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## **Gene Therapy in Transplantation: Towards Clinical Trials**

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**Abstract**

The genetic modification of organs or cells is an attractive approach to protect allogeneic transplants from acute rejection and other complications. The transplant setting offers a unique opportunity to utilise ex vivo gene therapy for modification of allogeneic organs and tissues prior to implantation. Significant challenges to applying this concept to human organ transplantation, however, include the large number of potential molecular targets, the diversity and safety profile of available vector delivery systems and the merging of gene-based therapies with existing immunosuppressive regimens. Accordingly many different therapeutic concepts and vector systems have been investigated in the pre-clinical area with a view to prolonging allograft survival but translation of promising gene therapy strategies to human clinical transplant studies has lagged behind the progress seen in other medical fields. In this article recent pre-clinical experimental applications of gene transfer to transplantation are outlined and the degree to which gene therapy has been clinically tested in organ transplant recipients is critically reviewed.

**Key words**

Transplantation, allograft rejection, gene therapy, viral vector, immune tolerance, clinical trials

## **1. Introduction:**

Solid organ transplantation is the therapy of choice for many patients suffering from end stage organ disease. Due to differences between donor and recipient major histocompatibility complex (MHC) class I and II genes allogeneic grafts are generally rejected by the recipient's immune response. Although several new immunosuppressive drugs have been developed over the last three decades (e.g. cyclosporine, tacrolimus, sirolimus, mycophenolate mofetil) and have led to significant prolongations of allogeneic graft survival, the long-term prognosis for many human organ transplants remains relatively poor. Moreover, drugs currently given routinely to transplant recipients (generally as triple or quadruple therapy) fail to induce immunologic tolerance against to allogeneic grafts and, therefore, must be given continuously in order to prevent rejection. In addition, long-term non-specific immunosuppression enhances the risk of re-activation of latent virus infections such as Cytomegalovirus (CMV) and Epstein-Barr Virus (EBV). Ideally, novel antigen-specific therapies will be developed which, under optimized conditions, need only be applied for a short period around the time of transplantation. The development of novel therapies, however, requires a fundamental understanding of the mechanisms of rejection of vascularised allogeneic grafts.

The immune system of the transplant recipient plays a pivotal part in the rejection process of vascularised grafts with both humoral and cellular immune responses contributing. Shortly after transplantation, passenger leukocytes leave the graft, migrate to the draining lymph node and present alloantigens via donor-derived MHC molecules to residing host CD4 and CD8 T cells. This strong stimulus leads to activation, proliferation and migration of recipient T cells into the graft and, ultimately, to its destruction. This pathway of activation of host immunity is called the direct pathway of T-cell activation and leads to acute graft rejection [1]. In addition to the direct activation of recipient T cells by donor-derived antigen-presenting cells (APCs), host APCs may contribute to the rejection process by collecting alloantigen from the graft and presenting it in the context of "self" MHC molecules to recipient T cells. This modality of alloantigen presentation is referred to as the indirect pathway of T-cell activation and also results in anti-graft cellular immunity although less strongly as compared to the direct pathway. Nonetheless there is evidence that the indirect pathway is primarily responsible for chronic rejection

of allogeneic grafts over a period of months or even years. Whereas acute rejection can be well controlled by current anti-inflammatory drugs there is often little influence of immunosuppressive drug administration on chronic graft rejection. Finally, in addition to MHC class I/II incompatibilities, ischemia/reperfusion injury (IRI) plays a critical role in the development of chronic graft rejection.

## **2. Gene therapy as novel therapeutic approach to prevent graft rejection**

The tremendous advances in molecular medicine and biology within the last decades have led to the development of novel therapeutic tools for the treatment of cancer, inherited and infectious diseases, autoimmune disorders and allogeneic transplantation. These include the generation of gene-based approaches for the correction of defective genes or for over-expression of therapeutic molecules [2,3]. To date however, the success of gene-based therapies has been limited. This is mainly due to as yet unsolved problems concerning the gene transfer vehicle (e.g. the viral vector), the efficiency of gene transfer, the level of therapeutic gene expression and the potential for unwanted immunologic responses directed against the vector or the transferred therapeutic gene. Despite these challenges, promising clinical trials using gene therapy are currently underway, an example being a recent study demonstrating its successful use in patients with Leber's congenital amaurosis [4].

### **a: Vectors used for gene therapy**

Viral vectors are generally characterized by high infectivity and broad tropism, although transduction efficiency may vary, depending on the cell type [5]. Therefore the selection of a gene therapy vector is critically dependent on the cell type or target tissue. Different virus families have been investigated for their potential as gene therapy vectors. These include vectors derived from retroviruses, adenoviruses (Ad), adeno-associated viruses (AAV) or herpesviruses. The most frequently used carriers for the transfer of genetic information in human gene therapy trials have been adenoviruses and retroviruses [6]. Adenoviral vectors have shown considerable efficiency in pre-clinical and clinical gene therapy studies but, when administered *in vivo*, induce an inflammatory cascade against the vector itself and against the transduced cells or tissues which can lead to strong,

destructive immune responses [5]. Despite this immunological activation numerous studies have been performed using Ad-vectors in gene therapy trials predominantly in patients with advanced malignancies.

Retroviral vectors have also been frequently used as gene transfer vehicles. It is believed that gene delivery with retroviral vectors leads to long-term expression of the therapeutic gene due to the integration of the viral genome into the cellular DNA. In addition, retroviral vectors do not seem to induce strong immune responses. However, integration into transcriptionally-active sites within the cellular DNA may lead to undesired side effects due to insertional mutagenesis [7]. For safety reasons and space constraints regarding the size of the therapeutic gene an in vitro triple/quadruple transfection system for the different components of the retroviral genome has been established.

Finally, AAV has been investigated for its potential as a gene therapy vector. To date ten serotypes (AAV 1-10) with different cell specificities have been identified and apparently lack pathologic effects upon infection in humans. In contrast to Ad-vectors, AAV does not induce a strong immune response due to the absence of immunogenic viral proteins. Clinical trials using AAV are in progress for the treatment of cystic fibrosis, hemophilia, muscular dystrophy and inherited blindness [4,8-10].

Other gene transfer techniques which do not use viruses as shuttle vector (non-viral gene transfer) include transfer of “naked” plasmid-DNA, use of DNA-liposomes complexes, electroporation or “gene gun”-mediated transfer of genetic material into cells or tissues.

#### **b: Genetic modification of the allograft to prevent immune-mediated rejection**

The genetic modification of organs or cells is an attractive approach to protect allografts from acute rejection. The transplant setting offers the unique advantage for gene therapy to modify allografts ex vivo prior to transplantation. Local expression of the therapeutic gene provides new and exciting prospects which may result in improved bioavailability of the immunomodulatory agent and additionally reduce or avoid systemic immune suppression. A significant problem in applying this concept to human organ transplantation, however, is that it remains unclear which specific therapeutic molecules should be expressed or which receptor should be blocked to most efficiently induce long-term allograft survival or even tolerance. Accordingly many therapeutic molecules and

different vector systems have been investigated in numerous pre-clinical studies aimed at demonstrating prolonged allograft survival. In this section of the review some of the more promising candidates are briefly described.

The full activation of alloantigen-specific T cells is one of the critical steps in transplant rejection. CTLA-4 (CD152), a protein homologous to CD28, is expressed on activated T cells and binds B7 on APC with high affinity. It has been shown that CTLA-4 interferes with the B7-CD28 interaction (negative signalling). Several studies have shown beneficial effects of systemic administration of CTLA-4Ig - a soluble fusion protein consisting of the extracellular domain of CTLA-4 fused to the immunoglobulin constant domain which inhibits CD28-B7 interaction - in allograft rejection and autoimmune diseases [11]. Interestingly, it has been speculated that localised expression of CTLA-4Ig through gene therapy techniques may also increase the number of regulatory T cells linking co-stimulatory blockade to the generation of regulatory T cell populations [12,13]. Although initial results from studies in rodent models were very encouraging, the translation of systemic or localised CTLA-4Ig therapy into the clinic has been hampered by disappointing results obtained in nonhuman primates showing much less efficacy in this setting [14,15]. However, since then a number of encouraging approaches have been undertaken to develop higher affinity forms of CTLA-4Ig [16]. In addition, other co-stimulatory pathways such as Inducible Costimulator (ICOS)/ICOSL [17], and PD-1/PD-1L [18] attracted the attention of various research groups as it became clear that T cell activation and transplant rejection can occur in the absence of CD28-B7 or CD40L-CD40 signals [19,20].

Besides targeting co-stimulatory events, over-expression of anti-inflammatory cytokines is another interesting option to modulate immune responses against allogeneic grafts. IL-10 is a potent immunomodulatory cytokine that interacts with APCs and inhibits production of monokines such as IL-1, IL-6, IL-8, and TNF- $\alpha$  [21]. It has been shown by various groups that gene transfer of IL-10 can lead to prolonged graft survival in different transplant models [22,23]. However gene transfer of cytokines may be problematic due to the pleiotropic effects of cytokines and to their short half-life in serum.

### **c: Genetic modification of the allograft to prevent ischemia-reperfusion injury**

Although the introduction of immunosuppressive regimens to prevent acute transplant rejection has significantly improved early allograft survival rates, the half-life of transplanted organs remains below expectations. Nevertheless, the success of transplantation has enhanced the disparity between the number of donor organs available and number of patients on transplant waiting lists. This donor shortage has led to an increase in the transplantation of organs from so-called “marginal” donors (i.e., from older individuals or those with a history of hypertension and cardiovascular disease) whose tissue may be more susceptible to chronic damage following ischemia-reperfusion injury (IRI).

IRI is a complex, antigen-independent event surrounding organ harvesting, storage, and reperfusion, which often leads to primary graft dysfunction as well increased incidence of acute and/or chronic rejection. Indeed, prolonged warm and cold ischemic time (>12h) correlates with an increase in primary graft dysfunction. The mechanism of IRI is multifaceted and includes impaired blood flow reconstitution, increased expression of adhesion molecules, neutrophil activation, activation of APCs, cytokine and chemokine release by infiltrating leukocytes, oxidative stress, and endothelial cell apoptosis [24]. Several gene therapy strategies to prevent IRI have been designed with the aim of blocking oxidative injury locally within the transplant itself. Among these strategies over-expression of anti-apoptotic molecules (Bcl-2, Bcl-xL, Bag-1) or graft-protective genes (Heme Oxygenase-1) reduced ischemia/reperfusion injury and in some pre-clinical studies prolonged graft survival. Interestingly it has also become clear that “danger” signals which are associated with innate immunity may contribute both to IRI and to initiation of transplant rejection [25]. The involvement of Toll-like receptor (TLR) activation in IRI has been documented by using liver transplants from TLR4 knock-out mice. Disruption of TLR4 signalling down-regulated early pro-inflammatory responses and ameliorated liver IRI [26]. In one recent study, over-expression of viral IL-10 (vIL-10) by adenovirus-mediated gene transfer was shown to prevent hepatic IRI in association with depressed expression of innate TLR4 and adaptive T helper type 1 cytokine/chemokine programs [27]. In summary these results indicate that both innate and adaptive immune responses may contribute to graft preservation and long-term survival and constitute important targets for gene therapy-mediated interventions.



**d: Use of genetically modified cells to prevent transplant complications**

Dendritic cells (DCs) are the most potent APC that respond to local injury by uptake and processing of antigenic material, migration to local lymphoid tissue and activation of antigen-specific T cells (for review see: [28,29]). In the normal steady state (absence of inflammation), DCs reside as interstitial immature APCs in most peripheral tissues. Immature DCs internalize exogenous antigens efficiently and exhibit low naive T cell stimulatory capacity (characterized by low MHC class II and CD80/CD86 expression) [30]. During inflammation, however, maturation of immature DCs is triggered by various stimuli including cytokines or bacterial and viral components via TLRs (for review see [25]). Activated DCs express high levels of MHC class II and co-stimulatory molecules (e.g. CD80 and CD86) that enable them to recruit and activate T cells in the lymph nodes. In addition, DCs have the potential to induce tolerance under defined conditions (for review see: [31]). Conditions that favour induction of tolerogenic DCs include exposure to Th2 cytokines such as IL-4, IL-10 or TGF- $\beta$ . The processes thought to be involved include a shift to a Th2-mediated immune response, induction of apoptosis or regulatory T cells. DCs which have been transduced with gene therapy vectors encoding for genes expressing IL-10, CTLA4Ig or TGF- $\beta$  significantly prolonged allogeneic heart graft survival in small animal models [32-34]. Gene-modified DCs expressing vIL-10 produced high levels of this cytokine in vitro with subsequent marked reduction of MHC antigen expression resulting in decreased T cell stimulation and induction of T cell hyporesponsiveness [32].

As previously discussed, a significant problem in transplantation remains the reactivation of latent viral infections such as EBV and CMV due to immunosuppressive medication. Conventional antiviral therapies for these pathogens may be associated with toxicity, viral resistance or triggering of graft rejection, therefore novel therapeutic regimens are required. One promising treatment strategy is the ex-vivo generation of recipient-derived virus-specific T cells which upon re-infusion into the patient remove virus-infected cells [35]. Interestingly, the generation of virus-specific T cells which were stimulated by ex-vivo adenovirally-modified DCs expressing CMV specific antigens has recently been successfully brought forward into the clinic [36].

The *ex-vivo* generation of T cells with regulatory capacity *in vivo* by repeated incubation of T cells with IL-10 has also been described experimentally [37]. Since then many groups have reported the generation of regulatory T cells (T<sub>reg</sub>) both *in vivo* and *in vitro* by various techniques including gene transfer. Recently, cell-based gene therapy using genetically modified T cells expressing the forkhead-winged helix transcription factor FOXP3 has been described [38]. The importance of this transcription factor for the generation of T<sub>reg</sub> first came to light in a mouse mutant strain called *scurfy* which exhibits an X-linked recessive autoimmune and inflammatory diseases as a result of uncontrolled activation and expansion of CD4<sup>+</sup> T cells [39]. A similar phenotype has been identified in humans with X-linked autoimmunity-allergic dysregulation syndrome [40]. Efforts to identify the defect revealed mutations in the gene encoding FOXP3 in affected mice and humans [40,41]. Subsequently it was shown that retrovirus-mediated gene transfer of FOXP3 cDNA in naïve T cells generates a phenotype similar to that of naturally occurring T<sub>reg</sub> and adoptive transfer prevents the onset of autoimmune disease in a model of inflammatory bowel disease [38]. Therefore the generation of FOXP3-expressing T cells has become an interesting option for the treatment of various diseases including allergy, autoimmunity and transplantation [38,42,43].

#### **e: Other niche applications of gene therapy in transplantation**

Other therapeutic opportunities for combining transplantation and gene transfer techniques in specific challenging patient groups have been identified and investigated in the pre-clinical arena. For example gene therapy represents an attractive strategy for increasing the therapeutic potency of isolated cells such as pancreatic islets or hepatocytes as an alternative to whole organ transplantation for metabolic diseases. Pre-clinical studies involving the transplantation of gene-modified islets into diabetic animals have shown promising results although this approach has not been clinically applied to date [44]. In contrast the *ex-vivo* genetic modification of hepatocytes has already shown its potential for clinical application in the correction of genetic defects (see below). Similarly, the association of certain malignancies with chronic organ failure (e.g. hepatocellular carcinoma, lung carcinoma) has generated interest in the administration of anti-tumor gene therapy in combination with organ transplantation and, as described in

the following section, this is one of the few strategies that has been directly tested in a clinical trial setting.

### **3. Clinical Application of Gene Therapy in Transplantation: The Reality**

Despite the enthusiasm that has been shown for gene therapy concepts and applications within the field of experimental transplantation [45] the translation of this work toward improving the outcome of human organ and tissue transplants has been surprisingly limited. This fact seems all the more unusual given the “bold steps” that have historically marked the progress of transplantation as a clinical intervention [46] but, as discussed later, there may be some specific explanations for the dearth of gene therapy trials among transplant recipients. In this section of the review, the limited but potentially important clinical reports involving gene therapy in recipients of non-haematological transplants or in patients groups that may be of direct relevance to transplantation are described.

*Registered clinical gene therapy trial in transplantation:* Clinical trial registries provide a valuable overview of the level and diversity of human translational research that has been inspired by basic and pre-clinical work in various emerging fields such as gene therapy. The most wide-reaching of these registries, ClinicalTrials.gov ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)), lists, as of April 1<sup>st</sup> 2009, over 1600 completed, active or planned human clinical trials involving *in vivo* or *ex vivo* viral and non-viral gene transfer techniques. A review of the protocol descriptions for these studies reveals that the largest number of registered gene therapy trials is targeted toward the therapy of malignant solid tumours while other indications for which multiple trials have been initiated include haematological malignancies and genetically-based immune deficiencies, non-immunological genetic disorders, chronic infections, cardiovascular diseases, neurological diseases and autoimmunity. Remarkably, while a search of this database for trials combining the terms “gene therapy (or gene transfer)” and “transplantation” returns over 100 studies, the large majority of these involve bone marrow/haematopoietic stem cell transplantation or non-interventional genetic studies of transplant recipients. Only one registered study (discussed below) represents a *bona fide* gene transfer technique in the context of solid organ transplantation [47]. Further searches of this database as well as other clinical trials registries and meta-registries (e.g. ISRCTN ([isrctn.org](http://isrctn.org)), WHO ICTRP

([www.who.int/ictcp/en/](http://www.who.int/ictcp/en/)) and Current Controlled Trials ([www.controlled-trials.com/mrct/](http://www.controlled-trials.com/mrct/)) revealed only one additional ongoing trial of a nucleic-acid based therapeutic product in organ transplant recipients. In this multi-centre phase I/II study (described at [www.quarkpharma.com/qbi-en/products/QPI-1002DGF/](http://www.quarkpharma.com/qbi-en/products/QPI-1002DGF/)) the effect of a small interfering RNA (siRNA) preparation directed against the stress/ischemia-activated protein p53 on renal allograft delayed function will be compared to placebo.

*Reported results for gene therapy trials in human transplant recipients:* In 1995, Grossman et al. reported the results of a novel clinical study in which primary hepatocytes, cultured from partial hepatectomy specimens of 5 patients with homozygous familial hypercholesterolemia, were retrovirally transfected *ex vivo* with the low density lipoprotein receptor and then transplanted back into the remaining liver through the portal vein [48]. In this pioneering auto-transplant trial, which followed an extensive pre-clinical research program, prolonged significant reduction in fasting serum cholesterol occurred in 3 of 5 treated patients and transgene-positive hepatocytes were detectable by liver biopsy in all 5. Although the protocol required substantial interventions, no severe detrimental effects occurred. While this study appeared to offer proof of principle for transplantation of virally-transfected primary cells in patients, it was not followed by additional similar human trials. Nonetheless, clinical interest in *ex vivo* gene therapy of human hepatocytes remains active, as evidenced by the recent report of Birraux et al. in which high-level lentiviral transduction of hepatocytes from a child with Crigler-Najjar type 1 syndrome was achieved and shown to result in expression of the deficient enzyme, diphosphate glucuronosyltransferase [49].

In the most significant clinical trial reported to date, Li and colleagues describe the results of an open-label clinical study comparing adenovirus-mediated delivery of herpes simplex virus thymidine kinase (n = 23) with no additional intervention (n = 22) in liver transplant recipients with large (>5cm), non-metastatic hepatocellular carcinoma [47]. Treated patients received a total of  $5 \times 10^{11}$  viral particles directly injected into the peri-hepatic and upper abdominal peritoneum during transplantation. This was followed by intravenous ganciclovir administered twice daily between days 1 and 10 post-transplant. The adenovirally-treated group experienced an increased rate of recurrence-free survival over a median follow-up of 26 months (44% vs. 9%) and of overall 2-year survival (70%

vs. 20%) despite very similar tumour number, tumour size and frequency of vascular invasion at baseline. Subgroup analysis indicated that, in those with documented vascular invasion at the time of transplant, adenoviral therapy delayed but did not prevent recurrence. In contrast, beneficial effects of gene therapy on recurrence-free survival were more striking in transplant recipients without vascular invasion with 100% patient survival up to 50 months. The only adverse effects described for adenovirus administration were mild catarrhal symptoms and low grade pyrexia limited to the first 5 days post-transplant. Systemically detectable adenoviral DNA peaked at 12 hours and disappeared by 7 days. Although confirmation of the beneficial effects will be essential, this well-conducted clinical study represents the best evidence to date that a viral gene therapy strategy can be successfully and safely applied as an adjuvant intervention at the time of organ transplant with the possibility of improved outcome for a challenging group of patients.

One other gene therapy strategy – the use of *ex vivo* hypothermic organ perfusion with preservation solutions containing viral vectors – has also been tested to a limited degree using human tissue. Within the past year, Henry et al. have reported superior transfection of human primary hepatocytes with VSV-G-pseudotyped lentiviral vectors under hypothermic conditions when virus was delivered in University of Wisconsin (UW) solution compared to histidine tryptophan ketoglutarate (HTK) solution and other vehicles. Viral transduction was shown to be specifically promoted by the presence of hydroxyethyl starch in UW preservation solution [50]. While not addressing safety or *in vivo* efficacy issues, this study does provide a step forward in the translation of a conceptual gene therapy strategy into practical protocols for delivering nucleic acid-based products directly into transplantable organs and tissues.

*Other human gene therapy trials with relevance to transplantation:* Given the apparent caution in proceeding to the clinic with gene therapy interventions in human organ transplant recipients as well as the diversity of experimental protocols that have arisen, it is worth briefly considering clinical gene transfer strategies that have been successfully pursued in other patient groups that share common therapeutic needs. A good example of such an experience is the successful delivery to hematopoietic stem cell recipients of cytomegalovirus (CMV)-specific T-cells generated *ex vivo* by stimulation with dendritic

cells adenovirally transfected to express viral antigens [36]. As CMV disease continues to be a common cause of morbidity following organ transplantation, the success of this therapeutic approach may well be reproducible in organ allograft recipients. Similarly, the development of viral antigen-specific T-cells against Epstein Barr virus (EBV)-associated post-transplant lymphoma represents a strategy for which clinical precedent exists in the haematological literature [51].

Although much has been made of the potential for gene therapy approaches to modify ischemia-reperfusion injury in human organs procured for transplantation [45], no clinical testing of this concept has been reported to date. In contrast, the field of cardiovascular medicine has recently seen the completion of sizeable clinical trials designed to examine the safety and therapeutic efficacy of virally or non-virally encoded protective factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), fibroblast growth factor 1 (FGF-1) and hypoxia-inducible factor 1 $\alpha$  (HIF-1 $\alpha$ ) in cardiac and limb ischemia [52-56]. It is beyond the scope of this review to provide a detailed summary of the results of these studies but a common emerging theme is that genetic vectors encoding protective factors against ischemic tissue injury can be safely and reliably delivered to complex patient groups often in combination with other well-established interventions. Some of these studies have also recruited large enough patient groups to control and treatment arms to convincingly demonstrate objective or subjective clinical benefits for specific gene therapies [52-55]. It is to be hoped that further clinical trial successes in the fields of cancer, haematological disease and cardiovascular diseases will provide a framework for new applications of gene transfer technologies to human transplant recipients.

#### **4. Conclusions: Has Gene Therapy in Transplantation Been Lost in Translation?**

**Figure 1** summarises the degree to which several major transplant-related gene therapy concepts have progressed from the laboratory to the clinic during the past two decades. As can readily be seen, the translation of pre-clinical experiments to human clinical trials has been minimal despite an abundance of promising animal model studies. It is reasonable, therefore, to reflect on the reasons for this “log jam” and on what barriers must be eliminated for robust clinical application to occur. A number of factors may have

contributed to what appears to be reluctance among transplant centres to initiate clinical studies involving gene therapy:

- (a) Current short-term results for most organ transplants leave relatively little room for improvement and would require large patient numbers to be recruited to any clinical trial designed, for instance, to show a reduction in acute rejection or an increase in 1-year graft survival.
- (b) Gene therapy approaches to treating chronic graft deterioration would likely require vector technology for long-term, stable intra-graft gene expression that is not yet available for clinical application.
- (c) Much of the externally-funded clinical trial activity in transplantation during the past two decades has focussed on comparisons of immunosuppressive regimens and, to a lesser degree, on the development of tolerogenic protocols. Specifically, there has been little interest from the pharmacologic industry in developing and testing gene-based therapies as an alternative to conventional drugs and biological agents.
- (d) In contrast to the early history of organ transplantation, the modern clinical transplant field has become highly regulated and increasingly risk-averse. With centres in several countries facing censure for “lower-than-expected” graft outcomes, safety concerns from early human gene therapy studies continue to exert a negative influence on the use of viral vectors in organ allograft recipients.

Although these factors will likely continue to engender caution there is also reason to believe that gene therapy trials in transplantation will become more common in the coming years. Successful results from other fields [51-56] should bring clinical confidence as well as renewed industry interest. The development of novel gene therapy vectors with less immunogenicity or toxicity will also be highly important to move this research closer to potential clinical applications for non-life threatening diseases. In particular the development of third and fourth generations of adenoviral or retroviral vectors will be essential for safe application of gene therapy to the prevention of allogeneic graft rejection. Significant progress in this area includes the development of third generation adenoviral vectors (also referred to as helper-dependent or gut-less Ad-vectors) which are devoid of any adenoviral DNA sequences save the inverted repeats

and the packaging signal required for efficient packaging into the Ad-capsids [57,58]. It has recently been shown that immune responses against third generation Ad after systemic injection are reduced and prolonged expression of the therapeutic gene has been reported [59]. This may even allow repeated application of Ad-vectors if required. Non-specific integration of retroviral vectors which may eventually lead to uncontrolled proliferation and tumour formation of transduced cells is a major concern for the application of retroviral vectors in gene therapy trials [7]. Consequently retroviral vectors which do not integrate but remain in an episomal location have been developed [60]. In addition, retroviral vectors with improved safety potential in terms of site-specific integration are under currently under investigation. Finally, combined cell-/gene therapy has the potential to move towards multiple clinical applications within the next few years and, in addition, is likely to be increasingly valuable as a tool to study the mechanisms of acute and chronic allograft loss in more detail.

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## References:

1. Game DS, Lechler RI: **Pathways of allorecognition: implications for transplantation tolerance.** *Transpl Immunol* (2002) **10**(2-3):101-108.
2. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, Gross F, Yvon E, Nusbaum P, Selz F, Hue C, Certain S, Casanova JL, Bousso P *et al*: **Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease.** *Science* (2000) **288**(5466):669-672.  
  
This article describes the first gene therapy trial for severe combined immunodeficiency-X1 using a replication-defective Moloney retrovirus-derived vector. Gene therapy was able to provide full correction of disease phenotype and resulted in clinical benefit.
3. Brantly ML, Spencer LT, Humphries M, Conlon TJ, Spencer CT, Poirier A, Garlington W, Baker D, Song S, Berns KI, Muzyczka N *et al*: **Phase I trial of intramuscular injection of a recombinant adeno-associated virus serotype 2 alphas-antitrypsin (AAT) vector in AAT-deficient adults.** *Hum Gene Ther* (2006) **17**(12):1177-1186.
4. Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, Viswanathan A, Holder GE, Stockman A, Tyler N, Petersen-Jones S *et al*: **Effect of gene therapy on visual function in Leber's congenital amaurosis.** *N Engl J Med* (2008) **358**(21):2231-2239.  
  
In this recent important article the treatment of three patients with loss of vision by AAV-mediated gene transfer of RPE65 is reported.
5. Kay MA, Glorioso JC, Naldini L: **Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics.** *Nat Med* (2001) **7**(1):33-40.
6. Edelstein ML, Abedi MR, Wixon J: **Gene therapy clinical trials worldwide to 2007--an update.** *J Gene Med* (2007) **9**(10):833-842.
7. Baum C, Kustikova O, Modlich U, Li Z, Fehse B: **Mutagenesis and oncogenesis by chromosomal insertion of gene transfer vectors.** *Hum Gene Ther* (2006) **17**(3):253-263.
8. Kay MA, Manno CS, Ragni MV, Larson PJ, Couto LB, McClelland A, Glader B, Chew AJ, Tai SJ, Herzog RW, Arruda V *et al*: **Evidence for gene transfer and expression of factor IX in haemophilia B patients treated with an AAV vector.** *Nat Genet* (2000) **24**(3):257-261.
9. Stedman H, Wilson JM, Finke R, Kleckner AL, Mendell J: **Phase I clinical trial utilizing gene therapy for limb girdle muscular dystrophy: alpha-, beta-, gamma-, or delta-sarcoglycan gene delivered with intramuscular instillations of adeno-associated vectors.** *Hum Gene Ther* (2000) **11**(5):777-790.
10. Wagner JA, Messner AH, Moran ML, Daifuku R, Kouyama K, Desch JK, Manley S, Norbash AM, Conrad CK, Friborg S, Reynolds T *et al*: **Safety and biological efficacy of an adeno-associated virus vector-cystic fibrosis transmembrane regulator (AAV-CFTR) in the cystic fibrosis maxillary sinus.** *Laryngoscope* (1999) **109**(2 Pt 1):266-274.
11. Clarkson MR, Sayegh MH: **T-cell costimulatory pathways in allograft rejection and tolerance.** *Transplantation* (2005) **80**(5):555-563.
12. Miao G, Ito T, Uchikoshi F, Akamaru Y, Kiyomoto T, Komodo H, Song J, Nozawa M, Matsuda H: **Development of donor-specific immunoregulatory T-cells after local CTLA4Ig gene transfer to pancreatic allograft.** *Transplantation* (2004) **78**(1):59-64.

13. Wei L, Wei-Min L, Cheng G, Bao-Guo Z: **Upregulation of CD4+CD25+ T lymphocyte by adenovirus-mediated gene transfer of CTLA4Ig fusion protein in experimental autoimmune myocarditis.** *Autoimmunity* (2006) **39**(4):289-298.
14. Levisetti MG, Padrid PA, Szot GL, Mittal N, Meehan SM, Wardrip CL, Gray GS, Bruce DS, Thistlethwaite JR, Jr., Bluestone JA: **Immunosuppressive effects of human CTLA4Ig in a non-human primate model of allogeneic pancreatic islet transplantation.** *J Immunol* (1997) **159**(11):5187-5191.
15. Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, Hong X, Thomas D, Fechner JH, Jr., Knechtle SJ: **CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates.** *Proc Natl Acad Sci U S A* (1997) **94**(16):8789-8794.
16. Larsen CP, Pearson TC, Adams AB, Tso P, Shirasugi N, Strobertm E, Anderson D, Cowan S, Price K, Naemura J, Emswiler J *et al*: **Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties.** *Am J Transplant* (2005) **5**(3):443-453.
17. Harada H, Salama AD, Sho M, Izawa A, Sandner SE, Ito T, Akiba H, Yagita H, Sharpe AH, Freeman GJ, Sayegh MH: **The role of the ICOS-B7h T cell costimulatory pathway in transplantation immunity.** *J Clin Invest* (2003) **112**(2):234-243.
18. Keir ME, Liang SC, Guleria I, Latchman YE, Qipo A, Albacker LA, Koulmanda M, Freeman GJ, Sayegh MH, Sharpe AH: **Tissue expression of PD-L1 mediates peripheral T cell tolerance.** *J Exp Med* (2006) **203**(4):883-895.
19. Yamada A, Salama AD, Sayegh MH: **The role of novel T cell costimulatory pathways in autoimmunity and transplantation.** *J Am Soc Nephrol* (2002) **13**(2):559-575.
20. Lin H, Rathmell JC, Gray GS, Thompson CB, Leiden JM, Alegre ML: **Cytotoxic T lymphocyte antigen 4 (CTLA4) blockade accelerates the acute rejection of cardiac allografts in CD28-deficient mice: CTLA4 can function independently of CD28.** *J Exp Med* (1998) **188**(1):199-204.
21. de Waal Malefyt R, Haanen J, Spits H, Roncarolo MG, te Velde A, Figdor C, Johnson K, Kastelein R, Yssel H, de Vries JE: **Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression.** *J Exp Med* (1991) **174**(4):915-924.
22. DeBruyne LA, Li K, Chan SY, Qin L, Bishop DK, Bromberg JS: **Lipid-mediated gene transfer of viral IL-10 prolongs vascularized cardiac allograft survival by inhibiting donor-specific cellular and humoral immune responses.** *Gene Ther* (1998) **5**(8):1079-1087.
23. David A, Chetritt J, Guillot C, Tesson L, Heslan JM, Cuturi MC, Soullillou JP, Anegon I: **Interleukin-10 produced by recombinant adenovirus prolongs survival of cardiac allografts in rats.** *Gene Ther* (2000) **7**(6):505-510.
24. Vardanian AJ, Busuttil RW, Kupiec-Weglinski JW: **Molecular mediators of liver ischemia and reperfusion injury: a brief review.** *Mol Med* (2008) **14**(5-6):337-345.
25. Kim IK, Bedi DS, Denecke C, Ge X, Tullius SG: **Impact of innate and adaptive immunity on rejection and tolerance.** *Transplantation* (2008) **86**(7):889-894.
26. Shen XD, Ke B, Zhai Y, Gao F, Tsuchihashi S, Lassman CR, Busuttil RW, Kupiec-Weglinski JW: **Absence of toll-like receptor 4 (TLR4) signaling in the donor organ reduces ischemia and**

**reperfusion injury in a murine liver transplantation model.** *Liver Transpl* (2007) **13**(10):1435-1443.

This study analyzes how toll-like receptor 4 (TLR4) signaling in the donor organ affects the ischemia and reperfusion injury (IRI) sequel following liver transplantation. Disruption of TLR4 pathway downregulated the early proinflammatory responses and ameliorated hepatic IRI.

27. Ke B, Shen XD, Tsuchihashi S, Gao F, Araujo JA, Busuttil RW, Ritter T, Kupiec-Weglinski JW: **Viral interleukin-10 gene transfer prevents liver ischemia-reperfusion injury: Toll-like receptor-4 and heme oxygenase-1 signaling in innate and adaptive immunity.** *Hum Gene Ther* (2007) **18**(4):355-366.

This pre-clinical study investigates how adenovirus mediated gene transfer of viral interleukin-10 transfer affects TLR4 and HO-1 signaling in host innate and adaptive immunity during liver IRI. Over-expression of vIL-10 prevents IRI in association with depressed TLR4 expression.

28. Morelli AE, Thomson AW: **Dendritic cells: regulators of alloimmunity and opportunities for tolerance induction.** *Immunol Rev* (2003) **196**:125-146.
29. Lechler R, Ng WF, Steinman RM: **Dendritic cells in transplantation--friend or foe?** *Immunity* (2001) **14**(4):357-368.
30. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K: **Immunobiology of dendritic cells.** *Annu Rev Immunol* (2000) **18**:767-811.
31. Morelli AE, Thomson AW: **Tolerogenic dendritic cells and the quest for transplant tolerance.** *Nat Rev Immunol* (2007) **7**(8):610-621.
32. Takayama T, Nishioka Y, Lu L, Lotze MT, Tahara H, Thomson AW: **Retroviral delivery of viral interleukin-10 into myeloid dendritic cells markedly inhibits their allostimulatory activity and promotes the induction of T-cell hyporesponsiveness.** *Transplantation* (1998) **66**(12):1567-1574.
33. Takayama T, Morelli AE, Robbins PD, Tahara H, Thomson AW: **Feasibility of CTLA4Ig gene delivery and expression in vivo using retrovirally transduced myeloid dendritic cells that induce alloantigen-specific T cell anergy in vitro.** *Gene Ther* (2000) **7**(15):1265-1273.
34. Takayama T, Kaneko K, Morelli AE, Li W, Tahara H, Thomson AW: **Retroviral delivery of transforming growth factor-beta1 to myeloid dendritic cells: inhibition of T-cell priming ability and influence on allograft survival.** *Transplantation* (2002) **74**(1):112-119.
35. Gottschalk S, Bollard CM, Straathof KC, Louis CU, Savoldo B, Dotti G, Brenner MK, Heslop HE, Rooney CM: **T cell therapies.** *Ernst Schering Found Symp Proc* (2006) (4):69-82.
36. Micklethwaite KP, Clancy L, Sandher U, Hansen AM, Blyth E, Antonenas V, Sartor MM, Bradstock KF, Gottlieb DJ: **Prophylactic infusion of cytomegalovirus-specific cytotoxic T lymphocytes stimulated with Ad5f35pp65 gene-modified dendritic cells after allogeneic hemopoietic stem cell transplantation.** *Blood* (2008) **112**(10):3974-3981.

This important clinical study documents successful immune reconstitution of anti-CMV cellular immunity in 12 allogeneic hematopoietic stem cell transplant recipients who received donor-derived T cells generated by *ex vivo* activation using dendritic cells transduced with adenovirus encoding the CMVpp65 protein.

37. Hara M, Kingsley CI, Niimi M, Read S, Turvey SE, Bushell AR, Morris PJ, Powrie F, Wood KJ: **IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo.** *J Immunol* (2001) **166**(6):3789-3796.
38. Hori S, Nomura T, Sakaguchi S: **Control of regulatory T cell development by the transcription factor Foxp3.** *Science* (2003) **299**(5609):1057-1061.  
  
In this seminal paper the authors show that retroviral gene transfer of Foxp3 converts naive T cells toward a regulatory T cell phenotype similar to that of naturally occurring CD4+ regulatory T cells indicating that Foxp3 is a key regulatory gene for the development of regulatory T cells.
39. Godfrey VL, Wilkinson JE, Russell LB: **X-linked lymphoreticular disease in the scurfy (sf) mutant mouse.** *Am J Pathol* (1991) **138**(6):1379-1387.
40. Wildin RS, Smyk-Pearson S, Filipovich AH: **Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome.** *J Med Genet* (2002) **39**(8):537-545.
41. Brunkow ME, Jeffery EW, Hjerrild KA, Paepfer B, Clark LB, Yasayko SA, Wilkinson JE, Galas D, Ziegler SF, Ramsdell F: **Disruption of a new forkhead/winged-helix protein, scurf, results in the fatal lymphoproliferative disorder of the scurfy mouse.** *Nat Genet* (2001) **27**(1):68-73.
42. Loser K, Hansen W, Apelt J, Balkow S, Buer J, Beissert S: **In vitro-generated regulatory T cells induced by Foxp3-retrovirus infection control murine contact allergy and systemic autoimmunity.** *Gene Ther* (2005) **12**(17):1294-1304.
43. Chai JG, Xue SA, Coe D, Addey C, Bartok I, Scott D, Simpson E, Stauss HJ, Hori S, Sakaguchi S, Dyson J: **Regulatory T cells, derived from naive CD4+CD25- T cells by in vitro Foxp3 gene transfer, can induce transplantation tolerance.** *Transplantation* (2005) **79**(10):1310-1316.
44. Kim YH, Lim DG, Wee YM, Kim JH, Yun CO, Choi MY, Park YH, Kim SC, Han DJ: **Viral IL-10 gene transfer prolongs rat islet allograft survival.** *Cell Transplant* (2008) **17**(6):609-618.
45. Sandovici M, Deelman LE, de Zeeuw D, van Goor H, Henning RH: **Immune modulation and graft protection by gene therapy in kidney transplantation.** *Eur J Pharmacol* (2008) **585**(2-3):261-269.
46. Groth CG, Brent LB, Calne RY, Dausset JB, Good RA, Murray JE, Shumway NE, Schwartz RS, Starzl TE, Terasaki PI, Thomas ED *et al*: **Historic landmarks in clinical transplantation: conclusions from the consensus conference at the University of California, Los Angeles.** *World J Surg* (2000) **24**(7):834-843.
47. Li N, Zhou J, Weng D, Zhang C, Li L, Wang B, Song Y, He Q, Lin D, Chen D, Chen G *et al*: **Adjuvant adenovirus-mediated delivery of herpes simplex virus thymidine kinase administration improves outcome of liver transplantation in patients with advanced hepatocellular carcinoma.** *Clin Cancer Res* (2007) **13**(19):5847-5854.  
  
The controlled clinical study reported in this publication represents an important milestone in the application of adenoviral gene therapy to liver transplant recipients. Patients with hepatocellular carcinomas conventionally considered too large for transplantation achieved very acceptable patient and graft survival following adjuvant therapy with adenovirally-encoded thymidine kinase and post-operative ganciclovir.
48. Grossman M, Rader DJ, Muller DW, Kolansky DM, Kozarsky K, Clark BJ, 3rd, Stein EA, Lupien PJ, Brewer HB, Jr., Raper SE, *et al*: **A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia.** *Nat Med* (1995) **1**(11):1148-1154.

49. Birraux J, Menzel O, Wildhaber B, Jond C, Nguyen TH, Chardot C: **A step toward liver gene therapy: efficient correction of the genetic defect of hepatocytes isolated from a patient with Crigler-Najjar syndrome type 1 with lentiviral vectors.** *Transplantation* (2009) **87**(7):1006-1012.
50. Henry SD, van der Wegen P, Metselaar HJ, Scholte BJ, Tilanus HW, van der Laan LJ: **Hydroxyethyl starch-based preservation solutions enhance gene therapy vector delivery under hypothermic conditions.** *Liver Transpl* (2008) **14**(12):1708-1717.
51. Bollard CM, Gottschalk S, Leen AM, Weiss H, Straathof KC, Carrum G, Khalil M, Wu MF, Huls MH, Chang CC, Gresik MV *et al*: **Complete responses of relapsed lymphoma following genetic modification of tumor-antigen presenting cells and T-lymphocyte transfer.** *Blood* (2007) **110**(8):2838-2845.
52. Stewart DJ, Hilton JD, Arnold JM, Gregoire J, Rivard A, Archer SL, Charbonneau F, Cohen E, Curtis M, Buller CE, Mendelsohn FO *et al*: **Angiogenic gene therapy in patients with nonrevascularizable ischemic heart disease: a phase 2 randomized, controlled trial of AdVEGF(121) (AdVEGF121) versus maximum medical treatment.** *Gene Ther* (2006) **13**(21):1503-1511.
53. Powell RJ, Simons M, Mendelsohn FO, Daniel G, Henry TD, Koga M, Morishita R, Annex BH: **Results of a double-blind, placebo-controlled study to assess the safety of intramuscular injection of hepatocyte growth factor plasmid to improve limb perfusion in patients with critical limb ischemia.** *Circulation* (2008) **118**(1):58-65.
54. Grines CL, Watkins MW, Mahmarian JJ, Iskandrian AE, Rade JJ, Marrott P, Pratt C, Kleiman N: **A randomized, double-blind, placebo-controlled trial of Ad5FGF-4 gene therapy and its effect on myocardial perfusion in patients with stable angina.** *J Am Coll Cardiol* (2003) **42**(8):1339-1347.
55. Nikol S, Baumgartner I, Van Belle E, Diehm C, Visona A, Capogrossi MC, Ferreira-Maldent N, Gallino A, Wyatt MG, Wijesinghe LD, Fusari M *et al*: **Therapeutic angiogenesis with intramuscular NV1FGF improves amputation-free survival in patients with critical limb ischemia.** *Mol Ther* (2008) **16**(5):972-978.
56. Rajagopalan S, Olin J, Deitcher S, Pieczek A, Laird J, Grossman PM, Goldman CK, McEllin K, Kelly R, Chronos N: **Use of a constitutively active hypoxia-inducible factor-1alpha transgene as a therapeutic strategy in no-option critical limb ischemia patients: phase I dose-escalation experience.** *Circulation* (2007) **115**(10):1234-1243.
57. Kochanek S, Clemens PR, Mitani K, Chen HH, Chan S, Caskey CT: **A new adenoviral vector: Replacement of all viral coding sequences with 28 kb of DNA independently expressing both full-length dystrophin and beta-galactosidase.** *Proc Natl Acad Sci U S A* (1996) **93**(12):5731-5736.
58. Palmer D, Ng P: **Improved system for helper-dependent adenoviral vector production.** *Mol Ther* (2003) **8**(5):846-852.
59. Barcia C, Jimenez-Dalmaroni M, Kroeger KM, Puntel M, Rapaport AJ, Larocque D, King GD, Johnson SA, Liu C, Xiong W, Candolfi M *et al*: **One-year expression from high-capacity adenoviral vectors in the brains of animals with pre-existing anti-adenoviral immunity: clinical implications.** *Mol Ther* (2007) **15**(12):2154-2163.

60. Yanez-Munoz RJ, Balaggan KS, MacNeil A, Howe SJ, Schmidt M, Smith AJ, Buch P, MacLaren RE, Anderson PN, Barker SE, Duran Y *et al*: **Effective gene therapy with nonintegrating lentiviral vectors.** *Nat Med* (2006) **12**(3):348-353.

## Figure Legends

### Figure 1

A diagram is shown representing current progress toward human clinical application of five different gene therapy concepts in the field of organ and tissue transplantation. Arrows represent the translational stage based on available current literature and clinical trials registries. The extended white arrows indicate that human case series or controlled trials have been reported for other patient groups (e.g. haematological stem cell transplant recipients, patients with acute MI) but not for organ transplant recipients. Relevant recent references (REFS) are indicated at the far right. \* = references pertaining to non-organ transplant recipients.

**Figure 1**

