REVIEW

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Gene therapy returns to centre stage

Luigi Naldini[1,2]

Recent clinical trials of gene therapy have shown remarkable therapeutic benefits and an excellent safety record. They provide evidence for the long-sought promise of gene therapy to deliver 'cures' for some otherwise terminal or severely disabling conditions. Behind these advances lie improved vector designs that enable the safe delivery of therapeutic genes to specific cells. Technologies for editing genes and correcting inherited mutations, the engagement of stem cells to regenerate tissues and the effective exploitation of powerful immune responses to fight cancer are also contributing to the revitalization of gene therapy.

Gene therapy has long fascinated scientists, clinicians and the general public because of its potential to treat a disease at its genetic roots. This is achieved by counteracting or replacing a malfunctioning gene within the cells adversely affected by the condition. As simple as the concept sounds, the hurdles to put it into practice are daunting. Gene transfer must overcome complex cellular and tissue barriers to deliver new genetic information into the target cell to drive proficient expression of a therapeutic molecule without disrupting essential regulatory mechanisms. The gene-corrected cells must be present in large enough quantities to reverse the condition, escape immunological recognition and survive in the long term, or be able to transmit the modification to their progeny to sustain the benefit. Several gene-therapy trials have been performed in the past two decades for inherited diseases, cancer and chronic infections, but only a few reported clear clinical benefits and in some, individuals experienced severe adverse events related to the vectors. Overall, concern and scepticism rose over the further deployment of these strategies. But these attitudes are radically changing. A number of phase I/II gene-therapy clinical trials have reported remarkable evidence of efficacy and safety for the treatment of various severe inherited diseases of the blood, immune and nervous systems, including primary immunodeficiencies, leukodystrophies, thalassaemia, haemophilia and retinal dystrophy, as well as cancers such as B-cell malignancies (Table 1). All of these trials exploit improved vector technologies to deliver therapeutic genes. In some trials, genetic material is transferred into haematopoietic stem cells (HSCs) or T lymphocytes (T cells) *ex vivo*, and in others hepatocytes in the liver or photoreceptors in the retina are targeted directly *in vivo*. Here I review the most relevant clinical results, highlight progress in gene-transfer technologies and in our understanding of the biological processes that underpin these advances and discuss the challenges and outlook for gene therapy.

Haematopoietic-stem-cell gene therapy

HSCs have long been a preferred target for *ex vivo* gene therapy[1]. Genetic modification of self-maintaining multipotent HSCs would ensure a steady supply of their gene-corrected progeny in the body. These cells have the potential to treat conditions that manifest when mature haematopoietic lineages fail to develop or to function correctly. Given the self-renewing nature of HSCs and the need to ensure that genetic modifications are passed on to their progeny, gene correction must be stably introduced into cellular chromatin, either by vector-mediated transgene insertion or by *in situ* gene editing.

Past trials of HSC gene therapy report clear benefits to people with selected conditions, which proved the therapeutic potential of the strategy[2–10]. However, they also highlight the limitations and risks of using early generation vectors that are based on gammaretroviruses (γ-RVs)[11–15]. For example, these techniques offered only a limited ability to transfer genes into the most primitive progenitor cells, giving rise to the low-level and transient appearance of gene-corrected haematopoietic cells *in vivo*. Leukaemia related to vector insertion near oncogenes also occurred in a fraction of patients during their long-term follow-up. The development of vectors with improved efficacy and safety in preclinical models, such as lentiviral vectors, has renewed interest in the approach[1].

HSC gene therapies that incorporate these new vectors have been tested in severe inherited diseases of the immune system (Wiskott–Aldrich syndrome (WAS) and X-linked severe combined immunodeficiency (SCID-X1))[16–18] and blood (β-thalassaemia)[19], and in neurodegenerative storage diseases (adrenoleukodystrophy and metachromatic leukodystrophy)[20–22] (see Table 1). In children with SCID-X1, the development and function of the immune system is impaired owing to deficiencies in the receptors for certain cytokines that are essential for immune-cell development, whereas those with WAS have deficiencies in a cytoskeletal adaptor that is required for assembling the immunological synapse[23]. Consequently, these children succumb to infection or, in the case of WAS, haemorrhage because of an accompanying platelet deficiency. People with β-thalassaemia major — the most severe form of β-thalassaemia — fail to express the haemoglobin-β chain, which leads to ineffective erythropoiesis and severe anaemia that requires frequent blood transfusions and an iron-chelation regimen. Children with early onset adrenoleukodystrophy or metachromatic leukodystrophy are affected by defective myelination and consequently experience glial- and neural-cell degeneration in the central and peripheral nervous systems. They are unable to break down some of the metabolites of myelin because of a deficiency in the peroxisomal ATP-binding cassette transporter in adrenoleukodystrophy[24], or in the lysosomal enzyme arylsulfatase A in metachromatic leukodystrophy[25]. Such patients undergo rapid and irreversible deterioration of their motor, sensory and cognitive performance, which leads to death within a few years. HSC transplantation, in which HSCs are transferred from a healthy donor to a recipient with a specific condition, can virtually cure SCID-X1, WAS and β-thalassaemia[26]. HSC transplantation can also

[1]San Raffaele Telethon Institute for Gene Therapy (TIGET), San Raffaele Scientific Institute, 20132 Milan, Italy. [2]Vita Salute San Raffaele University, 20132 Milan, Italy.

Table 1 | Gene-therapy clinical trials highlighted in this Review

Disease	Vector and strategy	Number of patients*	Follow-up (months)	Patient status and biological and clinical outcomes*	Clinical-trial identifier	References
HSC-based gene therapy						
Wiskott–Aldrich syndrome	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	7	10 to 60	All patients AAW; stable engraftment with transduced cells; persistent clinical benefit and safety.	NCT01515462	16 and L.N.†
Wiskott–Aldrich syndrome	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	7	9 to 42	6 patients AAW, 1 patient died of a pre-existing infection; stable engraftment with transduced cells; persistent clinical benefit and safety.	NCT01347242 NCT01347346 NCT02333760	17
X-linked severe combined immunodeficiency	Self-inactivating γ-RV; *ex vivo* gene transfer into $CD34^+$ cells.	9	12 to 39	8 patients AAW, 1 patient died of an infection; stable engraftment with transduced cells; persistent clinical benefit and safety in 7 patients; 1 patient failed to engraft and underwent HSC transplantation.	NCT01410019 NCT01175239 NCT01129544	18
β-Thalassaemia major	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	3‡	24 to 72	2 patients stably engrafted with transduced cells, 1 patient became transfusion independent; 1 patient failed to engraft and received rescue cells.	N/A	19, M. Cavazzana and BlueBird Bio†
β-Thalassaemia major	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	2‡	15	Stable engraftment with transduced cells; transfusion independence and safety in both patients.	NCT02151526	M. Cavazzana and BlueBird Bio†
β-Thalassaemia major	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	5§	1 to 6	Stable engraftment with transduced cells; safety and transfusion independence in the first 2 evaluable patients.	NCT01745120	BlueBird Bio†
Adrenoleukodystrophy	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	4	54 to101	Stable engraftment with transduced cells and safety in all patients; persistent clinical benefit in 3 patients.	N/A	20, 21 and P. Aubourg†
Metachromatic leukodystrophy	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	20	3 to 60	Stable engraftment with transduced cells and safety in all patients; persistent clinical benefit in all late-infantile patients who were treated when presymptomatic.	NCT01560182	22 and L.N.†
Liver-directed gene therapy						
Haemophilia B	AAV8 vector; intravenous administration.	10	16 to 48	No inhibitors; persistent FIX expression; in high-dose group, mean FIX levels of 5.1 ± 1.7% seen in all 6 treated patients.	NCT00979238	40
Haemophilia B	AAV8 vector; intravenous administration.	7	Up to 12	No inhibitors; persistent FIX expression in 1 patient.	NCT01687608	110
T-cell immunotherapy for cancer						
B-cell lymphoma or CLL	γ-RV; *ex vivo* gene transfer into T cells; CAR-modified anti-CD19 cells.	15	1 to 23	8 CRs, 4 PRs; ORR 80%.	NCT00924326	51
B-cell ALL	γ-RV; *ex vivo* gene transfer into T cells; CAR-modified anti-CD19 cells.	5	1 to 4	5 CRs; ORR 100%; 4 patients subsequently underwent allo-HSC transplantation as per clinical-study design, 1 patient was ineligible for HSC transplantation and relapsed.	NCT01044069	52
B-cell ALL	Lentiviral vector; *ex vivo* gene transfer into T cells; CAR-modified anti-CD19 cells.	30	1 to 24¶	27 CRs; ORR 90%; 19 patients remained in remission, 3 of these patients underwent HSC transplantation; 7 patients relapsed, 3 of these relapses occurred after loss of transduced T cells.	NCT01626495 NCT01029366	53
B-cell ALL or lymphoma	γ-RV; *ex vivo* gene transfer into T cells; CAR-modified anti-CD19 cells.	21	Median =10#	14 CRs (at day 28); ORR 67%; 10 patients subsequently underwent HSC transplantation.	NCT01593696	54
Retinal gene therapy						
Type 2 Leber congenital amaurosis	AAV2 vector; unilateral subretinal administration.	5	36	In all 5 patients, stable improvement in visual sensitivity seen.	NCT00516477	94, 97
Type 2 Leber congenital amaurosis	AAV2 vector; unilateral subretinal administration.	3	54 to 72	In all 3 patients, improvement in visual sensitivity seen at 6 months, which increased for 1 to 3 years and then declined.	NCT00481546	95
Type 2 Leber congenital amaurosis	AAV2 vector; unilateral subretinal administration.	12	36	In 6 patients, improvement in visual sensitivity seen, which peaked at 6 to 12 months and then declined.	NCT00643747	96

AAW, alive and well; ALL, acute lymphocytic leukaemia; CLL, chronic lymphocytic leukaemia; CR, complete response; N/A, not applicable; ORR, overall response rate; PR, partial response.

*As stated in the referenced publications or updated by personal communication. †Personal communication. ‡β^0/β^E genotype. §$2\beta^0/\beta^E$, $2\beta^0/\beta^0$, $1\beta^0/\beta^+$ genotypes. ¶Median = 7. #51 days to HSC transplantation median.

arrest the progression of leukodystrophies if performed before or near the time of onset, although the outcome of the procedure is more satisfactory in adrenoleukodystrophy[24] than in metachromatic leukodystrophy[27]. Allogeneic HSC transplantation confers a considerable risk of morbidity and death, particularly when performed between human leukocyte antigen (HLA)-mismatched individuals. Even in the few cases in which an HLA-matched family donor can be found, the risk of morbidity remains substantial[28]. HSC gene therapy can address this unmet medical need, especially when no matched HSC donor is available.

HSC gene therapy is administered by *ex vivo* gene transfer into haematopoietic progenitors. First, the cells are purified using the CD34 surface marker from other leukocytes harvested from the bone marrow or mobilized peripheral blood of the recipient. Next, they are cultured for 2–4 days in the presence of growth-stimulating cytokines while being exposed to vectors carrying an expression cassette for the corrective transgene. Before the modified cells can be administered, the recipient is treated with a preconditioning chemotherapy regimen. This depletes endogenous progenitors and differentiated cells in the bone marrow — as well as the lymphoid organs, in some cases — and favours engraftment of the *ex vivo* gene-corrected cells. Preconditioning results in considerable early morbidity owing to transitory blood-cell depletion, immunodeficiency and mucosal damage, which place the recipient at risk of severe infection. It also causes delayed morbidity owing to the risk of developing chemotherapy-induced secondary tumours and infertility[28]. Several HSC gene-therapy trials have attempted to alleviate the morbidity associated with preconditioning by lowering the dosage and combination of chemotherapeutic drugs that are administered in comparison to the regimens used in conventional HSC transplantation. However, the impact of changing these drug regimens on the risks and benefits of the therapy is yet to be determined in broader comparative studies and through long-term patient follow-up. Some preconditioning regimens also deplete endogenous microglia progenitors, which live in the central nervous system (CNS). In patients with leukodystrophies who have been treated with HSC gene therapy, these cells are replaced with their gene-corrected counterparts, which migrate to CNS tissues and produce functional progeny that can clear stored myelin metabolites. In the case of metachromatic leukodystrophy, these modified cells also release functional lysosomal enzyme for the cross-correction of other tissue-resident cells[29–31].

All HSC gene-therapy trials performed with lentiviral vectors report stable and high-level reconstitution of haematopoiesis with gene-corrected cells in most recipients, with the most recent trials observing up to 90% reconstitution in some patients[16,17,19–22]. Because all of the haematopoietic lineages tested (myelo–monocytic, megakaryocytic, erythroid, natural killer (NK), and B lymphoid and T lymphoid) contain gene-corrected cells and appear to receive a steady input of newly formed modified cells — most evident in the short-lived myeloid lineages — gene transfer must have occurred in self-renewing, multipotent progenitors that effectively engrafted to the recipients. The extent of haematopoietic reconstitution with gene-corrected cells varies among the patients and trials. This reflects the impact of the disease and its correction on the different haematopoietic lineages as well as the potency of each vector batch and the type of preconditioning regimen administered. Remarkably, most patients gain substantial benefits from gene therapy that can even exceed those observed after successful HSC transplantation from an allogeneic donor. This is especially true for a metachromatic leukodystrophy trial[22] based on the rationale that gene transfer could drive higher lysosomal enzyme expression in haematopoietic cells than in normal cells, which would enable these cells to provide an increased supply of enzyme while homing in to affected tissues. The strategy allowed the unsatisfactory outcome of HSC transplantation to be overcome in metachromatic leukodystrophy. Despite an increasing number of patients being treated in these HSC gene-therapy trials (see Table 1), there has been no report of adverse events related to lentiviral vectors. At the most recent follow-up of patients included in these trials, the first of whom was treated almost seven years ago in the adrenoleukodystrophy trial[20], those who had successfully engrafted with modified cells were reported to be in a good condition — that is, they are either disease free or their disease has been stable or in remission since the time of treatment — and are leading near-normal lives. These individuals would probably have already succumbed to their disease if untreated with gene therapy[24,25].

Liver-directed gene therapy

The liver has long been a preferred target for *in vivo* gene therapy[32]. This major internal organ and central metabolic hub receives an abundant blood supply through an extensive bed of sinusoids with highly permeable walls — a structure that facilitates the access of blood-borne particles, such as viruses, to hepatocytes. Hepatocytes are long-lived and robust protein factories that can efficiently release their products into the blood circulation. Stable transgene delivery to the liver could therefore provide a strategy for treating several inherited metabolic diseases and plasma-protein deficiencies, notably those of coagulation factors. Major hurdles to liver-directed gene therapy include the potential toxicity of an acute inflammatory response to the bolus administration of viral particles into the bloodstream, and the inactivation of these particles by pre-existing virus-specific antibodies and clearance by phagocytes that line the sinusoidal walls of the liver and spleen. If humoral or cellular immunity is triggered against the transgene product, the therapeutic activity of gene therapy could be inhibited in the circulation or the modified cells might be eliminated by cytotoxic T cells, respectively[33,34].

Liver-directed gene therapy has been tested mainly in the treatment of severe haemophilia B using vectors derived from the human parvovirus, adeno-associated virus (AAV)[35]. On intravascular injection, some AAV-vector serotypes effectively target hepatocytes and remain stably associated with the modified cells, mostly as nonintegrated episomes within the nucleus. In animal models, this delivery strategy does not induce an immune response against the transgene product and instead favours the development of transgene-specific tolerance[32]. The persistence of AAV-vector genomes that contain an expression cassette for coagulation factor IX (FIX) — a protein that is absent or defective in haemophilia B — within nonproliferating hepatocytes can give rise to a stable supply of functional FIX into the bloodstream[36]. FIX expression as low as 1% of the normal level can turn severe haemophilia B into a milder form of disease. This partial reconstitution alleviates the risk of spontaneous haemorrhages, which are detrimental to the joints and potentially lethal if they occur in the brain. It can also reduce the need for intravenous prophylactic factor-replacement therapy, a treatment that imposes a substantial burden on patients and is only available at high cost and in countries with well-developed health-care systems[37]. Although earlier trials demonstrated the safety of delivering AAV vectors into the human bloodstream, they reported only limited and transient FIX expression. This is probably because of low levels of gene transduction in the liver and a delayed cellular immune response that targeted AAV components persisting within modified hepatocytes and triggered their elimination[38,39]. More recently, a trial that used a new AAV serotype (AAV8) and an improved cassette design was able to overcome these limitations to demonstrate vector-dose-dependent FIX expression of up to 6% of the normal level after a single, well-tolerated vector infusion in a peripheral vein[40]. Three years later, FIX expression remained stable in most patients, although some had to be given a transient immunosuppressive corticosteroid treatment at the first sign of hepatocellular injury in the 4–8 weeks following treatment. Most treated patients were able to reduce or abrogate the need for prophylactic factor replacement, which substantially improved their quality of life.

T-cell immunotherapy for cancer

T cells are also popular targets for *ex vivo* gene therapy. Such therapies aim mostly at boosting the adaptive immune response against cancer and chronic infections such as HIV[41,42]. Autologous T cells can be harvested readily from the peripheral blood and expanded *ex vivo*. Cells are then transduced with a γ-RV or lentiviral vector expressing an exogenous T-cell antigen receptor (TCR) that is specific to a cancer-associated antigen or an antiviral molecule, and infused back into the patient. The use of T cells for cancer immunotherapy arose from seminal observations of objective

BOX 1

Lentiviral vectors

Major hurdles for haematopoietic-stem-cell (HSC) gene therapy include achieving efficient *ex vivo* gene transfer into long-term repopulating HSCs, preventing activation of oncogenes by the nearby integration of a vector and controlling transgene expression to avoid ectopic or constitutive expression that leads to toxicity[1]. As compared to early generation gammaretroviral vectors (γ-RVs), HIV-derived lentiviral vectors result in more efficient gene transfer and stable, robust transgene expression in HSCs and their multilineage progeny. Extensive preclinical work indicated important features in vector biology and design that affect genotoxicity and highlighted strategies to alleviate it[111–117]. The self-inactivating long terminal repeats (LTRs) and integration-site preferences of lentiviral vectors were shown to substantially alleviate insertional genotoxicity. When tested in γ-RVs, the self-inactivating LTR design was shown to improve the safety of this platform as well[18]. Retrospective analysis of several earlier trials suggests that disease background, transgene function, *ex vivo* culture and the efficiency of host repopulation can all influence the likelihood that insertional genotoxicity will manifest in a trial[13,14]. These data helped to shape the ideas that not all integrating vectors have the same effects and that genome-wide integration of improved vector designs, although still a mutagenic event, can be tolerated in the absence of aggravating circumstances[118].

Self-inactivating lentiviral vectors are also being used to engineer T cells with chimaeric antigen receptors (CARs) or T-cell antigen receptors for use in adoptive immunotherapy for the treatment of cancer. The advantages of this new platform in comparison to earlier-generation γ-RVs, which perform satisfactorily in this cell target, are yet to be fully established. Lentiviral vectors are thought to give rise to more robust and stable transgene expression in T cells *in vivo*, and could facilitate more efficient and versatile *ex vivo* gene transfer while supporting coordinated expression of multiple transgenes[41,42,55,119]. These advantages will become more relevant as the gene-therapy field implements refined strategies, such as improved T-cell manipulation to preserve T memory stem cells[59–61], or more demanding cell-engineering tasks, such as the co-expression of multiple CARs (to improve specificity) or a conditional safety switch/suicide gene (to improve safety)[120].

tumour responses, which sometimes led to complete tumour regression, after the infusion of *ex vivo*-expanded autologous tumour-infiltrating lymphocytes in patients with advanced melanoma[41]. The underlying mechanisms of these responses have been traced to the *ex vivo* activation and amplification — and subsequent *in vivo* persistence — of tumour-specific cytotoxic T cells that already exist in small numbers within some tumours but are suppressed by the local microenvironment. These data support the therapeutic potential of an adaptive immune response against some tumours when it is released from endogenous suppressive signals and the reactive T cells are infused at high numbers, which leads to a favourable effector–target ratio. Preconditioning patients with a lymphoid-depleting regimen before infusion of the *ex vivo*-expanded T cells improves their engraftment and activity *in vivo*[43].

The genetic transfer of an exogenous TCR to a T cell can bypass the need to find preexisting cancer specificities within the patient's T-cell population. It also allows the exploitation of synthetic high-affinity TCRs, which might otherwise be purged *in vivo* by thymic selection, to redirect the specificity of autologous cells. Ideally, such TCRs would target tumour-associated antigens from endogenous proteins that are not being expressed — at the same time or at detectable levels — by normal tissues, such as carcinoembryonic or cancer testis antigens. Alternatively, they could target tumour-driver mutations that are commonly found within a certain type of tumour and that cannot be lost, even to evade immune clearance. Recent studies, however, indicate that most spontaneous or elicited tumour-specific immune reactivity is instead directed against 'passenger' neoantigens, which uniquely originate from random mutations that accumulate within individual tumours[44–47]. This type of immunotherapy must therefore be highly personalized and will require both the identification of candidate neoantigens from the tumour exome or proteome and the retrieval or generation of the cognate TCR recognition sequences for *ad hoc* T-cell genetic engineering.

Early clinical trials of T-cell gene transfer with TCRs directed against tumour-associated antigens reported partial tumour responses and, in some instances, off-tumour reactivity that led to tissue damage and severe adverse events[48–50]. More recently, gene transfer was used to introduce synthetic chimaeric antigen receptors (CARs) to T cells. CARs combine the binding specificity of an antibody against a cancer-associated surface marker with one or more intracellular signalling domains from the TCR and costimulatory receptor complexes[42]. The strategy holds a number of advantages over the use of conventional TCRs. For instance, antigen recognition is not restricted by HLA, and antibody specificities previously validated for safe and specific cancer recognition *in vivo* can be exploited by the incorporation of single-chain antibody derivatives into the CARs. Furthermore, these engineered T cells can be fully activated when meeting their target. Trials that deployed CARs directed against the B-cell surface molecule CD19 reported dramatic benefits in patients with B-cell malignancies, who experienced durable clinical responses, including complete remission, with mostly manageable toxicity[51–54]. These results have spurred enormous interest in further developing this approach[55].

Reasons for success in recent clinical trials

The positive clinical outcomes discussed in this Review provide long-sought evidence for the elusive promise of gene therapy to deliver lasting therapeutic benefit, or even a 'cure', for an otherwise terminal or severely disabling condition after a single treatment. These results could represent the rewarding outcome of the effort to rationally improve vectors in the laboratory, as discussed in Box 1 and Box 2. Advances in vector manufacturing and characterization, leading to batches with higher potency and greater purity, could also be facilitating the increased transduction of target cells with lower adverse effects, even when using the same vector design as in earlier trials. A better understanding of the biological processes and specific cell types that are involved in the success or failure of each gene therapy has also helped to improve methods for *ex vivo* cell handling and led to more effective monitoring and management of patients. Indeed, increasing confidence in the positive outcome of recent trials is now built on both clinical observation and advanced molecular readouts that provide evidence for the efficacy and safety of gene therapy.

In recent lentiviral-vector-based HSC gene-therapy trials, vector insertional analysis was used to track individual clones in the reconstituted haematopoiesis and showed that polyclonal reconstitution by transduced stem cells had taken place without the emergence of dominant clones whose behaviour could be attributed to gain-of-function vector insertional mutagenesis. An in-depth molecular analysis of the reconstituted haematopoiesis now being performed in different diseases, including several that were treated with different types of vector, is facilitating the first reliable comparative assessment of vector-induced events in patients[16,17,20–22,56]. The emerging picture illustrates that the absence of adverse clinical events in recent trials is accompanied by a remarkably different landscape of vector-insertion distribution in patients. In the earlier trials, which were performed with long terminal repeat (LTR)-competent γ-RV vectors, there was frequent and early generation of dominant clones, whose expansion appeared to be driven by the altered expression of cancer genes that were targeted by vector insertion[11–13,14,57]. However, this finding is rarely observed when newer vectors, such as lentiviral vectors, with self-inactivating LTRs and different insertion-site preferences are used[16,17,19–22]. In parallel, retrospective reconstruction of multistep leukaemogenesis from the earlier trials supports the view that

vector insertion provided a first mutagenic hit that potentially led to the development of leukaemia. Progression of the disease was facilitated by accumulation of further vector-independent mutations in the expanding clone, which were favoured by concurrent precipitating factors such as oligoclonal reconstitution, selective pressure for a survival or growth advantage, transgene toxicity or stressed haematopoiesis resulting from the underlying disease[12,13,14]. Overall, these findings support the idea that improved efficiency of HSC transduction and transplantation, together with improved vector design, can substantially alleviate, but not eliminate, concerns about genotoxicity. Once it has been established that vector insertion is mostly neutral to cell behaviour, tracking clonal activity in the reconstituted haematopoiesis can highlight important biological features of haematopoietic regeneration. These include the pattern of activity and stability of the transduced HSCs, which provides the foundation for predicting long-term maintenance of the therapeutic benefit[16,22]. Tracking clones can also help to establish the reliability of current models of lineage relationship and haematopoietic hierarchy based on xenotransplantation studies[58].

In adoptive T-cell therapy, a subpopulation of T memory stem cells plays an important part in supporting long-term efficacy. This knowledge arose from an improved understanding and phenotypic characterization of T-cell differentiation pathways, and the identification of the cells that are responsible for the sustained generation of effector activity *in vivo*[59]. Optimization of *ex vivo* cell culture[60] and *in vivo* tracking of gene-marked T cells[61] also contributed to this discovery, which should facilitate the design of more effective trials. Epitope mapping and tracking of cancer-targeting T cells in patients who are undergoing adoptive T-cell therapies and checkpoint blockade drug treatment provide direct evidence for the potential to evoke cancer-specific effector T cells in some types of cancer, which could then be exploited to clear advanced metastatic disease after *ex vivo* amplification or genetic editing[44–47].

In liver-directed gene therapy, detailed epitope-specific immune monitoring of antiviral T-cell responses on *in vivo* AAV gene delivery is able to account partially for earlier failures to observe long-term stable FIX expression[34,39]. This also helped to uncover strategies for controlling a delayed cytotoxic adaptive response to viral components that persist within transduced cells, which include administering transient immunosuppressive drugs at the first occurrence of such a response[40] and engineering viral capsids to bypass dominant responses[62–64].

The enhanced efficacy observed in recent gene-therapy trials could also reflect improved study designs. These have been made possible by a deeper understanding of vector–host interactions and by the application of more rational patient-selection criteria, which are more easily adopted after the initial 'proof of safety' of administration by vector in humans has been obtained. Improved trial designs can enhance efficacy in several ways. For example, treating early symptomatic (or even presymptomatic) individuals instead of people with an advanced stage of a disease provides a greater chance for the therapy to work before tissue damage has become irreversible. Vector doses can be set at levels that approach therapeutic efficacy instead of minimal biological activity, as is usually done to alleviate risks when establishing the safety of a treatment in phase I/II trials. However, this cautionary approach often denies trial participants the chance to gain immediate benefit from the therapy, as well as any future benefit (because they become immune to the vector). By administering more aggressive preconditioning regimens, space can be made for the gene-corrected cells. Vector-induced adverse events can also be neutralized by preemptive or prompt pharmacological treatment, such as administering anti-inflammatory drugs or steroids to suppress innate or adaptive immune responses, respectively.

Gene-therapy trials often address rare diseases for which little is known about the natural history and genotype–phenotype relationships. Enriching such knowledge helps in the design of more effective trials because it becomes possible to recruit the patients who are most likely to benefit from the treatment. Efficacy can also be established according to validated endpoints within a timeframe that is compatible with further development of the therapy.

Challenges ahead and prospective developments

In HSC gene therapy, despite advances in vector design, determining the actual risk of insertional mutagenesis in clinical trials — and how it can be managed at the level of the individual patient or overcome by further improvements in vector design — remain major targets for future progress. Several experimental models have been developed to assess and rank the relative genotoxicity of different vector types and designs. Despite providing the rationale to advance new vectors to clinical testing, they fail to provide a quantitative prediction of actual oncogenic risk in the clinical setting. It is still difficult to establish the actual risk of genotoxicity for a given vector in a given trial, especially considering the low number of patients that have been treated with vectors to date, the longer follow-up periods that are required, and the possibility that the disease background could concur to increase the risk. In addition, it remains unclear whether molecular monitoring can predict the progression to malignancy of aberrant clones in individual patients. As long as the chosen vectors are able to integrate throughout the genome, subject to preferences dictated by the parental virus and particle composition, the risk of gene activation and inactivation at insertion sites can be mitigated but not abrogated. Although the improved vector types and designs that are currently being tested could alleviate some of the concerns that surround oncogene activation, which represents a high-risk dominant mutagenic event, the disruption of tumour-suppressor genes remains possible. These less frequent and recessive events might reveal their consequences only after a longer latency period or through testing in large-scale trials.

If efficacy and safety can be established in greater numbers of patients and over longer follow-up periods, HSC gene therapy might eventually challenge the dominance of allogeneic HSC transplantation as a first-line therapeutic option in monogenic diseases for which HSC transplantation is beneficial[5,65]. In fact, the use of autologous HSCs makes the treatment

BOX 2

New adeno-associated virus vector serotypes

Major hurdles for liver-directed gene transfer include alleviating the inflammatory response to intravenous administration of high-dose viral particles and bypassing pre-existing immunity to viral components. Hepatocyte targeting must also be improved and long-term transgene expression should be established[33,34]. In a recent clinical trial of liver-directed haemophilia B gene therapy, an adeno-associated virus (AAV) vector that had shown improved liver tropism (provided by the AAV8 serotype) and enhanced transduction efficiency (obtained by packaging a self-complementary genome) in animal models, resulted in the long-term expression of factor IX (FIX) in most patients[40] as long as a transient immunosuppressive corticosteroid treatment was promptly administered to those who were developing signs of hepatocellular injury. Whether the successful outcome can be attributed to the use of the new vector design remains to be established because the dose–response relationship and tropism of the different vector serotypes might differ between humans and animal models[64]. Another ongoing trial of haemophilia B gene therapy is using the same AAV8 serotype to express a hyperactive *FIX* transgene. Preliminary reports indicate that sustained FIX expression occurs only in some patients, despite the administration of oral corticosteroids on signs of hepatocellular injury[110]. It is possible that the immunosuppressive treatment was administered too late to be effective at preventing the clearance of modified hepatocytes. Alternatively, certain aspects of vector design and manufacturing might be missed by current methods of characterization, which could influence the robustness of the therapy.

potentially available to all patients. HSC transplantation, meanwhile, is available only to patients for whom an HLA-matched or compatible donor can be found. HSC gene therapy can also substantially lower morbidity because it abrogates the risk of graft-versus-host disease and abolishes the need for immune suppression after treatment. Moreover, it could permit the use of milder preconditioning treatments, as partial chimaerism through the presence of gene-corrected cells might be sufficient to correct the disease and spare the patients the risk of myelo- and lymphoablative preconditioning treatments. In addition, genetic engineering of HSCs might facilitate new modes of treatment if the present outcome of HSC transplantation is unsatisfactory. This could involve increasing the therapeutic gene dosage to a level that is higher than the level that is provided by normal donor cells (as previously described for metachromatic leukodystrophy) or delivering transgene activity to selected tissues or sites of disease (such as the CNS in metachromatic leukodystrophy). HSCs and their progeny could also be equipped with exogenous genetic information to better fight cancer or chronic infection. As therapeutic options become available for otherwise incurable diseases, the genomes of newborn babies could be screened to allow early treatment of these conditions. To fulfil these predictions, *ex vivo* genetic modification and culturing of HSCs and their progenitors must become more robust and reliable. This should be coupled with the improved maintenance and expansion of the treated cells, which would support faster haematopoietic recovery in preconditioned patients and increase the clonal composition, resilience and stability of the engineered graft — thereby improving the short-term and long-term safety of the procedure. When therapeutic benefit is reached on establishing a partial chimaerism, less-toxic conditioning regimens based on biological agents could also be applied, which would spare patients both the acute and long-term toxicity of current chemotherapy-based regimens[66].

In T-cell immunotherapy for cancer, the number of CARs in clinical testing is growing quickly, although most of the trials focus on treating B-cell malignancies using CARs that target B-cell surface antigens. Notably, this approach depletes not just the disease-causing malignant clones but also almost all B cells in the patient. Although the lack of B cells can be remedied by the infusion of immunoglobulins, depletion of other cell lineages might not be as manageable. This issue could limit the use of other lineage-specific antigens as CAR targets. In addition, the clearance of large tumour masses observed in these trials was accompanied by an acute and often severe syndrome — even requiring intensive care — that followed the massive release of cytokines from on-target activated T cells.

Given the remarkable benefits that have been observed in some patients, further CARs are likely to be designed and tested[42,55]. However, important questions still need to be addressed. For example, what CAR design will achieve the greatest efficacy with minimal toxicity? How can the toxicities deriving from the CAR-mediated recognition of healthy tissues that express target antigens at low levels be controlled in a timely and effective way? Can adoptive T-cell therapies be as effective in solid cancers as they seem to be in some lymphoid malignancies, or might features of the tumour stroma inhibit their activity? Although more challenging than transferring CAR genes, transferring TCR genes could be more effective in the long term. TCR gene transfer could also be a better fit for cancers that tend not to accumulate passenger mutations and might be less responsive to checkpoint blockade drugs. By targetting cancer-driver mutations, TCR gene transfer might be less prone to the induction of resistance than CAR-gene transfer, which has a more limited range of targets that must be surface antigens and are not always drivers of the disease. TCR gene editing[67,68] can redirect specificity of a T cell towards a new antigen by disrupting endogenous TCR genes then introducing an exogenous TCR or CAR. It could therefore become a powerful strategy to avoid TCR mispairing, which occurs when the same cell expresses two different TCRs. Moreover, in driving the biological responses of the genetically modified T cells, it suppresses confounding endogenous TCR signal transduction. When combined with growing evidence to support the substantial efficacy of immune checkpoint blockade therapy, the outcome of recent CAR T-cell-gene transfer trials suggests that immunotherapy could become a new pillar of cancer therapy that has the potential to eradicate the disease[69].

In some of the liver-directed, AAV-based, gene-therapy trials discussed in this Review, the consequences of a delayed cell-mediated immune response that targets transduced hepatocytes could be controlled. However, this remains an area of close scrutiny because the factors that might concur to trigger the response and regulate its timing are not fully understood[34]. For instance, how does the occurrence of this delayed hepatocellular toxicity relate to the administered vector dose, potency and type? And is it related to possible concomitant triggers such as transient subclinical liver injury, or inflammation that recruits memory T cells at a time when viral antigen is still being presented by the transduced hepatocytes? Another unresolved aspect concerns the durability of transgene expression over an extended period of time. Although AAV-mediated FIX expression remains stable over the life of treated dogs, it is difficult to predict whether this also applies to humans, who have longer lifespans. A clear understanding of the molecular forms that underlie the long-term association of AAV DNA with the hepatocyte nucleus — and their relative contribution to transgene expression — will surely provide insight. In preclinical studies, a fraction of the AAV genome is reported to have integrated into the chromatin of hepatocytes, although it is unclear how much it could contribute to long-term gene expression[35]. Although integration ensures long-term expression of genes, even in the face of cell replication, there are concerns over its safety[70,71,72]. Approaches must be developed that enable patients with high concentrations of AAV-neutralizing antibodies to access AAV-mediated gene therapy and to allow re-administration of gene therapy, possibly by exploiting alternative capsid composition[34,63,73,74].

As confidence grows in the choice of vector type and dosing, patient selection criteria can be used to target therapies to individuals who are less likely to mount AAV-directed immune responses. New vector serotypes will ensure that AAV-based gene therapy becomes more widely applicable. For instance, it should be possible to tackle haemophilia A — the most common and challenging form of haemophilia — as well as several other metabolic and storage diseases that affect the liver and other peripheral organs, including muscle and the heart. The remarkable ability of AAV gene therapy to establish a long-term clinical benefit through a simple, well-tolerated intravenous infusion, combined with the ease of manufacturing a vector-based medicinal product (instead of a personalized cell product that is necessary for *ex vivo* gene therapy), could set the stage for its rapid commercial development and broad market distribution. AAV gene therapy might also be suitable for addressing unmet medical needs in countries with less well-developed health-care systems because a single intervention could alleviate the burden of providing and accessing lifelong replacement therapy and medical care. Other vector platforms, including lentiviral vectors, are also being developed for liver-directed gene therapy[75] and could eventually complement AAV-based delivery to broaden the access of patients to — and the diseases targeted by — this promising type of gene therapy.

Targeted gene editing

Another important boost to the gene therapy renaissance has emerged from advances in gene-targeting technologies[76]. The ability to generate artificial DNA endonucleases that bind specifically to a DNA sequence of choice and induce a double-strand break (DSB) is making targeted genome editing more efficient and much easier to undertake, which brings the long-sought goals of somatic gene disruption, targeted transgene integration and *in situ* gene correction within the reach of gene therapy. All of these strategies entail a 'hit-and-run' mechanism that requires only transient expression of the nuclease complex and, in some cases, a repair template to modify the genome permanently. Because they target a selected region of the genome, these strategies abrogate the risk of insertional mutagenesis and poorly controlled transgene expression that is associated with conventional gene-replacement strategies.

Gene targeting has recently entered clinical testing through the adoptive T-cell therapy of patients infected with HIV[77]. Artificial zinc-finger nucleases (ZFNs) were used to disrupt the gene that encodes CCR5, a cellular

co-receptor for HIV, in T cells grown *ex vivo* from each patient — with the aim of making these cells resistant to infection with the virus before their reinfusion. Gene disruption was achieved by creating a DNA DSB in an exon of the *CCR5* gene because repair of DSBs by the non-homologous end-joining (NHEJ) pathway leads to the loss or insertion of bases during end rejoining, which inactivates the coding sequence. The trial reported both proof of safety and long-term persistence of the engineered cells *in vivo*. It also provided an indication of efficacy through a trend for the positive selection of cells with disrupted *CCR5* alleles, as well as an observation in some patients of improved control of viral replication on the scheduled interruption of antiviral therapy[77]. To achieve definitive viral clearance, however, the fraction and long-term maintenance of infused cells bearing biallelic *CCR5* disruption might need to be increased. This could be achieved by performing gene editing in self-renewing progenitor cells, such as HSCs[78] or T memory stem cells, because these cells offer better potential for achieving this level of enhanced and long-term reconstitution of the T-cell compartment, especially if combined with preconditioning to deplete endogenous non-modified cells. Targeted gene disruption can also be used to relieve the repression of an endogenous gene whose expression might compensate for the dysfunction of another gene. For instance, inactivation of the transcriptional repressor BCL11A in erythroid progenitor cells could reactivate fetal γ-globin expression to compensate for or counteract a dysfunctional β-globin chain in β-thalassaemia or sickle-cell disease, respectively[79]. However, a higher efficiency of biallelic knockout is required to fulfil these goals when there is no mechanism by which to amplify selectively the genetically modified cells.

Gene editing becomes even more ambitious when aiming to replace a target sequence with an exogenous version of choice by exploiting the homology-directed repair (HDR) pathway of DNA DSBs. To achieve this, artificial nucleases and an exogenous DNA template bearing homology to the target site and comprising the new sequence[76] must be delivered to the cell. The approach has great potential for use in *ex vivo* gene therapy because the targeted integration of an expression cassette into a preselected genomic 'safe harbour'[80,81] or the *in situ* reconstitution of a mutant gene would ensure robust and predictable expression — closely recapitulating the endogenous expression control in the latter — without the risk of insertional mutagenesis[82–84]. Several hurdles must be overcome before these strategies can be fully exploited. This is because the efficiency of HDR-mediated genome editing remains low in most primary cell types of relevance to gene therapy, such as HSCs[82]. In addition, it is challenging to achieve the safe and feasible clinical translation of cell-therapy products when having to rely on selection and extensive *ex vivo* amplification of a few edited cell clones. The cellular response to DNA DSBs varies according to cell type and cell cycle and growth statuses, and ranges from repair by the different pathways to differentiation or apoptosis[85]. Overall, how the cell chooses between NHEJ and HDR is poorly understood. Alternative mechanisms for HDR that might function outside the S/G2-phases of the cell cycle are also emerging.

Multiplexed, targeted genome editing is now easily achievable through the application of clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 RNA-based nucleases. These nucleases can be rapidly and easily adapted to seek out any DNA target site by designing an RNA guide instead of generating a protein-based sequence recognition motif for each target[86]. Consequently, multiple applications have been found for targeted genome editing in experimental and preclinical models. Translating these applications to the clinic will, however, require thorough assessment of the off-target activity of the selected nuclease[87,88] and optimization of the therapy. Gene addition by HDR or conventional vectors can also be combined with the targeted disruption of another cellular gene to augment effector function, such as by relieving inhibitory checkpoints when editing T-cell specificity[89].

Gene-editing strategies are also being developed for use in direct *in vivo* applications, including liver-directed gene therapy. Studies in mice have reported the reconstitution of FIX expression in haemophilia B mice by AAV-mediated delivery of ZFNs and a repair template[90], the targeted disruption of the cholesterol regulatory gene *Pcsk9* by AAV-mediated delivery of CRISPR/Cas9 (ref. 91), and the repair of the *Fah* mutation in tyrosinaemia (an inability to break down the amino acid tyrosine) by hydrodynamic plasmid delivery of CRISPR/Cas9 and a template[92]. Major hurdles to the further development of *in vivo* gene editing include the safe and clinically suitable delivery of the editing machinery, which should act transiently without inducing cellular toxicity and immunogenicity. All artificial nucleases in current use employ one or more domains derived from prokaryotes — in some cases, common bacterial pathogens or saprophytes. Therefore, when delivered *in vivo*, there is a risk that such nucleases will generate or encounter a preexisting cell-mediated immunity, especially if they are expressed over the long term. More recently, an endonuclease-independent gene-targeting strategy was demonstrated in mice. A transgene was targeted to the albumin locus of hepatocytes by administering a promoterless AAV vector with homology to the highly transcribed albumin gene[93]. A small proportion of the vector integrated at the albumin locus and became expressed from its endogenous promoter, which removed the requirement for both endonucleases and the transfer of a promoter within the vector. The efficiency and underlying mechanism of this and the other types of *in vivo* gene editing discussed in this Review are still to be fully determined.

BOX 3

Pricing gene therapy

The first gene therapy to be commercially approved in the Western world was alipogene tiparvovec (also known as Glybera). This muscle-directed adeno-associated virus 1-based gene therapy was granted marketing authorization in the European Union in 2012 for the treatment of a rare form of familial dyslipidaemia[121]. The market price was recently set at €1 million (US$1.1 million) per treatment[122]. If forthcoming gene therapies are also sold at such a high price, they will challenge the standard reimbursement policies of governments and insurance companies. This high price reflects the cost of preclinical development, manufacturing and distribution of the new medicine, especially for *ex vivo* gene therapies, which are highly personalized and require individualized manufacturing. However, gene therapies have the potential to deliver a substantial, long-lasting benefit to the patient on a single administration, which may offset the cost of the standard treatment of the condition and its complications. Nonetheless, a single upfront payment model for gene therapy may not be sustainable. Approaches that spread the payment over several years should be considered, which could be linked to the successful outcome of the therapy. For example, a pay-for-performance strategy has been proposed[123] that makes the payment of instalments dependent on improved patient health, as determined by objective biomarkers. The risks are then shared between the health-care provider and insurer and the cost of treatment is more closely commensurate to the actual benefits delivered to the patient. In addition, the sustainability of gene therapies could be improved by adapting regulatory and manufacturing requirements to accommodate the unique features of these medicines and by facilitating their accessibility and distribution without detriment to their safety. A flexible platform-based approval and registration strategy should be considered, especially when developing gene and cell therapies that must be adapted to each individual, as is the case for the transfer of T-cell antigen receptor genes that target tumour neoantigens into autologous T cells of people with cancer[44–47].

Retinal gene therapy

The remarkable benefits that gene replacement can provide to patients with severe degenerative diseases were first highlighted by retinal gene therapy. Subretinal administration of AAV serotype 2 (AAV2)-mediated

gene therapy in patients with type 2 Leber congenital amaurosis (LCA), an inherited retinal dystrophy that causes loss of vision at an early age, led to improved visual acuity in several young patients in three independent trials[94–96]. In two of these trials, the patients lost the benefit after a follow-up period of 2–3 years. However, sustained benefit was reported after a similar follow-up period in the third trial[97], which has now progressed to phase III testing. The reasons for the different outcome of these trials, all of which used an AAV2-derived capsid, remain unclear[98]. The progressive degenerative nature of type 2 LCA poses a challenge for delivering extended therapeutic benefits because the small number of photoreceptors that are rescued by the therapy can eventually be overcome by non-cell-autonomous changes in the tissue. As discussed for liver-directed therapy, there might also be subtle differences in vector design and manufacturing that affect the extent of *in vivo* gene transfer, the inflammatory response at the site of delivery and the level of transgene reconstitution in transduced cells. The availability of AAV vectors with higher potencies, which would allow safer dose escalation and enhanced transduction, and more stringent tropism for the relevant targets, should help to overcome these limitations.

Other relevant developments

There are several other advances in the gene-therapy field that could not be discussed in detail in this Review. These include applications in neurodegenerative diseases that have reached the clinical-testing stage. For example, good safety but limited efficacy has been demonstrated for the delivery of transgenes to the brain by AAV or lentiviral vectors[99–101]. Increasing the administered vector dose and optimizing the vehicle, cargo and study design are likely to lead to further advances.

Oncolytic viruses, which infect and kill cancer cells, have been in clinical use for some time. Although they can deliver robust and clinically relevant anticancer activity, these viruses are still being used as part of combination therapies[102]. Oncolytic therapies exploit viral replication and the induction of an immune response against infected cells — conditions that are normally offset in gene-therapy strategies. Intriguingly, their efficacy is likely to be augmented by adding a transgene cargo that improves the induction of anticancer immunity[103].

Adenoviral vectors of simian origin are also being assessed for their ability to induce humoral and cellular immunity through vaccination: encouraging results have already been seen in emerging or widespread infectious diseases that have long resisted conventional attempts[104,105]. In preclinical models, the gene-based delivery of antibody therapy or prophylaxis is being explored to establish an *in vivo* stable and robust source of large quantity of antibodies with optimal specificity to treat or prevent infection[106].

Future outlook

Gene therapy could be poised to become an important new approach for the third millennium because its reach extends well beyond that of conventional drugs. Gene therapy enables the targeted delivery of information-rich gene-based cassettes that facilitate the stable, sustained and regulated expression of biological agents. Furthermore, when combined with cell therapy, it turns cells into smart vehicles for targeted gene delivery. As exemplified in the studies discussed in this Review, gene therapy directs powerful biological processes towards the goals of disease correction, tissue repair and regeneration. For instance, the stability, fidelity and amplification of the delivered therapeutic can be guaranteed by transferring information by genetic mechanisms. The homing and trafficking mechanisms of cells in the human body can be used to target gene-based therapeutics to specific tissues and disease sites. Gene therapy also makes use of the regenerative potential of stem cells and transplantation as well as the biological weapon of immunity, which is exploited for the specific elimination of transformed or infected cells. By taking advantage of these inbuilt biological capabilities, gene therapy has the potential to address the substantial unmet medical needs of both rare and common severe diseases, which will benefit both patients and — more broadly — society.

Major challenges must still be addressed before this promise can be realized. For example, the efficacy and safety of gene-transfer vectors should be improved by further engineering their design and composition, which could include combining the biological features of different viruses with synthetic molecules. These advances will enable vectors to target tissues and cell types precisely[64,73,107], and overcome cellular restrictions on gene transduction and bypass sensors of exogenous nucleic acids. They will also help vectors to avoid activating the innate and adaptive immune system. Overall, the changes will also ensure that transgene expression is reproducible, robust, occurs over an extended period and closely mimics the endogenous pattern of expression (when gene replacement is performed). Improvements in vector manufacturing and characterization will allow the standardization and comparative assessment of vector performance between trials. The rate and specificity of *in situ* gene correction and editing, the integration of vectors at safe genomic harbours, and allele-specific silencing by artificial nucleases and epigenetic modifiers represent further opportunities for improving gene-therapy strategies. Deeper understanding of disease pathogenesis in inherited, multigenic or acquired conditions will enable the development of new gene-based treatment strategies . Because gene transfers employ 'live' biological drugs of unprecedented complexity that have the potential to induce extended effects on patients and their germ lines, long-term surveillance and precautionary measures must be taken while their use is being pioneered. Moreover, current — limited — understanding of the regulation of stem cells, tissue regeneration and immune-response checkpoints constrains the capacity for intervention and raises concerns about the untoward effects of manipulation.

From a clinical standpoint, the bedside delivery of gene and cell therapies calls for multidisciplinary expertise and, in some cases, advanced cell processing at the clinical-treatment site. Biological readouts must also be developed to monitor the safety and efficacy of therapies. As the first gene therapies progress from registration to marketing, both the pharmaceutical sector and regulatory agencies are being engaged to help define appropriate quality standards for manufacturing and release and to build suitable pipelines for supplying such highly personalized therapies. From a societal standpoint, the complexity and cost of manufacturing and supplying 'live' biological drugs in conventional health-care systems will challenge the sustainability of these therapies and require creative cost-reimbursement policies that enable all patients to benefit from them (Box 3).

Finally, from an ethics standpoint, it is important to consider whether medicine should surrender to the rule of technology or commit to a more responsible steering of the course of progress. For instance, the avenues that are being opened to intervention by emerging technologies could undermine our self-perception and self-determination as we end up viewing ourselves as the evolutionary product of DNA that has become self-conscious and can edit itself to shape its progeny as and when it desires. The call for a moratorium on applying genome editing to human germline cells highlights forthcoming ethical dilemmas[108,109]. Science might turn again to the ancient roots of Western culture to learn from its wisdom, such as the "Know thyself" inscription on the Temple of Apollo at Delphi in ancient Greece — a warning to recognize our limits. Modern philosophy has trained us to exercise criticism when assessing the truth and certainty of knowledge, which is limited a priori and enables learning relationships and making predictions but not uncovering the nature of things. Those predictions allow us to develop strategies that can alleviate human suffering from disease, thereby providing our endeavours with a translational framework that can guide our choices and justify them from an ethics standpoint. ■

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