Gene Therapy Studies

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Gene therapy: a definition

“Use of nucleic acid-based technology to deliberately alter gene expression in patient’s cells, with the aim of producing a therapeutic benefit"
Genomics Based Drug Development

Genes → Function →

Small molecule drug → Protein →

Manufacture → Pharmacology

Toxicology

Preclinical Testing (Animal Models/Transgenics)

Clinical Development (Phase I/II/III)
**Major considerations**

- Somatic versus germ-line gene therapy
- *Ex vivo* versus *in vivo* gene transfer
- Non-viral versus viral vectors
- Replication-defective viral vectors
- Episomal versus integrated transgene delivery
- Requirements for success
Major Ethical Issue

*Germline gene therapy* - the therapeutic genetic modification is introduced into cells of the body including the germ cells. The modified gene might be passed on to subsequent generations: the application in humans raises ethical questions; banned by law.

*Somatic gene therapy* - the therapeutic genetic modification is restricted to somatic cells with no effect on the germline.
The *in vivo* and *ex vivo* approaches

- **in vivo**: entails the genetic modification of the cells of a patient *in situ*
- **ex vivo**: cells are modified outside the body before re-implantation
Gene Therapy Vectors

Non-Viral
- Naked DNA
- Lipoplex/Polyplex
- Recombinant Cells (Microencapsulation)

Viral
- Retroviral vectors
- Adenoviral vectors
- Adeno-Associated Virus vectors
Naked DNA as a Delivery System

**Advantages**

- No infectious risk
- High capacity for insert size
- Low immune response
- Able to re-administer (no exogenous protein)
- Cheap to produce and easy to store

**Disadvantages**

- Very low transfection efficiency
- Immunostimulatory CpG sequence
- Limited range of target cells
- Transient expression
Viral vectors: the principle

Requirements for Successful Gene Therapy

*Cell targeting* -
transfer of the exogenous genetic material to the right cell type or tissue

*Efficient delivery* -
transfer of the exogenous genetic material in the appropriate quantity to as many cells possible

*Stable gene expression* -
long term expression of the transferred gene (choice of the right promoter)

*Avoidance of immune response* -
delivery system or the therapeutic agent should not provoke a strong immune response
Gene Therapy Strategies

• Targeted Cell Killing

• Gene Augmentation

• Targeted Modification of Gene Expression

• Targeted Gene Inactivation/Correction
Targeted cell killing: Control of GvHD

Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I–II study


Summary

Background Procedures to prevent severe graft-versus-host disease (GVHD) delay immune reconstitution secondary to transplants of haploidentical haemopoietic stem cells for the treatment of leukaemia, leading to high rates of late infectious mortality. We aimed to systematically add back genetically engineered donor lymphocytes to facilitate immune reconstitution and prevent late mortality.

Lancet Oncol 2009; 10: 489–500
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DOI:10.1016/S1470-2045(09)70074-9
Targeted cell killing: Control of GvHD

Bonini et al. Mol Ther 15, 1248-1252, 2007
**Targeted cell killing: Control of GvHD**

**Methods** In a phase I–II, multicentre, non-randomised trial of haploidentical stem-cell transplantation, we infused donor lymphocytes expressing herpes-simplex thymidine kinase suicide gene (TK-cells) after transplantation. The primary study endpoint was immune reconstitution defined as circulating CD3+ count of 100 cells per μL or more for two consecutive observations. Analysis was by intention to treat. This trial is registered with ClinicalTrials.gov, number NCT00423124.

**Findings** From Aug 13, 2002, to March 26, 2008, 50 patients (median age 51 years, range 17–66) received haploidentical stem-cell transplants for high-risk leukaemia. Immune reconstitution was not recorded before infusion of TK-cells. 28 patients received TK-cells starting 28 days after transplantation; 22 patients obtained immune reconstitution at median 75 days (range 34–127) from transplantation and 23 days (13–42) from infusion. Ten patients developed acute GVHD (grade I–IV) and one developed chronic GVHD, which were controlled by induction of the suicide gene. Overall survival at 3 years was 49% (95% CI 25–73) for 19 patients who were in remission from primary leukaemia at the time of stem-cell transplantation. After TK-cell infusion, the last death due to infection was at 166 days, this was the only infectious death at more than 100 days. No acute or chronic adverse events were related to the gene-transfer procedure.

**Interpretation** Infusion of TK-cells might be effective in accelerating immune reconstitution, while controlling GVHD and protecting patients from late mortality in those who are candidates for haploidentical stem-cell transplantation.
Gene Augmentation: X-SCID

Efficacy of Gene Therapy for X-Linked Severe Combined Immunodeficiency

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CONCLUSIONS

After nearly 10 years of follow-up, gene therapy was shown to have corrected the immunodeficiency associated with SCID-X1. Gene therapy may be an option for patients who do not have an HLA-identical donor for hematopoietic stem-cell transplantation and for whom the risks are deemed acceptable. This treatment is associated with a risk of acute leukemia. (Funded by INSERM and others.)

(Hacein-Bey-Abina et al. NEJM 363, 355-364, 2010)
Leukemia from insertional mutagenesis

Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

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CONCLUSIONS
Gene therapy, combined with reduced-intensity conditioning, is a safe and effective treatment for SCID in patients with ADA deficiency. (ClinicalTrials.gov numbers, NCT00598481 and NCT00599781.)
X-SCID and ADA-SCID: similar vector configurations
Gene therapy strategies: episomal vectors
Fate of an integration-deficient lentiviral vector

dLTR → dLTR → dsDNA in target cell cytoplasm

self-ligation

2-LTR circle

dsDNA circles in target cell nucleus

dLTR recombination

1-LTR circle

dsDNA in target cell nucleus

integrated provirus
Gene therapy strategies (with IDLVs)

- Gene augmentation
  - Functional gene
  - Therapeutic gene

- Repair by gene targeting

Therapeutic minigene integrates "at random" in target cell genome

Therapeutic minigene present in episomal vector

Therapeutic minigene integrates by site-specific recombination (or transposition)

Therapeutic genomic DNA corrects target cell genome by homologous recombination
Local restoration of dystrophin expression with the morpholino oligomer AVI-4658 in Duchenne muscular dystrophy: a single-blind, placebo-controlled, dose-escalation, proof-of-concept study

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Summary

Background Mutations that disrupt the open reading frame and prevent full translation of DMD, the gene that encodes dystrophin, underlie the fatal X-linked disease Duchenne muscular dystrophy. Oligonucleotides targeted to splicing elements (splice switching oligonucleotides) in DMD pre-mRNA can lead to exon skipping, restoration of the open reading frame, and the production of functional dystrophin in vitro and in vivo, which could benefit patients with this disorder.
Targeted modification of gene expression in DMD

Figure 1: Deletions and predicted results of exon skipping in the patients who were studied
**Targeted modification of gene expression in DMD**

**Methods** We did a single-blind, placebo-controlled, dose-escalation study in patients with DMD recruited nationally, to assess the safety and biochemical efficacy of an intramuscular morpholino splice-switching oligonucleotide (AVI-4658) that skips exon 51 in dystrophin mRNA. Seven patients with Duchenne muscular dystrophy with deletions in the open reading frame of DMD that are responsive to exon 51 skipping were selected on the basis of the preservation of their extensor digitorum brevis (EDB) muscle seen on MRI and the response of cultured fibroblasts from a skin biopsy to AVI-4658. AVI-4658 was injected into the EDB muscle; the contralateral muscle received saline. Muscles were biopsied between 3 and 4 weeks after injection. The primary endpoint was the safety of AVI-4658 and the secondary endpoint was its biochemical efficacy. This trial is registered, number NCT00159250.

**Findings** Two patients received 0.09 mg AVI-4658 in 900 μL (0.9%) saline and five patients received 0.9 mg AVI-4658 in 900 μL saline. No adverse events related to AVI-4658 administration were reported. Intramuscular injection of the higher-dose of AVI-4658 resulted in increased dystrophin expression in all treated EDB muscles, although the results of the immunostaining of EDB-treated muscle for dystrophin were not uniform. In the areas of the immunostained sections that were adjacent to the needle track through which AVI-4658 was given, 44–79% of myofibres had increased expression of dystrophin. In randomly chosen sections of treated EDB muscles, the mean intensity of dystrophin staining ranged from 22% to 32% of the mean intensity of dystrophin in healthy control muscles (mean 26.4%), and the mean intensity was 17% (range 11–21%) greater than the intensity in the contralateral saline-treated muscle (one-sample paired t test $p=0.002$). In the dystrophin-positive fibres, the intensity of dystrophin staining was up to 42% of that in healthy muscle. We showed expression of dystrophin at the expected molecular weight in the AVI-4658-treated muscle by immunoblot.

**Interpretation** Intramuscular AVI-4658 was safe and induced the expression of dystrophin locally within treated muscles. This proof-of-concept study has led to an ongoing systemic clinical trial of AVI-4658 in patients with DMD.
Gene Therapy Strategies

- Targeted Cell Killing
- Gene Augmentation
- Targeted Modification of Gene Expression
- Targeted Gene Inactivation/Correction
Zinc-finger nucleases

(Nat Biotech 21, 759-760, 2003)
Current model for the repair of DNA double-strand breaks

homologous recombination

nonhomologous recombination
Targeted gene inactivation: HIV control

Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo

Nathalia Holt¹, Jianbin Wang², Kenneth Kim², Geoffrey Friedman², Xingchao Wang³, Vanessa Taupin³, Gay M Crooks⁴, Donald B Kohn⁴, Philip D Gregory², Michael C Holmes³ & Paula M Cannon¹

CCR5 is the major HIV-1 co-receptor, and individuals homozygous for a 32-bp deletion in CCR5 are resistant to infection by CCR5-tropic HIV-1. Using engineered zinc-finger nucleases (ZFNs), we disrupted CCR5 in human CD34⁺ hematopoietic stem/progenitor cells (HSPCs) at a mean frequency of 17% of the total alleles in a population. This procedure produces both mono- and bi-allelically disrupted cells. ZFN-treated HSPCs retained the ability to engraft NOD/SCID/IL2γnull mice and gave rise to polyclonal multi-lineage progeny in which CCR5 was permanently disrupted. Control mice receiving untreated HSPCs and challenged with CCR5-tropic HIV-1 showed profound CD4⁺ T-cell loss. In contrast, mice transplanted with ZFN-modified HSPCs underwent rapid selection for CCR5⁻/⁻ cells, had significantly lower HIV-1 levels and preserved human cells throughout their tissues. The demonstration that a minority of CCR5⁻/⁻ HSPCs can populate an infected animal with HIV-1-resistant, CCR5⁻/⁻ progeny supports the use of ZFN-modified autologous hematopoietic stem cells as a clinical approach to treating HIV-1.

(Nat Biotech 2010, doi:10.1038/nbt.1663)
Targeted gene inactivation: HIV control

18th Conference on Retroviruses and Opportunistic Infections

Session 4: Sangamo
Obstacles to a Cure
Wednesday, 4-5 pm; Ballroom A

Paper # 106
Disruption of CCR5 in Zinc Finger Nuclease-treated CD4+ T Cells: Phase I Trials
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Background: HIV therapy with ARV drugs is effective to control viremia; however, it has not shown promise to eradicate the viral reservoir, leading to the necessity for life-long therapy. The unique observation that allogenic stem cell transplantation with a CCR5-deficient stem cell preparation led to eradication of replication-competent HIV raises the question as to whether autologous T cells or stem cells rendered CCR5-deficient could lead to a similar reduction in the HIV reservoir. We are testing the feasibility of zinc finger nucleases to create CCR5-deficient CD4+ T cells (SB-728-T) in an ongoing phase I clinical trial (NCT0083634). The protocol has 2 cohorts of patients: cohort 1 includes patients who have failed 2 or more HAART regimens and remain viremic; cohort 2 includes patients doing well on stable ARV medication, who are willing to undergo a single structured treatment interruption. All patients in cohort 2 have been enrolled.

Conclusions: interim results will be presented.

February 28, 2011

Sangamo BioSciences Announces Presentation of Positive Clinical Data From Novel ZFN Therapeutic Approach for the Treatment of HIV/AIDS at Conference for Retroviral and Opportunistic Infections


RICHMOND, Calif., Feb. 28, 2011 /PRNewswire/ -- Sangamo BioSciences, Inc. (Nasdaq: SGMO) announced today the presentation of positive preliminary clinical data from its Phase 1 trial (SB-728-902). The trial is being conducted in immunologic non-responders, HIV-infected subjects who are currently on highly active antiretroviral therapy (HAART) and have undetectable levels of virus but suboptimal CD4+ T-cell counts. The study is designed to evaluate safety and clinical outcomes of Sangamo's zinc finger nuclease (ZFN)-generated CCR5-modified, autologous T-cell product (SB-728-T) for the treatment of HIV/AIDS. CCR5 is the major co-receptor used by HIV to infect cells of the immune system.

(http://investor.sangamo.com/releasedetail.cfm?ReleaseID=553112)
Current model for the repair of DNA double-strand breaks

homologous recombination

nonhomologous recombination

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Gene therapy strategies: gene repair

(Lombardo et al., Nat Biotech 25, 1298-1306, 2007)
(Cornu and Cathomen., Mol Ther 15, 2107-2113, 2007)
Genetic Therapy Lab-2011

Dr Martin Broadstock
Dr Céline Rocca
Dr Anita Le Heron
Mr Sherif Ahmed
Mr Hayder Hafdh Abdul-Razak
Miss Tiziana Rossetti
Miss Gaby Boza
Miss Ngoc Lu Nguyen
Miss Hanna Kymäläinen
Miss Madeleine Oudin
Miss Kate Orton

Dr Klaus Wanisch
Dr Hugo Peluffo
Dr Mario Marotta
Mr F Javier Molina
Miss Raquel Cano
Miss Sara Olíván
Miss Alison Roberts
Miss Marta Muñoz-Alegre
Mr Victor Gan