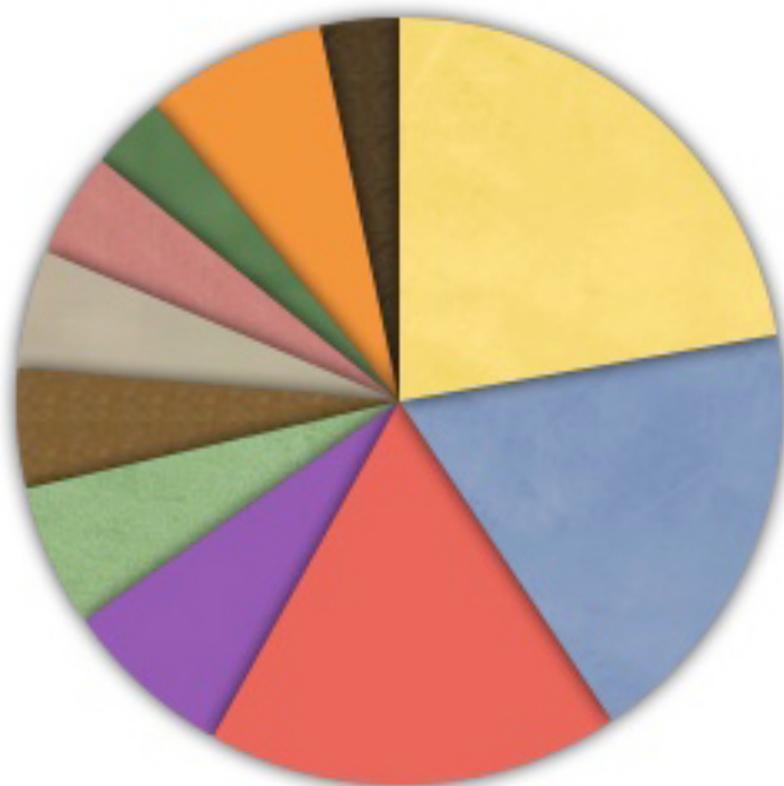


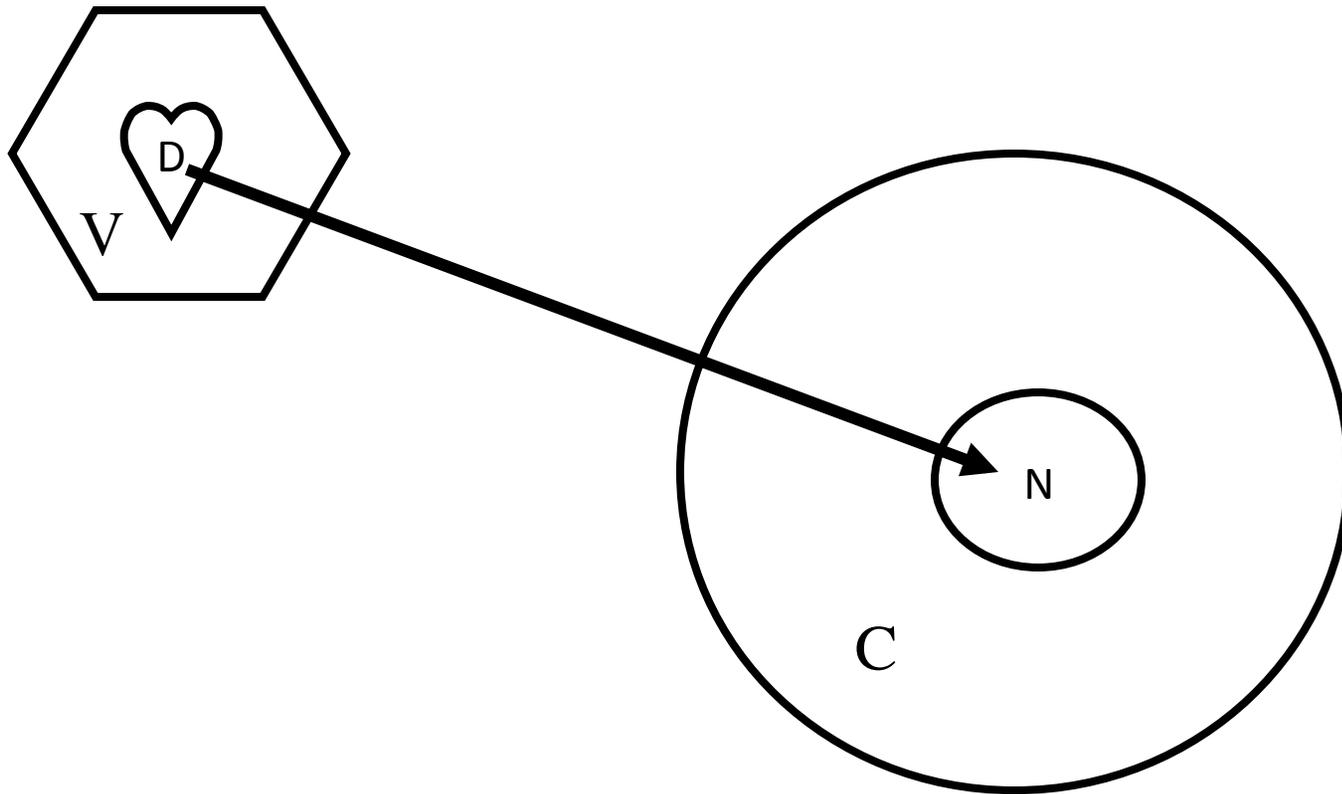
Which vectors for the genes

Vectors Used in Gene Therapy Clinical Trials

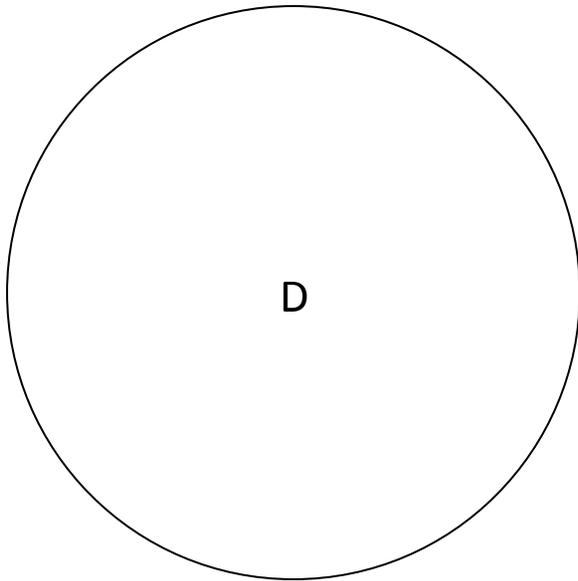


- Adenovirus 22.2% (n=506)
- Retrovirus 18.4% (n=420)
- Naked/Plasmid DNA 17.4% (n=397)
- Vaccinia virus 7.2% (n=165)
- Adeno-associated virus 6% (n=137)
- Lipofection 5% (n=115)
- Lentivirus 5% (n=114)
- Poxvirus 4.4% (n=101)
- Herpes simplex virus 3.2% (n=73)
- Other vectors 7.6% (n=174)
- Unknown 3.3% (n=76)

First step:
bring the transgene into the nucleus



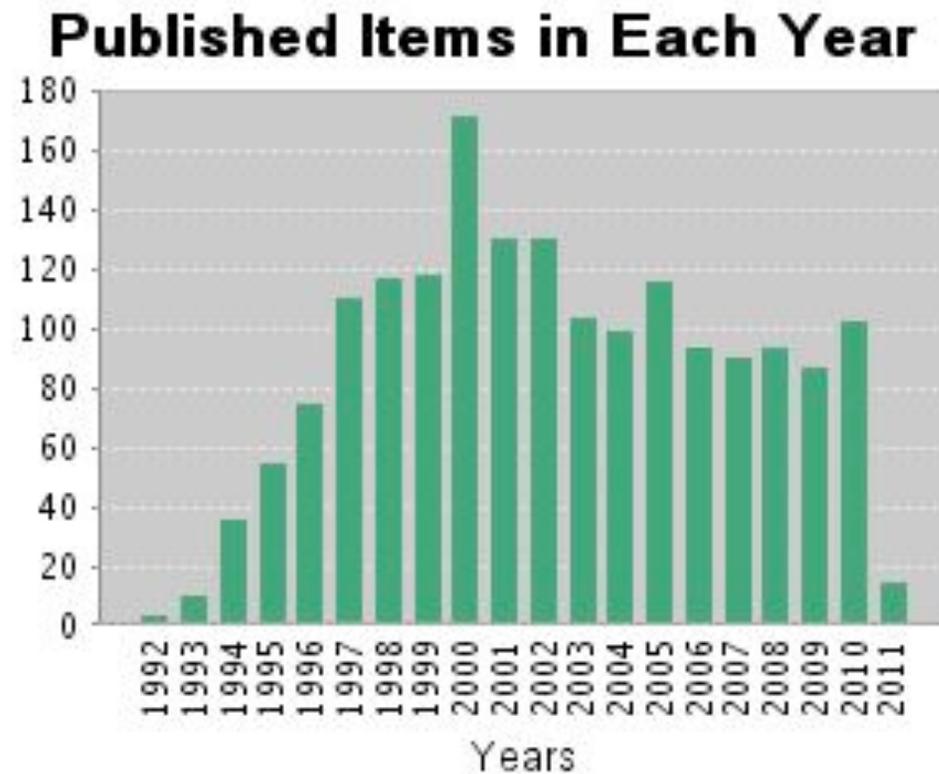
2nd step: the transgene is expressed



DNA must include:

- Antibiotic resistance
- *ORI*
- Eukaryotic expression cassette

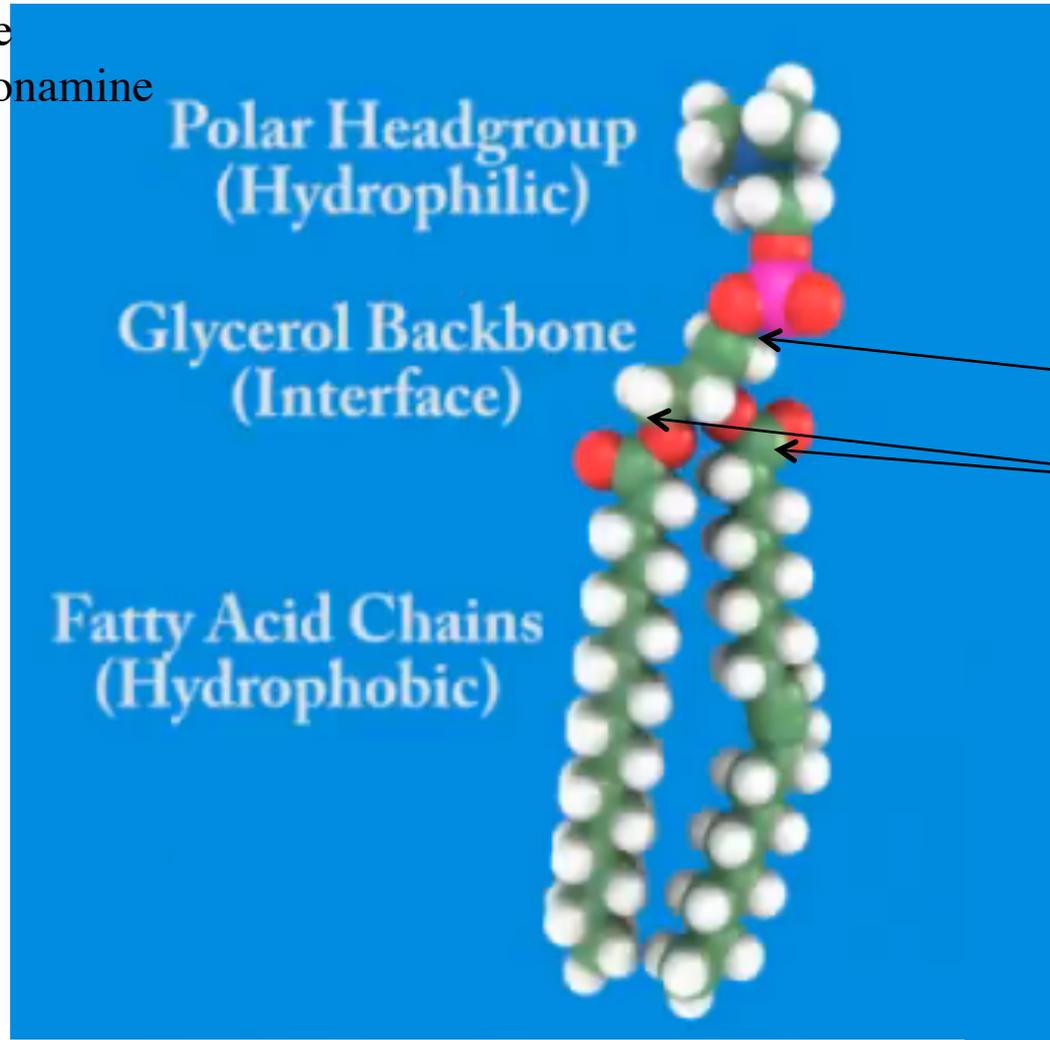
Liposome and gene therapy 1985 -2011; ISI



h index 103

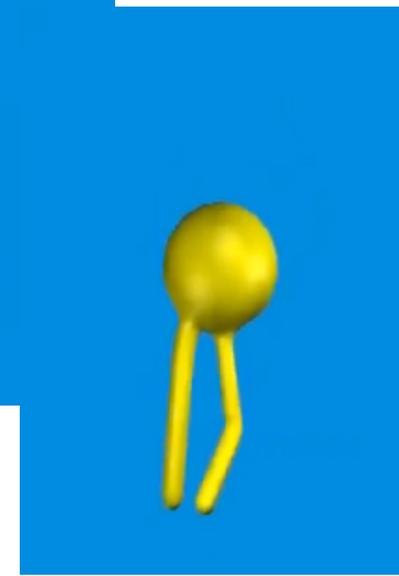
Phospholipids (membrane component)

Phosphoryl-coline
Phosphoryl-ethanolamine
-Glycerol
-Inositol
-Serine
....



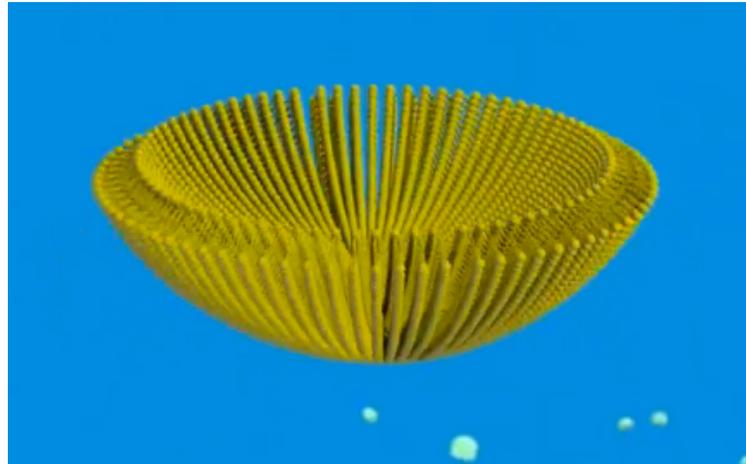
C3: polar headgro
C1 &2: fatty acids

Lauric acid
Myristic acid
Palmitic acid
Stearic acid
Oleic acid
...

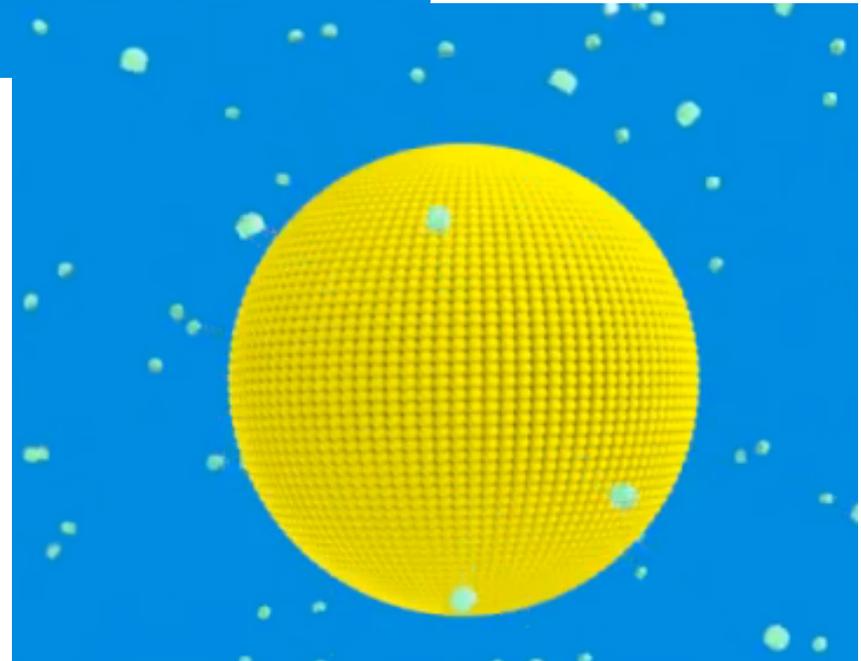


Liposomes

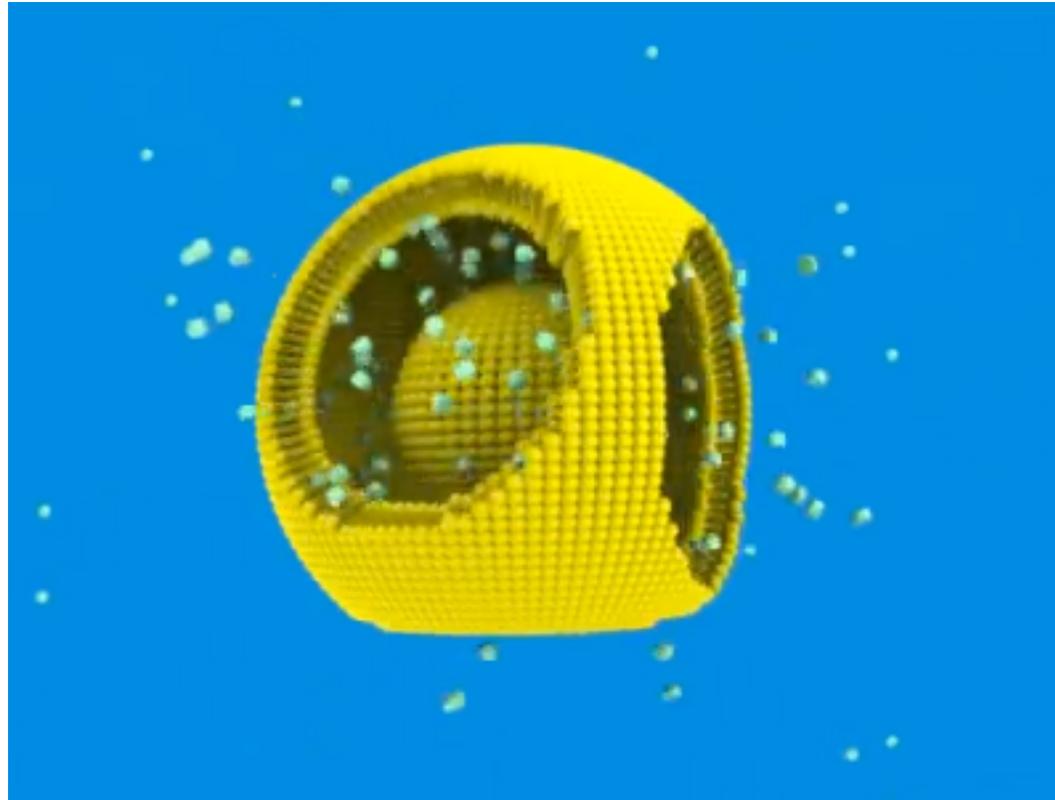
Nanometer size



In H₂O environment

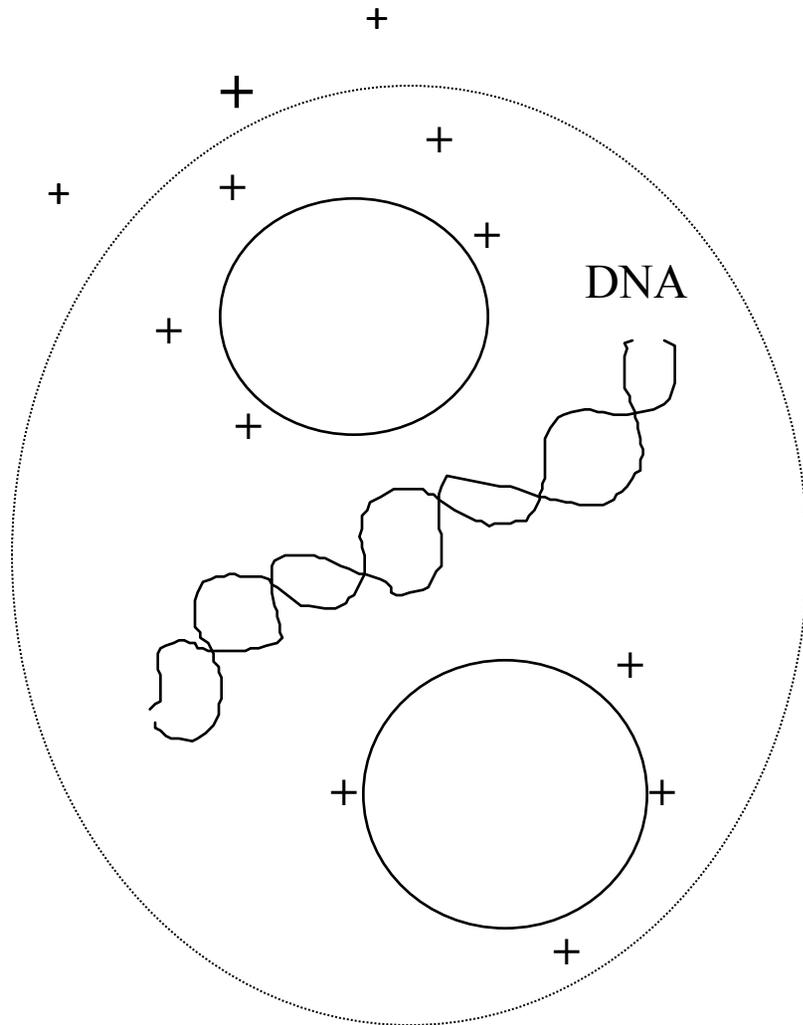


Enzymes and acids disrupt the liposome and let nucleic acids get out

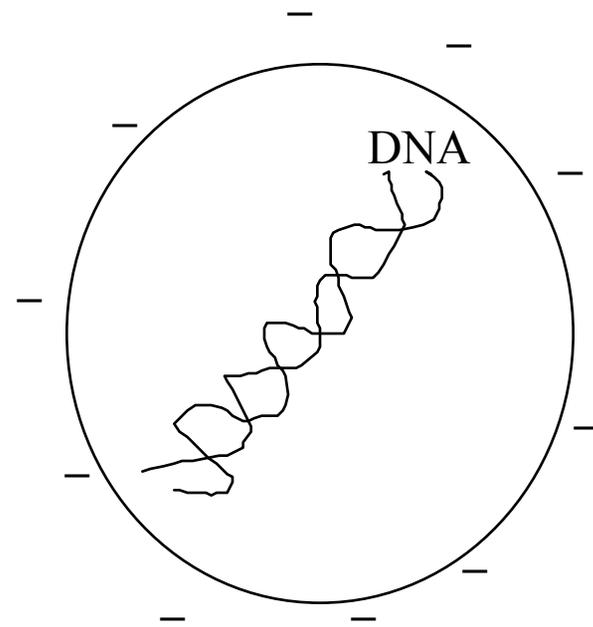


Liposomes/Lipoplexes

(12.1% clin tr.2003, 8.6% 2005, 7.1% 2008; 6.5 2010)



Successful



Unsuccessful, pH sensitive,
DOTAP

How does DNA-liposome enter the cell?

- Membrane fusion?
- Spontaneous endocytosis?
- mammalian cell surface is covered with negatively charged sialic acid

Liposome gene transfer in vivo (intraven.)

Organ distribution

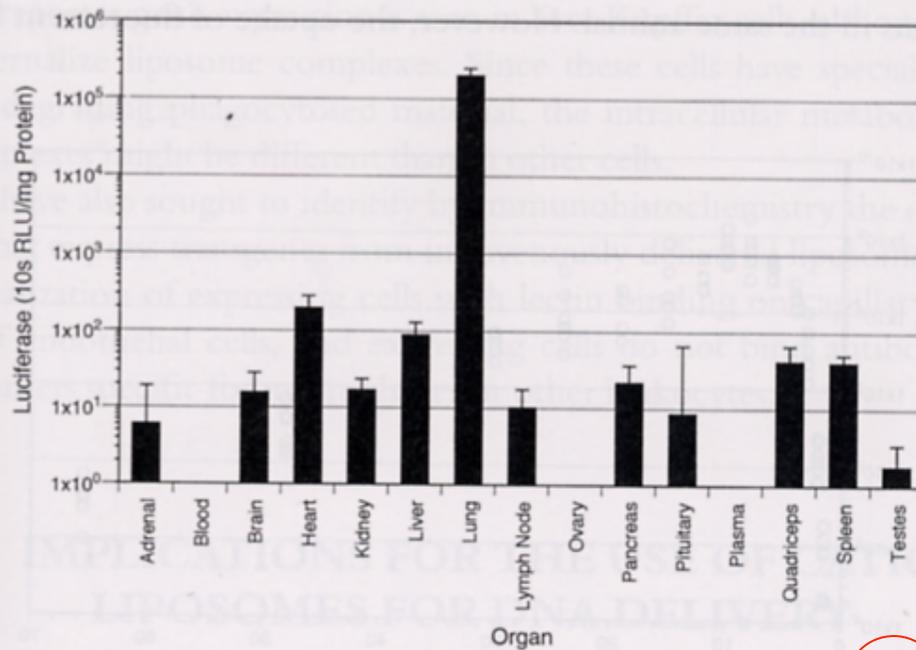


Figure 7 Luciferase expression in organs at 24 h after the intravenous injection of 60 μ g of luciferase plasmid complexed to 1440 nmoles of DOTAP/cholesterol (1/1) liposomes (mean \pm SD, $n = 6$). No detectable expression above background was seen in blood, ovary, or plasma.

Liposome gene transfer in vivo (intraven.)

Time course

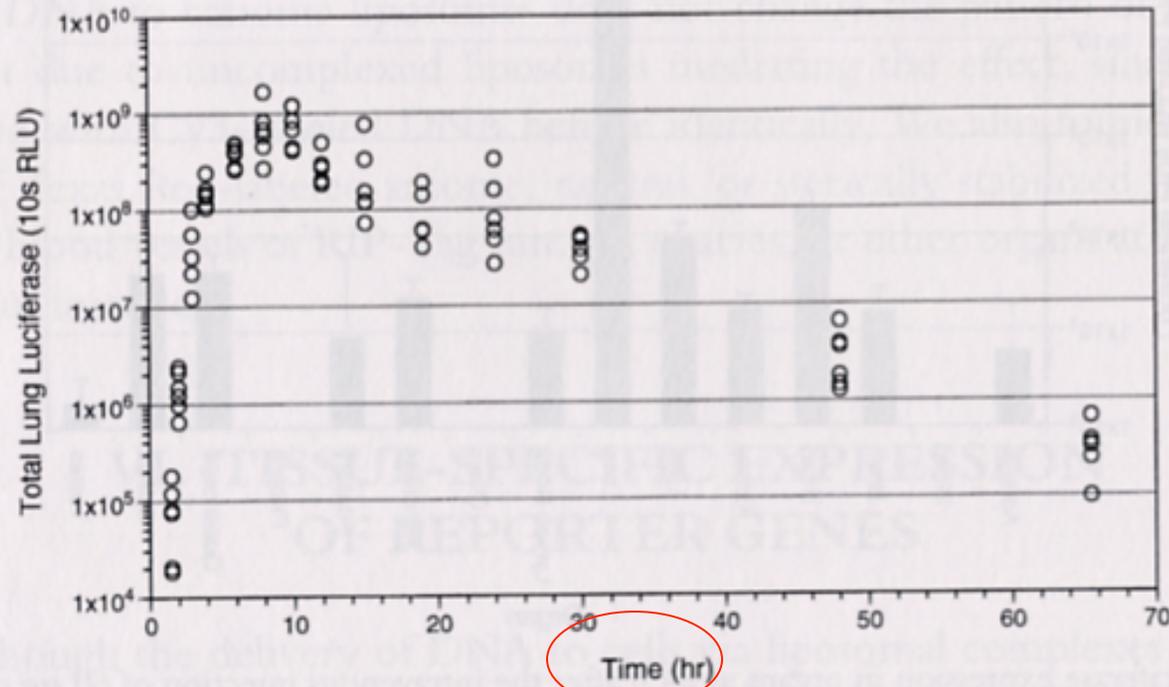


Figure 8 Time course of expression in lungs after the intravenous injection of 30 μg of luciferase plasmid complexed to 540 nmoles of DDAB/cholesterol (1/1) liposomes ($n = 6$ per time point).

Gene gun transfection in vivo, injection in mouse skin

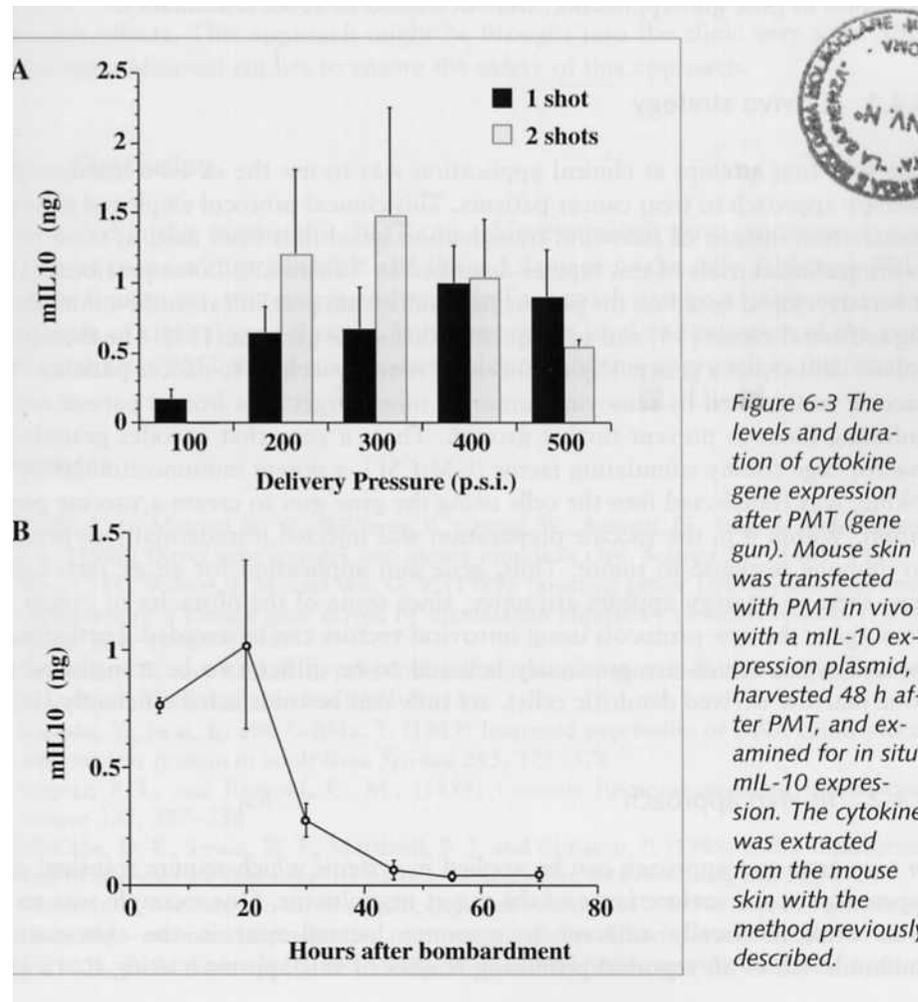
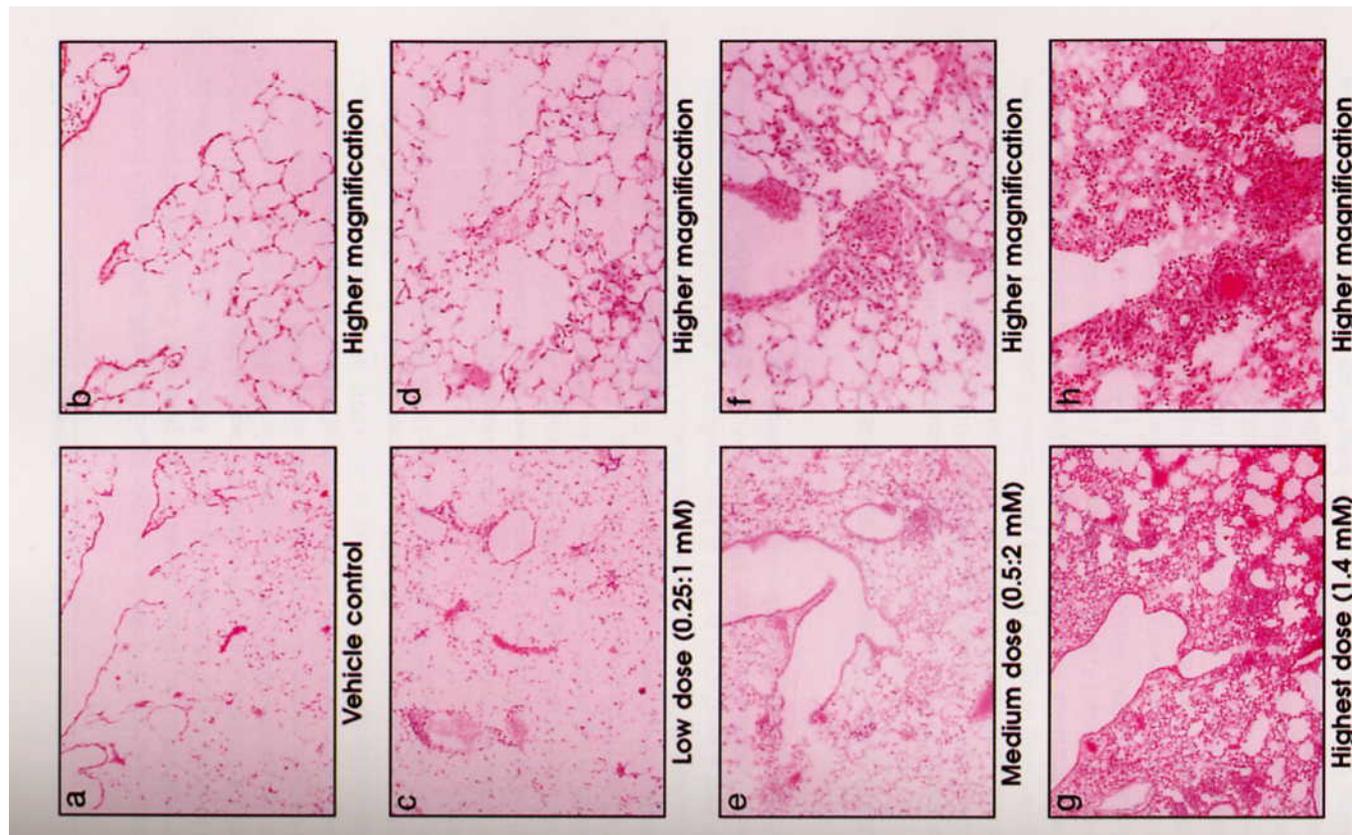


Figure 6-3 The levels and duration of cytokine gene expression after PMT (gene gun). Mouse skin was transfected with PMT in vivo with a mL-10 expression plasmid, harvested 48 h after PMT, and examined for in situ mL-10 expression. The cytokine was extracted from the mouse skin with the method previously described.

Dose dependent inflammation following intranasal instillation of lipoplexes into lungs of BALB/c mice. 48hrs after DNA inj.



NB toxicity reduced with aerosol

Intracellular transport and plasmid biodistribution after cationic lipid mediated transfection

- 1.000.000 plasmids/cell transfected
- 300.000 plasmids/cell in pellet
- 50.000 plasmids/ cell intracellular
- 1.000 plasmids/cell intranuclear (1/1000)

Delivery barriers that a vector must overcome

- Extracellular barriers

Opsonins (act as binding enhancers for the process of phagocytosis)

Phagocytic cells

Extra-cellular matrix

Digestive enzymes

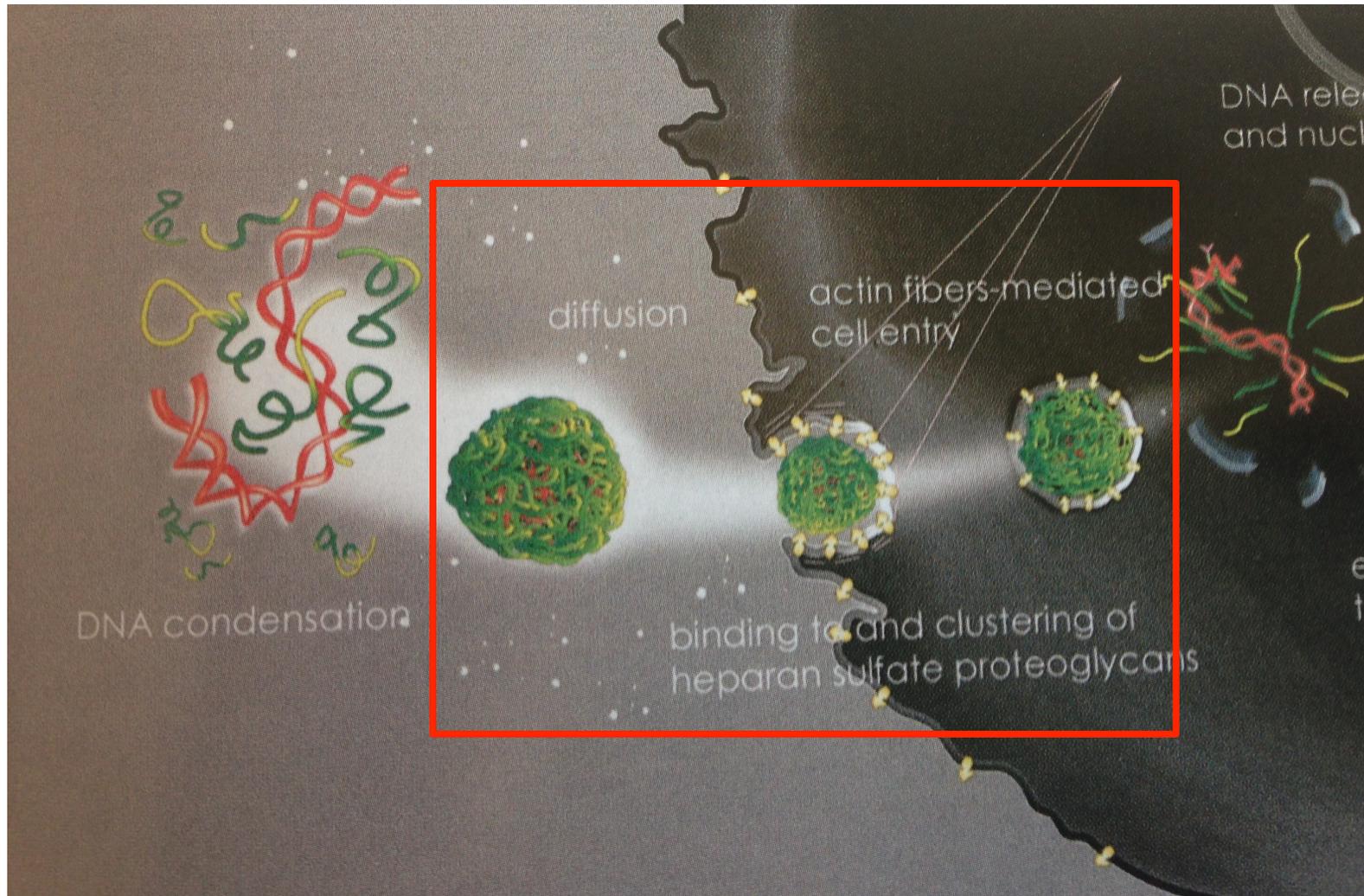
- Intracellular barriers

Plasma membrane

Endosome/lysosome

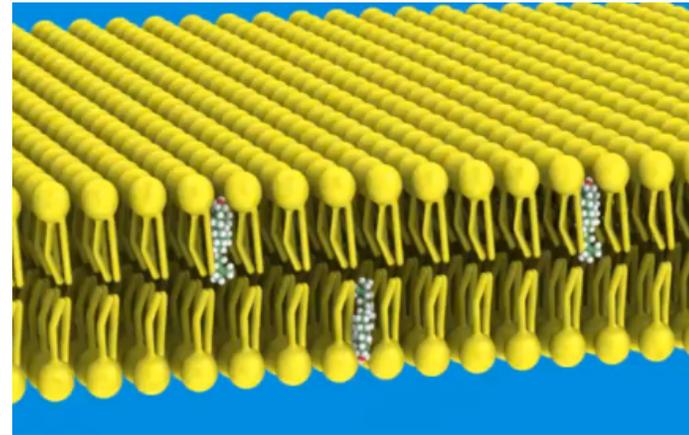
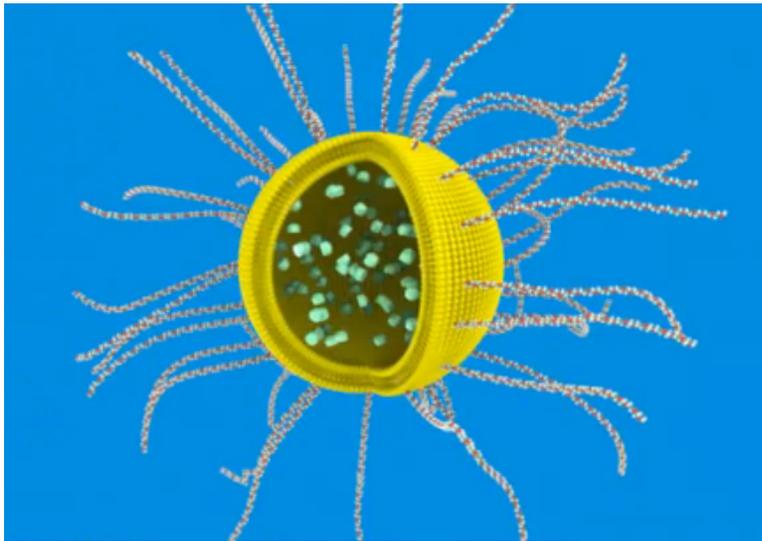
Nuclear membrane

Non viral vectors: the objectives of nanotech studies applied to gene transfer



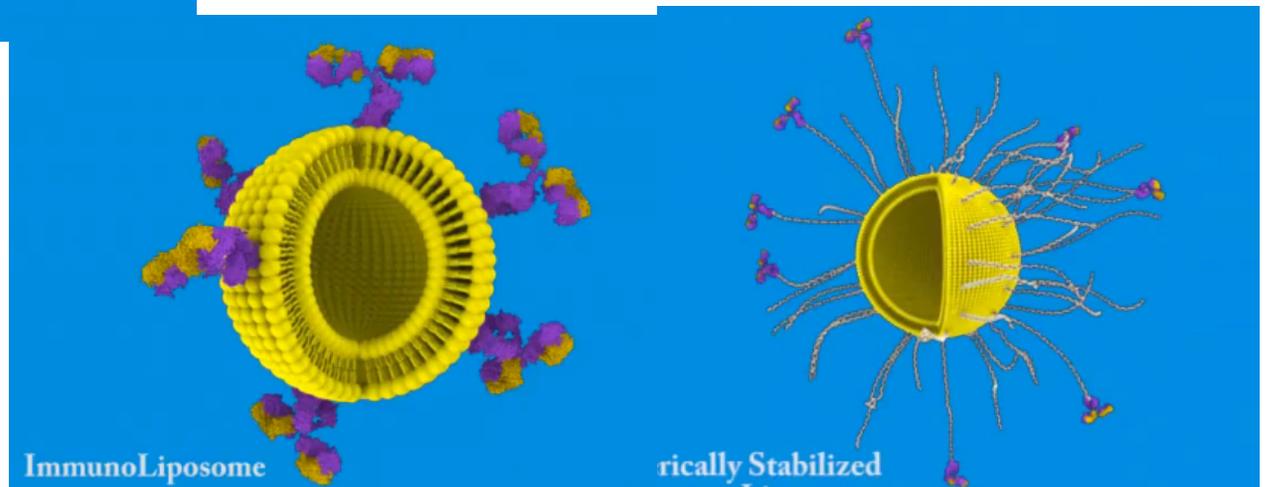
Liposomes/improvements

+cholesterol-increase stability in vivo

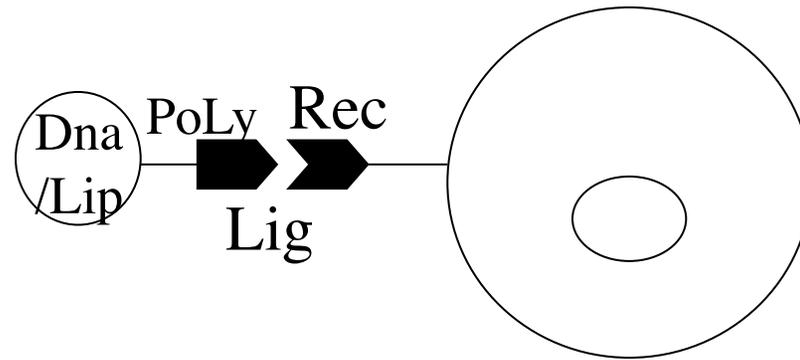


+PEG, more stable

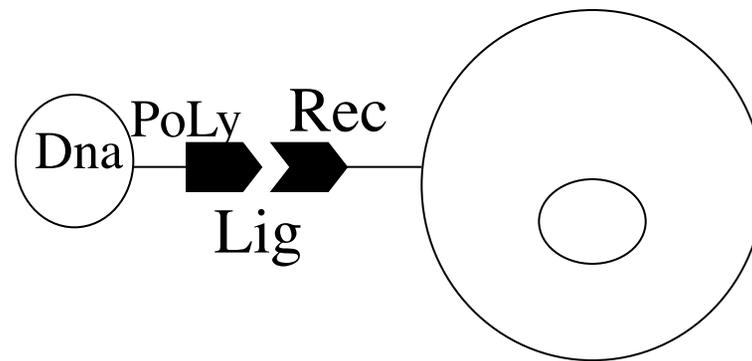
+Ab, targeting



Use of receptor ligands: to facilitate binding to cell membrane



or



Which receptor ligand?

Table 1 Ligands Used in Receptor-mediated Gene Transfer

Ligands	Refs.
Alpha2 macroglobulin	24,25
Anti-CD3	26,27
Anti-CD5	28
Anti-CD117	29
Anti-EGF	30
Anti-HER2	31
Anti-IgG	32,33
Antisecretory component Fab	34–36
Anti-Tn	37
Antithrombomodulin	38
Antibody ChCE7	39
Asialoglycoproteins	40–49
EGF	50–52
Fibroblast growth factor 2(FGF2)	9,53
Folate	54–56
Glycosylated synthetic ligands	57–69
IgG (FcR ligand)	32,70
Insulin	10,71
Invasin	72
Lectins	73–75
Malarial circumsporozoite protein	76
RGD-motif (integrin binding)	77
Steel factor (CD117 ligand)	78
Surfactant proteins A and B	79,80
Transferrin	13,81–87