Principles and perspectives of gene therapy

Prof. Józef Dulak, *PhD*, *DSc*
Department of Medical Biotechnology
Faculty of Biochemistry, Biophysics and Biotechnology
Room 3.025/3.07
Phone 664-63-75
Email: jozef.dulak@uj.edu.pl

Older versions of lectures can be downloaded from the web page –
Department of Medical Biotechnology
at **http://biotka.mol.uj.edu.pl/zbm/**

Conditions for positive outcome...

1. Learning and understanding the information delivered during the lectures

2. **Asking the questions during and after the lecture!**

3. Supplementary materials:
   a) articles distributed during the course
   b) „Gene transfer to animal cells" – several copies are in the library

For Polish students:

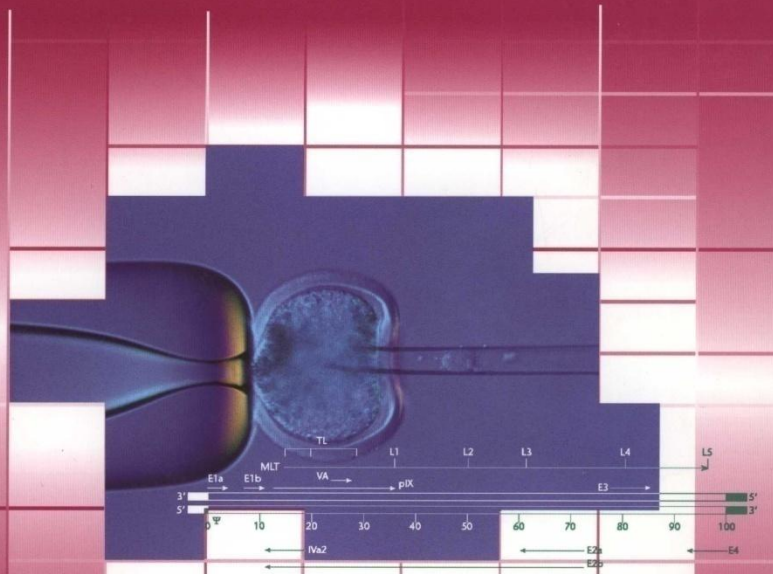
   b) „Terapia genowa" red. Stanisław Szala (PWN, 2003)
   c) „Biotechnologia" – No 3/2007 (several copies are available in the library)

4. Passing the final exam …

Test – multiple choice – questions will concern the information provided at the slides and those which will be explained in more details during the lectures – hence attending them is reasonable

Gene Transfer to Animal Cells

R.M. Twyman



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ADVANCED METHODS

Terapia genowa

Redaktor naukowy

Stanisław Szala

WYDAWNICTWO NAUKOWE PWN

FACULTY OF BIOCHEMISTRY, BIOPHYSICS AND BIOTECHNOLOGY
JAGIELLONIAN UNIVERSITY

DEPARTMENT OF MEDICAL BIOTECHNOLOGY

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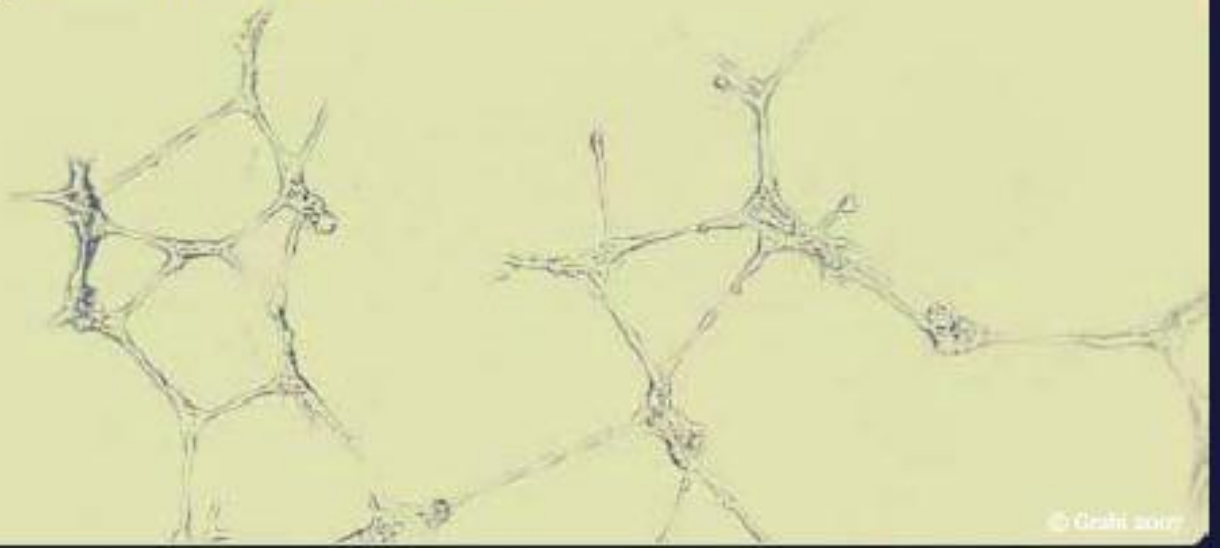
Department of Medical Biotechnology was established as a separate unit in June 2005. Our main field of research is vascular biology, with particular interest in molecular mechanisms of angiogenesis, vasculogenesis, inflammation and oxidative stress. We are also interested in tumor cell biology. In our lab we investigate gene and cell therapy approaches to treat vascular disorders.



http://biotka.mol.uj.edu.pl/zbm/

Parallel course

Gene transfer techniques in vitro

seminars and practical course

(Viral vectors in medical biotechnology)

Prof. Alicja Józkowicz – email: alicja.jozkowicz@uj.edu.pl

Magdalena Kozakowska – email: m.kozakowska@uj.edu.pl

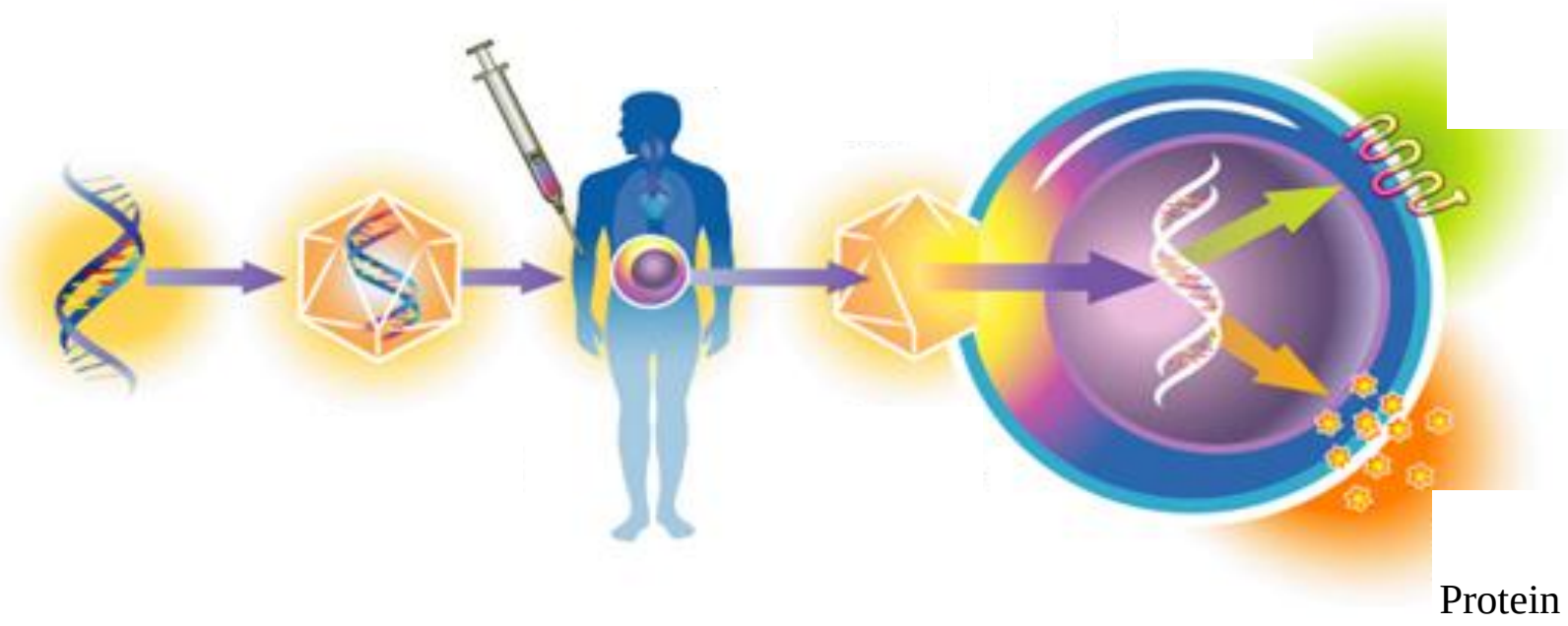
What is gene therapy?

Application of nucleic acids for treatment of diseases

Gene-based therapeutics is broadly defined as the introduction, using a vector, of nucleic acids into cells with the intention of altering gene expression to prevent, halt or reverse a pathological process.

M. Kay, Nature Reviewsb Genetics, 2011

Gene therapy



Therapeutic gene (transgene)

Vector

Patient

Expression of therapeutic gene

Which diseases could be cured with gene therapy?

Is gene therapy necessary?

Gene therapy was born in... 1962

Prof.Wacław Szybalski

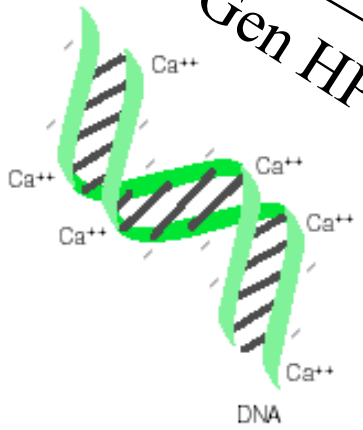
McArdle Laboratory
for Cancer Research,
Wisconsin, Madison,
USA

$HPRT^{-/-}$ cells

HAT medium

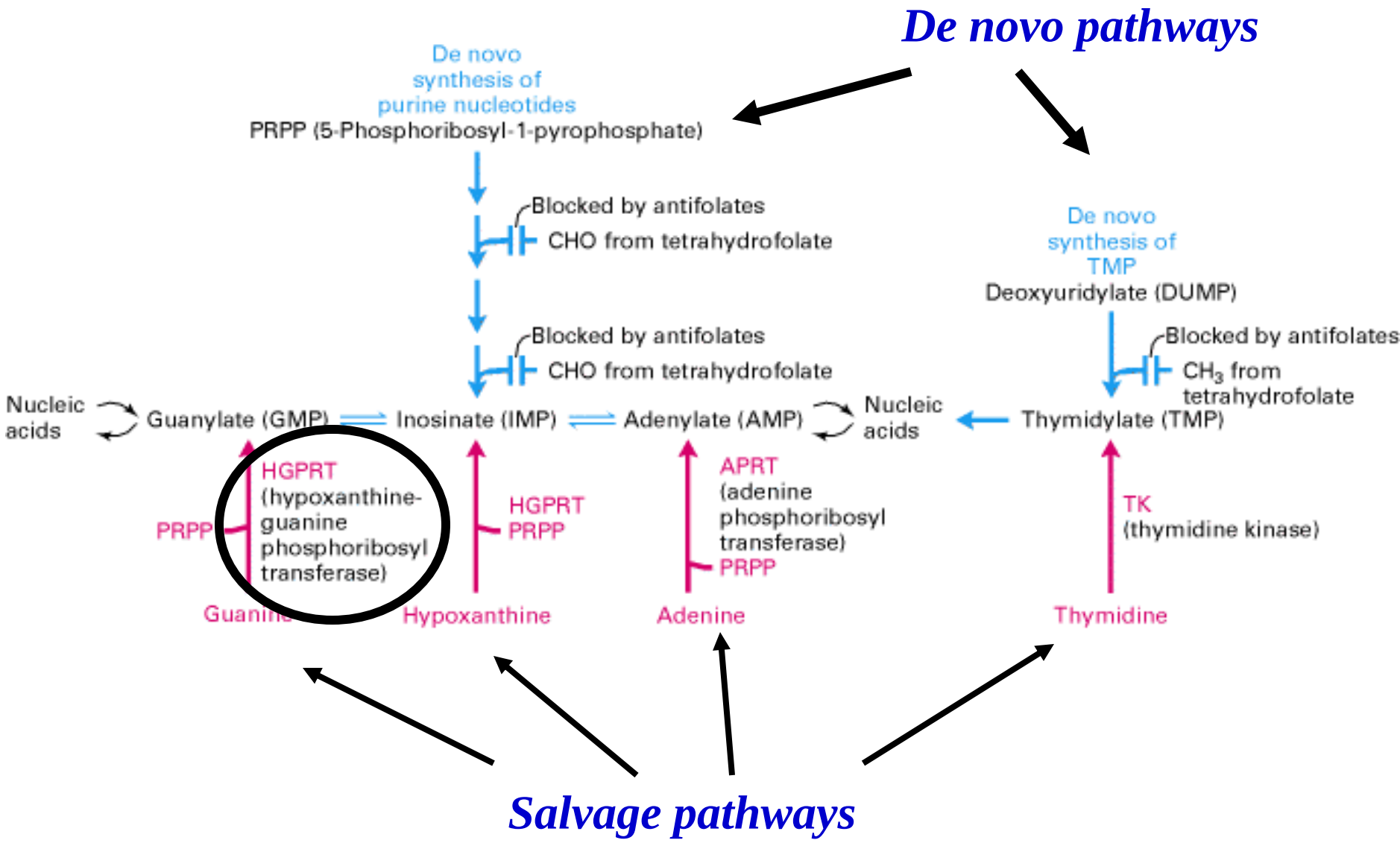
HAT medium

Gen HPRT



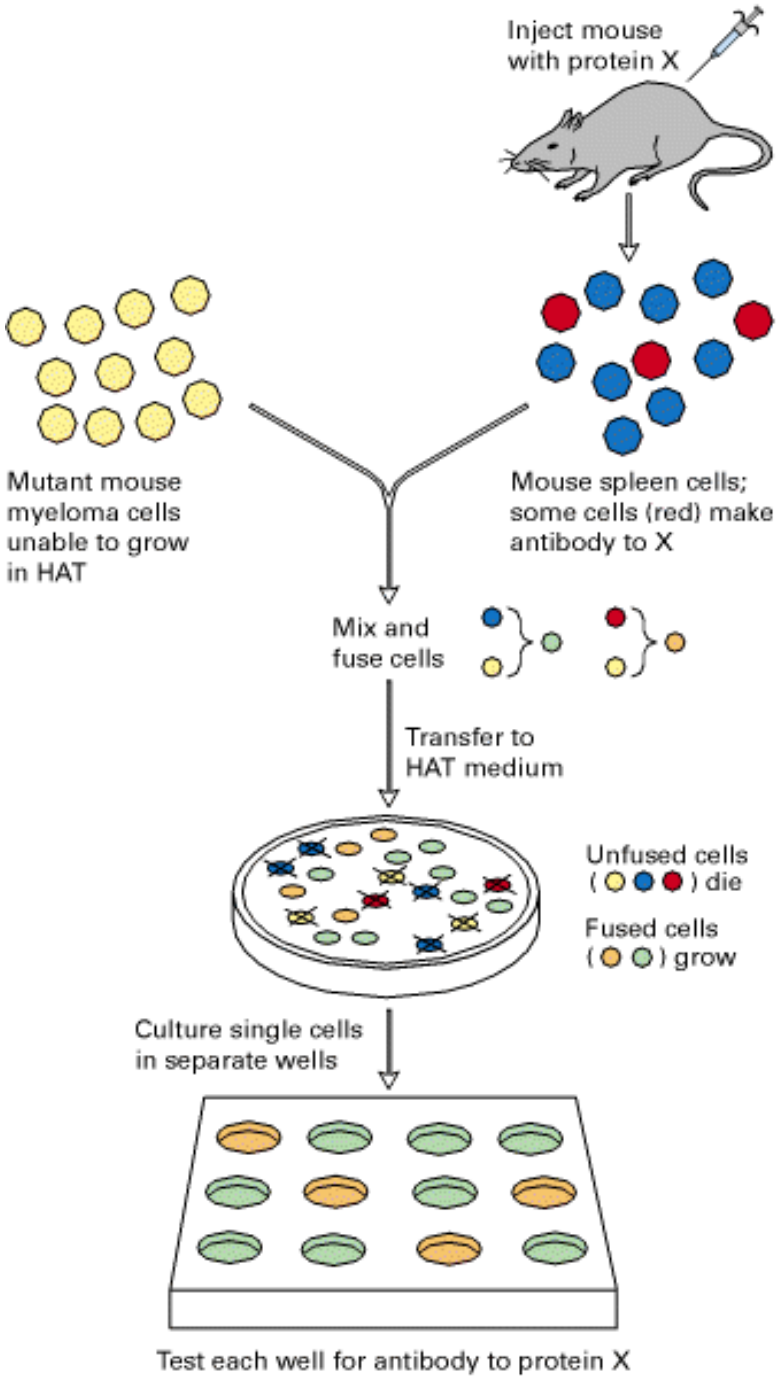
$HPRT^{+/+}$ cells

De novo and salvage pathways for nucleotide synthesis



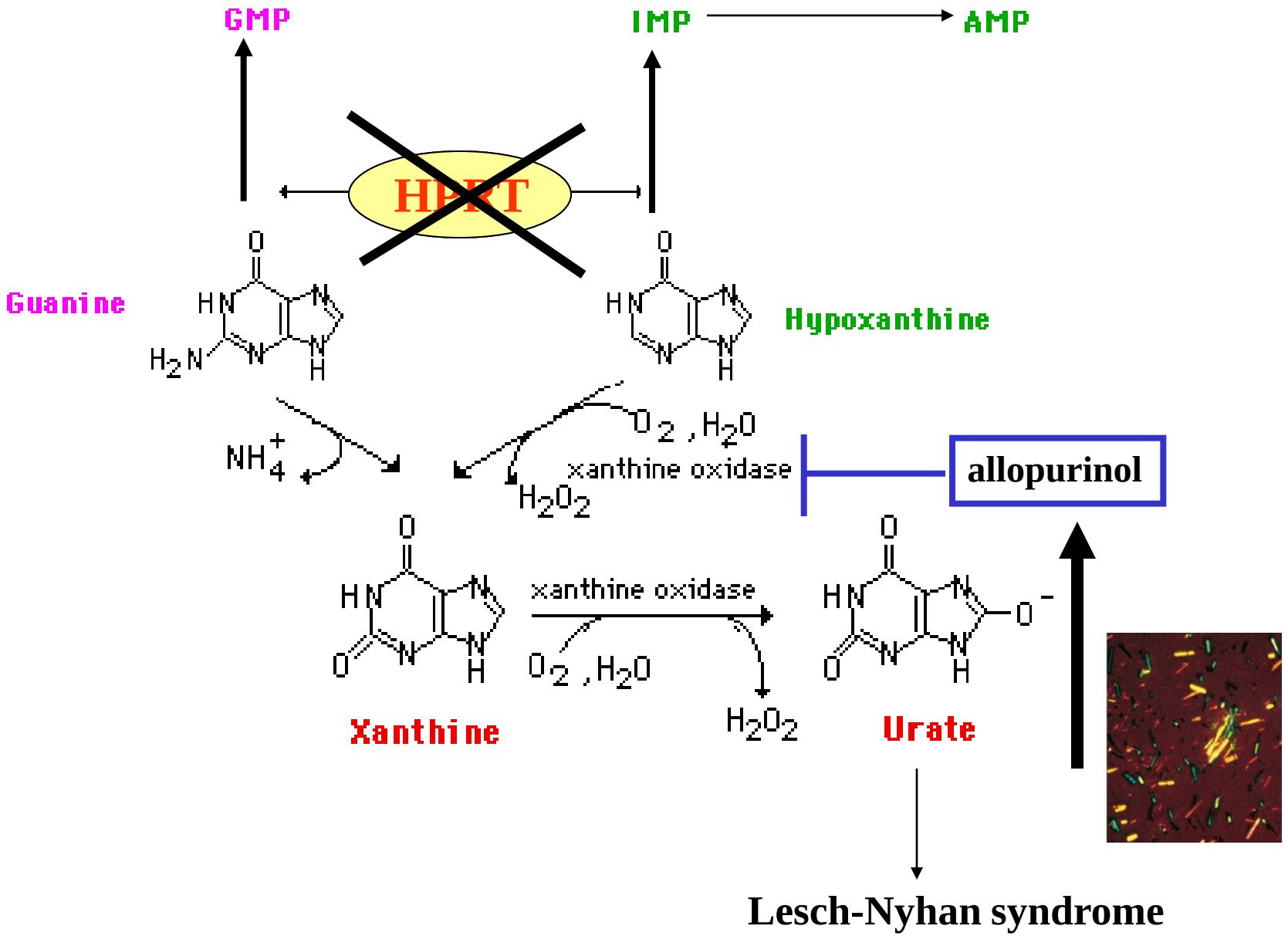
HAT medium

A selection medium for hybrid cell lines; contains **hypoxanthine; aminopterin; thymidine**. Only cell lines expressing both hypoxanthine phosphoribosyl transferase **(HPRT+)** and thymidine kinase **(TK+)** can survive in this medium. Aminopterin inhibits de novo synthesis of nucleosides, while HPRT and TK supply them from hypoxanthine and thymidine.



HAT medium is used to select hybridoma cells producing monoclonal antibodies

Inborn error of metabolism – deficiency of HPRT



What is Lesch-Nyhan Syndrome?

Lesch-Nyhan syndrome (LNS) is a rare, inherited disorder caused by a deficiency of the enzyme *hypoxanthine-guanine phosphoribosyltransferase* (HPRT). LNS is an X-linked recessive disease-- the gene is carried by the mother and passed on to her son. The lack of HPRT causes a build-up of uric acid in all body fluids, and leads to symptoms such as severe gout, poor muscle control, and moderate retardation, which appear in the first year of life. A striking feature of LNS is self-mutilating behaviors – characterized by lip and finger biting – that begin in the second year of life. Abnormally high uric acid levels can cause sodium urate crystals to form in the joints, kidneys, central nervous system, and other tissues of the body, leading to gout-like swelling in the joints and severe kidney problems. Neurological symptoms include facial grimacing, involuntary writhing, and repetitive movements of the arms and legs similar to those seen in Huntington's disease. Because a lack of HPRT causes the body to poorly utilize vitamin B12, some boys may develop a rare disorder called megaloblastic anemia.

Is there any treatment?

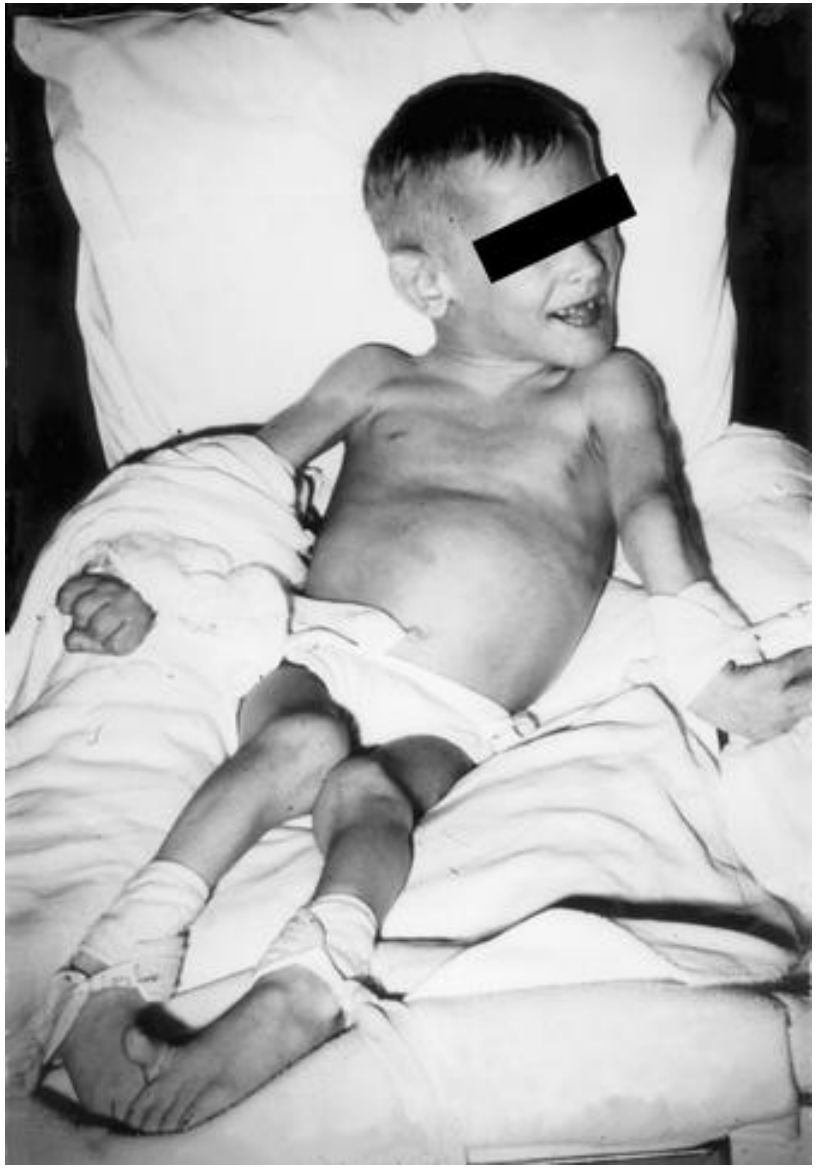
Treatment for LNS is symptomatic. Gout can be treated with allopurinol to control excessive amounts of uric acid. Kidney stones may be treated by breaking up kidney stones using shock waves or laser beams. There is no standard treatment for the neurological symptoms of LNS. Some may be relieved with the drugs carbidopa/levodopa, diazepam, phenobarbital, or haloperidol.

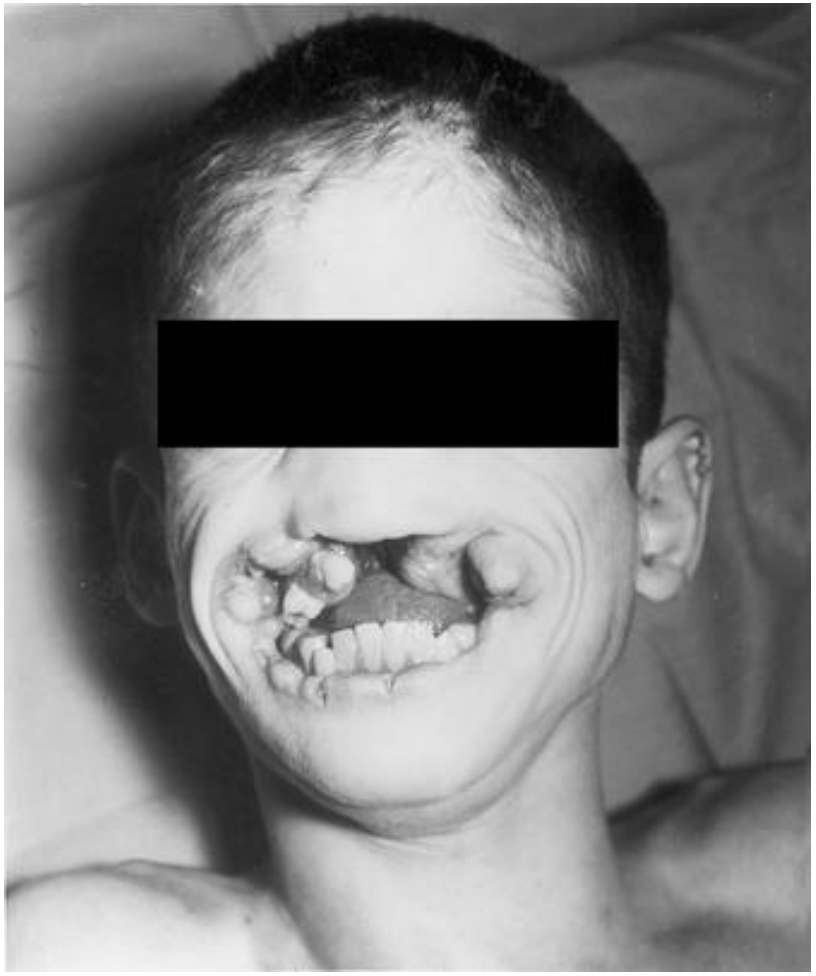
What is the prognosis?

The prognosis for individuals with LNS is poor. Death is usually due to renal failure in the first or second decade of life.

Children suffering from deficiency of HPRT-
Lesh-Nyhan syndrome

Children suffering from deficiency of HPRT- Lesh-Nyhan syndrome





Devlopment of gene therapy

- **Mechanisms of diseases: genes are known**

- **Tools: vectors**

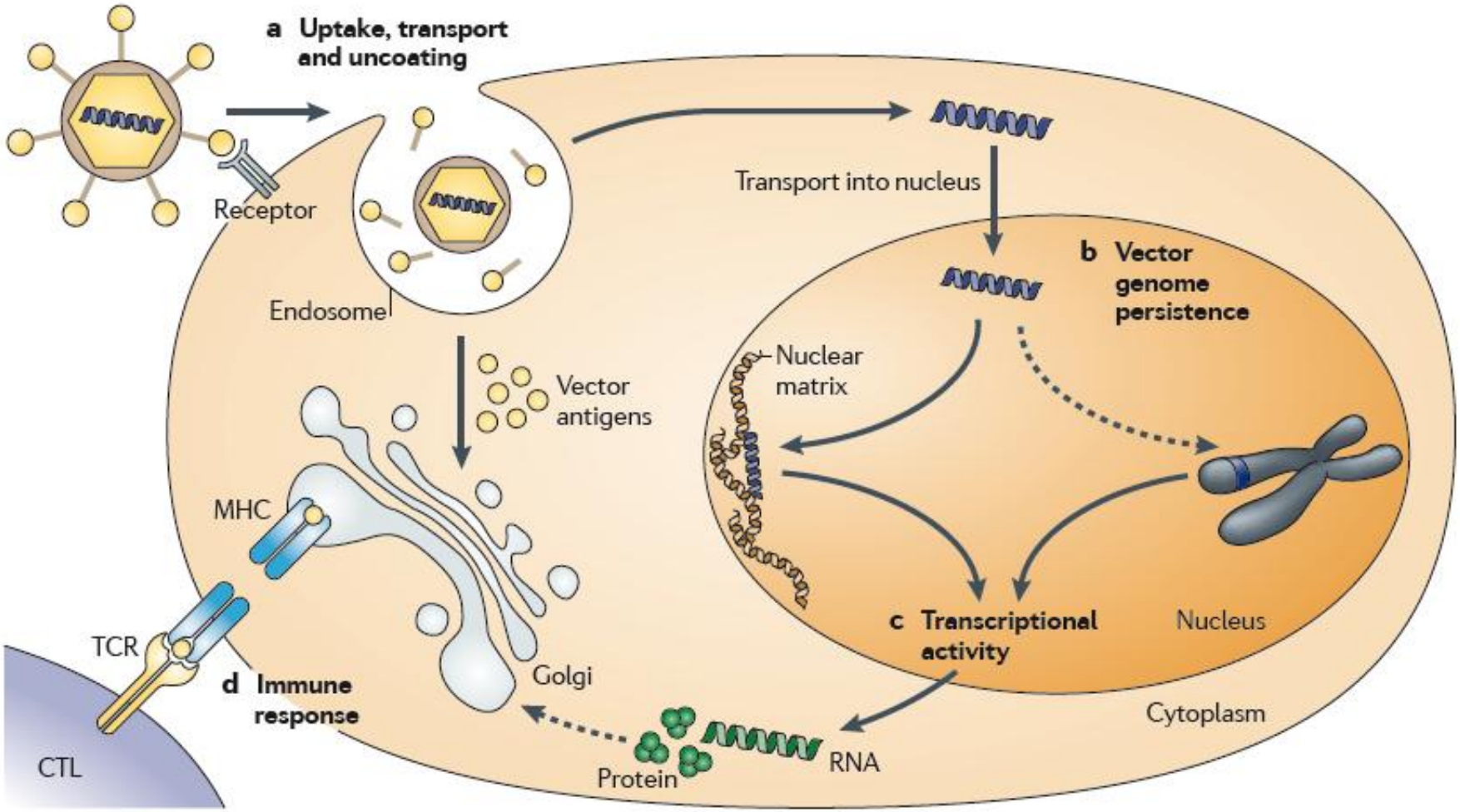
Main problems to solve in gene therapy

1. Efficient delivery of therapeutic gene

2. Safe delivery…

All is about vectors…

The fours barriers of successful gene therapy



Kay M, Nature Rev Genetics, 2011

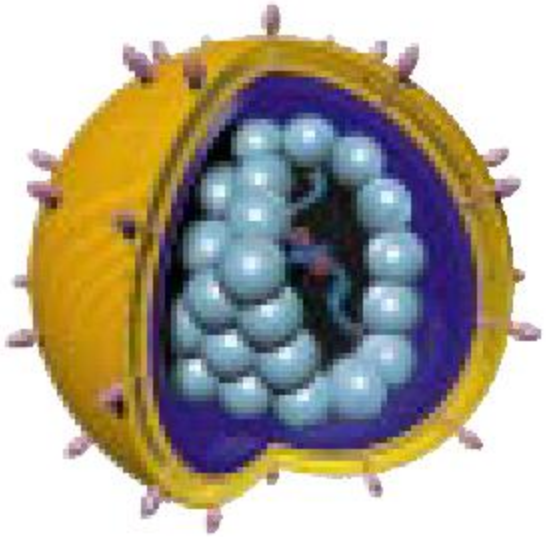
vector

nucleic acid, which is used to deliver the therapeutic gene/therapeutic nucleic acid

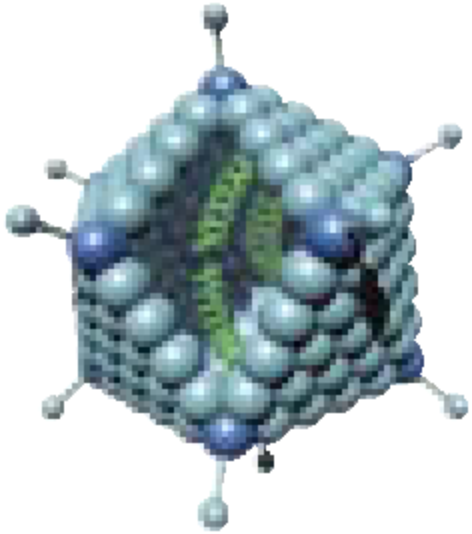
Vehicle

A chemical substance, which improves delivery of nucleic acid to the cells

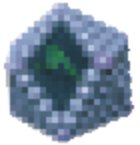
Genetic syringes - vectors



Retroviral vectors



Adenoviral vectors



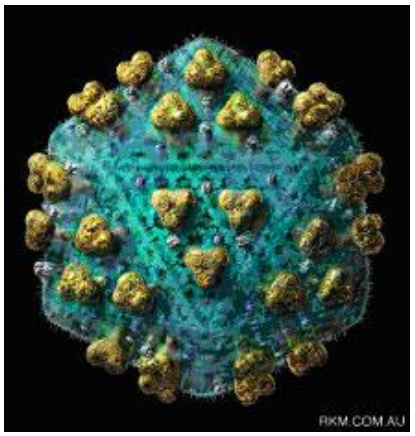
AAV vectors
(adeno-associated)

Plasmid DNA

Vectors

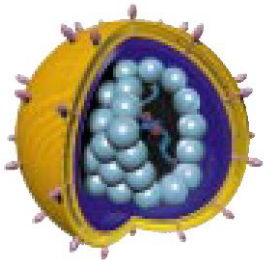
Carriers of the therapeutic nucleic acids

Retroviral expression system



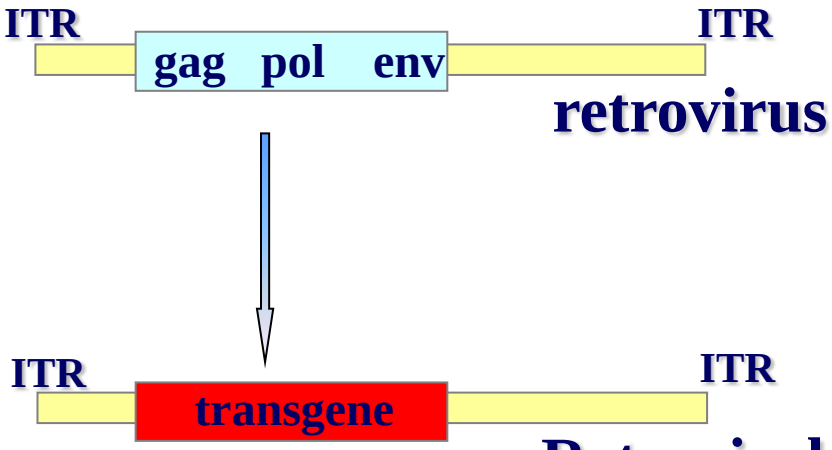
Gag – core proteins, matrix, nucleocapsid
Pol – reverse transcriptase and integrase
Env – envelope glycoproteins

Retroviral vectors

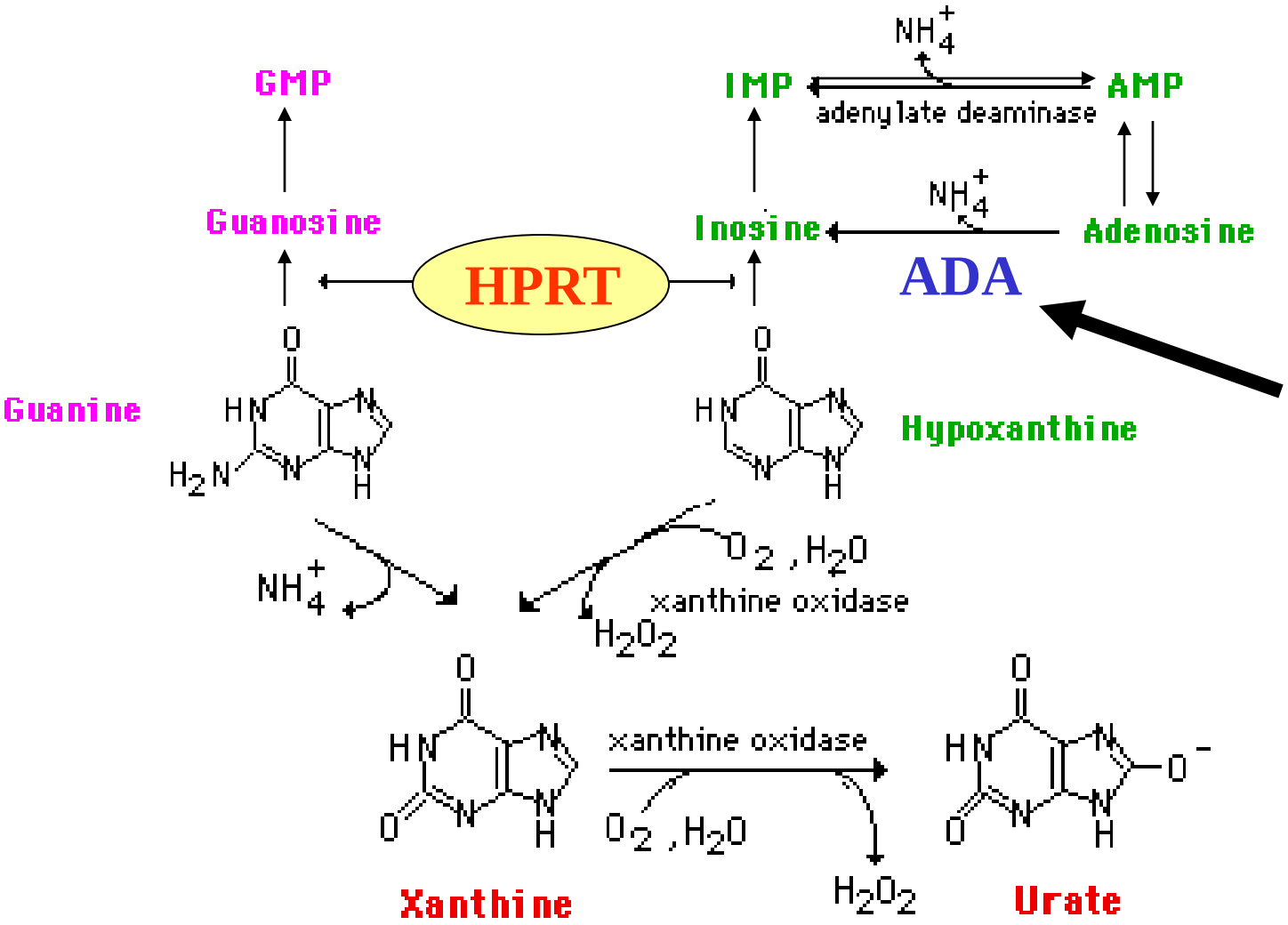


- *gag – structural proteins*
- *pol – reverse transcriptase*
- *env – envelope proteins*

- **long-term expression due to integration into cellular genome**

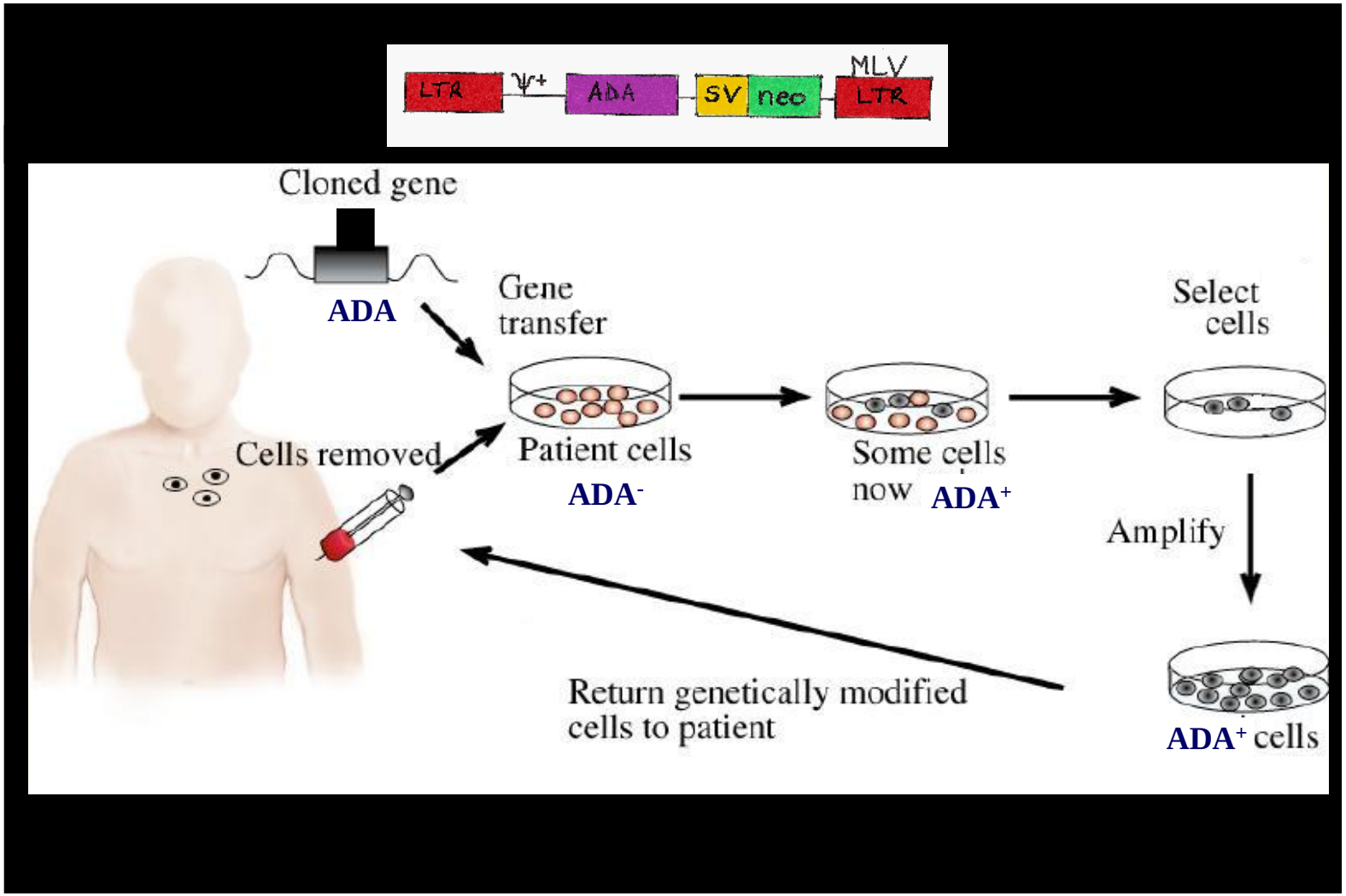


First controlled trial of gene therapy - 1991

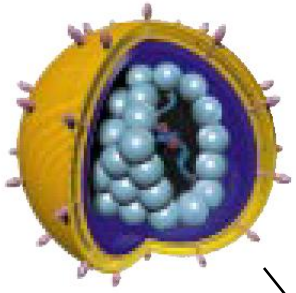


ADA deficiency– results in severe immunodeficiency syndrome

Gene therapy of ADA deficiency



First clinical trial of gene therapy - 1991



Retroviral vector containing correct ADA gene (cDNA) has been transduced into blood lymphocytes

Ashanti De Silva (patient)

This first clinical trial was not „pure" from the methodological point of view.

The patients have been treated concomitantly with enzyme injections – ADA-PEG.

Nevertheless, the marker transgene (neo) could be detected in the blood cells of the patients even more than 5 years after injection of modified cells.

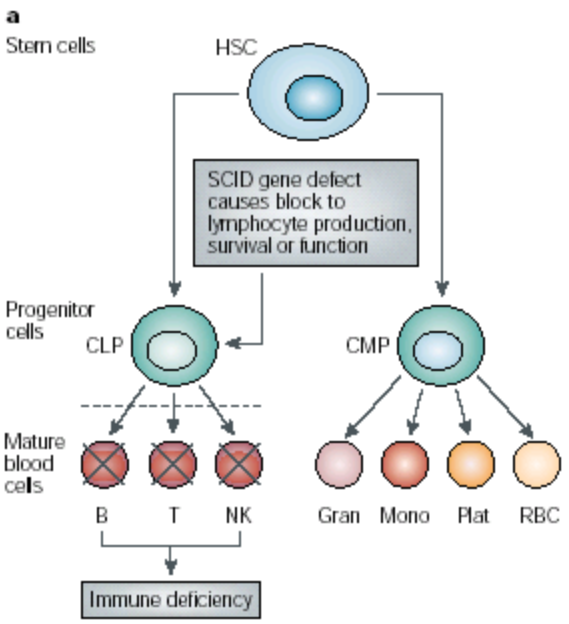
Succesful gene therapy

David Vetter - „Bubble Boy”

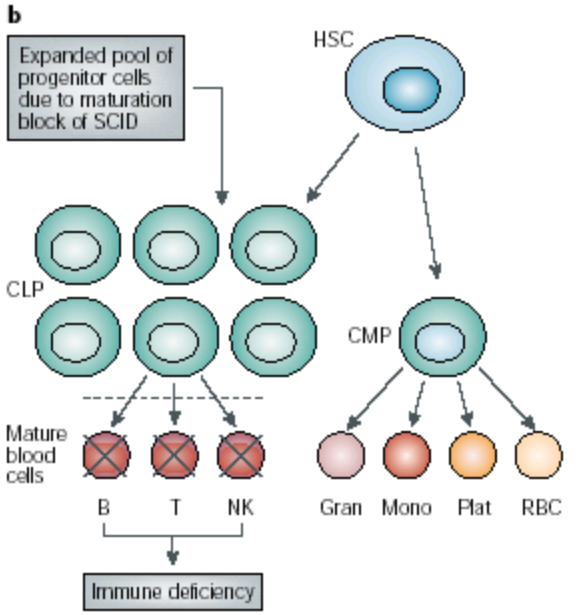
David has spent 12 years in a foil-protected environment. Finally has received the bone marrow transplantation from his sister, but unfortunately died due to Epstein-Barr virus infection

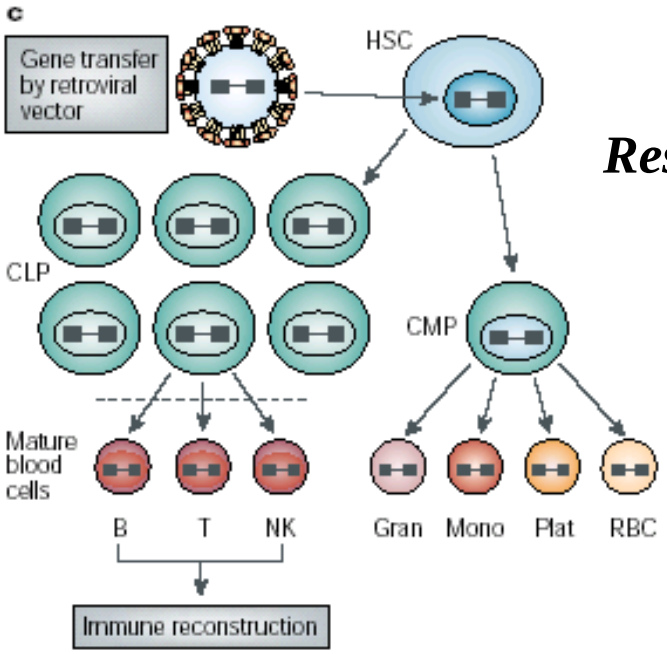
X-SCID deficiency

X-linked severe combined immunodeficiency (X- SCID)



Lack of γc gene

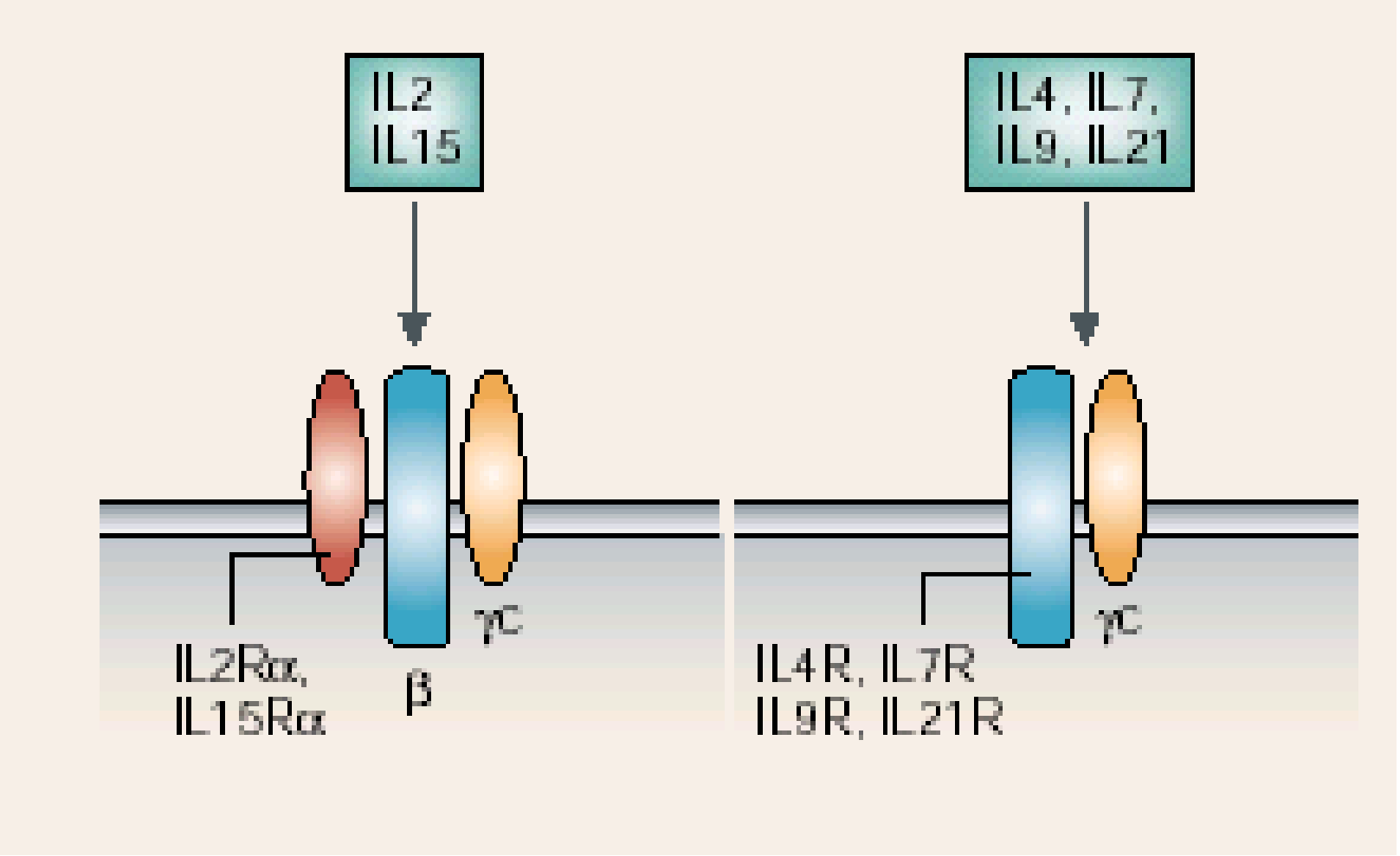




Restoration of B and T lymphocytes and NK cells

D. Kohn et al., Nature Rev Cancer July 2003

Cytokines receptors



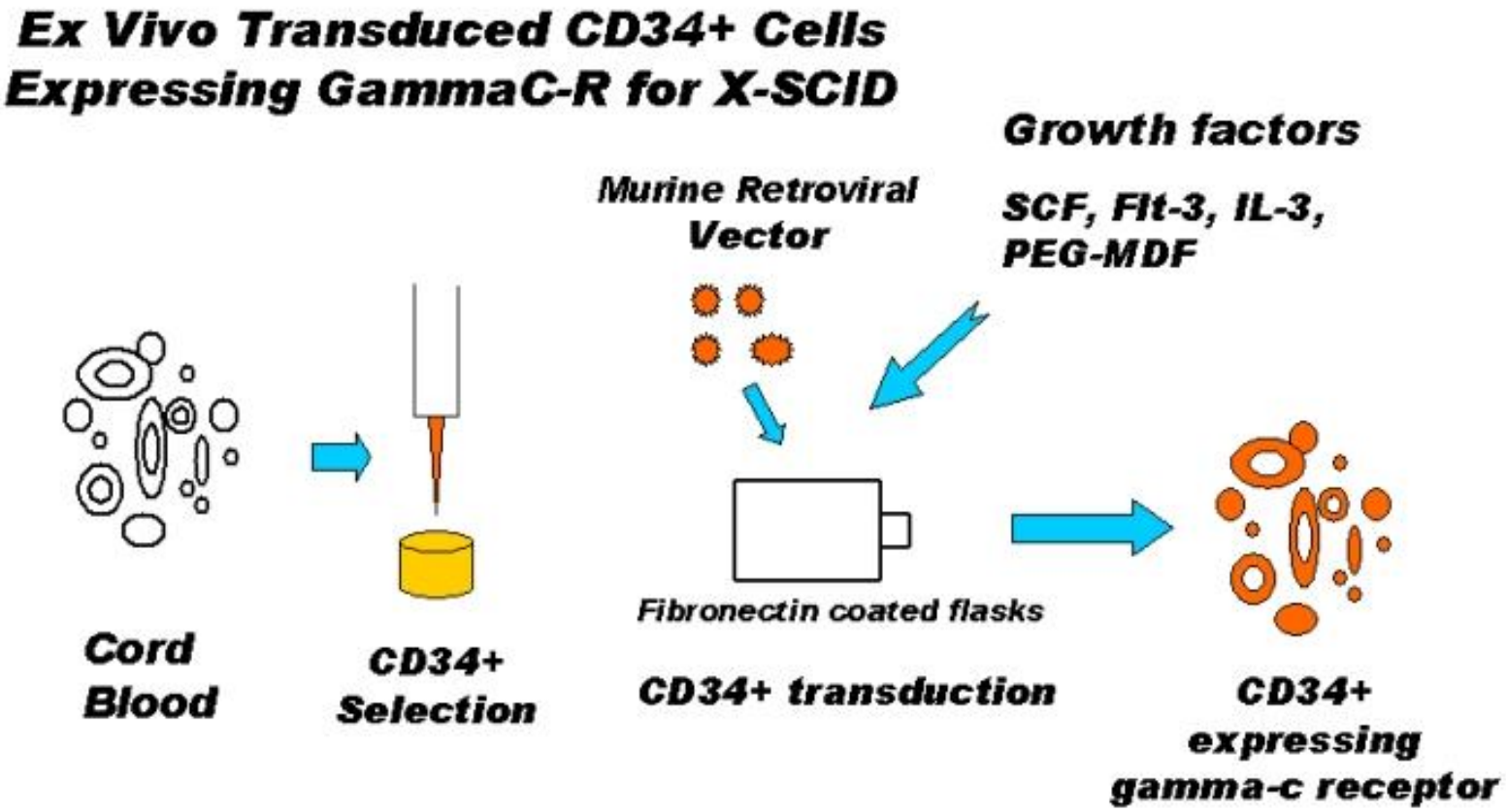
D. Kohn et al., Nature Rev Cancer July 2003

Cavazzana-Calvo M et al .

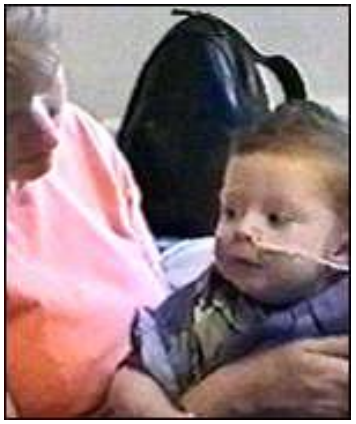
Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease

Science 2000: 28 April: 288: 669-672

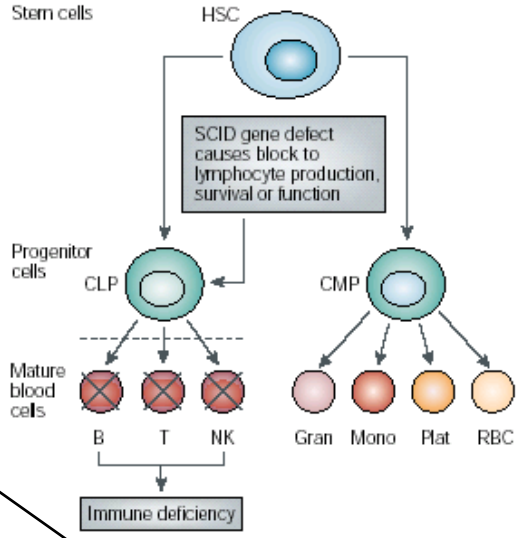
Gene therapy is efficient in treatment of X-SCID



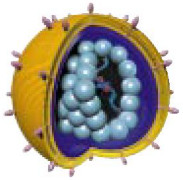
Gene therapy is efficient in treatment of X-SCID



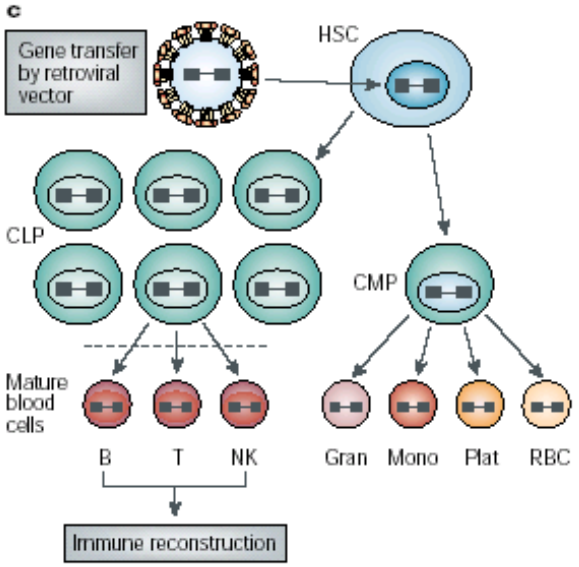
Stem cells without correct γc gene



Gene therapy



Retroviral vector with a correct γc gene



Combining stem cells and gene therapy

Future for treatment of some diseases?

Gene therapy is succesful in treatment of diseases

Some Gene Therapy Successes

Disorder	Disease type	Patients benefiting	First publication
X-SCID	Immunodeficiency	17/20	2000
ADA-SCID	Immunodeficiency	26/37	2002
Adrenoleukodystrophy	Neurologic	2/4*	2009
Leber's congenital amaurosis	Blindness	28/30	2008
Wiskott-Aldrich syndrome	Immunodeficiency	8/10	2010
β-thalassemia	Hemoglobinopathy	1/1	2010
Hemophilia	Coagulation	6/6	2011?

*Includes a patient treated too recently to see benefit

Science, 7th October 2011