

From DNA to Genes and Molecular Medicine



Human DNA: 3 billion nucleotides

Gene: DNA sequence codifying a protein

Molecular Medicine

Mechanisms of monogenic and polygenic diseases

Precision and accuracy in diagnosis

Susceptibility to develop diseases

Personalized Medicine

Gene Therapy



Rosalind Franklin



Francis Harry
Compton Crick

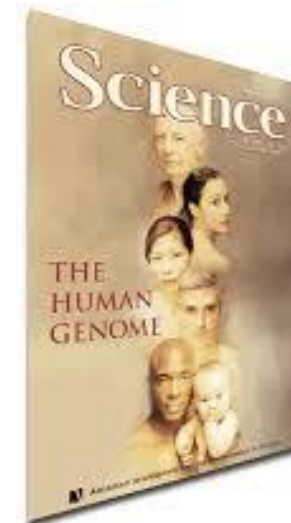


James Dewey
Watson



Maurice Hugh
Frederick Wilkins

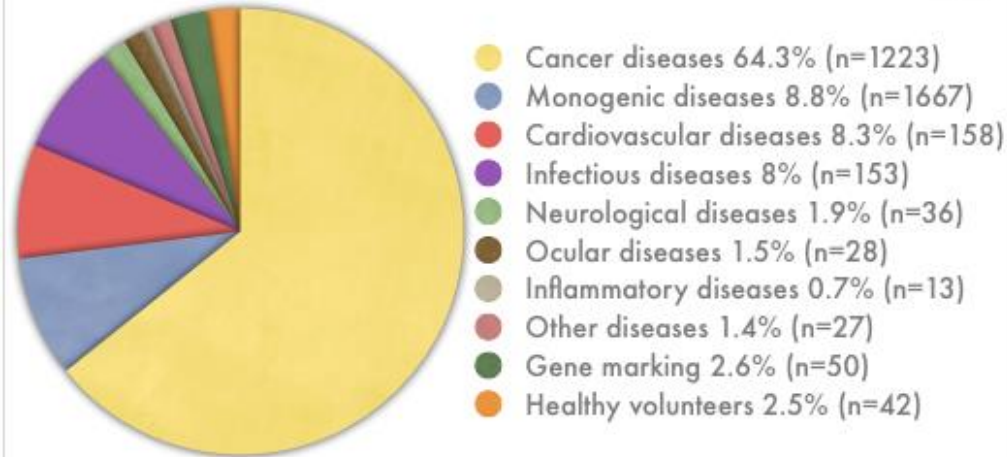
Nobel Prize 1962



Gene Therapy

Introduction of a therapeutic gene mediated by an expression vector able to penetrate in the target organ/cell

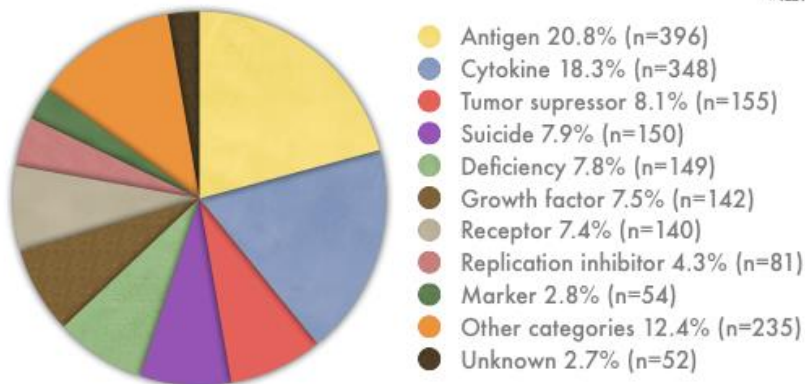
Indications Addressed by Gene Therapy Clinical Trials



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www.wiley.co.uk/genmed/clinical

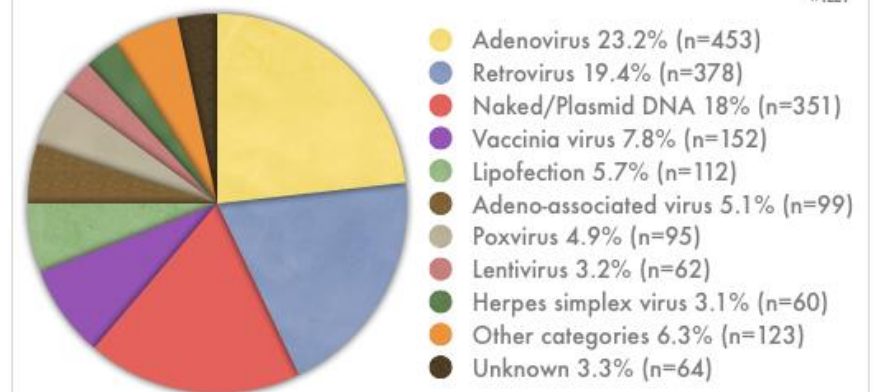
Gene Types Transferred in Gene Therapy Clinical Trials



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Vectors Used in Gene Therapy Clinical Trials



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Why a vaccination against cancer?

Immune Response and Tumors

**Immunodeficients or Immunosuppressed patients:
higher risk to develop tumors**

Lymphoma (x90), melanoma (x29), cervix (x 14)

Spontaneous regressions are possible:

Melanoma (69), gastric tumor (34), lung (25), breast (22)[#]

Antigens in tumor cells can be identified

Why a DNA Vaccine Development?

Naked DNA vaccination is emerging as a promising approach for introducing foreign antigens into the host, inducing protective immunity against infectious diseases and malignant tumours.

Antigen-specific DNA vaccination can induce both cellular and humoral immune responses

ADVANTAGES

Multiple or multi-gene vectors encoding several antigens/determinants and/or immune-modulatory molecules can be delivered **SIMPLY**

Long-lasting immune responses

Plasmid vectors can be constructed and tested with relative rapidity

Rapid and large-scale GFP manufacturing procedures at **LOW COST**

DNA is more **TEMPERATURE STABLE** than live or protein/peptide formulations so **EASY STORAGE** and **DISTRIBUTION**

DNA vaccination does not induce autoimmune disease in normal animals

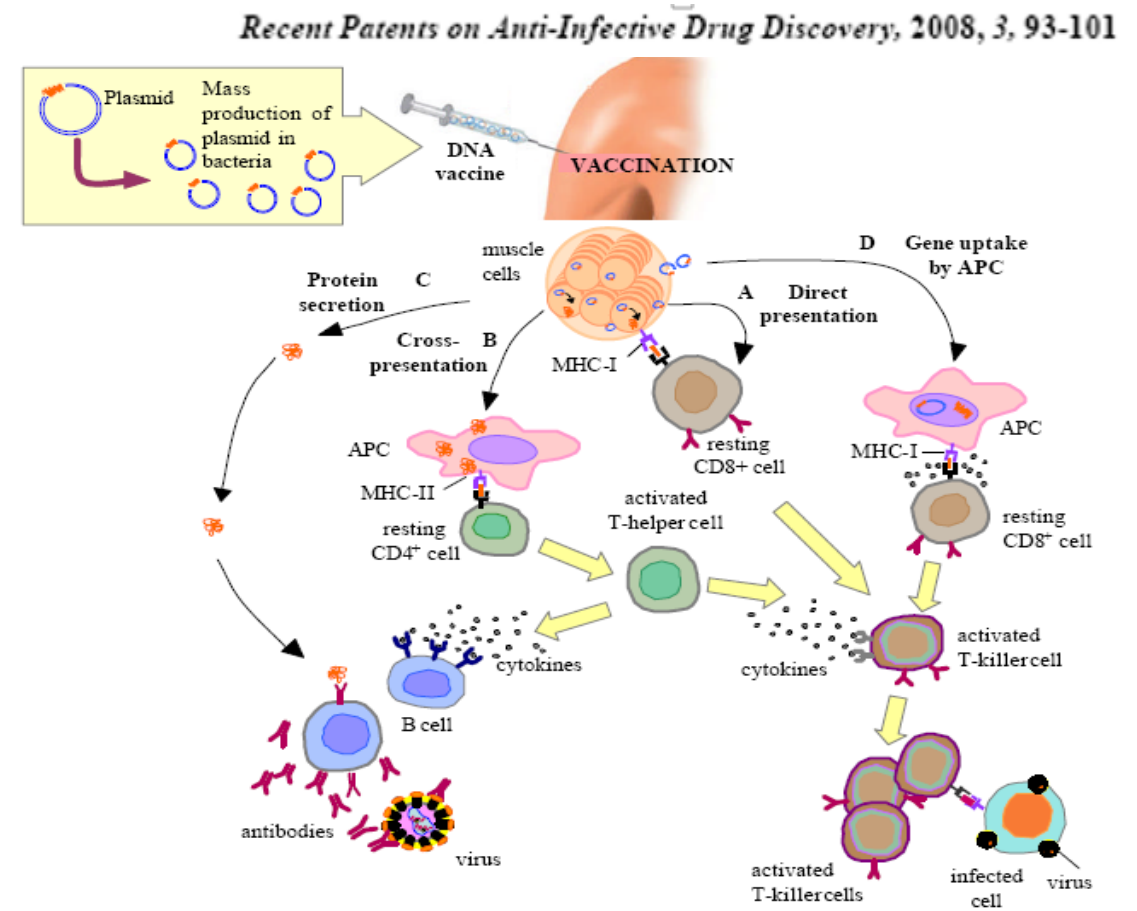


Fig. (1). Mechanism of action of naked DNA vaccine.

Viral antigen sequence is inserted in a bacterial plasmid. After mass production of plasmid in bacteria the naked DNA vaccine can be delivered by intramuscular injection. Plasmid enters in the nucleus of muscle cells, where the gene is transcribed, followed by protein production in the cytoplasm. Transfected muscle cells have the potential to activate T cells through direct presentation (A) as well as cross-presentation (B) allowing stimulation of both CD4+ T-helper and CD8+ cytotoxic T lymphocytes. Furthermore, secreted proteins (C) can induce the production of antibodies that will react with and eliminate virus. Professional APCs can directly uptake DNA vaccine (D), present peptides in context of the MHC-I and activate killer cells which lyse virus-infected cells.

DNA Vaccines: a Promising Approach

Considerations for Plasmid DNA Vaccines for Infectious Disease Indications

<http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/default.htm>

- **Toxicity studies suggest that DNA vaccines are safe**
- **Dozens of phase I clinical trials involving DNA vaccines (alone or as prime/boost) have been conducted**
- **Many hundreds of normal volunteers have been vaccinated**
- **Multi-milligram doses have been administered repeatedly to the same subjects.**

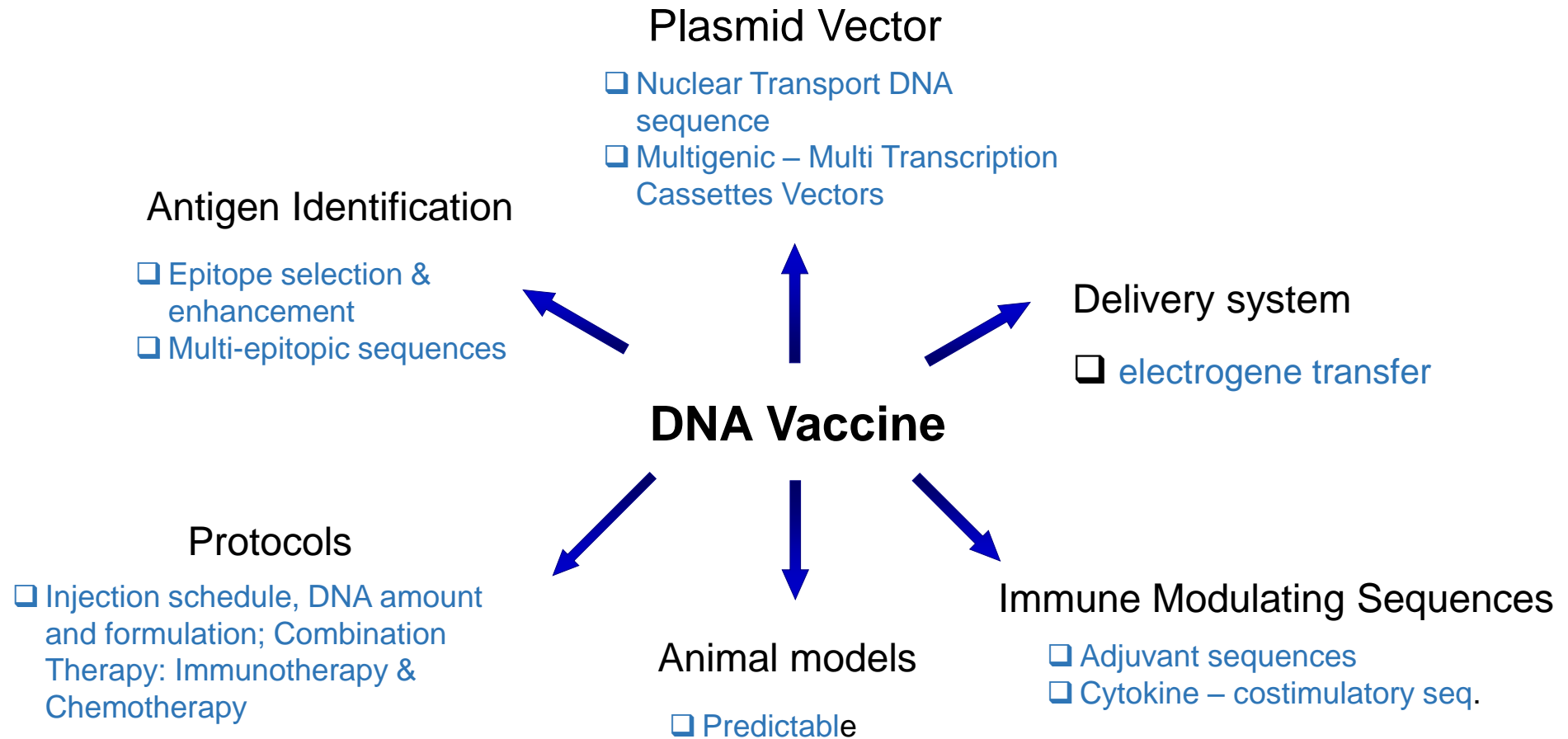
No serious adverse events have been reported

NEVERTHELESS...

Compared with many traditional vaccines, DNA vaccines induce low immune responses

Improvement of vaccine efficacy has become a critical goal in the development of DNA vaccination

Studies on Strategies to Improve DNA Vaccine Efficacy



MATHEMATICAL MODELS FOR OPTIMIZATION OF PLASMID DNA TRANSFER IN MUSCLE CELLS

we propose a new methodological approach based on the coupling of biology assays and predictive mathematical models in order to clarify the mechanism of the DNA uptake and expression into cells. Once better clarified these processes, we will be able to propose more efficient therapeutic gene transfer protocols for treating human patients.

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Gene Therapy: The Role of Cytoskeleton in Gene Transfer Studies Based on Biology and Mathematics

Maria G. Notarangelo¹, Roberto Natalini² and Emanuela Signori^{3,4,*}

Role of the Cytoskeleton in Gene Transfer

Current Gene Therapy, 2014, Vol. 14, No. 2 125

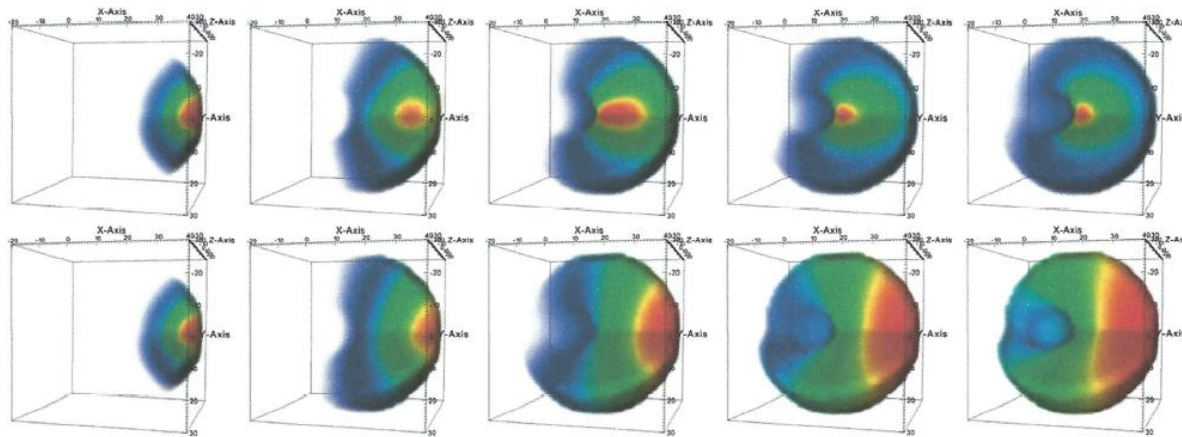


Fig. (1). Time evolution from the left to the right: Peripheral cargo activation. 3D-simulation of Rb nuclear import. Concentration of Tc : with active transport (above) and inhibited binding (below) (see [27]).

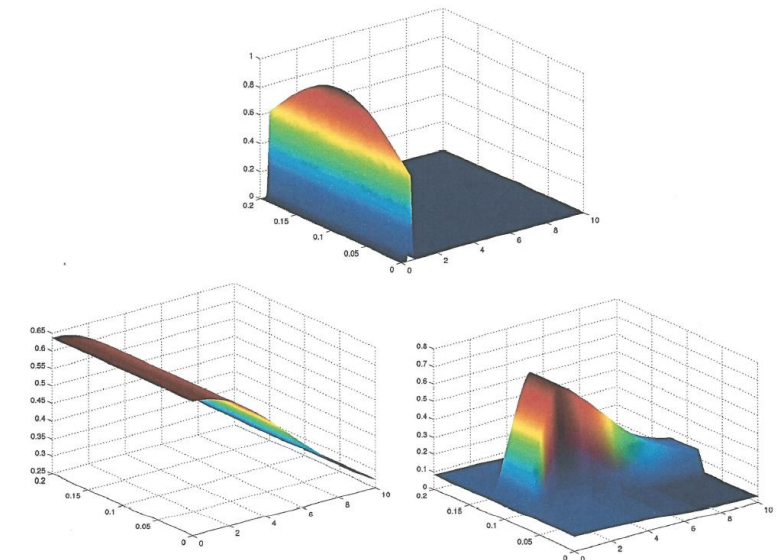


Fig. (2). Top: Initial cargo concentration in $\Omega=[0,10] \times [0,0.2](\mu m^2)$. Bottom: at left, final cargo concentration at time $T=2$, without microtubule support; at right, total concentration profile at time $T=2$ with microtubule activity.