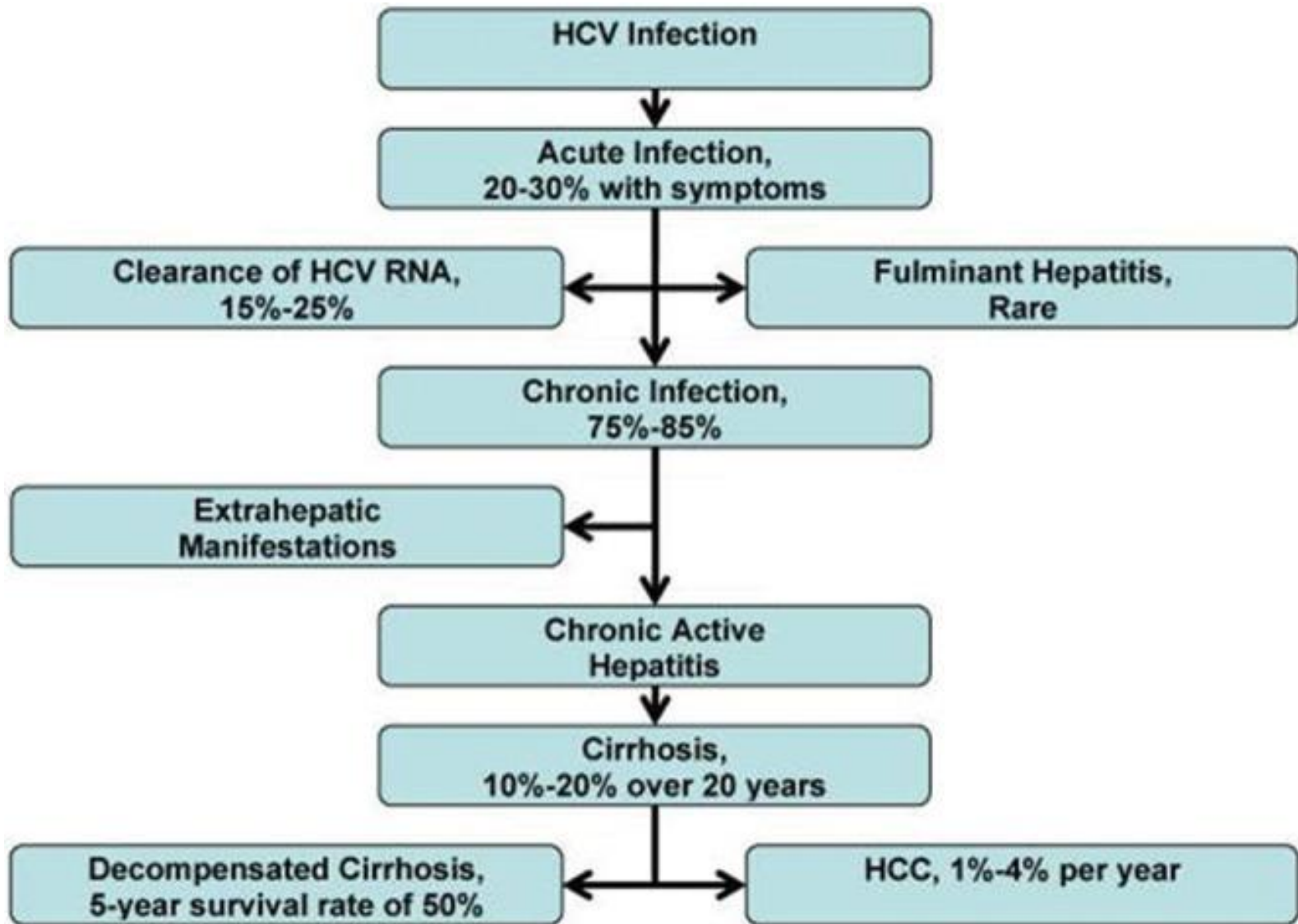
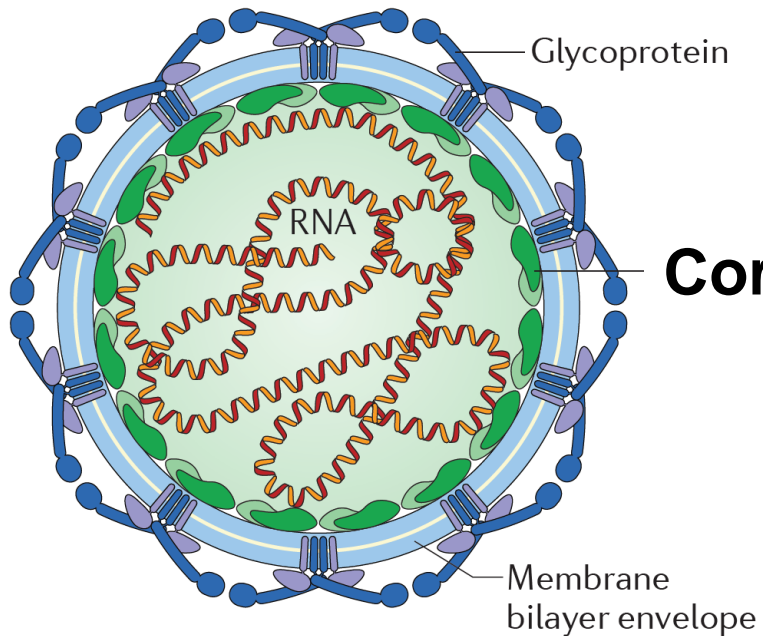


Optimization of DNA immunogens targeting nucleocapsid (core) protein of hepatitis C virus

Juris Jansons





Core protein

- is highly conserved among various HCV genotypes
- contains many T-cell epitopes and induces early antibody response

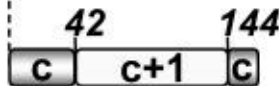
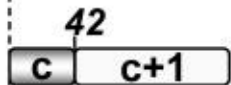
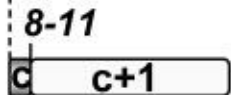
Schematic representations of the HCV genome depicting the position of the core+1 alternative reading frame

HCV



cd 126 - 162

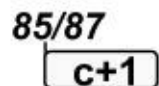
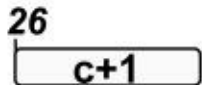
AUG



-2/+1 frameshift

Walewski et al 2001, Xu et al 2001, Varaklioti et al 2002
(HCV-1a, RRL, wheat germ extracts)

Boulant et al 2003
(HCV-1b, *E. coli*)



internal initiation at +1 cds

Baril et al 2005
(HCV-1a, HEK 293FT)

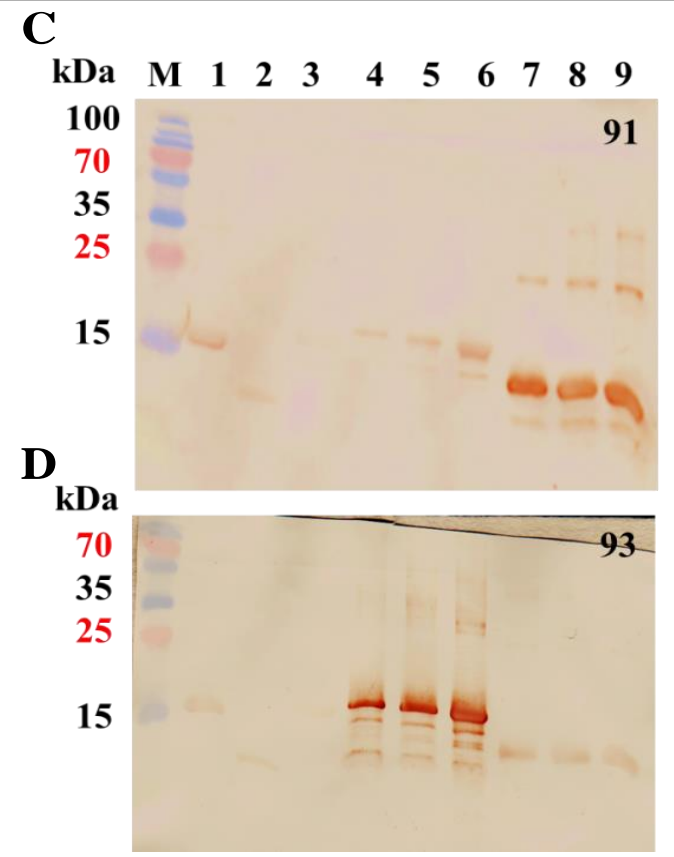
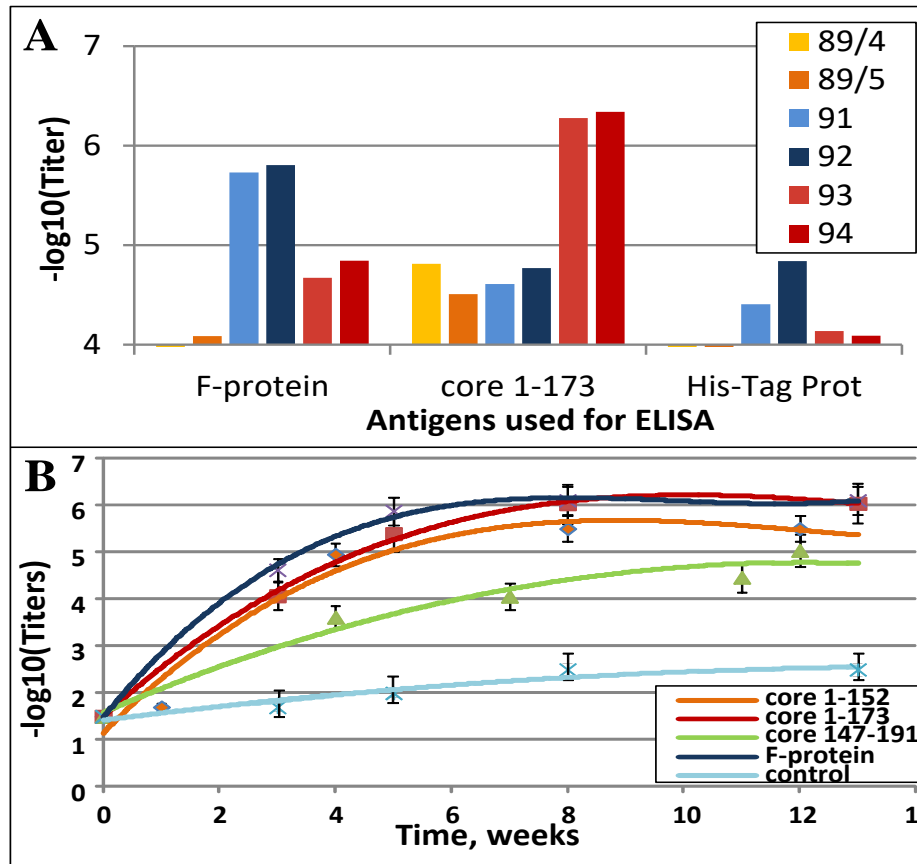
Vassilaki et al JBC 2003, FEBS 2007, JGV 2008, Virus Res 2008
(HCV-1a, -1b, Huh7)

Immunogenicity of HCV core and F-protein in rabbits

Core 1-152  89/4, 89/5

Core 1-173  93, 94

F-protein  91, 92



Immunogenicity of HCV core and F-protein mice

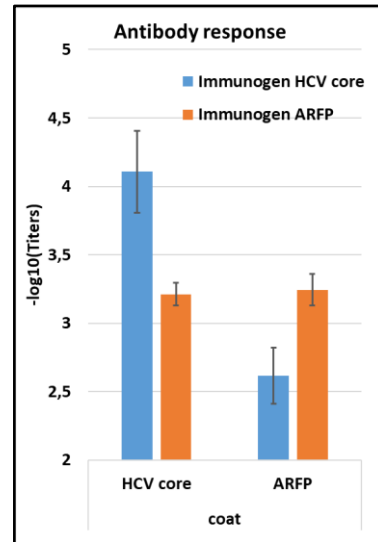
Core 1-159



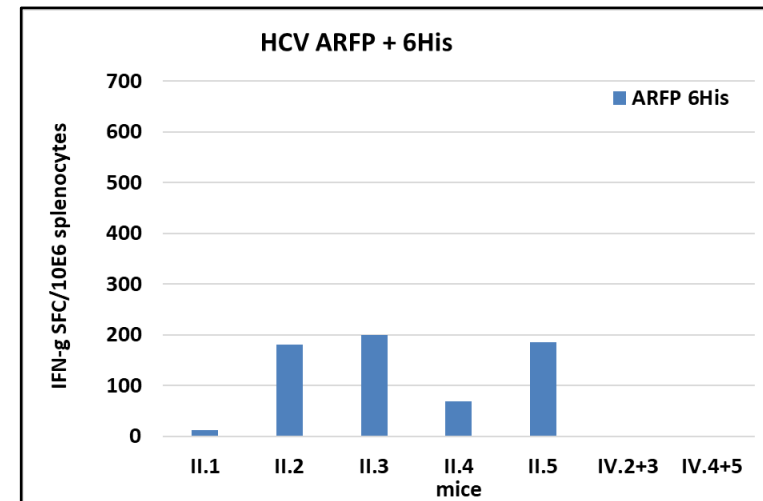
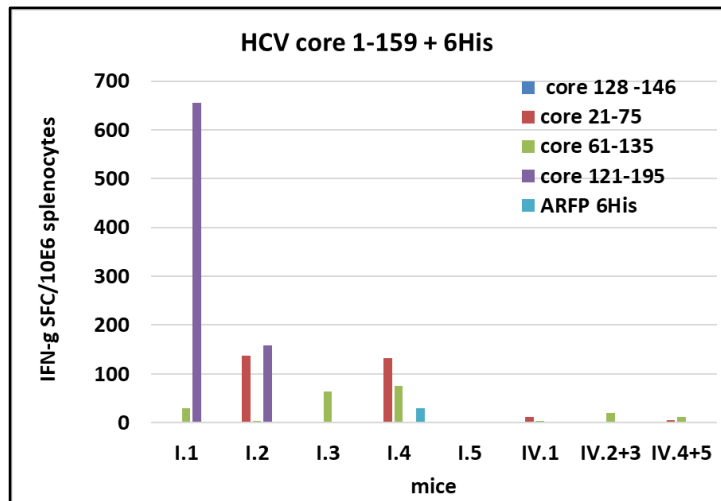
F-protein










B-cell response



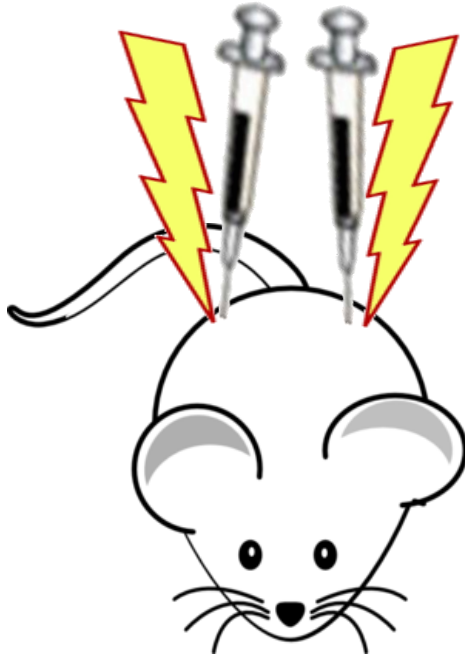
T-cell response



DNA immunogens used in study, based on pVAX1

Core 1-191 wt	
Core 1-191 mut	
ARFP frameshift expression	
Core 1-191 opt	
Core 1-152 opt	
Core 37-191	
ARFP direct expression	

Immunization procedure



Intradermal injection of plasmid DNA solution followed by electroporation

CUY21EDIT II pulse generator



In vivo and in vitro electroporation
Patterns of electroporation pulses

- Square
- Decaying
- Change of polarity

First constant current electroporator

- The user can set desired current



Electrodes used for skin electroporation

Multineedle array electrodes, BTX



Tweezers with plate electrodes, BEX

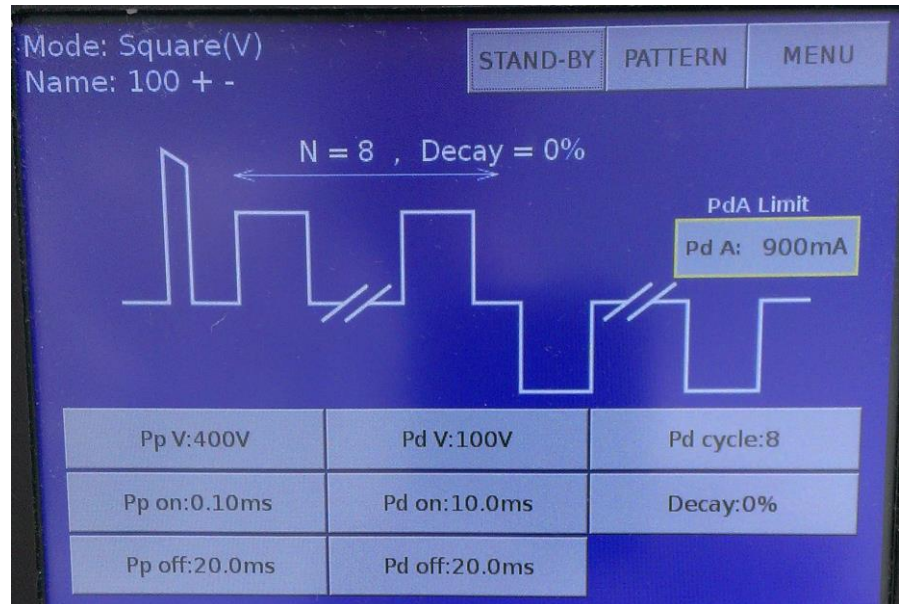
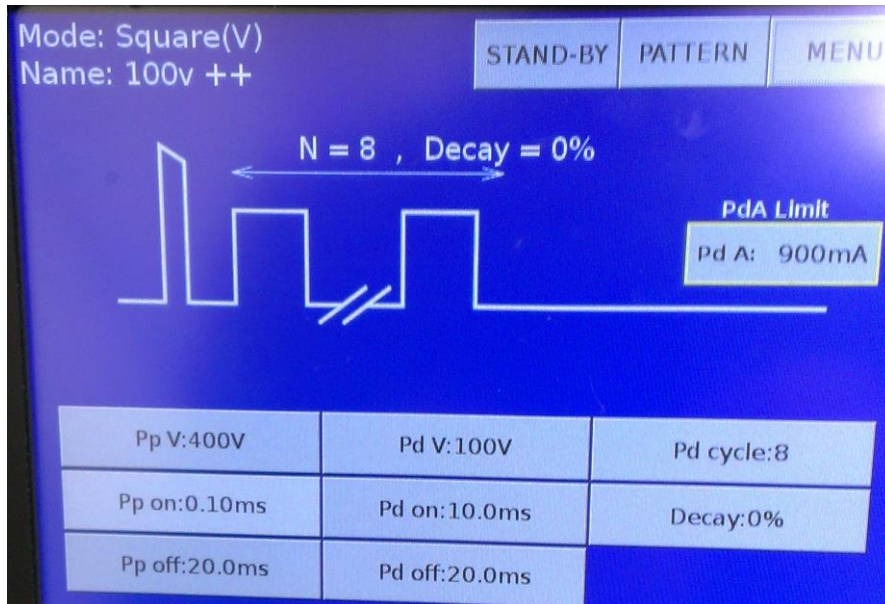


Tweezers with fork and a plate electrodes, BEX

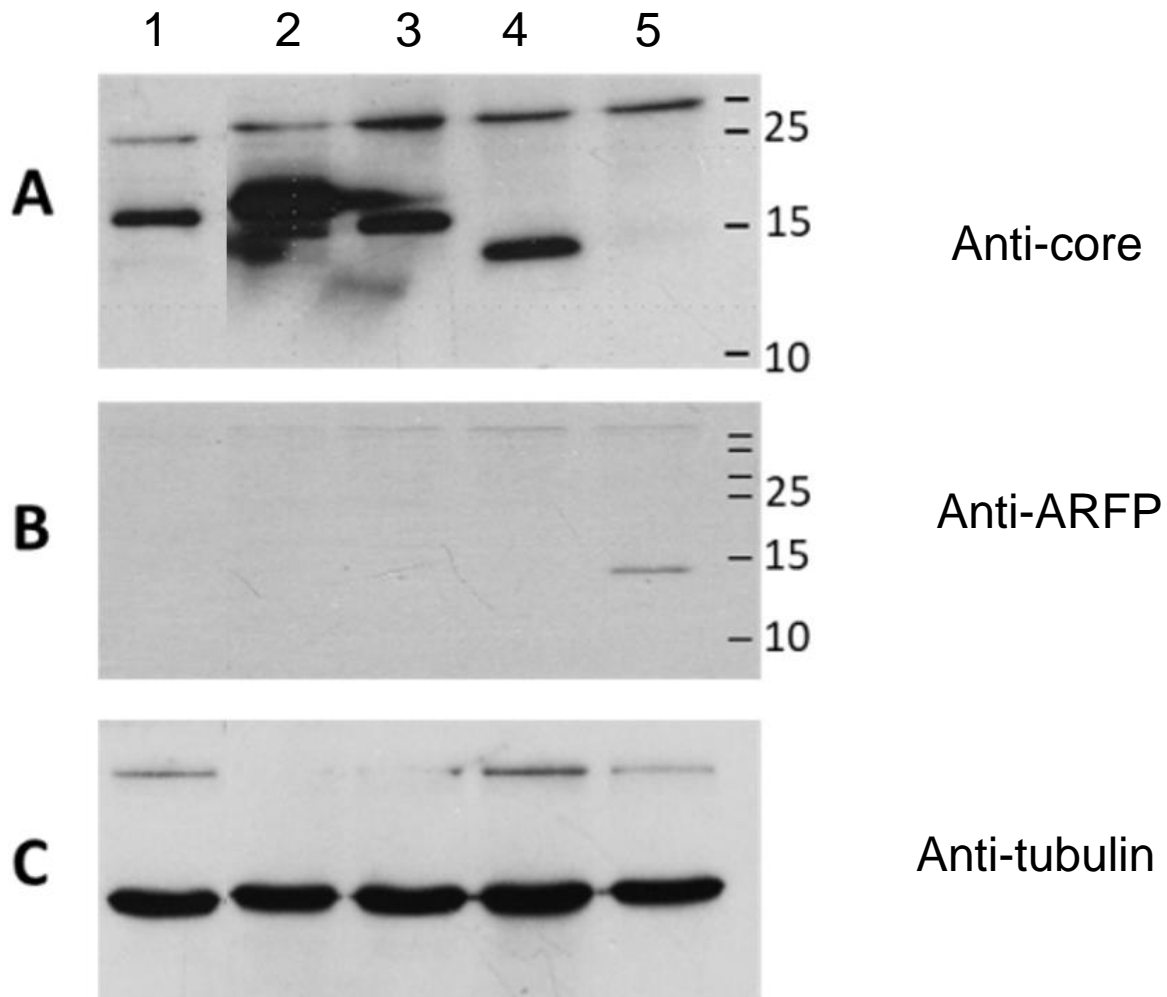


Electroporation parameters

- Electroporation immediately after injection
 - 1 poration pulse of 400 V (0.10 ms)
 - 8 driving pulses of 100V
 - All pulses had a 10 ms duration with 20 ms gaps

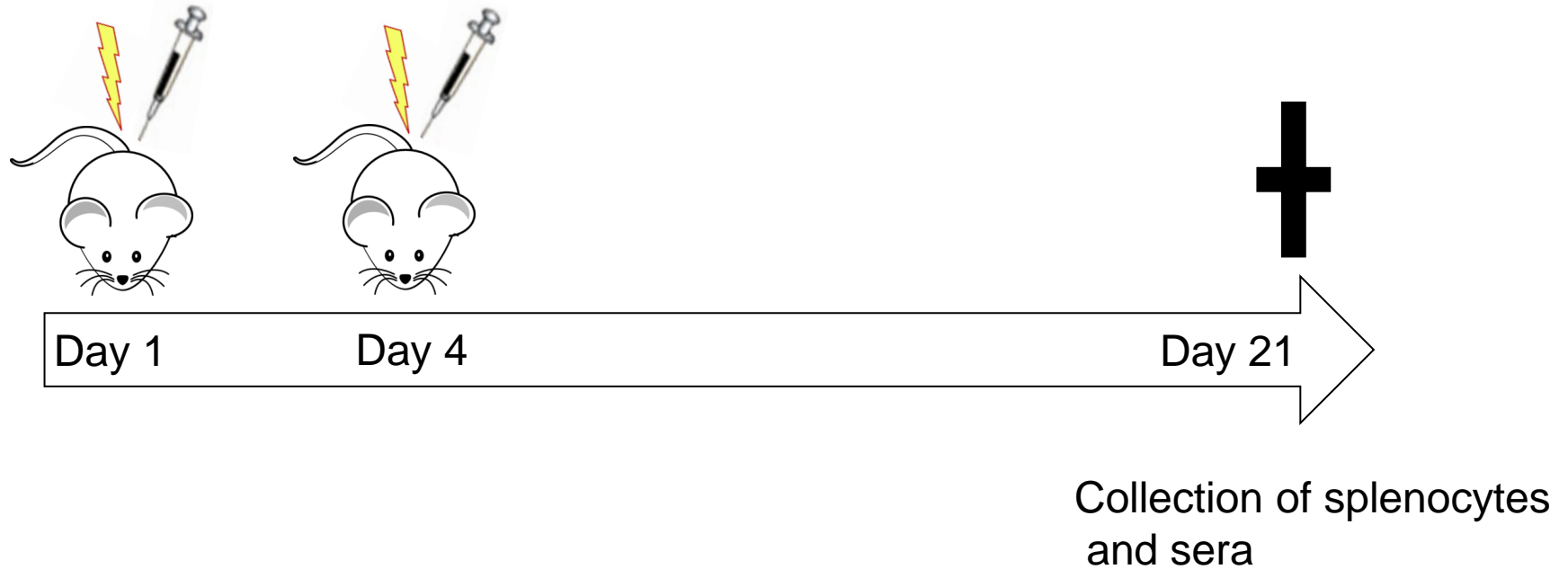


Expression of HCV core and ARFP in Huh7 cell culture

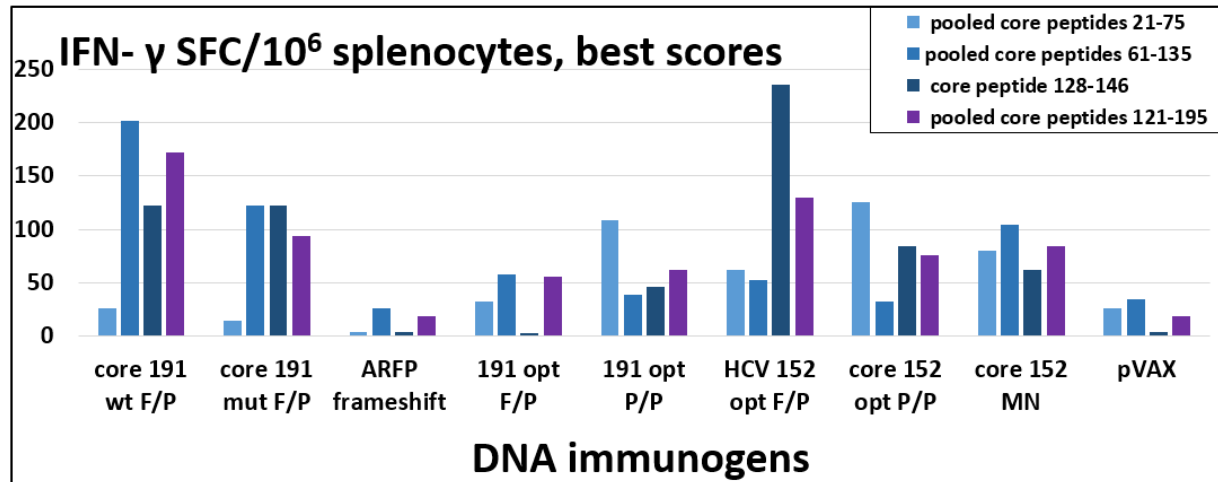
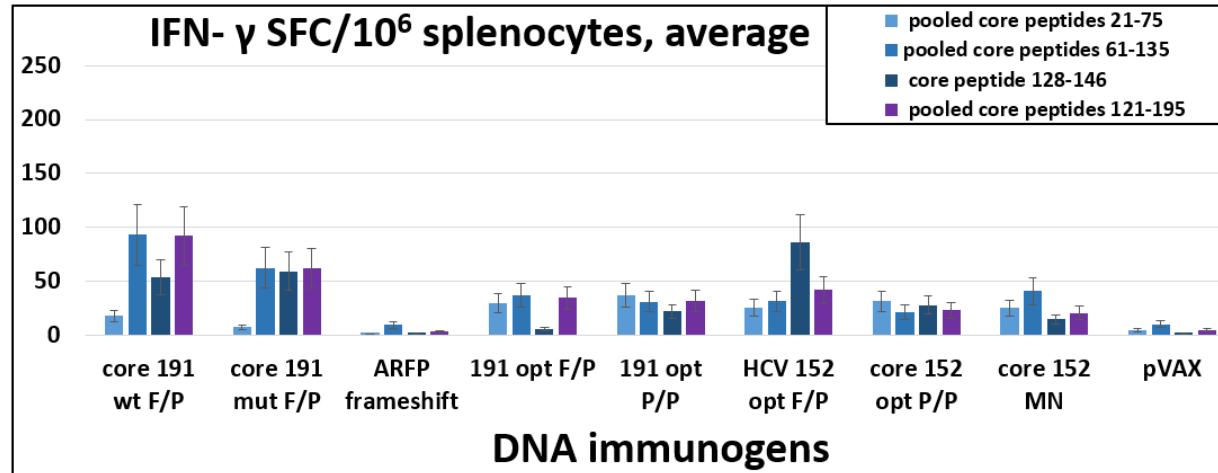


Lines: 1 - core 1-191 wide type, 2 - core 1-191 opt, 3 - core 1-152 opt, 4 - core 37-191, 5 - ARFP direct expression

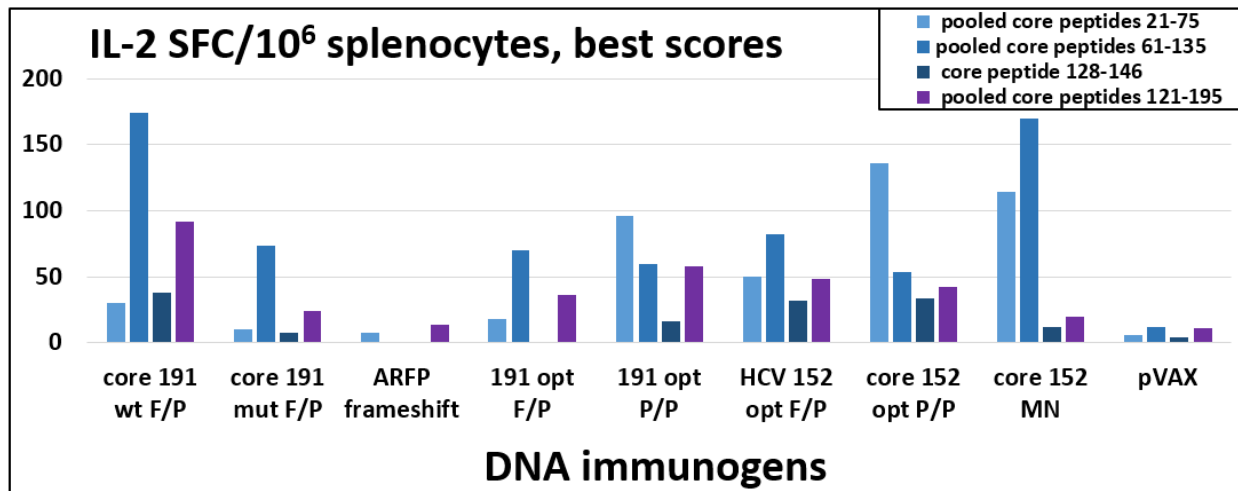
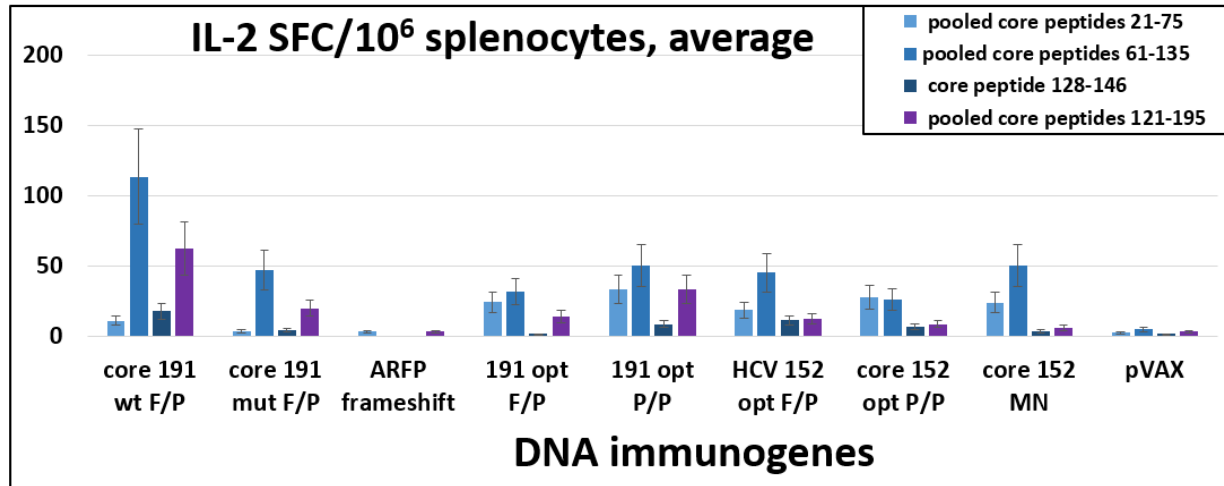
Scheme of immunization



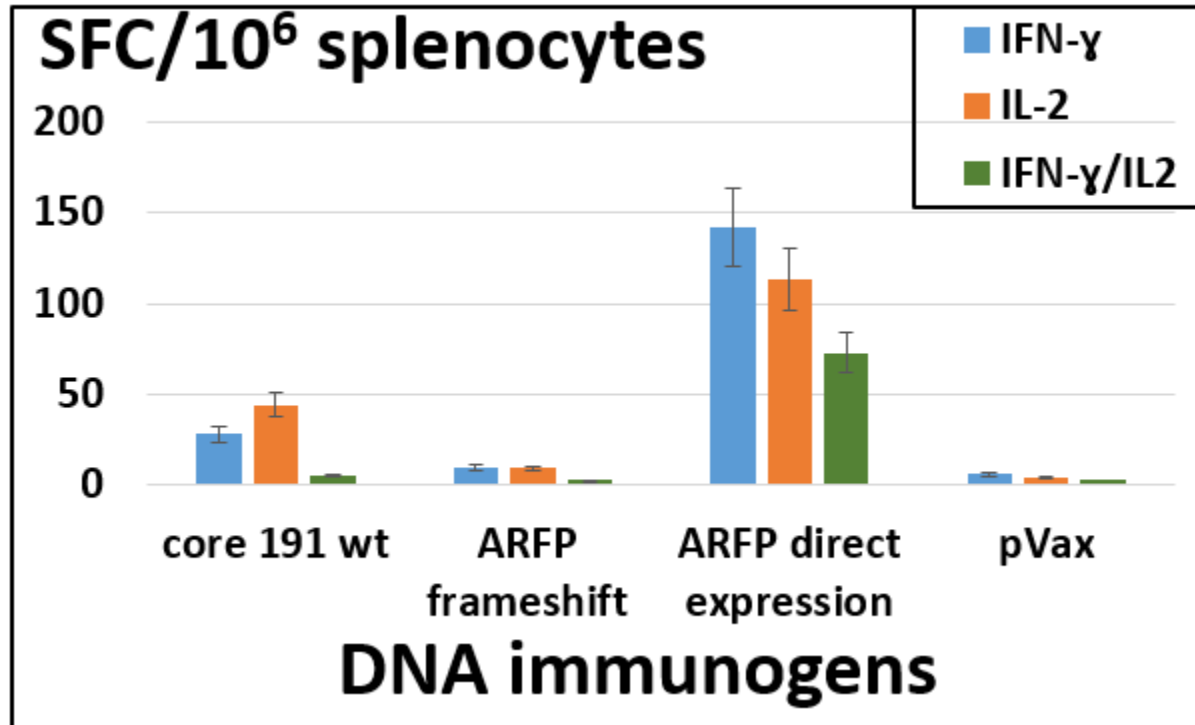
Analysis of immune response: Fluorospot



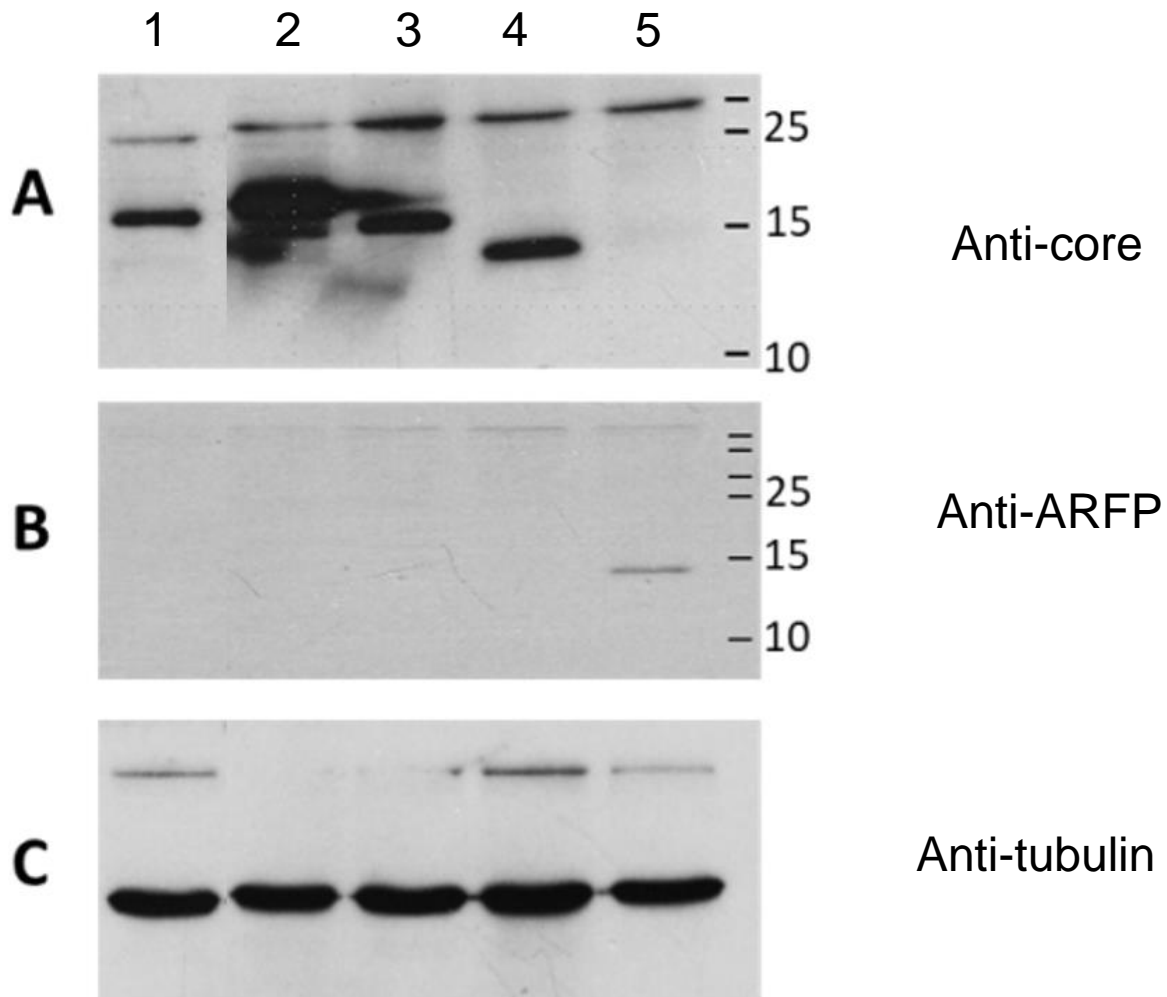
Analysis of immune response: Fluorospot



Analysis of immune response: Fluorospot



Expression of HCV core and ARFP in Huh7 cell culture



Lines: 1 - core 1-191 wide type, 2 - core 1-191 opt, 3 - core 1-152 opt, 4 - core 37-191, 5 - ARFP direct expression









cells

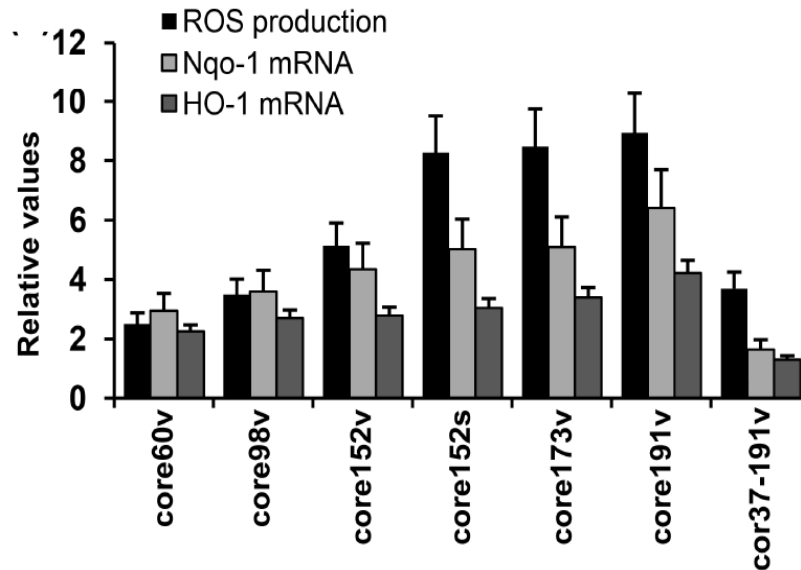


Article

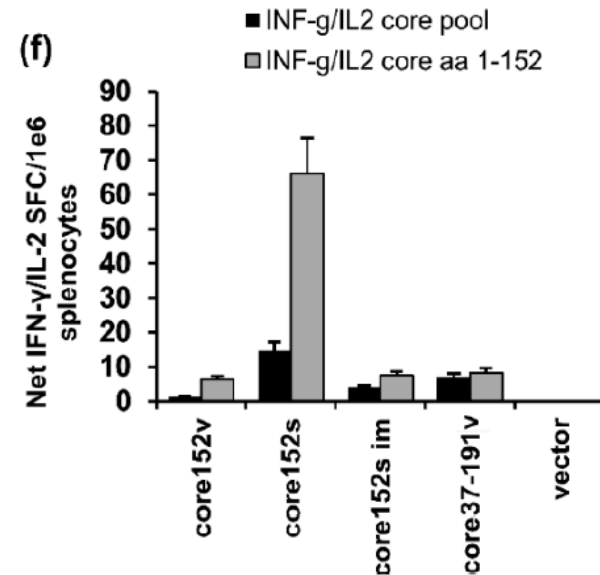
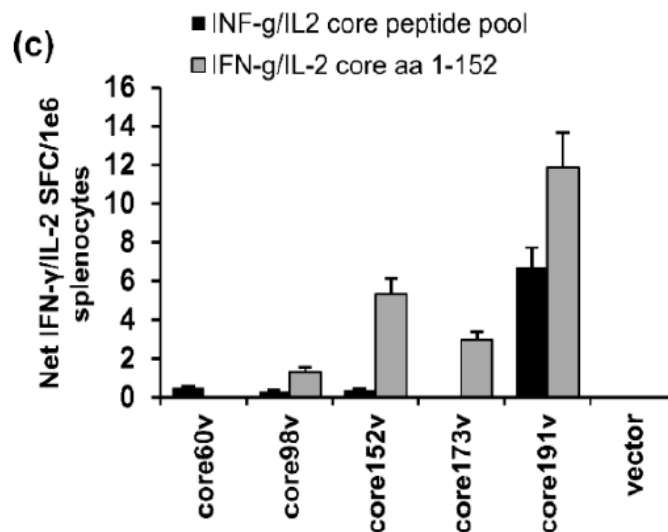
The Immunogenicity in Mice of HCV Core Delivered as DNA Is Modulated by Its Capacity to Induce Oxidative Stress and Oxidative Stress Response

Juris Jansons ^{1,2,3,†} , Irina Sominskaya ^{2,†} , Natalia Petrakova ⁴, Elizaveta S. Starodubova ^{4,5}, Olga A. Smirnova ⁵, Ekaterina Alekseeva ^{1,2}, Ruta Bruvere ², Olesja Eliseeva ⁴ , Dace Skrastina ^{1,2}, Elena Kashuba ^{3,6} , Marija Mihailova ² , Sergey N. Kochetkov ⁵, Alexander V. Ivanov ⁵  and Maria G. Isaguliants ^{1,3,4,7,*}

The induction of oxidative by variants of HCV core genes.



Cellular immune response of BALB/C mice to DNA-immunization with HCV core gene variants.



Acknowledgements

Dace Skrastina

Anastasija Dovbenko

Ivars Petrovskis

Stefan Petkov

Elizaveta Starodubova

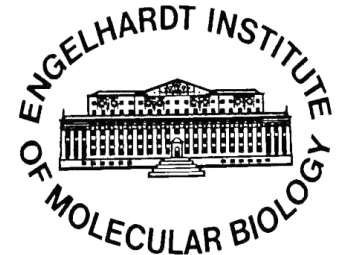
Alexander V. Ivanov

Maria Isaguliants

Irina Sominskaya



**Karolinska
Institutet**



**Chumakov Federal Scientific
Center for Research and
Development
of Immune-and- Biological
Products**



**Gamaleya
Research Institute
of Epidemiology
and Microbiology**

Financial support



VACTRAIN



TWINNING ON DNA-BASED
CANCER VACCINES

SI. Swedish
Institute

INNOVIMMUNE



FLPP

FUNDAMENTAL AND
APPLIED RESEARCH
PROJECTS

Thank you for attention!