Gene therapy approaches to cancer treatment

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JCCC Tumor Immunology

UCLA Clinical Pharmacology course (M263)
Gene Therapy

Intracellular delivery of genetic material to generate a therapeutic effect by correcting an existing abnormality or providing cells with a new function.

- Virus
- Naked DNA
- Liposomes
- Dendrimers
- Ex vivo
- In situ
- In vivo
- Gene replacement
- Inherited disorders
- Vascular diseases
- Neurodegenerative
- Infectious diseases
- Cancer
- ...
Viral Vectors
Life Cycle of Replicative Viruses

- Virion binding
- Uncoating
- Reverse transcription
- Proviral DNA
- Nuclear transport
- Integration
- Transcription/translation of viral gene products
- Assembly, budding, maturation of virion

Retroviridae
Viral Vector (C-type retrovirus)

Our favorite new gene

Production of the desired protein
Characteristics of the Ideal Vector

• Large carrying capacity of foreign DNA
• Safe
• Non-immunogenic
• Efficient transgene expression
• Long duration of expression
• Allow re-administration
# Summary of Vector Characteristics

<table>
<thead>
<tr>
<th>Vector</th>
<th>Expression</th>
<th>Infect Non-Dividing Cells</th>
<th>Transd. Efficiency</th>
<th>Immuno- genusity</th>
<th>Insert Size</th>
<th>Silencing</th>
<th>Safety In Humans</th>
<th>Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>γRetrovirus</td>
<td>Stable</td>
<td>No</td>
<td>Fair</td>
<td>No</td>
<td>&lt; 8 Kb</td>
<td>Variable</td>
<td>Fair</td>
<td>Easy</td>
</tr>
<tr>
<td>Lentivirus (HIV)</td>
<td>Stable</td>
<td>Yes</td>
<td>Fair</td>
<td>No</td>
<td>&lt; 10 Kb</td>
<td>No</td>
<td>Unkn</td>
<td>Fair</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>Transient</td>
<td>Yes</td>
<td>High</td>
<td>Yes</td>
<td>&lt; 10 Kb</td>
<td>n.a.</td>
<td>Fair</td>
<td>Easy</td>
</tr>
<tr>
<td>HSV</td>
<td>Transient</td>
<td>Yes</td>
<td>High (toxic)</td>
<td>Yes</td>
<td>&lt;50 Kb</td>
<td>n.a.</td>
<td>Yes</td>
<td>Difficult</td>
</tr>
<tr>
<td>Poxvirus (Vaccinia)</td>
<td>Transient</td>
<td>Yes</td>
<td>High (toxic)</td>
<td>Yes</td>
<td>&lt;25 Kb</td>
<td>n.a.</td>
<td>Yes</td>
<td>Easy</td>
</tr>
<tr>
<td>AAV</td>
<td>Stable</td>
<td>Yes</td>
<td>Fair</td>
<td>No</td>
<td>&lt;5 Kb</td>
<td>No</td>
<td>Yes</td>
<td>Difficult</td>
</tr>
<tr>
<td>Naked DNA</td>
<td>Transient</td>
<td>Yes</td>
<td>Low</td>
<td>No</td>
<td>&lt; 10 Kb</td>
<td>n.a.</td>
<td>Yes</td>
<td>Very Easy</td>
</tr>
</tbody>
</table>
Examples of Uses of Gene Therapy for Cancer
Delivery of tumor-suppressor genes

<table>
<thead>
<tr>
<th>Gene product</th>
<th>Function</th>
<th>Expression in cell lines</th>
<th>Expression in mouse models</th>
</tr>
</thead>
<tbody>
<tr>
<td>INK4A</td>
<td>Blocks cell cycle by inhibiting CDK4</td>
<td>Growth arrest (some evidence of resistance)</td>
<td>Tumour suppression</td>
</tr>
<tr>
<td>INK4A-KIP1 fusion</td>
<td>Blocks cell cycle by inhibiting CDK4 and CDK2</td>
<td>Apoptosis</td>
<td>Regression</td>
</tr>
<tr>
<td>RB</td>
<td>Blocks cell cycle by repressing E2F</td>
<td>Growth arrest</td>
<td>Tumour suppression</td>
</tr>
<tr>
<td>p130</td>
<td>Blocks cell cycle by repressing E2F</td>
<td>Growth arrest</td>
<td>Regression</td>
</tr>
<tr>
<td>ARF</td>
<td>Protects p53 by inhibiting MDM2</td>
<td>Growth arrest</td>
<td>Not done</td>
</tr>
<tr>
<td>p53</td>
<td>Promotes cell-cycle arrest and apoptosis</td>
<td>Growth arrest; increased radiosensitivity</td>
<td>Tumour suppression; reduced metastasis</td>
</tr>
<tr>
<td>PTEN</td>
<td>Degrades 3-phosphorylated phosphoinositides, which activate growth and survival pathways</td>
<td>Growth arrest; apoptosis; increased radiosensitivity</td>
<td>Tumour suppression or no effect</td>
</tr>
<tr>
<td>APC</td>
<td>Targets β-catenin for degradation</td>
<td>Apoptosis</td>
<td>Not done</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Genome Integrity</td>
<td>Growth arrest or apoptosis</td>
<td>Tumour suppression</td>
</tr>
</tbody>
</table>

APC, adenomatous polyposis coli; RB, retinoblastoma.
Inhibition of oncogene expression

<table>
<thead>
<tr>
<th>Gene product</th>
<th>Function of oncogene</th>
<th>Strategy</th>
<th>Expression in cell lines</th>
<th>Expression in mouse models</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERBB2</td>
<td>Receptor tyrosine kinase activated by EGF</td>
<td>Ribozyme</td>
<td>Blocks cell proliferation</td>
<td>Inhibition of tumour growth</td>
</tr>
<tr>
<td>ERBB4</td>
<td>Receptor tyrosine kinase activated by neuregulins</td>
<td>Ribozyme</td>
<td>Growth inhibition</td>
<td>Inhibition of tumour growth</td>
</tr>
<tr>
<td>KRAS</td>
<td>Small GTP-binding protein; activates growth and survival pathways</td>
<td>Antisense; ribozyme</td>
<td>Growth inhibition</td>
<td>Tumour regression; apoptosis; chemosensitization</td>
</tr>
<tr>
<td>HRAS</td>
<td>Small GTP-binding protein; activates growth and survival pathways</td>
<td>Ribozyme</td>
<td>Growth inhibition</td>
<td>Tumour regression</td>
</tr>
<tr>
<td>HPV E6/E7</td>
<td>E7 inhibits RB; E6 targets p53 for destruction</td>
<td>Antisense; ribozymes</td>
<td>Not done</td>
<td>No effect</td>
</tr>
<tr>
<td>BCL2</td>
<td>Inhibits mitochondrial apoptosis pathway</td>
<td>Ribozyme</td>
<td>Reduces BCL2 expression</td>
<td>Not done</td>
</tr>
<tr>
<td>Telomerase</td>
<td>Maintains telomere length to promote cellular immortality</td>
<td>Ribozyme</td>
<td>Chemosensitization</td>
<td>Not done</td>
</tr>
<tr>
<td>c-MET</td>
<td>Receptor tyrosine kinase activated by scatter factor</td>
<td>Ribozyme</td>
<td>Reduced migration; invasion</td>
<td>Not done</td>
</tr>
<tr>
<td>c-MYC</td>
<td>Transcription factor downstream of growth-factor signalling pathways</td>
<td>Ribozyme</td>
<td>Inhibits proliferation</td>
<td>Not done</td>
</tr>
</tbody>
</table>
# Suicide Gene Therapy

![Diagram](image)

## Enzyme-encoding gene

**Viral vector** → **Tumour cell**

**Enzyme** → **Prodrug** → **Toxin**

(by-stander effect)

## Table: Enzymes and Their Products

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Prodrug</th>
<th>Product</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV-tk</td>
<td>Ganciclovir</td>
<td>Ganciclovir triphosphate</td>
<td>Blocks DNA synthesis</td>
</tr>
<tr>
<td>Cytosine deaminase</td>
<td>5-Fluorocytosine</td>
<td>5-Fluorouracil (5-FU)</td>
<td>Pyrimidine antagonist: blocks DNA and RNA synthesis</td>
</tr>
<tr>
<td>Nitroreductase</td>
<td>Nitrobenzylxoycarbonyl anthracyclines</td>
<td>Anthracyclines</td>
<td>DNA crosslinking</td>
</tr>
<tr>
<td>Carboxylesterase</td>
<td>CPT-11</td>
<td>SN38</td>
<td>Topoisomerase inhibitor</td>
</tr>
<tr>
<td>Cytochrome P450</td>
<td>Cyclophosphamide</td>
<td>Phosphoramide mustard</td>
<td>DNA alkylating agent: blocks DNA synthesis</td>
</tr>
<tr>
<td>Purine nucleoside phosphorylase</td>
<td>6-Mercaptopurine-DR</td>
<td>6-Mercaptopurine</td>
<td>Purine antagonist: blocks DNA synthesis</td>
</tr>
</tbody>
</table>
Oncolytic viruses

Specificity to cancer is determined by tumor-specific genetic mutations.

Onyx 015
- adenovirus with deletion of the E1B 55kDa fragment (would facilitate replication in cells with a defective p53 pathway).

Telomelysin
- replication-competent Ad5
- incorporates a human telomerase reverse transcriptase gene (hTERT) promoter.

OncoVEX GM-CSF
- replication-competent HSV (HSV-1)
- coding sequence for GM-CSF under the control of the hCMV promoter
Genetic Immunotherapy

our experience
in
TCR engineered T cell adoptive transfer
to treat advanced melanoma patients
Autologous Tumor Infiltrating Lymphocytes

Genetically engineered

Cell infusion + IL-2
Preconditioning: chemotherapy

T cells

Tumor

TIL isolation

Viral vector

Engineered T cell

Peripheral blood lymphocytes
T Cell Receptor (TCR)-engineered T cells for Melanoma treatment

Human Melanoma

Subcloning anti-melanoma TCR into a viral vector

harvest T cells from new patient

Melanoma-specific T cell

Melanoma redirected T cell

Take the TCR genes from one patient who beat melanoma and use them to engineer a melanoma-fighting immune system in other patients.
UCLA Phase II F5 Clinical Trial Timeline

**Baseline:**
- Leukapheresis
- [¹⁸F]FDG PET
- Biopsy

**Adoptive Transfer**

**Blood draw**

**Follow up:**
- Leukapheresis
- [¹⁸F]FDG PET
- Biopsy

MSCV retroviral vector
MART-1 F5 TCR

UCLA GMP facility
Day 0: Stimulation
- AIMV media + 5% human AB serum
- OKT3
- IL-2

Day 2 & 3: Transduction (Txn)
- Mart-1 F5 TCR retrovirus

Day 4,5,6: Expansion
- AIMV media + 5% human AB serum
- IL-2

Leukapheresis
PBMC

Txn 1

Txn 2

Final Product
Mart-1 F5 TCR Tg PBMC

Cryopreservation in infusion bag
- 90% human serum albumin + IL-2
- 10% DMSO

Lot release testing of the clinical grade
PG13-F5af2aB C162D1 07-7-VP-1-164 Lot# 1.30/31/32

Day 7 Final Product Testing:
- Gram Stain
- Bacterial Culture
- Fungal Culture
- Mycoplasma Culture
- Endotoxin test
Untransduced T cell + melanoma M202GFP (A2.1+, MART1+)

F5 Transduced T cell + melanoma M202GFP (A2.1+, MART1+)

Light Field 10x

GFP 10x

Merged 10x

Thick arrow: melanoma cell; Thin arrow: T cell
Genetic Modification of Melanoma Patients’ Immune System Leading to Tumor Response

Effective targeting MART-1 \textit{in vivo} with TCR transgenic cells

- Skin rash
- Mole
- CD8 CTLs surrounding melanoma

May 09 (-2 mo)  Oct 09 (+3 mo)
Impressive Initial Responses with TCR Engineered ACT

Before Day +30

FDG PET

F5-1

F5-3

F5-10

F5-11
Antitumor activity and specific tumor targeting by Tyr-TCR engineered T cells

**Kinetic phases of distribution and tumor targeting by T cell receptor engineered lymphocytes inducing robust antitumor responses**

Richard C. Koya,¹ Stephen Mok,² Begoña Comin-Anduix,² Thinele Chodón,² Caius G. Radu,³ Michael I. Nishimura,⁴ Owen N. Witte,⁵,⁶,⁷, and Antoni Ribas⁸,⁹,¹¹

¹Department of Surgery, Division of Surgical Oncology; ²Department of Medicine, Division of Hematology/Oncology; ³Department of Molecular and Medical Pharmacology; ⁴Department of Microbiology, Immunology and Molecular Genetics; ⁵Howard Hughes Medical Institute; ⁶Broad Stem Cell Research Center; and ⁷Jonsson Comprehensive Cancer Center, University of California, Los Angeles, CA 90095; and ⁸Department of Surgery, Medical University of South Carolina, Charleston, SC 29403
Tumor Responses Correlate with TCR Transgenic Tumor Targeting

Day 1                      2                      3                      5                   7                     10

Day:  Day 1                      Days 2-4                      Days 5-7                      Days 8-10
Molecular Imaging of the *In Vivo* Kinetics of TCR Engineered T Lymphocytes

**Micro PET / CT**

[**F**$^{18}$]FHBG day 9

Melanoma specific engineered T cells accumulate in the melanoma-antigen-expressing tumor (right side)
Problems with Gene Therapy

- Suboptimal Gene Expression
- Vector Toxicity
- Insertional Mutagenesis
- Transgene Toxicity
History of Regulatory Issues for Human Gene Therapy Trials

- 1974 RAC Guidelines
- 1976 RAC Guidelines
- 1980 1st Gene Therapy Trial (unapproved)
- 1988 1st Gene Therapy Trial (approved)
- 1999 1st Gene Therapy Death
- 2002 1st Gene Therapy Cancer
Vector Toxicity

• Death of Jesse Gelsinger:
  – Due to large intra-arterial dose of adenoviral vector particles.
  – Temporary halt on all gene therapy trials.
  – Letter to IBCs and PIs requiring reporting of all SAE to RAC/ORDA (Office of Recombinant DNA Activities).
  – Nationwide audit of ongoing and completed gene therapy trials.
Insertional Mutagenesis

+/-

Transgene Toxicity

• Stem Cell Gene therapy as a cause of cancer (X-SCID clinical trials):
  – Five of the 20 patients (4 in the Paris trial and 1 in the London trial) had T-cell leukemia 2 to 5.5 years after gene therapy.
  – After chemotherapy, 4 patients survived and showed sustained remission and T-cell immunity.
  – One patient died from refractory leukemia.
  – In all cases it was found that the abnormal clone had 1 or 2 provirus integrations within a proto-oncogene locus. Many other genomic abnormalities were found.

  – Temporary halt on retroviral-mediated gene therapy trials.
Chimeric antigen receptor (CAR)-expressing T cells toxicity

Risk of “off-target” toxicity, resulting in autoimmune reaction against self-tissues

Two serious adverse events were reported:

-Anti-CD19-CD28-CD3z CAR into a patient with advanced CLL:
  -Acute sepsis, renal failure and expired 44 h following infusion.

-Anti-HER2/neu.CD28.4-1BB.CD3z in a patient with metastatic colon cancer:
  -Direct effect, cells targeted low levels of HER-2/neu expressed on pulmonary endothelium.
  -“Cytokine storm”
Human Gene Therapy Clinical Trial Regulatory Review

Federal

FDA (CBER)

OHRP

NIH (OBA) (RAC)

Local

IRB

ISPRC

IBC

G-CRC
Web site resources for more information

FDA: http://www.fda.gov

National Cancer Institute: http://www.nci.nih.gov

Cancer: http://cancernet.nci.nih.gov/index.html


Recombinant DNA Advisory Committee: http://www.nih.gov/od/oba