ASGCT 20th anniversary special issue of Molecular Therapy: Evolving Gene Therapy in Primary Immunodeficiency

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Abstract
Prior to the first successful bone marrow transplant in 1968, patients born with severe combined immunodeficiency (SCID) invariably died. Today, with a widening availability of new born screening, major improvements in the application of allogeneic procedures, and the emergence of successful haematopoietic stem and progenitor cell (HSC/P) gene therapy, the majority of these children can be identified and cured. Here we trace key steps in the development of clinical gene therapy for SCID and other primary immunodeficiencies (PIDs), and review the prospects for adoption of new targets and technologies.

Primary Immunodeficiencies as targets for gene therapy

Primary immunodeficiencies (PID) are a diverse group of rare largely monogenic disorders that result in variable susceptibility to infection, autoimmunity/inflammation, and in some cases malignancy. Over 300 genes have now been associated with syndromic and non-syndromic PID, including the most frequently occurring forms[1]. The severest PIDs present early in life with failure to thrive and severe infections (often opportunistic), and are usually fatal unless definitive therapy can be implemented rapidly[2]. Fortunately many of these immunological defects are intrinsic to the haematopoietic system making them tractable targets for allogeneic haematopoietic stem cell transplantation (HSCT). Since the first successful bone marrow transplants in two PIDs, X-linked SCID (SCID-X1) and Wiskott Aldrich Syndrome (WAS), HSCT methodologies and technologies (including conditioning regimens) have improved and the morbidity and mortality associated with this procedure have diminished considerably[3-6]. Even so, for patients without HLA-matched donors, graft-versus-host disease (GvHD), delayed immunological reconstitution, and graft rejection remain a significant problem. Some conditions are better candidates than others due to disease-specific characteristics, and risks are heightened in patients who are actively infected or who have developed chronic complications at the time of treatment. More than thirty years ago the demonstration of retroviral gene transfer to HSC/Ps led to the suggestion that this technology could provide an alternative platform for development of therapies in a number of diseases that were amenable to HSCT[7-11]. The advantages of autologous gene therapy were anticipated to lie in the lack of need to identify a suitable donor, obviation of GvHD, and the potential to reduce the risks of myelosuppressive and immunosuppressive pre-conditioning of the patient, which is required to make space for engrafting HSC/Ps. For some conditions it was expected that corrected cells would

have a profound growth and survival advantage, allowing reconstitution from relatively low numbers of cells. In addition, expression of transgenes at supraphysiological levels was anticipated to allow "cross correction" of other deficient non-haematopoietic cells in some diseases[12]. Over a number of years, successful correction of cellular and animal models boosted expectation that gene therapy would rapidly become mainstream, but clinical translation proved more difficult (Figure 1). This partly related to a limited understanding of human HSC/P culture conditions necessary to achieve high-level gene transfer *ex vivo* yet at the same time retaining *in vivo* engraftment capability, which was not well modelled in murine preclinical transplant assays[13]. Furthermore, the occurrence of clinically-manifesting insertional mutagenesis highlighted deficiencies in early retroviral vector technology that would have to be resolved[14].

Adenosine-deaminase-deficient (ADA)-SCID as a paradigm for development of HSC gene therapy

Adenosine deaminase (ADA) is a housekeeping enzyme of the purine metabolic pathway, and is widely expressed[15]. The metabolic environment resulting from deficiency of ADA and the accumulation of toxic purine metabolites causes variable skeletal, lung, liver, gastrointestinal, neurodevelopment and sensorineural defects, whereas the most consistent and profound abnormality is in the development of lymphocytes, including T, B and natural killer cells[16, 17]. Exogenous polyethylene glycol-conjugated ADA (PEG-ADA) can rescue the immunological defects through extracellular detoxification, but these effects are often partial and poorly sustained[18]. Even so, the use of PEG-ADA at diagnosis has proved a very useful way to stabilise patients before a definitive procedure can be implemented[19, 20]. Several clinical trials were implemented in the early 1990's using gammaretroviral vectors that had been evaluated in murine and primate model systems, and in which ADA cDNA transgene expression was regulated by the retroviral Long-Terminal-Repeat (LTR). It was also anticipated that the selective growth and survival advantage imparted to gene-corrected cells would obviate the need for pre-conditioning of the patient. However, these early studies using residual peripheral blood lymphocytes, umbilical cord blood, and bone marrow failed to provide clear clinical benefit over and above that of PEG-ADA, which the patients continued to receive[21-24]. Subsequent studies (again using LTR-intact gammaretroviral vectors) introduced some key changes, including the withdrawal of PEG-ADA to enhance the selective advantage of corrected cells, and most importantly the use of low/reduced intensity conditioning with alkylating agents (usually busulphan, in a few patients melphalan) to promote engraftment of HSC/Ps[25-32]. The combined overall survival of around 50 patients treated in these studies was 100%, with a disease-free survival (not requiring re-introduction of PEG-ADA or HSCT) of over 70%. Immunological reconstitution has in general been robust, with high-level gene marking in lymphocytes, and for the first time, sustained gene marking albeit at lower levels in myeloid cells, which is an effective surrogate for HSC/P marking in bone marrow. Permanent withdrawal of immunoglobulin supplementation was achieved in approximately 50% of patients, suggesting that further improvements are still possible. Recently, a collaboration between GSK, Fondazione Telethon and Ospedale San Raffaele secured European Marketing Authorisation for Strimvelis™ which is based on the original LTR-based vector studied in Milan[22]. From proof of concept in humans to this first ex-vivo market authorisation for a gene therapy product, vector technology has advanced considerably. HIV-1 based lentiviruses in particular have been adopted as the current vector of choice for HSC/P gene transfer as they appear to offer some advantages in terms of mutagenicity (because of a more favourable genome insertion profile within transcriptional units rather than at transcription start sites (TSS), a

theoretical advantage) lack of intrinsic retroviral LTR enhancer activity (a modification that has also been duplicated in a new generation of gamma retroviruses), and higher efficiency of gene transfer[33-35]. Following several years of preclinical development, clinical studies using lentiviral vectors are underway for ADA-SCID[36]. Early indications in over 32 treated patients, some of whom were identified by newborn screening, are of excellent efficacy, and no associated toxicity[37].

Interestingly, no clinically-manifesting vector-related toxicities have been observed in any of these studies to date. This is surprising because vectors with similar configurations have caused mutagenesis and malignancy in a number of other conditions as will be highlighted later[38, 39]. Several reports have now detailed a comprehensive analysis of retroviral integration sites (RIS) in cells from ADA-SCID patients treated with LTR-intact gammaretroviral vectors, and although the anticipated patterns of integration are observed, namely preference for transcriptional start sites (TSS), including sites near the proto-oncogenes Lim domain only 2 (*LMO2*), *BCL2* and *CCND2*, there are also indications of subtle mutagenic clonal disturbances, although apparently insufficient to drive a frank malignant programme[37, 40, 41]. The reason for this disease-specific difference in toxicity is at present unclear, but could relate to a lower replicative stress in reconstituting T cells on the non-haematopoietic environmental background of ADA-deficiency in the thymus, or the presence of competing non-transduced but detoxified thymocytes that successfully complete their maturation programme [42]. However, this still doesn't explain the lack of myeloid toxicity unless a similar argument can be applied to the bone marrow microenvironment or even during *ex vivo* culture.

Lessons learned from clinical trials in X-linked SCID (SCID-X1)

X-linked SCID (for SCID-X1) is the most common form of severe combined immunodeficiency, accounting for 40-50% of all cases[2]. Mutations in the *IL2RG* gene lead to defective expression of the common cytokine receptor gamma chain (gc), so-called because it is subunit shared by multiple cytokine receptors, including the IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 receptor complexes, which are variably involved in the development and function of all lymphocytes. As a consequence, patients show profound defects of adaptive immunity, resulting from the low number or absence of T and Natural Killer (NK) lymphocytes, and the loss of function of B lymphocytes. The first two clinical trials of gene therapy for SCID-X1 included a total number of 20 subjects and were conducted using virtually identical LTR-intact gammaretroviral vectors and very similar transduction protocols[43-46]. Neither study used preconditioning, with the expectation that the extremely high survival advantage of corrected lymphocyte precursors would allow the development of a highly diverse T cell repertoire (as observed following HLA-matched HSCT infusions, and in some variant patients with rare somatic reversion events) [47, 48]. Successful recovery of a functional T cell compartment occurred in most patients and has been sustained with a disease free survival over 80%, and excellent overall clinical outcome. In the absence of conditioning, sustained engraftment of HSCs did not occur, and consequently long-term B cell and NK cell marking was very low. Unfortunately 31-68 months after gene therapy, five patients who were clinically well and had successfully reconstituted, developed a T-cell acute lymphoblastic leukaemia (T-ALL)[49-51]. In four out of five patients enhancer-mediated upregulation of the *LMO-2* protooncogene was directly implicated in the leukaemic process, although an accumulation of more classical genetic changes (many of which have been previously described in *de novo* T-ALL) unrelated directly to retroviral vector insertion, were probably required for final evolution to acute T-ALL[51, 52]. It has recently been postulated that intra-thymic replicative stress in the absence of T cell

progenitor import from the bone marrow is a significant contributor to the establishment of a leukaemic programme as HSC engraftment is essentially absent[42, 53]. If this is the case, then use of conditioning would likely reduce risk of clinically manifesting mutagenesis. No convincing evidence could be confirmed for a pathogenic role of non-physiological expression of the transgene in leukaemogenesis through aberrant receptor signalling, although of course it allowed the T cell maturation to proceed[54]. All patients were treated with relatively standard chemotherapy, but one patient died of refractory leukaemia. Remarkably, the four surviving patients recovered their T cell immunity after completion of chemotherapy without need for any further intervention. This suggests that a very long lasting (possibly self-renewing), gene-corrected T cell precursors engrafted in the thymus soon after infusion of cells, and that these are capable of sustaining active thymopoiesis for many years.

Molecular studies in mice engrafted with cells transduced with replication-incompetant gammaretroviral vectors identified duplicated enhancer elements in the LTR as the major culprit driving dysregulated gene expression in the neighbourhood of the RIS[38, 55, 56]. This effect was also shown to have activity over quite long ranges, and to operate combinatorially with other molecular lesions[51, 52]. As part of an international collaboration, the Transatlantic Gene Therapy Consortium (TAGTC), a new generation of gammaretroviral technology was developed that deleted the LTR enhancer sequences, and used internal heterologous sequences to regulate transgene expression[57-59]. Assays were developed that demonstrated significantly reduced mutagenicity *in vitro*, while testing in animal models of disease, including SCID-X1 suggested that these new vectors designed for safety also retained efficacy[55, 60-62]. In a recently reported clinical study, 9 newly diagnosed patients with SCID-X1 were treated with a modified gammaretroviral vector using a very similar protocol to that previous implemented, and with similar outcomes in terms of immunological reconstitution[63]. More importantly, when RIS were compared to those retrieved from trials using LTR-intact gammaretroviruses, there was significantly less clustering within the *LMO-2* or *MECOM* loci, or at other proto-oncogenes implicated in leukaemogenesis. No vector-related adverse events have been observed to date, with a median follow up of over 5 years. Interestingly, the rate of early T cell reconstitution following autologous gene therapy has also been shown to be faster than that following haploidentical HSCT suggesting that there are other advantages of this approach[64].

As part of the first two studies, two older patients were also treated but did not reconstitute[65]. Similarly, in a third trial (with an LTR-intact gammaretroviral vector) using peripheral blood mobilised HSC/P cells rather than bone marrow-derived cells, two preadolescent subjects aged 10 to 14 years, who had previously been treated unsuccessfully with bone marrow transplantation, received transduced cells in the absence of conditioning[66]. Immune reconstitution was limited in these patients despite good engraftment, with only a slight improvement of T cell function in the youngest child. It has been proposed that there is an age-related decrease in plasticity and loss of thymopoietic capacity in older subjects particularly where thymopoiesis has been absent for extended periods. More recently a lentiviral vector approach has been used to treat 5 patients who have failed haploidentical HSCT, in a protocol that included reduced intensity conditioning with busulphan[67]. All 5 patients demonstrated expansion of gene marked T, B and NK cells, with clinical benefit. Interestingly these patients responded to therapy well despite their older age, and achieved successful recovery of humoral function. This suggests that the engraftment of corrected HSC, facilitated by the

lentiviral vector platform and pre-conditioning, allows superior recovery to that observed in gammaretroviral studies. Studies are currently ongoing in newly diagnosed infants.

All clinical approaches so far have utilised *ex vivo* manipulation of HSC/P cells, which although advantageous for product quality testing, does add some complexity in terms of manufacture. In an alternative approach, a clinically relevant canine model of SCID-X1 has been used to demonstrate partial correction through direct inject of a foamy virus vector in neonatal animals (again relying on selective growth and survival advantage of corrected cells)[68]. Whether this can be translated into a clinical protocol for newly diagnosed SCID patients or even for *in utero* application is unknown, but the efficiency of *ex vivo* gene therapy may be difficult to challenge.

Gene therapy for Chronic Granulomatous Disease: no selective advantage

Chronic Granulomatous Disease (CGD) results from deficiency of the multicomponent NADPH-oxidase enzyme complex in professional phagocytic cells[69, 70]. This oxidase is directly responsible for mitochondria-independent consumption of oxygen during phagocytosis (called the respiratory burst), which results in forced electron transport across the phagosome membrane, production of reactive oxygen species such as superoxide anion radical, hydrogen peroxide, hydroxylanion and hypochlorous acid (although the functional significance of these species *in vivo* is uncertain), liberation and activation of crucial microbicidal granule proteases through change in local pH and ionic balance, and effective formation of extracellular neutrophil nets[71, 72]. Patients with CGD are consequently susceptible to recurrent life-threatening infections by a spectrum of bacteria and fungi, particularly those which express high levels of catalase (so-called 'catalase-positive organisms' such as *Staphylococcus Aureus, Nocardia* and *Aspergillus*. In two thirds of cases, CGD is due to mutations in the X-linked *CYBB* gene (X-CGD), which encodes the $gp91^{phox}$ component of the NADPH-oxidase complex, although a significant proportion have mutations in *NCF1* which encodes $p47^{phox}$ (AR-CGD). Three other AR forms of CGD have been described, and while rare in Western populations, are much more prevalent in geographical areas of high consanguinity. The level of correction absolutely required for clinical benefit in CGD is unknown. However female carriers of X-CGD with over 10% of circulating functional neutrophils are usually well. The level of biochemical activity necessary per cell is also an important question, although patients with very low levels of natural activity have a significant survival advantage over those with no detectable activity, and animal studies predict that partial correction will be sufficient[73, 74]. One of the complexities of treating CGD through an HSC approach is that there is no selective advantage for corrected cells. Furthermore, chronic inflammation may have a detrimental effect on HSC/P numbers and viability after *ex-vivo* manipulation[75, 76]. Early trials did not use any preconditioning of patients, and engraftment was therefore at very low level and transient[77]. More recently, five early phase clinical studies, involving a total of 12 patients have been conducted in several centres worldwide, incorporating reduced intensity alkylating agent conditioning[75, 78-80]. Overall, these demonstrated biologically and clinically significant restoration of NADPH-oxidase activity (albeit partial, between 10-30% of normal) in circulating neutrophils for a short time after engraftment, allowing the majority of patients to clear pre-existing infections. However, clinical benefit was usually only transient, and gene marking rapidly decreased with only a few patients having significant marking after three months. Several patients treated with a Spleen Focus Forming Virus (SFFV)-derived LTR-based gammaretroviral vector developed an unexpected increase in the number of functionally corrected neutrophils over time as a result of insertional transactivation of myeloproliferative genes, particularly *PRDM16* and the *MECOM* locus[78, 81].

Interestingly, although the level of gene marking persisted, gene expression was silenced in the majority of these cells through CpG dinucleotide methylation at the retroviral promoter, even though enhancer activity and therefore mutagenic influence was preserved.

More recently, lentiviral gene therapy approaches for CGD have been developed in an attempt to reduce the risk of mutagenesis and enhance long-term efficacy. In the first approach a chimeric promoter (fusion between c-fes and Cathepsin G proximal regulatory sequences) was developed that contains binding sites for transcription factors that are active during terminal myeloid differentiation[82]. Consequently it confers higher levels of gene expression in differentiated myeloid cells rather than in non-myeloid lineages or multipotent primitive progenitors where mutageneic influences are least desirable. HSC gene therapy using this vector in mice resulted in high levels of $gp91^{phox}$ expression in committed myeloid progenitors and granulocytes with much less expression in other lineages, mimicking physiological patterns to a large degree. In accordance with these findings, high levels of NADPH oxidase activity was restored in all the transplanted animals and remained constant for the period of analysis. Secondary transplantation experiments also indicated that the chimeric regulatory sequences were not prone to silencing or indeed to epigenetic CpG methylation. A clinical trial is currently ongoing in US and Europe using myeloablative busulphan preconditioning, with encouraging early results, and evidence for the first time of sustained biochemical correction in the absence of mutagenesis[83]. A parallel strategy for AR-CGD has also recently been developed. A second approach has been to use vector-encoded micro-RNA recognition sequences to de-target gene expression away from HSC and therefore prevent any potential toxicity arising from transgene expression in these cells[84].

Gene therapy for Wiskott-Aldrich syndrome (WAS): complex multilineage disease

Wiskott-Aldrich syndrome (WAS) is a rare, X-linked, complex PID caused by mutations in the *WAS* gene[85, 86]. The Wiskott-Aldrich protein (WASp) is a major regulator of the actin cytoskeleton in most haematopoietic lineages and is consequently important for normal function of many immunological processes, and for normal platelet production[87]. Clinical manifestations of WAS include microthrombocytopenia, recurrent infections and eczema. Patients also display an increased incidence of autoimmunity and are at risk of developing lymphoreticular malignancy. In a study of 10 patients treated with an LTR-intact gammaretroviral vector and reduced intensity busulphan conditioning, 9 exhibited sustained improvement of immunological function with resolution of immunodeficiency and bleeding diatheses[88]. However, despite initial evidence for polyclonal RIS patterns, most patients subsequently developed leukaemia as a result of insertions at LMO-2 and MECOM loci[89]. This high frequency of leukaemia most likely occurred as a combinatorial result of the LTR enhancer activity and multiple vector insertions per cell, but also highlights the difficulty in using RIS analysis for prediction of dangerous clonal events. More recently, a lentiviral vector incorporating proximal WAS regulatory sequences has been tested in several centres, using a reduced intensity pre-conditioning regimen[90-93]. In over 20 patients treated worldwide there has been sustained correction of immunological parameters in most, with resolution of immunodeficiency and autoimmunity, and no evidence to date for mutagenesis. Recovery of platelet numbers has been more variable but surprisingly slow in most cases and dependent on higher vector copy numbers. One of the benefits of RIS analysis is the ability to track physiological haematopoiesis at a clonal level. Using treated WAS patients it was therefore possible to define cellular contributions to early and late phases of reconstitution, and to show that HSC/Ps manipulated *in vitro* for gene transfer retained

the ability to restore haematopoiesis *in vivo* in a way that mirrors normal HSC activity after transplantation[94].

Preclinical development of gene therapy for other candidate PIDs

The success of gene therapy for several PIDs using conventional gene addition, and the apparent improvement in safety through development of newer vector platforms suggests that similar strategies could be applied to other PIDs. Autosomal recessive Janus Kinase (JAK)-3 deficiency leads to a phenotype that is identical to SCID-X1 because JAK3 is a tyrosine kinase required for signal transduction by the common cytokine receptor gamma chain (deficient in SCID-X1)[95]. Similarly, Interleukin-7 receptor alpha deficiency results in the same T cell phenotype, with normal numbers of B and NK cells. Other tractable forms of SCID include defects in *RAG* (recombination activating genes) 1 and 2 genes, and in *DCLRE1C* wich encodes Artemis. RAG1/2 and Artemis proteins participate in V(D)J recombination, which is essential for the generation of functional B and T cell receptors and consequently for normal T and B cell development. In all cases, the transfer of genetically modified HSCs can effectively reconstitute murine models of the disease although with varying degree of difficulty[96-99]. X-linked Agammaglobulinemia (XLA) is caused by mutations in the Bruton's tyrosine kinase (*BTK*) gene, an enzyme of the TEC family of kinases expressed during B cell development and involved in pro-B/pre-B cell transition. The absence of BTK results in an accumulation of pro-B cells and a decrease in numbers of mature B cells and very low or absent serum immunoglobulins. Preclinical murine reconstitution experiments using both gammaretroviral and lentiviral-based gene transfer have demonstrated successful rescue of B cell differentiation and function[100, 101]. Similar results have been obtained using a more refined lentiviral vector encoding the immunoglobulin enhancer (Emu) and Igbeta (B29) minimal promoter to regulate lineage-specific Btk expression, again in mice[102]. As many XLA-associated mutations affect pre-mRNA splicing, attempts have also been made to model correction in a BAC-transgenic mouse model using splice-correcting oligonucleotides with some success[103]. X-linked hyper IgM syndrome (X-HIGM1) is a combined immunodeficiency characterised by defects in isotype switching from IgM to IgG, IgA and IgE, as well as impaired lymphocyte and myeloid functions. X-HIGM1 is associated with mutations in the gene (*TNFSF5*) encoding CD40 Ligand (CD40L), a member of the TNF superfamily, which is primarily expressed on activated T cells, and binds the CD40 receptor on the surface of B cells and antigen-presenting cells to regulate B cell function and inflammatory responses. Gammaretroviral gene transfer has previously been shown to successfully correct the phenotype of CD40L-deficient mice, but at the cost of pathological T-lymphoproliferation due to phenotoxicity of the expressed gene unrelated to insertional mutagenesis[104]. This is consistent with findings from another study, which reported atypical lymphoid proliferation in transgenic mice overexpressing CD40L, and highlights an important consideration for regulated gene expression in a number of these conditions[105]. An alternative strategy to counteract this obstacle by corrective trans-splicing was partially effective[106]. X-linked lymphoproliferative disease (XLP), a T and NK lymphoproliferative disorder caused by mutations in the signalling adaptor SLAM-associated protein (SAP), and familial hemophagocytic lymphohistiocytosis (FHLH), a hemophagocytic disease resulting from defective NK and $CD8^+$ T cell cytotoxicity are potential candidates for both HSC/P and peripheral T cell-based approaches[107-109]. SAP is involved in multiple signalling pathways affecting proliferation, apoptosis and differentiation, while perforin and Munc13-4, which are the most commonly deficient proteins in FHLH, are direct effectors of cytotoxicity. Lineage-restricted regulation of these genes might be required to avoid deleterious effects in cells

where these proteins are not normally expressed. Mature T cells may also be viable targets as long as sufficient numbers can be engrafted for long-term efficacy, or even for bridging to a more definitive HSC procedure. Leukocyte adhesion deficiency (LAD) type I, which is caused by mutations in the gene (*ITGB2*) encoding the CD18 integrin subunit, is primarily a neutrophil disorder characterized by recurrent life-threatening bacterial infections and poor wound healing. Experience from allogeneic transplant indicates that quite low numbers of normal donor neutrophils are sufficient to achieve disease control. A preliminary study, in which two patients were treated with gammaretroviral gene therapy in the absence of conditioning, achieved very low (<0.1%) levels of gene marking in neutrophils, with no corrected cells detectable in the periphery after two months[110]. More recently, the canine (CLAD) and murine models of disease have been successfully treated using HSCs transduced with either foamy or lentiviral vectors expressing CD18[111, 112]. In many of these conditions, preclinical efficacy and safety studies using improved viral vectors, including vector platforms developed from other classes of retrovirus such as foamy and avian viruses (which may have enhanced safety in terms of genetoxicity), are currently being undertaken in order to determine clinical applicability[113-115].

Gene editing approaches for PID

There has been an explosion of gene editing technologies over recent years, including homology repair platforms based on zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and more recently clustered regularly interspaced short palindromic repeats [CRISPR]/CRISPR-associated endonuclease [Cas]-9]. The advantages over gene addition include safety through precise editing of mutations (as long as off-target effects are minimal), targeting specific gene loci to harness natural regulatory elements, or targeting to 'safe harbours' within the genome, which are known to tolerate transgene integration without risk of mutagenesis. Considerable interest has been placed on editing in PID, particular where selective advantage is imparted to corrected cells (for example in SCID and WAS), or where precise gene regulation is desirable (XHIGM, XLA, RAG-SCID, JAK-3-deficient SCID, IL7R-deficient SCID). Proof of concept has now been demonstrated in HSC/Ps from SCID-X1 and CGD patients (at safe harbour and at natural gene locus), and for XHIGM in T cells[116-118]. One intriguing recent report has used a gene editing strategy to convert a mutant *NCF1* pseudogene into a functional gene thereby permitting correction of all molecular variants of p47phox-deficient CGD through a single approach[119]. Although functional correction through editing has now been demonstrated in iPSCs and human HSC/Ps, it still probably remains limited by efficiency in true HSCs, which is required for sustained clinical effect in many PIDs[120]. In contrast efficiency of editing in mature T cells can be quite high as demonstrated for XHIGM, although the challenge here will be to engraft sufficient numbers of T cells for long term effect. These strategies are exciting but some careful technology development is required before clinically applicable. Aside from obvious concerns about off-target DNA cleavage and illicit recombination, a much more pragmatic question exists regarding efficiency and ability to modify sufficient cells for clinical benefit. Even for conditions with selective advantages, the dose of cells required for immunological recovery is high as determined by clinical experience with gene addition and allogeneic HSCT. Where homologous recombination is required for effect this will be challenging. However, for dominant disorders where gene disruption is the desired effect (for example gain of function STAT1/STAT3-related disease), this strategy may be more immediately tractable, although specificity of editing of the variant allele would have to be carefully titrated, and depending on the disease, virtually all cells will have to be modified in order to prevent residual pathology.

Reflections

PIDs have played a significant part in the development of allogeneic HSC transplantation, and more recently have shown that somatic gene therapy is a viable and effective approach in patients. They undoubtedly will also feature prominently in the application of gene editing to human disease. Over 150 patients with various forms of PID have been treated worldwide by autologous gene therapy to date. More recent studies with refined vector technologies have demonstrated excellent safety profiles, and compelling evidence for clinical benefit in several diseases (Figure 2). Many challenges need to be overcome before gene therapy can be offered as a standard of care for all patients, although the recent market authorisation for Strimvelis™ indicates the direction of travel. There is now a steady increase in capitalization of gene therapy approaches in start-up biotechnology firms and some established pharmaceutical companies, reflecting a renewed appetite for commercialisation of therapies for these rare diseases. Future challenges lie in the design of safer vectors, in the incorporation of regulatory elements to achieve clinical efficacy, effective methodologies for gene editing, and in the optimisation of cell culture methods to preserve viability and function during manufacture of products. Application of pluripotent stem cells derived from somatic tissues by reprogramming (induced pluripotent stem (iPS) cells) may aid in gene editing applications if safe and effective means of successfully transplanting *in vitro* derived tissues can be developed. Somewhat surprisingly, a current bottleneck is the lack of capacity for clinical grade production of viral vectors, which is contributed to by the difficulties in massive scale-up of the lentivirus platform required for treatment of large numbers of patients. Logistics of delivery of cryopreserved cell products to the patient from a limited number of manufacturing centres will also need to be addressed. In the absence of *in-vivo* selection methodologies, for most PIDs there is a requirement for pre-conditioning. Less toxic strategies for example using serotherapy to deplete HSCs is one attractive option. At the same time that allogeneic HSCT has become much more sophisticated and flexible through use of alternative stem cell sources, which significantly attenuate the need for extended searches for donors, and more refined conditioning regimens, autologous gene therapy for PID has begun to deliver impressive returns in terms of efficacy and safety in patients. Alongside newborn screening for SCID, the outlook for patients diagnosed with severe PID is looking very good.

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Figure Legends

Figure 1. The development of viral vector technology and its application to human gene therapy. The blue time line shows the various ups and downs of the field based on successes and setbacks. References are included in the text.

Figure 2. A table of published and ongoing clinical trials for primary immune deficiencies. The disease, mutated gene, vector platform, use of conditioning and year are included.

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