Oncolytic Adenoviruses R&D in Russia

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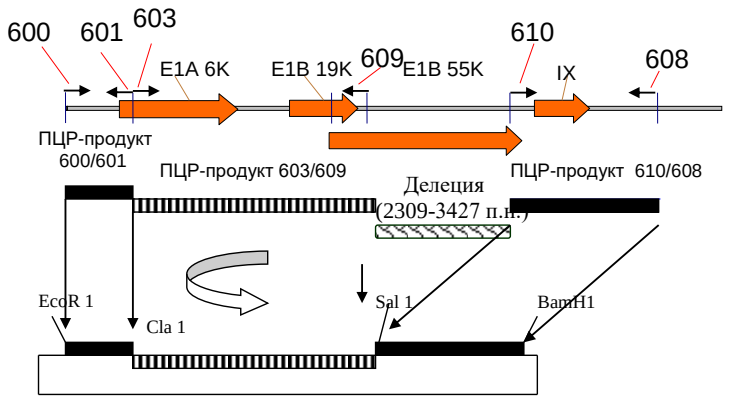
- In the end of 1980's I was the Principal Investigator of pioneer genomes sequencing projects of:

Marburg, Ebola, and Venezuelan Equine Encephalitis (VEEV), Eastern and Western Equine encephalitis viruses, tick-borne encephalitis and influenza viruses.

- I was also Involved in the development of inactivated **hepatitis A vaccine**, recombinant **hepatitis B vaccine** and recombinant **vaccine against VEE** virus based on vaccinia virus vector.

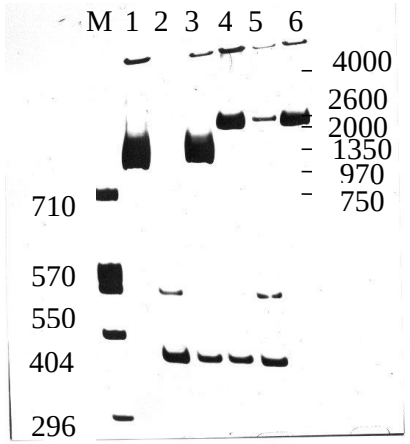
- **Cancerolysin** (analogue of ONYX-015) was developed here, too and was the subject of Phase 1 clinical trials in Russia

The Construction of Adenovirus Serotype 5 with deleted E1B55K gene



pAd5E1Bdel containing the 5’-region of Ad5 (3505 bp)

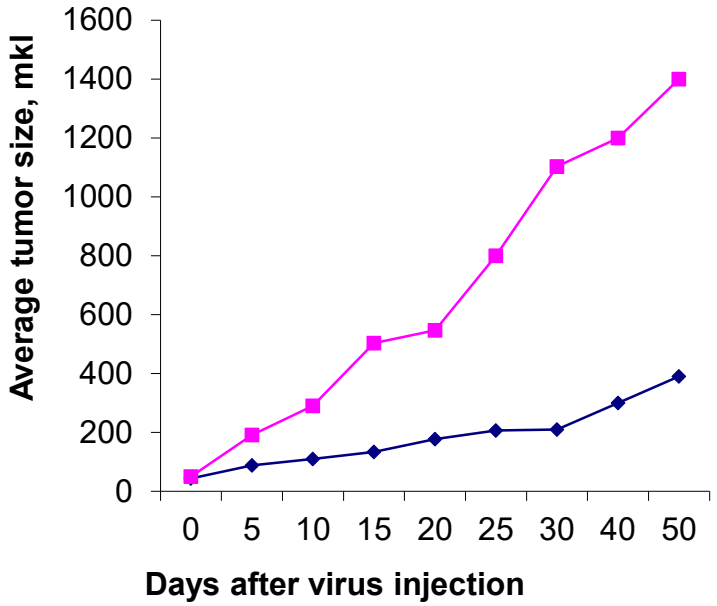
Construction of Adel2-strain from Adenovirus serotype 5 with E1B 55K deletion

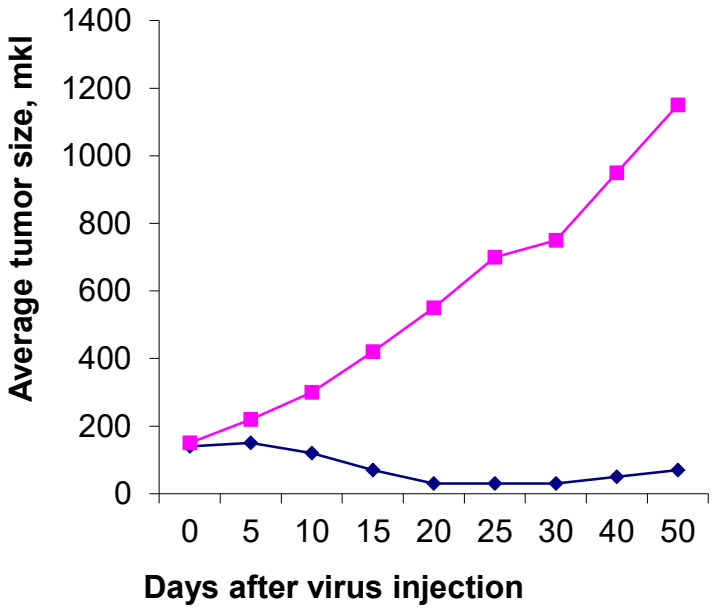


PCR analysis of Adel2 DNA.

1- Plasmid pAd5E1Bdel DNA; 2- ДНК from 293 cells control, 3- Adel2 DNA, 4,5,6- Ad5 wild type; M - marker.

Oncolytic activity of Cancerolysin (Adel2) preparation *in vivo*.





A) B)

A431 – human epidermoid carcinoma cells (A) and SW480 – human colon adenocarcinoma cells (Б) (10^7 cells in 150 μl DMEM) were injected subcutaneously to the lateral thigh of nu/nu mice. When tumors reached the size 40-60 mkl (n=5), the 10^9 PFU_{50} of cancerolysin preparation in 20 mkl of phosphate-saline buffer were injected every day during 5 days ( ). The same quantity of UV-inactivated cancerolysin preparation served as control ( ).

Conclusion - 1

The 1st phase of Cancerolysin clinical trials with its intratumoral administration to eight grade 4 oncological patients diagnosed with cancer in the head and neck region or disseminated skin melanoma had been organized and completed at the Oncological Center named AFTER N.N.Blokhin in Moscow during 2007-2008.

It was shown that in the absence of adenovirus in the blood, the titers of antibodies to it in the blood serum of patients who received two or three cycles of administration of the maximum dose of "Cancerolysin" moderately increased in comparison with the initial level.

In 2 out of 8 patients, the p53 gene mutations in tumor cells of the biopsy material were found. In 3 patients, the p53 gene mutations in DNA isolated from blood cells were found.

During the 1st phase of Cancerolysin clinical trials the drug was well tolerated in all patients and the process stabilization in at least one patient was registered. It has been shown that Cancerolysin does not express significant toxicity and reactogeneicity when used in minimum and maximum doses.

Based on the results of the 1st phase of clinical trials of the Cancerolysin anticancer drug, the candidate drug Cancerolysin was assessed as promising for further development and implementation of the 2nd phase of clinical trials.

Novosibirsk State University

Seroprevalence of Ad5 and Ad6

	Ad5	Ad6
African countries and Thailand	90.0-94.0%	66.7% to 78.8%
USA and Europe	38.0-69.1%	8.5% to 45.7%

Worldwide seroprevalence of alternative serotypes

Ad2 and Ad3	>	Ad6	>	Ad11 and Ad35
(44.7-92.0%) (18-100.0%)		(8.5-78.8%)		(3.0-40.0%) (0-41.0%)

Mennechet F.J.D. и др. A review of 65 years of human adenovirus seroprevalence // Expert Rev. Vaccines. Taylor & Francis, 2019. Т. 18, № 6. С. 597–613

Evaluation of wild type Ad6 oncolytic efficacy

prostate (LNCaP, DU145, PC3)
breast (SKBr-3, BT474, MDA-MB-231, MDA-MB-468)
hepatocellular (HepG2, Hep3B)
ovarian (SKOV-3, OVCAR-3)

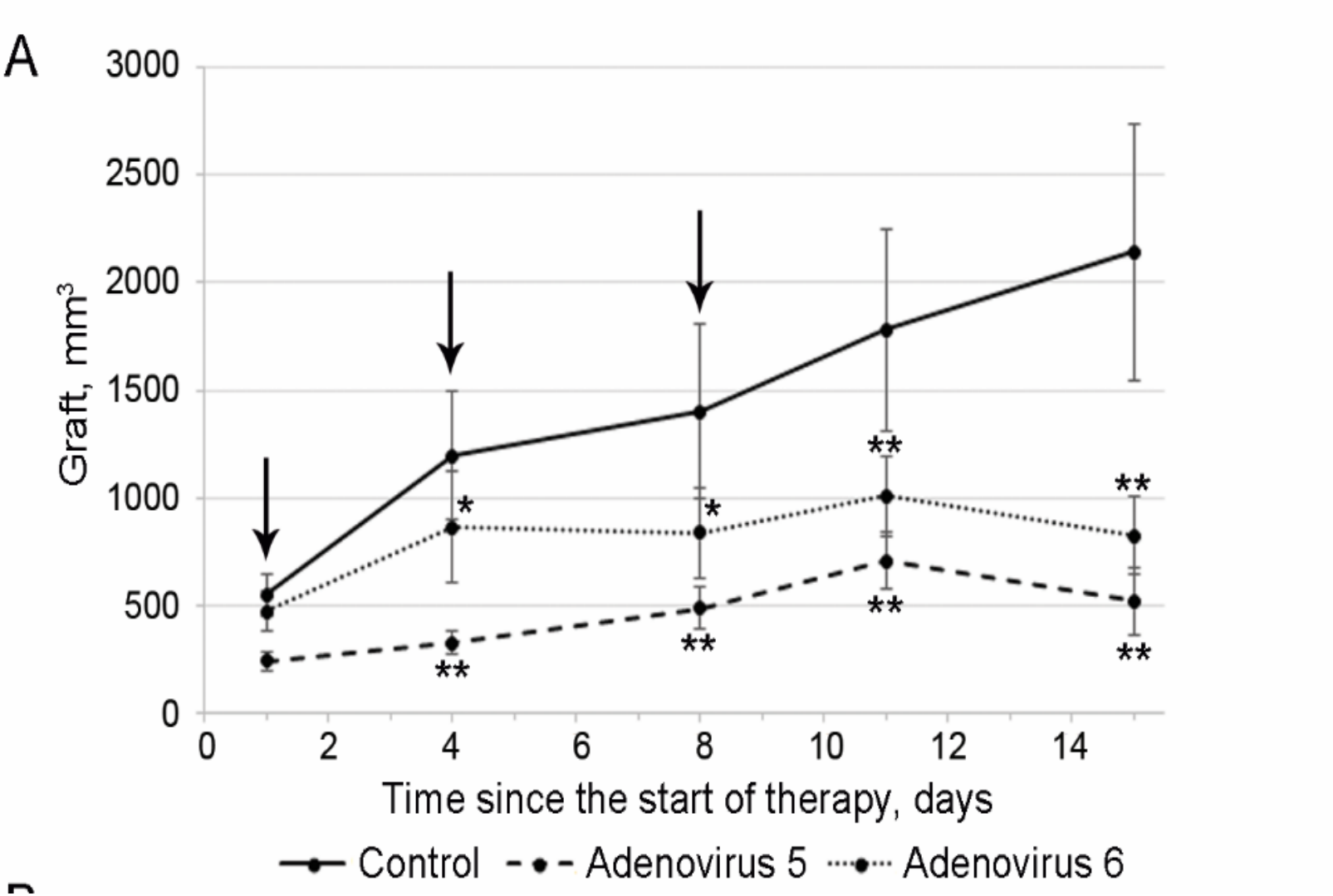
in vitro

prostate (DU14)
breast (MDA-MB-468),
ovarian (SKOV-3)

in vivo

Ad6 has the similar to Ad5 oncolytic efficacy

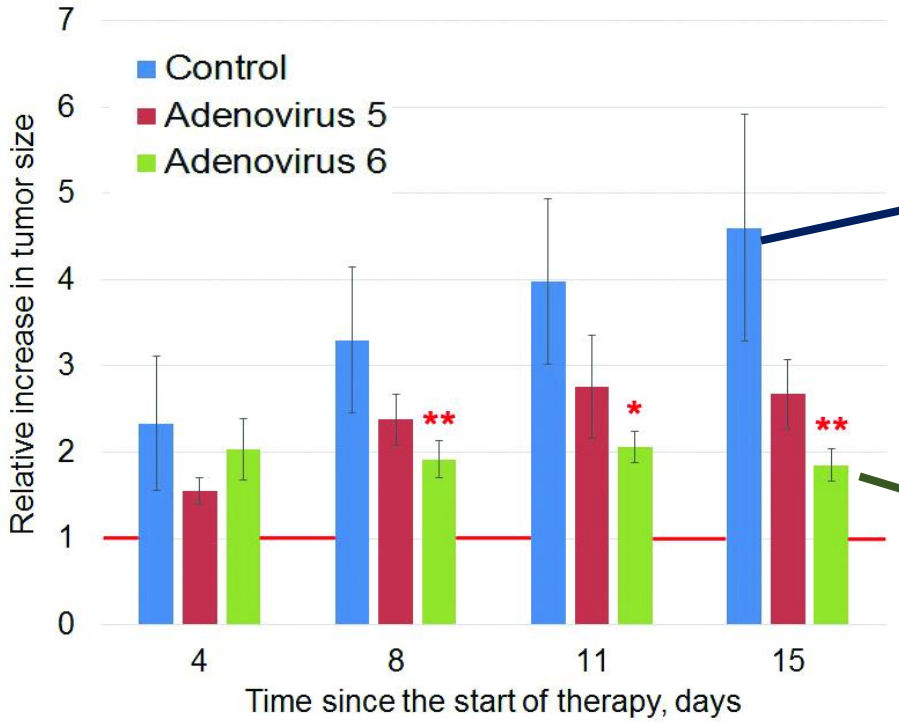
Regression of U87 tumors in SCID mice after the Ad6 and Ad5 i.t. injections



The graph represents the tumors growth rate (Median±SEM, n=6-8). Distinction confidence between the groups was estimated with the Mann-Whitney test, *p<0.05; **p<0.01.

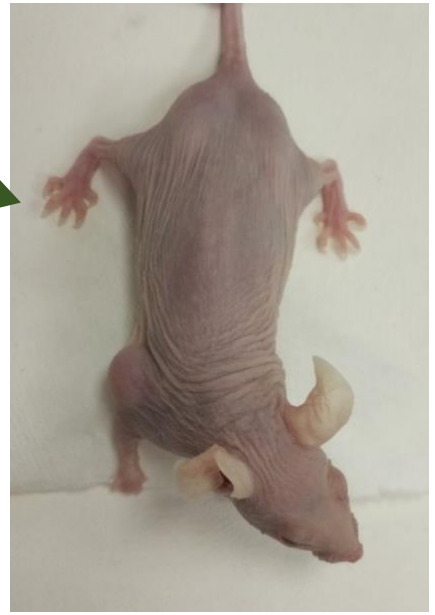
Arrows denote the days of adenovirus injections.

Regression of U87 tumors in SCID mice after triple intratumoral injection of the Ad6 and Ad5

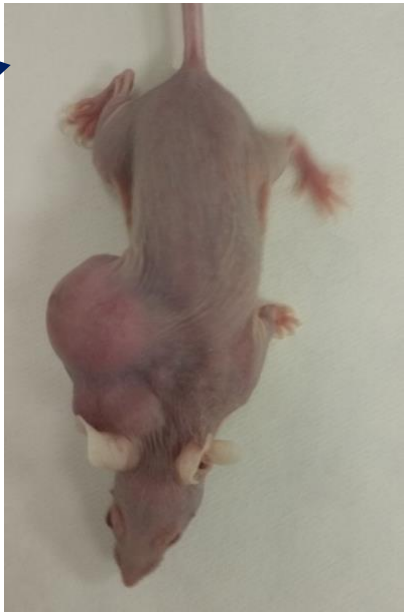


Y axis represents the ratio of the current tumor size to the initial one, measured prior to the start of therapy and taken as 1 (red baseline). Ad5 and Ad6 were administered at day 1, 4 and 8.

Distinction confidence between the groups was estimated with the Mann-Whitney test, *p<0.05, **p<0.01.



+ Ad6



Control

Cytotoxicity of Ad5 and Ad6 toward U87 and U251

ANTICANCER RESEARCH *39*: 6073-6086 (2019)
doi:10.21873/anticanres.13815

Oncolytic Effect of Adenoviruses Serotypes 5 and 6 Against U87 Glioblastoma Cancer Stem Cells

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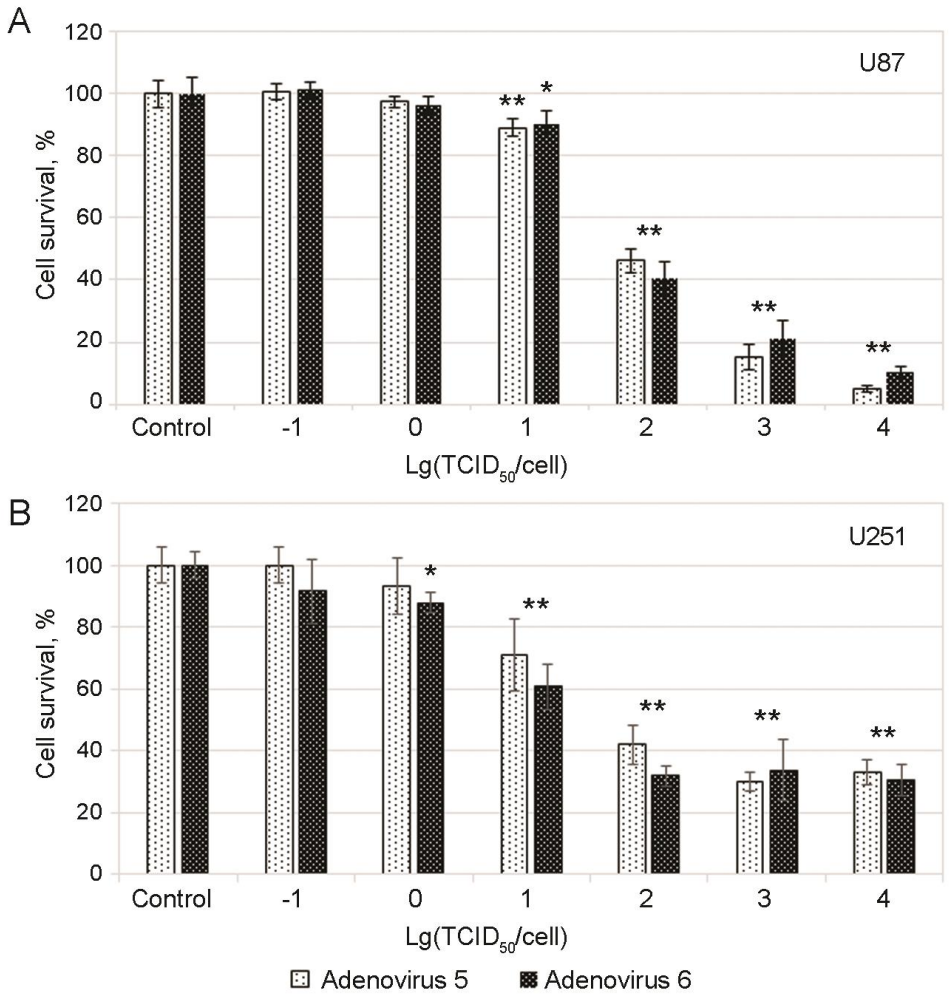
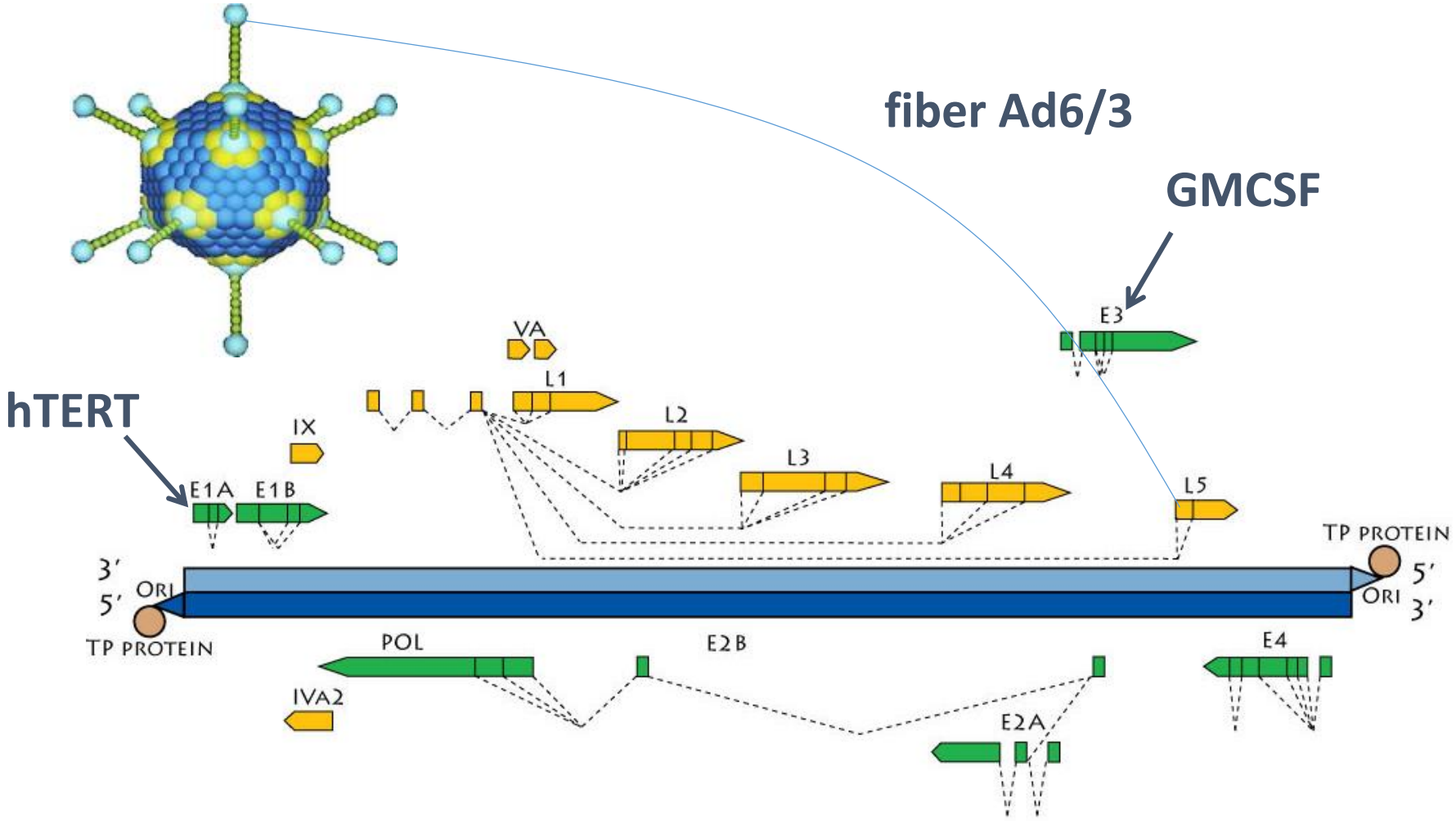
Abstract. *Background/Aim: Oncolytic adenoviruses are promising therapeutic agents against both the bulk of tumor cells and cancer stem cells. The present study intended to test the oncolytic capability of adenovirus serotype 6 (Ad6), which has a lower seroprevalence and hepatotoxicity relatively to adenovirus 5 (Ad5), against the glioblastoma and its cancer stem cells. Materials and Methods: Oncolytic efficacy of Ad6 was compared to widespread Ad5 both in vitro and in vivo, using the U87 and U251 human glioblastoma cell lines and subcutaneously transplanted U87 cells in SCID mice, respectively. Results: Ad6 had a dose-dependent cytotoxicity toward glioblastoma cells in vitro and its intratumoral injections lead to a significant ($p<0.05$) decrease in volume of U87 xenografts, similarly*

Despite the relatively low occurrence, central nervous system malignancies yield extremely high risks of lethal outcomes. Glioblastoma (GB; grade IV tumor according to the WHO classification) is the most frequent and poorly curable type of brain tumors (1). In contrast to other types of solid tumors, GB extensively invades the surrounding tissues while it rarely metastasizes to other organs (2).

Currently, the medical treatment of GBs involves the maximal surgical resection of the tumor followed by radio and/or chemotherapy. Despite the progress in neurosurgery, the invention of new promising chemotherapeutic agents (for example, temozolomide), the active development of immunotherapy methods as well as the methods of molecular diagnostics, the survival rate of patients with GB has not

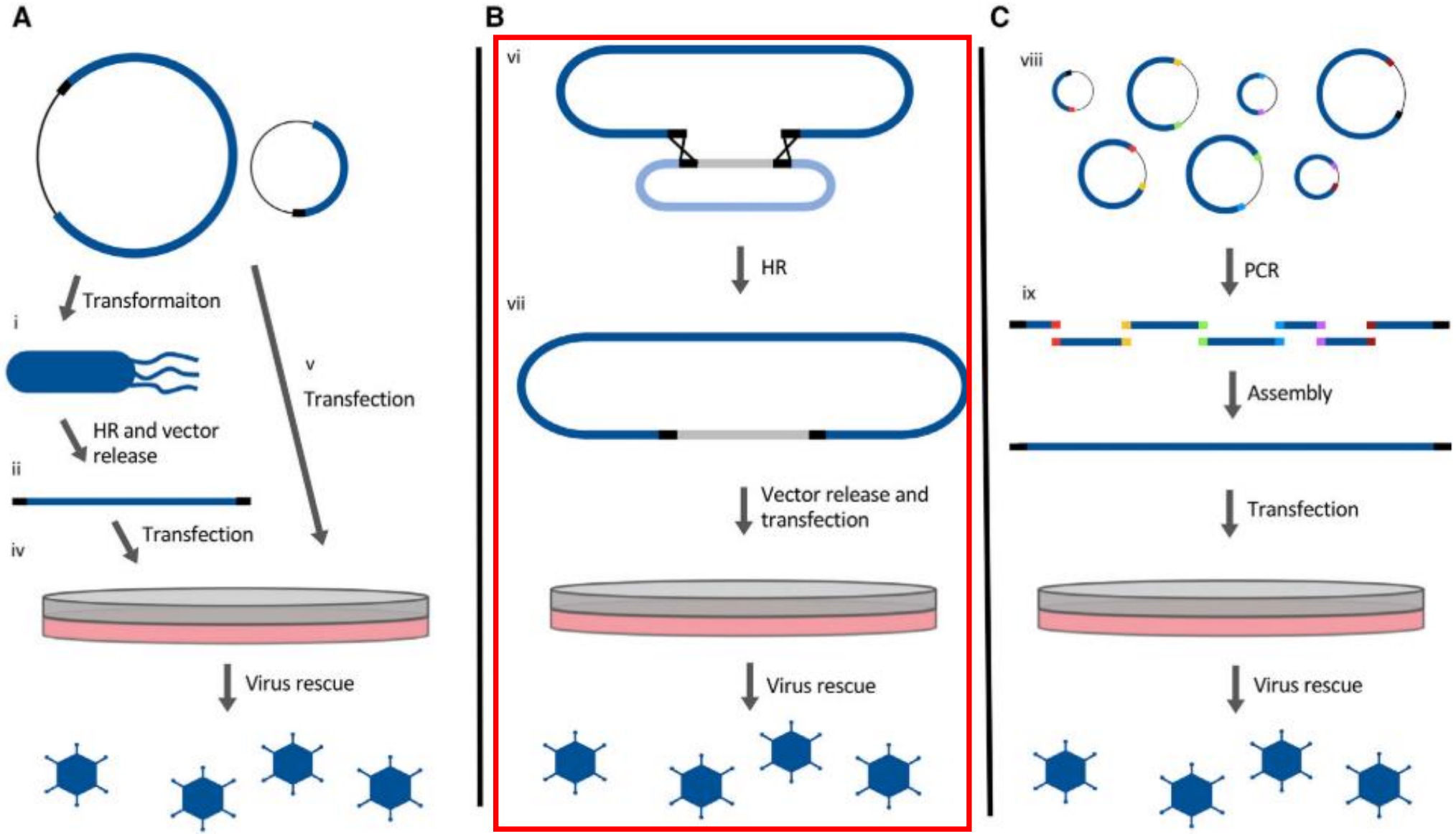


Ad6 modifications



http://www.lookfordiagnosis.com

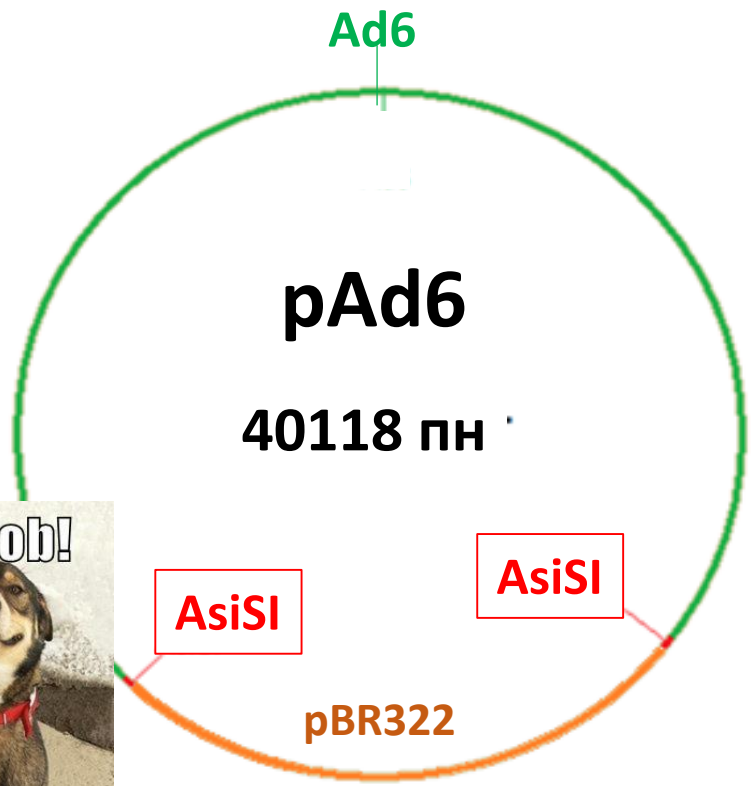
Ad vectorisation strategies



Gao J, Mese K., Bunz O., Ehrhardt A. FEBS Lett . 2019 Dec;593(24):3609-3622. doi: 10.1002/1873-3468.13691.

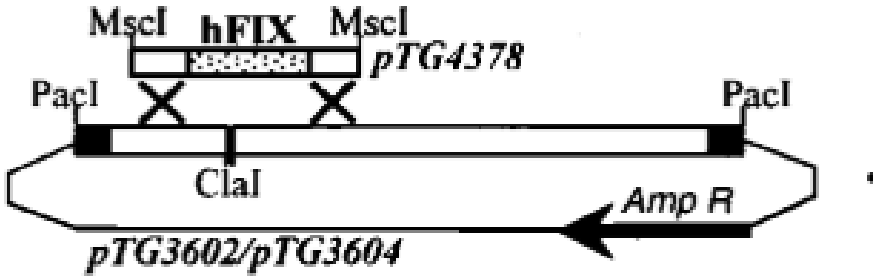
Ad6 vectorisation: *in vivo* recombination

In vivo recombination in BJ5183



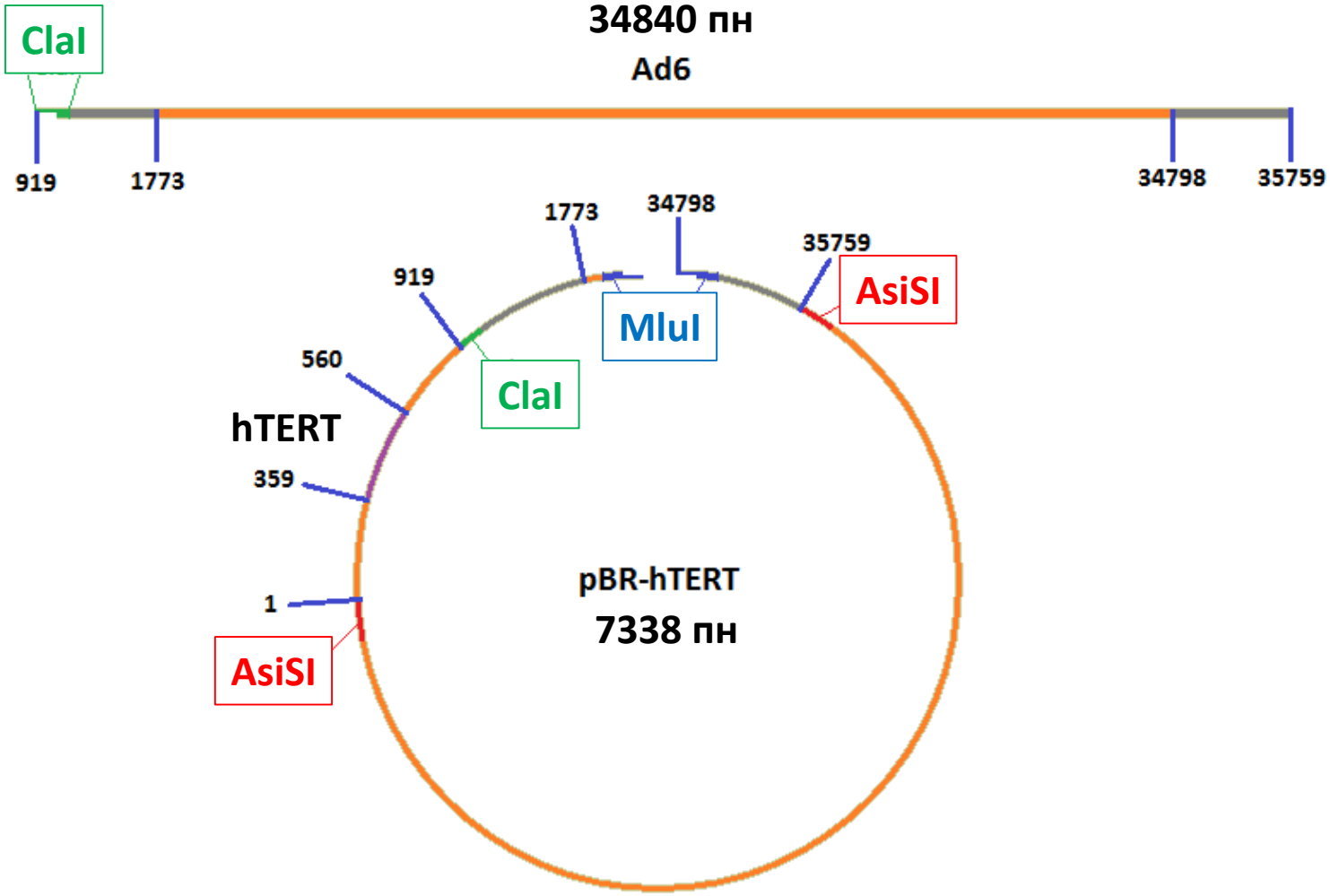
ClaI needs dam- dcm- plasmid

Ad5 E1 region targeting



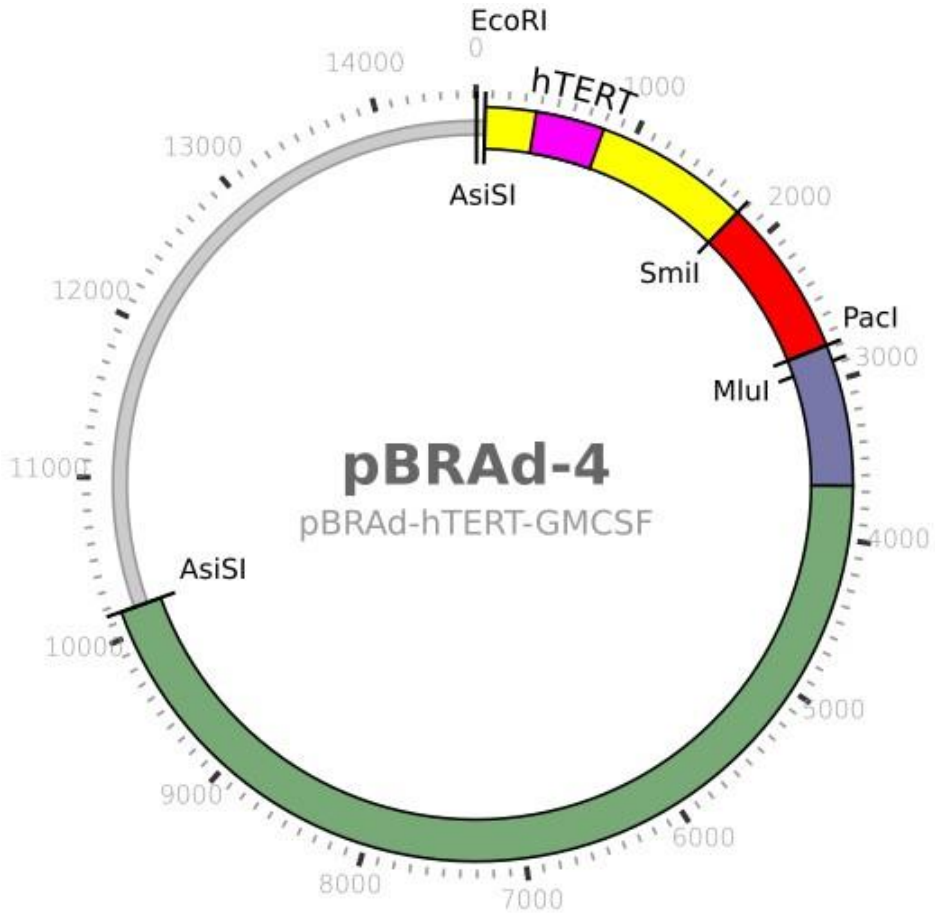
Chartier et al., J Virol. 1996 Jul;70(7):4805-10.

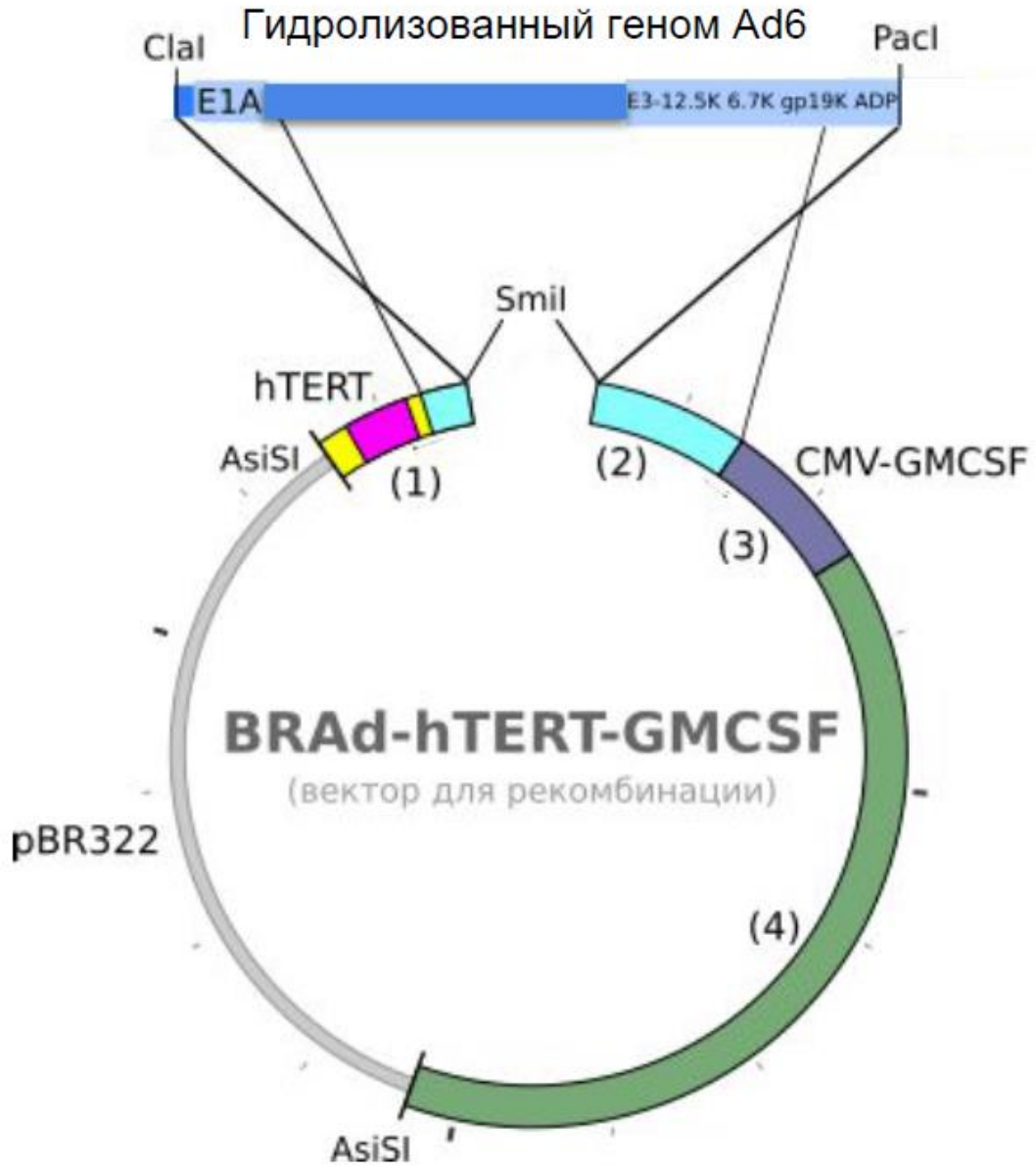
Ad6 vectorisation: hTERT insertion



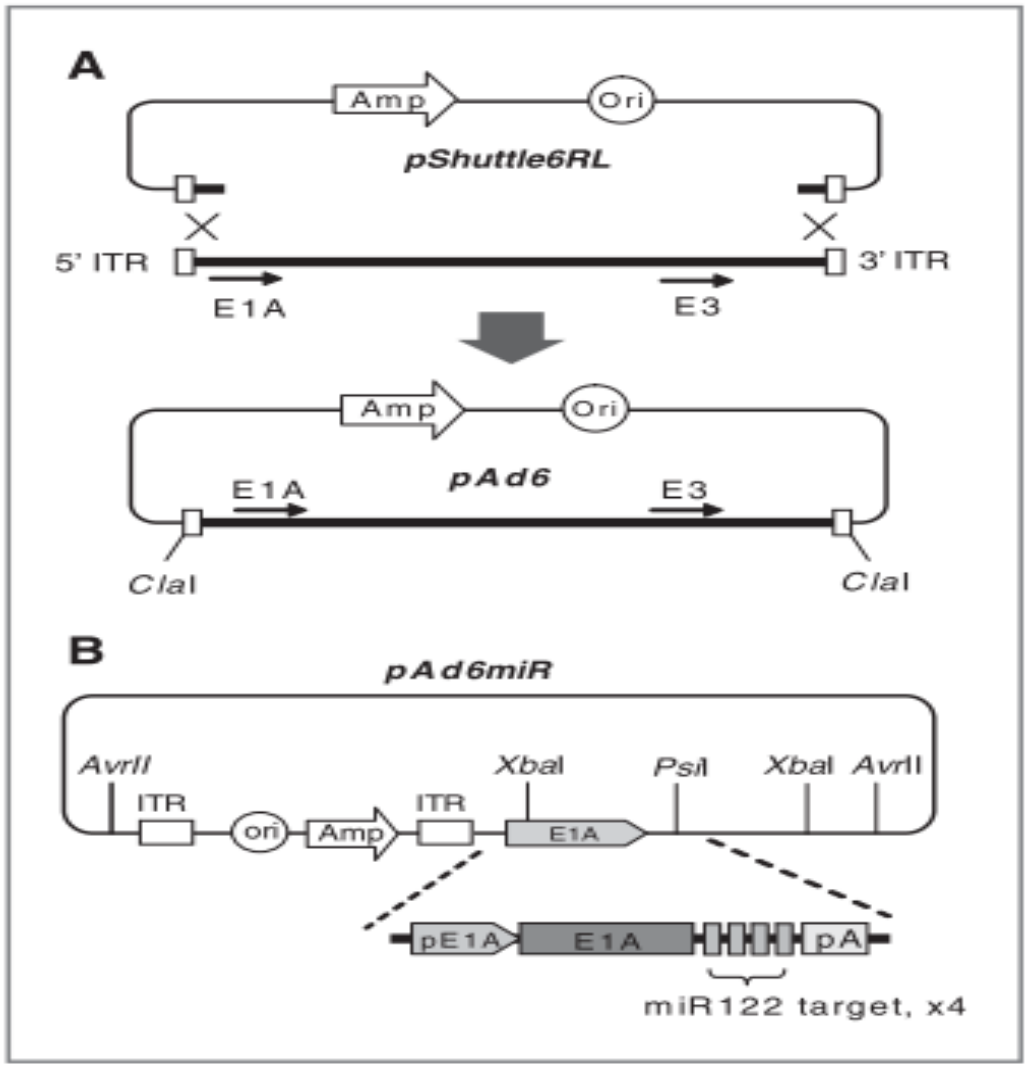
5

Ad6 vectorisation: hTERT and GMCSF insertion

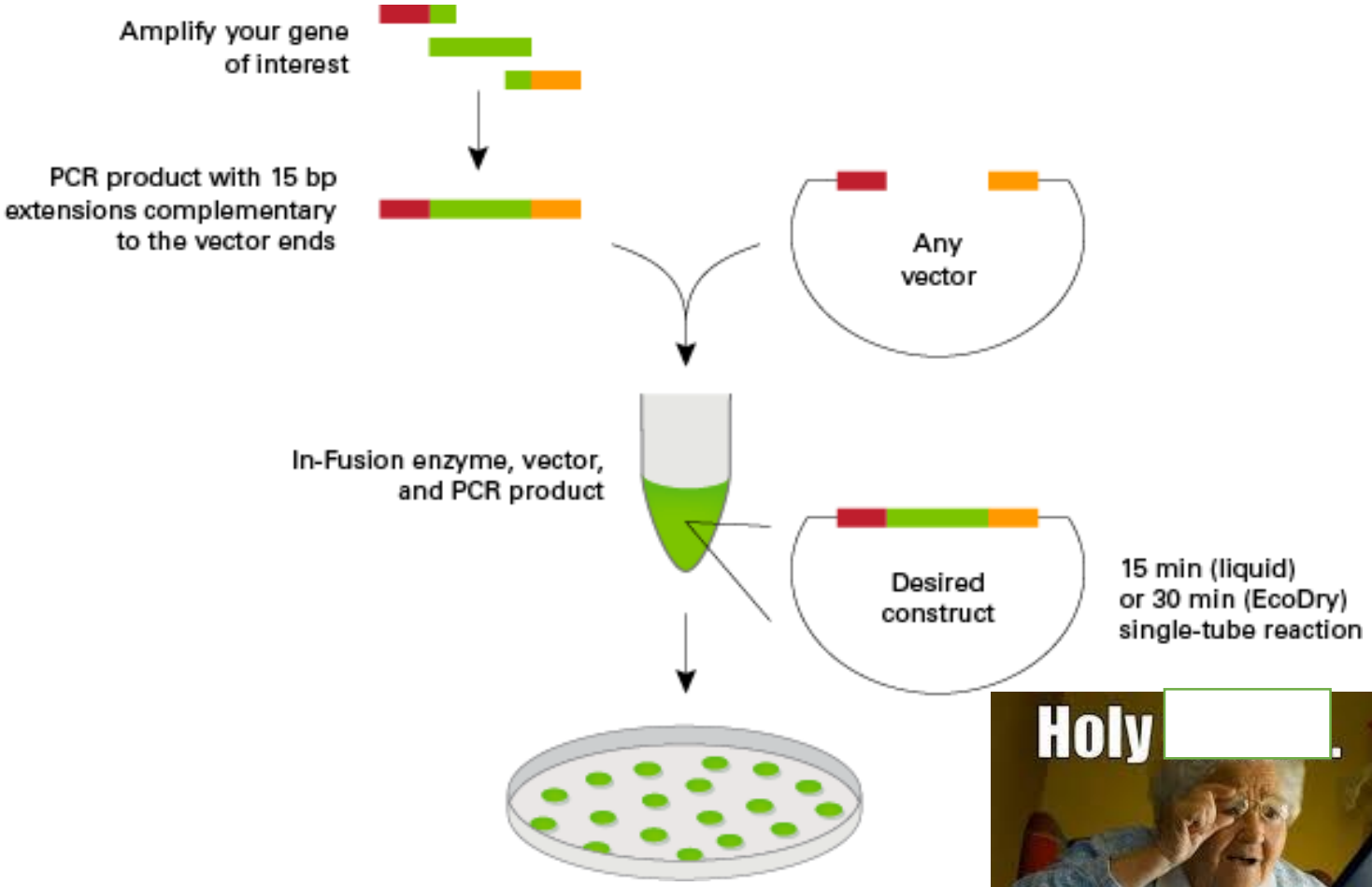




Ad6 vectorisation: *in vitro* recombination

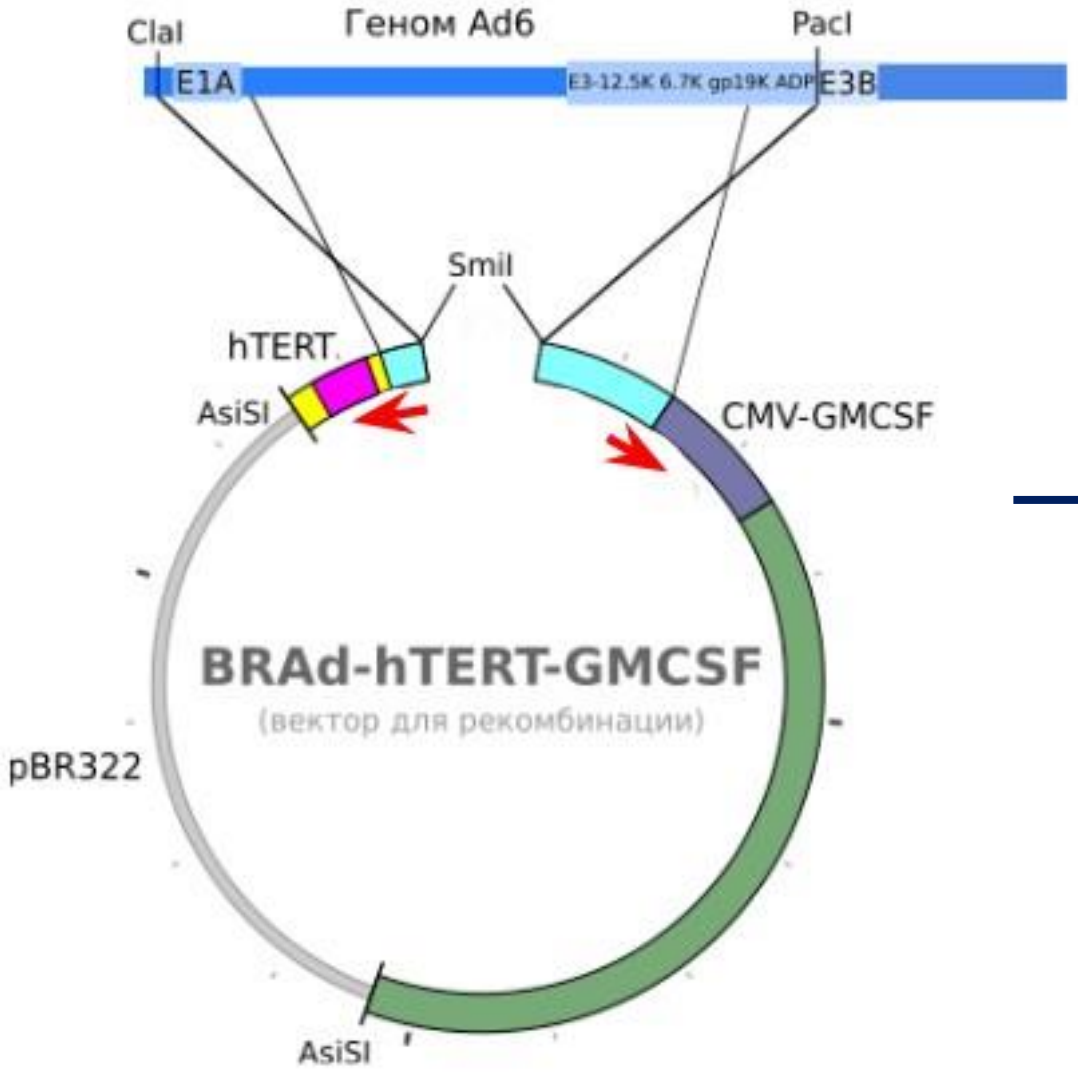


***In vitro* recombination**
(In-Fusion, Clontech)



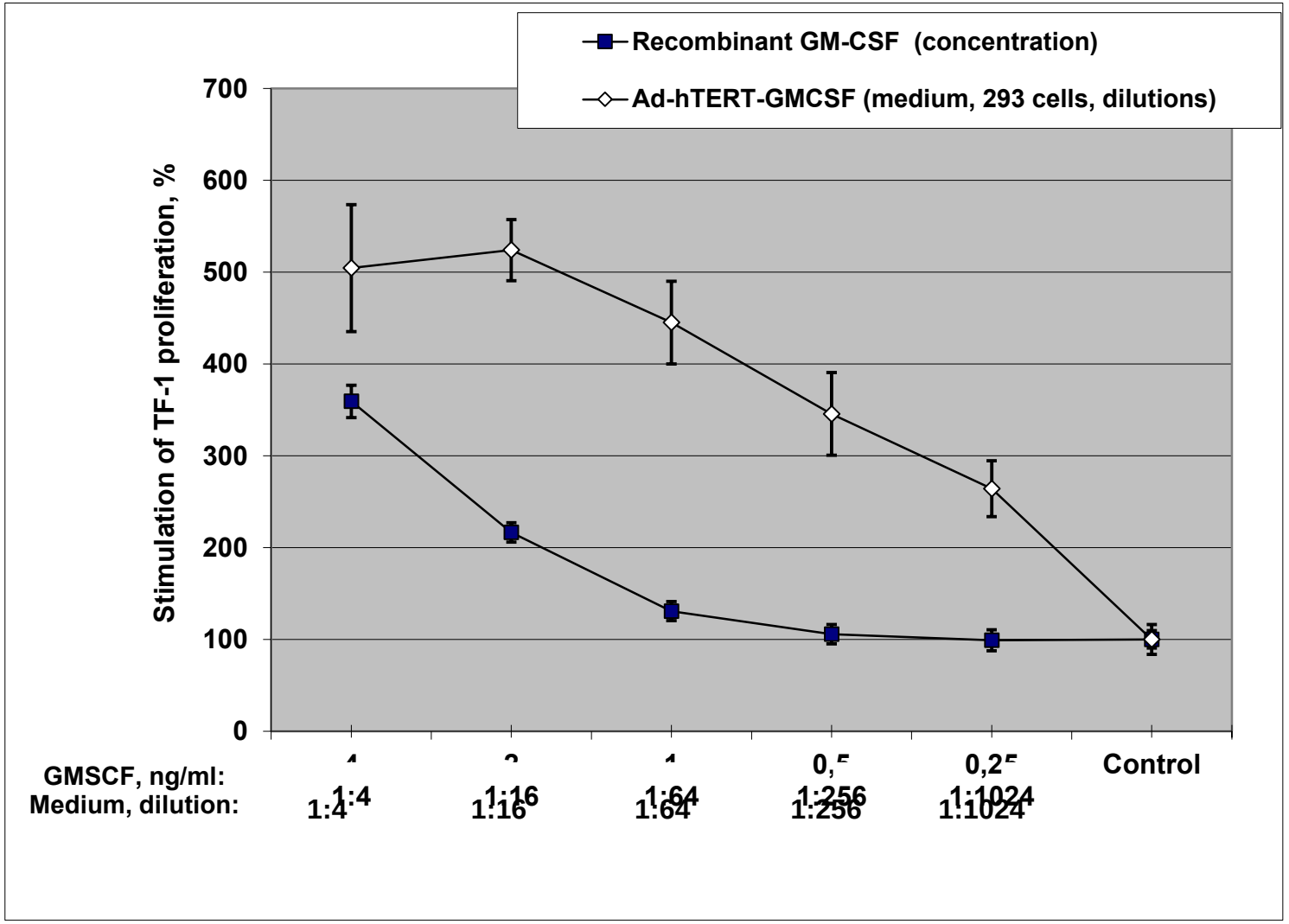
Zhang et al., Mol Cancer Ther . 2012 Nov;11(11):2410-8

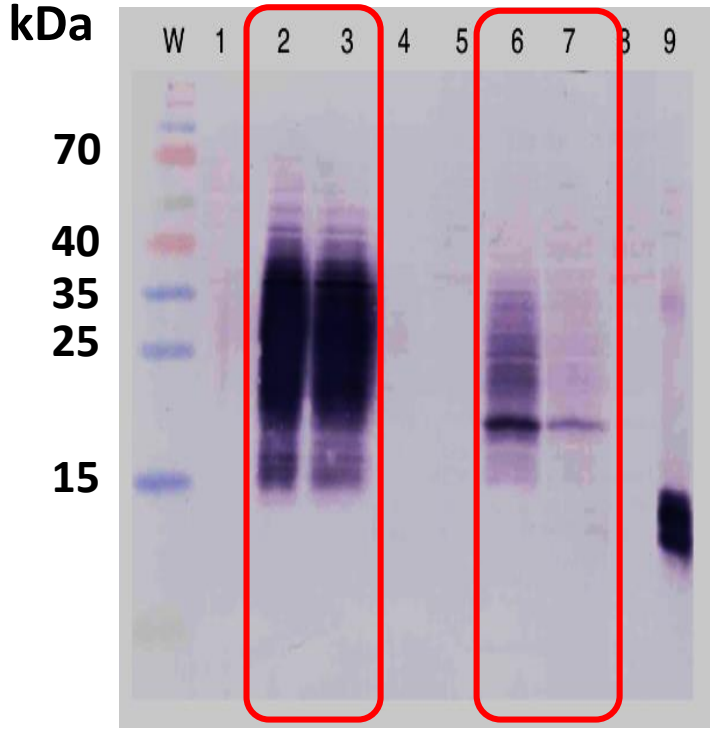
Ad6 vectorisation: *in vitro* recombination



Ad6-hTERT-GMCSF

Evaluation of GMSCF activity





1 — medium with Ad6

2, 3 — medium with Ad6-hTERT-GMCSF

4 — medium control

5 — lysate with Ad6

6, 7 — lysate after Ad6-hTERT-GMCSF

8 — lysate control

9 — recombinant non-glycosylated GMCSF from *E. coli*.

Ladder - Spectra Multicolor Broad Range Protein, Thermo Scientific

Conclusions

- Adenovirus serotype 6 has a dose-dependent cytotoxicity toward the U87 and U251 cell lines *in vitro,* which is similar to that of serotype 5. The intratumoral injections of both adenovirus serotypes lead to a significant ($p<0.05$) decrease in volume of the U87 xenografts in immunodeficient mice.
- The approach based on In-Fusion recombination was proposed for vectorisation of alternative Ad serotypes.
- The recombinant Ad6-hTERT, Ad6-hTERT-GMCSF (double recombinant) and Ad6/3-hTERT-GMCSF (triple recombinant) were successfully constructed, characterized by restriction analysis and full-length sequencing and are under biological characterization now.
- The preclinical trials are proposed in 2021.

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