



ORIGINAL ARTICLE

Efficacy and safety of gene therapy in pediatric patients with Fanconi anemia: a systematic review



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KEYWORDS

Fanconi anemia;
Anemia;
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Abstract

Introduction: Fanconi anemia is a genetic disorder characterized by high risk of hematological and oncological disease. The aim of this review was to summarize the evidence on the safety and efficacy of gene therapy in pediatric patients with Fanconi anemia.

Methods: Systematic review (PROSPERO registration record CRD420251152704). We searched PubMed/MEDLINE, Scopus, Web of Science, ClinicalTrials.gov, ICTRP, and CENTRAL. The study outcomes included safety, engraftment and persistence, and hematologic outcomes. The risk of bias was assessed with the RoB 2 tool.

Results: The review included seven studies, all conducted in patients with *FANCA* variants, that evaluated autologous hematopoietic stem cell-based gene therapy from mobilization and collection to lentiviral transduction and reinfusion without conditioning. Safety profile: favorable, with no serious adverse events attributable to the vector/cells and no evidence of lentiviral replication, clonal dominance, or genotoxicity in follow-up periods of up to 7 years. Engraftment/persistence: detectable vector copy numbers and stable polyclonal patterns, more durable engraftment with higher cell doses. Hematological outcomes: stabilization/improvement of cytopenia; transfusion independence in some patients and deferral of bone marrow transplantation. The efficacy of gene therapy was greater in younger patients and in those without advanced bone marrow failure. The overall risk of bias was high.

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Conclusions: Gene therapy showed a favorable safety profile, with no serious adverse events or evidence of genotoxicity. Efficacy was greater in younger children, in those without advanced marrow failure, and with higher doses of corrected CD34+ cells. However, as the included studies were phase I/II trials, the results should be interpreted cautiously; nevertheless, gene therapy appears to be a promising and safe treatment option in the early stages of the disease.

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PALABRAS CLAVE

Anemia de fanconi;
Anemia;
Terapia génica;
Revisión sistemática;
Pediatría

Eficacia y seguridad de la terapia génica en pacientes pediátricos con anemia de Fanconi: una revisión sistemática

Resumen

Introducción: La anemia de Fanconi es un trastorno genético con alta morbilidad hematológica y oncológica. Nuestro objetivo es sintetizar la evidencia sobre la seguridad y eficacia de la terapia génica en población pediátrica con anemia de Fanconi.

Metodología: Revisión sistemática (protocolo PROSPERO CRD420251152704). Búsqueda en PubMed/MEDLINE, Scopus, Web of Science, ClinicalTrials.gov, ICTRP y CENTRAL. Desenlaces: seguridad, injerto/persistencia y evolución hematológica. Medición del riesgo de sesgos según RoB 2.

Resultados: Se incluyeron siete estudios, todos en pacientes FANCA, que evaluaron terapia génica basada en células madre hematopoyéticas autólogas, desde la movilización y recolección hasta la transducción lentiviral y reinfusión sin acondicionamiento. Seguridad: perfil favorable; sin eventos adversos graves atribuibles a vector/células; sin evidencia de replicación lentiviral, dominancia clonal ni genotoxicidad en seguimientos de hasta 7 años. Injerto/persistencia: número de copias vector detectables y patrones policlonales estables; mayor durabilidad con dosis celulares altas. Hematología: estabilización/mejoría de citopenias; independencia transfusional en una fracción de pacientes y diferimiento del trasplante de médula ósea. La eficacia de la terapia génica es mayor en pacientes más jóvenes y sin falla medular avanzada. El riesgo global de sesgo fue alto.

Conclusiones: La terapia génica mostró un perfil de seguridad favorable, sin eventos adversos graves ni evidencia de genotoxicidad. La eficacia fue mayor en niños más jóvenes, sin falla medular avanzada y con dosis más altas de células CD34+ corregidas. Sin embargo, los estudios incluidos corresponden a ensayos fase I/II, por lo cual los resultados deben interpretarse con cautela; no obstante, la terapia génica constituye una alternativa prometedora en etapas tempranas de la enfermedad.

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Introduction

Fanconi anemia (FA) is a rare genetic disorder characterized by impaired DNA repair mechanisms resulting in genomic instability.¹ It is caused by germline pathogenic variants in any of the 22 *FANCA* genes identified to date.² Clinically, it is characterized by developmental defects, early-onset pancytopenia secondary to bone marrow failure, and a predisposition to leukemia and solid tumors.^{3,4} Its global incidence is estimated at 1 per 360 000 live births.⁵ At present, allogeneic hematopoietic stem cell transplantation (HSCT) is the only treatment available for FA that can achieve a cure. However, it is associated with significant short- and long-term complications, such as severe infection, organ failure and graft-vs-host disease, in addition to a substantial increase in the risk of solid tumors.⁶ Given the genetic etiology of FA, theoretically, the optimal therapeutic approaches would be those aimed at correcting the underlying defect, as would be the case of gene therapy (GT) aimed at repairing the defective hematopoietic stem cells (HSCs) of the patient.⁷ This approach has demonstrated its

potential in several hereditary diseases, such as primary immunodeficiencies and hemoglobinopathies, and, more recently, clinical trials have shown that lentiviral-mediated GT can be used in the management of complex diseases like FA.⁸ In this context, GT via infusion of gene-corrected peripheral blood autologous HSCs has emerged as a promising alternative that circumvents the limitations associated with the availability of suitable donors, conditioning regimens and complications of allogeneic HSCT.⁹ In this context, the aim of the review was to summarize and systematically appraise the available evidence on the use of GT for FA in the pediatric population, analyzing its efficacy, safety and feasibility, as well as the reported hematologic and clinical outcomes.

Material and methods

Study design and protocol registration

We conducted a systematic review that adhered to the recommendations of the PRISMA 2020 reporting guideline and the Cochrane

Handbook for Systematic Reviews of Interventions, and the protocol was registered prior to its initiation in PROSPERO with accession number CRD420251152704.

Eligibility criteria

We included clinical trials evaluating the use of GT in patients aged less than 18 years with a confirmed diagnosis of FA, irrespective of genetic subtype, and with any degree of cytopenia, considering both controlled trials and single-arm studies. We excluded studies conducted exclusively in adults, studies other than clinical trials, and studies that only analyzed allogeneic transplantation or hematopoietic agents and not GT.

Sources of evidence

We conducted a literature search in the PubMed/Medline, Scopus and Web of Science databases using the following search strategy: ("Fanconi anemia" [Title/Abstract]) AND ("gene therapy" [Title/Abstract]), using both as MeSH terms. We also searched the references of the studies identified through the search. In addition, we consulted clinical trial databases, including ClinicalTrials.gov, the International Clinical Trials Registry Platform (ICTRP) and the Cochrane Central Register of Controlled Trials (CENTRAL). We limited the search to sources published between January 2015 and October 2025, with no language restrictions.

Study selection process

The selection was performed using the Rayyan Beta management platform, with three authors (SECR, MADC and CRF) performing the initial screening of titles and abstracts blinded to the others, labeling studies as "Include" "Maybe" or "Exclude". The same authors read the full text of studies labeled as "Include" or "Maybe" to make the definitive decision whether to "Include" or "Exclude" them. In the case of disagreement, a fourth author (SCPJ) made the final decision. The Cohen kappa was used to assess the agreement between the authors. The entire search and selection process was represented graphically with a PRISMA 2020 flow diagram.

Data extraction

Three blinded authors extracted the data (SECR, MADC and SCPJ), and, in the case of discrepancies, a fourth author made the final decision (NSSO). The data were extracted directly to Excel spreadsheets (Microsoft Office 2019).

List of data and outcomes

Data on the following variables were extracted from each study: first author, date of publication, study period/date, journal, study design, number of participants, studied intervention, sociodemographic variables, objectives, and outcomes. The primary outcomes were the safety of the intervention and of the vector, evaluated according to the following: adverse events associated with the treatment; absence of reactivation of lentiviral clones, clonal dominance, dysplasia or leukemia attributable to the procedure; engraftment and persistence of the corrected cells, assessed through the vector copy number (VCN) in bone marrow or peripheral blood; hematologic stabilization or improvement assessed by means of neutrophil and platelet counts and hemoglobin concen-

tration; and transfusion independence and deferral or avoidance of allogeneic HSCT.

Assessment of risk of bias

The risk of bias in the included clinical trials was assessed with the Risk of Bias 2 (ROB 2) tool. This tool is structured into five main domains according to the source of bias: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result. Each of these domains was assessed independently by three authors (SECR, NSSO or SCPJ), and, in the case of disagreement, a fourth author (CRF) made the final decision, which allowed the general classification of each trial into the following categories: low risk of bias, some concerns, or high risk of bias.

Results

Fig. 1 presents the PRISMA 2020 flow diagram of the trial identification and selection process. The initial search yielded 384 records: PubMed (61 records), Web of Science (134 records), Scopus (157 records), CENTRAL (7 records), ClinicalTrials.gov (18 records), and ICTRP (7 records). Before screening, 134 duplicates were eliminated, leaving 250 records to screen based on the title and abstract. At this stage, 232 were eliminated because they were not pertinent, and 18 remained for retrieval and reading of the full text.⁹⁻²⁶ Another 11 were excluded after reading the full text because they did not meet the eligibility criteria,¹⁰⁻²⁰ so only seven records retrieved from the databases were finally included in the qualitative review.⁹⁻²⁶ Other searches were performed in parallel: Google Scholar (200 records) and backward citation search of the previously identified sources (76 references), which yielded another 276 records. Of these records, only 12 were considered sufficiently relevant to attempt their retrieval, and the full text was obtained for all. However, those 12 trials were duplicates of records already identified in the database search¹¹⁻²² (kappa = 0.82).

Table 1 summarizes the general characteristics of the included clinical trials that evaluated GT in patients with FA. The review included a total of seven articles published between 2016 and 2025, chiefly conducted in Spain and the United States.^{9,21-26} Adair et al.²¹ conducted a phase I trial in two patients with FA type A that underwent harvesting of HSCs for lentivirus-mediated *FANCA* gene transfer and subsequent reinfusion of the edited cells without pre-infusion conditioning. Both patients tolerated the harvesting and infusion procedures but exhibited low and declining levels of transduced cells in peripheral blood after infusion.²¹ Subsequent trials conducted by Czechowicz et al.^{22,23} consolidated the use of the phosphoglycerate kinase-Fanconi anemia complementation group A woodchuck hepatitis virus posttranscriptional regulatory element (PGK-FANCA-WPRE) lentiviral vector (known as RP-L102) and its application to the pediatric population. The first trial, which included two patients, achieved effective mobilization with granulocyte-colony stimulating factor (G-CSF) and plerixafor, in absence of severe adverse events and with hematologic stabilization at six months of follow-up. The article for the second trial, which included nine patients, reported confirmed functional engraftment in most cases, with resistance to mitomycin-C and a favorable safety profile, as there was no evidence of toxicity or significant clonal abnormalities.^{22,23} Complementing this evidence, the studies by Sevilla et al.²⁵ and Diana et al.⁹ focused on optimizing mobilization and harvesting of HSCs. Sevilla et al.²⁵ evaluated 11 pediatric patients treated with filgrastim and plerixafor, which achieved the CD34+ levels required for apheresis in nine of them without serious complications and with only transient and mild adverse events.²⁵ Similarly, Diana et al.⁹ confirmed the

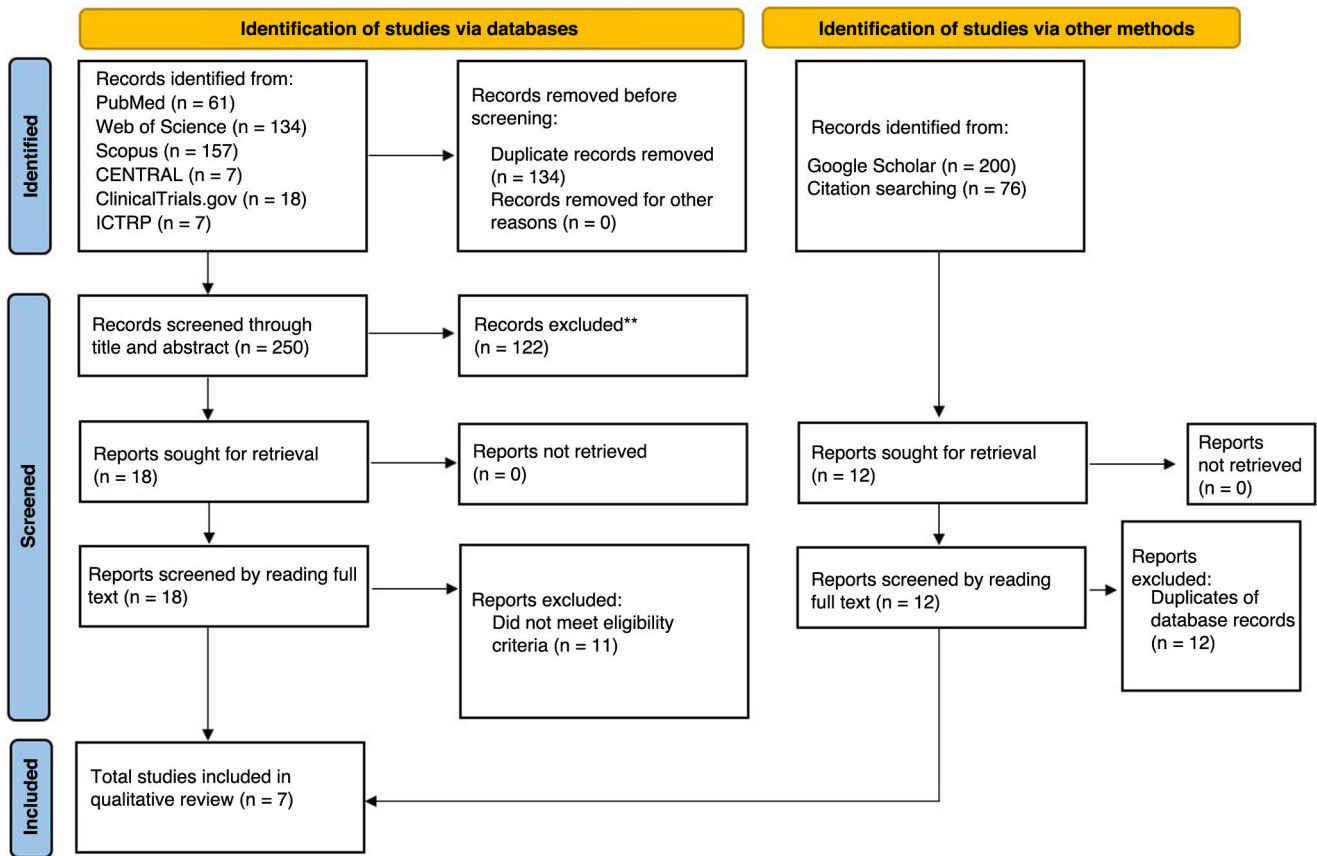


Fig. 1 PRISMA 2020 flow diagram of the identification, screening and selection of studies included in the review.

safety and efficacy of mobilization with combined G-CSF and plerixafor, observing that the response was better in younger patients, with a number of collected cells adequate for subsequent GT.⁹ In more recent trials, Czechowicz et al.²⁴ and Río et al.²⁶ consolidated evidence on long-term clinical outcomes. The first study, which included 14 patients treated with FANCA-cel, found sustained genetic and phenotypic correction in more than 60% of participants, associated with sustained hematologic stability and in absence of serious adverse events or genotoxicity.²⁴ The pioneering trial conducted in Spain by Río et al.²⁶ in nine patients with a follow-up of up to seven years was the first to establish the persistence of engraftment and genetic correction, the long-term viability of the graft, the preservation of clonal diversity and the absence of dysplasia or malignant progression.

Outcomes

Safety of the procedure and the vector

All reviewed trials found a favorable safety profile for GT conducted with lentiviral vectors carrying the *FANCA* gene.^{9,21–26} None reported serious adverse events attributable to the administration of the vector or the genetically corrected cells.^{9,21–26} The described adverse events were mild or moderate, consistent with the use of mobilizing agents such as filgrastim or plerixafor, and included fever, headache, nausea and mild abdominal pain.^{9,25}

The phase I/II trial conducted by Adair et al.²¹ was groundbreaking, as it established the safety of the procedure, demonstrating the absence of significant hematologic or systemic toxicity and that prior conditioning with chemotherapy is

not required.²¹ Subsequent studies, such as those by Czechowicz et al.^{22–24} and Río et al.,²⁶ confirmed that the use of the PGK-FANCA-WPRE lentiviral vector (RP-L102) and the FANCA-cel product was not associated with the presence of replication-competent lentivirus, clonal dominance, dysplasia or progression to leukemia.^{22,26} In addition, these studies had prolonged follow-ups of up to 7 years post-infusion, during which there were no genotoxic events nor evidence of preferential insertion in genetic risk loci.^{24,26}

Engraftment and persistence of corrected cells

Adair et al.²¹ were the first to demonstrate the ability of transduced CD34+ cells to engraft in the bone marrow without need of myeloablative conditioning, but also reported declining levels of transduced cells in peripheral blood after infusion.²¹ The studies conducted by Czechowicz et al.^{22–24} contributed evidence on the persistence of corrected cells, with detectable VCN values in bone marrow and peripheral blood through 3 years post-infusion.^{22–24} In the 2024 study, more than 60% of the patients exhibited sustained and progressive genetic correction, indicative of a stable and functional polyclonal graft.²⁴ The pioneering study by Río et al.²⁶ found that the efficacy of the graft persisted at 7 years of follow-up, particularly in patients who received higher cell doses ($\geq 240\,000$ corrected CD34+ cells/kg). Clonal diversity was preserved, with no evidence of dominant clones.²⁶

Hematologic and clinical outcomes

In terms of hematologic outcomes, several studies reported stabilization or improvement of cytopenias after infusion of corrected

Table 1 Description of clinical trials of gene therapy in pediatric patients with Fanconi anemia.

First author	Year	Study design	Number of participants	Intervention	Demographic characteristics	Objectives	Outcomes
Adair et al. ²¹	2016	Phase I trial	2	Bone marrow harvest; collection of HSCs (CD34+ or unfractionated cells); <i>ex vivo</i> transduction with lentiviral vector carrying <i>FANCA</i> gene; autologous reinfusion without prior conditioning; monitoring of graft and hematological parameters.	Ages 10 and 22 years; both male.	Evaluate safety and feasibility/biological activity of gene therapy with <i>FANCA</i> as alternative to transplantation	Both patients tolerated procedures well, without serious complications; both displayed low and declining levels of transduced cells in peripheral blood after infusion.
Czechowicz et al. ²²	2019	Open-label phase I non-randomized clinical trial (preliminary results)	2 (reported)	Mobilization with G-CSF + plerixafor; leukapheresis; enrichment of CD34+; culture with cytokines and transduction with PGK- <i>FANCA</i> -WPRE vector; infusion of fresh product; no conditioning regimen.	Ages 5 and 6 years; unspecified sex	Evaluate the feasibility and safety of <i>ex vivo</i> gene therapy (PGK- <i>FANCA</i> -WPRE) in pediatric FA-A with the goal of stabilizing cytopenias without prior conditioning	No severe adverse events during mobilization/apheresis and production process; successful infusion; stabilization of cytopenia progression at 6 months of follow-up; no serious adverse events associated with infusion.
Czechowicz et al. ²³	2021	Ongoing clinical trial (TG RP-L102, no conditioning)	9	Key eligibility criteria: confirmed mutation in <i>FANCA</i> , age ≥ 1 year; absence of HLA-matched donor, ≥ 30 CD34+/ μ L in bone marrow. Mobilization with G-CSF + plerixafor; leukapheresis (2 days); CD34+ enrichment; transduction with PGK- <i>FANCA</i> -WPRE; autologous cell reinfusion without conditioning; administration of fresh product; follow-up of up to 3 years.	Ages 2–6 years, unspecified sex	Evaluate safety and efficacy of RP-L102 through gene repair of autologous CD34+ in FA-A	Confirmed engraftment in six patients ≥ 6 months of follow-up (increased VCN in peripheral blood). Two of three pts with follow-up of ≥ 12 months showed mitomycin-C resistance. One patient developed blood marrow failure following infection by influenza B and required transplantation. Favorable safety profile (one grade 2 transient RP-L102 infusion-related reaction).

Table 1 (Continued)

First author	Year	Study design	Number of participants	Intervention	Demographic characteristics	Objectives	Outcomes
Sevilla et al. ²⁵	2021	Phase I clinical trial (mobilization/collection)	11	Mobilization with filgrastim (12 µg/kg c/12 h hasta 8 días) + plerixafor (240 µg/kg/día días 4–8); repeated measurement of CD34+; threshold for initiation of leukapheresis of ≥ 5 CD34+/ μ L; up to 4 procedures with a target of $\geq 4 \times 10^6$ CD34+/kg.	Ages 3–16 years; 10 male, 1 female	Evaluate the safety and efficacy of filgrastim + plerixafor for mobilization and collection of HSC in pediatric FA	Threshold for leukapheresis initiation achieved in 9 of the 11 patients; median 21.8 CD34+/ μ L at mobilization peak. Patients aged 15–16 years did not reach the threshold. Median number collected of 4.8×10^6 CD34+/kg over 2–3 procedures. No serious adverse events; frequent mild adverse events.
Diana et al. ⁹	2022	Open-label phase I/II, clinical trial (mobilization/collection)	5 initially; 4 included	Selection: mutation in <i>FANCA</i> , age 2–12 years, >10 kg, no prior transplantation and absence of matched HLA donor. Mobilization with G-CSF (12 µg/kg/day in 2 doses, days 1–5) + plerixafor (0.24 mg/kg from day 5, 2 h before each apheresis); leukapheresis (Optia); target 5×10^6 CD34+/kg; immunoselection CD34+ (CliniMACS) and cryopreservation for future use in GT.	Ages 2, 5, 8 and 12 years; 3 male, 1 female	Primary: safety/feasibility of G-CSF + plerixafor in FA. Secondary: mobilization kinetics and possibility of collecting sufficient doses for GT	Safer and more efficacious mobilization in younger patients without advanced bone marrow failure. Two patients reached the threshold, with a higher volume collected in the patient aged 2 years (11.7×10^6 CD34+/kg). No clinically relevant AEs during the procedure or in 12 months of follow-up.

Table 1 (Continued)

First author	Year	Study design	Number of participants	Intervention	Demographic characteristics	Objectives	Outcomes
Czechowicz et al. ²⁴	2024	Phase I/II, multicenter	14	Selection: mutation in <i>FANCA</i> , age ≥ 1 year, no matched-HLA sibling donor, ≥ 30 CD34+/ μ L in bone marrow. Mobilization with G-CSF + plerixafor; leukapheresis; CD34+ enrichment; lentiviral transduction of functional <i>FANCA</i> copy; autologous reinfusion of fresh product, without conditioning; planned 3-year follow-up.	Ages 1.8–7.0 years (mean 4.05; SD, 1.65); 57.1% female	Evaluate efficacy and safety of FANCA-cel (GT with lentivirus-transduced autologous CD34+) in FA-A	For 12 patients with follow-up ≥ 12 months: progressive/sustained genetic repair in 8/12; phenotypic correction (resistance to mitomycin-C) in 7 at 12 months and one other patient at 36 months; stabilization of cytopenia associated with genetic/phenotypic correction. Mild-moderate AEs, without major safety problems (no associated dysplasia, clonal dominance, or leukemia).
Río et al. ²⁶	2025	Open-label phase I/II, single arm	9 treated (8 evaluable)	Mobilization with filgrastim + plerixafor; apheresis to collect CD34+; ex vivo transduction through a lentiviral vector carrying the <i>FANCA</i> gene; IV infusion of product without conditioning. Modification to the protocol: fresh infusion prioritized to avoid losses due to cryopreservation. Prolonged follow-up, up to 7 years in some patients.	Median age 5 years (IQR, 3.5–6.5); range, 3–7; 7 male, 2 female	Evaluate efficacy (vector/engraftment at 24 months) and safety (AEs/severe AEs within 3 years) of hematopoietic GT without conditioning in pediatric FA-A	Primary efficacy endpoint in 5/8 (62.5%). High doses (≥ 240 000 corrected CD34+ /kg): stable graft through 7 years with hematologic stabilization/improvement. Low doses or delayed GT: progression to bone marrow failure and need of rescue therapy/transplantation. Preserved polyclonal integration with no signs of significant genotoxicity; mostly mild-moderate AEs; 1q-gain in uncorrected cells unrelated to insertion of therapeutic provirus.

Abbreviations: AE, adverse event; FA, Fanconi anemia; FA-A, Fanconi anemia, subtype A/complementation group A; GT, gene therapy; HSC, hematopoietic stem cell; CD34+, CD34-positive hematopoietic stem cell; G-CSF, granulocyte colony-stimulating factor; HLA, human leukocyte antigen; PGK-FANCA-WPRE, lentiviral vector with PGK promoter that expresses the *FANCA* gene and includes the WPRE element; RP-L102, gene therapy product/protocol based on the PGK-FANCA-WPRE vector; VCN, vector copy number.

leukapheresis: apheresis for collection of peripheral blood mononuclear cells; "without conditioning": no prior chemotherapy or radiotherapy; "fresh infusion": administration of the final product without cryopreservation.

Table 2 Evaluation of risk of bias in the clinical trials that used gene therapy in pediatric patients with Fanconi anemia using the RoB 2tool.

Study / Year	ID	Bias arising from randomization process	Bias due to deviation from intervention	Bias due to missing data	Bias in outcome measurement	Bias in selection of reported result	Overall risk of bias
Adair et al. ²¹	NCT01331018 (Seattle)	High (no randomization, only 2 treated patients)	Alto (open-label, uncontrolled, deviation from CD34+purification protocol)	High (very short follow-up, loss of transduced cells)	Low (objective blood cell counts)	High (partial results, abstract)	High
Czechowicz et al. ²²	Early RP-L102 trial (Stanford/Madrid)	High (no randomization, single cohort)	High (early stage, no masking)	High (preliminary data for few patients)	Low (VCN and CFU are objective measures).	High (abstract with interim results)	High
Czechowicz et al. ²³	RP-L10	High (no randomization)	High (no masking, flexible apheresis procedure)	High (transplantation required in one patient, incomplete data in another).	Bajo (objective laboratory outcomes)	High (publication of abstract)	High
Sevilla et al. ²⁵	FANCOSTEM-I (NCT02931071)	High (phase I, open-label, no randomization)	High (adjustments based on achieved mobilization)	Low (11/13 treated patients, few excluded)	Low (clear CD34+ and hematological counts)	Moderate (full-text article, more detailed than abstract)	High
Diana et al. ⁹	EUDRACT2014-005264-14	High (open-label phase I/II trial)	High (no masking, coadministration of G-CSF + plerixafor).	Low (4 treated patients, no losses in short-term follow-up).	Low (objective CD34+ count)	Moderate (peer-reviewed article)	High
Czechowicz et al. ²⁴	Global RP-L102, FANCA-cel	High (non-randomized, open-label)	High (no conditioning, adapted interventions)	Moderate (≥ 1 year follow-up, incomplete data for some patients).	Low (objective VCN, MMC, CFU measurements)	Moderate (ASH abstract, no full article)	High
Río et al. ²⁶	NCT03157804; EudraCT 2011-006100-12	High (phase I/II; open-label)	Moderate (more standardized protocol, no conditioning)	Low (8 evaluable/9 total, follow-up of 3-7 years)	Low (objective measurement of engraftment and VCN)	Low (full-text article in <i>Lancet</i>)	High

Abbreviations: ASH, American Society of Hematology; CFU, colony-forming units; EudraCT/EUDRACT, European Union Drug Regulating Authorities Clinical Trials; G-CSF, granulocyte colony-stimulating factor; LV, lentiviral vector; MMC: mitomycin C; VCN, vector copy number.

cells.^{9,22–26} The studies conducted by Czechowicz et al.^{22–24} and Río et al.²⁶ found progressive improvement of hematologic parameters associated with sustained genetic correction and resistance of stem cells to genotoxic agents such as mitomycin-C.^{22,26} In the study by Río et al.,²⁶ GT achieved transfusion independence and prolonged hematologic stabilization in five out of eight evaluable participants (62.5%), who did not require allogeneic HSCT during the follow-up.^{22–24}

Secondary outcomes and exploratory endpoints

The studies conducted by Sevilla et al.²⁵ and Diana et al.⁹ included a detailed analysis of the efficacy of mobilization with G-CSF and plerixafor. Both demonstrated that this drug combination could achieve adequate concentrations of CD34+ cells in peripheral blood, enabling harvesting of sufficient volumes for lentiviral transduction, with optimal average yields of 4.8×10^6 CD34+ cells/kg in absence of serious adverse events.^{9,25} These results suggest that this strategy is safe, efficacious and reproducible, especially in younger children, who responded better to mobilization.

As regards molecular correction, Czechowicz et al.^{22–24} and Río et al.²⁶ reported sustained increases in VCN values in both bone marrow and peripheral blood, which evinced the persistence of the FANCA vector in the different hematopoietic lineages. In addition, functional analyses demonstrated the restoration of the response to genotoxic agents—mainly, resistance to mitomycin-C, the characteristic marker of phenotypic correction in Fanconi cells.^{22,26} From a genomic perspective, studies with long-term follow-up, chiefly the one by Río et al.,²⁶ confirmed polyclonal reconstitution and absence of dominant lentiviral integration, with a random insertion site distribution.

The predominance of open-label phase I/II trials without a control group and marked clinical heterogeneity in this review precluded the performance of a quantitative synthesis of the evidence.

Assessment of risk of bias

Table 2 presents the assessment of the risk of bias using the RoB 2 tool. We found an overall high risk of bias in the selected studies due to the open-label, nonrandomized phase I/II design and the small sample sizes^{9,21–26} ($\kappa = 0.90$).

Discussion

In this systematic review, we summarized the clinical experience with ex vivo HSC GT in children with FA, all of them with subtype A and treated with lentiviral vectors carrying the FANCA gene without prior myeloablative conditioning.^{9,21–26} Although some patients were duplicated in different publications,^{22–24} the overall evidence indicated that a sequential approach of mobilization with G-CSF and plerixafor, apheresis and CD34+ enrichment, and reinfusion of transduced autologous cells is feasible in pediatric patients^{9,21–26} with a favorable safety profile, as adverse events were mainly mild to moderate and there was no evidence of an increased risk of leukemia or of clonal dominance in the available follow-up data.^{26–28} With regard to the biological outcomes of GT, the included trials show that the infusion of autologous CD34+ cells transduced with FANCA-carrying lentiviral vectors results in stable engraftment of corrected cells, with low but sustained vector loads and a clear corrective effect at the functional level.^{22,26} In the Spanish cohort, patients who received higher doses of corrected CD34+ cells achieved a sustained proliferative advantage of the repaired clones, slowing the progression of bone marrow failure, with stabilization of hematologic parameters for several years and no evidence of genotoxic integrations or monoclonal

expansion; the outcomes were best in younger patients without advanced bone marrow failure.^{26,28} Previous reviews indicate that the switch from gammaretroviral vectors to self-inactivating lentiviral vectors has significantly reduced the risk of insertional mutagenesis observed in early GT trials for combined immunodeficiencies, while achieving sustained and stable expression of the transgene.^{29–31} In addition, functional studies with single-cell sequencing for transcriptomic profiling have demonstrated that GT not only corrects the hypersensitivity to cross-linking agents, but also restores the transcriptional program of hematopoietic stem cells in FA, which then resembles the transcriptional program of healthy donors, with normalization of the TGF- β , p21 and telomere maintenance pathways, among others.⁸ When considering these findings in the context of potential therapies for FA, it is important to recognize that lentiviral-based GT competes with and may be complemented by new-generation gene editing strategies.^{11,32–34} Preclinical trials of adenine base editors and CRISPR/Cas9 gene editing have demonstrated the efficient correction of common FANCA variants, restoring function in the interstrand cross-link repair pathway and resistance to alkylating agents in HSCs without depending on homology-directed repair, a mechanism that is deficient in FA.^{11,32–34} These advances suggest that, in the future, clinical platforms based on targeted editing could be developed to prevent semi-random vector integration.

Among the strengths of the review are the broad search strategy for recent sources, the exclusive inclusion of studies on GT, the adherence to the PRISMA 2020 guidelines and the specific focus on the pediatric population.³⁵ On the other hand, it also has several limitations: it included a small number of children and the selected studies were open-label phase I/II trials without control groups and with a high risk of bias.

Conclusion

Lentiviral-based ex vivo GT without conditioning is a promising strategy that may be disease-modifying in children with FA type A in the early stages of bone marrow failure. Studies in larger samples with long-term follow-up and prospective comparison with bone marrow transplant are required to define its place in the treatment sequence for pediatric FA.

Patient data

This study did not involve the use of patient data, so it did not require informed consent.

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Declaration of competing interest

The authors have no conflicts of interest to declare.

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