Gene therapy matures in the clinic

To the Editor:

Gene therapy has promised much and delivered little for patients over many years, but a range of clinical projects are now showing clear signs of efficacy, giving considerable reassurance that technology from this field will soon enter into mainstream medicine. The studies showing clinical promise cover several different diseases and use a variety of vectors, ranging from simple oligonucleotides to replicating lytic viruses. However, one crucial unifying feature is that they all focus on realistic and achievable objectives, particularly in terms of effective delivery of the therapeutic agent to target cells, but also using levels and durations of transgene expression commensurate with the desired treatment outcome. Careful selection of disease targets on the basis of the vector systems available is an essential prerequisite to success. Here we highlight some of the more notable recent advances and try to put them into the context of the underpinning technological improvements; details of selected recent clinical studies are summarized in **Table 1**. These and many other areas of scientific and clinical progress were recently updated at a collaborative congress held in Brighton, UK, between the European Society of Gene & Cell Therapy (ESGCT) and the British Society for Gene Therapy (BSGT)[1] (http://www.esgct.eu/index.php/en/congress2/previous-congress/congres-2011).

Technological landscape

Development of better genetic vectors over the last two decades has produced a number of therapeutic reagents that have now successfully transferred from laboratory study to clinical application. Most clinical studies now use viral-based vector systems that benefit from high efficiency but may be compromised by immunogenicity and toxicities specific to the type of vector employed. For example, integrating retroviral vectors mediate long-term expression of transgenes in replicating and expanding cell populations, but in some circumstances have produced toxicity from insertional mutagenesis. Whereas retroviral vectors are often used to mediate long-term stable gene expression, other viral vectors, such as those based on adeno-associated viruses (AAV), are being widely explored to mediate extended transgene expression in non-replicating cells, where they are thought to persist as non-integrated non-replicating episomes. However, all viral vectors are limited by their packaging capacity—in other words, by the size of the transgenic cassette, which may restrict capacity for incorporation of large genes or complex regulatory elements. Furthermore, very large-scale bio-production remains challenging for some viral vectors. Non-viral technologies based on different chemistries, targeting strategies and genetic constructs are being developed to remove some of these problems, and although efficiency has remained problematic, for some applications there are signs of genuine efficacy. Emerging successful strategies are therefore a 'best fit' based on knowledge of the target disease, the desired regulatory pattern of gene expression and the bioactivity of different vector systems.

Gene therapies for genetic disease

Congenital retinal blinding conditions affect approximately 1 in 2,000 people worldwide, yet are without effective treatments. They are clinically heterogeneous, with mutations in over 165 different genes identified as causing disease largely through dysfunction of photoreceptor, bipolar or retinal pigment epithelium cells. Although conventional medicine has little to offer, the eye presents one of the most accessible targets for localized delivery and therefore for application of gene (and cellular) therapies. Furthermore, there may be advantages in terms of immunological privilege and ability to monitor local outcomes and potential toxicities. Several studies have recently reported remarkable clinical improvements after treatment of patients with Leber's congenital amaurosis (LCA), for which alternative therapies are unavailable. Highly efficient delivery of AAV to retinal pigment epithelial cells was achieved by precise surgical subretinal injection. In one study, AAV serotype 2 (AAV2) expressing RPE65 (the retinal pigment protein that is lost in LCA) under transcriptional control of its physiological promoter was administered to three patients (**Table 1**)[2]. Although none of the recipients showed any change in retinal responses measured by electroretinography in this trial, one patient had significant improvement in visual function measured by microperimetry and dark-adapted perimetry, and also showed improvement in a subjective test of visual mobility. In another study, each patient showed a modest improvement in measures of retinal function in subjective tests of visual acuity, although normal vision was not achieved[3]. Finally, a dose-escalation study of AAV2 expressing RPE65 under control of the chicken β-actin promoter produced sustained improvements in subjective and objective measurements of vision, with at least a 2-log-unit increase in pupillary light responses in all 12 patients. The greatest improvement was noted in children, all of whom gained ambulatory vision[3,4]. The fact that maximal benefit occurred in younger recipients indicates that regenerative therapies for this and many other disorders, whether through gene or stem cell approaches, should be targeted as early as possible, before functional recovery becomes irretrievable. As a corollary to this, clinical trials should not necessarily dwell on extended study in patients for whom clinical benefit is unlikely, as these populations may not be predictive of efficacy or even safety. In many diseases, carefully structured studies in children are therefore of paramount importance.

As these highly encouraging trials progress into phase II and eventually phase III, trials are planned for other genetic forms of inherited blindness, as well as for use of the same or similar technologies to treat acquired disease such as vascular retinopathies and age-related macular degeneration. At the same time, the technologies are continually being refined to enhance efficacy and safety—for example, through the use of alternative AAV serotypes shown to transduce retinal cells more efficiently *in vivo*. The small size of the target organ

Table 1 Some recent advances in clinical gene therapy

	Vector, dose range, and number and ages of patients	Transgene and promoter	Route of administration and cell target	Scientific and clinical outcomes	Reference
Gene therapy for genetic disease					
Leber's congenital amaurosis	AAV2; 1.5×10^{10} vg per patient; three patients (19–26 years old)	RPE65 under chicken β-actin promoter	Subretinal injection to retinal epithelial cells	All patients showed improved visual acuity and modest improvements in pupillary light reflexes.	3
	AAV2; 10^{11} vg per patient; three patients (17–23 years old)	RPE65 under cognate promoter	Subretinal injection to retinal epithelial cells	No change in visual acuity or retinal responses to flash or pattern electroretinography; microperimetry and dark-adapted perimetry showed no change in retinal function in patients 1 and 2 but showed improved retinal function in patient 3.	2
	AAV2; 1.5×10^{10}, 4.8×10^{10} or 1.5×10^{11} vg per patient; 12 patients (8–44 years old)	RPE65 under chicken β-actin promoter	Subretinal injection to retinal epithelial cells	All patients showed sustained improvement in subjective and objective measurements of vision (dark adaptometry, pupillometry, electroretinography, nystagmus and ambulatory behavior).	4
Hemophilia B	AAV8; 2×10^{11}, 6×10^{11} or 2×10^{12} vg per kg body weight; six patients (27–64 years old)	FIX gene, regulated by the human apolipoprotein hepatic control region and human α-1-antitrypsin promoter	Intravenous delivery targeting hepatocytes	Durable circulating FIX at 2–11% normal levels; decreased frequency (two of six patients) or cessation (four of six) of spontaneous hemorrhage	11
X-linked severe combined immunodeficiency (SCID-X1)	Gammaretrovirus; ten patients (4–36 months old); $CD34^+$ cells were infused (without conditioning) at doses of 60×10^6 to 207×10^6 cells per patient	Interleukin-2 receptor common γ-chain, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Functional polyclonal T-cell response restored in all patients; one patient developed acute T-cell lymphoblastic leukemia	23
	Gammaretrovirus; nine patients (1–11 months old); $CD34^+$ cells were infused (without conditioning) at doses of 1×10^6 to 22×10^6 cells per kg	Interleukin-2 receptor common γ-chain, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Functional T-cell numbers reached normal ranges. Transduced T cells were detected for up to 10.7 years after gene therapy. Four patients developed acute T cell lymphoblastic leukemia, one died.	24
Adenosine deaminase deficiency resulting in severe combined immunodeficiency (ADA-SCID)	Gammaretrovirus; six patients (6–39 months old); $CD34^+$ cells were infused (after non-myeloablative conditioning with melphalan (Alkeran), 140 mg per m^2 body surface area, or busulfan (Myleran), 4 mg per kg) at doses of $<0.5 \times 10^6$ to 5.8×10^6 cells per kg	Adenosine deaminase gene, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Restoration of immune function in four of six patients; three of six taken off enzyme-replacement therapy; four of six remain free of infection	25
	Gammaretrovirus; ten patients (1–5 months old); $CD34^+$ cells were infused (after non-myeloablative conditioning with busulfan, 4 mg per kg) at doses of 3.1×10^6 to 13.6×10^6 cells per kg	Adenosine deaminase gene, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Nine of ten patients had immune reconstitution with increases in T-cell counts (median count at 3 years, 1.07×10^9 l^{-1}) and normalization of T-cell function. Eight of ten patients do not require enzyme-replacement therapy.	26
Chronic granulomatous disorder	A range of studies, using gammaretrovirus vectors pseudotyped either with gibbon ape leukemia virus envelope or with an amphotrophic envelope; various non-myeloablative conditioning strategies	Gp91phox, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Twelve of twelve patients showed short-term functional correction of neutrophils with resolution of life-threatening infections. Three patients developed myeloproliferative disease.	27*
Wiskott-Aldrich syndrome	Gammaretrovirus; ten patients; $CD34^+$ cells were infused (after non-myeloablative conditioning with busulfan, 4 mg per kg)	WAS gene, retroviral LTR	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Nine of ten patients showed improvement of immunological function and platelet count. Two patients developed acute T-cell lymphoblastic leukemia.	28, 29
β-thalassemia	Self-inactivating HIV-1–derived lentivirus; one patient (18 years old) received fully myeloablative conditioning with busulfan; 3.9×10^6 $CD34^+$ cells per kg	Mutated adult β-globin ($\beta^{A(T87Q)}$) with anti-sickling properties, LCR control	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	Patient has been transfusion independent for 21 months. Blood hemoglobin is maintained between 9 and 10 g dl^{-1}, of which one-third contains vector-encoded β-globin.	30
Adrenoleukodystrophy	Self-inactivating HIV-1–derived lentivirus; two patients (7 and 7.5 years old) received myeloablative conditioning with cyclophosphamide (Cytoxan) and busulfan; transduced $CD34^+$ cells, 4.6×10^6 and 7.2×10^6 cells per kilogram, respectively	Wild-type *ABCD1* cDNA under the control of the MND viral promoter	*Ex vivo*, $CD34^+$ hematopoietic stem and progenitor cells	9–14% of granulocytes, monocytes, and T and B lymphocytes expressing the ALD protein; beginning 14–16 months after infusion of the genetically corrected cells, progressive cerebral demyelination in the two patients attenuated.	8

(continued)

Table 1 Some recent advances in clinical gene therapy (continued)

	Vector, dose range, and number and ages of patients	Transgene and promoter	Route of administration and cell target	Scientific and clinical outcomes	Reference
Gene therapy for genetic disease					
Duchenne muscular dystrophy	Phosphorodiamidate morpholino antisense oligodeoxynucleotides; dose escalation from 0.5 to 20.0 mg per kg; 19 patients (5–15 years old)	Oligonucleotide promotes skipping of spliceosome across diseased exon 51 of dystrophin gene	i.v., aiming to promote exon skipping in muscle cells	No serious treatment-related toxicities; muscle biopsies showed exon 51 skipping in all cohorts and dose-dependent expression of new dystrophin protein at doses of 2 mg per kg and above. Best responder had 18% normal muscle dystrophin levels.	9
Gene therapy for degenerative disease					
Heart failure	AAV1; 6×10^{11}, 3×10^{12} or 1×10^{13} DNase-resistant particles per patient	Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), CMV immediate early promoter	Antegrade epicardial coronary artery infusion over a 10-min period, targeting cardiac myocytes	High dose showed significant improvement in symptoms, functional status, biomarker (N-terminal prohormone brain natriuretic peptide) and left ventricular function, plus significant improvement in clinical outcomes.	11
Gene therapy for cancer					
B-cell leukemia and lymphoma	Self-inactivating lentivirus expressing a chimeric T cell receptor; a single patient was conditioned with pentostatin (Nipent; 4 mg per m^2) and cyclophosphamide (600 mg per m^2) before receiving 1.5×10^5 transduced T cells per kg (total 3×10^8 T cells, of which 5% were transduced)	Anti-CD19 scFv derived from FMC63 murine monoclonal antibody, human CD8α hinge and trans-membrane domain, and human 4-1BB and CD3ζ signaling domains	*Ex vivo*, autologous T cells, i.v. infusion, split over 3 d	Transduced T cells expanded more than 1,000 times *in vivo*, with delayed development of the tumor lysis syndrome and complete remission, ongoing 10 months after treatment. Engineered cells persisted at high levels for 6 months in the blood and bone marrow.	31
	Murine stem cell virus–based splice-gag (retroviral) vector expressing CD19 CAR; eight patients (47–63 years old) with progressive B-cell malignancies received cyclophosphamide and fludarabine (Fludara) before CAR-transduced autologous T cells and interleukin 2. Patients received 0.3×10^7 to 3.0×10^7 CAR^+ T cells per kg, of which an average of 55% were transduced.	Anti-CD19 scFv derived from the FMC63 mouse hybridoma, a portion of the human CD28 molecule and the intracellular component of the human TCR-ζ molecule	*Ex vivo*, autologous T cells, single i.v. infusion, followed (3 h) by a course of IL2	Varied levels of anti–CD19-CAR–transduced T cells could be detected in the blood of all patients. One patient died on trial, with influenza A pneumonia, nonbacterial thrombotic endocarditis and cerebral infarction. Four patients had prominent elevations in serum levels of IFNγ and TNF, correlating with severity of acute toxicities. Six of the eight patients treated obtained objective remissions.	32
Acute leukemia	SFG retrovirus expressing an inducible suicide system for improved safety of stem cell transplantation to prevent graft-versus-host disease (GVHD); transduced haploidentical T cells (1×10^6 to 1×10^7 T cells per kg); five patients (3–17 years old)	FK506-binding protein linked to modified human caspase 9 with truncated CD19 as a selectable marker; in the presence of the drug, the iCasp9 promolecule dimerizes and activates apoptosis; retroviral LTR	*Ex vivo*, allodepleted haploidentical T cells, infused i.v. into recipients of allogeneic bone marrow transplants.	The genetically modified T cells were detected in peripheral blood from all five patients and increased in number over time. A single dose of dimerizing drug, given to four patients in whom GVHD developed, eliminated more than 90% of the modified T cells within 30 min after administration and ended the GVHD without recurrence.	33
Squamous-cell carcinoma of the head and neck	Oncolytic vaccine based on herpes virus combined with chemotherapy and chemoradiotherapy; patients with stage III, stage IVA or stage IVB disease; four doses of virus, 10^6–10^8 p.f.u. per dose	Clinical isolate of HSV-1 from which the proteins ICP34.5 and ICP47 have been deleted	Intratumoral injection into nodules of squamous head and neck carcinoma	14 patients (82.3%) showed tumor response by RECIST criteria, and pathologic complete remission was confirmed in 93% of patients at neck dissection. Prolonged progression-free survival was seen in two-thirds of the patients.	34
Melanoma	Oncolytic vaccine based on herpes virus; patients with stage IIIc and IV disease; 4×10^6 p.f.u. followed 3 weeks later by up to 4×10^8 p.f.u. every 2 weeks for up to 24 treatments	Clinical isolate of HSV-1 from which the proteins ICP34.5 and ICP47 have been deleted	Intratumoral injection into melanoma nodules	The overall response rate by RECIST was 26%, with regression of both injected and distant (including visceral) lesions. 92% of the responses had been maintained for 7 to 31 months. Ten additional patients had stable disease for >3 months, and two additional patients had surgical complete response.	35
Advanced or metastatic solid tumors refractory to standard of care treatment, or for which no curative standard therapy existed	25 adult patients received 75 mg per m^2 docetaxel (Taxotere; day 1) and escalating doses of reovirus up to 3×10^{10} $TCID_{50}$ (days 1–5) every 3 weeks	Reovirus type 3 Dearing, a wild-type double-stranded RNA virus	Intravenous delivery to treat advanced and/or disseminated cancer	Of 16 evaluable patients, dose-limiting toxicity of grade 4 neutropenia was seen in one patient but the maximum tolerated dose was not reached. Antitumor activity was seen with one complete response and three partial responses. A disease-control rate (combined complete response, partial response and stable disease) of 88% was observed.	18

being treated means that production of vector for large numbers of patients will be relatively straightforward. Furthermore, the prospect of a one-time treatment for what is essentially a lifetime illness with significant healthcare and social costs implies that this novel therapeutic approach will be extremely cost-effective. The potential for the development of gene therapies into mainstream medicine for ocular disorders appears remarkably good.

While localized delivery of gene therapeutics has clear applications for some disorders, preclinical studies have suggested that some AAV serotypes injected intravascularly will predominantly transduce hepatocytes, providing a useful platform for local or systemic expression of a wide range of therapeutic proteins from this tissue. Although attempts to use this strategy for treatment of metabolic disease and hemophilia have previously been disappointing as immunological responses to the viral proteins caused toxicity or prevented long-term expression, new data from an experimental treatment of hemophilia B has demonstrated that AAV serotype 8 (AAV8) can be administered intravenously to deliver a codon-optimized gene encoding Factor IX (FIX) into liver[5]. AAV8 was selected for this study partly because of its hepatic tropism, but also because most individuals have not been previously exposed to the virus, diminishing the likelihood of virus-specific memory T cells that might attack transduced hepatocytes, as noted in a previous study using AAV2. Eligible patients were selected on the basis of having only low levels of antibodies against AAV8. Transgene expression was regulated by core domains from the human apolipoprotein hepatic control region and the human α-1-antitrypsin gene promoter. The approach has now been explored in six patient volunteers, and all have shown signs of durable clinical benefit associated with recovery of 2–11% of normal levels of FIX. There were signs of transient inflammation at higher AAV doses, perhaps reflecting immune recognition of transduced hepatocytes, although this was managed effectively using glucocorticoids with only small effects on FIX levels.

Unlike inherited blinding disorders, hemophilias and some metabolic diseases can be treated by exogenous-factor or enzyme-replacement therapy. However, the prospect of a one-time treatment as opposed to lifelong drug administration is clearly attractive on a number of counts, not only from the quality-of-life perspective but also economically. It is also important to remember that a significant proportion of the global population with these diseases does not have access to what are regarded as conventional therapies. Other tissues may also provide tractable targets for a similar approach. For example, systemic injection of AAV serotype 9 has been shown to result in widespread transduction of neurones and is soon to be evaluated in patients with spinal muscular atrophy, among others. The highly promising early data from the hemophilia B study therefore raises the real possibility of successful long-term treatment by gene therapy of a large number of patients with inherited disease[5,6].

Gene transfer to hematopoietic stem cells (HSCs), first shown to have major therapeutic effects in severe combined immunodeficiency (SCID) over 10 years ago, continues to show particular promise, with applications now broadening to other hematological (including Wiskott-Aldrich syndrome (WAS) and B thalassemia) and metabolic disorders (X-linked adrenoleukodystrophy and metachromatic leukodystrophy)[7]. The long-term data in over 90 patients with a number of inherited primary immunodeficiencies who have received gene therapy using conventional γ-retroviral vectors over the last decade show well over 90% overall survival, with the vast majority experiencing significant clinical benefit, despite insertional mutagenesis in around ten patients to date[7]. These striking results become more impressive when compared with published outcomes for alternative therapies, bearing in mind that the patients selected for these trials were usually without human leukocyte antigen (HLA)–matched hematopoietic stem cell donors. The observed toxicities in these trials are now known to share a common mechanism, namely upregulated expression of proto-oncogenes induced by powerful enhancer sequences within the long terminal repeats (LTRs) of the early-generation γ-retroviral vectors that were used. During the last decade, conventional γ-retroviral vectors have been largely replaced by so-called 'self-inactivating' γ-retroviral vector systems and particularly lentiviral vector systems, which can tolerate larger transgene sequences. These are designed to limit the risks of insertional mutagenesis through use of regulatory sequences that are much less likely to interfere with expression of host-cell genes adjacent to the vector integration site. Some benefits may accrue from changing the class of vector from gammaretrovirus to lentivirus, as the intrinsic integration strategy of the latter is less prone to targeting regulatory regions of the genome. Lentiviral vectors may also be more efficient for transduction of HSCs, as they do not require breakdown of the nuclear membrane during mitosis to access the chromosomes and can therefore transduce HSCs through minimal *ex vivo* culture times, thereby preserving engraftment capability. One of the first studies using a lentivirus to transduce HSCs expressed the *ABCD1* gene and showed successful protection from demyelination in two boys with adrenoleukodystrophy[8], establishing the impressive potential of this approach. As examples of the ongoing process of technological refinement, new trials for X-linked SCID, WAS and chronic granulomatous disease have recently entered the clinical arena. However, large-scale production of these vectors to Good Manufacturing Practice–grade remains a biotechnological challenge that will need to be addressed before widespread application to large numbers of patients can be contemplated.

Aside from viral vectors, antisense oligonucleotides based on relatively simple chemistries have recently been used to promote exon skipping in some patients with Duchenne muscular dystrophy (DMD). By allowing the spliceosome to 'skip' a frameshift mutation in exon 51 of the dystrophin gene (which characterizes a major subset of patients with DMD), this approach has successfully induced expression of a short, though 'in frame' and partly functional dystrophin protein[9]. A real step forward here is the observation that oligonucleotide treatments may be given systemically to access dystrophic muscle systems throughout the body, thereby obviating the very significant difficulties associated with multiple local deliveries. In principle, exon skipping could be useful to induce production of in-frame though truncated proteins for other genetic diseases such as the dysferlinopathies[10] as well as for disrupting aberrant expression of proteins in cancer. However, the successful tissue uptake of antisense oligonucleotides in DMD is thought to reflect unusually high membrane permeability of dystrophic muscle cells, so widespread use of this technology may require continued focus on delivery mechanisms, both viral and non-viral.

Regenerative gene therapy

Outside the context of Mendelian genetic disorders, very similar approaches to those described above are being evaluated in a number of 'degenerative' conditions. After observations that chronic heart failure is associated with low levels of sarcoplasmic calcium, the coronary arteries of patients with

advanced heart failure were injected with AAV vectors (serotype 1, AAV1) encoding the enzyme responsible for reloading the sarcoplasmic reticulum with calcium during relaxation, the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a), under transcriptional control of the cytomegalovirus immediate early promoter and enhancer. Patients with high levels of AAV1-specific antibodies were excluded from the study, given that the route of delivery was intravenous, and a total of 39 patients were randomized into the placebo and three treatment groups[11]. At the highest dose ($n = 9$ for test and $n = 14$ for placebo), the treatment resulted in a positive outcome for several predefined functional parameters: high-dose treatment, compared with placebo, led to substantial increases in time to clinical events, lower frequency of cardiovascular events observed at 12 months, and shorter mean duration of cardiovascular hospitalizations (0.4 versus 4.5 days). Similarly, lentiviruses engineered to express dopamine-biosynthetic enzymes are being injected stereotactically directly into the corpus striatum of patients with Parkinson's disease. Encouraging and objective signs of efficacy have been recorded using the Unified Parkinson's Disease Rating Scale and quality-of-life measures, with some patients now approaching 3 years since treatment. These very encouraging results make a strong case for conducting larger trials.

Infectious disease and cancer

Recombinant viruses provide a powerful vaccine platform for infectious diseases, allowing expression of proteins and epitopes from target pathogens in the context of strong background immune stimulation by the viral vector. A range of vaccine strategies are being explored and extensive trials conducted for prophylaxis of malaria, tuberculosis and HIV[12]. Particularly exciting are new innovations using gene-based approaches as a treatment to alleviate disease in people who are already infected. Among the most advanced are clinical studies using 'locked nucleic acid' oligonucleotides targeting microRNA 122 as anti–hepatitis C agents. There are several anti-infective approaches on the horizon, including strategies based on transfer of viral restriction factors. As an example, long-term cellular protection against HIV infection might be achieved through modification of hematopoietic stem cells to express a TRIM5a–cyclophilin A fusion protein, designed to bind incoming HIV virus and target it for proteasomal degradation before reverse transcription can occur[13].

Targeted endonucleases are being developed to facilitate repair of DNA mutations through homologous recombination and have already shown some efficacy in preclinical models including hemophilia[14]. Although promising, this approach may not yet be efficient enough to enter the clinical arena, and some questions remain regarding selectivity and off-target activity. However, endonucleases also provide the possibility of selective disruption of specific genes through induction of double-strand DNA breakages and host-cell repair by nonhomologous end-joining. Following the observation that HSCs lacking expression of the CCR5 coreceptor for HIV produce $CD4^+$ T cells that are HIV resistant, early trials in patients with HIV are showing signs of progress after infusion of autologous $CD4^+$ T cells subjected to genome editing *ex vivo* with zinc-finger nucleases to prevent CCR5 expression[15]. Although this is one example of current clinical application, endonuclease-based technologies are extremely versatile, and their use to modify the cellular genome may eventually find a broad array of applications in many different inherited and acquired diseases.

In the field of cancer, cytotoxic T cells modified to recognize specific tumor targets using engineered T-cell receptors, or chimeric antigen receptors (CARs) formed using single-chain antibodies (as the extracellular domain) fused to internal signaling and additional costimulatory domains, are showing increasing promise as the technology matures[16]. Tumor-killing 'oncolytic' viruses, where lytic viruses replicate selectively in cancer cells and lyse them before spreading to adjacent cells, are also showing promise. Virus-mediated cytotoxicity is particularly attractive for cancer treatment as it may be able to overcome deficiencies in cellular apoptosis mechanisms that characterize many drug-resistant tumors. Wild-type reovirus has been studied in over 20 clinical trials treating a range of tumor types and has shown signs of therapeutic activity both as a single agent[17] and in combination with chemotherapy[18]. Several trials are ongoing, and the agent is now in a phase III trial with paclitaxel (Abraxane) and carboplatin (Paraplatin) for treatment of squamous-cell carcinoma of the head and neck. Conditionally replicating vaccinia virus modified to express granulocyte-macrophage colony-stimulating factor (GM-CSF) has shown a good toxicology profile and signs of activity in a range of phase I and phase II trials, including extended survival after direct injection of the virus into non-resectable liver cancer[19]. A conditionally replicating herpes virus, also expressing GM-CSF and designed to stimulate an anticancer immune response after direct injection into tumor nodules, has likewise performed well in early-phase trials. It is now in phase III trials for treatment of melanoma and seems to be another promising candidate for product licensure[20]. Oncolytic viruses can spread from cell to cell, providing the possibility of increased penetration into tumor nodules after the initial infection; nevertheless, it remains essential that the initial delivery can reach enough tumor cells to allow productive infection and adequate virus replication. For viruses with poor stability in human blood, the greatest success to date has been achieved after direct injection into tumor nodules[21], although recently a great deal of attention has been given to exploring the feasibility of using even these oncolytic viruses systemically to treat metastatic disease[22].

Conclusions

The clinical benefits seen from a variety of gene therapy approaches, coupled with growing enthusiasm for applying genetic technologies in medicine, strongly indicate that gene therapy has now passed beyond the proof-of-principle stage and may soon provide realistic approaches for several previously intractable medical disorders. Strategies to enable transgene expression in a sufficient proportion of target cells are central to progress, together with achieving an appropriate duration of transgene expression without unwanted immune response. This may present significant challenges, particularly where very high-level or tightly regulated gene expression is necessary, although technology is evolving quickly and the regulatory climate may be changing to facilitate testing of mechanism and molecular performance as well as toxicology in early-phase clinical trials. Some of the best candidate diseases for gene supplementation therapy are those in which the transgenic cells are endowed with specific growth and survival advantages owing to functional correction, allowing physiological factors to regulate their repopulation of the diseased environment. However, in other settings a degree of host manipulation may be used to ensure sufficient engraftment or survival of transduced cells (e.g., through chemotherapeutic or immunosuppressive conditioning of the bone marrow or host immunity).

No real consensus has yet emerged on whether transgene expression must

be controlled by cognate promoters to allow physiological regulation of activity. Indeed, for many disorders the profile of gene expression required to achieve significant therapeutic effect is achievable with relatively simple expression systems. For example, the frequency of spontaneous hemorrhage is greatly reduced in hemophilia B with a small fraction of normal circulating levels of FIX. Similarly, a fraction of functional circulating phagocytes is sufficient to offer substantial protection against infection in some inherited immunodeficiencies. Diseases most amenable to effective treatment with current gene therapy approaches may therefore be those where the therapeutic window for functional protein expression is relatively broad, and where a large number of target cells are accessible to transduction.

Previous expectations that gene therapy would produce a 'cure-all' solution for intractable diseases were unrealistic. The agents in question are a diverse spectrum of nucleic-acid based medicines that are formulated in very different ways, yet can be used to prevent, alleviate and provide long-lasting treatments for a wide variety of diseases both inherited and acquired. In some cases they can now realistically provide physicians and patients with new therapeutic options where more conventional approaches have failed, a testament to the huge amount of scientific research in the field over the last 10–20 years. By decreasing the requirement for frequent repeated interventions, many gene therapy approaches can lead to substantial savings in the costs of lifetime medication. However, it is also noteworthy that without clinical trials, the field would not have progressed nearly so rapidly. The fact that some gene therapy strategies are finally beginning to deliver on their potential may well herald a raft of new and imaginative interventional approaches designed to exploit recent insights into cell biology and disease processes.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available at http://www.nature.com/doifinder/10.1038/nbt.2290.

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TAL effector RVD specificities and efficiencies

To the Editor:

The DNA-binding domain of transcription activator–like effectors (TALEs) has become an important tool for the programmable and specific targeting of genome-editing nucleases[1–4]. We determined specificities of TALE DNA-binding modules and discovered varying efficiencies to promote TALE activity. This prompted us to study the basis of TALE efficiency. Natural TALEs from plant-pathogenic *Xanthomonas* sp. bacteria function as eukaryotic transcription factors. TALEs bind to DNA via tandem near-identical 34-amino-acid repeats, each of which recognizes one base pair via two adjacent amino acids (12 and 13) termed repeat-variable diresidues (RVDs)[5,6]. Three-dimensional structures of TALE–DNA complexes revealed that only amino acid 13 mediates specific recognition of the sense-strand DNA base, whereas amino acid 12 stabilizes the repeat structure[7,8]. *In vitro* assembly of repeat units yielded TALEs with new and predictable DNA specificities[2,9]. Different DNA-recognition specificities of individual RVDs have been shown[4,5,10]. Specific recognition of guanine is difficult because the common RVD NN (Asn-Asn) recognizes guanine and adenine, whereas the guanine-specific RVD NK (Asn-Lys) apparently functions less well than NN does[11]. Here we describe new RVD specificities including NH (Asn-His), which is highly specific for guanine. We show that efficiency of RVDs varies and that strong repeats (HD (His-Asn) or NN) are key for overall activity of TALEs.

First, we constructed artificial TALEs (ArtTALs) composed of an unusual arrangement of repeats with predominantly one type of RVD to decipher RVD specificities (**Supplementary Methods**). With this highly artificial setup, we first aimed to study properties of each RVD type separately. These ArtTALs contained the RVDs NI (Asn-Ile) and NG (Asn-Gly) in the first and last repeat, respectively, to properly position the TALE on the target DNA. We transfected *ArtTAL* expression constructs together with β-glucuronidase (*GUS*) reporter constructs via *Agrobacterium* into leaf cells of *Nicotiana benthamiana*. As expected, TALEs with poly(HD) ($ArtTAL_{polyHD}$) and poly(NN) ($ArtTAL_{polyNN}$) RVDs efficiently induced the polycytosine and polyguanine reporters, respectively (**Fig. 1a** and **Supplementary Fig. 1**). To our surprise, $ArtTAL_{polyNN}$ did not induce expression of the polyadenine reporter construct, which is in contrast to the known specificity of the RVD NN for guanine and adenine. Similarly, ArtTALs with poly(NI) (specificity of NI is for adenine),