Sandro Rusconi

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Geneva 30.09.03
AISTS
'genes & sport' workshop

1972-75	Primary school teacher (Locarno, Switzerland)
1975-79	Graduation in Biology UNI Zuerich, Switzerland
1979-82	PhD curriculum UNI Zuerich, molecular biology
1982-84	Research assistant UNI Zuerich
1984-86	Postdoc UCSF, K Yamamoto, (San Francisco)
1987-94	Group leader, UNI Zuerich (mol. bio., PD)
1994-today	Professor Biochemistry UNI Fribourg
1996-02	Director Swiss National Research Program 37 *'Somatic Gene Therapy'*
2001-today	Swiss Natl. Res. Program 50 *'Endocrine disruptors'*
2002-03	Sabbatical, Tufts Med. School Boston and Univ. Milano, Pharmacology Department
2002-05	President Union of Swiss Societies for Experimental Biology (USGEB)

Gene transfer: limits and potential as doping vehicle

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Schedule

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- Basic understanding of 'genes':
  what is a gene, how many genes, molecular biology dogma
  genetic diseases, environmental factors, ageing
- Essential concepts on 'molecular medicine' & molecular doping:
  applications and problems,
- Techniques of gene transfer (Gene Therapy)
  problems and solutions, vectors, clinical achievements
- Gene-based doping
  applications, comparison with other doping, detection
- Conclusions
  plausibility table

1 Gene -> 1 or more functions

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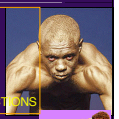
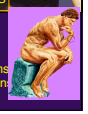
DNA → RNA (Transcription / translation) → Protein

Gene expression

GENE → 2-5 FUNCTIONS





100 '000 genes (50 '000 genes?) → >300 '000 functions (>150 '000 functions)

What is in fact a gene?: a segment of DNA acting as a regulated machine for RNA production

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DNA → RNA (Transcription / translation) → Protein

GENE → FUNCTION

RNA

DNA

spacer | regulatory | coding | spacer

1 Organism -> more than 10^5 genetically-controlled Functions

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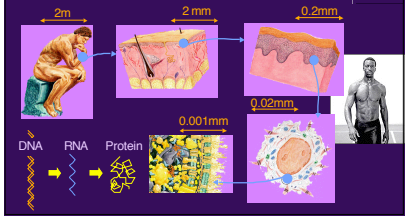

Reductionistic molecular biology paradigm (gene defects and gene transfer)

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DNA → Protein

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GENE	FUNCTION(s)
GENE OK	FUNCTION OK
GENE KO	FUNCTION KO
GENE transfer	FUNCTION transfer

Not only the genome determines the health status...

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genetics behaviour environment

Muscle distrophy
Familial Breast Cancer
Sporadic Breast Cancer
Lung Cancer
Obesity
Artherosclerosis
Alzheimer
Parkinson 's
Drug Abuse
Homosexuality

also acquired conditions may have a genetic component that modulates their healing
- trauma
- fractures
- burns
- infections

Gene amplification / manipulation techniques (genetic engineering, recombinant DNA) are simple

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segments of genomic DNA can be specifically cut and isolated

isolated s

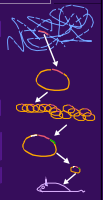
plasmid v

isolated re
the final desired molecule

Science-grade material
can be essentially prepared in your cellar

...not so clinical-grade material!

Final molecule is transferred into cells or organisms



The THREE missions of medicine, impact of molecular techniques

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Prevention +

Diagnosis +

+ Therapy

'Molecular Medicine'
Application of the know-how in molecular genetics to medicine

The FOUR eras of molecular medicine

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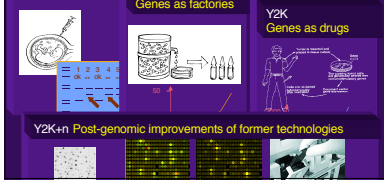
Eighties
Genes as probes

Nineties
Genes as factories

Y2K
Genes as drugs



Y2K+n Post-genomic improvements of former technologies

The major disease and medical challenge of the 21st century: Ageing

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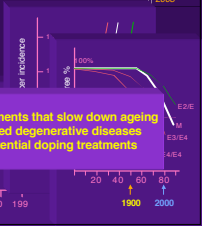

NB:
many treatments that slow down ageing or age-related degenerative diseases are also potential doping treatments

Now, let's talk about Somatic Gene Therapy (somatic gene transfer)

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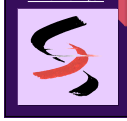
Definition of GT:
'Use genes as drugs':
Correcting disorders by somatic gene transfer

Chronic treatment
Acute treatment
Preventive treatment

NFP37 somatic gene therapy
www.unifr.ch/nfp37



Hereditary disorders
Acquired disorders

Loss-of-function
Gain-of-function

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Why 'somatic'?

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- Germ Line Cells: the cells (and their precursors) that upon fertilisation can give rise to a descendant organism

Ergo:
somatic gene transfer is a post-natal treatment aiming at somatic cells and consequently does not lead to a hereditary transmission of the genetic alteration
--> is NOT a GENETIC SELECTION!

- Somatic Cells: all the other cells of the body

Somatic gene therapy's (gene transfer) four fundamental questions

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Efficiency of gene transfer

Specificity of gene transfer

Persistence of gene transfer

Toxicity of gene transfer

Remember!

Efficiency

Specificity

Persistence

Toxicity

Pharmacological considerations for DNA transfer

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Classical Drugs	Protein Drugs	Nucleic Acids
• Mw 50- 500 Daltons • Synthetically prepared • Rapid diffusion/action • Oral delivery possible • Cellular delivery: - act at cell surface - permeate cell membrane - imported through channel • Can be delivered as soluble molecules Angstromism size • rapidly reversible treatment	• Mw 20 '000- 100 '000 Da • Biologically prepared • Slower diffusion/action • Oral delivery not possible • Cellular delivery: - act extracellularly • Can be delivered as soluble molecules nm size • rapidly reversible treatment	• Mw N x 1'000'000 Da • Biologically prepared • Slow diffusion • Oral delivery inconceivable • Cellular delivery: - no membrane translocation - no nuclear translocation - no biological import • Must be delivered as complex carrier particles 50-200 nm size • slowly or not reversible

Therapy with nucleic acids
- requires particulated formulation
- is much more complex than previous drug deliveries
- has a different degree of reversibility (dosage problem)

THREE classes of physiological gene delivery

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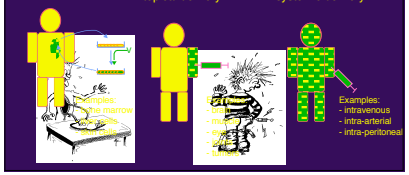
Ex-vivo

In-vivo topical delivery

In-vivo systemic delivery



Examples:
- intravenous
- intra-arterial
- intra-peritoneal

TWO classes of gene transfer vehicles: non-viral & viral

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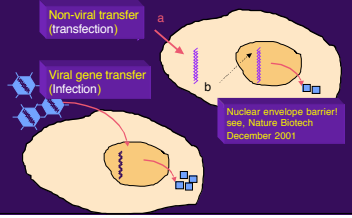
Non-viral transfer (transfection)

Viral gene transfer (Infection)



Nuclear envelope barrier!
see, Nature Biotech December 2001

Transfection with recombinant DNA Vs Infection with recombinant viruses

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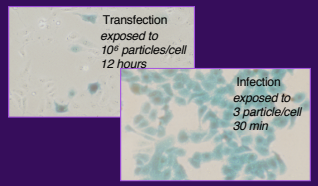

List of popular vectors/methods

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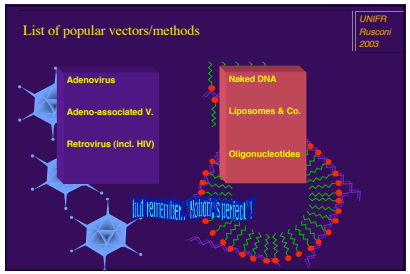

Recap: current limitations of popular gene transfer vectors

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Adenovirus
- no persistence
- limited packaging
- toxicity
- immunogenicity

Retrovirus (incl. HIV)
- limited package
- random insertion
- unstable genome

General
- antibody response
- limited packaging
- gene silencing

Solutions:
- synthetic viruses ("Virosomes")

Biolistic bombardment or local direct injection
- limited area

Electroporation
- limited organ access

Liposomes, gene correction & Co.
- very inefficient transfer

General
- low transfer efficiency
- 1/10`000 of viruses' in vivo

Solutions:
- improved liposomes
- with viral properties ("Virosomes")

Gene Therapy in the clinic: Trials Worldwide

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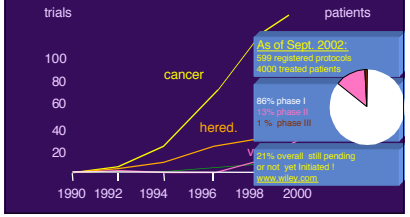

Gene Therapy Milestones

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1990, 1993, 2000 // ADA deficiency
1997, 2000, Critical limb ischemia
1998, Restenosis
1999, Crigler Njiar (animal)
2000, Hemophilia
2000, SCID
A Fischer, Science April 2000
2000, correction Apo E4 (animal model)
G. Dickson, ESGT congress, 7.10.2000 Stockho
2000, correction Parkinson (animal model)
2001, ONYX oncolytic Viruses
D Kirn (Gene Ther 8, p 89-98)

Kirn, 2001

Clinical trials with ONYX-015, what we learned?

(Review)

The most feared potential side-effects of gene transfer

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- Immune response to vector
- immune response to new or foreign gene product
- General toxicity of viral vectors
- Adventitious contaminants in recombinant viruses
- Random integration in genome
  -> insertional mutagenesis (-> cancer risk)
- side effects of newly acquired gene product
- Contamination of germ line cells

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Ergo
- many effects are due to 'primitiveness' of the today's protocols
- for the moment side effects would (should) ethically limit GT to serious diseases without valid alternatives

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Four bitter lessons, but only one treatment-related death so far

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NY May 5, 1995, R. Crystal:
in a trial with adenovirus mediated gene transfer to treat cystic fibrosis (lung) one patient developed a mild pneumonia-like condition and recovered in two weeks. The trial interrupted and many others on hold.

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

Paris, Oct 2, 2002, A Fischer:
in a trial with retrovirus mediated gene transfer to treat SCID (bone marrow) one patient developed a leukemia-like condition. The trial has been suspended to clarify the issue of insertional mutagenesis. Similar trials in US and Germany have been put on hold.

Paris, Jan 14, 2003, A Fischer:
a second patient of the cohort of 9 comes up with a similar leukemia than the one reported in october 2002. 30 trials in USA are temporarily suspended

!! Most Recent Paris' Trial News
www.unifr.ch/nfp37/adverse.html

Ups and Downs and current status of Gene Therapy: a true roller coaster ride!

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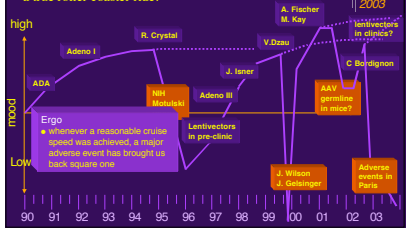

The THREE levels of doping

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Before the competition (anabolic enhancers) +

During the competition (performance enhancers) +

After the competition (repair enhancers) +

Molecular treatments
Application of the know-how in molecular genetics to doping

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Which gene transfer approaches would be compatible with doping strategies

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- *ex vivo*, hematopoietic tissue: pro hematopoietic (Epo receptor, oxygen transport...)
- *in vivo* local (example muscle): metabolic enhancers, growth factors, muscular fiber changers, cardio-modulators (glucose/oxygen, MGF, anti-myostatin,...)
- *in vivo* local (example joints): pain reducers, inflammation inhibitors, recovery and repair factors (anti-TNF, BMPs, ...)
- *in vivo* systemic: anabolic enhancers, endocrine factors, pain killers, vascular controllers, (hormone metabolising enzymes, proenkephalins, ...)

Which would be the objective current limitations in gene-based doping strategies

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Viral gene transfer
- immune problems
- limited readministration possibilities
- general toxicity, genotoxicity

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Nonviral gene transfer
- generally inefficient
- lack of persistence, requires readministration

Strategy-independent problems
- laborious, not readily available
- long term gene expression difficult to control
- irreversible effects or permanent tagging

Which side effects could be feared in gene-based doping strategies

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Short -mid term
- Autoimmunity
- Hyperimmunity
- Toxic shock

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Long term
- Fibrosis
- Cancer
- conventional side- effects of administered factors
- Inaccessibility to future gene therapy interventions (immunity

Intrinsic to reckless application (probably the biggest danger)
- malpractice (unsuitable vector/administration route)
- non-clinical grade material (pathogens or allergens)
- lack of follow-up

Putative detection methods for gene-transfer-based doping strategies and their linked problems

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- Antibody detection (viral antigens)
- r-nucleic acids detection (PCR)
- recombinant protein / post-translational modification detection (MALDI-TOF)

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- Anatomically difficult to detect (if locally administered) -> but leaves permanent genetic marking

- Detection of nucleic acids cannot be performed in body fluids (except in early phase after systemic administration) -> might require specific tissue biopsy

Final side-by-side comparison: gene-based doping versus drug- or protein-based doping

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Category	Drug/protein	Gene-based

Ergo:
The odds would speak currently rather against the adoption of gene-based doping,

but:
this applies to common-sense clinical practice, and this aspect is not guaranteed in the doping field

and:
... there are several sporting disciplines where doping is not rigourously (or not at all) verified.

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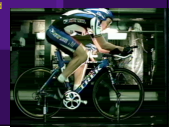
Somatic gene transfer: conclusions

- somatic gene transfer has been originally developed for the treatment of diseases (genetical or acquired)
- must be distinguished from genetic selection
- has the potential to be applied for pre- during- and post-performance enhancement
- currently still experimental and not technically mature for applications in non-lethal conditions
- has already raised the interest of doping field
- major risk linked with premature application
- single gene transfer for enhancement will create more problems than it could solve



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...Thanks, and let's hope that fair sports will continue to rise genuine emotions: yesterday, today and tomorrow!

AISTS, MSA program

My collaborators at UNIFR

Swiss National Research Foundation

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if you are too shy to ask
send an e-mail to:
sandro.rusconi@unifr.ch
or visit:
www.unifr.ch/nfp37

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