Master in Cellular and Molecular Biology

Medical and Cancer Genetics course

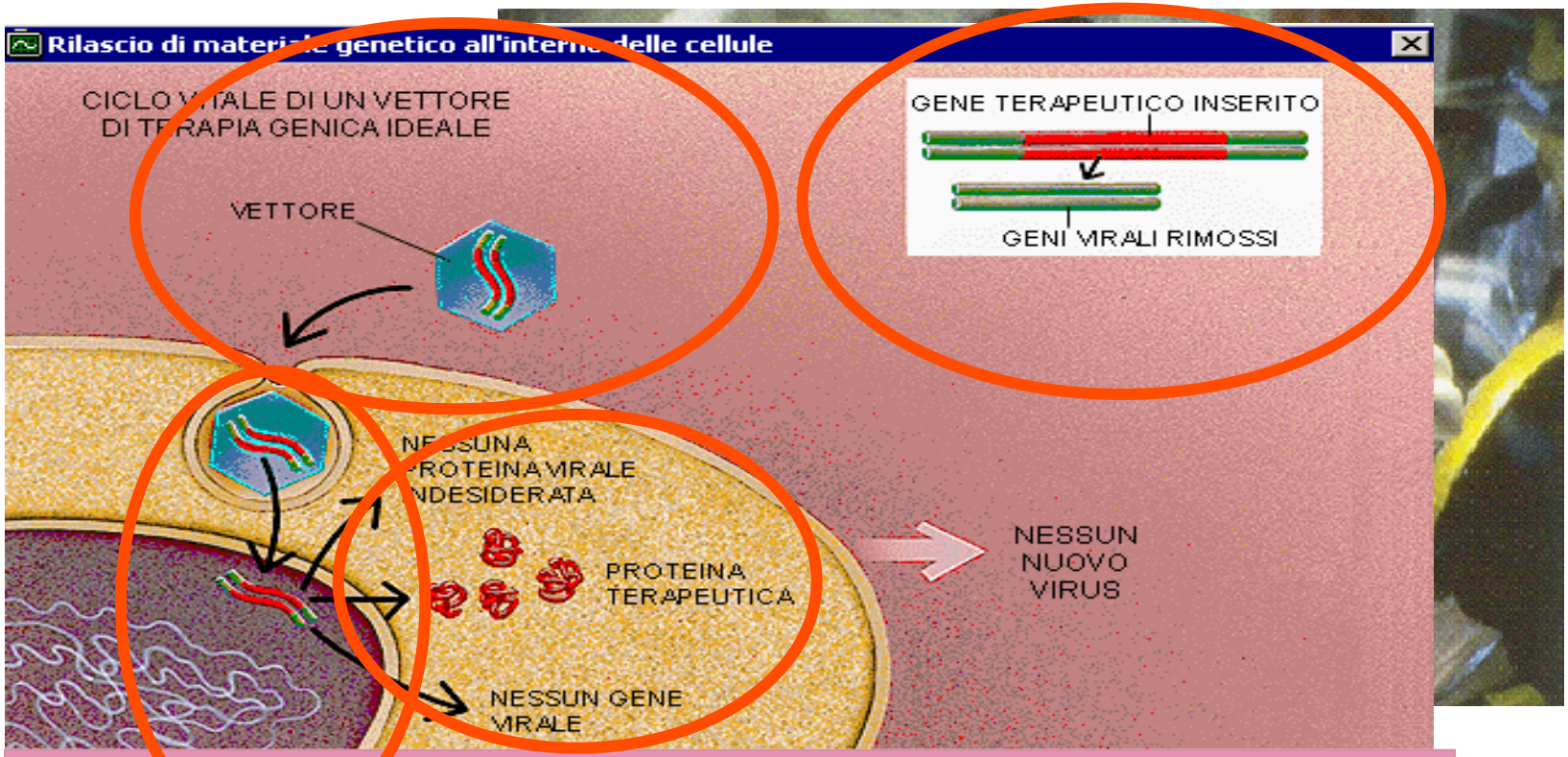
MEDICAL GENETICS

Teacher: Claudia Giachino

Lesson 5

Gene therapy

1990: Michael Blaese in the United States applies the first gene therapy procedure on a child with a SCID, a hereditary severe combined immunodeficiency



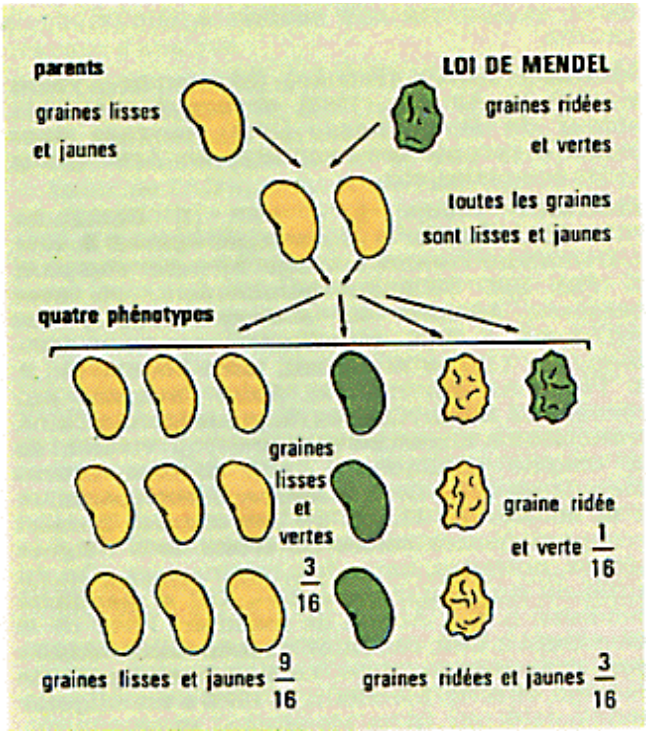
Gene Therapy

The Forefront of Medicine

Gene Therapy - Background

- **1865** - Mendel's experiments described the laws of heredity, and that features are inherited by a defined and predictable mechanism

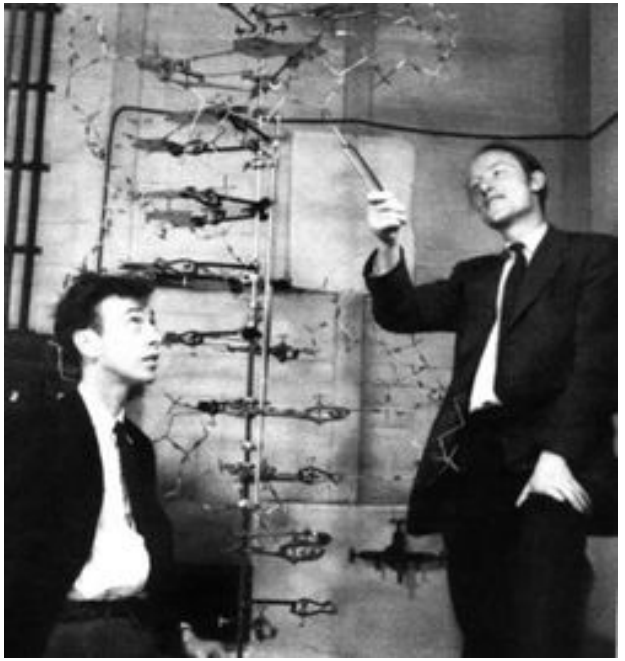
- **1940s** - Avery and colleagues identified carrier of genetic information, demonstrated that the information is encoded by DNA



Gregor Mendel's Heredity Experiment

Gene Therapy - Background

- **1953** –Watson and Crick proposed that DNA is a double helix, suggesting how this structure could be used to replicate and inherit genetic information
- **1961** –Nirenberg deciphered triplets in the genetic code
- **1978** – Arber, Nathans and Smith discovered restriction enzymes and applied it to problems of molecular genetics



James Watson and Francis Crick

Gene Therapy - Background

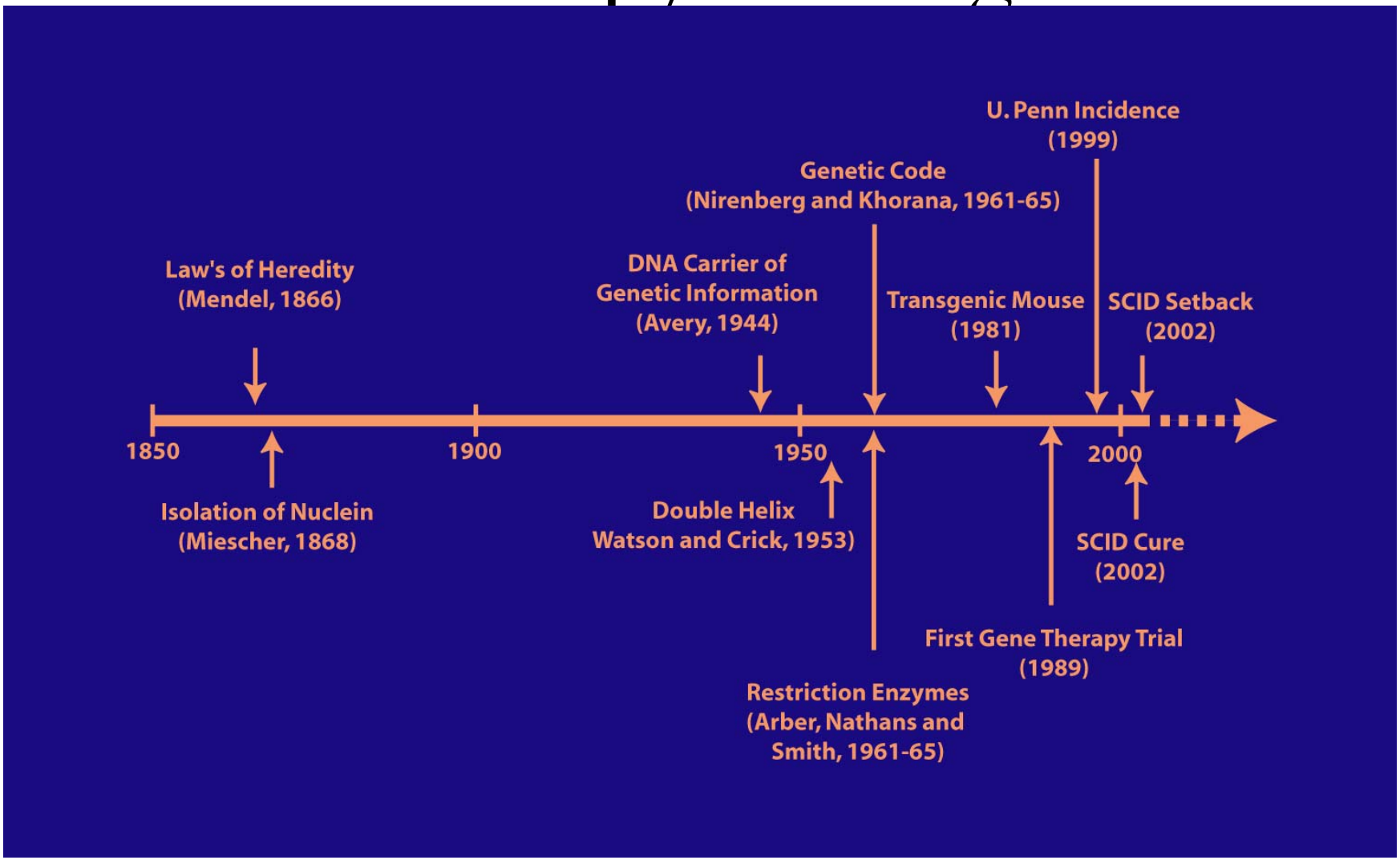
- **1990** - The first gene therapy journal published, *Human Gene Therapy*

- **1990** - The first approved gene therapy clinical trial took place when Ashanthi DeSilva, a 4 year old girl with ADA-deficient Severe Combined Immunodeficiency, was given her own T cells engineered with a retroviral vector carrying a normal ADA gene

- **2000** - The first gene therapy cure was reported when Alain Fischer (Paris) succeeded in totally correcting children with SCID-X1, or "bubble boy" syndrome

"Bubble Boy"

Gene Therapy - Background



GENE THERAPY

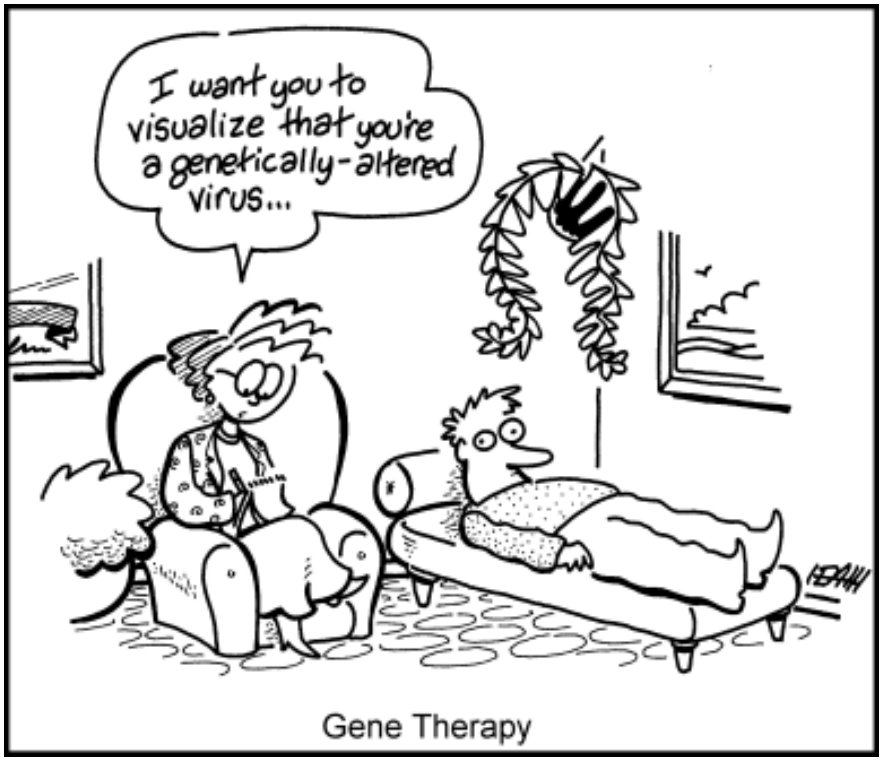
Any procedure intended to treat or alleviate disease by genetically modifying the cells of a patient

What is Gene Therapy?

- Researchers may use one of several approaches for correcting faulty genes:
  - A normal gene may be inserted into a location within the genome to replace a nonfunctional gene. Most common approach.
  - An abnormal gene could be swapped for a normal gene through homologous recombination.
  - An abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.
  - The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.

How Does Gene Therapy Work?

- In most gene therapy studies, a "*normal*" gene is inserted into the genome to replace an "*abnormal*," disease-causing gene.
- A carrier molecule called a *vector* must be used to deliver the therapeutic gene to the patient's target cells.
- The most common *vector* is a *virus* that has been genetically altered to carry normal human DNA.
- Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner.
- Scientists manipulate the virus genome to remove disease-causing genes and insert therapeutic ones.
- Target cells, such as the patient's liver or lung cells, are infected with the viral vector.



GENE THERAPY

- **Delivery mechanism**
  - *Ex vivo*
  - *In vivo*
- **Type of cells modified**
  - Germ-line cells
  - Somatic cells
- **Mechanism of modification**
  - Gene augmentation/supplementation
  - Gene replacement
  - Targeted inhibition of gene expression
  - Targeted killing of specific cells

DELIVERY MECHANISMS

in vivo

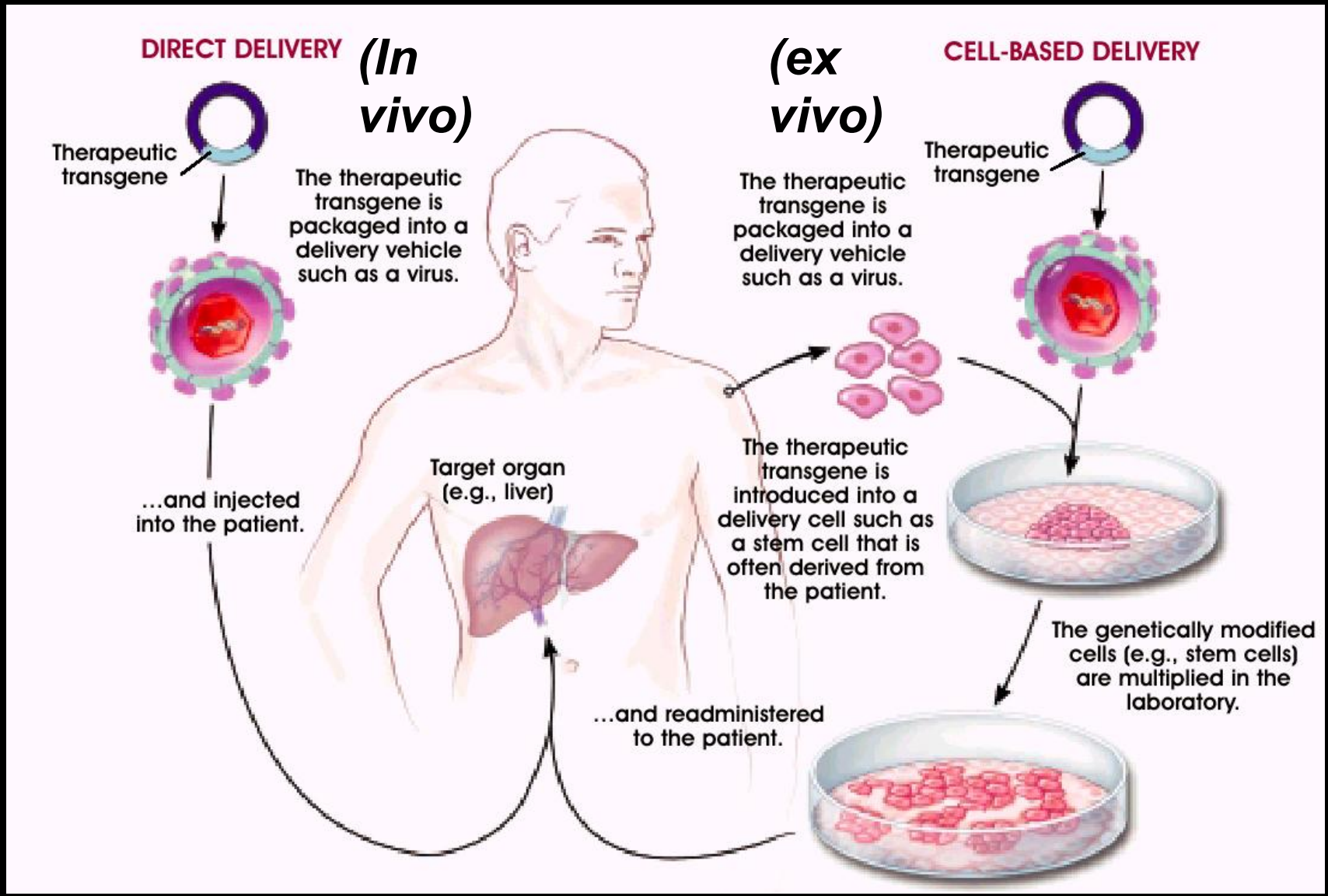
genetic material transferred directly into cells within a patient

ex vivo

cells are removed from the patient, genetically modified and transplanted back into the patient

- *In vivo* gene therapy: delivery of new genetic material directly to target cells within the body
  - The challenge lies in ensuring the specificity and in reaching the correct target cells within the body
- *Ex vivo* therapy: target cells are removed from the body and then genetically modified
  - The cells are then returned to the body after selection and amplification
  - This is a safe method but dependent on the type of cells being targeted

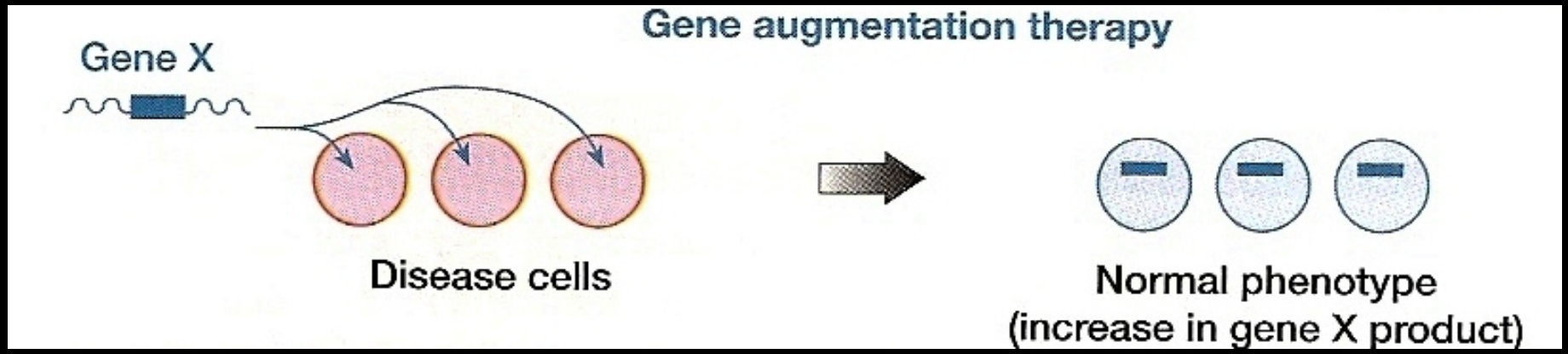
DELIVERY MECHANISMS



TYPES OF CELLS MODIFIED

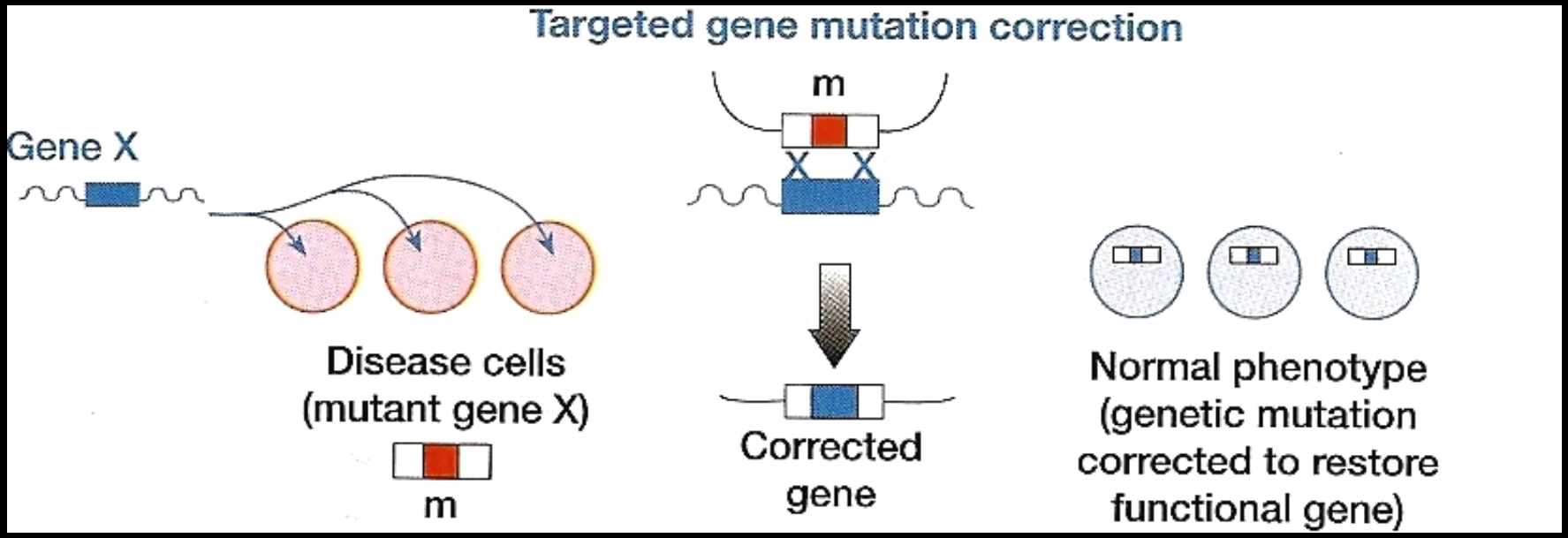
- **Germ-line gene therapy**
  - Modification of gametes, zygote or early embryo
  - Permanent and transmissible
  - Banned due to ethical issues
- **Somatic cell gene therapy**
  - Modification of somatic cells, tissues etc
  - Confined to the patient

Mechanism of modification
1. Gene augmentation

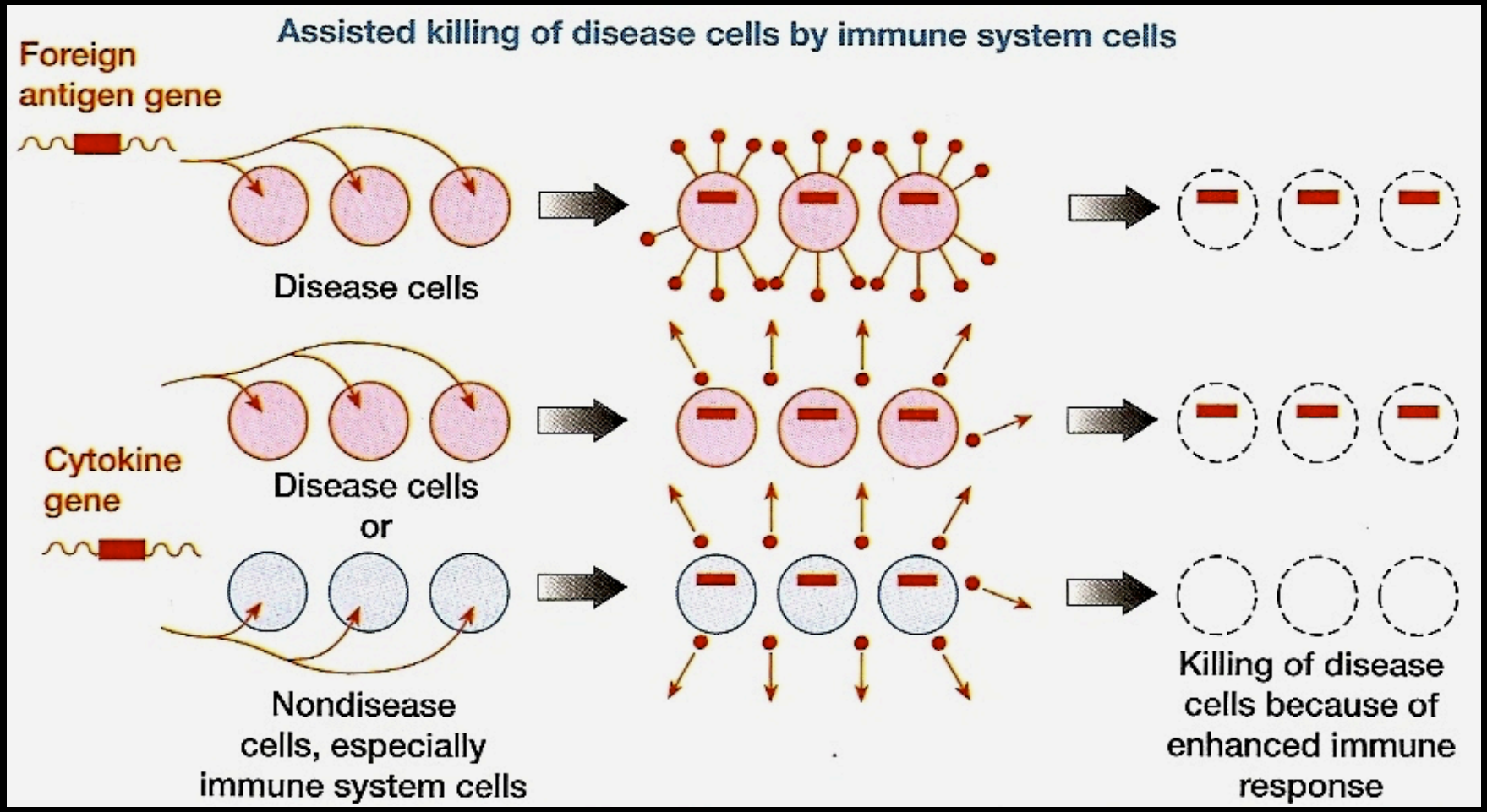


- **Targeted at disorders where <u>pathogenesis is reversible</u>**
- **<u>Recessive disorders</u> are more amendable to treatment than dominant disorders**
- **Gain-of-function mutations are untreatable by GAT**

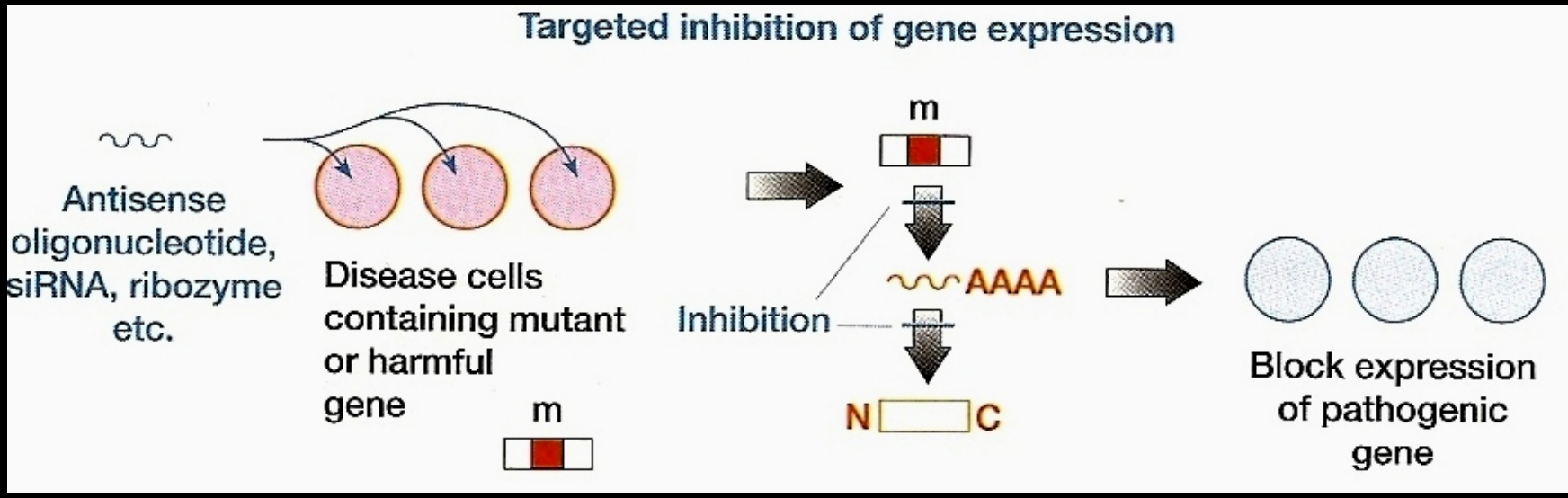
Mechanism of modification
2. Gene replacement



Mechanism of modification
3. Targeted killing of specific cells



Mechanism of modification
4.Targeted inhibition of gene expression



Gain-of-function diseases where mutant gene is producing a harmful protein

RNA Interference

- RNA interference, also known as RNAi presents a new approach to gene therapy by targeting specific genes and down-regulating gene expression
- One of the most potent forms of RNAi is small interfering RNA, or siRNA
- Small fragments of double stranded RNA, specific for a particular gene target, are introduced to the cell
- Specific hybridization between the naturally occurring transcript and the induced siRNA (antisense portion) instigates the destruction of the message.
- This form of RNAi acts directly on the transcriptional level of gene expression.
- Therapeutically speaking, siRNA efficacy would be determined by percent knock-down (gene is still present, some product is still made).
- Also, this method is transient, requiring readministration within the system.

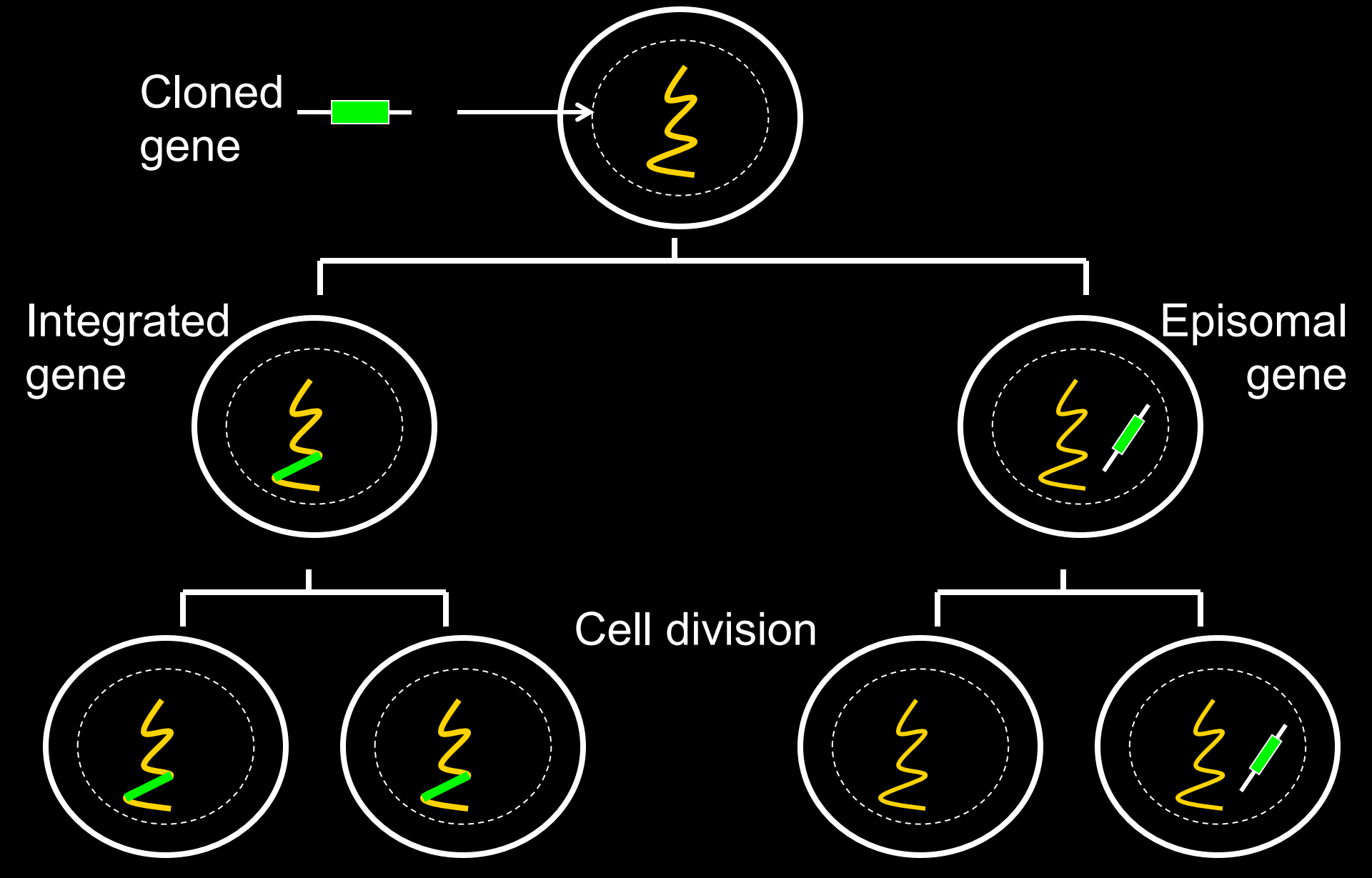
“The principal issue in turning RNAi from an effective functional genomics tool into a therapy remains one of delivery. RNAi primarily acts within the cytoplasmic compartment, which is easier to access using nonviral methods than the nucleus, but ensuring efficient uptake and long-term stability in vivo in disease relevant tissues is still likely to be difficult.”

–NJ Kaplen

Amenability to gene therapy

- Mode of inheritance
- Identity of molecular defect
- Nature of mutation product
- Accessibility of target cells and amenability to cell culture
- Size of coding DNA
- Control of gene expression

Gene transfer

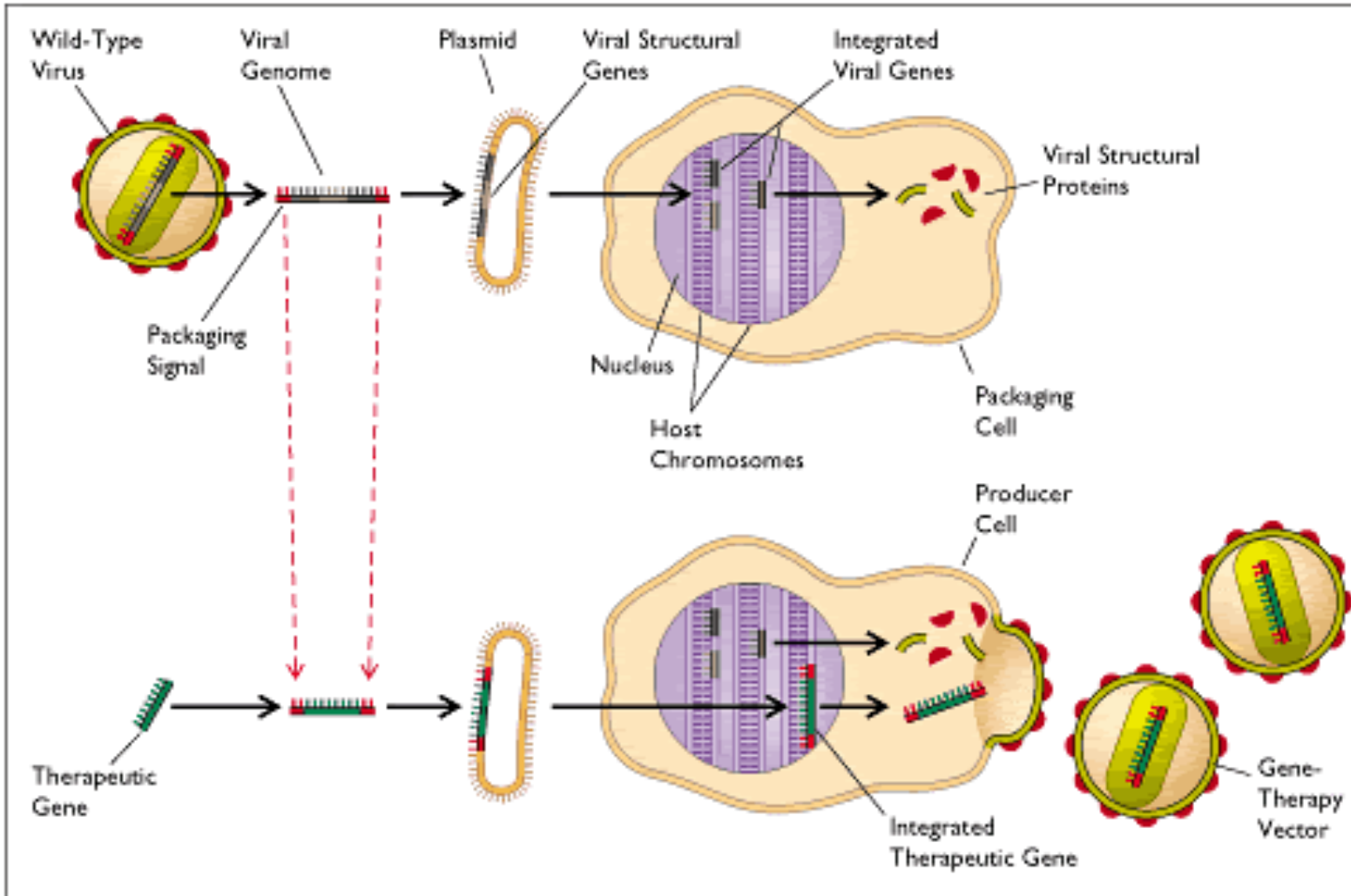


Vectors

- Vectors are carrier molecules which are employed to enhance gene transfer efficiency in gene therapy
- In optimizing a particular vector, one must consider:
  - Host immune response
  - Must target specific tissues for long term gene expresssion
  - Regulation of the gene after insertion
- Both viral and non-viral vectors have been used, though non-viral have a decreased transfer efficiency

Viral vector strategy

Replication & virulence genes can be substituted with therapeutic genes



Vectors in use

- **Viral**
  - Retro-
  - Adeno-
  - Adeno-associated-
  - Herpes simplex-
- **Non-viral**
  - Naked DNA/Plasmid
  - liposomes

Retroviruses

- create cDNA copies from the viral RNA genome
- integrate into the human genome
- Maximum insert size 7-7.5 kb
- Preexisting host immunity unlikely

- Can only transduce dividing cells
- May cause insertional mutagenesis

Retroviruses

Lentiviruses (e.g HIV)

- **Maximum insert size 7-7.5 kb**
- **Can transduce non-dividing cells**
- **May cause insertional mutagenesis**

Adenoviruses

- Are double stranded DNA genome that cause respiratory, intestinal, and eye infections in humans
- Maximum insert size > 30 kb
- Can transduce dividing and non-dividing cells
- Extensive unwanted immunological responses
- Episomal- do not integrate
  - Have to be reinserted when more cells divide
- Pre-existing host immunity

Adeno-associated Viruses

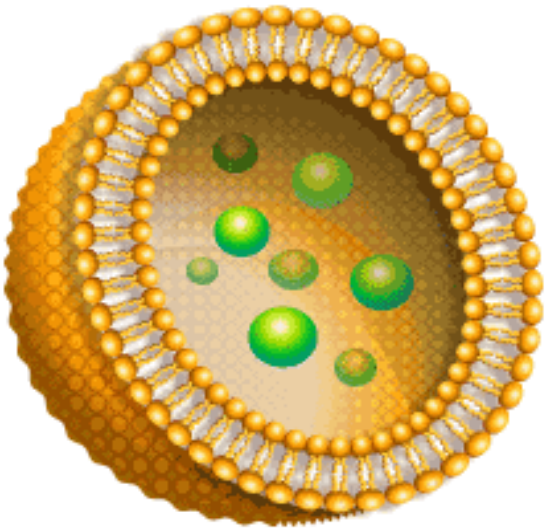
- small, single stranded DNA viruses
- productive infection only with co-infection by another virus
- insert genetic material at a specific point on chromosome 19
- Low information capacity- 4.0 Kb

Herpes simplex Viruses

- Complex ds DNA
- Establish life long latent infections as non-integrated extra chromosomal elements
- information capacity 30 Kb

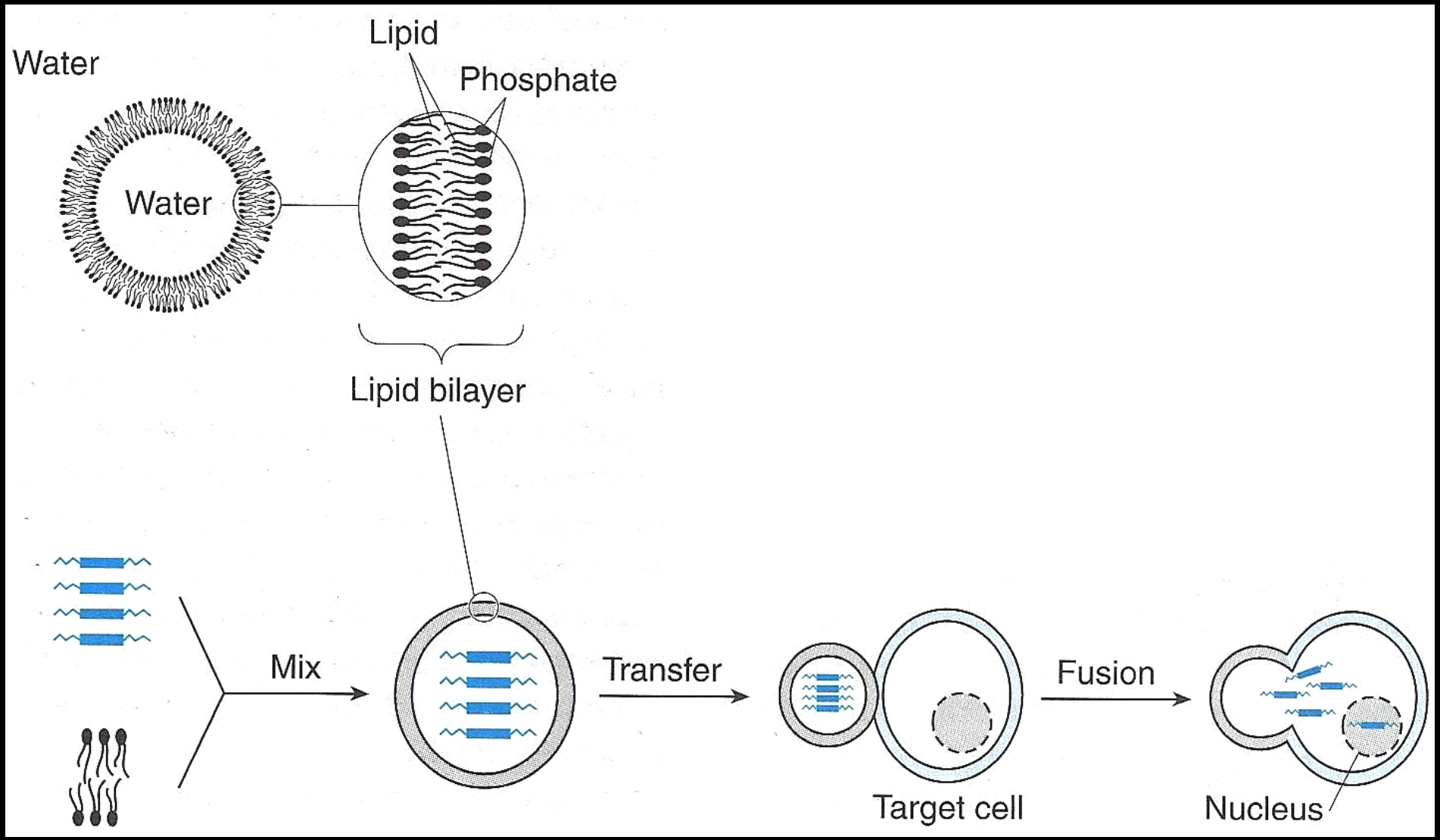
Non-viral options

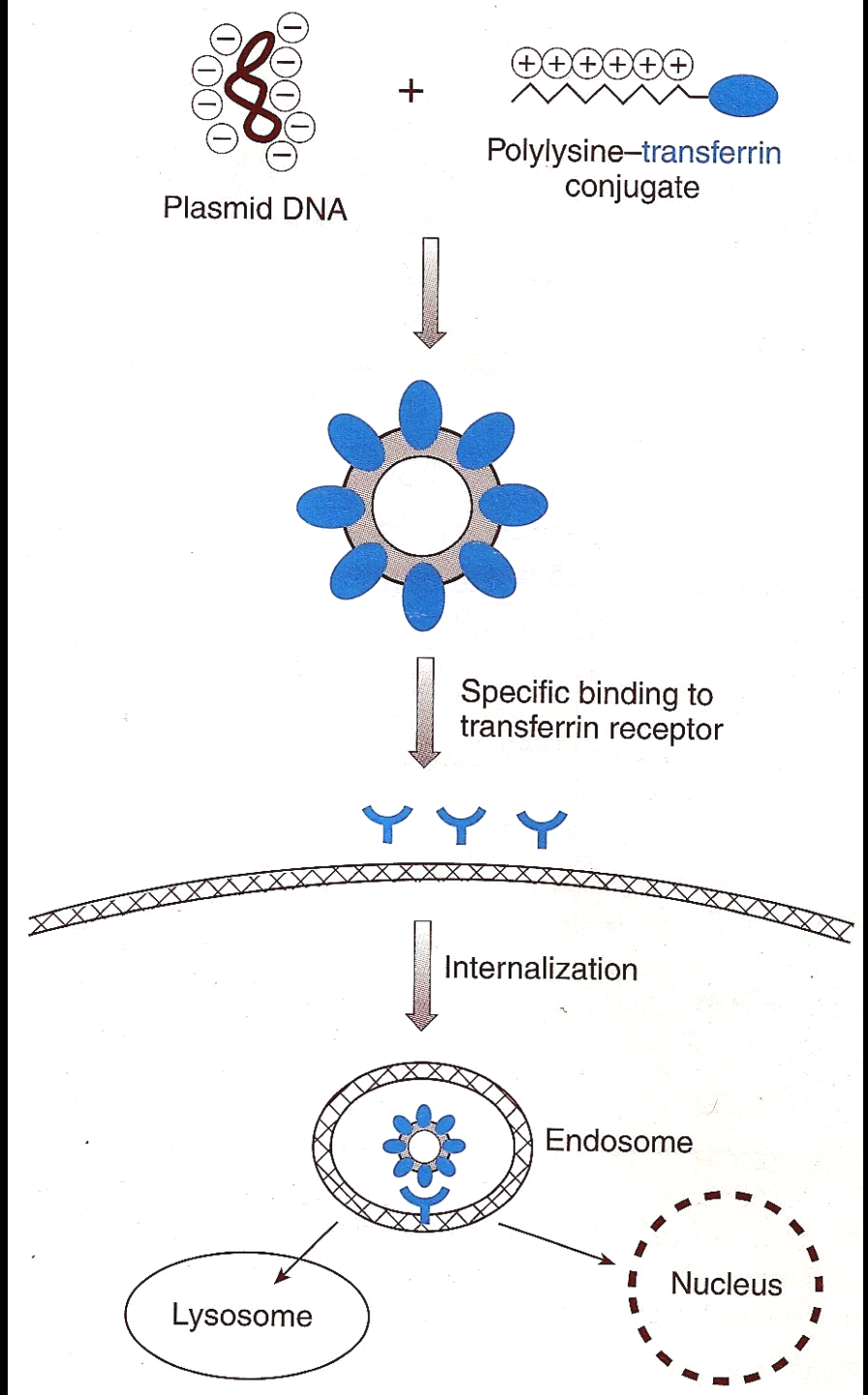
- Non-viral options:
  - Direct introduction of therapeutic DNA into target cells. Can be used only with certain tissues and requires large amounts of DNA.
  - An artificial lipid sphere with an aqueous core, called a *liposome*, which carries the therapeutic DNA, is capable of passing the DNA through the target cell's membrane.



Liposome

Liposomes


Water
Lipid
Phosphate
Water
Lipid bilayer
Mix
Transfer
Fusion
Target cell
Nucleus


Plasmid DNA
+
Polylysine–transferrin conjugate
Specific binding to transferrin receptor
Internalization
Endosome
Lysosome
Nucleus

Plasmids

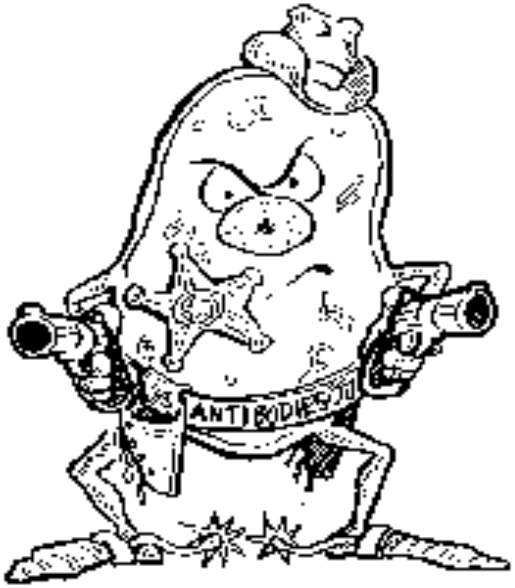
The Ethics and Social Concerns Surrounding Gene Therapy

Risks of Gene Therapy

- New gene might be inserted into wrong location in the DNA (misfire)
- Immune system complications
- Vector viruses can infect more than one type of cell
- Over-expression of missing protein
- DNA could accidentally be introduced into reproductive cells (germ-line gene therapy)

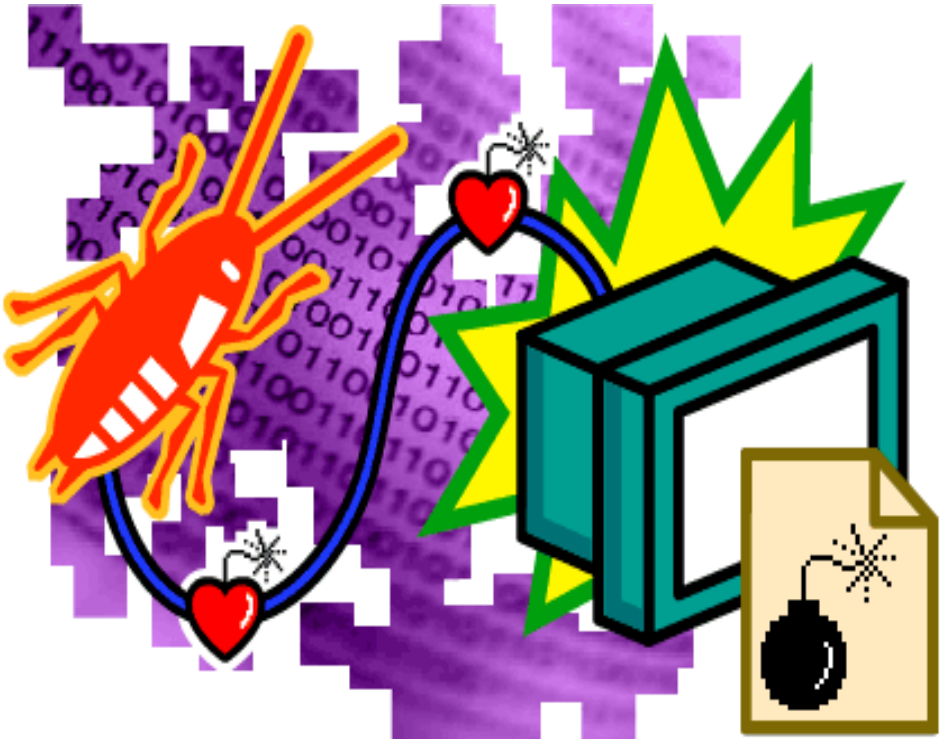
Immune System Complications

- Immune and Inflammatory responses
  - Immune system designed to attack foreign invaders
  - Shutting defense system down risks further advance of illness
  - Difficulty for gene therapy to be repeated



Viral Vectors

- Virus could be transmitted from the patient to other individuals
- Could disrupt vital genes, causing another disease or a predisposition to cancer



Over-Expression

- Overexpression can contribute to oncogenesis
- Overexpression contributes to cancer growth by removing controls on normal cell cycle regulation.

Risks associated with gene therapy

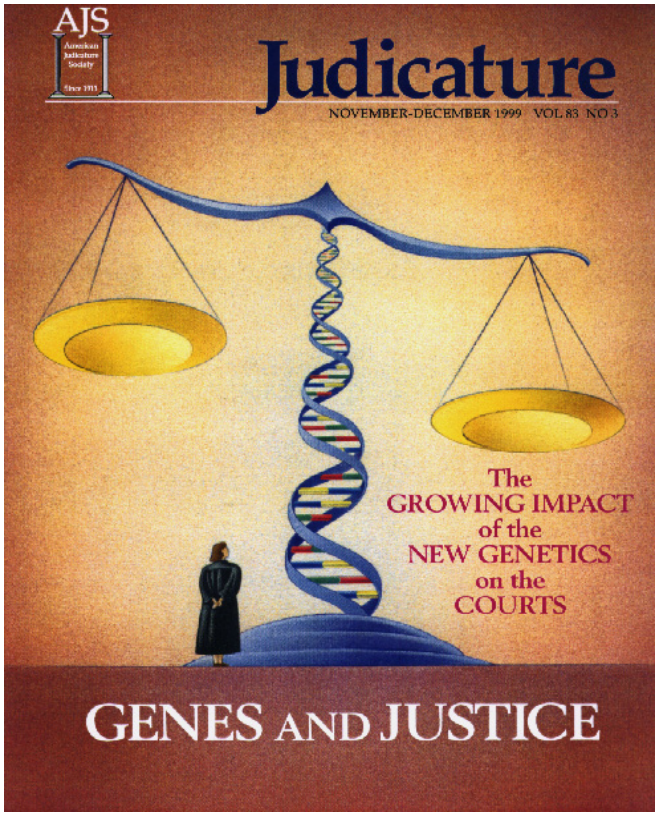
- Adverse response to the vector
- Insertional mutatgenesis resulting in malignant neoplasia
- Insertional inactivation of an essential gene
- Viruses may infect surrounding health tissues
- Overexpression of the inserted gene may lead to so much protein that it may become harmful

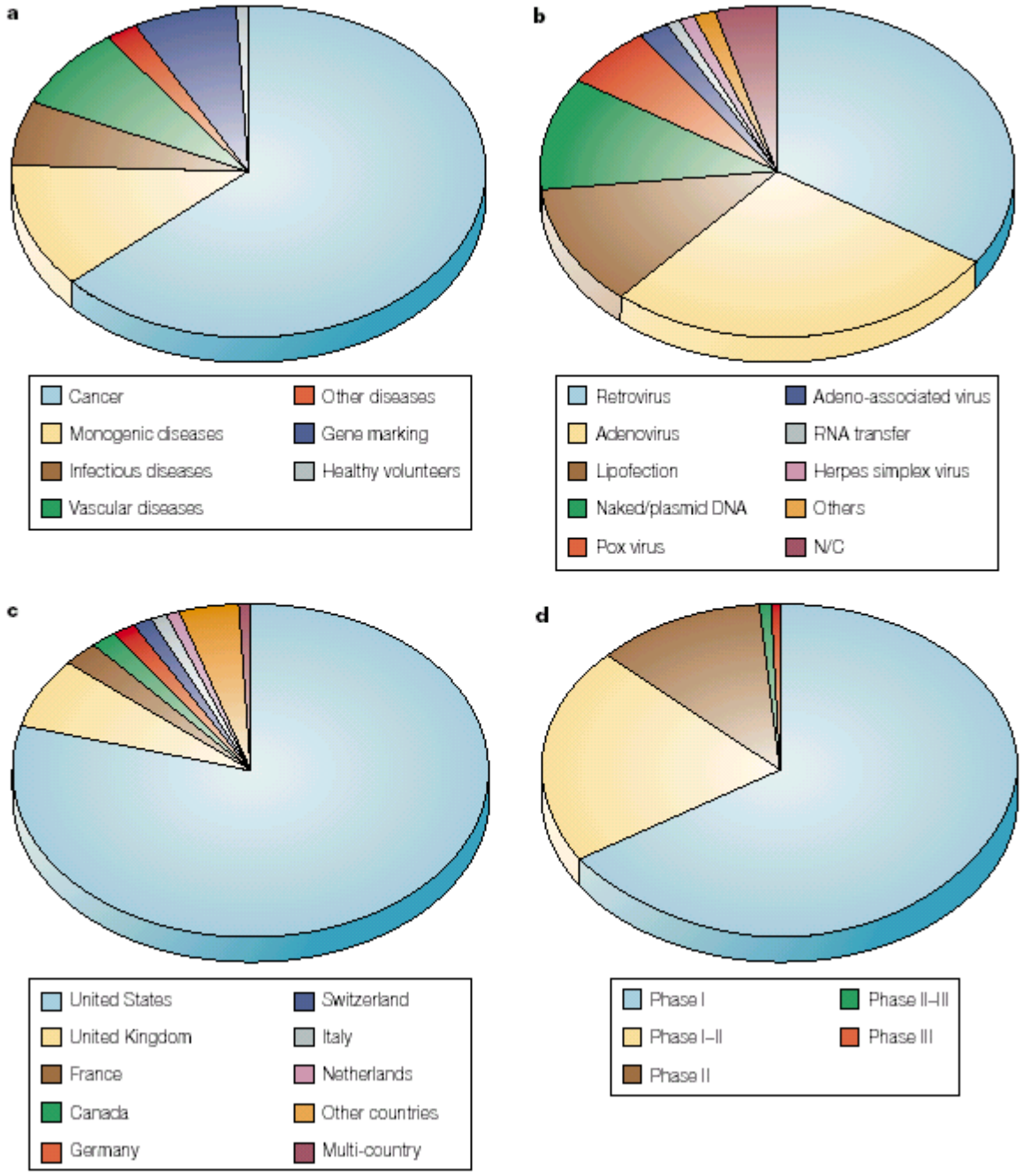
Problems With Gene Therapy?

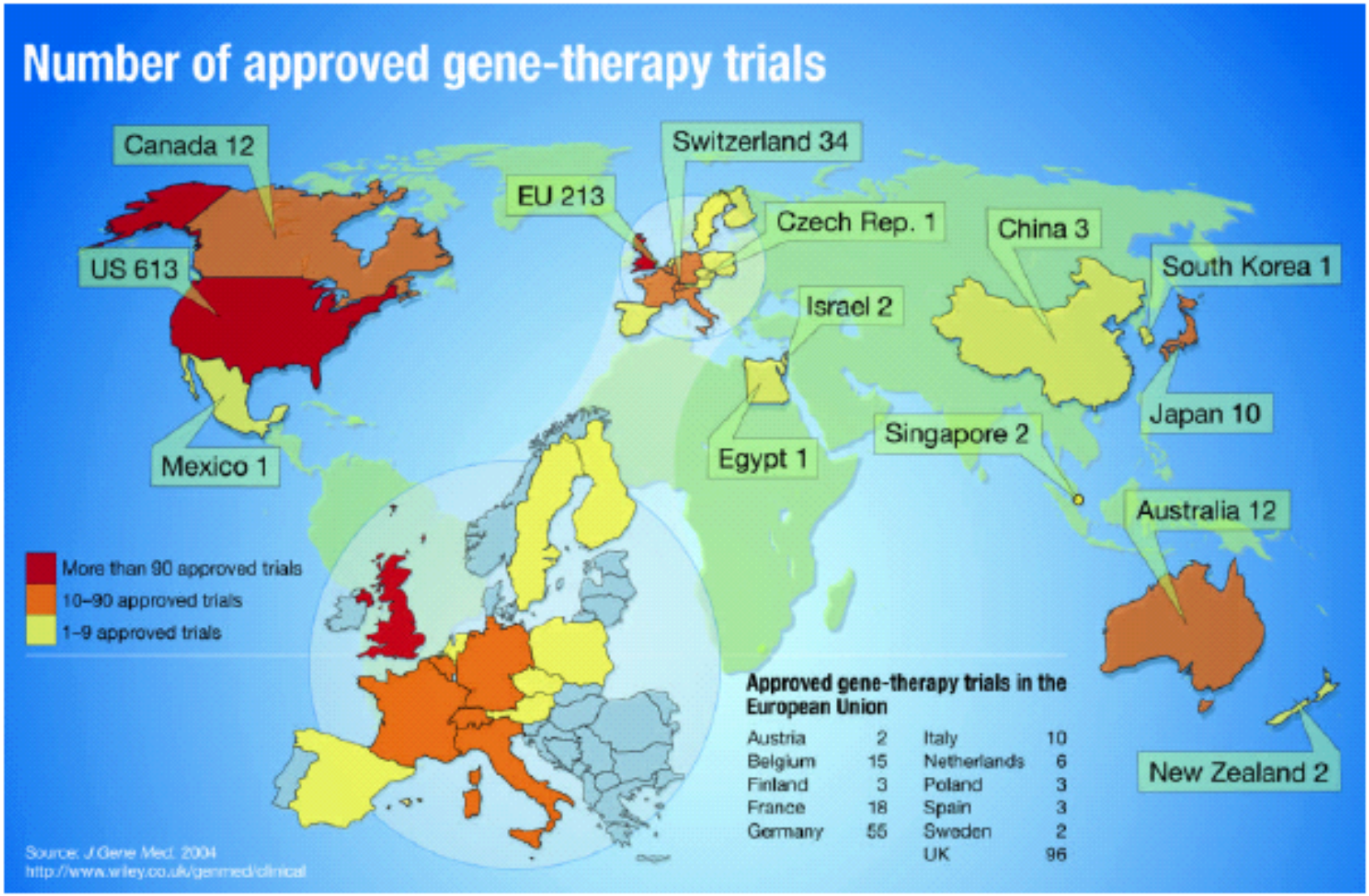
- **Short-lived nature of gene therapy-** patients will have to undergo multiple rounds of gene therapy.
- **Immune response-** risk of stimulating the immune system in a way that reduces gene therapy effectiveness is always a potential risk.
- **Problems with viral vectors-** viruses, the carrier of choice, present potential problems to the patient, like toxicity, immune and inflammatory responses, and gene control and targeting.
- **Multi-gene disorders-** most common disorders, such as heart disease, high blood pressure, Alzheimer's disease, arthritis and diabetes, are caused by the combined effects of variations in many genes.

Is Gene Therapy Ethical?

- Questions we will consider:
  - What is normal and what is a disability or disorder, and who decides?
  - Who will have access to your genetic information?
  - Is *somatic gene therapy* (done in the adult cells of people known to have the disease) more or less ethical than *germline gene therapy* (done in egg and sperm cells and prevents the trait from being passed on to further generations)?
  - Preliminary attempts at gene therapy are expensive. Who will have access to these therapies? Who will pay for their use?

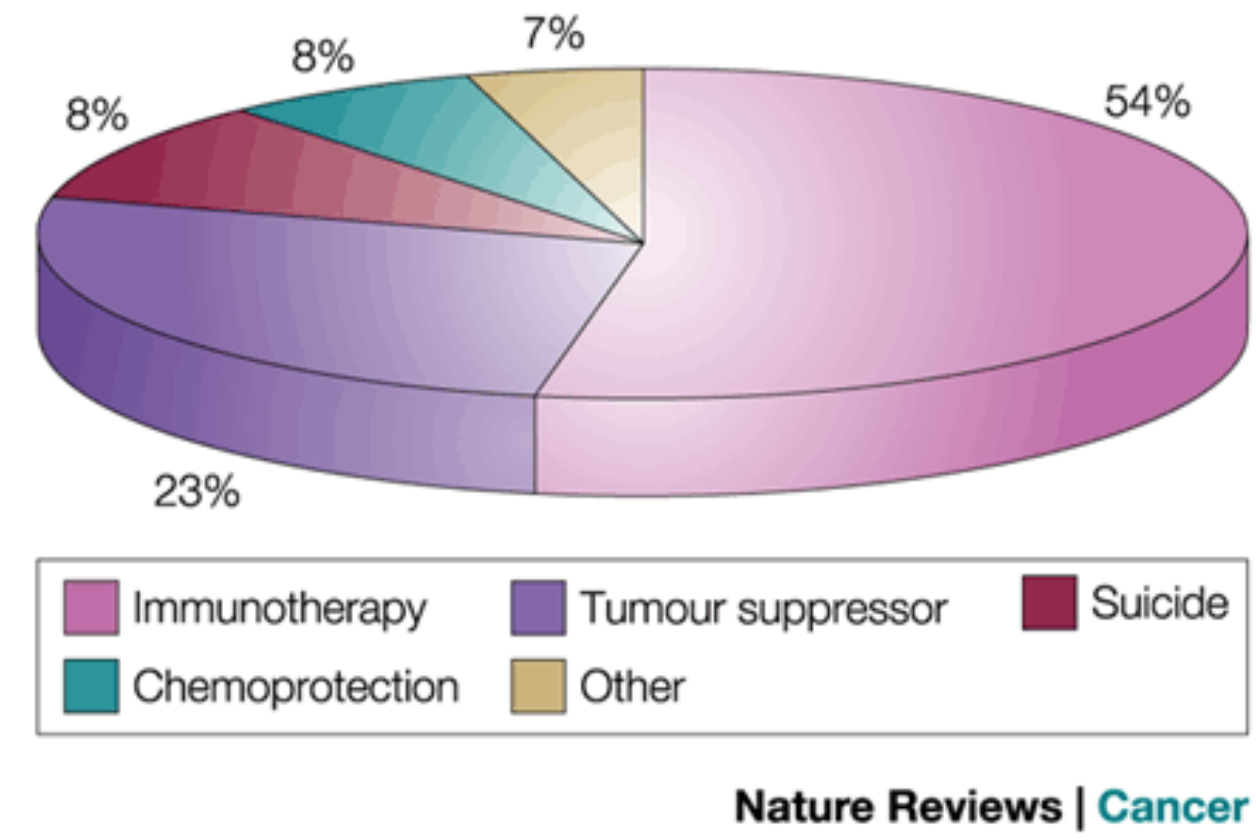



a
Cancer
Monogenic diseases
Infectious diseases
Vascular diseases
Other diseases
Gene marking
Healthy volunteers
b
Retrovirus
Adenovirus
Lipofection
Naked/plasmid DNA
Pox virus
Adeno-associated virus
RNA transfer
Herpes simplex virus
Others
N/C
c
United States
United Kingdom
France
Canada
Germany
Switzerland
Italy
Netherlands
Other countries
Multi-country
d
Phase I
Phase I–II
Phase II
Phase II–III
Phase III


Number of approved gene-therapy trials
Canada 12
US 613
Mexico 1
EU 213
Switzerland 34
Czech Rep. 1
Israel 2
Egypt 1
China 3
South Korea 1
Japan 10
Singapore 2
Australia 12
New Zealand 2
More than 90 approved trials
10–90 approved trials
1–9 approved trials
Approved gene-therapy trials in the European Union
Austria 2
Belgium 15
Finland 3
France 18
Germany 55
Italy 10
Netherlands 6
Poland 3
Spain 3
Sweden 2
UK 96
Source: J Gene Med. 2004
http://www.wiley.co.uk/genmed/clinical

Cancer gene therapy

Direct genetic modification of cells in patients



3 challenges in cancer gene therapy

delivery ***delivery*** ***delivery***

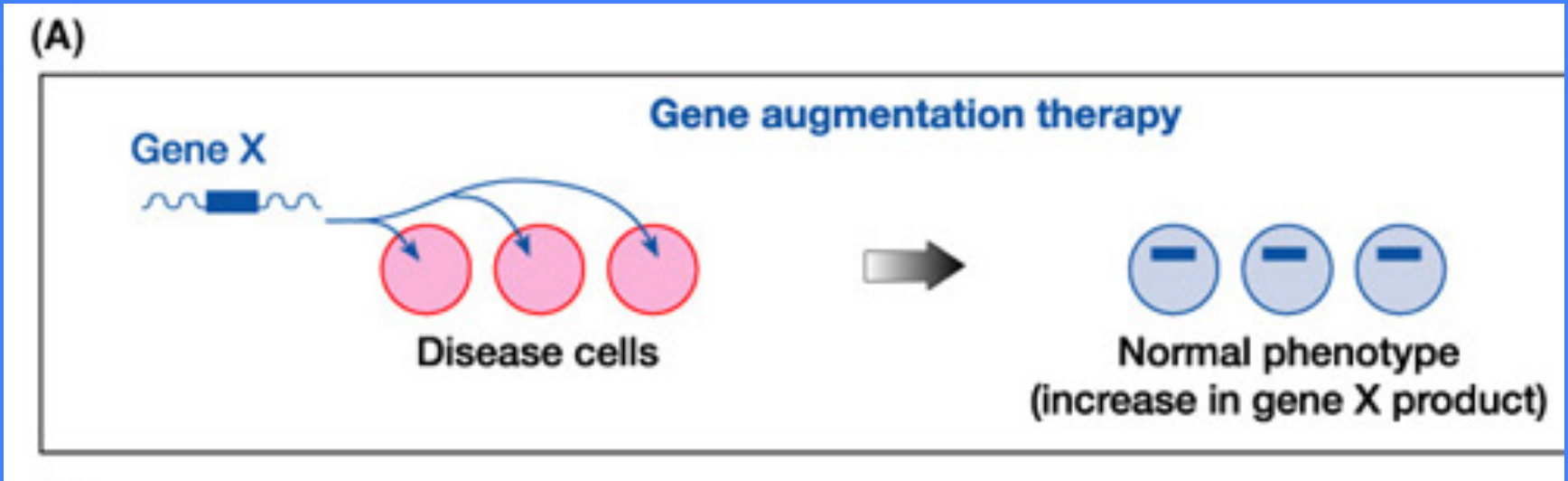
1) Package the gene
2) Protect the gene
3) targeted delivery to the nucleus and release in an active form

Vectors

‘Trojan horses’ that sneak the gene into the cell

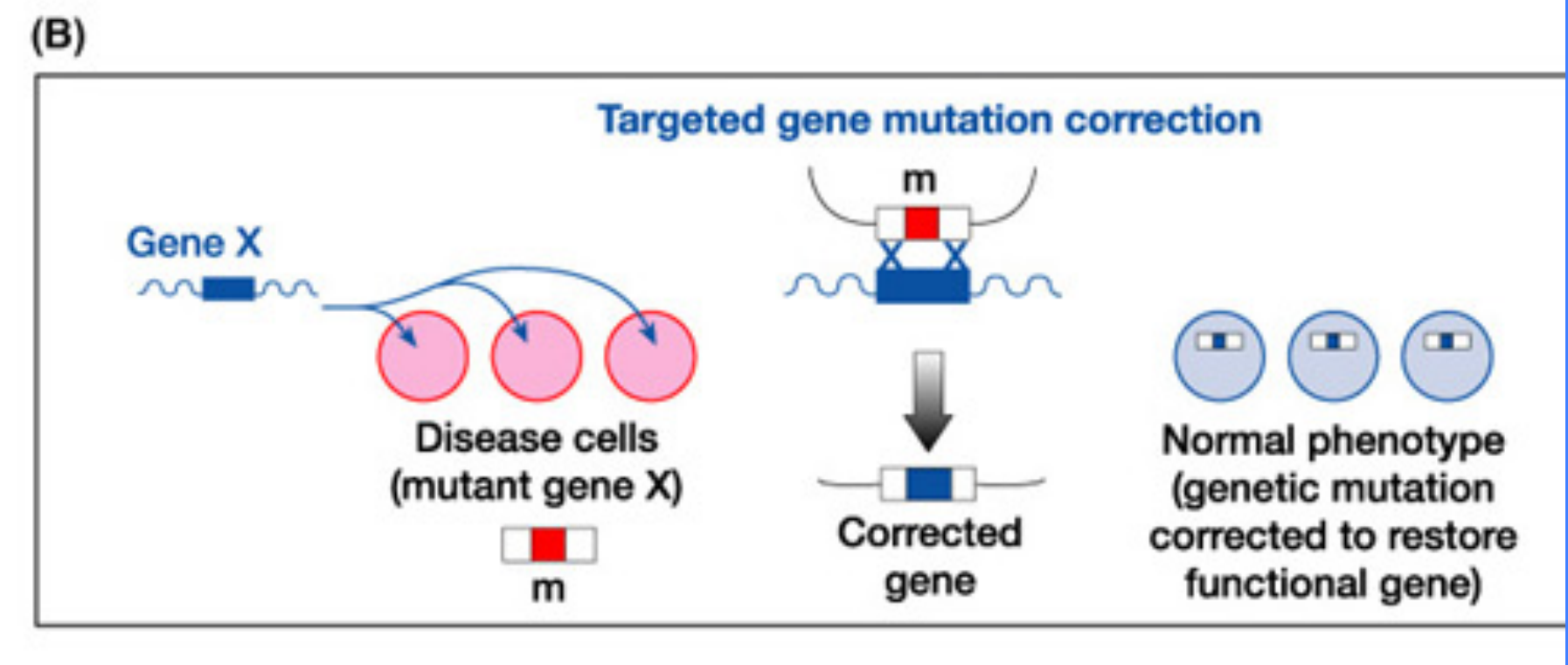
Gene augmentation

most therapies simply add a useful gene into a selected cell type to compensate for the missing or flawed version. Useful in treating loss of function mutations such as Tumour Genes



Gene replacement

This strategy replaces the mutant copy with a correctly functioning copy in situ. Useful for gain of function mutations such as oncogenes

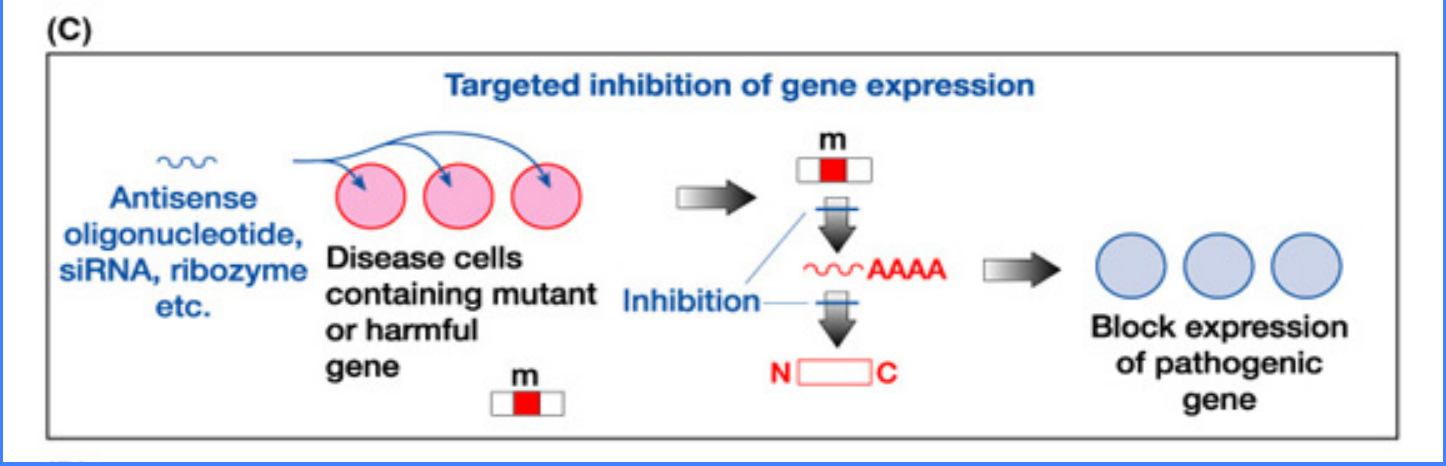


Specific inhibition of gene expression

Involves silencing of specific genes like activated oncogenes, by using molecules that degrade RNA transcripts.

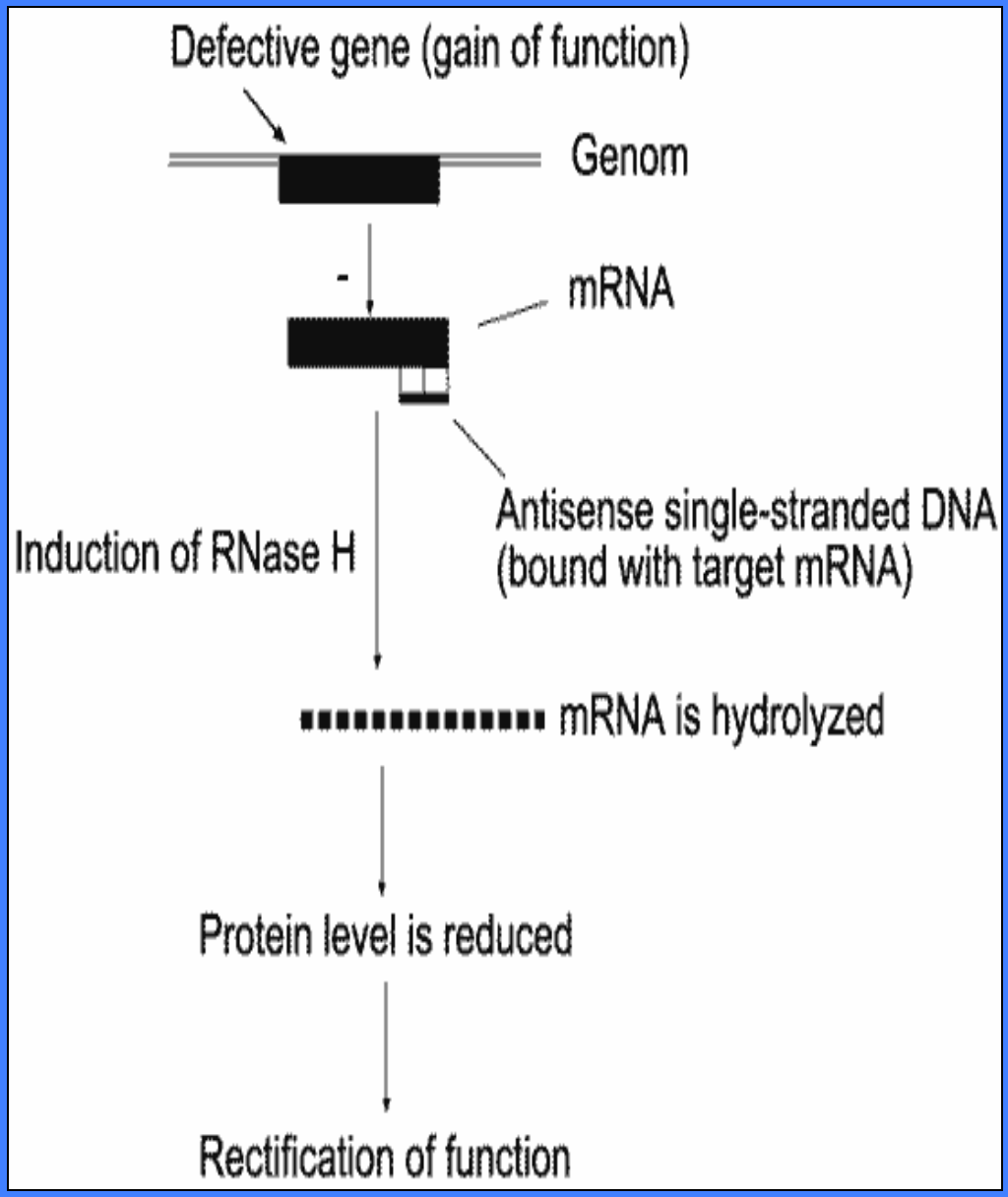
Strategies include

- **Antisense therapy**
- **siRNA (small interfering RNA)**
- **Ribozymes etc**



Antisense therapy

short stretches of synthetic ssDNA that target the mRNA transcripts of abnormal proteins preventing its translation



siRNA therapy

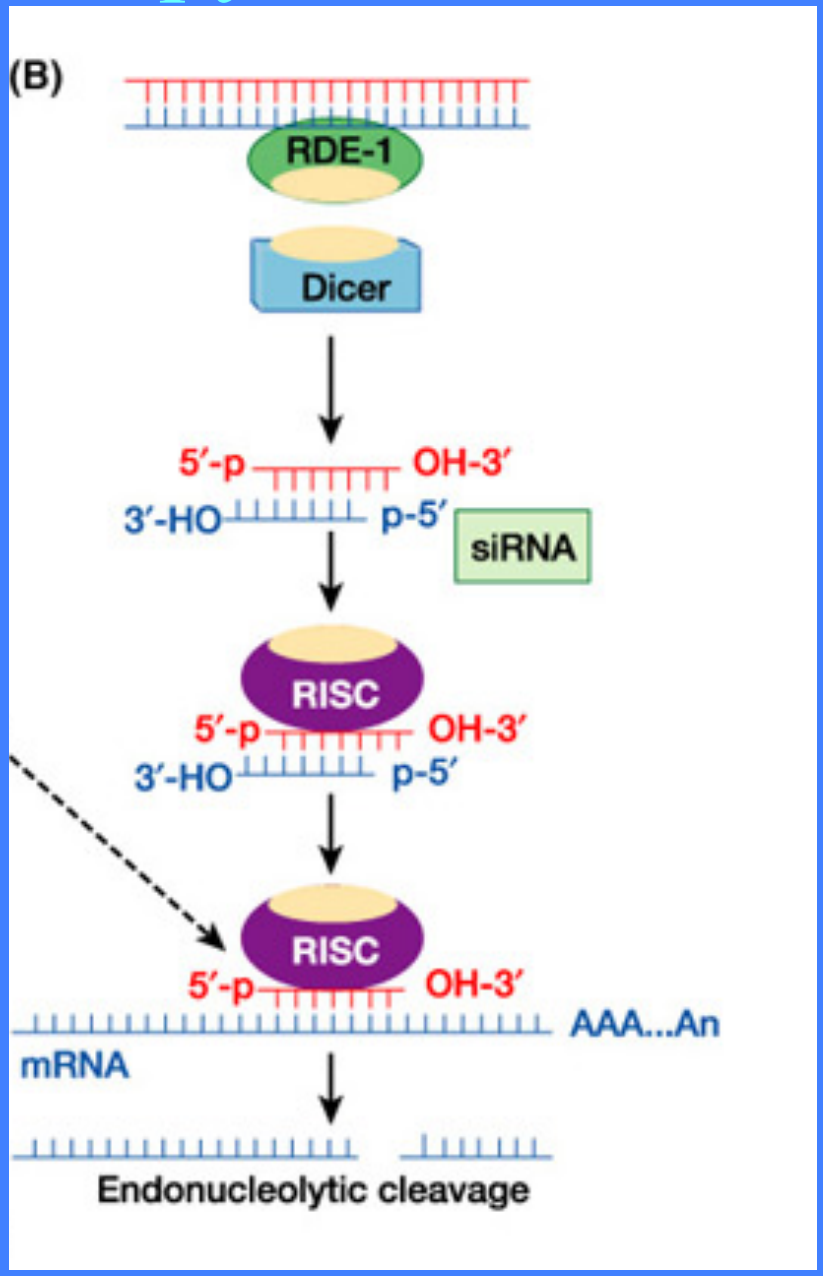
Small interfering RNAs

short stretches (21-23nt) of synthetic dsRNA

Has 3' overhangs of 2 nt

Incorporates into RISC (RNA induced silencing complex)

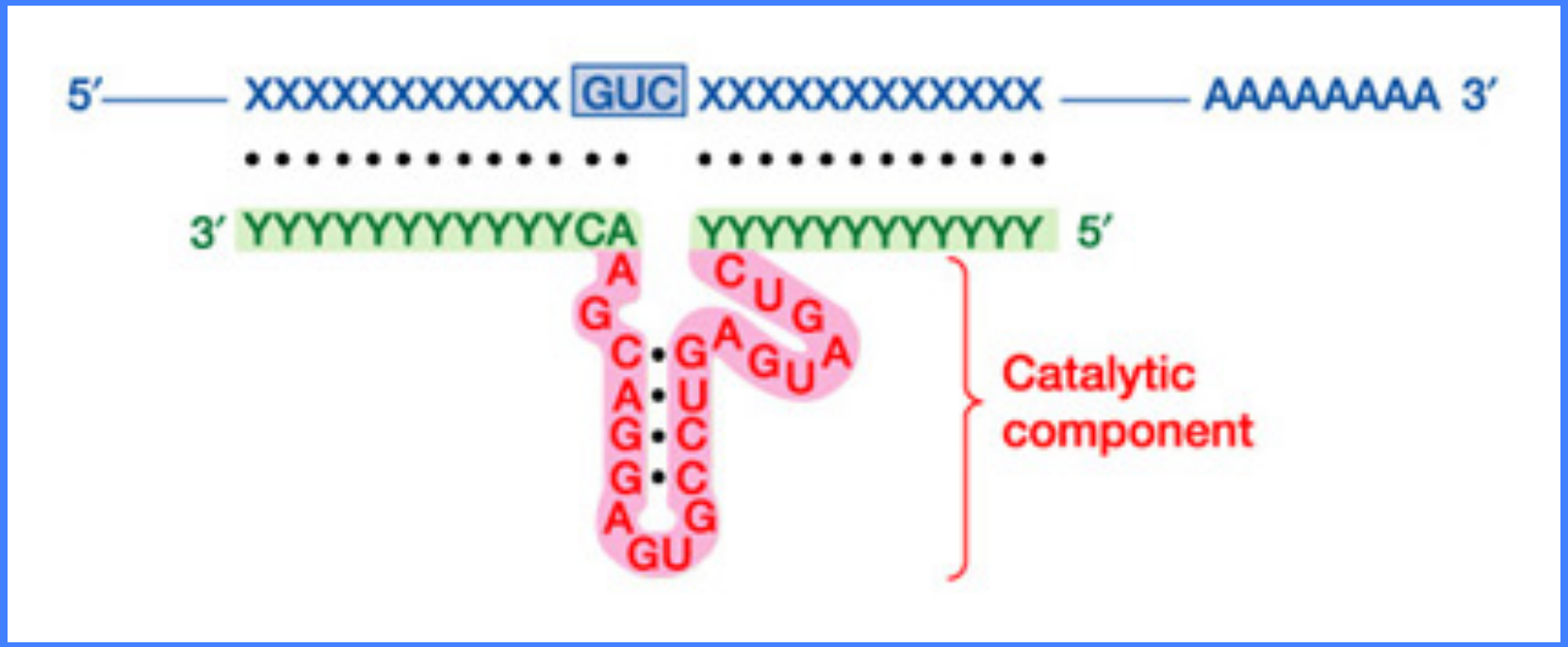
Target mRNA cleaved in the middle



Ribozymes

Catalytic RNAs that cleave target mRNAs in a sequence-specific manner

e.g. hammerhead ribozymes are engineered to recognise specific sequences and made resistant to nucleases



Targeted cell death

Tissue specific toxicity as a result of gene therapy. Useful in cancer therapy

direct approach

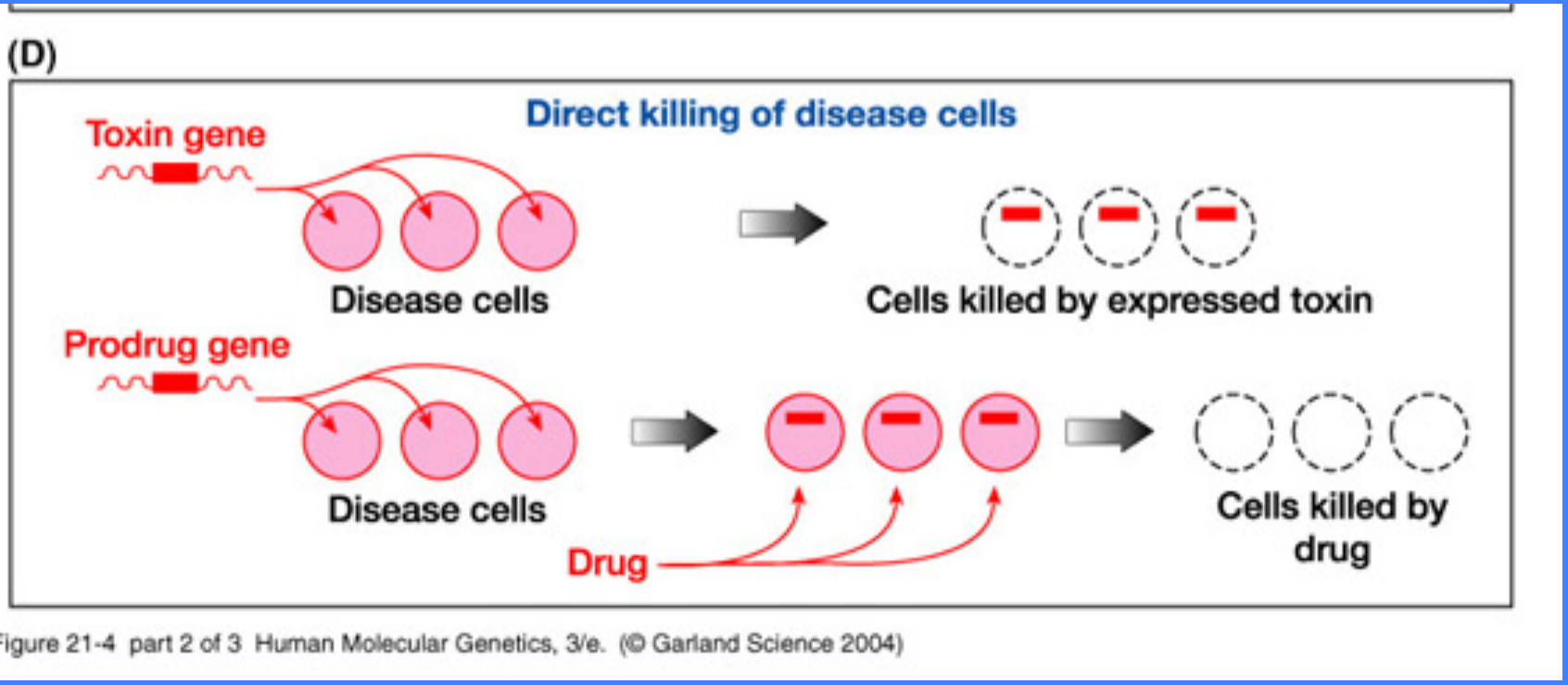


Figure 21-4 part 2 of 3 Human Molecular Genetics, 3/e. (© Garland Science 2004)

Targeted cell death

Indirect approach

stimulating an immune response against selected cells or eliminating the blood supply.

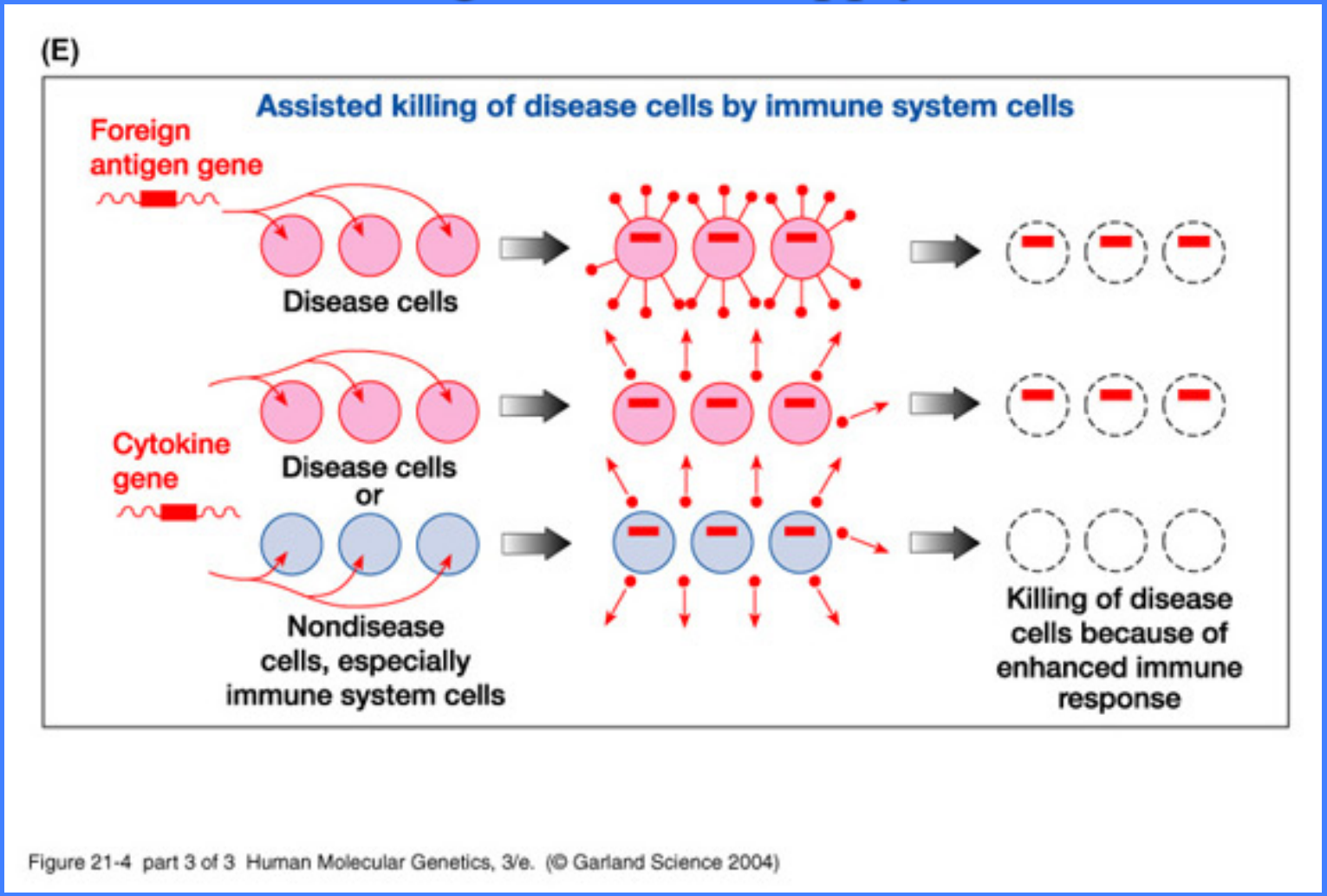
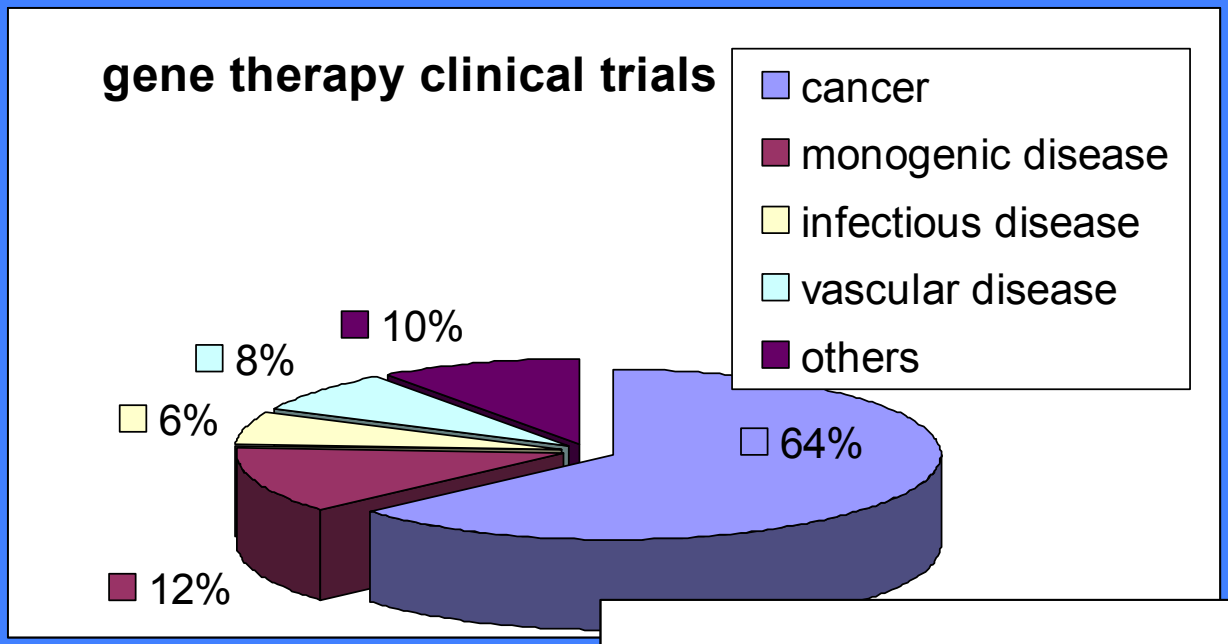
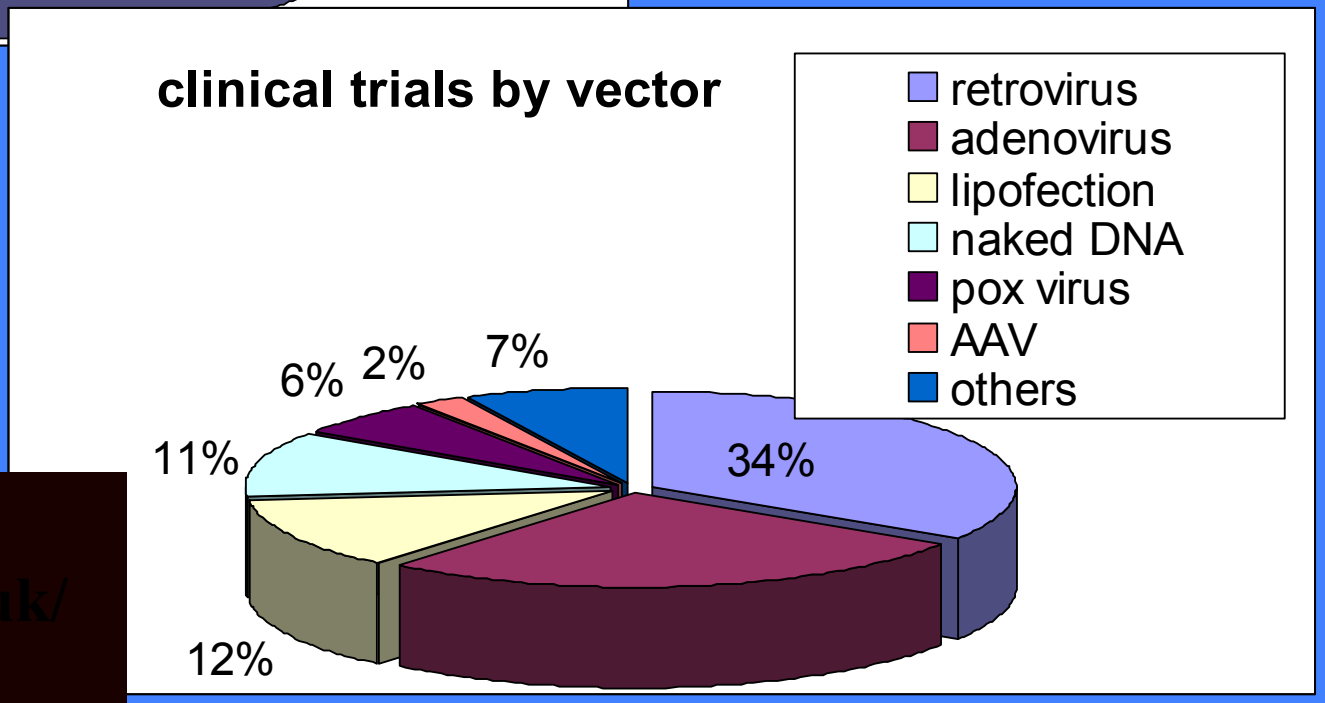


Figure 21-4 part 3 of 3 Human Molecular Genetics, 3/e. (© Garland Science 2004)

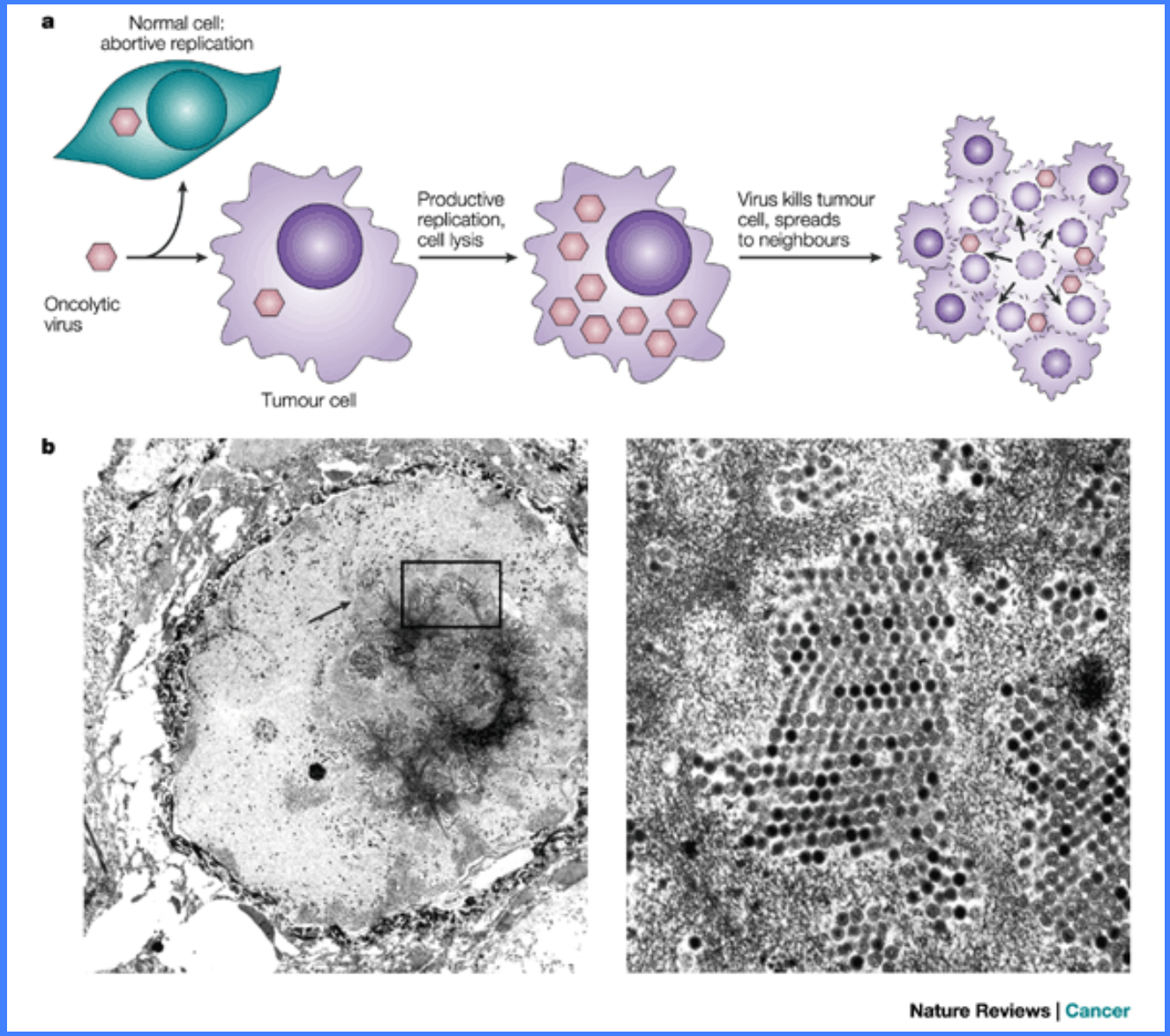
Gene therapy in cancer





Based on http://www.wiley.co.uk/genetherapy/clinical/

Conditionally replicating viruses

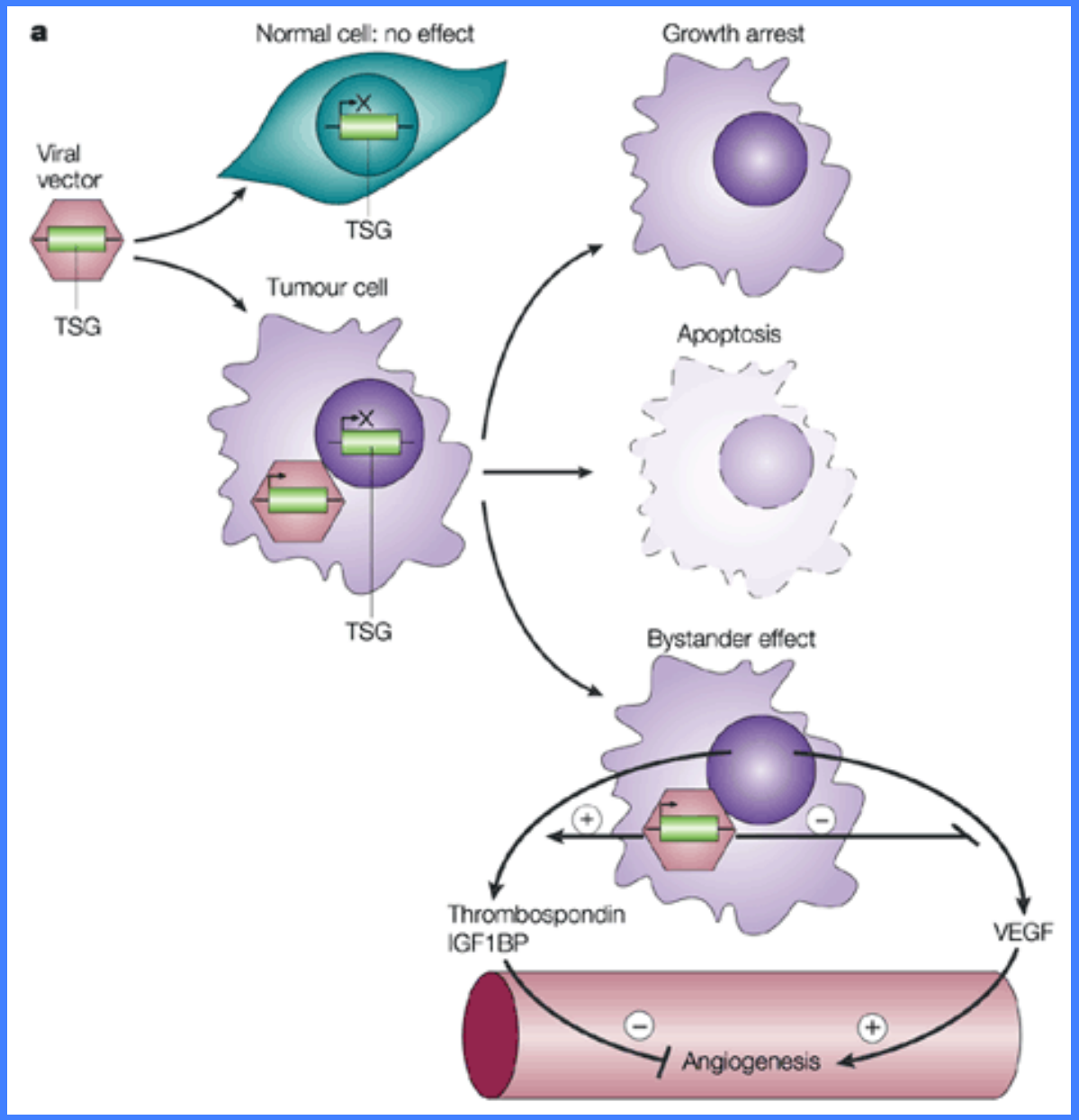


Replication of a conditionally replicating virus, **ONYX-015**, in a cancer cell from a patient with head and neck cancer during Phase II clinical testing.

Conditionally replicating viruses.

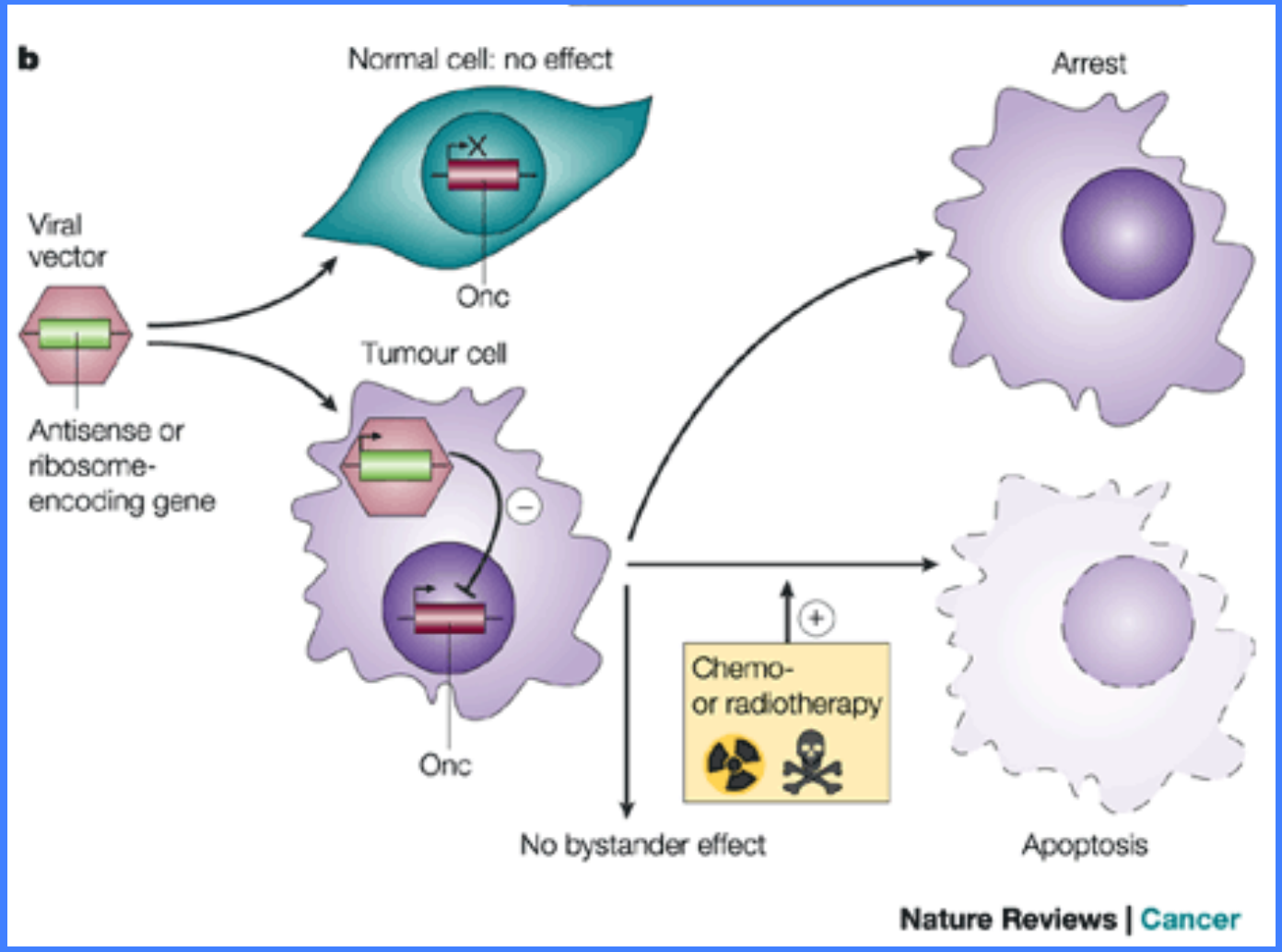
- **a** | Mechanism of action. The viruses infect both normal and tumour cells, but can only replicate in tumour cells. The progeny then go on to kill surrounding tumour cells.
- **b** | Replication of a conditionally replicating virus, ONYX-015, in a cancer cell from a patient with head and neck cancer during Phase II clinical testing. 109 infectious particles were injected over a 5-day period. After 8 days, biopsy was performed and analysed by electron microscopy. The inset on the left panel is magnified on the right. Clearly, this cell is doomed to die: presumably the new virus particles it produces will infect its neighbours.

Tumour-suppressor gene delivery



Nature Reviews Cancer (2001) Vol **1**; 130-141

Delivery of agents that block oncogene expression



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Cancer gene therapy by delivery of tumour-suppressor genes or inhibition of oncogene expression

- **a** | Vectors encoding the tumour suppressor of choice are assumed to infect normal cells and tumour cells. In tumour cells they induce either growth arrest or apoptosis, whereas in normal cells they are assumed not to have any detrimental effects. Some tumour suppressors might also exert unexpected bystander effects. For example, p53 blocks angiogenesis by downregulating the production of vascular endothelial growth factor (VEGF) and by upregulating two anti-angigogenic molecules, thrombospondin and insulin-like growth factor 1 binding protein (IGF1BP).
- **b** |Delivery of agents that block oncogene (Onc) expression. These include genes that encode antisense oligonucleotides, which block oncogene expression, and ribozymes, which cleave oncogene transcripts. Again, they are expected to have no detrimental effects on normal cells, which don't express oncogenes. By contrast, they should cause cancer cells to arrest or undergo apoptosis. In some cases, they also sensitize radio- or chemo-resistant tumour cells to radiotherapy or chemotherapy. No bystander effects have been reported for antioncogenic gene-therapy agents.

Suicide gene delivery

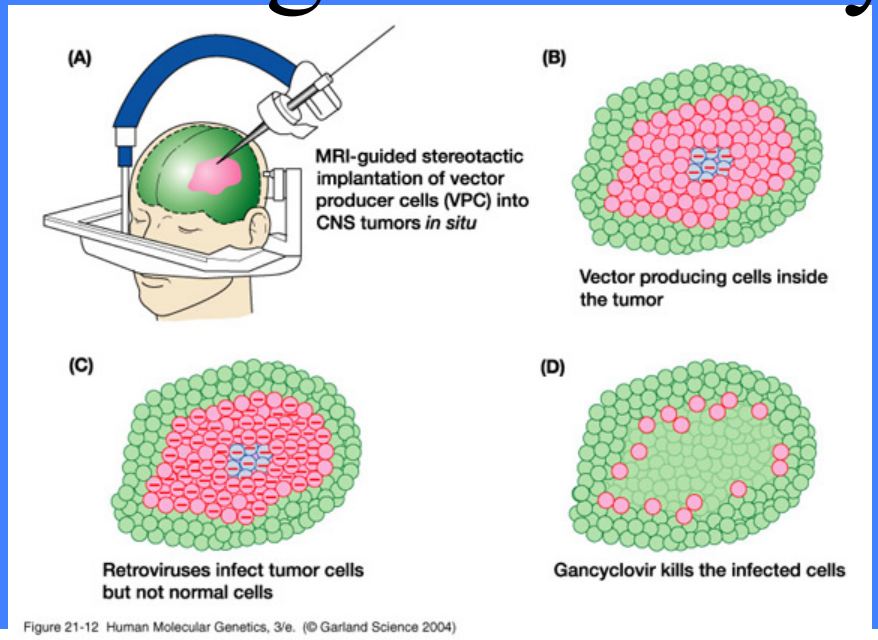
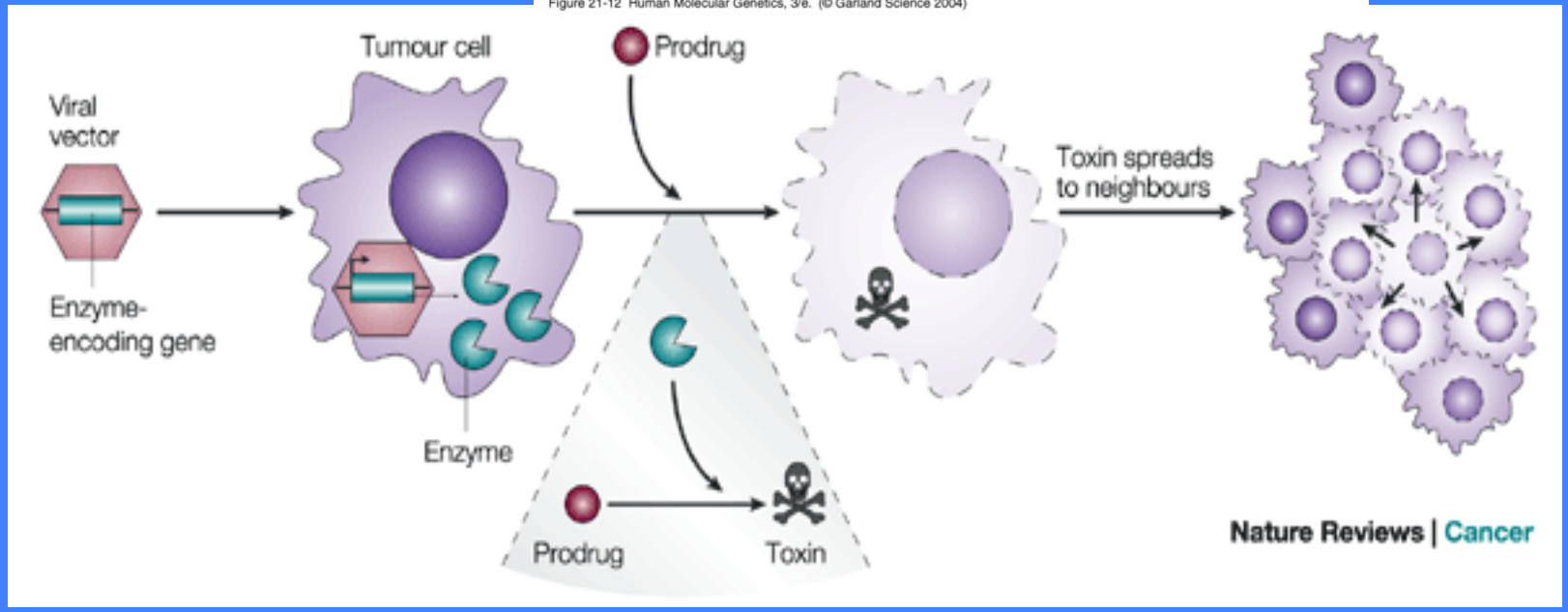


Figure 21-12 Human Molecular Genetics, 3/e. (© Garland Science 2004)



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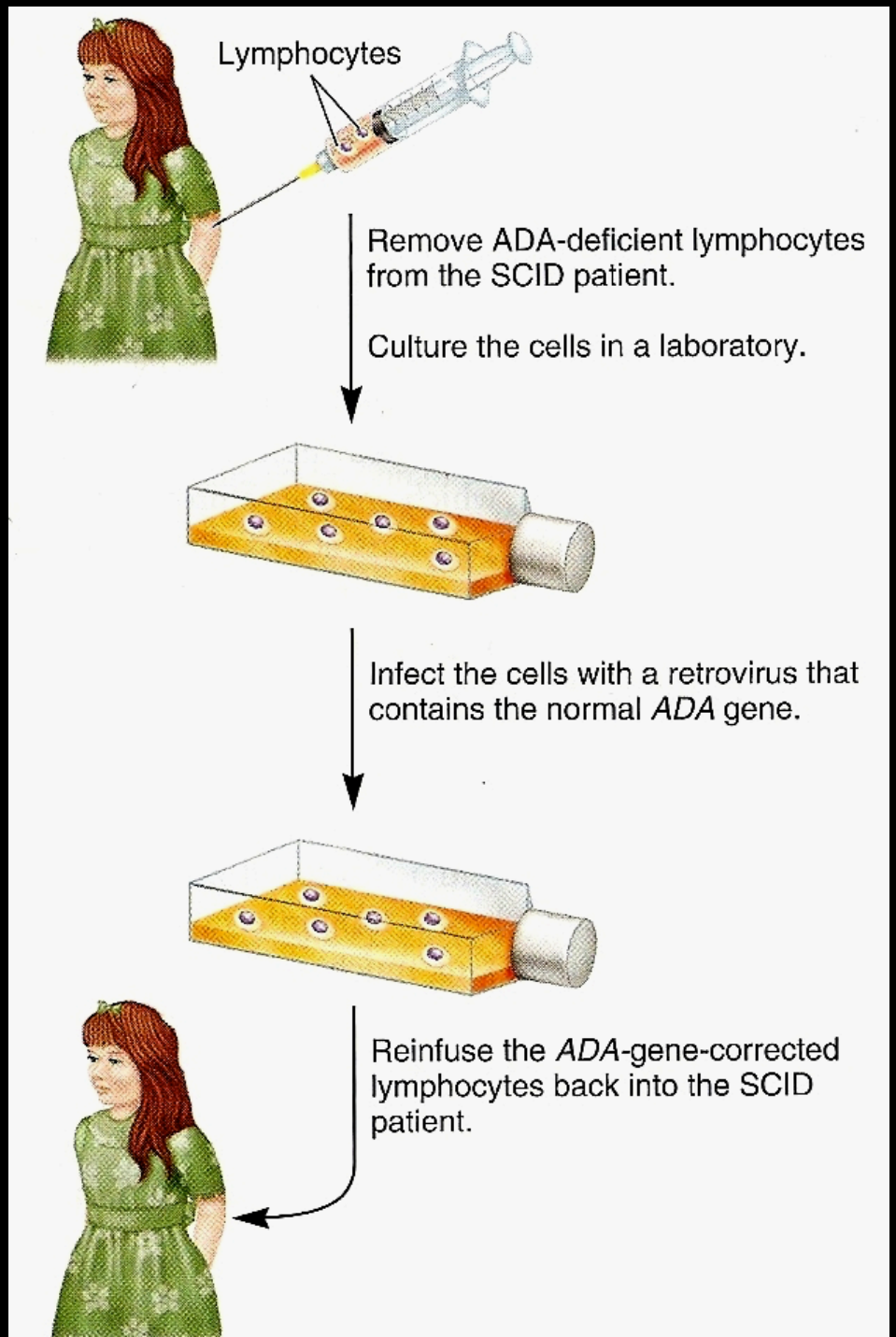
Suicide gene delivery.

- | The vector delivers a gene that encodes a prodrug-converting enzyme, such as herpes simplex virus thymidine kinase (HSV-tk), to tumour and normal cells alike. Local delivery of either the prodrug (in this case, ganciclovir) or the vector to the tumour provides specificity. The prodrug is converted to the active, cytotoxic metabolite in the tumour cell, and diffusion to neighbouring cells confers a potent bystander effect.

Gene therapy for ADA deficiency

- Three approaches for treatment
  - Bone marrow transplant
  - Enzymatic replacement
  - Gene therapy
    - ADA small gene
    - Cloned
    - T-cells accessible and easy to culture *in vitro*
    - Recessive inheritance
    - Gene expression is not tightly controlled



First gene therapy trial for ADA deficiency -1990

Box 2 | **Lessons from gene-therapy trials for adenosine-deaminase deficiency**

- Transducing billions of T cells was harmless
- Need for preclinical testing in an animal model
- Longevity of transduced T cells
- In the absence of a selective advantage provided to transduced cells, the available gene-transfer technology does not result in a sufficiently high level of correction

2002: new trial using a retroviral vector and transduction of bone-marrow precursors, without PEG-ADA administration.
Efficacy demonstrated.

Gene therapy for OTC deficiency

In 1999, 18-year-old Jesse Gelsinger died from multiple organ failure 4 days after treatment for ornithine transcarbomylase deficiency.

- Death was triggered by severe immune response to the adenoviral vector

Gene therapy for SCID-X1

- Mutation in gene for γ_c-cytokine receptor
- 10 month follow-up two patients' T-cells expressed normal γ_c-cytokine receptor
- However, in a French study 3/10 patients developed leukemia within three years
  - Integration of retroviral DNA next to an oncogene

Gene therapy for SCID-X

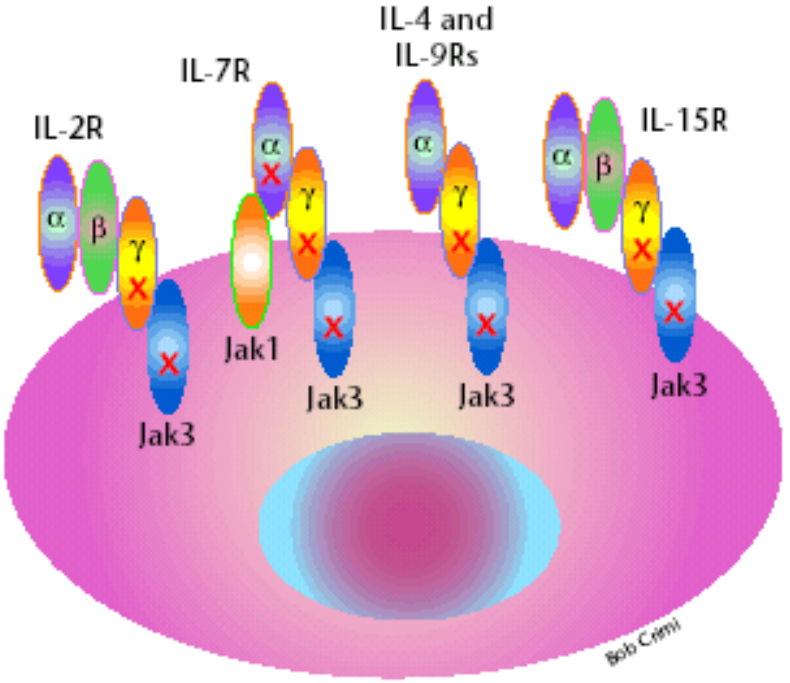


Fig. 1 Cytokine receptor defects known to cause SCID. Mutations in the gene encoding the common gamma chain (γc) for all five receptors (Rs) result in SCID-X. Mutations in the gene for Janus kinase 3 (Jak3), which transduces the receptor signal from γc, cause autosomal recessive SCID that is phenotypically identical to SCID-X1. Finally, mutations in the gene encoding the α chain of the IL-7 receptor also cause autosomal recessive SCID.

Gene Therapy as a Treatment for SCID-X

- X-linked severe combined immunodeficiency (X-SCID) is a disease that affects young children and is usually fatal within their first year of life.
- Bone marrow transplants are usually the best option for treatment, but with the difficulty in finding a donor who matches the patient, gene therapy has become a new alternative.
- Clinical trials for treating X-SCID patients have been marked by mixed results

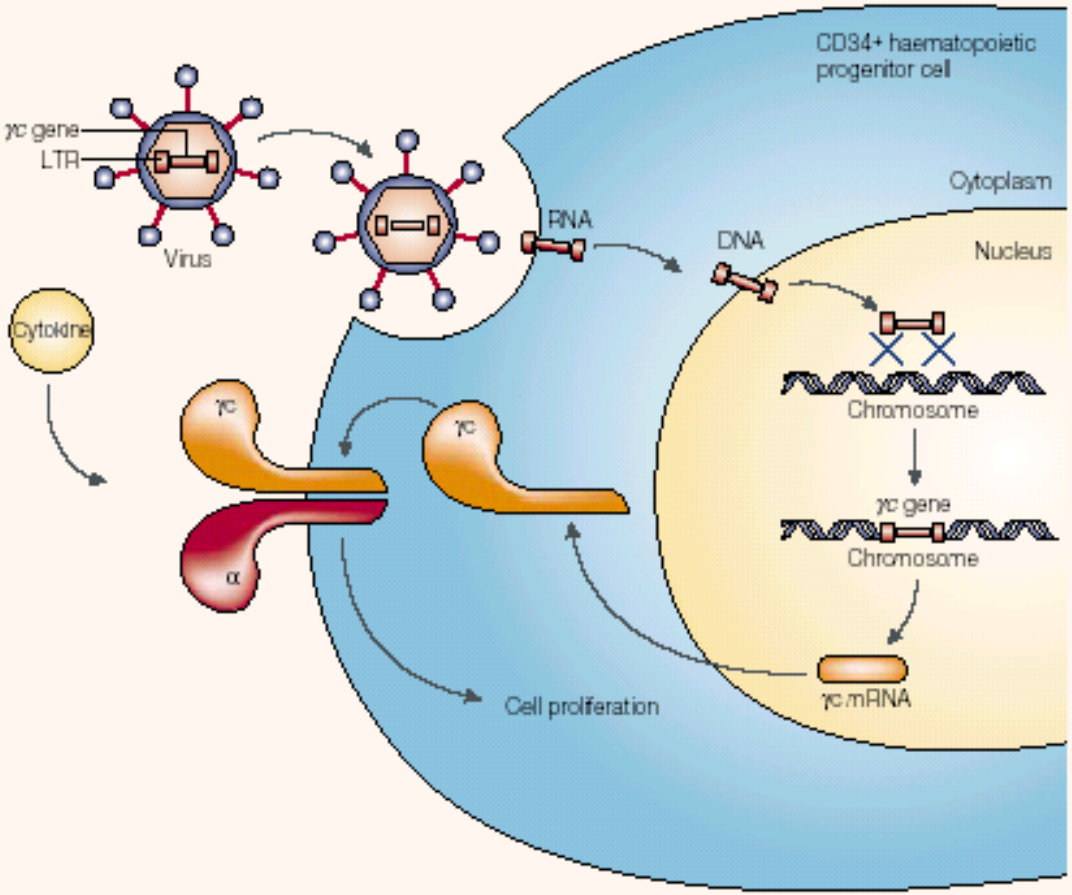


Figure 3 | **Principle of *ex vivo* gene therapy of the SCID-X condition.** CD34+ cells are incubated *ex vivo* with supernatant that contains retrovirus encoding the common cytokine-receptor γ-chain (γc) gene. Binding of the virus to a cell is followed by viral entry. Viral RNA is retrotranscribed into DNA, which, as a preintegration complex, can recombine with the cell's genome. The γc gene can be transcribed, being under the control of the viral long terminal repeat (LTR), which leads to protein synthesis, membrane expression and function. mRNA, messenger RNA.

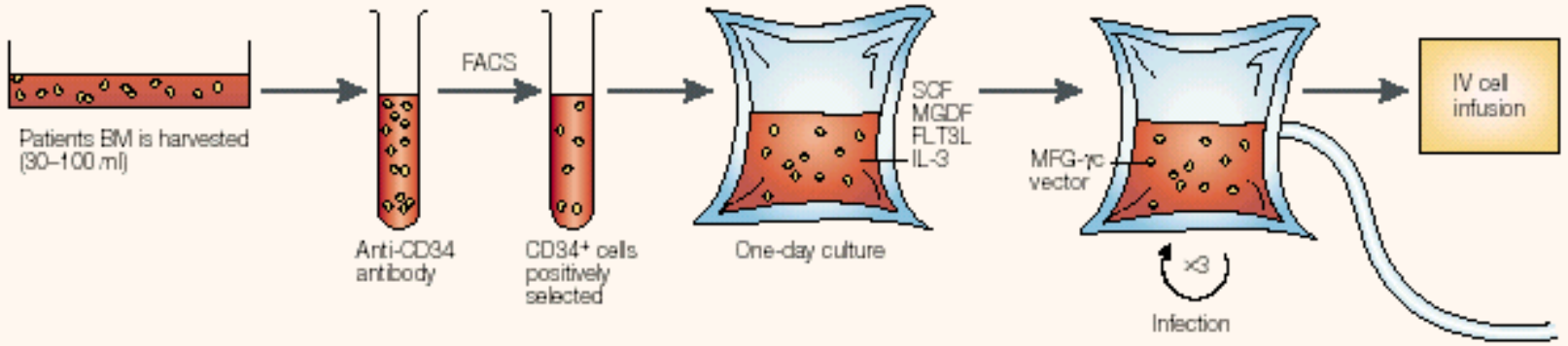


Figure 4 | **Scheme of the transduction protocol for the SCID-X gene-therapy trial.** The patient's bone marrow (BM) was harvested and the CD34+ cells were selected. These cells were first incubated with cytokines that can provide survival and proliferative signals, before they were infected (three one-day cycles of infection) with the retroviral vector. Cells were contained in bags coated with a fibronectin fragment (CH-296), which facilitated cell–virus interaction. After completion of the four-day procedure, cells were washed and injected back into the patient intravenously (IV) without additional therapy. FACS, fluorescence-activated cell sorting; FLT3L, FLT3 ligand; IL-3, interleukin-3; MFG, retroviral vector; MGDF, megakaryocyte growth and development factor; SCF, stem-cell factor.

- One of the patients involved in the Fischer trials has developed leukemia two and a half years after the initial gene therapy treatment (*Gene Therapy)*.
- Two of eleven patients involved in a similar study in France have also developed leukemia (*Trends in Biotechnology)*.
- The gene therapy treatments have resulted in the overexpression of the a gene that may be an oncogene and is located at the site of the retroviral insertion.
- The site of insertion is the first intron of the LMO-2 gene, which is located on chromosome eleven. LMO-2 is also the site of a translocation that occurs in leukemia. This observation clearly correlates the retroviral insertion as the cause of leukemia in the patients.

- Law of Unintended Consequences?
- It is still unknown whether the development of leukemia in these clinical studies was the result of a premature treatment (which could be eliminated with further research and development) or if it is a permanent risk.
- Despite the fact that without treatment, X-SCID is a fatal disease, there is still an ethical question of whether or not it is right to subject a sick child to the possibility of developing another disease through the risks of gene therapy treatments.

Regulatory responses in Europe and the United States

United States

The FDA allows gene-therapy trials for X-SCID if no other therapy is available. Clinical hold on other stem-cell gene-therapy trials may be lifted after case-by-case review.

www.fda.gov/ohrms/dockets/ac/03/minutes/3924M2.doc

United Kingdom

Approved clinical SCID trials are assessed on a case-by-case basis and are ongoing.

www.doh.gov.uk/genetics/gtac/recommendationsGTAC-CSM.PDF

France

After a temporary hold, the French reopened clinical studies for X-SCID in January 2004.

afssaps.sante.fr

Italy

Moratorium on any clinical trial involving the use of retroviruses until 31 December 2003. New ruling is currently awaited.

www.iss.it/sitp/scf1/comu/index.html

Germany

After a temporary hold on all trials involving retroviruses, gene-therapy trials for SCIDs and other diseases restarted in February 2003.

www.bundesaerztekammer.de/30/Ethik/80Themen/85KomSomGen

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 29, 2009 VOL. 360 NO. 5

Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

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CONCLUSIONS

Gene therapy, combined with reduced-intensity conditioning, is a safe and effective treatment for SCID in patients with ADA deficiency. (ClinicalTrials.gov numbers, NCT00598481 and NCT00599781.)

Related Commentary, page 2083

Research article

Vector integration is nonrandom and clustered and influences the fate of lymphopoiesis in SCID-X1 gene therapy

Annette Deichmann,[1,2,3] Salima Hacein-Bey-Abina,[4,5] Manfred Schmidt,[1,2,3] Alexandrine Garrigue,[4] Martijn H. Brugman,[6] Jingqiong Hu,[1] Hanno Glimm,[1,2] Gabor Gyapay,[7] Bernard Prum,[8] Christopher C. Fraser,[9] Nicolas Fischer,[10] Kerstin Schwarzwaelder,[1,3,11] Maria-Luise Siegler,[1] Dick de Ridder,[12,13] Karin Pike-Overzet,[12] Steven J. Howe,[14] Adrian J. Thrasher,[14,15] Gerard Wagemaker,[6] Ulrich Abel,[3,16] Frank J.T. Staal,[12] Eric Delabesse,[17] Jean-Luc Villeval,[18] Bruce Aronow,[19] Christophe Hue,[4,5] Claudia Prinz,[1] Manuela Wissler,[1,2] Chuck Klanke,[20] Jean Weissenbach,[7] Ian Alexander,[21] Alain Fischer,[4,22] Christof von Kalle,[1,2,3,20] and Marina Cavazzana-Calvo[4,5]

Research article

Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1

Salima Hacein-Bey-Abina,[1,2] Alexandrine Garrigue,[2] Gary P. Wang,[3] Jean Soulier,[4] Annick Lim,[5] Estelle Morillon,[2] Emmanuelle Clappier,[5] Laure Caccavelli,[1] Eric Delabesse,[6] Kheira Beldjord,[7,8] Vahid Asnafi,[7,8] Elizabeth MacIntyre,[7,8] Liliane Dal Cortivo,[1] Isabelle Radford,[8] Nicole Brousse,[9] François Sigaux,[4] Despina Moshous,[10] Julia Hauer,[2] Arndt Borkhardt,[11] Bernd H. Belohradsky,[12] Uwe Wintergerst,[12] Maria C. Velez,[13] Lily Leiva,[13] Ricardo Sorensen,[13] Nicolas Wulffraat,[14] Stéphane Blanche,[10] Frederic D. Bushman,[3] Alain Fischer,[2,10] and Marina Cavazzana-Calvo[1,2]

REVIEWS

OCCURRENCE OF LEUKAEMIA FOLLOWING GENE THERAPY OF X-LINKED SCID

Donald B. Kohn, Michel Sadelain‡ and Joseph C. Glorioso§*

Recombinant viral vectors have allowed gene transfer to be developed as a promising approach to the treatment of genetic diseases. Recently, gene therapy of children with X-linked severe combined immune deficiency resulted in impressive levels of immune reconstitution — a triumph that was later overshadowed by the development of leukaemia in two patients. What were the causes of this cancer, and how can the therapeutic benefits of gene therapy be achieved while minimizing risk to the patient?

Research article

Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients

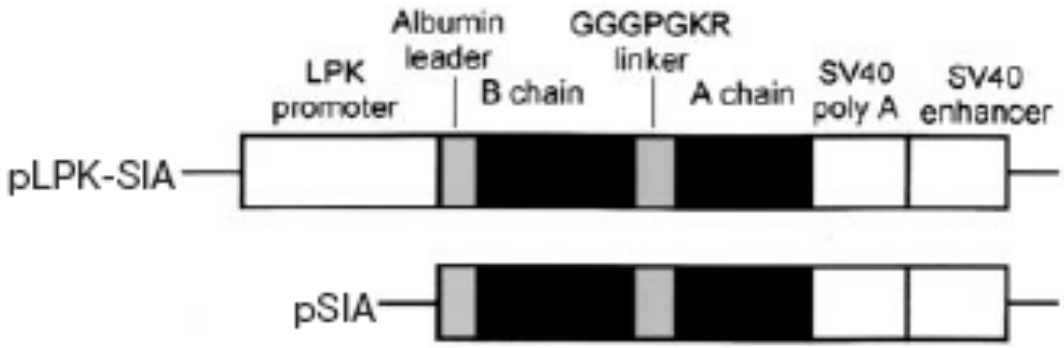
Steven J. Howe,[1] Marc R. Mansour,[2] Kerstin Schwarzwaelder,[3] Cynthia Bartholomae,[3] Michael Hubank,[4] Helena Kempski,[4,5] Martijn H. Brugman,[6] Karin Pike-Overzet,[7] Stephen J. Chatters,[5] Dick de Ridder,[7,8] Kimberly C. Gilmour,[9] Stuart Adams,[9] Susannah I. Thornhill,[1] Kathryn L. Parsley,[1,9] Frank J.T. Staal,[7] Rosemary E. Gale,[2] David C. Linch,[2] Jinhua Bayford,[9] Lucie Brown,[9] Michelle Quaye,[1] Christine Kinnon,[1] Philip Ancliff,[9] David K. Webb,[9] Manfred Schmidt,[3] Christof von Kalle,[3,10] H. Bobby Gaspar,[1,9] and Adrian J. Thrasher[1,9]

[1]Centre for Immunodeficiency, Molecular Immunology Unit, UCL Institute of Child Health, and [2]Department of Haematology, University College London, London, United Kingdom. [3]Department of Translational Oncology, National Center for Tumor Diseases (NCT), German Cancer Research Center (DKFZ), Heidelberg, Germany. [4]Molecular Haematology and Cancer Biology Unit, Institute of Child Health, University College London, London, United Kingdom. [5]Paediatric Malignancy Cytogenetics Unit, Great Ormond Street Hospital, London, United Kingdom. [6]Department of Experimental Hematology, Hannover Medical School, Hannover, Germany. [7]Department of Immunology, Erasmus Medical Center, Rotterdam, The Netherlands. [8]Information and Communication Theory Group, Faculty of Electrical Engineering, Mathematics, and Computer Science, Delft University of Technology, Delft, The Netherlands. [9]Great Ormond Street Hospital for Children NHS Trust, London, United Kingdom. [10]Division of Experimental Hematology and Cancer Biology, Cincinnati Children's Research Foundation, Cincinnati, Ohio, USA.

Gene Therapy used to Treat Type I Diabetes

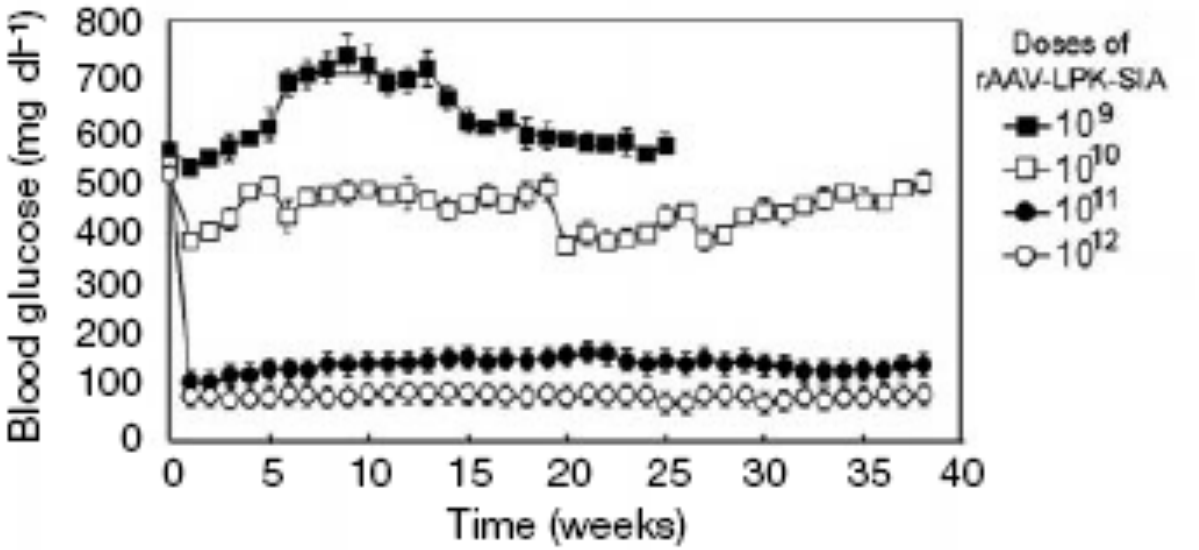
- Study by Lee, Kim, Kim, Shin, and Yoon performed in Korea (2000). Their results were published in *Nature*
- Type I diabetes is caused by the destruction of insulin-producing pancreatic β cells by an inappropriate autoimmune response

- This experiment was performed on mice and rats but the results may result in future implications for humans
- Scientists used a recombinant adeno-associated virus (rAAV) to insert a gene that results in the expression of a single-chain insulin analogue (SIA) into streptozotocin-induced diabetic rats and autoimmune diabetic mice.
- First, the gene was cloned under the L-type pyruvate kinase (LPK) promoter, which regulates the expression of SIA in response to glucose levels



- The LPK-SIA gene was then attached to a recombinant adeno-associated virus and integrated into the host chromosomal DNA

- After insertion of the rAAV-LPK-SIA, the rats displayed a drop in glucose levels that reached a range of normoglycaemia within one week of treatment. The rates remained in this range for more than eight months.



- In addition to eight months of controlled glucose levels, there were no visible side affects from the gene therapy.
- While the results did not show permanent remission, the control of glucose levels from the insertion of the SIA gene was promising.
- This form of gene therapy may provide a cure for type I diabetes for humans in the future (but a lot more research would be required before that can happen).

Gene Therapy: A Scientific Perspective

Gene Therapy has been defined as: nucleic-acid based treatment, or transfer of DNA/RNA to somatic target cells in the intention to treat serious illness' (1).

In somatic gene therapy, new genes are introduced to the body

In germ line therapy, the human germ line is modified, conferring heritable modifications to the offspring

However, germ line therapy is not permitted in any country, on the basis that it is unethical

Essential to the progression of gene therapy is a comprehensive understanding of the human genome and various genetic diseases

Types of Gene Therapy

- Prominent forms include postnatal gene delivery via viral vectors for insertion within the genome, imparting expression of the newly incorporated gene, and so-called “gain of function”
- RNA interference, or RNAi, borrows from the principals of naturally occurring process within biological systems, used to affect relative levels of expression of certain genes.
- Present research and ongoing efforts are also being made in the development of human prenatal gene therapy

Postnatal Gene Therapy

- **Purpose**: Correction of the deleterious effects of genetic disease via long term integration of gene sequences into a patient's genome
- This property makes the use of retroviral vectors particularly attractive when considering effective gene delivery to correct inherited monogenetic disorders

Types of Postnatal Gene Therapy

- Gene replacement: non-functional or defective gene is replaced by a new, functional copy of the gene
  - Can be accomplished by homologous recombination, although efficiency is low
- Gene addition: introduction of a gene that is able to produce a protein not normally expressed in the cell
  - i.e. Introduction of a so-called “suicide gene” into cancer cells

Gene Therapy Progress and Prospects

Fetal gene Therapy:

Also known as prenatal or *in utero* gene therapy

Targets genetic diseases which require lifelong correction

The concept of fetal gene therapy is based on the following aims:

- avoiding early-onset manifestation of life-threatening genetic conditions
- achieving permanent correction of such diseases by stable transduction of relevant fetal progenitor cell populations
- Avoiding immune reactions against the therapeutic vector and transgene by induction of tolerance.

First proofs of principle for therapeutic in utero gene application

- First successful therapeutic application of gene transfer *in utero* was carried out in 2003 by Seppen et al.
- This was achieved by direct injection of a lentiviral vector expressing the human bilirubin UDP-glucuronyltransferase (UGT1A1) gene under control of the phosphoglycerate kinase promoter into the liver of Gunn rat fetuses.

Benefits of prenatal gene therapy

- Provides early phenotypic correction, reducing or avoiding otherwise devastating effects of genetic disease
- Demonstration of long-term postnatal therapeutic protein production
- Tolerance to the transgenic protein can be induced by *in utero* expression

“Although fetal gene therapy will not replace postnatal gene therapy, it is essentially a preventive approach to the management of otherwise predominantly incurable diseases and would therefore – if successful and safe – be most effectively conducted in conjunction with prenatal screening programmes.”

Progress in Prenatal Gene Therapy

- Disparity between species must be taken into account when considering administration of human fetal gene therapy
- Minimally invasive methods of ultrasound guided gene delivery are being devised in large animal models

Adverse Effects of Gene Therapy

- Vector induced oncogenesis
- Germline transfer of transgenic DNA sequences
- Developmental aberrations caused by expression of the transgenic proteins and vector induced oncogenesis
- Without proper specificity, delivery to the right cell type in the right organ, at the right time, there could be detrimental immunological effects.

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"[illegible]" Davies