Disclosure

- Equity interest in Genetix Pharm. Inc.
- Exclusive license of retroviral cell lines from Columbia
- No direct participation in MDR clinical trials
- Columbia U. annual reporting
- FDA

Gene Therapy

- Transfer of genes into cells
- Expression of transferred genes
  - To correct a defect
  - To provide a new function

Gene Replacement/Homologous Recombination

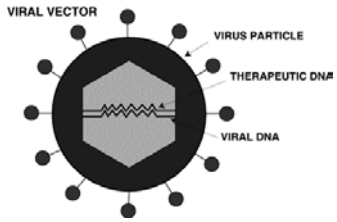
- Best theoretical approach
- Very low efficiency
- Useful in ES cells
- Not practical at present

Gene Addition

- Best practical approach
- High efficiency possible
- Used most often

Vectors for Gene Transfer

- Naked DNA
- DNA in lipid complexes
- Adenoviruses
- Adeno-associated viruses (AAV)
- Retroviruses
- Lentiviruses



Adenoviruses

- Very high titers
- Can be used in vivo
- Do not integrate; episomal
- Are immunogenic and provoke inflammatory responses

Adeno-associated Viruses

- Hiigh titers
- Can be used in vivo
- Variable integration
- Are immunogenic

Retroviruses

- Advantages: Acceptable titers and gene expression; chromosomal integration; stable producer lines available; safety known
- Disadvantages: Require cell division for stable integration
- Uses: Bone marrow stem cell gene therapy
- Lentiviruses better

Uses of Gene Therapy

- Correct genetic defects-ADA, hemophilia, sickle cell, Gaucher's disease
- Add new gene functions-angiogenesis, cancer

Gene Therapy Versus Protein Therapy

- Potentially permanent correction with gene as opposed to daily requirement for drug
- Must be effective in level of expression and expression must be regulatable

Systems to Study Gene Transfer

- Tissue culture cells: relatively easy
- Mice
- Larger animals - dogs, primates
- Humans

Factor 8 and 9 Deficiencies

- Hemophilia A and B
- Factor 8 and 9 concentrates and recombinant proteins effective
- Factor 8 and 9 genes in AAV or adenovirus injected into muscle raises levels in mice and dogs
- Human Factor 9 AAV trial into muscle underway (High)
- Evidence for immune responses

Ischemic Vascular Disease

- Angioplasty, bypass surgery available
- VEGFs can grow new blood vessels
- VEGF gene as naked DNA injected into ischemic legs relieves ischemia
- VEGF gene in AAV and adenovirus injected into ischemic cardiac muscle being tested

Anti-Cancer Gene Therapy

- Add a toxic gene to tumor cells (HSVTK)
- Add normal tumor suppressor gene-p53 or Rb
- Add anti-sense oligonucleotide to oncogenes (bcr-abl)
- Provoke immune response to tumor using CD34+ or dendritic cells transduced with antigens

Adding a Toxic Gene

- Herpes simplex thymidine kinase (HSVTK)gene:
  - Specifically phosphorylates gancyclovir and converts it to a toxic product
  - End result is tumor cell killing
  - Injected into brain tumors post-operatively
  - Patients treated with gancyclovir
  - Results equivocal

Anti-Sense to Oncogenes

- Oligonucleotides with anti-sense to:
  - BCR-Abl in CML
  - Mutated Ras
  - BCL
  - Results to date equivocal

Tumor Suppressor Genes

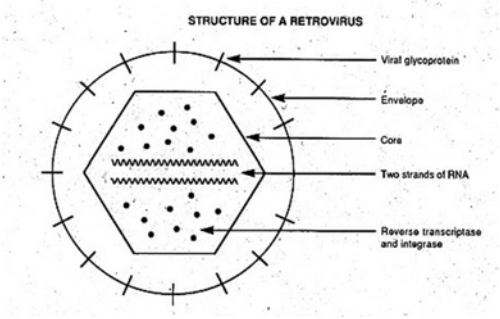
- P53
- Retinoblastoma (RB)

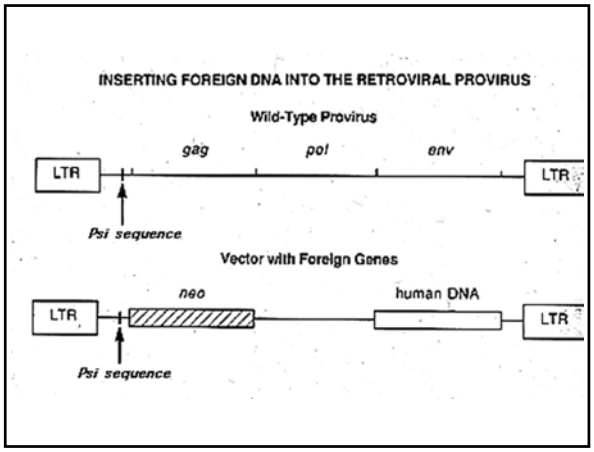
Increase Anti-tumor Immune Responses

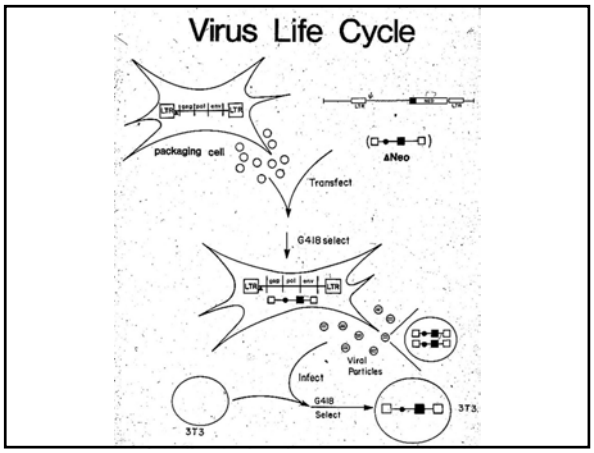
- Injecting cytokine genes into tumors and using as vaccines
- Adding tumor antigens to antigen presenting cells (dendritic cells) and using as vaccines

Cancer Gene Therapy

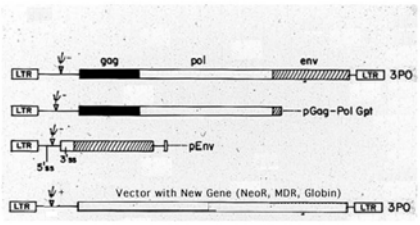
- Protecting marrow cells from the toxic effects of chemotherapy
- Use of the multiple drug resistance gene





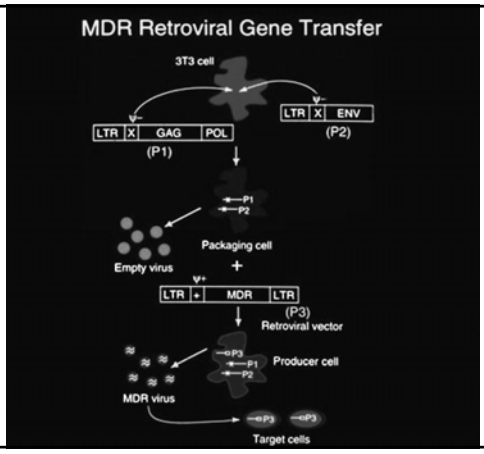


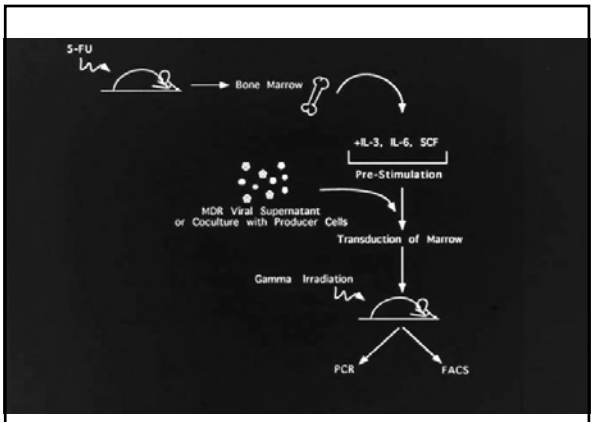
Critical Plasmids for Safe Retroviral Production

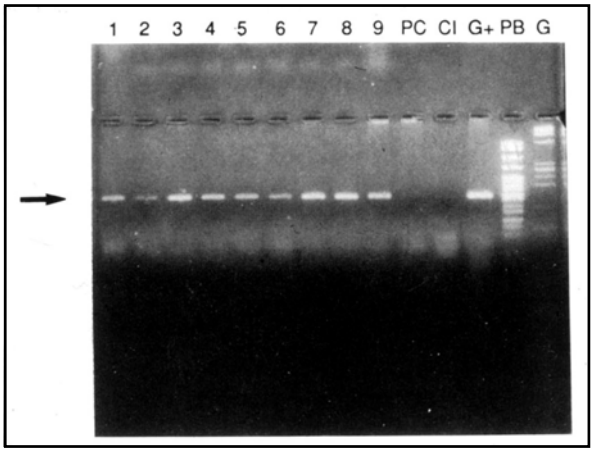


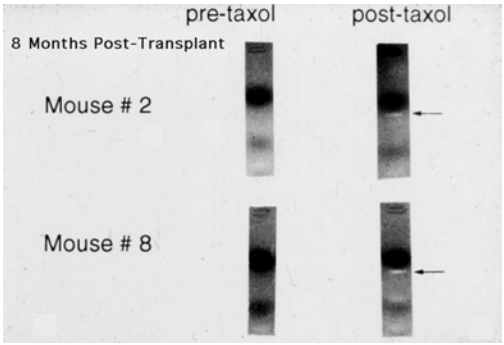
MDR Gene Therapy

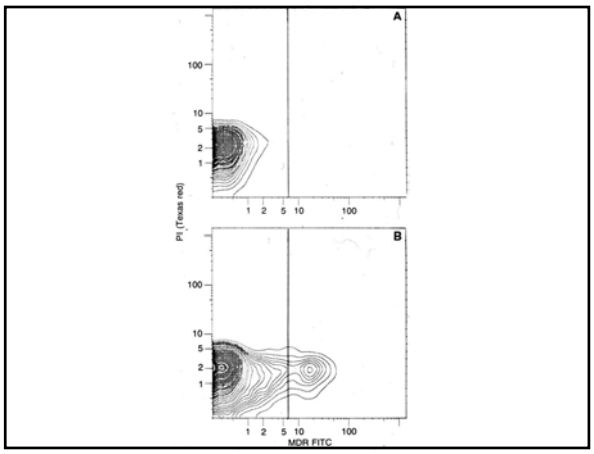
- MDR gene product is a p-glycoprotein
- Pumps natural compounds out of cells
- Many classes of anti-cancer drugs require MDR pump for removal
- Normal marrow cells have little or no MDR gene function
- Add a normal MDR gene to marrow stem cells
- Provides drug resistance
- Can also be used to select transduced cells






1 2 3 4 5 6 7 8 9 PC CI G+ PB G


pre-taxol
post-taxol
8 Months Post-Transplant
Mouse # 2
Mouse # 8

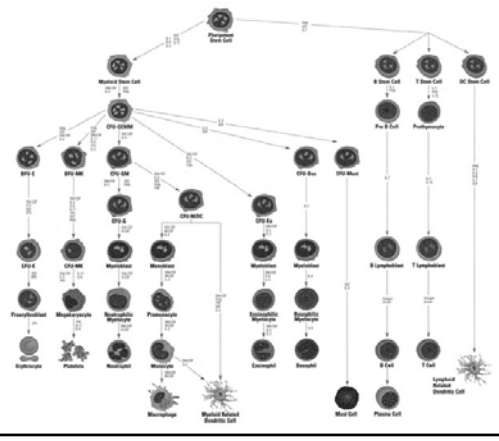

A
B
100
10
5
2
1
1 2 5 10 100
PI (Texas red)
MDR FITC

MDR Transduction in Mice

- MDR gene present and expressed up to one year
  - Evidence for stem cell transduction
- Taxol selects MDR-transduced cells

Challenges of Human Gene Therapy

- Complete safety
- Unique receptors on human HSC
- High level and efficient gene transfer



Autotransplantation

- Harvest stem cells from patient
- Transduce stem cells with vector containing gene of interest
- Return transduced stem cells to patient

Peripheral Blood Stem Cells

- Capable of marrow reconstitution
- Easily harvested by out-patient apheresis
- Mobilized with chemotherapy/growth factors
- Efficiently transduced
- Repeated harvesting and use
- Cells of choice for marrow transplantation

Progenitor Assays

- Methylcellulose plates
- Measure BFU-E and CFU-GM
- PCR-positive colonies
- Colonies with and without taxol

Transduction Protocol

- CD34+ cells cultured on fibronectin plates with IL-3, IL-6 and SCF
- 48 hr pre-incubation
- Two changes of retroviral supernatant over 24 hrs
- Successful MDR transduction of methylcellulose colonies
  - Resistance to taxol

Summary

- These results indicated the feasibility of using CD34+ PBPC MDR transduction to provide drug resistanceof marrow in Phase 1 clinical trials

Columbia MDR Phase1Clinical Trial

- Safety demonstrated: no delayed engraftment or RCR
- Feasibility shown: Large scale retroviral supernatants and CD34+ cells used in scale-up
- Pre-infusion: High-level CD34+ transduction in BFU-E and CFU-GM
- Post-infusion: 2/5 patients with low level MDR PCR + cells

Requirements for HSC Gene Transfer

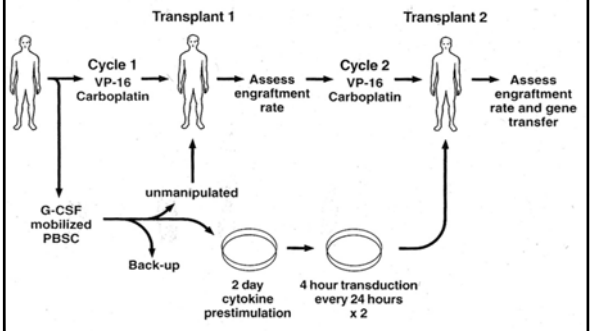
- Stem cells required for short- and long-term marrow repopulation
- Progenitors (BFU-E and CFU-GM) are irrelevant to repopulation
- True stem cells (NOD-SCID) required for marrow homing, marrow repopulation and expansion

Murine Studies-Qin 1999

- Untransduced (fresh) cells outcompete transduced cells for marrow engraftment both short- and long-term
- Two to 4 day delay in infusing untransduced cells after infusing transduced cells increases short- and long-term repopulation of transduced cells

Indiana Trial- MDR Gene Therapy

- Pts with relapsed germ cell tumors
- Intensive carboplatin and etoposide therapy followed by either MDR-transduced or untransduced HSC
- Three cycles of oral etopside
- CH-296 fibronectin fragment (Retronectin)
- Abonour-Nature Medicine 2000



Indiana Gene Therapy Trial

- **Best results reported to date of HSC gene therapy**
- **MDR-transduced cells persist up to 1 year and are selectable with drug**
- **TPO, SCF and G-CSF are best growth factor combination**
- **Retronectin fragment used**

Table 3 Percentage of transgene-containing colonies in the graft and at 4 time points after transplantation

Subject	Graft	1 month	Pre cycle 2[a]	Post cycle 3[b]	1 year
1	15	ND	5	NA	NA
3	31	14	10	9	3
4	4	0	8	10	0
5	6	28	15	9	5
6	8	9	14	8	4
7	12	8	NA	NA	NA
8	5	14	7	4	15
9	9	8	NA	8	13
10	52	8	ND	3	7
11	31	78	NA	NA	NA
12	27	31	9	7	NA

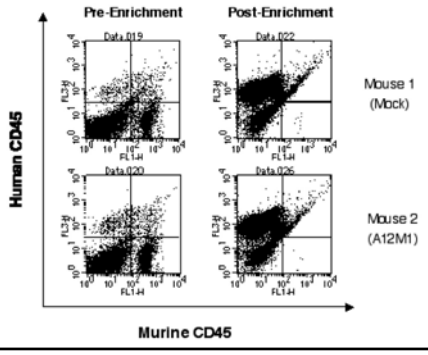
Indiana Trial: Summary

- Best HSC gene transfer and expression to date
- MDR-transduced cells selected by chemotherapy
- Retronectin effect positive
- TPO, SCF, G-CSF growth factors best
- Lack of competition of fresh and transduced cells critical

NOD-SCID Mouse Assay

- Only valid assay for human HSC
- MDR-transduce human cord blood CD34+ cells
  - 5 cytokines, Retronectin
- Plate for MDR PCR +colonies in MC
- Inject cells into NOD-SCID
- Analyze NOD-SCID 5-6 weeks later

NOD-SCID Mouse Engraftment



MDR-Transduced HSC in NOD-SCID Mouse - MDR PCR

- Methylcellulose colonies: PCR+
  - Pre- NOD-SCID: 20/30 (66%)
  - Post-NOD-SCID:
    - Mock: 0/50 + (0%)
    - A12M1: 16/168 + (10%)

Summary: MDR-Transduced HSC in NOD-SCID Mouse

- MDR transduction of human HSC achieved
- Transduction efficiency comparable to that of clinical trial:1-10% of human cells
- Conditions: 5 cytokines, no polybrene, Retronectin, multiple viral exposures

Amphotropic Retroviral Packaging Lines

- AM12 et al
- Titers between 10^4 and10^6
- Limited receptor expression on human HSC
- Cannot be concentrated
- Safety and scale-up documented in human clinical trials
- Low-level transduction efficiency in human clinical trials

VSV-G Envelope Packaging Lines

- High-titer
- Virus can be concentrated
- Transient packaging due to VSV-G toxicity
  - Adding plasmids to 293T cells
  - Plasmids require SV40 T antigen expression
- Variable packaging and titers
- Potential recombinational events
- Difficult to scale-up as compared to stable lines

RD114 Envelope Packaging Lines

- Transient supernatants produced
- High-titer
- Can be concentrated
- Efficiently transduce human HSC as tested in NOD-SCID mice (Kelly et al 2000, Gatlin et al 2001)

Stable RD114 Packaging Line (M. Ward)

- Moloney gag-pol in 3T3 cells
- Add RD114 gene with phleomycin selection
- Isolate high titer clones with NeoR gene and G418
- Make retroviral supernatants
- Concentrate virus by centrifugation
- Can transfer G418 resistance to human CD34+ cells
- Can transfer normal β globin gene into sickle CD34+ cells

Current Bank lab GT Goals-2003

- Better HSC transduction - new envelopes (RD114); transient VSV-G packaging lines
- Concentrate on human globin gene therapy using Leboulch lentiviral vector
- Use NOD-SCID mouse model to predict human HSC transduction

Cure of Children with X-SCID

- Most successful human trial to date
- T cells lack γC cytokine receptor required for lymphoid proliferation
- Retroviral transfer of γC cytokine receptor gene into CD34+ cells
- Autotransplantation
- Selection of corrected cells
- Normal immune function in 7/9 patients
- T cell leukemia (clonal) in 2/9 patients 3 years post-transduction

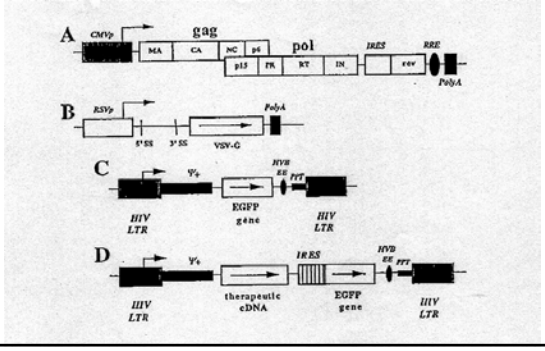
Leukemia in Children with X-SCID

- Similar insertional mutagenesis events in both children
- Unregulated γC cytokine receptor gene inserted into LMO2 locus
- Activation of LMO2, a proliferative gene
- A rare event in an early T cell/HSC that leads to a leukemic transformation
- Slow growth and eventual proliferation of the clone
- May be prevented by regulated γC cytokine receptor gene

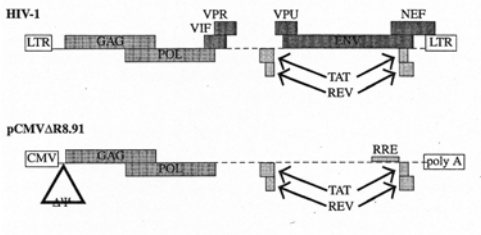
Lentiviral Vectors

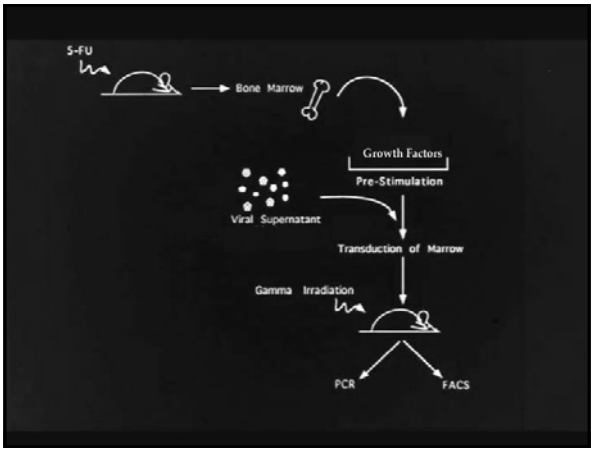
- Transduce non-dividing cells
- Can transduce murine and human HSC efficiently
- Very high titers
- Better for globin gene therapy
- Can cure mouse models of human sickle and thalassemia
- Safety issues

Lentiviral Vector Plasmids



Lentiviral Plasmids





Successful β Thal Gene Therapy

- May et al: Nature 2000
- β globin gene correction in β thalassemic mice
- Lentiviral vectors with extensive β -LCR elements used
- Gene-modified cells produce β globin in vivo
- Correction of thalassemia phenotype

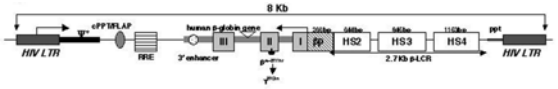
Successful Sickle Gene Therapy

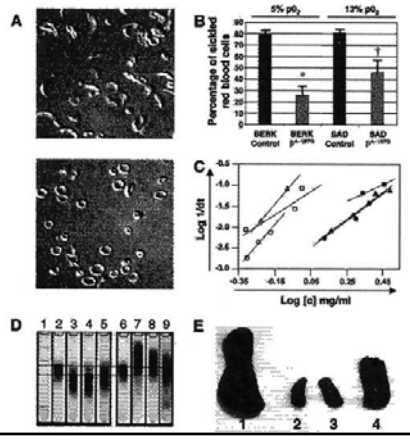
- Pawliuk et al: Science 2001
- β globin gene correction in two mouse models of sickle cell
- Lentiviral vectors with extensive β -LCR elements used
- Gene-modified cells produce β globin in vivo
- Correction of sickle phenotype

Sickle Mouse Models

Variable	Transgenic Mouse	Transgenic Mouse	Transgenic Mouse	Knockout–Transgenic Mouse
Human globin genes expressed	β^S, α	β^{SAD}, α	$\beta^S, \beta^{S\ Antilles}, \alpha$	β^S, α
Mouse globin genes expressed	β, α	β, α	β, α	None
Expression of sickle or sickle-related genes	β^S, 75%	β^{SAD}, 26%	β^S, 42% $\beta^{S\ Antilles}$, 38%	β^S, 100%
Hemolytic anemia	No	Compensated	Compensated	Severe
Micro-occlusive disease	Mild	Moderate	Moderate	Severe

Leboulch Globin Lentiviral Vector





Current Gene Therapy Experiments - 4/03

- Viruses with new envelopes - RD114
- New incubation conditions- BIT media, new cytokines
- NOD-SCID mouse assay for true HSC - CD34+ CD38- cells
- Use of lentiviral vectors in human globin gene therapy