A génterápia alkalmazási lehetőségei

Gene therapy: A 15-years hailstorm of highly emotionalised good and bad news

Feb 1990 First trial ADA deficiency

Dec 1988 IL-2 cancer tre

Mar 1994 SAE cystic fib

Jun 1995 Motulsky NIH

Feb 1996 r-lentiviruses

Oct 1998 VEGF ischemia

BBC, NBC, CNN,...

New York Tim

Washingto

Jesse Gelsinger Oct 1999

F Thrasher Paris & UK Dec 2000

AV germline Sept 2000

on, Milano trial May 2002

First SAE Paris Sep 2002

second SAE Paris Feb 2003

Nature

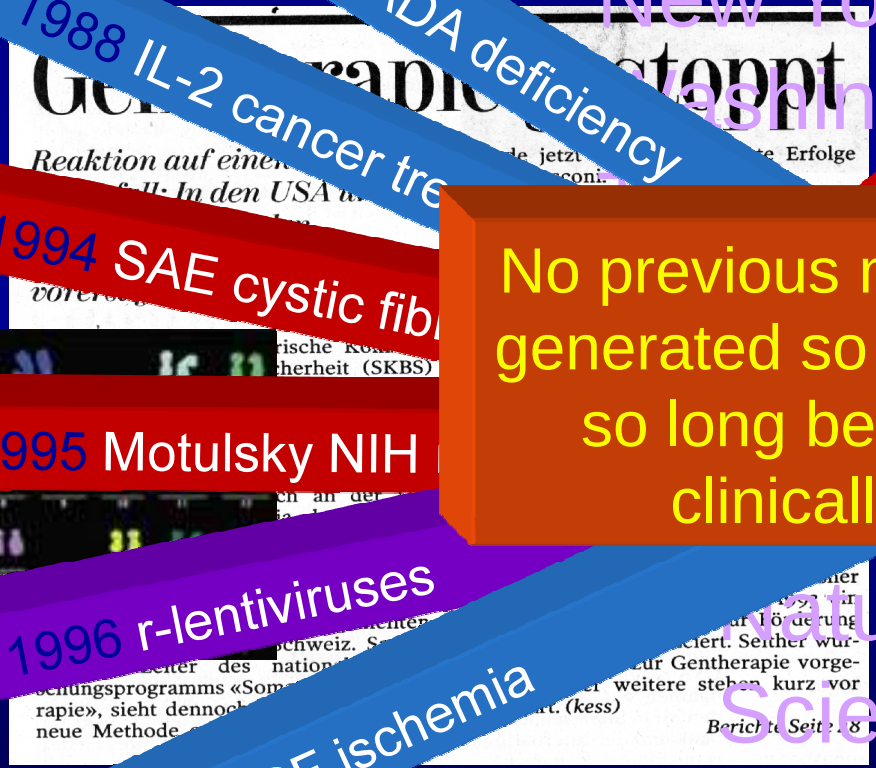
Science

NEJM

Internet

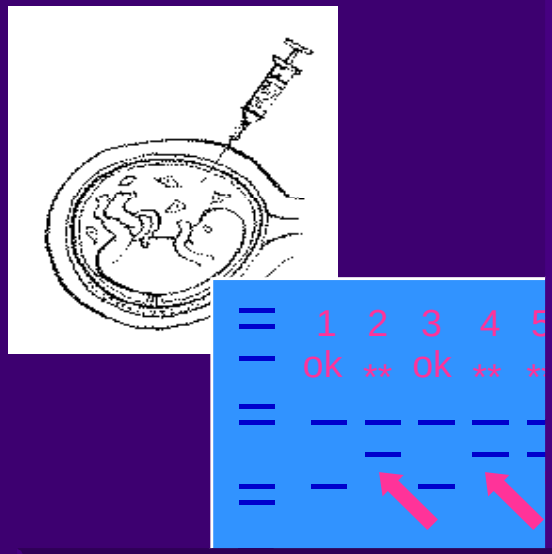
...

No previous medical procedure generated so many discussions so long before being ever clinically applicable

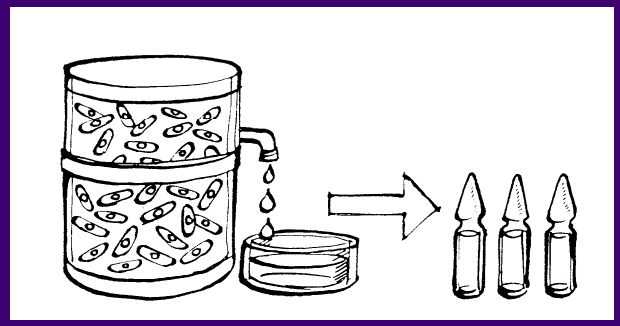


The FOUR eras of molecular medicine

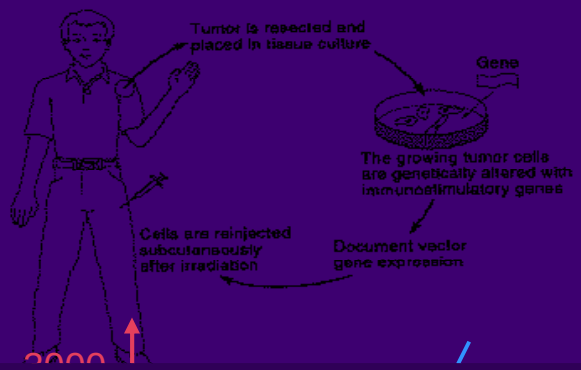
Genes as probes



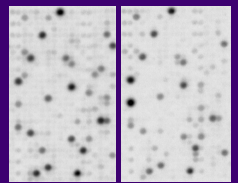
Genes as factories

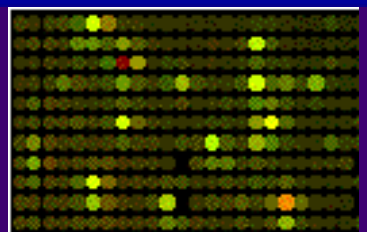


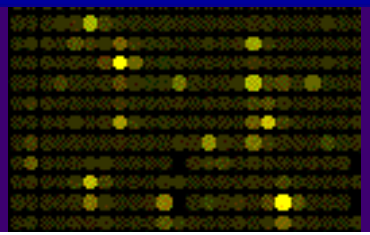
Genes as drugs



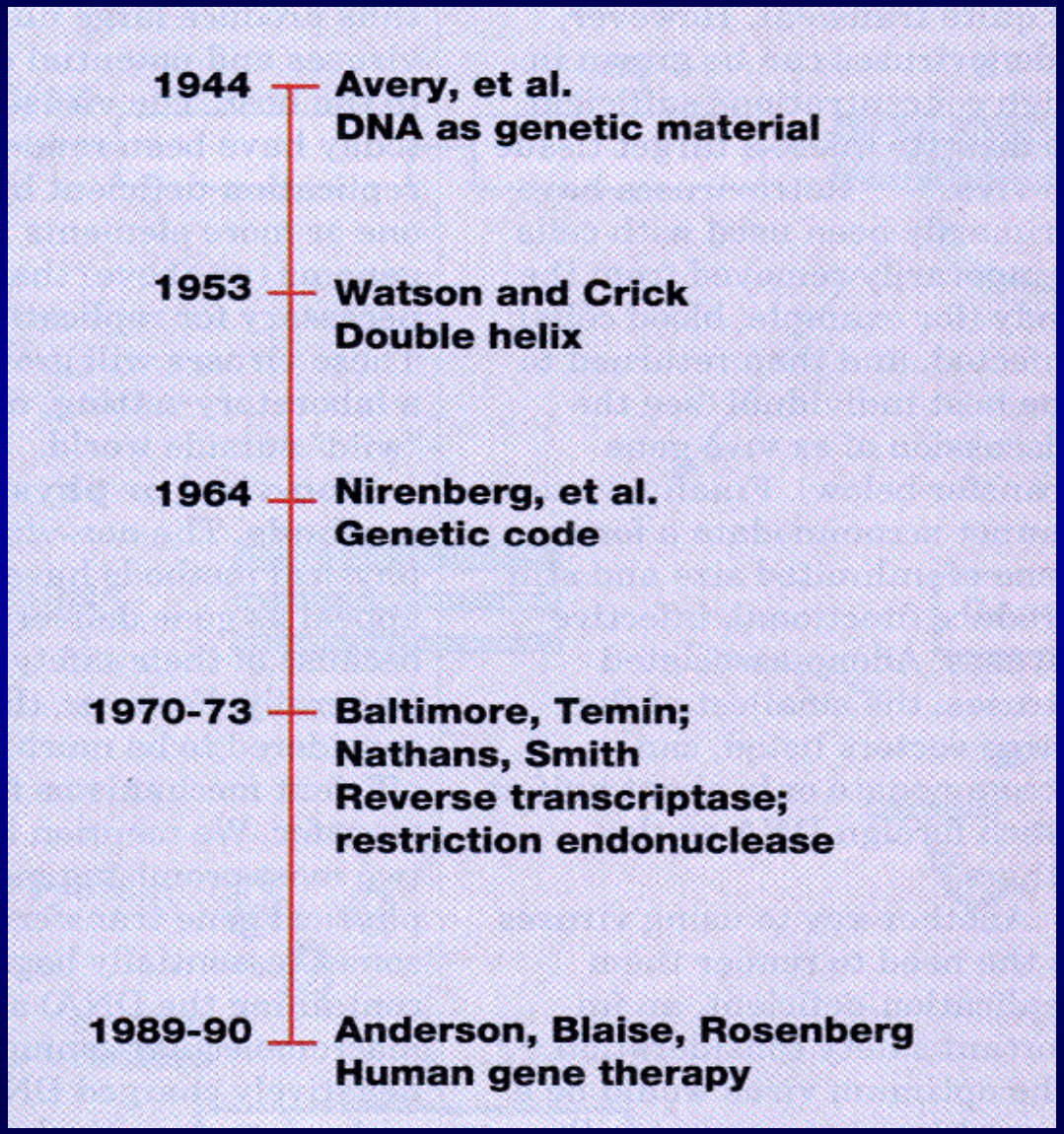
Post-genomic improvements of former technologies







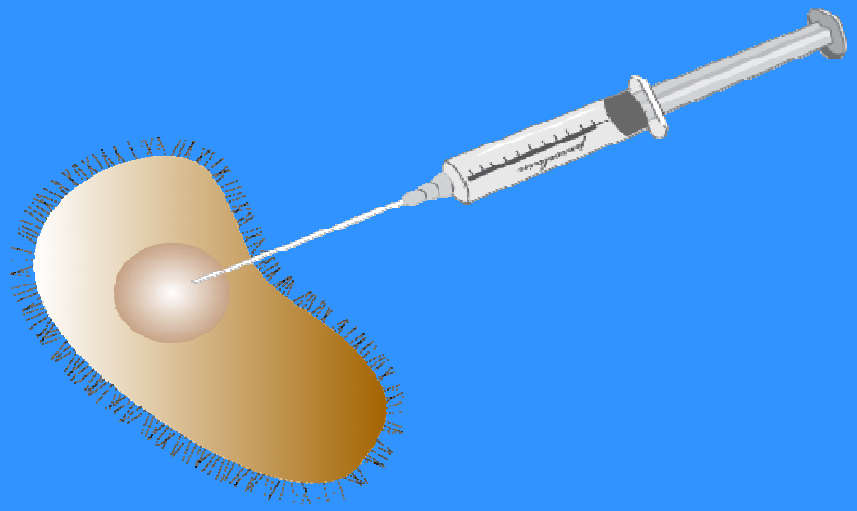
Major Scientific Advances



- Tools of molecular biology
  - Reverse transcriptase
  - Restriction endonucleases

What is gene therapy?

- A way to treat diseases caused by mutated genes
- Adding a normally functioning copy of the gene(s) to en ough affected cells to restore normal function.



Genetic Science Learning Center

Gene therapy:
transfer of genetic material into cells

- To displace or correct an inherited defective gene (cystic fibrosis, hemophilia, etc.)
- To alter or repair an acquired genetic disorder (cancer, ischaemic disease)
- To provide a new or changed function to a cell (introduce resistance to HIV, program an immune cell to attack cancer

Gene Therapy in the clinics: Trials Worldwide (cumulative)

UNIFR
Rusconi
2005

trials

patients

100
80
60
40
20

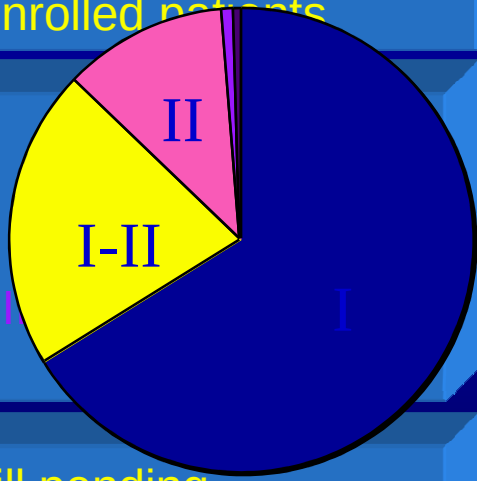
1990 1992 1994 1996 1998 2000

Ergo
- in spite of 13 year- research only less than 2% of the trials has reached phase III
- not necessarily due to the «novel» 'fail early, fail fast' paradigm

! As of Jan 1, 2004:
1 approved product in China
(*Gendicine*, by Sibiono Inc. 2004

As of Jan 2005:
938 cumulative protocols (90-2004)
4600 treated /enrolled patients

66% phase I
19% phase I-II
13% phase II
0.8% phase II-III
1.7% phase III



21% overall still pending
or not yet Initiated !
www.wiley.com/genetherapy

Overwiev

I. Types of gene therapy
II. Vector systems
III. Therapeutic genes
IV. Ethical points

Types of gene therapy

Somatic gene therapy

Germline gene therapy

Germline gene therapy

Gene therapy in reproductive cells of a patient so that genetic defect would be corrected in the offspring.

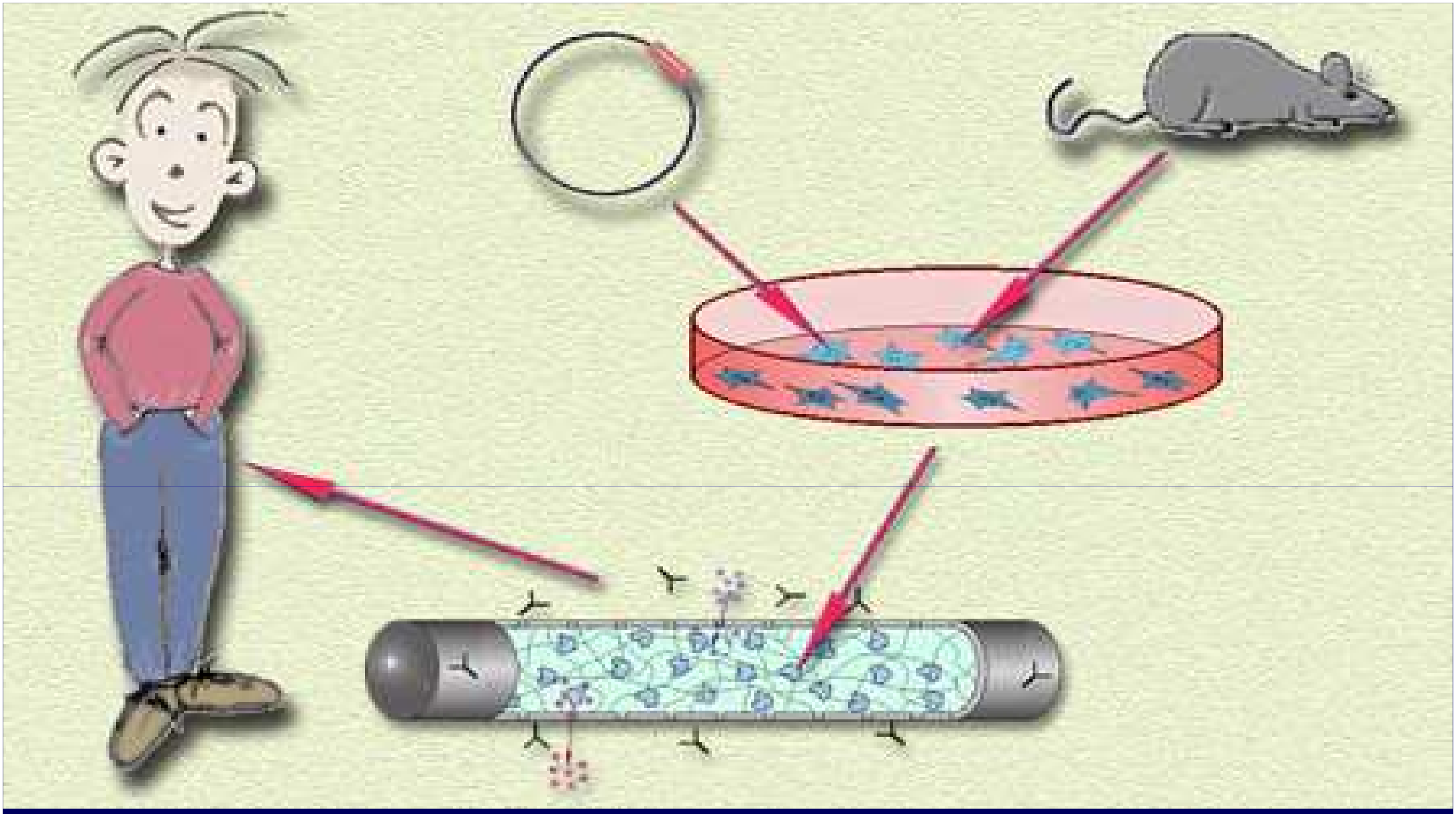
Currently banned in most countries.

Clinical applications of somatic gene therapy

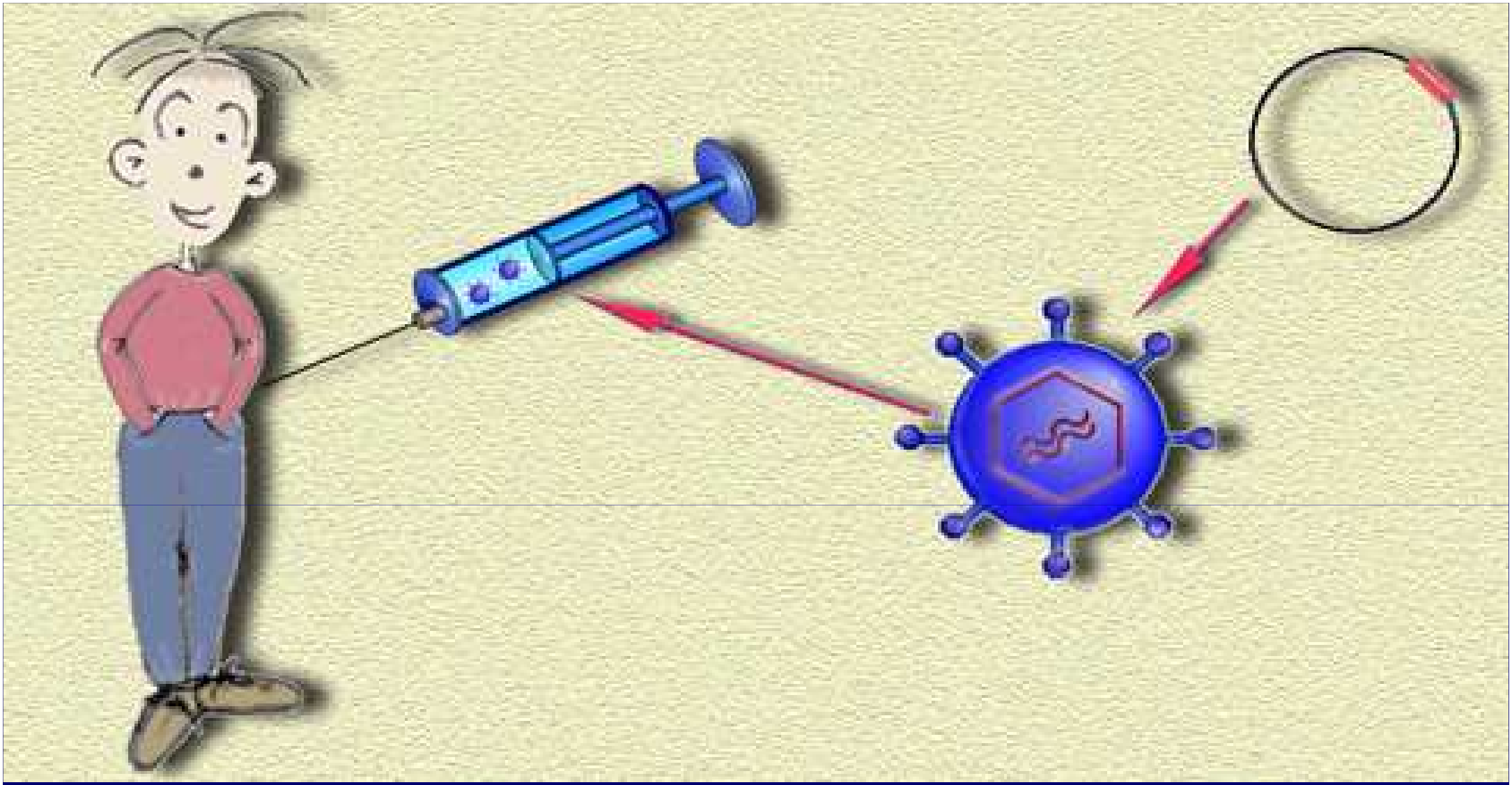
- Cancers
- Inherited disorders
- Infectious diseases (viral or bacterial) – HIV, Hepatitis B
- Immune system disorders
- Acute illnesses
- Vaccination

Diseases for applying gene therapy

Disease	Defect	Target cell (Strategy)
Severe combined immunodeficiency	Adenosine deaminase 4	Bone marrow cells or T-lymphocytes
Heamophilia	Factor VIII, Factor IX deficiency	Liver, muscle, fibroblasts
Cystic fibrosis	Loss of CFTR gene	Airspaces in the lung
Haemoglobulinpathies	α or β globulin gene	Bone-marrow cells, macrophages
α1-antitrypsin deficiency	α1-antitrypsin	Lung or liver cells
Cancer	Many causes	Many cell types
Neurological diseases	Parkinson's, Alzheimers	Direct injection into the brain
Cardiovascular	Restinosis, arteriosclerosis	Vascular endothelial cells, Arteries
Infectious diseases	AIDS, hepatitis B	T cells, macrophages, liver
Liver cirrhosis	Fibrogenesis	Hepatocyte growth factor
Autoimmune disease	Lupus, diabetes	MHC, β2-microglobulin



Ex vivo



In vivo

Approaches for gene therapy

Ex vivo:

- The relevant target cells are removed from the body, transduced *in vitro* and subsequently the modified cells are re-introduced into the body.
- patient specific
- the problems in cost and the labor of the surgery
- tumor cells, skin fibroblasts, haematopoietic cells

In vivo:

- The genetic material is directly transferred into the cells of the patient
- the low transfection efficiency, transient gene expression
- i.v. or i.m. injectable

Ideal vector

- Non-toxic
- Non-immunogenic even after repeated administration
- High transfection efficiency
- Injectable
- Regulation possible
- Site-specific targeting possible
- Reliable and longer duration of gene expression
- Ease of production on a large scale
- High capacity in DNA insertion size
- Ability to remove/replace defective genes
- Reproducible
- Stable
- Cost-effective

Methods of Gene delivery

- **Physical methods-**

  electroporation, microinjection, direct DNA injection, DEAE-dextran, Ca phosphate and etc.

- **Non-viral vectors-**

  liposomes, emulsion, peptides, cationic polymers etc.

- **Viral vectors-**

  adenoviruses, retroviruses and etc.

Non-Viral vector

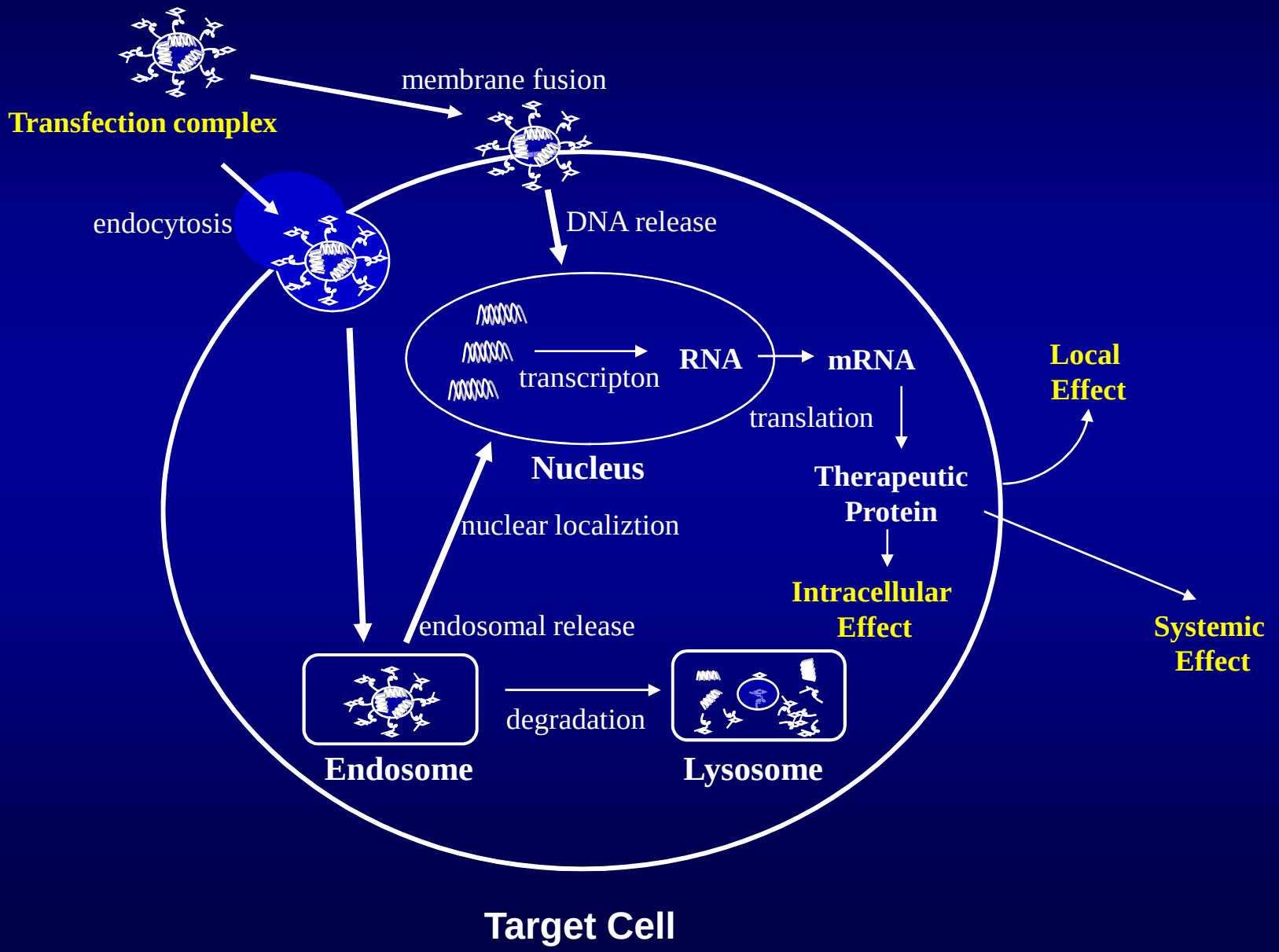
Advantages:

- Less risk for inflammatory or immune reactions
- Easier manipulation and large scale production
- Flexibility in the DNA insertion size
- More specific targeting
- Safety, non-immunogenecity
- Non-pathogenecity

Disadvantages:

- Lower transfection efficiency
- Expression is transient
- Instabilization by serum components in vivo

Nonviral vector mediated gene delivery



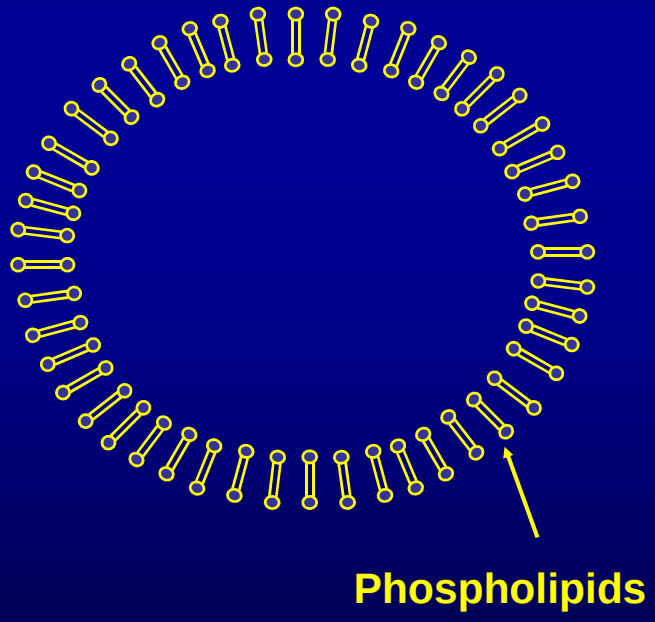
Non-viral vector system

- Lipid based system
  - Liposomes
  - Emulsions
  - Solid-lipid nanoparticle
- Polymers

Liposome

- Small vesicles of bipolar phospholipids with an aqueous interior

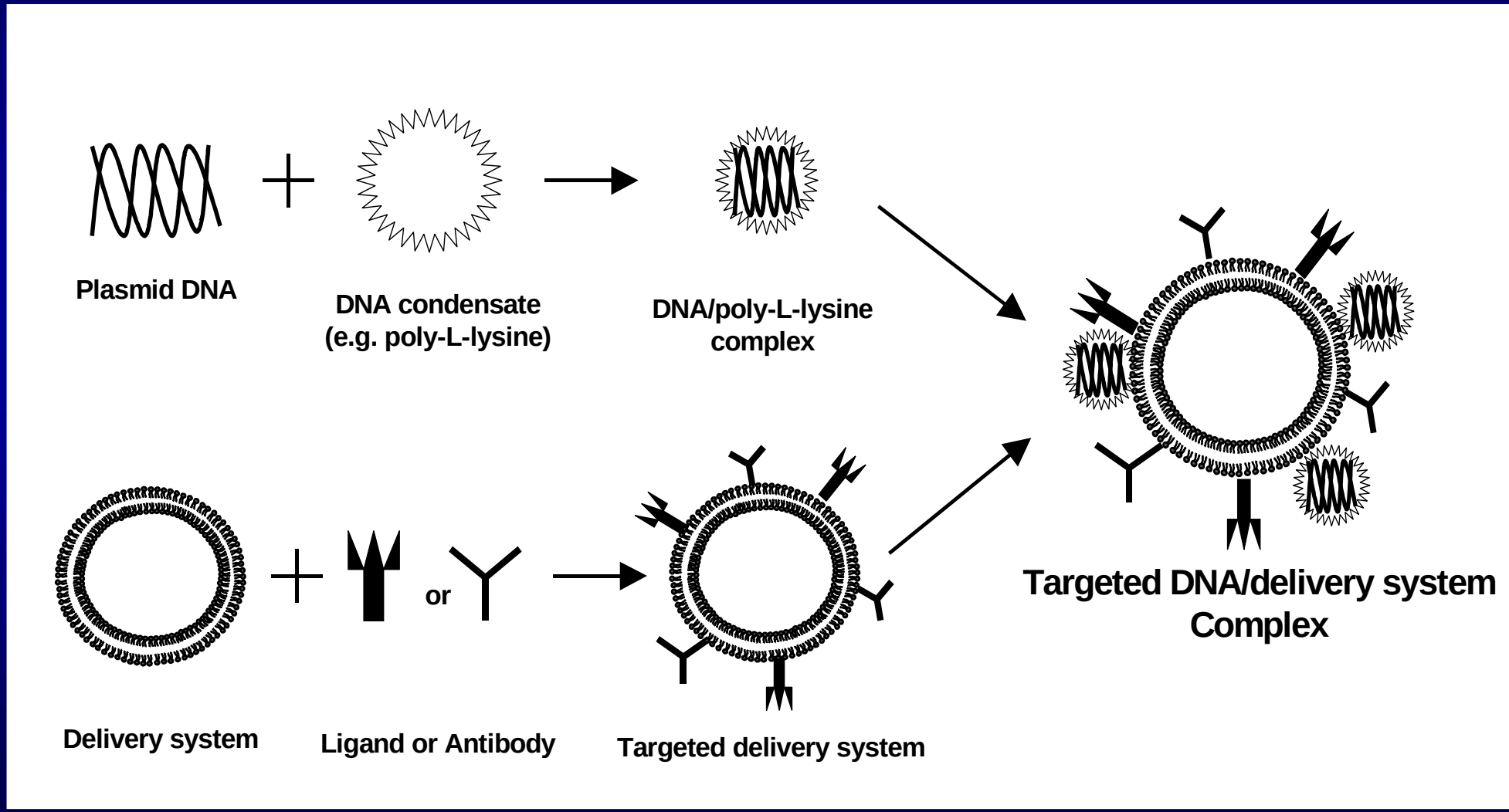
- - cationic liposome
  - pH-sensitive liposome
  - fusogenic liposome
  - stealth liposome
  - thermosenstive liposome
  - immunoliposome



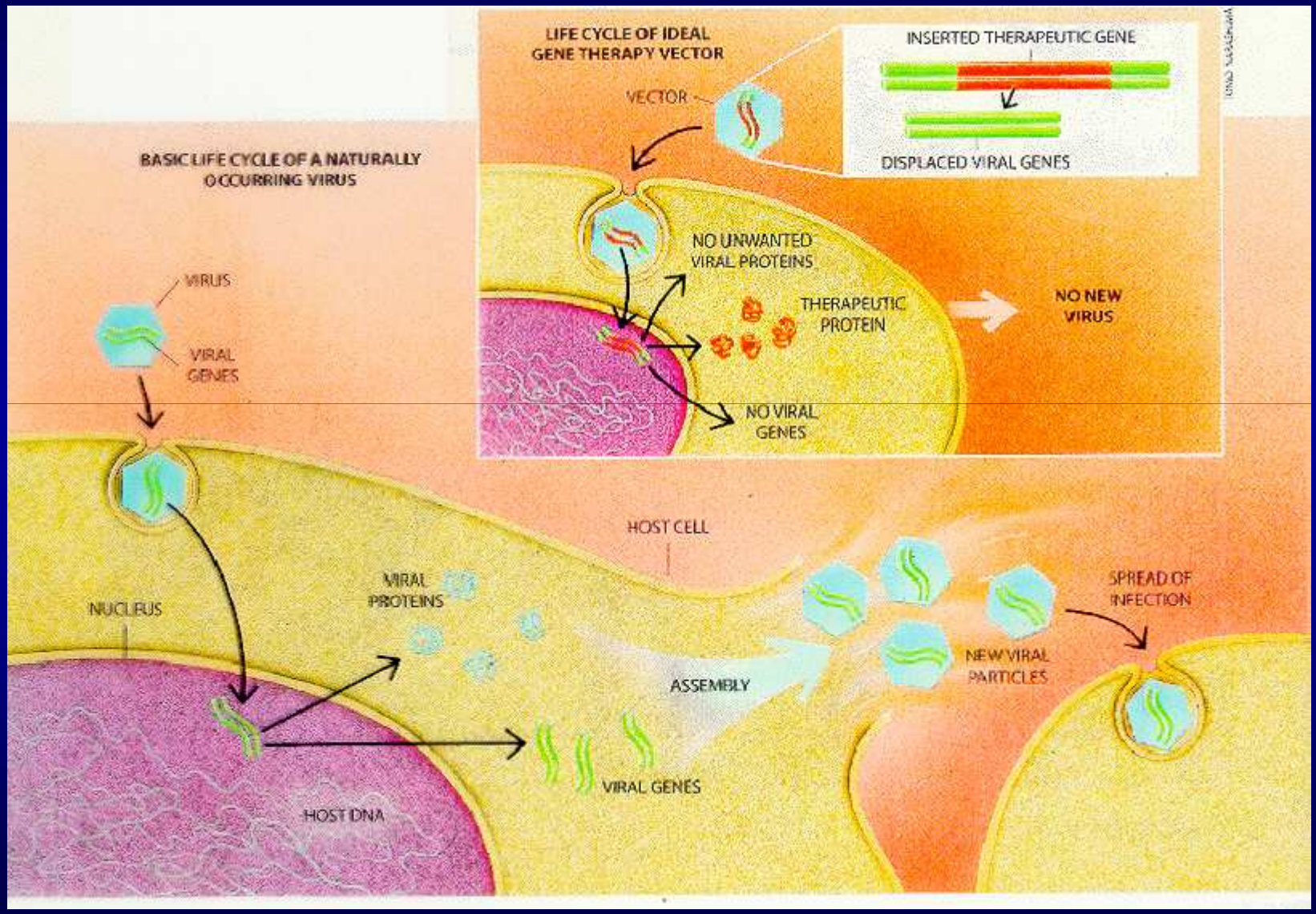
Cationic liposome

- Quaternary ammonium detergents, cationic derivatives of cholesterol and diacylglycerol, lipid derivative of polyamines
- Electrostatic interaction with DNA
- DOPE or cholesterol are added to as helper lipid
- Successfully deliver DNA to lung, brain, tumor and skin

Non-viral vector



Viral vectors



Viral vectors

Advantages:

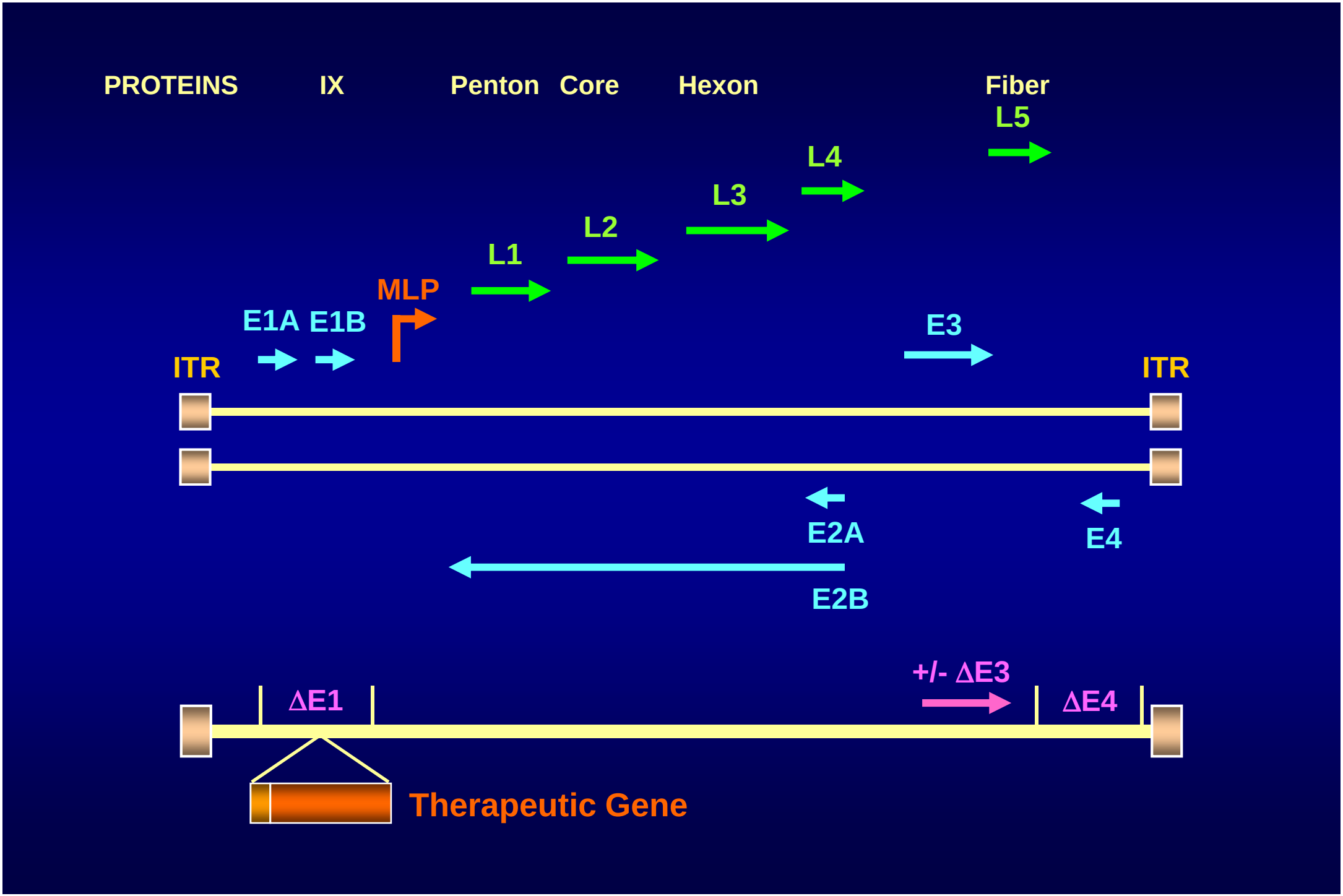
- Large transduction efficiency
- Expressing large amounts of gene products

Disadvantages:

- Expensive, long procedure
- Risk of viral infection
- Triggers immune response
- Restriction in the size and structure of gene to be inserted
- Lack of target specificity
- Risk of mutagenesis and tumorigenesis

Currently used gene therapy vectors

- Adeno-associated virus
- Adenovirus
- Alphaviruses
- Herpes Simplex Virus
- Retrovirus
- Lentivirus


PROTEINS
IX
Penton
Core
Hexon
Fiber
L5
L4
L3
L2
L1
MLP
E1A
E1B
E3
ITR
ITR
E2A
E4
E2B
+/- ΔE3
ΔE1
ΔE4
Therapeutic Gene

Moloney Murine Leukemia virus structural scheme

structure proteins: **GAG**

- MAtrice
- CApside
- NucleoCapside

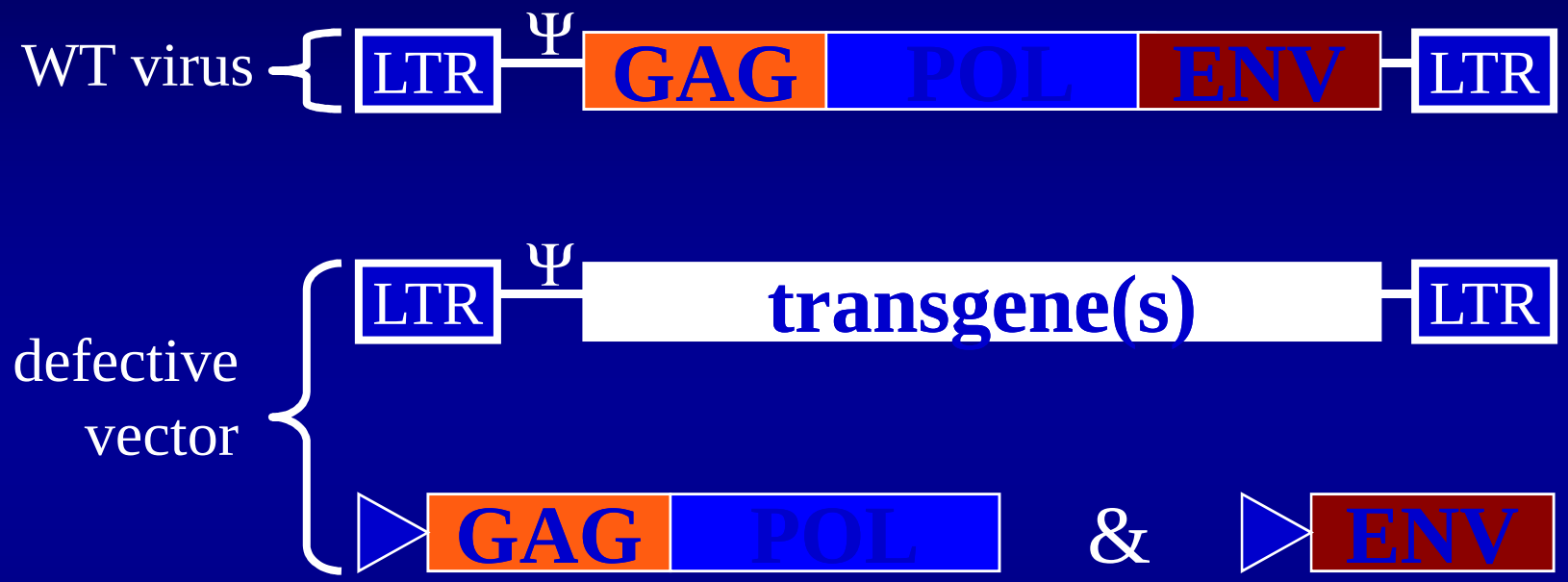
replication enzymes: **POL**

- PRotease
- INtegrase
- Reverse-Transcriptase

Envelope:

- Cellular-derived membrane
- Viral **ENV** protein: Surface & TransMembrane

Moloney Murine Leukemia virus Vectors structure



Lentivirus

The viral genome is a dimer of linear, positive-sense, single-stranded RNA

Genome size is ~10 kb

Require reverse transcription for replication and integration

The genome contains three large genes, gag (group-specific antigen), Pol (polymerase) and env (envelope). In addition, six additional viral proteins Are the primary translation products of spliced mRNA

Eg. HIV-1

Lentivirus

Pros

Ability to infect both dividing and non-dividing cells

Long-term expression possible, genome integration

Ability to pseudotype with other viral envelope proteins to increase Target range

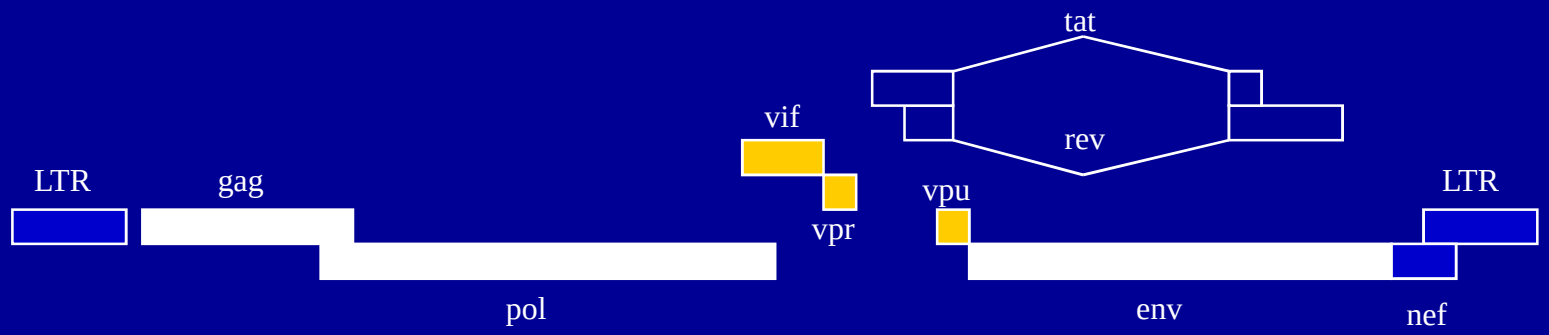
Cons

Safety concerns

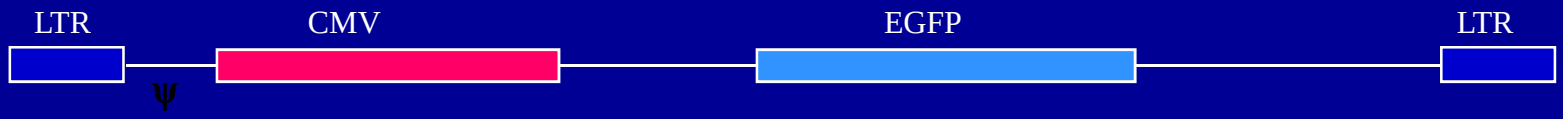
Integration

HIV-1 based vectors- Safety

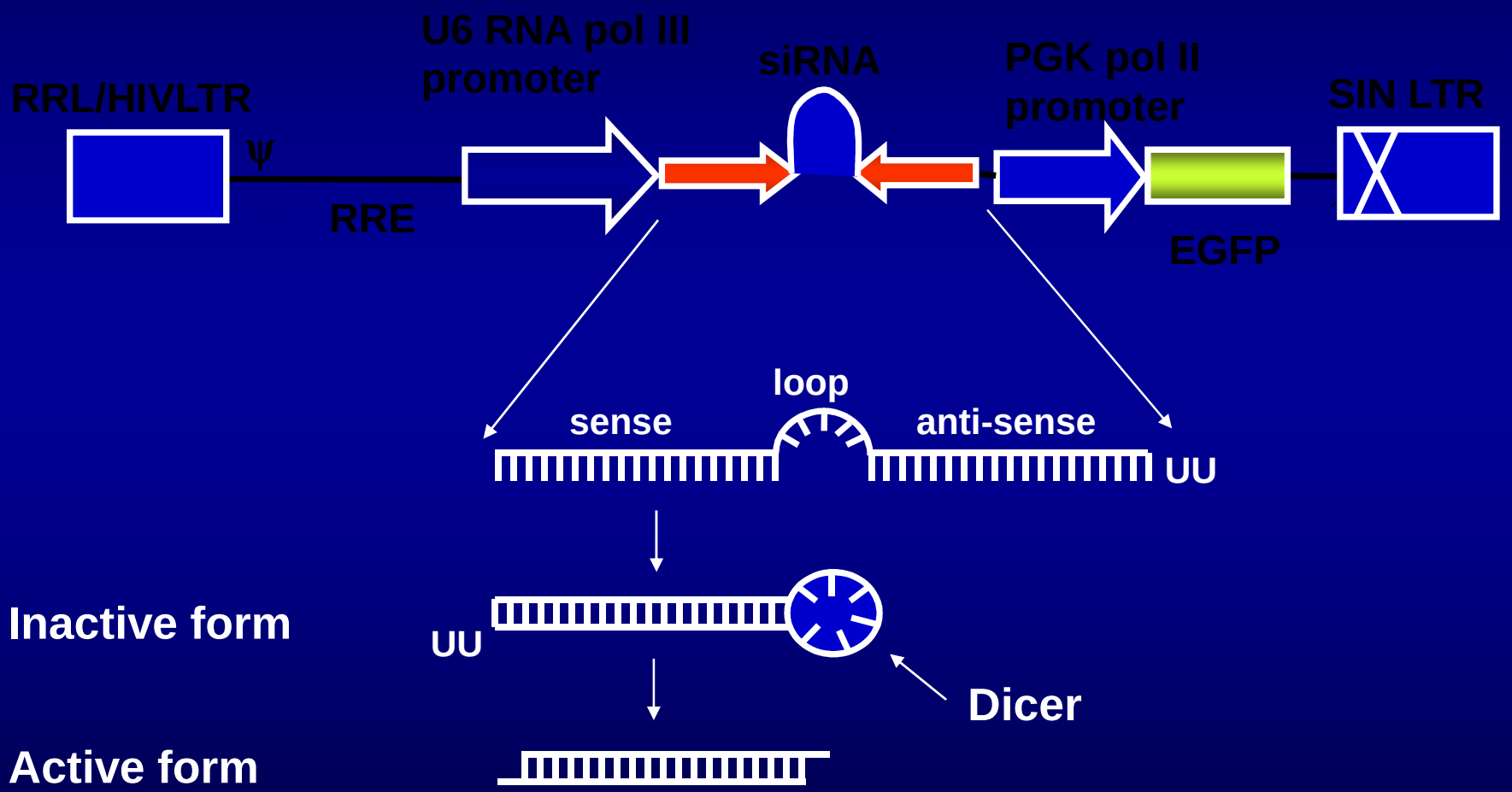
HIV-1



HR'CMVEGFP



Lentivirus vector for siRNA delivery



Viral vectors used for gene delivery

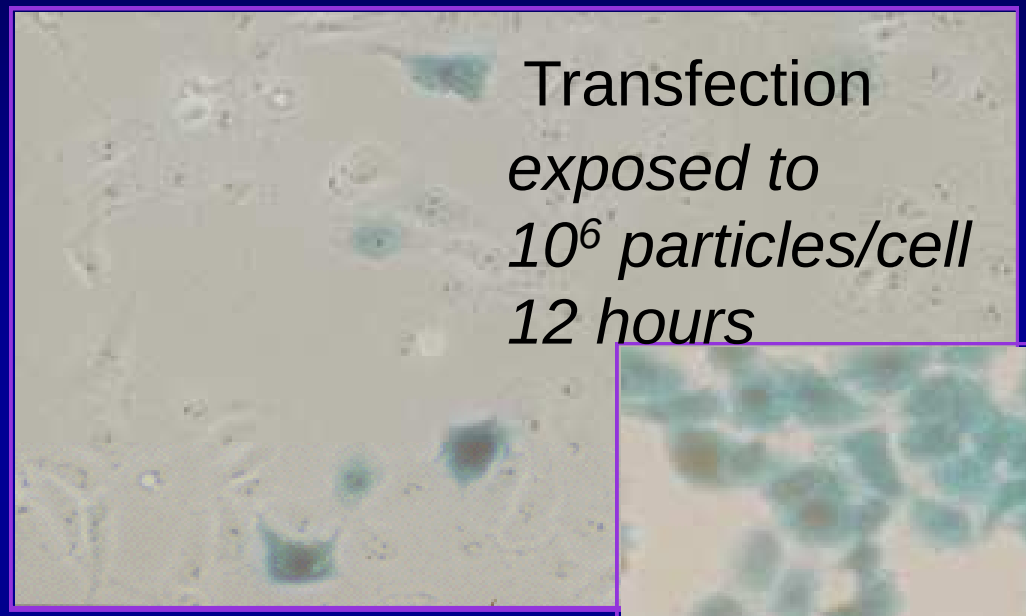
Vector	Packaging capacity	Host range	Clinical trials
AAV	Low <4 kb	Broad, infects both non-dividing and dividing cells	+
Adenovirus	Medium <7.5 kb	Broad low transduction of neurons	+
Alphaviruses	Medium <7.5 kb	Broad, neuron and glial cell-specific strains	+
Herpes simplex virus	High >30 kb	Broad, neurons, stem cells, muscle cells	–
Lentivirus	Medium 8 kb	Broad, dividing and non-dividing cells	–
Retrovirus	Medium 8 kb	Restricted, dividing cells only	+

Viral vectors used for gene delivery

Vector	Features
AAV	Slow expression onset, genome integration (±), long-term expression, inefficient large-scale virus production
Adenovirus	Transient expression, strong immunogenicity
Alphaviruses	Transient, but extreme, expression levels; low immunogenicity
Herpes simplex virus	Latent infection, long-term expression, low toxicity (mutants)
Lentivirus	Genome integration, long-term expression, safety concerns low titers, production inefficient
Retrovirus	Genome integration, long-term expression

Transfection versus Infection

UNIFR
Rusconi
2005



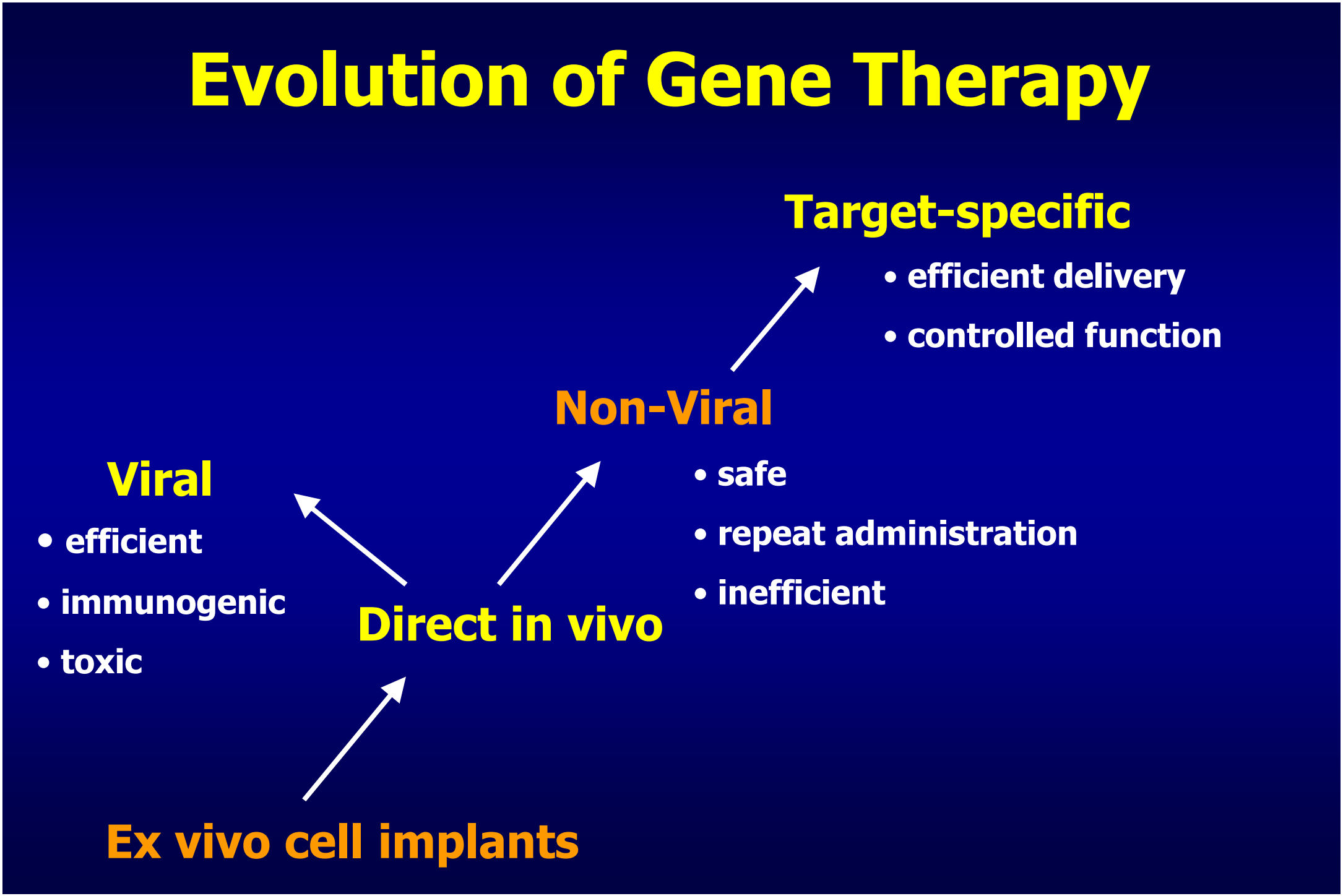
Transfection
exposed to
10^6 particles/cell
12 hours

Infection
exposed to
1 particle/cell
30 min

Ergo

- virally mediated gene transfer is millions of times more efficent than nonviral transfer (when calculated in terms of transfer/particle)

Evolution of Gene Therapy



Methods for Gene Delivery

	Transduction efficiency	Integration efficiency
Chemical		
Calcium-phosphate transfection	Low	Low
DEAE-dextran transfection	Low	Low
Physical		
Electroporation	Low	Low
Microinjection	High	Low
Particle bombardment	High	Low
Fusion		
Liposomes	Low	Low
Receptor-mediated endocytosis		
DNA-protein complexes	High	Low
Viral envelope/capsid-DNA complexes	High	Low
Recombinant viruses		
Adenovirus	High	Low
Adeno-associated virus (AAV)	High	High
Herpes simplex virus	Low	Low
Human immunodeficiency virus (HIV)	High	High
Moloney murine leukemia virus (MoMLV)	High	High
Vaccinia virus	High	Low

Gene therapy for cancer I

- Therapeutic genes
  - Immunomodulatory genes
  - Gene directed enzyme prodrug therapy
  - Cell cycle control and apoptosis
  - Down regulation of oncogenes
- Selective gene expression
- Viral oncolysis

Immunomodulatory genes

Cytokines

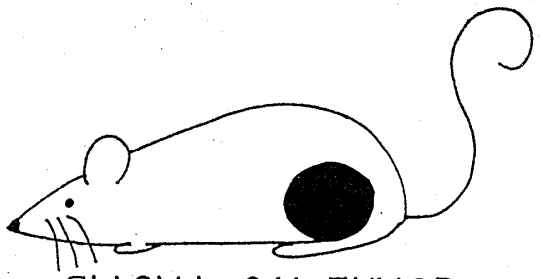
IL-2, IL-4, GM-CSF, etc.

Costimulatory genes

B7-1, B7-2

Tumor-associated antigen genes

prostate specific antigen, p53, etc



GLIOMA 261 TUMOR
GROWN S.C.

EXCISED,

TRYPSINIZED,
CULTURED
IN VITRO

GLIOMA 261
CELLS
GROWN IN
CELL CULTURE

TRANSDUCTION

OF CYTOKINE
GENES

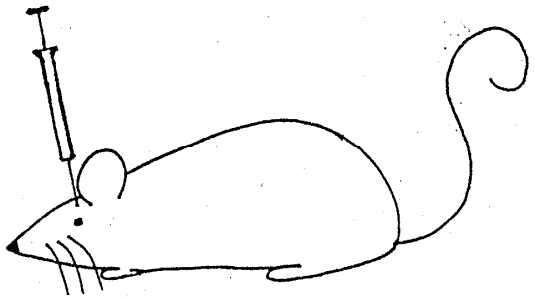
MEASURING
CYTOKINE LEVEL
IN MEDIUM
OF CULTURED
GLIOMA CELLS

20 Gy

γ-IRRADIATION

CELLS
COLLECTED

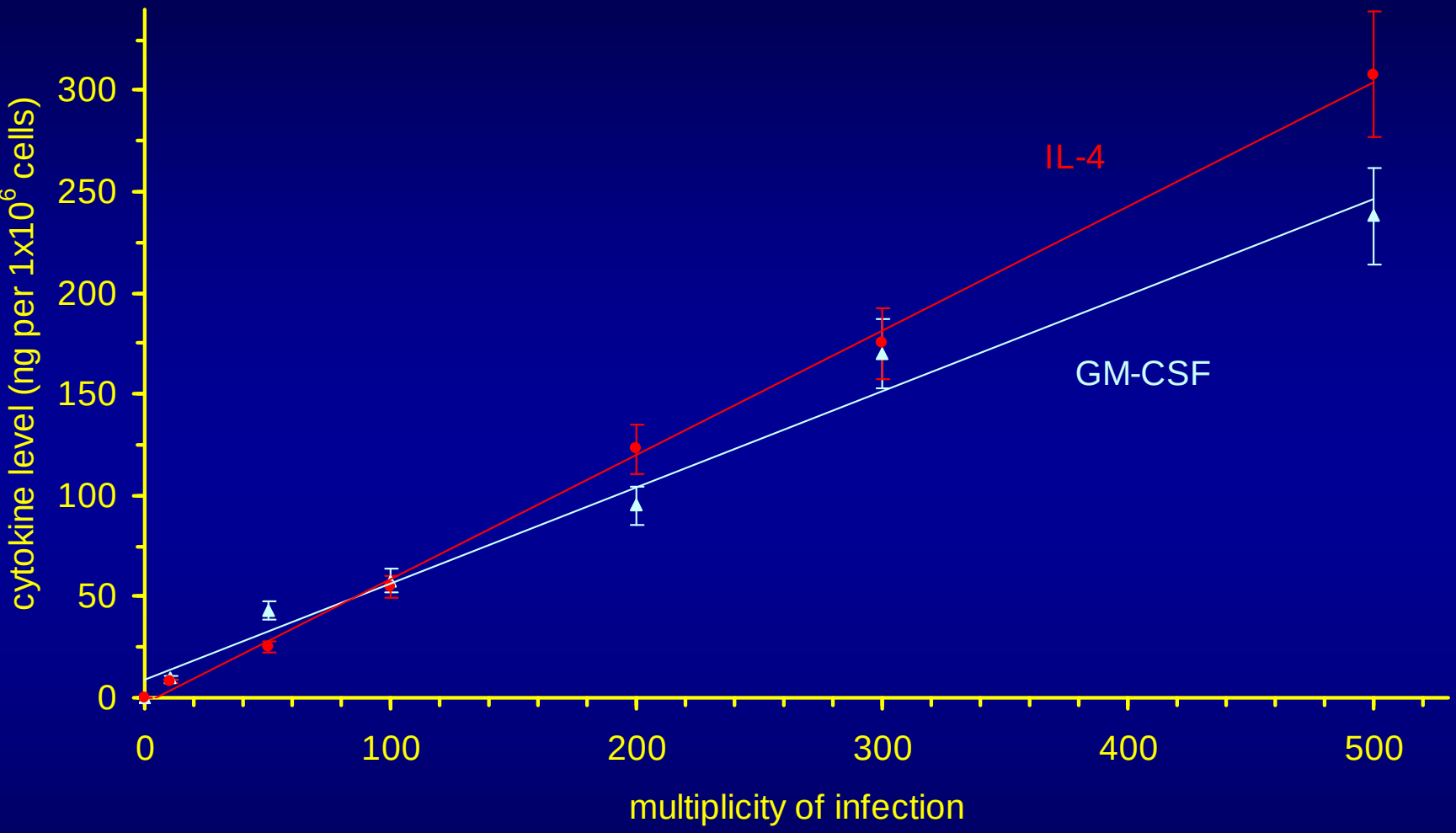
VACCINATION



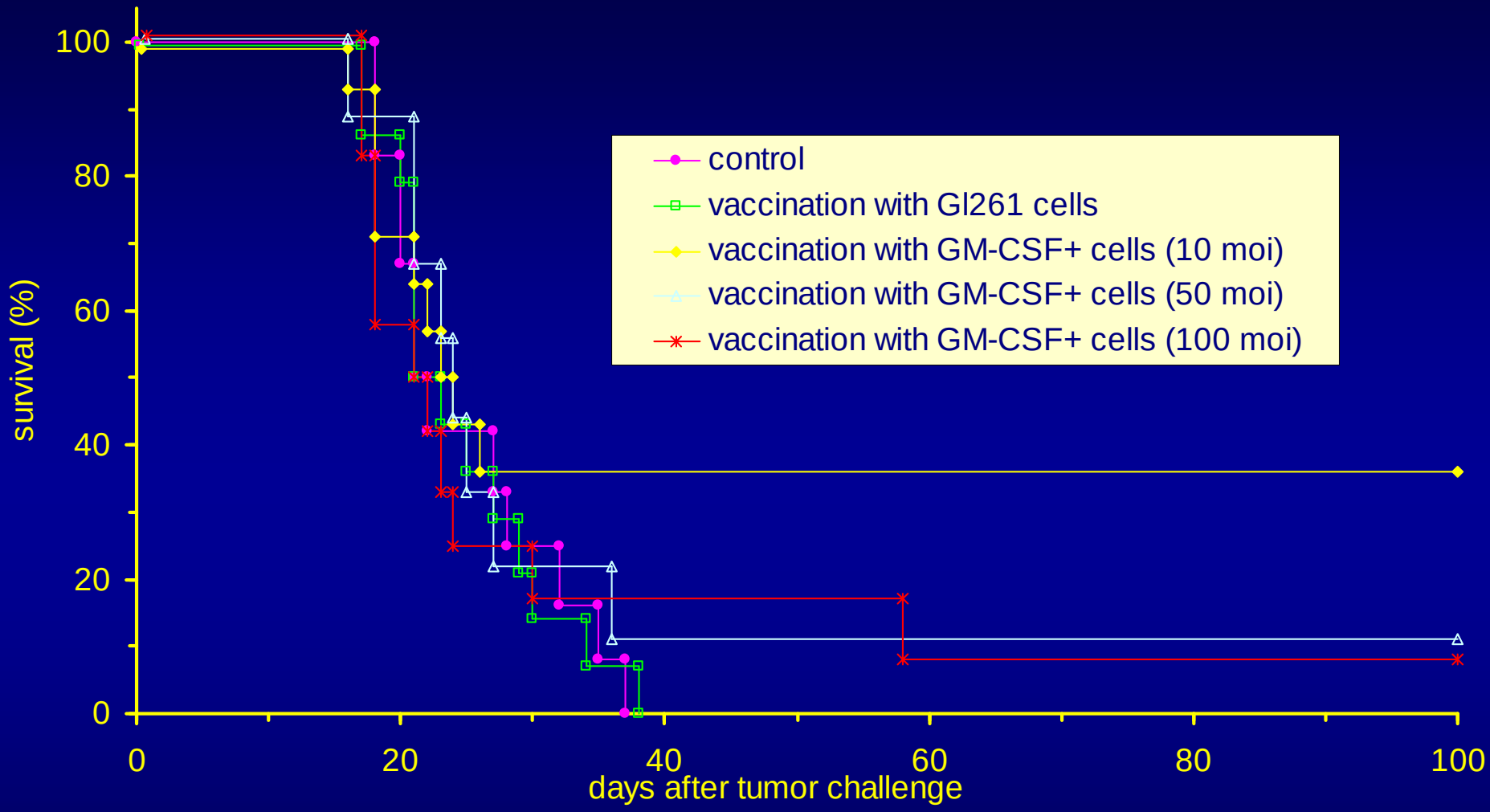
TRANSPLANTATION
GLIOMA CELLS I.C.

GLIOMA BEARING MICE

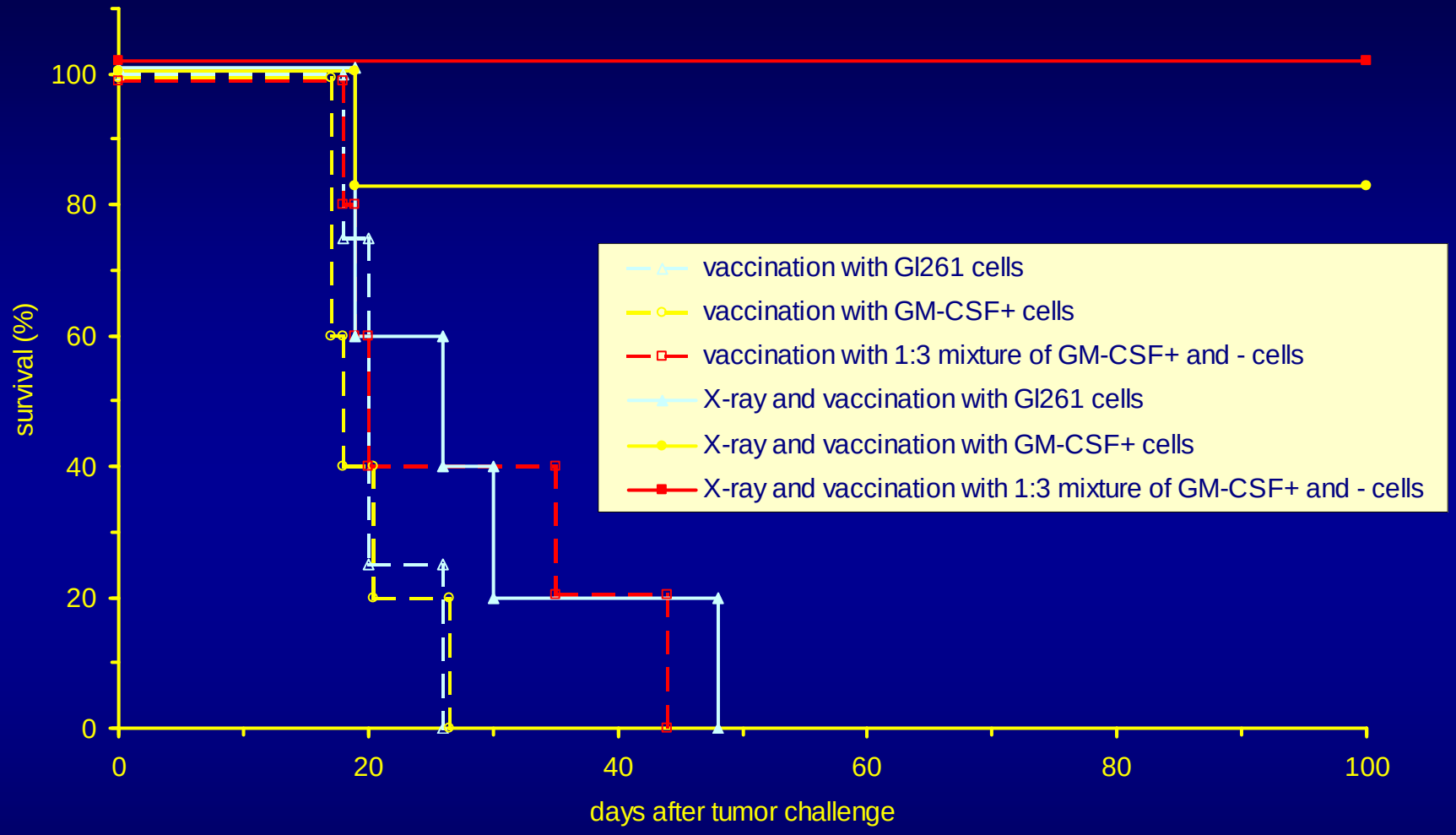
SURVIVAL OF ANIMALS
FOLLOWED



Cytokine levels in the medium of Gl261 cells 24 hrs after infection with either AdexmIL-4 or AdexmGM-CSF at different moi.

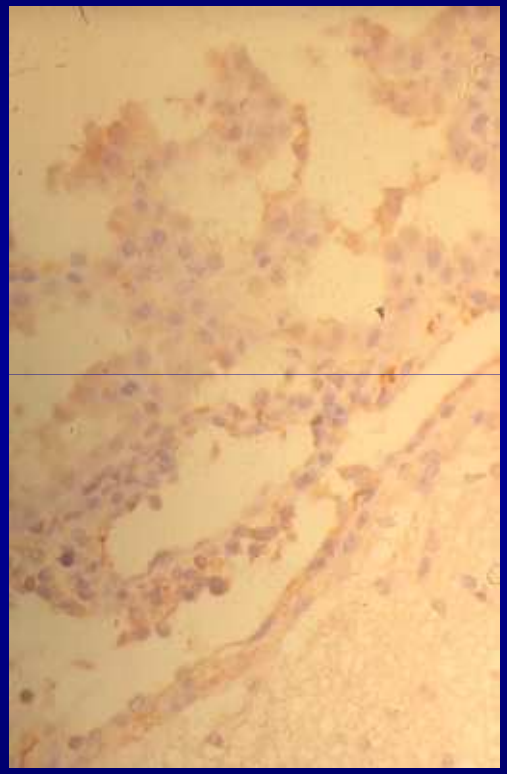


Survival of glioma bearing mice after treatment with mGM-CSF producing cancer vaccines. Gl261 cells were transduced at different moi with AdexmGM-CSF. Cells were harvested and used 48 h after transduction.

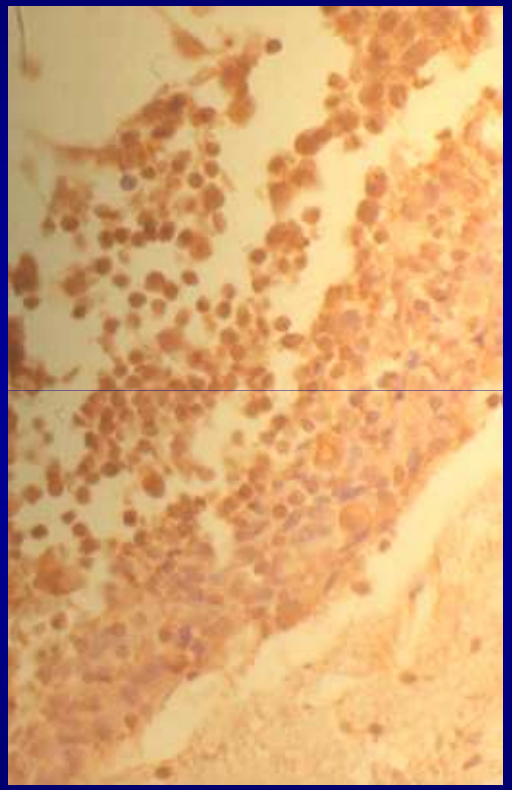


Survival of brain tumor bearing mice after combined treatment with mGM-CSF-producing vaccine and local radiotherapy. Mice were treated with 6 Gy X-ray and mixtures of mGM-CSF plus and minus cells.

Infiltration of mouse gliomas by CD4+ cells after vaccination with cytokine producing Gl261 cells

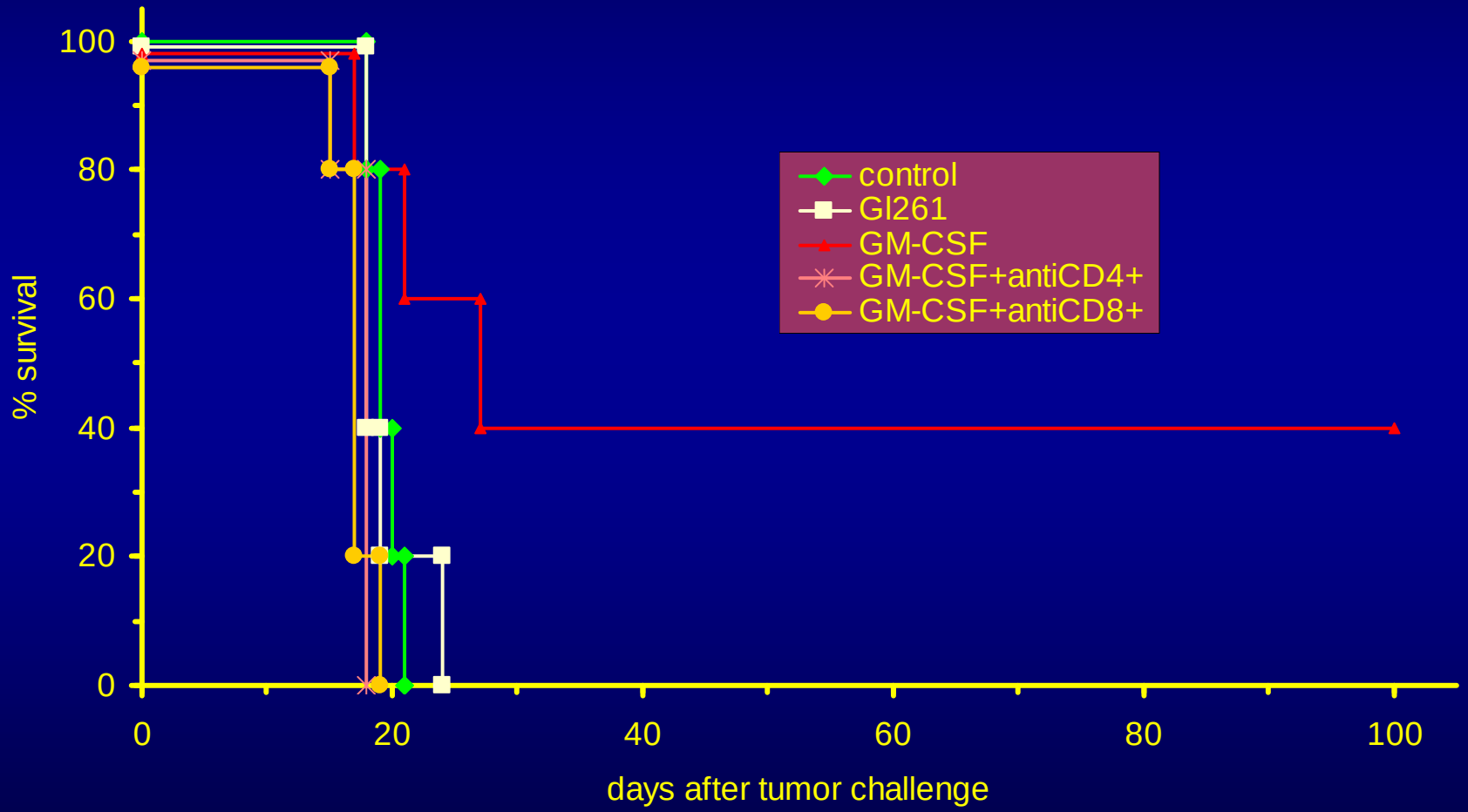


Vaccination with
Gl261 cells



Vaccination with
GM-CSF expressing
Gl261 cells

Survival of brain tumor bearing mice after vaccination with GM-CSF-producing Gl261 cells and after depletion of $CD4^+$ and $CD8^+$ cells



Gene directed enzyme prodrug therapy

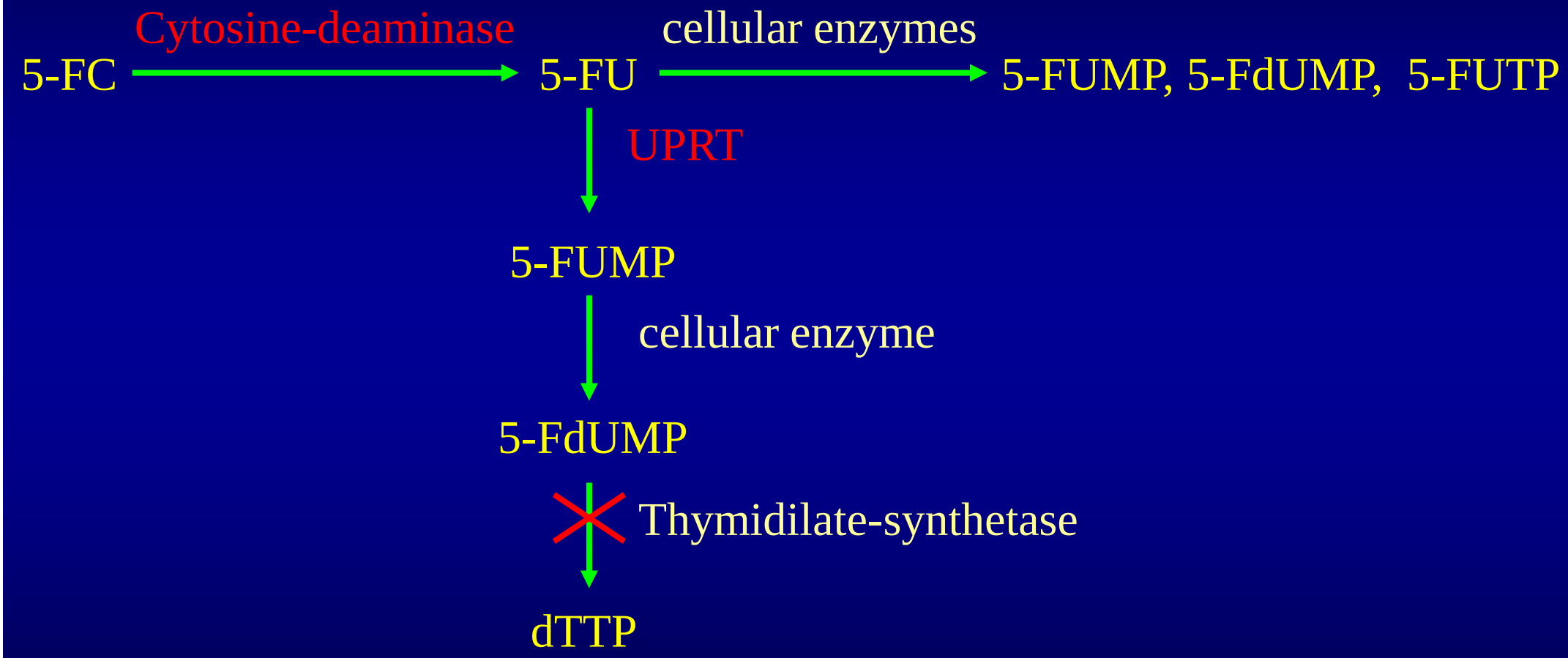
Most of the anticancer chemotherapeutic agents are undergoing metabolic activation in the cells.

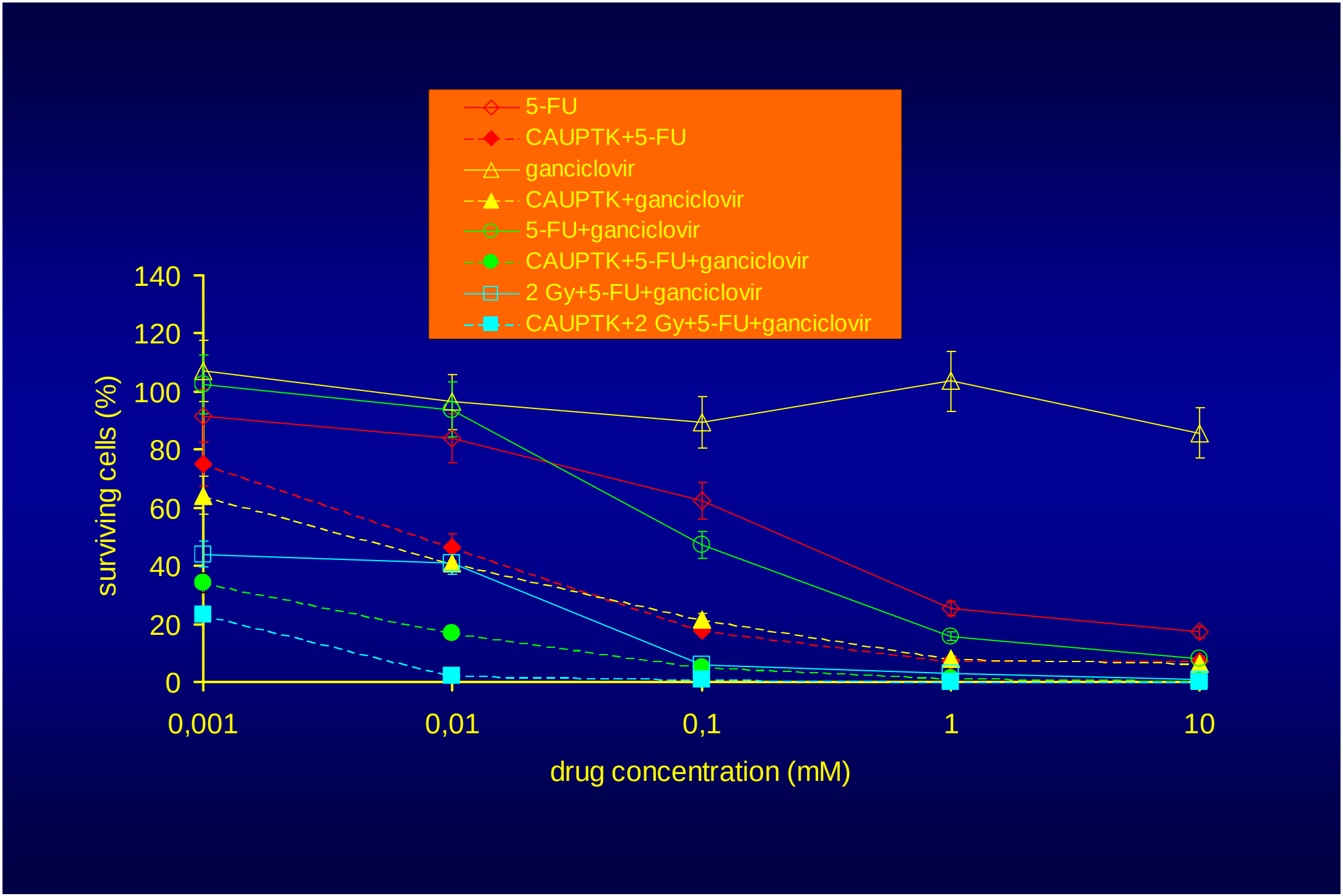
After selective introduction of the metabolic enzymes into the cancer cells an enhanced local toxicity is expected.

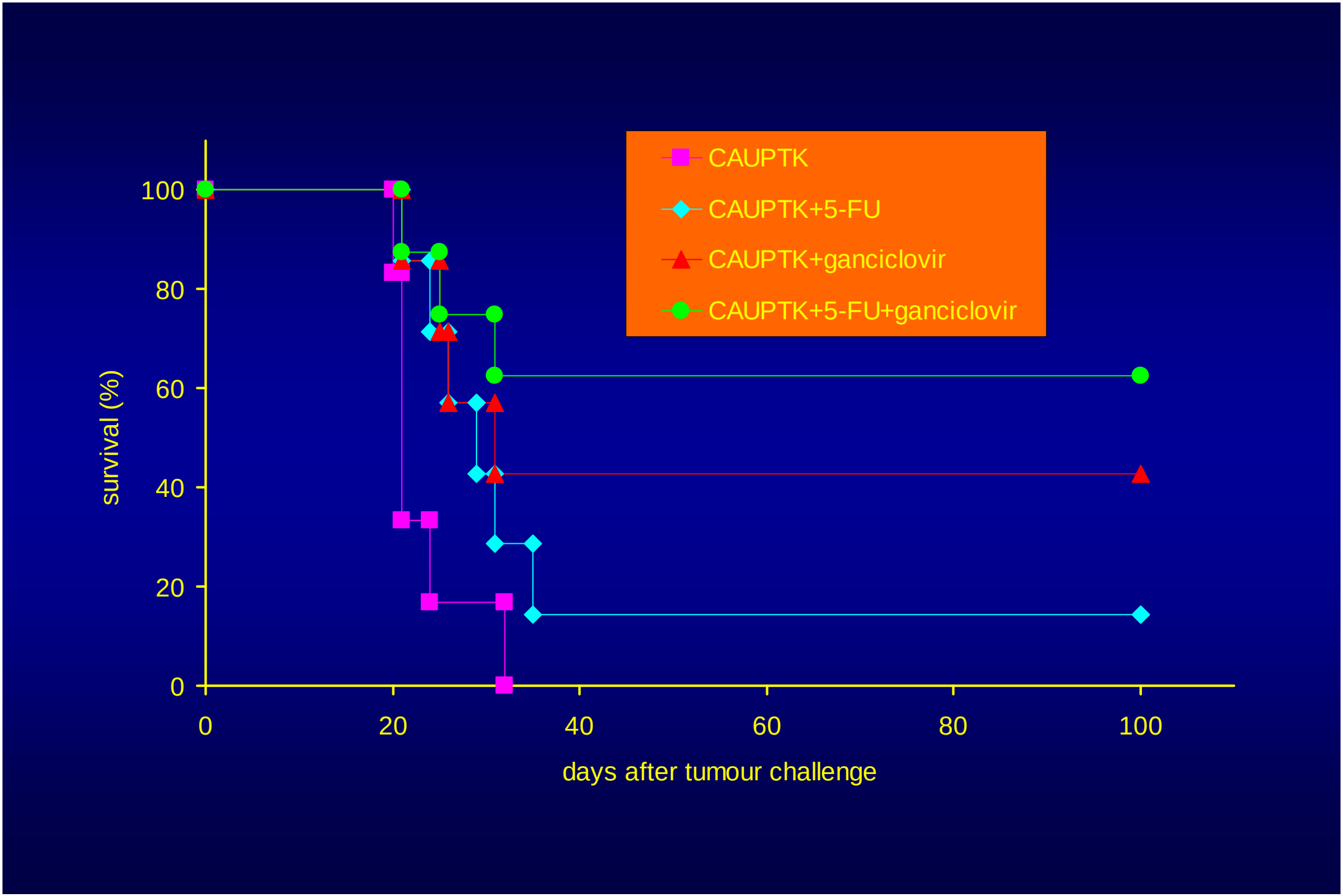
Gene-directed enzyme prodrug therapy (GDEPT)

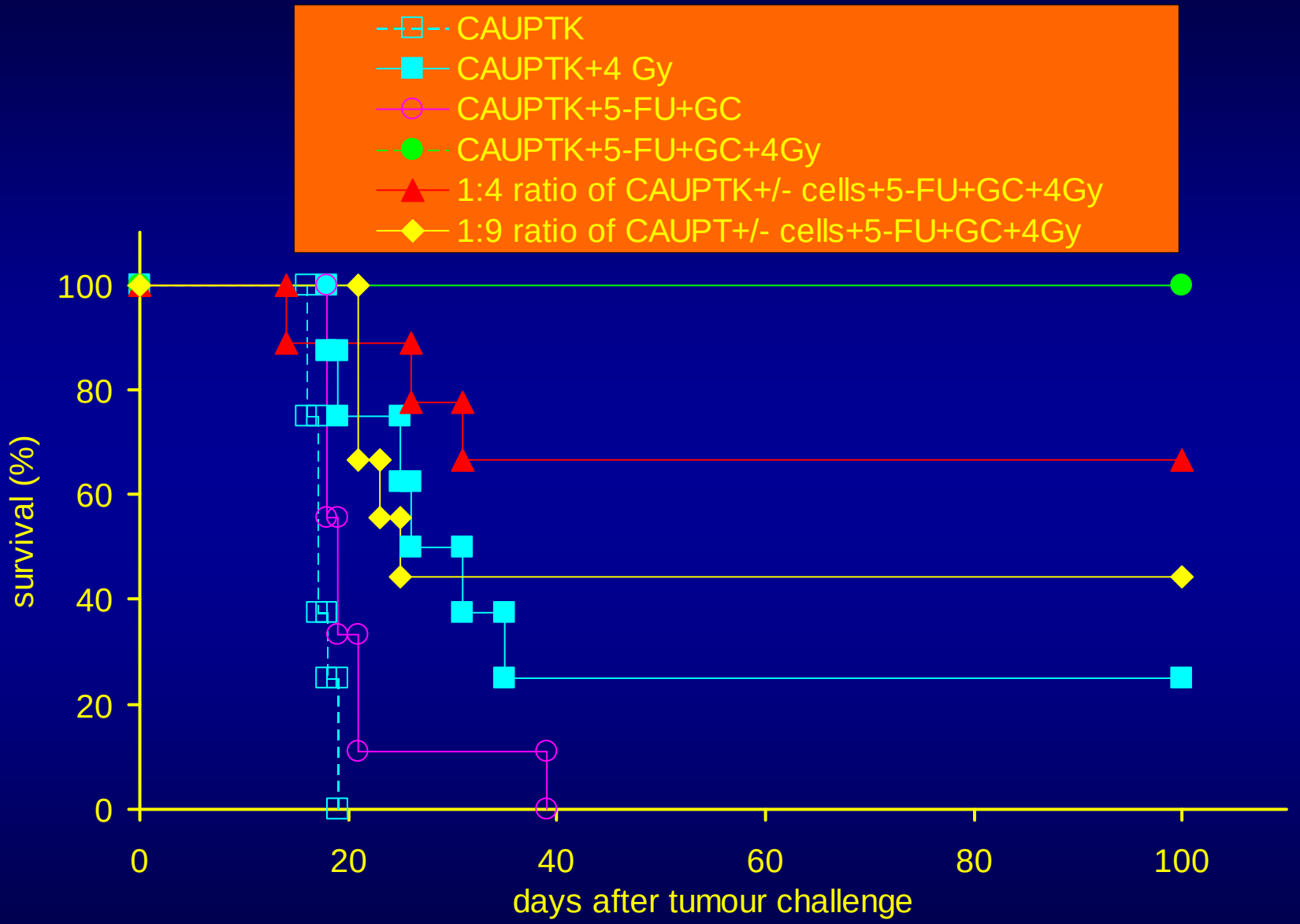
Thymidine-kinase (TK) – ganciclovir (guanosine derivative, GC) pathway:

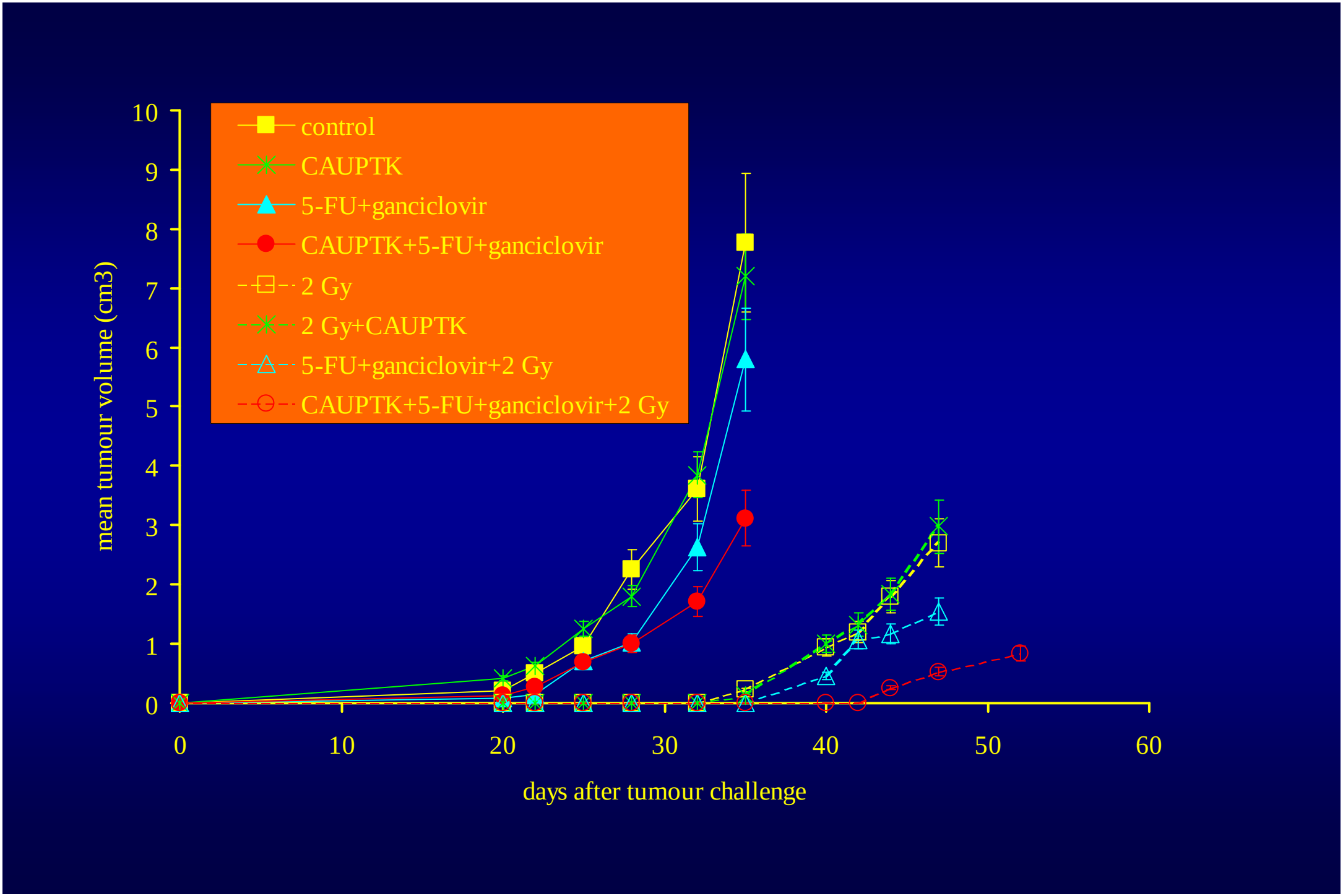
Uracyl-phosphoribosyl-transferase (UPRT) + 5-FU system




5-FU
CAUPTK+5-FU
ganciclovir
CAUPTK+ganciclovir
5-FU+ganciclovir
CAUPTK+5-FU+ganciclovir
2 Gy+5-FU+ganciclovir
CAUPTK+2 Gy+5-FU+ganciclovir
surviving cells (%)
140
120
100
80
60
40
20
0
0,001
0,01
0,1
1
10
drug concentration (mM)


CAUPTK
CAUPTK+5-FU
CAUPTK+ganciclovir
CAUPTK+5-FU+ganciclovir
survival (%)
100
80
60
40
20
0
0
20
40
60
80
100
days after tumour challenge


CAUPTK
CAUPTK+4 Gy
CAUPTK+5-FU+GC
CAUPTK+5-FU+GC+4Gy
1:4 ratio of CAUPTK+/- cells+5-FU+GC+4Gy
1:9 ratio of CAUPT+/- cells+5-FU+GC+4Gy
survival (%)
100
80
60
40
20
0
0
20
40
60
80
100
days after tumour challenge


control
CAUPTK
5-FU+ganciclovir
CAUPTK+5-FU+ganciclovir
2 Gy
2 Gy+CAUPTK
5-FU+ganciclovir+2 Gy
CAUPTK+5-FU+ganciclovir+2 Gy
mean tumour volume (cm3)
days after tumour challenge
0
1
2
3
4
5
6
7
8
9
10
0
10
20
30
40
50
60

TREATMENT OF EXPERIMENTAL GLIOMAS WITH RADIOTHERAPY, RADIOSENSITIZING AND CHEMOSENSITIZING GENE THERAPY

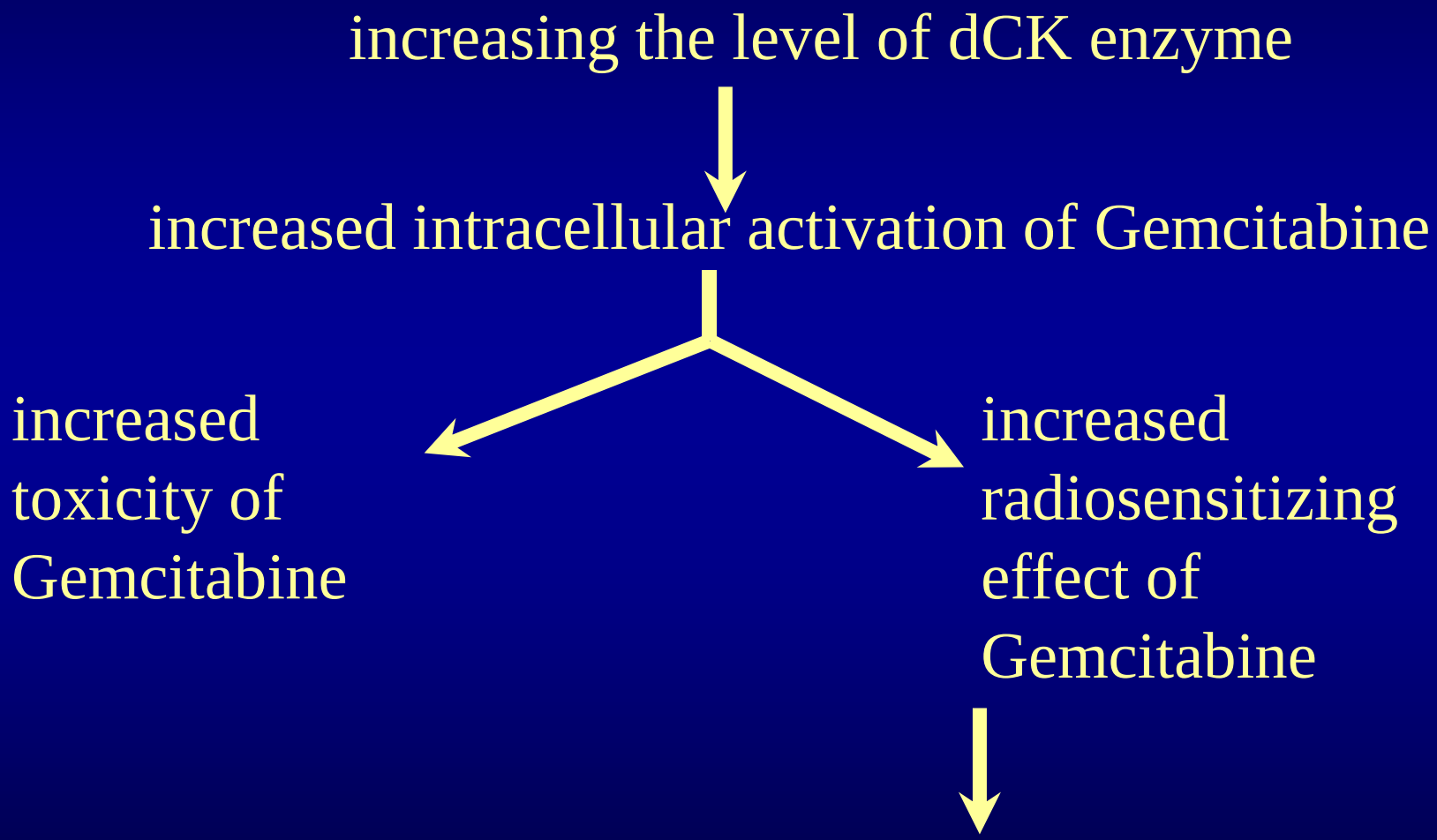
Theoretical background:

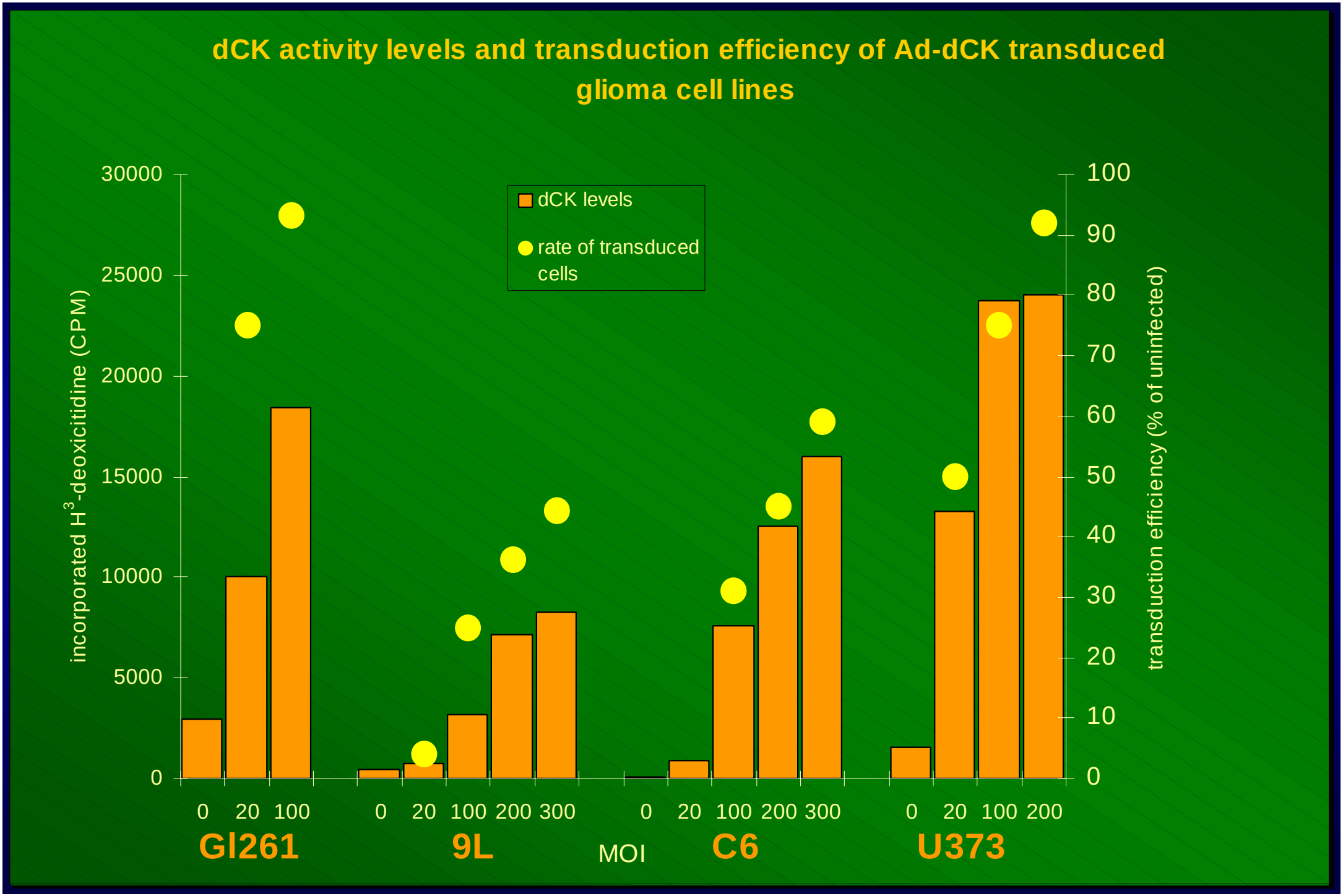
Gene-directed enzyme pro-drug therapy approach

Pro-drug: **Gemcitabine** (2',2'-difluorodeoxycytidine, Gemzar)

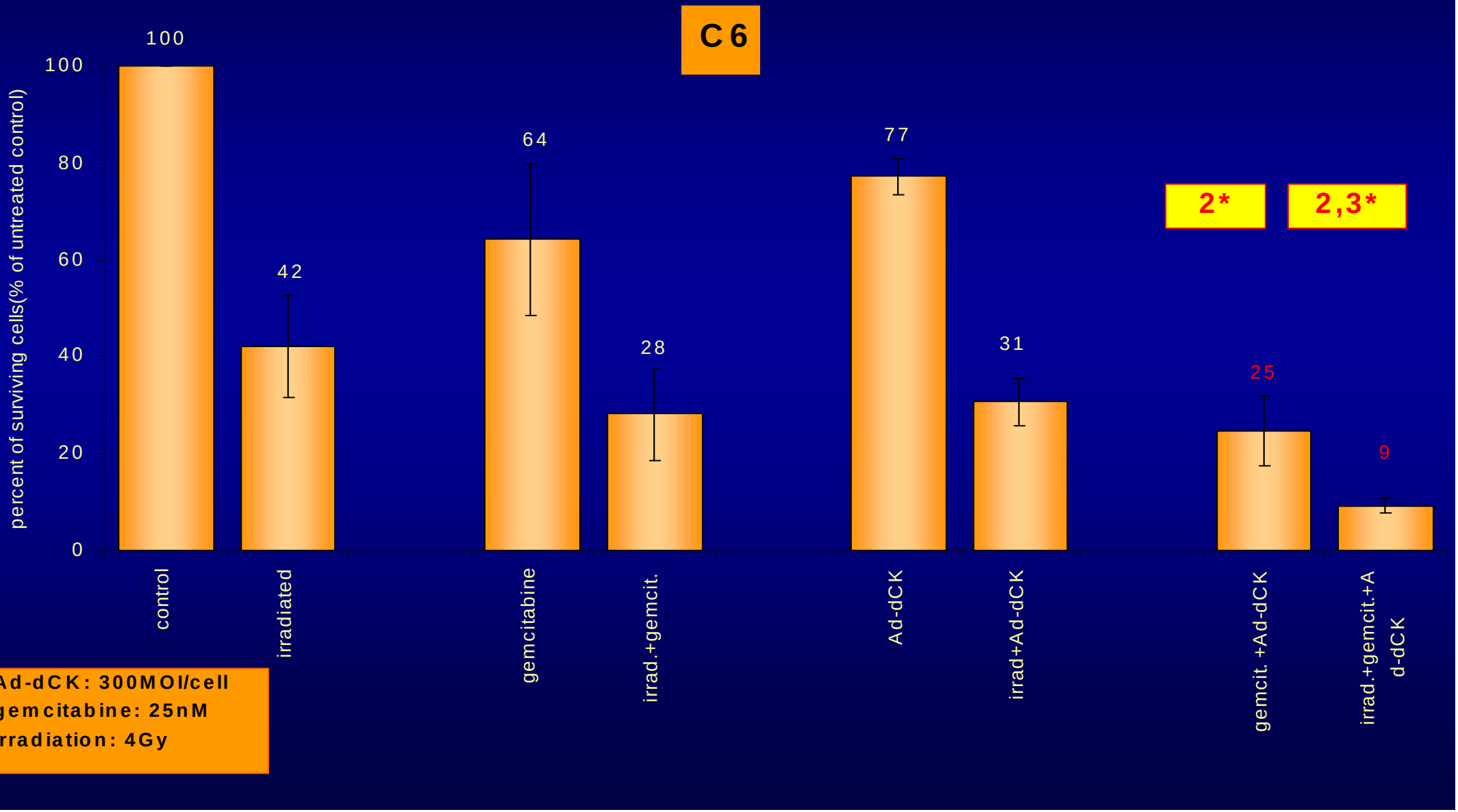
Gene encoding for the pro-drug activating enzyme: **deoxicytydine-kinase** (dCK)

HYPOTHESIS

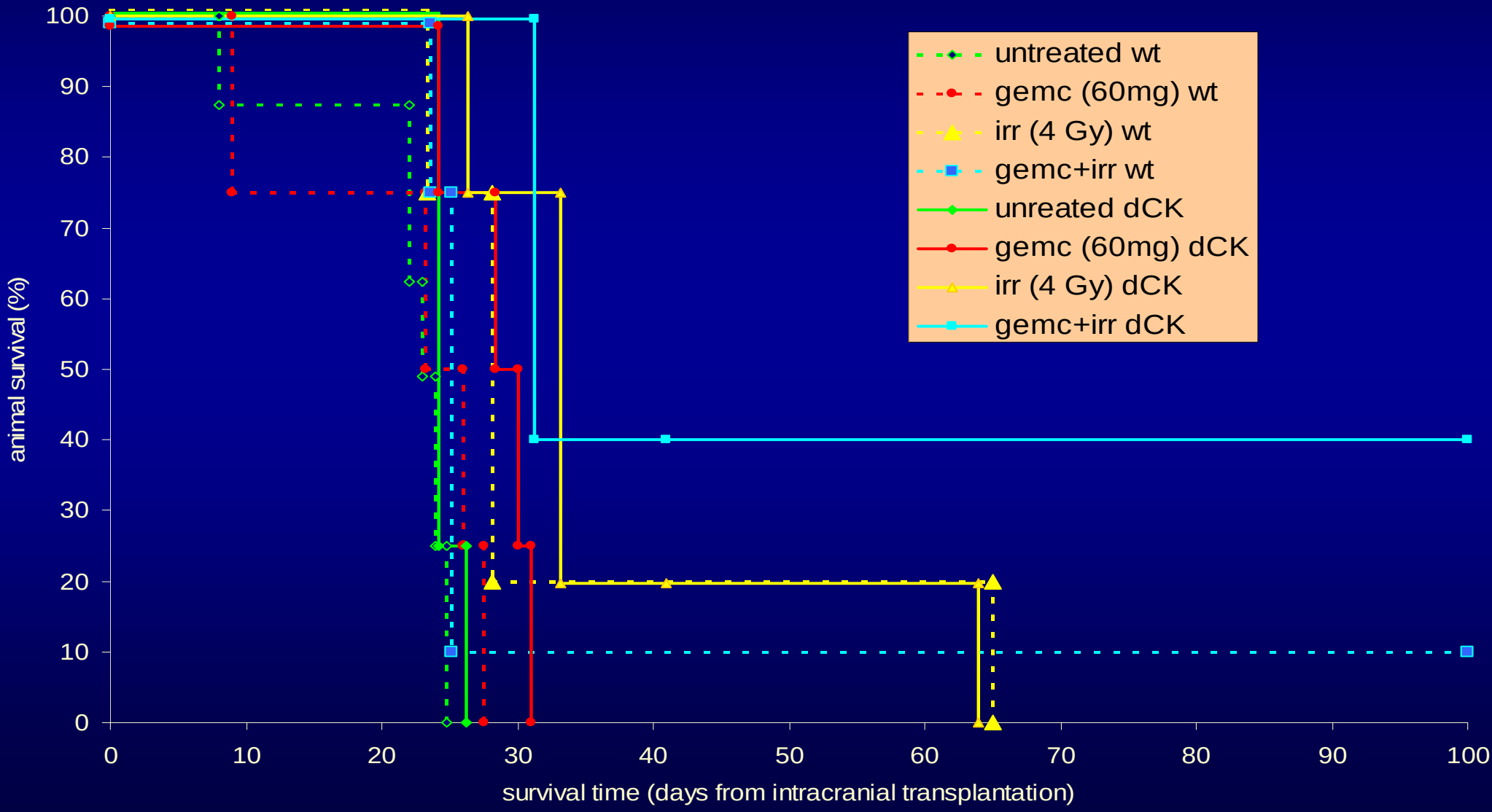

increasing the level of dCK enzyme
increased intracellular activation of Gemcitabine
increased toxicity of Gemcitabine
increased radiosensitizing effect of Gemcitabine
improved efficacy of radiotherapy


dCK activity levels and transduction efficiency of Ad-dCK transduced glioma cell lines
dCK levels
rate of transduced cells
incorporated H3-deoxicitidine (CPM)
transduction efficiency (% of uninfected)
30000
25000
20000
15000
10000
5000
0
100
90
80
70
60
50
40
30
20
10
0
0 20 100
0 20 100 200 300
0 20 100 200 300
0 20 100 200
Gl261
9L
MOI
C6
U373

Combined effect of dCK overexpression, Gemcitabine treatment and irradiation in the four glioma cell lines in vitro



Survival of tumor bearing rats transplanted intracranially with wild type and dCK overexpressing C6 glioma cells and treated with Gemcitabine and local irradiation



Cell cycle control and apoptosis

p53

E2F-1

p21

p53

- tumor suppressor gene
- keep cell numbers down by stopping cells from multiplicity or by promoting cell death
- Inactivated in most human cancers

A major goal of molecular oncology:

Identification of means to kill cells lacking p53 function

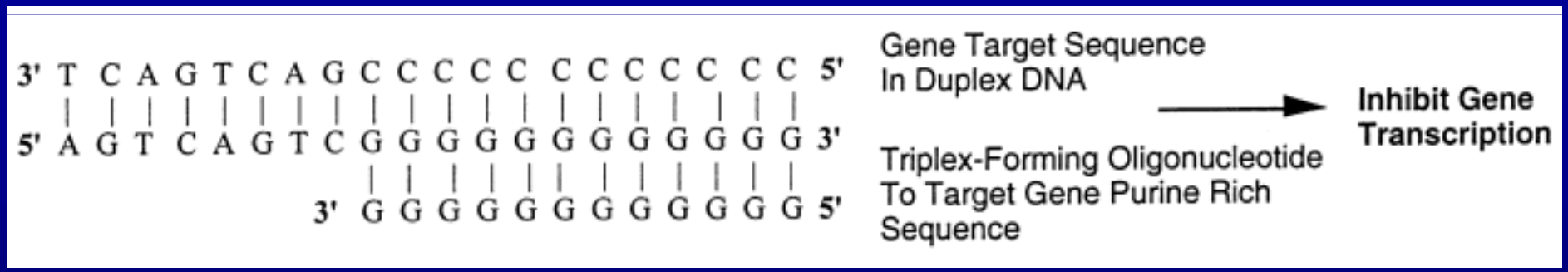
Using p53 to kill cancer cells

- Introduce normal p53 genes into a cancer cell with mutant p53.
- Introduce a small compound that converts mutant p53 proteins from an abnormal to a normal shape.
- Add a protein that attaches itself to mutant p53 and kills cells.
- Stimulate the host's immune response to mutant p53 peptides.
- Introduce drugs that disrupt the interaction between the MDM2 or E6 proteins and p53. (MDM2 and E6 negatively regulate p53; they are present at abnormally high levels in some cancer cells, so 'quench' any normal p53.)

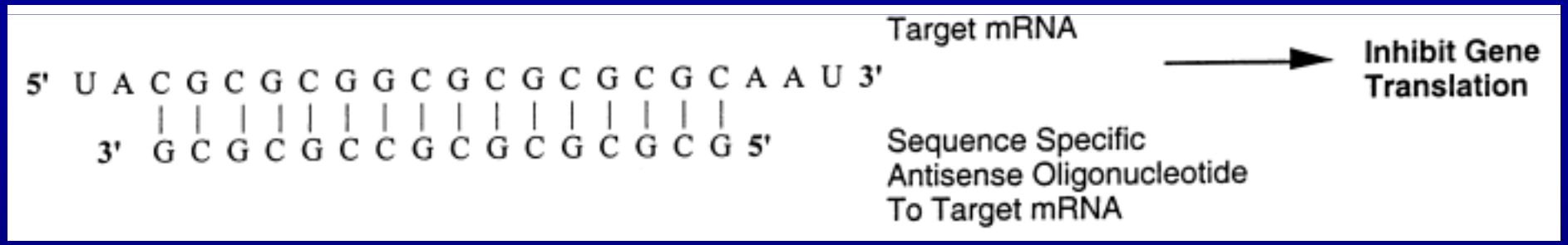
Down-regulation of genes involved in tumor progression

- Triple –helix formation
- Antisense oligonucleotides
- Ribozymes

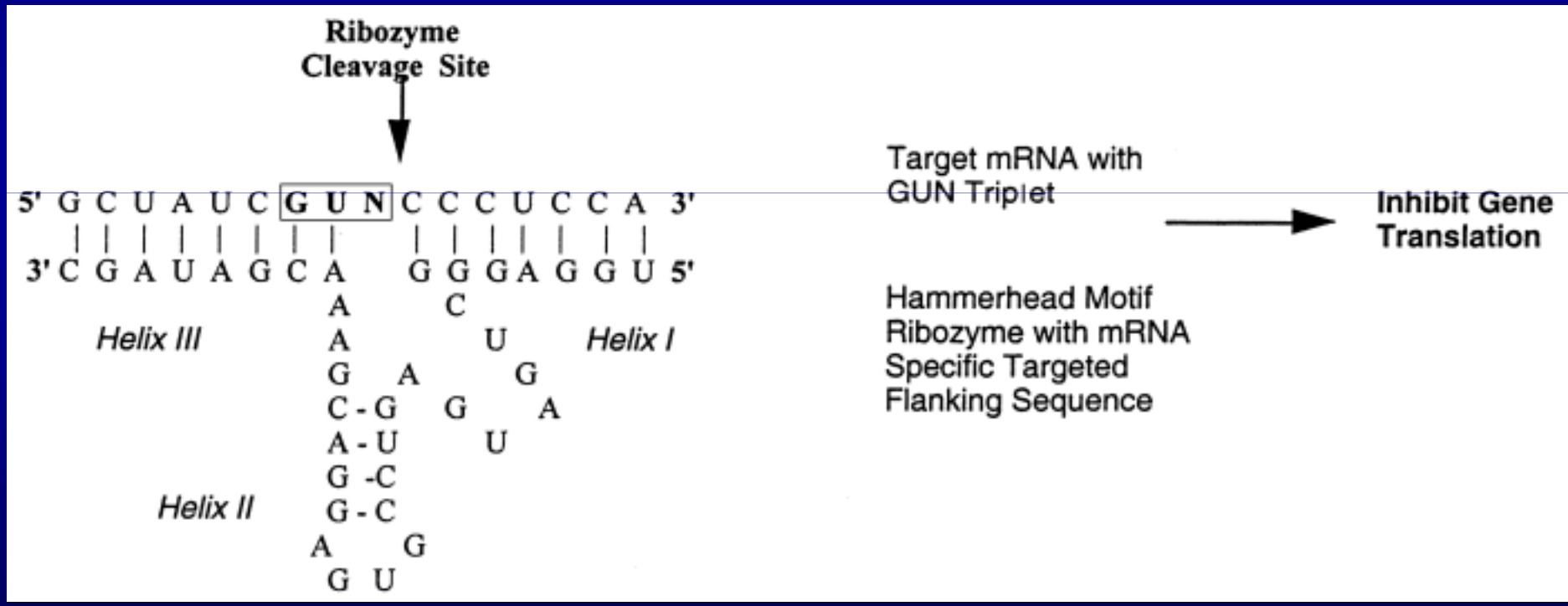
Triple-helix formation to block gene transcription



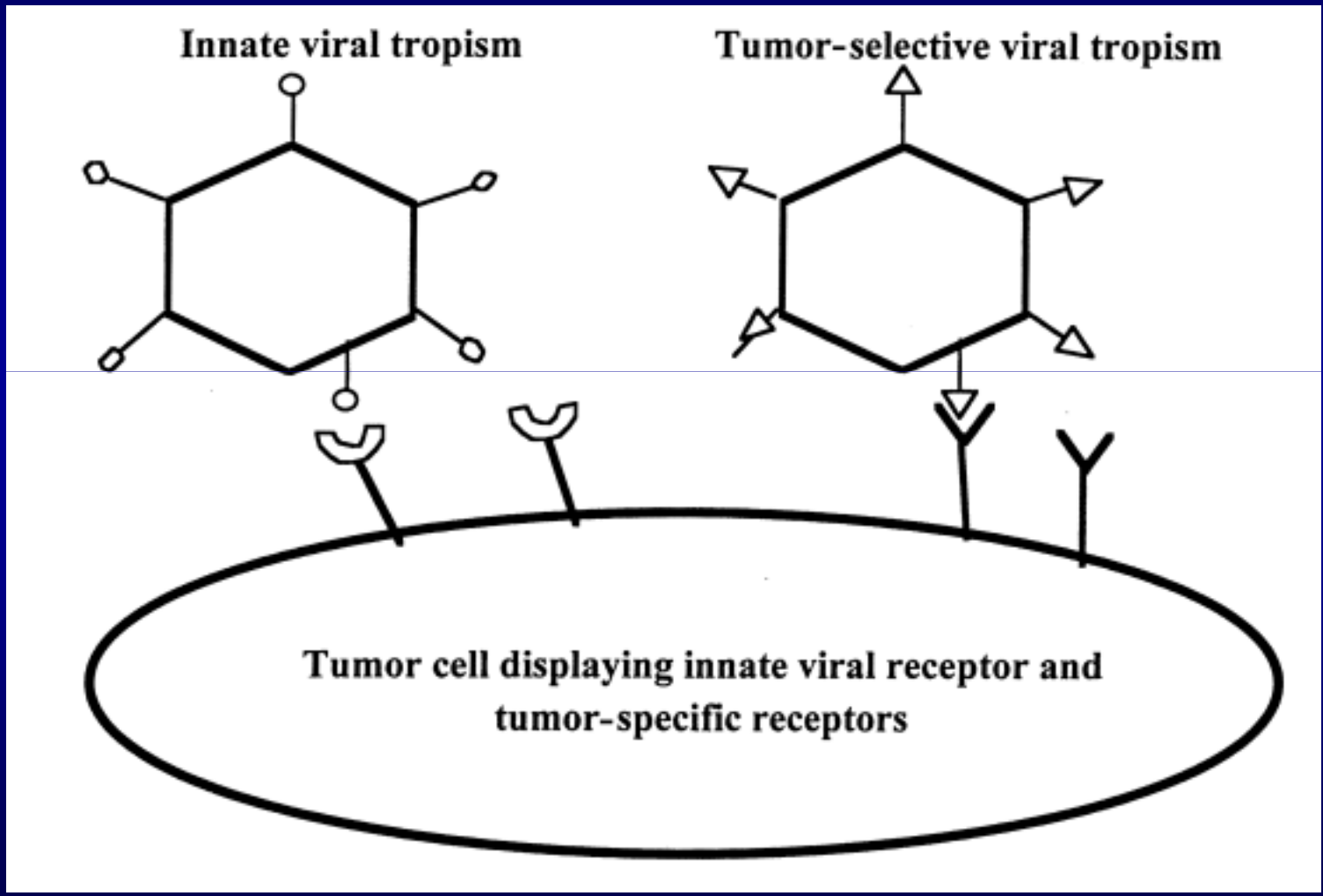
Antisense oligonucleotide to block mRNA translation



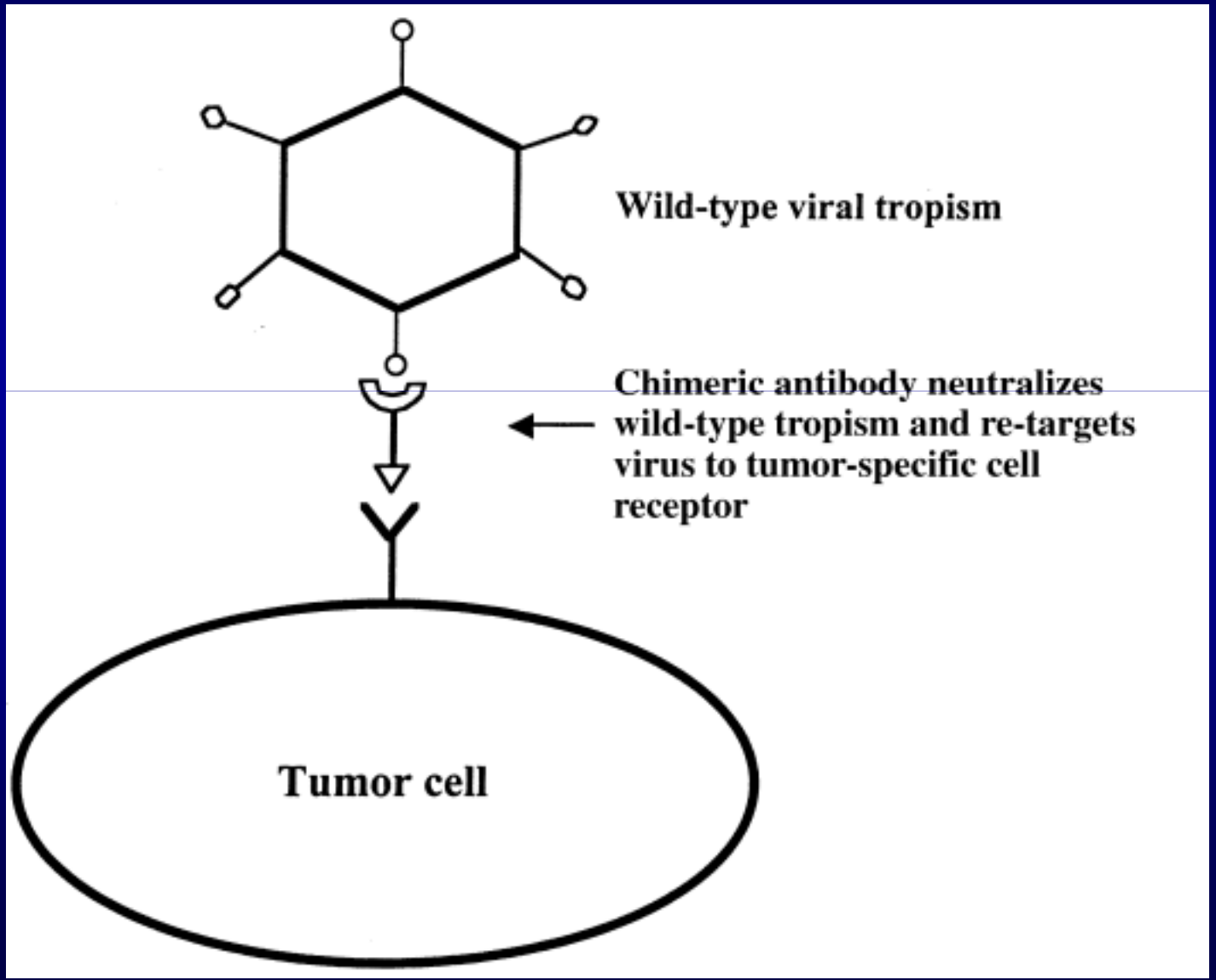
Ribozyme mediated cleavage of mRNA



Genetic modification of viral receptor proteins to target tumor-specific receptors



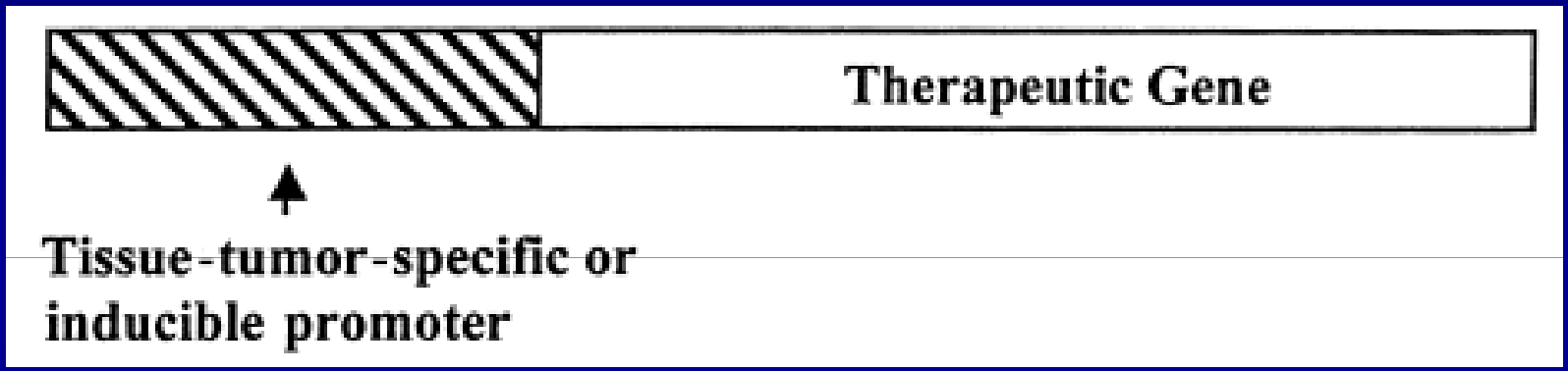
Chimeric antibodies to alter viral tropism for tumor cells



Receptor-mediated targeting

Ligand	Type of Receptor	Cells	Disease
antibody Herceptin®	Antigen HER2 receptor	Breast cancer cells	Cancer
Folate	Folate receptor	Cancer cells	Cancer
Transferrin	Transferrin receptor	Infected RBC	Infection
Galactose	Asialoglycoprotein receptor	Hepatocyte cells	Liver disease
hormone EGF	EGF receptor	Cancer cells	Cancer
tuftsin	Tuftsin receptor	Macrophages of RES	Infectious disease

Transcriptional regulation through selective promoters



Alfa-fetoprotein (hepatoma)
Thyroglobulin (thyroid carcinoma)
Prostate-specific antigen (prostate carcinoma)
Carcinoembryonic antigen (breast, lung, colorectal)

Replicative viral vectors for cancer gene therapy?

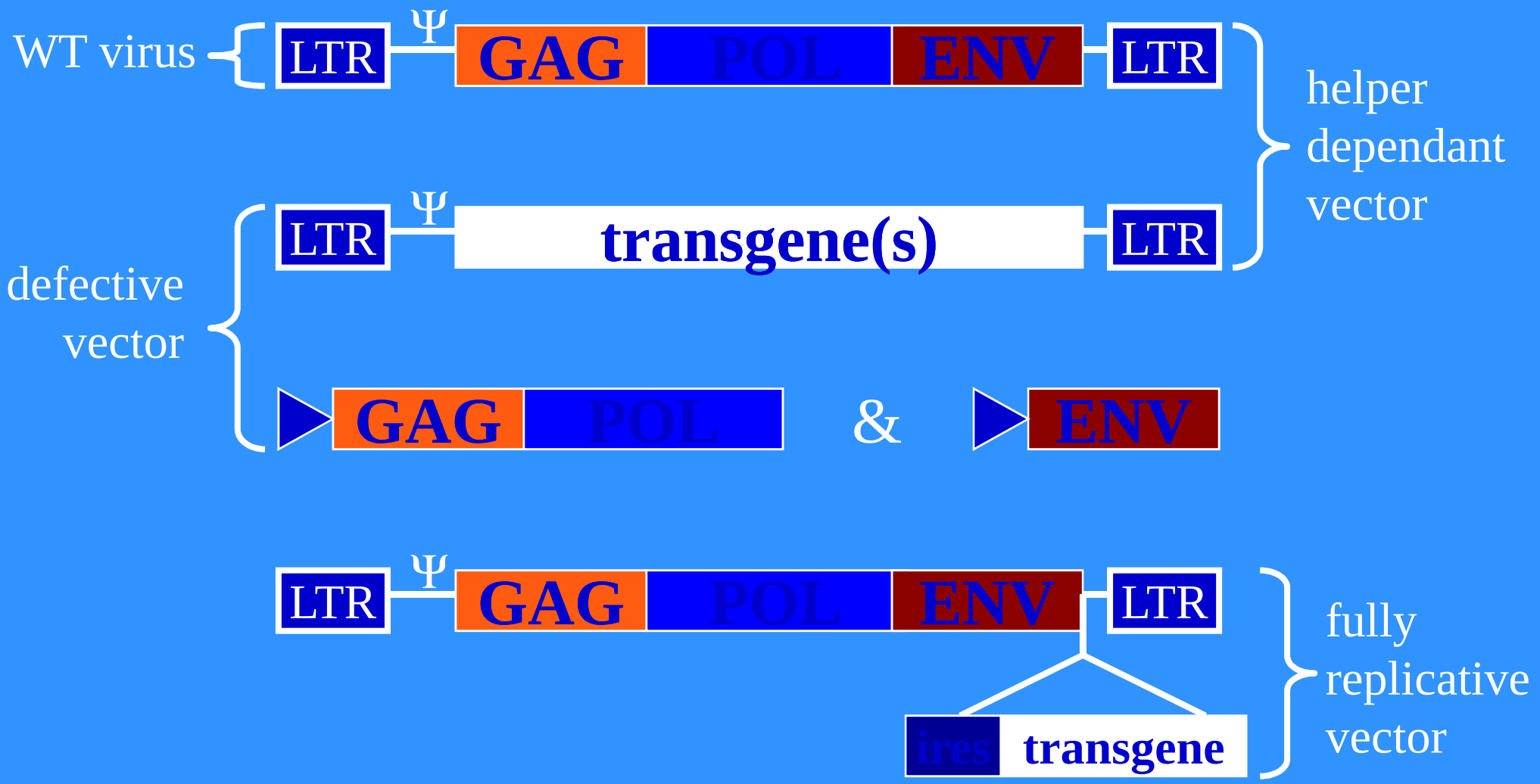
• There is a need for improving gene transfer efficacy, the main current limitation for cancer gene therapy

• Vector propagation should increase gene transfer efficacy by disseminating the transgene within the tumor

• The recombinant replicative viral vectors developed for transgene propagation are mainly based on:

- ✞ Adenoviruses
- ✞ Herpes viruses
- ✞ **Oncoretroviruses (MLV)**

Moloney Murine Leukemia virus
Vectors structure



Viral oncolysis

- Oncolytic adenoviruses
  - ONYX-015 - E1B deleted, replicates in p53 defficient cells
  - Other viruses – Vaccinia, NDV, Herpes simplex
- Conditionally replicating viral vectors
  - Tumor specific promoter drives virus replication

Discussion: An effective cancer molecular treatment :
Oncolytic viruses on the example of ONYX-015

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2002

A) Normal Adenovirus
- can propagate in virtually all cells

B) ONYX-015
- deleted E1B function
- can propagate efficiently only in P53 -deficient cells (e.g. most cancer cells)
- Clinical success Head & Neck Cancer
- Awaiting for further successes (currently in Phase II and III)
- expected to be useful in combination with conventional therapy

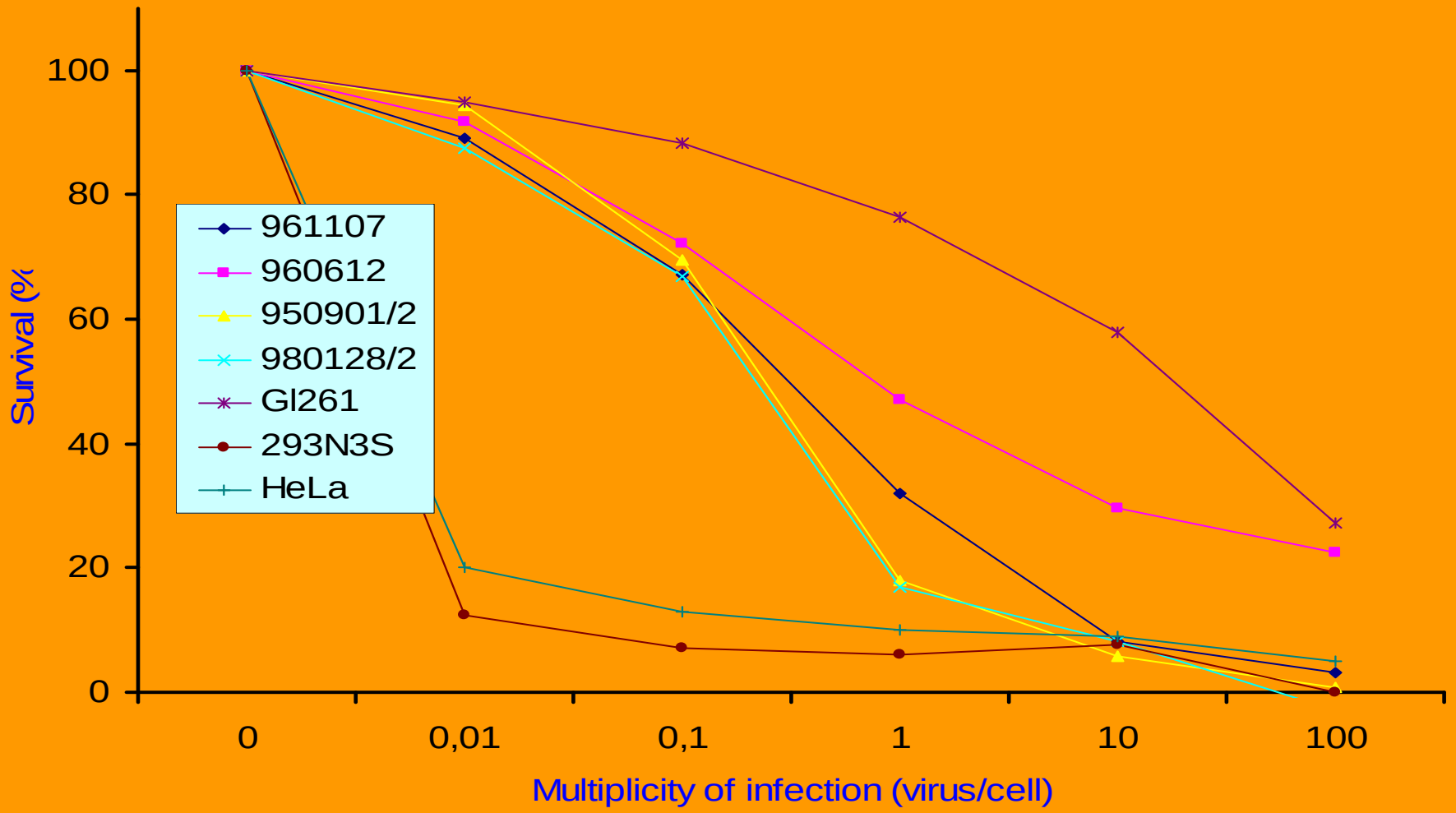
ADVANTAGE:
- the 'drug' has its own dynamics

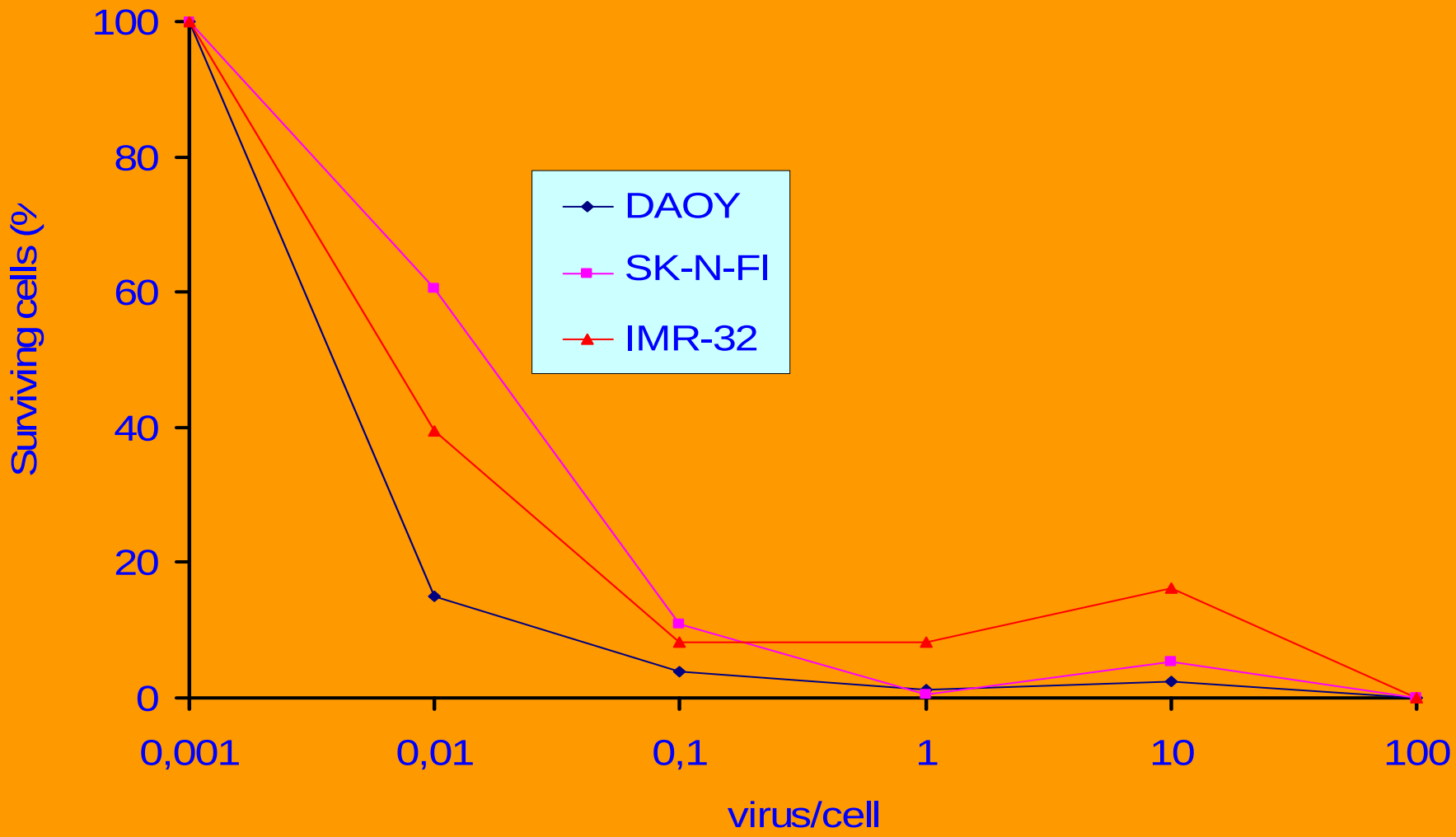
DISADVANTAGE:
- danger of evolving viruses
- unclear if it works in adeno-immune patients
- unclear if if works in immuno-compromised patients (chemotherapy)

TREATMENT OF EXPERIMENTAL GLIOMAS WITH MTH-68/H, CHEMOTHERAPY AND LOCAL TUMOR IRRADIATION

G. Safrany[1], K. Lumniczky[1], C. Csatary[2], L.K. Csatary[2]
[1]National Research Institute for Radiobiology and Radiohygiene, Budapest, Hungary
[2]United Cancer Research Institute, Alexandria, VA, USA

Cytotoxic effect of MTH68 on various cell lines




The effect of MTH68 on DAOY, SK-N-FI and IMR-32 cells
Surviving cells (%
100
80
60
40
20
0
0,001
0,01
0,1
1
10
100
virus/cell
DAOY
SK-N-FI
IMR-32

Heart Disease

In 1995:

- 481,000 deaths related to Coronary Artery Disease (CAD)
- 1,100,000 new or recurrent cases of CAD
- Estimated that 7.2 million people experienced angina to some degree

Treatment

- 434,000 angioplasties performed
- 573,000 Bypasses performed

- 60,000-100,000 patients not good candidates for bypass/angioplasty

  (Possibly up to 250,000 patients a year)

Current Treatments for CAD

- Percutaneous Transluminal Coronary Angio plasty or PTCA (434,000)
- Coronary Artery Bypass Graft (CABG) “cab bage” (573,000)
- Vascular Stents (wire props for an artery)
- Rotational Atherectomy (much like a drill)

Problems with Current Treatments

- Restenosis
- Graft disease
- Arterial puncture
- Coronary thrombosis

How can we help people who don't respond well or are not good candidates for conventional treatments?

Why use VEGF to Promote Angiogenesis?

VEGF *(vascular endothelial growth factor)*

- Specific for only endothelial cells
- May inhibit smooth muscle growth…reduce restenosis

FGF *(fibroblast growth factor)*

- Associated with tumor angiogenesis
- Can stimulate growth in other cells besides endothelial cells
- Not as specific as VEGF

TGF-β *(transforming growth factor ß)*

- Indirect angiogenesis effect
- Possibly induces VEGF expression (Protein Kinase C pathway)

PDGF *(platelet derived growth factor)*

- Not well characterized in angiogenesis

Other VEGF Characteristics

- VEGF expressed by Macrophages, fibroblasts, smooth muscle cells, endothelial cells (all are present in the heart)
- Action is direct because of the exclusive specificity for receptors (flt-1 and flk-1)
- Receptors only found on endothelial cells
- Causes activation of many other genes involved in angiogenic r esponse

How to Deliver VEGF

Protein Therapy

- Direct injection of protein
- Time delay delivery
- Local intercoronary bolus

Gene Therapy

Adenovirus vector

- Excellent specificity for endothelial cells
- Extended expression of VEGF

Direct gene transfer

- Involves direct injection of eukaryotic plasmid DNA containing VEGF cDNA

Should VEGF administration prove effective, it is likely that VEGF/VEGF DNA will be delivered on a catheter platform

Case Studies

Injection of naked VEGF cDNA contained in an Eukaryotic Expression Vector

Jeffery Isner *et al.* St. Elizabeth's Medical Center

Phase I clinical trial…designed to assess safety and bioactivity of treatment methods

Limited sample…only 5 patients involved

- Prior Bypass and/or angioplasty
- Class 3-4 Angina
- No longer respond to additional treatment

Results

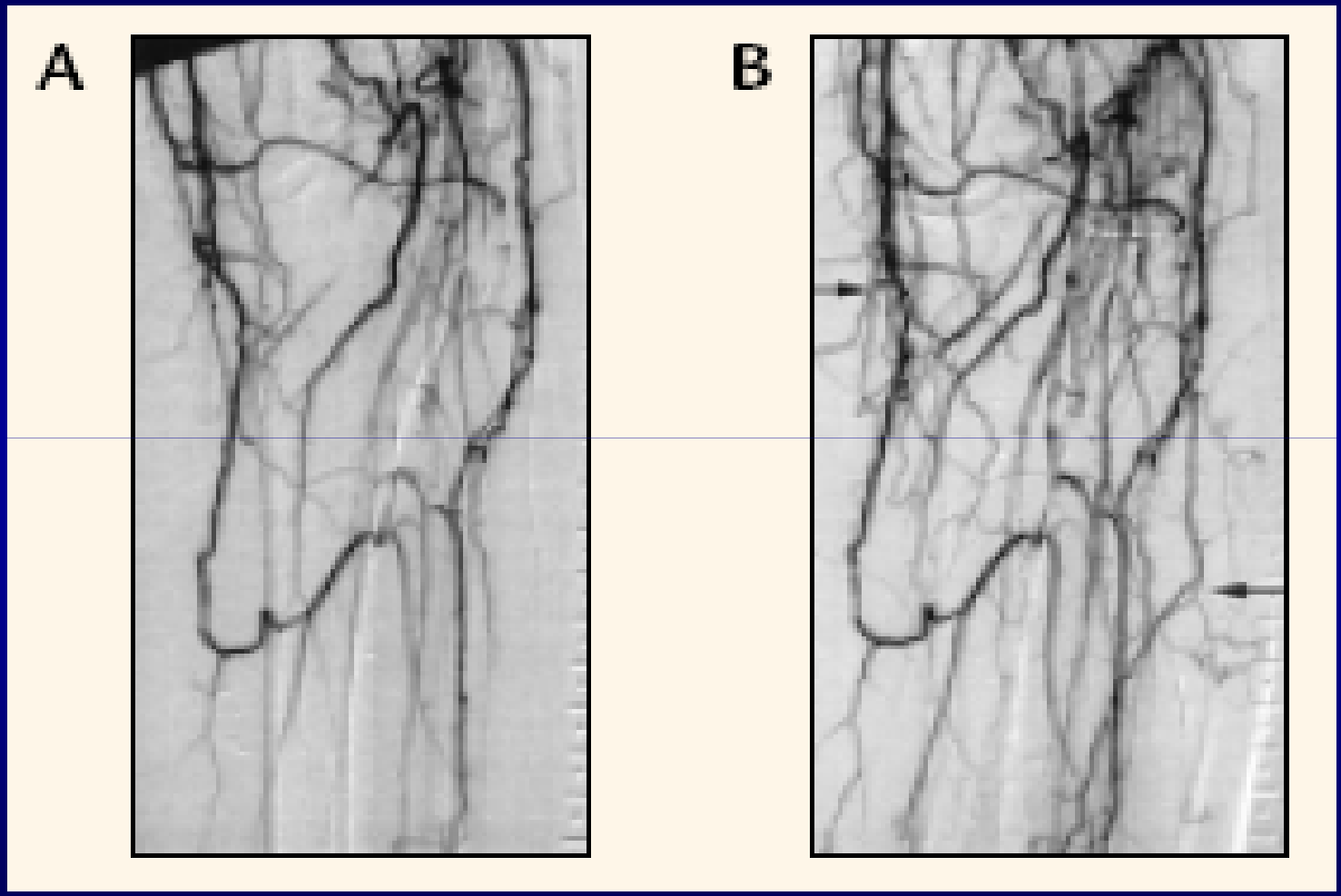
Age	Lifestyle Before Treatment	Lifestyle After Treatment
67	Angina from Mild activity	• Angina virtually gone • Able to resume swimming • Nitroglycerin (NTG) no longer needed
69	Angina after walking 10 yards	• 30 days post needed very little NTG • 60 days post could exercise for 30 minutes on a stationary bike
53	Angina after walking 50 yards	• 60 days post could walk ½ mile • Claims to have felt beneficial effects after only two weeks
71	Angina from walking 100 yards	• 30 days NTG use decreased dramatically • Returned to work part time
59	Daily Angina	• 30 days later could walk up to ¼ mile without pain • Less need for supplemental oxygen • 2 episodes of angina/month

Also notable:

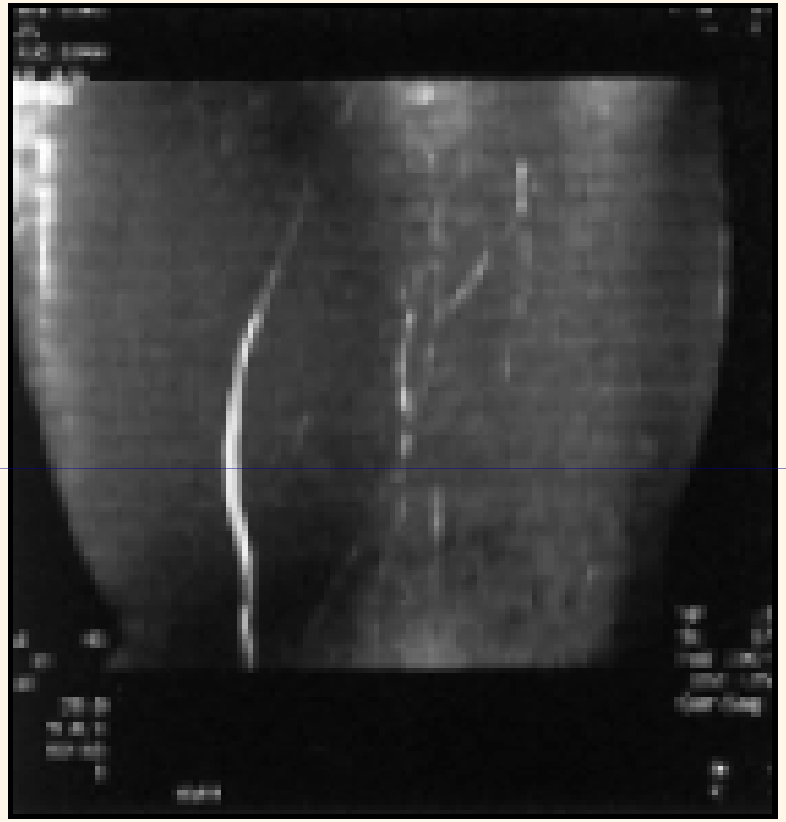
- Nitroglycerin usage dropped from 7.7 pills per day to 1.4 p er day for the group (60 days post)
- Effective biological outcomes despite low transfection rate s
- Because of the condition of the patients in the study, the i mprovements to health were not likely random events

All 5 patients had remarkable gains in quality of lif e post procedure

Perifériás ischaemiás megbetegedések

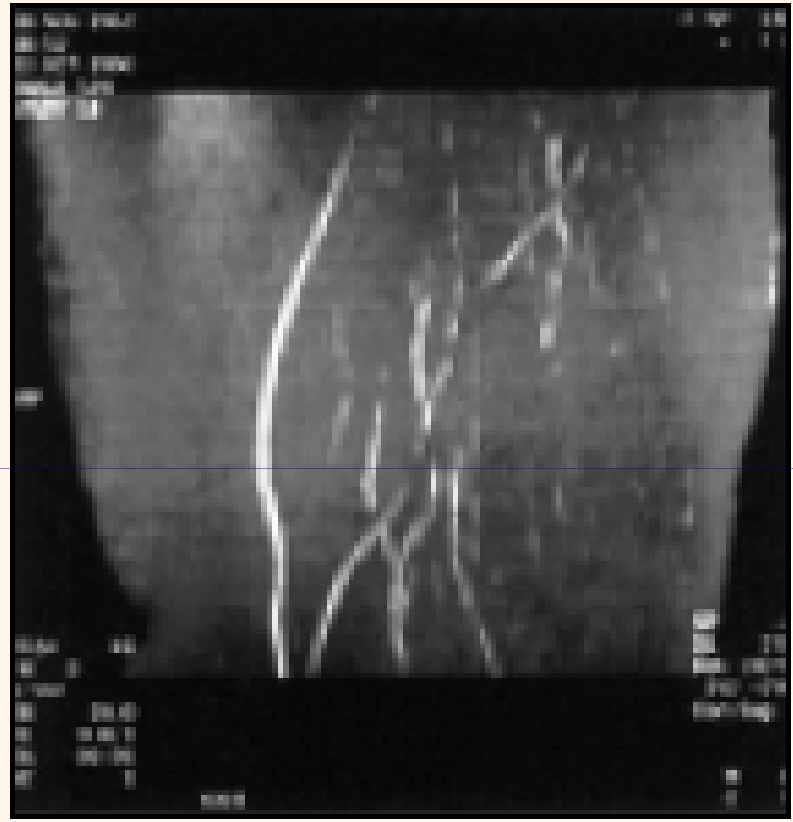

A
B

C



Before gene therapy
(baseline)

D



After gene therapy
(8 weeks)

Gene Therapy Successes

Photo courtesy of Van de Silva

Ashanti de Silva successfully treated for ADA deficiency - 1990

Photo: Courtesy of Jeans for Genes

Rye Evans successfully treated for SCID - 2001

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Gene Therapy Problems

Jesse Gelsinger died of complications due to an immune system response while participating in a clinical trial

Two boys treated for SCID developed leukemia due to disruption of a gene that regulates cell division

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SAEs1: best documented cases: acute and long ter m SAEs: from Gelsingers' death to Paris' Leukaem ias caused by insertional mutagenesis

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NY May 5, 1995, R. Crystal:
adenovirus, cystic fibrosis (lung)
one patient mild pneumonia-like condition
Trial interrupted and many others on hold.

UPenn, Sept. 19, 1999, J. Wilson:
adenovirus , OTC deficiency (liver)
one patient (Jesse Gelsinger) died of a severe septic shock.
Many trials were put on hold for several months (years).

Paris, Oct 2, 2002, A Fischer:
retrovirus , x-SCID (bone marrow)
one patient developed a leukemia-like condition.
Trial suspended and some trials in US and Germany on hold until 2003

Paris, Jan 14, 2003, A Fischer:
retrovirus X-SCID (bone marrow) same cohort
a second patient developed a similar leukemia
30 trials in USA were temporarily suspended

Paris, Jan 24, 2005, A Fischer:
retrovirus X-SCID (bone marrow) same cohort
a third patient developed a similar leukemia
what will happen?

Most Recent Paris' Trial News discussed at:
www.unifr.ch/nfp37/adverse03.html

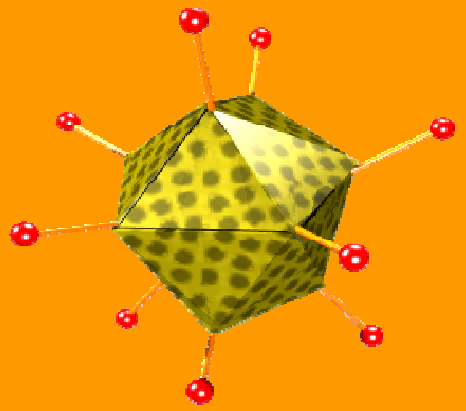
it is now rather established (2004) that the Paris' leukaemia events were caused by treatment-specific circumstances (type of transferred gene, dosing, type of vector, predisposition)

The third SAE might delay the nextly planned restart of patients recruitment

Ergo
gene therapy can produce both short-term and long-term severe side effects through acute immunogenicity or insertional mutagenesis (cancer risk)

Ethical and Social Issues

- Patient safety while participating in clinical tri als
- Which applications are therapies and which a re enhancements?
  - “Designer” babies
- Access to gene therapies



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'Classical' SGT models and strategies

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Disease	transferred function	Clinical Results
ADA deficiency (Immunodeficiency)	ADA normal gene (enzyme) retrovirus, ex-vivo BM	1990 F. Anderson, 2002 C. Bordignon
Cystic Fibrosis (Lung, Pancreas)	CFTR gene (chlorine transporter), retrov., aav, adenoII, local	no significant results in spite of several trials
Haemophilia B (Blood)	Factor IX gene (clotting factor), aav, adenoIII, intramuscular	1999-2000 M. Kay, K. High
SCID (Immunodeficiency)	IL2R gene (gamma-C receptor) retrov., ex vivo BM	2000 A. Fischer 2002, UK trials
Limb ischaemia (Hands, Feet)	VEGF gene (vascular growth factor), plasmid, intramuscular	1998 J. Isner
Cardiac ischaemia (Heart)	VEGF gee (vascular growth factor), plasmid, intracardiac	2000 J. Isner

additional 'popular' and emerging examples:
Morbus Gaucher, Morbus Parkinson, Crigler Njiar, OTC deficiency, Duchenne's MD, Restenosis control

Gene Therapy Clinical and Preclinical Milestones

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2005

1990, 1993, 2000, 2004 // ADA deficie
F Anderson, M Blaese // C Bordignon

1997, 2000, Critical limb ischemia
J Isner († 4.11.2001), I Baumgartner, Ci

1998, Restenosis
V Dzau, HGT 1998

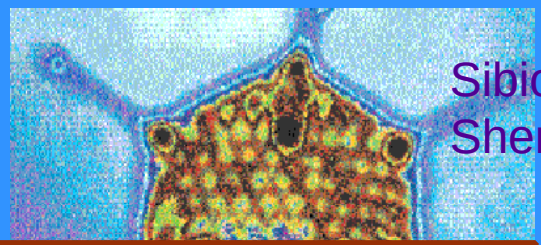
2000, Hemophilia
M Kay, K High

2000, 2002, X-SCID
A Fischer, Science April 2000, UK

2001, 2003 ONYX oncolytic Viruse
D Kirn (Cancer Gene Ther 9, p 979

2004, Chronic Granulomatous Dis

2004, Gendicine (adeno-p53 vector)
L Peng, Sibiono Inc, Shenzen, China



Sibiono
Shenzen

21 lives saved
21 lives were so far documentedly saved by GT in european trials (x-SCID, ADA, CGD) (France, UK, Italy) (all in phase I)
~200 lives quality-improved
in several other phase I and II trial
~xxx lives saved or quality-improved ?
by Gendicine (still undocumented)

Conclusions 1: in spite of the many hurdles, GT has already saved >20 condemned lives and keeps pro ducing positive signals

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2004

X- SCID trials

- France: 9/10 patients permanently cured of the lethal disease X-SCID
- UK: 6/6 patients cured of X-SCID lethal condition

ADA deficiency

- C Bordignon trials 4/4 patients permanently corrected + detoxified

Others

- significant amelioration of CLI condition in Phase II trials
- important therapeutic benefit with oncolytic viruses
- promising amelioration in hemophilia patients
- promising results from Chronic Granulomatosis treatment
- First gene medicine product registered in China by Sibiono Inc. (see www.unifr.ch/sibiono.html)

Ergo

- gene therapy's principle works
- we better know limitations and potential of individualvectors