Molecular Therapies: False Hope or the Future of Medicine?

Ruth Foley
Institute of Molecular Medicine
Trinity College Dublin

Advances in medicine and science 1500-present!

- 1543 First anatomy textbook
- 1798 Smallpox vaccine
- 1846 Anaesthetics: surgery developed
- 1866 Mendel discovers genes
- 1880s Micro-organisms isolated
- 1899 X-rays used in cancer diagnosis and treatment
- 1940s Antibiotics become available
- 1953 Structure of DNA
- 1956 Metastatic cancer first cured by chemotherapy
- 1972 Recombinant DNA
- 1990 First gene therapy trial
- 2003 Human genome sequence completed

Molecular therapy and the media

Molecular medicine in action

- Identify disease specific target or pathway - a molecular abnormality implicated in the disease
- Develop approach to interfere with the gene, its product or its pathway
- *In vitro* and *in vivo* tests followed by clinical trials and licencing

The ideal molecular therapy

- Effective
- Non-toxic
- Stable *in vivo*
- Easy administration
- Manufactured in bulk
- Cost

Range of molecular therapies

- Protein inhibitors
  - Monoclonal antibodies
  - Tyrosine kinase inhibitors
  - Antiviral molecules
- Nucleic acid therapeutics
  - Therapeutic genes
  - Oligonucleotides

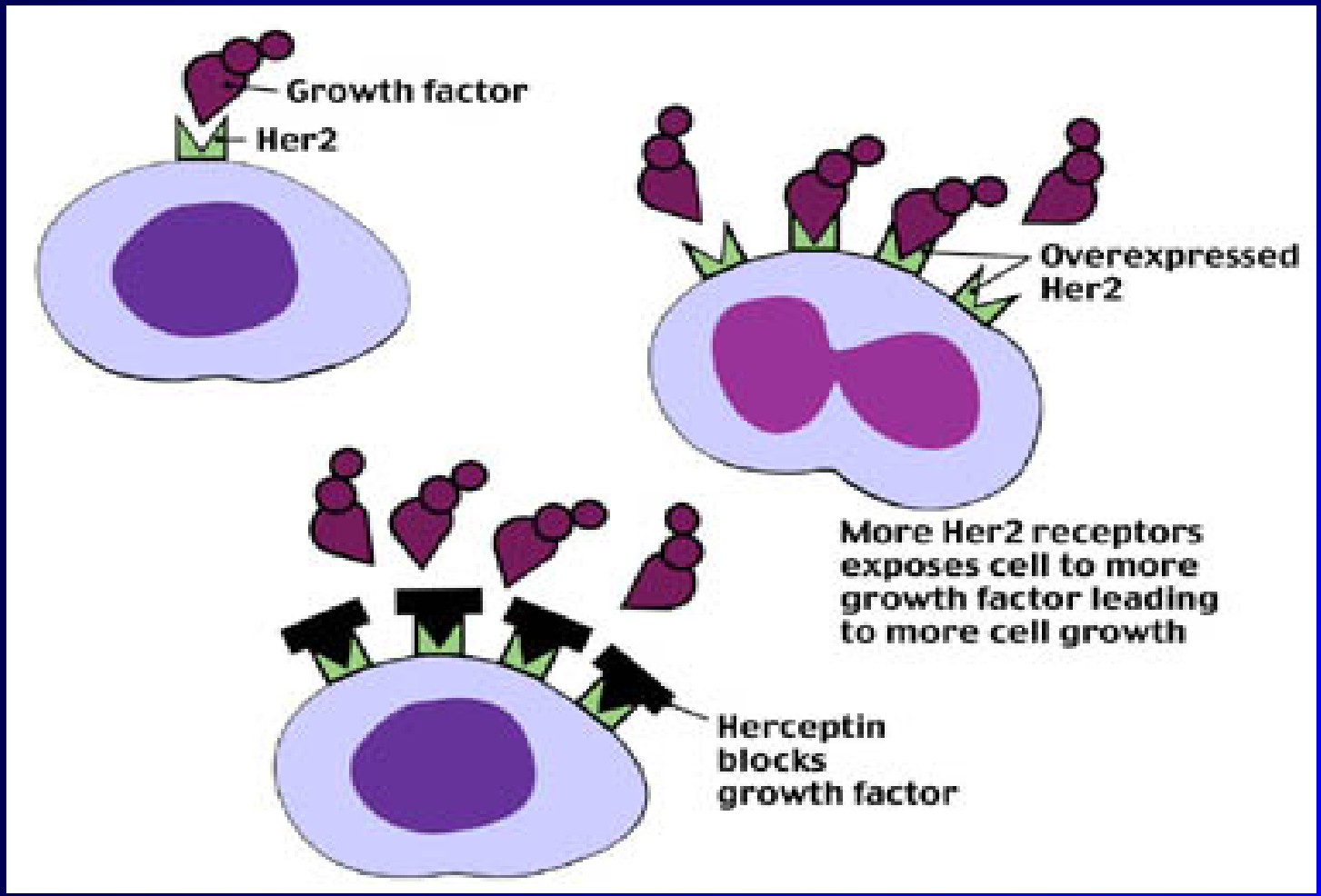
Targeted protein inhibitors

- Herceptin targets HER2 oncogene in breast cancer
- Imatinib mesylate targeted bcr-abl in CML
- Antiretroviral drugs target HIV gene products

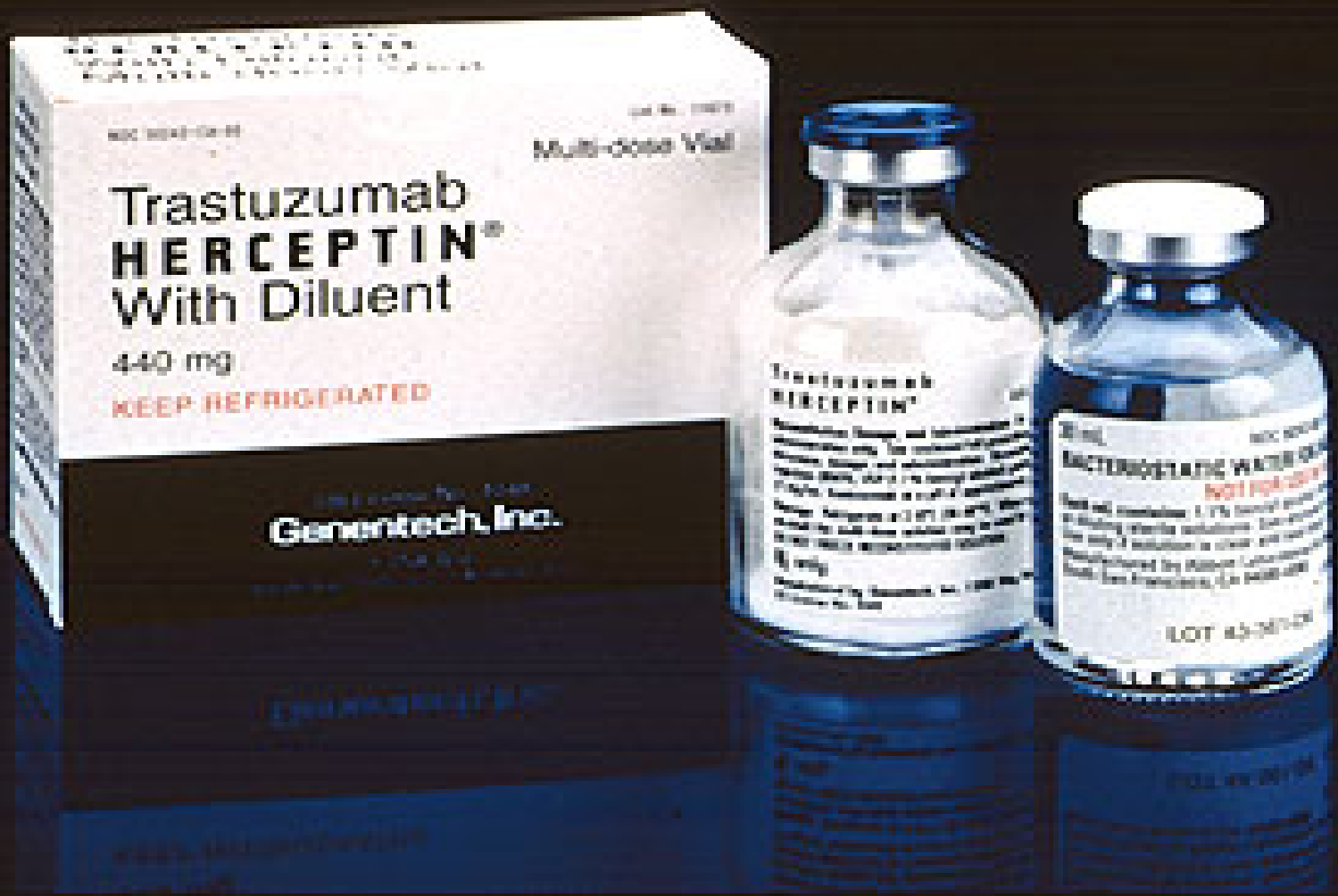
Molecular targets in breast cancer

- ErbB-2 gene overexpression occurs in 30% of invasive ductal adenocarcinomas
- Chromosome 17q21, encodes a 1255 amino acid, 185kD class I transmembrane tyrosine kinase
- Patients who overexpress c-erbB-2/HER-2 have aggressive tumors refractory to conventional therapy
- New therapeutic strategies for management of these high risk groups are essential
  - Herceptin hMoAb

HER2 pathway in breast cancer



Herceptin – Molecular medicine in action

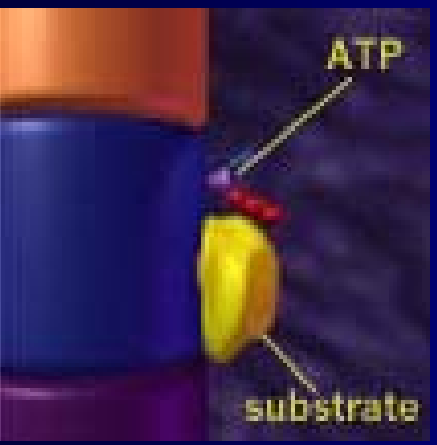


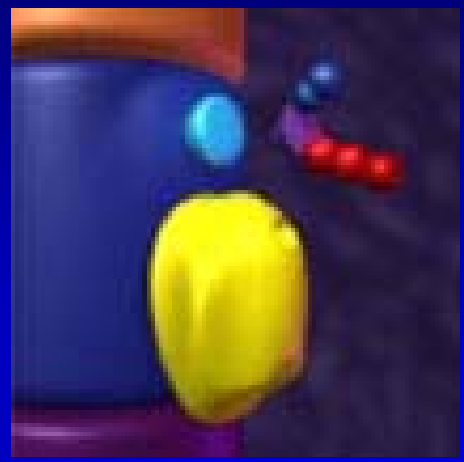
Chronic Myeloid Leukaemia (CML)

- Acquired genetic abnormality t(9;22) generates a chimaeric leukaemia specific gene BCR-ABL
- Results in production of a leukaemia specific protein P210
- Enhanced tyrosine kinase activity leads to abnormal signaling, adhesion defect and resistance to apoptosis
- Molecular target!

Stopping the cancer gene working

Cancer gene active **Cancer gene blocked**



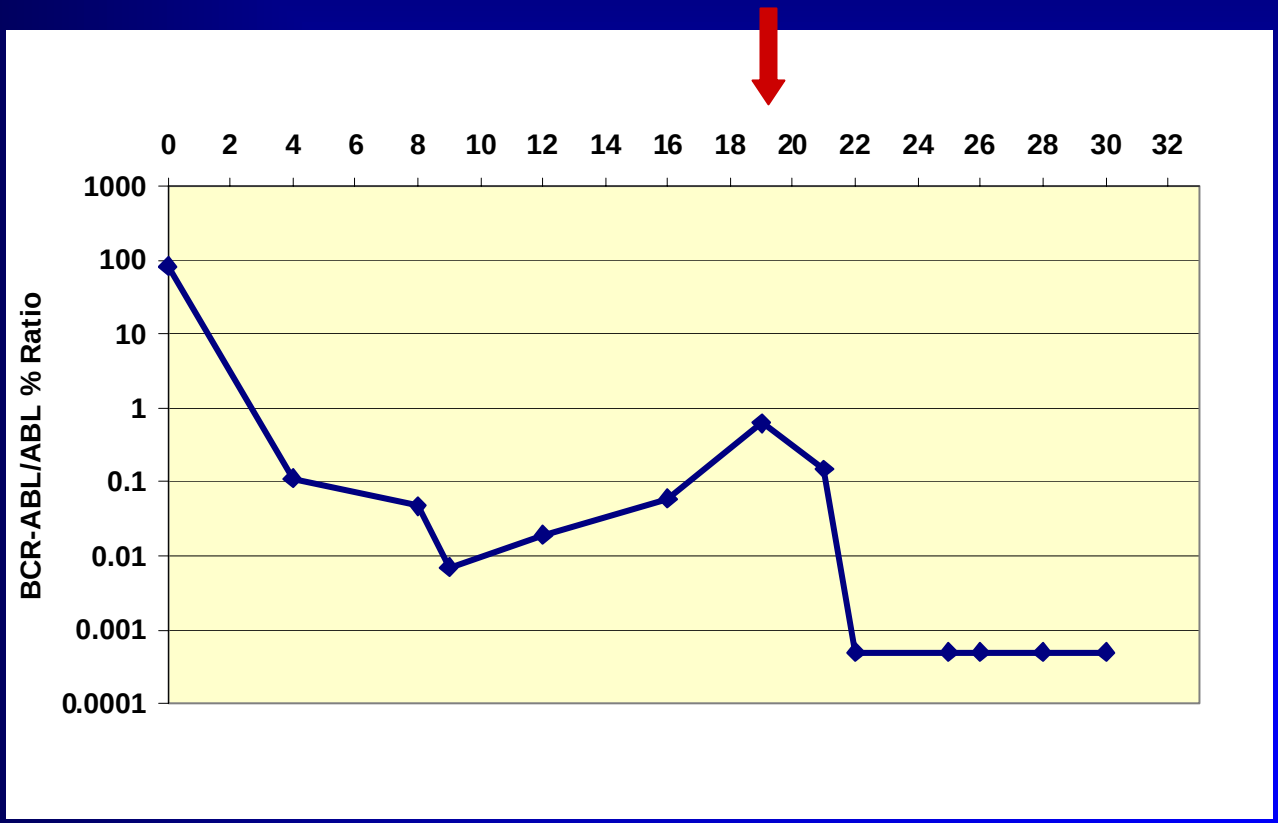


- Molecular Therapy (Imatinib Mesylate)
- Revolutionised treatment of CML: orally available

Why does Imatinib not always work?

- Primary or secondary resistance
- Mutations in the BCR-ABL kinase domain that impair drug binding (50–90% of cases)
- Amplification of the BCR-ABL gene (5– 10% of cases)
- Other postulated mechanisms
  - bypass BCR-ABL through SRC kinase activation
  - drug transporter proteins: hOCT1
- Resistance to IM has led to development of second generation TK inhibitors

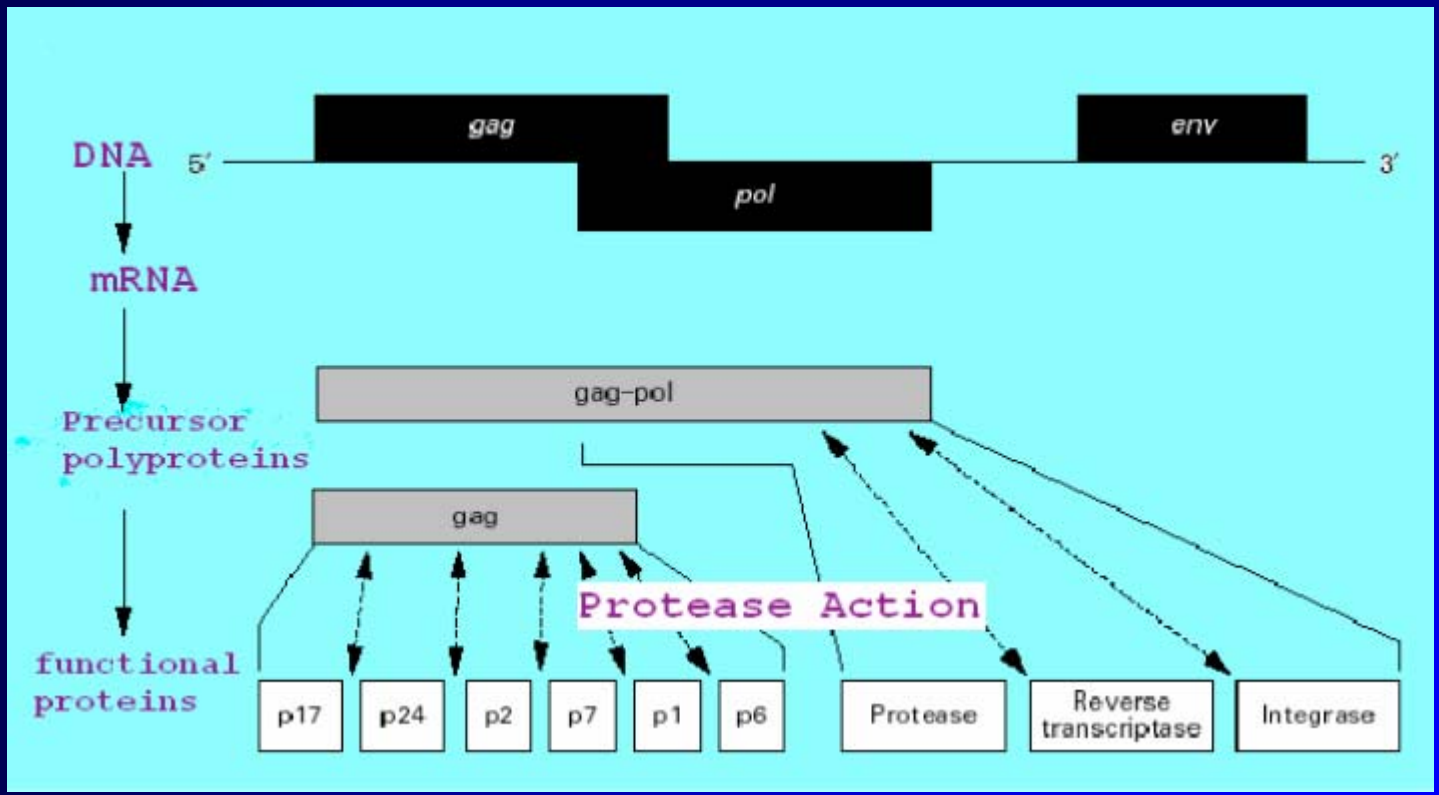
Acquired resistance to Imatinib and use of Dasatinib



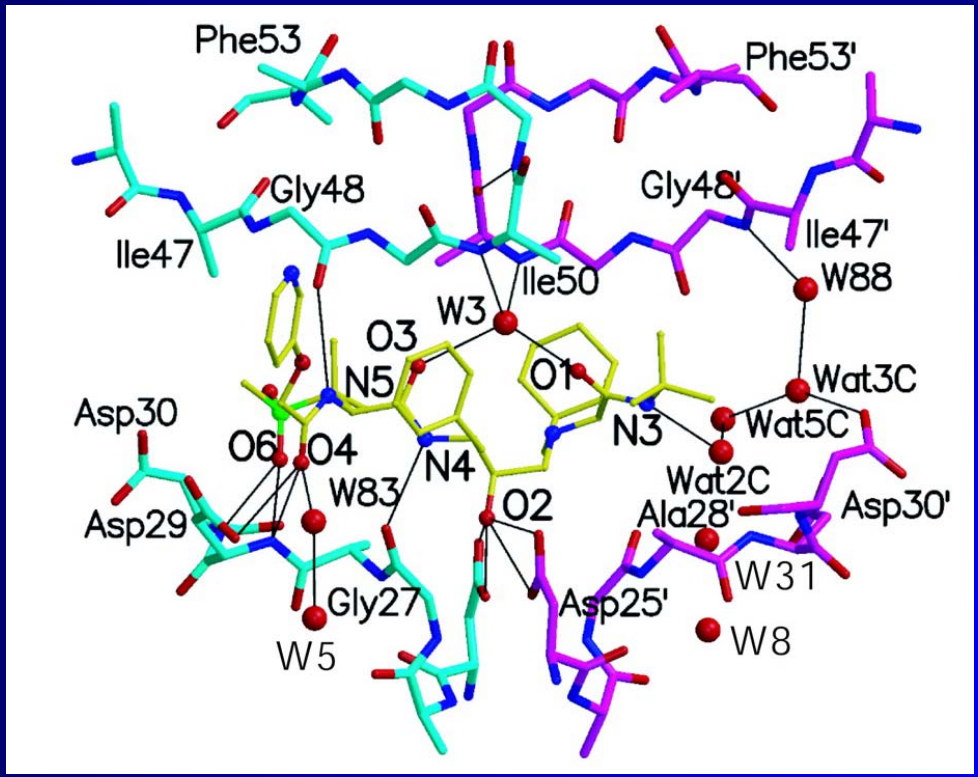
HIV: molecular targets

- Most drugs have targeted reverse transcriptase and protease
- Problems with resistance including cross-resistance
- Recently drugs developed to prevent entry into cells: enfuvirtide

HIV-1 genome


DNA
5′
gag
pol
env
3′
mRNA
Precursor polyproteins
gag-pol
gag
Protease Action
functional proteins
p17
p24
p2
p7
p1
p6
Protease
Reverse transcriptase
Integrase

Protease inhibitors

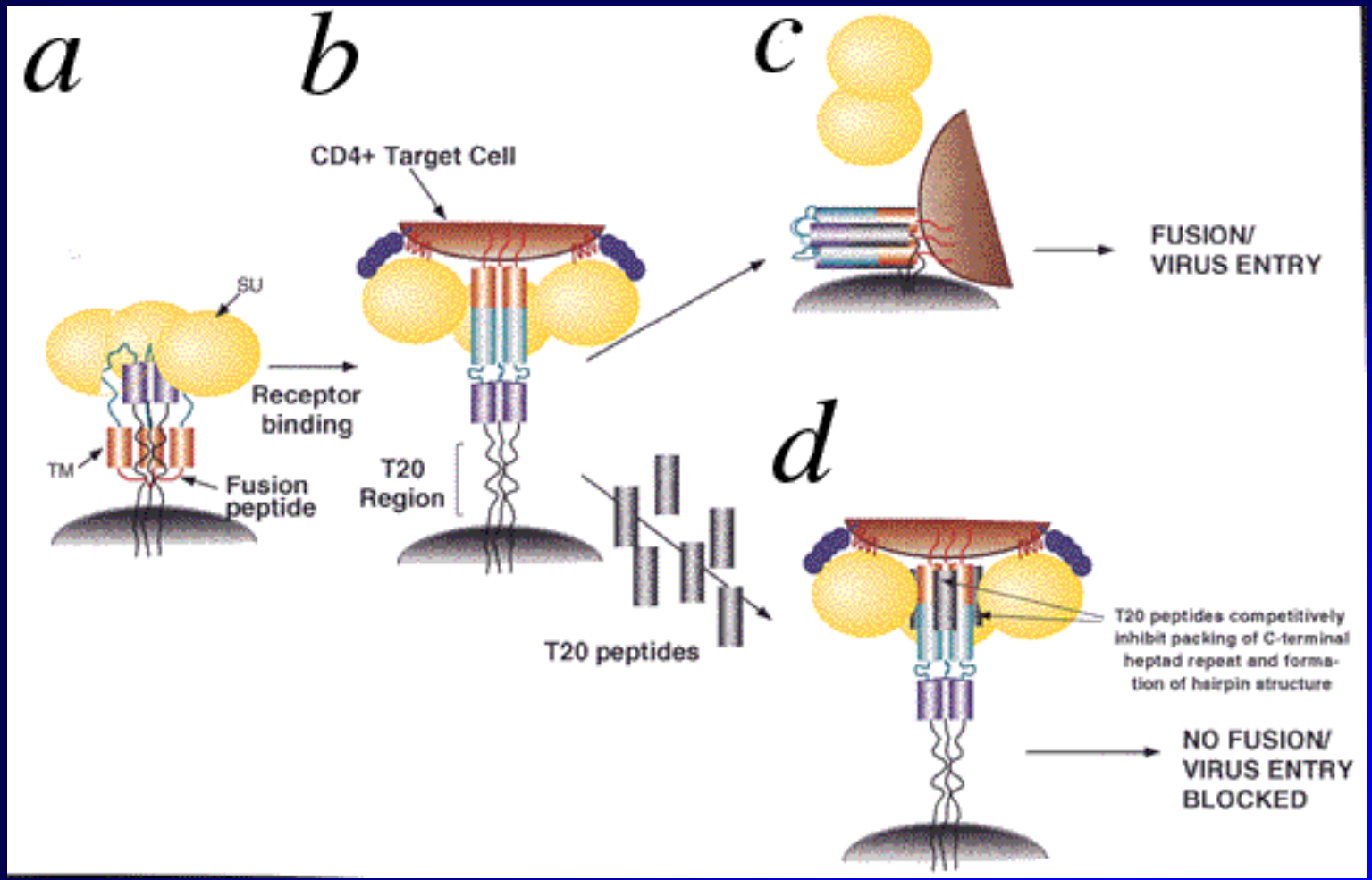


Prabu-Jeyabalan M *et al.* Antimicrobial Agents and Chemotherapy, April 2006, p. 1518-1521, Vol. 50, No. 4

Enfuvirtide

- Synthetic 36-amino-acid peptide
- Binds to envelope glycoprotein 41 and inhibits fusion with cell membrane
- Developed from protein function study
  - Peptide fragment of gp41 synthesised to examine possible oligomer formation
  - Inhibition of HIV entry not expected but observed
- Second-line combination therapy
- Effective in reducing viral load
- Licenced in US and Europe

HIV entry and enfuvirtide



Kilby JM et al, *Nature Medicine* 4, 1302 - 1307 (1998)

Range of molecular therapies

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  - Antiviral molecules
- Nucleic acid therapeutics
  - Therapeutic genes
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Gene therapy issues...

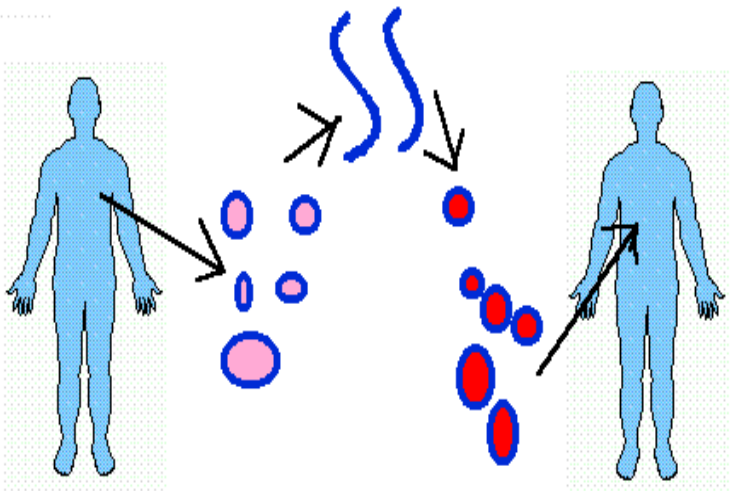
- How does it work?
- How has it been used?
- Is it effective?
- Is it safe?

Delivery to patients

- Ex vivo: removal of cells, introduction of genetic material and reintroduction of gene modified cells
- In situ: localised delivery of gene to specific tissue
- In vivo: systemic delivery with movement of genetic material to site(s) of action

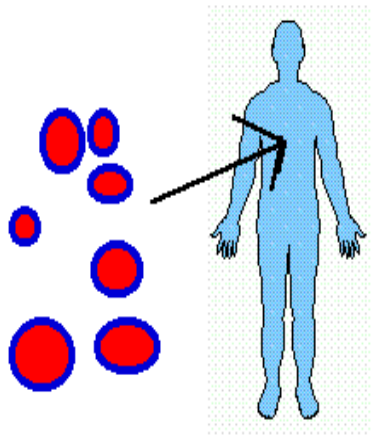
Delivery to patients

a) Ex vivo



Cells are extracted, transfected and then replaced

b) In vivo

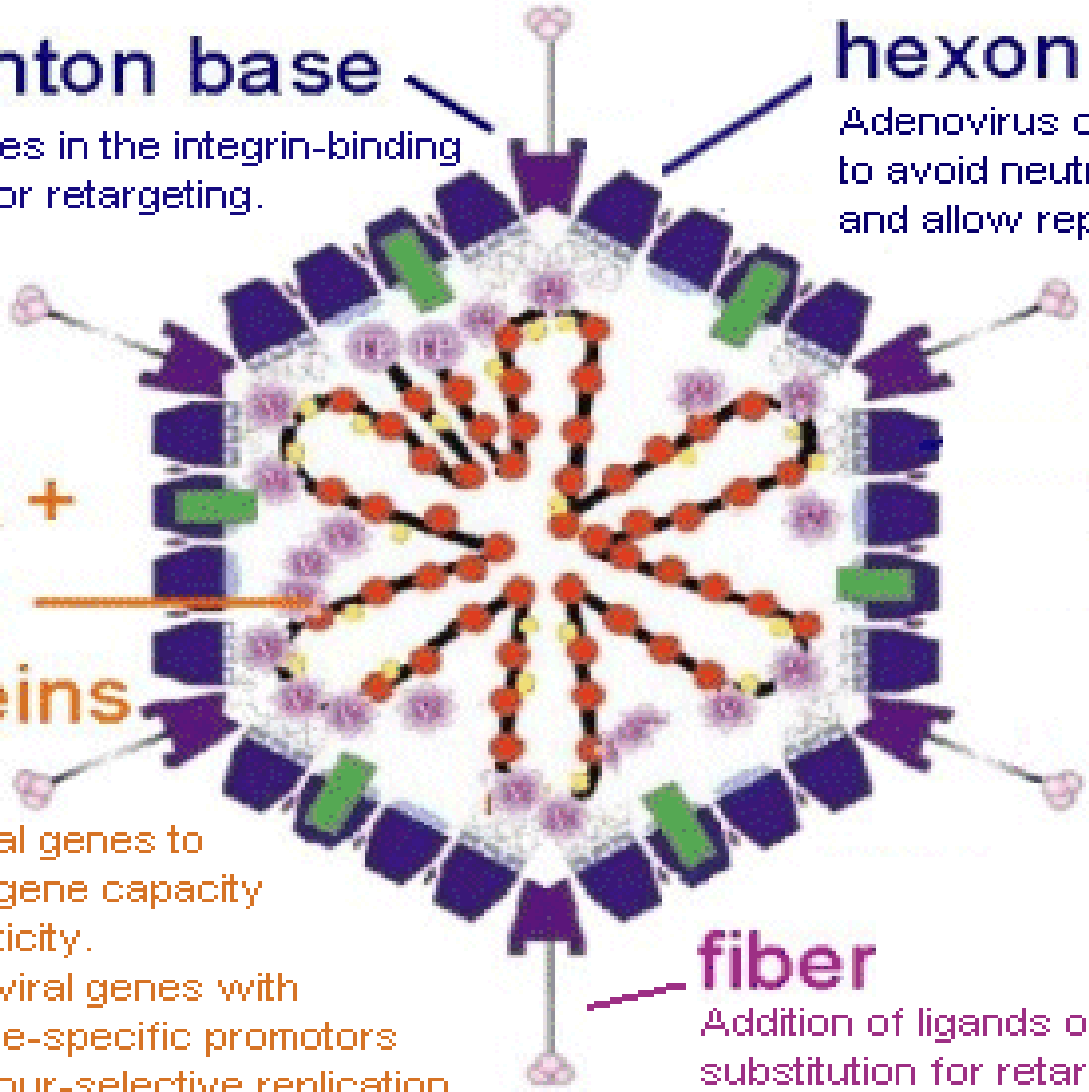


Viruses are inject into the patient directly

Gene delivery vectors: viral

- Retrovirus
- Adenovirus / adeno associated virus
- Herpes virus
- Lentivirus
- Other

A Schematic of the Adenovirus Showing the Main Modifications Ongoing to Increase the Efficacy of the Virus as a Vector



penton base

Changes in the integrin-binding motif for retargeting.

hexon

Adenovirus of different isotypes to avoid neutralization by antibodies and allow repeated administration.

DNA + core proteins

-Deletion of viral genes to increase transgene capacity and reduce toxicity.
-Regulation of viral genes with tumour or tissue-specific promotors to achieve tumour-selective replication.
-Mutations to achieve tumour-selective replication.

fiber

Addition of ligands or complete substitution for retargeting.

Viral vectors: key points

- Consider target cells, size of construct, duration of expression, efficiency, safety
- Viruses generally more efficient than non-viral delivery
- Some viruses infect dividing cells; lentiviral vectors (HIV) infect non-dividing cells
- Adenoviruses elicit immune response and allow only transient expression
- Retroviruses integrate into genome and can be maintained long-term – but risk of insertional mutagenesis
- Adeno-associated viruses: smaller but safer

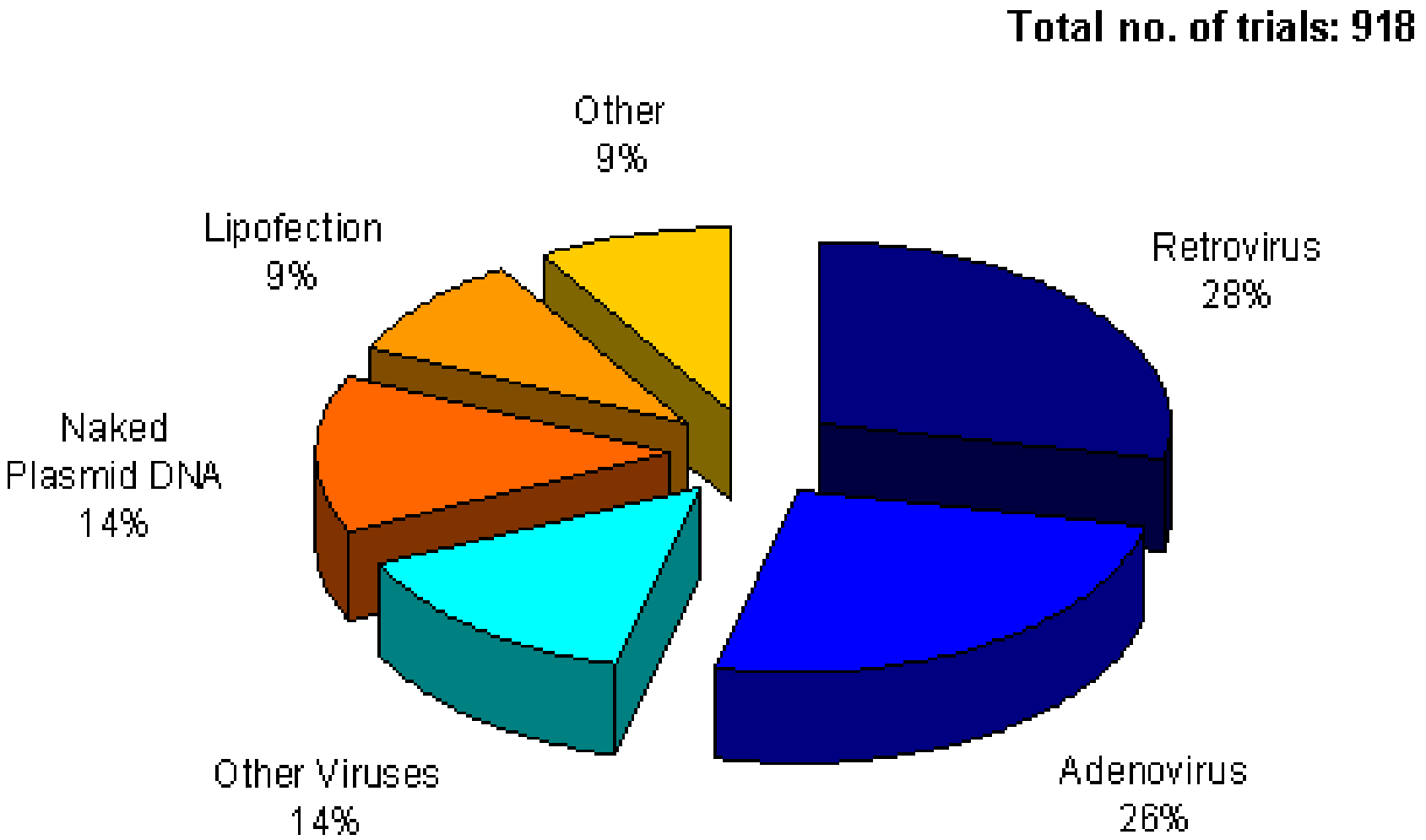
Gene delivery vectors: nonviral

- Receptor mediated
- Carrier (liposomes etc)
- Direct injection/naked DNA
- Electroporation
- Nanotechnology

Non-viral vectors: key points

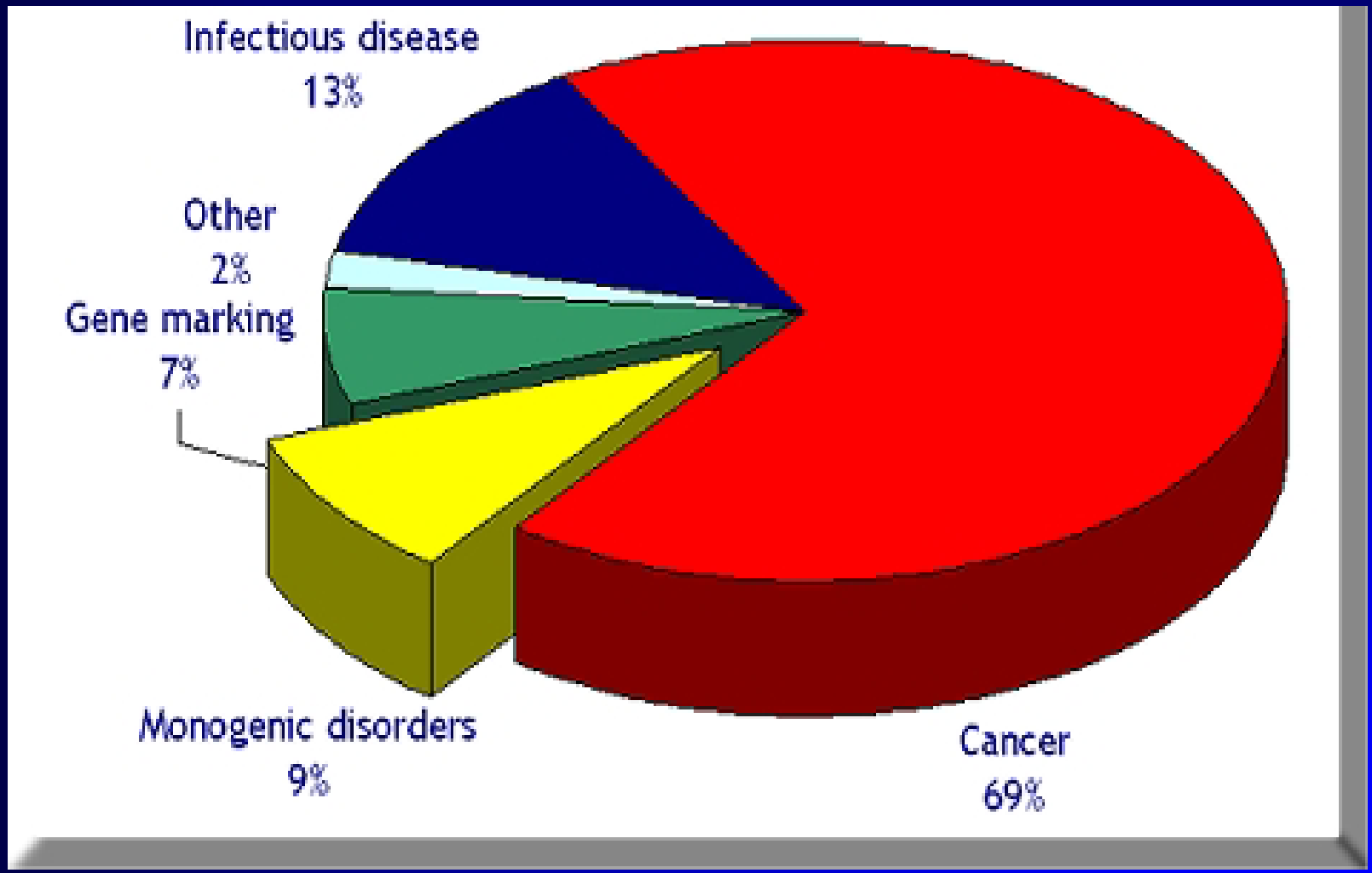
- Consider target cells, efficiency, safety
- Generally less efficient though this could change
- Liver uptake, reduced by designing vectors targeted to tissue-specific receptors
- Generally transiently maintained

Use of vectors in gene therapy

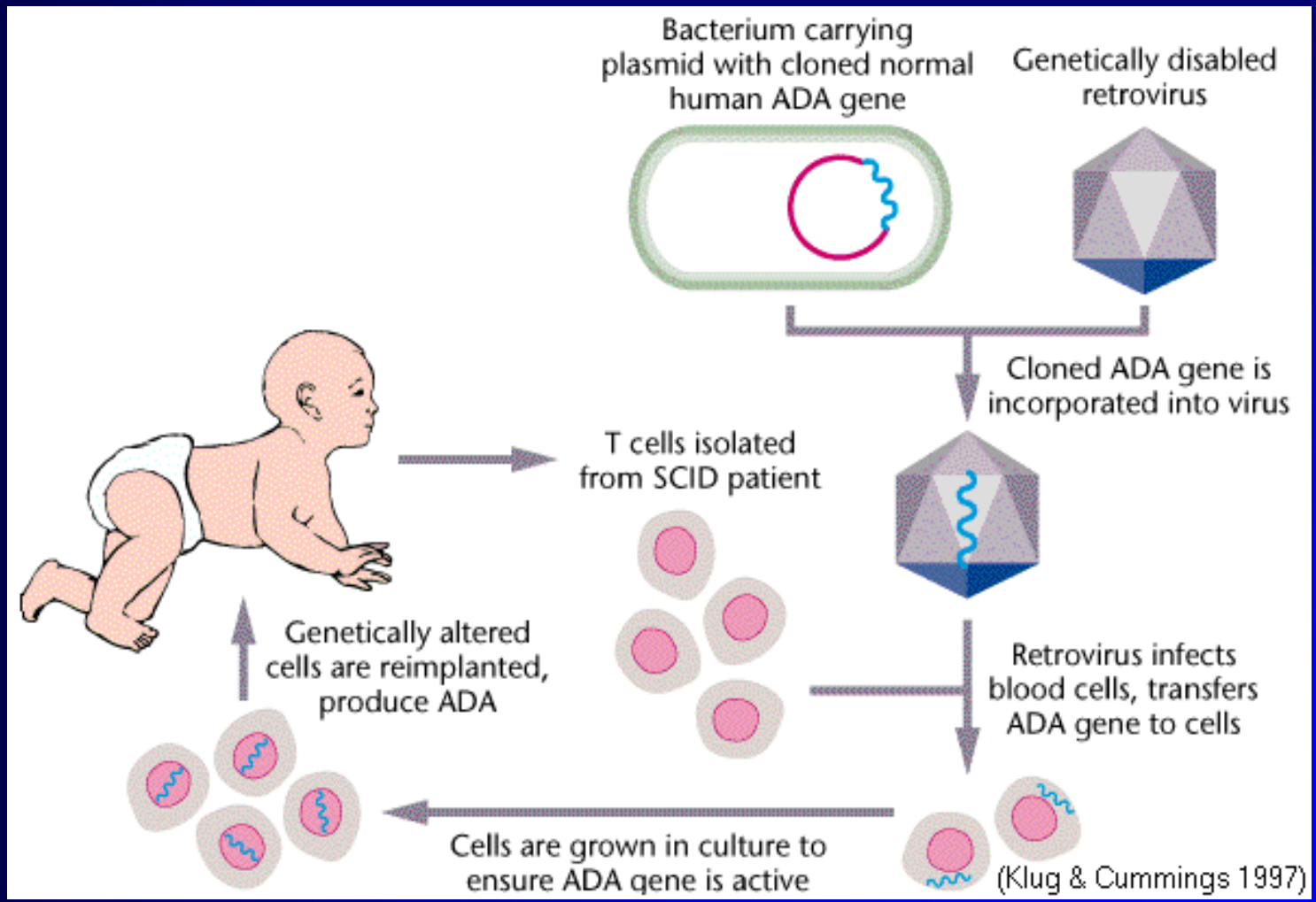


Source: The Journal of Gene Medicine (2004 John Wiley and Sons Ltd), Billam AG

Diseases in gene therapy clinical trials



The First "Therapeutic" Gene Therapy: Adenosine deaminase (ADA) deficiency



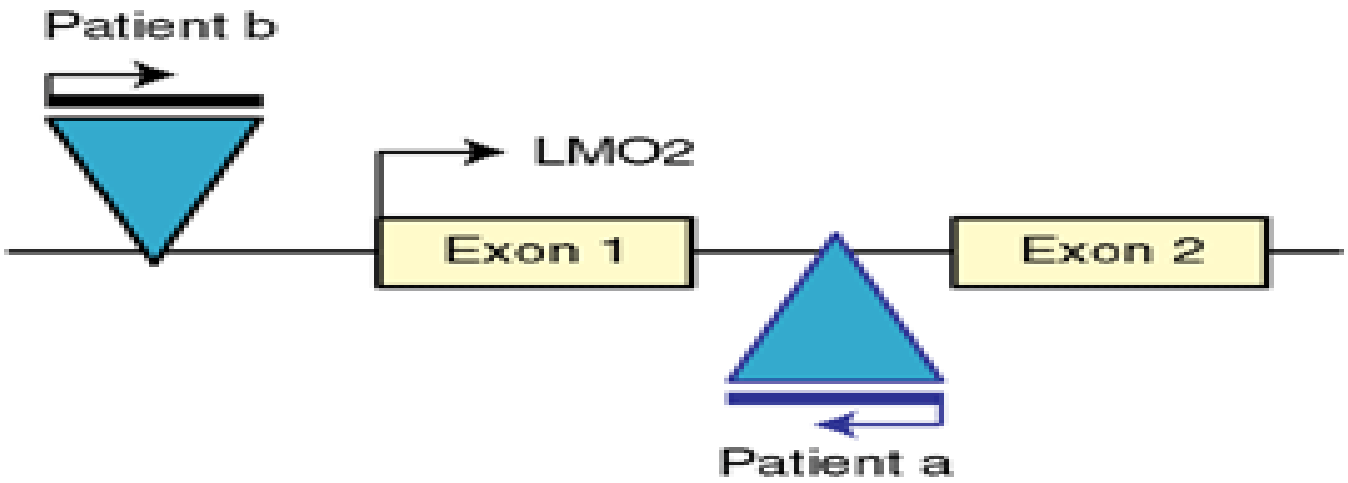
ADA gene therapy

- September 14 1990
- 4 year old girl with no immune function on PEG-ADA
- Isolate T-cells + expand
- Infect with ADA-neo vector
- Series of infusions over a 1 year period
- 2nd patient treated in January 1991
- Initial increase in ADA production - detection of gene modified cells
- Disappointingly, removal of PEG-ADA caused loss of immune function

Gene therapy successful, but insertional mutagenesis

- French X-SCID trial restoring gamma-c receptor
- 9/11 showed immune reconstitution, some long-term
- Three of the children developed leukaemia 2-3 years later, one since died
- Two of these showed insertional mutagenesis at LMO2 proto-oncogene
- Some similar trials suspended

Insertional mutagenesis site identified



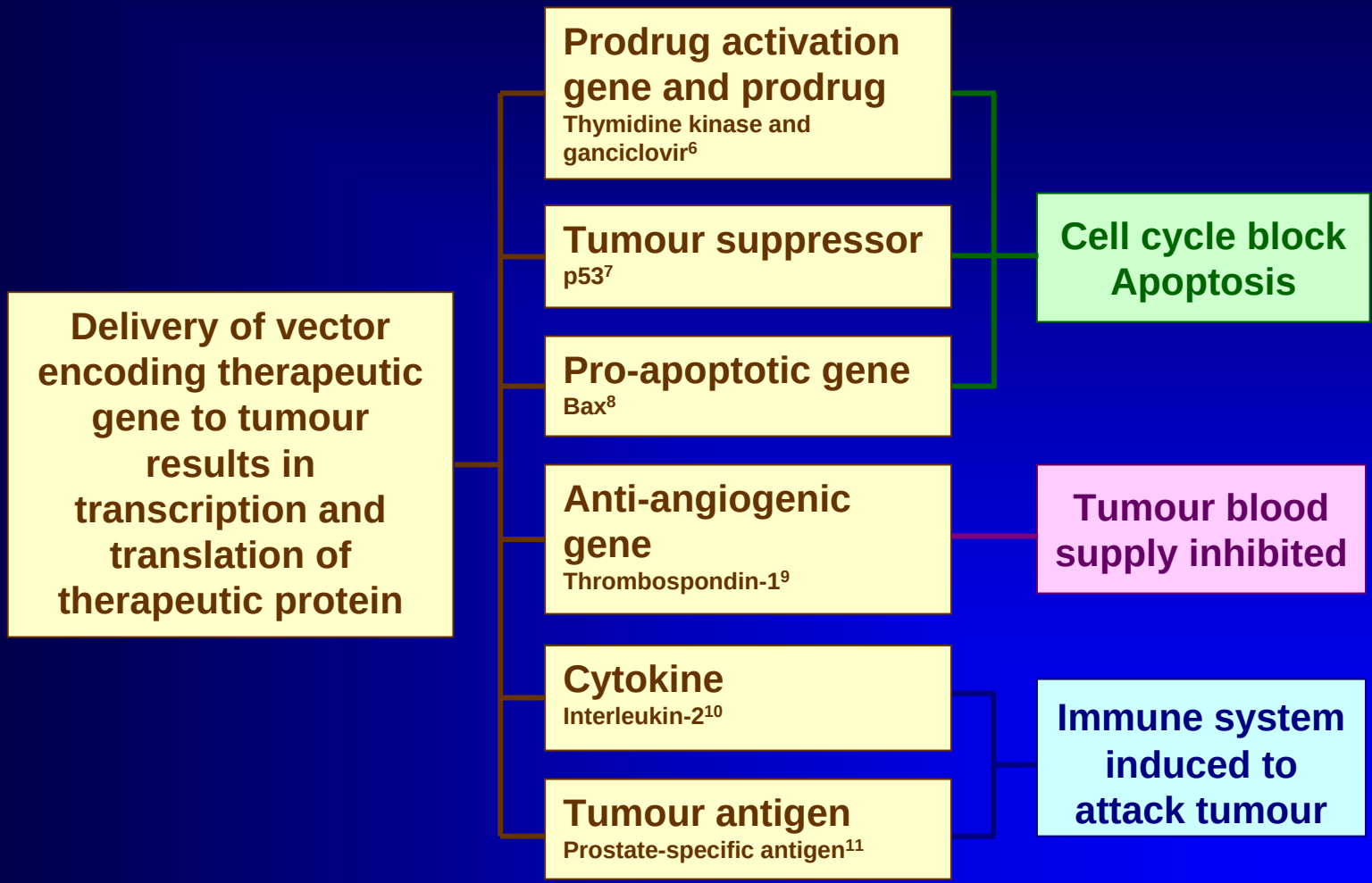
Molecular mechanism for insertional mutagenesis following gene therapy for X-linked severe combined immune deficiency (SCID-X1)

Expert Reviews in Molecular Medicine

Adenoviral gene therapy safety failure

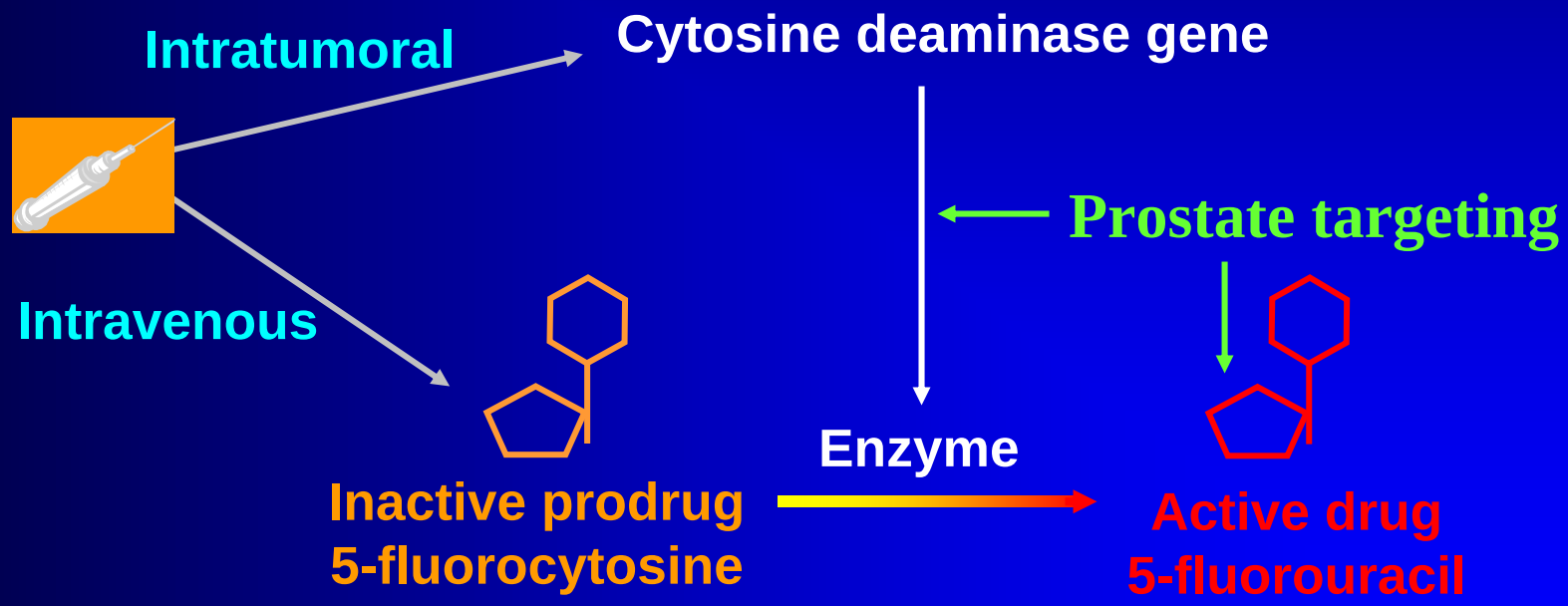
- Ornithine transcarbamylase deficiency
- Unable to digest nitrogen in food protein
- Adenoviral gene therapy trial
- A patient on the trial developed fever following vector infusion
- Trial protocol had not been adhered to
- Four days later, on September 17, 1999, Jesse Gelsinger died, the first fatality definitely linked to gene therapy

Therapeutic genes for cancer

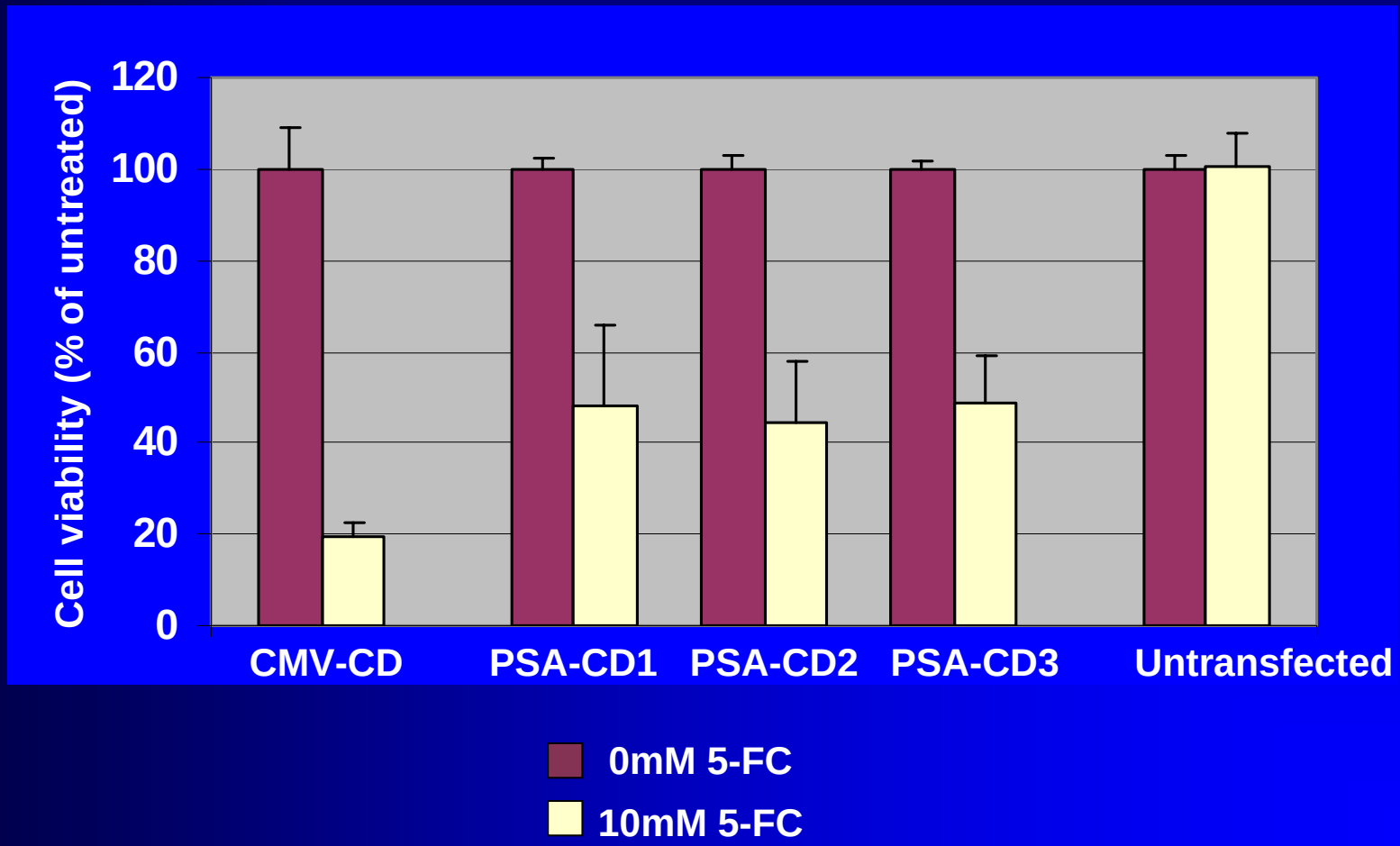


Foley R, Lawler M, Hollywood D. Lancet Oncol 2004, Vol. 5, Pages 469-479

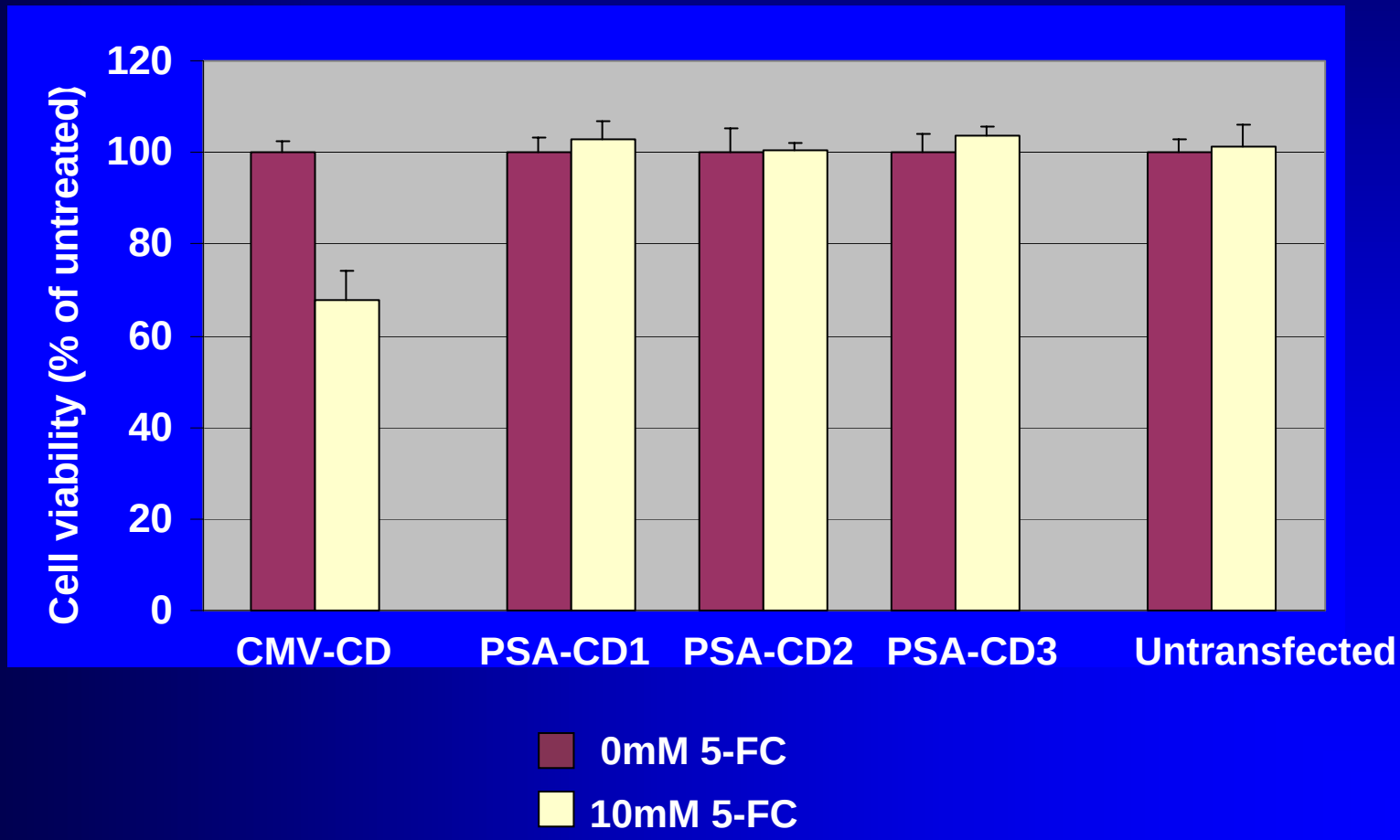
Genetic prodrug activation therapy (Suicide Gene Therapy)



Prodrug activation strategy results in significant cell kill in prostate cancer cells



PSA-CD vectors inactive in colon cancer cells



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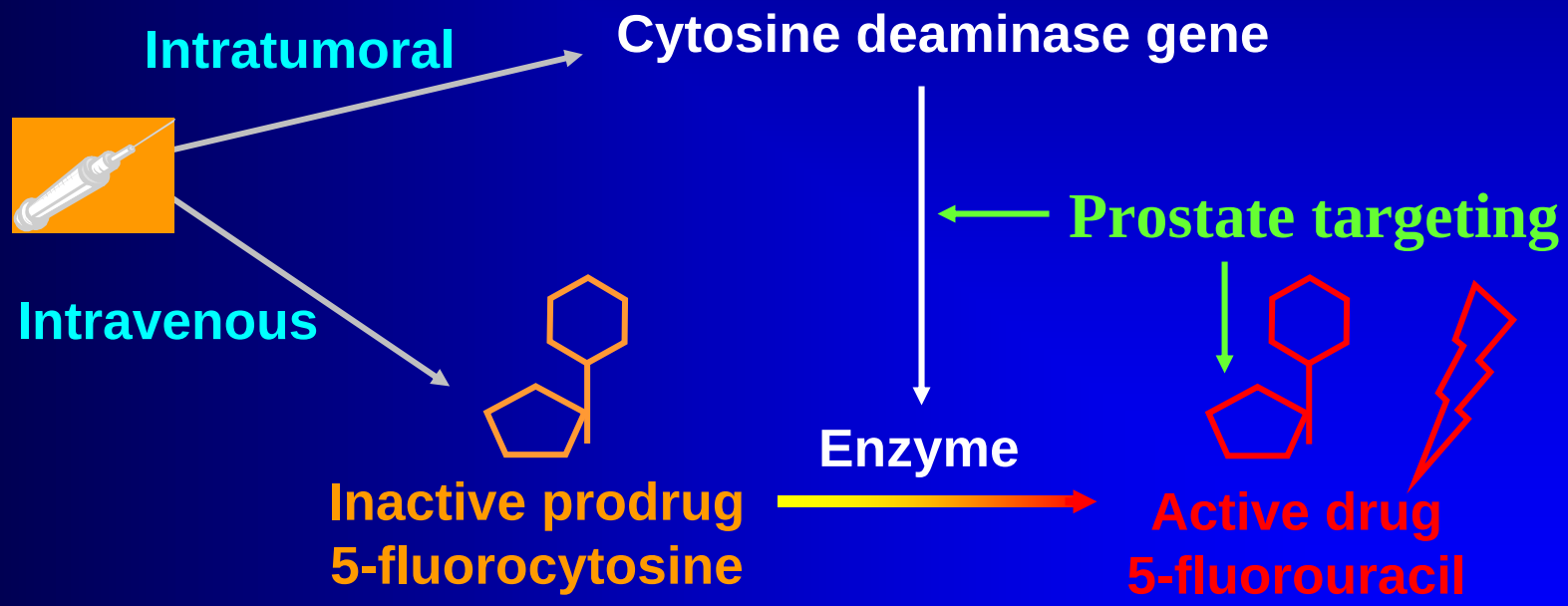
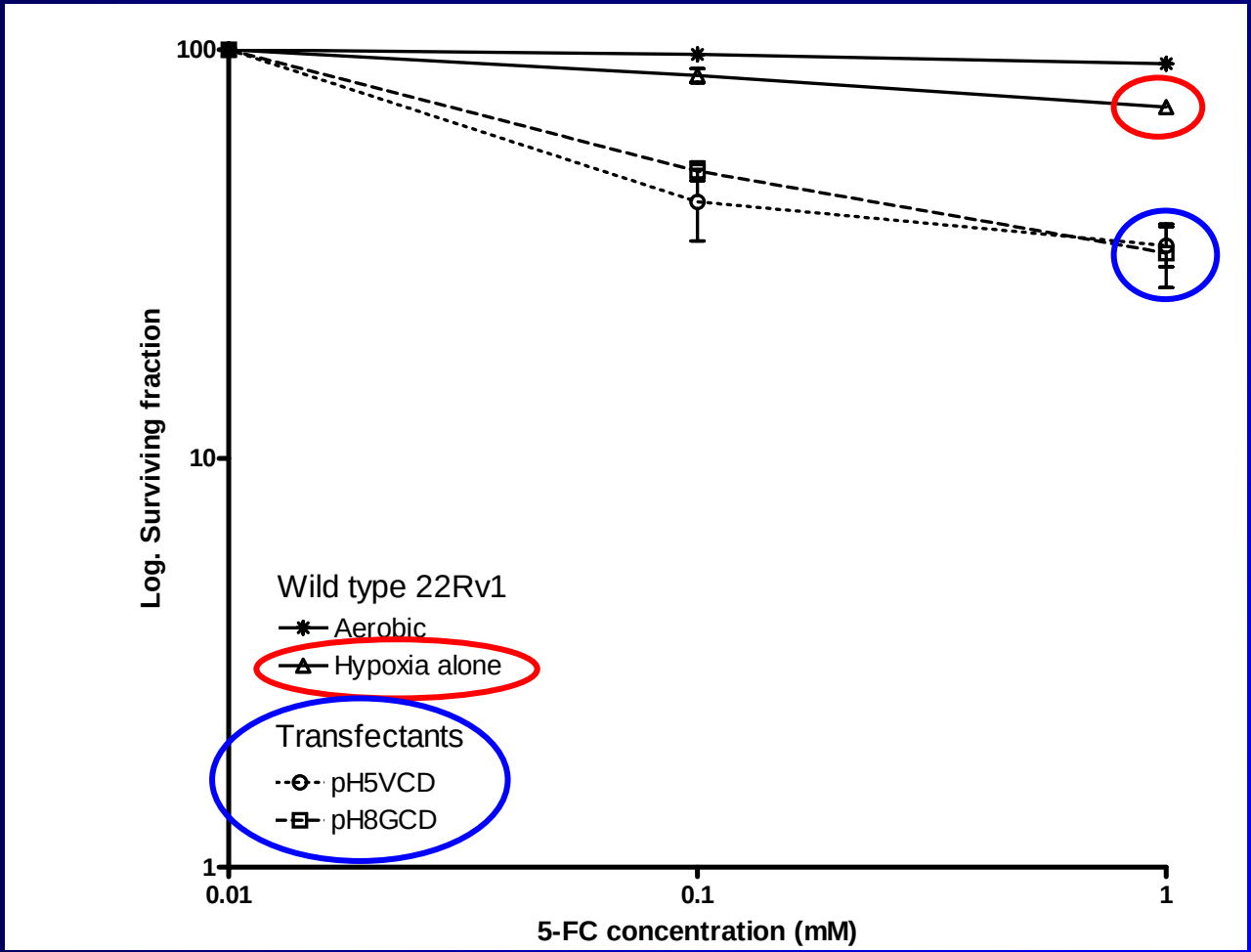
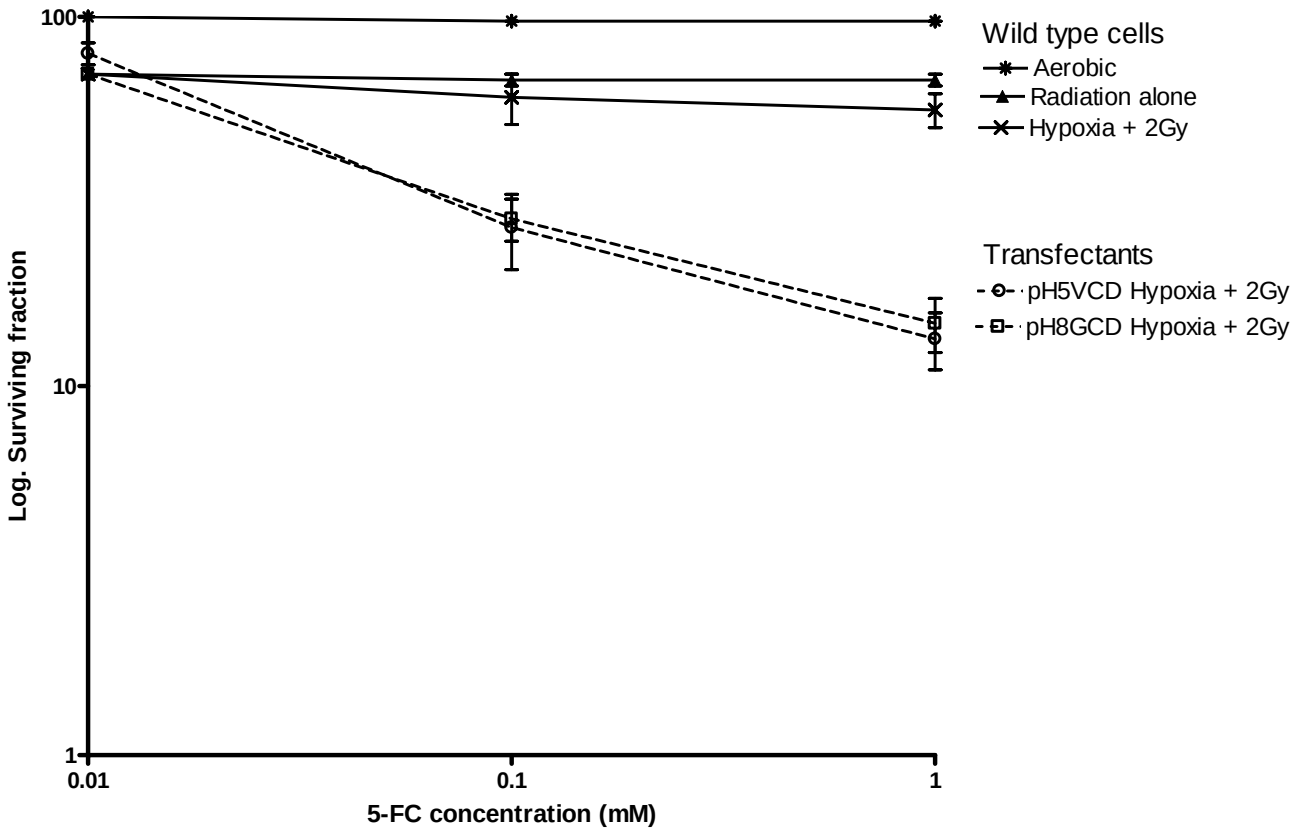


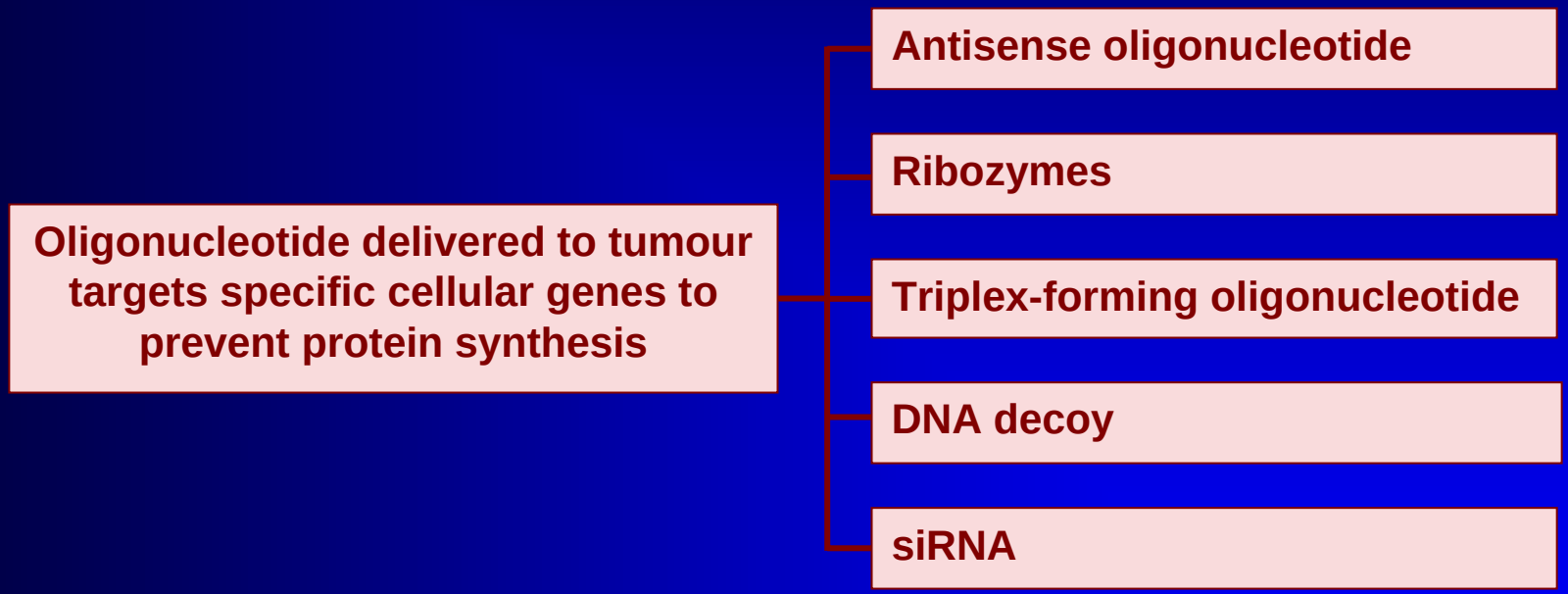
Transcriptional tumour targeting (Hypoxia)



Transcriptional tumour targeting (Hypoxia with radiation)



Oligonucleotide-based therapeutic strategies



Oligo-based therapies

- Potential for high specificity though may also have non-specific effects
- Wide range of targets available
- Difficulty with *in vivo* stability and delivery

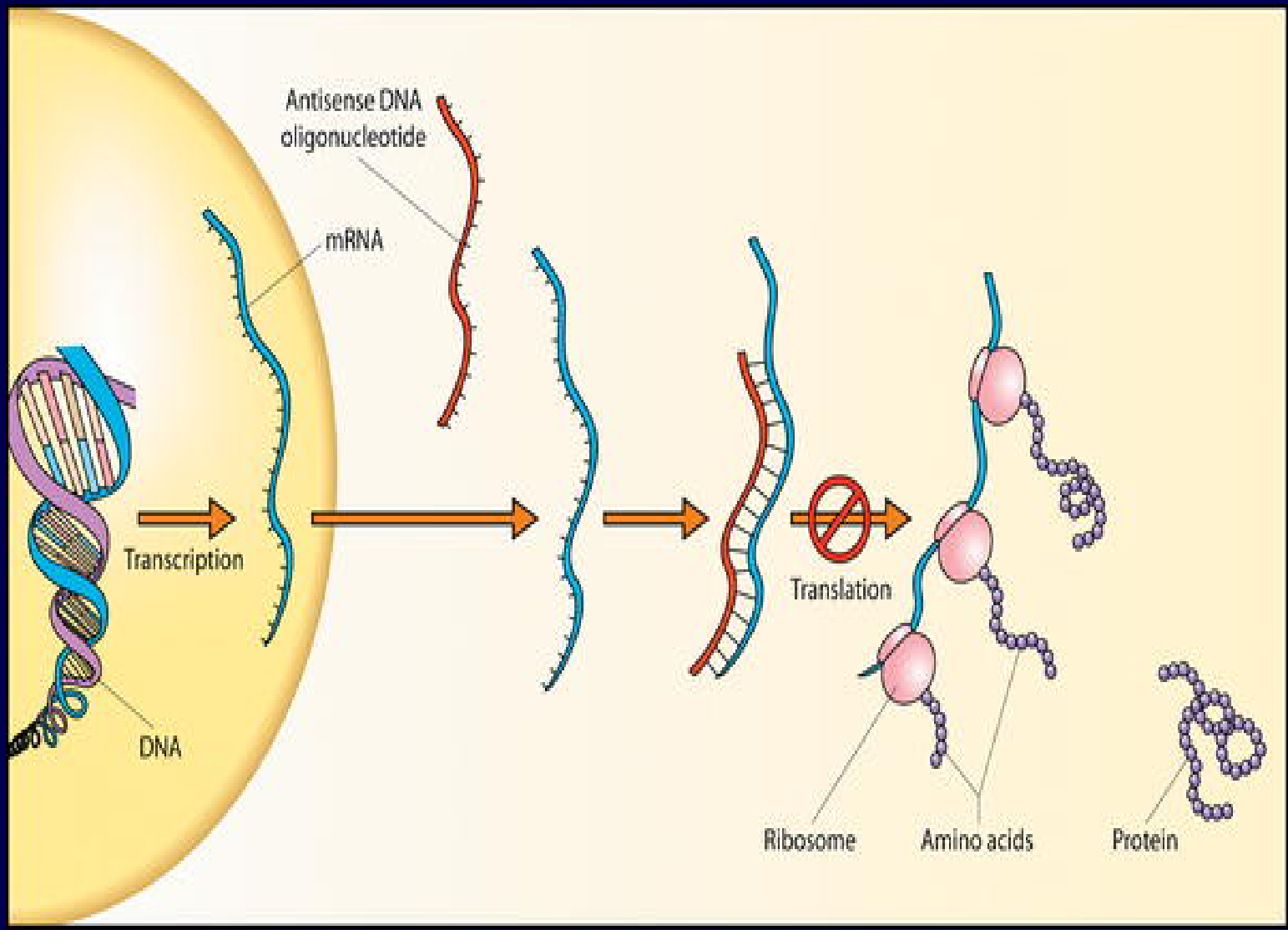
Gene expression as a therapeutic target

- Downregulate expression of genes implicated in malignancy
- Downregulate expression of genes that provide resistance to chemotherapy induced apoptosis
- Downregulate pathogenic viral genes

Antisense

- Antisense - Inhibition of mRNA expression
- Antisense oligonucleotides target and bind to specific regions of mRNA
- Binding inhibits mRNA translation by
  - Steric hindrance
  - Enzymatic destruction of transcript: RNase H

Antisense mechanism



Antisense in the Clinic: Vitravene

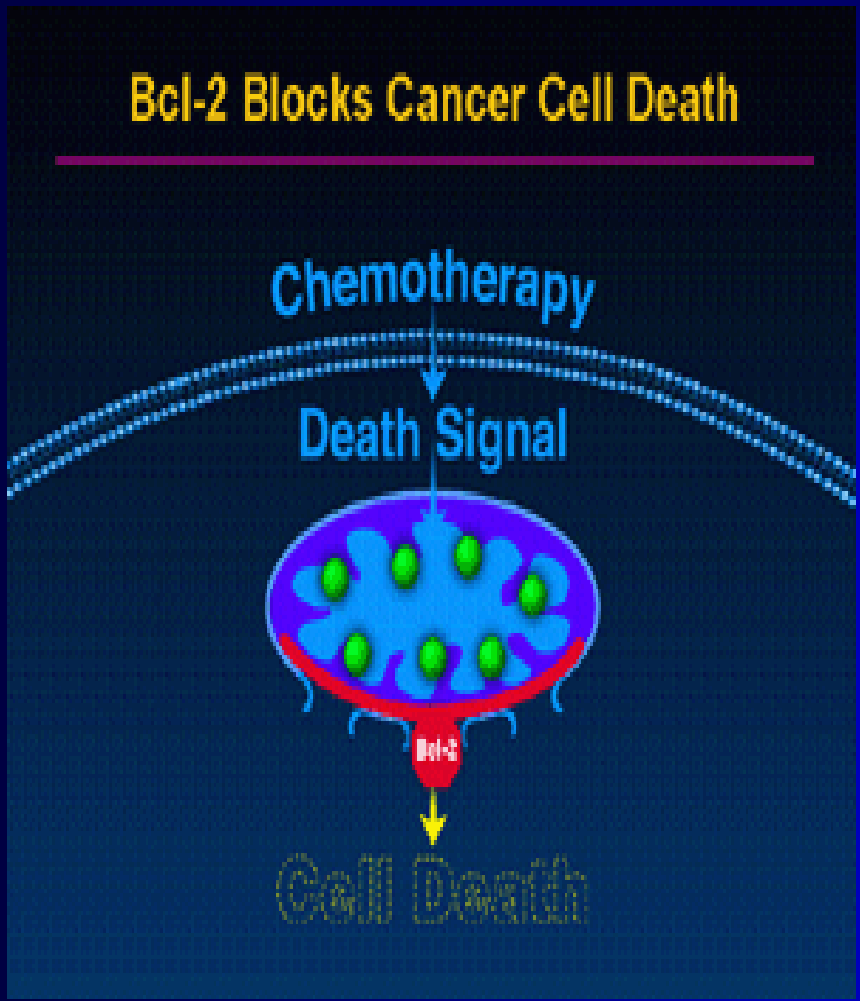
- Antisense inhibitor of human CMV retinitis in AIDS patients (Isis)
- Administered once monthly by intravitreal injection, providing local therapy for retinal infection
- 2nd and 3rd generation drugs
- Efficacy with good safety profile in patients who undergo repeated intravitreal injection
- Vitravene (fomivirsen) is the first (and only) antisense drug to achieve marketing clearance

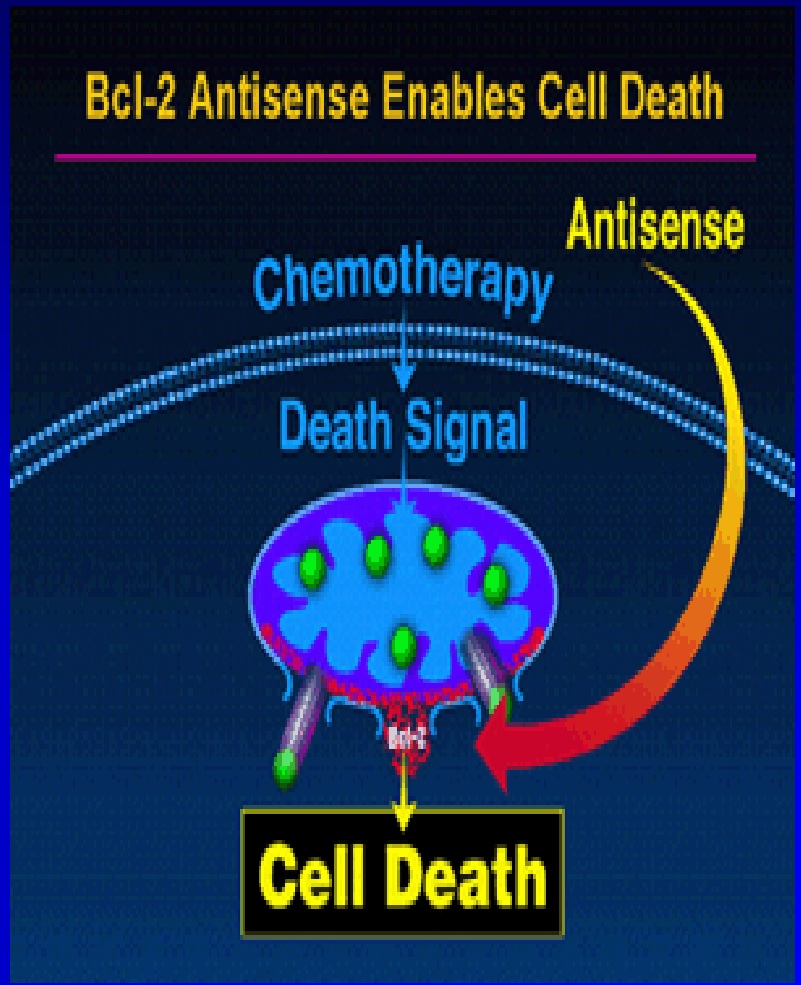
Antisense works!!

...sometimes, but slow progress

- Phase III trials of Genasense (Genta) and other antisense oligonucleotides
- No others licensed to date

Genasense and bcl-2 targeted cancer therapy

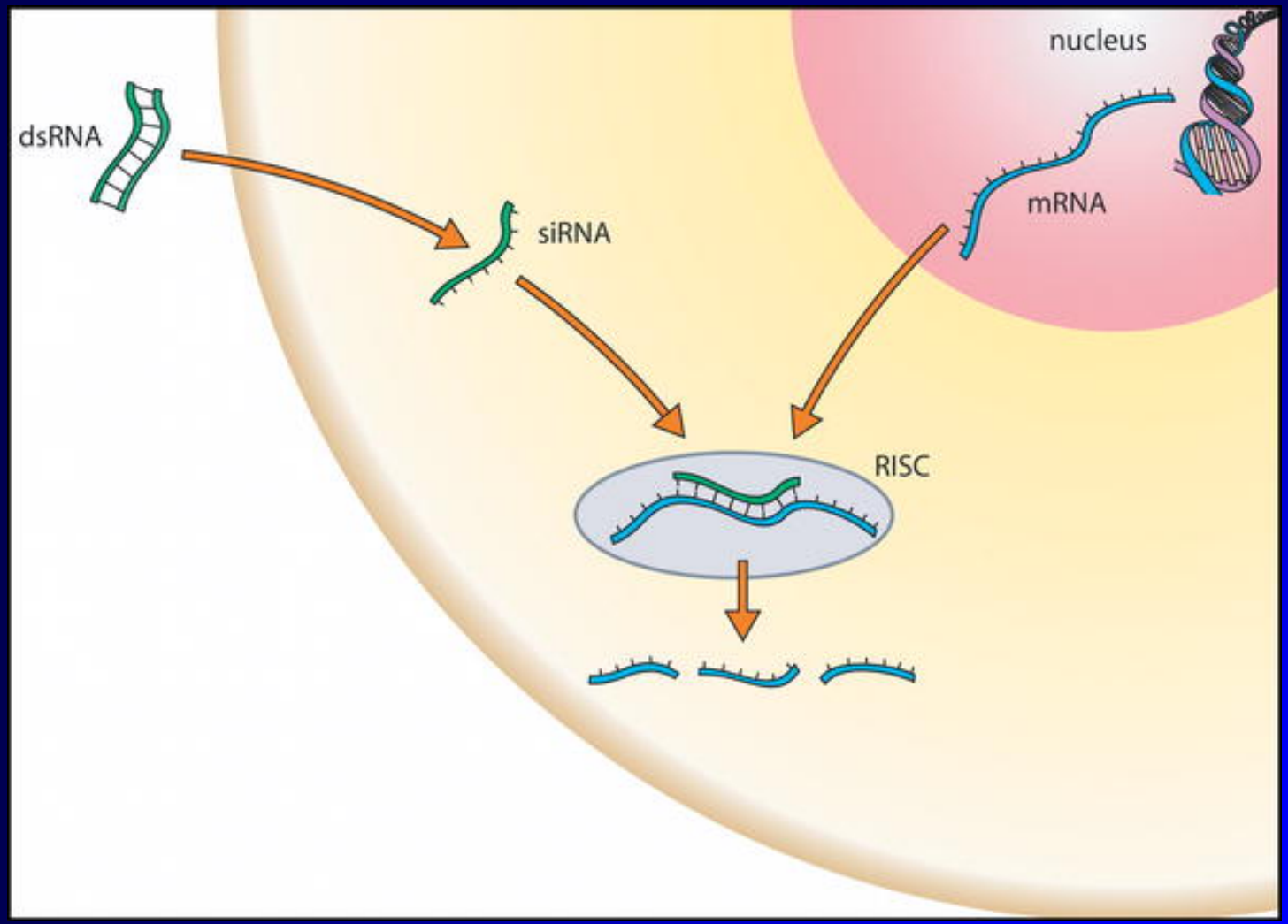




siRNA

- Natural intracellular process
- Enzymatic activity: one molecule can cause degradation of many
- Specificity (20-25bp long)
- Highly potent *in vitro*
- Challenges:
  - How to deliver efficiently
  - Off-target effects
  - Effect of occupying RISCs artificially

siRNA



Robinson R (2004) PLoS Biol 2(1): e28

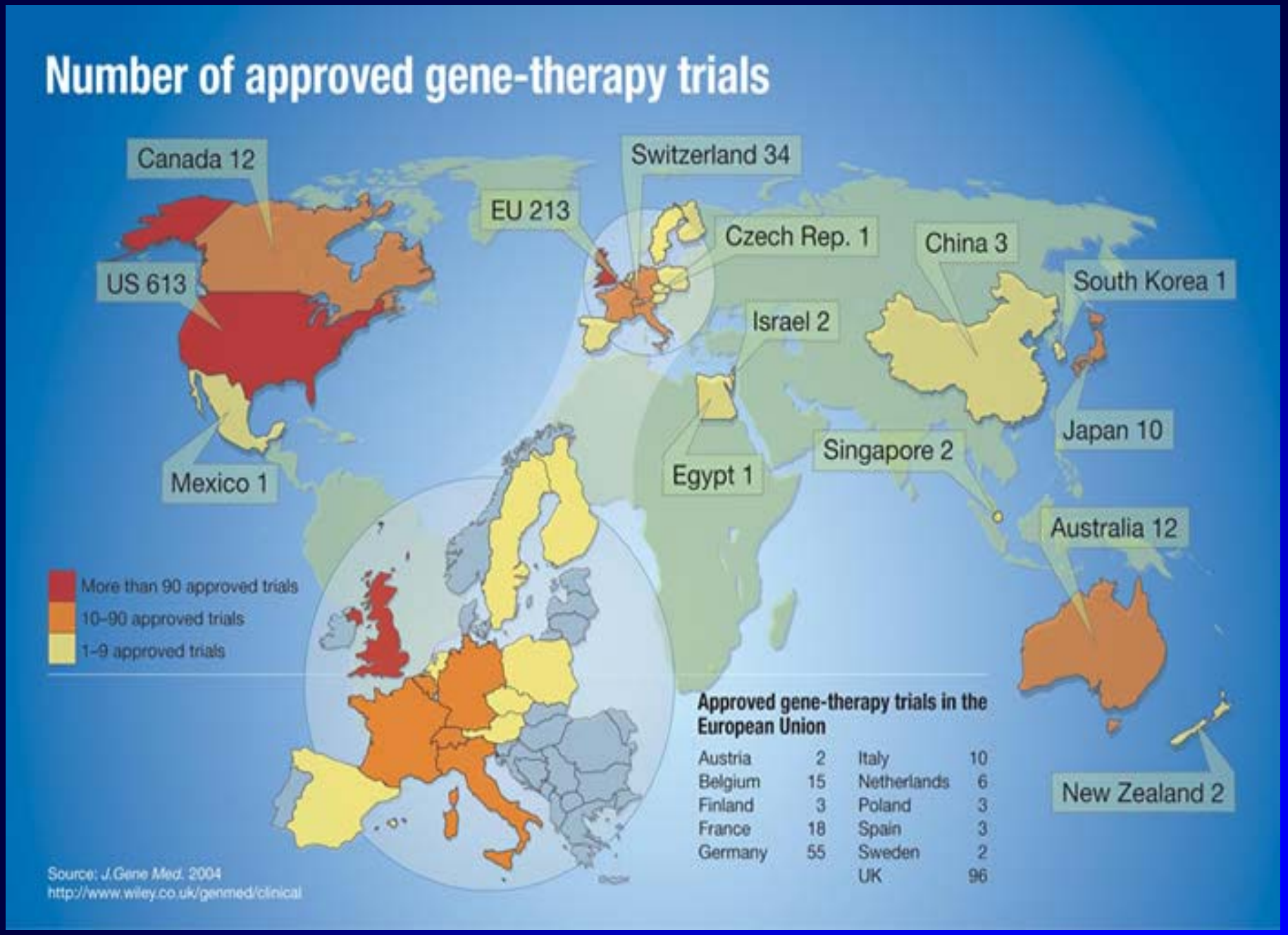
siRNA: not just another variation on a theme

- 2002 Science's Breakthrough of the Year
- 2006 Andrew Fire and Craig Mello win Nobel Prize for discovery of siRNA
- Over 13000 papers published involving siRNA

siRNA in clinical trials for macular degeneration

- VEGF induces blood vessel formation at retina which obscures vision
- Delivery issues less challenging – direct injection
- First siRNA clinical trial started 2004, bevasiranib (Opko) targeting VEGF
- Bevasiranib also in first phase III clinical trial for siRNA 2007
- Other clinical trials for macular degeneration and other conditions ongoing

Number of approved gene-therapy trials



Approved gene-therapy trials in the European Union

Austria	2	Italy	10
Belgium	15	Netherlands	6
Finland	3	Poland	3
France	18	Spain	3
Germany	55	Sweden	2
		UK	96

Source: *J.Gene Med.* 2004
http://www.wiley.co.uk/genmed/clinical

Future molecular therapy targets in development

- Malaria: few new drugs available to date but genome sequencing completed 2002
- Alzheimer's disease: hybrid/dimeric drugs in development targeting two disease-related pathways
- Cystic fibrosis: clinical trials in progress of small molecule modifiers of mutant CFTR function

Molecular therapy: prospects for the future

- More molecular targets being identified
  - Primary disease pathology
  - Drug resistance
- Molecular testing to identify patients likely to benefit
- Rational drug design
- Continuing vector development
- Groundbreaking technology eg siRNA

Molecular therapy: future challenges

- Translation of *in vitro* and *in vivo* efficacy to clinical use
- Safer and more convenient therapies
- Gene therapy: sustained adequate transgene expression
- Novel approaches needed to overcome resistance

Molecular therapeutics: towards the future

- Milestones in molecular medicine
  - 1987 Antiretroviral drugs for HIV
  - 1990 First gene therapy
  - 1998 First targeted cancer therapy
  - 2001 Orally available cancer drug
  - 2004 siRNA in clinical trials
  - 2008... ??
- Already a crucial therapeutic option in specific areas
- siRNA on the brink??
- Here to stay and expand!