Lecture 9

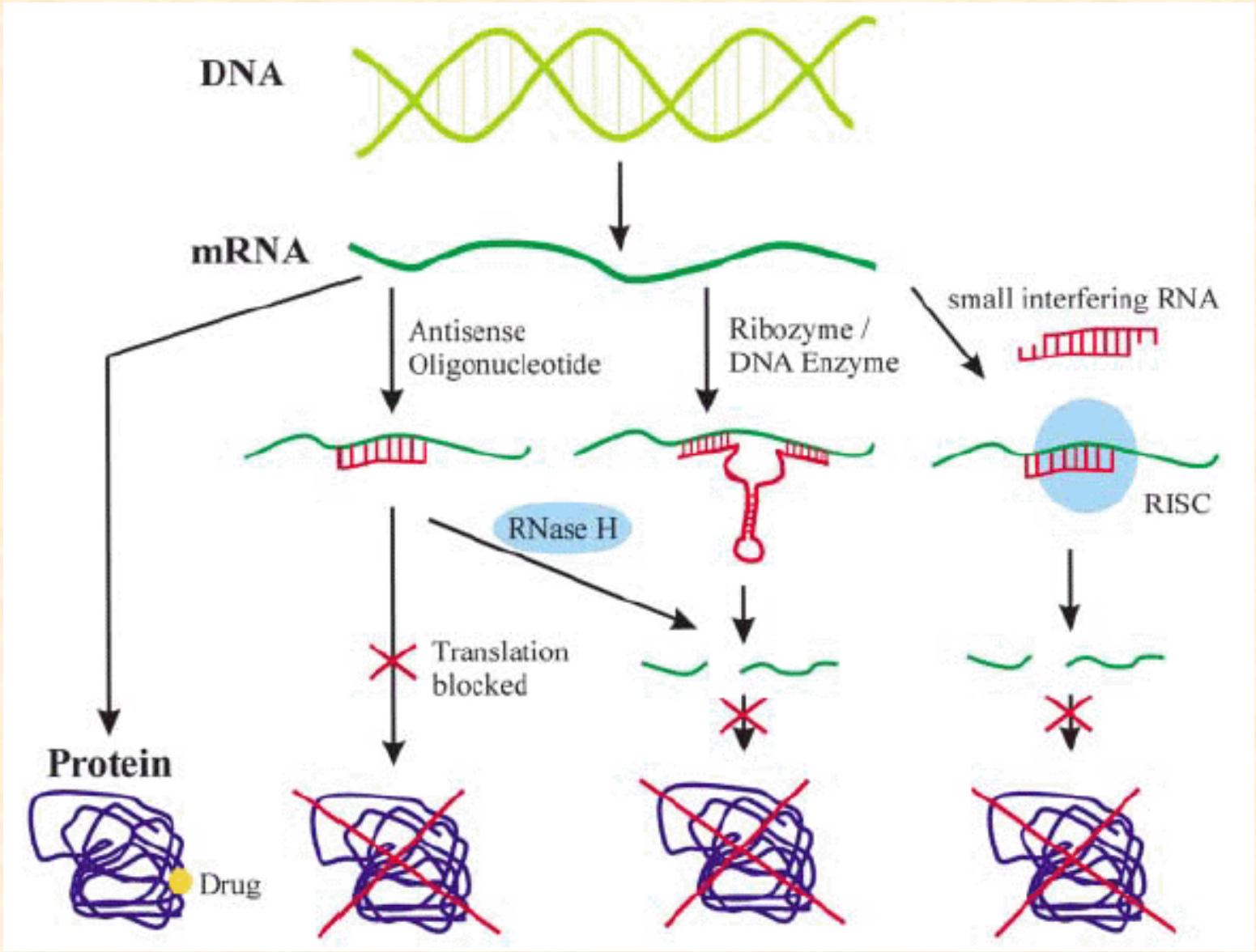
Regulation of gene expression in gene therapy

DNA decoys

3th December 2007

Date of exam

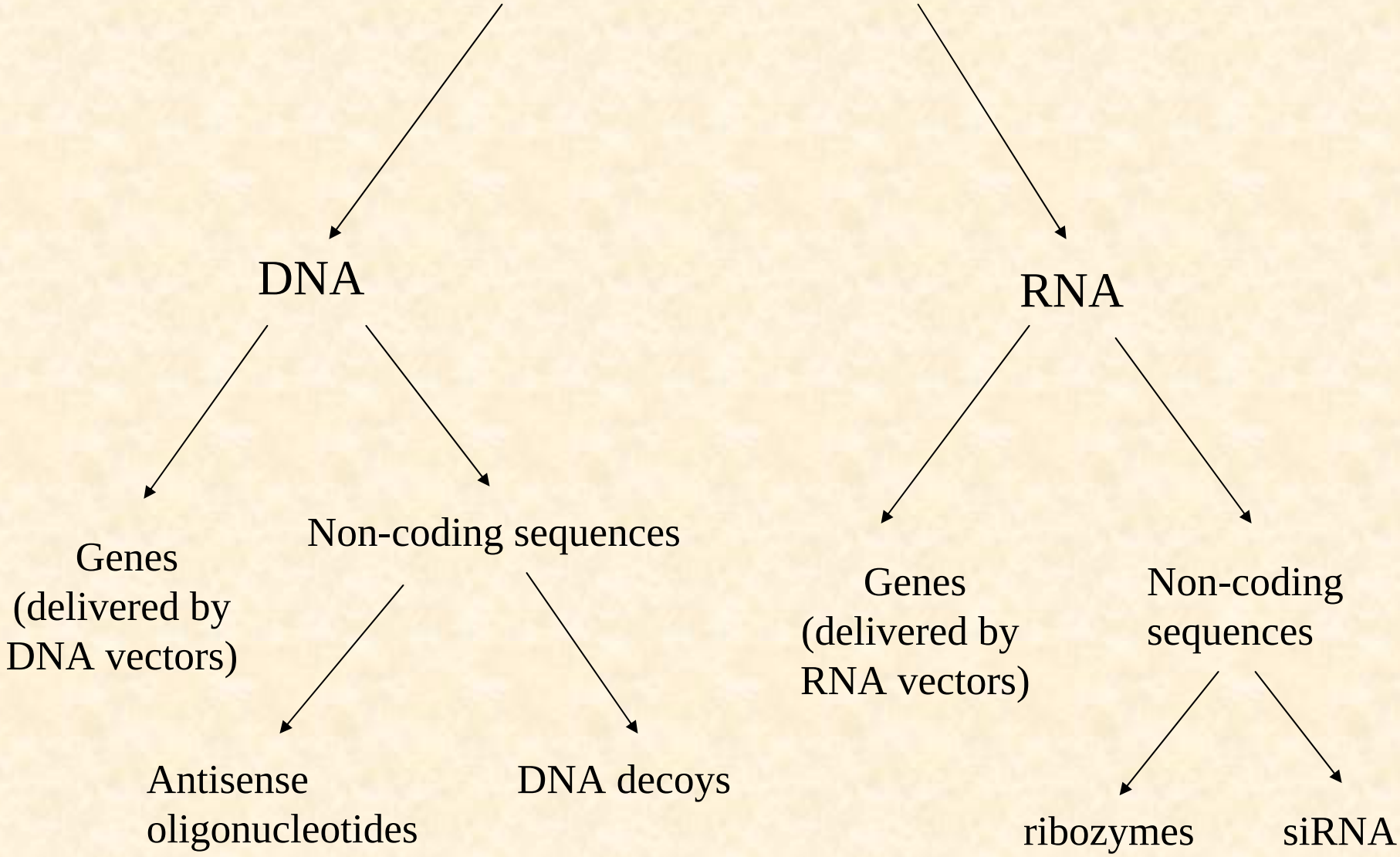
Different ways of inhibition of gene expression



Protein therapy

Gene therapy

Therapeutic nucleic acids



Aptamers

Nucleic acids that bind proteins

Exist naturally – produced by viruses (HIV, adenoviruses)

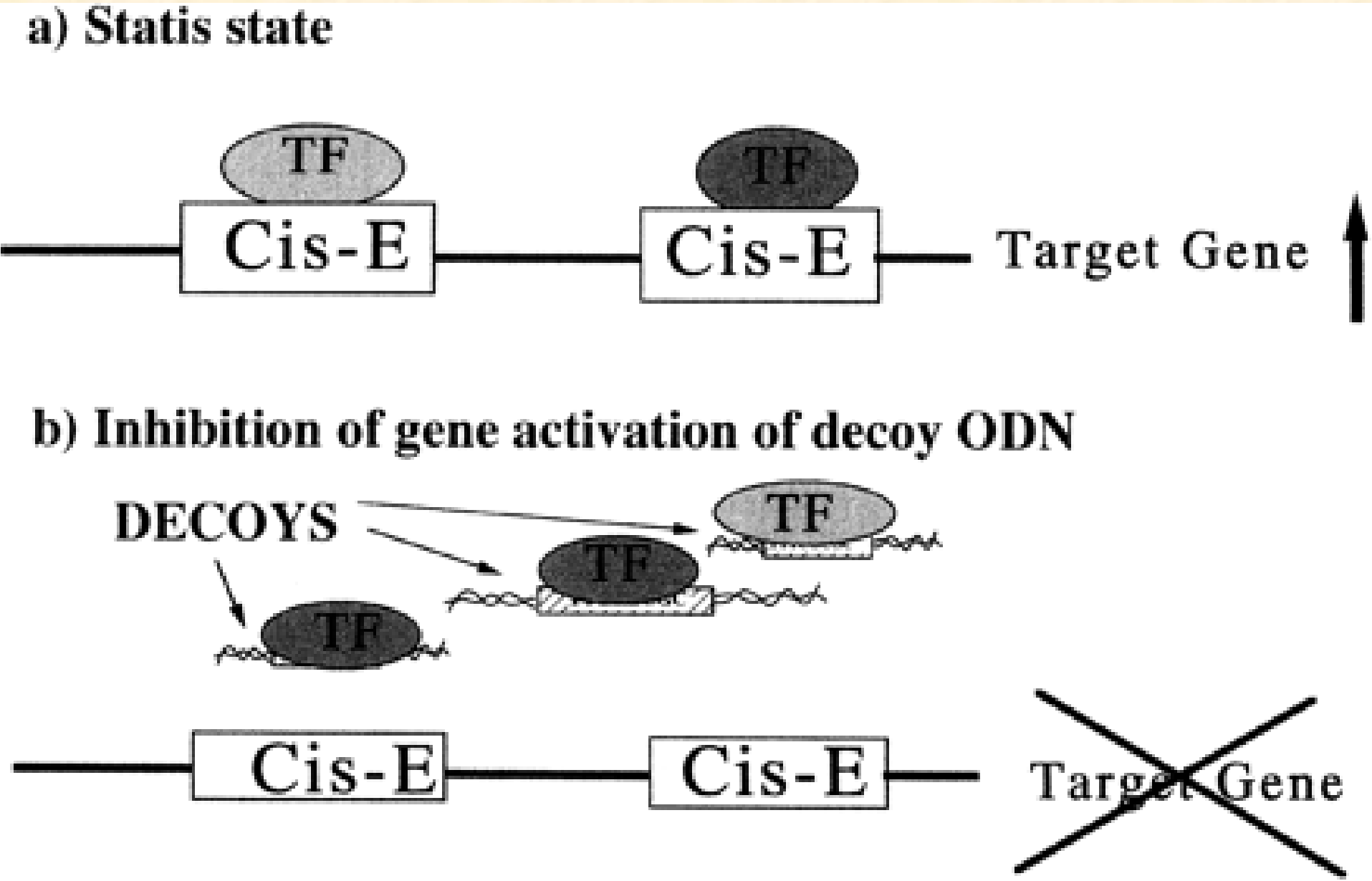
1. DNA decoys – bind transcription factors

2. Pegaptinib sodium – a pegylated oligonucleotide binding $VEGF_{165}$

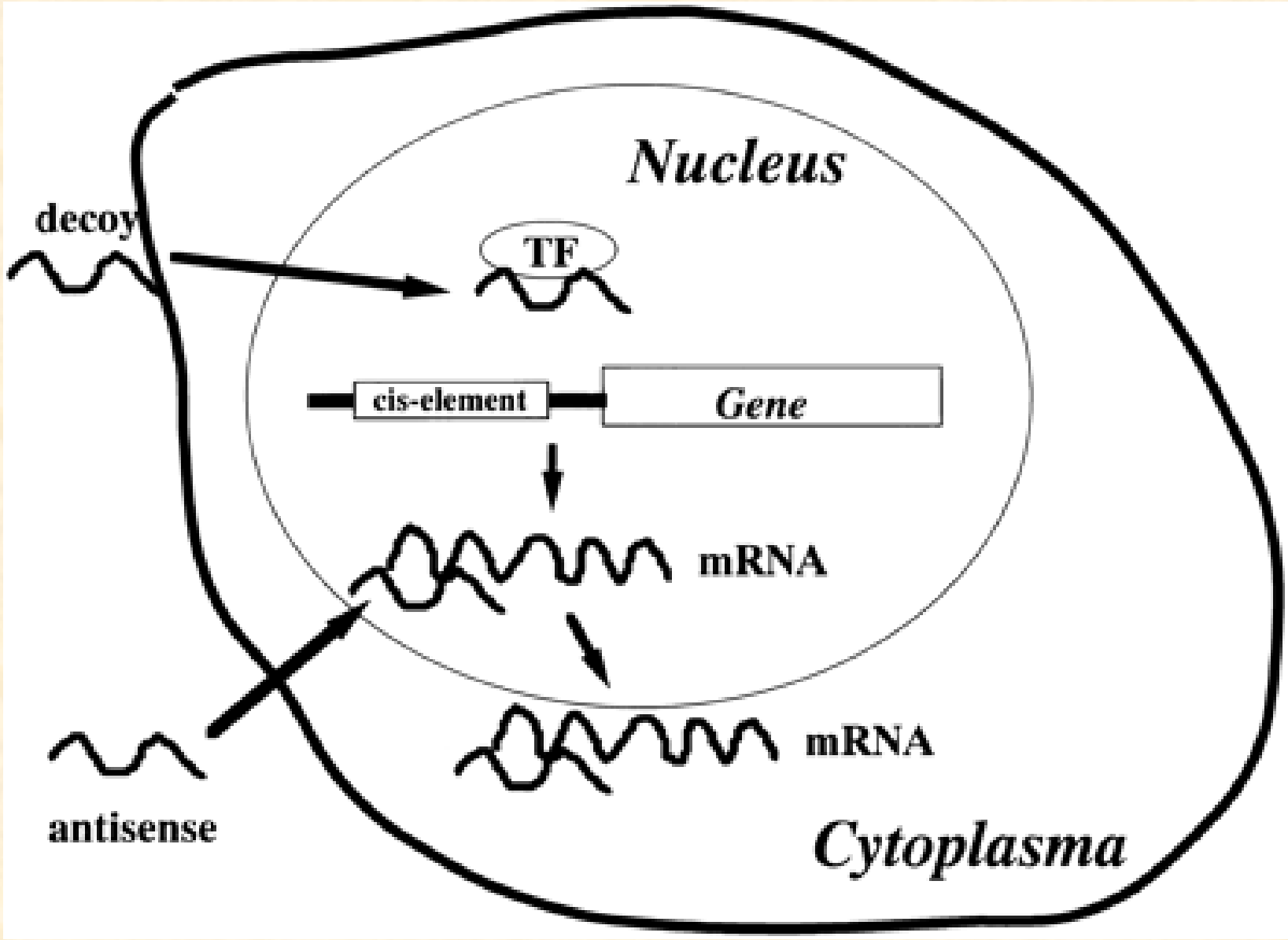
DNA decoys

Pułapki oligonukleotydowe

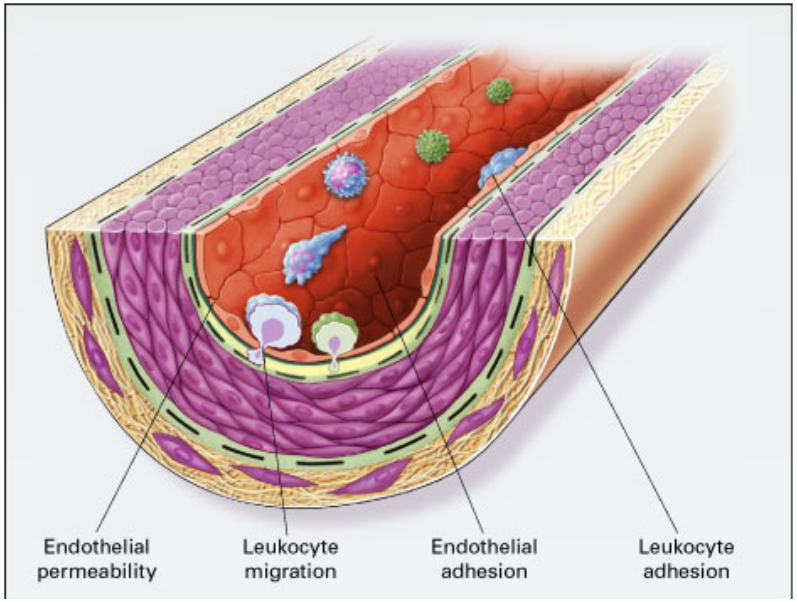
DNA decoys

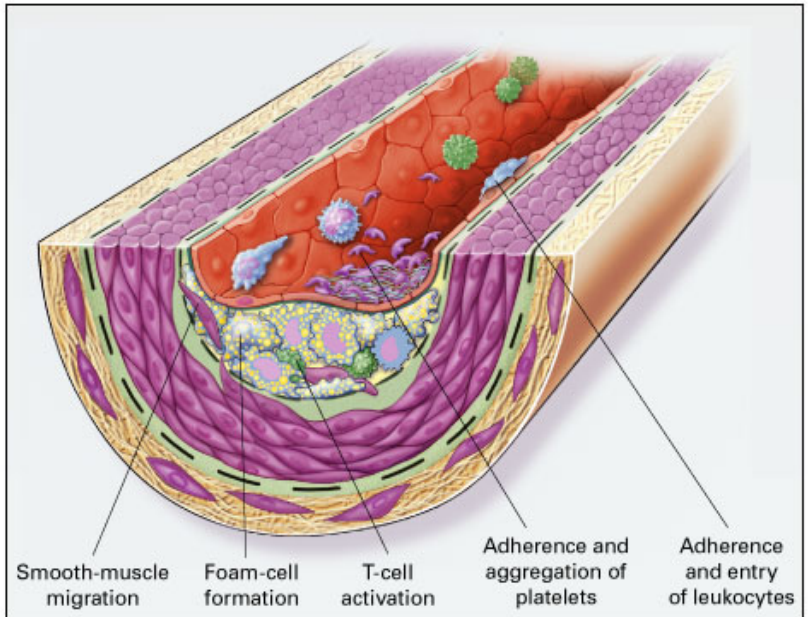

a) Statis state
TF
TF
Cis-E
Cis-E
Target Gene
b) Inhibition of gene activation of decoy ODN
DECOYS
TF
TF
TF
Cis-E
Cis-E
Target Gene

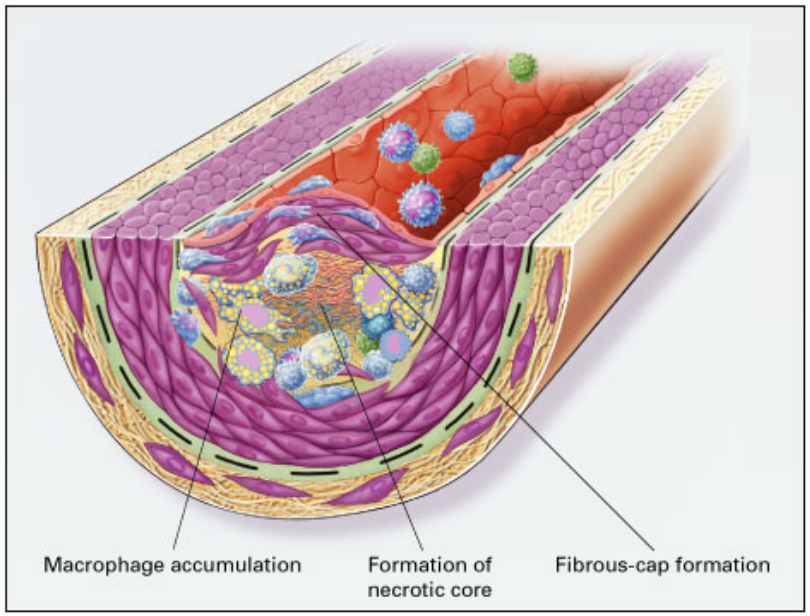
DNA decoys

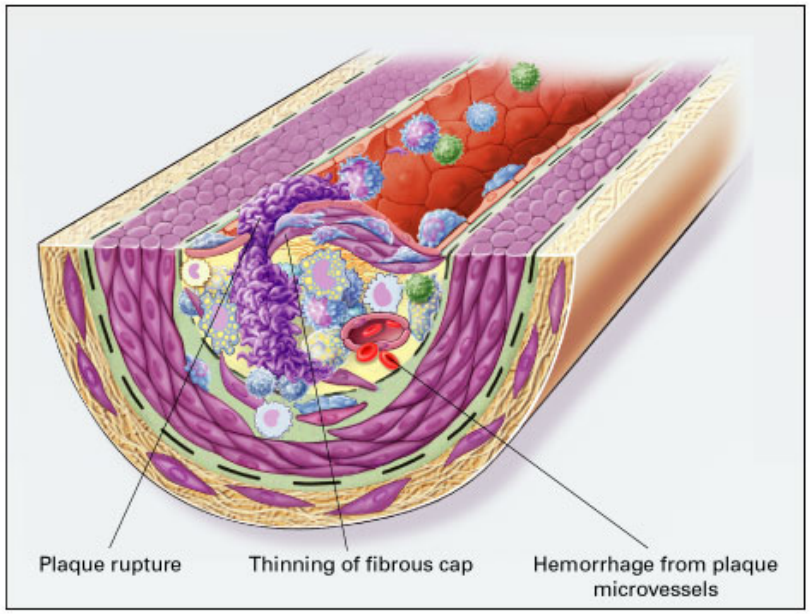

Nucleus
decoy
TF
cis-element
Gene
mRNA
mRNA
antisense
Cytoplasma

Progression of atherosclerosis

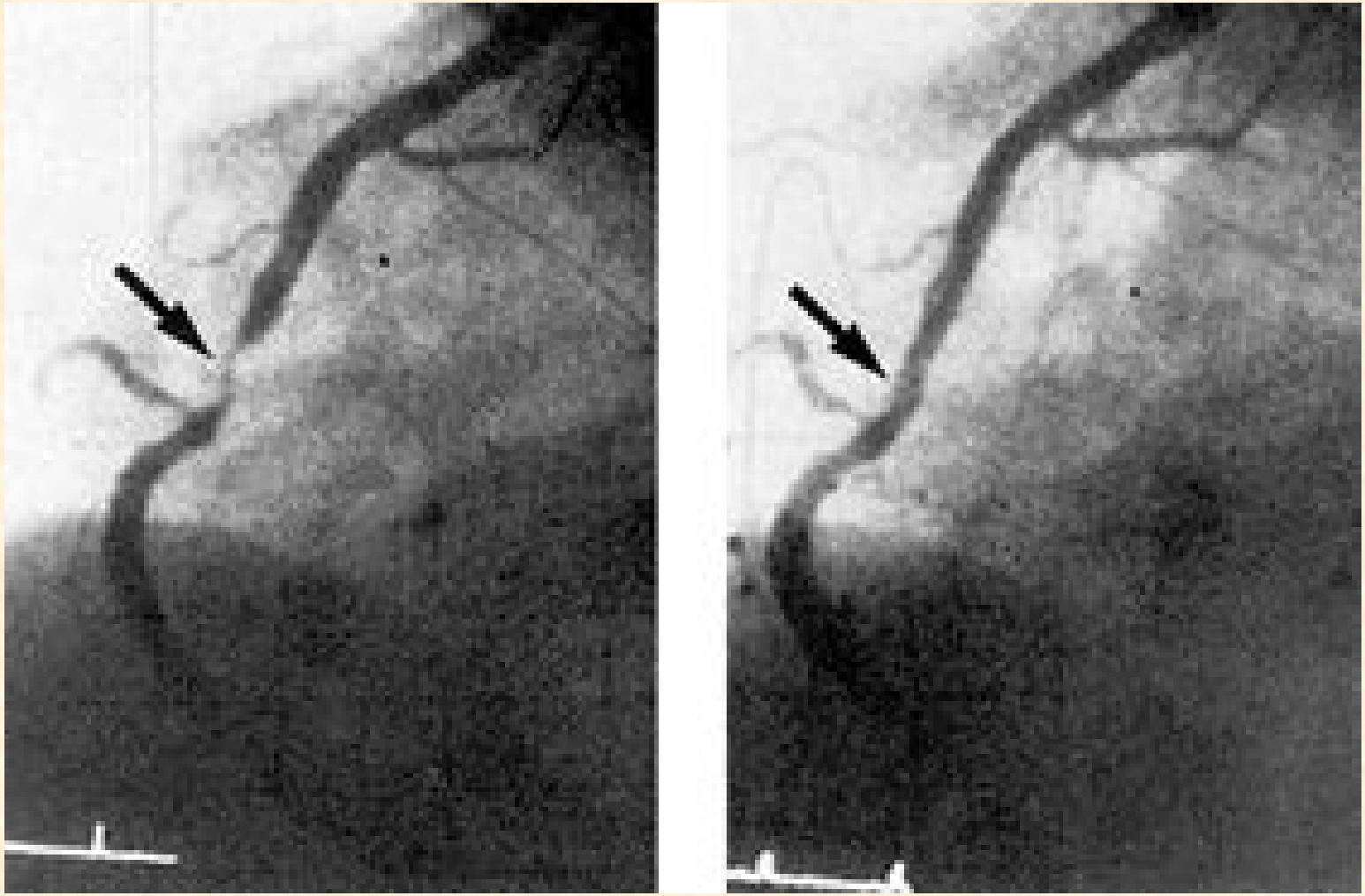


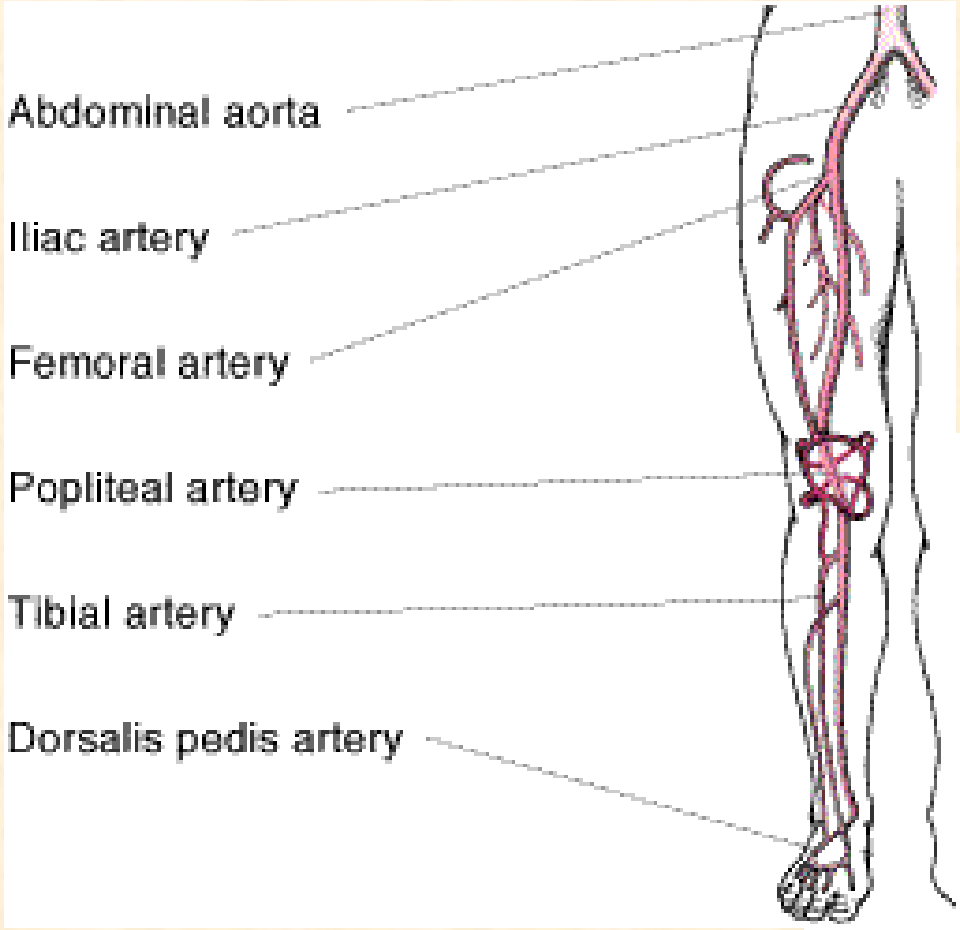


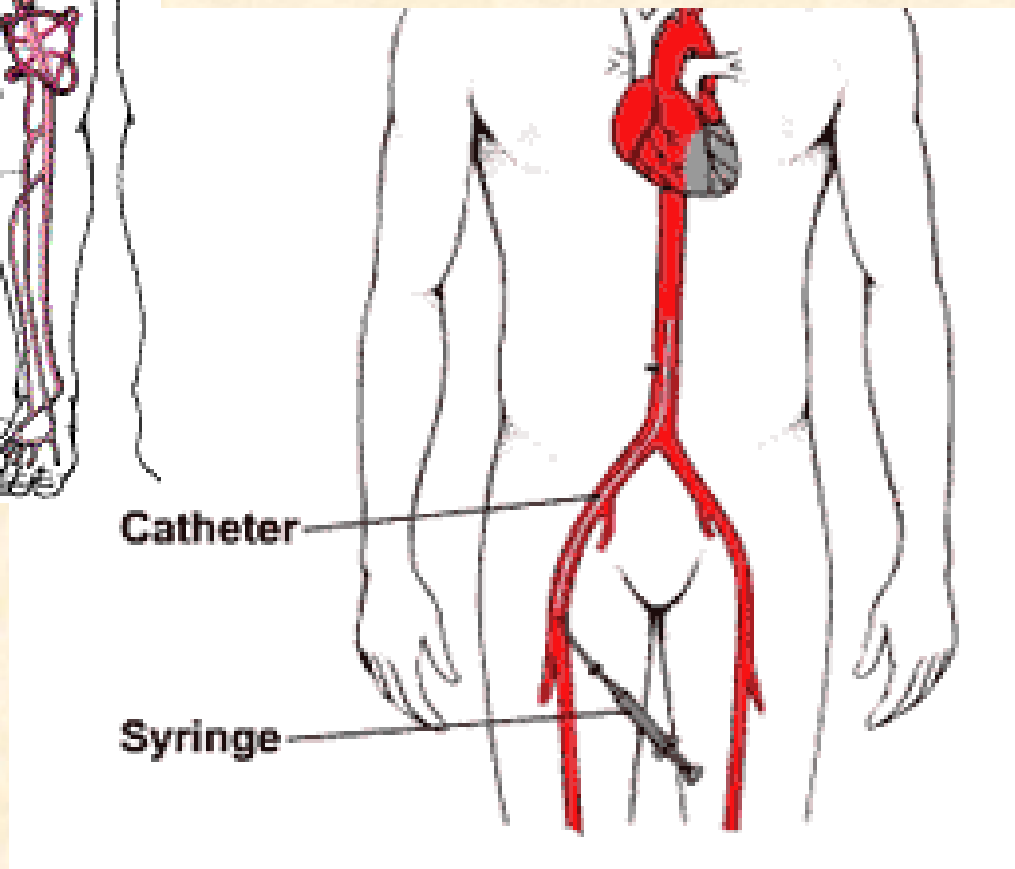




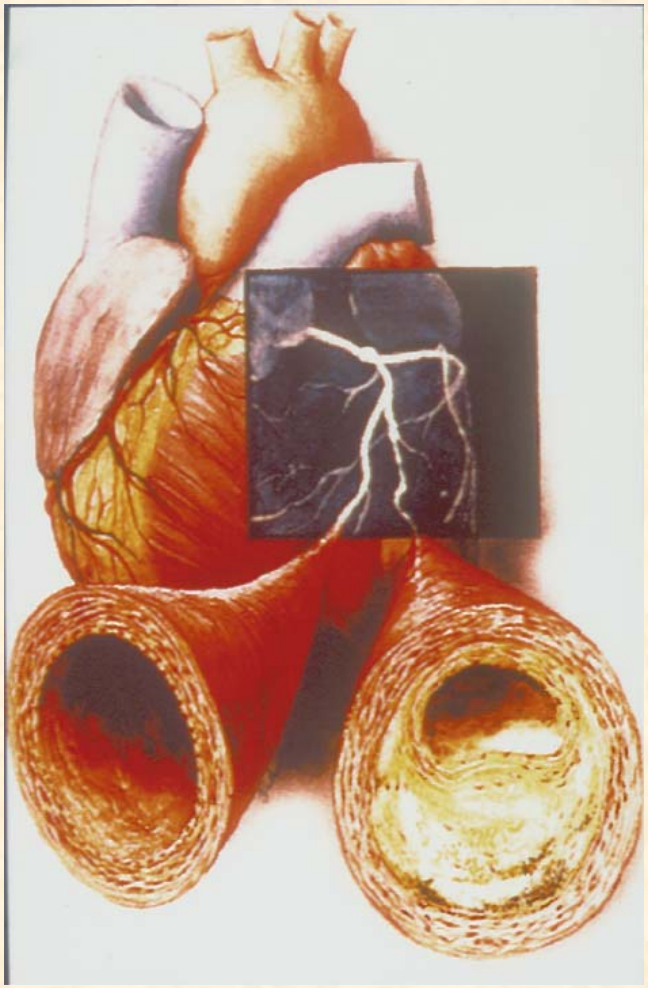
Ross, NEJM, 1999

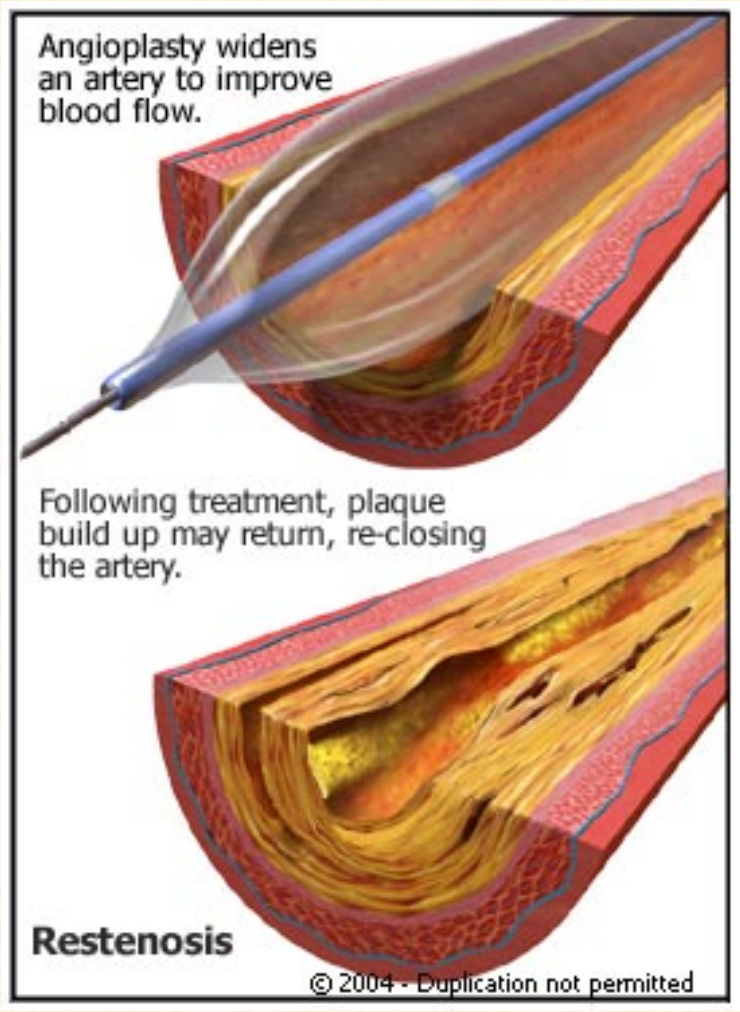



Abdominal aorta
Iliac artery
Femoral artery
Popliteal artery
Tibial artery
Dorsalis pedis artery


Catheter
Syringe

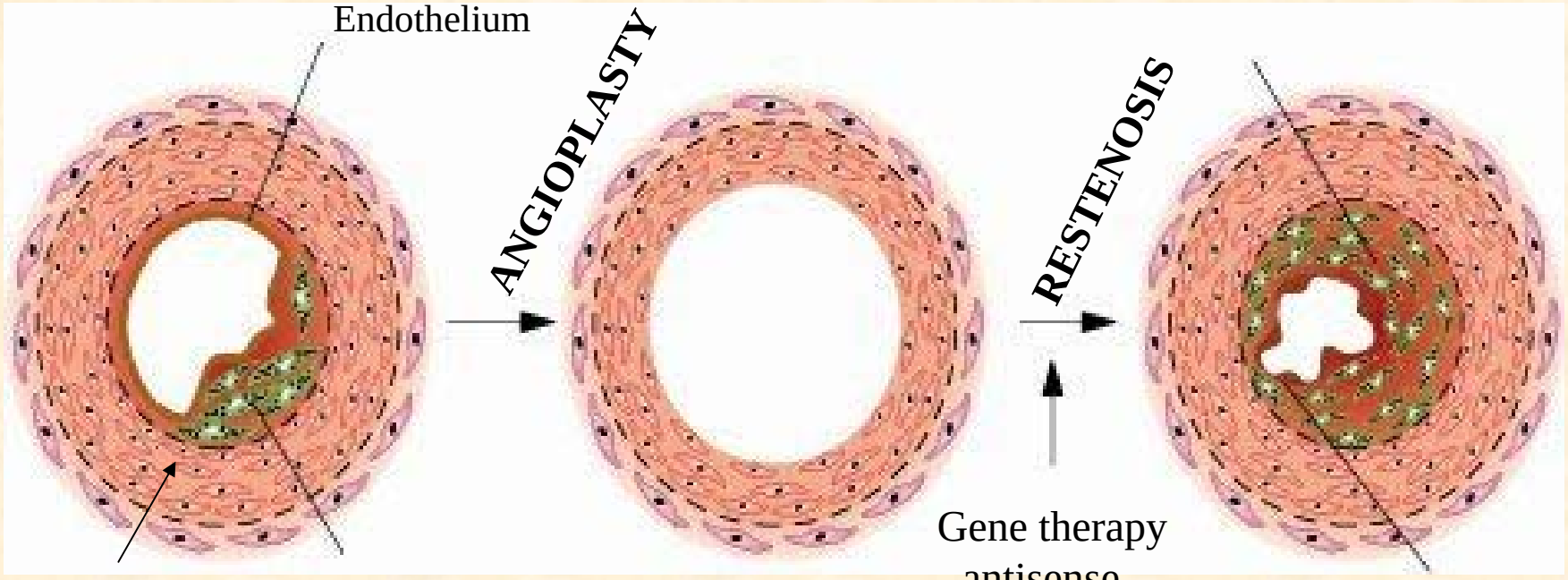
Gene therapy for atherosclerosis



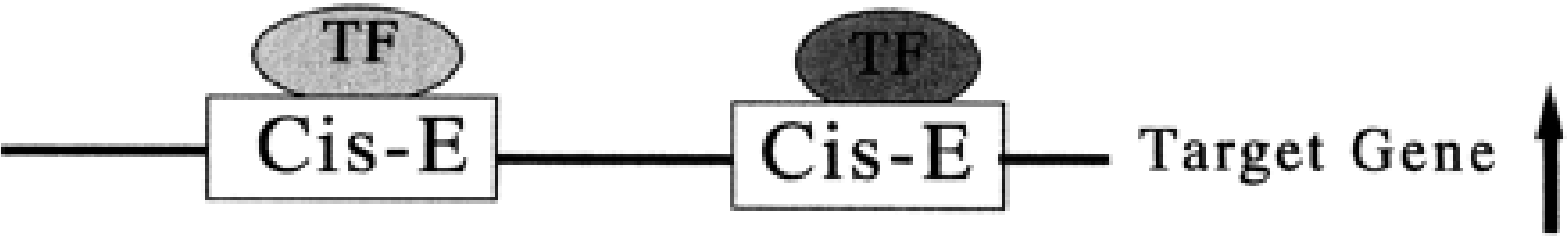


narrowing occurs also in vessels used for by-pass grafting

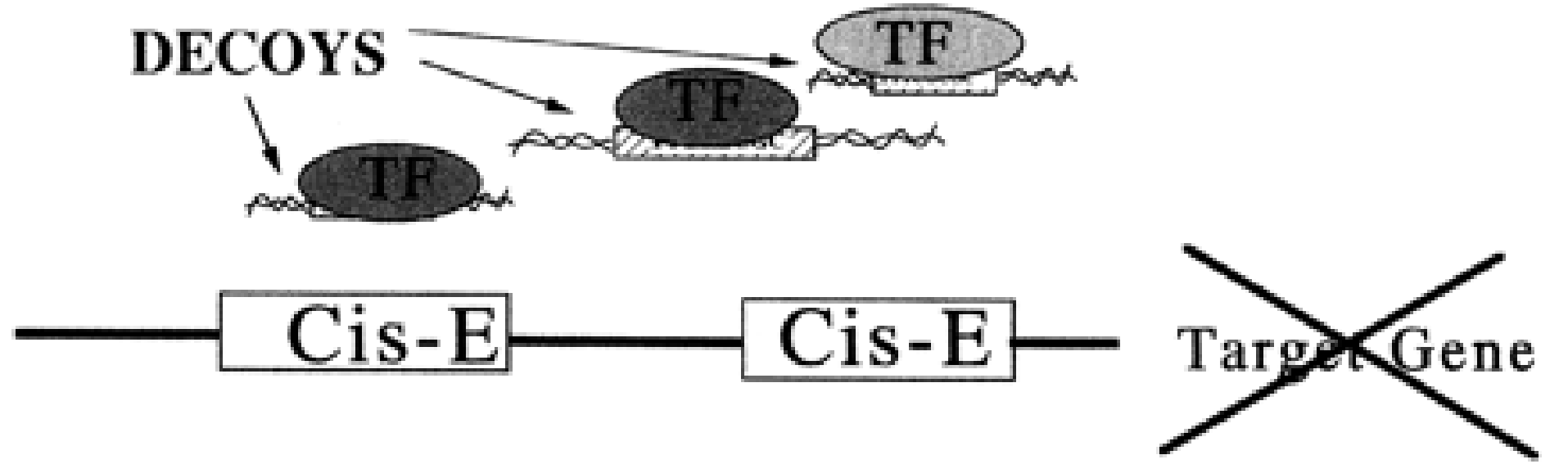
Gene therapy for treatment of neointima formation after balloon angioplasty



a) Statis state



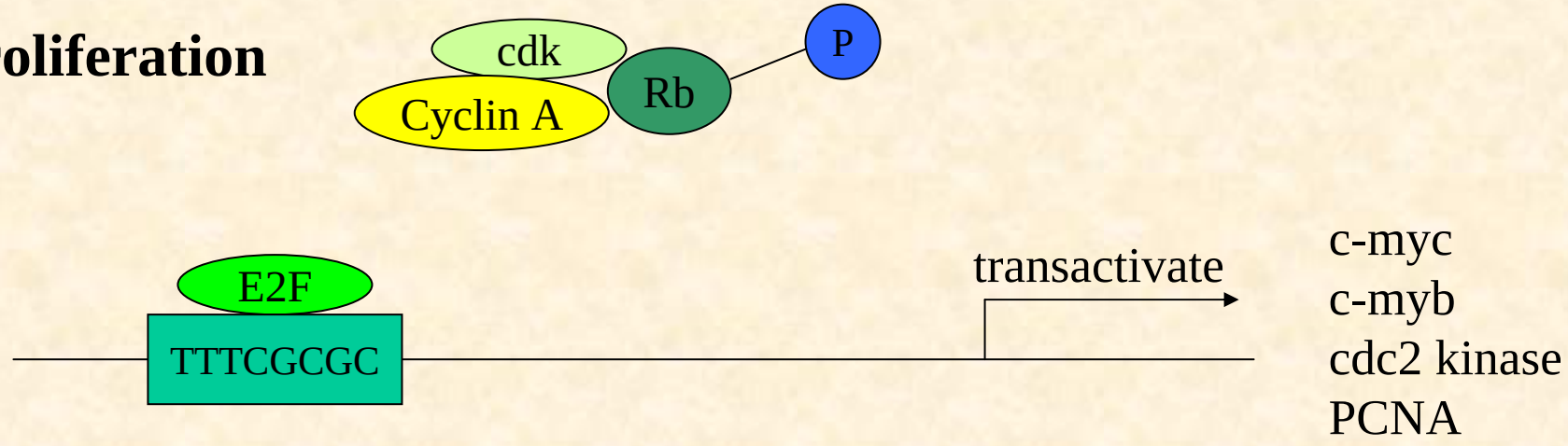
b) Inhibition of gene activation of decoy ODN



Transcription factor E2F

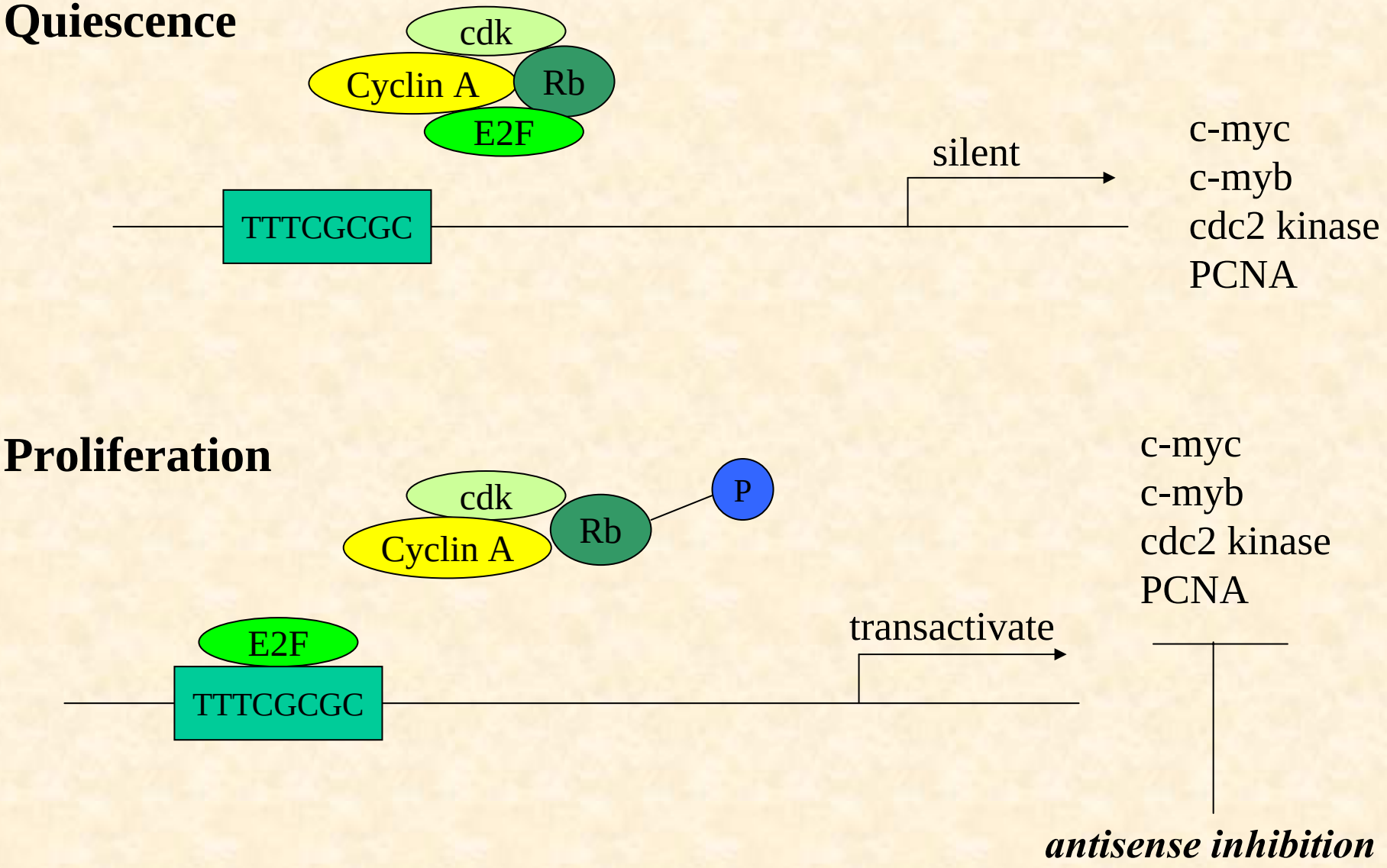
Quiescence

Proliferation



Mann MJ, Mol Med. Today 2000

Inhibition of E2F dependent cell proliferation by antisense oligonucleotides against E2F-downstream genes



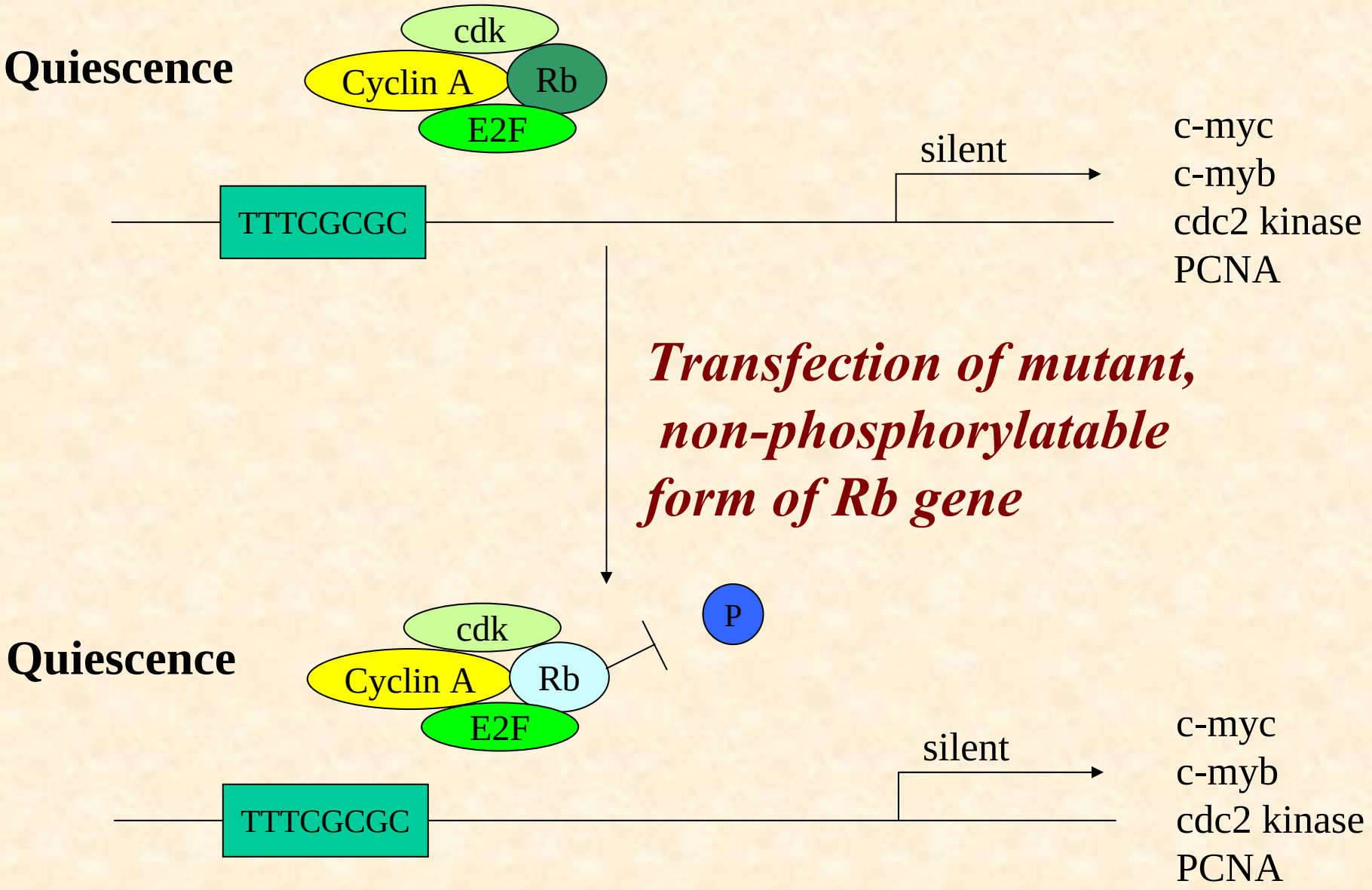
J Clin Invest 1997 Mar 15;99(6):1295-301

Cell cycle inhibition preserves endothelial function in genetically engineered rabbit vein grafts

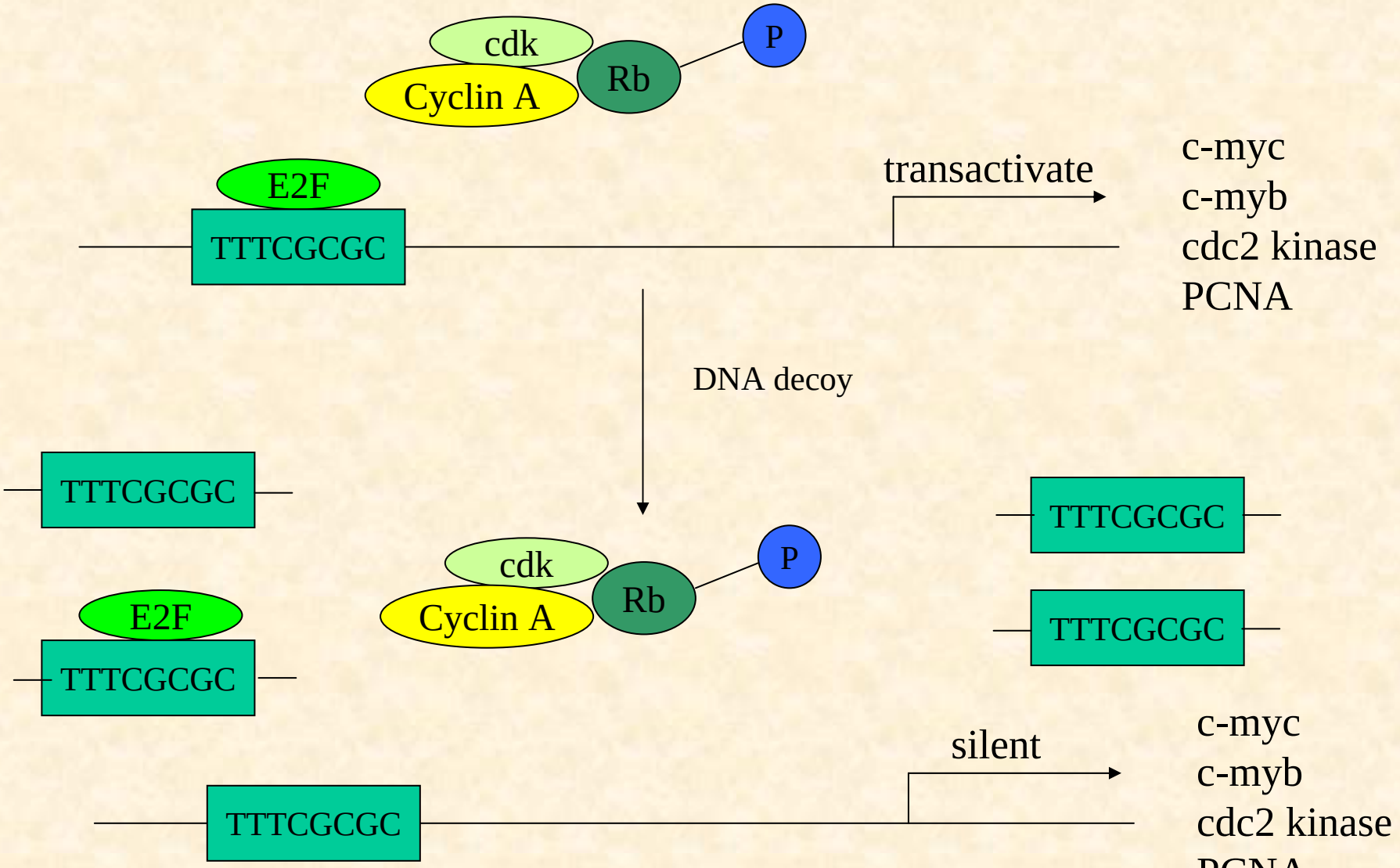
Mann MJ, Gibbons GH, Tsao PS, von der Leyen HE, Cooke JP, Buitrago R, Kernoff R, Dzau VJ.

We have recently shown that ex vivo gene therapy of rabbit autologous vein grafts with antisense oligodeoxynucleotides (AS ODN) blocking cell cycle regulatory gene expression inhibits not only neointimal hyperplasia, but also diet-induced, accelerated graft atherosclerosis. **We observed that these grafts remained free of macrophage invasion and foam cell deposition.** Since endothelial dysfunction plays an important role in vascular disease, the current study examined the effect of this genetic engineering strategy on graft endothelial function and its potential relationship to the engineered vessels' resistance to atherosclerosis. **Rabbit vein grafts transfected with AS ODN against proliferating cell nuclear antigen (PCNA) and cell division cycle 2 (cdc2) kinase elaborated significantly more nitric oxide and exhibited greater vasorelaxation to both calcium ionophore and acetylcholine than did untreated or control ODN-treated grafts**. This preservation of endothelial function was associated with **a reduction in superoxide radical generation, vascular cell adhesion molecule-1 (VCAM-1) expression, and monocyte binding activity in grafts in both normal and hypercholesterolemic rabbits**. Our data demonstrate that AS ODN arrest of vascular cell cycle progression results in the preservation of normal endothelial phenotype and function, thereby influencing the biology of the vessel wall towards a reduction of its susceptibility to occlusive disease.

Inhibition of cell proliferation by overexpression of Rb mutant gene



Inhibition of cell proliferation by E2F decoys



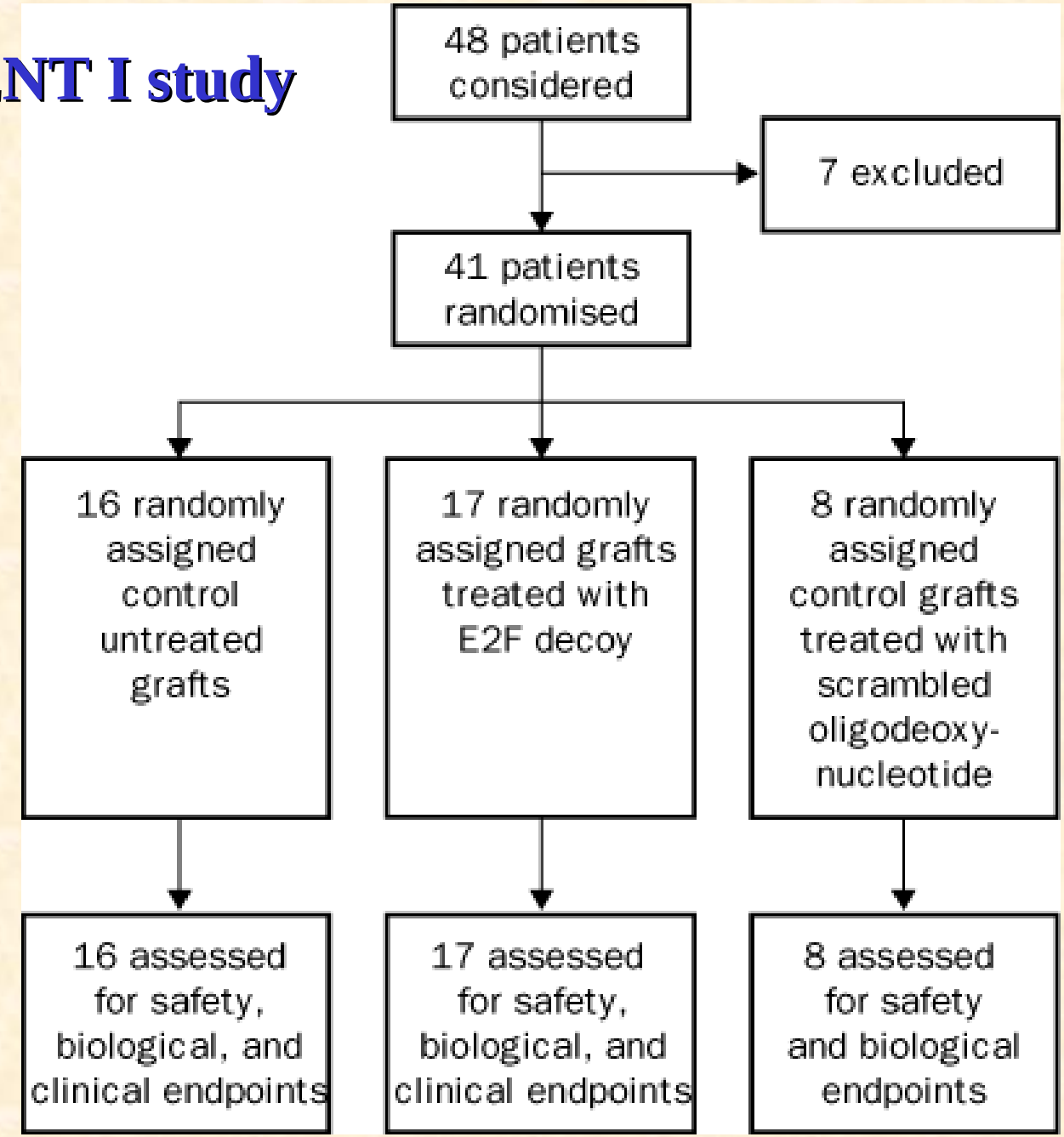
Mann MJ, Mol Med. Today 2000

Edifoligide

A commercial E2F decoy oligonucleotide

Corgentech

PREVENT I study

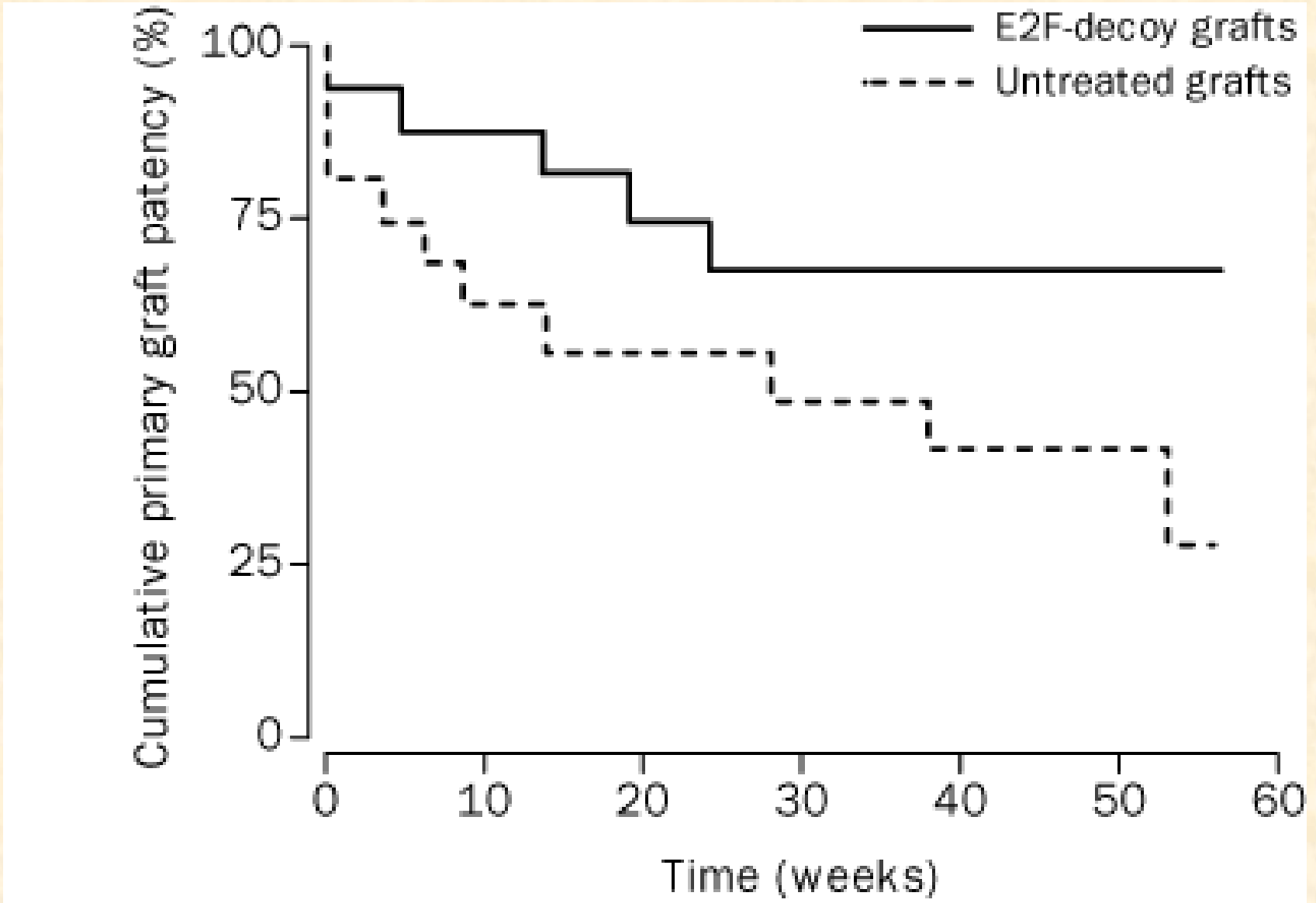


Lancet 1999 Oct 30;354(9189):1493-8

Ex-vivo gene therapy of human vascular bypass grafts with E2F decoy: the PREVENT single-centre, randomised, controlled trial.

Mann MJ, Whittemore AD, Donaldson MC, Belkin M, Conte MS, Polak JF, Orav EJ, Ehsan A, Dell'Acqua G, Dzau VJ.

BACKGROUND: Cell-cycle blockade by ex-vivo gene therapy of experimental vein grafts inhibits the neointimal hyperplasia and subsequent accelerated atherosclerosis that lead to human bypass-graft failure. In a prospective, randomised, controlled trial, we investigated the safety and biological efficacy of intraoperative gene therapy in patients receiving bypass vein grafts. METHODS: We studied gene therapy that uses decoy oligodeoxynucleotide, which binds and inactivates the pivotal cell-cycle transcription factor E2F. **41 patients were randomly assigned untreated (16**), **E2F-decoy-treated (17), or scrambled-oligodeoxynucleotide-treated (eight) human infrainguinal vein grafts. Oligonucleotide was delivered to grafts intraoperatively by ex-vivo pressure-mediated transfection**. The primary endpoints were safety and inhibition of target cell-cycle regulatory genes and of DNA synthesis in the grafts. Analysis was by intention to treat. FINDINGS: **Mean transfection efficiency was 89.0% (SD 1.9). Proliferating-cell nuclear antigen and c-myc mRNA concentrations and bromodeoxyuridine incorporation were decreased in the EF2-decoy group by medians of 73% [IQR 53-84], 70% [50-79], and 74% [56-83], respectively) but not in the scrambled-oligodeoxynucleotide group (p<0.0001).** Groups did not differ for postoperative complication rates. At 12 months, fewer graft occlusions, revisions, or critical stenoses were seen in the E2F-decoy group than in the untreated group (hazard ratio 0.34 [95% CI 0.12-0.99]). INTERPRETATION: Intraoperative transfection of human bypass vein grafts with E2F-decoy oligodeoxynucleotide is safe, feasible, and can achieve sequence-specific inhibition of cell-cycle gene expression and DNA replication. Application of this genetic-engineering strategy may lower failure rates of human primary bypass vein grafting.



Patients at risk

Untreated	16	10	8	7	6	5	4
Treated	17	14	10	8	7	6	6

Genetic Manipulation of Human Coronary Artery Bypass Grafts With E2F Decoy (GT003) Reduces Clinical Graft Failure: Results of the Randomized, Controlled PREVENT II Trial

E Grube, T Felderhoff, PJ Fitzgerald, M Terashima, U Gerckens, EJ Orav, TJ Lorenz, S Iversen

Background: Human coronary artery bypass grafting is limited by neointimal hyperplasia and subsequent accelerated atherosclerosis that lead to vein graft failure rates of 30 - 40%. Gene suppression with E2F decoy (CGT003) that blocks vascular smooth muscle proliferation has prevented vein graft disease in previous pre-clinical and clinical studies (Dzau and Mann, 1999). We examined the effect of E2F blockade on coronary vein graft patency at 12 months in a randomized, double blind study. **Methods:** Vein grafts in 200 CABG patients received either non-distending pressure-mediated transfection (6 psi for 10 minutes) with CGT003 or placebo. Quantitative angiography and intravascular ultrasound (IVUS) was performed 12 months after enrollment in 136 patients (61 placebo, 75-CGT003) who received 309 grafts. Graft failure was defined as =" border=0 src="/math/ge.gif" 75% stenosis. **Results: CGT003 was associated with a 30% relative reduction in a composite index of vein graft failure and death** (*P* = 0.034). Furthermore, vessel wall volume/50mm measured by IVUS was similarly reduced by 30% (78.6±45.6 mm^3 vs 114.8±78.3 mm^3, *P* = 0.031), reflecting an alteration of graft wall adaptation that parallels the inhibition of atherosclerosis seen in animal models. **Conclusions:** The results from this first randomized, controlled study of genetic manipulation of coronary bypass grafts suggest that E2F decoy may reduce the long term morbidity and mortality associated with currently high rates of human coronary artery vein graft failure.

PRVENT Clinical Trials

In the phase I, or **PREVENT I, trial**, 33 patients who were undergoing lower-extremity bypass with an autologous vein received grafts treated with either edifoligide or saline. The randomized, double-blind trial showed that about 90% of the cells in small segments of the vein treated with edifoligide took in the decoy molecule. Edifoligide also inhibited specific cell-cycle genes and reduced the proliferation of smooth muscle cells in the graft *(Lancet 1999;354:1493-8*

The drug continued to show potential in **PREVENT II**, a double-blind, randomized, phase II study of 200 patients who were undergoing a cardiac artery bypass graft. Grafts treated with edifoligide had a 30% lower rate of vessel lumen occlusion of 75% or more, compared with those treated with saline, according to results from coronary angiography. In intravascular ultrasound images, total wall volume of the grafts treated with edifoligide declined by a significant 30%, compared with those that received saline.

Based on the results of those trials and the significant unmet clinical need, the Food and Drug Administration gave a fast-track designation for phase III trials to test edifoligide for the prevention of vein graft failure

Efficacy and safety of edifoligide, an E2F transcription factor decoy, for prevention of vein graft failure following coronary artery bypass graft surgery: PREVENT IV: a randomized controlled trial.

Alexander JH et al., PREVENT IV Investigators

JAMA 2005, November, 294: 2495-2497

Failure of phase III trials with E2F decoys...

according to recent statements issued by Corgentech, edifoligide failed to show any benefit for primary and secondary end points in two phase III trials...

In the **PREVENT III** trial of 1,404 patients with critical limb ischemia who needed peripheral artery bypass graft surgery, there was no difference between edifoligide and placebo on the primary end point of limb amputation. No differences were seen in secondary end points of critical graft stenosis, recurrent limb ischemia, or quality of life.

Similar results were reported in the **PREVENT IV trial**, which tested edifoligide against placebo in 3,014 patients for the prevention of vein graft failure after coronary artery bypass surgery.

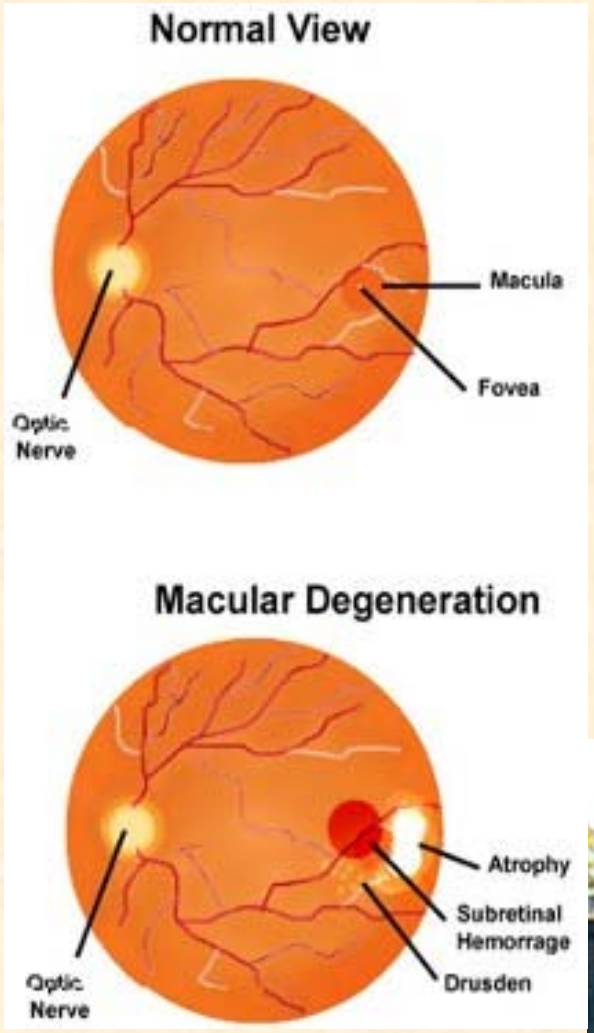
Reasons?

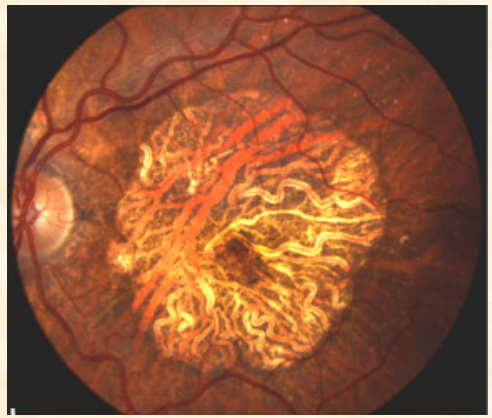
Multiple isoforms of E2F exist, and the drug may not have inhibited them all. Edifoligide's pharmacokinetics may not have allowed it to inhibit E2F adequately

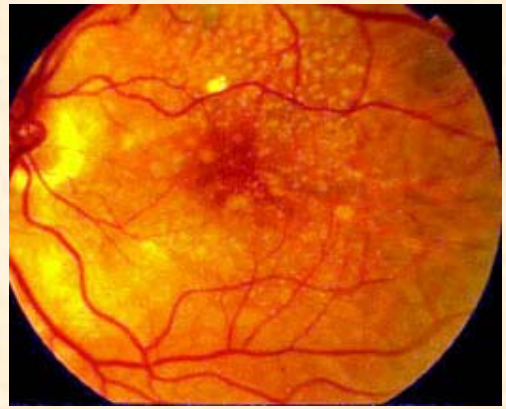
Anti-angiogenic therapy

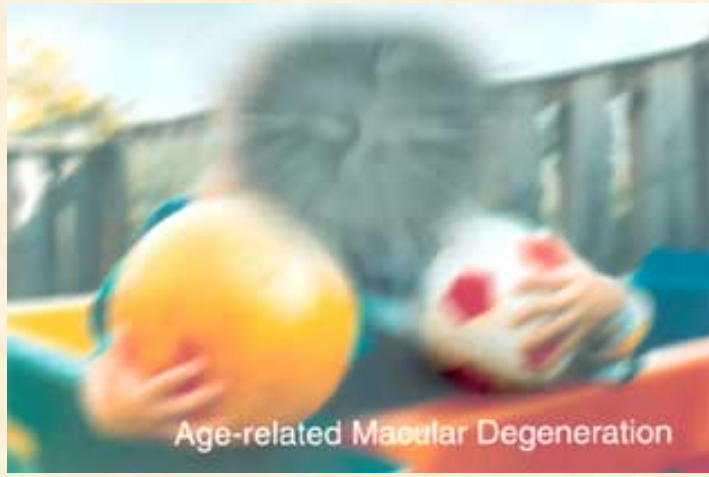
Macular degeneration

DMB

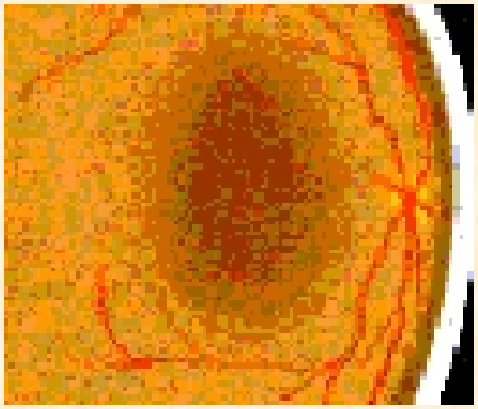




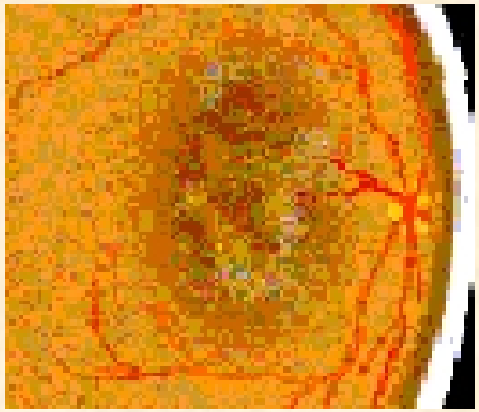




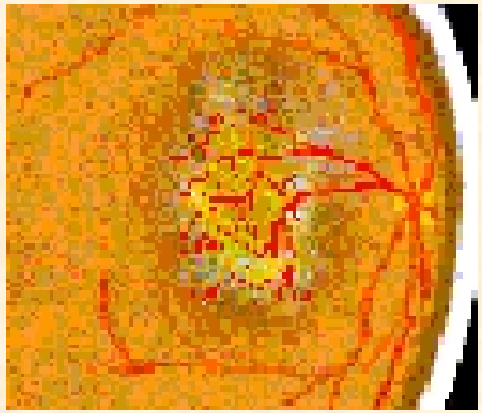
Age-related macular degeneration (AMD)



Normal Macula



Dry AMD:Drusen formation under the Macula



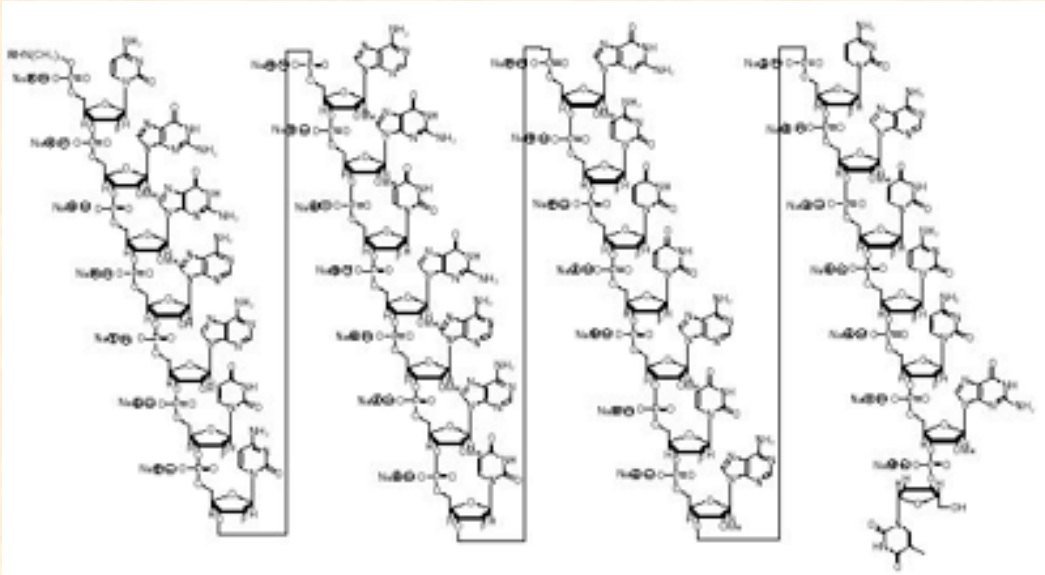
Wet AMD:Macula with abnormal blood vessels

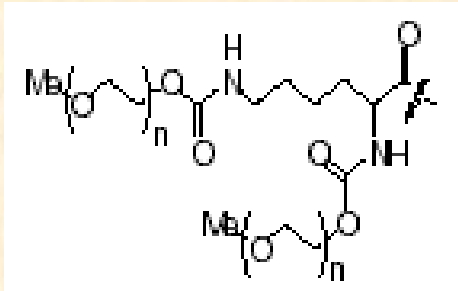
MACUGEN (PEGAPTANIB)

Pegaptinib sodium is a pegylated oligonucleotide aptamer that binds to and inactivates $VEGF_{165}$

Pegaptanib sodium

Pegaptanib sodium is a covalent conjugate of an oligonucleotide of twenty-eight nucleotides in length that terminates in a pentylamino linker, to which two 20-kilodalton monomethoxy polyethylene glycol (PEG) units are covalently attached via the two amino groups on a lysine residue.





Pegaptanib sodium

The chemical name for pegaptanib sodium is as follows: RNA, ((2'-deoxy-2'-fluoro)C-Gm-Gm-A-A-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2' -fluoro)C-Am-Gm-(2'-deoxy-2-fluoro)U-Gm-Am-Am-(2'-deoxy-2' -fluoro)U-Gm-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)U-Am-(2'-deoxy-2' -fluoro)U-Am-(2'-deoxy-2'-fluoro)C-Am-(2'-deoxy-2'-fluoro)U-(2'-deoxy-2'-fluoro)C-(2'-deoxy-2' -fluoro)C-Gm-(3'® 3')-dT), 5'-ester with a ,a '-[4,12-dioxo-6-[[[5-(phosphoonoxy)pentyl]amino]carbonyl]-3,13-dioxa-5,11-diaza-1,15-pentadecanediyl]bis[w -methoxypoly(oxy-1,2-ethanediyl)], sodium salt.

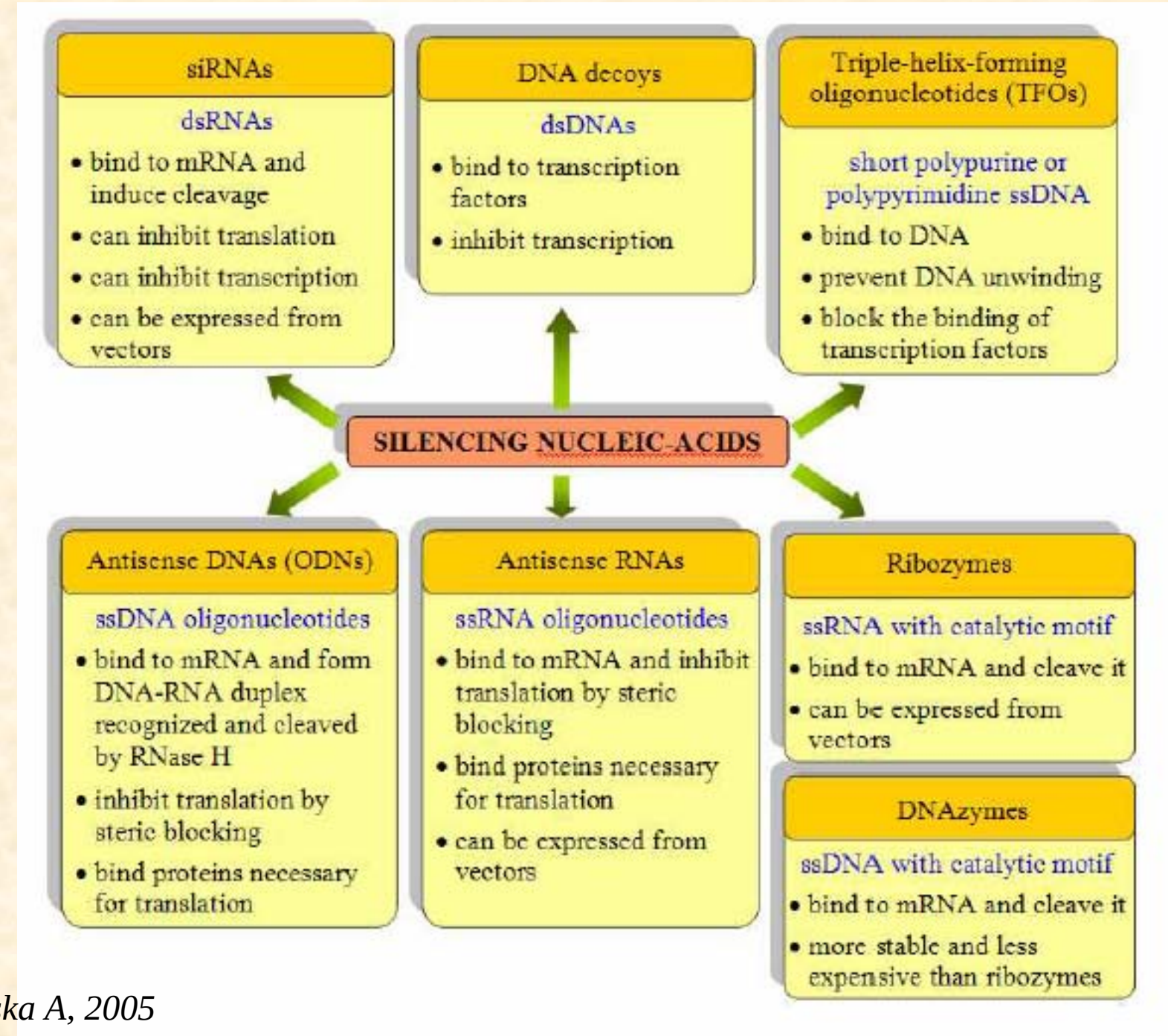
The molecular formula for pegaptanib sodium is
$C_{294}H_{342}F_{13}N_{107}Na_{28}O_{188}P_{28}$ $[C_2H_4O]n$

(where n is approximately 900)
and the molecular weight is approximately 50 kilodaltons.

Macugen was demonstrated to be effective in prevention of vision loss in two large clinical trials in patients with AMD

Clinical Studies of Anti-VEGF Therapy for Neovascular AMD: Pegaptinib and Ranibizumab

Pegaptinib sodium injection (Macugen®) and ranibizumab (Lucentis®) are the first ocular anti-VEGF treatments evaluated in large, randomized, controlled clinical trials for the treatment of neovascular AMD. Both are administered locally by intravitreal injection into the back of the eye.



Zagórska A, 2005