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ORIGINAL RESEARCH REPORT

Role of gene therapy in Fanconi anemia: A systematic and literature review with future directions

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Abstract
Gene therapy (GT) has been reported to improve bone marrow function in individuals with Fanconi anemia (FA); however, its clinical application is still in the initial stages. We conducted this systematic review, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, to assess the long-term safety and clinical outcomes of GT in FA patients. Electronic searches from PubMed, Web of Science, Cochrane Library, and Google Scholar were conducted and full texts of articles meeting our inclusion criteria were reviewed. Three clinical trials were included, with a total of nine patients and mean age of 10.7 ± 5.7 years. All patients had lentiviral-mediated GT. A 1-year follow-up showed stabilization in blood lineages, without any serious adverse effects from GT. A metaregression analysis could not be conducted, as very little long-term follow-up data of patients was observed, and the median survival rate could not be calculated. Thus, we can conclude that GT seems to be a safe procedure in FA; however, further research needs to be conducted on the longitudinal clin-

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ical effects of GT in FA, for a better insight into its potential to become a standard form of treatment.

Introduction

Fanconi Anemia (FA) is an inherited bone marrow failure (BMF) syndrome, characterized by congenital malformations, pancytopenia, cancer predisposition, and sensitivity to cross-linking agents [1]. To date, 22 genes have been implicated in FA, which code for ''FANC" proteins that recognize and repair DNA damage. Disease results from homozygous mutations in both alleles (autosomal recessive) of the specific *FANC (A-W)* gene, except for *FANCB* (X-linked), and *FANCR* (dominantly inherited), which encodes RAD51 [2,3].

Although androgens have been used to improve cytopenias caused by FA, the only current cure available is a hematopoietic cell transplant (HCT) [4,5]. Another evolving management strategy is gene therapy (GT), which can potentially improve BM function in FA patients, and help overcome limitations of HCT such as relapse and graft-versus-host disease (GVHD), which are associated with a high mortality and morbidity [6,7].

The regenerative nature of hematopoietic stem cells (HSCs) has potential for maximum regain of function and elimination of hematological abnormalities after genetic correction in FA [8]. In addition, mosaicism in FA patients allows for a natural reversion to normal hematopoiesis by providing these corrected stem cells with a strong repopulation and survival advantage in vivo over FA stem cells, making FA compelling for GT [9].

In gene addition, functional copies of the gene are added with the help of viruses, such as adenovirus or retrovirus. Retroviruses have the potential to transfer a specific FA gene, for example, *FANCC*, to HSCs [10,11]. Although gamma-retroviruses have been associated with genotoxicity [12,13], recent developments in lentiviral (LV) vectors have shown excellent safety profiles, with optimized transcriptional activity required for correction of hematopoietic progenitors in FA [14–16].

We undertook a systematic review to evaluate the totality of evidence for the role of GT in FA with respect to clinical and long-term outcomes.

Methods

This review follows the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) guidelines [17].

Selection criteria

Inclusion criteria

For types of studies: randomized control trials (RCT), clinical trials (CTs) or quasi-randomized trials, as well as retrospective studies, case reports, and case controls. For types of participants: individuals with FA of any age or sex, regardless of geography. For types of interventions: GT, with or without standard treatment of HCT. For time of publishing: studies published from 2005 to 2019.

Exclusion criteria

Papers were excluded if they were non-English literature, murine studies, or review articles.

Types of outcomes (attributable to and after GT)

Primary outcomes

The primary outcomes included: symptom-free survival (individuals alive and free of FA symptoms); serious adverse events (SAEs), including genotoxic events, death, inpatient admissions, life-threatening complications, or significant disability and impairment; long-term survival probability of individuals with FA.

Secondary outcomes

Secondary outcomes were post-infusion blood cell counts [hemoglobin (Hb), platelets (Plts), and neutrophils]; need for blood transfusions or HCT; and quality of life (QoL).

Search methods for identification of studies

Electronic searches implemented for each search engine included PubMed, Web of Science, Cochrane Library, and Google Scholar using the following search strategies:

PubMed: ((''Fanconi Anemia"[Mesh]) AND (''Genetic Therapy"[Mesh] OR ''Gene Editing"[Mesh] OR ''Gene Transfer"[Mesh])).
Web of Science: (''Fanconi Anemia") AND (''Genetic Therapy" OR ''Gene Editing").
Cochrane Library: (''Fanconi Anemia") AND (''Gene Editing" OR ''Gene Therapy").
Google Scholar: (''Fanconi Anemia") AND (''Genetic Therapy") and (''Fanconi Anemia") AND (''Genetic Editing").

The date of the most recent search was December 30, 2019.

Search for other resources

Other resources were screened through the reference lists of included studies to identify potentially relevant studies.

Data collection and analysis

Two authors independently undertook searches for eligible studies.

Selection of studies

Search results were compiled in referencing software (EndNote X8) and duplicates excluded. Two authors independently screened and filtered through the search results using the titles of the studies. A consecutive second screening using abstracts was performed, and full texts of articles that met our inclusion criteria were reviewed. Upon disagreement regarding the inclusion of an article, a consensus meeting with a third author was held.

Data extraction and management

Two authors independently extracted data from the included studies, using a structured data form, which included:

1. Study characteristics: title; authors; year of publication; journal name; study type; and sample size of patients.
2. Patients: gender; age at GT (years); mutation and specific protein type; baseline blood cell counts [Hb (g/dl), neutrophils (/μL) and Plts (10^3/μl)]; and baseline bone marrow CD34+ cells collected (10^6/kg) and colony forming cells (CFCs) (/μL).
3. Intervention: cryopreservation; cell mobilization; transduced/infused CD34+ cells; total nucleated cells (TNCs) infused; transduced CFCs; vector used and its specific subtype; number of transductions; vector copy number (VCN).
4. Follow-up (FU) after GT: post-infusion blood cell counts; serious adverse effects (SAEs) from the investigational therapy; CFC survival to mitomycin-C (MMC) pre- and post-infusion (%); and cytogenetic abnormalities post-infusion.

Dealing with missing data

If numerical data was not reported, or full texts did not provide sufficient information, efforts were made to contact the authors to request for necessary data for further analysis.

Assessment of risk of bias in the included studies

Two authors independently planned to assess the risk of bias for RCTs and other CTs from the Cochrane Risk of Bias (RoB) tool, using bias from randomization process, deviations from the intended interventions, missing outcome data, in measurement of the outcome, in selection of the reported result, and overall bias [18]. In case of controls or cohort studies, we planned to use the Newcastle Ottawa Scale (NOS), with the use of a ''star" system [19].

Measures of treatment effect

For continuous outcome data, we calculated the means and standard deviations (SD), using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA). If outcomes had different units, the standardized mean difference (SMD) and their associated 95% confidence intervals (CI) would be used.

Data synthesis

We planned to assess for clinical homogeneity between results, to see if a meta-analysis can be conducted. If not, a descriptive/qualitative analysis was considered to be the default.

Assessment of reporting biases

Assessment for publication bias was planned if at least 10 trials were included in our systematic review, using the funnel plot to check for presence of asymmetry. If it was present, then publication bias would be considered a possible factor in skewing the results [20].

Assessment of heterogeneity

Statistical heterogeneity would be calculated using the chi-square test for homogeneity, with $p < .1$ considered significant. The impact of statistical heterogeneity was quantified using I^2, which describes the percentage of total variation across studies attributable to heterogeneity rather than chance [21]. An I^2 value of $\geq$ 75% was considered as significant heterogeneity.

Results

Study selection

A total of 298 studies were identified from the resources described above. With duplicate removal, title screening, and a rigorous abstract and full-text review as per our inclusion criteria, a total of three open-label CTs met requirements for qualitative review (Fig. 1) [22—24]. We could not proceed for a quantitative review (meta-analysis) because of substantial heterogeneity between articles.

Study characteristics

Of the three CTs, two were available as full-text articles, whereas one was presented as a meeting abstract. A total of nine patients were therefore included in our study, with a mean age of 10.7 ± 5.7 years. Mean pre-infusion Hb, neutrophils, and Plts were 11.0 ± 1.6 (2 studies), 1,181 ± 525, and 61.1 ± 28.7, respectively. An average of 0.75 ± 0.4 (median, 0.71; 0.2—1.3) CD34+ cells were collected (2 studies) and transduced with an overall VCN of 0.59 ± 0.6 (2 studies). Shared features between these studies included patients with a *FANCA* mutation using an LV vector for GT; gene editing was not reported in any study. A limited follow-up (FU) period was reported in two of the studies. None of the three articles reported any SAEs from the investigational therapy, and there were no cytogenetic abnormalities reported in two studies after infusion. Neither symptom-free survival nor median survival rates were determined as no mortality was reported for any of the nine patients during their FU; also, no patients required HCT after GT, indicating a positive response to GT as a safe and low toxicity management solution for FA.

FIGURE 1: PRISMA FLOWCHART:

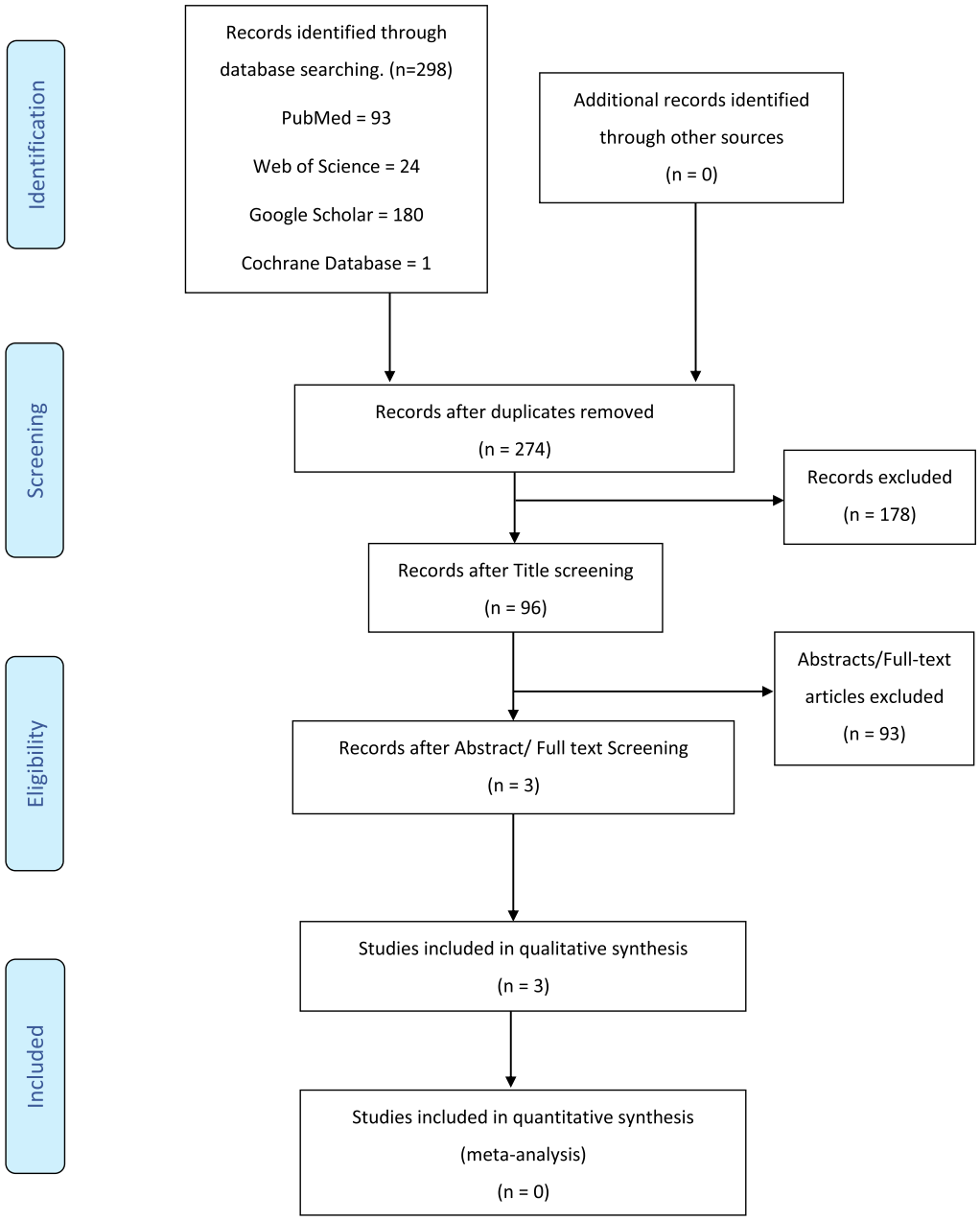

Fig. 1 PRISMA flowchart. *Note*. PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

The age range of participants in the study of Rio et al. [22] and Kelly et al. [23] was narrower compared with that in the study cohort of Adair et al. [24], with a gap of 2.4 years (7.6—5.2), 2.4 (15.5—13.1), and 12 (22—10), respectively. Whereas Kelly et al. [23] used two cycles of GT, others used one cycle of transduction, demonstrating an improvement in the methods and efficacy of GT over the years.

A common finding was increased survival of CFCs to MMC, which reflects chromosomal stability after transduction, as chromosomal fragility to this DNA cross-linking agent is a gold standard test for FA [25]. Kelly et al. [23] reported an average CFC survival after MMC (10 nM) administration of approximately 45%, in comparison to 31% in Rio et al.'s [22] study; however, these could not be compared statistically. All three studies had used different cell lines for the transduction process; hence, a true comparison of CFC survival could not be made. Also, age did not seem to be a factor in considering comparison of CFC survival after MMC administration.

FU hematological parameters were not available numerically in the work of Rio et al. [22] and Adair et al. [24], so it was not possible to statistically compare improvements in individual parameters. However, a 1-year FU of most patients showed stabilization in blood lineages. Table 1 summarizes all three studies.

Table 1 Summary of the three included studies.

Variables	Study 1	Study 2	Study 3
Title	Successful engraftment of gene-corrected hematopoietic stem cells in nonconditioned patients with Fanconi anemia	Stem cell collection and gene transfer in Fanconi anemia	Gene therapy for Fanconi anemia in Seattle: clinical experience and next steps
Authors	Rio et al.	Kelly et al.	Adair et al.
Year of publication	2019	2007	2016
Journal of publication	*Nature Medicine*	*Molecular Gene Therapy*	*Blood*. Conference: 58th Annual Meeting of the American Society of Hematology, ASH 2016
Study design	Clinical trial	Clinical trial	Clinical trial
Full text available	Yes	Yes	No (Abstract)
Sample size	4	3	2
Mean age (y)	5.85 ± 1.6	13.7 ± 1.6	16.0 ± 8.5
Cryopreservation	Yes (2 patients)	Yes (1 patient)	No
Cell mobilization	Yes (all; G-CSF and Plerixafor)	Yes (1 patient; G-CSF)	No
Baseline mean			
Hb (g/dL)	11.3 ± 0.9	10.7 ± 2.5	NP
Neutrophils (/μL)	1,235 ± 472	1,423 ± 672	710 ± 56.7
Plts ($\times 10^3$/μL)	48.0 ± 21.8	76.0 ± 40.1	61.0 ± 29.7
CD34+ **cells (10^6/kg)**	0.833 ± 0.3	0.183 ± 0.2	NP
CFC survival to 10 nM MMC (%)	0.025	0.0	NP
Vector used (specific subtype)	Lentiviral (PGK-FANCA-WPRE lentiviral)	Lentiviral (MSCV-FANCA lentiviral)	Lentiviral (NP)
LV transduction	3×10^8 IU/mL	NP	10 IU/cell
VCN	0.35 ± 0.2	NP	1.08 ± 1.1
Transduced/infused mean			
CD34+ **cells (10^5/kg)**	2.6 ± 1.07	NP	NP
CFCs (/kg)	$46.0 \times 10^3 \pm 76.1 \times 10^3$	NP	30.7%
TNC (10^5/kg)	NP	2.83 ± 2.1	NP
No. of transductions	1	2	1
Mean CFC survival after MMC administration after gene therapy (10 nM) (%)	31.0	44.7	NP
Follow-up period	18—30 mo	12 mo	NP
Follow-up cytogenetic abnormalities	None	None	NP
Serious adverse effects of investigational therapy	None	None	None
FU: blood cell lines improvement: (numerical/ description):	No	Yes (transient) 6 mo after infusion: 13.5 ± 0.7	NP
(Hb (g/dL)		NP	
Neutrophils (/μL)		90.0 ± 28.3	
Plts ($\times 10^3$/μL)	Yes	Yes	Yes
Stabilization			

Note. CFCs = colony forming cells; FU = follow-up; Hb = hemoglobin; LV = lentiviral; mo = month; NP = not provided; Plts = platelets; TNC = total nucleated cells; VCN = vector copy number.

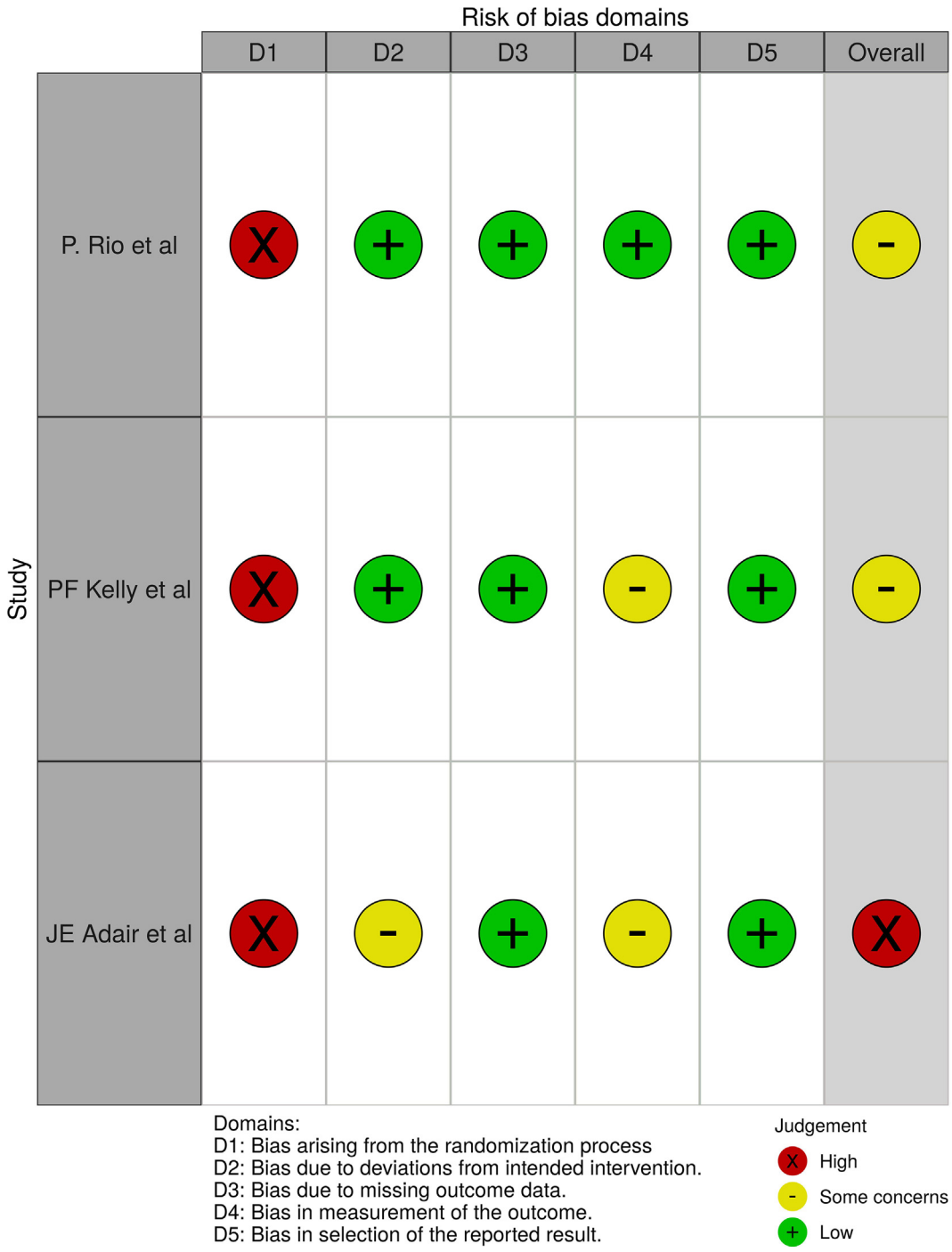

Fig. 2 Risk of bias assessment.

Risk of bias

With no randomized, case–control, or cohort studies available, assessment of risk of bias showed a poor score in many domains, with the overall quality of our review being low, typical of newer strategies (Fig. 2). Furthermore, as we only had three articles, we were unable to assess the publication bias of our studies.

Results of individual studies

Rio **et al.** [22] *(NCT03157804, ClinicalTrials.gov)*

In this study [22], four male patients, with a mean age of 5.85 ± 1.6 years, participated, as they met the inclusion criteria of at least one hematological parameter: Hb > 8, neutrophils > 750, or Plts > 30,000. No patient had been transfused for 3 months prior to being treated with mobilizing drugs. The mean baseline values for Hb, neutrophils, and

Table 2 Details of Rio et al. [22] study.

Variables	Patient 1	Patient 2	Patient 3	Patient 4
Age at HSC gene therapy	5.2	7.6	4.0	6.6
Mutation (protein)	FANCA:c295C > T; p. Gln99* (truncated protein)	FANCA; c.1115_1118delTTGG; p.V372AfsX42	FANCA; c295C > T;p. Gln99* (truncated protein)	FANCA; exon38: c.3788_3790del; p.Phe1263del & exon29: c.2851C > G; p. Arg951Gly)
Baseline Hb (g/dL)	10.5	10.8	12.5	11.3
Baseline neutrophils (/μL)	1,600	900	1,680	760
Baseline Plts ($\times 10^3$/μL)	29	46	38	79
Baseline bone marrow				
CD34+ **cells (/μL) at GT**	135	25.1	276.1	34.1
CD34+ **cells (10^6)/kg**	0.55	0.71	1.30	0.77
Baseline CFCs (/μL)	2.81	0.8	5.25	5.05
Survival to MMC (10 nM) (%)	0.1	0.0	0.0	0.0
Cryopreservation	Yes	Yes	No	No
Cell mobilization	G-CSF and Plerixafor			
Transduced/infused: CD34+ **cells (10^5)/kg**	2.5	1.6	2.2	4.1
LV transduction (IU/mL)	3.0×10^8			
Transduced CFCs (10^3)/kg	14.0 ± 2.9	7.3 ± 0.88	2.8 ± 0.4	160 ± 12.0
VCN/cell (total colonies)	0.45	0.24	0.17	0.53
CFC survival after MMC administration (10 nM) (%)	70 (24 mo)	20 (24 mo)	30 (24 mo)	4 (12 mo)
Cytogenetics	No abnormalities (24 mo for Patients 1 and 3; 12 mo for Patients 2 and 4)			
Gene marking at follow-up (latest)	55% in PBL and 70% in BL (30 mo) 43.5% in BM (24 mo)	2—9% in PBL and 15—25% in BM (24 mo)	4—6% in PBL (30 mo) 8—20% in BM (24 mo)	5—17% in PBL (18 mo) 4—8% in BM (12 mo)

Note. BL = B-lymphocytes; BM = bone marrow; mo = month; CFCs = colony forming cells; G-CSF = granulocyte colony-stimulating factor; Hb = hemoglobin; LV = lentiviral; PBL = peripheral blood leukocytes; Plts = platelets.

Plts were 11.3 ± 0.9, 1,235 ± 472, and 48.0 ± 21.8, respectively. The mean baseline BM CD34+ cells and CFCs were 0.833 ± 0.3 and 3.48 ± 2.1, respectively. In addition, 118.0 ± 117.0 bone marrow CD34+ cells/μL were available at GT. A mean of 2.6 ± 1.07 CD34+ cells (10^5)/kg and 46.0 ± 76.1 CFCs (10^3)/kg were infused, with LV transduction of 3×10^8 IU/mL and an average VCN/cell of 0.35 ± 0.2. There were no cytogenetic abnormalities at 12 and 24 months FU. Patient 3 received prophylactic platelet transfusion after GT, at days 32 and 36 and at months 12 and 18, whereas other patients had stable, although low, Plt counts. Gene markers in peripheral blood leukocytes, B-lymphocytes, and BM increased progressively throughout FU at regular intervals as shown in Table 2.

In a recent update, a higher level of gene marking was associated with a higher survival of CFC to MMC, up to 70% at 3-year FU, and also lower levels of chromosomal breakage. Hb levels for two patients had increased to a normal healthy range, and no genotoxic events had been reported. An additional five patients were recruited in this study, who were transduced with 50,000—1.6×10^6 CD34+ cells/kg. Preliminary results include confirmation of gene corrected PB cells, with a similar pattern in the levels of gene marking with the initial four patients. Two of these patients, after ≥ 1-year FU, have demonstrated evidence of engraftment, as shown by increases in PB VCNs [26—28].

Kelly et al. [23] *(NCT00272857 ClinicalTrials.gov)*

In this study [23], three patients (mean age, 13.7 ± 1.6 years) were recruited. Eligibility criteria included a diagnosis of FA genotype A (FA-A), age > 1 year, no evidence of leukemia or myelodysplastic syndrome, and > 1×10^5 CD34+ cells/kg available for in vitro gene correction. One patient underwent cryopreservation, whereas another received granulocyte colony-stimulating factor (G-CSF) for stem cell mobilization. The mean baseline values for Hb, neutrophils, and Plts were 10.7 ± 2.5, 1,423 ± 672, and 76.0 ± 40.1, respectively. This study reported a mean post-

Table 3 Details of Kelly et al. [23] study.

Variables	Patient 1	Patient 2	Patient 3
Age at HSC gene therapy	13.1	12.5	15.5
Cryopreservation	No	Yes	No
Cell Mobilization	G-CSF	NR	None
Baseline Hb (g/dL)	11.8	7.8	12.5
Baseline neutrophils (/μL)	780	2,120	1,370
Baseline Plts ($\times 10^3$/μL)	94	30	104
Baseline CFC survival to MMC (10 nM) (%)	0	0	0
Post-harvest and post-CD34+ selection: total CD34 (10^6)/kg	0.4	1.3	0.2
Cell yield post-transduction and 84 hours in culture (%)	82	5	110
Total nucleated cells infused (10^5)/kg:	4.5	0.5	3.5
Pro-virus detected after infusion	No	N/A[a]	(At 2 and 4 weeks after infusion; none in the BM)
CFC Survival after MMC administration (10 nM) (%)	40	46	48
Peak improvements in blood cell counts (months after GT):			
Hb (g/dL)	Between 13 and 14 (2)	N/A	Between 14 and 15 (3)
Plts ($\times 10^3$/μL)	Between 110 and 120 (1)		Between 130 and 140 (1)
6 mo after infusion		N/A	
Hb (g/dL)	13		14
Plts (×/μL)	70		110
1 y follow-up	Approximately 1 g increase in Hb for 10 mo	N/A	Variable increase in Hb, that peaked at 3 mo
After infusion	Transient increase in platelets No changes in WBCs No cytogenetic abnormalities (including FISH studies) No morphological changes		Increase in platelets that peaked at 1 mo after infusion No changes in WBCs No cytogenetic abnormalities (including FISH studies) No morphological changes

Note. FISH = fluorescent *in situ* hybridization; G-CSF = granulocyte colony-stimulating factor; Hb = hemoglobin; GT = gene therapy; N/A = not applicable; Plts = platelets; WBC = white blood cells.

[a] Patient 2 who underwent cryopreservation was not transduced with autologous products due to the poor in vitro survival of previously cryopreserved cells.

Table 4 Details of Adair et al. [24] study.

Variables	Patient 1	Patient 2
Age at HSC gene therapy	22	10
Mutation	FANCA; c1827-1 G > A	FANCA; exon6-31del
Conditioning (prior to infusion)	No	
Baseline Hb (g/dL)	NP	NP
Baseline neutrophils (/μL)	500—1,000 750 (mean)	670
Baseline Plts ($\times 10^3$/μL)	40	82
Total CD 34 + cells collected/10^6 (BM volume in L)	9.4 (1.1)	30.6 (0.4)
LV transduction (IU/cell)	10	
VCN (/cell)	0.33	1.83
Transduced CFC/kg (%)	18.4	43.0
Post-infusion cell counts	NP	NP

Note. BM = bone marrow; CFCs = colony forming cells; Hb = hemoglobin; HSCs = hematopoietic stem cells; LV = lentiviral; VCN = vector copy number.

harvest and CD34+ selection of 0.183 ± 0.2 cells (10^6)/kg, and an average of 65.7% cell yield after transduction and 84 hours in culture. All patients received a TNC infusion of approximately 2.83 ± 2.1 (10^5)/kg, whereas the mean CFC survival after MMC administration (10 nM) was 44.7%. On FU, there were no 12-month cytogenetic abnormalities in the two patients infused, and 6 months mean post-infusion Hb and Plts values were 13.5 ± 0.7 and 90 ± 28.3, respectively, as shown in Table 3.

Adair **et al.** **[24]** *(NCT01221018 ClinicalTrials.gov)*

As this was an abstract [24], only limited data were available. The mean age of the two patients included was 16.0 ± 8.5 years. Bone marrow was harvested to collect unmanipulated HSCs and did not include either cryopreservation or stem cell mobilization prior to cell infusion. Baseline neutrophils and Plts were 710 ± 56.7 and 61.0 ± 29.7, respectively. A mean of 20.0 ± 15 total ($\times 10^6$) CD34+ cells were collected from 0.75 L of BM. LV transduction was 10 IU/cell, and an average of 30.7% transduced CFC/kg was achieved with a mean VCN/cell of 1.08 ± 1.1 as shown in Table 4. The infusion was tolerated well, and stable blood cell counts were maintained post GT, but showed progressively lower levels of transduced cells in peripheral blood.

Discussion

We aimed to review the impact of GT reported for FA patients. Only three studies met our inclusion criteria, which highlights the need for further research on the effects of GT for FA. Another CT identified was conducted two decades ago, hence considered beyond the scope of our a priori inclusion criteria [29]. In this trial, four patients were recruited, out which three patients received three or four cycles of gene transfer, each with two or three infusions of HSCs transduced ex vivo with normal *FANCC* genes based retroviral vectors. There was a significant increase in HSC colonies in vitro and a transient improvement in BM cellularity.

All published and ongoing GT CTs for FA have used retroviral, for example, LV, mediated gene transfer. However, there have been rapid developments in other methods of GT, with a specific focus on gene editing. Site-specific DNA double-strand breaks are introduced using specific endonuclease enzymes, after which they are repaired by either non-homologous end joining (NHEJ) through gene mutations via insertion—deletion (indel) disruptions, or homology-dependent repair (HDR), which uses donor DNA templates for precise gene modification. Currently used enzymes include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and canonical clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein-9 nuclease (Cas9). Thus, the specific mutation is corrected in its original genomic locus, allowing for targeted delivery and tissue-specific regulatory response [30—32].

Both ZFNs and TALENs-based gene editing in FA have reported that integration of the transgene adeno-associated virus integration site 1 (AASV1) can correct MMC hypersensitivity, and therapeutic gene editing is possible in CD34+ cells from FA-A patients [33,34]. Meanwhile, CRISPR-Cas9-based gene editing, with some advantages over ZFNs and TALENs [35], has demonstrated immense therapeutic potential by generating induced pluripotent stem cells for gene correction and differentiation into adult stem cells [36]. Moreover, murine studies have shown that CRISPR-Cas9 mediated gene editing is effective in restoring FANCF function with increased survival in the presence of MMC, whereas NHEJ-mediated gene editing via CRISPR-Cas9 has been very efficient in restoring mutant coding frames across multiple FA groups, with corrected FA-HSCs having a proliferative advantage [37,38]. Although this approach does appear promising, there has been an association of imperfect DNA repair with deleterious indels, along with a limited editing efficiency [39]. Most recently, transfusion independence was achieved in two patients of transfusion-dependent β-thalassemia at 15 and 9 months after GT (NCT036655678, ClinicalTrials.gov), and in one patient of severe sickle cell disease, who was also free of vaso-occlusive crises at 9 months after GT (NCT03745287, ClinicalTrials.gov) [40,41].

In addition, cytosine and adenine base editors as well as novel prime editors, both versions of the DNA double-strand breaks free CRISPR-Cas9 genomic editing tool, were developed. Prime editors, as a ''search-and-replace" method, are CRISPR-Cas9 nickase/nCas9 (H840A)-reverse transcriptase fusions that use designed prime editing RNAs (pegRNAs) [42,43]. When comparing base editing (CRISPR) versus prime editing, the preclinical work by Lui's team [44] has demonstrated prime editing to offer more targeting flexibility and a greater editing precision, while having a higher or similar editing efficiency compared with HDR and a lower indel by-product generation [45]. Thus, we hope this newer technique of prime editing will prove to be potentially efficacious and safe for the treatment of monogenic diseases, including FA, and emphasize the essential need for and urgency of gene edited human trials for FA.

Besides a small number of patients, absence of long-term FU was the major limitation of our study. We were also unable to assess QoL after GT. In addition, a metaregression analysis could not be conducted because of the limited availability of patient data, and absence of RCTs for GT versus HCT for FA, which would have provided a more reliable insight into benefits and adverse effects of this intervention. Meanwhile, HCT has evolved significantly over the years, with longer survival rates and long-term mortality benefits, attributable to improved grafting techniques, T-cell-depleted grafts, and reduced intensity conditioning regimens [46]. Advancements in the approach to HCT for FA have allowed for 100% engraftment, acute GVHD of < 10%, and a 94% survival; meanwhile, Anur et al. [47] demonstrated 5- and 10-year survival rates to be 100% and 84%, respectively [48].

With promising initial results, multiple GT CTs have been initiated, from the FANCOLEN-2 trial in the United States (NCT04248439, ClinicalTrials.gov) [49], to a global Phase 2 study aiming to treat patients in earlier stages of BMF with infusion of higher numbers of corrected HSCs (NCT04069533, ClinicalTrials.gov) [50]. Early Phase 1 data (NCT03814408, ClinicalTrials.gov) described two patients (aged 5 and 6 years) with CD34+ cells transduction from an LV carrying the *FANCA* gene. No adverse events related to GT occurred. Both patients had stabilizing cytopenias

6 months after infusion, as well as increasing BM-MMC resistance. Continuous monitoring is being done to assess the safety and efficacy of GT [51]. Despite multiple challenges, there is an essential need for future genetic-edited HSC CTs for FA (Table 5), with translational relevance of prime importance since it can be life-changing for FA patients.

Conclusion

In summary, GT seems to be a safe and promising management strategy for FA, with beneficial clinical outcomes in the future. However, further research must be conducted to study the long-term effects of GT, for conclusive evidence of the potential to become a standard form of treatment. Novel studies involving gene editing techniques also need to be explored, in which HCT is the only currently known curative therapy.

Authors' contributions

SS and ET: wrote the first draft of the manuscript and are the first authors, under the supervision and guidance of SKH. All authors contributed substantially to the conception, acquisition, analysis, and interpretation of the data for the work and approved the final approval of the version to be published.

Declaration of Competing Interest

The authors declare the following disclosures, which **may not be** considered as potential competing interests: SKH has received honoraria from Novartis, Pfizer, Mallinckrodt, and Janssen. SKH has received travel grants from Sanofi, Gilead, MSD, and GSK. ADP has received research funding from Genentech and Takeda, has participated in Advisory Boards for Bayer, Shire/Takeda, Sigilon, and UniQure, and consulted for I-Mab and Sunovion. The other authors declare no conflicts of interest.

References

[1] De Rocco D, Bottega R, Cappelli E, Cavani S, Criscuolo M, Nicchia E, et al. Molecular analysis of Fanconi anemia: the experience of the bone marrow failure study group of the Italian association of pediatric onco-hematology. Haematologica 2014;99:1022—31.
[2] Río P, Navarro S, Bueren JA. Advances in gene therapy for Fanconi anemia. Hum Gene Ther 2018;29:1114—23.
[3] Kreutmair S, Erlacher M, Andrieux G, Istvanffy R, Mueller-Rudorf A, Zwick M, et al. Loss of the Fanconi anemia—associated protein NIPA causes bone marrow failure. J Clin Invest 2020;130:2827—44.
[4] Mehta PA, Tolar F. Fanconi anemia. Synonym: Faconi Pancytopenia. Seattle: University of Washington, https://www.ncbi.nlm.nih.gov/books/NBK1401/; 2002 (updated 2018) [accessed 1 March 2021].
[5] Paustian L, Chao MM, Hanenberg H, Schindler D, Neitzel H, Kratz CP, et al. Androgen therapy in Fanconi anemia: a retrospective analysis of 30 years in Germany. Pediatr Hematol Oncol 2016;33:5—12.
[6] Hanenberg H, Roellecke K, Wiek C. Stem cell genetic therapy for Fanconi Anemia-a new hope. Curr Gene Ther 2016;16:309—20.
[7] Henig I, Zuckerman T. Hematopoietic stem cell transplantation–50 years of evolution and future perspectives. Rambam Maimonides Med J 2014;5.
[8] Balistreri CR, De Falco E, Bordin A, Maslova O, Koliada A, Vaiserman A. Stem cell therapy: old challenges and new solutions. Mol Biol Rep 2020;47:3117—31.
[9] Gross M, Hanenberg H, Lobitz S, Friedl R, Herterich S, Dietrich R, et al. Reverse mosaicism in Fanconi anemia: natural gene therapy via molecular self-correction. Cytogenet Genome Res 2002;98:126—35.
[10] Walsh CE, Grompe M, Vanin E, Buchwald M, Young NS, Nienhuis AW, et al. A functionally active retrovirus vector for gene therapy in Fanconi anemia group C. Blood 1994;84:453—9.
[11] Müller LU, Milsom MD, Kim M-O, Schambach A, Schuesler T, Williams DA. Rapid lentiviral transduction preserves the engraftment potential of Fanca(−/−) hematopoietic stem cells. Mol Ther 2008;16:1154—60.
[12] Howe SJ, Mansour MR, Schwarzwaelder K, Bartholomae C, Hubank M, Kempski H, et al. Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest 2008;118:3143—50.
[13] Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, et al. Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest 2008;118:3132—42.
[14] Kohn DB. Historical perspective on the current renaissance for hematopoietic stem cell gene therapy. Hematol Oncol Clin North Am 2017;31:721—35.
[15] González-Murillo Á, Lozano ML, Álvarez L, Jacome A, Almarza E, Navarro S, et al. Development of lentiviral vectors with optimized transcriptional activity for the gene therapy of patients with Fanconi anemia. Hum Gene Ther 2010;21:623—30.
[16] Río P, Navarro S, Guenechea G, Sánchez-Domínguez R, Lamana ML, Yañez R, et al. Engraftment and in vivo proliferation advantage of gene-corrected mobilized CD34+ cells from Fanconi anemia patients. Blood 2017;130:1535—42.
[17] Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med 2009;151(264—9):W64.
[18] Higgins JP, Savović J, Page MJ, Elbers RG, Sterne JA. Assessing risk of bias in a randomized trial. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, editors. Cochrane handbook for systematic reviews of interventions. Chichester, UK: John Wiley; 2019. p. 205—28.
[19] The Newcastle-Ottawa scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa: Ottawa Hospital Research Institute, http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp; 2011 [accessed 1 March 2021].
[20] Page MJ, Higgins JP, Sterne JA. Assessing risk of bias due to missing results in a synthesis. In: Higgins JP, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, editors. Cochrane handbook for systematic reviews of interventions. Chichester, UK: John Wiley; 2019. p. 349—74.
[21] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557—60.
[22] Rio P, Navarro S, Wang W, Sánchez-Domínguez R, Pujol RM, Segovia JC, et al. Successful engraftment of gene-corrected hematopoietic stem cells in non-conditioned patients with Fanconi anemia. Nat Med 2019;25:1396—401.
[23] Kelly PF, Radtke S, Von Kalle C, Balcik B, Bohn K, Mueller R, et al. Stem cell collection and gene transfer in Fanconi anemia. Mol Ther 2007;15:211—9.

[24] Adair JE, Becker PS, Chandrasekaran D, Choi G, Woolfrey AE, Burroughs L, et al. Gene therapy for Fanconi anemia in Seattle: clinical experience and next steps. Blood 2016;128:3510.
[25] Castella M, Pujol R, Callén E, Ramírez MJ, Casado JA, Talavera M, et al. Chromosome fragility in patients with Fanconi anaemia: diagnostic implications and clinical impact. J Med Genet 2011;48:242–50.
[26] Rio P, Navarro S, Sanchez-Dominguez R, Segovia JC, Wang W, Merino E, et al. Hematopoietic engraftment of Fanconi anemia patients through 3 years after gene therapy. Blood 2019;134:4627.
[27] Rio P, Navarro S, Sanchez-Dominguez R, Segovia JC, Wang W, Merino E, et al. Updated results of a European gene therapy trial in Fanconi anemia patients, subtype A. Mol Ther 2020;28:57.
[28] Rocket Pharmaceuticals Presents Positive Updates on FA and LAD-I Gene Therapy Programs at the 23rd Annual Meeting of the American Society of Gene and Cell Therapy. Rocket Pharmaceuticals, Inc., https://www.businesswire.com/news/home/20200512005280/en/Rocket-Pharmaceuticals-Presents-Positive-Updates-FA-LAD-I; 2020 [assessed 1 March 2021].
[29] Liu JM, Kim S, Read EJ, Futaki M, Dokal I, Carter CS, et al. Engraftment of hematopoietic progenitor cells transduced with the Fanconi anemia group C gene (FANCC). Hum Gene Ther 1999;10:2337–46.
[30] Demirci S, Uchida N, Tisdale JF. Gene therapy for sickle cell disease: an update. Cytotherapy 2018;20:899–910.
[31] Yin H, Kauffman KJ, Anderson DG. Delivery technologies for genome editing. Nat Rev Drug Discov 2017;16:387–99.
[32] Fanconi Anemia: Guidelines for Diagnosis and Management. Fanconi Anemia Research Fund, Inc., https://www.fanconi.org/images/uploads/other/FARF_Guidelines_Book_interior_lo-res.pdf; 2014 [accessed 1 March 2021].
[33] Diez B, Genovese P, Roman-Rodriguez FJ, Alvarez L, Schiroli G, Ugalde L, et al. Therapeutic gene editing in CD34+ hematopoietic progenitors from Fanconi anemia patients. EMBO Mol Med 2017;9:1574–88.
[34] Pino-Barrio MJ, Giménez Y, Villanueva M, Hildenbeutel M, Sánchez-Dominguez R, Rodríguez-Perales S, et al. TALEN mediated gene editing in a mouse model of Fanconi anemia. Sci Rep 2020;10:1–4.
[35] Nemudryi A, Valetdinova K, Medvedev S, Zakian S. TALEN and CRISPR/Cas genome editing systems: tools of discovery. Acta Naturae 2014;6:19–40.
[36] Sürün D, von Melchner H, Schnütgen F. CRISPR/Cas9 genome engineering in hematopoietic cells. Drug Discov Today Technol 2018;28:33–9.
[37] Román-Rodríguez FJ, Ugalde L, Álvarez L, Díez B, Ramírez MJ, Risueño C, et al. NHEJ-mediated repair of CRISPR-Cas9-induced DNA breaks efficiently corrects mutations in HSPCs from patients with Fanconi anemia. Cell Stem Cell 2019;25:607–21.
[38] van de Vrugt HJ, Harmsen T, Riepsaame J, Alexantya G, van Mil SE, de Vries Y, et al. Effective CRISPR/Cas9-mediated correction of a Fanconi anemia defect by error-prone end joining or templated repair. Sci Rep 2019;9:768.
[39] Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: a review of the challenges and approaches. Drug Deliv 2018;25:1234–57.
[40] Corbacioglu S, Cappellini MD, Chapin J, Chu-Osier N, Fernandez CM, Foell J, et al. Initial safety and efficacy results with a single dose of autologous CRISPR-CAS9 modified CD34+ hematopoetic stem and progenitor cells in transfusion-dependent B-thalassemia and sickle cell disease. HemaSphere 2020;4:101 (abstract s280).
[41] CRISPR Therapeutics and Vertex Announce New Clinical Data for Investigational Gene-Editing Therapy CTX001™ in Severe Hemoglobinopathies at the 25th Annual European Hematology Association (EHA) Congress. CRISPR Therapeutics 2020; https://www.globenewswire.com/news-release/2020/06/12/2047260/0/en/CRISPR-Therapeutics-and-Vertex-Announce-New-Clinical-Data-for-Investigational-Gene-Editing-Therapy-CTX001-in-Severe-Hemoglobinopathies-at-the-25th-Annual-European-Hematology-Associ.html, 2020 [accessed 1 March 2021]
[42] Cohen J. Prime editing promises to be a cut above CRISPR. Science 2019;366:406.
[43] Rees HA, Liu DR. Base editing: precision chemistry on the genome and transcriptome of living cells. Nature Rev Genet 2018;19:770–88.
[44] Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 2019;576:149–57.
[45] Flotte TR, Gao G. Prime editing: a novel Cas9-reverse transcriptase fusion may revolutionize genome editing. Hum Gene Ther 2019;30:1445–6.
[46] Chaudhury S, Auerbach AD, Kernan NA, Small TN, Prockop SE, Scaradavou A, et al. 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;140:644–55.
[47] Anur P, Friedman DN, Sklar C, Oeffinger K, Castiel M, Kearney J, et al. Late effects in patients with Fanconi anemia following allogeneic hematopoietic stem cell transplantation from alternative donors. Bone Marrow Transpl 2016;51:938–44.
[48] Ebens CL, MacMillan ML, Wagner JE. Hematopoietic cell transplantation in Fanconi anemia: current evidence, challenges and recommendations. Expert Rev Hematol. 2017;10:81–97.
[49] Gene Therapy for Fanconi Anemia, Complementation Group A, NCT04248439. ClinicalTrials.gov, https://ClinicalTrials.gov/show/NCT04248439; 2020 [accessed 1 March 2021].
[50] Lentiviral-mediated Gene Therapy for Pediatric Patients With Fanconi Anemia Subtype A, NCT04069533. ClinicaTrials.gov, https://ClinicalTrials.gov/show/NCT04069533; 2019 [accessed 1 March 2021].
[51] Czechowicz A, Rio P, Bueren JE, Beard B, Nicoletti E, Schwartz J, et al. Changing the natural history of Fanconi anemia complementation Group-À with gene therapy: early results of US Phase I study of lentiviral-mediated ex-vivo FANCA gene insertion in human stem and progenitor cells. Biol Blood Marrow Transplant 2020;26:S39–40 (abstract 50).