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24

2011

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1990 . (4-
(Onodera et
al., 1998).

(Eliahu et al., 2005; Kichler et
al., 2004).

(HSVtk)/ HSVtk -

1.

()-

()

2.

3.

8

(Salazar-Onfray et al., 2002).

: 1) «
», 10-14 2009 ., ; 2) «
», 29 - 4 2009 ., ; 3)
, 6-8 2009 ., ; 4)
« - 2010», 12-25 2010 ., ; 5) International Symposium
«Control of Gene Expression», 21-25 2010 ., ; 6)
«
», 6-9 2010 ., ; 7)
2010, 15 2010 ., ; 8)
, 1-3 2010 .,
(2010 .).

12 . 2
10
104 , 7
36 ,
169 .

1.

().

in vivo

() (N- -24).

GRKKKRRQRC,

(Rudolph C., 2003).

1 :

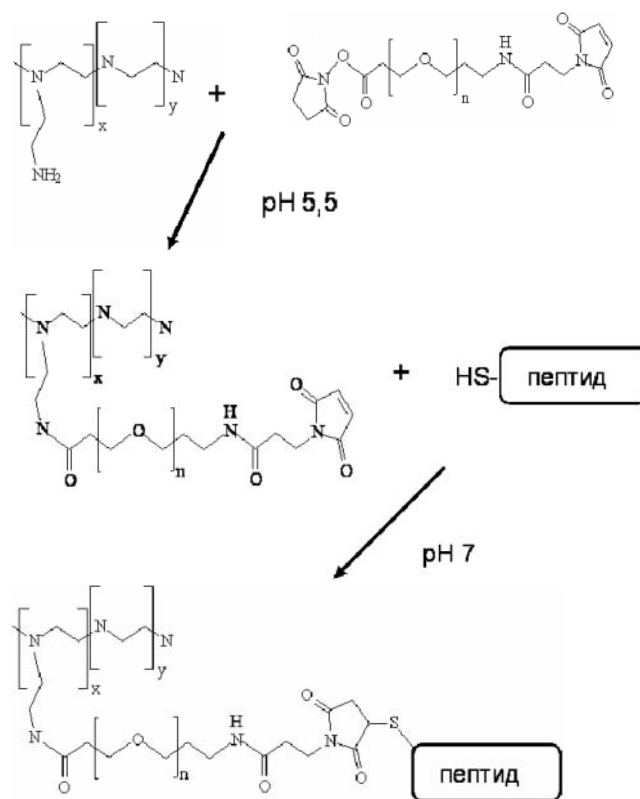
CGYGPKKKRKVSGSGSSIISHFRWGKPV,

(NLS)

SV-40.

(. 1),

. (Kleemann et al., 2005).

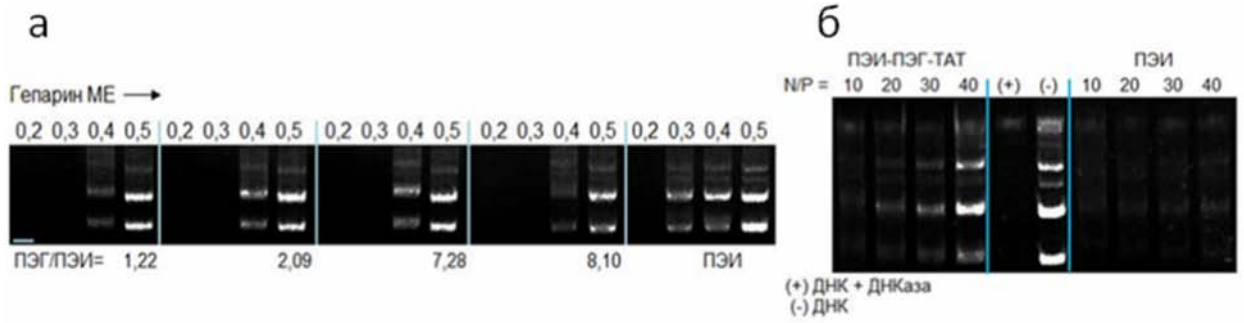


. 1.

2.

(. 2).

/ (N/).



. 2.

. ().

. ().

(,),

(Ho et al., 2006).

(ζ)-

ζ-

ζ-

ζ-

(Fisher et al., 2000, Kircheis et al., 2001).

Zeta PALS

pEGFP-N3 450

26-61
(Kleemann et al., 2005).

: (Grayson et al., 2006)

150

50-75

200

in vivo,

vivo (Smrekar et al., 2003).

in

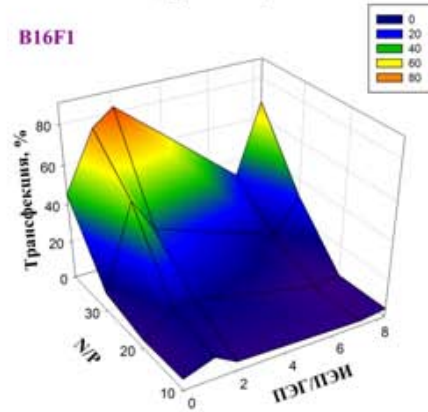
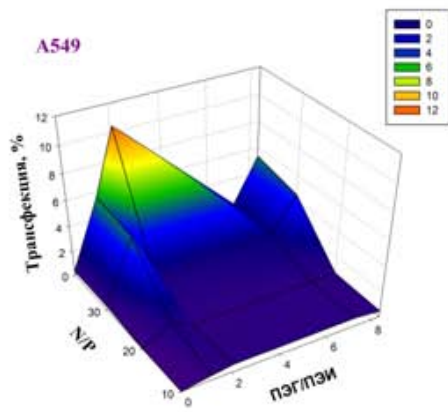
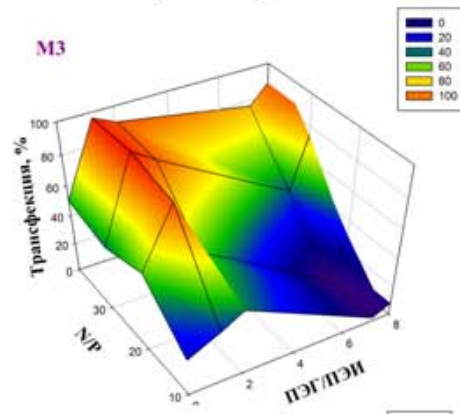
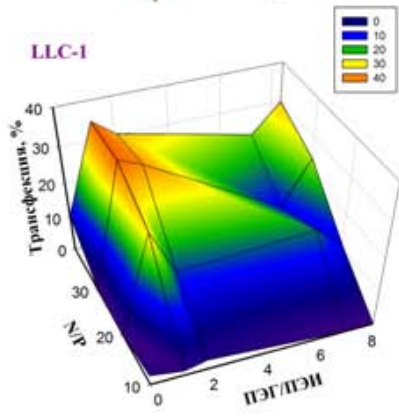
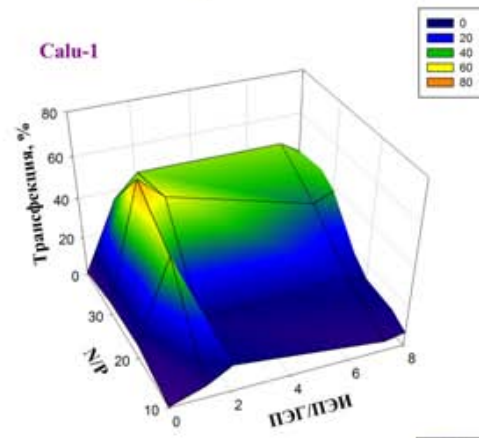
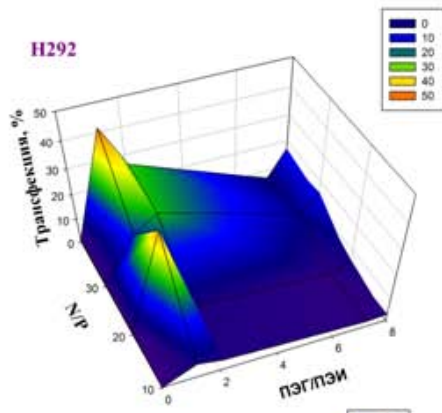
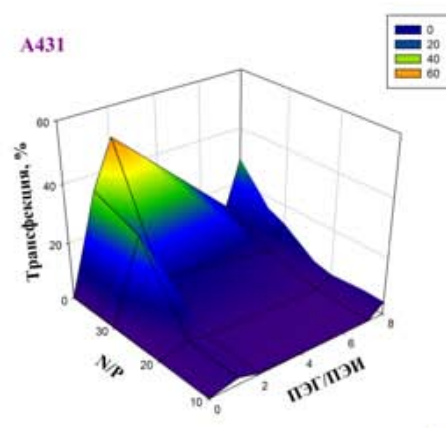
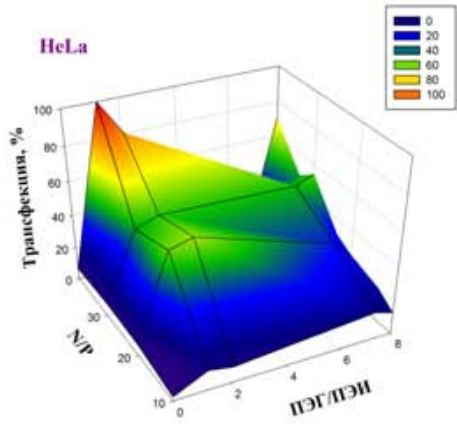
3.

pEGFP-N3,
(EGFP)

EGFP,

) « » (. 3),

/ N/P ()



.3.

/

80-100% - / N/P
(. 1).

(> 50%)

CMV-

CAGGS (Shoji et al., 1997). CAGGS

CMV-

. 1.

1	HeLa	100
2	S91 (M3)	98
3	HEK 293	96
4	B16F1	83
5	Calu-1	63
6	A431	50
7	NCI-H358	46
8	NCI-H292	42
9	LLC-1	35
10	549	17,7
11	BT-474	4,4

4.

FRET (Förster Resonance Energy

Transfer):

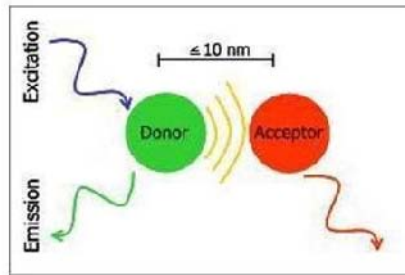
(, Qd605),
(AlexaFluor647) -
FRET Qd

Qd, Alexa,

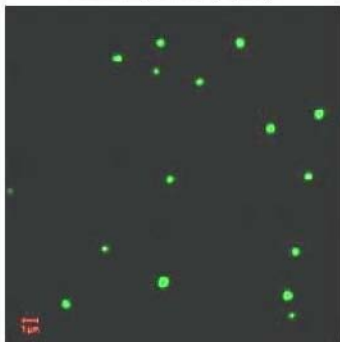
100%

FRET.

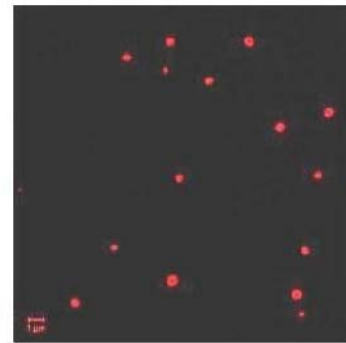
FRET (Förster Resonance Energy Transfer) Резонансный перенос энергии по Фёрстеру



канал Qd605 (ДПК)



канал Alexa-647 (полиплекс)



4.

FRET.

Qd (), Alexa
2
1

3

(): 549 Calu-1,
S91 (M3) (17,7, 63,2 98,0%).
: 1)

Cy3,

; 2) LysoTracker Green DND-26

3) Hoechst

(30 16).

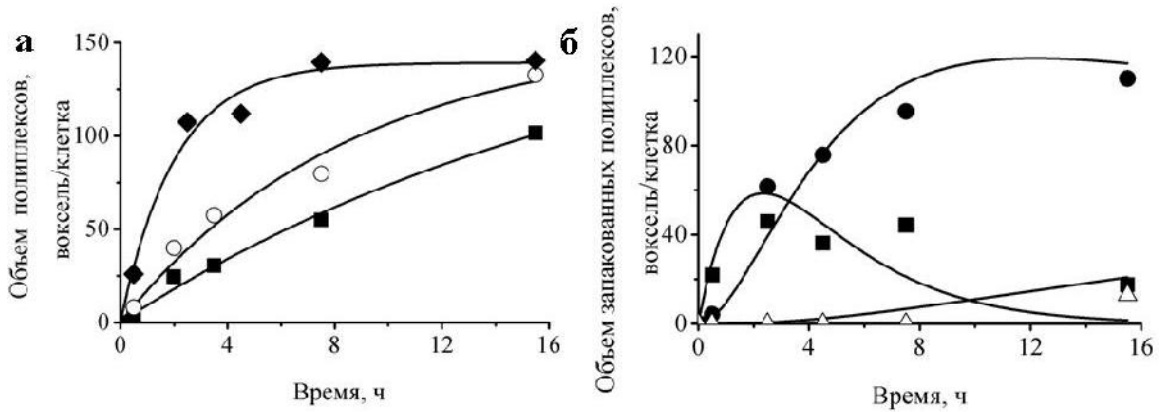
Z- (20), 30

(. 5),

ObjectCounter3D plugin (Institut Curie, Orsay, France)

Colocalization plugin (Institut Jacques Monod, Service Imagerie, Paris, France)

ImageJ (1.43i; <http://rsb.info.nih.gov/ij>).



. 5.

()

S91 (M3); -

549. ()

S91 (M3)

: - Calu-1; -

. - ;

(- . .),

()

549 3

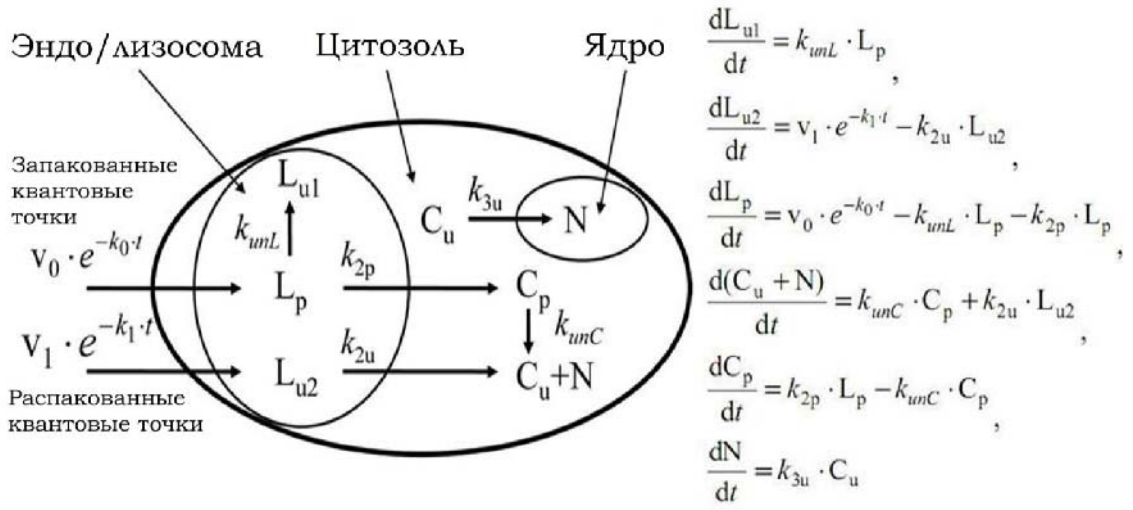
(. 6),

(),

(

0.95).

16



. 6.

FRET: (N), (u) / (L_{u1} L_{u2}), (p)

/ (L_p)

FRET: (p)

: v₀ -

FRET) , k₀ -

, v₁ -

FRET) , k₁ -

, k_{unL} -

/ , k_{un} -

, k_{2p}, k_{2u} -

/ , ; k_{3u} -

(. 2).

$$1/v \quad T_{max}$$

$$k_{unL}$$

2.

	549	Calu-1	M3
(%)	17,7 ± 5,2	63,2 ± 1,8	98,0 ± 0,8
v (/)	9,4 ± 1,0	18 ± 2	68 ± 10
1/v (/)	0,107 ± 0,011	0,055 ± 0,008	0,015 ± 0,002
k _{unL} (⁻¹)	0,49 ± 0,05	0,217 ± 0,007	0,063 ± 0,006

v 1/v - ; k_{unL} -

(/) ;

±

S91 (M3).

Calu-1,
in vitro.

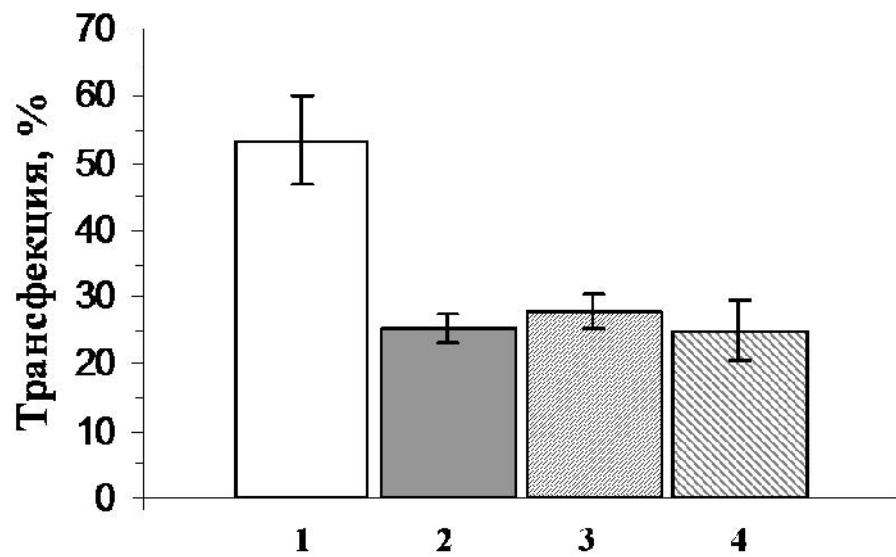
5.

S91 (3).

(Szardenings et al., 2000).

6

(.7).



. 7.

(α -)

α -

- 1 (N/P=30)

S91 (M3). 1-

- 1 ; 2 -

- 1 + α - ; 3-

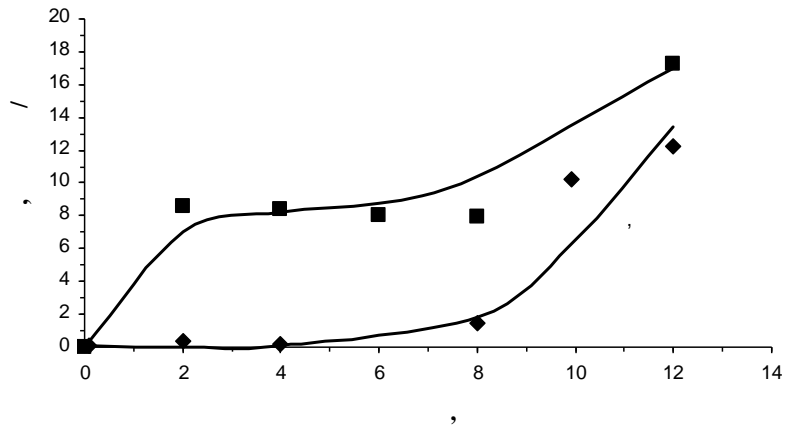
- ; 4-

- + α - . α -

1

5

S91 (M3).



8.

- - - 1 ; - - .

in vivo

8.

in vivo.

in vitro,

in vivo.

1 .

$(66,3 \pm 17,8) \cdot 10^3$. / (N=7, \pm .),

2,6 : $(25,7 \pm 6,6) \cdot 10^3$.

./ (N=5).

in vivo

2 (3) DBA/2

S91 (3),

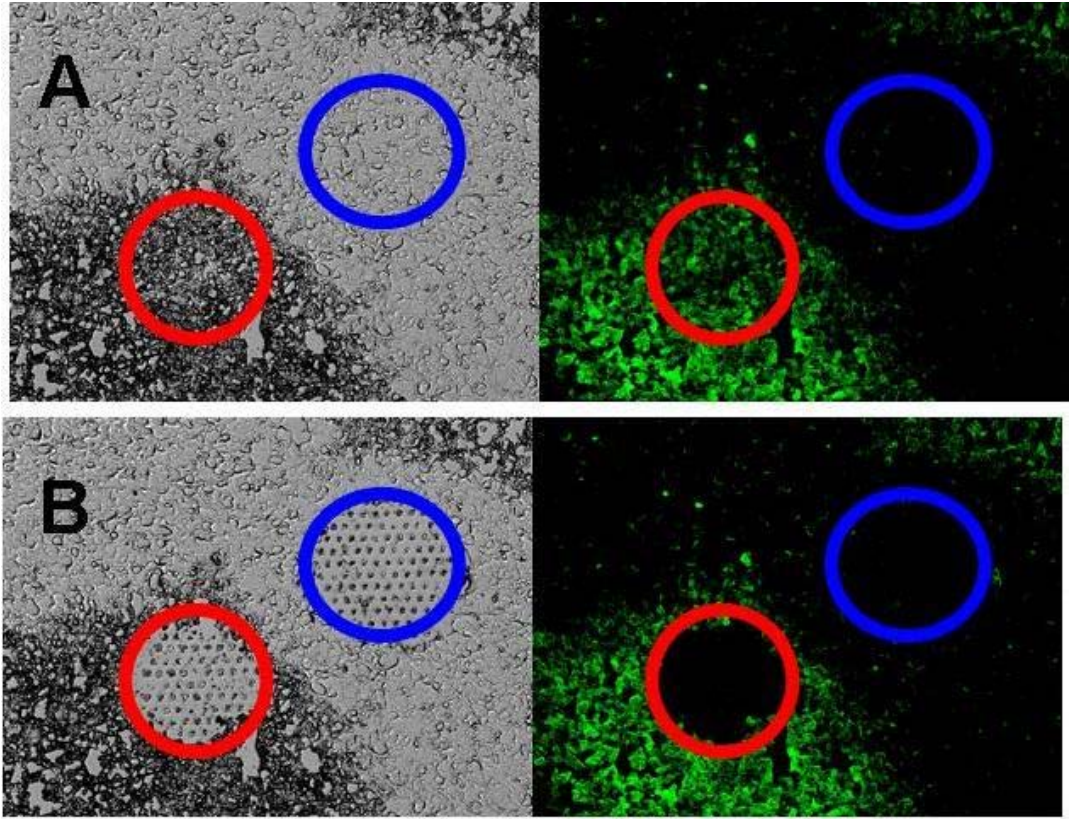
(. 9)

(

)

(-

).



. 9.
3),

S91 (

GFP.

6

PALM laser capture microscope (P.A.L.M. Microlaser Technologie).

- 450-490

- 515-565

(×20;

-2

- (-)

(.3).

3.

in vivo.

				T	T
	1	24,70 ± 0,33	32,82 ± 0,32	-8,12	-8,31
	2	19,34 ± 0,56	29,31 ± 0,53	-9,97	
	3	17,03 ± 0,14	23,88 ± 0,56	-6,85	
	4	20,32 ± 0,06	21,35 ± 0,03	-1,04	-1,66
	5	24,98 ± 0,19	28,17 ± 0,14	-3,19	
	5	20,04 ± 0,29	20,79 ± 0,15	-0,75	

-2
PALM

T

S91 (

3).

(Schmittgen and Livak, 2008),
 « ... »
 -2-
 94% 98%
 (Schmittgen and Livak, 2008).

$2^{-T} = 2^{-(T_1 - T_2)} = 100,7$ ($< 0,05$).
in vivo, 2

in vivo

- - 1

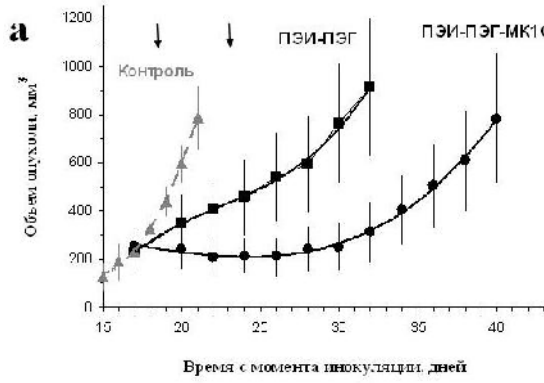
HSVtk (

),

6

3

(. 10).



. 10.

. ().

DBA/2

1
HSVtk

().)

: 8

HSVtk

() ± 1
1, 6

HSVtk

1

1 (),

1 ().

HSVtk

2-

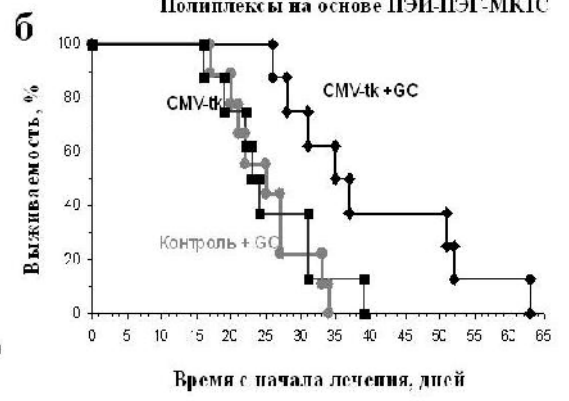
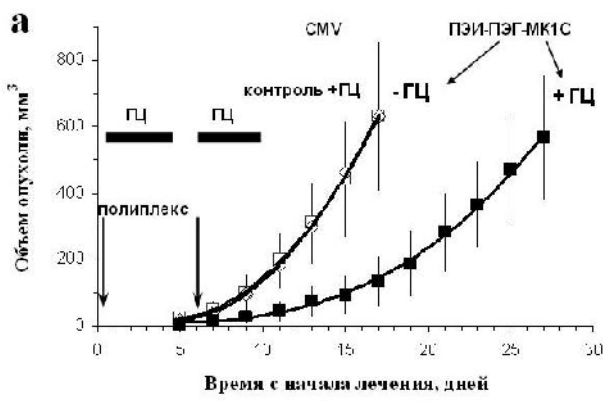
- - 1

3,6

1,6

. 11

- - 1 +



. 11

DBA/2

()

-

-

-

1

HSVtk

3

DBA/2y.

(25 /)

(

±

8

()

HSVtk

(GC),

in vitro, *in vivo*.

