Comparative immunogenicity in mice of DNA encoding HIV enzymes: Step towards control of drug resistance in HIV infection

Isaguliants Maria

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

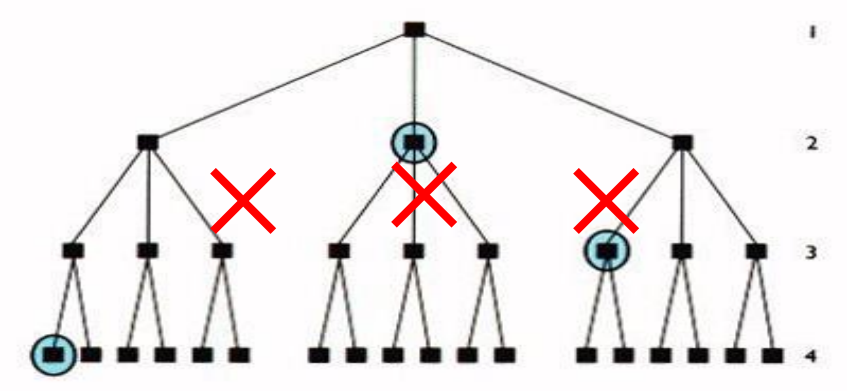
- **How long clinical benefit will last?**
  the emergence of multiple drug-resistant viral strains (drHIV)
  primary infections with drHIV
  failures on HAART regimens

- **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!

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.

We suggest: No unsafe procedures

Therapeutic immunization + HAART, NO STIs →

Measure frequency of drug-resistance conferring mutations

Choice of vaccine vehicle - naked DNA

PLASMID BACKBONE

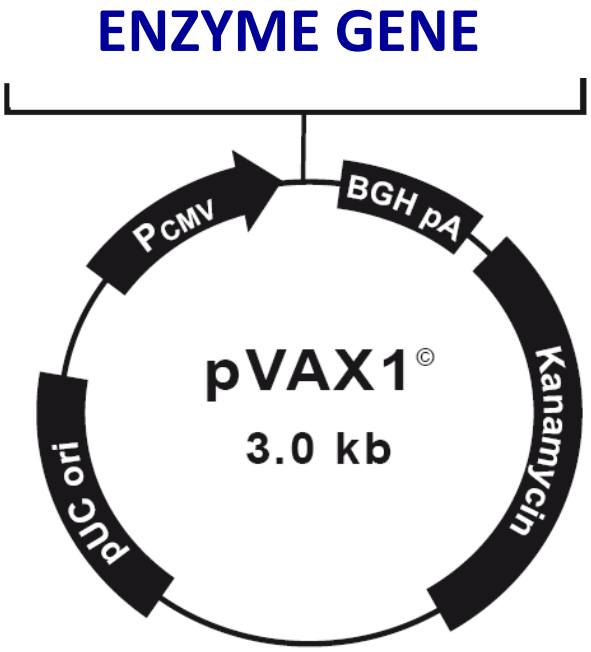
pVax 1 (Invitrogen)

HIV ENZYME GENES

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

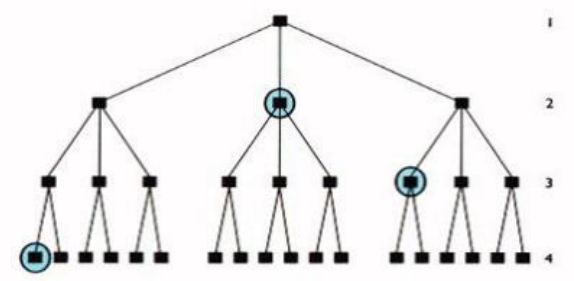
MULTI-GENE SET GENERATED: RTPRIN-A

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



Choice of components
Antigens involved in HIV resistance – 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

HIV-1 FSU-A Pol polyprotein amino acid sequences from treatment-naïve patients selected from databases 1999-2015 (Los Alamos, Genbank).

Average number of new HIV cases per 100 000 people:

6.6 | 1.9 | 22.0

www.euro.who.int/aids

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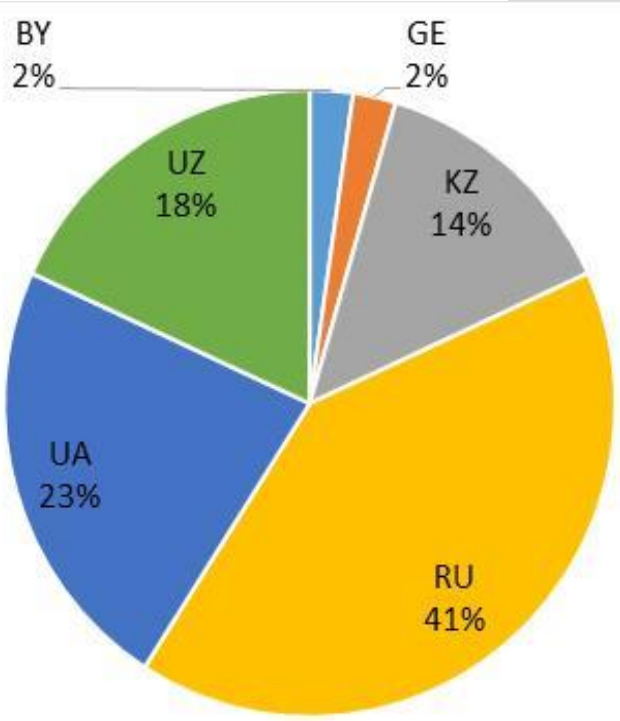
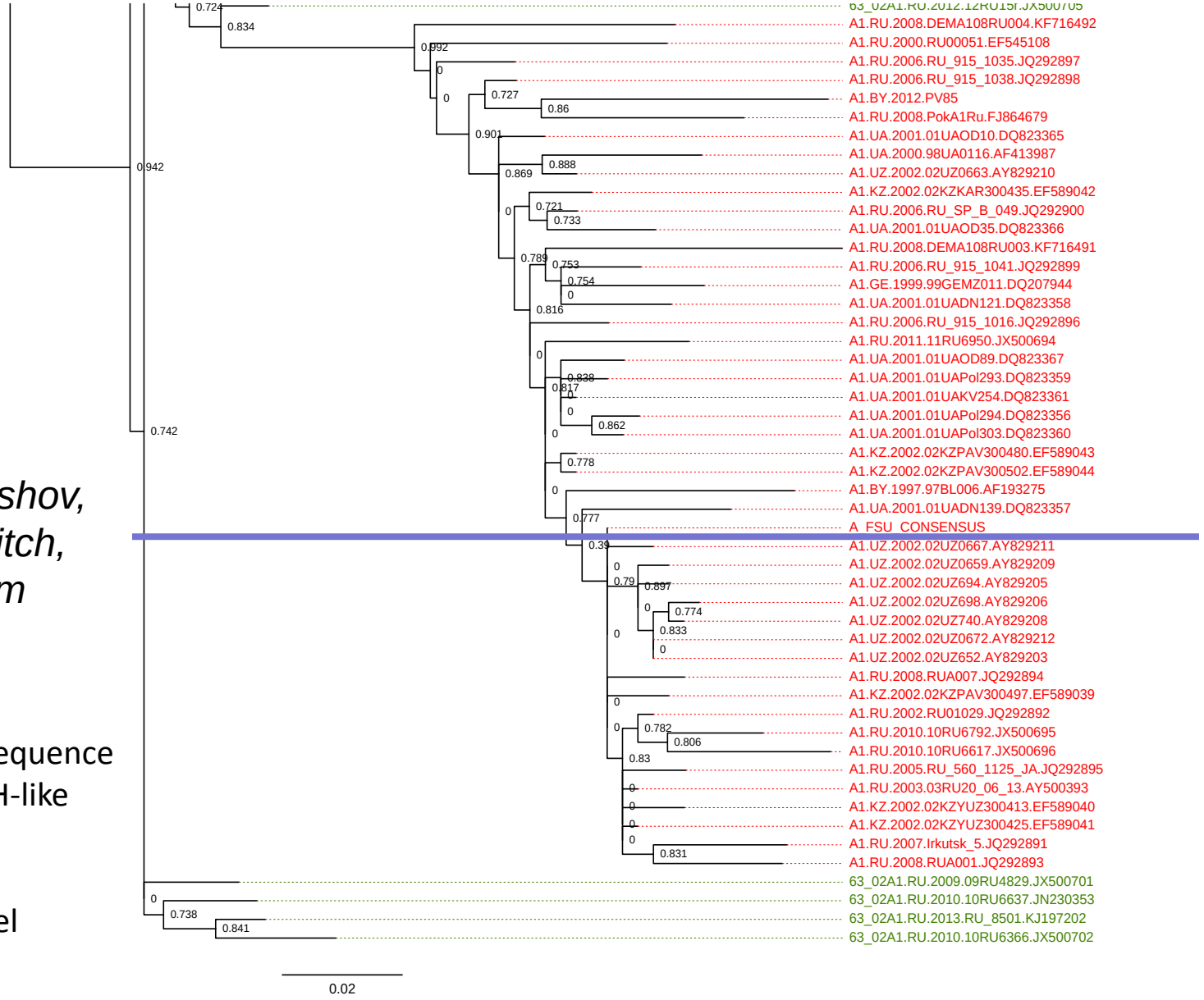
World Health Organization Europe

ecdc



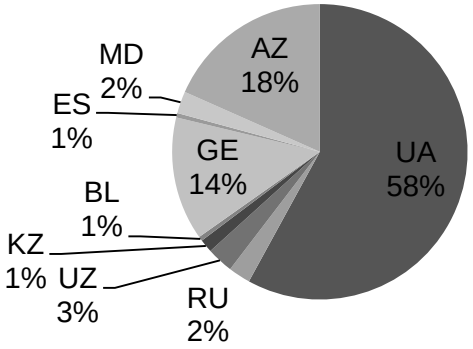
Maximum Likelihood tree with A_FSU CONSENSUS



Vladimir Loukashov, Sviat Sasinovitch, Per Warholm

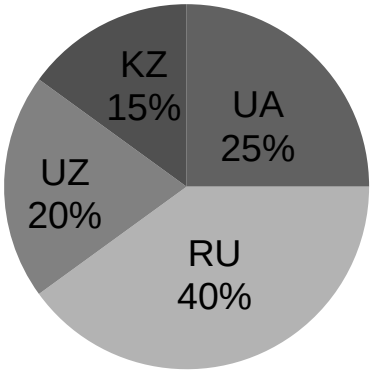
PhyML multiply sequence alignment with SH-like branch support,
LG (Le Gascuel) substitution model

Sequence set 1 (n=218)



Example of protease

Sequence set 2 (n=40)



Consensus sequence:

PQITLWQRPLVTVRIGGQLKEALLDTGADDTVLEDINLPGKW KPKMIGGIGGFIKVRQYDQILIEICGKKAIGTVLVGPTPVNIIG RNMLTQLGCTLNF

Consensus sequence:

PQITLWQRPLVTVRIGGQLKEALLDTGADDTVLEDINLPGKW KPKMIGGIGGFIKVRQYDQILIEICGKKAIGTVLVGPTPVNIIGR NMLTQLGCTLNF

Score	Expect	Method	Identities	Positives	Gaps
196 bits(499)	7e-71	Compositional matrix adjust.	99/99(100%)	99/99(100%)	0/99(0%)

```
Query  1   PQITLWQRPLVTVRIGGQLKEALLDTGADDTVLEDINLPGKWKPKMIGGIGGFIKVRQYD  60
           PQITLWQRPLVTVRIGGQLKEALLDTGADDTVLEDINLPGKWKPKMIGGIGGFIKVRQYD
Sbjct  1   PQITLWQRPLVTVRIGGQLKEALLDTGADDTVLEDINLPGKWKPKMIGGIGGFIKVRQYD  60

Query  61  QILIEICGKKAIGTVLVGPTPVNIIGRNMLTQLGCTLNF  99
           QILIEICGKKAIGTVLVGPTPVNIIGRNMLTQLGCTLNF
Sbjct  61  QILIEICGKKAIGTVLVGPTPVNIIGRNMLTQLGCTLNF  99
```

Two consensuses built on sequences collected 1996-2003 and 2000-2011 were 100% identical indicating prototype vaccine adequacy in years to come.

Choice of primary drug resistance mutations for incorporation

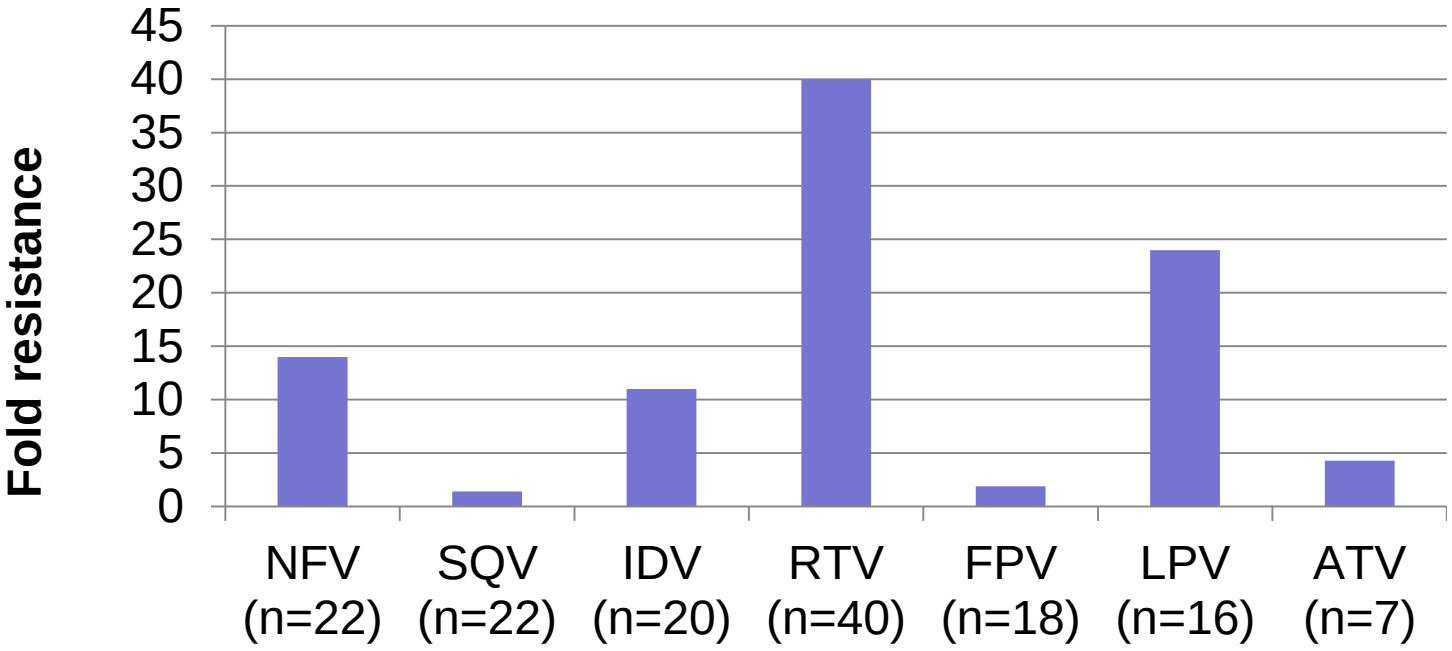
- No primary DR present in the Consensus sequence.
- Common mutations for drugs and drug combinations used in the Russian Federation (Stanford Resistance Database)

Mutation	ATV	DRV	FPV	IDV	LPV	NFV	SQV
L24I	X			X	X		X
V32I		X	X		X		
L33F	X	X	X		X		
M46I	X		X	X	X	X	
I47V	X	X	X	X	X		
I54L	X	X	X	X	X		X
G73S/T	X	X	X	X			X
V82A	X		X	X	X		X
I84V/A/C	X	X	X	X	X	X	X
L90M	X	X	X	X	X	X	X

Choice of drug resistance mutations for HIV FUS_A protease

Primary DR pattern - 46I, 54V and 82A

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



PR_A

PR_A2mut – at 46, 54

PR_A3mut – at 46, 54 and 82

Primary DR mutations for HIV FSU_A reverse transcriptase RT)

RT_A

RT_An - pattern I (NRTI) aa 65 , 184

RT_Ann - pattern II (NNRTI): aa 103 , 190

(Bobkova MR et al, 2011-2015)

DR mutations for HIV FSU_A integrase (IN)

IN_A

IN_Ar1 - Raltegravir I phenotype

Primary at aa 92;

Secondary at aa: 155, 74, 92, 151, 163

IN_Ar2 - Raltegravir II phenotype

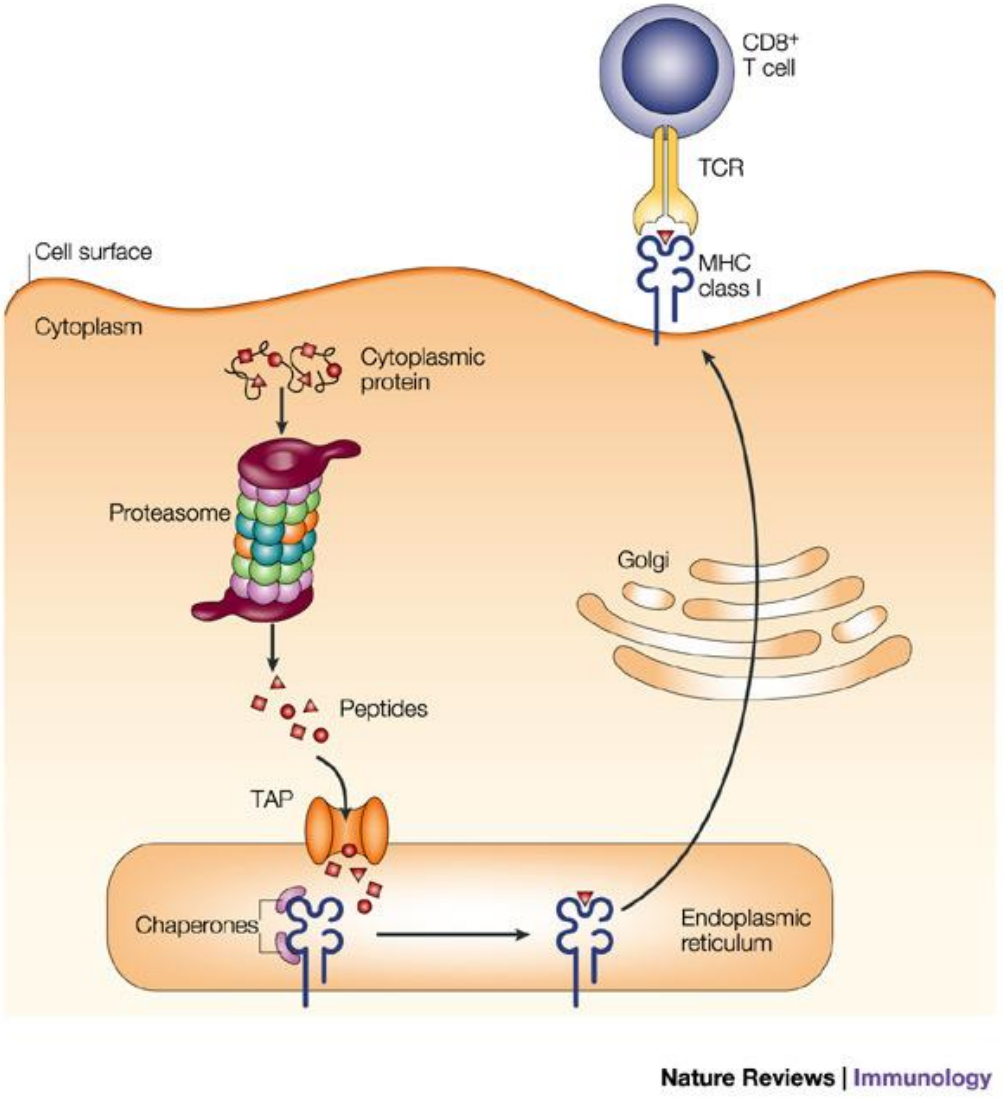
Primary at aa 148; Secondary at aa 138, 140

(Myers RE, Pillay D 2008)

Effects of drug resistance mutations effects on immunogenicity

Prediction programs were used to predict changes in immunogenicity.

- MHC-I processing parameters (*Nielsen M et al, 2007; Hoof I et al, 2009*).
  - Proteasomal cleavage
  - TAP transport
  - Peptide-MHC affinity
- Peptide-MHC II binding (*Wang P et al, 2008; Wang P et al, 2010*)
- Alignment of all known epitopes



DNA-immunization protocol

GENE injection +
Electroporation

DAY -4

DAY 0

DAY 28

COLLECTION OF
PREIMMUNE SAMPLES

COLLECTION OF
HYPERIMMUNE SAMPLES
(SERA, splenocytes)

SAMPLE ANALYSIS: ELISA, ELISpot, FLUOROSpot, FACS

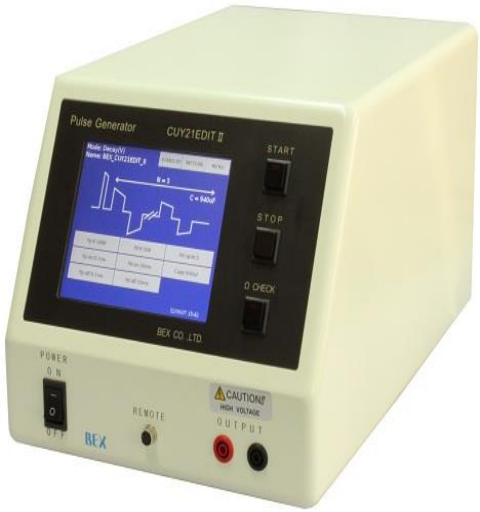
Several injection/EP protocols in circulation.

Electrodes, polarity, duration of pulses differ.

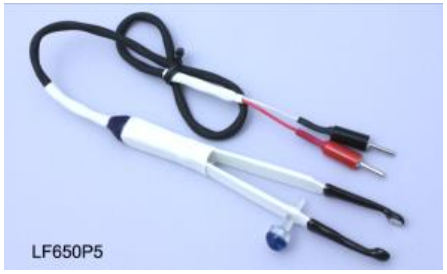
QUESTION №1:
How to deliver to get the strongest immune response??

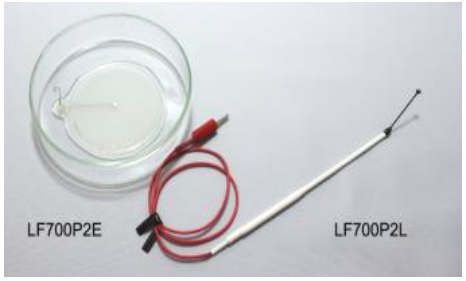
Optimization of gene delivery

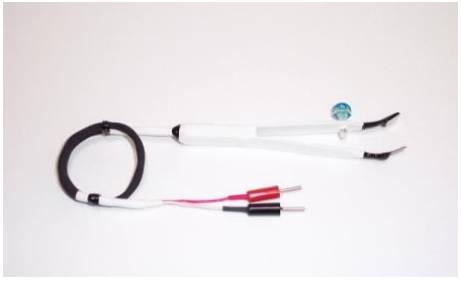
CUY21EDIT II pulse generator, BEX Ltd



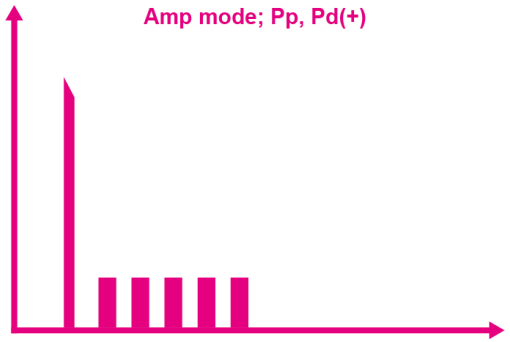
Electrodes

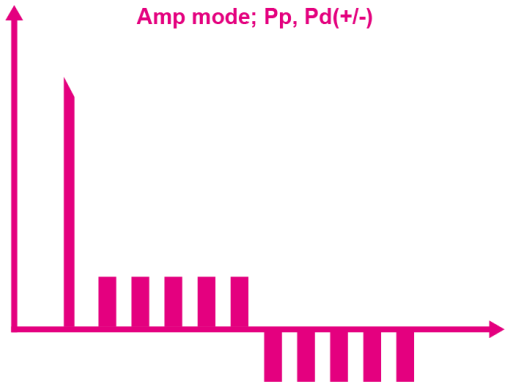


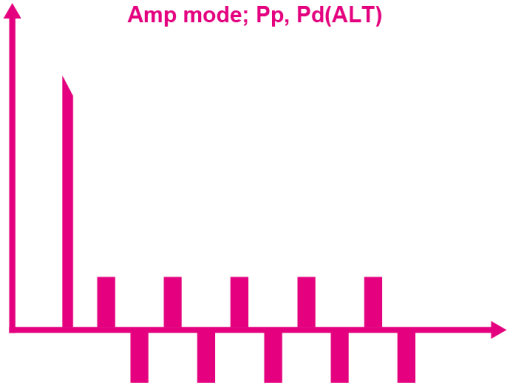




Pulse configuration

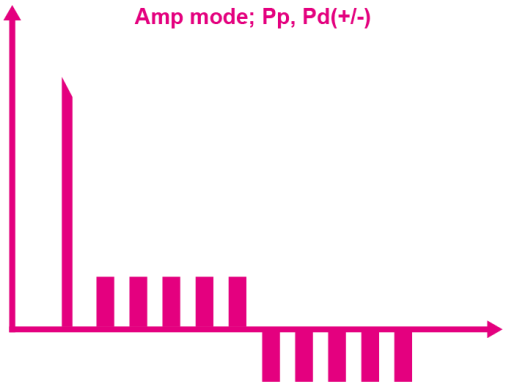






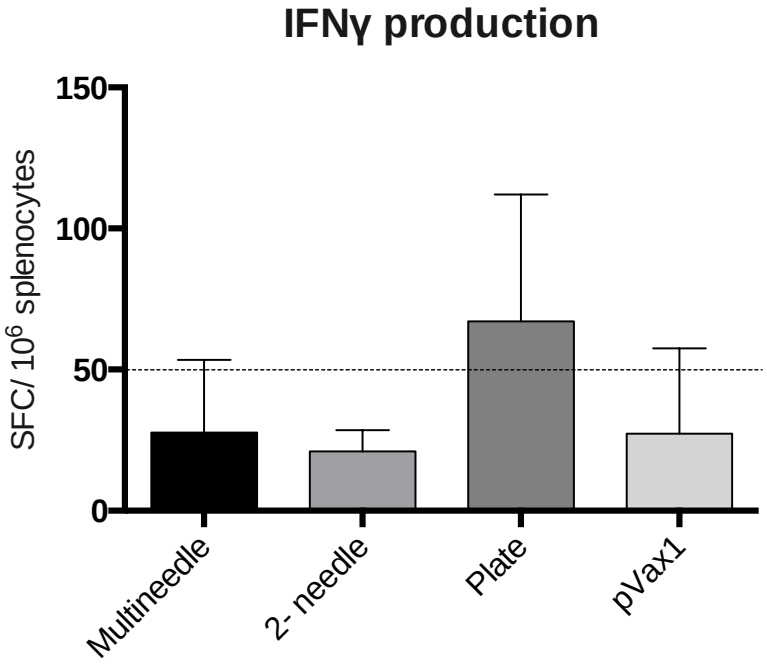
Defining optimal regimen by expression in explanted skin + induction of IFN-g response against the reporter protein

Platinum tweezers plate electrodes



Altering polarity of pulses

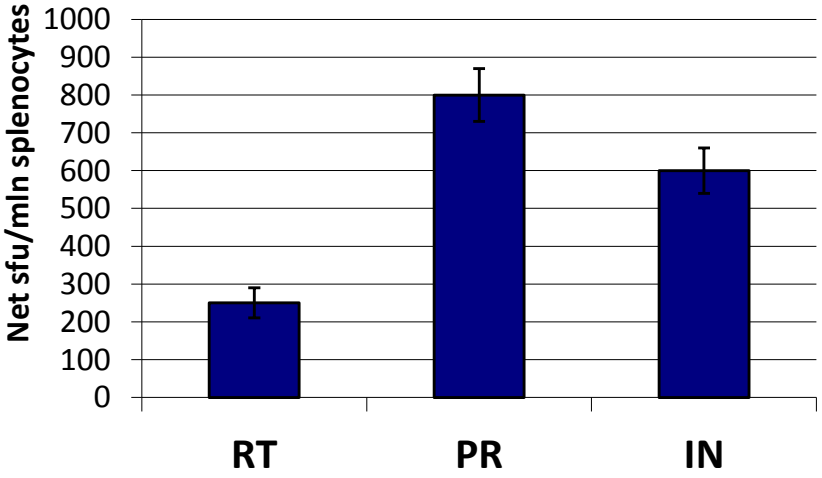
Model immunization by pVaxLuc



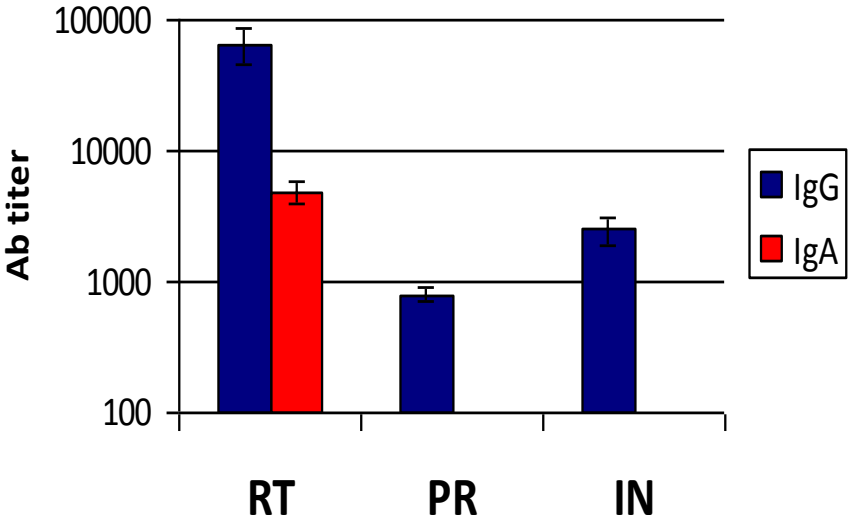
Immune response to CTL epitope of Luciferase

Immune response in BALB/C mice after one immunization

T-cell response
IFN-g (Elispot)

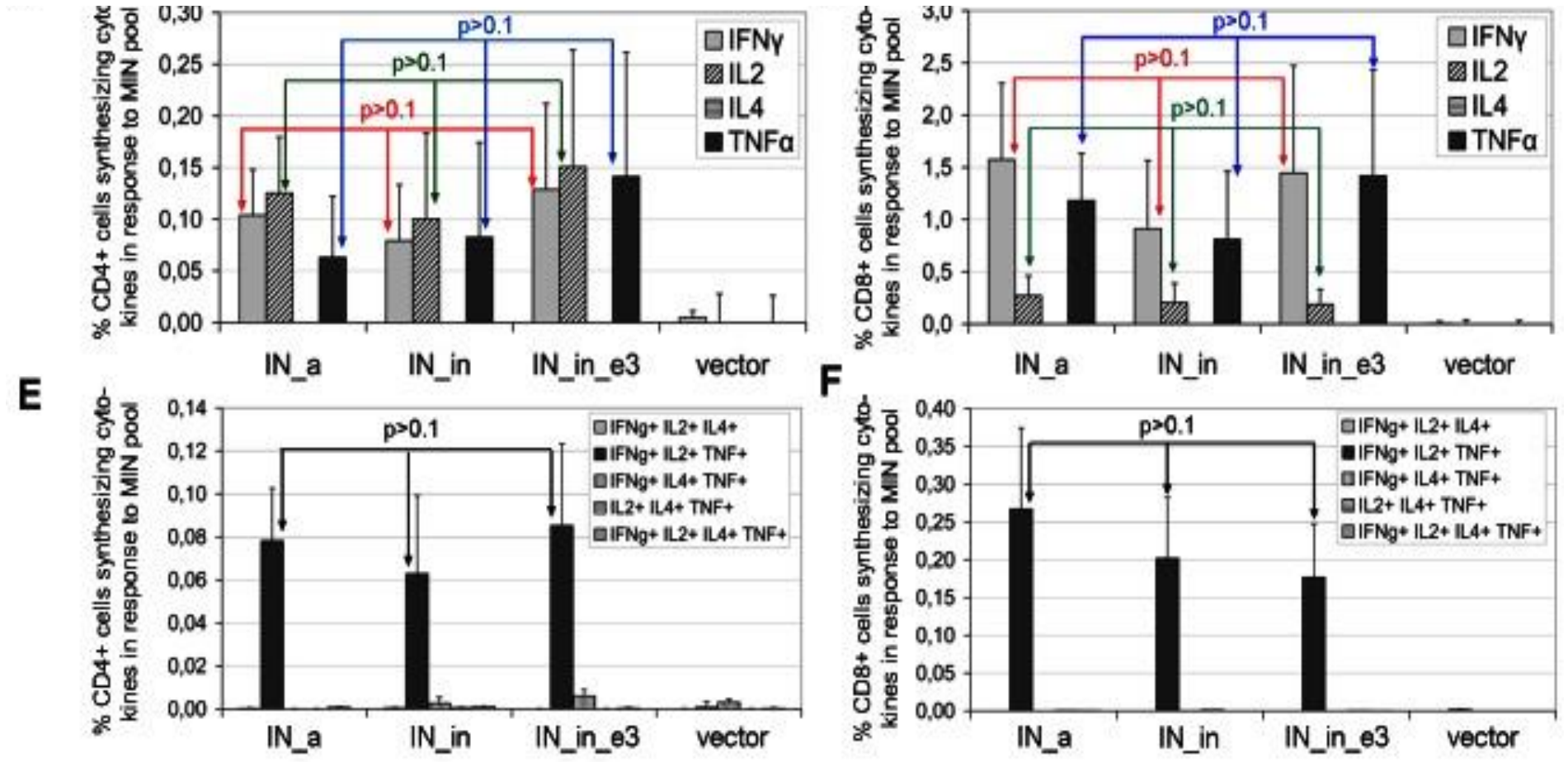


Humoral response
IgG and IgA titers
(ELISA)



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; Kilpeläinen A et al HIV Nordic 2014; Petkov S, Lic thesis, 2015

Multi-functional CD4+ and CD8+ T cell responses elicited by FSU-A IN genes in BALB/c mice.



Olga Krotova, PLoS One 2013

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;

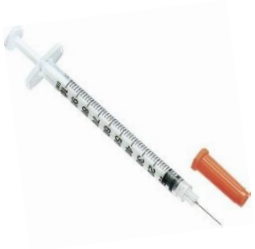
Start of non-human primate safety & immunogenicity trial planned early 2017

?

QUESTION №1:
Which injection/EP regimens are the best of DNA immunogen delivery in mouse, marmoset (and human) skin? - Regimens defined.

QUESTION №2:
Can we mix our plasmids? – YES, they work well in a mixture; PR responses are significantly enhanced in a mix.

QUESTION №3:
Which of RT, IN,PR variants should be mixed for marmoset trial? - THREE most immunogenic plasmids were selected based on ”antigen challenge” model and Fluorospot tests.



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Thank you for your attention!