



Gene Therapy in Hemophilia

Update April 28, 2001 Regional Meeting

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Gene Therapy in Hemophilia

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■ Terminology

- Gene Therapy - transferring a specific gene into a patient
- Vector - A vehicle for transferring genes into a cell, usually a viral derivative
- Transgene - the therapeutic gene
- Transduction - process of introduction of gene into the cell



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- ▶ Demonstration of Gene Transfer and Expression
 - Tissue Culture
 - Small animal - mice
 - Large animal - dog
 - IM injection
 - Expression persists for life of dog
 - Humans with disease



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- Why is Hemophilia Considered “Best” Candidate for Gene Therapy?
 - Genetic basis of disease is known
 - Levels of VIII and IX are not tightly regulated
 - A little is good and too much is not too bad
 - Variety of organs can secrete clotting factor
 - liver, muscle, spleen, etc.
 - Excellent animal models - Mice, Dogs
 - Gene Rx represents advance over current Rx
 - Ease of marker recognition \Rightarrow Factor Level



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- ▶ Differences Between Hemophilia A & B
 - Size of DNA
 - F IX 1.5 KB
 - F VIII 4.4 KB
 - Prevalence of inhibitory antibodies (lower in B)
 - Levels of protein expression required
 - F IX 5 μ g/ml
 - F VIII 300 nanograms



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- | | | |
|-----------------------|--------|-------------------------|
| ▶ Hemophilia A | versus | Hemophilia B |
| ▶ Gene - 7000 bp | | Gene - 1500 bp |
| ▶ Hard to express | | Easy to express |
| ▶ Needs vWF | | Secretion into blood |
| ▶ Muscle may not work | | Expressed in many cells |



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- ▶ Complexities of Gene Therapy (in vivo)
 - Vector must reach the correct cell
 - Vector must bind to the correct cell
 - Vector must be taken in by the cell
 - Vector must get into the nucleus
 - Cell must be able to express the transgene



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- ▶ Intact Genome is critical to normal health
- ▶ It is well protected - problems for gene Rx
 - Vector destruction by immune system
 - Some cells “turn-off” vectors by inactivating genetic material that allows gene expression
 - Cell expressing transgene may be recognized as abnormal and destroyed by the immune system



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- ▶ Two Primary Approaches
- ▶ Ex Vivo (popular in the late 1980s)
 - Take cells from body - fibroblasts, BM, muscle
 - Modify outside body and culture
 - Re-infuse genetically modified cells
- ▶ In Vivo (current primary technique)
 - Direct injection of vector carrying gene
 - Allows same vector prep for all patients
 - Vector is injected IV or into specific site



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- ▶ Five Systems to Introduce DNA
 - Retroviral Vectors
 - Adenoviral Vectors
 - Adeno-Associated Viral (AAV) Vectors
 - Lentiviral Vectors
 - Naked DNA



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■ Retroviral Vectors

- Advantages
 - They integrate into the host DNA
 - Will (hopefully) express gene for life-span of cells
 - If cell divides - gene is copied into daughter cells
- Disadvantages
 - Can only transduce dividing cells
 - Are sometimes “turned off” for unclear reasons
 - Theoretical concern insertion-induced mutation



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► Retroviral Vectors

- Use Ex Vivo
 - Used to transduce FIX gene into mouse fibroblasts
 - When injected - Good initial expression- gone day 7
 - Longer but lower expression in muscle cells
 - Even less encouraging results in larger animals
- Potential In Vivo Application
 - Co-injection with protein that induces cell division
 - Can be used in younger animals- more dividing cells
 - Very high level injections



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■ Adenoviral Vectors

- Advantages
 - Extremely efficient transduction even nondividing cells
 - Achieve high level transgene expression
- Disadvantages
 - Do not integrate into genome - eventually disappear
 - Adenovirus are common cold virus - many people will have neutralizing antibodies



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- ▶ Adenoviral Vector - Use In Vivo
 - Mouse
 - Target liver cells- virtually 100% transduced
 - High initial level - fall off with time - but therapeutic at one year
 - Dog
 - Expression detected for only 10 days
 - Significant liver toxicity



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- ▶ Adenoviral Vector - Use In Vivo
 - “Gutless” Adenoviral Vectors
 - Adenoviral genes are removed
 - High level expression in mice and baboons
 - Less liver toxicity
 - May represent a major advance in gene therapy



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- Adeno-Associated Viral Vector (AAV)
 - Parvovirus Family - 4.7 KB ss DNA genome
 - Integration status not determined
 - Does result in long term expression
 - No obvious toxicity
 - Target muscle and liver
 - Problem is size - IX ok, VIII +/-
 - In Vivo Treatment
 - IX AAV Vector -- Expression in mice > one year
 - Recently high level expression of VIII in mice



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■ Lentiviral

- Advantages
 - Integrates into genome
 - Transduces both dividing and nondividing cells
 - Multi. targets-muscle,liver,spleen,endothelium,BM
- Disadvantages
 - Derived from HIV- virtually all viral genes removed
 - Potential for mutagenesis (theoretical)
 - Difficult to manufacture
 - Newest vector - least amount of knowledge



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▶ Lentiviral Vector

- Studies
 - Expression of genes delivered to liver or muscle
 - Factor VIII containing vector under development



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• Naked DNA

- Advantages
 - No Vehicle Required
 - Avoids problems associate with viruses
- Disadvantages
 - Must work ex Vivo
 - Ex Vivo techniques are labor intensive
 - DNA may be broken down prior to reaching cell
 - DNA may be inefficiently taken up or expressed



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■ Naked DNA

- Studies

- Transkaryotic Therapies -- DNA into fibroblasts
- Electrical technique creates temporary “holes”
- Known as electroporation



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**▶ THREE HEMOPHILIA
TRIALS WERE ONGOING IN
2000**



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- ▶ AVIGEN - HEMOPHILIA B TRIAL
- ▶ AAV for hemophilia B: muscle vs. liver
 - In muscle procedure is safe and familiar
 - IM versus intrahepatic injection
 - Most adults would be candidates for muscle
 - Toxicity of muscle injection much better defined



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- ▶ What should be the clinical endpoints?
- ▶ Primary endpoint
 - Factor Level > 1%
 - Would like level of 20-40% but 1-5% is good
- ▶ Secondary endpoint - Clinical Status
 - Number of bleeds
 - Severity of bleeds
- ▶ Prevalence of inhibitory antibodies



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- ▶ Phase I Trial of AAV in Hemophilia B
 - 3 patients each in 3 cohorts
 - Low dose
 - Medium dose
 - High dose



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► Phase I Trial of AAV in Hemophilia B

- Subjects

- Adult males with severe hemophilia B
- No history of inhibitor
- > 20 exposures to factor concentrate
- Life expectancy of at least one year
- Must have made the decision not to have children



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▶ Phase I Trial of AAV in Hemophilia B

- Exclusions:
 - Acute infectious disease
 - End stage renal or liver disease
 - Inflammatory muscle disease
 - Must be willing to stop prophylactic treatment



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- ▶ Phase I Trial of AAV in Hemophilia B
 - Procedure
 - Site Vastus Lateralis muscle of thigh
 - Multiple 250-500 μ L IM injections
 - Each injection site is tattooed
 - Biopsy of injection sites performed to document gene incorporation
 - Plasma Factor IX levels performed to document gene expression



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- ▶ Phase I Trial of AAV in Hemophilia B
 - Results
 - Nearing completion
 - Gene incorporation documented
 - Gene expression documented (in most)
 - No Significant safety issues
 - At least two patients have had a marked reduction in the number of bleeding episodes
 - *(Remember - Clinical Improvement was not a goal of the Phase I trial)*



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- ▶ Good chance Avigen Muscle Trial will not go on to Phase II
- ▶ WHY?
 - Technological Advances
 - Change of AAV target from muscle to liver
 - Avigen has applied to initiate separate Phase I
 - Discovery (UNC) of more efficient “Serotypes”



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- ▶ Chiron - FVIII Trial (began June 1999)
 - Seman sample showed traces of viral vector
 - Raised potential of germ line transmission
 - FDA suspended trial to investigate
 - Sept 2000 FDA lifted Suspension
 - Chiron decided not to enroll additional patients
 - Trial appears to have been safe
 - Benefit gained (if any) still unclear



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- ▶ Transkaryotic Therapies (TKT)-
Hemophilia A Trial
 - Ongoing for two years
 - Excellent safety results
 - Most impressive therapeutic results - patients with less bleeding and decreased infusions
 - Maximum circulating level of 4% in one patient
 - Company has a number of modified vectors that may increase efficacy in Phase II trial



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- ▶ Other News - Increased Corporate Support
- ▶ Bayer-----Avigen
 - AAV for hemophilia B
- ▶ Genetics Institute-----Targeted Genetics
 - AAV for hemophilia A
- ▶ Baxter-----GenStar Therapeutics
 - Adenoviral for hemophilia A



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▶ Hemophilia Gene Trials 2001

▶ Company	Hemophilia	Vector	Method	Status
▶ TKT	A	Plasmid	ex vivo	PII 2001
▶ AVIGEN	B	AAV	in vivo Mus.	Complete
▶ CHIRON	A	RetroV	in vivo	Terminated
▶ AVIGEN	B	AAV	in vivo liver	Start 2001
▶ GENSTAR	A	AdenoV	in vivo liver	Start 2001
▶ TARGET GEN	A	AAV	in vivo liver	Start 2001
▶ CELL GENESY	A	LentiV	in vivo	Start 2001?
▶ CELL GENESY	B	AAV	in vivo	Start 2001?



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- ▶ Considering a Trial?
- ▶ DON'T
 - Enroll unless you understand the process
 - Enroll because your hematologist or favorite nurse is involved
 - Expect significant benefit
 - Let the Trial Choose YOU, YOU choose the trial



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- ▶ Considering a Trial?
- ▶ DO
 - Ask Questions
 - Consider all trials - ongoing and planned
 - Examine animal results
 - Ask about other research results with the vector
 - Read NHF's guidelines for hemophilia gene trials