Published in final edited form as:
Mutat Res. 2009 July 31; 668(1-2): 141. doi:10.1016/j.mrfmmm.2009.03.010.

Finding the needle in the hay stack: Hematopoietic stem cells in Fanconi anemia

Lars U.W. Müller[1] and **David A. Williams**[1,*]

[1] Department of Medicine, Division of Pediatric Hematology Oncology, Children's Hospital Boston, and Dana-Farber Cancer Institute, Harvard Stem Cell Institute, Harvard Medical School, Boston, Massachusetts

Abstract

Fanconi anemia is a rare bone marrow failure and cancer predisposition syndrome. Childhood onset of aplastic anemia is one of the hallmarks of this condition. Supportive therapy in the form of blood products, androgens, and hematopoietic growth factors may boost blood counts temporarily. However, allogeneic hematopoietic stem cell transplantation (HSCT) currently remains the only curative treatment option for the hematologic manifestations of Fanconi anemia (FA). Here we review current clinical and pre-clinical strategies for treating hematopoietic stem cell (HSC) failure, including the experience with mobilizing and collecting CD34+ hematopoietic stem and progenitor cells as target cells for somatic gene therapy, the current state of FA gene therapy trials, and future prospects for cell and gene therapy.

Keywords

Fanconi anemia; HSC; gene therapy; bone marrow failure

1. Introduction

Fanconi anemia (FA) is an inherited cancer predisposition and bone marrow failure syndrome, affecting about 1 in 100,000 births. The clinical presentation of FA is highly heterogeneous. Approximately two thirds of patients present with physical anomalies, which may vary greatly in number and severity. Most commonly skin hypo-or hyper-pigmentation, short stature, microphthalmia, radial ray or thumb abnormalities are observed. Other organ systems involved include the cardiac, renal, and auditory systems[1] (in press, Mutation Research, 2009). Bone marrow failure occurs nearly universally with a median age of onset of eight years and remains the primary cause of morbidity and mortality in FA. By age 40, 90% of FA patients will have developed bone marrow failure [2]. In addition, the risk of myelodysplasia or acute myeloid leukemia (AML) is markedly increased, with an AML incidence of 33% by age 40[2,3]. FA patients are also at increased risk of other cancers, particularly squamous cell carcinomas of the anogenital region and head and neck region[2,3]. In contrast to idiopathic aplastic anemia, bone marrow failure in FA poses unique challenges. While the majority of pediatric patients with idiopathic aplastic anemia may be cured with medical management consisting of immune

*Correspondence should be addressed to: David A. Williams, MD, Children's Hospital Boston, 300 Longwood Avenue, Karp 08125.4, Boston, MA 02115, E-mail: DAWilliams@childrens.harvard.edu, Telephone: 617.919.2027, Fax: 617.730.0934.

suppression [4], bone marrow failure in FA patients can sometimes be delayed but never be halted or reversed by medical treatment.

In recent years, major advances have been achieved with regard to elucidating the genetic basis of FA. Thirteen genes (*FANCA*, *FANCB*, *FANCC*, *FANCD1/BRCA2*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCJ/BACH1/BRIP1*, *FANCL*, *FANCM*, and *FANCN/PALB2*) have been identified and cloned. Resultant products interact with the FA/BRCA biochemical pathway and studies on the function of FA proteins indicate that they participate in the recognition of and protection against genotoxic stress via the formation of multi-protein complexes [5–7] reviewed in [8]). FA mutation are sometimes also organized into complementation groups, in reference to the initial classification based on cell-fusion techniques [9]. Mutations in *FANCA*, *FANCC*, and *FANCG* are the most common in humans, accounting for >80% of FA patient mutations[2]. Mutations in different FA genes result in similar clinical manifestations, even though there is some suggestion that patients with the FA-D2 complementation type (3–6% of all cases) are more severely affected in terms of malformations and onset of hematopoietic failure [10].

The clinical challenges posed by hematopoietic stem cell failure have informed basic and translational research efforts. In this review, we provide an overview of hematopoietic stem cell failure in FA with a focus on current results and future directions of cell and gene therapy.

2. The effect of pro-apoptotic cytokines on Fanconi anemia HSC

While the etiology of marrow failure in FA remains unclear, some evidence points towards an intrinsic hypersensitivity of FA bone marrow cells to **pro-apoptotic** cytokines such as tumor necrosis factor-α (TNF-α) and interferon-γ (INF-γ). Both cytokines are known late effectors of damage to the hematopoietic stem cell compartment in idiopathic aplastic anemia, a condition that bears some similarity to bone marrow failure in FA[11,12]. Several investigators have noted increased serum levels of both these cytokines in FA patients[13,14]. In one series, TNF-α was detected in the plasma of 16 of 18 FA patients with concentrations ranging from 6 to 131 pg/ml (mean 31 pg/ml). TNF-α was detected in only one of 25 control (healthy donor) plasma samples, and at low levels (7 pg/ml)[13]. Increased production of TNF-α and increased intra-cellular levels of TNF-α and INF-γ have also been noted in lymphocytes and bone marrow mononuclear cells of FA patients[14,15]. Importantly, the addition of the anti-TNF-α fusion decoy protein etanercept to human bone marrow cultures was associated with a significantly increased number and size of erythroid colonies grown *in vitro* in FA but not in normal control samples, indicating that TNF-α inhibits FA erythropoiesis *in vitro* [15]. Sensitivity of FA cells to **pro-apoptotic** cytokines has also been demonstrated in *Fanca*−/− and *Fancc*−/− gene-targeted mouse models. In *Fancc*−/− mice, TNF-α and INF-γ induce apoptosis in hematopoietic progenitor cells, even at low cytokine levels[16–18]. In addition, continuous infusion of INF-γ for seven days in *Fancc*−/− mice by micro-osmotic pumps at doses of 0, 120 and 400 μg/kg/day has led to a significant reduction of the peripheral blood white blood cell count and bone marrow cellularity. TUNEL assays conducted on BM sections showed increased apoptosis in the treated *Fancc*−/− but not in the wild type (wt) bone marrow. Moreover, *Fancc*−/− but not wt mice treated in this fashion engrafted congeneic **CD45.1**+ bone marrow cells robustly without additional conditioning [19].

Recently, Li et al. described the emergence of myeloid leukemic clones following the prolonged exposure of *Fancc*−/− bone marrow hematopoietic stem and progenitor cells (HSC/P) to TNF-α [20]. As expected, compared to wt bone marrow cells, ***Fancc***−/− cells were initially hypersensitive to TNF-α exposure *in vitro*. However, after three-week exposure to TNF-α *in vitro*, a rapidly expanding population of *Fancc*−/− cells emerged. This cell population could be maintained in culture with IL-3 stimulation for another 3 weeks and gave rise to large

colonies in semi-solid media that could be re-plated up to four times, a result consistent with transformation of the colony forming cells. When transplanted into lethally irradiated congenic mice, 12/12 mice receiving TNF-α exposed *Fancc*−/− bone marrow cells developed myeloid leukemia. No leukemia was observed in the case of TNF-α treated wt cells[20]. Although the relevance of these observations to the human system is unknown, taken together, these data suggest that **pro-apoptotic** cytokines are potential contributing factors to HSC failure and leukemogenesis in FA.

3. Clinical challenges of hematopoietic stem cell failure in Fanconi anemia

The onset of marrow failure can be insidious and is initially suspected based on peripheral blood cytopenia and red blood cell macrocytosis. These cytopenias worsen over time and according to current consensus opinion, treatment of marrow failure is generally indicated for hemoglobin less than 8mg/dl, platelets less than 50,000/mm^3, or absolute neutrophil counts below 500/mm^3 [21]. Supportive care is provided with judicious use of red cell and platelet transfusions. Avoidance of blood transfusions is critical whenever possible, since even relatively modest blood component exposure has been shown to have a negative impact on the outcomes of allogeneic bone marrow transplantation[22]. Hematopoietic growth factors such as granulocyte-colony forming factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF) or androgens may also be used. Oxymethalone is the most widely used androgen in FA patients, although oxandrolone is currently under investigation in a phase I clinical trial. The predominant effect of androgens is an increase of hemoglobin, though increases in platelets and, to a lesser degree, neutrophils have also been observed[23,24]. There is no evidence that androgens cure bone marrow failure, so treatment is instituted once anemia reaches a critical threshold but prior to the complete depletion of the bone marrow of hematopoietic stem cells, which may be stimulated by androgens.

The use of GM-CSF and G-CSF has been studied in single-arm prospective clinical trials with small numbers of FA patients[25,26]. The short term use of GM-CSF resulted in satisfactory increase of peripheral blood neutrophil counts in seven patients treated for 12 weeks[25]. Similarly, G-CSF has been shown to be effective in a series of ten patients. All ten patients showed an increase of their absolute neutrophil count (ANC) after eight weeks of treatment. Interestingly, four patients were also found to have an increase in their platelet count or hemoglobin level, indicating a potential multilineage effect of G-CSF. This effect was primarily observed in patients with the least severe bone marrow failure and was generally transient[26]. There have been no randomized studies comparing GM-CSF versus G-CSF in FA patients. In any case, over time, current supportive measures fail, necessitating allogeneic hematopoietic stem cell transplantation (HSCT) as definitive therapy.

The early experience with bone marrow transplantation in FA was less than optimal due to excessive toxicity when similar preparative conditioning regimens as those used in acquired aplastic anemia were applied to FA patients. These failures led to *in vitro* laboratory studies that confirmed the sensitivity of FA cells to cyclophosphamide and irradiation and subsequently to the development of low dose cyclophosphamide/thoracoabdominal irradiation conditioning protocol[27,28]. Currently, the 5-year survival rate after matched sibling transplant is approximately 85%[29,30]. However, taking into account the incidence of FA in siblings, <30% of FA patients have an HLA-identical sibling donor available. Results of unrelated donor transplantation, while traditionally less satisfactory than sibling donor transplants, have improved with the introduction of the anti-metabolite fludarabine to the preparative chemotherapy regimen[31,32] A retrospective review of 98 patients found significantly improved survival of patients who received fludarabine-containing regimens, achieving 52% overall survival at 3 years[22]. Fludarabine is a non-genotoxic anti-metabolite

and its profound immunosuppressive properties provide a significant reduction of the incidence of graft failure without increasing regimen-related end organ toxicity[22].

However, despite these advances, there continues to be the need for novel therapeutic approaches. Autologous hematopoietic stem cells, genetically corrected via the introduction of the appropriate wt FA gene, offer the potential of hematopoietic reconstitution in the absence of immune barriers. The success of gene therapy approaches to FA in a clinical setting has been limited by a combination of factors, including the paucity of autologous CD34+ cells available as targets for gene correction, reservations about the use of cytoreductive therapy to "make space" for the autologous graft, and potentially defective engraftment of FA HSC following prolonged *in vitro* processing[33–35].

4. The coming of age of gene therapy: From the bench to the bed-side and back

When the notion of using viral gene transfer vectors as delivery vehicles for "therapeutic" genes of interest was first conceived over two decades ago, it was met with a high level of enthusiasm and the expectation that this technology could be harnessed to cure many monogenic diseases in the future. As with most novel therapeutic modalities, gene therapy has produced mixed results of success and failure as promising pre-clinical approaches were translated into early phase I clinical trials. The general principal of gene therapy targeting the hematopoietic system involves the isolation of CD34+ HSC/P from the patient, followed by *ex vivo* gene transfer and re-infusion of the cells into the patient. Re-infusion has been described with[36,37]and without[38,39]the prior administration of myelosuppressive chemotherapy, aimed at "making space" in the bone marrow to facilitate the engraftment of the gene-modified cells.

Most published gene therapy trials to date have utilized vectors derived from the moloney murine leukemia virus (MLV), a gammaretrovirus with strong enhancer-promoters in the long terminal repeats (LTRs), driving the expression of the therapeutic gene of interest[36–40]. The first success in clinical gene therapy was reported in 2000 by a French group, detailing the reconstitution of near normal immunity in two patients with X-linked SCID (SCID-X1), a severe immunodeficiency caused by a defective gene encoding the common cytokine-receptor γ chain[38]. This was followed by other reports from Italy and the United Kingdom, detailing successful gene therapy trials involving patients affected by SCID due to adenosine desaminase-deficiency (ADA) and SCID-X1[37,39]. However, following an initial period of successful reconstitution of immune function in the vast majority of the now >30 patients treated on these clinical trials, unexpected severe adverse events have occurred in 5 patients with SCID-X1. Initially, two patients developed a T-cell acute lymphoblastic leukemia (T-ALL)-like lymphoproliferative disease nearly three years following cell infusion[41]. Two additional patients in the French trial and one patient in the British SCID-X1 trial have subsequently been reported as having experienced similar adverse events[42,43]. These severe adverse events are now understood to result from "side effects" of viral integration into the genome. The LTR harbors strong promoter/enhancer activity and shows a propensity to the up-regulate genes neighboring the integration site[44–46]. Retroviral vectors may thus "turn-on" cellular proto-oncogenes adjacent to their integration site in the genome. Multiple studies now indicate that gammaretroviral vectors such as the vectors used in the two SCID-X1 trials preferentially integrate into the 5′ end of genes, near the transcription start sites[47]. Gammaretroviral vectors have also been shown to integrate in or near a number of proto-oncogenes that are actively expressed in human CD34+ cells. For example, when human CD34+ cells were transduced with retroviral vectors *in vitro*, 21% of retroviral integrations occurred at recurrent insertion sites ("hot spots"), which were highly enriched for proto-oncogenes and growth-controlling genes[48]. In this regard, retroviral integration in the proximity of proto-

oncogenes, particularly the LIM domain only 2 (LMO2) promoter, has been shown to be involved in leukemogenesis in 3 of the French patients and one British patient[41,43,49]. The occurrence of insertional mutagenesis in these clinical trials has led to a concerted effort to develop a newer generation of gene transfer vectors with enhanced safety features. Emerging evidence indicates that advanced retroviral gene transfer vector design may be able to reduce the incidence of transforming genotoxic events. Specifically, removing the strong retroviral enhancer/promoter sequences from the LTR and placing the promoter sequence into an internal position (self inactivating (SIN) vector), has been shown to reduce the insertional transformation of target cells in an *in vitro* immortalization assay. [45]. Additionally, for applications that do not require very high levels of transgene expression, weaker (cellular) internal enhancer/promoters may further reduce the incidence and severity of insertional side effects[50]. Insulator elements, incorporated into the LTR, may also reduce the genotoxic potential of retroviral vectors[51]. Finally, a retroviral vector system based on the human immunodeficiency virus, the lentiviral vector system, is capable of delivering the therapeutic pay load to non-dividing cells[52]. This offers the possibility of reduced cytokine stimulation of HSC *in vitro* and shortened transduction protocols[33,53,54]. Lentiviral vectors have been shown to integrate less frequently into promoter-proximal regions, providing a theoretic safety advantage[55]. While these modifications hold the promise of reduced genotoxicity, their efficacy and safety has yet to be established in clinical trials.

5. Rational for Fanconi anemia gene therapy

As a monogenic disorder with primary disease manifestation in hematopoietic system, Fanconi anemia is viewed as a candidate disease for gene therapy. In contrast to allogeneic hematopoietic stem cell transplantation (HSCT), the use of genetically corrected autologous HSC/P would be predicted to result in fewer consequences of immunological barriers such as delayed engraftment and graft versus host disease. Lessons from successful clinical gene therapy trials for immunodeficiencies indicate that a selective advantage of corrected HSC or the HSC progeny over uncorrected cells promotes the efficacy of gene therapy[37–39]. In FA, the high incidence of somatic mosaics in up to 10% of patients may point towards a selective advantage of genetically fit wt HSC[56–59]. In several FA patients harboring somatic reversions in hematopoietic cells, improved blood counts have been described. This form of "natural gene therapy" may occur though one of several mechanisms, including back-mutation, intragenic recombination, or second site compensation mutations[57,58]. In the context of gene therapy, these observations suggest that the engraftment of a very limited number of corrected, long term repopulating hematopoietic stem cells may confer a therapeutic effect.

The development of clinical FA gene therapy trials has been aided by the availability of murine models of FA. Mice with targeted disruptions of *Fanca* and *Fancc*, the two most prevalent genotypes in humans, have been studied in this regard[33,60–62]. While these models fail to faithfully recapitulate all aspects of the human disease, bone marrow cells do show the characteristic hypersensitivity towards DNA damaging agents such as diepoxybutane (DEB), mitomycin C (MMC), cyclophosphamide, and ionizing irradiation. Unlike humans, mice with disruptions of FA genes do not develop a bone marrow failure phenotype under steady state conditions[63]. *Ex vivo* gene transfer into HSC of gene targeted FA mice has been shown to correct the cellular FA phenotype at the level of long term repopulating stem cells [33,60–62]. Genetically corrected cells have been shown to achieve enhanced engraftment and a modest selective advantage over uncorrected cells[64–66]. Pharmacologic *in vivo* selection of corrected HSC with MMC and cyclophosphamide has been demonstrated to confer powerful selection *in vivo* but has limited translational relevance due to the increased sensitivity of FA patients to DNA damaging agents[62,65]. The use of *in vivo* selection conferred by the O^6-methylguanine-DNA-methyltransferase (MGMT) chemoresistance gene as well as nongenotoxic agents such as interferon-γ (INF-γ) has also been proposed[19,33]. While limited

in their ability to recapitulate the complex phenotype and clinically challenges of FA patients, FA knock-out mice continue to provide a powerful platform for assessing and optimizing novel gene therapy approaches.

6. Mobilization of FA hematopoietic stem cells

Current gene therapy strategies require harvest and purification of autologous hematopoietic CD34+ stem and progenitor cells (HSC/P) as targets for gene transfer. Both peripheral blood and bone marrow human CD34+ cells are enriched for HSC/P and capable of trilineage hematopoietic reconstitution upon transplantation [67,68]. Mobilization and collection of peripheral blood and bone marrow CD34+ cells in FA patients poses particular challenges related to the nature of this bone marrow failure disease [34,35,69]. Overall, the CD34+ collection yields in FA patients are below the expected yield of healthy individuals with a high degree of intra-patient variability. Increasing evidence demonstrates that the bone marrow HSC/P content is reduced in FA patients, even prior to the onset of overt bone marrow failure. This notion is supported by the observation of a profound reduction in colony forming progenitors in the cord blood of a child with FA[70]. Rackoff et al. prospectively investigated the use of G-CSF to mobilize HSC/P in 10 patients with FA. While the primary goal of this study was to demonstrate the feasibility of treatment of cytopenia in FA, the investigators also observed an increase of the bone marrow and peripheral blood contents of CD34+ cells in 8 patients. This response was variable, with a 3- to 18-fold increase in peak CD34+ detected in the peripheral blood[26]. This study demonstrated the feasibility of using G-CSF at a dose range of 5–10 μg/kg/day for the treatment of neutropenia and was followed by other studies aiming to procure CD34+ cells for re-infusion and as targets for gene therapy. With regard to high-dose G-CSF, doses of 16–20 μg/kg daily for four days has been well tolerated by healthy volunteer donors undergoing leukapheresis for granulocyte transfusions[71]. Still higher G-CSF doses of 24–32 μg/kg/day have been used in children with severe congenital neutropenia (Kostmann's Syndrome) or patients who have been heavily pre-treated with myeloablative chemotherapy[72–76]. In a second study of patients with FA, treatment with 10 μg/kg G-CSF for 6–14 days, resulted in the goal level of ≥ 6 CD34+ cells per μl peripheral blood being mobilized in six patients and the collection of 2×10^6 CD34+ cells/Kg of target weight in four of eight patients[69]. However, mobilization and collection was achieved only with extended G-CSF treatment and multiple days of apheresis, reducing the practical feasibility and raising questions regarding the ability of the CD34+ cells to engraft upon transplantation. Liu et al. enrolled four FA patients with complementation group C into a clinical gene therapy trial, seeking to collect CD34+ cells for gene therapy in four cycles of mobilization and collection. Each collection cycle involved a 5-day treatment with 10 μg/kg/day G-CSF followed by leukapheresis. CD34+ yields were highly variable in this study ranging from a failure to mobilize in one patient to the collection of 1.1, 1.9, and 1.3×10^6/kg CD34+ cells in separate mobilization cycles, values within the range used for autologous hematopoietic rescue following high-dose chemotherapy, in another study subject[34].

More recently, Kelly et al. undertook a prospective analysis of the bone marrow CD34+ content of eighteen FA patients, aged 1.5–26 years, with mild peripheral blood cytopenias (absolute neutrophil count >750/mm^3, Hb > 8 mg/dl, platelets > 30,000/mm^3). Seven patients were enrolled in the collection arm with the dual intention of isolating CD34+ cells for future autologous re-infusion and potentially corrective gene transfer into CD34+ of complementation group A patients. An initial diagnostic bone marrow aspirate indicated that the goal of 2×10^6 CD34+ cells per Kg (based on estimated weight 5 years in the future) could be achieved in four of seven subjects. The actual number of nucleated cells in the bone marrow collection ranged from 1.4 to 3×10^8/kg and was within the range predicted by the bone marrow analysis at study entry. However, the frequency of CD34+ cells in the marrow collections, which ranged from 0.14 to 1.5%, was lower than the entry marrow aspirate analysis suggested

in six of seven subjects. Thus, the target CD34+ cell dose of 2×10^6 per kg future weight was not obtained in any patient. However, three of four subjects achieved >80% of the collection goal based on future weight and all four attained collection of 2×10^6 CD34+ cells/kg based on their weight at study entry. Of note, two patients treated with G-CSF (16μg/kg of G-CSF for four days followed by an additional four days of 32μg/kg) yielded bone marrow CD34+ cell collections that exceeded the predicted collection based on entry marrow aspirate analysis. G-CSF was well tolerated and the peripheral blood ANC increased in these two study subjects (from 780/mm^3 to 14,000/mm^3 and from 1000/mm^3 to 11,700/mm^3, respectively). Overall, compared to normal controls, FA patients had a 2–fold reduction in marrow cellularity and a 6-fold reduction in CD34+ cells. Accordingly, a significant reduction in the bone marrow progenitor content was noted *in vitro*[35]. Taken together, these findings highlight the relative paucity of CD34+ cells in FA patients as a major rate limiting factor for successful implementation of gene therapy strategies.

7. Clinical experience with Fanconi anemia gene therapy

Despite considerable challenges related to the procurement of autologous CD34+ HSC/P, two clinical gene therapy trials involving a total of 7 patients with *FANCA* and *FANCC* mutations have been conducted and these studies established the feasibility of gammaretroviral gene transfer of FA genes into human hematopoietic progenitor cells[34,35]. In both trials, transduction rates *in vitro* were judged to be adequate based on experience in other successful human gene therapy trials, ranging between 10–20% in the initial trial[34] and 42–62% in a more recent trial, which employed state of the art gene transfer protocols, including the use serum free medium and Retronectin™ [35]. Transduced autologous cells were infused into the patients without prior conditioning chemotherapy. While vector sequences were detectable transiently in peripheral blood and bone marrow mononuclear cells of several patients in both trials, neither trial achieved prolonged engraftment of gene modified HSC[34,35]. Interestingly, despite this lack of engraftment, transient improvements in bone marrow cellularity and peripheral blood counts were noted in several patients. However, it is likely that these hematologic improvements are the result of re-infusion of autologous “expanded” CD34 + derived progenitor cells rather than a result of gene therapy. Of note, the cell doses infused in both trials were up to 50-fold less than in previous clinically efficacious gene therapy trials for immunodeficiencies[37–39]. Combined with the lack of preparative chemotherapy, the relatively modest number of transduced CD34+ cells infused almost certainly contributed to the lack of long term engraftment of gene modified cells in both trials. In addition, the predicted selective advantage of genetically corrected HSC over *FANC*-deficient HSC has not been observed in either trial. Nonetheless, Liu et al. reported the emergence of detectable vector sequences in the peripheral blood and bone marrow of a 29 year-old study patient who developed a squamous carcinoma of the vulva and received 4500 cGy of fractionated pelvic irradiation. This malignancy occurred after the gene therapy treatment but was thought to be unrelated. The radiation treatment induced prolonged cytopenia and following hematopoietic recovery, approximately 220 days after the cell infusion, gene-positive peripheral blood and bone marrow nuclear cells were detected by sensitive PCR methods for the first time. This observation might suggest the potential for positive selection, mediated by ionizing radiation, of a transduced progenitor cell[34].

8. Future directions

Fanconi anemia HSCs have proven to be the proverbial needle in the hay stack. Improving cell-based therapies for FA will likely require a combination of several strategies (Table 1). Despite evidence pointing to the role of **pro-apoptotic** cytokines [14,15], the precise mechanism by which HSC are progressively lost in children with FA remains an enigma. Supportive care using hematopoietic growth factors or androgens to boost blood cell production

provides transient benefit in some patients, delaying but not obviating the need for more definitive treatment [23–26]. Thus far, allogeneic hematopoietic stem cell transplantation remains the gold standard approach to advanced marrow failure in FA patients. A better understanding of the extra-hematopoietic toxicity of the preparative chemotherapy regimen, enhanced supportive care, and increasing experience of specialized transplant centers has led to improved outcomes of allogeneic transplantation [22].

Evidence implicating **pro-apoptotic** cytokines such as TNF-α and INF-γ in the pathogenesis of marrow failure and leukemic transformation in FA points towards a potential opportunity for therapeutic intervention. Etanercept is a dimeric form of the TNF-α receptor, consisting of a fusion protein of the extra-cellular ligand-binding domain of the human TNF-α receptor with the Fc portion of human IgG1[77]. Etanercept has been shown to be efficacious in rheumatologic diseases involving TNF-α dysregulation, including rheumatoid arthritis and juvenile rheumatoid arthritis[78,79]. It is conceivable that long term treatment of FA patients with TNF-α antagonists such as etanercept might delay or abrogate the development of marrow failure and acute myeloid leukemia. Long term etanercept administration in FA patients is currently under investigation in at least one phase I trial.

FA HSCs appear to be reduced in number early in life, even prior to the onset of overt marrow failure[35,70]. This poses substantial challenges to the collection of autologous HSC/Ps for gene therapy. However, advances in the technology of gene transfer in recent years highlight the potential utility of this approach. To date, >30 patients with primary immunodeficiencies have been treated with good results[80]. While some of the immunodeficiency gene therapy trials have been complicated by adverse events related to insertional mutagenesis[41–43], the understanding of these events has grown considerably in recent years and this knowledge is now leading to the next generation of gene therapy trials with safety modified gene transfer vectors. It is likely that a specific subgroup of Fanconi anemia patients will benefit from cell and gene therapy with autologous hematopoietic stem cells in the future as this approach avoids the immunologic barriers associated with allogeneic HSCT. Strategies that will aide the realization of successful FA gene therapy are likely to involve enhanced mobilization, preservation, engraftment, and expansion of HSC/Ps.

As it relates to HSC/P mobilization, G-CSF remains the gold-standard in clinical transplantation. However, broad inter-individual variability of efficacy exists in normal healthy subjects, patients pre-treated with chemotherapy, and patients with Fanconi anemia[34,35, 69,81]. This has led to the investigation of alternative mobilization agents. One such agent, AMD 3100, is a competitive small-molecule antagonist of the interaction of stromal derived factor 1 (SDF-1) and the chemokine receptor CXCR4 [82]. SDF-1, a chemoattractant produced by BM stromal cells, is a ligand for CXCR4, expressed on HSC/P, and is implicated in chemotaxis, homing, and survival of HSC/P[83–85]. Thus, antagonizing the interaction of SDF-1 with CXCR4 on HSCs has been shown to result in HSC mobilization in healthy human volunteers and in patients with multiple myeloma and non-Hodgkin's lymphoma who had received previous chemotherapy[82,86]. In addition, AMD3100 plus G-CSF has been shown to successfully mobilize CD34+ cells from cancer patients previously failing mobilization with chemotherapy and/or cytokine treatment[87]. In a murine model of FA genotypes A and C, the combination of G-CSF and AMD3100 has been shown to synergistically mobilize HSC with a greater than additive effect on long-term BM repopulation and self-renewal capacity [88]. The combination of AMD3100 and G-CSF may prove to be advantageous with regard to mobilizing sufficient numbers of CD34+ cells for gene therapy applications and is currently in phase I trial in FA patients.

With regard to HSC engraftment, recent work has focused on the role of the peptidase CD26 (DPPIV/dipeptidylpeptidase IV). CD26 is a membrane bound extracellular peptidase which is

found on a subset of hematopoietic cells and cleaves dipeptides from the N-terminus of polypeptide chains, including SDF 1, rendering the chemokine inactive[89–91]. The tri-peptide Diprotin A (Ile-Pro-Ile) is a specific inhibitor of CD26[89] and the inhibition of CD26 by short term exposure of the donor cells to Diprotin A *in vitro* has been shown to increase transplantation efficiency in syngeneic murine and human xenotransplantation models[91, 92]. Diprotin A has also been shown to enhance the engraftment of murine HSC following *ex vivo* retroviral gene transfer[93].

Similarly, North et al. has recently identified chemicals that enhance prostaglandin (PG) E2 synthesis which appear to preserve or expand HSC in a screening assay utilizing the zebra fish system[94]. Exposing murine whole bone marrow *in vitro* to a long acting derivative of prostaglandin E2 (dmPGE2) prior to transplantation resulted in a 3-fold increase of day-12 spleen colony forming units, indicative of an increase in HSC, and a 2.3-fold increase in long term engraftment in competitive limiting –dilution transplantation assays in the mouse[94]. Neither Diprotin A or dmPGE2 have been evaluated in humans or in the context of FA, but it is conceivable that the use of pro-HSC engraftment compounds may prove beneficial in diseases with limited cells available for gene therapy and transplantation.

We recently reported the advantages of rapid lentiviral over conventional gammaretroviral transduction in a murine model of FA type A[33]. This approach was informed by the observation that murine FA HSCs are prone to excessive apoptosis and the development of cytogenetic aberrations during *in vitro* culture under conditions that promote stem-cell/ progenitor proliferation[64]. This is particularly relevant as a disease-specific challenge in FA gene therapy, because a common feature of conventional gammaretroviral transduction protocols employed in previous gene transfer protocols involve cytokine prestimulation with the goal of inducing HSC proliferation, an essential prerequisite for gammaretroviral integration [95]. In both previous FA gene therapy trials, *in vitro* culture of FA HSCs lasted up to 84 hours[34,35]. Lentiviral vectors are capable of transducing nondividing cells, allowing for shortened *ex vivo* culture durations while maintaining efficient gene transfer to long-term repopulating cells[96]. We demonstrated significantly enhanced engraftment of transduced long term repopulating HSCs following rapid lentiviral transduction transduction[33]. Our observation was recently confirmed by Si at al. in a report demonstrating the superior engraftment of *Fancc*−/− HSC/P following rapid transduction with a foamy virus gene transfer vector [97].

Thus, improvements in FA gene therapy are likely to result from a combination of advances, including the use of compounds that enhance HSC mobilization, expansion and engraftment in addition to optimized shortened transduction with lentiviral gene transfer vectors.

Finally, the use of pluripotent embryonic stem (ES) cells to generate HSC provides a potential means of obtaining limitless numbers of HSC [98]. More recently, the introduction of four reprogramming factors into somatic cells has been demonstrated to yield cells that act like ES cells, termed induced pluripotent stem (IPS) cells. IPS cells have been generated from somatic murine [99] and human[100–102] cells. IPS cells offer the potential opportunity for tissue engineering, including hematopoietic stem cells, in a patient specific manner and without many of the ethical concerns surrounding other approaches, which require the use of human oocytes or embryos[103]. A recent paper by Hanna et al. establishes **prove**-of- principal for the correction of an inherited blood disorder with this approach by demonstrating the derivation of engraftable hematopoietic stem cells generated *in vitro* from autologous iPS cells in a humanized sickle cell anemia mouse model[104]. This approach may proof useful in the context of genetic bone marrow failure syndromes like Fanconi anemia, as fibroblast-derived IPS cells present an unlimited source of material from which patient specific HSC could be derived. The feasibility of deriving IPS cells from a panel of human subjects with various

genetic diseases has recently been shown[103]. However, no data on the applicability of IPS technology to Fanconi anemia has been published to date and many technological barriers remain[99].

In summary, the outlook for FA patients has improved dramatically in recent years, mostly as a result of advances in clinical stem cell transplantation. As our understanding of the complex molecular and cellular pathophysiology of FA continues to increase, novel cell and gene therapy approaches may hold additional promise for patients affected by this disorder.

Acknowledgments

This work was supported by a fellowship grant from the St. Baldrick's Foundation (to L.M.) and NIH R01 HL081499 and DK074310 (to D.A.W.).

References

1. Alter BP, Rosenberg PS. Granulocyte colony-stimulating factor and severe aplastic anemia. Blood 2007;109:4589. author reply 4589–4590. [PubMed: 17483301]
2. Kutler DI, Singh B, Satagopan J, Batish SD, Berwick M, Giampietro PF, Hanenberg H, Auerbach AD. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). Blood 2003;101:1249–1256. [PubMed: 12393516]
3. Butturini A, Gale RP, Verlander PC, Adler-Brecher B, Gillio AP, Auerbach AD. Hematologic abnormalities in Fanconi anemia: an International Fanconi Anemia Registry study. Blood 1994;84:1650–1655. [PubMed: 8068955]
4. Scheinberg P, Wu CO, Nunez O, Young NS. Long-term outcome of pediatric patients with severe aplastic anemia treated with antithymocyte globulin and cyclosporine. J Pediatr 2008;153:814–819. [PubMed: 18672253]
5. Smogorzewska A, Matsuoka S, Vinciguerra P, McDonald ER 3rd, Hurov KE, Luo J, Ballif BA, Gygi SP, Hofmann K, D'Andrea AD, Elledge SJ. Identification of the FANCI protein, a monoubiquitinated FANCD2 paralog required for DNA repair. Cell 2007;129:289–301. [PubMed: 17412408]
6. Andreassen PR, D'Andrea AD, Taniguchi T. ATR couples FANCD2 monoubiquitination to the DNA-damage response. Genes Dev 2004;18:1958–1963. [PubMed: 15314022]
7. Taniguchi T, Garcia-Higuera I, Xu B, Andreassen PR, Gregory RC, Kim ST, Lane WS, Kastan MB, D'Andrea AD. Convergence of the fanconi anemia and ataxia telangiectasia signaling pathways. Cell 2002;109:459–472. [PubMed: 12086603]
8. Taniguchi T, D'Andrea AD. Molecular pathogenesis of Fanconi anemia: recent progress. Blood 2006;107:4223–4233. [PubMed: 16493006]
9. D'Andrea AD, Grompe M. The Fanconi anaemia/BRCA pathway. Nat Rev Cancer 2003;3:23–34. [PubMed: 12509764]
10. Kalb R, Neveling K, Hoehn H, Schneider H, Linka Y, Batish SD, Hunt C, Berwick M, Callen E, Surralles J, Casado JA, Bueren J, Dasi A, Soulier J, Gluckman E, Zwaan CM, van Spaendonk R, Pals G, de Winter JP, Joenje H, Grompe M, Auerbach AD, Hanenberg H, Schindler D. Hypomorphic mutations in the gene encoding a key Fanconi anemia protein, FANCD2, sustain a significant group of FA-D2 patients with severe phenotype. Am J Hum Genet 2007;80:895–910. [PubMed: 17436244]
11. Dufour C, Rondelli R, Locatelli F, Miano M, Di Girolamo G, Bacigalupo A, Messina C, Porta F, Balduzzi A, Iorio AP, Buket E, Madon E, Pession A, Dini G, Di Bartolomeo P. Stem cell transplantation from HLA-matched related donor for Fanconi's anaemia: a retrospective review of the multicentric Italian experience on behalf of AIEOP-GITMO. Br J Haematol 2001;112:796–805. [PubMed: 11260086]
12. Nistico A, Young NS. gamma-Interferon gene expression in the bone marrow of patients with aplastic anemia. Ann Intern Med 1994;120:463–469. [PubMed: 8311369]
13. Schultz JC, Shahidi NT. Tumor necrosis factor-alpha overproduction in Fanconi's anemia. Am J Hematol 1993;42:196–201. [PubMed: 8438880]

14. Rosselli F, Sanceau J, Gluckman E, Wietzerbin J, Moustacchi E. Abnormal Lymphokine Production: A Novel Feature of the Genetic Disease Fanconi Anemia. II. In Vitro and In Vivo Spontaneous Overproduction of Tumor Necrosis Factor Alpha. Blood 1994;83:1216–1225. [PubMed: 8118026]
15. Dufour C, Corcione A, Svahn J, Haupt R, Poggi V, Beka'ssy AN, Scime R, Pistorio A, Pistoia V. TNF-alpha and IFN-gamma are overexpressed in the bone marrow of Fanconi anemia patients and TNF-alpha suppresses erythropoiesis in vitro. Blood 2003;102:2053–2059. [PubMed: 12750172]
16. Rathbun RK, Faulkner GR, Ostroski MH, Christianson TA, Hughes G, Jones G, Cahn R, Maziarz R, Royle G, Keeble W, Heinrich MC, Grompe M, Tower PA, Bagby GC. Inactivation of the Fanconi anemia group C gene augments interferon-gamma-induced apoptotic responses in hematopoietic cells. Blood 1997;90:974–985. [PubMed: 9242526]
17. Whitney MA, Royle G, Low MJ, Kelly MA, Axthelm MK, Reifsteck C, Olson S, Braun RE, Heinrich MC, Rathbun RK, Bagby GC, Grompe M. Germ cell defects and hematopoietic hypersensitivity to gamma-interferon in mice with a targeted disruption of the Fanconi anemia C gene. Blood 1996;88:49–58. [PubMed: 8704201]
18. Haneline LS, Broxmeyer HE, Cooper S, Hangoc G, Carreau M, Buchwald M, Clapp DW. Multiple inhibitory cytokines induce deregulated progenitor growth and apoptosis in hematopoietic cells from Fac−/− mice. Blood 1998;91:4092–4098. [PubMed: 9596654]
19. Li X, Yang Y, Yuan J, Hong P, Freie B, Orazi A, Haneline LS, Clapp DW. Continuous in vivo infusion of interferon-gamma (IFN-gamma) preferentially reduces myeloid progenitor numbers and enhances engraftment of syngeneic wild-type cells in Fancc−/− mice. Blood 2004;104:1204–1209. [PubMed: 15113761]
20. Li J, Sejas DP, Zhang X, Qiu Y, Nattamai KJ, Rani R, Rathbun KR, Geiger H, Williams DA, Bagby GC, Pang Q. TNF-alpha induces leukemic clonal evolution ex vivo in Fanconi anemia group C murine stem cells. J Clin Invest 2007;117:3283–3295. [PubMed: 17960249]
21. Fanconi Anemia: Standards for Clincial Care. Fanconi Anemia Research Fund, Inc; Eugene, OR: 2003.
22. Wagner JE, Eapen M, MacMillan ML, Harris RE, Pasquini R, Boulad F, Zhang MJ, Auerbach AD. Unrelated donor bone marrow transplantation for the treatment of Fanconi anemia. Blood 2007;109:2256–2262. [PubMed: 17038525]
23. Shahidi NT, Diamond LK. Testosterone-induced remission in aplastic anemia of both acquired and congenital types. Further observations in 24 cases. N Engl J Med 1961;264:953–967. [PubMed: 13750594]
24. Diamond LK, Shahidi NT. Treatment of aplastic anemia in children. Semin Hematol 1967;4:278–288. [PubMed: 4294607]
25. Guinan EC, Lopez KD, Huhn RD, Felser JM, Nathan DG. Evaluation of granulocyte-macrophage colony-stimulating factor for treatment of pancytopenia in children with Fanconi anemia. J Pediat 1994;124:144–150. [PubMed: 8283365]
26. Rackoff WR, Orazi A, Robinson CA, Cooper RJ, Alter BP, Freedman MH, Harris RE, Williams DA. Prolonged administration of granulocyte colony-stimulating factor (filgrastim) to patients with Fanconi anemia: a pilot study. Blood 1996;88:1588–1593. [PubMed: 8781414]
27. Gluckman E, Devergie A, Dutreix J. Radiosensitivity in Fanconi anaemia: application to the conditioning regimen for bone marrow transplantation. Br J Haematol 1983;54:431–440. [PubMed: 6344915]
28. Berger R, Bernheim A, Gluckman E, Gisselbrecht C. In vitro effect of cyclophosphamide metabolites on chromosomes of Fanconi anaemia patients. Br J Haematol 1980;45:565–568. [PubMed: 7426437]
29. Gluckman E, Auerbach AD, Horowitz MM, Sobocinski KA, Ash RC, Bortin MM, Butturini A, Camitta BM, Champlin RE, Friedrich W, et al. Bone marrow transplantation for Fanconi anemia. Blood 1995;86:2856–2862. [PubMed: 7670120]
30. Farzin A, Davies SM, Smith FO, Filipovich A, Hansen M, Auerbach AD, Harris RE. Matched sibling donor haematopoietic stem cell transplantation in Fanconi anaemia: an update of the Cincinnati Children's experience. Br J Haematol 2007;136:633–640. [PubMed: 17367413]
31. Guardiola P, Pasquini R, Dokal I, Ortega JJ, van Weel-Sipman M, Marsh JC, Ball SE, Locatelli F, Vermylen C, Skinner R, Ljungman P, Miniero R, Shaw PJ, Souillet G, Michallet M, Bekassy AN, Krivan G, Di Bartolomeo P, Heilmann C, Zanesco L, Cahn JY, Arcese W, Bacigalupo A, Gluckman

E. Outcome of 69 allogeneic stem cell transplantations for Fanconi anemia using HLA-matched unrelated donors: a study on behalf of the European Group for Blood and Marrow Transplantation. Blood 2000;95:422–429. [PubMed: 10627445]

32. Chaudhury S, Auerbach AD, Kernan NA, Small TN, Prockop SE, Scaradavou A, Heller G, Wolden S, O'Reilly RJ, Boulad F. Fludarabine-based cytoreductive regimen and T-cell-depleted grafts from alternative donors for the treatment of high-risk patients with Fanconi anaemia. Br J Haematol 2008;140:644–655. [PubMed: 18302713]
33. Muller LU, Milsom MD, Kim MO, Schambach A, Schuesler T, Williams DA. Rapid Lentiviral Transduction Preserves the Engraftment Potential of Fanca(−/−) Hematopoietic Stem Cells. Mol Ther. 2008
34. Liu JM, Kim S, Read EJ, Futaki M, Dokal I, Carter CS, Leitman SF, Pensiero M, Young NS, Walsh CE. Engraftment of hematopoietic progenitor cells transduced with the Fanconi anemia group C gene (FANCC). Hum Gene Ther 1999;10:2337–2346. [PubMed: 10515453]
35. Kelly PF, Radtke S, Kalle C, Balcik B, Bohn K, Mueller R, Schuesler T, Haren M, Reeves L, Cancelas JA, Leemhuis T, Harris R, Auerbach AD, Smith FO, Davies SM, Williams DA. Stem cell collection and gene transfer in fanconi anemia. Mol Ther 2007;15:211–219. [PubMed: 17164793]
36. Ott MG, Schmidt M, Schwarzwaelder K, Stein S, Siler U, Koehl U, Glimm H, Kuhlcke K, Schilz A, Kunkel H, Naundorf S, Brinkmann A, Deichmann A, Fischer M, Ball C, Pilz I, Dunbar C, Du Y, Jenkins NA, Copeland NG, Luthi U, Hassan M, Thrasher AJ, Hoelzer D, von Kalle C, Seger R, Grez M. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. Nat Med 2006;12:401–409. [PubMed: 16582916]
37. Aiuti A, Slavin S, Aker M, Ficara F, Deola S, Mortellaro A, Morecki S, Andolfi G, Tabucchi A, Carlucci F, Marinello E, Cattaneo F, Vai S, Servida P, Miniero R, Roncarolo MG, Bordignon C. Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. Science 2002;296:2410–2413. [PubMed: 12089448]
38. Cavazzana-Calvo M, Hacein-Bey S, de Saint Basile G, Gross F, Yvon E, Nusbaum P, Selz F, Hue C, Certain S, Casanova JL, Bousso P, Deist FL, Fischer A. Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease [see comments]. Science 2000;288:669–672. [PubMed: 10784449]
39. Gaspar HB, Parsley KL, Howe S, King D, Gilmour KC, Sinclair J, Brouns G, Schmidt M, Von Kalle C, Barington T, Jakobsen MA, Christensen HO, Al Ghonaium A, White HN, Smith JL, Levinsky RJ, Ali RR, Kinnon C, Thrasher AJ. Gene therapy of X-linked severe combined immunodeficiency by use of a pseudotyped gammaretroviral vector. Lancet 2004;364:2181–2187. [PubMed: 15610804]
40. Kohn DB, Hershfield MS, Carbonaro D, Shigeoka A, Brooks J, Smogorzewska EM, Barsky LW, Chan R, Burotto F, Annett G, Nolta JA, Crooks G, Kapoor N, Elder M, Wara D, Bowen T, Madsen E, Snyder FF, Bastian J, Muul L, Blaese RM, Weinberg K, Parkman R. T lymphocytes with a normal ADA gene accumulate after transplantation of transduced autologous umbilical cord blood CD34+ cells in ADA- deficient SCID neonates. Nat Med 1998;4:775–780. [PubMed: 9662367]
41. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, Lim A, Osborne CS, Pawliuk R, Morillon E, Sorensen R, Forster A, Fraser P, Cohen JI, de Saint Basile G, Alexander I, Wintergerst U, Frebourg T, Aurias A, Stoppa-Lyonnet D, Romana S, Radford-Weiss I, Gross F, Valensi F, Delabesse E, Macintyre E, Sigaux F, Soulier J, Leiva LE, Wissler M, Prinz C, Rabbitts TH, Le Deist F, Fischer A, Cavazzana-Calvo M. LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 2003;302:415–419. [PubMed: 14564000]
42. Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, Clappier E, Caccavelli L, Delabesse E, Beldjord K, Asnafi V, Macintyre E, Dal Cortivo L, Radford I, Brousse N, Sigaux F, Moshous D, Hauer J, Borkhardt A, Belohradsky BH, Wintergerst U, Velez MC, Leiva L, Sorensen R, Wulffraat N, Blanche S, Bushman FD, Fischer A, Cavazzana-Calvo M. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest. 2008
43. Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempski H, Brugman MH, Pike-Overzet K, Chatters SJ, de Ridder D, Gilmour KC, Adams S, Thornhill SI, Parsley KL, Staal FJ, Gale RE, Linch DC, Bayford J, Brown L, Quaye M, Kinnon C, Ancliff P, Webb DK, Schmidt M, von Kalle C, Gaspar HB, Thrasher AJ. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest. 2008

44. Li Z, Dullmann J, Schiedlmeier B, Schmidt M, von Kalle C, Meyer J, Forster M, Stocking C, Wahlers A, Frank O, Ostertag W, Kuhlcke K, Eckert HG, Fehse B, Baum C. Murine leukemia induced by retroviral gene marking. Science 2002;296:497. [PubMed: 11964471]
45. Modlich U, Bohne J, Schmidt M, von Kalle C, Knob S, Schambach A, Baum C. Cell culture assays reveal the importance of retroviral vector design for insertional genotoxicity. Blood 2006;108:2545–2553. [PubMed: 16825499]
46. Kustikova O, Fehse B, Modlich U, Yang M, Dullmann J, Kamino K, von Neuhoff N, Schlegelberger B, Li Z, Baum C. Clonal dominance of hematopoietic stem cells triggered by retroviral gene marking. Science 2005;308:1171–1174. [PubMed: 15905401]
47. Wu X, Li Y, Crise B, Burgess SM. Transcription start regions in the human genome are favored targets for MLV integration. Science 2003;300:1749–1751. [PubMed: 12805549]
48. Cattoglio C, Facchini G, Sartori D, Antonelli A, Miccio A, Cassani B, Schmidt M, von Kalle C, Howe S, Thrasher AJ, Aiuti A, Ferrari G, Recchia A, Mavilio F. Hot spots of retroviral integration in human CD34+ hematopoietic cells. Blood 2007;110:1770–1778. [PubMed: 17507662]
49. Cavazzana-Calvo M, Fischer A. Gene therapy for severe combined immunodeficiency: are we there yet? J Clin Invest 2007;117:1456–1465. [PubMed: 17549248]
50. Zychlinski D, Schambach A, Modlich U, Maetzig T, Meyer J, Grassman E, Mishra A, Baum C. Physiological promoters reduce the genotoxic risk of integrating gene vectors. Mol Ther 2008;16:718–725. [PubMed: 18334985]
51. Emery DW, Yannaki E, Tubb J, Stamatoyannopoulos G. A chromatin insulator protects retrovirus vectors from chromosomal position effects. Proc Natl Acad Sci U S A 2000;97:9150–9155. [PubMed: 10908661]
52. Naldini L, Blomer U, Gallay P, Ory D, Mulligan R, Gage FH, Verma IM, Trono D. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector [see comments]. Science 1996;272:263–267. [PubMed: 8602510]
53. Mostoslavsky G, Kotton DN, Fabian AJ, Gray JT, Lee JS, Mulligan RC. Efficiency of transduction of highly purified murine hematopoietic stem cells by lentiviral and oncoretroviral vectors under conditions of minimal in vitro manipulation. Mol Ther 2005;11:932–940. [PubMed: 15922964]
54. Kurre P, Anandakumar P, Kiem HP. Rapid 1-hour transduction of whole bone marrow leads to long-term repopulation of murine recipients with lentivirus-modified hematopoietic stem cells. Gene Ther 2006;13:369–373. [PubMed: 16208421]
55. Montini E, Cesana D, Schmidt M, Sanvito F, Ponzoni M, Bartholomae C, Sergi LS, Benedicenti F, Ambrosi A, Di Serio C, Doglioni C, von Kalle C, Naldini L. Hematopoietic stem cell gene transfer in a tumor-prone mouse model uncovers low genotoxicity of lentiviral vector integration. Nat Biotechnol 2006;24:687–696. [PubMed: 16732270]
56. Lo Ten Foe JR, Kwee ML, Rooimans MA, Oostra AB, Veerman AJ, van Weel M, Pauli RM, Shahidi NT, Dokal I, Roberts I, Altay C, Gluckman E, Gibson RA, Mathew CG, Arwert F, Joenje H. Somatic mosaicism in Fanconi anemia: molecular basis and clinical significance. Eur J Hum Genet 1997;5:137–148. [PubMed: 9272737]
57. Gross M, Hanenberg H, Lobitz S, Friedl R, Herterich S, Dietrich R, Gruhn B, Schindler D, Hoehn H. Reverse mosaicism in Fanconi anemia: natural gene therapy via molecular self-correction. Cytogenet Genome Res 2002;98:126–135. [PubMed: 12697994]
58. Mankad A, Taniguchi T, Cox B, Akkari Y, Rathbun RK, Lucas L, Bagby G, Olson S, D'Andrea A, Grompe M. Natural gene therapy in monozygotic twins with Fanconi anemia. Blood 2006;107:3084–3090. [PubMed: 16397136]
59. Waisfisz Q, Morgan NV, Savino M, de Winter JP, van Berkel CG, Hoatlin ME, Ianzano L, Gibson RA, Arwert F, Savoia A, Mathew CG, Pronk JC, Joenje H. Spontaneous functional correction of homozygous fanconi anaemia alleles reveals novel mechanistic basis for reverse mosaicism. Nat Genet 1999;22:379–383. [PubMed: 10431244]
60. Liu G, Excoffon KJ, Benoit P, Ginzinger DG, Miao L, Ehrenborg E, Duverger N, Denefle PP, Hayden MR, Lewis ME. Efficient adenovirus-mediated ectopic gene expression of human lipoprotein lipase in human hepatic (HepG2) cells. Hum Gene Ther 1997;8:205–214. [PubMed: 9017424]

61. Yamada K, Ramezani A, Hawley RG, Ebell W, Arwert F, Arnold LW, Walsh CE. Phenotype correction of Fanconi anemia group A hematopoietic stem cells using lentiviral vector. Mol Ther 2003;8:600–610. [PubMed: 14529833]
62. Galimi F, Noll M, Kanazawa Y, Lax T, Chen C, Grompe M, Verma IM. Gene therapy of Fanconi anemia: preclinical efficacy using lentiviral vectors. Blood 2002;100:2732–2736. [PubMed: 12351379]
63. Cheng NC, van de Vrugt HJ, van der Valk MA, Oostra AB, Krimpenfort P, de Vries Y, Joenje H, Berns A, Arwert F. Mice with a targeted disruption of the Fanconi anemia homolog Fanca. Hum Mol Genet 2000;9:1805–1811. [PubMed: 10915769]
64. Li X, Le Beau MM, Ciccone S, Yang FC, Freie B, Chen S, Yuan J, Hong P, Orazi A, Haneline LS, Clapp DW. Ex vivo culture of Fancc−/− stem/progenitor cells predisposes cells to undergo apoptosis, and surviving stem/progenitor cells display cytogenetic abnormalities and an increased risk of malignancy. Blood 2005;105:3465–3471. [PubMed: 15644418]
65. Battaile KP, Bateman RL, Mortimer D, Mulcahy J, Rathbun RK, Bagby G, Fleming WH, Grompe M. In vivo selection of wild-type hematopoietic stem cells in a murine model of Fanconi anemia. Blood 1999;94:2151–2158. [PubMed: 10477746]
66. Haneline LS, Gobbett TA, Ramani R, Carreau M, Buchwald M, Yoder MC, Clapp DW. Loss of FancC function results in decreased hematopoietic stem cell repopulating ability. Blood 1999;94:1–8. [PubMed: 10381491]
67. Collins RH Jr. CD34+ selected cells in clinical transplantation. Stem Cells 1994;12:577–585. [PubMed: 7533577]
68. Henon PR. Peripheral blood stem cell transplantations: past, present and future. Stem Cells 1993;11:154–172. [PubMed: 8100462]
69. Croop JM, Cooper R, Fernandez C, Graves V, Kreissman S, Hanenberg H, Smith FO, Williams DA. Mobilization and collection of peripheral blood CD34+ cells from patients with Fanconi anemia. Blood 2001;98:2917–2921. [PubMed: 11698271]
70. Auerbach AD, Liu Q, Ghosh R, Pollack MS, Douglas GW, Broxmeyer HE. Prenatal identification of potential donors for umbilical cord blood transplantation for Fanconi anemia. Transfusion 1990;30:682–687. [PubMed: 2219253]
71. Martinez C, Urbano-Ispizua A, Marin P, Merino A, Rovira M, Carreras E, Montserrat E. Efficacy and toxicity of a high-dose G-CSF schedule for peripheral blood progenitor cell mobilization in healthy donors. Bone Marrow Transplant 1999;24:1273–1278. [PubMed: 10627634]
72. Kroger N, Rauhoft C, Zeller W, Renges H, Hassan HT, Kruger W, Schuch G, Wacker-Backhaus G, Colberg H, Kuse R, Gutensohn K, Loliger C, Zander AR. Efficacy of further attempts to mobilize CD34+ peripheral stem cells with alternative procedures after primary failure. Acta Haematol 2000;102:144–147. [PubMed: 10692678]
73. Kroger N, Zeller W, Fehse N, Hassan HT, Kruger W, Gutensohn K, Lolliger C, Zander AR. Mobilizing peripheral blood stem cells with high-dose G-CSF alone is as effective as with Dexa-BEAM plus G-CSF in lymphoma patients. Br J Haematol 1998;102:1101–1106. [PubMed: 9734664]
74. Jonkhoff AR, De Kreuk AM, Franschman G, Van Der Lelie J, Schuurhuis GJ, Drager AM, Zweegman S, Huijgens PC, Ossenkoppele GJ. Granulocyte colony-stimulating factor mobilized whole blood containing over 0.3 × 106/kg CD34+ cells is a sufficient graft in autologous transplantation for relapsed non-Hodgkin's lymphoma. Br J Haematol 2002;118:90–100. [PubMed: 12100131]
75. Perez-Duenas B, Alcorta I, Estella J, Rives S, Toll T, Tuset E. Safety and efficacy of high-dose G-CSF (24 microg/kg) alone for PBSC moblization in children. Bone Marrow Transplant 2002;30:987–988. [PubMed: 12476297]
76. Gazitt Y, Freytes CO, Callander N, Tsai TW, Alsina M, Anderson J, Holle L, Cruz J, Devore P, McGrath M, West G, Alvarez R, Montgomery W. Successful PBSC mobilization with high-dose G-CSF for patients failing a first round of mobilization. J Hematother 1999;8:173–183. [PubMed: 10349911]
77. Mohler KM, Torrance DS, Smith CA, Goodwin RG, Stremler KE, Fung VP, Madani H, Widmer MB. Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. J Immunol 1993;151:1548–1561. [PubMed: 8393046]

78. Moreland LW, Baumgartner SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL, Ettlinger RE, Cohen S, Koopman WJ, Mohler K, Widmer MB, Blosch CM. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. N Engl J Med 1997;337:141–147. [PubMed: 9219699]

79. Lovell DJ, Giannini EH, Reiff A, Cawkwell GD, Silverman ED, Nocton JJ, Stein LD, Gedalia A, Ilowite NT, Wallace CA, Whitmore J, Finck BK. Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. N Engl J Med 2000;342:763–769. [PubMed: 10717011]

80. Kohn DB. Gene therapy for childhood immunological diseases. Bone Marrow Transplant 2008;41:199–205. [PubMed: 17994122]

81. Steensma DP, Pardanani A, Stevenson WS, Hoyt R, Kiu H, Grigg AP, Szer J, Juneja S, Hilton DJ, Alexander WS, Roberts AW. More on Myb in myelofibrosis: molecular analyses of MYB and EP300 in 55 patients with myeloproliferative disorders. Blood 2006;107:1733–1735. author reply 1735. [PubMed: 16461764]

82. Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, Liles WC, Li X, Graham-Evans B, Campbell TB, Calandra G, Bridger G, Dale DC, Srour EF. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. J Exp Med 2005;201:1307–1318. [PubMed: 15837815]

83. Aiuti A, Webb IJ, Bleul C, Springer T, Gutierrez-Ramos JC. The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. J Exp Med 1997;185:111–120. [PubMed: 8996247]

84. Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, Nagler A, Ben-Hur H, Many A, Shultz L, Lider O, Alon R, Zipori D, Lapidot T. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science 1999;283:845–848. [PubMed: 9933168]

85. Broxmeyer HE, Kohli L, Kim CH, Lee Y, Mantel C, Cooper S, Hangoc G, Shaheen M, Li X, Clapp DW. Stromal cell-derived factor-1/CXCL12 directly enhances survival/antiapoptosis of myeloid progenitor cells through CXCR4 and G(alpha)i proteins and enhances engraftment of competitive, repopulating stem cells. J Leukoc Biol 2003;73:630–638. [PubMed: 12714578]

86. Devine SM, Flomenberg N, Vesole DH, Liesveld J, Weisdorf D, Badel K, Calandra G, DiPersio JF. Rapid mobilization of CD34+ cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin's lymphoma. J Clin Oncol 2004;22:1095–1102. [PubMed: 15020611]

87. Calandra G, McCarty J, McGuirk J, Tricot G, Crocker SA, Badel K, Grove B, Dye A, Bridger G. AMD3100 plus G-CSF can successfully mobilize CD34+ cells from non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma patients previously failing mobilization with chemotherapy and/or cytokine treatment: compassionate use data. Bone Marrow Transplant 2008;41:331–338. [PubMed: 17994119]

88. Pulliam AC, Hobson MJ, Ciccone SL, Li Y, Chen S, Srour EF, Yang FC, Broxmeyer HE, Clapp DW. AMD3100 synergizes with G-CSF to mobilize repopulating stem cells in Fanconi anemia knockout mice. Exp Hematol. 2008

89. Christopherson KW 2nd, Cooper S, Broxmeyer HE. Cell surface peptidase CD26/DPPIV mediates G-CSF mobilization of mouse progenitor cells. Blood 2003;101:4680–4686. [PubMed: 12576320]

90. Christopherson KW 2nd, Hangoc G, Broxmeyer HE. Cell surface peptidase CD26/dipeptidylpeptidase IV regulates CXCL12/stromal cell-derived factor-1 alpha-mediated chemotaxis of human cord blood CD34+ progenitor cells. J Immunol 2002;169:7000–7008. [PubMed: 12471135]

91. Campbell TB, Hangoc G, Liu Y, Pollok K, Broxmeyer HE. Inhibition of CD26 in human cord blood CD34+ cells enhances their engraftment of nonobese diabetic/severe combined immunodeficiency mice. Stem Cells Dev 2007;16:347–354. [PubMed: 17610364]

92. Christopherson KW 2nd, Hangoc G, Mantel CR, Broxmeyer HE. Modulation of hematopoietic stem cell homing and engraftment by CD26. Science 2004;305:1000–1003. [PubMed: 15310902]

93. Tian C, Bagley J, Forman D, Iacomini J. Inhibition of CD26 peptidase activity significantly improves engraftment of retrovirally transduced hematopoietic progenitors. Gene Ther 2006;13:652–658. [PubMed: 16341058]

94. North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM, Weber GJ, Bowman TV, Jang IH, Grosser T, Fitzgerald GA, Daley GQ, Orkin SH, Zon LI. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature 2007;447:1007–1011. [PubMed: 17581586]

95. Miller DG, Adam MA, Miller AD. Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. Mol Cell Biol 1990;10:4239–4242. [PubMed: 2370865]

96. Reiser J, Harmison G, Kluepfel-Stahl S, Brady RO, Karlsson S, Schubert M. Transduction of nondividing cells using pseudotyped defective high-titer HIV type 1 particles. Proc Natl Acad Sci U S A 1996;93:15266–15271. [PubMed: 8986799]

97. Si Y, Pulliam AC, Linka Y, Ciccone S, Leurs C, Yuan J, Eckermann O, Fruehauf S, Mooney S, Hanenberg H, Clapp DW. Overnight transduction with foamyviral vectors restores the long-term repopulating activity of Fancc−/− stem cells. Blood 2008;112:4458–4465. [PubMed: 18684868]

98. Kyba M, Perlingeiro RC, Daley GQ. HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. Cell 2002;109:29–37. [PubMed: 11955444]

99. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–676. [PubMed: 16904174]

100. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131:861–872. [PubMed: 18035408]

101. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin JA II, Thomson. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318:1917–1920. [PubMed: 18029452]

102. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 2008;451:141–146. [PubMed: 18157115]

103. Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, Lensch MW, Cowan C, Hochedlinger K, Daley GQ. Disease-Specific Induced Pluripotent Stem Cells. Cell. 2008

104. Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP, Beard C, Brambrink T, Wu LC, Townes TM, Jaenisch R. Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science 2007;318:1920–1923. [PubMed: 18063756]

105. Pasquini R, Carreras J, Pasquini MC, Camitta BM, Fasth AL, Hale GA, Harris RE, Marsh JC, Robinson AJ, Zhang MJ, Eapen M, Wagner JE. HLA-matched sibling hematopoietic stem cell transplantation for fanconi anemia: comparison of irradiation and nonirradiation containing conditioning regimens. Biol Blood Marrow Transplant 2008;14:1141–1147. [PubMed: 18804044]

106. Macmillan ML, Weisdorf DJ, Brunstein CG, Cao Q, Defor TE, Verneris MR, Blazar BR, Wagner JE. Acute graft-versus-host disease after unrelated donor umbilical cord blood transplantation: Analysis of risk factors. Blood. 2008

Table 1

Future directions for stem cell therapy of Fanconi anemia

Treatment approach	Status of pre-clinical or clinical development
Optimization of allogeneic bone marrow transplantation	Total body irradiation dose de-escalation is under investigation in unrelated donor HSCT [105,106]
Blocking of pro-apoptotic cytokines	Etanercept, a TNF-α decoy receptor [15], is under investigation in phase I clinical trial in GVHD following allogeneic transplantation
Use of alternative hematopoietic stem cell mobilization agents	AMD3100, a competitive small-molecule antagonist of the interaction of SDF-1 and CXCR4 [82,88], is currently under investigation in a phase I trial of CD34+ cell mobilization in FA
Enhancing HSC/P expansion or engraftment	Diprotin A [92] or prostaglanding E2 [94] have been shown to enhance engraftment or confer HSC expansion, respectively. A phase I clinical trial of PGE2 is currently ongoing.
Optimization of gene transfer technology	Shortened transduction has shown superior engraftment in pre-clinical studies [33,97]
Development of patient-specific pluripotent stem cells	Disease-specific induced pluripotent stem (iPS) cells closely resemble embryonic stem cells. iPS technology will require more pre-clinical development prior to clinical translation [100,101, reviewed in Müller LU et al., MTHE, in press]

HSCT: hematopoietic stem cell transplantation; TNF-α: Tumor necrosis factor alpha; SDF-1: stromal-derived-factor 1; HSC/P: hematopoietic stem and progenitor cells;