Molecular & Gene Therapy

Definitions:
- Molecular Therapy (aka biologics):
  - Treatment that targets specific molecular features of cancer
    - Targets: oncogenes, tumor suppressor genes, apoptotic pathways, telomerase, angiogenesis
    - Drugs: small molecule inhibitors, antibodies, siRNA, viruses
- Gene therapy:
  - specific type of molecular therapy that seeks to complement a genetic defect through gene replacement

History of Gene Therapy
- 1944 DNA is determined to be the hereditary material
- 1953 The structure of DNA is determined
- 1961-7 The genetic code is deciphered
- 1968 Restriction endonucleases are discovered
- 1973 The technique for recombining different genes in living cells is established
- 1976 The first cancer gene is identified
- 1986 Tumor suppressor genes are identified
- 1990 The international human genome project is created
- 1991 The first human gene therapy experiment is initiated
- 1992 The first gene therapy trial using in vivo gene transfer for cancer is initiated
- 1993 The first gene therapy trials for autoimmune and vascular disease are approved
- 1994 More in vivo gene transfer trials than ex vivo methods are approved
- 1999 More than 1000 individuals have received experimental gene therapy
- 1999 Death of a patient after adenoviral gene therapy
- 2000 Successful treatment of X-linked severe immunodeficiency
- 2002 Murine leukemia induced by retroviral insertion
- 2003 Human leukemia develops after retroviral gene therapy
- 2004 First commercialization of clinically approved p53 gene therapy (China)
Curing SCID with retroviral gene therapy

The γc partners with many interleukin receptors to bind cytokines (growth factors for cells of the immune system). Mutations in γc results in severe combined immunodeficiency (SCID).

Gene Therapy Approaches

According to target tissue

Somatic: targeted to non-reproductive cells. All current gene therapies belong to this category.

Germ-line: targeted to reproductive cells and may eliminate disease for later generations. Currently not feasible and ethically controversial.

According to location of gene transfection

Ex vivo: carried out for tissues or cells that can be removed.

In vivo: carried for all tissues and organs.

According to delivery vehicle

Non-viral: DNA, RNA or their mixture in combination with other physical or chemical agents.

Viral: human or animal viruses that have been genetically engineered to carry therapeutic genes.
Gene therapy: protocols by therapeutic gene:

![Gene Types Transferred in Gene Therapy Clinical Trials](image1)

Vectors Used in Gene Therapy Clinical Trials:

![Vectors Used in Gene Therapy Clinical Trials](image2)

Protocols by trial phase:

![Phases of Gene Therapy Clinical Trials](image3)
protocols by disease type:

Methods to deliver genes into human cells take advantage of various advances in molecular biology:

Non-viral gene delivery methods
- Naked DNA or RNA injection
- Chemical approaches
calcium phosphate, cationic liposomes, nanoparticles, polymeric matrices
- Physical approaches
electroporation, microinjection, particle bombardment
- Biological approaches
DNA-protein complexes, viral envelope-DNA complexes, nuclear localization peptides

Viral gene delivery vectors
- Adenovirus
- Adeno-associated virus (AAV)
- Herpes simplex virus (HSV)
- Poxvirus (vaccinia)
- Lentivirus (HIV, FELV)
- Moloney murine leukemia virus (MoMLV)  Red = retrovirus
Cancer gene therapy by delivery of tumor suppressor genes (TSG)

p53 is mutated in half of all human cancers. Loss of p53 function is required for tumor maintenance. Several forms of gene therapy attempt to express p53 in p53 mutant human cancer cells.

Cancer therapy with viruses that do not transfer genes

- Oncolytic adenovirus that replicates only in cells lacking p53/or Rb function

By deleting the E1A and E1B regions of the adenovirus genome, this mutant virus can only replicate in cells lacking Rb and p53.
ONYX-015: adenovirus that lacks E1B and (selectively?) replicates in p53 null cells

10^9 infectious particles were injected into a patient with head and neck cancer over 5 days

Cancer therapy with bacteria that replicate in hypoxic tumor cells

- Anaerobic bacteria: Clostridium infected into xenograft tumors

Dang et al PNAS 2001

Perhaps anaerobic bacteria could be used with radiation to attack the hypoxic (and radio-resistant) regions of cancers?
Other approaches to increase specificity of gene therapy to tumor cells

- Express suicide genes from a promoter that is specific for tumor cells:
  - PSA promoter (prostate)
  - Telomerase
  - Promoter that responds to radiation therapy or heat

Heat-induced gene therapy

- Heat-shock protein (HSP) promoter drives expression of a toxin
Gene therapy to deliver an enzyme that turns a non-toxic prodrug into a toxin

Gene therapy to activate the immune system

Genome Editing: Rather than transferring in a gene, what about “editing” the mutant genome to correct the mutant gene

Zn Finger Nucleases: Combine DNA binding specificity of Zn Finger Protein with a cleavage domain of a restriction enzyme
Targeted genome editing in human repopulating haematopoietic stem cells

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TALENs: Transcription activator-like effector nucleases, which are artificial restriction enzymes with specificity for a particular DNA sequence

Zn Finger Nucelases and TALENs engineer proteins to recognize specific sequences of DNA (Very Difficult)

CRISPR-associated Cas9 nuclease (from S. pyogenes): targeted to specific DNA sites for single guide RNA (sgRNA) that is complimentary to a specific sequence (Relatively Easy)
Molecular & Gene Therapy

a. Gene disruption: silence a pathogenic gene
   - Nuclease
   - Pathogenic protein

b. NHEJ gene correction: deletion of a pathogenic insertion
   - DSB
   - NHEJ
   - Nonfunctional protein
   - Functional protein

c. HDR gene correction: correct a deleterious mutation
   - DSB
   - Corrective HDR template
   - Corrected protein

d. HDR gene addition: introduce a therapeutic gene
   - DSB
   - Corrective HDR template
   - Functional protein
Major Limitations to gene therapy including genome editing for cancer:

• Delivery
  – Very difficult to infect/deliver the gene into every cancer cell
  • Even when the “bystander effect” is at play, hard to reach the last clonongen
  • Perhaps in combination with standard therapies (radiation therapy) may be useful
• Potential toxicity

Molecular Therapy (aka biologics)

• Treatment that targets specific molecular features of cancer
  – Effective molecular therapies target proteins or pathways that are required for tumor maintenance
  – Ideal therapy has minimal toxicity, because the target or pathway is not essential for normal cells
• In contrast to “non-specific” cytotoxic therapy
**Targets of Molecular Therapies**

- **Angiogenesis:**
  - VEGF with **Bevacizumab (Avastin)**, a monoclonal antibody approved for colorectal cancer. Avastin can “normalize” tumor vasculature and appears to improve the response of tumors to radiation therapy.

- **Oncogenes:**
  - Her2/neu with **Trastuzumab (Herceptin)**, Lapatinib (Tykerb) for breast cancer
  - EGFR with **Cetuximab (Erbitux)** for Head and Neck Cancer, Erlotinib (Tarceva) for non-small cell Lung Cancer
  - B-Raf with Sorafenib (Nexavar) for renal cancer
  - B-Raf with PLX4032 (Flaherty et al NEJM 2010)

Red = monoclonal antibodies  
Black = small molecule inhibitors
• Signaling Pathways:
  – CD20 with Rituximab (Rituxan) is a monoclonal antibody that is used to treat B-cell lymphomas. Rituximab binds to CD20 and blocks intracellular signaling that can alter apoptosis.

• Proteosome Function:
  – Proteosome Inhibitor: Bortezomib (Velcade) for myeloma. The proteosome is the cells trash compactor and degrades proteins. Velcade blocks entry of proteins into the proteosome, so that excess proteins are present in cancer cells, which causes cell death
• Apoptotic Machinery:
  – Bcl-2: Oblimersen (Genasense) testing in melanoma
  • Goal: decrease Bcl-2 levels and lower the threshold for apoptosis
  – ABT-737 and ABT-263 (Navitoclax) are BH3 mimetic that blocks Bcl-2, Bcl-xL, and Bcl-w (but not Mcl-1)

Substantial Susceptibility of Chronic Lymphocytic Leukemia to BCL2 Inhibition: Results of a Phase I Study of Navitoclax in Patients With Relapsed or Refractory Disease (JCO 2011)

Summary:

1. Gene therapy (particularly with genome editing tools like CRISPR) is likely to become an important approach for treating human disease in the near future. Applications to cancer therapy are likely, particularly for engineering T-cells to target a specific antigen. However, limited efficiency of targeting most/all cancer cells remains a major limitation.
2. Molecular therapies are now part of the armamentarium for treating cancer.