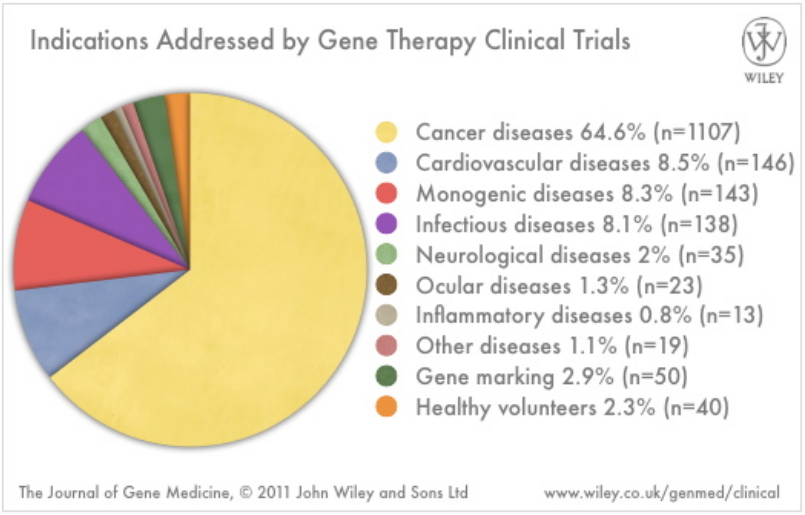
JAGIELLONIAN UNIVERSITY
IN KRAKÓW

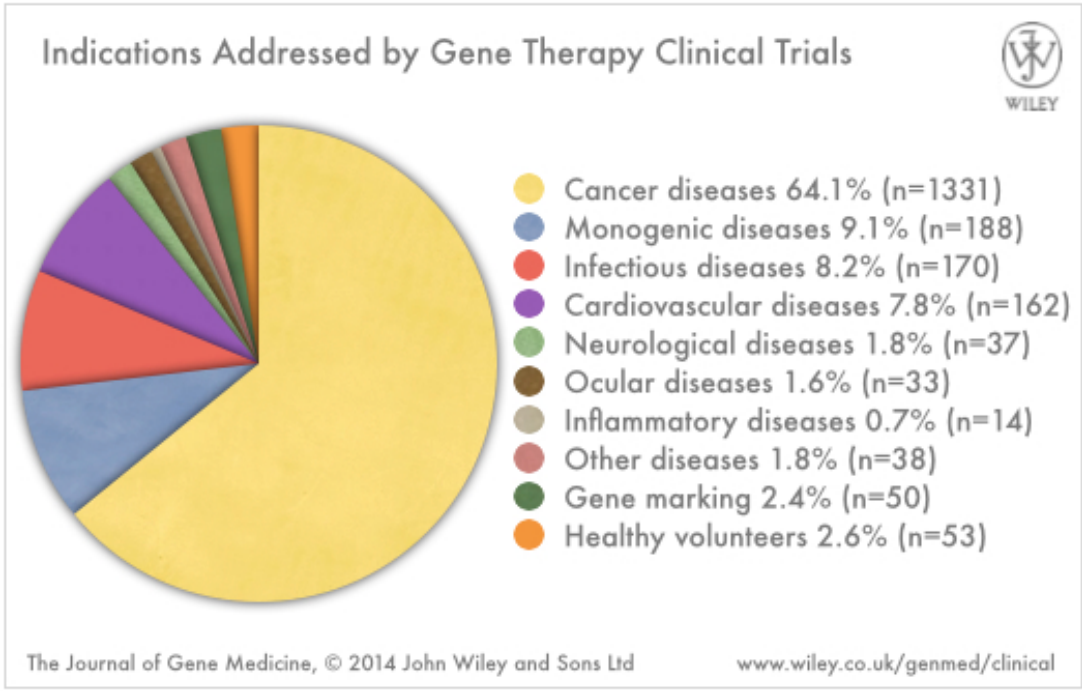
Gene therapy for cancer

Lecture 11

19th January 2015

Department
of Medical
Biotechnology


Indications Addressed by Gene Therapy Clinical Trials
WILEY
Cancer diseases 64.6% (n=1107)
Cardiovascular diseases 8.5% (n=146)
Monogenic diseases 8.3% (n=143)
Infectious diseases 8.1% (n=138)
Neurological diseases 2% (n=35)
Ocular diseases 1.3% (n=23)
Inflammatory diseases 0.8% (n=13)
Other diseases 1.1% (n=19)
Gene marking 2.9% (n=50)
Healthy volunteers 2.3% (n=40)
The Journal of Gene Medicine, © 2011 John Wiley and Sons Ltd
www.wiley.co.uk/genmed/clinical


Indications Addressed by Gene Therapy Clinical Trials
WILEY
Cancer diseases 64.1% (n=1331)
Monogenic diseases 9.1% (n=188)
Infectious diseases 8.2% (n=170)
Cardiovascular diseases 7.8% (n=162)
Neurological diseases 1.8% (n=37)
Ocular diseases 1.6% (n=33)
Inflammatory diseases 0.7% (n=14)
Other diseases 1.8% (n=38)
Gene marking 2.4% (n=50)
Healthy volunteers 2.6% (n=53)
The Journal of Gene Medicine, © 2014 John Wiley and Sons Ltd
www.wiley.co.uk/genmed/clinical

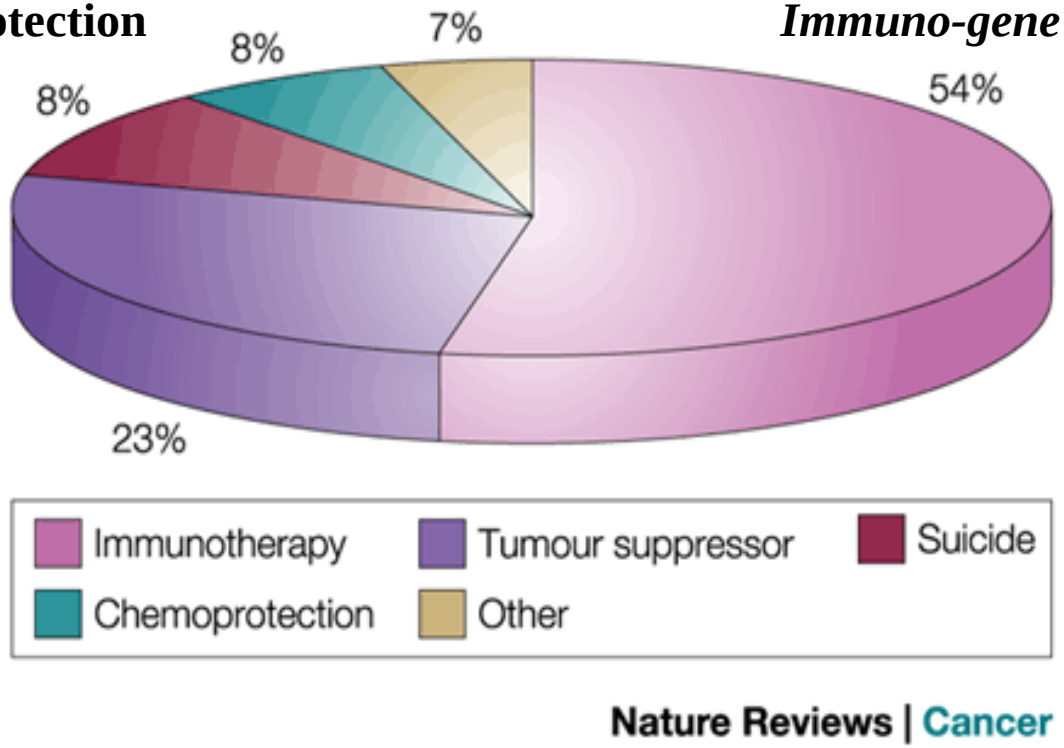
Types of cancer gene therapy

chemoprotection

Immuno-gene therapy

Suicide gene therapy

Tumor suppressor (p53)



Cancer gene therapy

1. Direct attack on tumor cells
    a) transfer of tumor suppressor gene – p53
    b) inhibition of oncogenes
        - antisense therapy
        - ribozymes
        - microRNAs
    c) suicide genes
    d) oncolytic viruses (replication-competent viruses)
2. Harnessing immune response to tumor antigens
3. Chemoprotection
4. Anti-angiogenic therapy

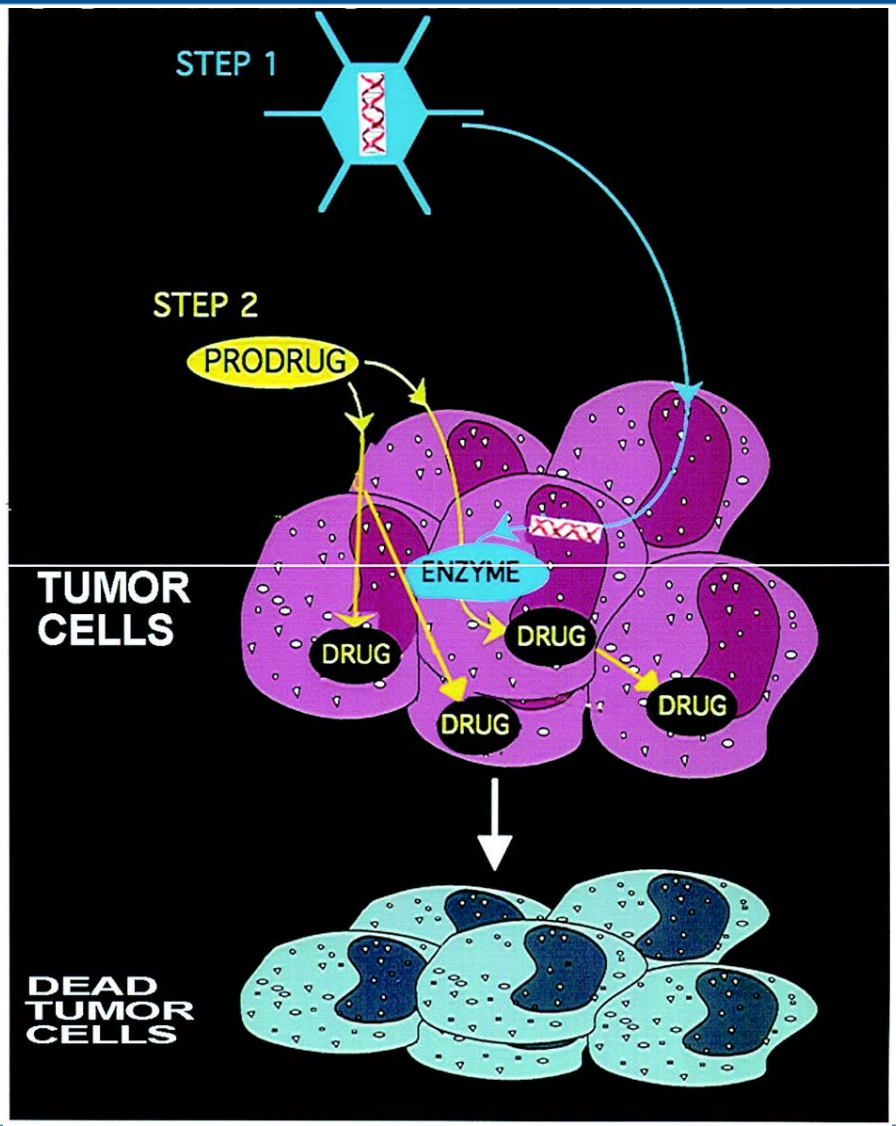
When Bad Gene Transfer Is Good...

Suicide gene therapy –pro-drug activation

Gene-directed enzyme prodrug therapy

The objective of pro-drug activation therapy is to express an activating enzyme within the tumor, which will then activate a systemically delivered, inactive pro-drug at the target site only

Suicide gene therapy –pro-drug activation

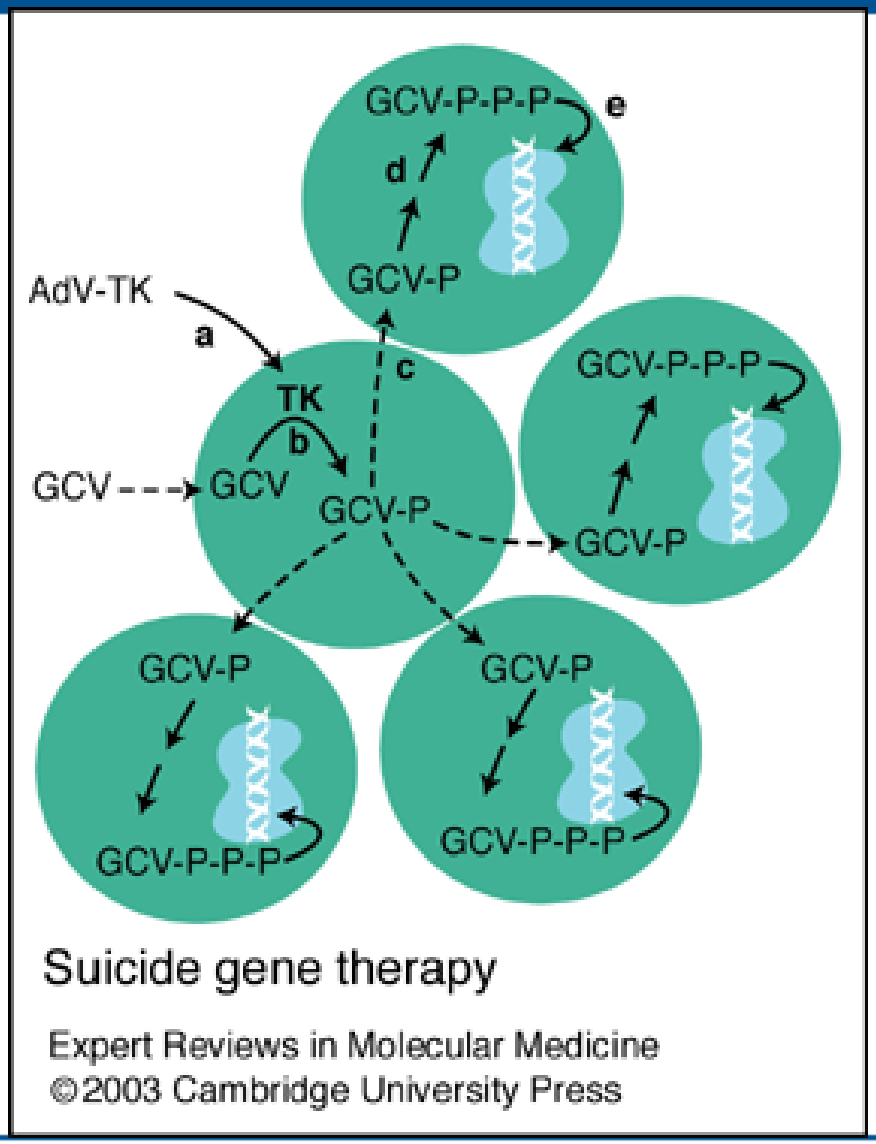


Bystander effect
(efekt sąsiedztwa)

Enzyme-prodrug combination for suicide gene therapy

Enzyme	Prodrug	Product	Mechanism
HSV-tk	ganciclovir acyclovir valacylcovir	ganciclovir triphosphate	blocks DNA synthesis
cytosine deaminase	5-fluorocytosine	5-fluorouracil (5-FU)	blocks DNA and RNA synthesis (pyrimidine antagonist)
cytochrome P450	cyclophosphamide	phosphoramide mustard	DNA alkylating agent; blocks DNA synthesis

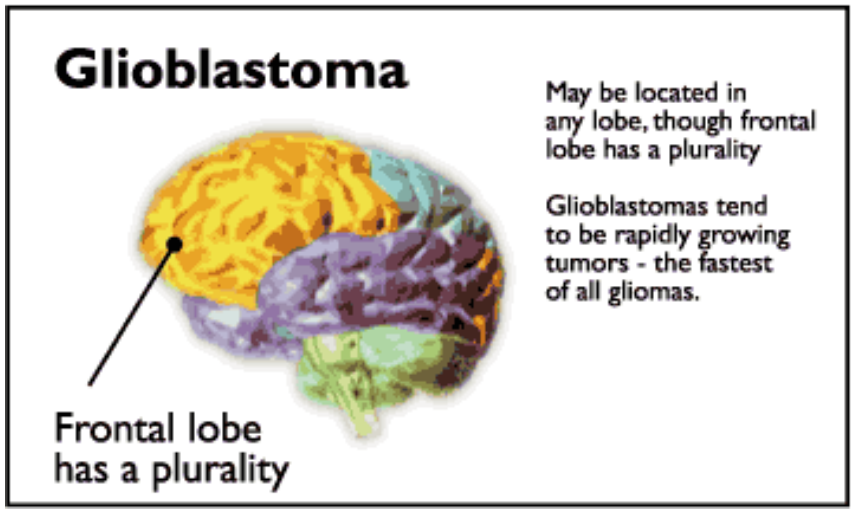
Herpes virus thymidine kinase-based suicide gene therapy



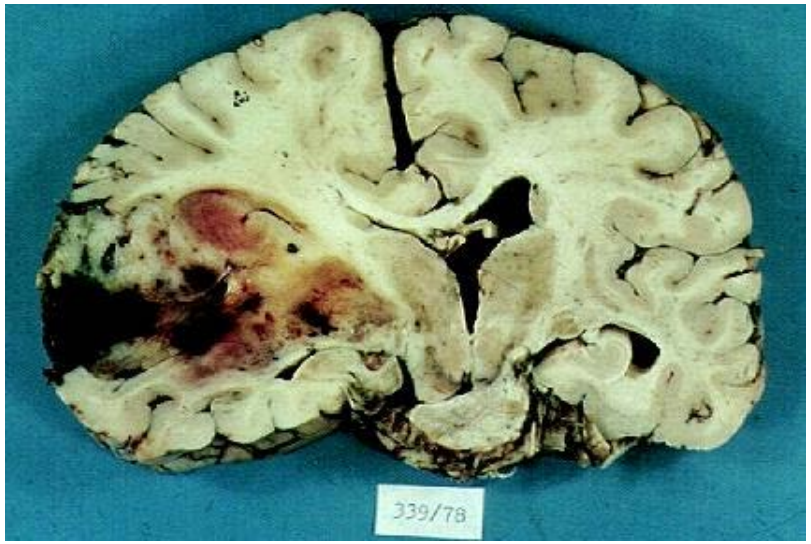
Suicide gene therapy

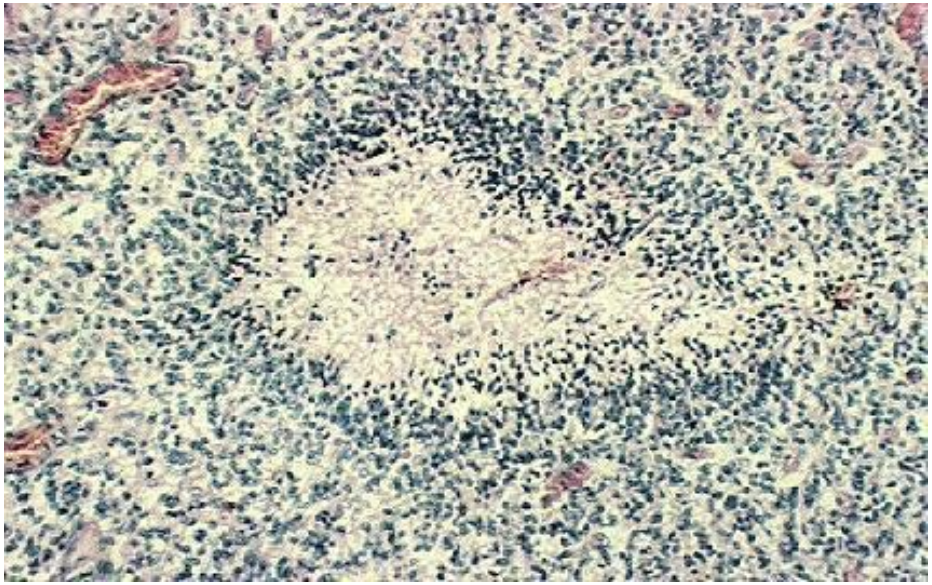
Expert Reviews in Molecular Medicine

Glioblastoma multiforme



Glioblastoma multiforme - Infiltrative; rapid-growing; occurs: most frequently in mid-aged; apt to involve both cerebral hemispheres via the corpus callosum; Average Survival: 1 year





Gene therapy for glioblastoma multiforme

Kuopio University

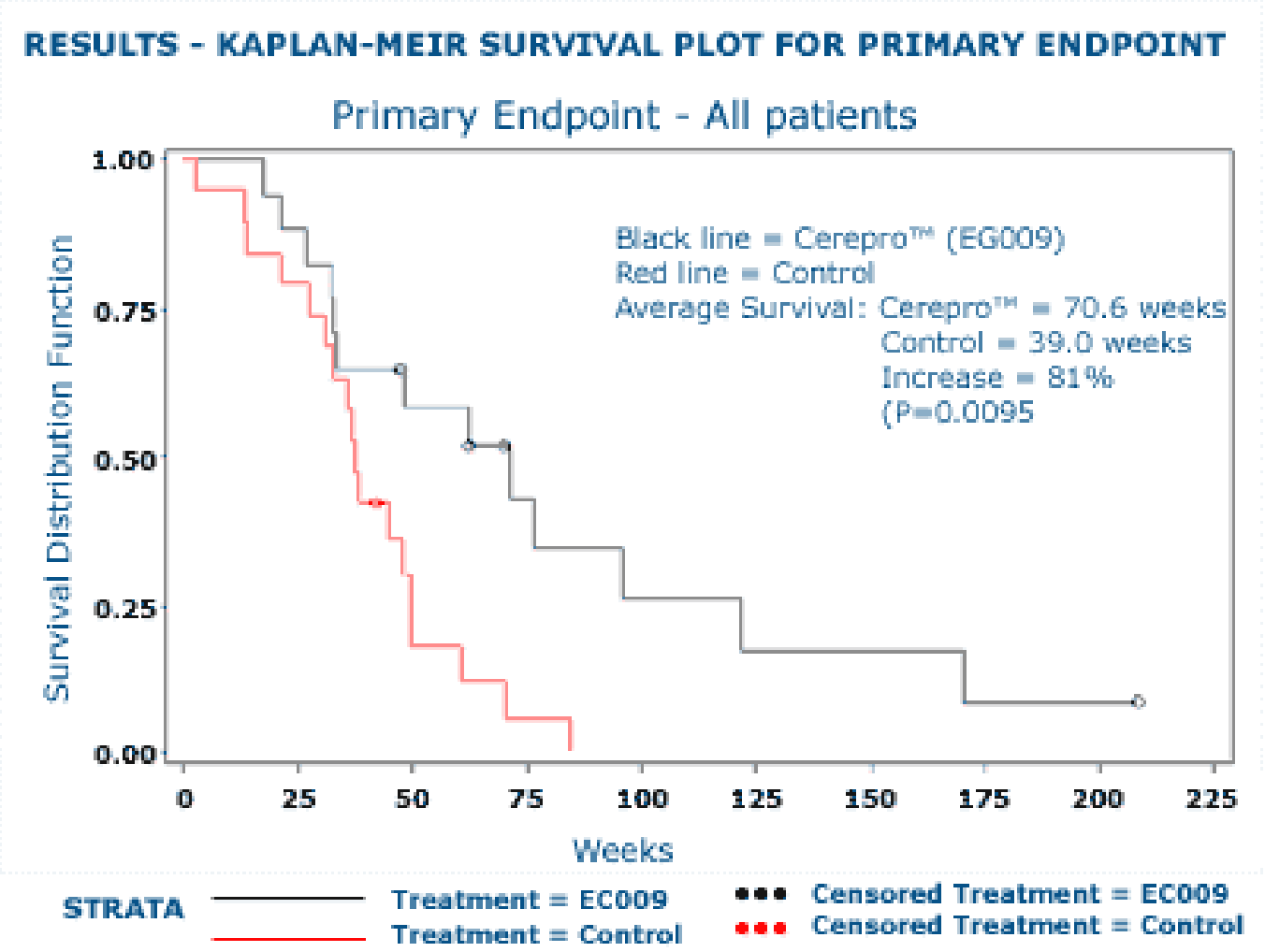
- surgical resection of tumor followed by radiotherapy – all patients

- delivery of AdvHSV-tk (3 x 10(10) pfu) by local injection into the wound bed after tumor resection, followed by intravenous ganciclovir 5 mg/kg twice daily for 14 days - starting 5 days after surgery

- Cell division is a key characteristics of cancer, and the normal brain cells are not dividing, hence tumor cells that try to divide around the site of the removal of the original tumor are targeted for destruction

AdvHSV-tk treatment produced a clinically and statistically significant increase in mean survival from 39.0 +/- 19.7 (SD) to 70.6 +/- 52.9 weeks (P = 0.0095, log-rank regression vs. randomized controls). **The median survival time increased from 37.7 to 62.4 weeks.**

Gene therapy for glioblastoma multiforme



Mol Ther. 2004 Nov;10(5):967-72

Cerepro™ has been granted Orphan Drug Status by the European Committee for Orphan Medicinal Products and by the Office of Orphan Products Development, FDA

Cerepro – sitimagene ceradenovec

1. 1998 – establishing the dose and method of administration using a marker gene

2. 2000 – results of the second, open label efficacy and safety study showed that Cerepro doubled mean survival time and was well tolerated

3. 2004 – results of the third study presented and published (Molecular Therapy)

4. ASPECT study – a phase III, randomised, controlled, multicentre evaluation that recruited 250 patients

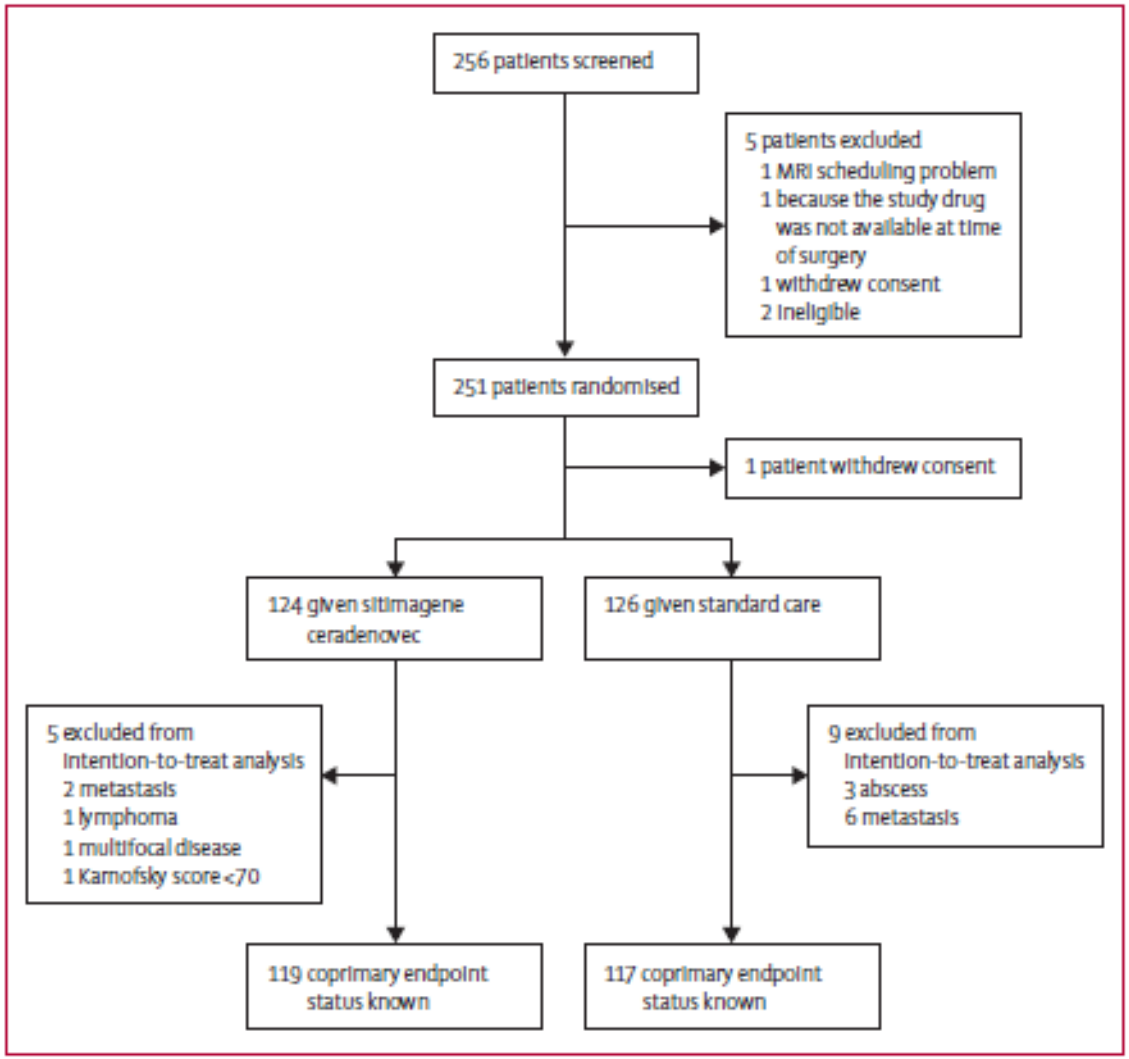
December 2009 - the European Medicines Agency has not agreed to market Cerepro

Adenovirus-mediated gene therapy with sitimagene ceradenovec followed by intravenous ganciclovir for patients with operable high-grade glioma (ASPECT): a randomised, open-label, phase 3 trial

Manfred Westphal, Seppo Ylä-Herttuala*, John Martin, Peter Warnke, Philippe Menei, David Eckland, Judith Kinley, Richard Kay, Zvi Ram, for the ASPECT Study Group†*

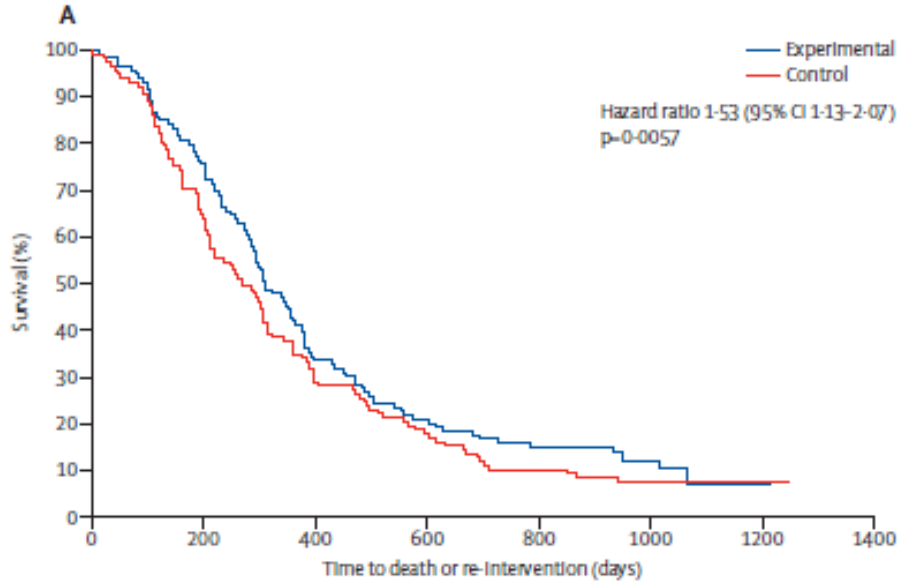
Lancet Oncol
August 2013

ASPECT trial of glioblastoma multiforme



Lancet Oncol
August 2013

Kaplan-Meier estimates of time to death or reintervention



Day	0	183	365	548	730	913	1096	1278
Number at risk								
Control	117	82	41	25	12	8	4	0
Experimental	119	94	49	28	19	12	2	0

Findings Between Nov 3, 2005, and April 16, 2007, 250 patients were recruited and randomly allocated: 124 to the experimental group and 126 to the standard care group, of whom 119 and 117 patients, respectively, were included in the ITT analyses. Median time to death or re-intervention was longer in the experimental group (308 days, 95% CI 283–373) than in the control group (268 days, 210–313; hazard ratio [HR] 1·53, 95% CI 1·13–2·07; p=0·006). In a subgroup of patients with non-methylated *MGMT*, the HR was 1·72 (95% CI 1·15–2·56; p=0·008). However, there was no difference between groups in terms of overall survival (median 497 days, 95% CI 369–574 for the experimental group *vs* 452 days, 95% CI 437–558 for the control group; HR 1·18, 95% CI 0·86–1·61, p=0·31). More patients in the experimental group had one or more treatment-related adverse events those in the control group (88 [71%] *vs* 51 [43%]). The most common grade 3–4 adverse events were hemiparesis (eight in the experimental group *vs* three in the control group) and aphasia (six *vs* two).

Lancet Oncol
August 2013

Ad-HSC-Tk is tested as therapy in various cancer

There is a hope that new methods of delivery might provide better therapeutic outcome

Cancer gene therapy

1. Direct attack on tumor cells
    a) transfer of tumor suppressor gene
    b) inhibition of oncogenes
        - antisense therapy
        - ribozymes
    c) suicide genes
    d) oncolytic viruses (replication-competent viruses)
2. Harnessing immune response to tumor antigens
3. Anti-angiogenic therapy

Oncolytic viruses

Unhindered by interferon-mediated antiviral defence, which is compromised in many tumours, these viruses **specifically attack** cancer cells by:

1. gaining entry through receptors that are overexpressed in these cells and/or
2. exploiting molecular pathways associated with malignant transformation for their replication

Replication competent viruses

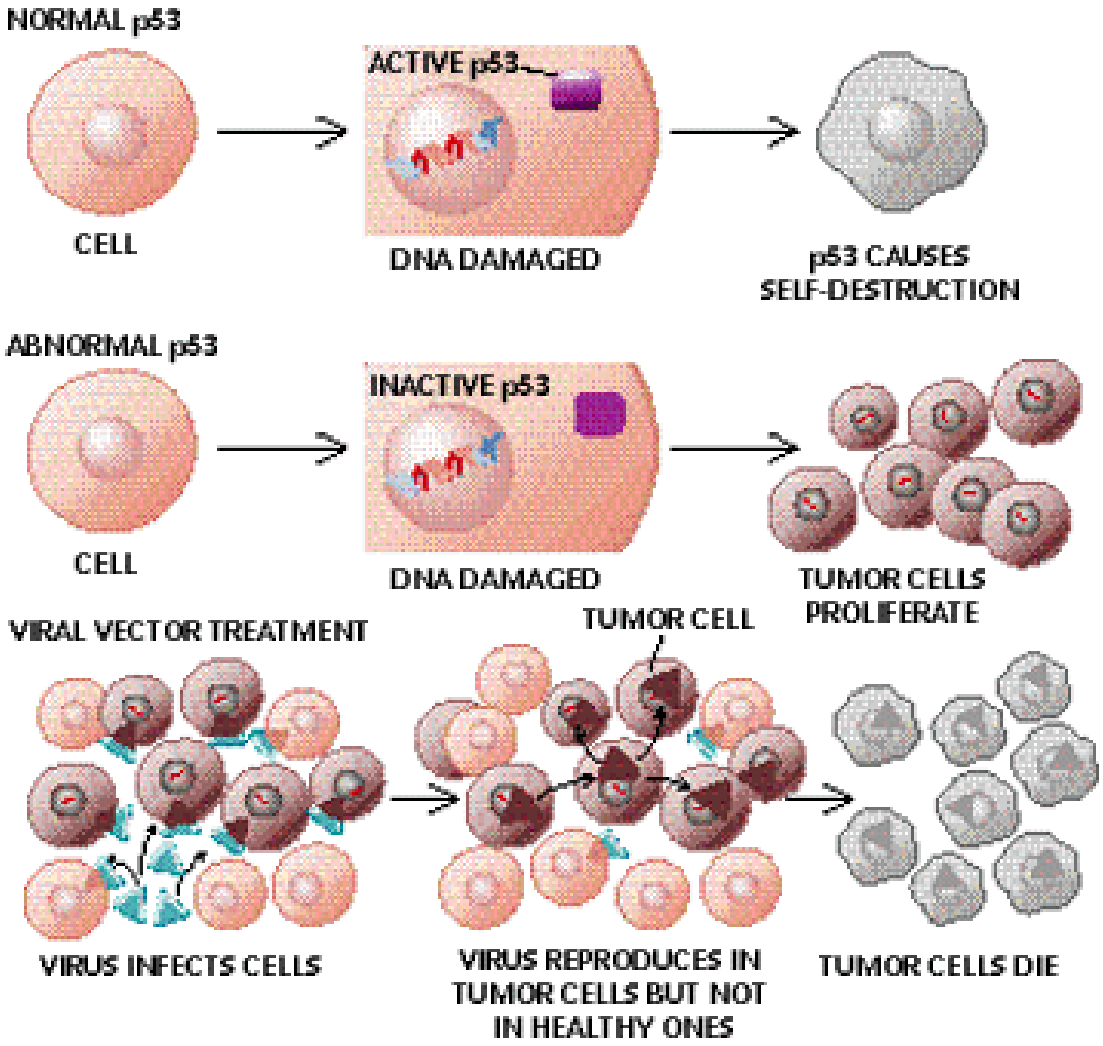
Naturally occuring viruses

- autonomously replicating parvoviruses
- human reoviruses
- vesicular stomatitis virus
- Newcastle disease virus

Engineered oncotropic viruses

- conditionally replicating adenoviruses (such as ONYX-15)

Oncolytic viruses replicate in cells deficient of p53

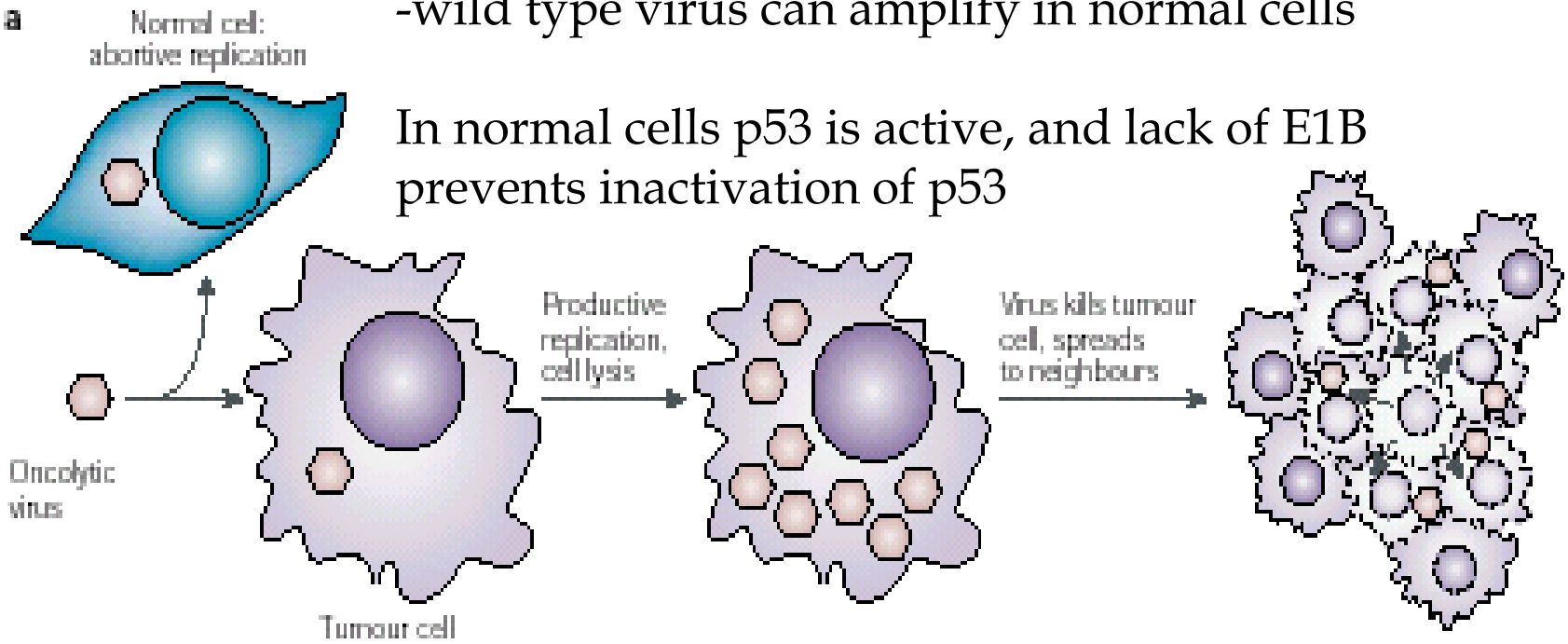


Oncolytic viruses

Lyse only tumor cells,
Adenovirus ONYX-015 , with deletion in E1B gene, may amplify only in cells with mutated p53 gene

E1B 55K protein binds to p53 and blocks its function
-wild type virus can amplify in normal cells

In normal cells p53 is active, and lack of E1B prevents inactivation of p53



Ongoing trials with oncolytic viruses

1. soon-to-be-completed phase III trial of an attenuated strain of herpes simplex virus-1 that encodes GM-CSF in patients with metastatic melanoma;
2. the recently activated phase III trial testing addition of reovirus to paclitaxel/carboplatin chemotherapy in patients with recurrent head and neck cancer;
3. and a randomized phase II trial comparing JX-594 with the best supportive care in patients with hepatocellular carcinoma for whom treatment with the drug sorafenib has failed.

Nature, 1st September 2012

Cancer gene therapy

1. Direct attack on tumor cells
    a) transfer of tumor suppressor gene
    b) inhibition of oncogenes
        - antisense therapy
        - ribozymes
    c) suicide genes
    d) oncolytic viruses (replication-competent viruses)
2. **Harnessing immune response to tumor antigens**
    - tumor vaccines
    - genetically engineered lymphocytes

3. Anti-angiogenic therapy

Genetically modified tumor vaccines

- Immune system may play a role in controlling tumor growth and development
- However, antigens present on tumor cells are not sufficient to boost immune response:
  - they are weak
  - lack the co-stimulatory molecules necessary for full recruitment of cellular immunity
- Many tumors also employ active immune evasion strategies

hence

Modification of tumor cells – i.e, overexpressing certain genes, may stimulate immune system to respond to tumor cells

Strategy of genetically modified tumor vaccines

1. Isolate tumor cells from a patient – however, it is often not possible to use autologic cells

2. Alternative – culture other tumor cells- eg, cell line of the same type – i.e. allogeneic cell line

3. Transduce such cells with vector – eg. retroviral vector harboring cytokine gene

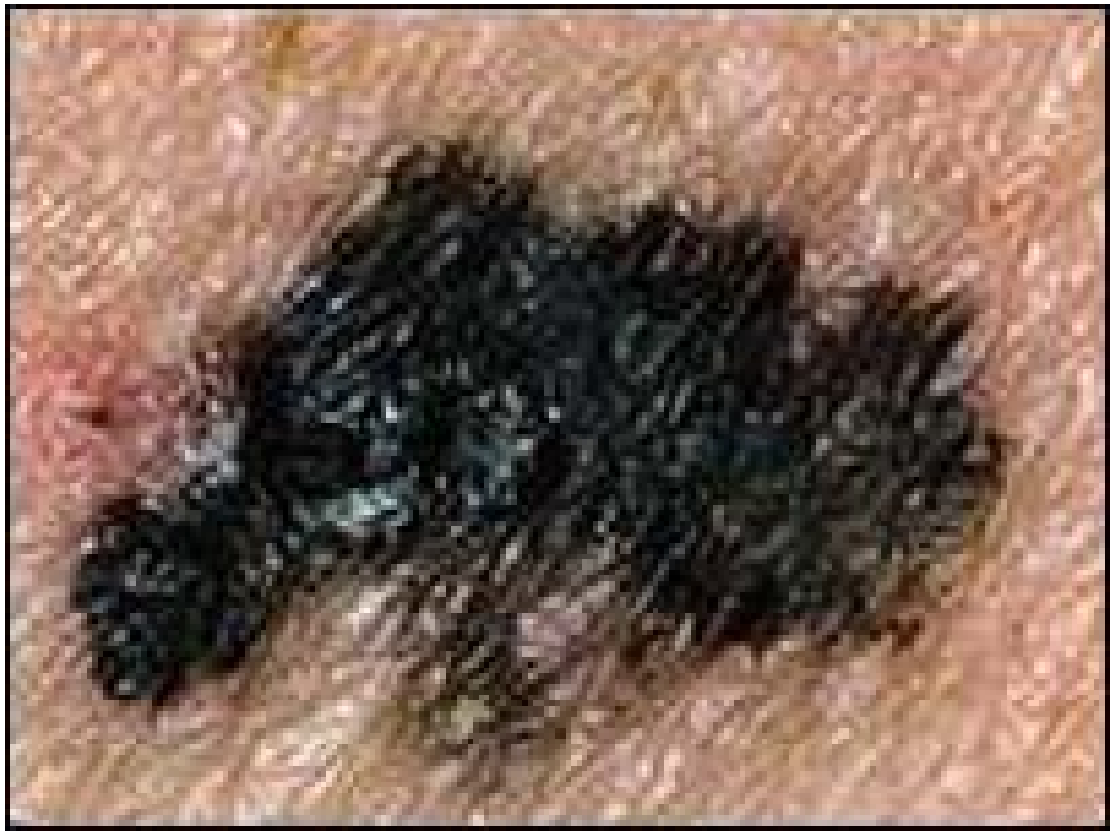
4. Inject such modified cells into patients

5. Antigens present on allogeneic tumor cells stimulate immune system, which respond to the same antigens present on patient's tumor
   - Cytokines enhance the response

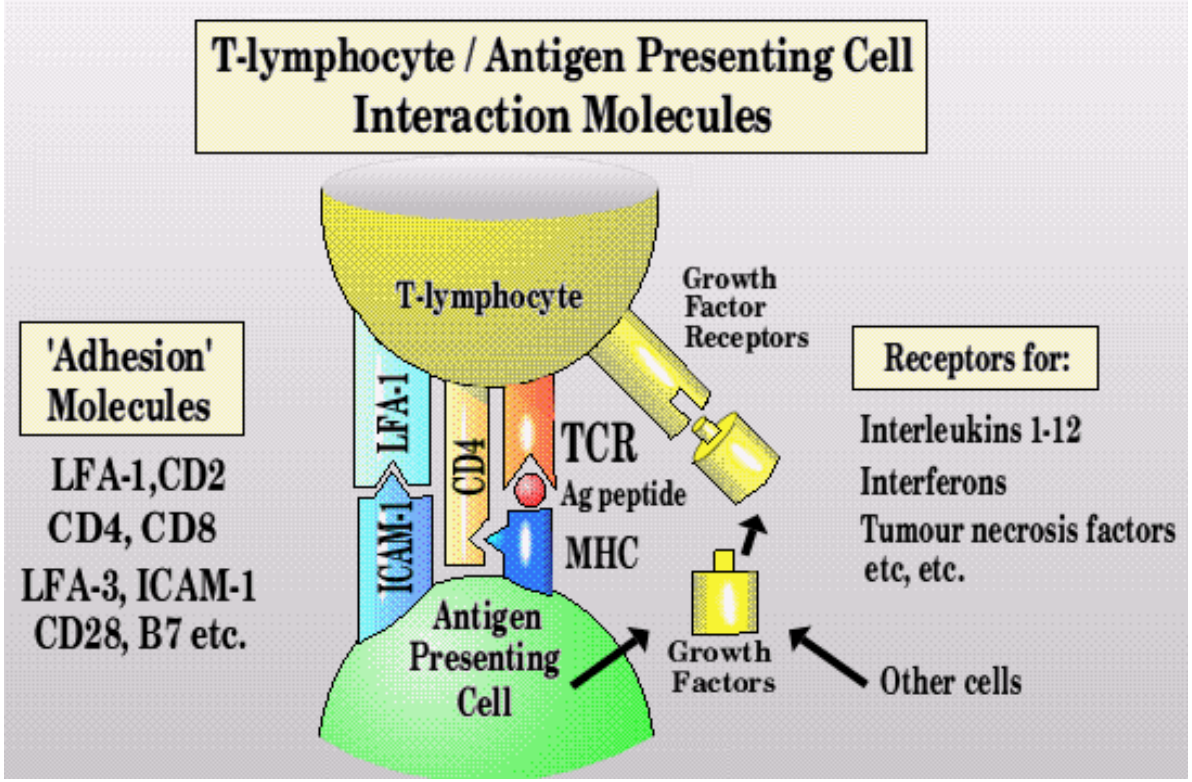
Gene therapy for treatment of melanoma

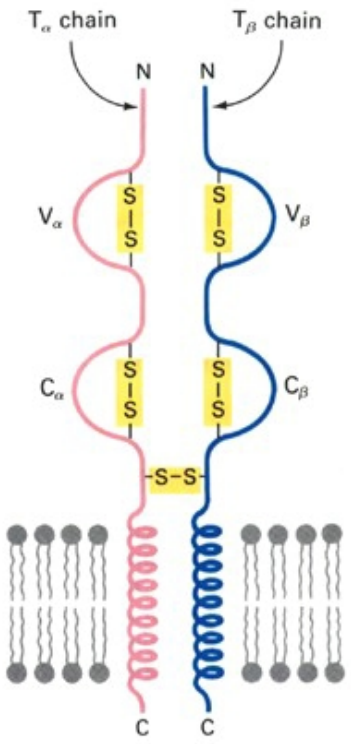
Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes.

Morgan at al.. (Rosenberg), Science, 31 August 2006



Tumor associated antigens (TAA) are recognized by T cell receptor (TCR) on the T lymphocyte surface, which is composed of the TCR a and b-chains





Genes encoding TCR specific for a variety of TAA have now been cloned and these include:

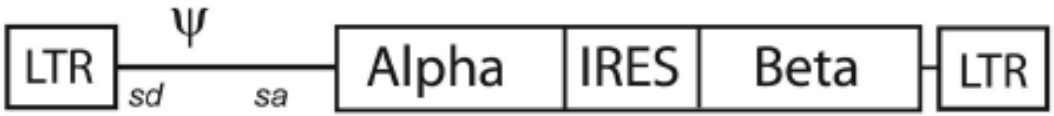
1) the MART-1 and gp100 melanoma/melanocyte differentiation antigens
2) the NY-ESO-1 cancer-testis antigen present on many epithelial cancers
3) the epitope from the p53 molecule, expressed on the surface of approx. 50% of cancers of common epithelial origin

Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes

Morgan at al.. (Rosenberg), Science, 31 August 2006

Peripheral blood lymphocytes (PBL) of the patients with refractory melanoma were transduced ex vivo with retroviral vector encoding T cell receptor (TCR), recognizing:

1) the MART-1 and gp100 melanoma/melanocyte differentiation antigens



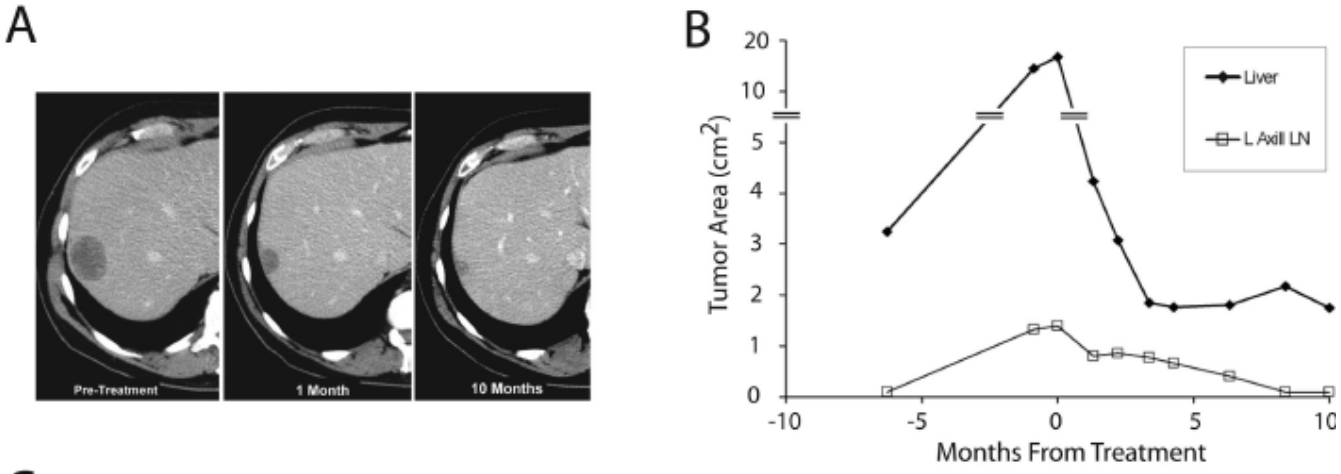
Transduction with these TCR encoding retroviral vectors converted normal PBL to cells capable of specifically recognizing and destroying both fresh and cultured cells from multiple common cancers

Those TCR transduced T cells secreted IFN-γ following co-culture with melanoma cells

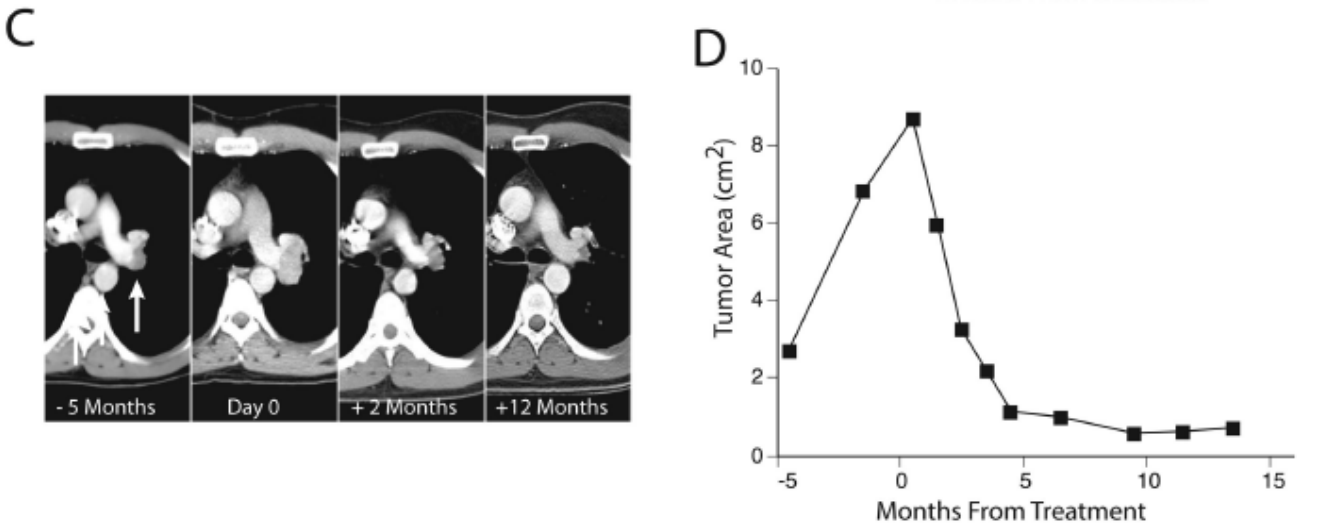
Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes

Morgan at al.. (Rosenberg), Science, 31 August 2006

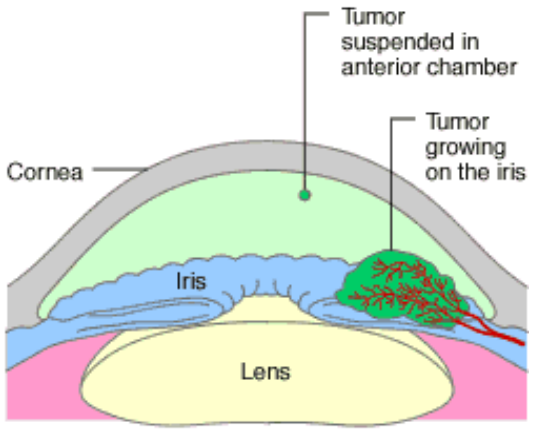
Patient 4
liver metastasis

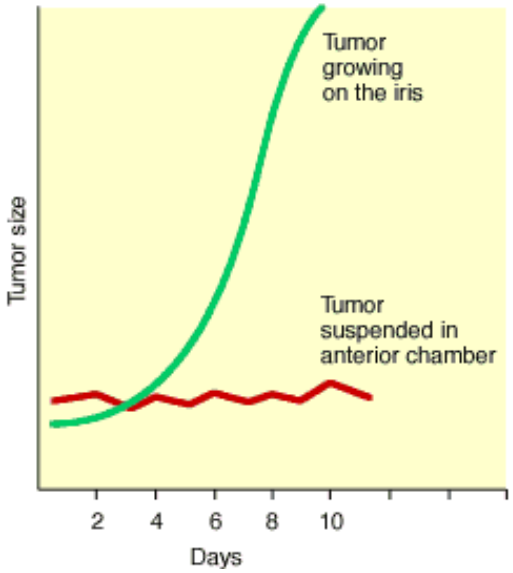


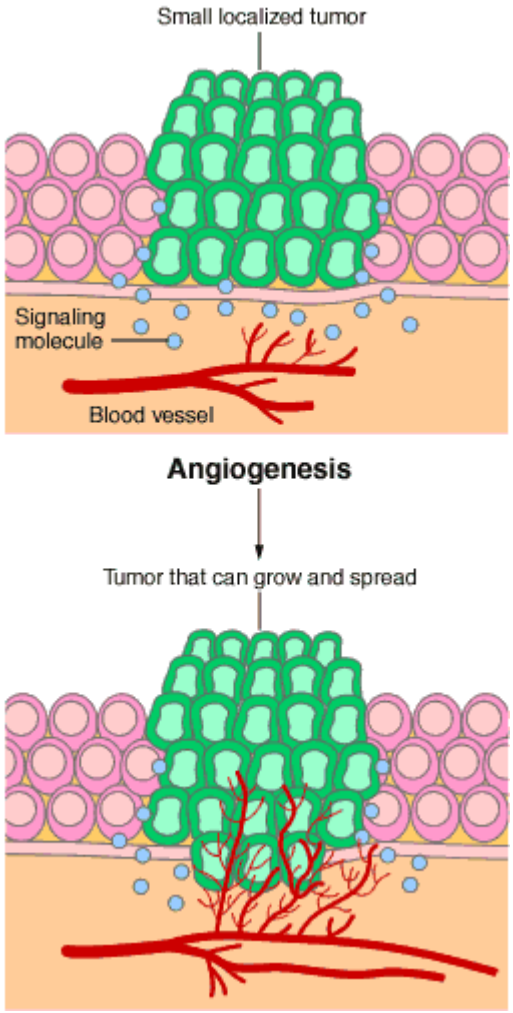
Patient 14
lymph node
metastasis



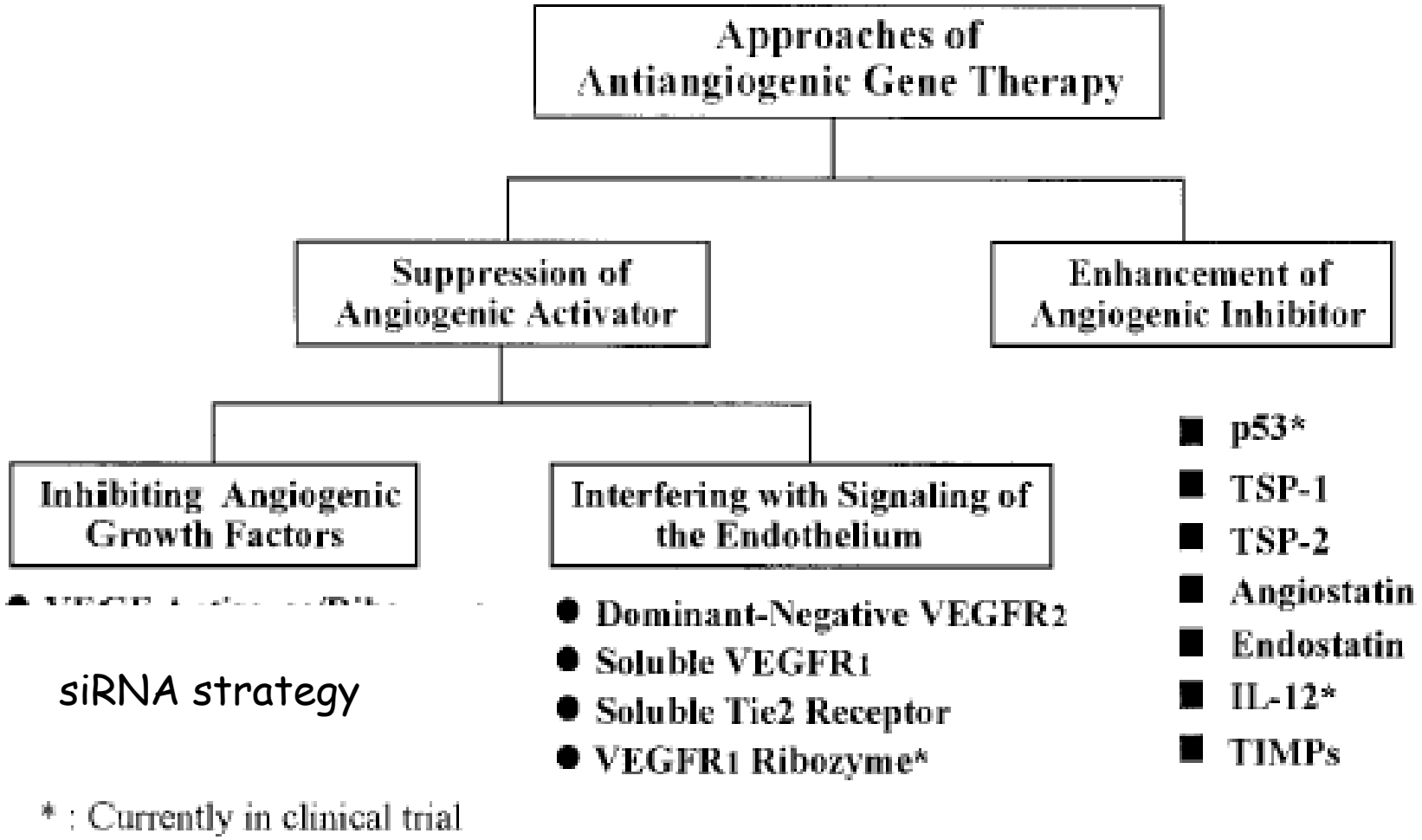
Tumor growth is dependent on angiogenesis







Strategies of anti-angiogenic gene therapy



Hypoxia-regulated overexpression of sVEGFR2 inhibits melanoma growth

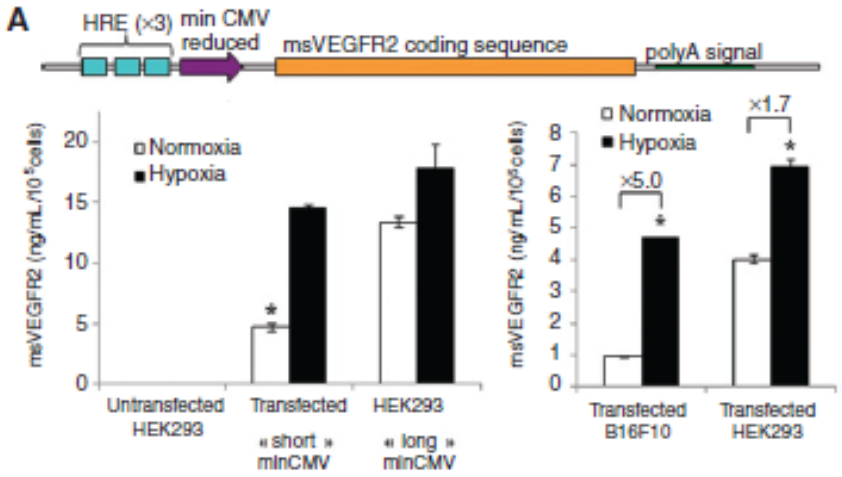
Published OnlineFirst October 29, 2013; DOI: 10.1158/1535-7163.MCT-13-0637

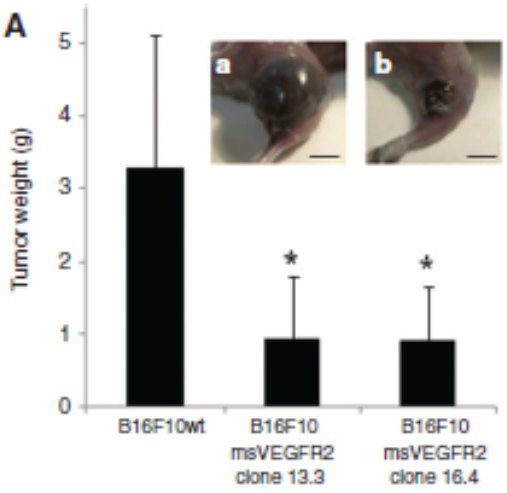
Molecular Cancer Therapeutics

Cancer Biology and Signal Transduction

Hypoxia-Regulated Overexpression of Soluble VEGFR2 Controls Angiogenesis and Inhibits Tumor Growth

Guillaume Collet[1,2], Nathalie Lamerant-Fayel[1], Magdalena Tertil[1,2], Bouchra El Hafny-Rahbi[1], Jacek Stepniewski[2], Alan Guichard[1], Alexandra Foucault-Collet[1], Krzysztof Klimkiewicz[1,2], Stéphane Petoud[1], Agata Matejuk[1,3], Catherine Grillon[1], Alicja Jozkowicz[2], Jozef Dulak[2], and Claudine Kieda[1]





Gene therapy of cancer: anti-cancer vs anti-angiogenic

Anti-cancer cells- introduction of genes that:

1. Permit tumor cells to express toxic molecules
2. Prevent or correct genetic defects
3. Increase the immunogenecity of tumor cells
4. Increase the sensitivity of tumor cells to drugs

Anti-angiogenic - anti-endothelial cells: introduction of genes that:

1. Specifically target tumor endothelial cells
2. Block tumor-derived angiogenic activity

They may be employed together or separately

Cancer gene therapy

1. The largest number of clinical trials in gene therapy are for cancer

2. Different strategies are employed

3. Experimental and early clinical trials indicate for the potential benefit of this type of therapy

4. Unfortunately, the large clincial trials have not yet shown the real effectiveness of the anti-cancer gene therapy