Latvijas Biomedicīnas
pētījumu un studiju centrs
biomedicīnas pētījumi un izglītība no gēniem līdz cilvēkam

Alfavīrusu onkolitisko īpašību izpēte kombinācijā ar ķīmijas terapijas preparātiem

Anna Zajakina, Dr. Biol.

anna@biomed.lu.lv

MĒRĶIS: Izmantojot alfavīrusu vektorus izstrādāt optimālu gēnu piegādes stratēģiju peļu modeļu audzējos un izpētīt alfavīrusu onkolītiskas īpašības.

OPEN ACCESS Freely available online

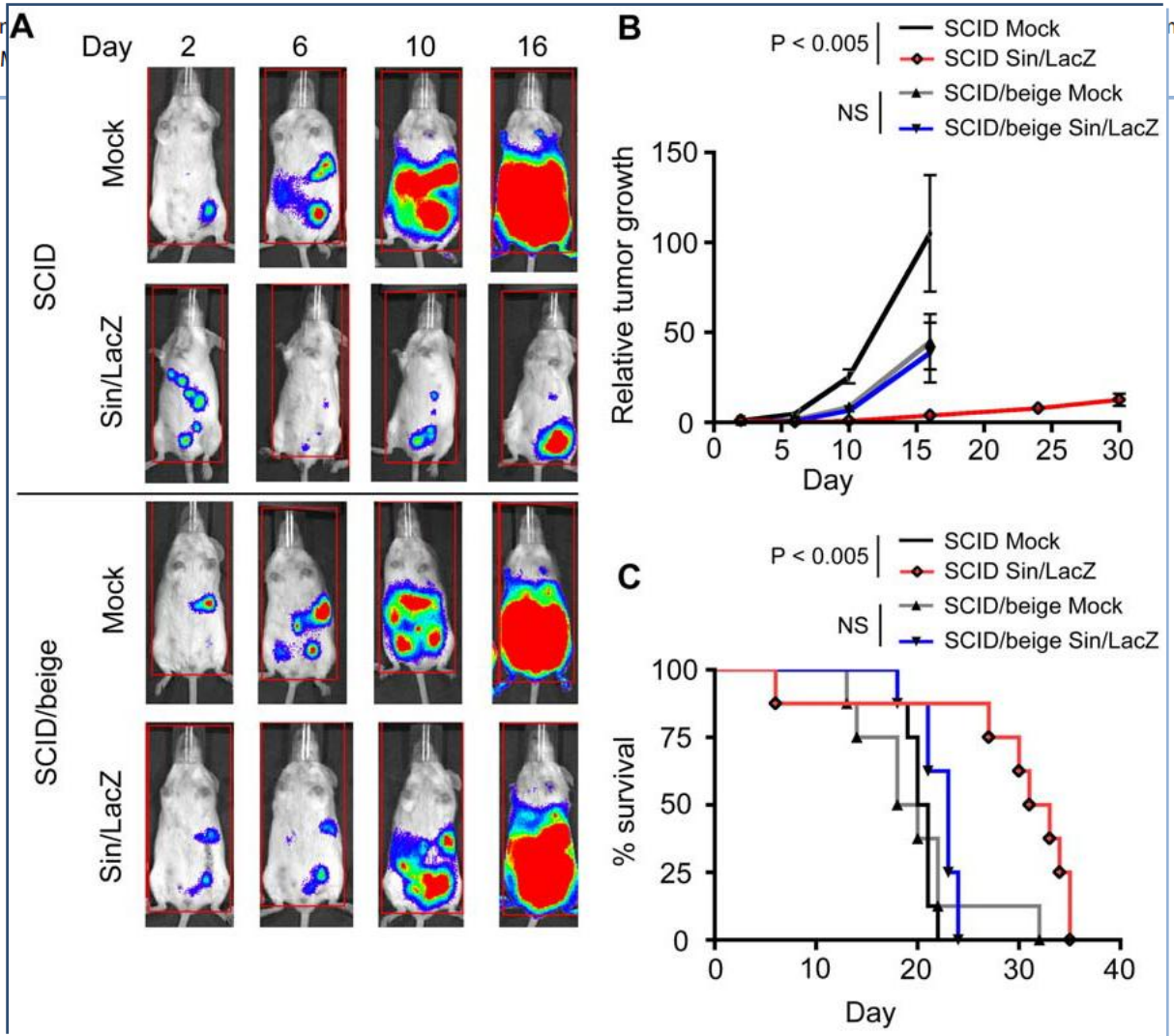
PLoS one

Activation of Cytotoxic and Regulatory Functions of NK Cells by Sindbis Viral Vectors

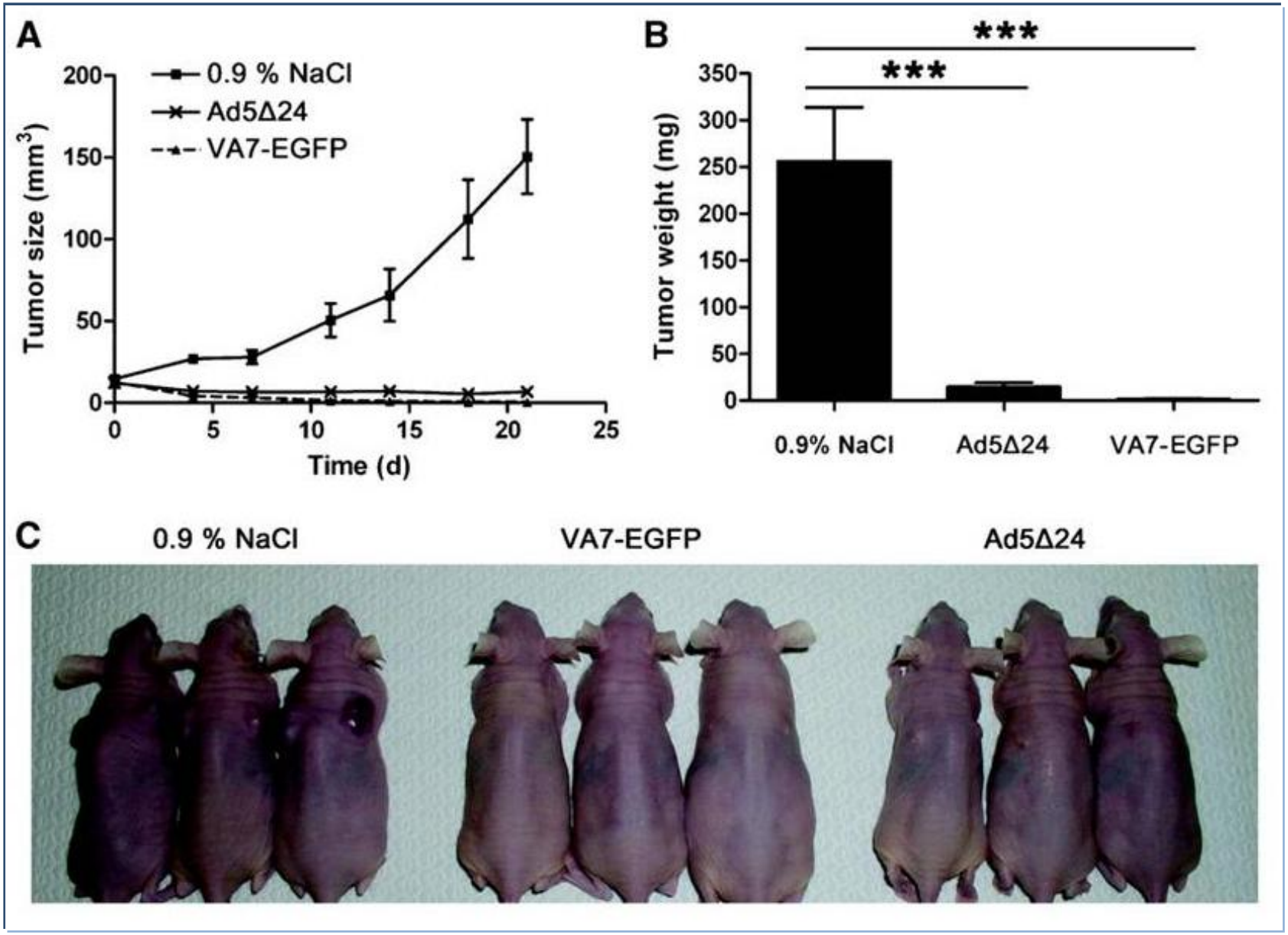
Tomer Granot[1], Lisa Venticinque[1], Jen-Chieh Tseng[2], Daniel Meruelo[1]*

1 Gene Therapy Center, Cancer In... nerica, **2** Dana-Farber Cancer Institute, Boston, M...

2011



Therapeutic effect of both VA7-EGFP and Ad5Δ24 viruses was tested in subcutaneous Saos2LM7 human osteosarcoma tumors implanted into nude mice.



Ketola A et al. Cancer Res 2008;68:8342-8350

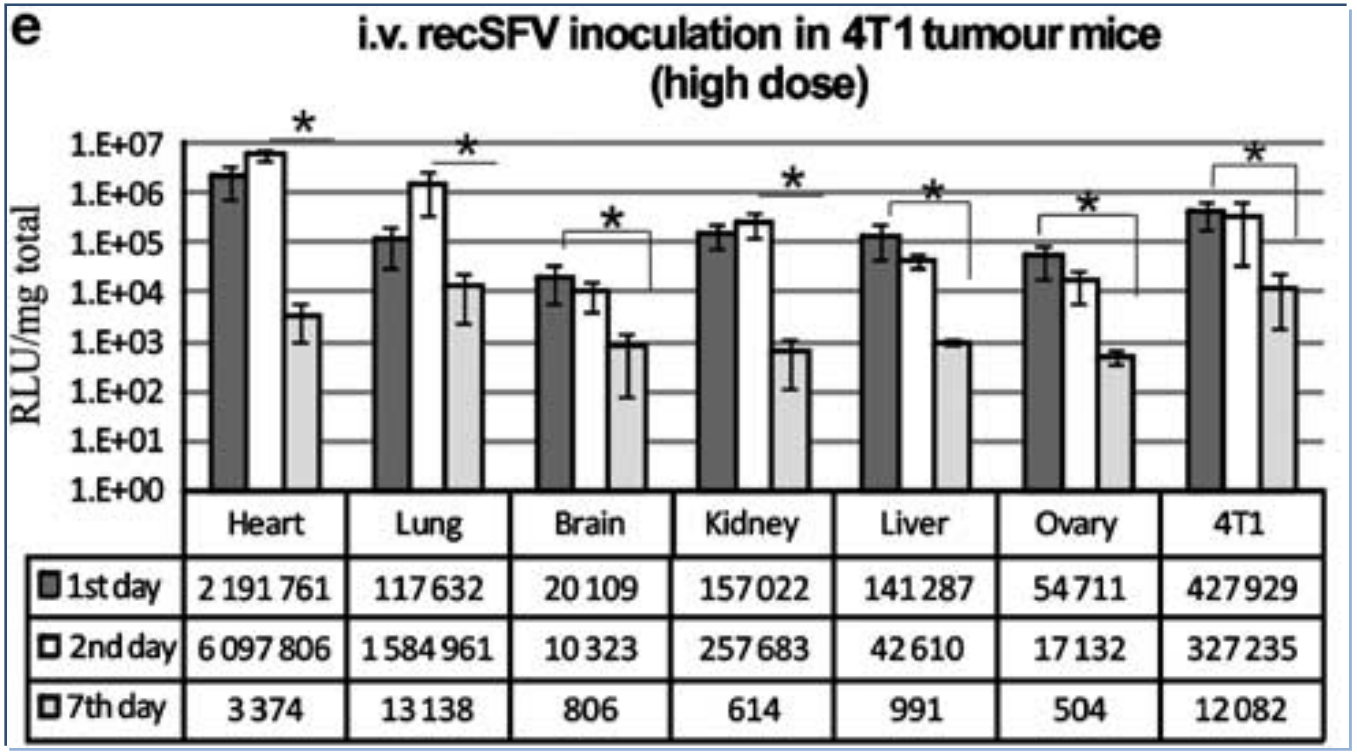
Cancer Gene Therapy (2012) 19, 579 – 587

www.nature.com/cgt

ORIGINAL ARTICLE

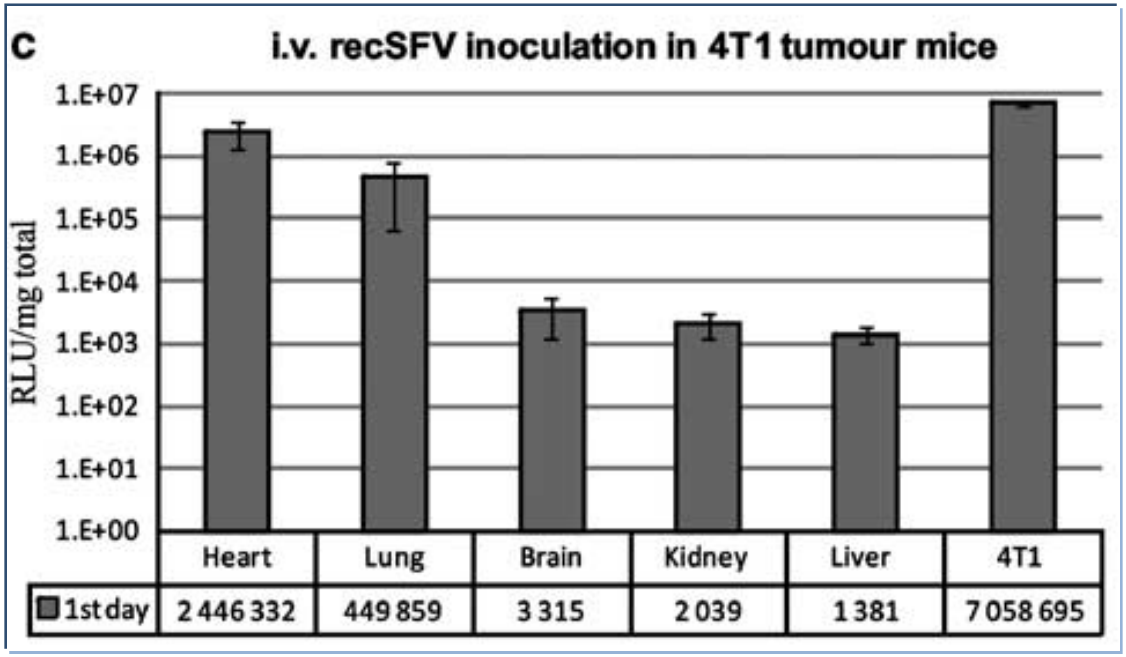
Semliki Forest virus biodistribution in tumor-free and 4T1 mammary tumor-bearing mice: a comparison of transgene delivery by recombinant virus particles and naked RNA replicon

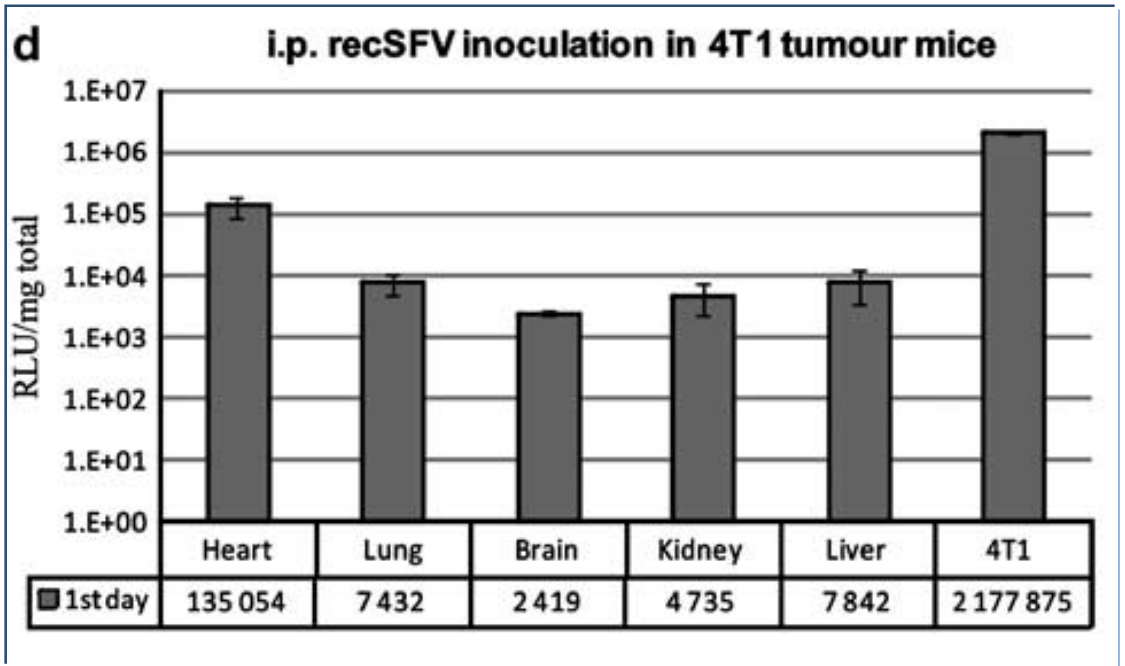
J Vasilevska[1], D Skrastina[1], K Spunde[1], H Garoff[2], T Kozlovska[1] and A Zajakina[1]



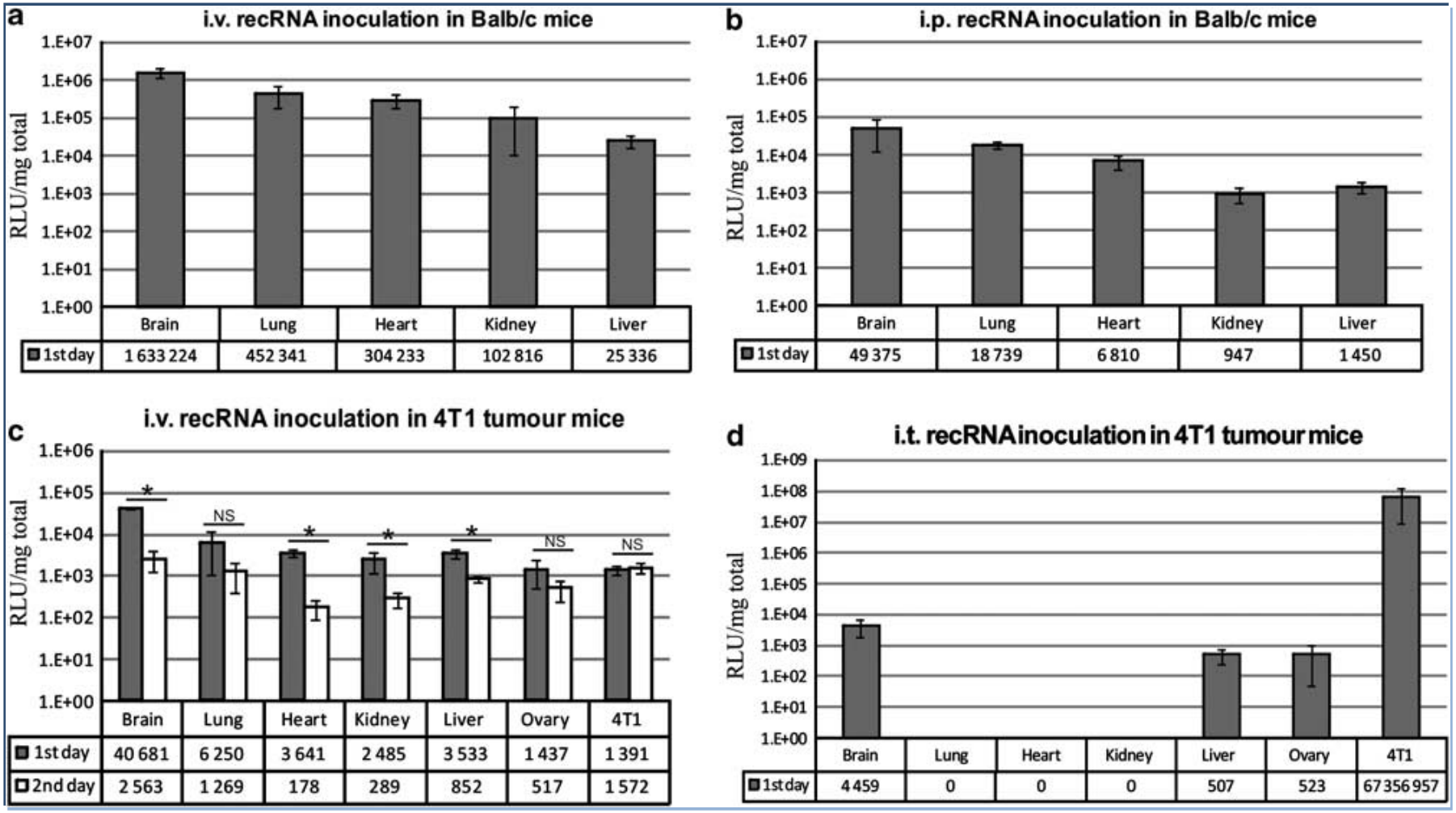
	Heart	Lung	Brain	Kidney	Liver	Ovary	4T1
1st day	2 191 761	117 632	20 109	157 022	141 287	54 711	427 929
2nd day	6 097 806	1 584 961	10 323	257 683	42 610	17 132	327 235
7th day	3 374	13 138	806	614	991	504	12 082

Nekoncentrēts vīruss: $3{,}3\times10^{8}$ v.d

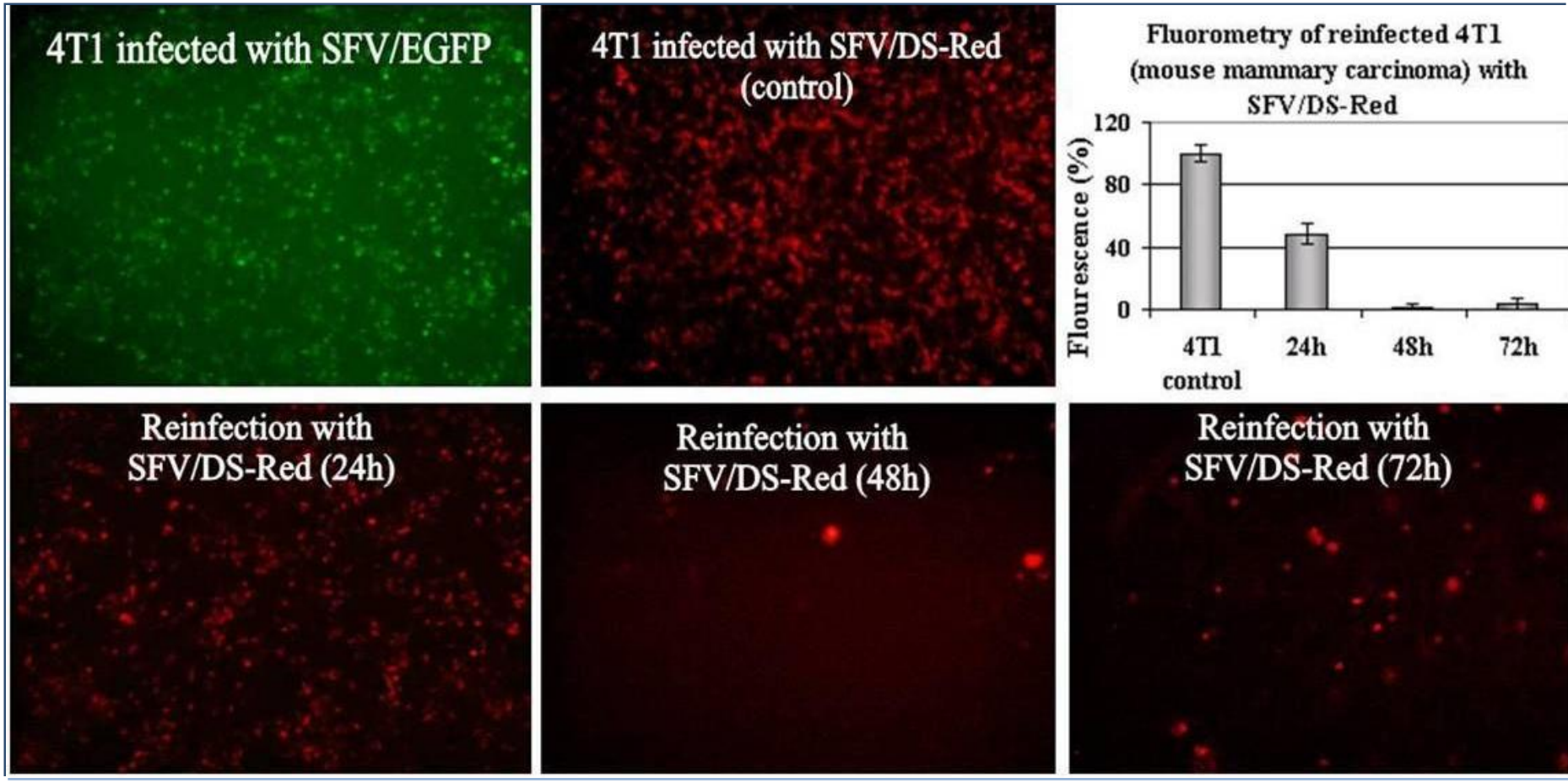




RNA inoculation



J Vasilevska, D Skrastina, K Spunde, H Garoff, T Kozlovska and A Zajakina, Cancer G Ther., 2012



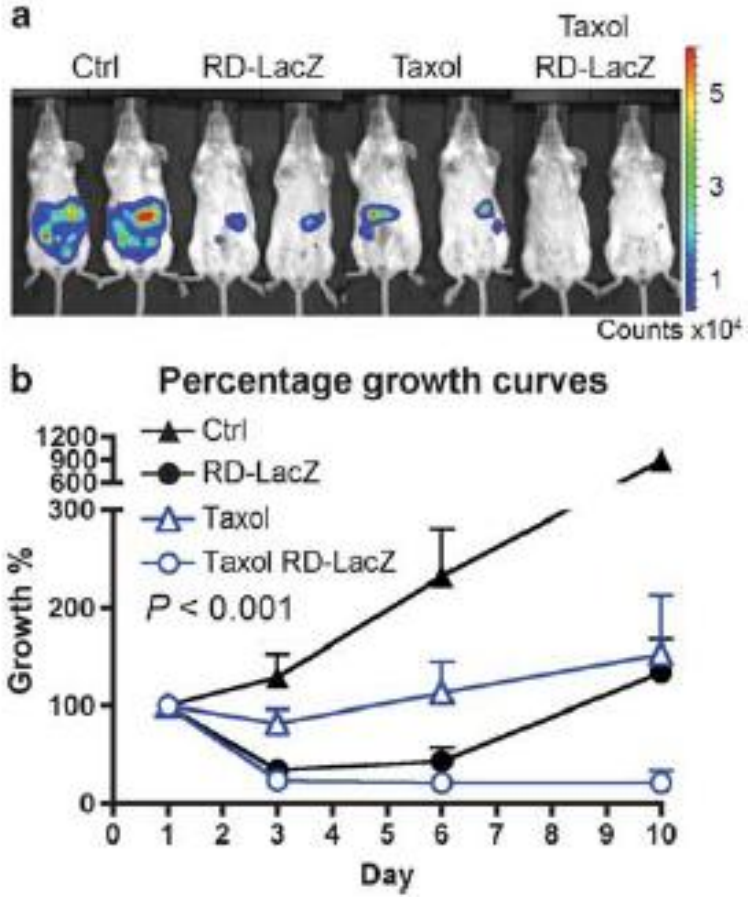
Reinfection of 4T1 (mouse mammary tumor) cell lines with recSFV particles producing EGFP and DS-Red fluorescence proteins *(prepared for publication in Gene Ther. J.)*

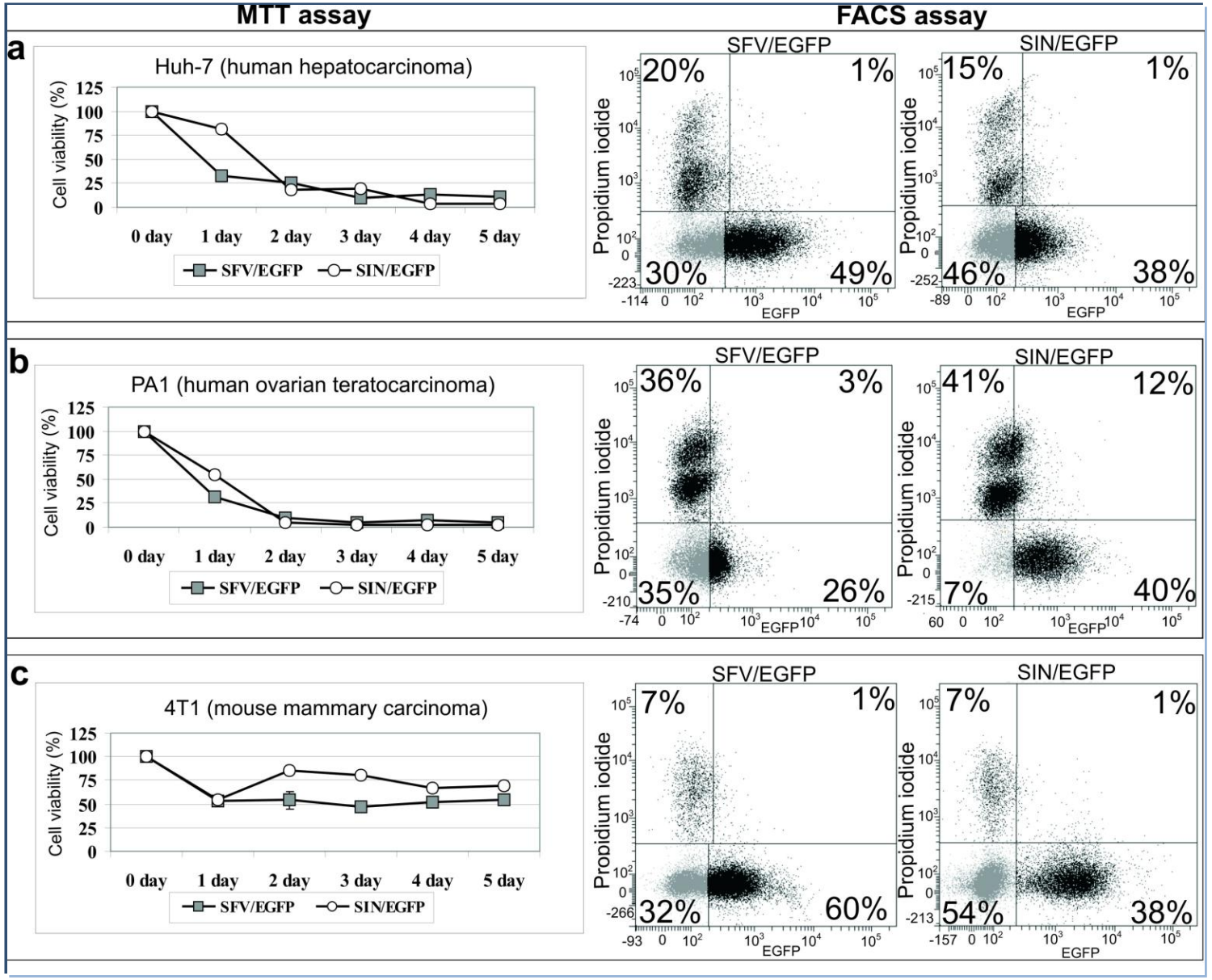
Cancer Gene Therapy (2010) 17, 244–255
 0929-1903/10 $32.00
www.nature.com/cgt

ORIGINAL ARTICLE

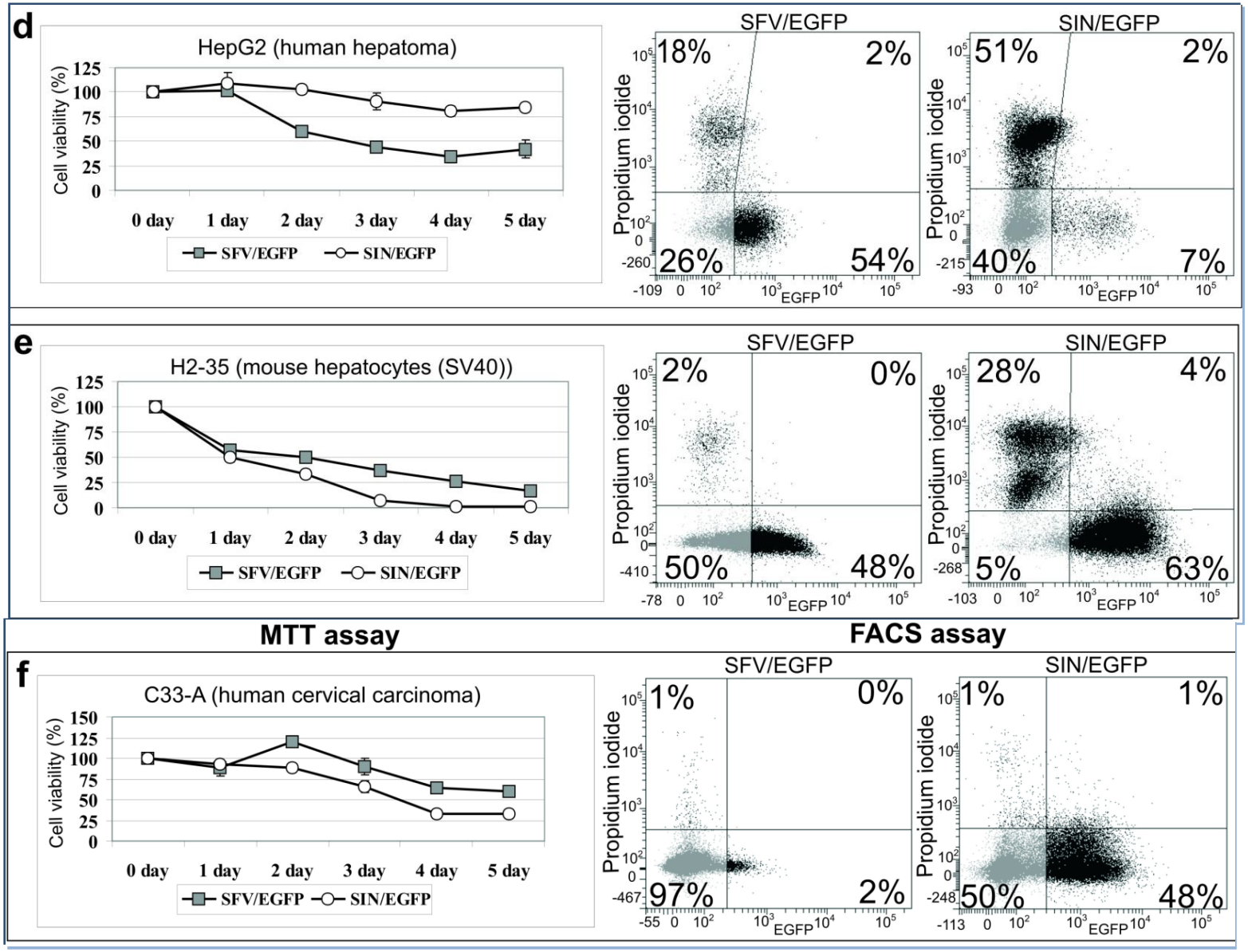
Enhanced specific delivery and targeting of oncolytic Sindbis viral vectors by modulating vascular leakiness in tumor

J-C Tseng[1], T Granot[1], V DiGiacomo[1], B Levin[1] and D Meruelo[1]

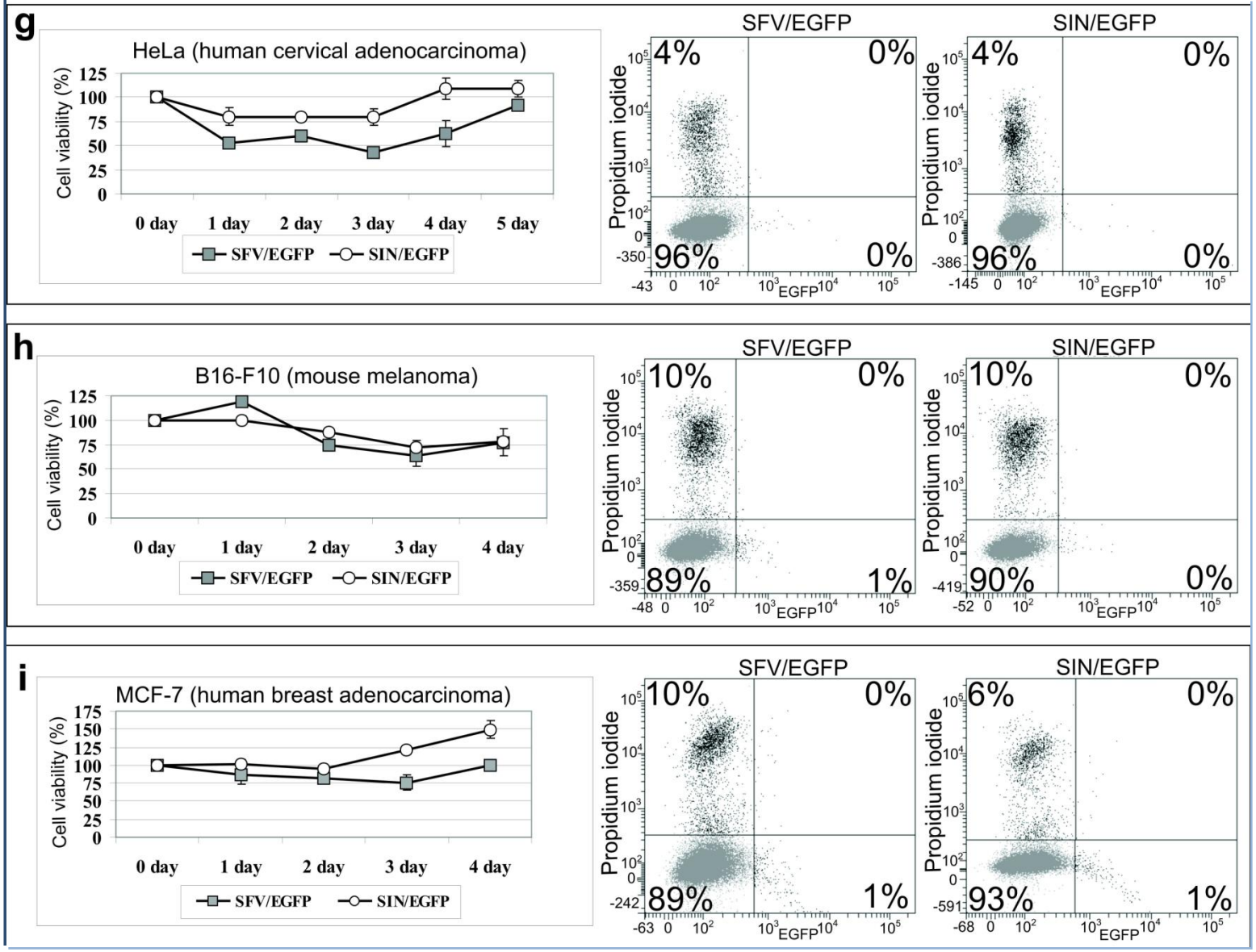




Transduction efficiency and cytotoxicity of alphaviral vectors in cancer cell lines *(prepared for publication in Gene Ther. J.)*



Transduction efficiency and cytotoxicity of alphaviral vectors in cancer cell lines *(prepared for publication in Gene Ther. J.)*



Transduction efficiency and cytotoxicity of alphaviral vectors in cancer cell lines *(prepared for publication in Gene Ther. J.)*

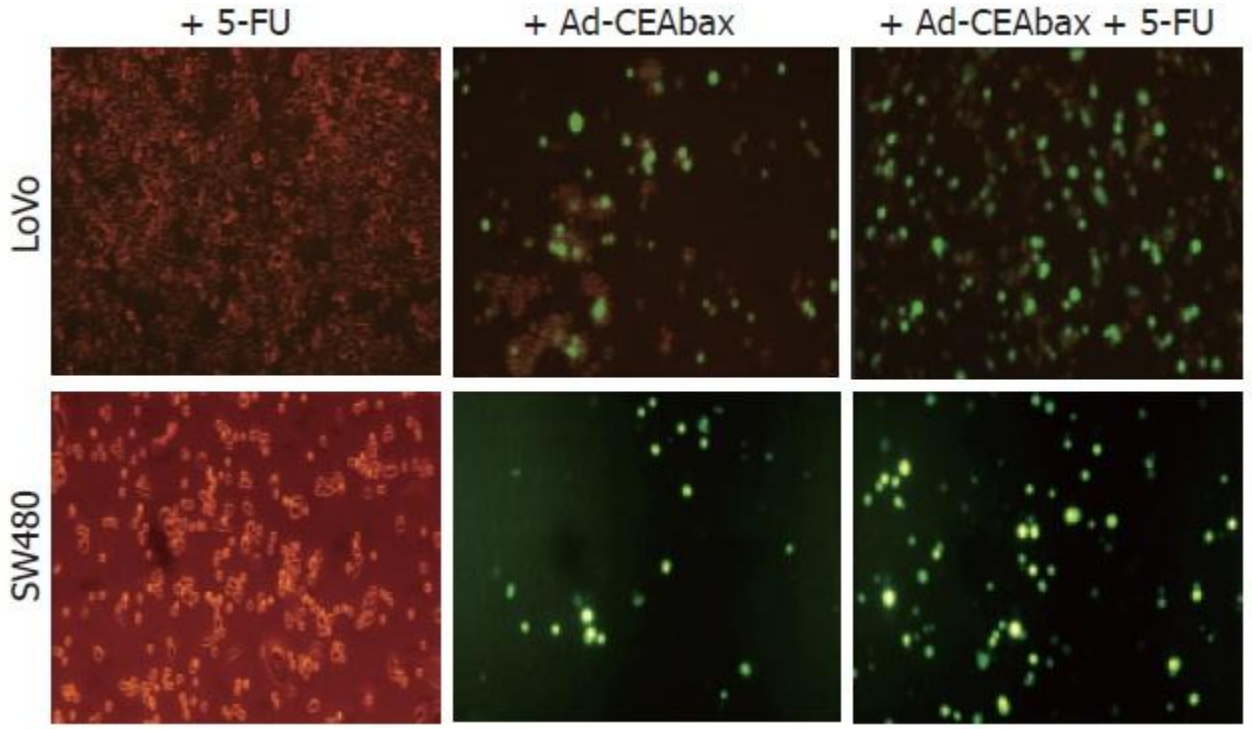
PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

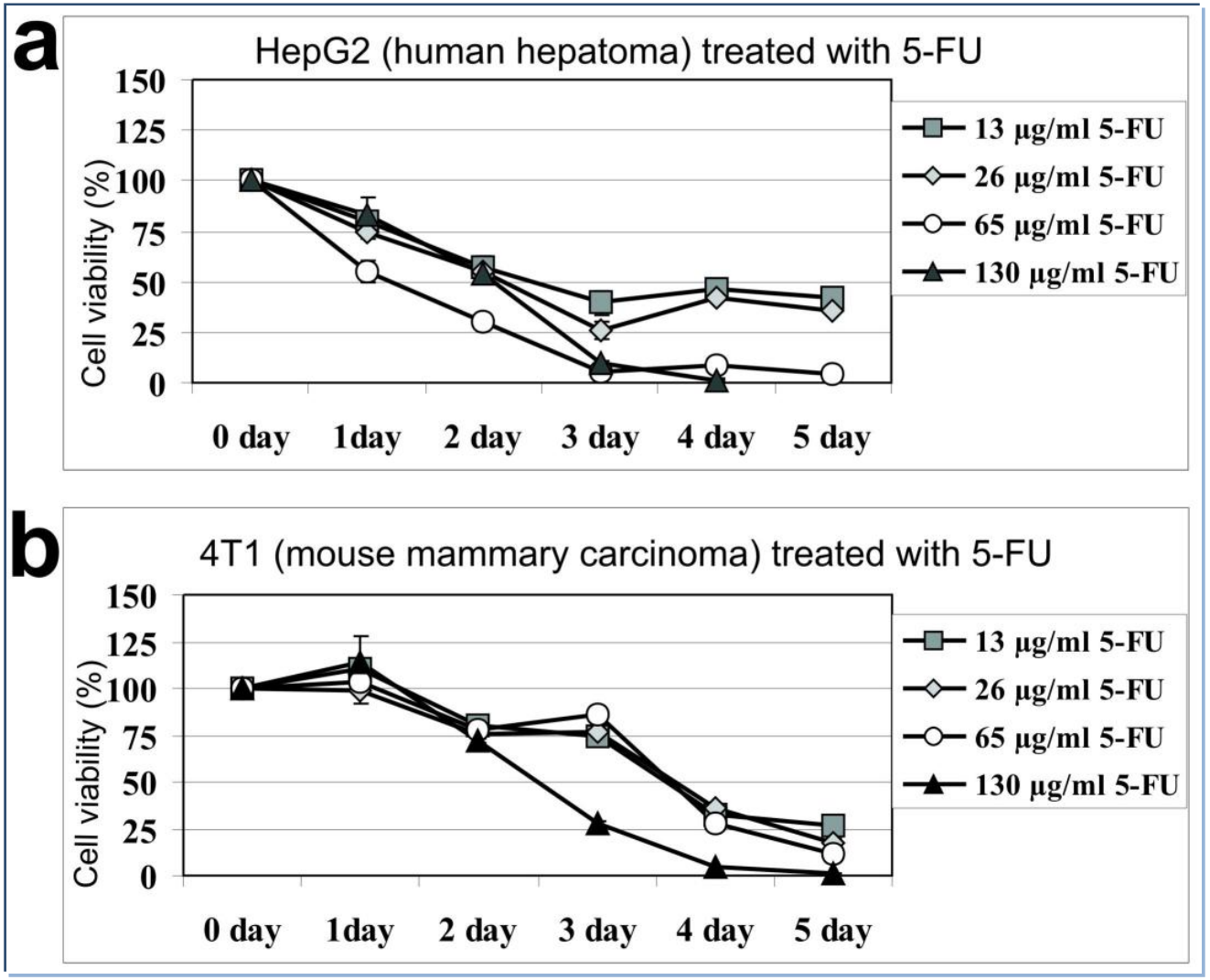
World J Gastroenterol 2006 August 28; 12(32): 5168-5174
World Journal of Gastroenterology ISSN 1007-9327

BASIC RESEARCH

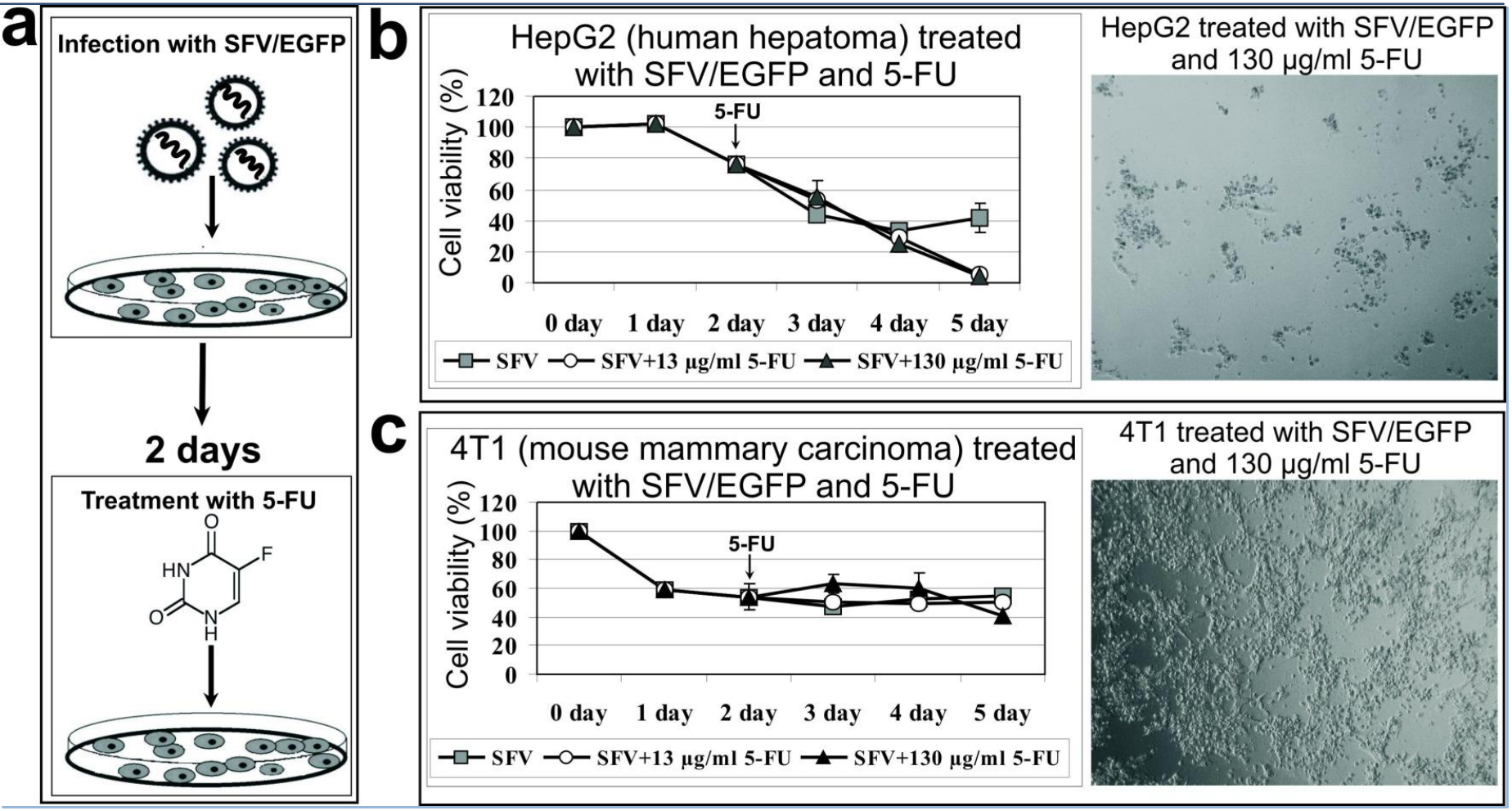
5-Fluorouracil-related enhancement of adenoviral infection is Coxsackievirus-adenovirus receptor independent and associated with morphological changes in lipid membranes

Chiara Cabrele, Mandy Vogel, Pompiliu Piso, Markus Rentsch, Josef Schröder, Karl W Jauch, Hans J Schlitt, Alexander Beham

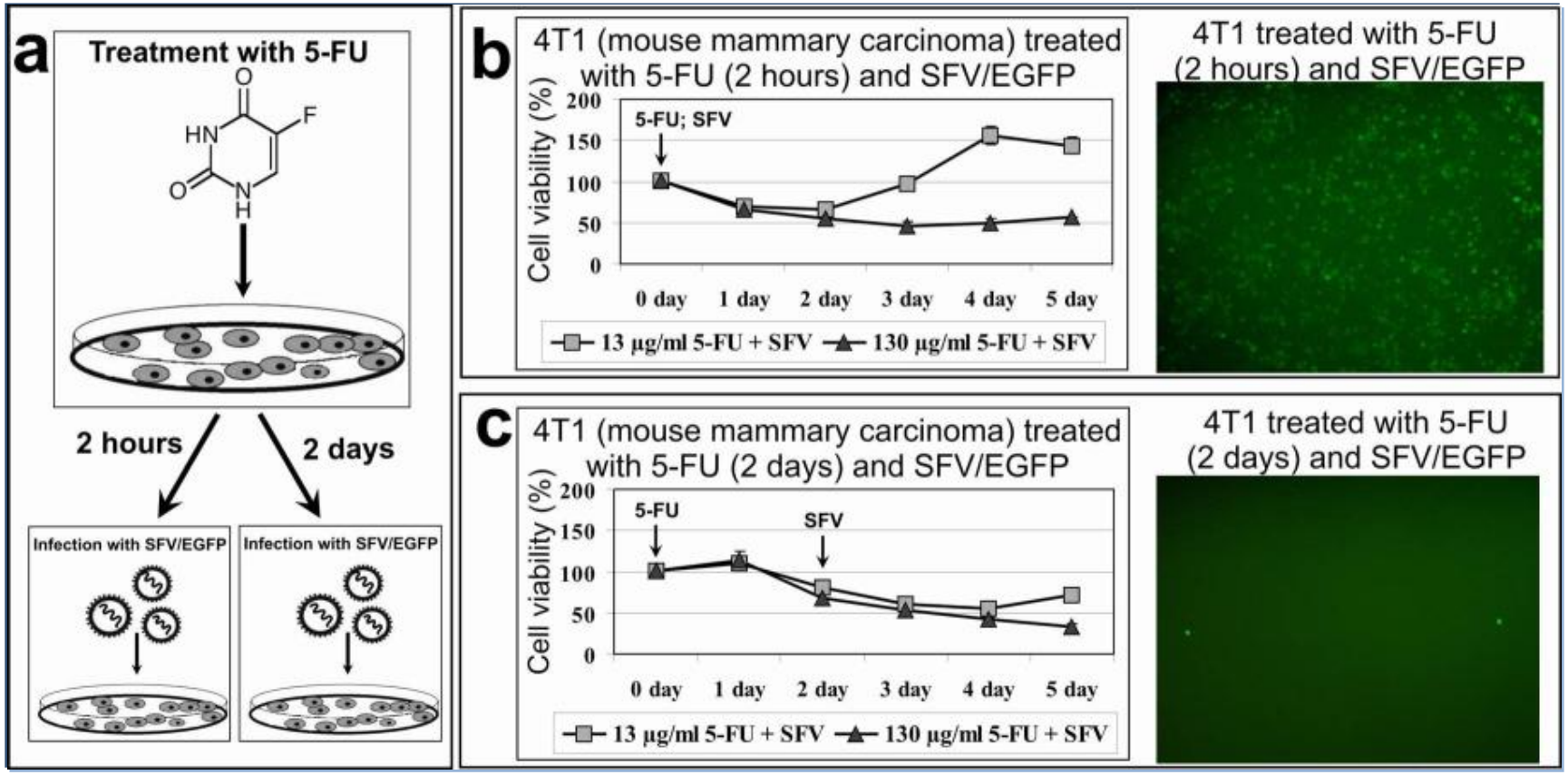




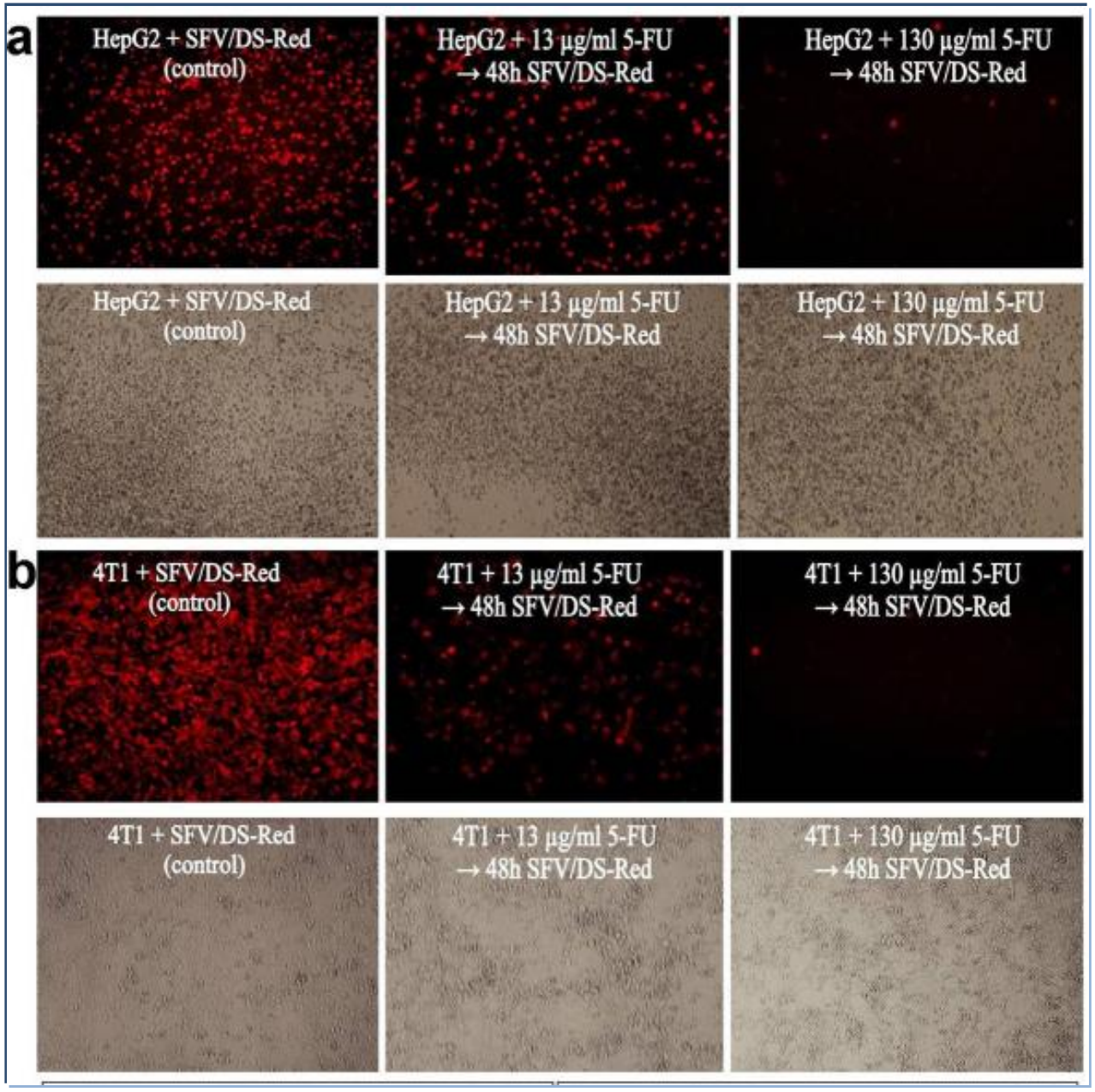
Inhibiting effect of 5-floururacil (5-FU) for proliferation of HepG2 and 4T1 cell lines.



Combined treatment of HepG2 and 4T1 cells with recSFV/EGFP virus particles and 5-floururacil (5-FU) drug.

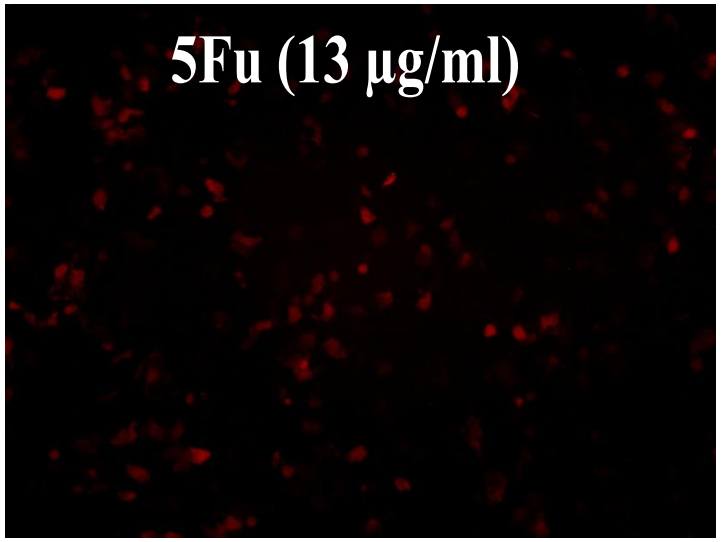


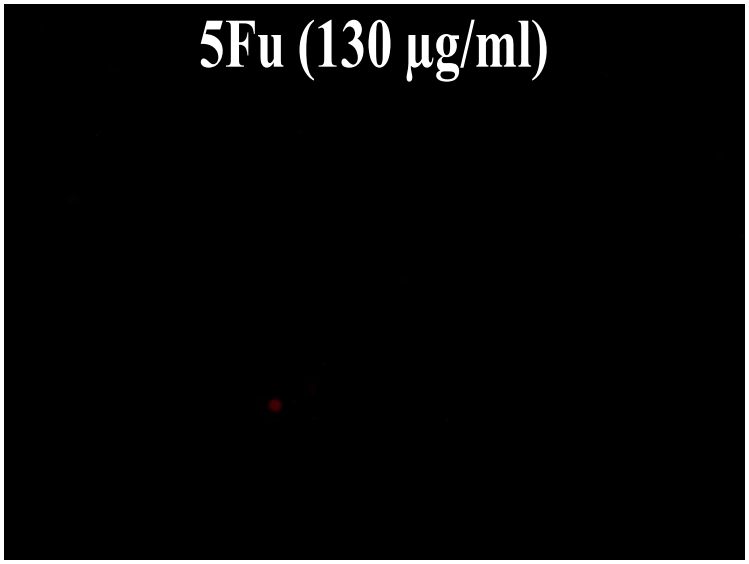
recSFV/EGFP infection of 4T1 cells pretreated with 5-floururacil (5-FU) drug: infectivity and citotoxicity evaluation.

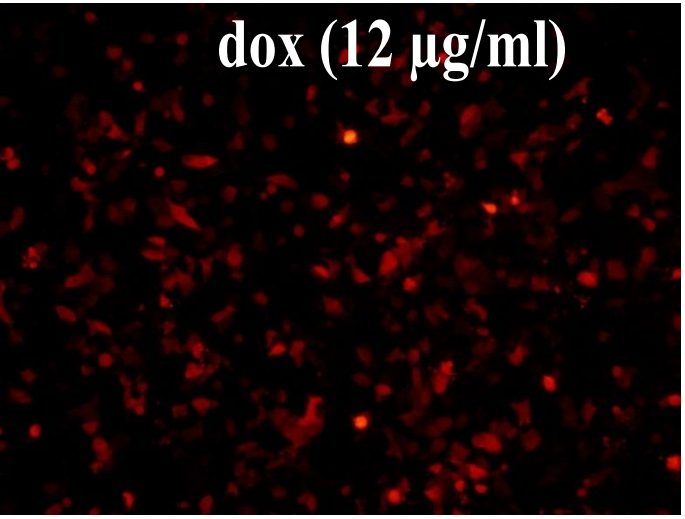

a
HepG2 + SFV/DS-Red (control)
HepG2 + 13 µg/ml 5-FU → 48h SFV/DS-Red
HepG2 + 130 µg/ml 5-FU → 48h SFV/DS-Red
HepG2 + SFV/DS-Red (control)
HepG2 + 13 µg/ml 5-FU → 48h SFV/DS-Red
HepG2 + 130 µg/ml 5-FU → 48h SFV/DS-Red
b
4T1 + SFV/DS-Red (control)
4T1 + 13 µg/ml 5-FU → 48h SFV/DS-Red
4T1 + 130 µg/ml 5-FU → 48h SFV/DS-Red
4T1 + SFV/DS-Red (control)
4T1 + 13 µg/ml 5-FU → 48h SFV/DS-Red
4T1 + 130 µg/ml 5-FU → 48h SFV/DS-Red

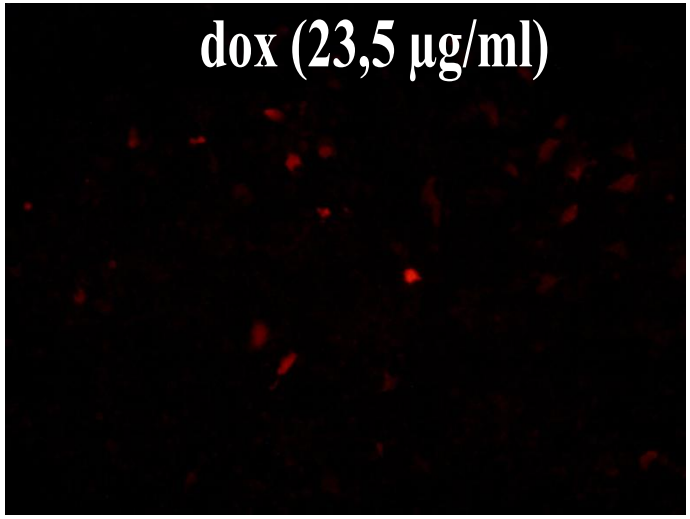
4T1 cells

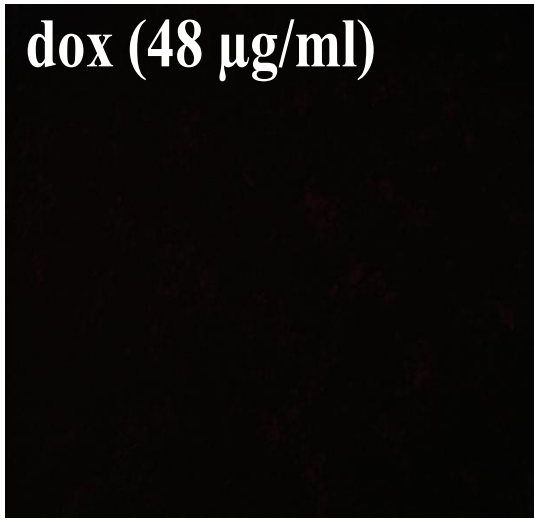
Infection with SFV-Red after 2 days incubation with chemical drug

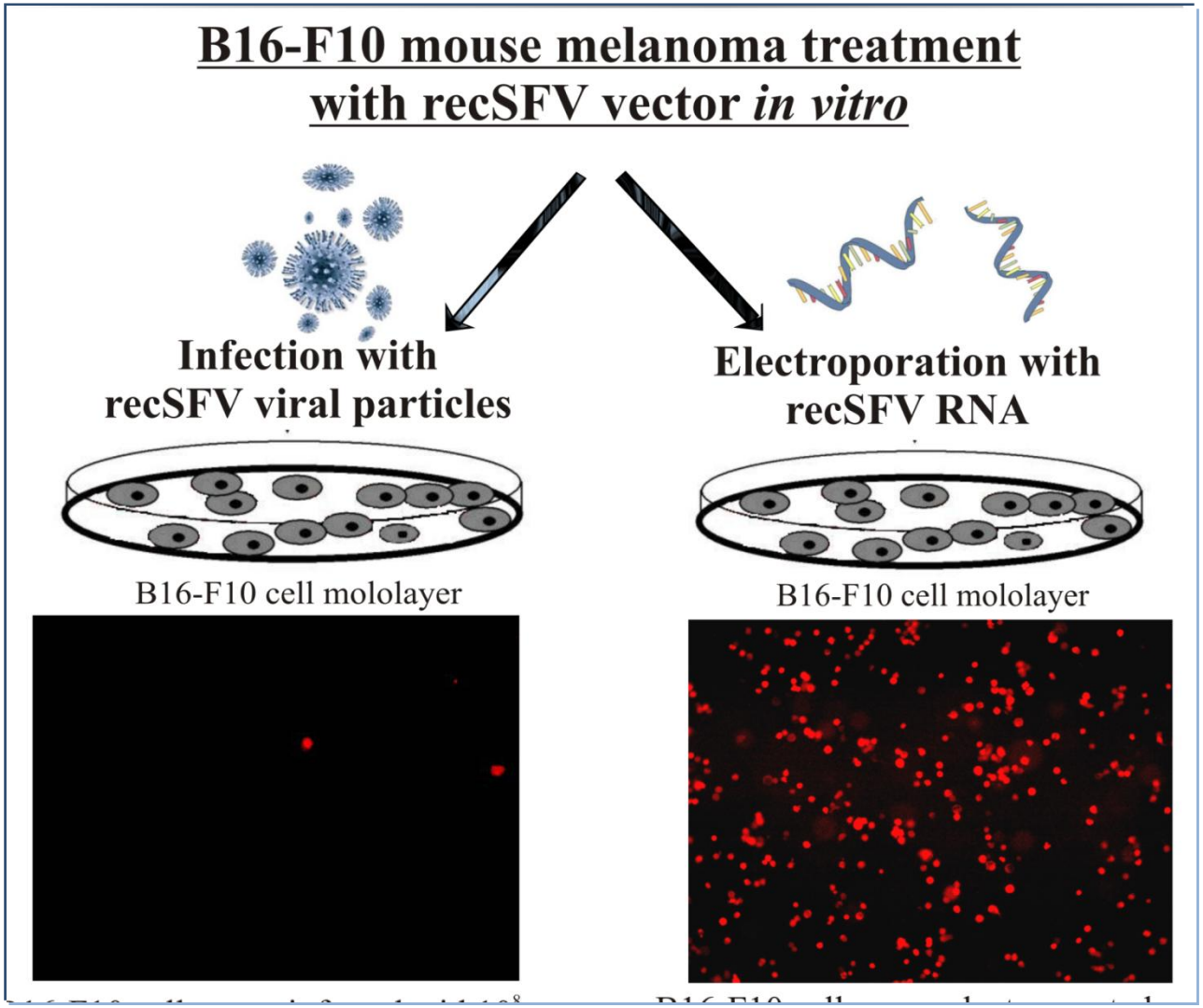






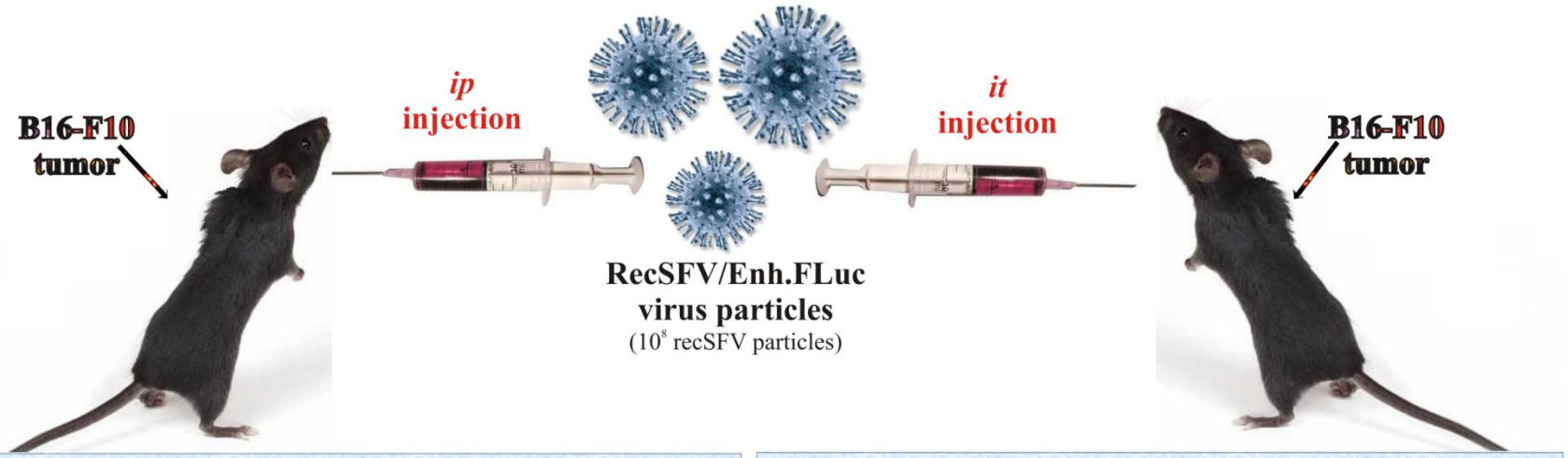


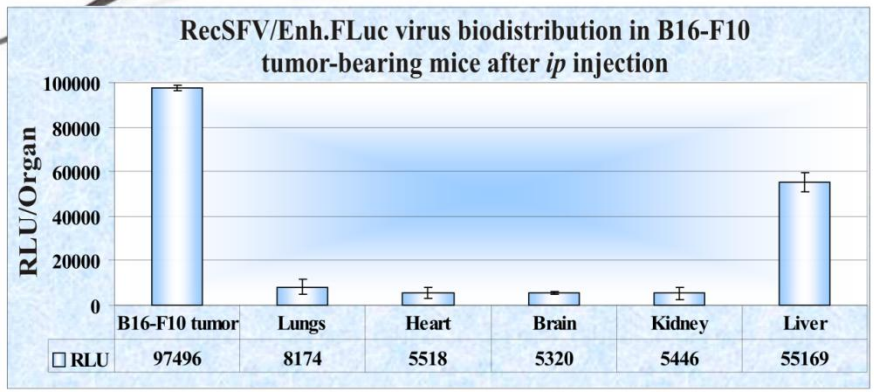


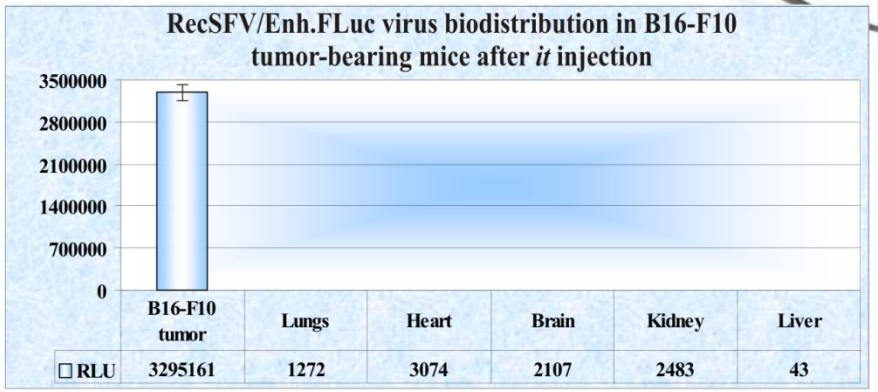


2012. October 25-29: **The European Society of Gene and Cell Therapy Congress.**
"Intratumoral delivery and biodistribution of oncolytic alphaviral vectors in mouse melanoma model" authors: Jelena Vasilevska, Dace Skrastina, SvetlanaLubina, Tatjana Kozlovska, Anna Zajakina. France, Versailles.

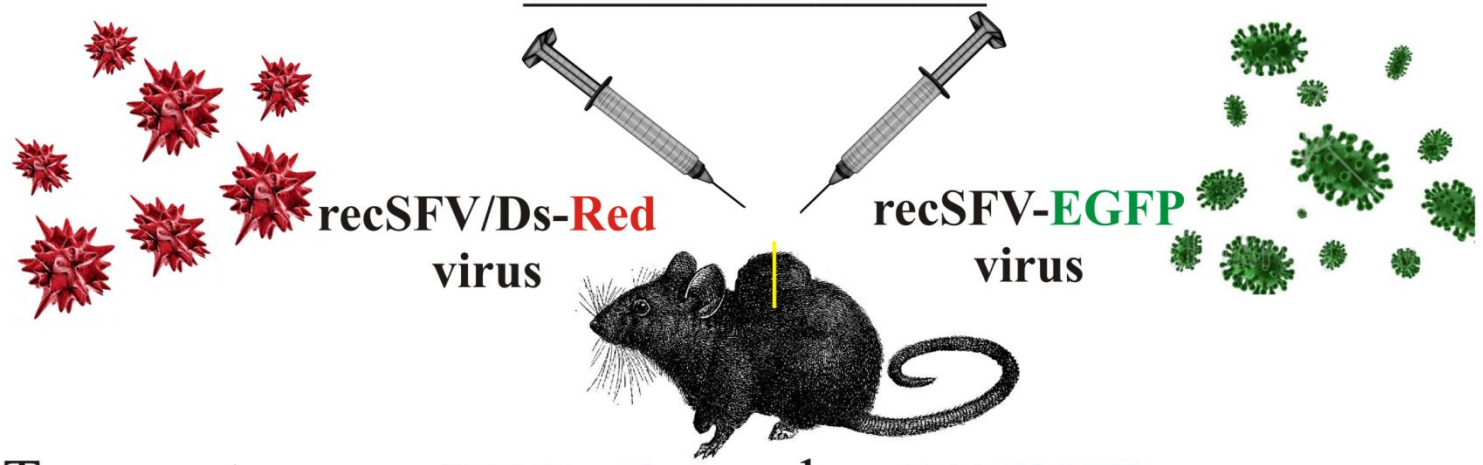
RecSFV virus biodistribution in melanoma B16-F10 tumor-bearing mice



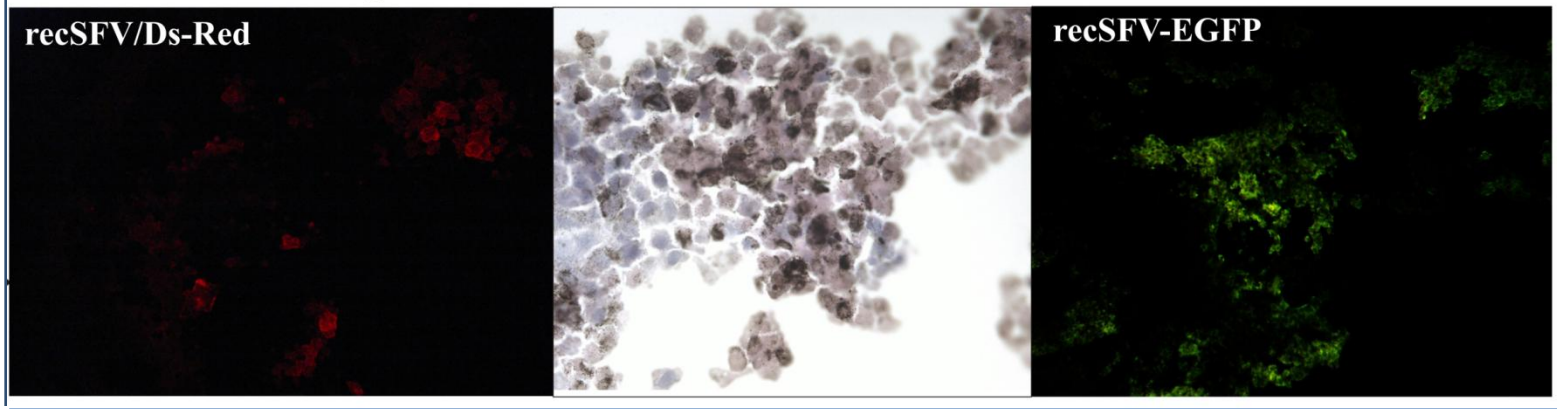




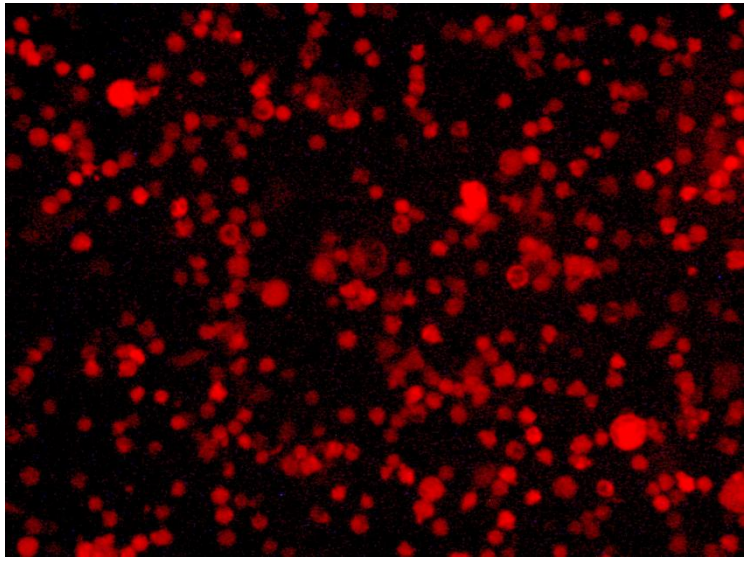
RecSFV virus distribution inB16-F10 within the tumor

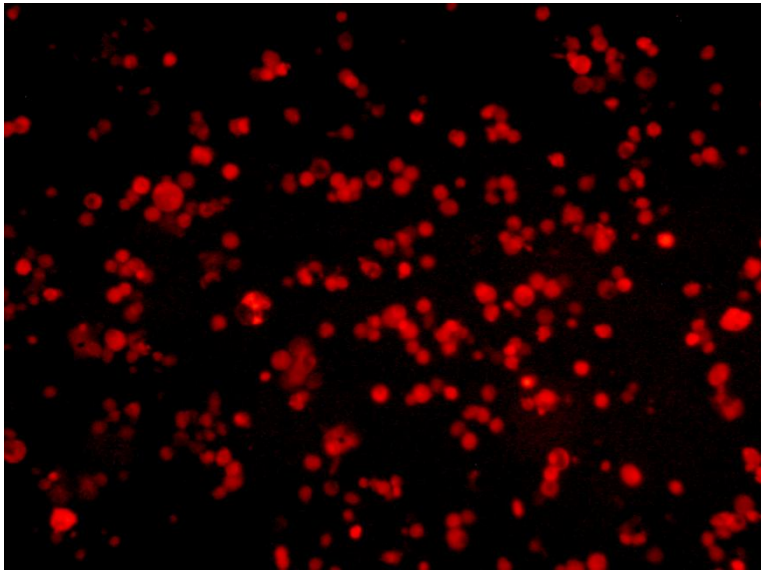


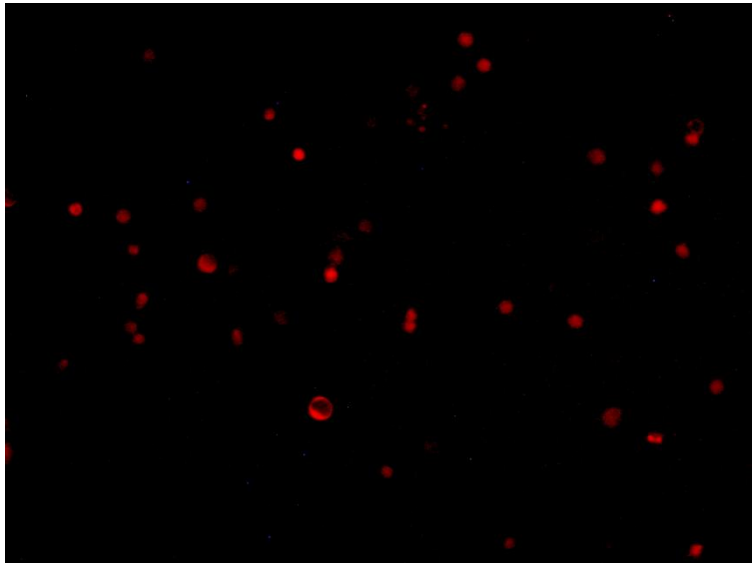
Two vectors **recSFV/Ds-Red** and **recSFV-EGFP** were inoculated into different points of tumor nodule on the left and right side correspondingly. The vector distribution was analyzed by fluorescence microscopy of tumor **cryosections**.

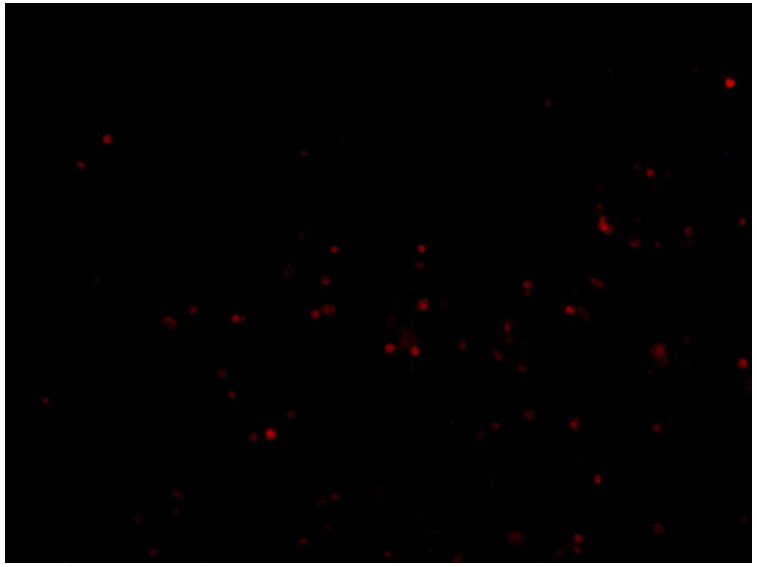


B16 melanoma *ex vivo* infection









Secinājumi:

Tika izpētīta rekombinanta SFV vīrusa izplatīšanās Balb/C peļu modelī un 4T1 peļu krūts vēža modelī; Vasilevska et all 2012, Semliki Forest virus biodistribution in tumor-free and 4T1 mammary tumor-bearing mice: a comparison of transgene delivery by recombinant virus particles and naked RNA replicon. *Cancer Gene Ther.* 2012 Aug;19(8):579-87)

Tika izpētīta SFV un Sindbis vīrusa transdukcijas spēja un citotoksiskais efekts, inficējot dažādas peļu un cilvēka vēža šūnu kultūras. Šūnu proliferācijas inhibēšanai tika izanalizētas dažādas šūnu apstrādāšanas stratēģijas kombinējot SFV vīrusu ar 5-FU (publikācija ir sagatavota *Gene Therapy Journal*).

Tika uzsākti eksperimenti lai izpētītu SFV vīrusa transdukcijas spējas un citotoksisko efektu kombinācijā ar doksorubicīnu

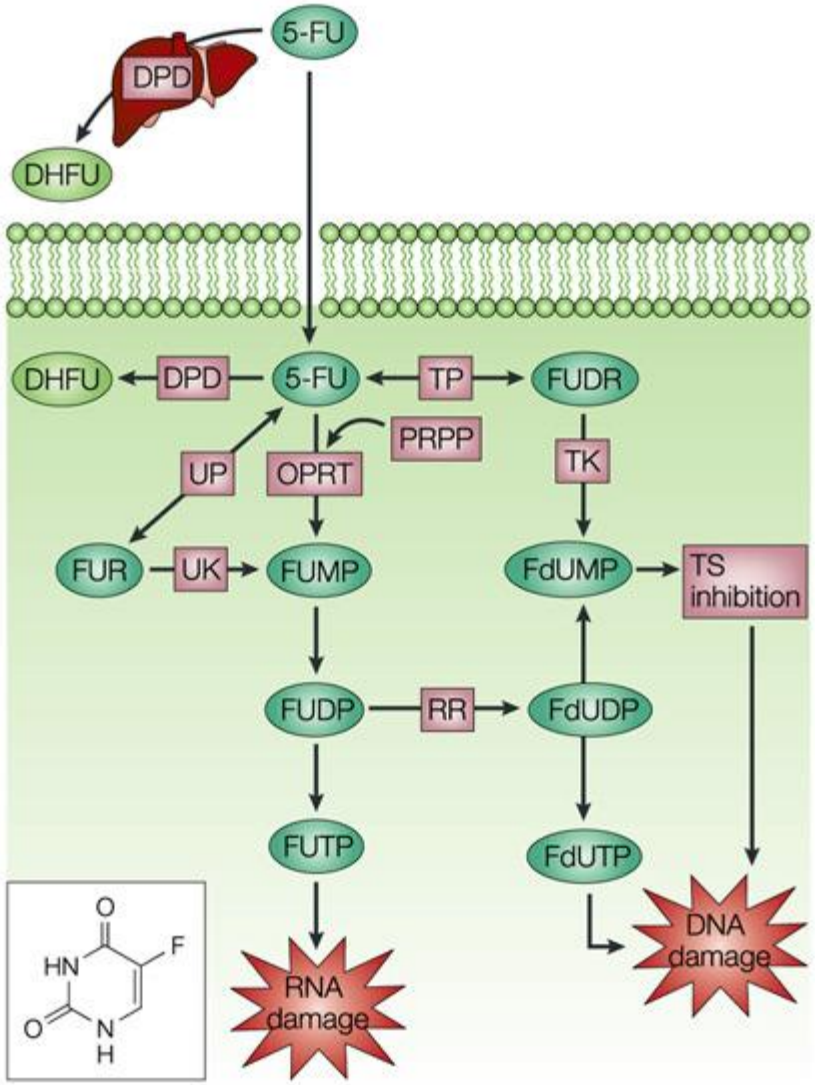
Tika uzsākti eksperimenti lai izpētītu SFV vīrusa izplatīšanos B16-F10 peļu audzēju modelī (melanoma) (2012, tēzes Francija).

Pateicības:

BMC
Jeļena Vasiļevska
Dace Skrastiņa
Baiba Ķūrēna

OSI
Aiva Plotniece
Gunārs Duburs

Projekta vad.
Tatjana Kozlovska



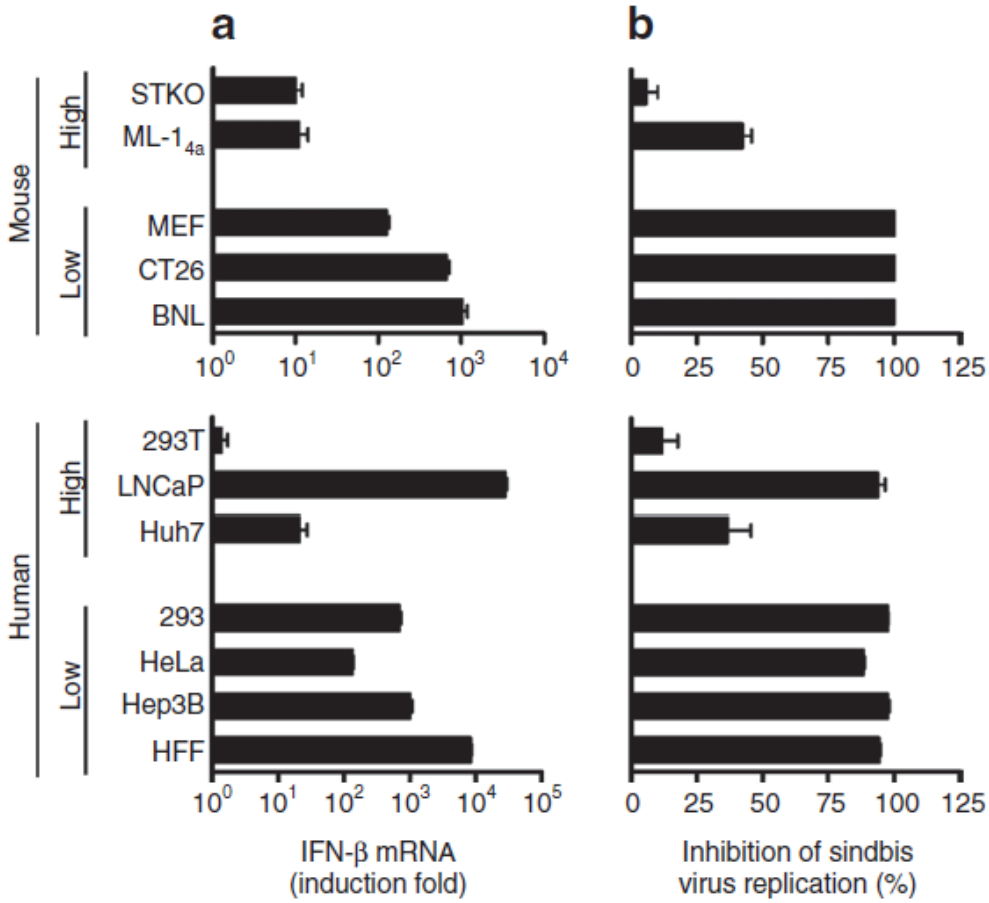
Nature Reviews | Cancer

Oncolytic Sindbis Virus Targets Tumors Defective in the Interferon Response and Induces Significant Bystander Antitumor Immunity *In Vivo*

Pong-Yu Huang[1], Jih-Huong Guo[1] and Lih-Hwa Hwang[1,2]

[1]*Graduate Institute of Microbiology, National Taiwan Univ*
National Yang-Ming University, Taipei, Taiwan

www.moleculartherapy.org vol. 20 no. 2, 298–305 feb. 2012

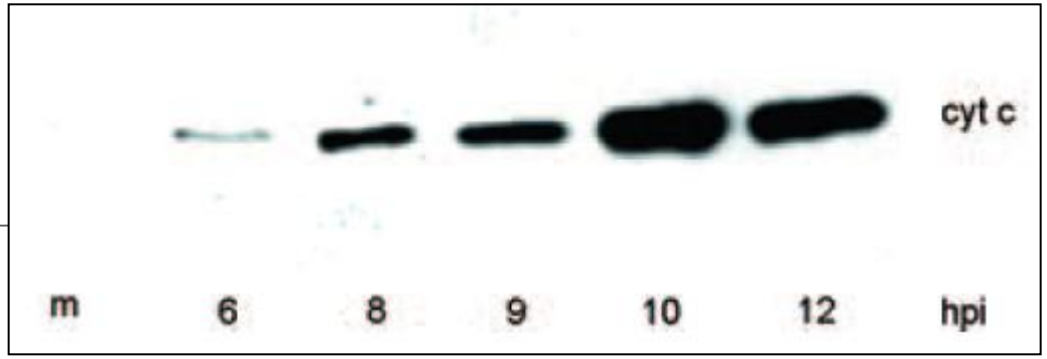


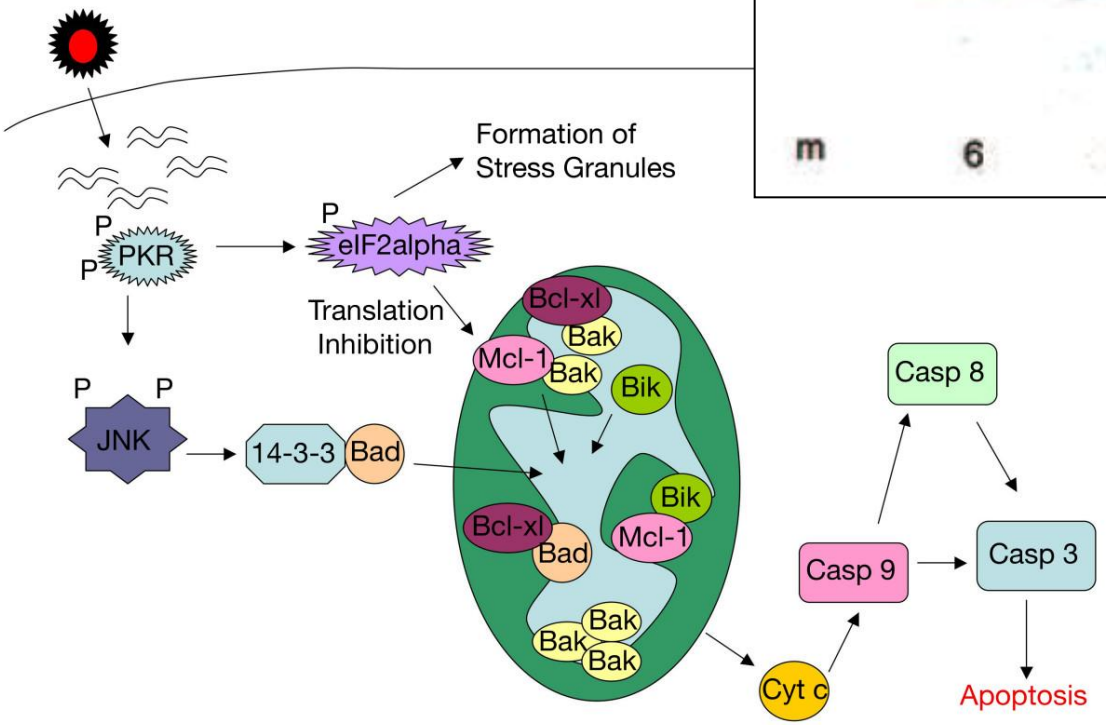
Exp Physiol 90.1 pp 45–52

Alphaviral cytotoxicity and its implication in vector development

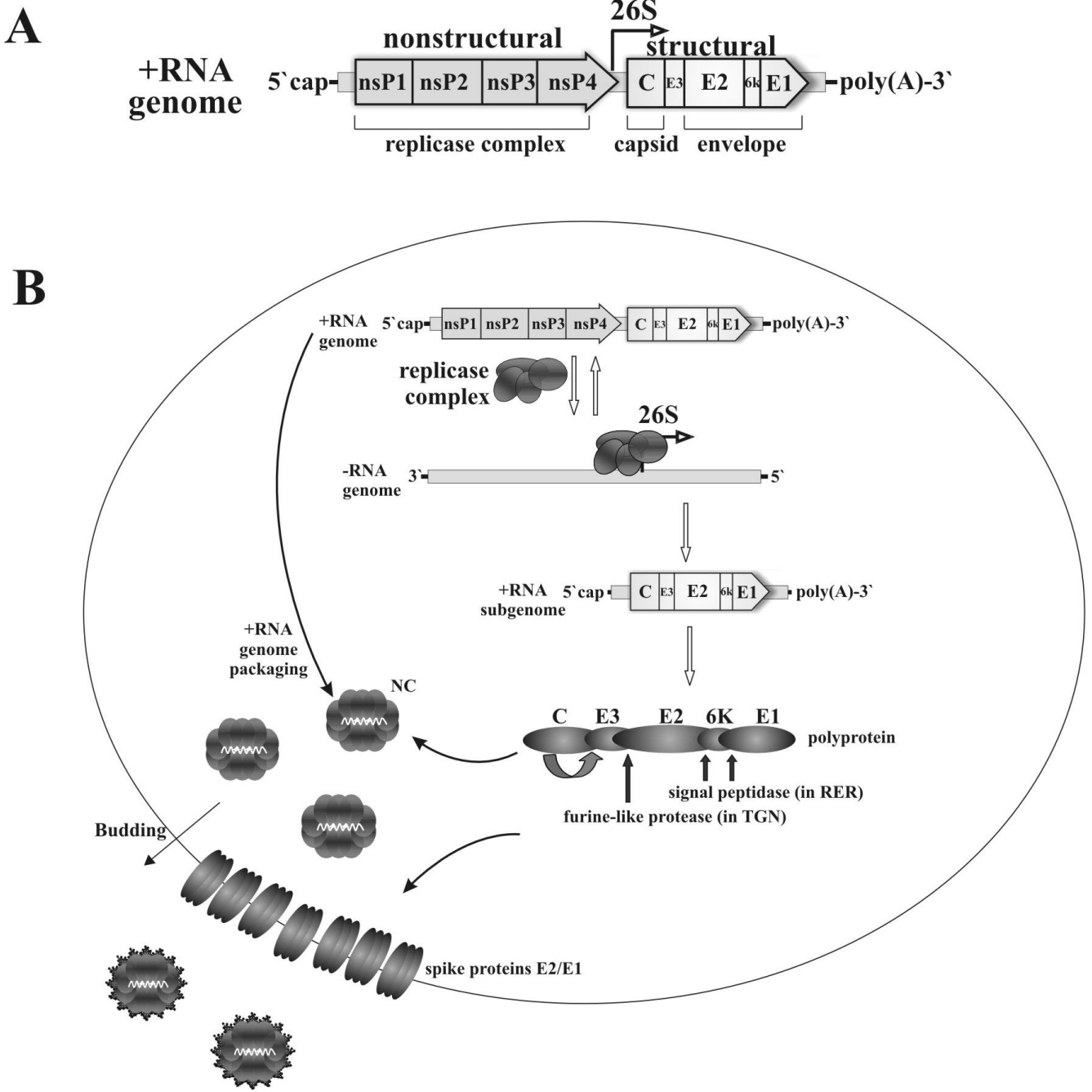
Céline Rhême[1], Markus U. Ehrengruber[2] and Denis Grandgirard[3]

2004

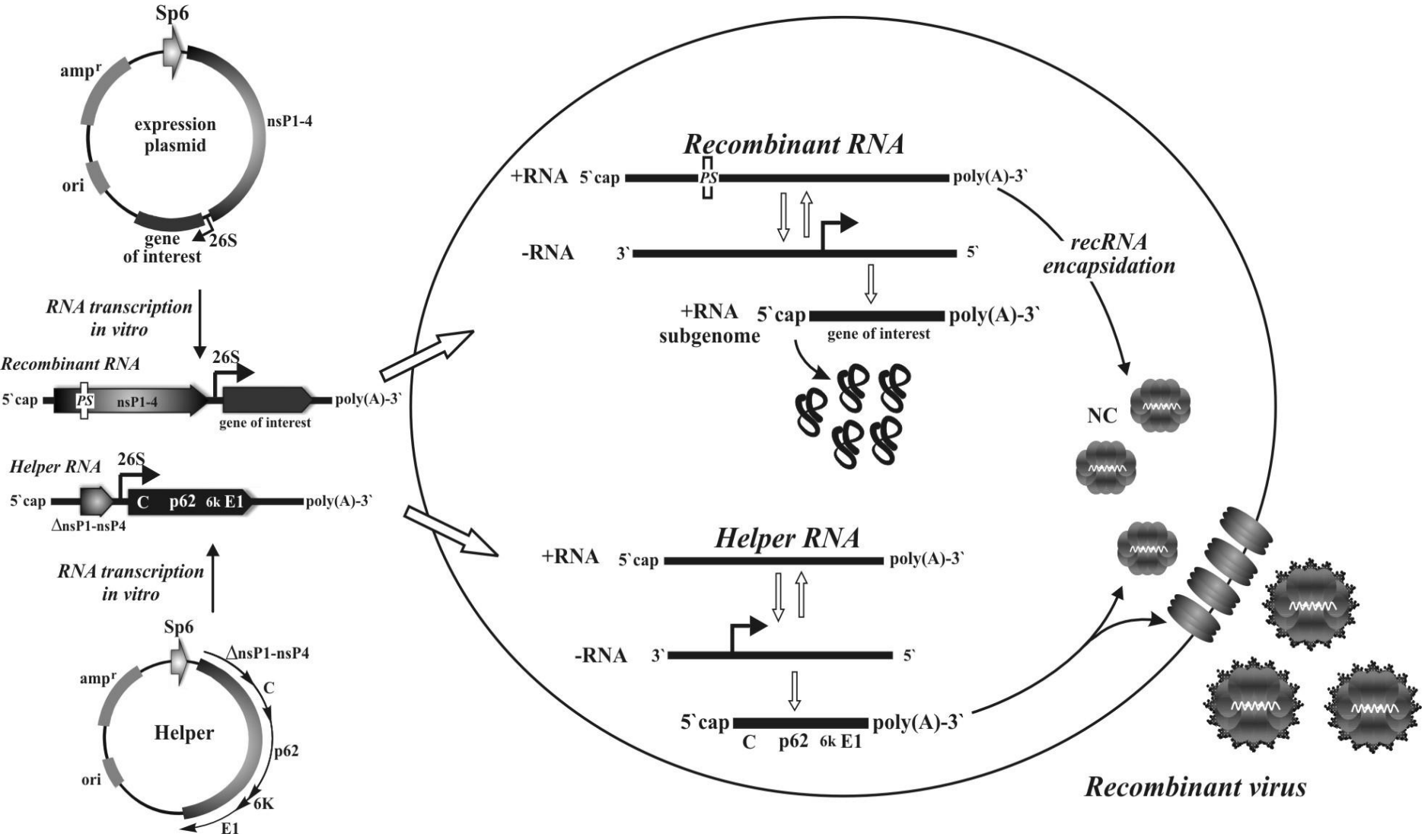




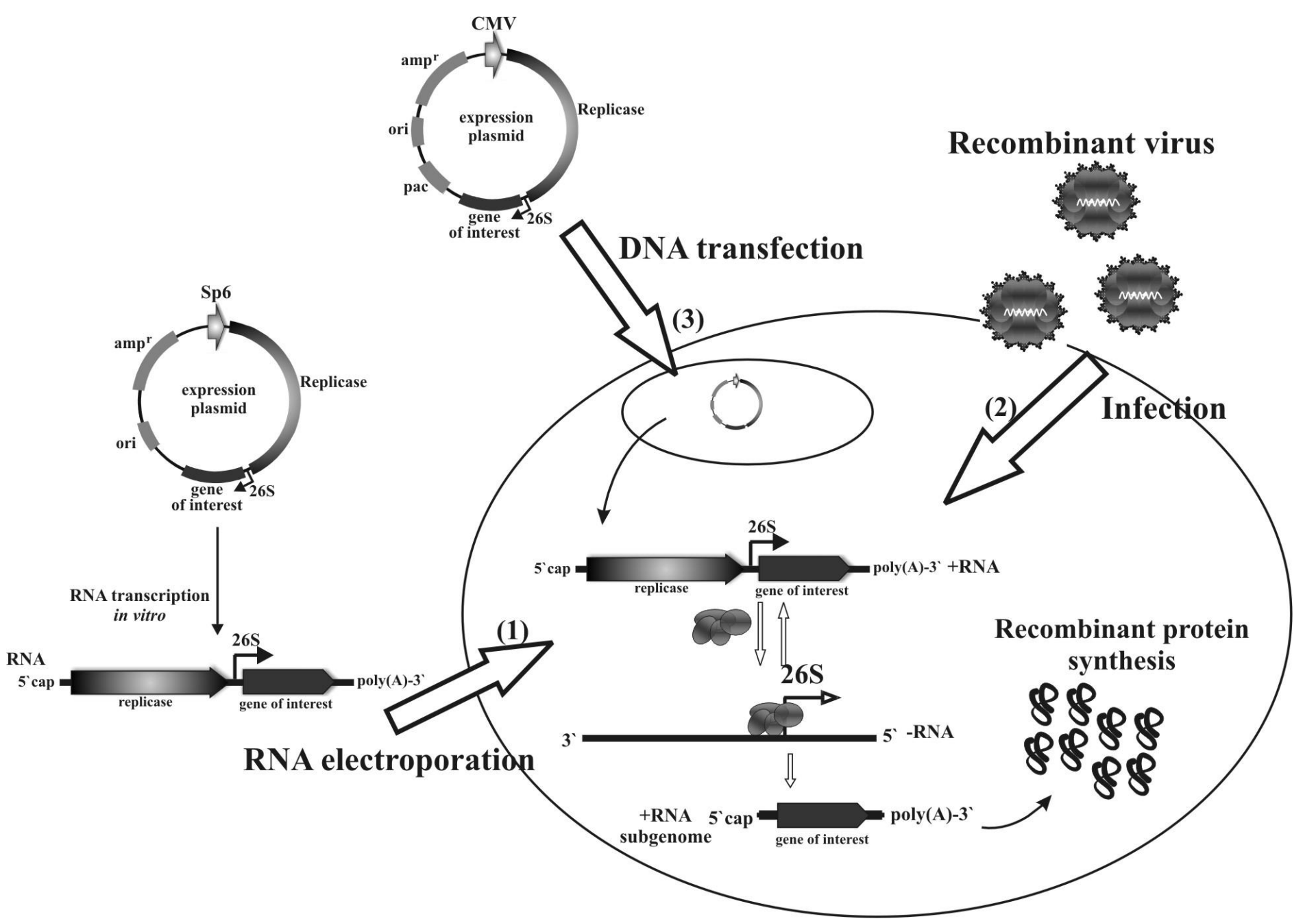
Venticinque and Meruelo *Molecular Cancer* 2010 9:37



(A) Genome structure and (B) replication cycle of alphaviruses (Zajakina *et* al, 2009)



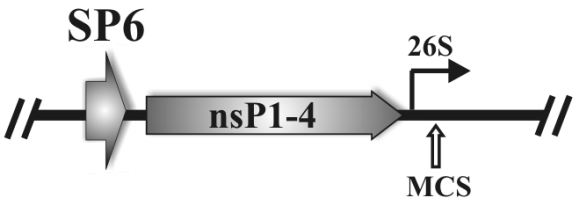
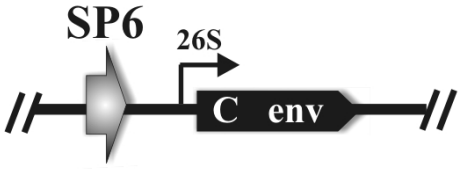
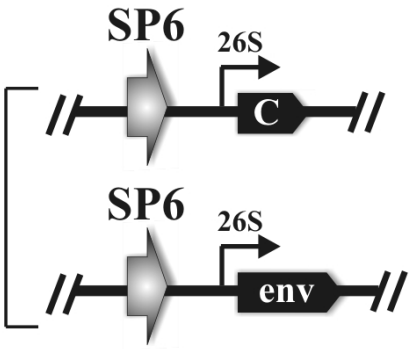
Recombinant alphavirus production.



Three ways of the expression of gene of interest by alphaviruses

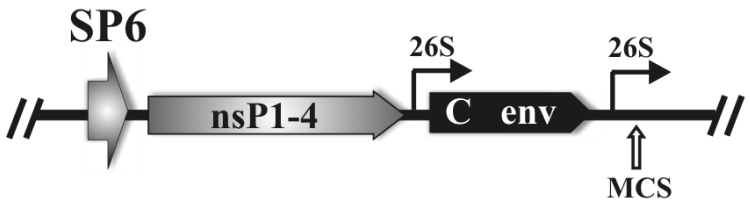
RNA vectors:

Examples:

	SFV	SIN
Replication-deficient		
	***Cytopathic***	
	pSFV-1 (Liljestrom and Garoff, 1991)[31] **pSFV-3** (Liljestrom and Garoff, 1991)[31]	**pSinRep5** (Xiong et al., 1989)[32]
	Non-cytopathic	
	SFV(PD) (Lundstrom et al., 2003)[51] **SFV(PD713P)** (Lundstrom et al., 2003)[51]	**pSINrep19** (Agapov et al., 1998)[50]
Helper		
	**Helper-1** (Liljestrom and Garoff, 1991)[31] **Helper-2** (Berglund et al., 1993)[41]	**DH-EB(5'SIN)** **DH-EB** **DH-BB(5'SIN)** **DH-BB** **DH(26S)** (Bredenbeek et al., 1993)[39]
Split-Helper		
	**SFV-helper-C** **SFV-helper-S** (Smerdou and Liljestrom, 1999)[45]	**DH-BB-Csin** **DH-BB-Crrv** (Frolov et al., 1997)[47]

Schematic diagram of recombinant constructs developed on the basis of alphaviruses.

Replication-competent

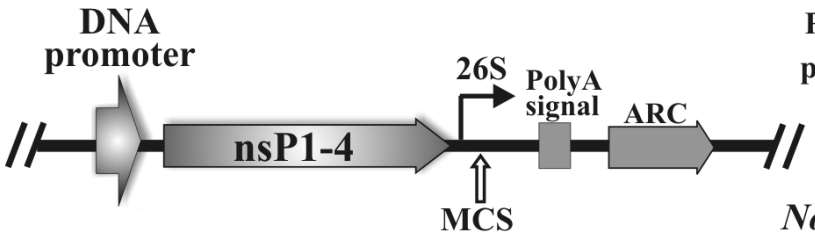


VA7-EGFP
(Vaha-Koskela et al., 2003)[37]

dsSIN (Hahn et al., 1992)[36]

DNA vectors:

Suicidal



PBK-SFV (Berglund et al., 1998)[57]

pSCAβ (DiCiommo and Bremner, 1998)[38]

pDCMVSIN, pDLTRSIN
(Dubensky, Jr. et al., 1996)[56]

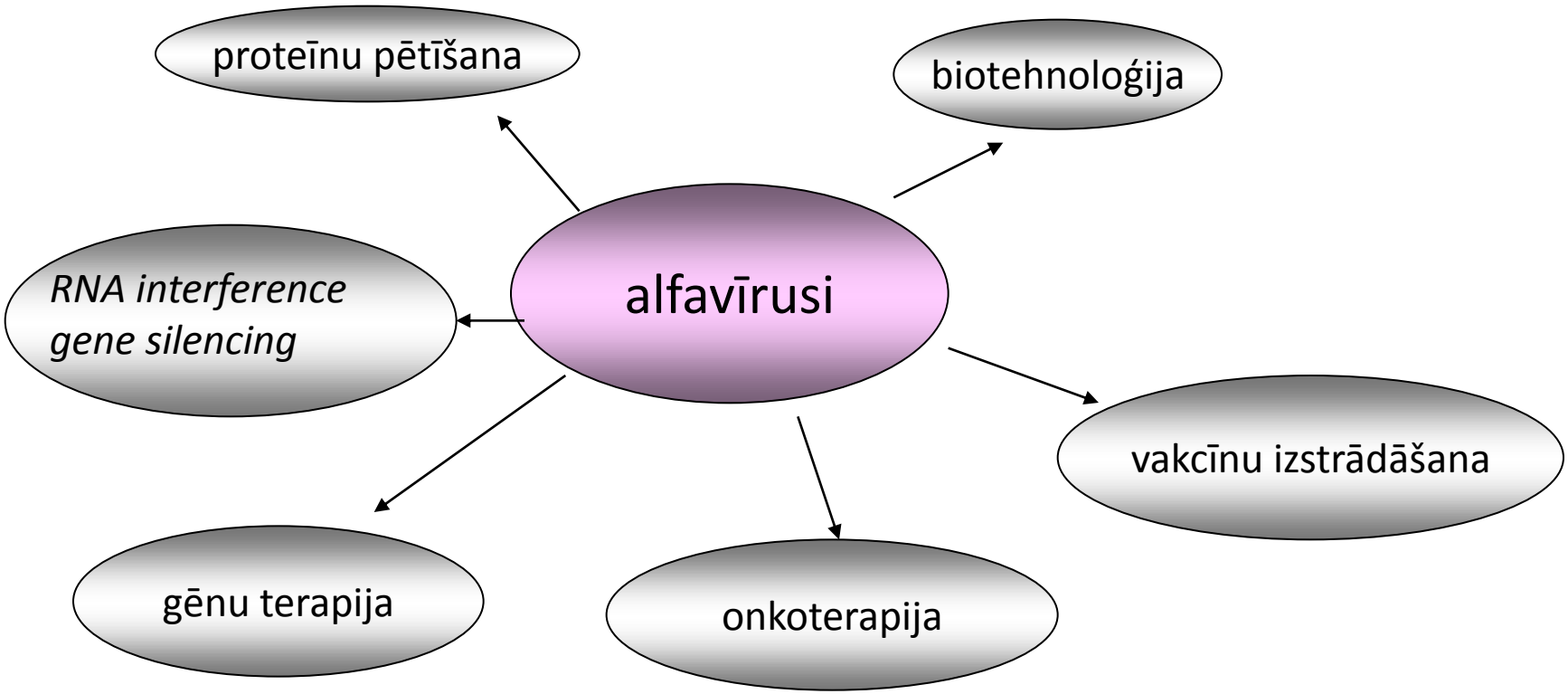
pSin-SV40-HDV-SV40pA
(Yamanaka and Xanthopoulos, 2004)[59]

Non-cytopathic

pSINrep21 (Agapov et al., 1998)[50]

pCytTS (Boorsma et al., 2000)[54]

Schematic diagram of recombinant constructs developed on the basis of alphaviruses.



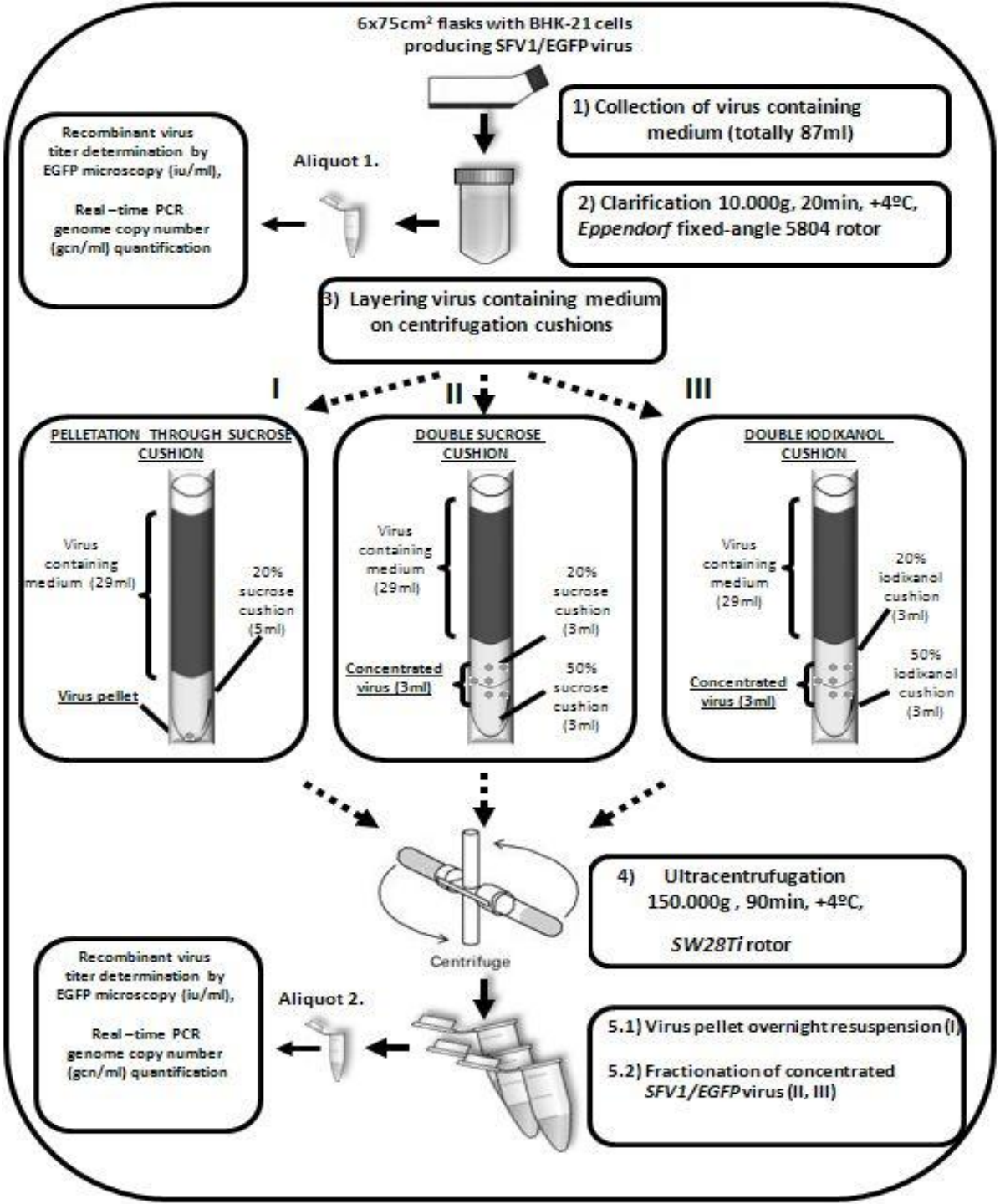
- ✓ Semliki Forest virus (SFV)
- ✓ Sindbis virus (SIN)
- ✓ Venezuelan Equine Encephalitis virus (VEE)

Alfavīrusu vektoru **priekšrocības** gēnu terapijas mērķiem

- ✓augsts transgēna produkcijas līmenis
- ✓plašs šūnu tropisms
- ✓pietiekošā vektora kapacitāte
- ✓biodrošs cilvēkiem
- ✓citopatiskais efekts
- ✓nav vektora pre-imunitātes
- ✓ir iespējams iegūt augstu vīrusa titru

Comparison of recombinant hepatitis B virus core (HBc) gene expression by three vectors: pSFVC, pSFV1, pCytTS. (Zajakina *et* al, 2009)

Kinetic of GFP protein expression in BHK cells transfected with pCytTS/EGFP. Induction time is indicated (3, 6, and 9 days), unpublished.



Comparison of ultracentrifugation methods for concentration of recombinant alphaviruses: iodixanol and sucrose cushions

Table 1. Application of alphavirus vectors for generation of tumor vaccines.

Target	Gene	Vector/Delivery	Response	Ref
Brain tumor	IL-12	SFV/particles	Immunogenicity	[19]
Cervical cancer	HPV E6-E7	SFV/particles	Tumor protection	[20]
Glioma	B16, 203	SFV/particles	Tumor protection	[28]
Tumor	β-gal	SFV/RNA	Tumor protection	[26]
Tumor	HPV E7	VEE/particles	Tumor protection	[27]
Tumor	HPVE7-VP22	SIN/particles	$CD8^+$ T-cell response	[21]
Tumor	P815A	SFV/particles	Tumor protection	[22]
Tumor antigen	MHC Class II	SFV/particles, DNA	Immunogenicity	[23]
Tumor antigen	P185	SFV/particles	CTL, tumor protection	[25]
Tumor antigen	Tyr-related prot-1	SIN/DNA	Antitumor activity	[24]
Melanoma	MUC18	SIN/DNA	Tumor protection	[29]
Tumor	Neu	VEE/particles	Tumor protection	[30]
Prostate cancer	PSMA	VEE/particles	Immunogenicity	[31]

β-gal, β-galactosidase; CTL, Cytotoxic T-lymphocyte activity; HPV, human papilloma virus; IL, interleukin; MHC, major histocompatibility complex; MCAM, melanoma cell adhesion molecule; PSMA, prostate-specific membrane antigen; SFV, Semliki Forest virus; SIN, Sindbis virus; VEE, Venezuelan equine encephalitis virus.

Lundstrom, 2009

Alphavirus vectors

induce protective and therapeutic immune responses
against many tumor associated antigens

A phase I/II clinical trial for a CEA
(Carcinoembryonic Antigen) tumor vaccine

Colorectal cancer
Colorectal liver metastases
Breast cancer
Lung cancer
Skeletal metastases
Nonmalignant liver disease
Pancreatic disease
Smoking
Ageing
Atherosclerosis

antitumoral efficacy by expressing
antitumoral molecules in tumor cells:
cytokines, antiangiogenic factors
or toxic proteins

LipoVIL12 are in Phase I/II Clinical trials
in Europe against seven major
human malignancies
(lung, breast, prostate, pancreatic, head & neck,
melanoma and kidney carcinomas).

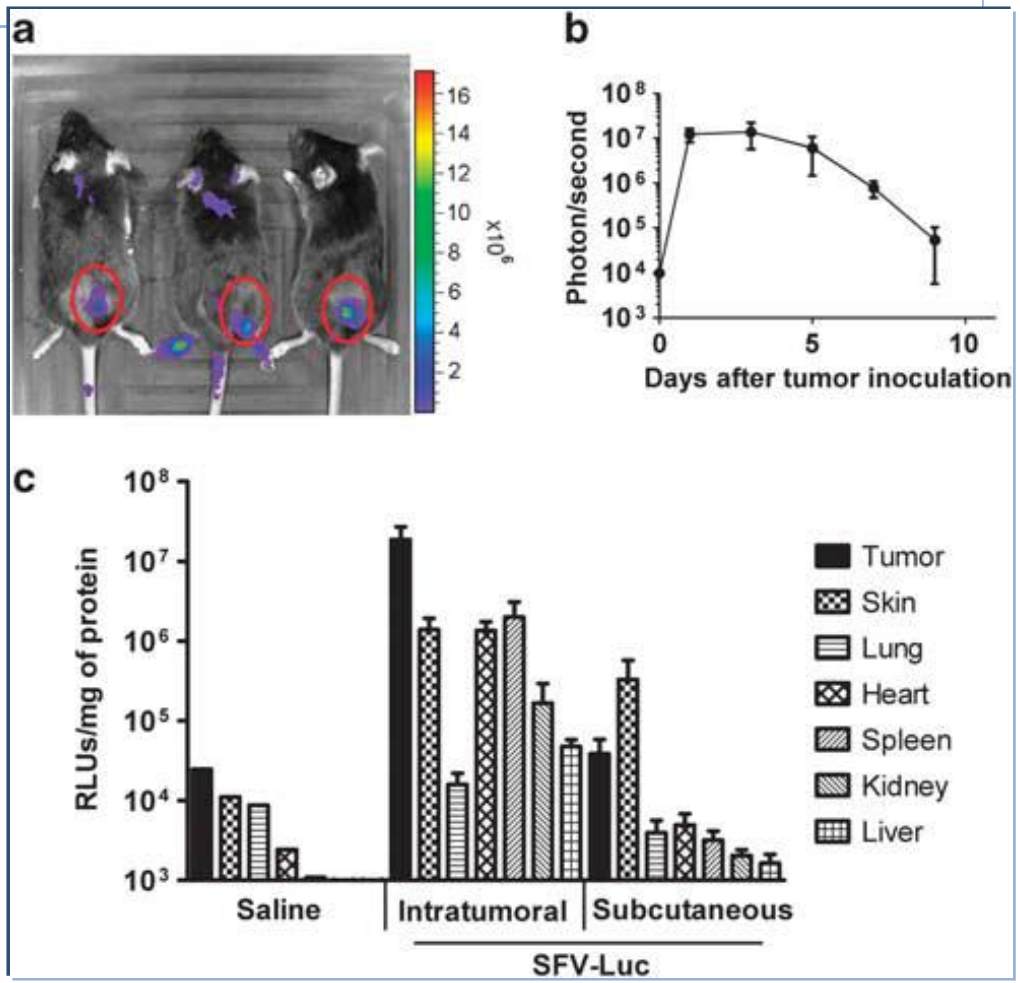
Gene Therapy (2012) 19, 271–278

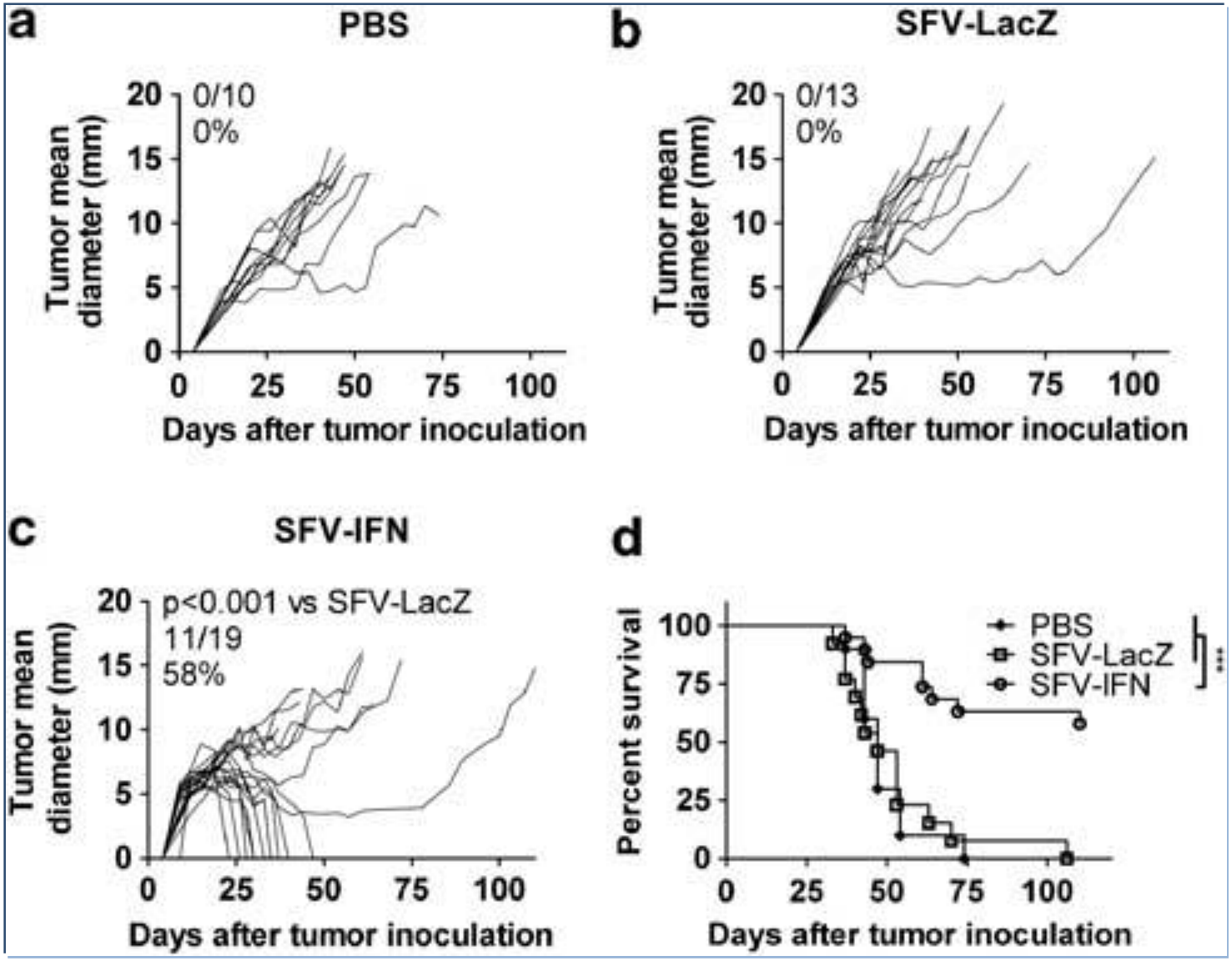
www.nature.com/gt

ORIGINAL ARTICLE

A Semliki Forest virus vector engineered to express IFNα induces efficient elimination of established tumors

JI Quetglas[1,3], J Fioravanti[1,3], N Ardaiz[1], J Medina-Echeverz[1], I Baraibar[1], J Prieto[1,2], C Smerdou[1,4] and P Berraondo[1,4]

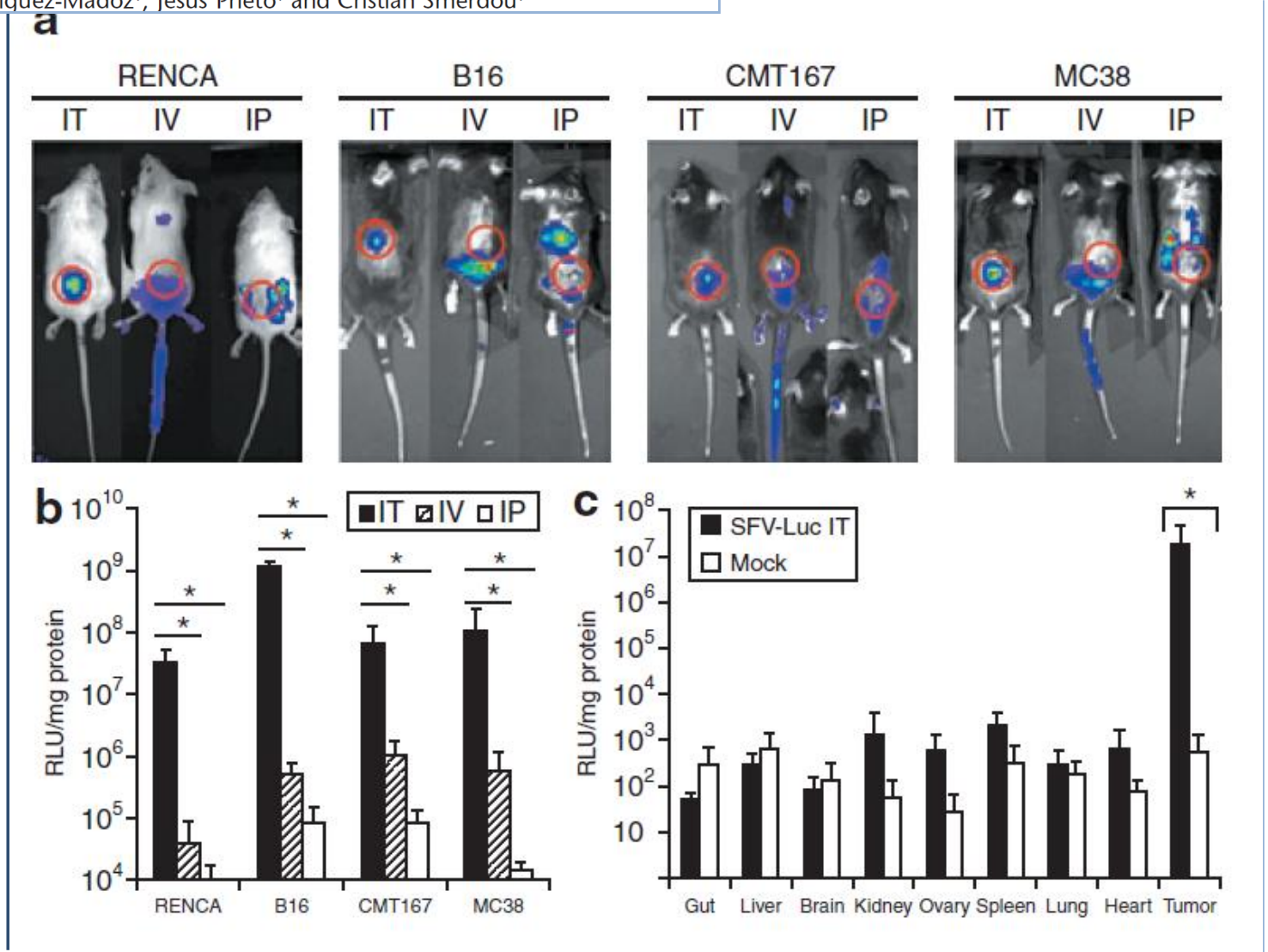



a
PBS
0/10
0%
Tumor mean diameter (mm)
Days after tumor inoculation
b
SFV-LacZ
0/13
0%
Tumor mean diameter (mm)
Days after tumor inoculation
c
SFV-IFN
p<0.001 vs SFV-LacZ
11/19
58%
Tumor mean diameter (mm)
Days after tumor inoculation
d
Percent survival
PBS
SFV-LacZ
SFV-IFN
***
Days after tumor inoculation

Biodistribution and Tumor Infectivity of Semliki Forest Virus Vectors in Mice: Effects of Re-administration

Mol Ther. 2007 Dec;15(12):2164-71. 2007

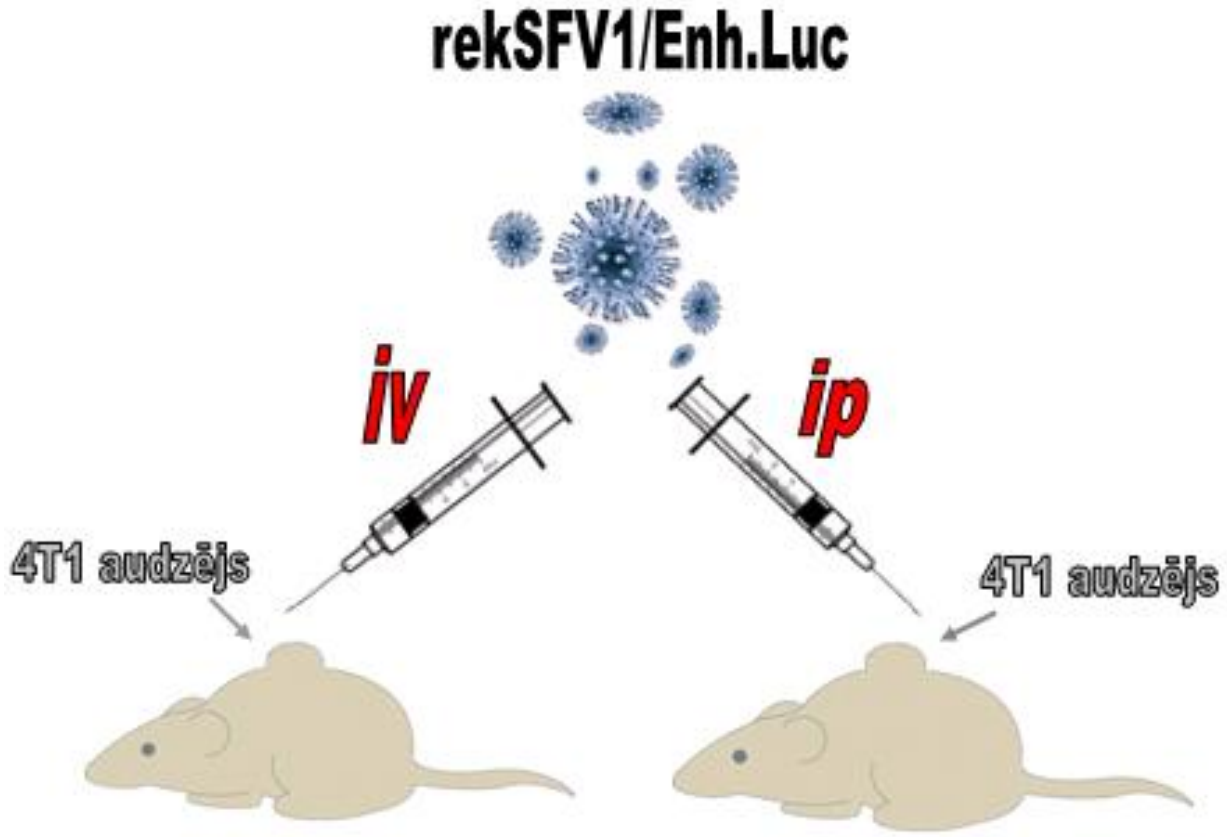
Juan R Rodriguez-Madoz[1], Jesus Prieto[1] and Cristian Smerdou[1]



Alfavīrusa rekSFV1/Enh.Luc ekspresija peļu krūts vēža modelī

vīrusa daudzums:

- Nekoncentrēts vīruss: $3{,}3\times10^8$ v.d
- Koncentrēts vīruss: $1{,}6\times10^9$ v.d.



Gēnu terapijas problēmas:

1.gēnu piegāde (*biodistribution*)
2.biodrošība
3.efektivitāte

Alfavīrusu vektori

gēnu piegāde
- nespecifiskā piegāde
- pret vektora imunitāte atkārtotas infekcijas gadījumā
+ inficē dendrītu šūnas
+ nav preimunitātes

biodrošība
- relatīvi bīstami
+ neintegrējās genomā
+ replikācijas gadījumā neizraisa smagu saslimšanu

efektivitāte
- ierobežots ekspresijas laiks
+ augstais produkcijas līmenis
+ inducē apoptozi
+ ātra terapeitiskā gēna ekspresija

In vivo pētījuma uzdevums:

Pārbaudīt un optimizēt SFV vadītas Luciferāzes gēna ekspresiju *in vivo*

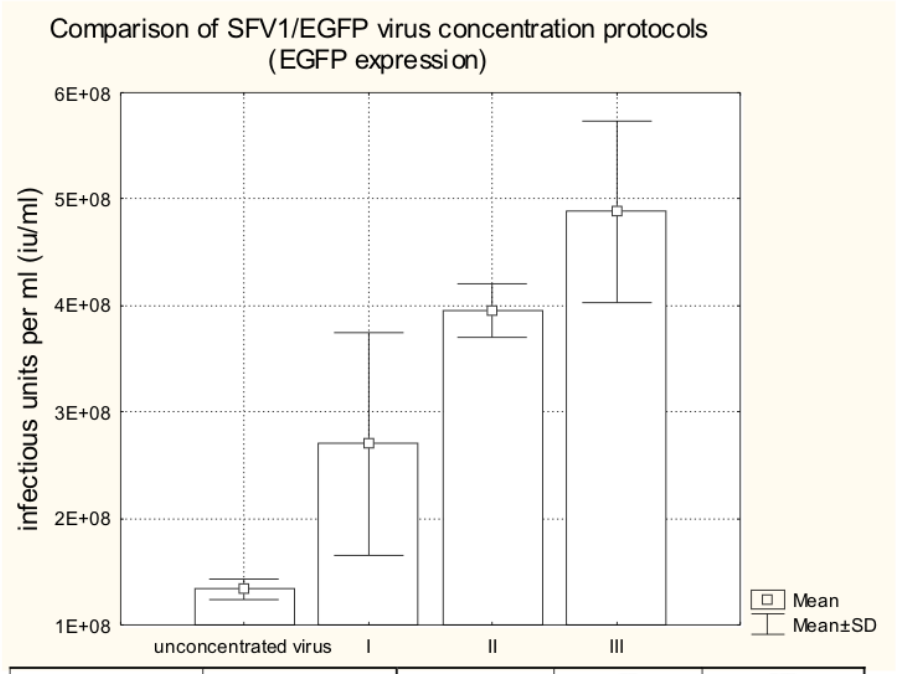
Stratēģija

1.Izpētīt transgēna biosadale *in vivo* izmantojot:
✓Vīrusu daļiņas
✓Brīvas RNS ievadīšanu
✓RNS/liposomu kompleksu ievadīšanu

2. Modelēt transgēna ekspresiju *in vivo* izmantojot dažādas transgēna ievadīšanas kombinācijas:
✓Infekcija+brīvā RNS
✓Infekcija + RNS/liposomu komplekss + brīvā RNS....

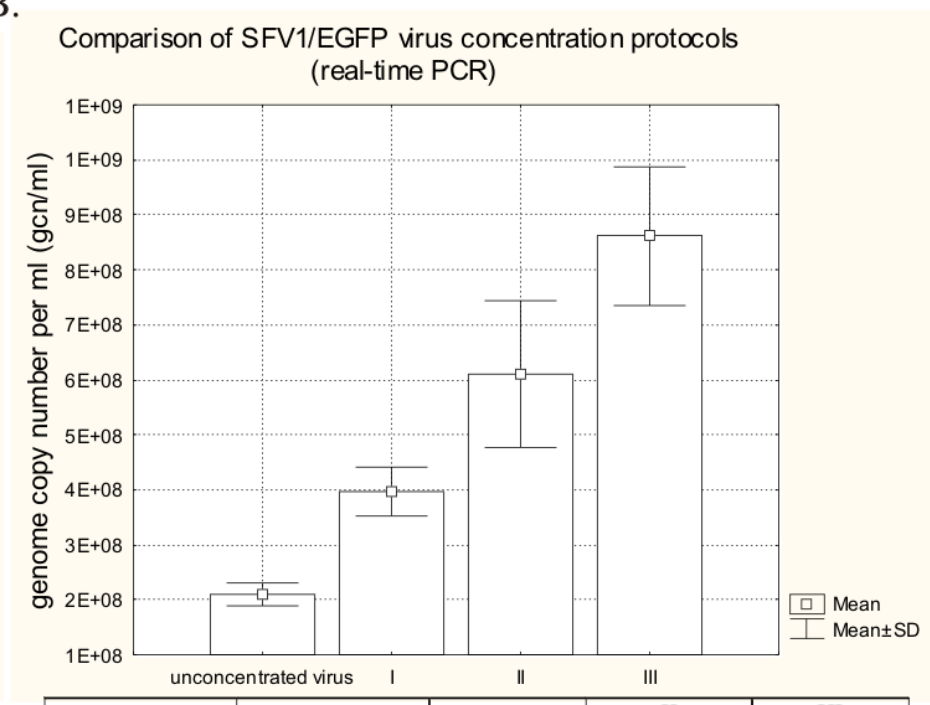
3. Alfavīrusu gēnu piegādes un onkolitiskās īpašības raksturošana peļu audzēju modeļos

A.



	unconcentrated virus	I (pelletation)	II (double sucrose cushion)	III (double iodixanol cushion)
concentration factor (times)	× 1	× 2.02	× 2.95	× 3.64
SD of *iu*/ml values	+/- 6.57 %	+/-34.46 %	+/-5.86 %	+/-15.59 %
recovery yield	-	6.9 %	30.5 %	37.7 %

B.



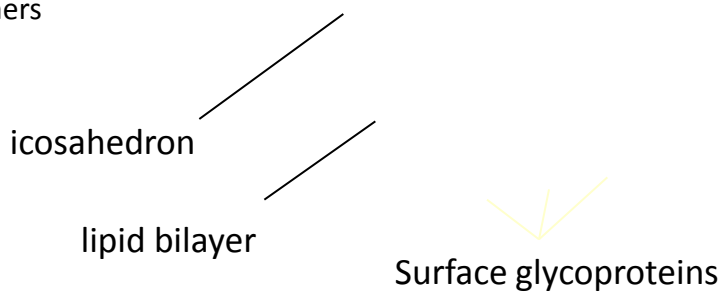
	unconcentrated virus	I (pelletation)	II (double sucrose cushion)	III (double iodixanol cushion)
concentration factor (times)	× 1	× 1.88	× 2.91	× 4.10
SD of *gcn*/ml values	+/- 8.43 %	+/-9.19 %	+/-17.83 %	+/-12.78 %
recovery yield	-	6.5 %	30.1 %	42.5 %

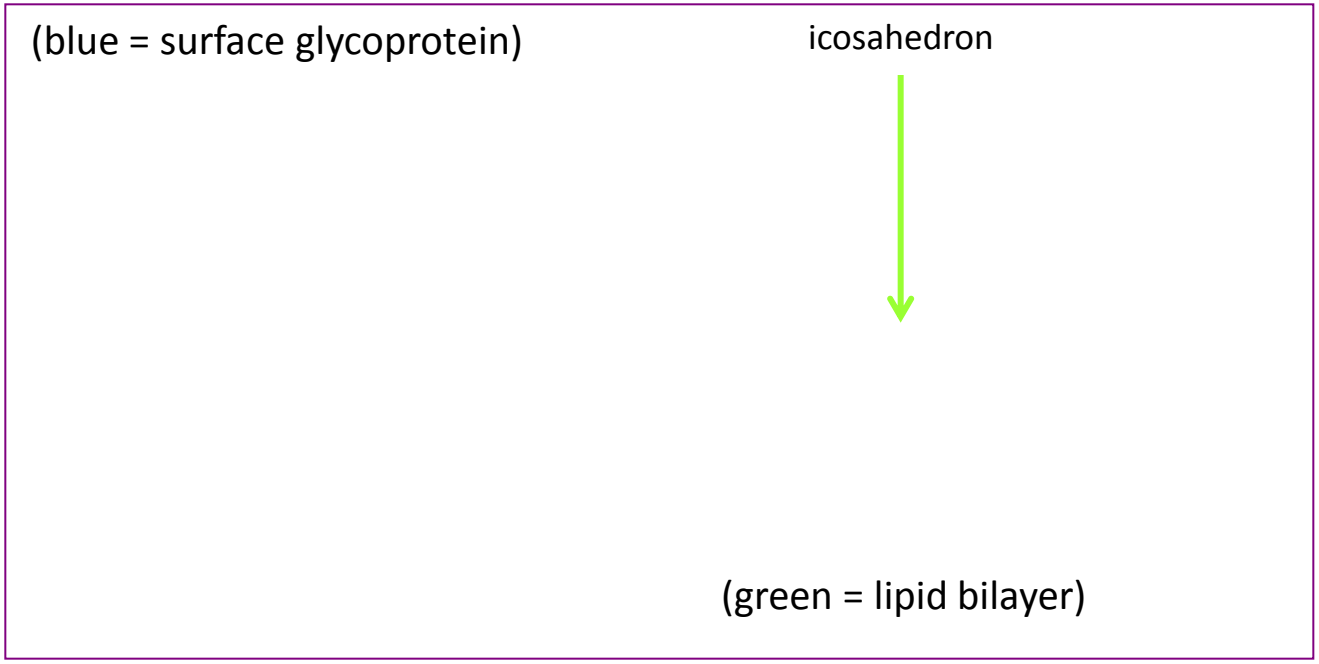
Comparison of ultracentrifugation methods for concentration of recombinant alphaviruses: iodixanol and sucrose cushions
(Hutornojs *et* al, 2011, submitted at *Biol. Pocedures online*)

Alphaviruses

Enveloped virus
Icosahedron : 240 copies of 1 protein
Spherical : 65-70nm
Envelope : 80 trimer spikes

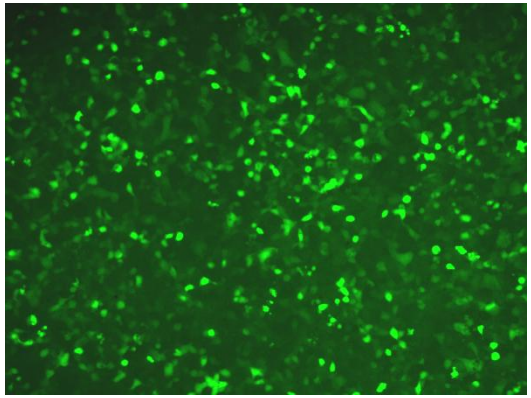
each spike = 3 x E1/E2 heterodimers



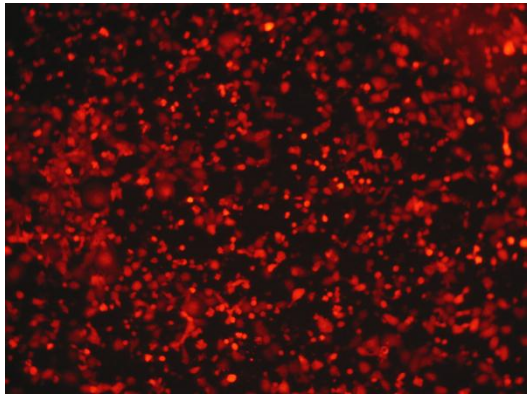


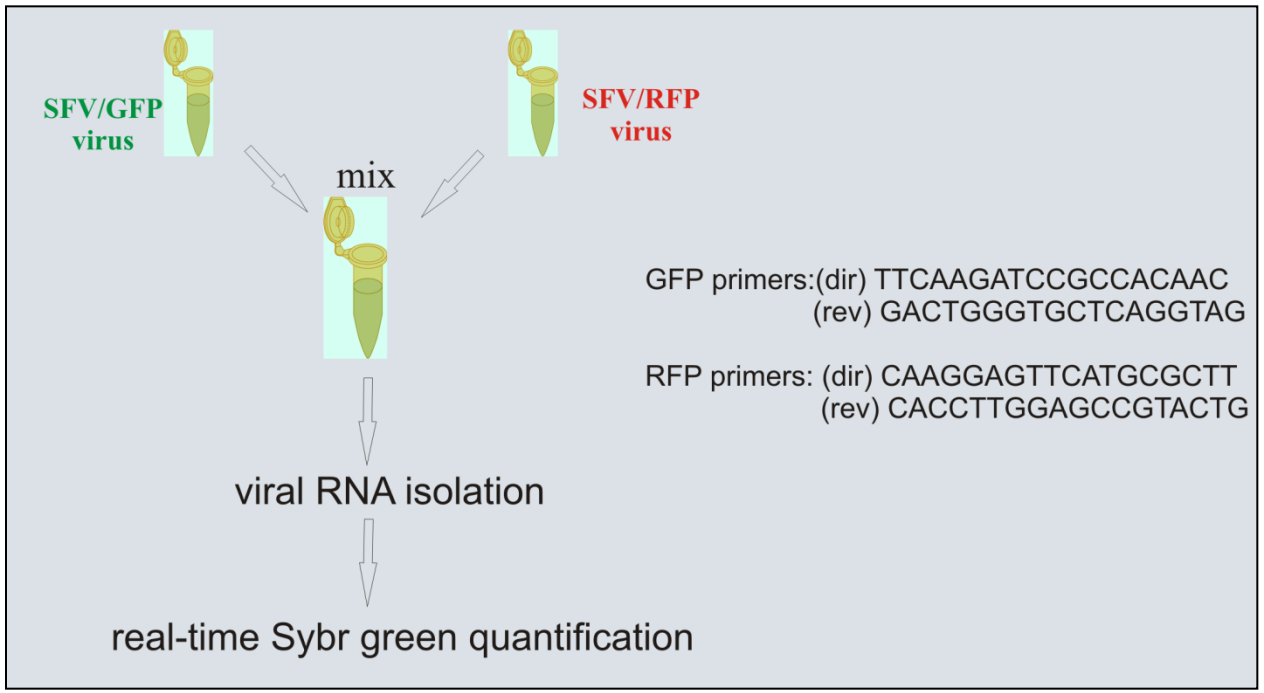
Mancini *et* al, 2000

SFV1/GFP



SFV1/Red





Reference virus as an internal standard for Semliki Forest virus real-time PCR quantification (Zajakina *et* al, 2011, *Current Opinion in Biotechnology*, Vol: 22: S113-114)

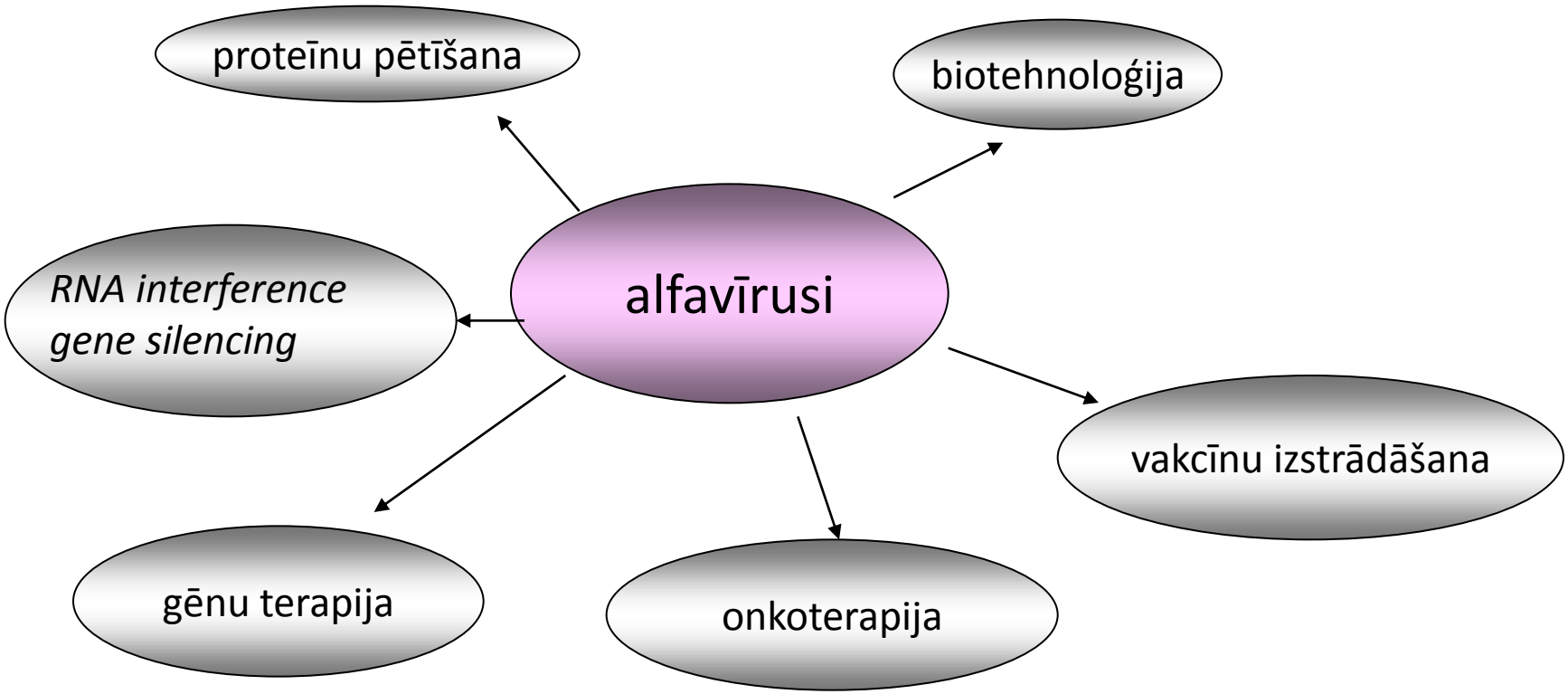
SFV1/GFP

SFV1/Red

$2{,}60 \times 10^7$ i.u./ml

$0{,}56 \times 10^7$ i.u./ml

Reference virus as an internal standard for Semliki Forest virus real-time PCR quantification (Zajakina *et* al, 2011, *Current Opinion in Biotechnology*, Vol: 22: S113-114)



- ✓ Semliki Forest virus (SFV)
- ✓ Sindbis virus (SIN)
- ✓ Venezuelan Equine Encephalitis virus (VEE)

ELISA of HBV antibodies induced in BALB/c mice by immunization with rSFV vectors. (Niedre-Ottomere, *et* al 2011, *J. Viral Hepatitis, in press*)

Markers of HBV infection in PTH cultures after inoculation with virus/antibody mixtures. (Niedre-Ottomere, *et* al 2011, *J. Viral Hepatitis, in press*)