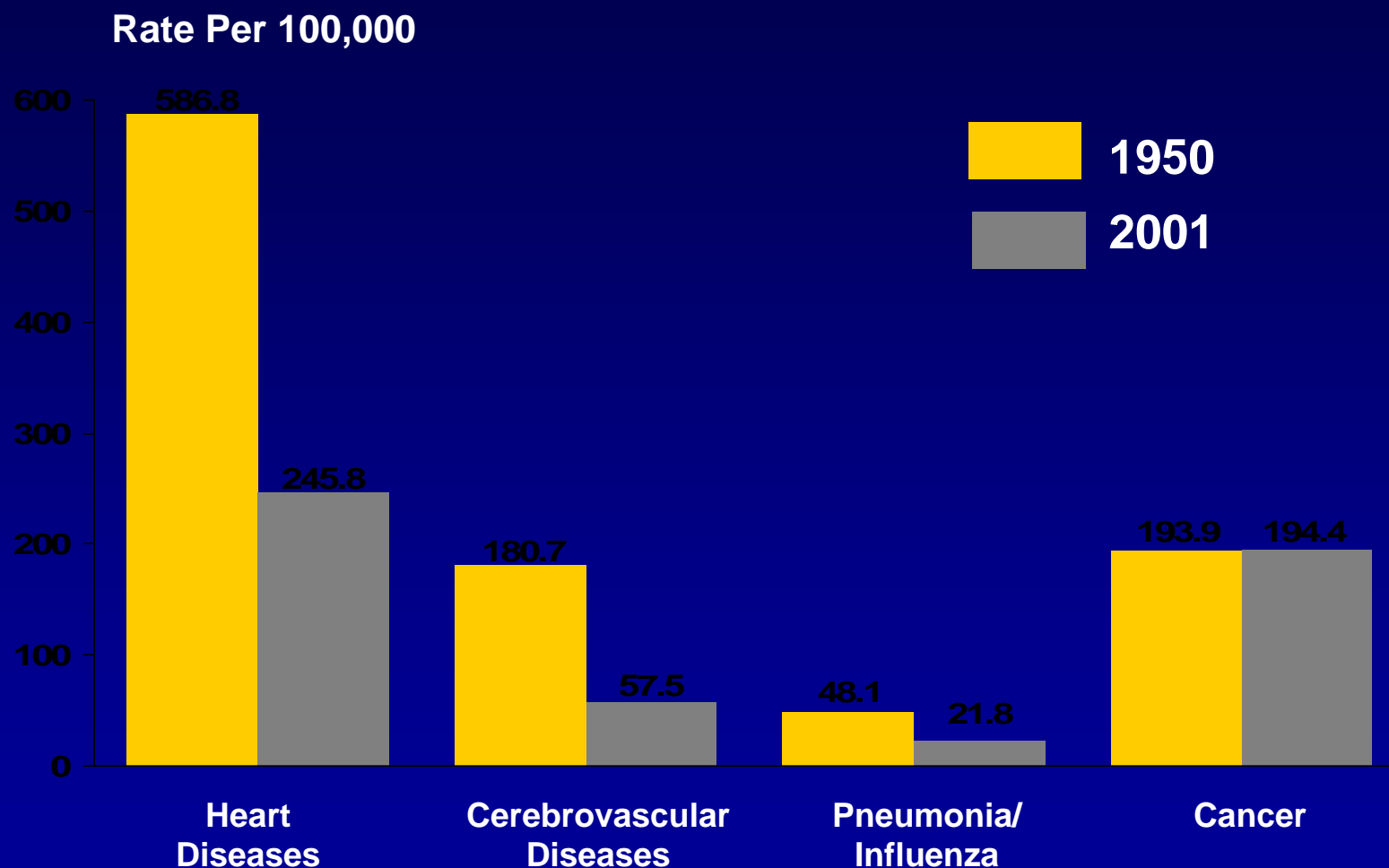


New Treatments for Lung Cancer

Donald M. Miller, M.D., Ph.D.

10/30/04

Change in the US Death Rates* by Cause, 1950 & 2001



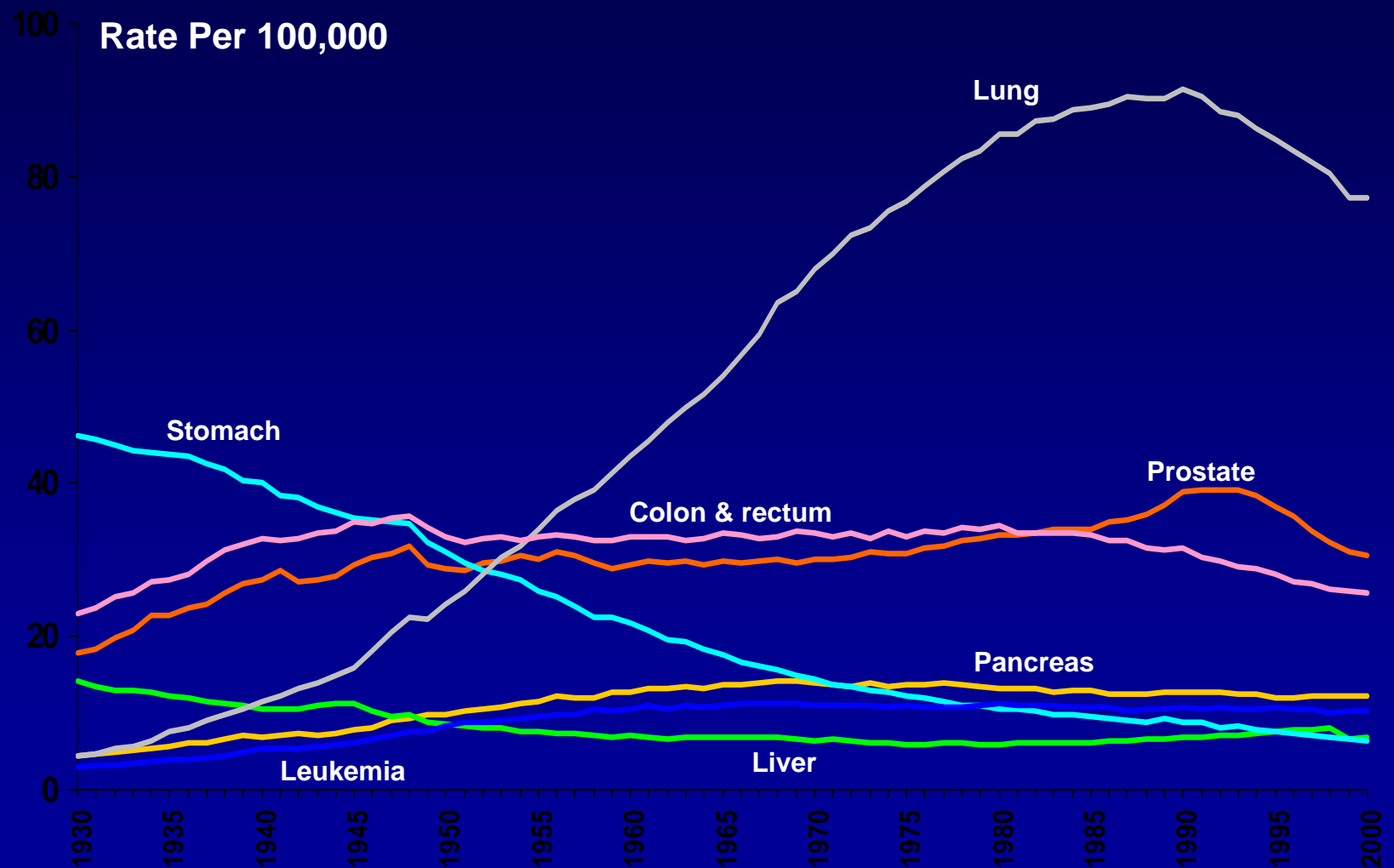
* Age-adjusted to 2000 US standard population.

Sources: 1950 Mortality Data - CDC/NCHS, NVSS, Mortality Revised.

2001 Mortality Data - NVSR-Death Final Data 2001 - Volume 52, No. 3.

http://www.cdc.gov/nchs/data/nvsr/nvsr52/nvsr52_03.pdf

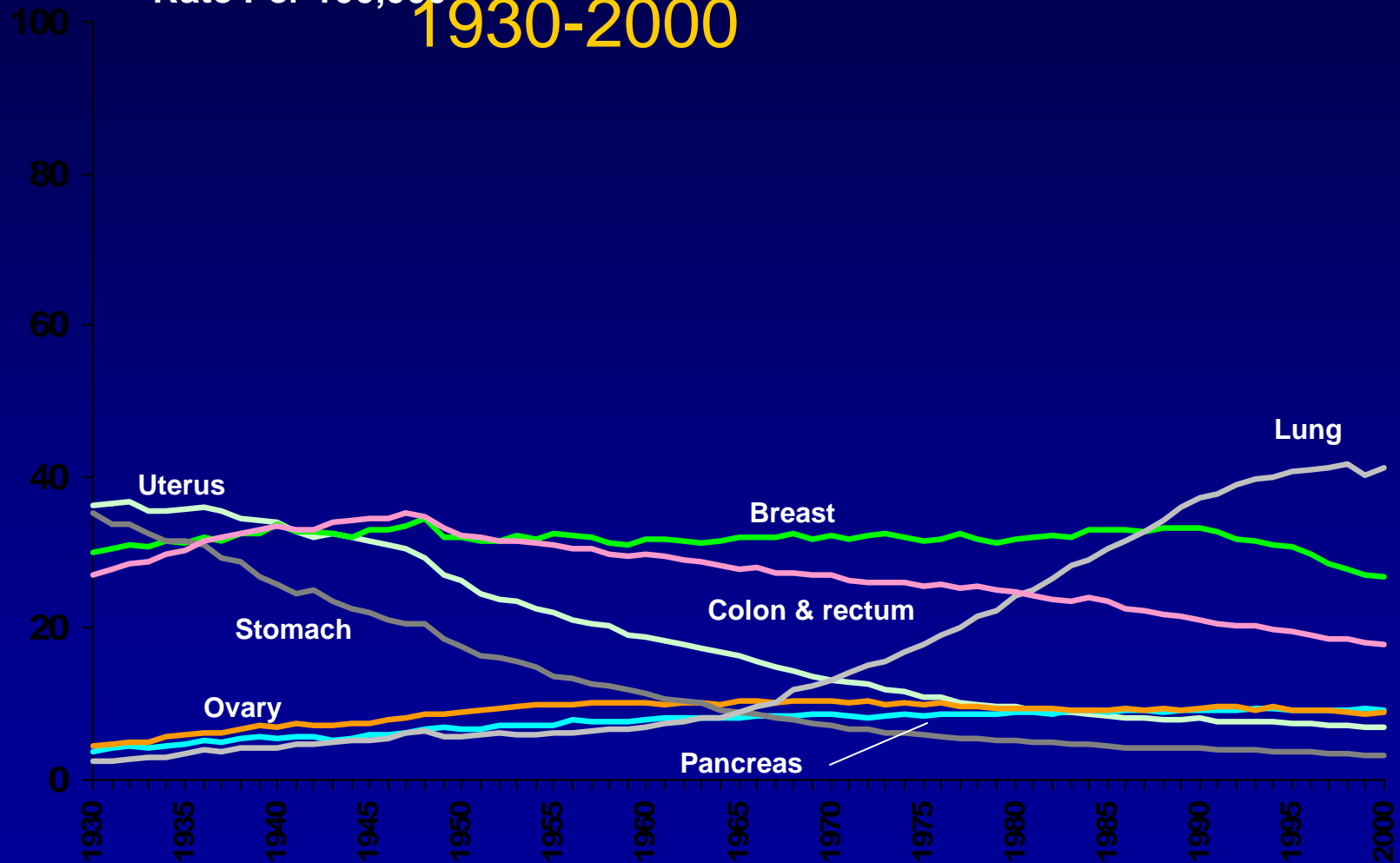
Cancer Death Rates*, for Men, US, 1930-2000



*Age-adjusted to the 2000 US standard population.

Source: US Mortality Public Use Data Tapes 1960-2000, US Mortality Volumes 1930-1959, National Center for Health Statistics, Centers for Disease Control and Prevention, 2003.

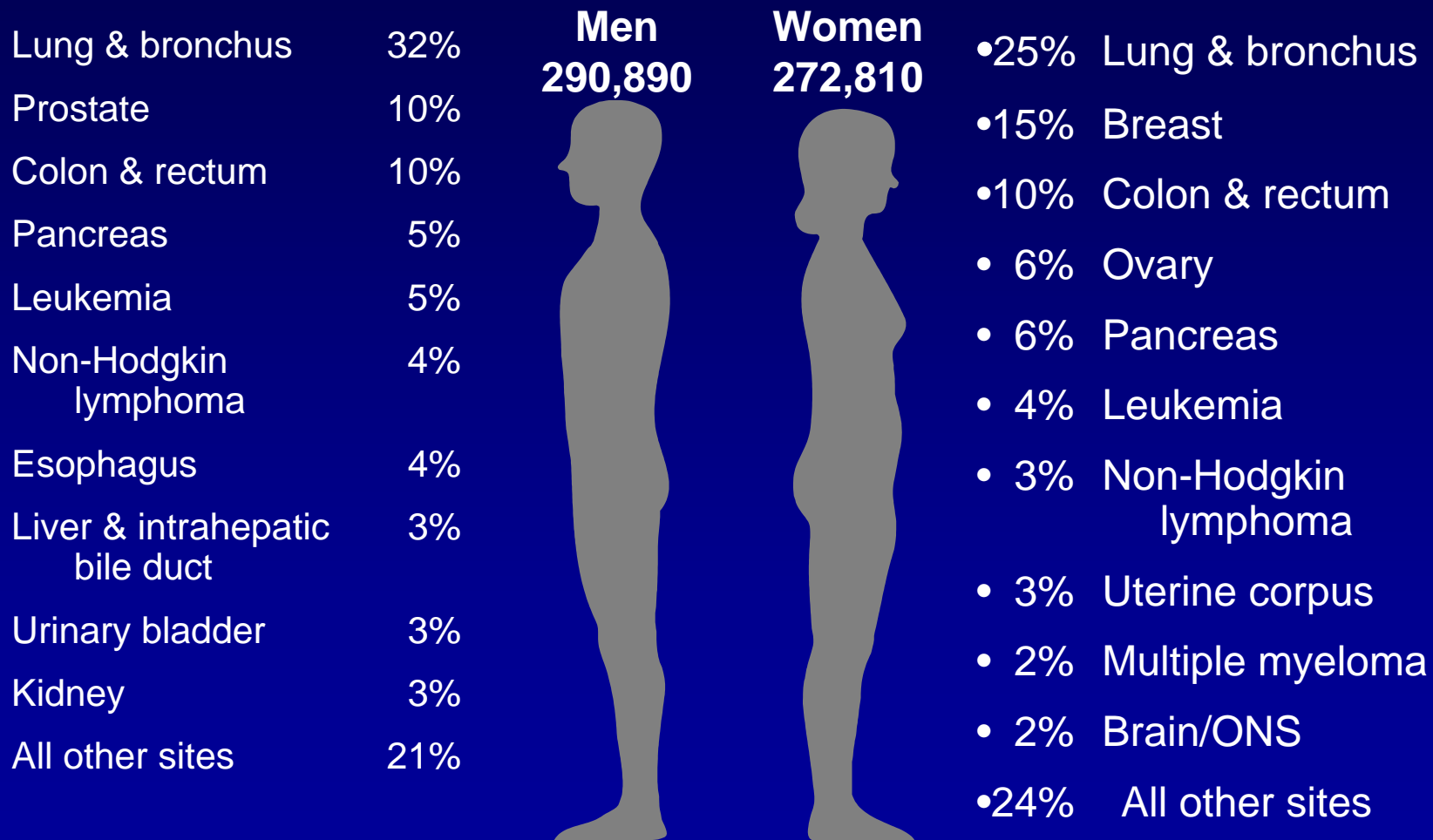
Cancer Death Rates*, for Women, US, Rate Per 100,000 1930-2000



*Age-adjusted to the 2000 US standard population.

Source: US Mortality Public Use Data Tapes 1960-2000, US Mortality Volumes 1930-1959, National Center for Health Statistics, Centers for Disease Control and Prevention, 2003.

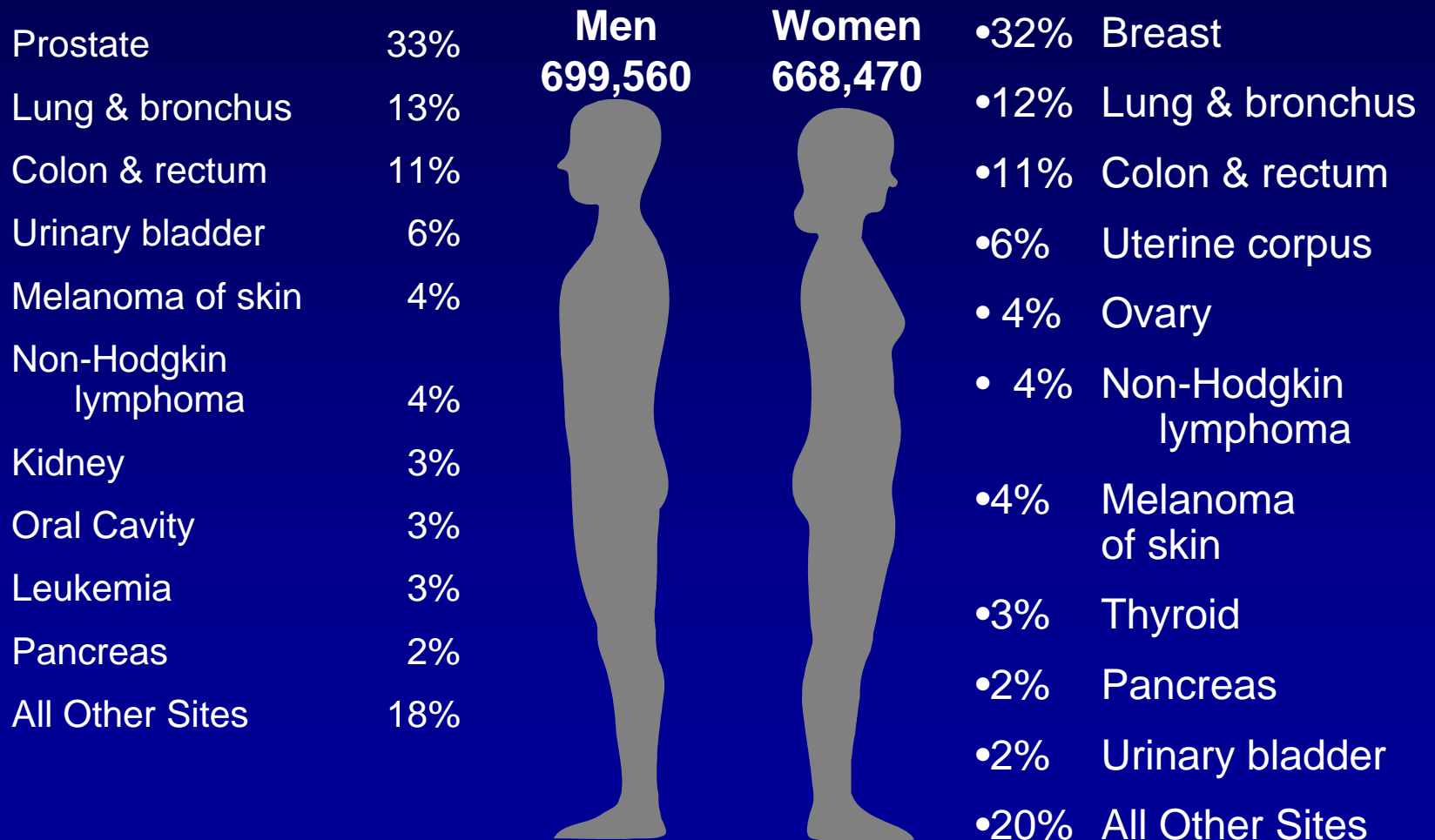
2004 Estimated US Cancer Deaths*



ONS=Other nervous system.

Source: American Cancer Society, 2004.

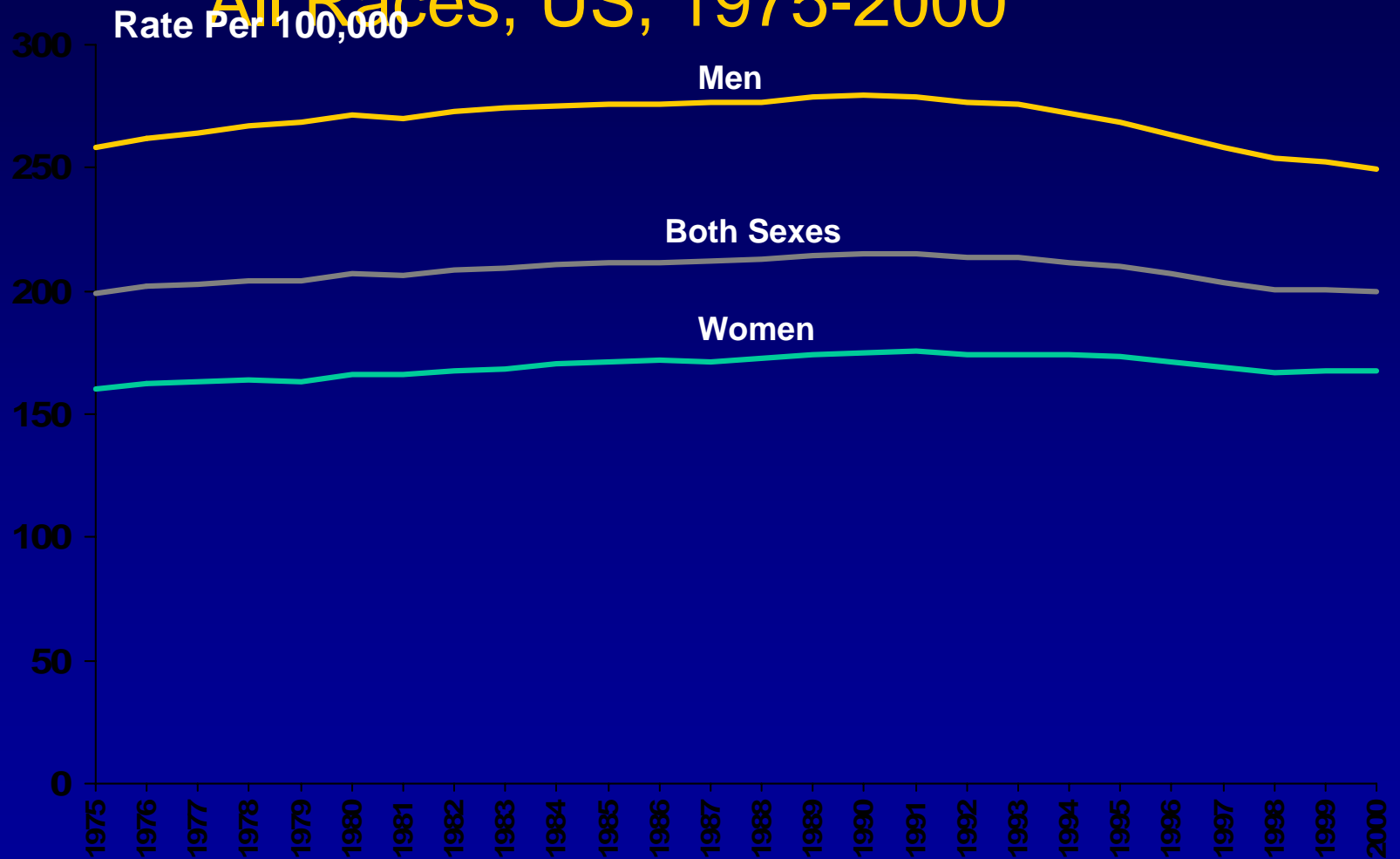
2004 Estimated US Cancer Cases*



*Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.

Source: American Cancer Society, 2004.

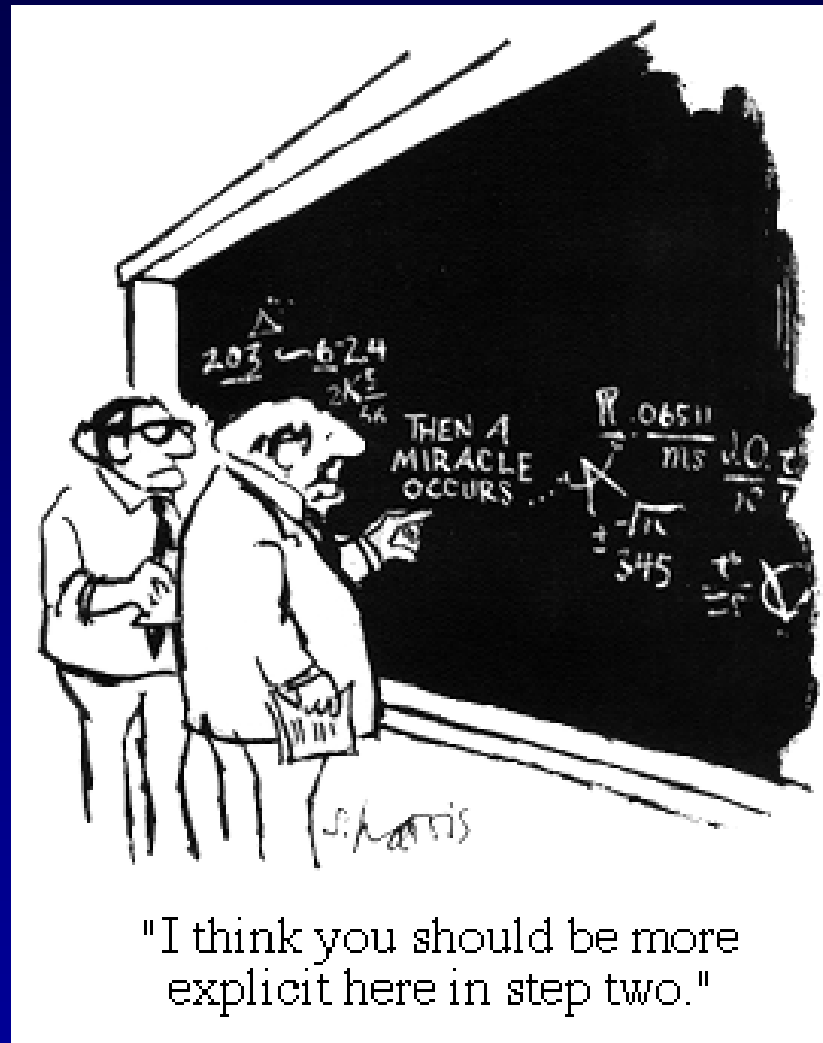
Cancer Death Rates*, All Sites Combined, All Races, US, 1975-2000



*Age-adjusted to the 2000 US standard population.

Source: Surveillance, Epidemiology, and End Results Program, 1975-2000, Division of Cancer Control and Population Sciences, National Cancer Institute, 2003.

Cytotoxic Drugs



"They do more good than harm so use them"
most of the time

New Targeted Therapies

- EGFr Targeted Treatments
 - Iressa
 - Erbitux
- Antiangiogenic Therapies
 - Avastin
- Gene Therapies
 - P53 Adenovirus (Invitrogen)

Oligonucleotide therapeutics

Antigene Strategy

- Targets genomic DNA, *e.g. c-myc, c-ras, HIV genes*
- Sequence-specific tripe helix formation

Decoy Strategy

- Targets DNA binding protein, *e.g. E2F, NF κ B*
- Sequestration of protein by oligonucleotide containing consensus sequence

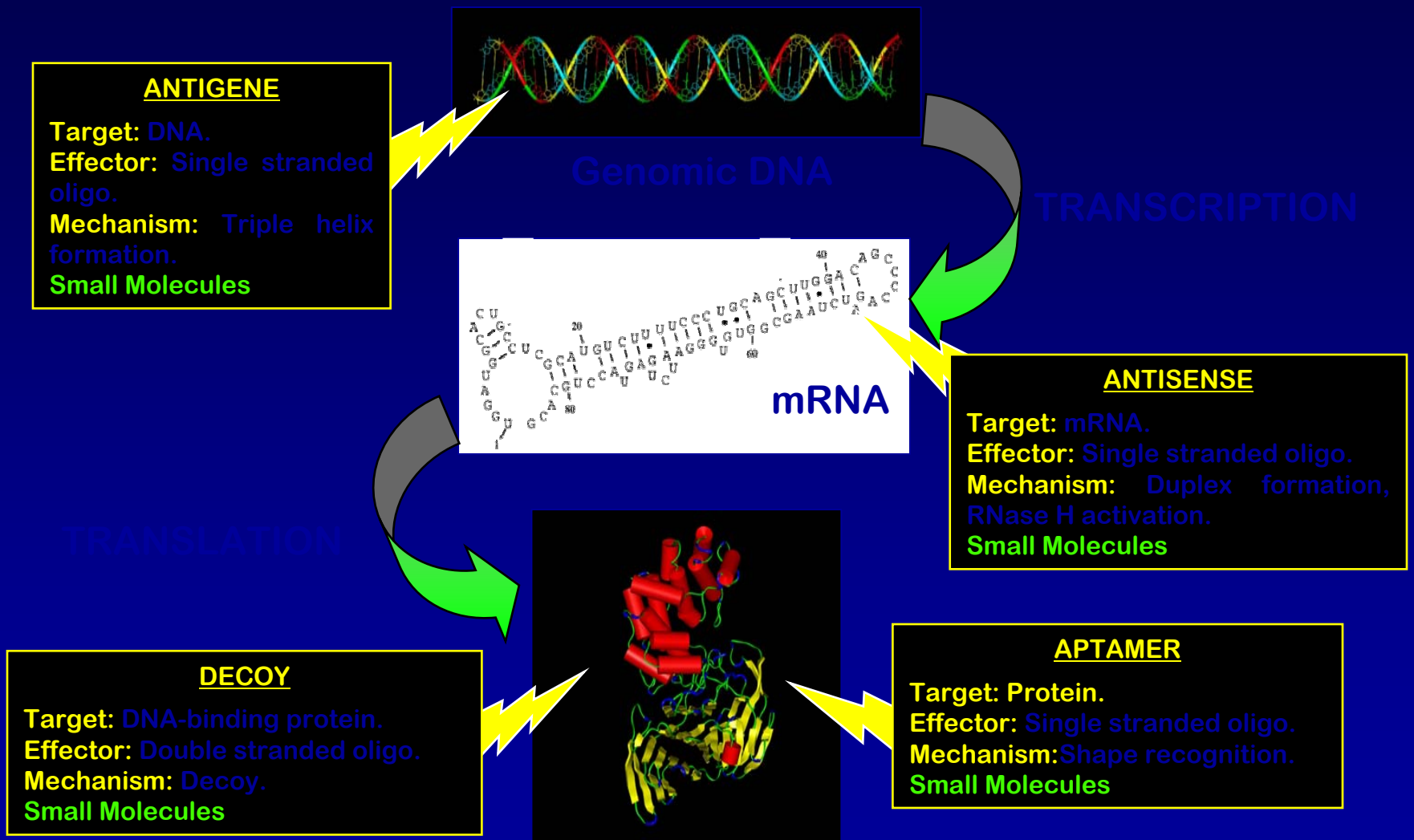
Aptamer Approach

- Targets small molecules, proteins, *e.g. thrombin*
- Shape-specific recognition by combinatorially selected oligonucleotides

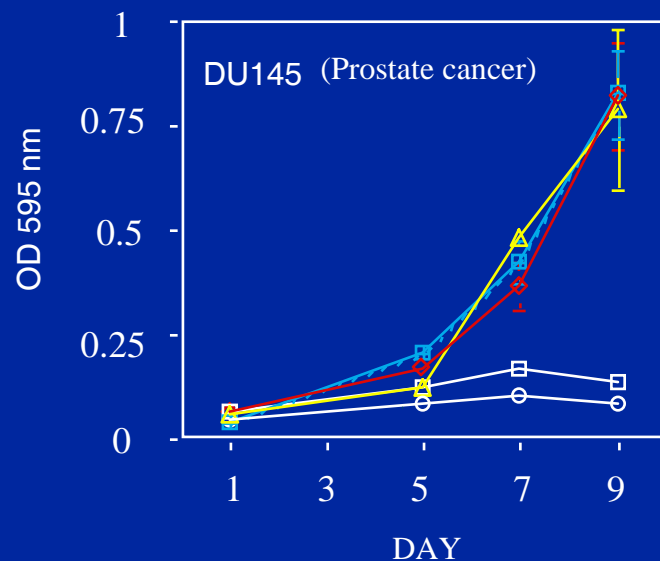
Immunomodulatory Oligonucleotides

- Vaccine adjuvants, allergy therapy
- Oligonucleotides containing 5'-CG motifs

Therapeutic Strategies



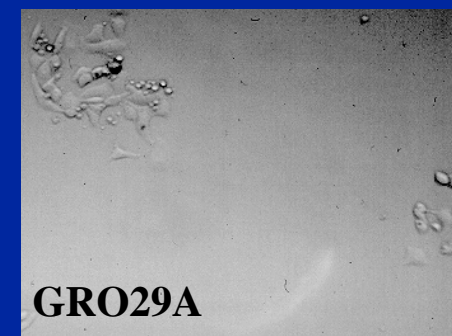
Antiproliferative properties of G-rich oligonucleotides



MTT Assay:

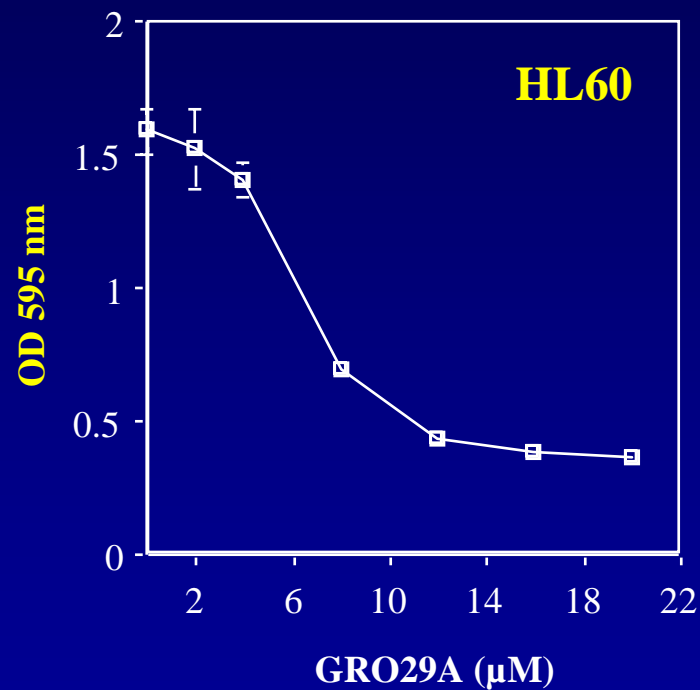
Showing growth of cells treated with four doses (days 1-4) of GRO.

Photographs of growth inhibited (GRO29A) and untreated MDA-MB-231 cells on day 7 after initial treatment (10x mag)



- **GRO15A** 5' -GTTGTTTGGGGTGGT-3'
- ◇- **GRO15B** 5' -TTGGGGGGGGTGGGT-3'
- ...○... **GRO29A** 5' -TTTGGTGGTGGTGGTTGTGGTGGTGGTGG-3'
- ...△... **GRO26A** 5' -GGTTGGGGTGGGTGGGGTGGGTGGGG-3'
- ...□... **WATER**

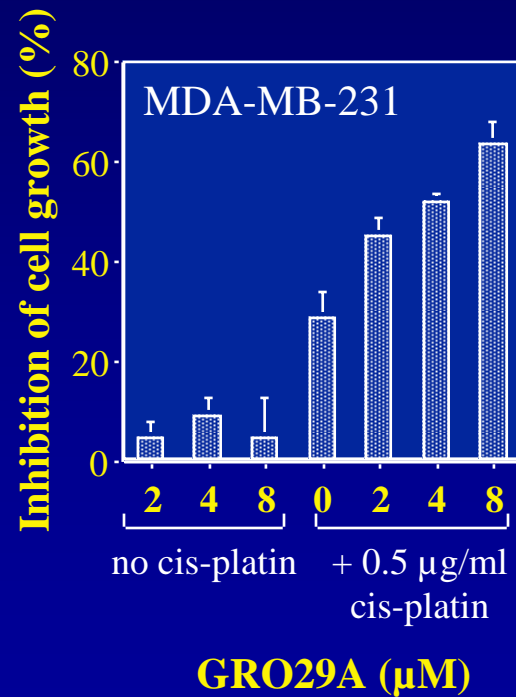
Inhibition is dose dependent



HL60 cells treated with a single dose of GRO29A on day 1 and assessed by MTT assay on day 7

Therapeutic potential of nucleolin-binding oligonucleotides

- Synergy of GROs with conventional treatments?

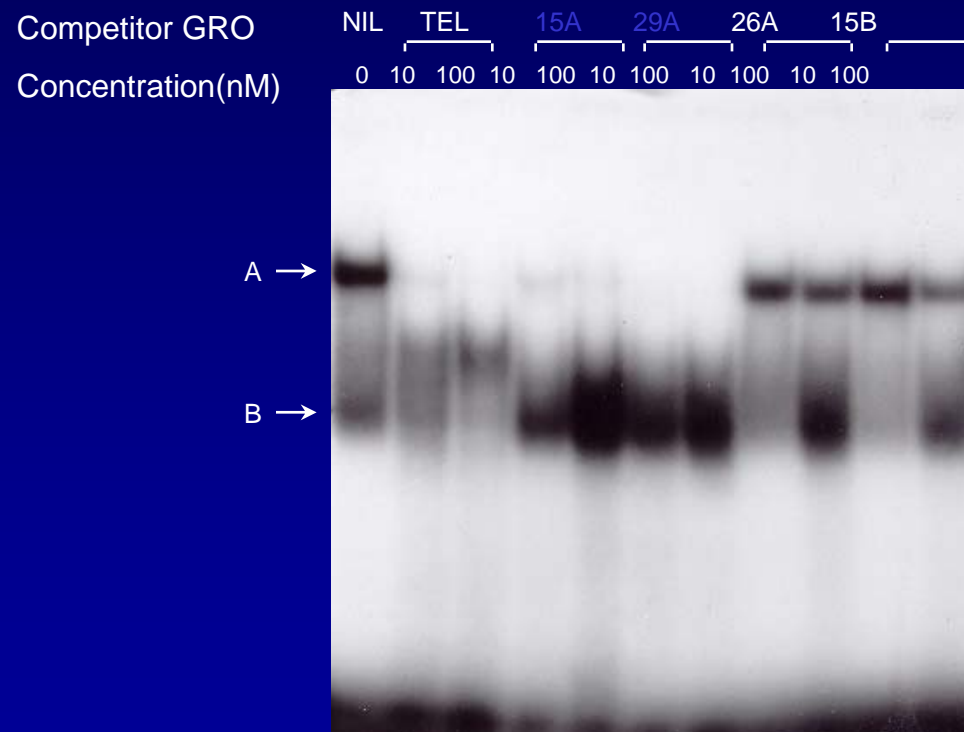


← Many Types of Cancer Cells are Responsive to GRO Treatment →

<u>Cell Line</u> ^a	<u>Origin & Description</u>	<u>GRO response?</u>
MDA-MB-231	Breast adenocarcinoma, tumorigenic ^b , hormone ind.	Yes
MCF7	Breast adenocarcinoma, non-tumorigenic, hormone dep.	Weak ^d
DU145	Prostate adenocarcinoma, tumorigenic, hormone ind.	Yes
PC3	Prostate adenocarcinoma, tumorigenic, hormone ind.	Yes
CaLu1	Epidermoid carcinoma, lung, tumorigenic	Yes
HeLa	Adenocarcinoma of cervix, non-tumorigenic	Yes
HS27	Foreskin fibroblasts, derived from <u>normal</u> tissue	Weak ^d
'MTX'	Methotrexate-resistant MCF7 derivative ^c	No ^e
B16-F0	Mouse melanoma, tumorigenic	No
U937	Histiocytic lymphoma	Yes
K562	Chronic myelogenous leukemia	Yes
MEG01	Chronic myelogenous leukemia	Yes
RS4[11]	Acute lymphoblastic leukemia	Yes
10P2	Mast cell (represents <u>normal</u> hematopoietic precursor)	Weak ^f

Footnotes: (a) all human cells except 10P2 and B16-F0; (b) in nude mice; (c) K. H. Cowan et al., J. Biol. Chem. 257:15079 (1982); (d) compared to solid tumor cell lines; (e) may be due to unusual resistance of this cell line to cell cycle arrest; (f) compared to leukemia cell lines.

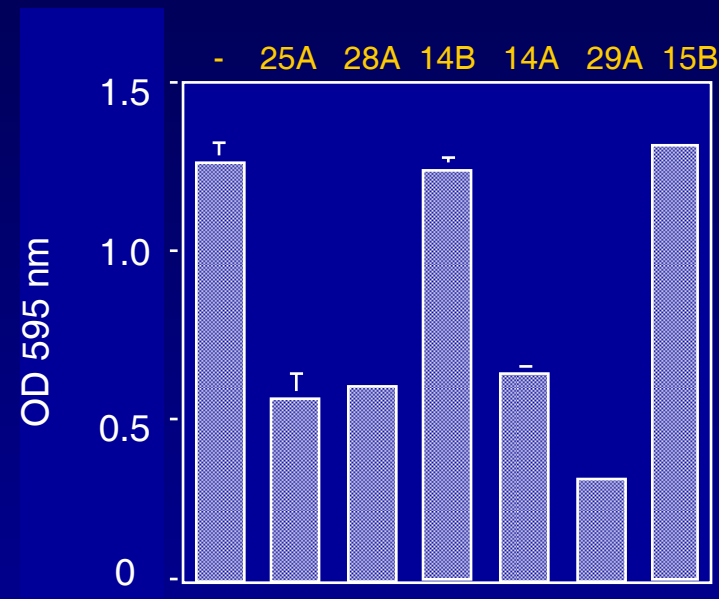
Antiproliferative oligonucleotides compete for binding to a protein that binds a G-quartet forming telomere sequence



Electrophoretic Mobility Shift Assay:

^{32}P -TEL (5'-(TTAGGG)₄)
+ HeLa nuclear extracts
+ cold competitor GRO

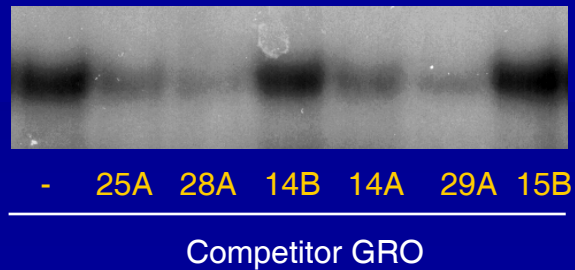
Activity of G-rich oligonucleotides correlates with protein binding



MTT Assay:

MDA-MB-231 treated with single dose (10 μ M) GRO. Day 7 after treatment.

TEL-binding protein A →



Competition Mobility Shift Assay:

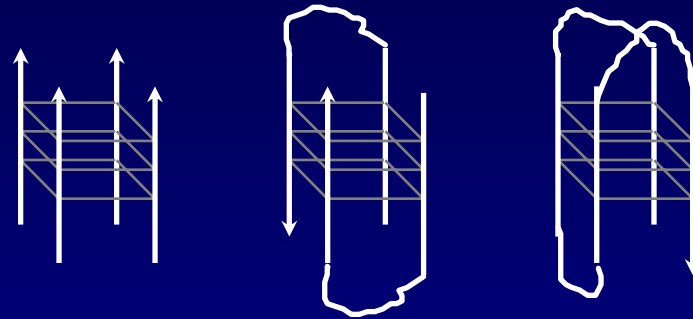
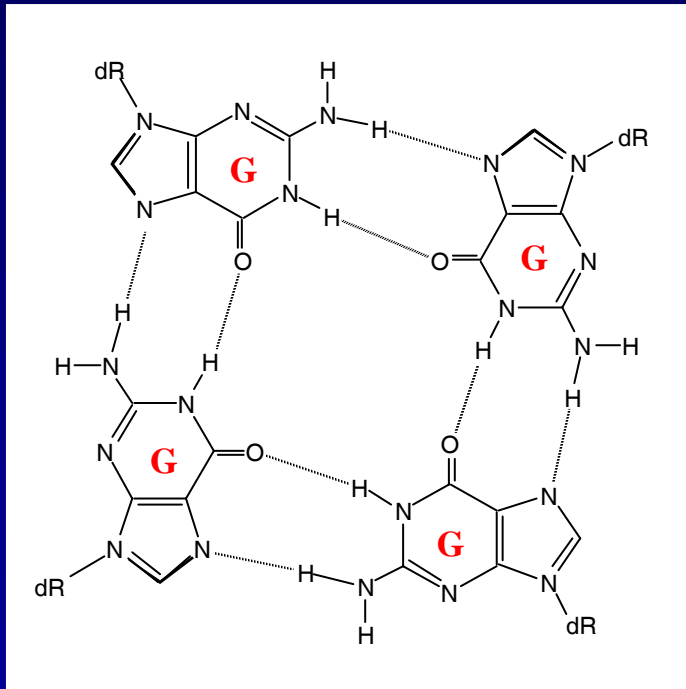
MDA-MB-231 nuclear ext. + ³²P-TEL + cold GRO

Nucleolin - structure

- **Abundant, multifunctional phosphoprotein -707 aa**
- **Major non-ribosomal protein located in nucleoli**



G-quartet Formation



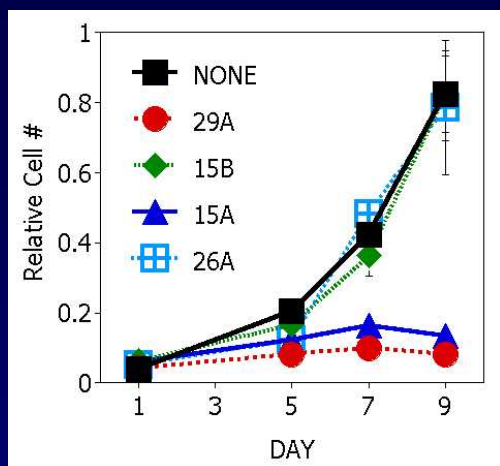
Tetramer Dimer Monomer

G-quartets in vivo?

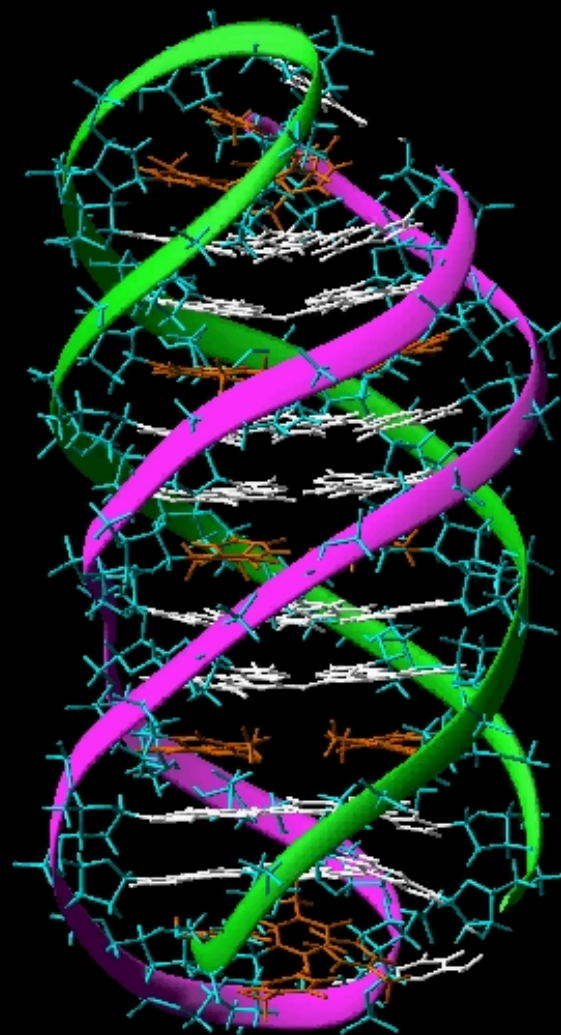
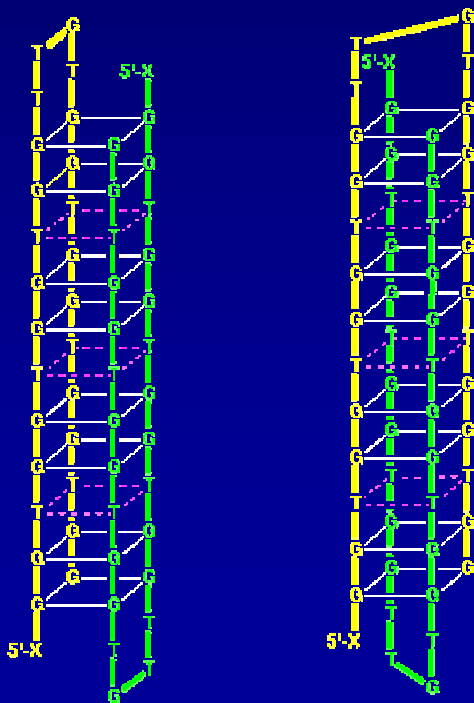
- **Telomeres**
- **Immunoglobulin switch regions**
- **HIV1 RNA**
- **Fragile X repeat**
- **Ribosomal DNA**

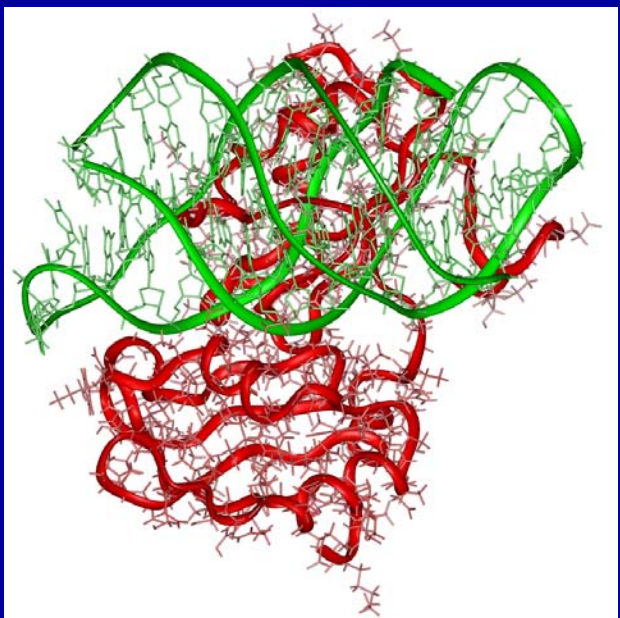
"The Aptamera Story"

GROs: A new cancer treatment



DU145 Cells

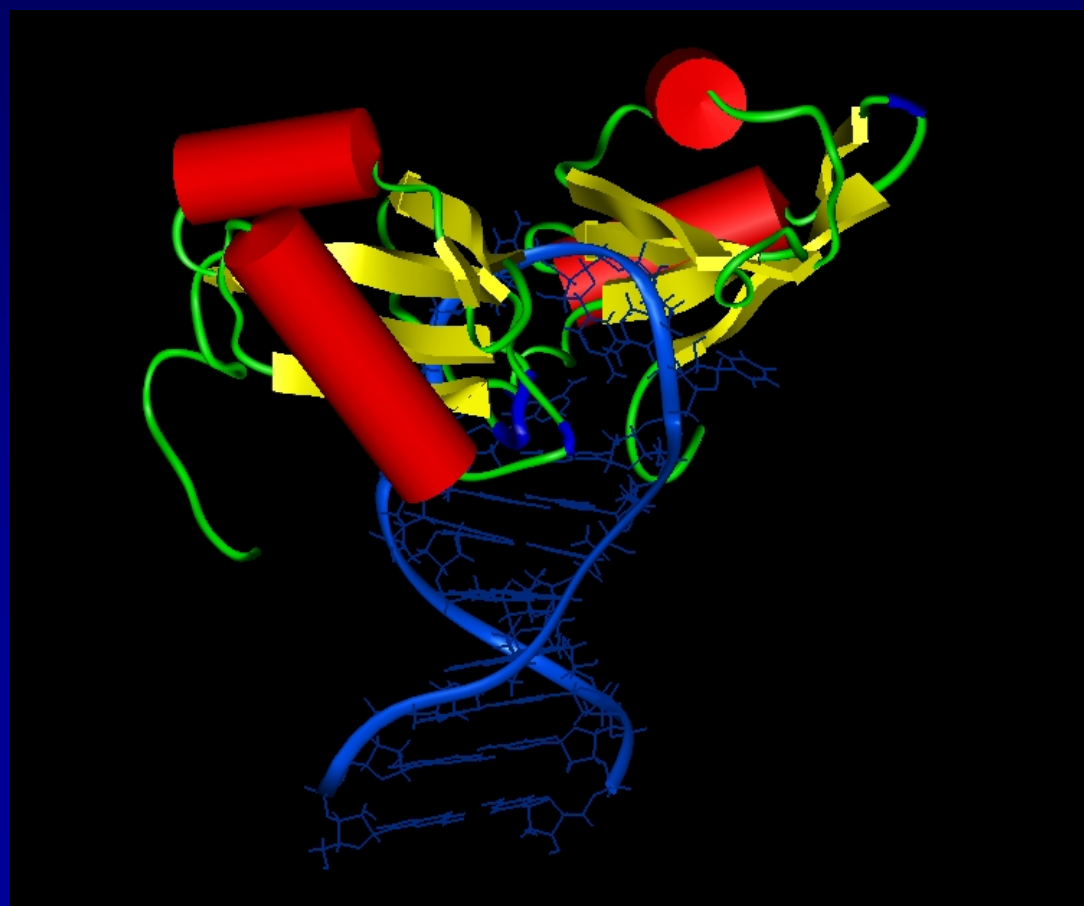
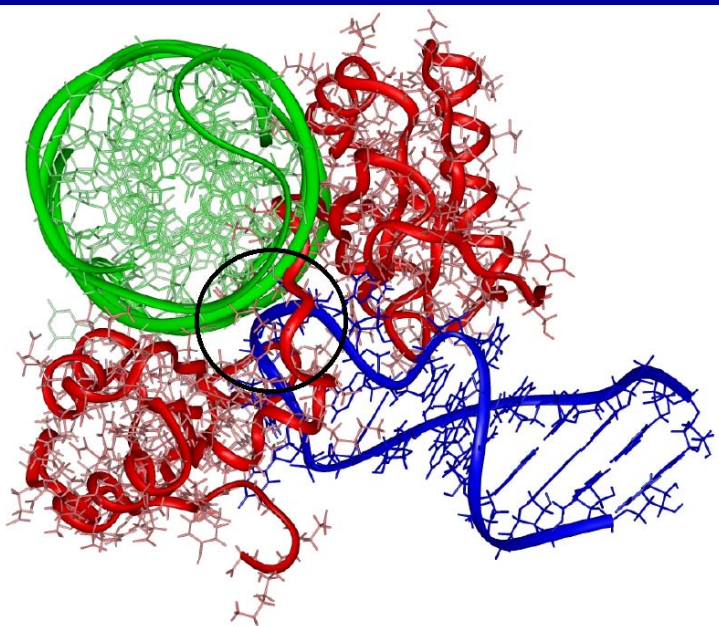




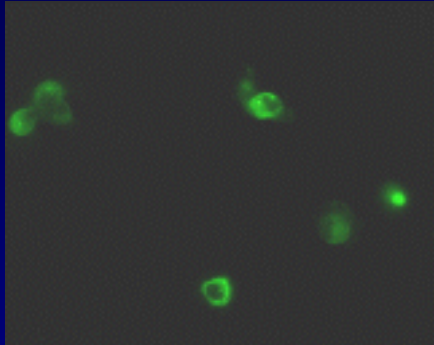
New Cancer Specific Target

PJ Bates, JO Trent, DM Miller

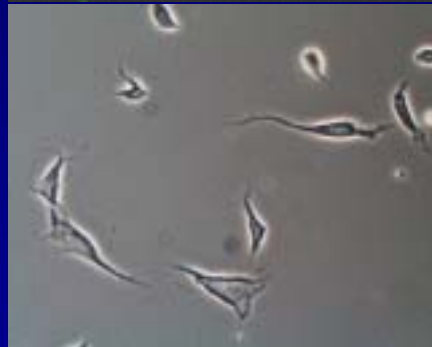
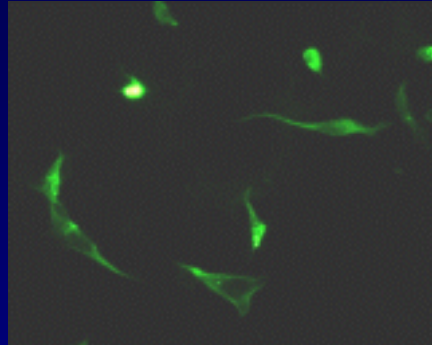
Nucleolin is on the surface of tumor cells but not “normal” cells



Nucleolin is Present on the SURFACE of Cancer Cells



**DU145 cells
(prostate cancer)**

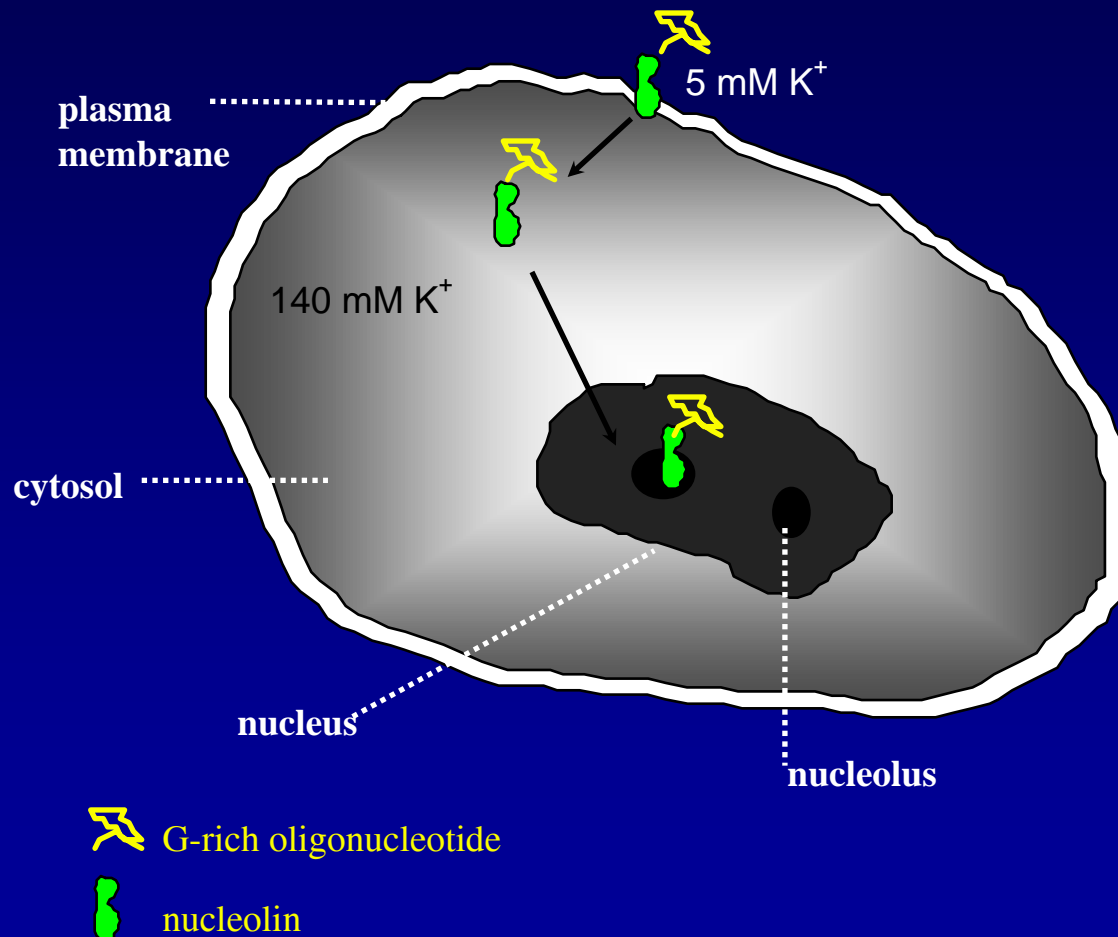


**MDA-MB-231 cells
(breast cancer)**

Immunofluorescent staining
of non-permeabilized cells
with nucleolin antibody.

Phase contrast micrograph
of same cells.

Speculative model of G-rich oligonucleotide action



Functions of Nucleolin

- Cell entry?
- Nuclear-cytosolic shuttle
- Ribosome biogenesis
- Chromosome condensation
- DNA helicase?

Regulation of Nucleolin

- Phosphorylation
- Self-proteolysis

Nucleolin - a G-quartet binding protein?

- GROs
- Telomeres
- Ig switch regions (Maizels et al.)
- rDNA

Phase I Clinical Trial

- 17 patients with end stage cancer were given a single four day infusion
- If there was evidence of response the patients were retreated
- There was no toxicity
- More than half the patients had stable disease for up to six months

Structure-Based Drug Design

Biophysics

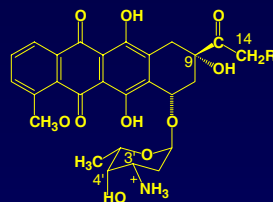
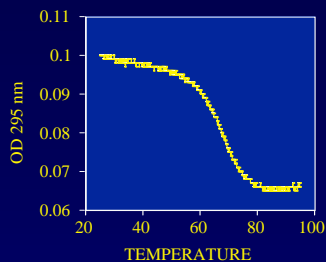
Molecular Biology

X-ray Crystallography

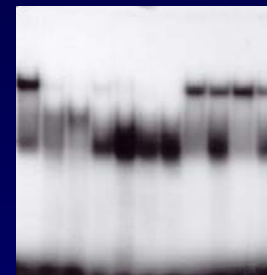
NMR

Molecular Modeling Drug Design

Synthesis

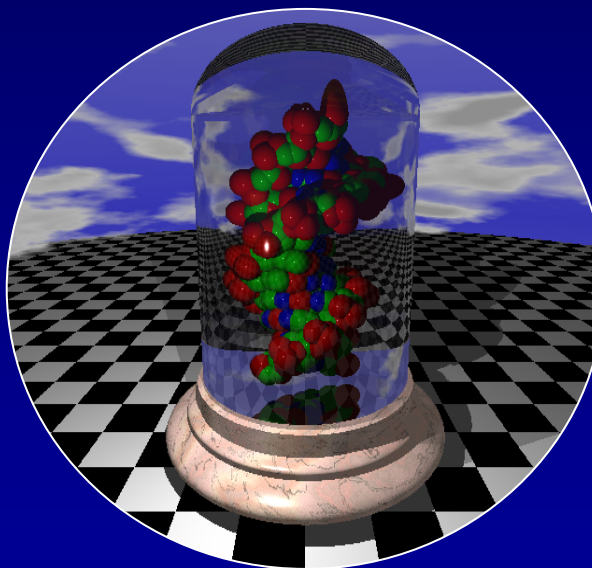


Synthesis



Biophysics

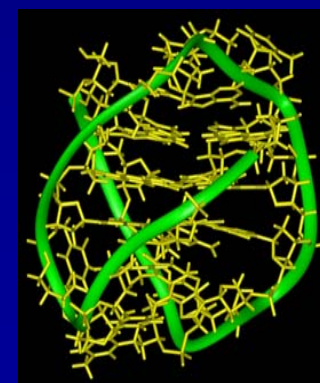
Molecular Biology



Molecular Modeling Drug Design



X-ray Crystallography



NMR

Investigator: JASON CHESNEY

Target: Tumor cell metabolism/
Inducible phosphofructokinase-2
which acts as a supercharger of
anaerobic glycolysis

Progress: In collaboration with
John Trent and Andrew Lane, has
designed inhibitors, some of which
have significant activity at low
micromolar concentrations against
neoplastic cell lines.



Investigator: NICK DELAMERE

Target: Disruption of tumor cell cation balance.

Progress: Inhibitors of Na/K ATPase (ouabain and derivatives) slow growth of transformed cells in sub-micromolar concentrations (i.e., lower than required for typical Na/K ATPase inhibition).



Investigator: GLENN McGREGOR

Target: Cancer prevention through targeted inhibition of error-prone DNA repair

Progress: Has developed anti-sense strategies for in vitro (and possibly in vivo) inhibition of error-prone DNA repair enzyme which, in vitro, suppresses UV-mediated mutagenesis. Possible application in sunscreens.



Investigator: Bob Mitchell

Target: Macrophage/monocyte migration inhibitor factor (MIF) a cytokine with enzymatic activity which is necessary for cell transformation.

Progress: With John Trent, he has designed and tested small-molecule inhibitors of MIF which selectively arrest the migration, invasion and survival of transformed cells.



DNA contains all of the secrets of nature. If we could only sequence the human genome we could understand every human disease. Unfortunately, that will not happen in any of our lifetimes.

- James Watson,
Durham, NC, 1967

Owensboro Cancer Research Center

- Large Scale Biology
 - Company based in Vacaville, CA
 - Production facility in Owensboro, KY
 - Produces GMP grade peptide pharmaceuticals from tobacco
 - Use TMV vectors to infect adult plants
 - Have produced antibodies for clinical trial with Stanford
 - Ben Jenson is developing an HPV vaccine to be produced in tobacco with LSBC

Owensboro Cancer Research Center

- Collaboration between JGBCC and Owensboro community
- To be funded by federal grants to Owensboro/UofL
- \$30 M/ten year project to develop research facility to develop cancer peptide therapeutics from tobacco

Owensboro Cancer Research Center

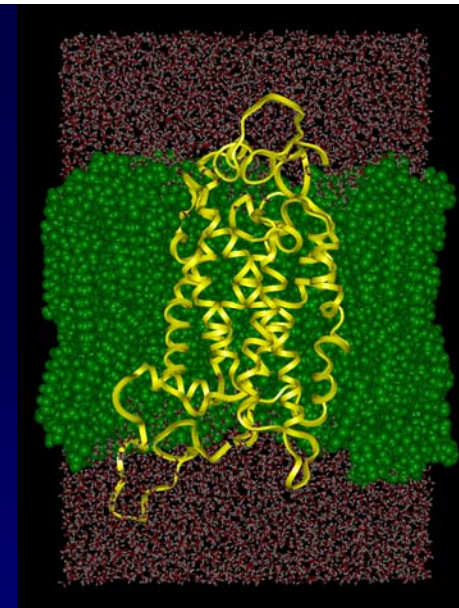
- Ten UofL faculty based in Owensboro
- Will include plant molecular biologists, virologists, translational scientists
- One goal will be to translate small molecule therapeutics to peptide therapeutics
- Construction has begun

Drug Design at JBCC

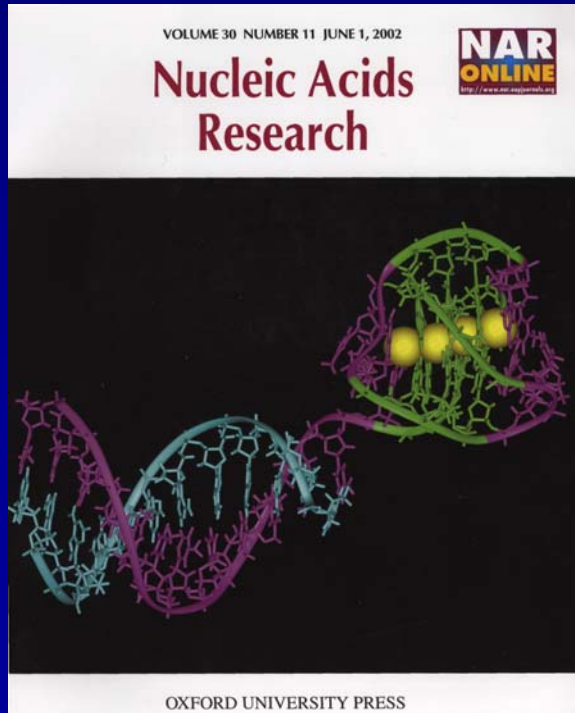
- Molecular Modeling Core
- NMR Facility
- Xray Crystallography
- Biophysics core lab
- Protein Expression and purification
- Micropet Imaging Facility

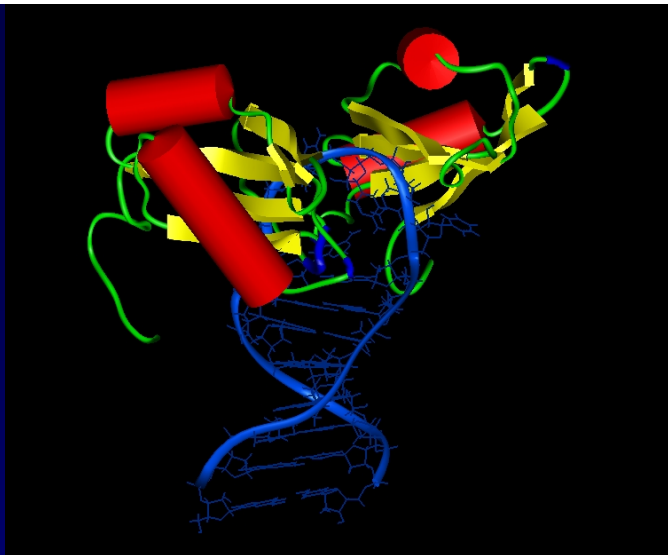


James Graham Brown Cancer Center

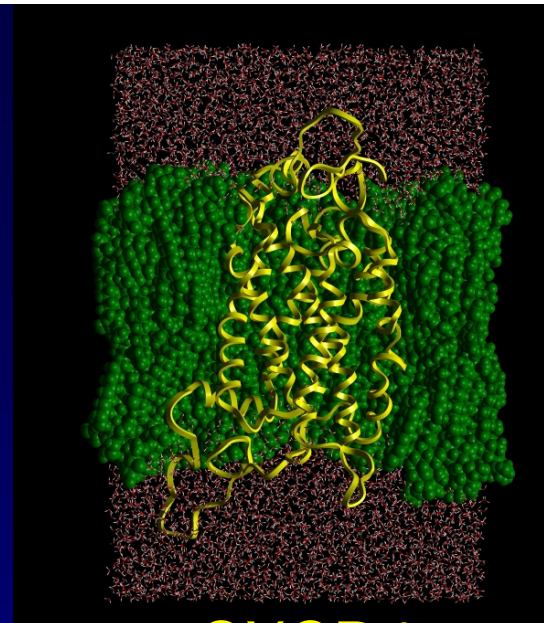


BCC Computational Laboratory



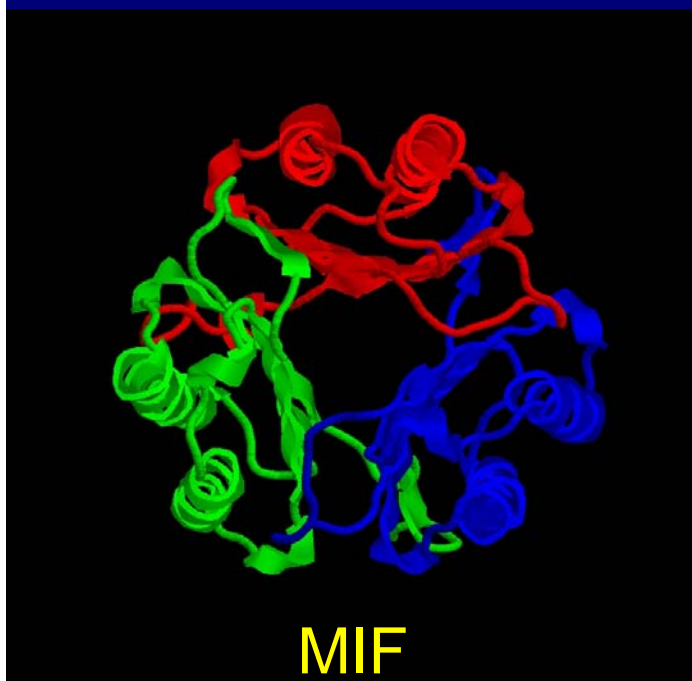


Nucleolin



CXCR4

New Targets



MIF



iPKF-2

NMR Center Facilities

- **600 and 800 MHz 4-channel NMR spectrometers**
- **Proteins and nucleic acids**
- **Target-drug interactions**
- **Automation for metabolomics and screening**

14.1 T magnet with SMS robot



14.1 T spectrometer

**14.1 T Oxford shielded
magnet-51 mm bore
(4.2 K)**

**Varian Inova 4-channel
console
+ Robot**

Drug Discovery

Virtual Screening of Small Molecules

Computationally screen databases of small molecules (6,000,000)

To physically test these at 1000 molecules per day = 16.4 years

To *in silico* screen these = 20 days

Nucleolin

In silico screen of 300,000 compounds = 48 hours

50 out of 200 “hits” were selected

25 biologically tested

Compounds “G”, “N”

Cancer selective

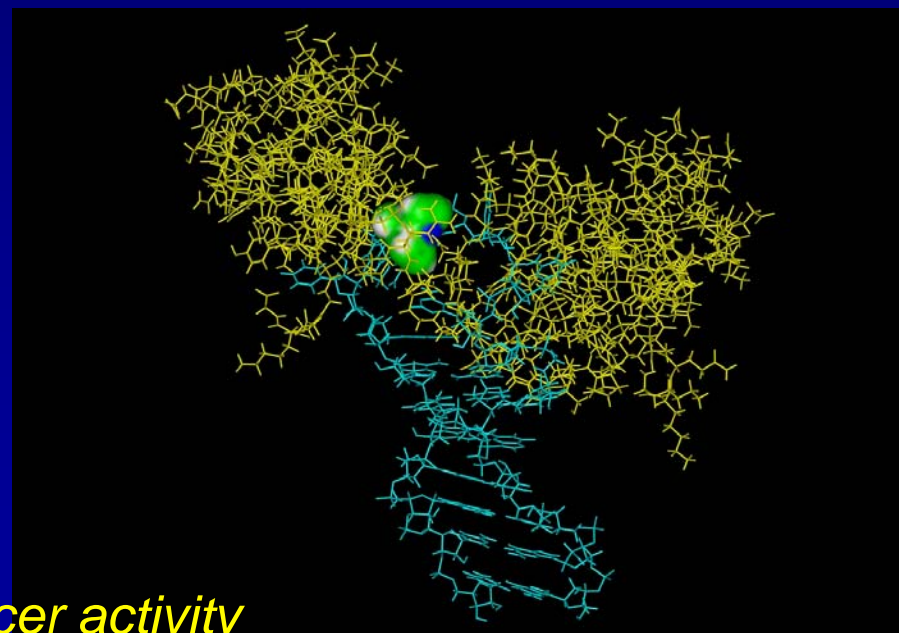
Competes with GROs

Active in lead compound range

Never been tested in cancer before

Can buy in 100kg drums

National Cancer Institute confirms cancer activity



Center for Macromolecular Interactions

A Biophysical Core Facility for the BCC

(Supported by a Kentucky New Economy Grant)

Dr. J. Brad Chaires, Director

Dr. Nichola Garbett, Assistant Director

State-of-the-art instrumentation for:

Calorimetry

- Differential Scanning Calorimeter (DSC)
- Isothermal Titration Calorimeter (ITC)
- Automated High-throughput DSC (1 of only 4 in academia)

Spectroscopy

- UV/Vis Spectrophotometer
- Fluorescence Spectrometer
- Circular Dichroism (simultaneous fluorescence; auto titrator)
- Surface Plasmon Resonance (SPR – Biacore)
- Rapid Scan Stopped-Flow Spectrometer