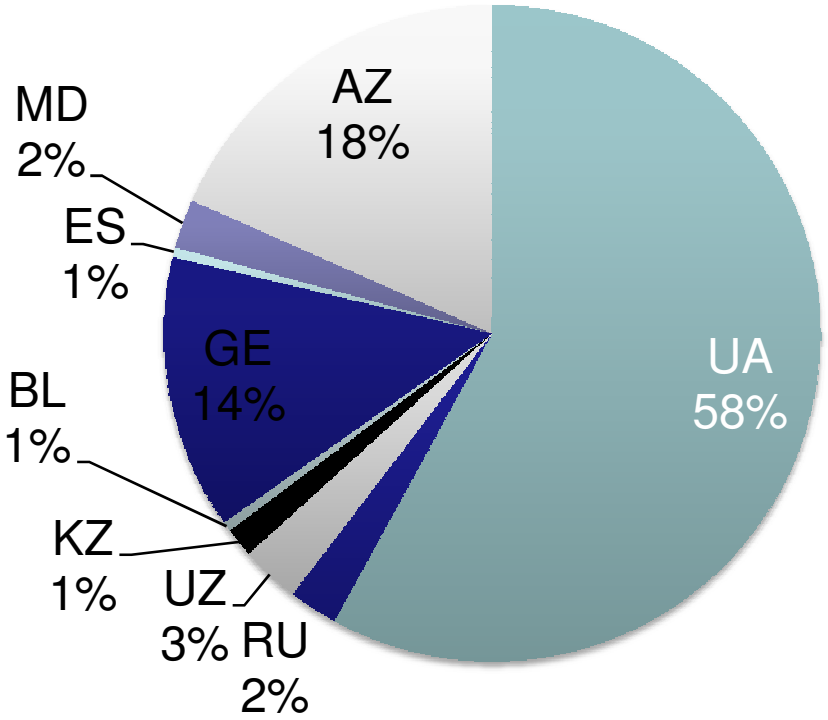
Louis et al. 2011

Slide by Athina Kilpeläinen

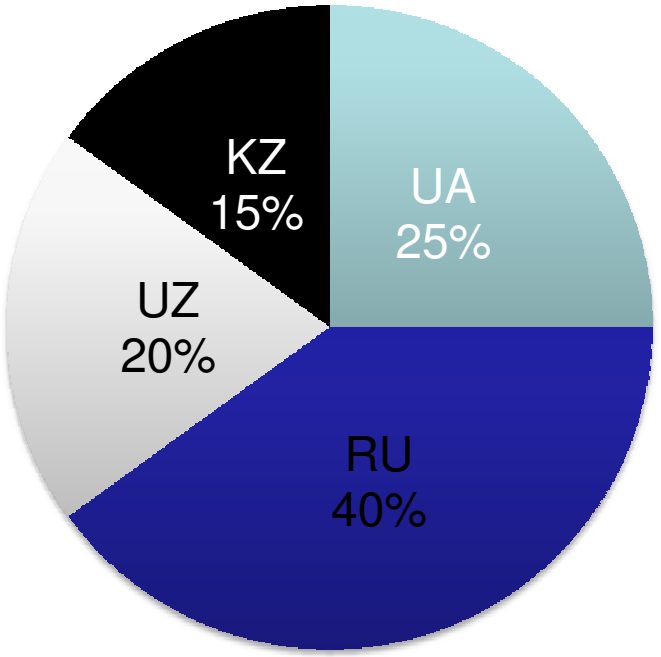
DESIGN OF IMMUNOTHERAPEUTICALS: sequence selection

- HIV-1 FSU-A Protease amino acid sequences from treatment-naïve patients were selected from databases (Los Alamos, Genbank).

Sequence set 1 (n=218)



Sequence set 2 (n=40)



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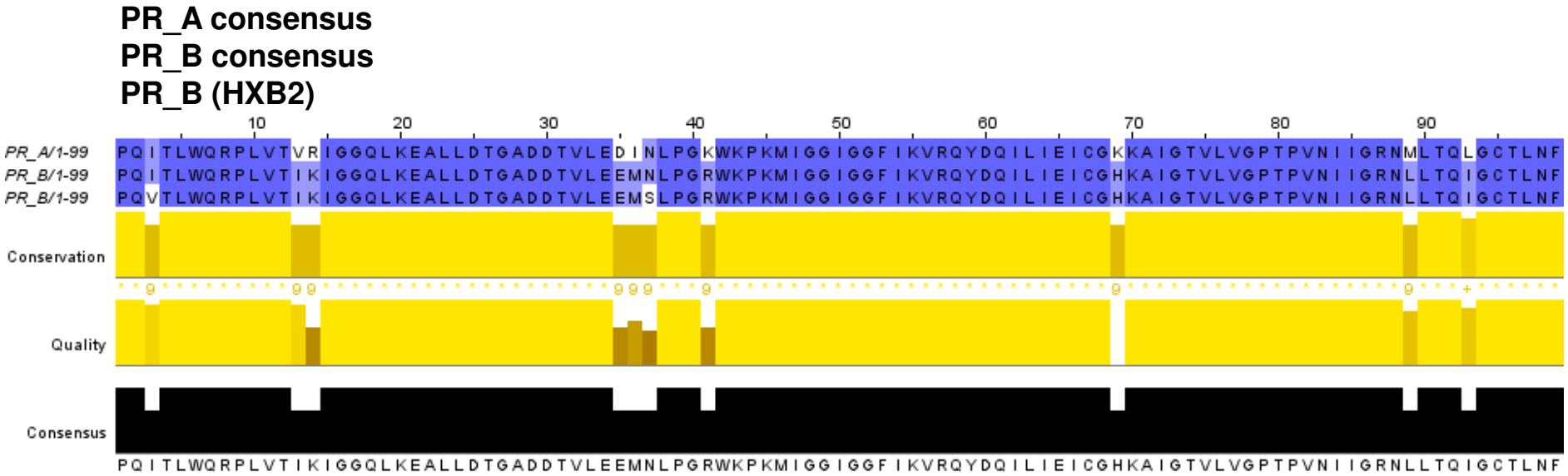
DESIGN OF IMMUNOTHERAPEUTICALS: creation of consensus

Consensus protein sequence

Consensus sequences built on 1996-2003 and 2000-2011 selections were **100% identical**

Consensus adequately represent FSU-A PR in (some) years to come



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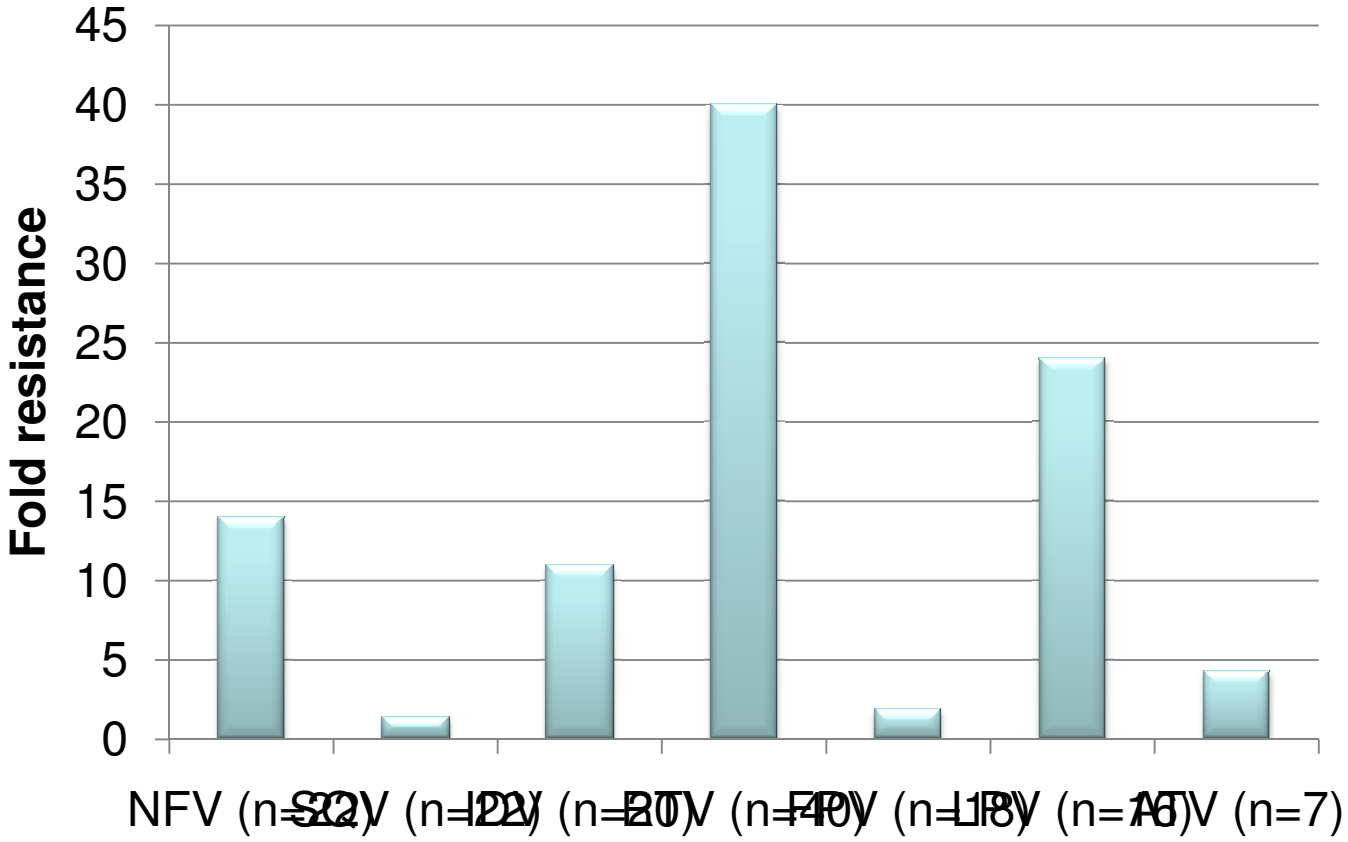
DESIGN OF IMMUNOTHERAPEUTICALS: selection of DR mutations

Selection of drug resistance mutations:

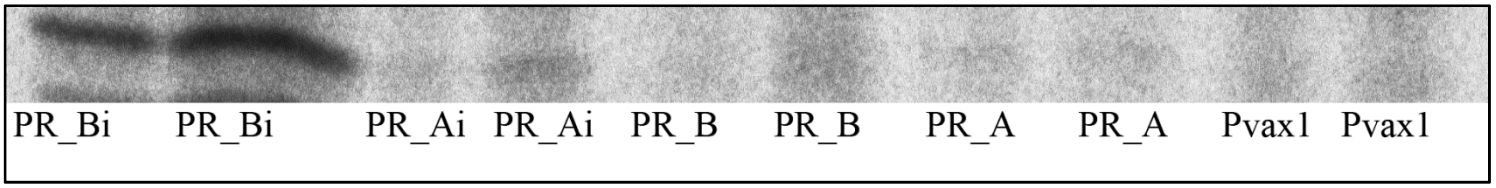
A set of primary DR mutations 46I, 54V and 82

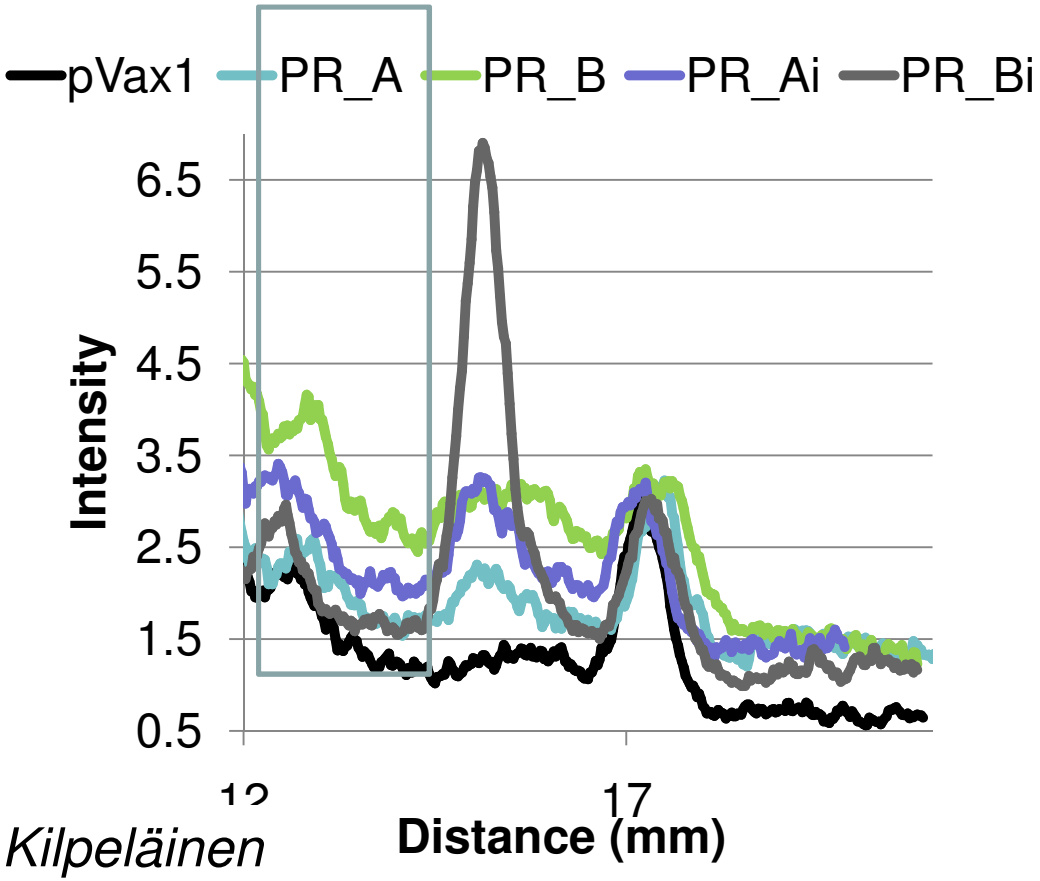


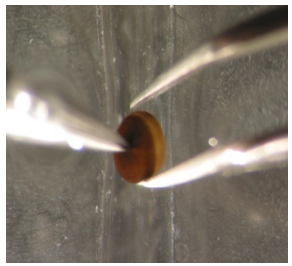
http://hivdb.stanford.edu/cgi-bin/PositionPhenoSummary.cgi

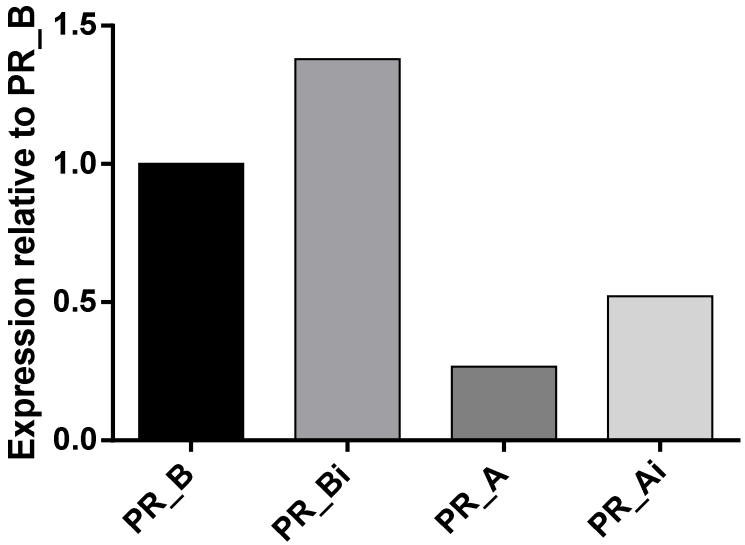
Immunogens: In vitro, cell line tests

PR_A and PR_Ai are expressed in oocytes of *X laevis*









A Kilpeläinen

Immunogens: Evolution of RT-based immunogens

Modification of HIV-1 reverse transcriptase gene

Modification	Codon Optimization	Enzyme inactivation	Drug Resistance
RT wt	-	-	-
RT wt opt	+	-	-
RT wt opt-in	+	+	-
RT 1.14	-	-	+
RT 1.14 opt	+	-	+
RT 1.14 opt-in	+	+	+

PRECLINICAL PERFORMANCE OF VACCINE COMPONENTS

DNA-immunization protocol

GENE Injection + Electroporation

DAY -4

COLLECTION OF PREIMMUNE SAMPLES

DAY 0

DAY 21

COLLECTION OF HYPERIMMUNE SAMPLES (SERA, splenocytes)

SAMPLE ANALYSIS: ELISA, ELISpot, FLUOROSpot, FACS

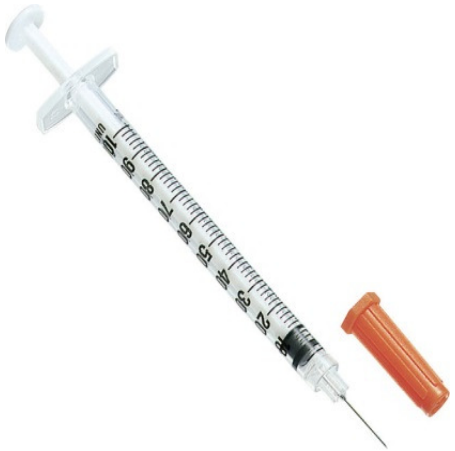
Optimized protocol of vaccine delivery

- **Needle injection** → - most commonly used

- **Electroporation over the injection site** →
  - 1000-times enhanced gene delivery & expression
  - 100-times enhanced immune response
  - widely used in clinical trials

Optimization of gene delivery – vaccine inoculation

29G needles OR **Microneedles**



Micronjet (Nanopass Technologies)

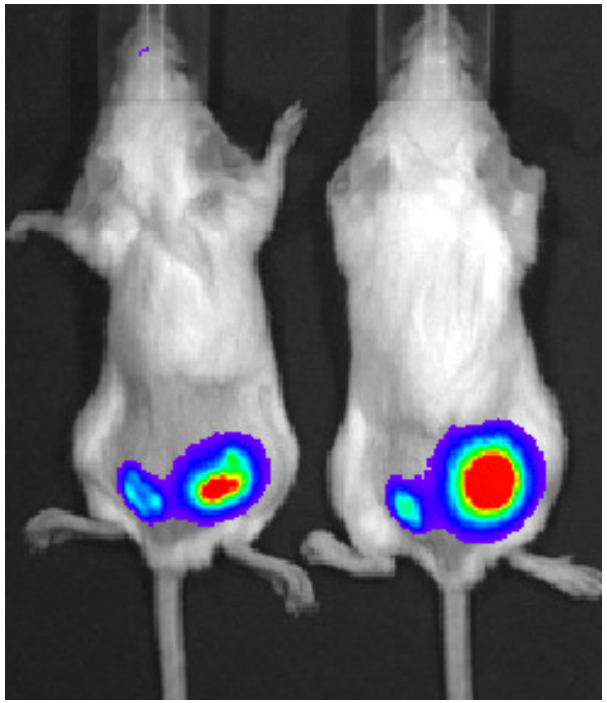
Biojector (delivery by gas pressure)

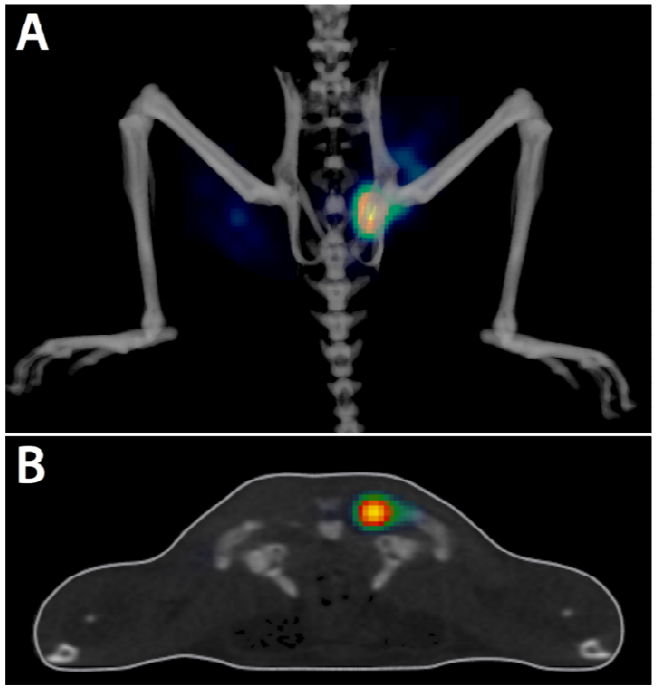
Optimization of gene delivery - electroporation

Petkov SP, Heuts F, Krotova OA, Kilpelainen A, Engström G, Starodubova ES, Isaguliants MG. Hum Vaccin Immunother. 2013 Jul 3;9(10). Evaluation of immunogen delivery by DNA immunization using non-invasive bioluminescence imaging

Optimization of plasmid delivery

Immunization of BALB/c mice with viral DNA mixed with DNA encoding Luciferase followed by *in vivo* imaging





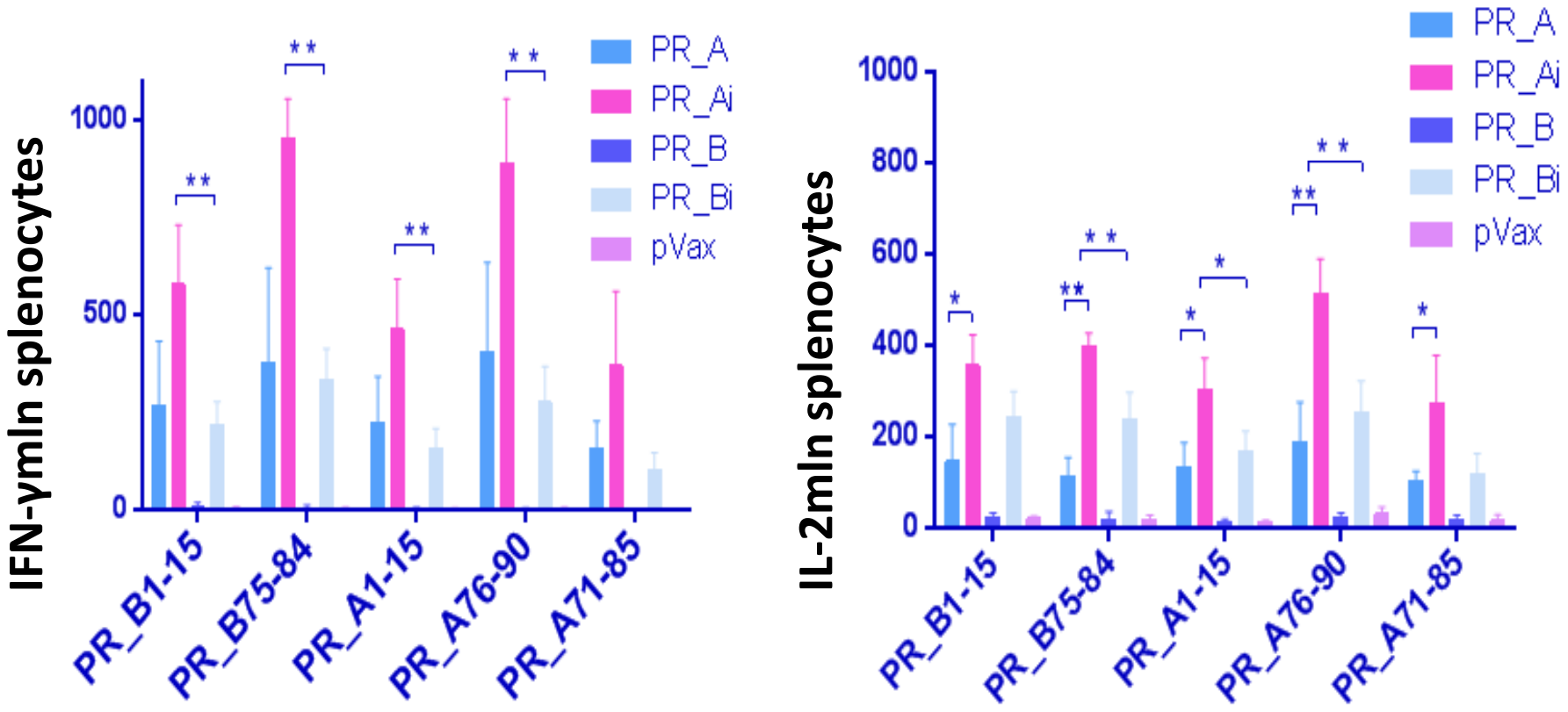
2D- and 3D-imaging quantifies gene delivery with each injection

Stefan Petkov

Immunogenicity of candidate immunotherapeuticals in mice

Inactive consensus protease of HIV FSU-A was highly immunogenic in BALB/c mice

- PR-specific secretion of IFN-γ/IL-2 by splenocytes of PR_Ai ne immunized mice. DR variants of PR_Ai are under trial



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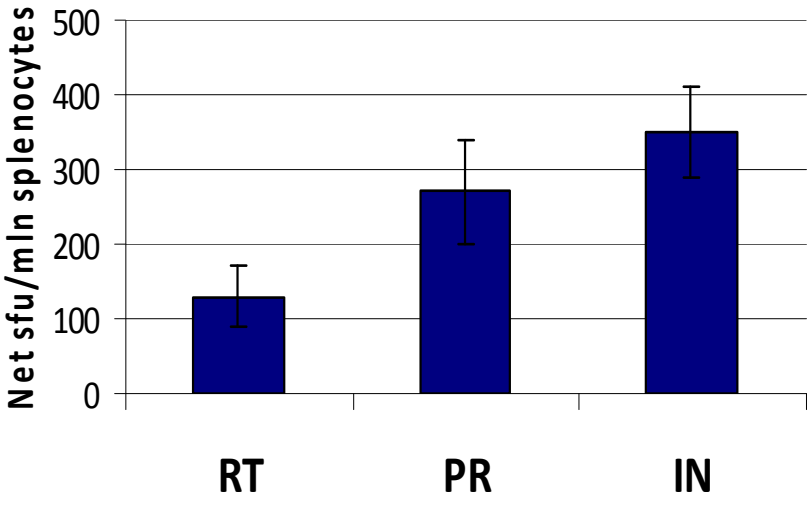
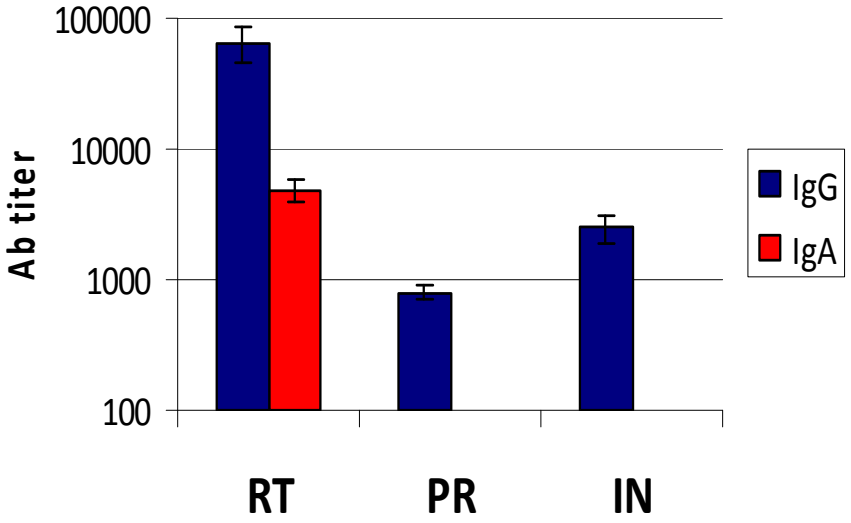
PRECLINICAL PERFORMANCE OF VACCINE COMPONENTS

Immune response in BALB/C mice after one immunization

T-cell response
Dual IFN-g/IL-2 production
(Fluorospot)



Humoral response
IgG and IgA titers
(ELISA)



Starodubova E. Et al Mol Imaging 2012; Isaguliants M et al, Human Vaccines & Immunotherapeuticals 2013; Hallengärd D et al, Vaccine 2011; Krotova OA et al, PLoS One 2013

SUMMARY ON WHAT WE OFFER TO DEVELOP FOR THERAPEUTIC USE

PROTOTYPE VACCINE COMPONENTS

Plasmids encoding HIV enzymes

- reverse transcriptase, protease and integrase that are:
- contain primary mutations of drug resistance;
- no enzymatic activity;
- consensus sequence of subtype A and B (region specific).

Plasmids tested for expression in cell culture and mouse tissues

DELIVERY METHOD

Effective protocols of needle injections followed by electroporation

STRONG IMMUNOGENIC PERFORMANCE IN PRECLINICALS

All components induce strong polyfunctional immune response in rodents; Rabbit experiments are on-going.

SUMMARY ON WHAT WE OFFER TO DEVELOP FOR THERAPEUTIC USE

OUTLINE FOR A CLINICAL TRIAL:

Standard in therapeutic HIV-1 vaccine trials:
Immunization → Drug treatment off → Measure viral loads

Structured Therapy Interruption (STI) can be harmful!

We suggest: No unsafe procedures

Therapeutic immunization + HAART, NO STIs →
Measure frequency of drug-resistance conferring mutations

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