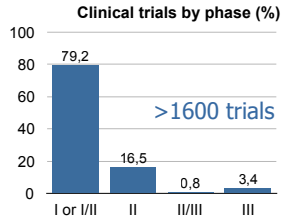
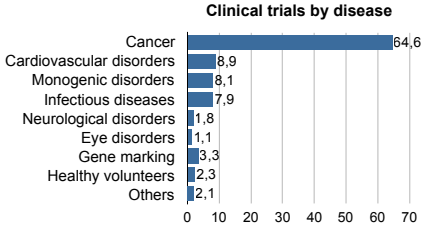
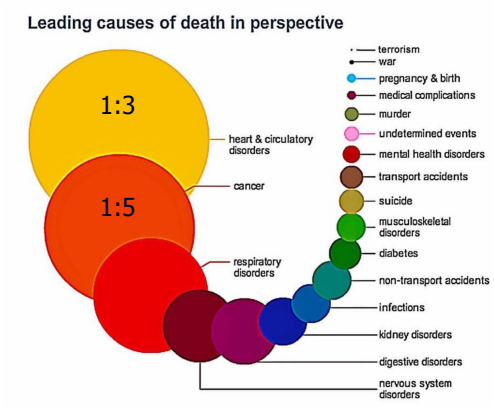
# Gene therapy clinical trials



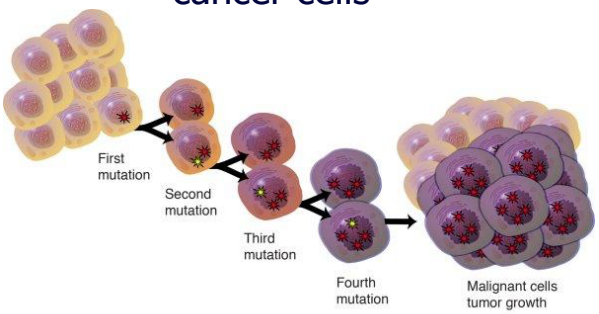




Giacca, M. 2010. Gene Therapy. Springer

# Burden of diseases

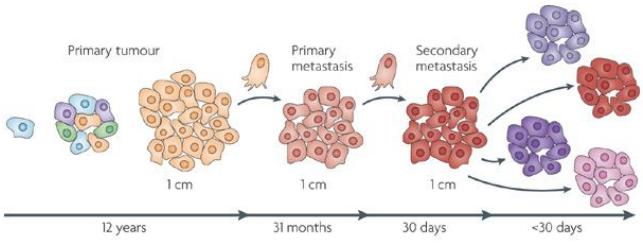



# The pathway from normal to cancer cells




Evolution of cancer is more complex than the straightforward linear accumulation of oncogenic mutations. Potentially oncogenic proliferative signals are coupled to a variety of growth-inhibitory processes, such as the induction of apoptosis, differentiation or senescence, each of which restricts subsequent clonal expansion and neoplastic evolution. Tumour progression occurs only in the very rare instances where these growth-inhibitory mechanisms are thwarted by compensatory mutations.

# The pathway from normal to cancer cells




Nature Reviews | Cancer

# The *Cancer Gene Atlas*

## Discovery and saturation analysis of cancer genes across 21 tumour types

Michael S. Lawrence[1], Petar Stojanov[1,2], Craig H. Mermel[1,3], James T. Robinson[1], Levi A. Garraway[1,2,4], Todd R. Golub[1,2,4,5], Matthew Meyerson[1,2,4], Stacey B. Gabriel[1], Eric S. Lander[1,4,6]* & Gad Getz[1,3,5]*

Although a few cancer genes are mutated in a high proportion of tumours of a given type (>20%), most are mutated at intermediate frequencies (2–20%). To explore the feasibility of creating a comprehensive catalogue of cancer genes, we analysed somatic point mutations in exome sequences from 4,742 human cancers and their matched normal-tissue samples across 21 cancer types. We found that large-scale genomic analysis can identify nearly all known cancer genes in these tumour types. Our analysis also identified 33 genes that were not previously known to be significantly mutated in cancer, including genes related to proliferation, apoptosis, genome stability, chromatin regulation, immune evasion, RNA processing and protein homeostasis. Down-sampling analysis indicates that larger sample sizes will reveal many more genes mutated at clinically important frequencies. We estimate that near-saturation may be achieved with 600–5,000 samples per tumour type, depending on background mutation frequency. The results may help to guide the next stage of cancer genomics.

"As a reference set, we used the Cancer Gene Census (CGC), which is a manually curated catalogue of cancer genes. The current version (v65) contains 130 cancer genes driven by somatic point mutations (as well as additional genes mutated by other mechanisms), of which 82 are associated with 1 or more of the 21 tumour types studied here."

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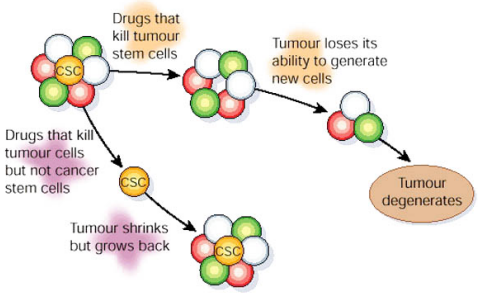
# Cancer stem cells




Conventional therapies may shrink tumours by killing mainly cells with limited proliferative potential. If the putative cancer stem cells are less sensitive to these therapies, then they will remain viable after therapy and re-establish the tumour. By contrast, if therapies can be targeted against cancer stem cells, then they might more effectively kill the cancer stem cells, rendering the tumours unable to maintain themselves or grow. Thus, even if cancer stem cell-directed therapies do not shrink tumours initially, they may eventually lead to cures.

## Strategies for gene therapy of cancer

| Target cell | Strategy | Goal | Therapeutic gene |
|---|---|---|---|
| Cancer cells | Inhibition of cancer cell proliferation | Restoration of cell cycle control | Tumor suppressors (p53, Rb, BRCA1) |
| | | | Antisense oligonucleotides, ribozymes, siRNAs or intracellular antibodies against oncogenes, cdc2, cyclins, PCNA, tyrosine kinase receptors, signal transducers, etc. |
| | Transfer of suicide genes into cancer cells | Specific induction of cytotoxicity in the suicide gene-expressing cells | Gene activating a cytotoxic pro-drug, for example HSV-TK |
| | Oncolytic viruses | Selective lysis of cancer cells by viral replication | |
| Cells of the immune system | Immunotherapy | Increase of antigenic stimulation by cancer cells (active immunization, cancer vaccination) | Tumor-specific antigens (TSAs and TAAs |
| | | | Genes coding for cytokines increasing antigen stimulation (IL-2, IL-12, IFN-γ, GM-CSF) |
| | | Increase of the cytotoxic T-cell response against cancer cells | Genes coding for immunoregulatory cytokines (IL-2, IL-12, IL-7, GM-CSF, IFN-γ, IL-6, TNF-α) |
| | | | Genes coding for co-stimulatory proteins (B7, ICAM-1, LFA-3) |
| | | | Genes coding for immunogenic proteins (MHC I and II alloantigens) |
| | | Genetic modification of effector T cells to redirect them towards cancer cells (adoptive immunotherapy) | TCR genes |
| Hematopoietic stem cells (HSCs) | Increase of the therapeutic index of cancer chemotherapy | Transfer of genes preventing toxicity of chemotherapy into HSCs | Mdr-1 |

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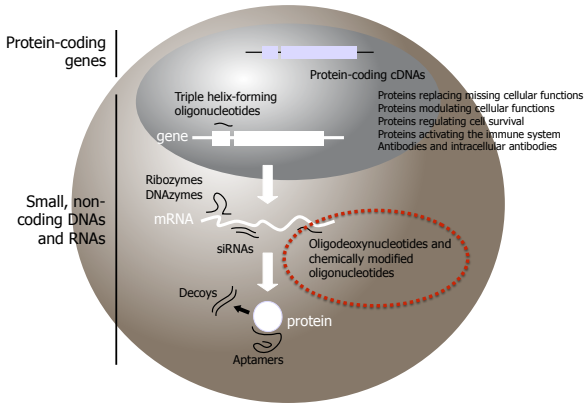
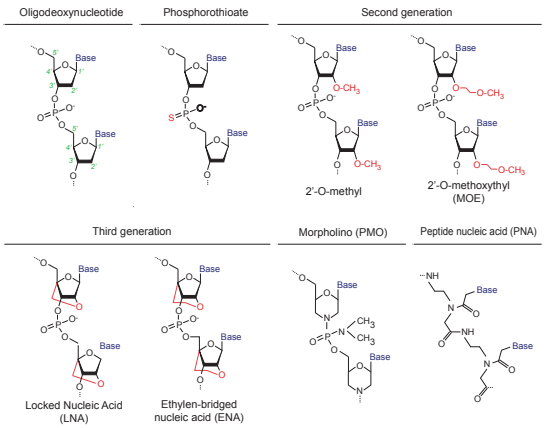
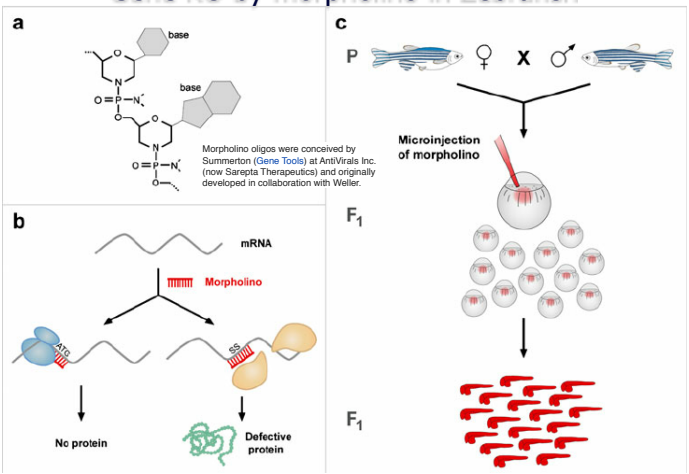
## Therapeutic nucleic acids for somatic gene therapy




## Modified oligonucleotides



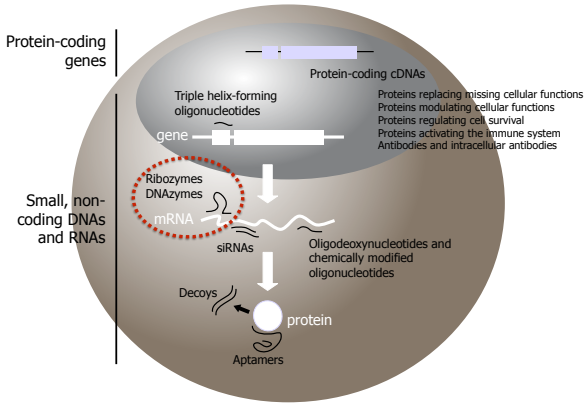

## Gene KO by morpholino in Zebrafish




## Oligonucleotidi per la terapia genica dei tumori

| Gene bersaglio | Funzione del gene | Nome del farmaco | Struttura dell'oligonucleotide | Tipo di tumore |
|---|---|---|---|---|
| Bcl2 | Inibitore dell'apoptosi | G3139 (Oblimersen) | Fosforotioato | Melanoma, leucemia linfatica cronica, mieloma multiplo, carcinoma del polmone non a piccole cellule (NSCLC) |
| Clusterina | Chaperon delle proteine | OGX-011 | Fosforotioato con modificazioni 2'-metossietile (gapmer) | Carcinoma della prostata, carcinoma della mammella, carcinoma del polmone non a piccole cellule (NSCLC) |
| Protein-chinasi Cα (PKCα) | Trasduttore del segnale | ISIS 3621 | Fosforotioato | Carcinoma del polmone non a piccole cellule (NSCLC) |
| Survivina | Inibitore dell'apoptosi | LY2181308 | Fosforotioato con modificazioni 2'-metossietile | Tumori solidi |
| Myb | Oncogene, fattore di trascrizione | LR3001 | Fosforotioato con modificazioni 2'-metossietile | Leucemia mieloide cronica (purging del midollo osseo prima del trapianto) |
| XIAP (X-linked inhibitor of apoptosis) | Inibitore dell'apoptosi | AEG35156 | Fosforotioato con modificazioni 2'-metossietile | Leucemia mieloide cronica |
| HSP27 | Heat shock protein, inibitore dell'apoptosi | OGX-427 | Fosforotioato con modificazioni 2'-metossietile | Carcinoma della prostata |
| STAT-3 | Trasduttore del segnale e fattore di trascrizione | ISIS 345794 | Fosforotioato con modificazioni 2'-metossietile | Diversi tumori |

# Therapeutic nucleic acids for somatic gene therapy




# Ribozymes

**i** and **ii)** Group I and II introns, which undergo splicing through an autocatalytic process.

**iii)** The RNA subunit of **E. coli** ribonuclease P (RNase P), which is responsible of the maturation of the tRNA 5' ends. In bacteria, this enzyme consists of an RNA subunit (M1 RNA), with catalytic activity, and of a protein subunit, having structural function (in humans, RNase P is composed by an RNA subunit, the H1 RNA, whose enzymatic activity is only apparent under specific circumstances, and by 10 protein subunits).

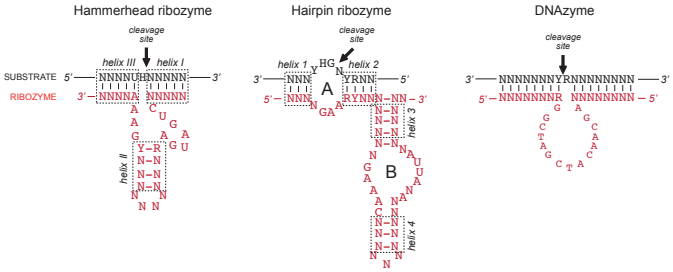
**iv)** Hammerhead ribozymes, present in the RNA genome of different plant viroids and virusoids, where they are essential for rolling circle RNA replication.

**v)** Hairpin ribozymes, also naturally present in the satellite RNAs of some plant viruses, where they participate in viral genome RNA replication.

**vi)** The **hepatitis virus** (HDV) pseudoknot ribozyme.

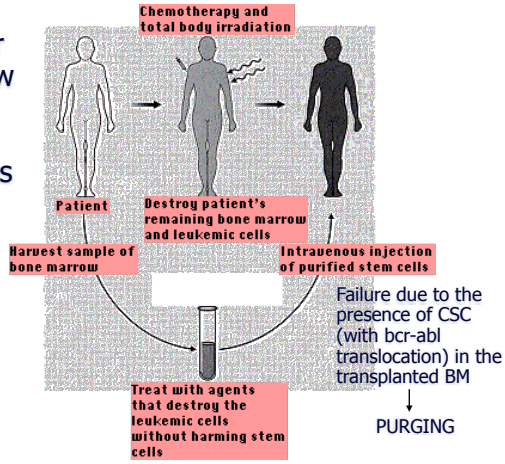
**vii)** The **Neurospora** VS satellite RNA ribozyme.

# Ribozymes




- enzymatic activity: they bind, cut and move to the next target
- difficult in vivo delivery, rapidly degraded by RNase in serum
- conceived for ex vivo applications (i.e. to block infection targeting viral receptors)

# Anti-bcr/abl ribozyme for bone marrow purging in chronic myelogenous leukemia

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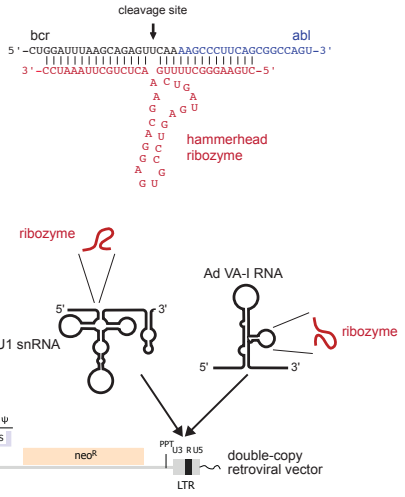



## DISCOVERIES LEADING TO FDA APPROVAL OF STI571/Gleevec FOR TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA

**1960**

**1960 –** Abnormal chromosome 22 (Philadelphia Chromosome) observed in CML patients

**1970**

**1973 –** Chromosome 22 and 9 translocation observed by new staining techniques

**1980**

**1982 –** *abl* Proto-oncogene identified in chromosome 22 translocation

**1984-1987 –** BCR-ABL protein identified as possible cause of CML

**1990**

**1990 –** *bcr-abl* Gene identified as cause of leukemia in mice

**1993 –** First STI571/Gleevec laboratory studies begin

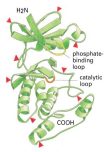
**1998 –** First human tests begin

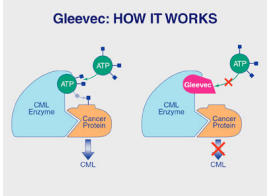
**1999 –** First human results reported

**2000**

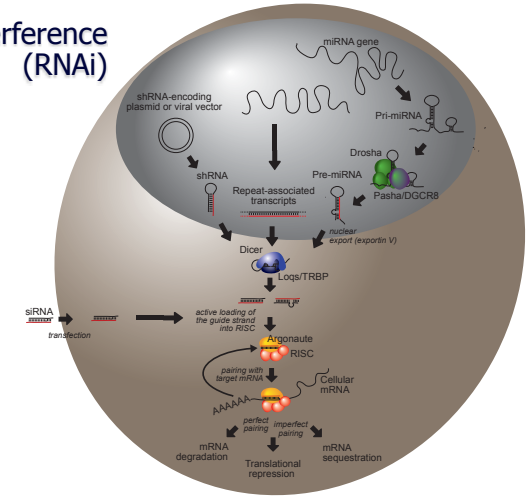
**2001 – April:** Larger study confirms earlier findings

**2001 – May:** FDA approves STI571/Gleevec for treatment for CML

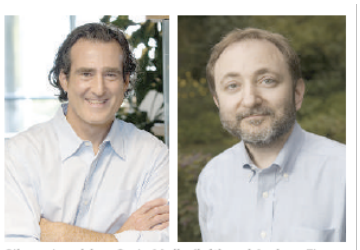


## RNA interference (RNAi)




# Youthful duo snags a swift Nobel for RNA control of genes

Nobel prize 2006 Physiology and Medicine to Craig Mello and Andrew Fire for their report on RNAi.



Silence is golden: Craig Mello (left) and Andrew Fire.

## Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*


**Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡**

* *Carnegie Institution of Washington, Department of Embryology, 115 West University Parkway, Baltimore, Maryland 21210, USA*
† *Biology Graduate Program, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA*
‡ *Program in Molecular Medicine, Department of Cell Biology, University of Massachusetts Cancer Center, Two Biotech Suite 213, 373 Plantation Street, Worcester, Massachusetts 01605, USA*



Experimental introduction of RNA into cells can be used in certain biological systems to interfere with the function of an endogenous gene[1,2]. Such effects have been proposed to result from a simple antisense mechanism that depends on hybridization between the injected RNA and endogenous messenger RNA transcripts. RNA interference has been used in the nematode *Caenorhabditis elegans* to manipulate gene expression[3,4]. Here we investigate the requirements for structure and delivery of the interfering RNA. To our surprise, we found that double-stranded RNA was substantially more effective at producing interference than was either strand individually. After injection into adult animals, purified single strands had at most a modest effect, whereas double-stranded mixtures caused potent and specific interference. The effects of this interference were evident in both the injected animals and their progeny. Only a few molecules of injected double-stranded RNA were required per affected cell, arguing against stochiometric interference with endogenous





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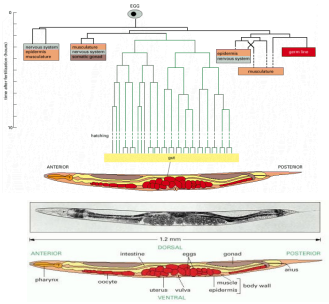
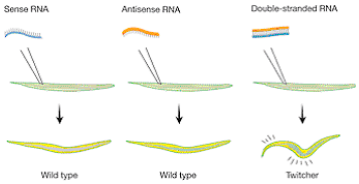
mRNA and suggesting that there could be a catalytic or amplification component in the interference process.



C. elegans is a precious tool in developmental biology:
- it is tiny and grow rapidly
- females are composed of 956 cells
- males are composed of 1031 cells
- the fate of every cell is characterized

## Conclusions of Fire&Mello's study:




*C. elegans*

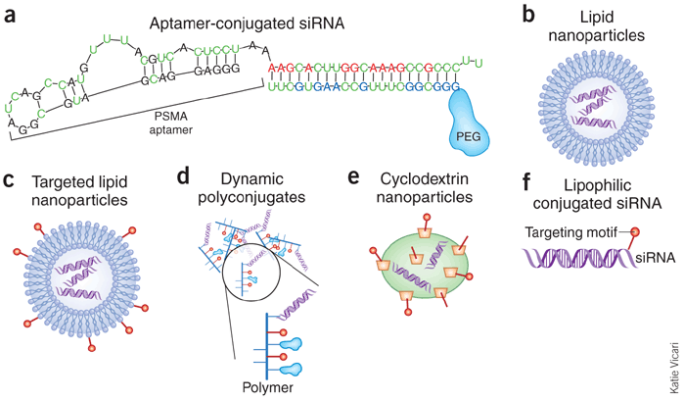
*Phenotypic effect after injection of single-stranded or double-stranded unc-22 RNA into the gonad of C. elegans. The **unc-22** gene encodes a myofilament protein. Decrease in unc-22 activity is known to produce severe twitching movements. Injected double-stranded RNA, but not single-stranded RNA, induced the twitching phenotype in the progeny.*

1) silencing was triggered efficiently by injected **dsRNA**, but weakly or not at all by sense or antisense single-stranded RNAs.
2) silencing was **specific** for an mRNA homologous to the dsRNA; other mRNAs were unaffected
3) the dsRNA had to correspond to the mature mRNA sequence; neither intron nor promoter sequences triggered a response. This indicated a **post-transcriptional**, presumably **cytoplasmic** mechanism
4) the targeted mRNA disappeared suggesting that it was **degraded**
5) only a few dsRNA molecules per cell were sufficient to accomplish full silencing. This indicated that the dsRNA was amplified and/or acted **catalytically** rather than stoichiometrically
6) the dsRNA effect could spread between tissues and even to the progeny, suggesting a **transmission** of the effect between cells

## siRNA-based gene therapy

| | Disease | Target gene |
|---|---|---|
| **Monogenic or multifactorial diseases (also dominant!!!)** | Familial hypercholesterolemia | Apolipoprotein B |
| | Age-related macular degeneration (AMD) | VEGF, VEGFR1, RTP801 |
| | Amyotrophic lateral sclerosis (ALS) | SOD1 |
| | Spinocerebellar ataxia type 1 | Ataxin 1 |
| | Alzheimer's disease | Tau, APP |
| | Huntington's disease | Mutated huntingtin allele |
| | Parkinson's disease | α-synuclein |
| **Cancer** | Different tumors | Bcl-2 |
| | Acute myeloid leukemia (AML) | AML1/MTG8 |
| | Chronic myelogenous leukemia (CML) | Bcr-Abl |
| | Glioblastoma | MMP-9, uPAR |
| **Infectious diseases** | Hepatitis B | HBsAg |
| | Hepatitis C | NS3, NS5B, E2 |
| | Influenza | Nucleoprotein, polymerase |
| | HIV-1 infection | Viral or cellular genes required for viral replication |
| | HSV-1 infection | Glycoprotein E |
| | Syncytial respiratory virus (RSV) | P, N, L genes |

## Strategies for systemic delivery of siRNAs




Katie Vicari

## Strategies for gene therapy of cancer

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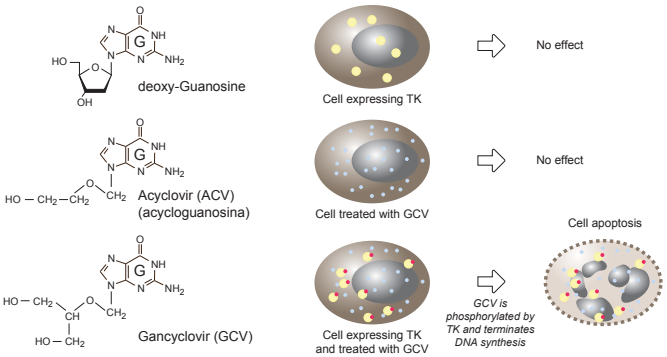
## Pro-drug gene therapy




## Terapia genica mediante l'attivazione di profarmaci

| Gene suicida | Profarmaco | Meccanismo di azione |
|---|---|---|
| Timidino-chinasi del virus dell'herpes simplex-1 (HSV-TK) | Ganciclovir (GCV), aciclovir (ACV), valaciclovir | Inibizione della sintesi del DNA |
| Citosina deaminasi (CD) di E. coli | 5-fluorocitosina (5-FC) | Inibizione della sintesi del DNA e dell'RNA |
| Enzimi del ciitocromo P450 umano CYP2B e CYP3A | Ciclofosfamide ed ifosfamide | Agenti alchilanti del DNA |
| Xantina-guanina fosforibosiltrasferasi (XGPRT) di E. coli | 6-tioxantina (6-TX) | Inibizione della sintesi del DNA |
| Purina-deoxynucleoside fosforilasi (PNP) di E. coli (gene deoD) | 6-metilpurina-2'deossiribonucleoside (MeP) | Inibizione della sintesi del DNA |
| Nitroreduttasi di E. coli | 5-aziridina-1-il-2,4-dinitrobenzamide (CB1954) | Agente alchilante |

[CANCER RESEARCH 46, 5276–5281, October 1986]

**Tumor Chemosensitivity Conferred by Inserted Herpes Thymidine Kinase Genes: Paradigm for a Prospective Cancer Control Strategy[1]**

**Frederick L. Moolten[2]**

*Veterans Administration Medical Center, Bedford, Massachusetts 01730, and the Department of Microbiology, Boston University School of Medicine, Boston, Massachusetts 02118*

**ABSTRACT**

**The lack of highly exploitable biochemical differences between normal tissues and some tumors can theoretically be circumvented by a strategy utilizing gene insertion prophylactically to create tissue mosaicism for drug sensitivity, thereby ensuring that any tumor arising clonally will differ from part of the normal cell population. Elements of the strategy were tested with neoplastic BALB/c murine cell lines bearing the herpes thymidine kinase gene. Exposure to the herpes thymidine kinase-specific substrate 9-[[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl]guanine ablated the clonogenic potential of the cells *in vitro*, and administration of this drug to BALB/c mice bearing tumors produced by the cell lines uniformly induced complete regression of the tumors. The observed responses to therapy imply that the strategy may prove valuable when the genetic technology needed for its human implementation becomes available.**

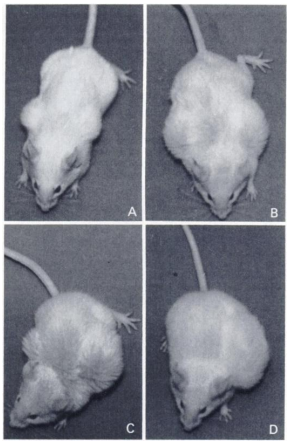



**Fig. 4. Differential effects of HHEMG on HSV-TK-positive and -negative tumors in the same mouse. *A*, Day 13 after $PK_1$ tumor inoculation into the right flank and TK(−) into the left flank. Small tumors are visible at each site. *B*, Day 16. Both tumors are growing progressively. An 8-day course of HHEMG therapy is begun. *C*, Day 23. The $PK_1$ tumor has shrunk, while the TK(−) tumor has enlarged. *D*, Day 37. The $PK_1$ tumor has regressed completely; the TK(−) tumor has continued to grow.**

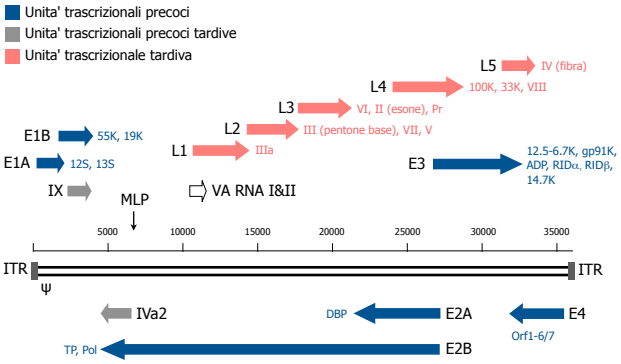
## Randomized Multicenter Trial Comparing the Efficacy of Surgery, Radiation, and Injection of Murine Cells Producing Herpes Simplex Thymidine Kinase Vector Followed by Intravenous Ganciclovir Against the Efficacy of Surgery and Radiation in the Treatment of Newly Diagnosed Previously Untreated Glioblastoma

- Brain tumors are the **third** leading cause of death from cancer in persons **15 to 34** years of age. Despite aggressive therapy, the prognosis is very grim (10 months survivals).
- The strategy consists of injection of **murine cells producing replication-incompetent retroviral vectors containing the HSV-Tk gene**. The mechanism of action is that the Tk protein can phosphorylate nucleoside analogs, such as GCV, to form nucleotide-like precursor that will block replication of DNA, thereby killing the cell.
- The central nervous system has several advantages of safety and efficacy for retroviral-mediated gene transfer. In the brain the tumor is the **most mitotically active** cell, with only macrophages, blood and endothelial cells at minimal risk. Moreover, the brain is a partially **immunologically privileged** site, which should allow a longer survival of the xenogenic cells.
- A particularly attractive feature of using HSV-Tk is the "**bystander effect**", probably due to the transfer of the cytotoxic metabolite, phosphorylated GCV, through cell communication networks such as gap junctions. This phenomenon obviates the necessity for transducing every cell in order to eradicate or reduce the tumor.

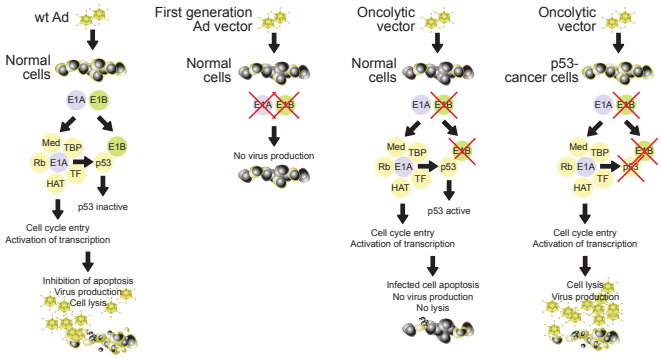
## Strategies for gene therapy of cancer

| Target cell | Strategy | Goal | Therapeutic gene |
|---|---|---|---|
| Cancer cells | Inhibition of cancer cell proliferation | Restoration of cell cycle control | Tumor suppressors (p53, Rb, BRCA1) |
| | | | Antisense oligonucleotides, ribozymes, siRNAs or intracellular antibodies against oncogenes, cdc2, cyclins, PCNA, tyrosine kinase receptors, signal transducers, etc. |
| | Transfer of suicide genes into cancer cells | Specific induction of cytotoxicity in the suicide gene-expressing cells | Gene activating a cytotoxic pro-drug, for example HSV-TK |
| | Oncolytic viruses | Selective lysis of cancer cells by viral replication | |
| Cells of the immune system | Immunotherapy | Increase of antigenic stimulation by cancer cells (active immunization, cancer vaccination) | Tumor-specific antigens (TSAs and TAAs |
| | | | Genes coding for cytokines increasing antigen stimulation (IL-2, IL-12, IFN-γ, GM-CSF) |
| | | Increase of the cytotoxic T-cell response against cancer cells | Genes coding for immunoregulatory cytokines (IL-2, IL-12, IL-7, GM-CSF, IFN-γ, IL-6, TNF-α) |
| | | | Genes coding for co-stimulatory proteins (B7, ICAM-1, LFA-3) |
| | | | Genes coding for immunogenic proteins (MHC I and II alloantigens) |
| | | Genetic modification of effector T cells to redirect them towards cancer cells (adoptive immunotherapy) | TCR genes |
| Hematopoietic stem cells (HSCs) | Increase of the therapeutic index of cancer chemotherapy | Transfer of genes preventing toxicity of chemotherapy into HSCs | Mdr-1 |

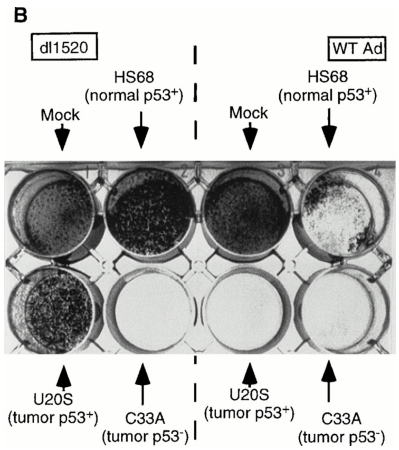
# Organizzazione del genoma di Adenovirus




# Oncolytic adenoviruses




## An adenovirus E1B mutant (ONYX-015) that replicates selectively in p53 deficient human tumor cells




F. McCormick and coll. 1996. Science 274, 373-376

Gene Therapy (2001) 8, 1618–1626

www.nature.com/gt

***RESEARCH ARTICLE***

## *Intra-arterial administration of a replication-selective adenovirus (dl1520) in patients with colorectal carcinoma metastatic to the liver: a phase I trial*

T Reid[1], E Galanis[2], J Abbruzzese[3], D Sze[1], J Andrews[2], L Romel[4], M Hatfield[4], J Rubin[2] and D Kirn[5]

[1]*Palo Alto Veterans Administration Hospital and Stanford University Medical Center, Palo Alto, CA;* [2]*Mayo Clinic, Rochester, MN;* [3]*MD Anderson Cancer Center, Houston, TX;* [4]*Onyx Pharmaceuticals, Richmond, CA, USA; and* [5]*Imperial Cancer Research Fund, Imperial College School of Medicine, London, UK*

**ONYX-015 is a first generation replication-selective adenovirus with a deletion in the E1B-55kDa gene, which is responsible for p53 inactivation. Thus, this mutant should be unable to overcome the p53-mediated blockade of viral replication in normal cells. In contrast, in a tumor cell lacking p53 function, the E1B-p53 protein should be expendable for p53 inhibition and replication should proceed.**
**ONYX-015 has shown promise in phase I and II clinical trials following direct intratumoral injection into recurrent head and neck cancers.**

| (Study day) | Pre | 1 | 4 | 8 | 22 | 50 | 78+ |
|---|---|---|---|---|---|---|---|
| *Treatment* | | | | | | | |
| • ONYX-015 h.a.i. | | X | | X | X | X | X |
| • 5-FU/leucovorin i.v. | | | | | X | X | X |
| *Assessment* | | | | | | | |
| • Pharmacokinetics | | X | | | X | | |
| • Viral replication, shedding | X | | X* | | | | |
| • Cytokine assessment | X | X | | | | | |
| • Neutralizing antibodies | X | | | | X | X | X |
| • Efficacy (CT scan, serologic) | X | | | | X | X | X |

- **Moderate fever, rigors and fatigue were the most common adverse events**
- **Antibody titers increased significantly in all patients**
- **Viral replication was detectable in patients receiving the highest doses**
- **An objective response was demonstrated in combination with chemotherapy in a patient who was refractory to 5-FU**

**Hepatic artery infusion of dl1520 was well-tolerated at doses resulting in infection, replication and chemotherapy-associated antitumoral activity**

## A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer

Fadlo R. Khuri[1], John Nemunaitis[2], Ian Ganly[4], James Arseneau[3], Ian F. Tannock[7], Larry Romel[8], Martin Gore[5], Janet Ironside[6], R.H. MacDougall[8], Carla Heise[8], Britta Randlev[8], Ann M. Gillenwater[2], Patricia Bruso[2], Stanley B. Kaye[4], Waun Ki Hong[1] & David H. Kirn[9]

*The University of Texas M. D. Anderson Cancer Center,*
*[1]Divisions of Cancer Medicine and [2]Surgery, Houston, Texas;*
*[3]U.S. Oncology, Dallas, Texas,*
*[4]Beatson Oncology Institute, University of Glasgow, Glasgow Scotland*
*[5]Royal Marsden Hospital, London, England,*
*[6]Western General Hospital, Edinburgh, Scotland;*
*[7]Princess Margaret Hospital, Toronto, Ontario; [8]ONYX Pharmaceutical, Richmond, California;*
*[9]Imperial Cancer Research Fund, London, England*
*Correspondence should be addressed to: F.R.K.; email: fkhuri@mdanderson.org*

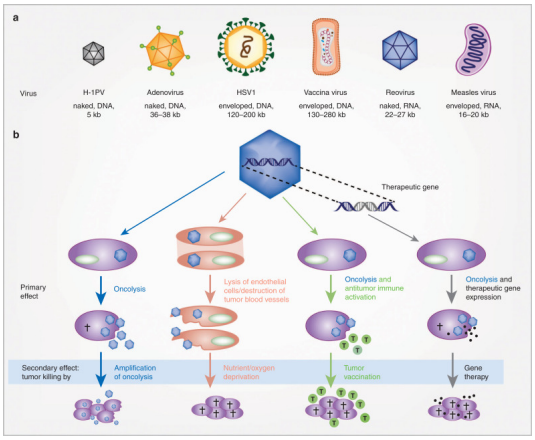
**ONYX-015 is an adenovirus with the E1B 55-kDa gene deleted, engineered to selectively replicate in and lyse p53-deficient cancer cells while sparing normal cells. Although ONYX-015 and chemotherapy have demonstrated anti-tumoral activity in patients with recurrent head and neck cancer, disease recurs rapidly with either therapy alone. We undertook a phase II trial of a combination of intratumoral ONYX-015 injection with cisplatin and 5-fluorouracil in patients with recurrent squamous cell cancer of the head and neck. There were substantial objective responses, including a high proportion of complete responses. By 6 months, none of the responding tumors had progressed, whereas all non-injected tumors treated with chemotherapy alone had progressed. The toxic effects that occurred were acceptable. Tumor biopsies obtained after treatment showed tumor-selective viral replication and necrosis induction.**

**Table 1. Summary of a selection of completed oncolytic virus clinical trials discussed in this review.**

| Name of agent | Virus | Indications | Phase | Outcome and comments | Ref. |
|---|---|---|---|---|---|
| G207 | Engineered conditionally replicative HSV1 | Glioma | I | No adverse events that could be unequivocally related to HSV. Some cases had radiologic and histologic signs of tumor response | [68] |
| HSV 1716 | Engineered conditionally replicative HSV1 | Glioma | I | No evidence of encephalitis or other adverse events. Four of nine patients alive 14–24 months after OV administration | [69] |
| Onyx-015 | E1B-deleted adenovirus | Head and neck cancer | I | Dose-limiting toxicity not reached, mild flu-like symptoms observed. No objective responses recorded | [70] |
| PV701 | Naturally attenuated strain of Newcastle disease virus | Advanced solid tumors | I | Primarily mild flu-like symptoms recorded. 100-fold intensification from starting dose achieved with objective responses recorded for higher doses | [72] |
| MV-CEA | Edmonston strain of measles virus engineered to express CEA as a marker | Ovarian carcinoma | I | Dose-limiting toxicity not reached. Dose-dependent disease stabilization in 14 of 21 patients | [73] |
| JX-594 | Thymidine kinase deleted Vaccinia expressing GM-CSF | Advanced solid tumors | I | Dose-limiting toxicity not reached. Mild flu-like symptoms were the most common adverse effects reported. 87% of tumor biopsies positive for JX-594 | [75] |
| JX-594 | Thymidine kinase deleted Vaccinia expressing GM-CSF | Hepatocellular carcinoma | II | Randomized dose-finding study, significantly longer survival times with higher dose (14.1 vs 6.7 months) | [76] |
| Reolysin | Reovirus | Malignant melanoma | II | No objective responses, but treatment well tolerated. Trials in combination with cytotoxic therapies are ongoing | [77] |
| T-VEC (originally called OncoVEX-GM-CSF) | HSV expressing GM-CSF | Malignant melanoma | II | Overall response rate of 26%. 1- and 2-year survivals of 58 and 52%, respectively | [78] |
| T-VEC (originally called OncoVEX-GM-CSF) | HSV expressing GM-CSF | Malignant melanoma | III | Significant improvement of durable response rate compared with GM-CSF alone (16 vs 2%). Trend towards increased survival data collection ongoing | [79] |

*OV: Oncolytic virus.*

# Modern oncolytic viruses




Therapeutic genes:
IL-12
IL-18

Cancer-specific promoters (i.e. PSA)

# HSV-1 and HSV-1 vectors

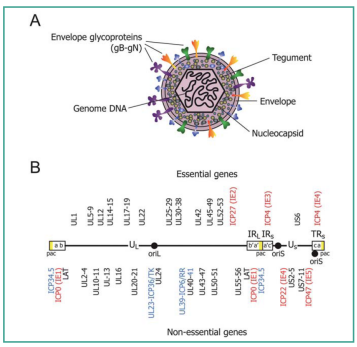
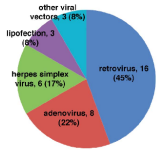



**Fig. 3.29** HSV-1 and HSV-1 vectors. **A** Schematic representation of the structure of an HSV-1 virion. **B** HSV-1 genome organization. The HSV-1 genome consists of a linear, double-stranded DNA molecule of 152 kb containing more than 80 genes. The genome is composed of unique long ($U_L$) and unique short ($U_S$) segments which are flanked by inverted repeats. These are designated as $TR_L$ and $IR_L$ (terminal and internal repeat of the long segment, respectively) and $TR_S$ and $IR_S$ (terminal and internal repeat of the short segment). The repeats surrounding $U_L$ are designated ab and b'a', while those surrounding $U_S$ are designated a'c' and ca. There are two different origins of replication, oriL in the long segment and oriS in the short segment. OriS is duplicated, along with ICP4, because it is found in the inverted repeats surrounding the long segment. Approximately half of the genes are essential for viral replication in cell culture (*listed on top*); the other half are non-essential for viral replication in cultured cells (*bottom*). Genes in blue are non-essential genes that are mutated in the replication-competent viruses so far developed and described in the text; genes in red are immediate early (IE) genes that are mutated in the replication-defective viruses. The genome contains three pac signals (shown in *yellow*) that assist in packaging the viral genome DNA into virions




**Gene Therapy for High-Grade Glioma**

# Strategies for gene therapy of cancer

| Target cell | Strategy | Goal | Therapeutic gene |
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| Cancer cells | Inhibition of cancer cell proliferation | Restoration of cell cycle control | Tumor suppressors (p53, Rb, BRCA1) |
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| Hematopoietic stem cells (HSCs) | Increase of the therapeutic index of cancer chemotherapy | Transfer of genes preventing toxicity of chemotherapy into HSCs | Mdr-1 |

# Tumor Infiltrating Lymphocytes (TIL)

Cancer Gene Ther. 1995 Jun;2(2):125-36.

**Genetically marking human cells--results of the first clinical gene transfer studies.**

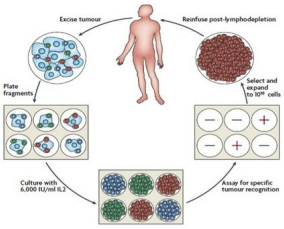
Cai Q[1], Rubin JT, Lotze MT

Author information

1 Department of Surgery, School of Medicine, University of Pittsburgh Medical Center, Pennsylvania, USA.

**Abstract**

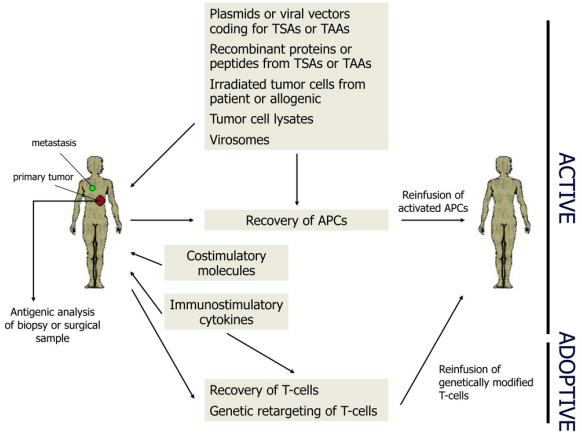
The rapid development of both knowledge and techniques in molecular biology have made it possible to engineer genetic constructs and transfer them into cells of individuals with various diseases. Such **gene** therapies may alleviate or perhaps even cure diseases for which no adequate treatment now exists. One potential application is to treat genetic disease by inserting a normal **gene** into cells in individuals with a "malfunctioning" **gene**. The added genetic information could allow these cells to function properly and might reduce or eliminate the sequelae of the disease. Such genetic manipulation could also be used to combat other diseases using the same general technique. For example in cancer patients, various cytokine **genes** inserted into **tumor** cells may serve as components of a **tumor** vaccine because such cytokine-secreting **tumor** cells can induce a significant T-cell response in experimental animal models when compared with non-**gene**-modified tumors, ultimately leading to a systemic immune response. In addition to treating patients, transferred **genes** also can serve as markers to obtain important information about the fate of otherwise indistinguishable cells. For example, we used a genetic marker to label **tumor-infiltrating lymphocytes** (TILs) to monitor their in vivo survival and ability to "home" to **tumor** sites. **Gene** markers also were transferred into autologous bone marrow cells to study the mechanism of **tumor** relapse. This review will focus primarily on studies using **gene** markers to track TILs after transfer. We will focus on the following issues: (a) that TILs are potent antitumor cells, mediating partial and complete responses in patients with melanoma; (b) the importance of the initial **gene** marked TIL study; (c) safety considerations in the use of **gene marking/gene** therapy; (d) results of **gene**-marked TIL studies; and (e) other **gene**-marked cells.




# Antigeni delle cellule tumorali

| | | Antigene | Tumore |
|---|---|---|---|
| Antigeni presenti esclusivamente nelle cellule tumorali (tumor specific antigen, **TSA**) | Antigeni specifici delle cellule tumorali | **Idiotipo** dell'anticorpo espresso dalle cellule tumorali | Linfomi a cellule B |
| | | T-cell receptor (TCR) espresso dalla cellule tumorali | Linfomi a cellule T |
| | Proteine cellulari mutate che partecipano al processo di trasformazione tumorale | Proteina p21$^{ras}$ mutata | ~10% dei tumori |
| | | Proteina di fusione **p210**$^{bcr-abl}$ | Leucemia mieloide cronica |
| | | Proteina p53 mutata | >50% dei tumori |
| | Proteine di origine virale espresse dalle cellule tumorali | Proteine **E6**, **E7** del virus del papilloma umano (**HPV**) | Carcinoma della cervice uterina |
| | | Proteina EBNA-1 del virus di Epstein-Barr (EBV) | Morbo di Hodgkin<br>Linfomi EBV-positivi |
| Proteine normali espresse abnormemente dalle cellule tumorali (tumor-associated antigens, **TAA**) | Proteine normali espresse a livelli molto elevati | **PSA**, HER2/neu, MUC-1 | Diversi carcinomi |
| | Antigeni oncofetali | **CEA**, **AFP** | Diversi carcinomi |
| | Antigeni di differenziamento | Melan-A/MART-1, tirosinasi, gp100 | >50% dei melanomi |
| | Antigeni CTA (cancer-testis antigens) | Proteine delle famiglie MAGE, BAGE, GAGE, LAGE, PRAME, NY1-ESO-1, etc. | Melanoma, tumore della vescica, tumore del polmone non a piccole cellule, ed altri tumori |

# Immunotherapy of cancer



## Vaccinazione con DNA tramite "Gene Gun"

### DNA vaccination for HIV

**Studies in chimpanzees provide preliminary evidence that DNA vaccines may protect against experimental infection with HIV-1 (pages 526–532).**

WHO has been in the IDS vaccine research for who, together with col-

RONALD C. KENNEDY

products. These invest ously characterized th mune responses to

NEWS & VIEWS

### Naked DNA: New shots for allergy?

**Injection of an allergen gene into rat muscle prevents specific IgE production and asthmatic reactions (pages 540–544).**

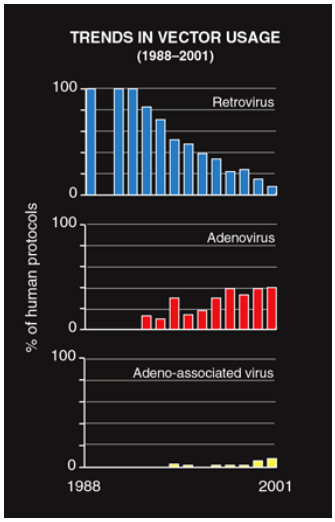
of asthma and allergic it the most frequent af- n society. The medical

MIRIAM MOFFATT & WILLIAM COOKSON

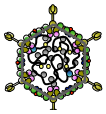
muscle tissues. A trans to *Der p* V was detectec no IgE response was nc

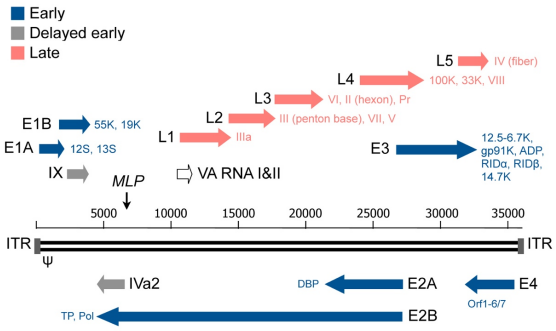
### Genetic vaccination: The advantages of going naked

**Manipulating the immune system with DNA vaccines shows promise for protecting against pathogens and suppressing autoimmune disease (pages 888–905).**

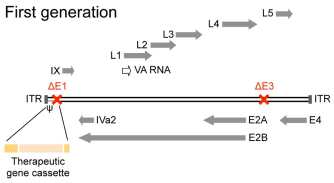


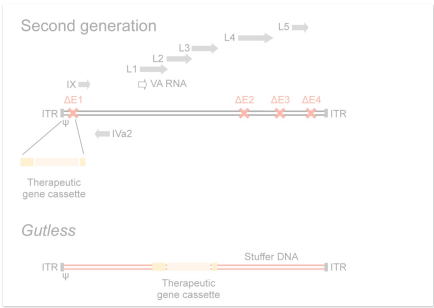

## Adenovirus genome organization






## Adenoviral vectors




## First generation Adenoviral vectors

### Advantages

**Broad target cell repertoire:**

i) Natural tropism for a variety of cell types.
ii) Ability to infect proliferating and quiescent cells.

**High efficiency of in vivo transduction**

**Remain episomal**

*Technically:*

High titre production levels (up to $10^{11}$-$10^{12}$ pfu/ml).
Quite stable, manipulation friendly genome.
Well understood molecular biology and host cell interactions.

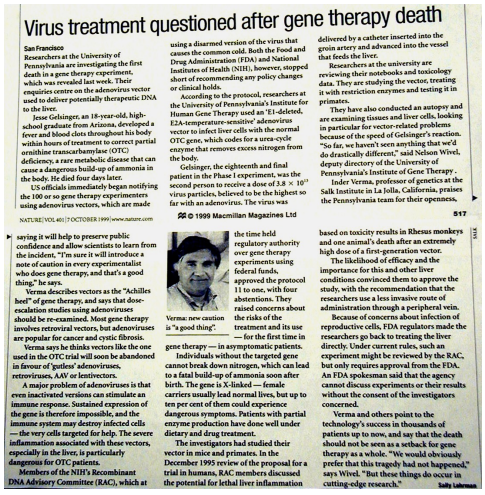
### Limitations

**Transient gene expression and problematic re-administration**

**Strong host immune response to viral proteins and cytotoxicity (CD4+, CD8+ T-cell activation, neutralising antibodies)**

*Technically:*

Limited cloning capacity (<8 kb)
RCA generation



### Virus treatment questioned after gene therapy death

San Francisco

Researchers at the University of Pennsylvania are investigating the first death in a gene therapy experiment, which was revealed last week. Their enquiries centre on the adenovirus vector used to deliver potentially therapeutic DNA to the liver.

Jesse Gelsinger, an 18-year-old, high-school graduate from Arizona, developed a fever and blood clots throughout his body within hours of treatment to correct partial ornithine transcarbamylase (OTC) deficiency, a rare metabolic disease that can cause a dangerous build-up of ammonia in the body. He died four days later.

US officials immediately began notifying the 100 or so gene therapy experimenters using adenovirus vectors, which are made

NATURE | VOL 401 | 7 OCTOBER 1999 | www.nature.com

using a disarmed version of the virus that causes the common cold. Both the Food and Drug Administration (FDA) and National Institutes of Health (NIH), however, stopped short of recommending any policy changes or clinical holds.

According to the protocol, researchers at the University of Pennsylvania's Institute for Human Gene Therapy used an 'E1-deleted, E2A-temperature-sensitive' adenovirus vector to infect liver cells with the normal OTC gene, which codes for a urea-cycle enzyme that removes excess nitrogen from the body.

Gelsinger, the eighteenth and final patient in the Phase I experiment, was the second person to receive a dose of $3.8 \times 10^{13}$ virus particles, believed to be the highest so far with an adenovirus. The virus was

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delivered by a catheter inserted into the groin artery and advanced into the vessel that feeds the liver.

Researchers at the university are reviewing their notebooks and toxicology data. They are studying the vector, treating it with restriction enzymes and testing it in primates.

They have also conducted an autopsy and are examining tissues and liver cells, looking in particular for vector-related problems because of the speed of Gelsinger's reaction. "So far, we haven't seen anything that we'd do drastically different," said Nelson Wivel, deputy directory of the University of Pennsylvania's Institute of Gene Therapy.

Inder Verma, professor of genetics at the Salk Institute in La Jolla, California, praises the Pennsylvania team for their openness,

517

saying it will help to preserve public confidence and allow scientists to learn from the incident. "I'm sure it will introduce a note of caution in every experimentalist who does gene therapy, and that's a good thing," he says.

Verma describes vectors as the "Achilles heel" of gene therapy, and says that dose-escalation studies using adenoviruses should be re-examined. Most gene therapy involves retroviral vectors, but adenoviruses are popular for cancer and cystic fibrosis.

Verma says he thinks vectors like the one used in the OTC trial will soon be abandoned in favour of 'gutless' adenoviruses, retroviruses, AAV or lentivectors.

A major problem of adenoviruses is that even inactivated versions can stimulate an immune response. Sustained expression of the gene is therefore impossible, and the immune system may destroy infected cells — the very cells targeted for help. The severe inflammation associated with these vectors, especially in the liver, is particularly dangerous for OTC patients.

Members of the NIH's Recombinant DNA Advisory Committee (RAC), which at



Verma: new caution is "a good thing".

the time held regulatory authority over gene therapy experiments using federal funds, approved the protocol 11 to one, with four abstentions. They raised concerns about the risks of the treatment and its use — for the first time in gene therapy — in asymptomatic patients.

Individuals without the targeted gene cannot break down nitrogen, which can lead to a fatal build-up of ammonia soon after birth. The gene is X-linked — female carriers usually lead normal lives, but up to ten per cent of them could experience dangerous symptoms. Patients with partial enzyme production have done well under dietary and drug treatment.

The investigators had studied their vector in mice and primates. In the December 1995 review of the proposal for a trial in humans, RAC members discussed the potential for lethal liver inflammation

based on toxicity results in Rhesus monkeys and one animal's death after an extremely high dose of a first-generation vector.

The likelihood of efficacy and the importance for this and other liver conditions convinced them to approve the study, with the recommendation that the researchers use a less invasive route of administration through a peripheral vein.

Because of concerns about infection of reproductive cells, FDA regulators made the researchers go back to treating the liver directly. Under current rules, such an experiment might be reviewed by the RAC, but only requires approval from the FDA. An FDA spokesman said that the agency cannot discuss experiments or their results without the consent of the investigators concerned.

Verma and others point to the technology's success in thousands of patients up to now, and say that the death should not be seen as a setback for gene therapy as a whole. "We would obviously prefer that this tragedy had not happened," says Wivel. "But these things do occur in cutting-edge research."

Sally Lehrman

## Replication-defective Adenoviral vectors

### First generation (E1 deletion)

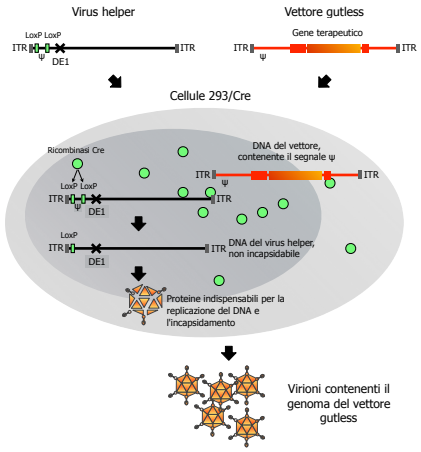
- Cloning capacity < 6 kb
- Blocking of virus genetic program can be leaky (cytopathic effects)
- High level expression of transgene in transduced cells

### Second generation (e.g. E1 + E4 deletions)

- Cloning capacity extended to 9 kb
- Profound blockage of viral gene expression
- Reduced vector-induced cytopathic effects
- Vector persists longer in transduced cells
- Expression of transgene impaired

## Gutless Adenoviral vectors

I vettori gutless, che contengono solo le sequenze terminali invertite (ITR, Inverted Terminal Repeats), il segnale di incapsidazione e la cassetta d'espressione, richiedono un virus helper (difettivo per l'incapsidazione) che fornisca in trans le proteine virali necessarie per la sintesi della particella virale infettiva nella cellula produttrice. Il virus helper può poi essere rimosso dalla preparazione di vettore mediante un processo di purificazione, con efficienza superiore al 99,9%.




## Helper-dependent Adenoviral vectors

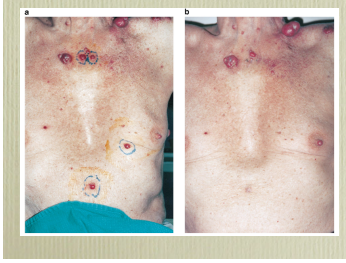
| Advantages | Limitations |
|---|---|
| Reduced toxicity and nearly eliminated immune responses | Low but significant helper virus contamination |
| Higher levels and prolonged transgene expression | Error prone not robust production system (susceptible to recombination and instability) |
| Increased cloning capacity (up to 36 kb) | RCA and defective viral particle production (1:10 or 1:200 ratio) |
| All benefits of F.G adenoviral vectors | Massive-scale production restrictions for clinical use due to purification restrictions |

Gene Therapy (1999) 6, 350–363

http://www.stockton-press.co.uk/gt

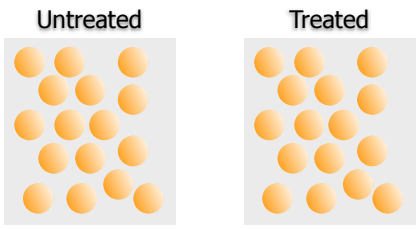
*Adenovector-mediated gene delivery of interleukin-2 in metastatic breast cancer and melanoma: results of a phase 1 clinical trial*

Direct injection into subcutaneous deposits of melanoma or breast cancer (23 patients injected at 7 dose levels)



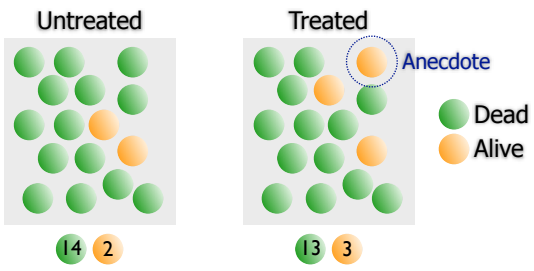

- 60% local inflammation
- 24% incomplete **local tumor regression,** but no conventional clinical responses
- Tumor necrosis and lymphocytic infiltration at biopsy
- IL-12 mRNA and protein detectable at 48 hrs (only transcript at day 7)
- This trial therefore confirms the **safety of use of adenoviral vectors for gene delivery in humans** and demonstrates **successful transgene expression** even in the face of pre-existing immunity to adenovirus

## Science vs. Anecdote




Survival

## Science vs. Anecdote

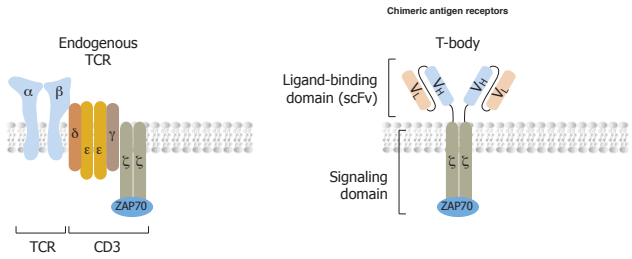



P (probability that difference is by chance) > 0.05 (5%)

## Strategies for gene therapy of cancer

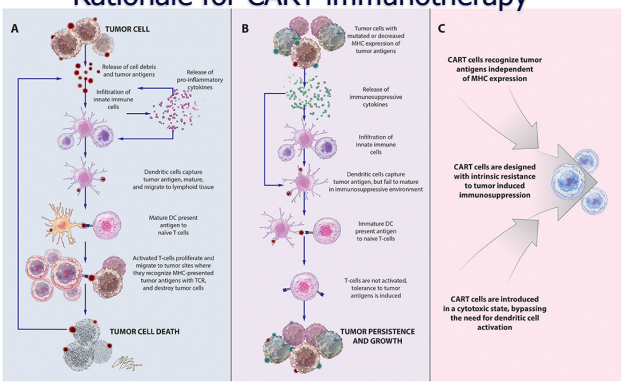
| Target cell | Strategy | Goal | Therapeutic gene |
|---|---|---|---|
| Cancer cells | Inhibition of cancer cell proliferation | Restoration of cell cycle control | Tumor suppressors (p53, Rb, BRCA1) |
| | | | Antisense oligonucleotides, ribozymes, siRNAs or intracellular antibodies against oncogenes, cdc2, cyclins, PCNA, tyrosine kinase receptors, signal transducers, etc. |
| | Transfer of suicide genes into cancer cells | Specific induction of cytotoxicity in the suicide gene-expressing cells | Gene activating a cytotoxic pro-drug, for example HSV-TK |
| | Oncolytic viruses | Selective lysis of cancer cells by viral replication | |
| Cells of the immune system | Immunotherapy | Increase of antigenic stimulation by cancer cells (active immunization, cancer vaccination) | Tumor-specific antigens (TSAs and TAAs |
| | | | Genes coding for cytokines increasing antigen stimulation (IL-2, IL-12, IFN-γ, GM-CSF) |
| | | Increase of the cytotoxic T-cell response against cancer cells | Genes coding for immunoregulatory cytokines (IL-2, IL-12, IL-7, GM-CSF, IFN-γ, IL-6, TNF-α) |
| | | | Genes coding for co-stimulatory proteins (B7, ICAM-1, LFA-3) |
| | | | Genes coding for immunogenic proteins (MHC I and II alloantigens) |
| | | Genetic modification of effector T cells to redirect them towards cancer cells (adoptive immunotherapy) | TCR genes |
| Hematopoietic stem cells (HSCs) | Increase of the therapeutic index of cancer chemotherapy | Transfer of genes preventing toxicity of chemotherapy into HSCs | Mdr-1 |

## Recombinant T-cell Receptor




**Chimeric antigen receptors** (**CARs**, also known as **chimeric immunoreceptors**, **chimeric T cell receptors**, **artificial T cell receptors** or **CAR-T**) are engineered receptors which graft an arbitrary specificity onto an immune effector cell (T cell). Typically, these receptors are used to graft the specificity of a monoclonal antibody onto a T cell, with transfer of their coding sequence facilitated by retroviral vectors. The receptors are called chimeric because they are composed of parts from different sources.

## Rationale for CART immunotherapy




**(A)** Release of cell debris and tumor antigens from malignant cells activates a cascade of host antitumor immune responses, initiated by innate immune cells that release pro-inflammatory cytokines and contribute to tumor cell destruction. Among these cells are dendritic cells, which capture tumor antigens, mature in response to the pro-inflammatory cytokines in the environment, and travel to lymphoid tissues to stimulate T-cell proliferation and activation of antigen-specific adaptive immune responses leading to tumor death. **(B)**. Tumors often develop adaptations to evade detection and destruction by the host immune system. Through the recruitment of suppressive leukocytes and elaboration of immunosuppressive cytokines, tumors inhibit the function of infiltrating immune cells, including dendritic cells. Incompletely matured DCs are unable to effectively activate naïve T cells, instead inducing T-cell anergy, apoptosis, or tolerance to tumor-associated antigens. Downregulation of antigen-presenting machinery and the development of antigen-loss variants enable tumor cells to escape detection by infiltrating immune cells. **(C)** CAR T-cells, which recognize antigens via a mechanism distinct from TCR stimulation, bypass the need for DC antigen presentation and are unaffected by MHC downregulation. CAR structure and culture conditions can also be optimized to create CART populations with superior cytotoxicity and resistance to tumor-induced suppressive influences.

| Target antigen | Disease | CAR signaling domain | ClinicalTrial.gov identifier | Clinical center |
|---|---|---|---|---|
| CD19 | B-CLL | CD28-CD3ζ | NCT00466531 | MSKCC |
| CD19 | B-ALL | CD28-CD3ζ | NCT01044069 | MSKCC |
| CD19 | Leukemia | CD28-CD3ζ | NCT01416974 | MSKCC |
| CD19 | Leukemia/lymphoma | CD28-CD3ζ | NCT00924326 | NCI |
| CD19 | Leukemia/lymphoma | CD28-CD3ζ | NCT01087294 | NCI |
| CD19 | Leukemia/lymphoma | CD28-CD3ζ vs. CD3ζ | NCT00586391 | BCM |
| CD19 | B-NHL/CLL | CD28-CD3ζ vs. CD3ζ | NCT00608270 | BCM |
| CD19 | Advanced B-NHL/CLL | CD28-CD3ζ vs. CD3ζ | NCT00709033 | BCM |
| CD19 | ALL post-HSCT | CD28-CD3ζ | NCT00840853 | BCM |
| CD19 | Leukemia/lymphoma | CD137-CD3ζ | NCT01029366 | UP |
| CD19 | B-lymphoid malignancies | CD28-CD3ζ | NCT00968760 | MDACC |
| CD19 | B-lineage malignancies | CD28-CD3ζ | NCT01362452 | MDACC |
| CD20 | Mantle cell lymphoma/indolent B-NHL | CD28-CD137-CD3ζ | NCT00621452 | FHCRC |
| PMSA | Prostate cancer | CD28-CD3ζ | NCT01140373 | MSKCC |
| CEA | Breast cancer | CD28-CD3ζ | NCT00673829 | RWMC |
| CEA | Colorectal cancer | CD28-CD3ζ | NCT00673322 | RWMC |
| Her2/neu | Lung cancer | CD28-CD3ζ | NCT00889954 | BCM |
| Her2/neu | Osteosarcoma | CD28-CD3ζ | NCT00902044 | BCM |
| Her2/neu | Glioblastoma | CD28-CD3ζ | NCT01109095 | BCM |
| Kappa light chain | B-NHL and B-CLL | CD28-CD3ζ vs. CD3ζ | NCT00881920 | BCM |

*MSKCC, Memorial Sloan-Kettering Cancer Center; NCI, National Cancer Institute; BCM, Baylor College of Medicine; RWMC, Roger Williams Medical Center; UP University of Pennsylvania; MDACC, M.D. Anderson Cancer Center; FHCRC, Fred Hutchinson Cancer Research Center.*

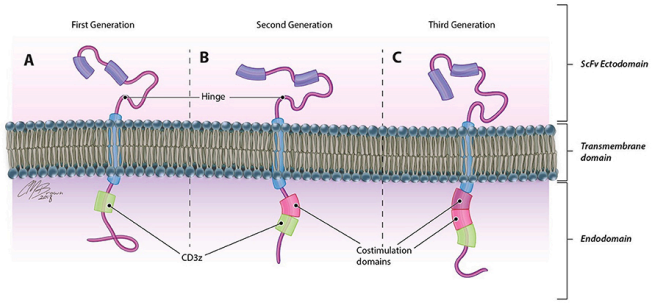
## Ideal antigens for CAR generation

1)tumor exclusive
2)expressed by all malignant cells
3)function crucial to tumor growth and survival

## Results

1)maximize tumoricidal capacity
2)prevent immune evasion
3)reduce the risk of toxicity stemming from CART destruction of antigen-expressing healthy cells

## Evolution of CAR structure




Originally derived from the CD3ζ chain of the traditional TCR, CAR endodomains have undergone generational changes to include one or more costimulatory domains, most commonly CD28 and 41BB, to enhance the persistence and cytotoxicity of CAR-expressing cells

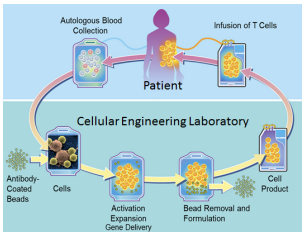
NOVARTIS

**Basel, August 30, 2017**

# Novartis receives first ever FDA approval for a CAR-T cell therapy, Kymriah(TM) (CTL019), for children and young adults with B-cell ALL that is refractory or has relapsed at least twice

ALL prognosis is poor. Patients often undergo multiple treatments including chemotherapy, radiation, targeted therapy or stem cell transplant, yet less than 10% of patients survive five years

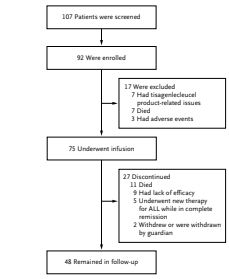
Kymriah is an innovative immunocellular therapy that is a one-time treatment. Kymriah uses the 4-1BB costimulatory domain in its chimeric antigen receptor to enhance cellular expansion and persistence.


ORIGINAL ARTICLE

## Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia

S.L. Maude, T.W. Laetsch, J. Buechner, S. Rives, M. Boyer, H. Bittencourt, P. Bader, M.R. Verneris, H.E. Stefanski, G.D. Myers, M. Qayed, B. De Moerloose, H. Hiramatsu, K. Schlis, K.L. Davis, P.L. Martin, E.R. Nemecek, G.A. Yanik, C. Peters, A. Baruchel, N. Boissel, F. Mechinaud, A. Balduzzi, J. Krueger, C.H. June, B.L. Levine, P. Wood, T. Taran, M. Leung, K.T. Mueller, Y. Zhang, K. Sen, D. Lebwohl, M.A. Pulsipher, and S.A. Grupp

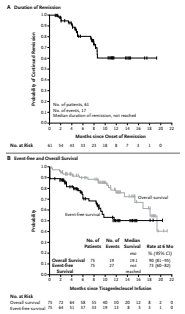



Overall remission rate of 81% among 75 patients with at least 3 months of follow-up after a single infusion of tisagenlecleucel

The remissions were durable, with a 6-month relapse-free survival rate of 80%

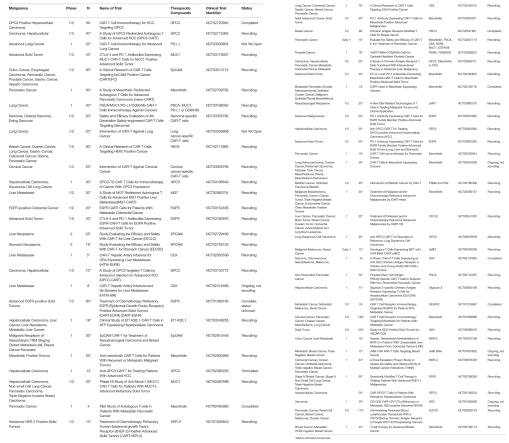
Tisagenlecleucel was administered as a single infusion, and most toxic effects were observed only during the first 8 weeks after infusion.

**Cytokine release syndrome**
A condition that may occur after treatment with some types of immunotherapy, such as monoclonal antibodies and CAR-T cells. Cytokine release syndrome is caused by a large, rapid release of cytokines into the blood from immune cells affected by the immunotherapy. Cytokines are immune substances that have many different actions in the body. Signs and symptoms of cytokine release syndrome include fever, nausea, headache, rash, rapid heartbeat, low blood pressure, and trouble breathing. Most patients have a mild reaction, but sometimes, the reaction may be severe or life threatening.




N ENGL J MED 378;5 NEJM.ORG FEBRUARY 1, 2018

## CART and Solid Malignancies



## CAR for glioblastoma multiforme

Strong positive correlation between the degree of intratumoral infiltration with antigen-specific cytotoxic T-cells (CTLs) and overall patient survival

Antigen targets: EGFRvIII, IL-13Rα2, and HER2

EGFRvIII is a mutated form of the epidermal growth factor receptor (EGFR), resulting from a tumor-specific in-frame deletion creating a constitutively active surface receptor protein. Present in approximately 30% of GBMs, this mutant receptor enhances glioma cell proliferation, angiogenesis, and invasiveness and is independently associated with a poor prognosis

RESEARCH ARTICLE

IMMUNOTHERAPY

### Rational development and characterization of humanized anti–EGFR variant III chimeric antigen receptor T cells for glioblastoma

Laura A. Johnson,[1,2*] John Scholler,[3*] Takayuki Ohkuri,[4] Akemi Kosaka,[4] Prachi R. Patel,[1] Shannon E. McGettigan,[1] Arburn K. Nace,[1] Tavata Dentchev,[4] Pramod Thokkat,[4] Andreas Loew,[4] Alina C. Boesteanu,[1] Alexandria P. Cogdill,[1] Taylor Chen,[1] Joseph A. Fraietta,[1] Christopher C. Kloss,[1] Avery D. Posey Jr.,[1] Boris Engels,[5] Rashma Singh,[6] Tucker Endt,[6] Neeraja Idamakanti,[6] Melissa H. Ramnoney,[6] Na Li,[6] Li Zhou,[6] Gabriela Plesa,[7] John T. Sarkeva,[4] Hidaho Okada,[4] Carl H. June,[1,2] Jennifer L. Brogdon,[6] Marcela V. Maus[1,2*]

Chimeric antigen receptors (CARs) are synthetic molecules designed to redirect T cells to specific antigens. CAR-modified T cells can mediate long-term durable remissions in B cell malignancies, but expanding this platform to solid tumors requires the discovery of surface targets with limited expression in normal tissues. The variant III mutation of the epidermal growth factor receptor (EGFRvIII) results from an in-frame deletion of a portion of the extracellular domain, creating a neoepitope. We chose a vector backbone encoding a second-generation CAR based on efficacy of a murine scFv–based CAR in a xenograft model of glioblastoma. Next, we generated a panel of humanized scFvs and tested their specificity and function as soluble proteins and in the form of CAR-transduced T cells; a low-affinity scFv was selected on the basis of its specificity for EGFRvIII over wild-type EGFR. The lead candidate scFv was tested in vitro for its ability to direct CAR-transduced T cells to specifically lyse, proliferate, and secrete cytokines in response to antigen-bearing targets. We further evaluated the specificity of the lead CAR candidate in vitro against EGFR-expressing keratinocytes and in vivo in a model of mice grafted with normal human skin. EGFRvIII-directed CAR T cells were also able to control tumor growth in xenogeneic subcutaneous and orthotopic models of human EGFRvIII+ glioblastoma. On the basis of these results, we have designed a phase 1 clinical study of CAR T cells transduced with humanized scFv directed to EGFRvIII in patients with either resected or recurrent glioblastoma (NCT02209376).

www.ScienceTranslationalMedicine.org 18 February 2015 Vol 7 Issue 275 275ra22

Preclinical studies have established
- the ability of T-cells targeting this unique, tumor-specific epitope to proliferate and release cytokines in response to stimulation with the mutant EGFRvIII antigen, but not wild-type EGFR
- EGFRvIII-targeting CARTs effectively traffic to tumor sites and suppress the growth of glioma xenografts in murine models

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

### A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma

Donald M. O'Rourke,[1] MacLean P. Nasrallah,[2*] Arati Desai,[2*] Jan J. Melenhorst,[3*] Keith Mansfield,[3*] Jennifer J. D. Morrissette,[4] Maria Martinez-Lage,[2] Steven Brem,[1] Eileen Maloney,[1] Angela Shen,[1] Randi Isaacs,[1] Suyash Mohan,[1] Gabriela Plesa,[5] Simon F. Lacey,[5] Jean-Marc Navenot,[6] Zhaohui Zheng,[6] Bruce L. Levine,[5] Hidaho Okada,[4] Carl H. June,[5] Jennifer L. Brogdon,[6] Marcela V. Maus[1,5]*

We conducted a first-in-human study of intravenous delivery of a single dose of autologous T cells redirected to the epidermal growth factor receptor variant III (EGFRvIII) mutation by a chimeric antigen receptor (CAR). We report our findings on the first 10 recurrent glioblastoma (GBM) patients treated. We found that manufacturing and infusion of CAR-modified T cell (CART)–EGFRvIII cells are feasible and safe, with evidence of off-tumor toxicity or cytokine release syndrome. One patient has had residual stable disease for over 18 months of follow-up. All patients demonstrated detectable transient expansion of CART-EGFRvIII cells in peripheral blood. Seven patients had post-CART-EGFRvIII surgical intervention, which allowed for tissue-specific analysis of CART-EGFRvIII trafficking to the tumor, phenotyping of tumor-infiltrating T cells and the tumor microenvironment in situ, and analysis of post-therapy EGFRvIII target antigen expression. Imaging findings after CART immunotherapy were complex to interpret, further reinforcing the need for pathologic sampling in infused patients. We found trafficking of CART-EGFRvIII cells to regions of active GBM, with antigen decrease in five of these seven patients. In situ evaluation of the tumor environment demonstrated increased and robust expression of inhibitory molecules and infiltration by regulatory T cells after CART-EGFRvIII infusion, compared to pre–CART-EGFRvIII infusion tumor specimens. Our initial experience with CAR T cells in recurrent GBM suggests that although intravenous infusion results in on-target activity in the brain, overcoming the adaptive changes in the local tumor microenvironment and addressing the antigen heterogeneity may improve the efficacy of EGFRvIII-directed strategies in GBM.

O'Rourke et al., Sci. Transl. Med. 9, eaaa0984 (2017) 19 July 2017



Analysis of pre- and post-treatment tumor samples revealed post-treatment decreases in antigen expression and an increased presence of inhibitory immune checkpoint molecules and regulatory T-cell infiltrates, indicative of evasive tumor responses

The median overall survival was approximately 8 months, with one patient experiencing residual stable disease at 18 months

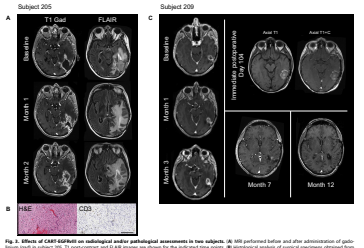



Fig. 3. Effects of CART-EGFRvIII on radiological and/or pathological assessments in two subjects. (A) MR performed before and after administration of gadolinium (gad) in subject 205. T1 post-contrast and FLAIR images are shown for the indicated time points. (B) Histological analysis of surgical specimens obtained from subject 205, 2 months after CART-EGFRvIII infusion. Hematoxylin and eosin (H&E)–stained sections and immunohistochemistry for CD3 to demonstrate T cells are shown. Scale bar, 200 μm. (C) MRI (T1 post-contrast images) shown at the indicated time points for subject 209. This subject underwent surgical resection of one portion of the tumor after the 3-month scan.

THE NEW ENGLAND JOURNAL of MEDICINE

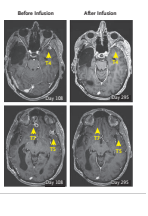
BRIEF REPORT

## Regression of Glioblastoma after Chimeric Antigen Receptor T-Cell Therapy

Christine E. Brown, Ph.D., Darya Alizadeh, Ph.D., Renate Starr, M.S., Lihong Weng, M.D., Jamie R. Wagner, B.A., Ascelli Naranjo, B.A., Julie R. Ostberg, Ph.D., M. Suzette Blanchard, Ph.D., Julie Kilpatrick, M.S.N., Jennifer Simpson, B.A., Anita Kurien, M.R.S., Saul J. Priceman, Ph.D., Xiuli Wang, M.D., Ph.D., Todd L. Harshbarger, M.D., Massimo D'Apuzzo, M.D., Julie A. Ressler, M.D., Michael C. Jensen, M.D., Michael E. Barish, Ph.D., Mike Chen, M.D., Ph.D., Jana Portnow, M.D., Stephen J. Forman, M.D., and Behnam Badie, M.D.

SUMMARY

A patient with recurrent multifocal glioblastoma received chimeric antigen receptor (CAR)–engineered T cells targeting the tumor-associated antigen interleukin-13 receptor alpha 2 (IL13Rα2). Multiple infusions of CAR T cells were administered over 220 days through two intracranial delivery routes — infusions into the resected tumor cavity followed by infusions into the ventricular system. Intracranial infusions of IL13Rα2-targeted CAR T cells were not associated with any toxic effects of grade 3 or higher. After CAR T-cell treatment, regression of all intracranial and spinal tumors was observed, along with corresponding increases in levels of cytokines and immune cells in the cerebrospinal fluid. This clinical response continued for 7.5 months after the initiation of CAR T-cell therapy. (Funded by Gateway for Cancer Research and others; ClinicalTrials.gov number, NCT02208362.)




N ENGL J MED 375;26 NEJM.ORG DECEMBER 29, 2016

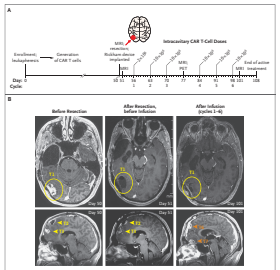
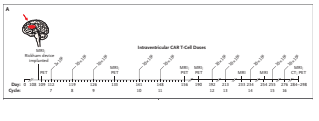
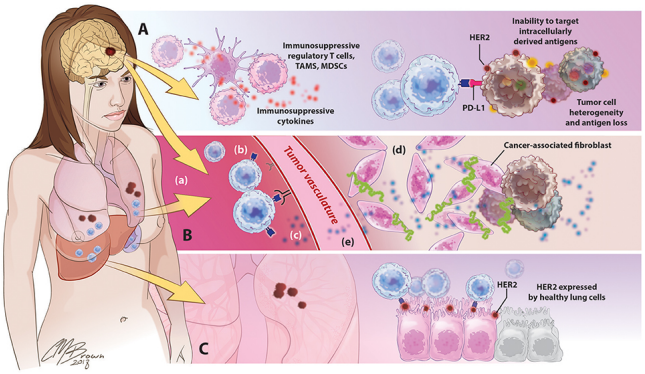



Figure 1. Local Tumor Control after Intracavitary Delivery of IL13Rα2–Chimeric Antigen Receptor (CAR) T Cells.
Panel A shows an overview of the intracavitary administration of six cycles of IL13Rα2–CAR T cells according to dose schedule 1 (see Table S1 in the Supplementary Appendix). CAR T cells were delivered through a Rickham catheter device into the right temporal–occipital region (tumor 1; red arrow) from day 56 to day 98 after enrollment, with 1 week of rest after cycles 3 and 6 for evaluation of safety and disease. MRI denotes magnetic resonance imaging, and PET positron-emission tomography. Panel B shows axial MRI (T1-weighted with gadolinium enhancement) of the brain highlighting the site of the resected tumor at which the catheter was placed for delivery of CAR T cells (tumor 1 [T1]; yellow circles), as well as the resected-only tumor sites in the frontal lobe (tumors 2 and 3 [T2 and T3]; yellow arrowheads) and the sites of tumors that developed during the intracavitary treatment period (tumors 4 and 7 [T4 and T7]; orange arrowheads). The CAR T-cell injection site (T1) remained stable without evidence of disease recurrence, whereas other disease foci, including T4 and T7, which were adjacent to resected T2 and T3 but distant from the CAR T-cell injection site, continued to progress.



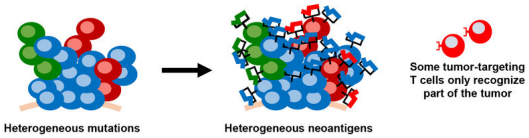
## Barriers for CART in solid tumors




## Barriers for CART in solid tumors

### 1. Selection of TAA

Solid tumors are comprised of highly molecularly heterogeneous subpopulations expressing a diverse, overlapping profile of unique TAAs




Enhancing CART cytotoxicity against epitopes not restricted to malignant cells is limited by the danger of simultaneously promoting CAR recognition of target antigen expressed by healthy tissues. Toxicities secondary to unintentional destruction of non-cancerous cells has been observed to varying degrees following CART therapy targeting overexpressed self-antigens like CEA, a tumor-associated antigen that is also expressed in normal gastrointestinal epithelium (severe inflammatory colitis in all treated patients, due to the destruction of healthy epithelial cells).

## Barriers for CART in solid tumors

### 2. Lymphocyte trafficking

In contrast to the simplicity and ease of encountering of malignant cells in hematologic cancers, CARTs for solid tumors face the additional challenge of migrating to and infiltrating tumor sites. In humans and mice, CART persistence and intratumoral accumulation following systemic adoptive transfer is characteristically poor, with some studies showing initial trafficking to organs such as the lung, spleen, and liver, without any preferential accumulation in tumor sites

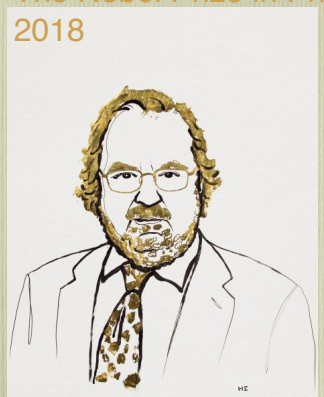
### 3. Tumor-induced immunosuppression

Immune checkpoints

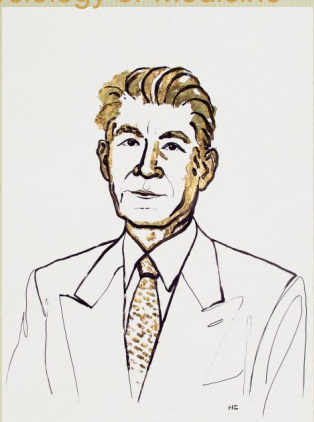
1) programmed cell death-1 (PD-1)
2) cytotoxic T-lymphocyte antigen-4 (CTLA-4)

Activation of CTLA-4 receptors expressed by naïve T cells prevents their initial activation and stimulation of PD-1 on activated T-cells induces anergy, apoptosis, or development of immunosuppressive regulatory T-cells (Tregs). By upregulating PD-L1 and enhancing T-cell CTLA-4 and PD-1 expression, tumor cells are able to suppress the activity of incoming immune cells

## The Nobel Prize in Physiology or Medicine 2018

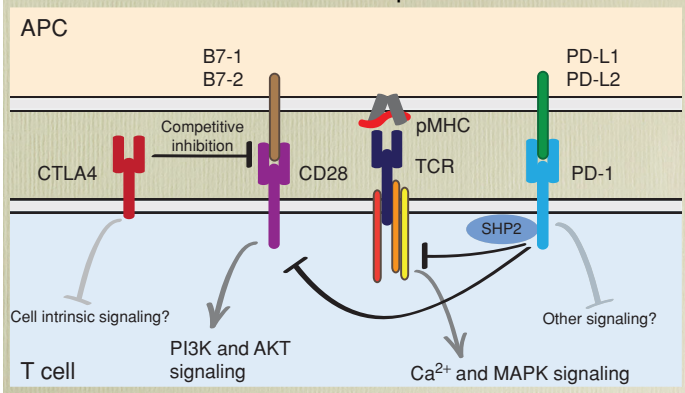


James P. Allison



Tasuku Honjo

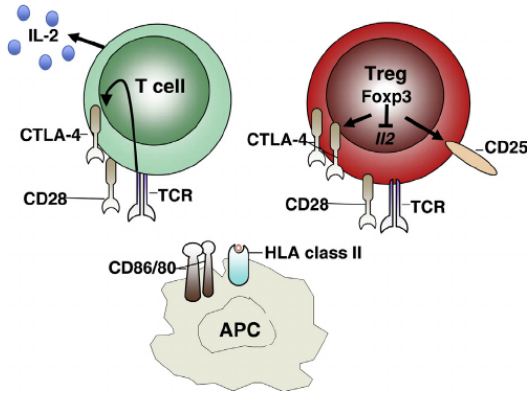
## Immune checkpoints




Immune checkpoints normally maintain immune responses within a desired physiologic range and protect the host from autoimmunity.

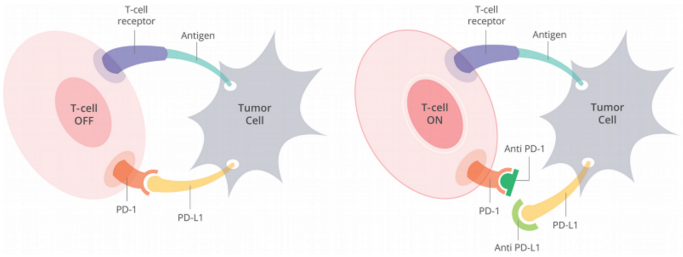
Immune checkpoint blockade removes inhibitory signals of T-cell activation, which enables tumor-reactive T cells to over- come regulatory mechanisms and mount an effective antitumor response

## CTLA-4




CTLA4 is immediately upregulated following T-cell receptor (TCR) engagement. It dampens TCR signaling through competition with the costimulatory molecule CD28 for the B7 ligands B7-1 (CD80) and B7-2 (CD86), for which CTLA4 has higher avidity and affinity. Because both B7-1 and B7-2 provide positive costimulatory signals through CD28, competitive inhibition of both molecules by CTLA4 is necessary to effectively attenuate T-cell activation.

# PD-1




The primary biological functions of PD-1 are to maintain peripheral tolerance and to maintain T-cell responses within a desired physiologic range. Because the PD-1/PD-L1 regulatory system is induced by immune responses, this forms a negative feedback loop to attenuate local T-cell responses and minimize tissue damage.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

## Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma

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**BACKGROUND**

Nivolumab is programmed death 1 (PD-1) checkpoint inhibitor) and ipilimumab (a cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) checkpoint inhibitor) have been shown to have complementary activity in metastatic melanoma. In this randomized, double-blind, phase 3 study, nivolumab alone or nivolumab plus ipilimumab was compared with ipilimumab alone in patients with metastatic melanoma.

**METHODS**

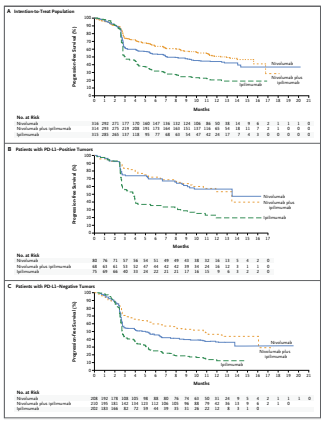
We assigned, in a 1:1:1 ratio, 945 previously untreated patients with unresectable stage III or IV melanoma to nivolumab alone, nivolumab plus ipilimumab, or ipilimumab alone. Progression-free survival and overall survival were coprimary end points. Results regarding progression-free survival are presented here.

**RESULTS**

The median progression-free survival was 11.5 months (95% confidence interval [CI], 8.9 to 16.7) with nivolumab plus ipilimumab, as compared with 2.9 months (95% CI, 2.8 to 3.4) with ipilimumab (hazard ratio for death or disease progression, 0.42; 99.5% CI, 0.31 to 0.57; P<0.001), and 6.9 months (95% CI, 4.3 to 9.5) with nivolumab (hazard ratio for the comparison with ipilimumab, 0.57; 99.5% CI, 0.43 to 0.76; P<0.001). In patients with tumors positive for the PD-1 ligand (PD-L1), the median progression-free survival was 14.0 months in the nivolumab-plus-ipilimumab group and in the nivolumab group, but in patients with PD-L1–negative tumors, progression-free survival was longer with the combination therapy than with nivolumab alone (11.2 months [95% CI, 8.0 to not reached] vs. 5.3 months [95% CI, 2.8 to 7.1]). Treatment-related adverse events of grade 3 or 4 occurred in 16.3% of the patients in the nivolumab group, 55.0% of those in the nivolumab–plus-ipilimumab group, and 27.3% of those in the ipilimumab group.

**CONCLUSIONS**

Among previously untreated patients with metastatic melanoma, nivolumab alone or combined with ipilimumab resulted in significantly longer progression-free survival than ipilimumab alone. In patients with PD-L1–negative tumors, the combination of PD-1 and CTLA-4 blockade was more effective than either agent alone. (Funded by Bristol-Myers Squibb; CheckMate 067 ClinicalTrials.gov number, NCT01844505.)






## Fundamental Mechanisms of Immune Checkpoint Blockade Therapy

Spencer C. Wei[1], Colm R. Duffy[1], and James P. Allison[1,2]

**Table 1. Summary of the tumor types for which immune checkpoint blockade therapies are FDA-approved**

| Tumor type | Therapeutic agent | FDA approval year |
|---|---|---|
| Melanoma | Ipilimumab | 2011 |
| Melanoma | Nivolumab | 2014 |
| Melanoma | Pembrolizumab | 2014 |
| Non–small cell lung cancer | Nivolumab | 2015 |
| Non–small cell lung cancer | Pembrolizumab | 2015 |
| Melanoma (BRAF wild-type) | Ipilimumab + nivolumab | 2015 |
| Melanoma (adjuvant) | Ipilimumab | 2015 |
| Renal cell carcinoma | Nivolumab | 2015 |
| Hodgkin lymphoma | Nivolumab | 2016 |
| Urothelial carcinoma | Atezolizumab | 2016 |
| Head and neck squamous cell carcinoma | Nivolumab | 2016 |
| Head and neck squamous cell carcinoma | Pembrolizumab | 2016 |
| Melanoma (any BRAF status) | Ipilimumab + nivolumab | 2016 |
| Non–small cell lung cancer | Atezolizumab | 2016 |
| Hodgkin lymphoma | Pembrolizumab | 2017 |
| Merkel cell carcinoma | Avelumab | 2017 |
| Urothelial carcinoma | Avelumab | 2017 |
| Urothelial carcinoma | Durvalumab | 2017 |
| Urothelial carcinoma | Nivolumab | 2017 |
| Urothelial carcinoma | Pembrolizumab | 2017 |
| MSI-high or MMR-deficient solid tumors of any histology | Pembrolizumab | 2017 |
| MSI-high, MMR-deficient metastatic colorectal cancer | Nivolumab | 2017 |
| Pediatric melanoma | Ipilimumab | 2017 |
| Hepatocellular carcinoma | Nivolumab | 2017 |
| Gastric and gastroesophageal carcinoma | Pembrolizumab | 2017 |
| Non–small cell lung cancer | Durvalumab | 2018 |
| Renal cell carcinoma | Ipilimumab + nivolumab | 2018 |

NOTE: A summary of the tumor indications, therapeutic agents, and year of FDA approval for immune checkpoint blockade therapies. FDA approval includes regular approval and accelerated approval granted as of May 2018. Ipilimumab is an anti-CTLA4 antibody. Nivolumab and pembrolizumab are anti-PD-1 antibodies. Atezolizumab, avelumab, and durvalumab are anti-PD-L1 antibodies. Tumor type reflects the indications for which treatment has been approved. Only the first FDA approval granted for each broad tissue type or indication for each therapeutic agent is noted. In cases where multiple therapies received approval for the same tumor type in the same year, agents are listed alphabetically.

Abbreviations: MSI, microsatellite instability; MMR, mismatch repair.