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International Centre for Genetic Engineering and Biotechnology (ICGEB)
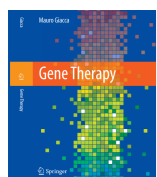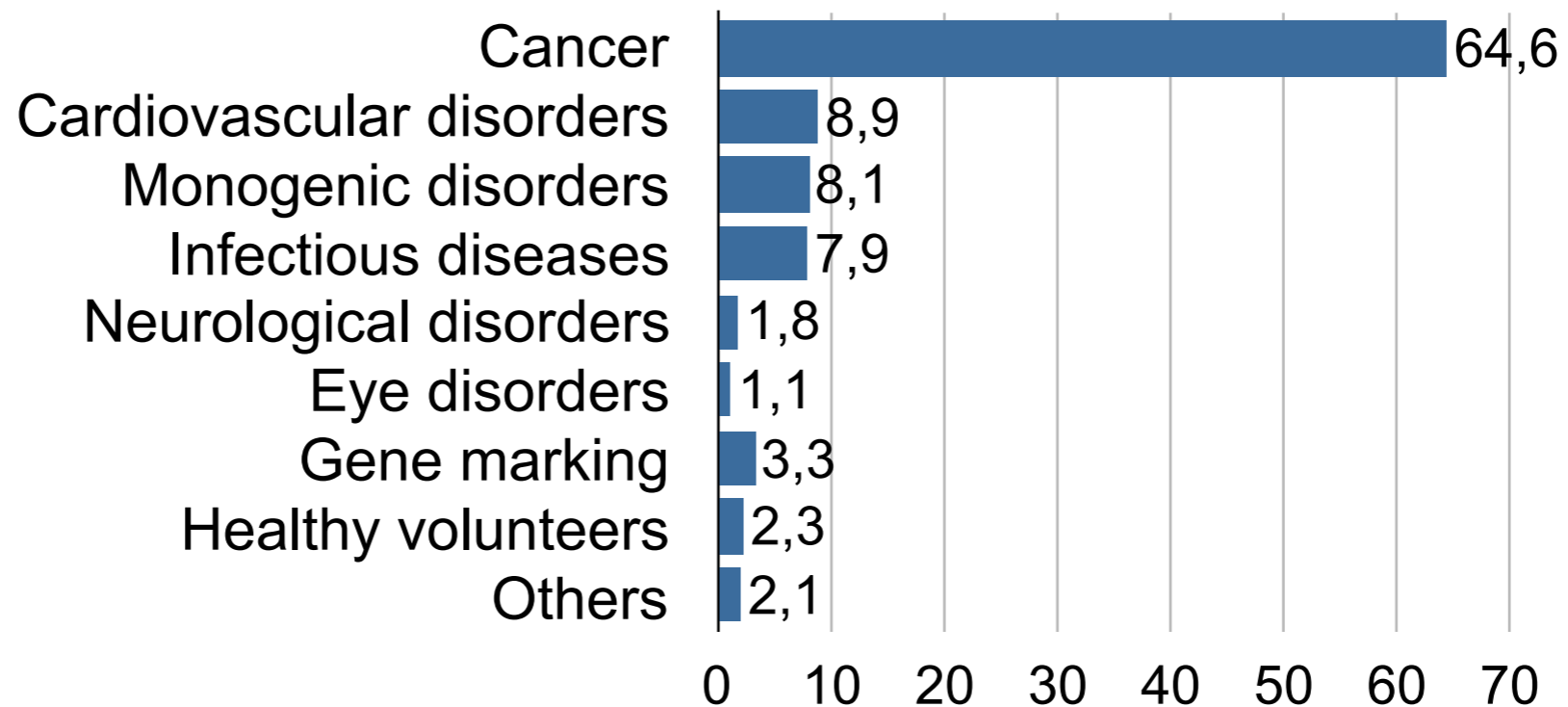
Trieste

Adriatic sea

Gene therapy clinical trials

Clinical trials by phase (%)

>1600 trials

Phase	%
I or I/II	79,2
II	16,5
II/III	0,8
III	3,4
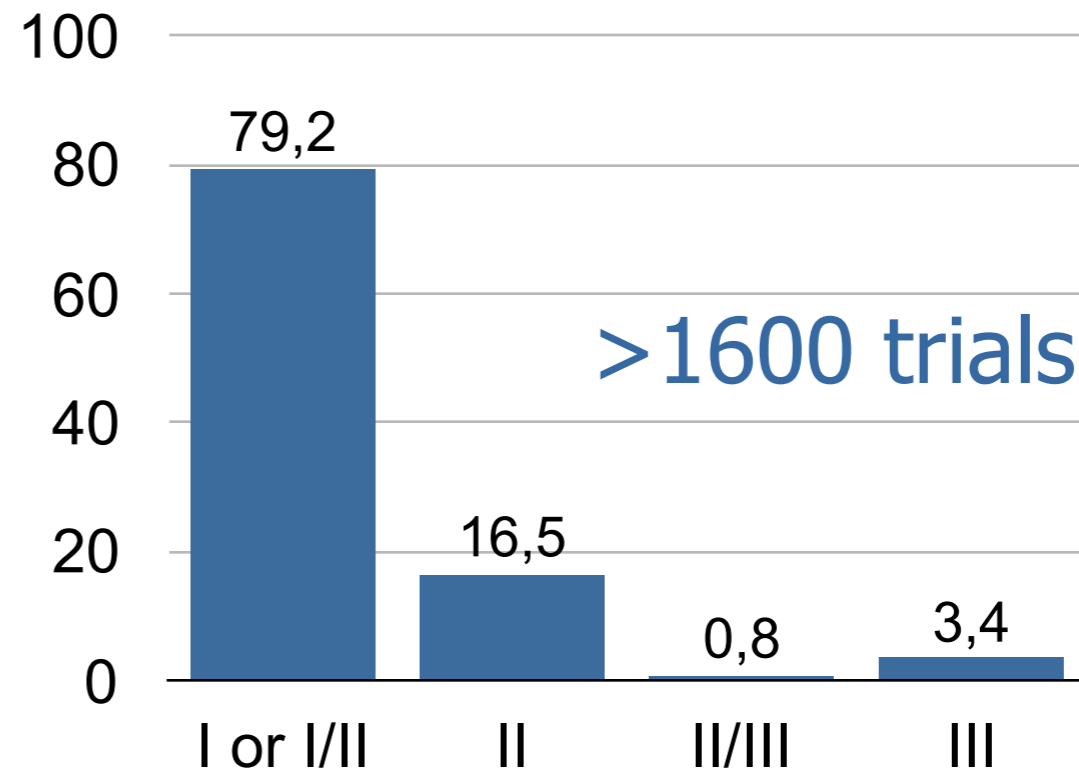
Clinical trials by disease (%)

Disease	%
Cancer	64,6
Cardiovascular disorders	8,9
Monogenic disorders	8,1
Infectious diseases	7,9
Neurological disorders	1,8
Eye disorders	1,1
Gene marking	3,3
Healthy volunteers	2,3
Others	2,1

Giacca, M. 2010. Gene Therapy. Springer

Gene Therapy

Genetic modification of human somatic cells via transfer of nucleic acids

European Guidelines for the Production of Gene Therapeutics, 1994

ICGEB

Therapeutic nucleic acids

Protein-coding cDNAs

Proteins replacing missing cellular functions

Burden of genetic disease

Disorder type	Population %
Single gene	2
Congenital abnormalities	3
Chromosomal abnormalities	0.5
maternal age >35	4
Behavioral & CNS	10
Adult onset multifactorial	60

Gene therapy of monogenic inherited disorders

e.g.
Immunodeficiencies (ADA, SCID-X1)
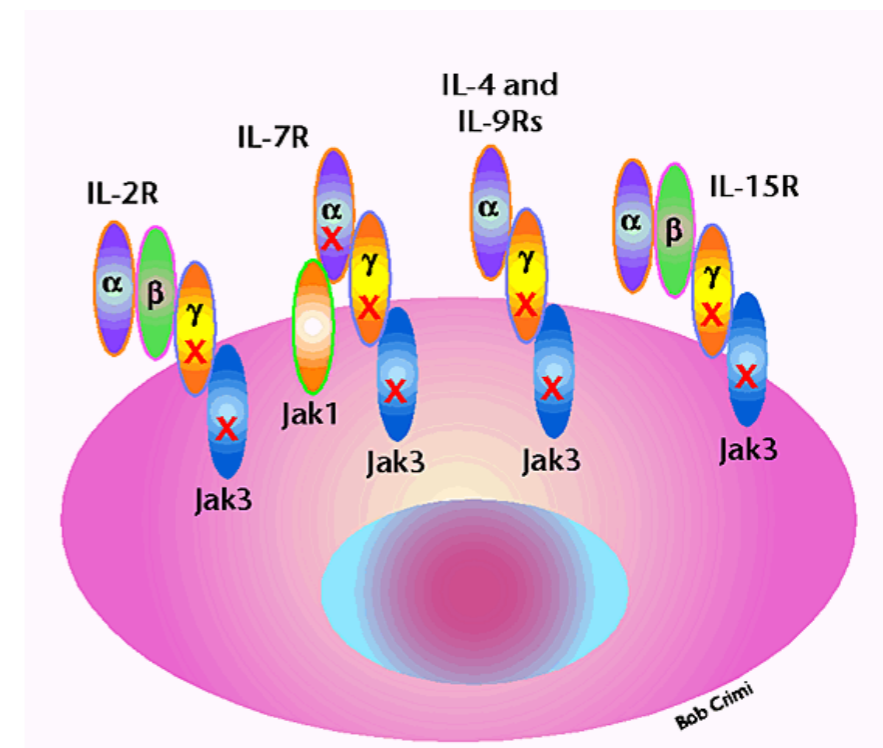Hemophilia
Leber's congenital amaurosis
Muscular dystrophy
Cystic fibrosis
Lysosomal storage disease
.....
several others

Therapeutic nucleic acids

Protein-coding cDNAs

Proteins replacing missing cellular functions
Proteins modulating cellular functions
Proteins regulating cell survival

Gene therapy for neurodegenerative or traumatic disorders

Clinical trials of growth factors for neurological disease

Ref	Disease	Growth factor
45–47	Amyotrophic lateral sclerosis	CNTF, BDNF, CNTF + BDNF, GDNF, IGF-1
48	Spinal muscular atrophy	BDNF
49	Alzheimer's disease	NGF
50–53	Peripheral neuropathy	NGF, BDNF, NT-3
54	Stroke	FGF-2

CNTF=ciliary neurotropic factor; BDNF=brain-derived neurotropic factor; GDNF=glial cell line-derived neurotropic factor; IGF-1=insulin-like growth factor 1; NGF= nerve growth factor; NT-3=neurotropin 3; FGF-2=fibroblast growth factor 2.

Therapeutic nucleic acids

Protein-coding cDNAs

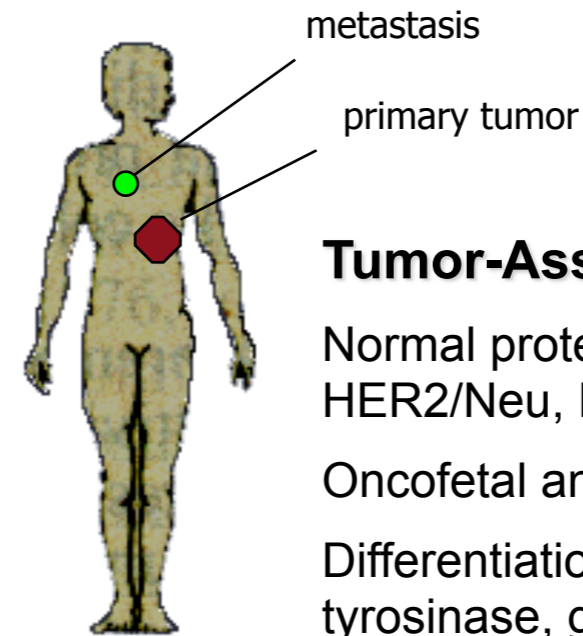Proteins replacing missing cellular functions

Proteins modulating cellular functions

Proteins regulating cell survival

Proteins activating the immune system

Antibodies and intracellular antibodies

Anti-tumor vaccination

metastasis

primary tumor

Tumor-Associated Antigens (TAA)

Normal proteins overexpressed (PSA, HER2/Neu, MUC-1

Oncofetal antigens (CEA, AFP)

Differentiation antigens (Melan A/MART-1, tyrosinase, gp100)

Cancer-testis antigens (members of the MAGE, BAGE, GAGE, NY1-ESO-1 families)

Tumor-Specific Antigens (TSA)

Antibody or TCR idiotypes

Mutated cellular proteins (eg. p53, p21)

Viral poteins (HPV E6 and E7, EBV EBNA-1)

Therapeutic nucleic acids

Protein-coding cDNAs

Proteins replacing missing cellular functions

Proteins modulating cellular functions

Proteins regulating cell survival

Proteins activating the immune system

Antibodies and intracellular antibodies

Small, non-coding DNAs and RNAs

Oligonucleotides and modified oligonucleotides

   Phosphorothioate oligonucleotides

   Oligonucleotides modified in 2' ribose

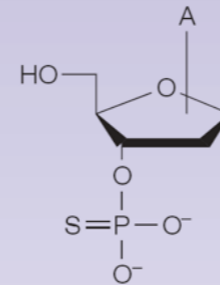   Locked Nucleic Acids (LNA) and Ethylene Bridged Nucleic Acids (ENA)

   Morpholino (PMO)

   Peptide Nucleic Acids (PNA)

a Antisense 1st generation chemistry	b Antisense 2nd generation chemistries	c Antisense 3rd generation chemistries
• Nuclease resistant	• Greater potency due to enhanced affinity for RNA	• Further improvement in potency?
• Acceptable tissue distribution	• Increased stability	• Improved tissue distribution?
• RNase H activity	• Decreased toxicity	• Further reductions in toxicity?
		• Oral bioavailability?
Phosphorothioate oligodeoxynucleotide	2-O-methoxyethyl (MOE)	Morpholina
	2-O-methyl	Peptide nucleic acid (PNA)
	Locked nucleic acid (LNA)	
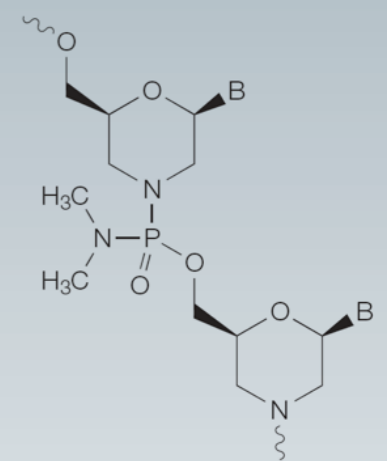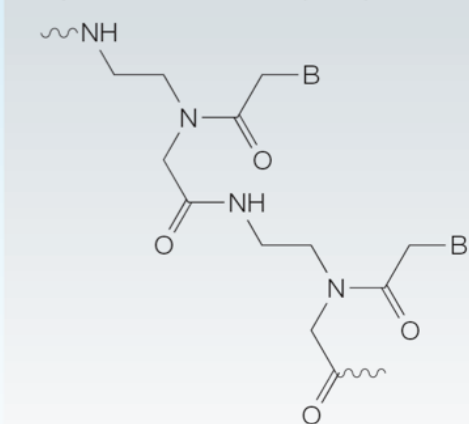
Chemical modifications to modify in vivo pharmacokinetics of oligonucleotides

Gleave et al. Nat. Rev. Cancer 2005

Therapeutic nucleic acids

Protein-coding cDNAs

Proteins replacing missing cellular functions
Proteins modulating cellular functions
Proteins regulating cell survival
Proteins activating the immune system
Antibodies and intracellular antibodies

Small, non-coding DNAs and RNAs

Oligonucleotides and modified oligonucleotides
    Phosphorothioate oligonucleotides
    Oligonucleotides modified in 2' ribose
    Locked Nucleic Acids (LNA) and Ethylene
    Bridged Nucleic Acids (ENA)
    Morpholino (PMO)
    Peptide Nucleic Acids (PNA)

Cancer gene therapy clinical trials using oligonucleotides

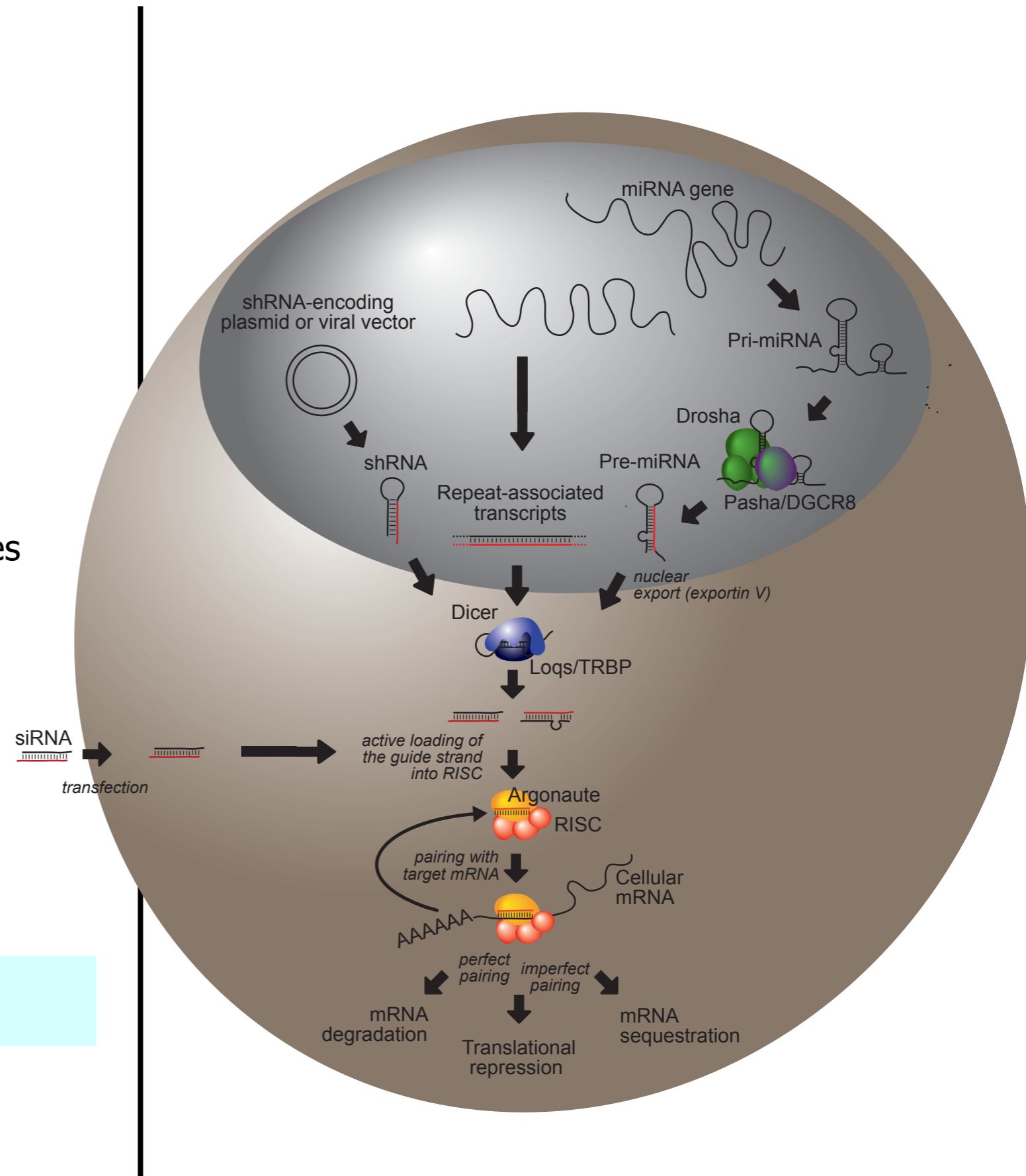| ODN	Chemistry	Target gene	Gene function
G3139 (Oblimersen)	Phosforothioate	Bcl2	Apoptosis inhibitor
OGX-011	Phosforothioate, 2'-methoxyehtyl	Clusterin	Protein chaperon
ISIS 3621	Phosforothioate	Protein kinase C alpha	Signal transduction
LY2181308	Phosforothioate, 2'-methoxyehtyl	Survivin	Apoptosis inhibitor
LR3001	Phosforothioate, 2'-methoxyehtyl	Myb	Oncogene, transcription factor
AEG35156	Phosforothioate, 2'-methoxyehtyl	XIAP	Apoptosis inhibitor
OGX-427	Phosforothioate, 2'-methoxyehtyl	Hsp27	Heat shock protein
ISIS 345794	Phosforothioate, 2'-methoxyehtyl	STAT-3	Transcriptional activator

Therapeutic nucleic acids

Protein-coding cDNAs

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Proteins modulating cellular functions
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Antibodies and intracellular antibodies

Small, non-coding DNAs and RNAs

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    Peptide Nucleic Acids (PNA)
Catalytic RNAs and DNAs (ribozymes and DNAzymes)

Small catalytic RNAs

A Hammerhead ribozyme

B Hairpin ribozyme

C HDV pseudoknot ribozyme

Therapeutic nucleic acids

Protein-coding cDNAs

Proteins replacing missing cellular functions

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Small, non-coding DNAs and RNAs

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Catalytic RNAs and DNAs (ribozymes and DNAzymes)

Small regulatory RNAs (siRNAs, shRNAs, microRNAs)

Therapeutic nucleic acids

Protein-coding cDNAs

Proteins replacing missing cellular functions

Proteins modulating cellular functions

Proteins regulating cell survival

Proteins activating the immune system

Antibodies and intracellular antibodies

Small, non-coding DNAs and RNAs

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    Morpholino (PMO)

    Peptide Nucleic Acids (PNA)

Catalytic RNAs and DNAs (ribozymes and DNAzymes)

Small regulatory RNAs (siRNAs, shRNAs, microRNAs)

Therapeutic approaches using siRNAs

Condition	Disease	Target gene
Hereditary and multifactorial disorders	Familial hypercholesterolemia (FH)	Apolipoprotein B
	Age-related macular degeneration (AMD)	VEGF, VEGFR1, RTP801
	Lateral amyotrophic sclerosis (LAS)	SOD1
	Spinocerebellar ataxia	Ataxin 1
	Alzheimer's disease	Tau, APP
	Parkinson's disease	-sinucleina
Cancer	Several cancers	Bcl-2
	Acute myeloid leukemia (AML)	AML1/MTG8
	Chronic myelogenous leukemia (CML)	BCR-Abl
	Glioblastoma	MMP-9, uPAR
Infectious disorders	Hepatitis B	HBsAg
	Hepatitis C	NS3, NS5B, E2
	Influenza	Nucleoprotein, polymerase
	HIV-1	Different viral genes
	HSV-1	Glicoprotein E
	RSV	Genes P, N and L

Therapeutic nucleic acids

Protein-coding cDNAs

Proteins replacing missing cellular functions

Proteins modulating cellular functions

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Catalytic RNAs and DNAs (ribozymes and DNAzymes)

Small regulatory RNAs (siRNAs, shRNAs, microRNAs)

DNA and RNA decoys

Aptamers

Age-related macular degeneration (AMD)

Most frequent cause of blindness >30% of people +75 y
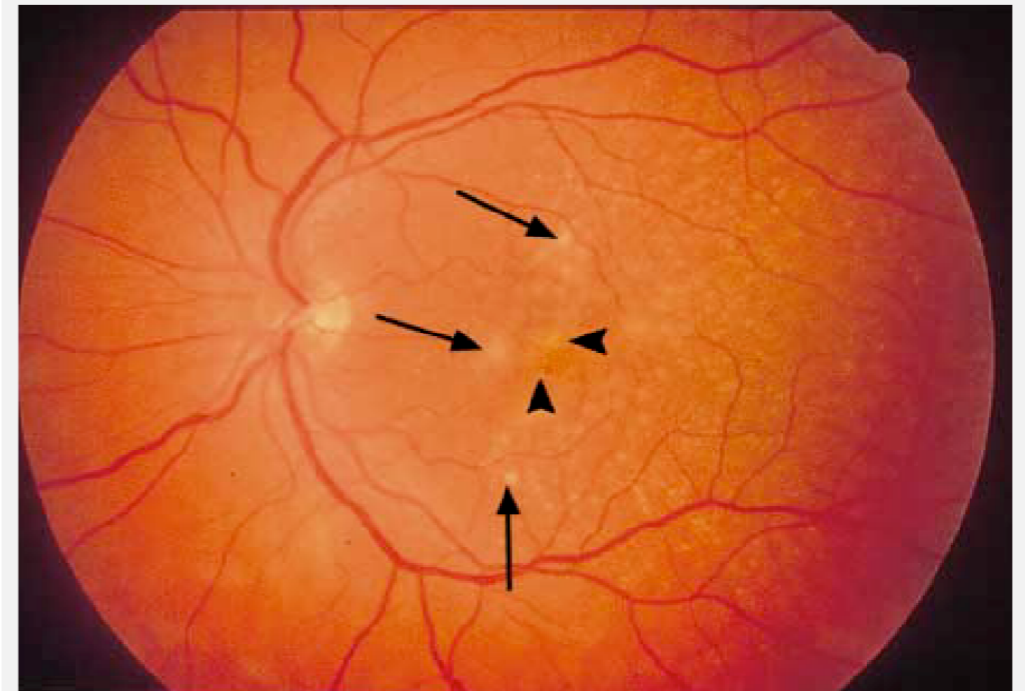
Figure 2. Early Age-Related Macular Degeneration, Characterized by Large Drusen (Arrows) and Clumps of Pigment (Arrowheads) in the Macula.

This eye has normal visual acuity but is at risk for late age-related macular degeneration and loss of vision.

Gene therapy of AMD

Anti-VEGF antibodies (bevacizumab, ranibizumab)

Soluble VEGFR (VEGF Trap-Eye)

Anti-VEGF aptamer (pegaptanib)

Anti-VEGF siRNA (bevasiranib)

In vivo gene therapy
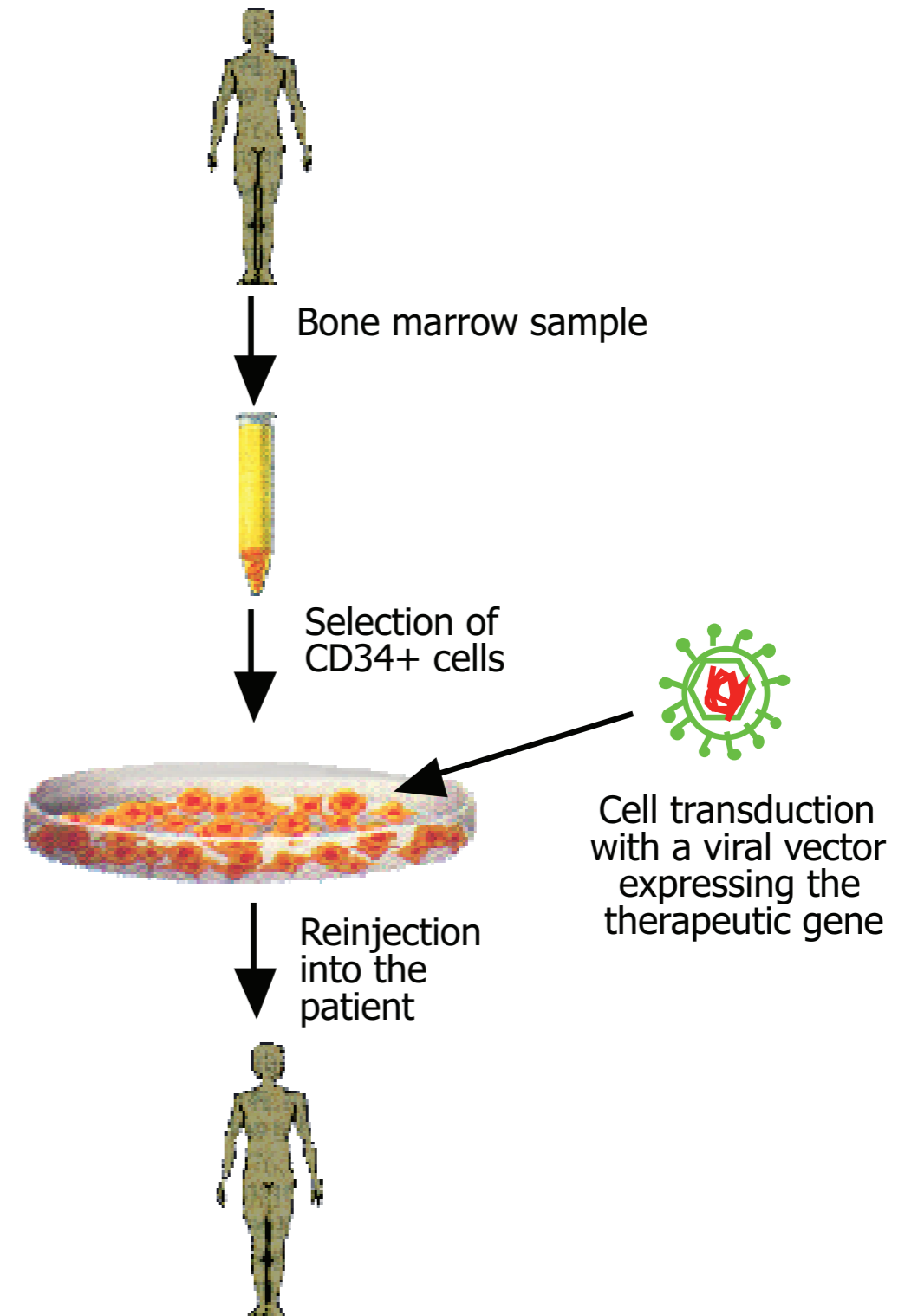
Prevention of restenosis after angioplasty

Arterial obstruction due to an atheromatous plaque

Baloon angioplasty

Prevention of restenosis by gene therapy of the artherial wall
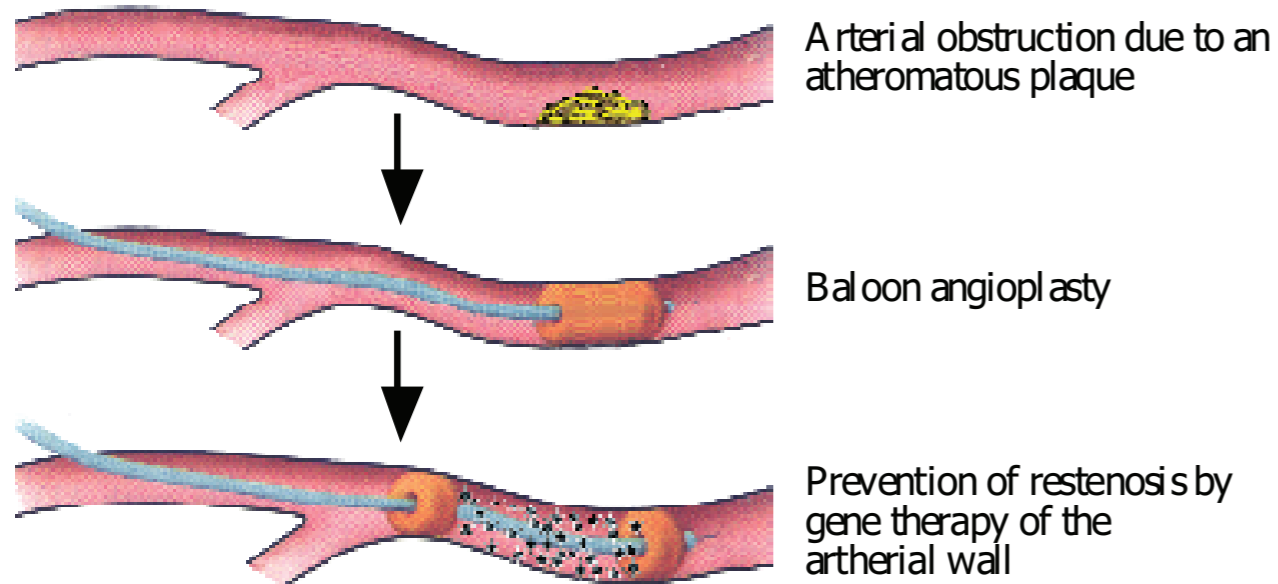
Gene therapy of brain tumors

Brain with tumor

Injection of a viral vector (or of vector-producing packaging cell lines) expressing a toxic gene

Reduction of the tumoral mass
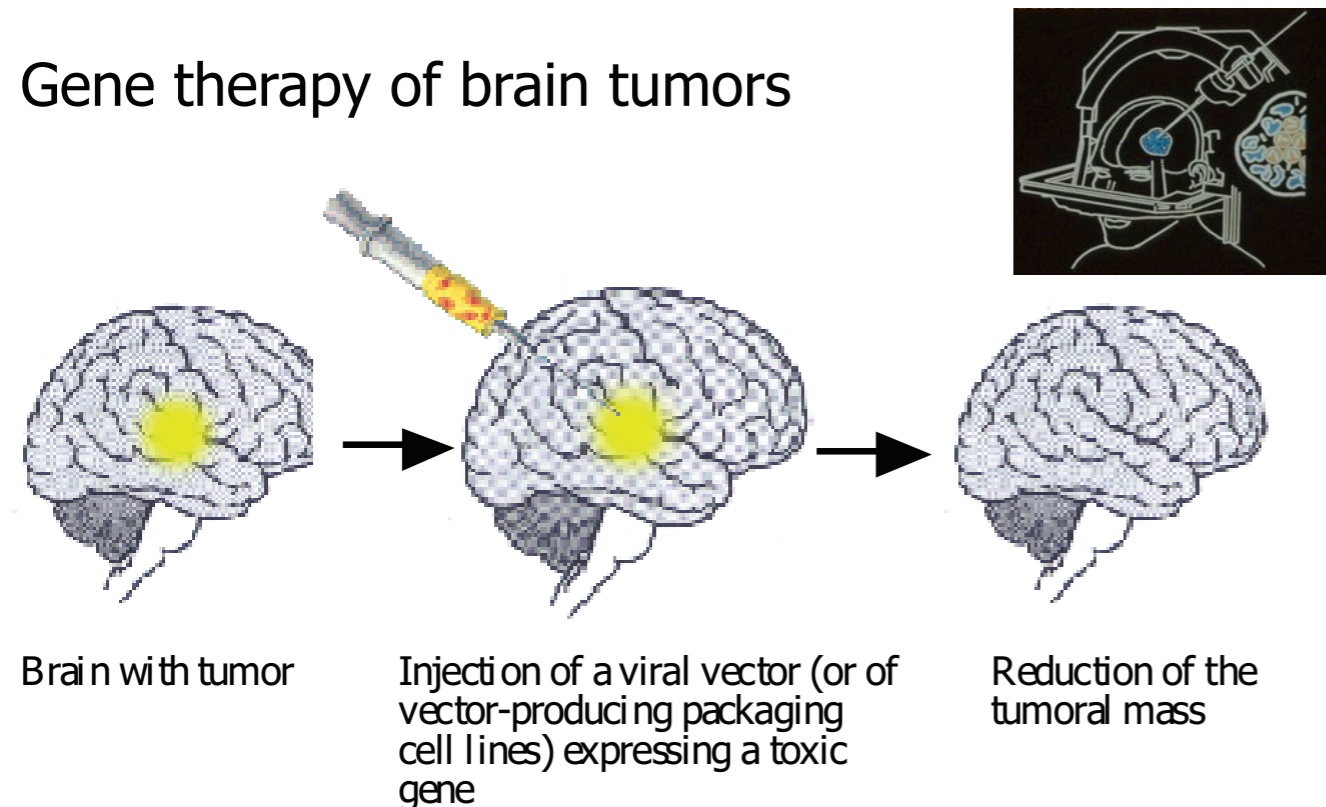
Ex vivo gene therapy

Gene therapy of hematopoietic stem cells

Bone marrow sample

Selection of CD34+ cells

Cell transduction with a viral vector expressing the therapeutic gene

Reinjection into the patient

Somatic gene therapy:
appropriate candidate genetic diseases

Single-gene disorder, recessive or X-linked inheritance

Significant morbidity or mortality

Current therapy inadequate or unavailable

Accessible cellular site of genetic defect causing phenotype

LARRY
THOMPSON
CORREGGERE
IL CODICE
Le nuove terapie geniche

GARZANTI
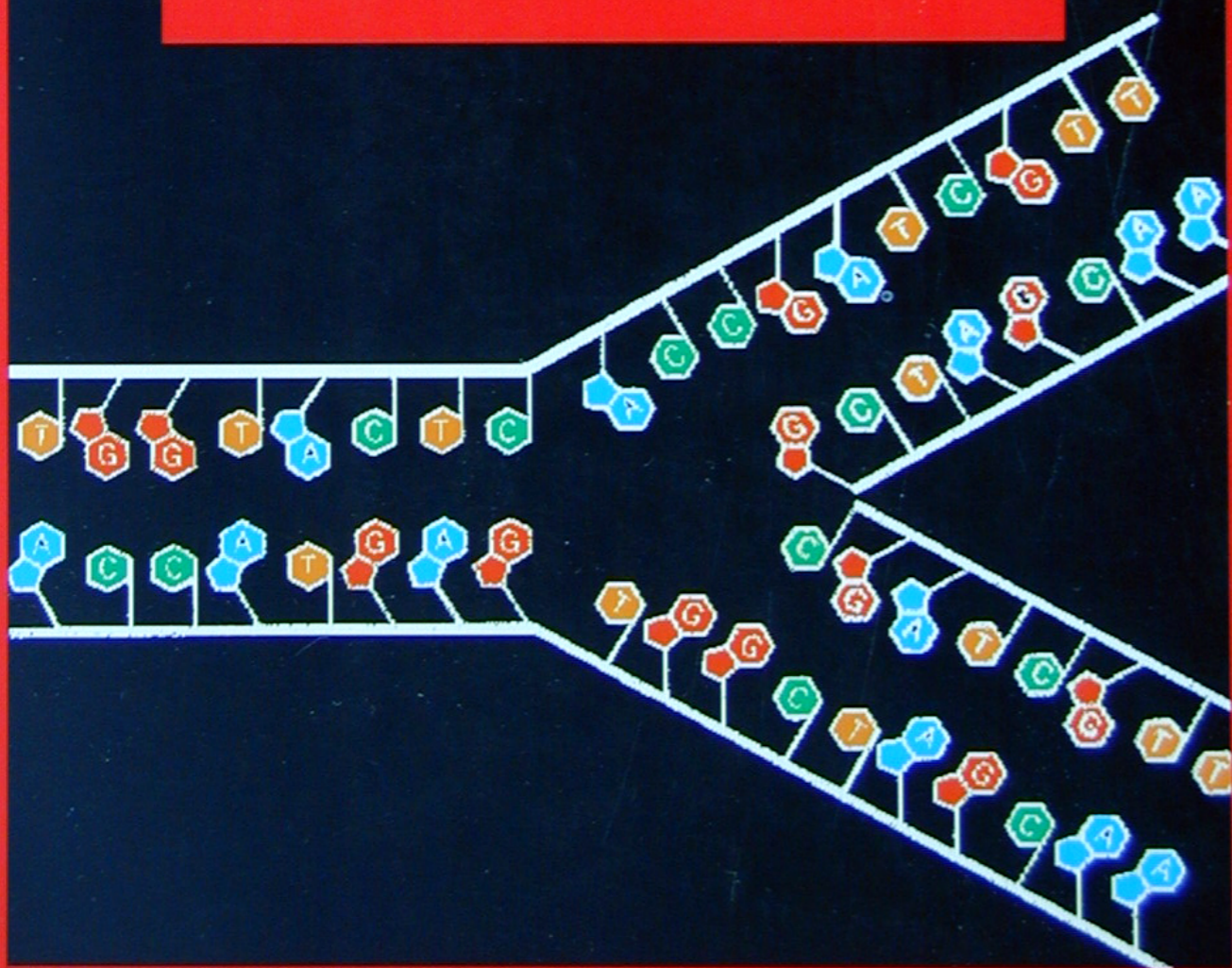
ALTERED FATES

GENE THERAPY AND THE
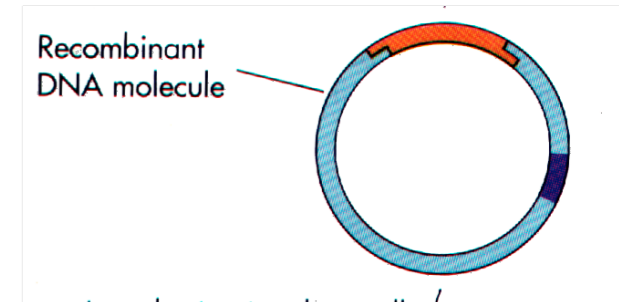RETOOLING OF HUMAN LIFE
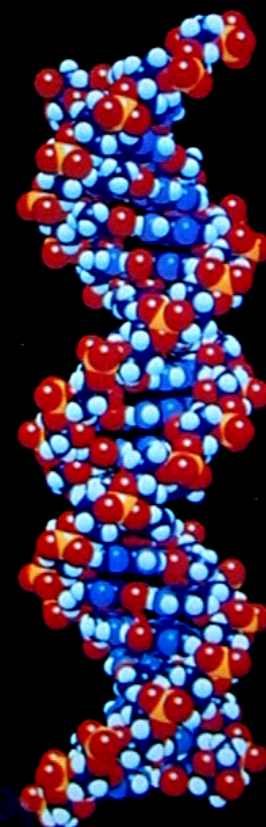
JEFF LYON AND PETER GORNER

The plasma membrane is an impermeable barrier to large polyanions such as DNA
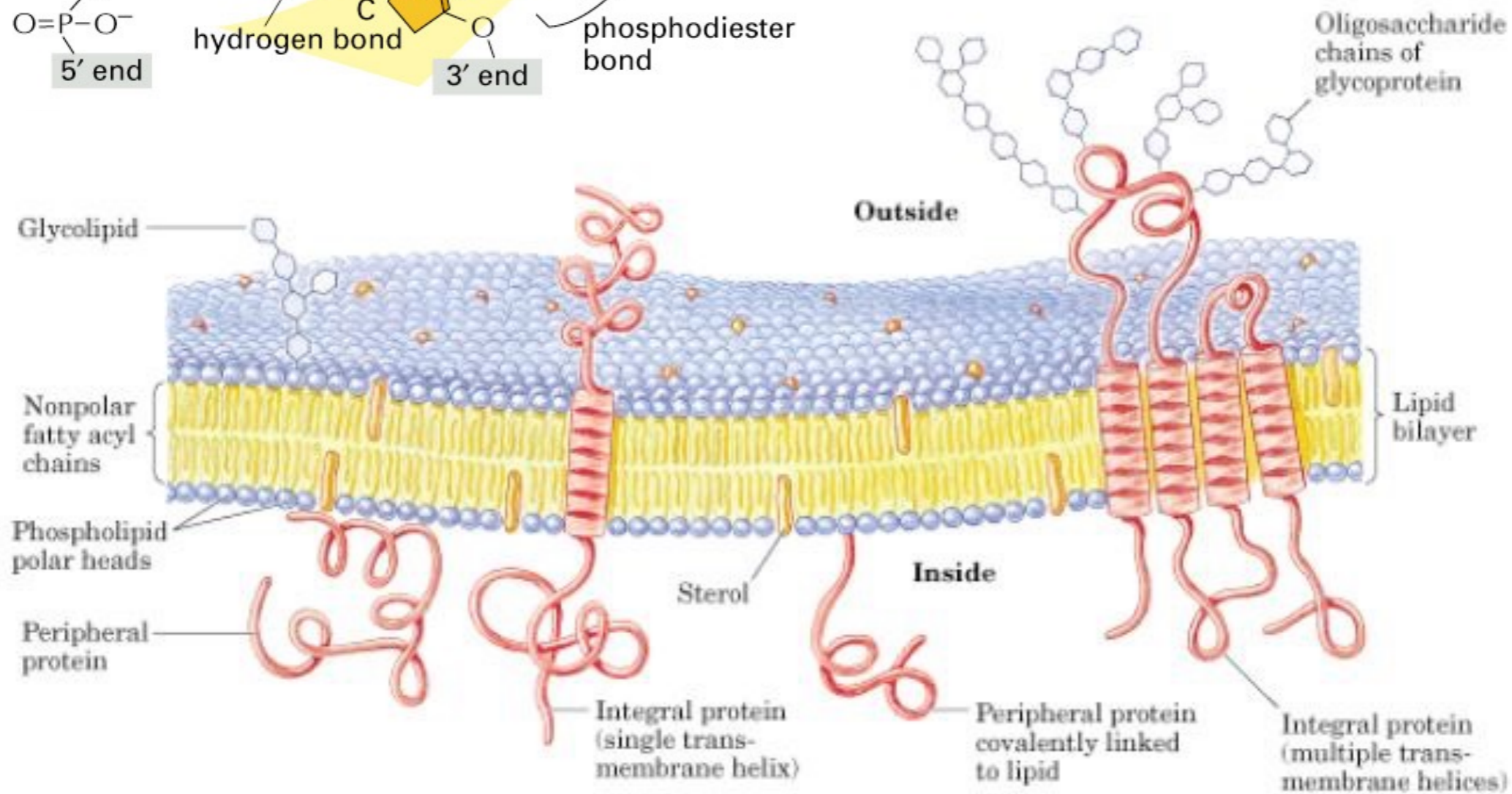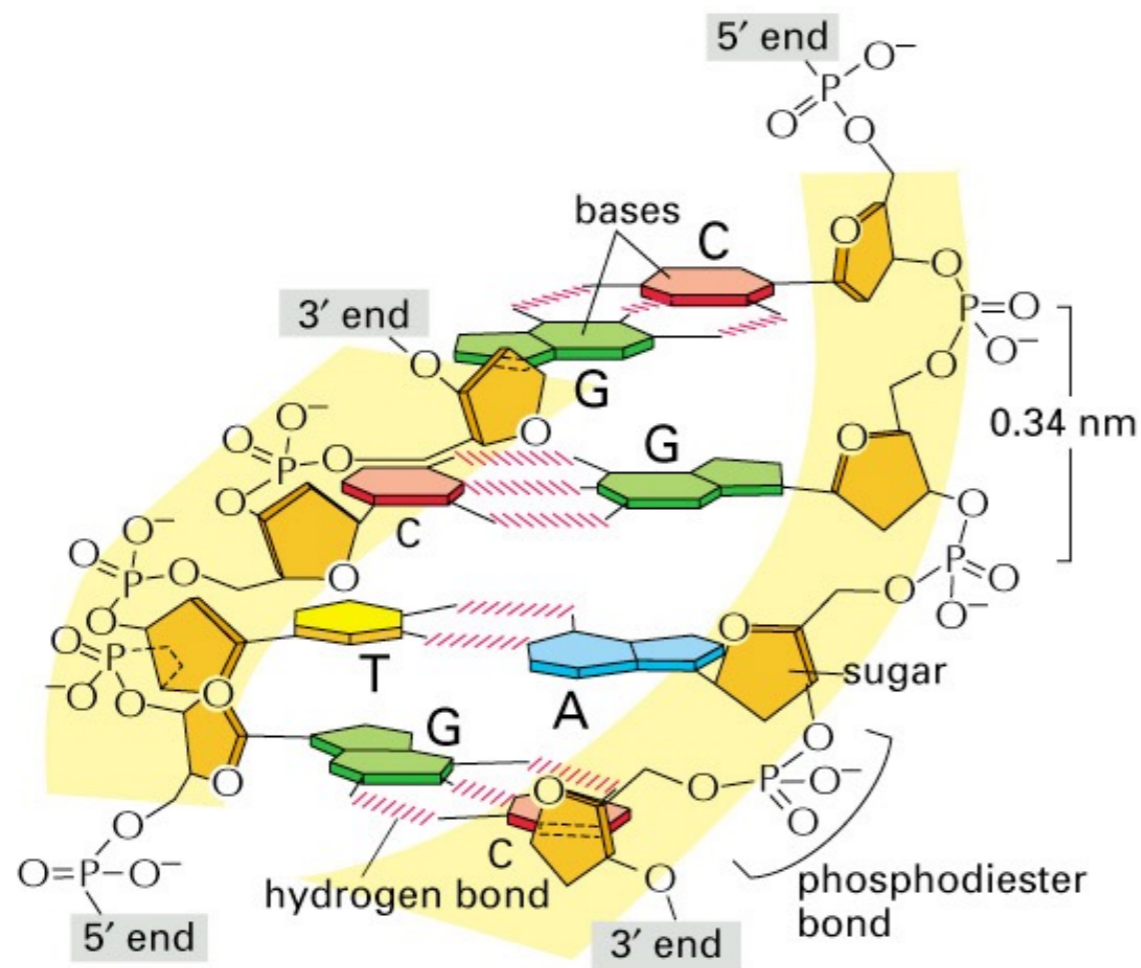
Delivery systems for gene therapy

I. Naked DNA or RNA

**Direct uptake
of plasmid DNA**

Recombinant
DNA molecule

- limited to muscle cells and APCs
- very low efficiency

**Uptake of
oligonucleotides, siRNAs
and other small RNAs**

- very low efficiency

Delivery systems for gene therapy

I. Naked DNA or RNA

II. Physical methods

Electroporation
- skeletal muscle and skin mainly

Bombardment with DNA-coated gold microparticles ("gene gun") and **jet injection**
- limited to the skin

High hydrodynamic pressure
- usually very invasive

Ultrasound and **microbubble-aided ultrasound**
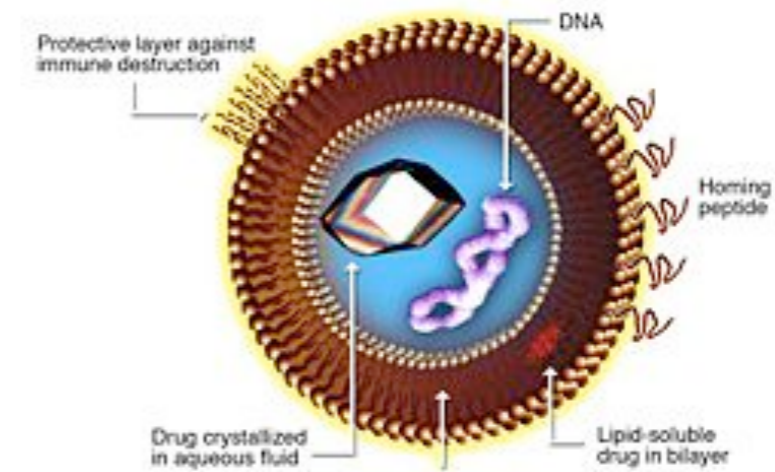- difficult to standardize
- vascular or perivascular applications

Delivery systems for gene therapy

I. Naked DNA or RNA

II. Physical methods

III. Chemical methods

Liposomes and cationic lipids (**lipoplexes**)
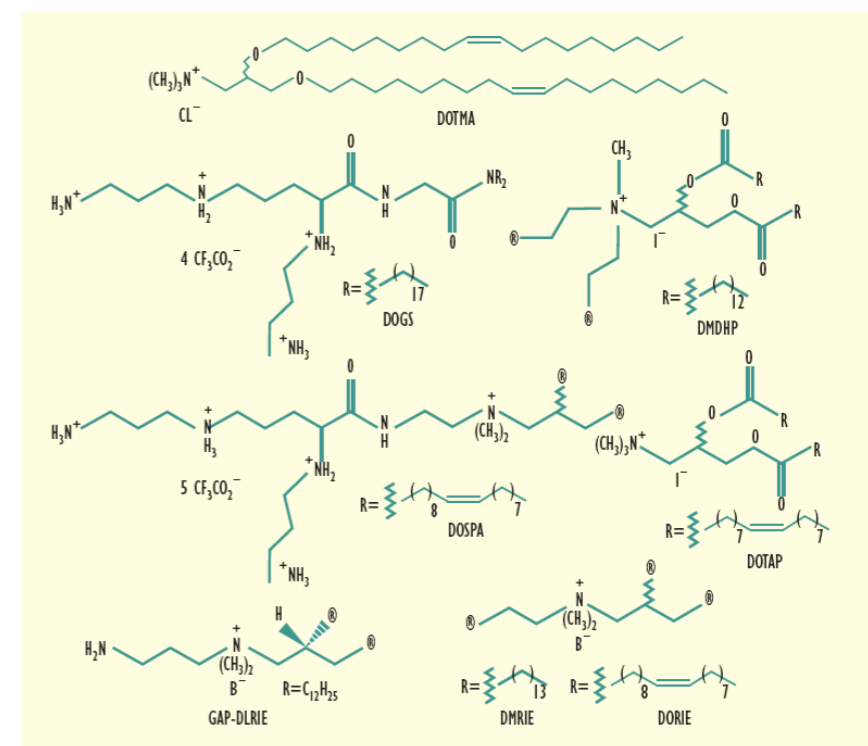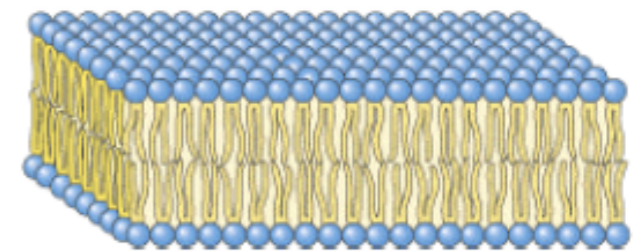
Delivery systems for gene therapy

I. Naked DNA or RNA
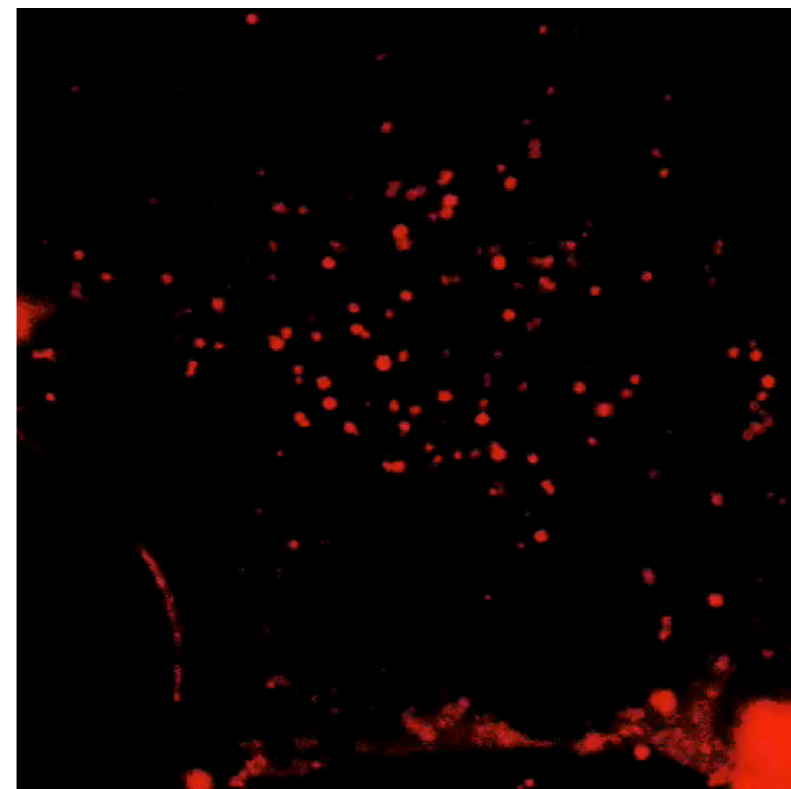
II. Physical methods

III. Chemical methods

Proteins

To induce passage through membranes (e.g. HIV-1 Tat, Antennapedia, VP22)

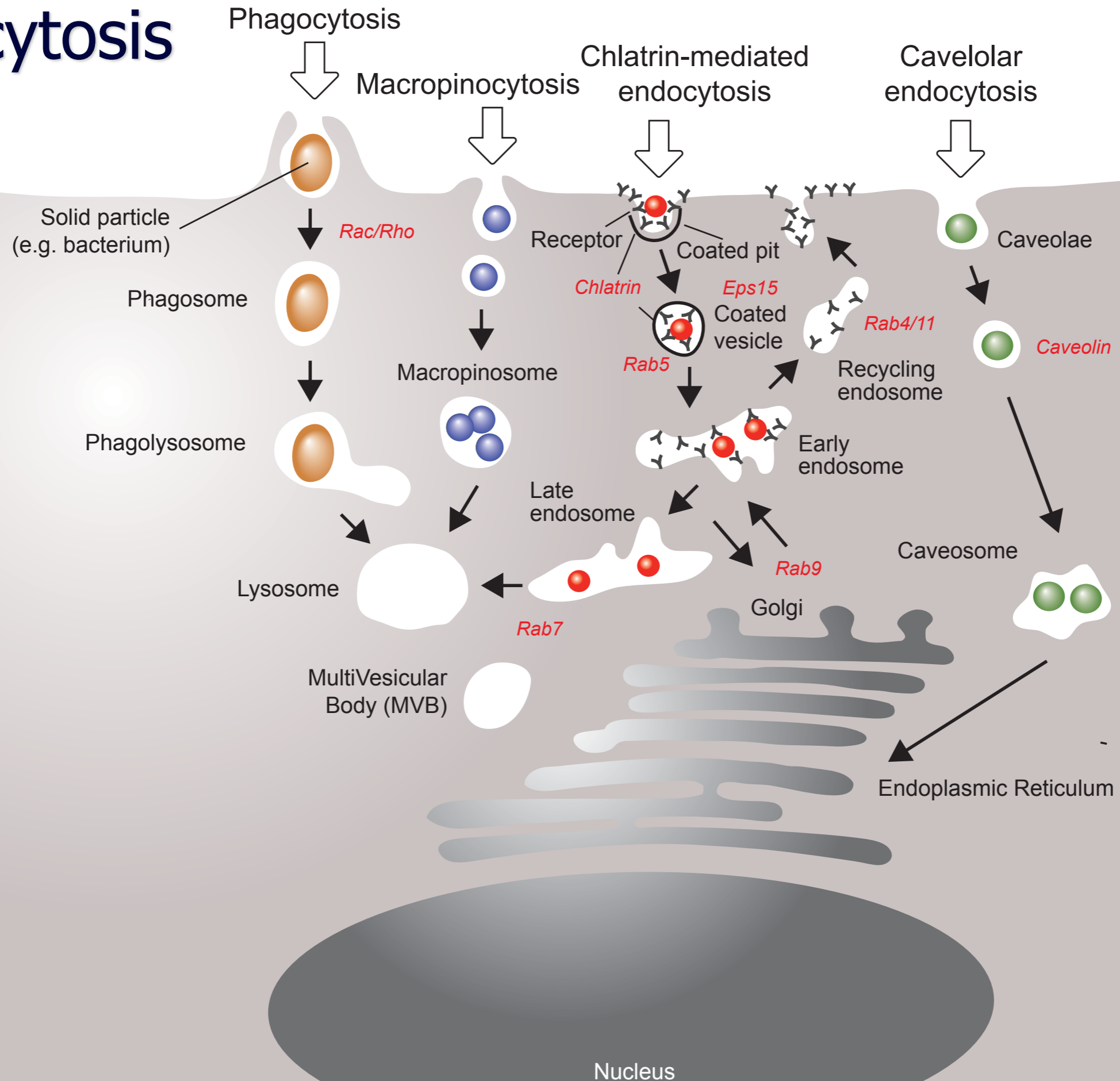To confer cell targeting (e.g. asialoglycoproteins, transferrin. RGD peptide, antibodies)

To induce DNA condensation (e.g. protamine, histones, poly-L-lysine)

To promote endosomal escape (e.g. influenza hemoagglutinin, Ad capsid)

HIV-1 Tat-rhodamine in endosomes

Delivery systems for gene therapy

I. Naked DNA or RNA

II. Physical methods

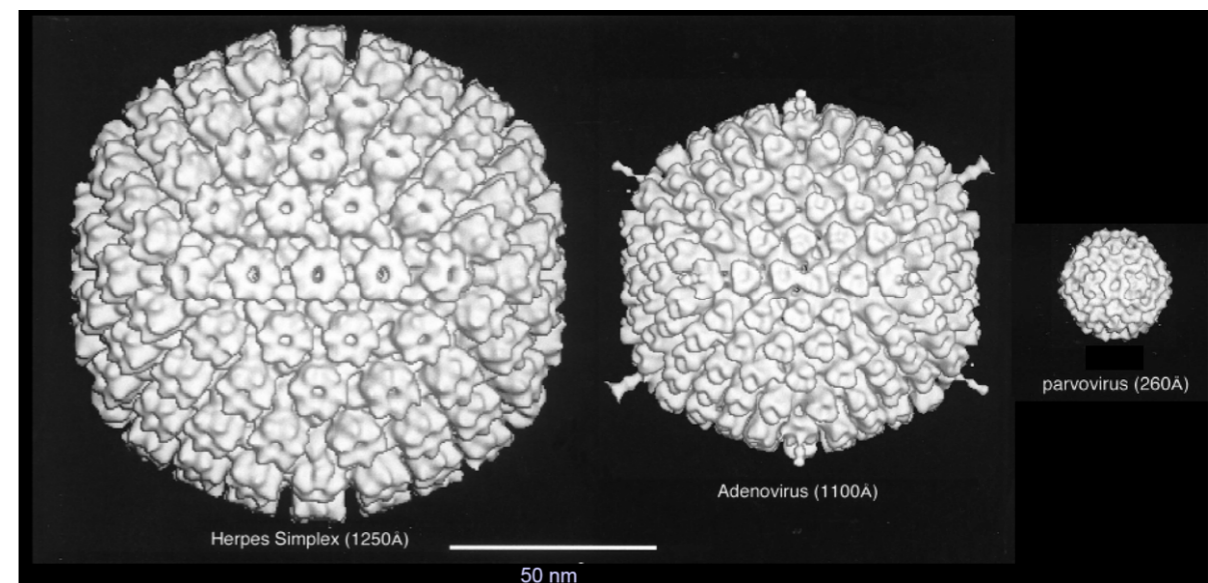III. Chemical methods

IV. Viral vectors

Gammaretroviruses
Lentiviruses
Adenovirus
Adeno-associated virus (AAV)
Herpes simplex virus type 1

Vaccinia (for genetic vaccination)

Herpes Simplex (1250A)   Adenovirus (1100A)   parvovirus (260A)
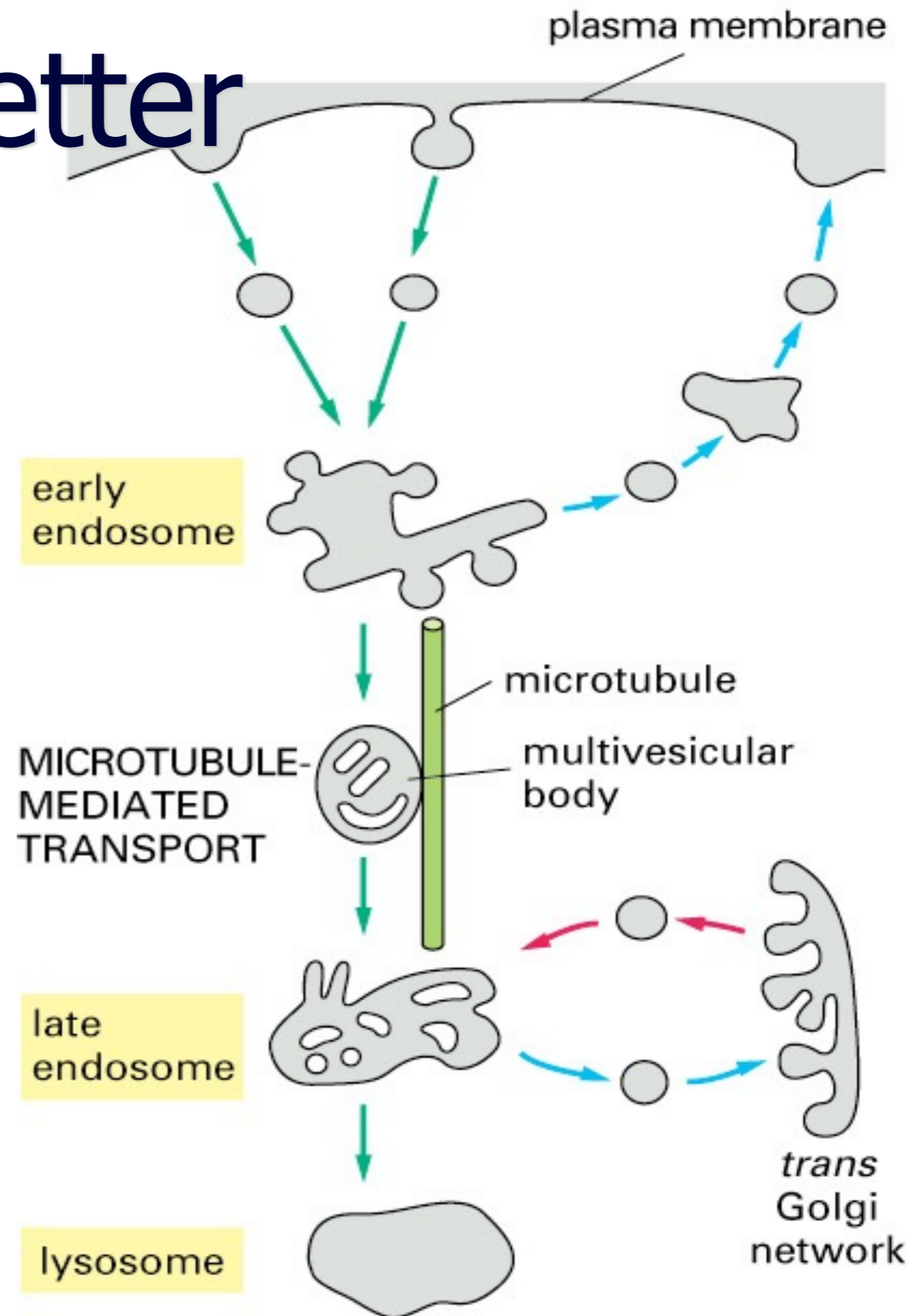
50 nm

Viruses do it better

Targeting to specific receptors

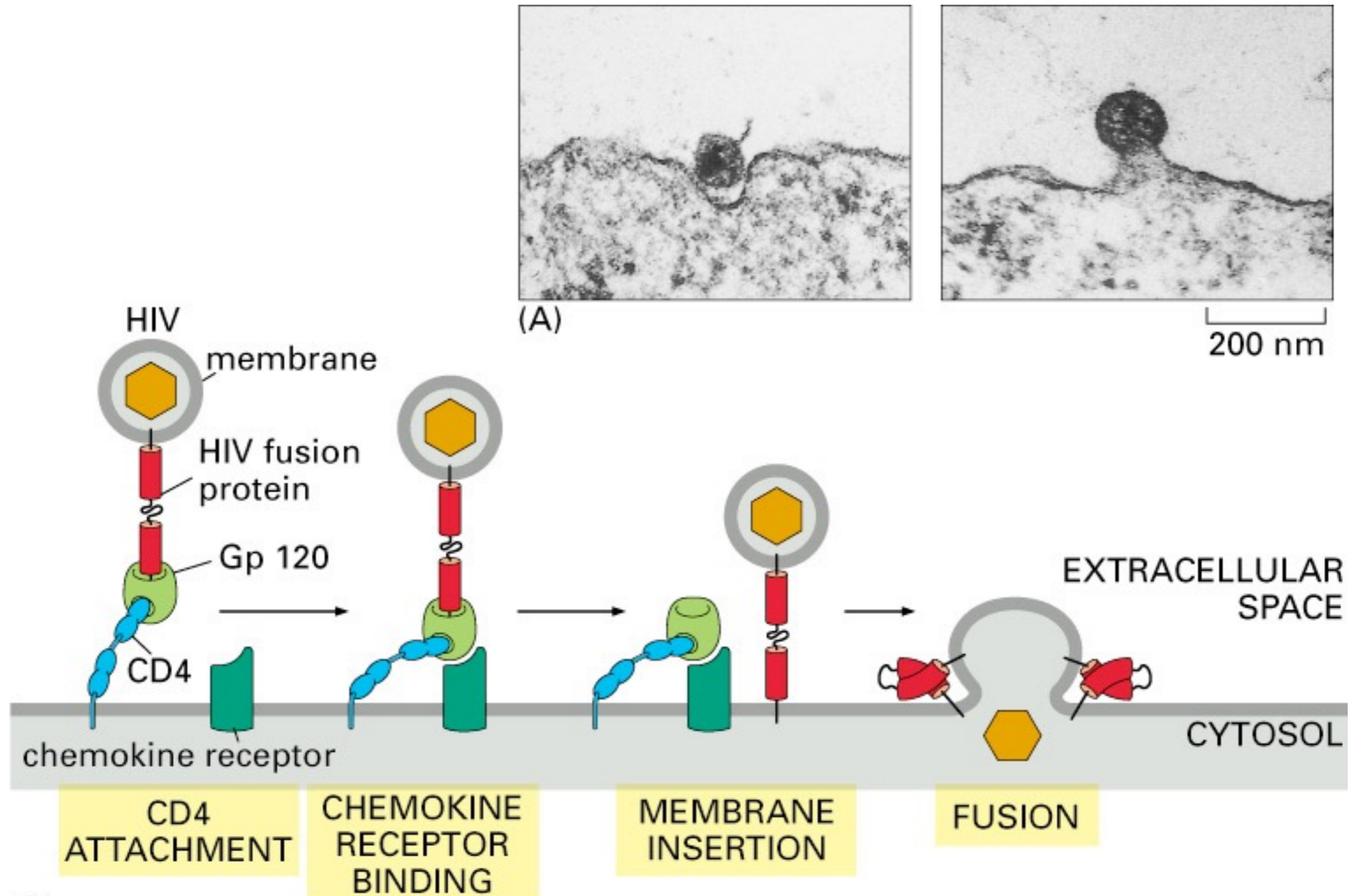Direct fusion of envelope at the cell membrane or escape from endosomes

Transfer of nucleic acids to the nucleus
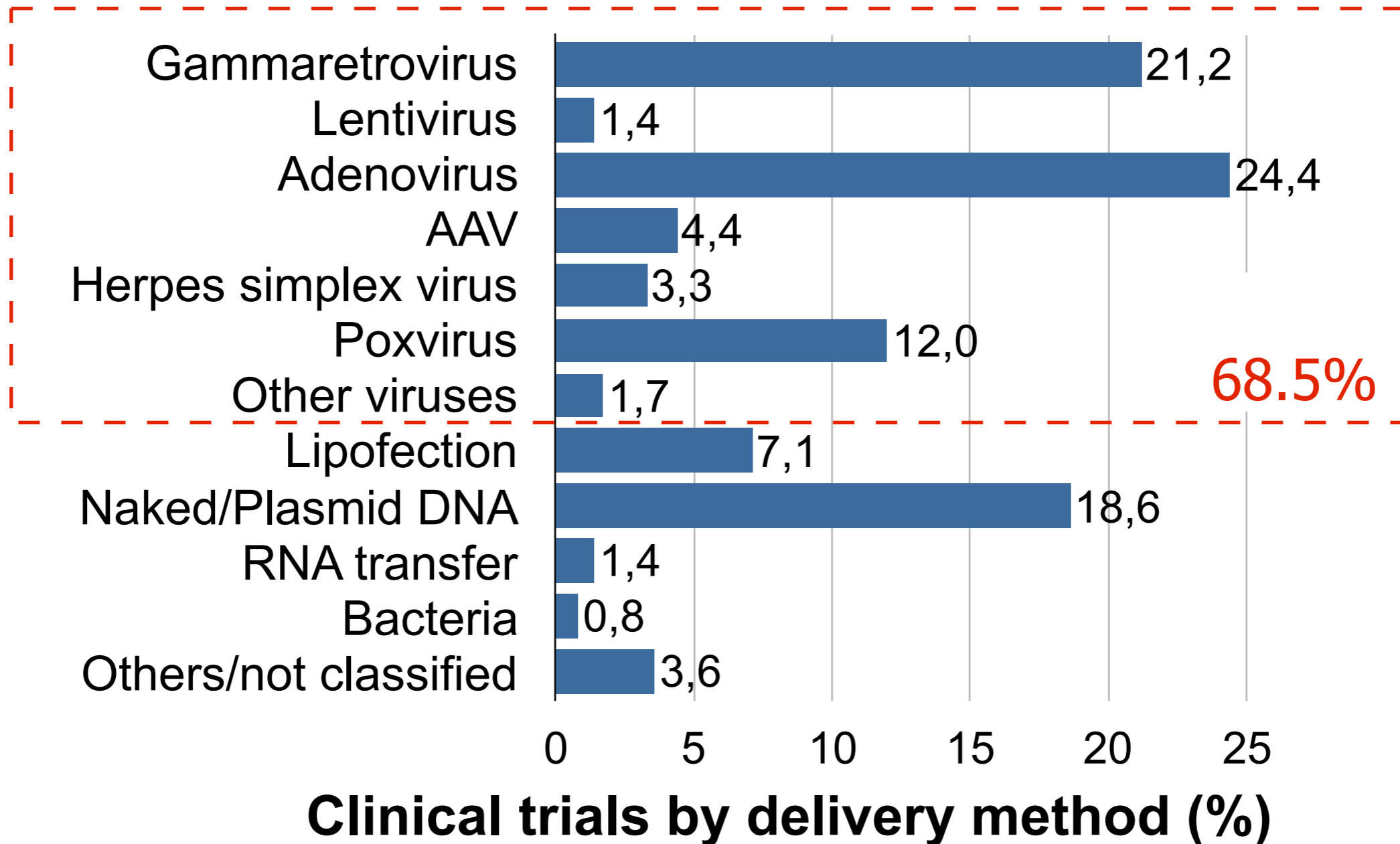
Protection of nucleic acids from degradation

Prolonged (permanent) expression of therapeutic gene

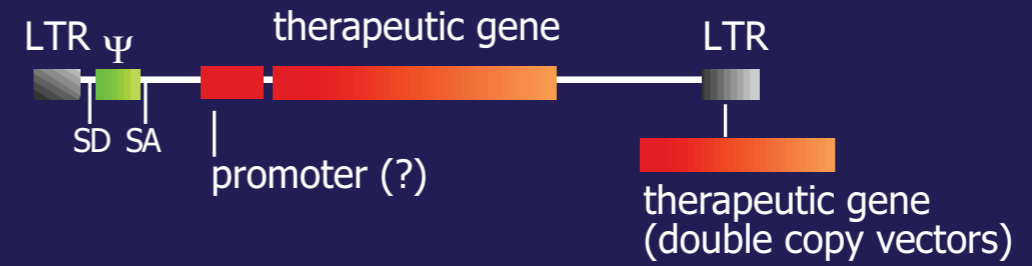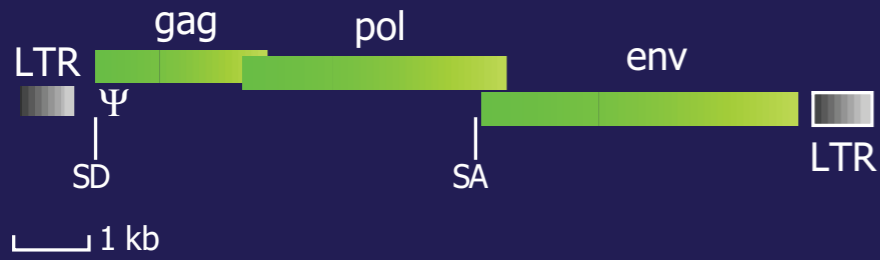# Retrovirus internalization by fusion at the plasma membrane

Gene therapy clinical trials by delivery method

Clinical trials by delivery method (%)

Delivery method	%
Gammaretrovirus	21,2
Lentivirus	1,4
Adenovirus	24,4
AAV	4,4
Herpes simplex virus	3,3
Poxvirus	12,0
Other viruses	1,7
Lipofection	7,1
Naked/Plasmid DNA	18,6
RNA transfer	1,4
Bacteria	0,8
Others/not classified	3,6

68.5%

Giacca, M. 2010. Gene Therapy. Springer

Virus

Murine/avian retroviruses

LTR
gag
pol
env
Ψ
LTR
SD
SA
1 kb

AAV

p5
p19
p46
polyadenylation site
ITR
ITR
rep
cap
1 kb

Adenovirus

E1A
E1B
L1
L2
L3
L4
L5
E3
E2A
E4
E2B
1 kb

HIV-1

LTR
gag
pol
vif
vpr
vpu
tat
rev
env
RRE
nef
LTR
Ψ
1 kb

Vector

LTR
Ψ
therapeutic gene
LTR
SD
SA
promoter (?)
therapeutic gene
(double copy vectors)

promoter
therapeutic gene
TR
TR

promoter
therapeutic gene
L1
L2
L3
L4
L5
E3
E2A
E4
E2B

LTR
Ψ
RRE
promoter
therapeutic gene
SD
SA
LTR