Thérapie génique pour la maladie de Fanconi ou l'Anémie de Blackfan-Diamond Mythe ou réalité?

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9ème Journée du CRMR

Aplasies médullaires acquises & constitutionnelles

4 Octobre 2024

URP 3518

Plan

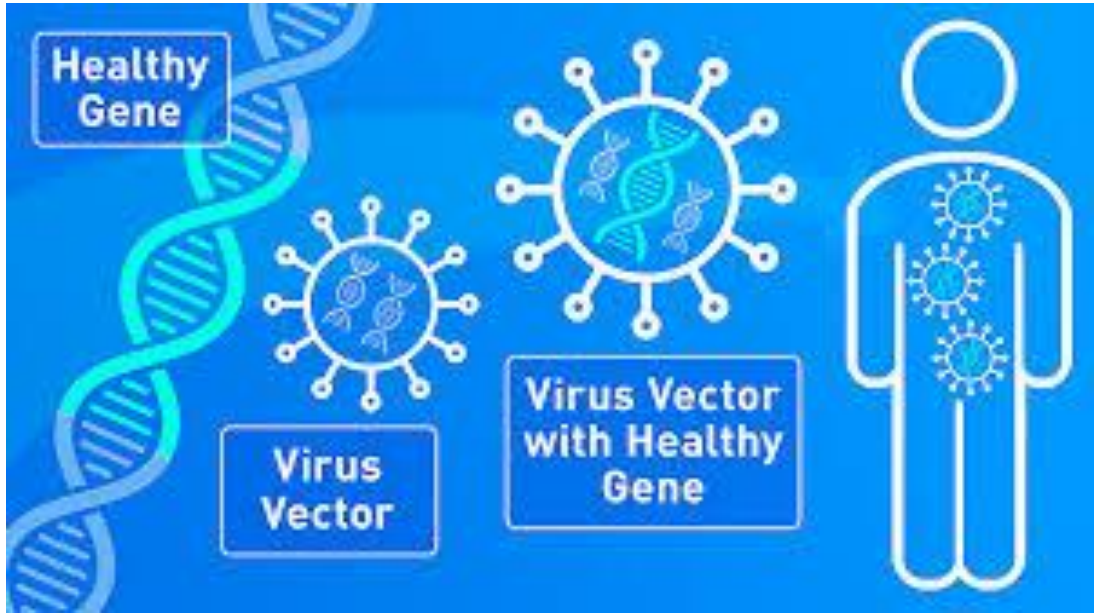
FANCONI

FancoMob results

FANCOLEN-I results
FANCOLEN-II

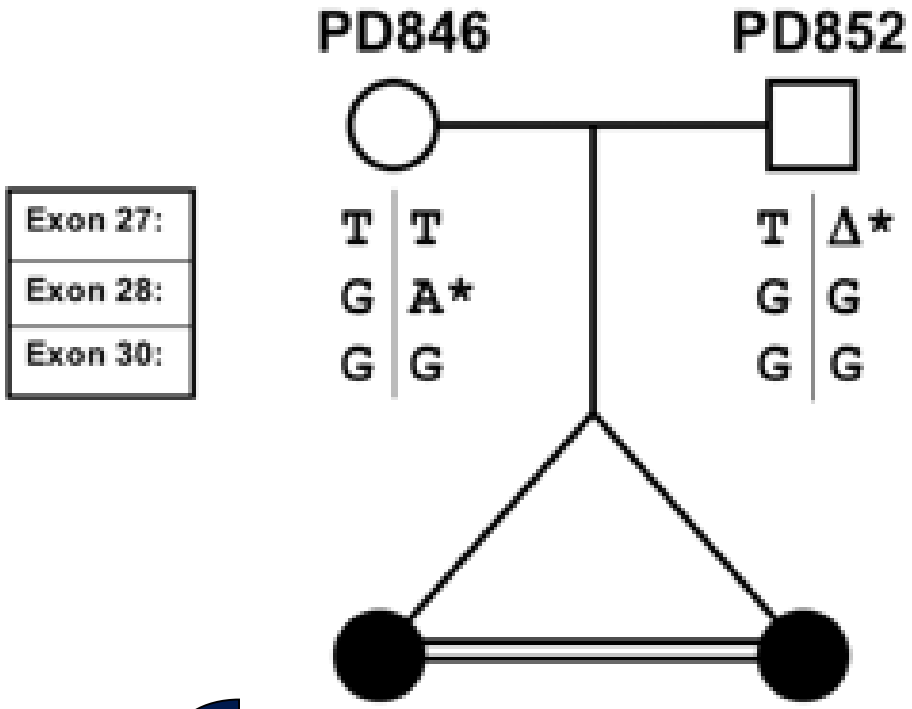
DBAS

Pre-clinical data



Natural gene therapy in monozygotic twins with Fanconi

Mankad & al, Blood, 2004

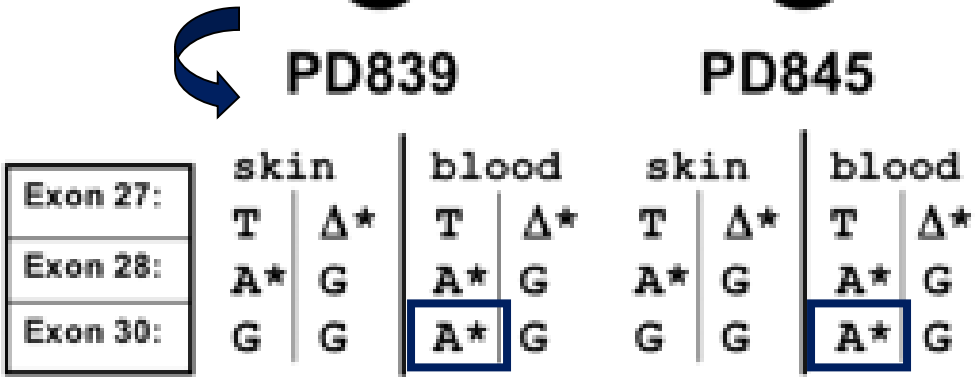


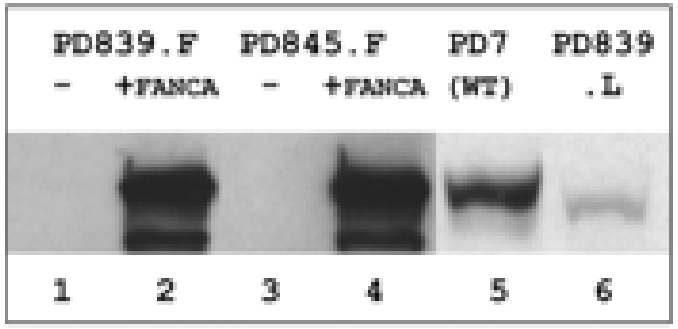
Age at study: 28 yr

*** mutant allele:**

Exon 27; 2555 ΔT
Exon 28; 2670G>A; R880Q
Exon 30; 2927G>A; E966K

Acquired somatic mutation (in utero); exon 30: corrective of germinal variant in cis (exon 28)



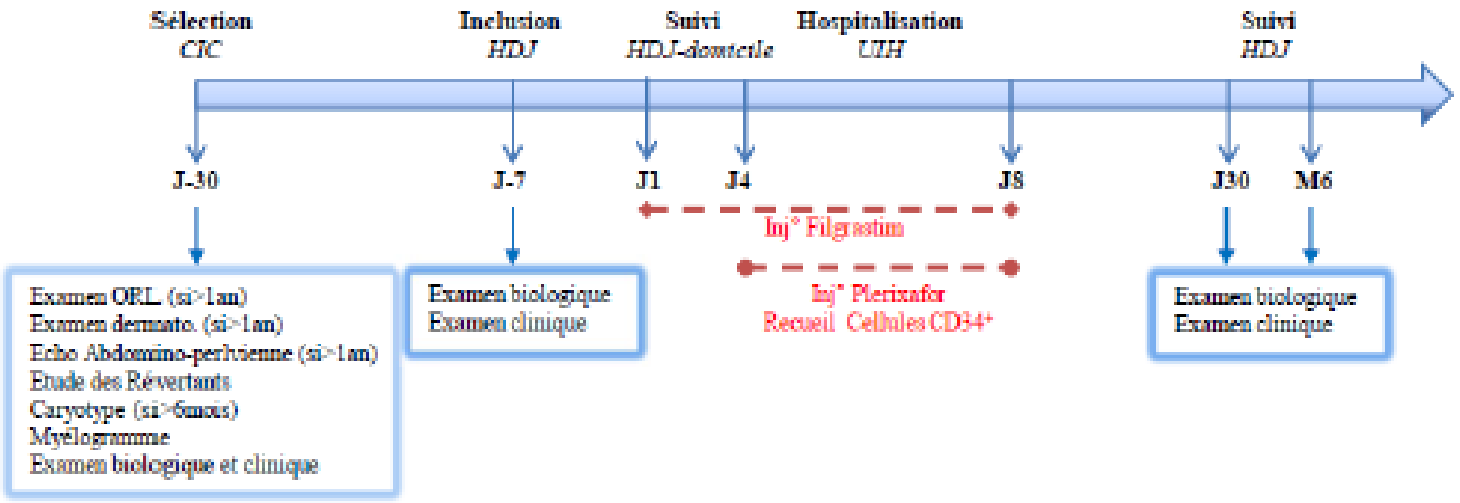


FancoMob

Inclusion criteria
FA / FANCA
HSCT indication but no MSD
Age: > 4 and < 18 yr

<u>Goal:</u> preselection of 8 pts ☞ 5 only

<u>Minimal N of cells to be collected:</u>
- Post stimulation: 10 CD34/µl
- Collection: 5.10^6 CD34/kg (Weigh estimated at 5 yrs)
(+ rescue: 5.10^6 CD34/kg)



CIC : Centre d'Investigation Clinique (Necker, Bât. Imagine)
HDJ : Hôpital De Jour de Biothérapie (Necker, Bât. Hamburger)
UIH : Unité d'Immunologie et Hématologie Pédiatrique (Necker, Bât. Hamburger)

G-CSF: 12 µg/kg x 2/d for 6-7 d
Plerixaflor: 240 µg/kg/d for 2-3 d

A new step in understanding stem cell mobilization in patients with Fanconi Anemia: a bridge to gene therapy

5 pts included: **1: excluded after workup: abnormal karyotype**

2: failure of mobilization (low peripheral CD34)

2: collected *(brother: 5 yr & sister: 2 yr)* ☞ **successful: 1**

		FA3				FA4
Procedures		**(*n* = 4)**				**(*n* = 1)**
Collected cells	Total nucleated cells (10e8)	167	203	163	120	450
	Total CD34 (10e6)	11.0	8.1	3.2	4.1	117.0
	Total CD34/kg (10e6)	0.7	0.5	0.2	0.3	11.7
	CD34 content (%)	0.07	0.04	0.02	0.03	0.26
CD34 + Selection	CD34+ recovery (%)	11	35	38	59	8.6
	Cryopreserved CD34+ cells (10e6/kg)	0.08	0.19	0.08	0.16	1.01
	CD34+ purity (%)	8.4	9.3	12.6	7.4	20
	CD3+ (%)	0.34	0.15	0.63	0.42	0.04
	CD19+ (%)	4.07	5.50	11.11	7.52	6
	Granulocytes (%)	39.64	78	35	58	66
	Monocytes (%)	48	7.2	39	31	9

Diana & al, Transfusion 2021

Equipe de Juan Bueren (Madrid)

Essai FANCOLEN-I

Ciemat

Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas

LETTERS

https://doi.org/10.1038/s41591-019-0550-z

nature medicine

Successful engraftment of gene-corrected hematopoietic stem cells in non-conditioned patients with Fanconi anemia

Outcome in 4 pts

☞ Proof of concept

Rio & al, Nat Medecine, 2019

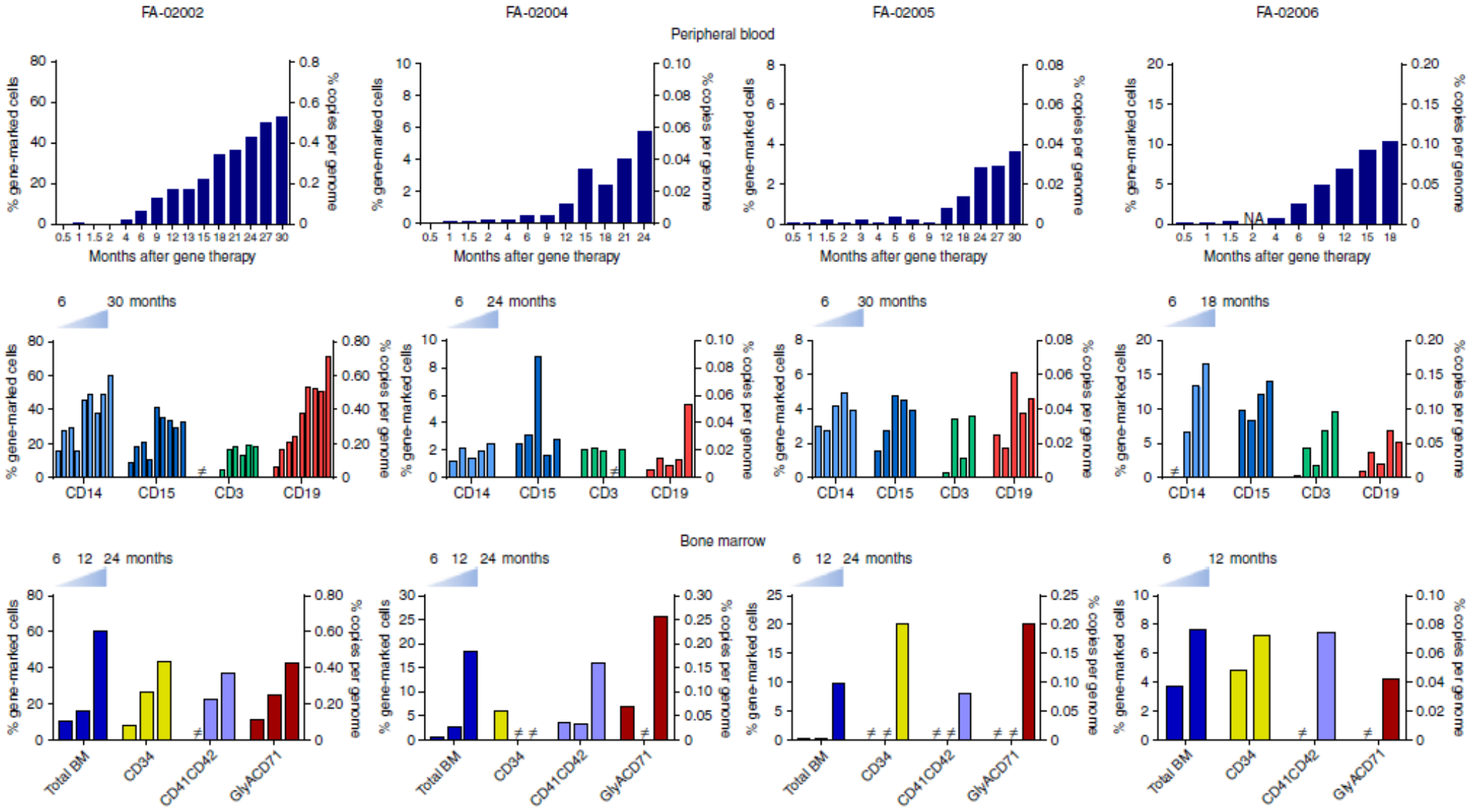
Patients

Patient ID	Cryopr. CD34$^+$ cells	Screening visit	Age (years)	Hb (g/dL)	Neutroph/ µL	Platelets/ µL	BM CD34$^+$ cells/µL	BM CD34$^+$/ CD38$^-$cells/µL	CFCs/µL	CFCs Surv. to MMC (10 nM)	% aberrant T cells (DEB test)
FA-02002	Yes	HSC Collection	3.4	12.7	2,400	84,000	545.8	13.26	13.87	0.0 %	80%
		HSC Gene therapy	5.2	10.5	1,600	29,000	135.0	12.29	2.81	0.1 %	86%
FA-02004	Yes	HSC Collection	5.9	12.3	1,000	68,000	35.3	1.56	2.70	3.9 %	66%
		HSC Gene therapy	7.6	10.8	900	46,000	25.1	0.99	0.80	0.0 %	80%
FA-02005	No	HSC Collection/ Gene Therapy	4.0	12.5	1,680	38,000	276.1	0.39	5.25	0.0 %	74%
FA-02006	No	HSC Collection/ Gene Therapy	6.6	11.3	760	79,000	34.1	3.15	5.05	0.0 %	64%

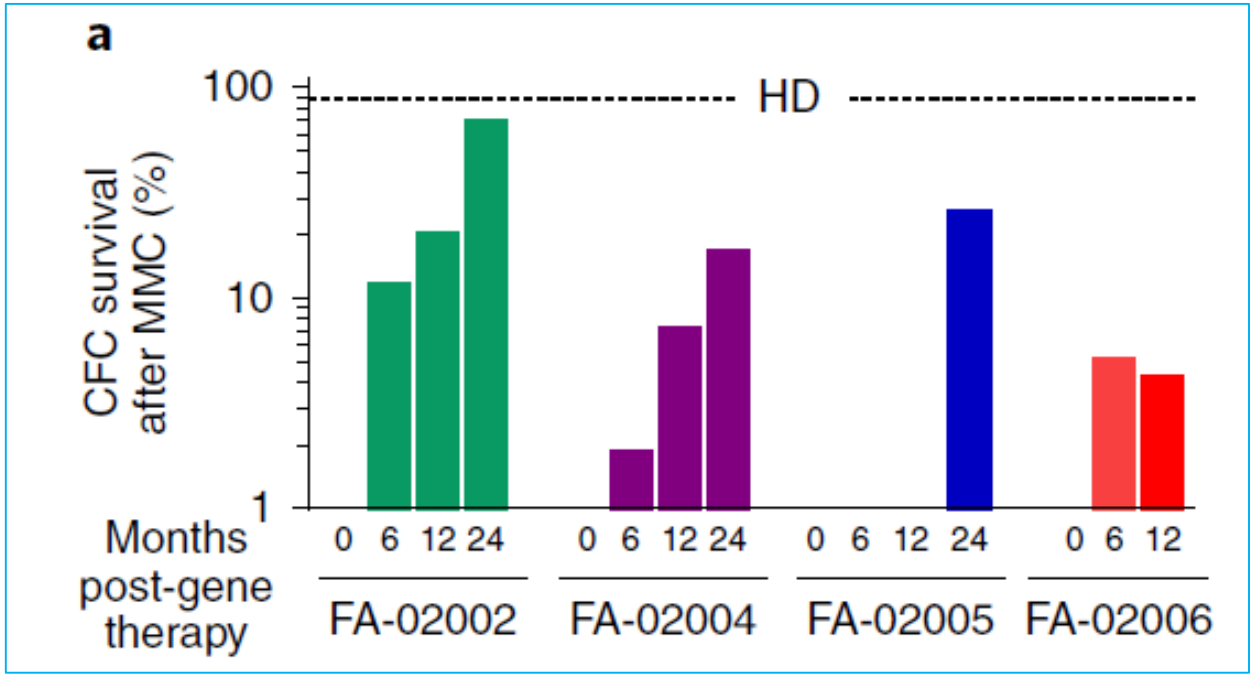
Patients:
4 boys (2 from Gypsy community).
Characterised homozygous FANCA biallelic variants

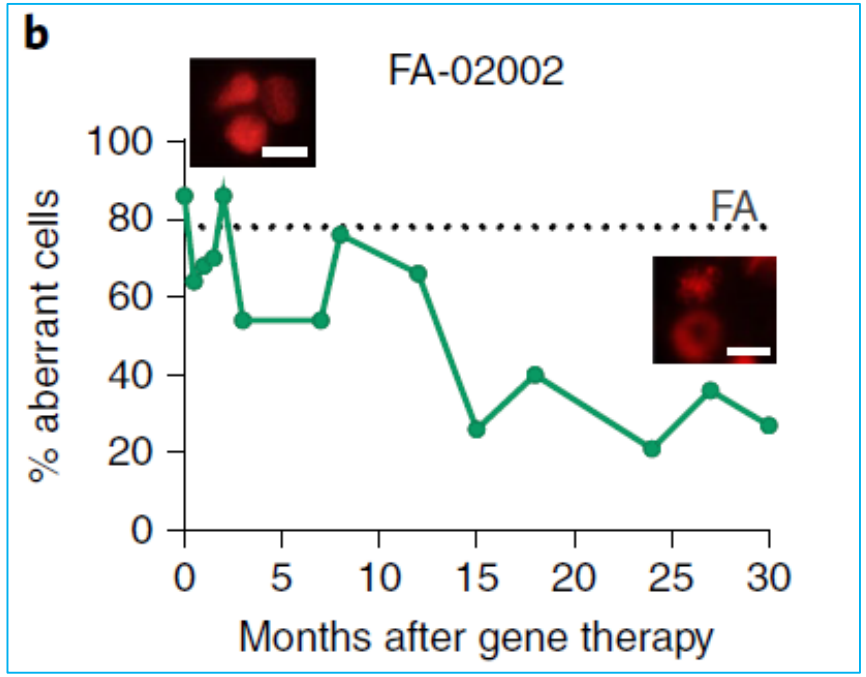
To be included: at least 1 cytopenia: Hb < 8g, Neutrophils < 750 (⇨ 1000) or platelets < 30,000 (⇨ < 50,000)

Progressive engraftement



Phenotypic correction: MMC sensitivity





Phenotypic correction *in vivo*: CBC

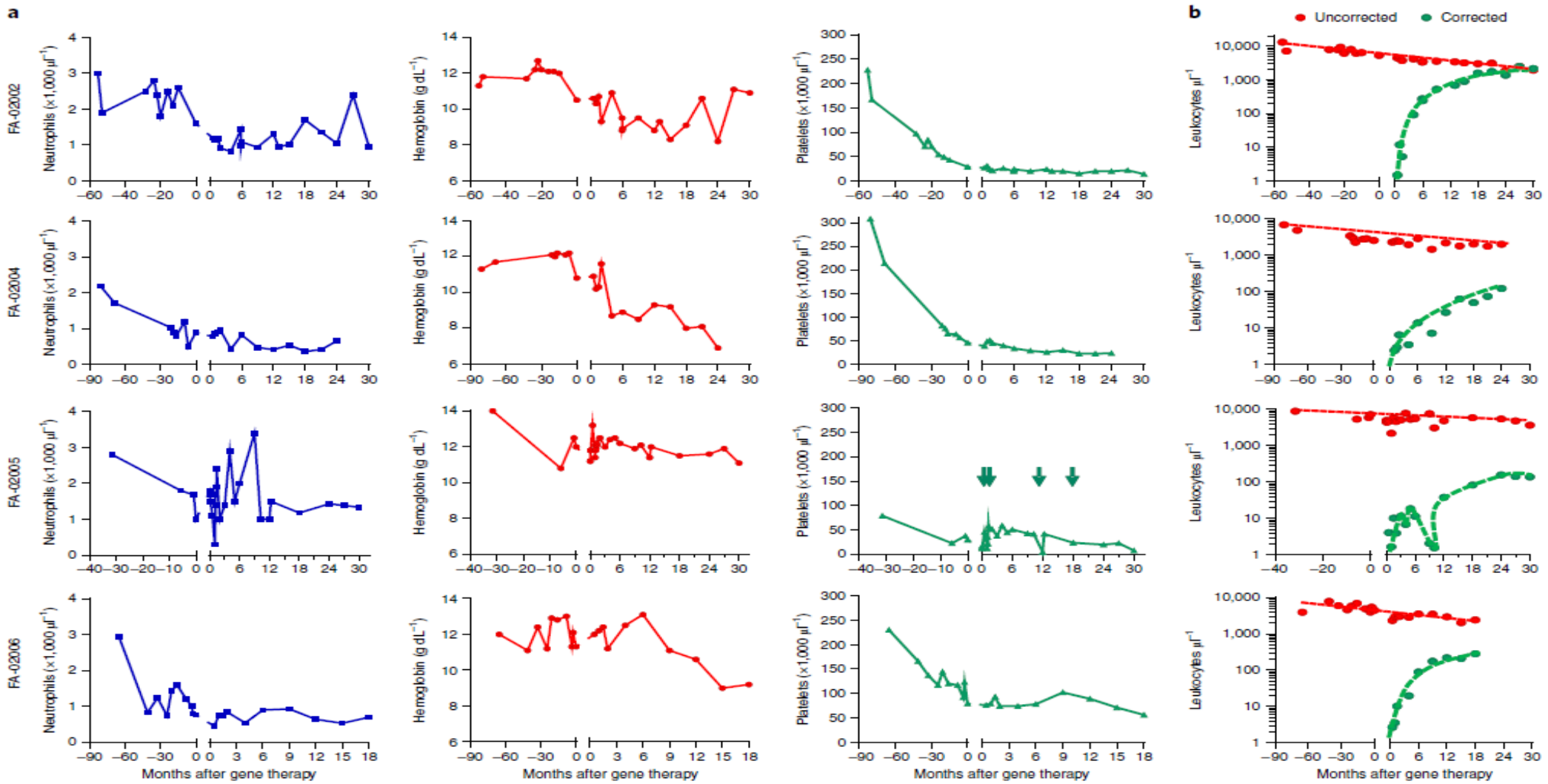


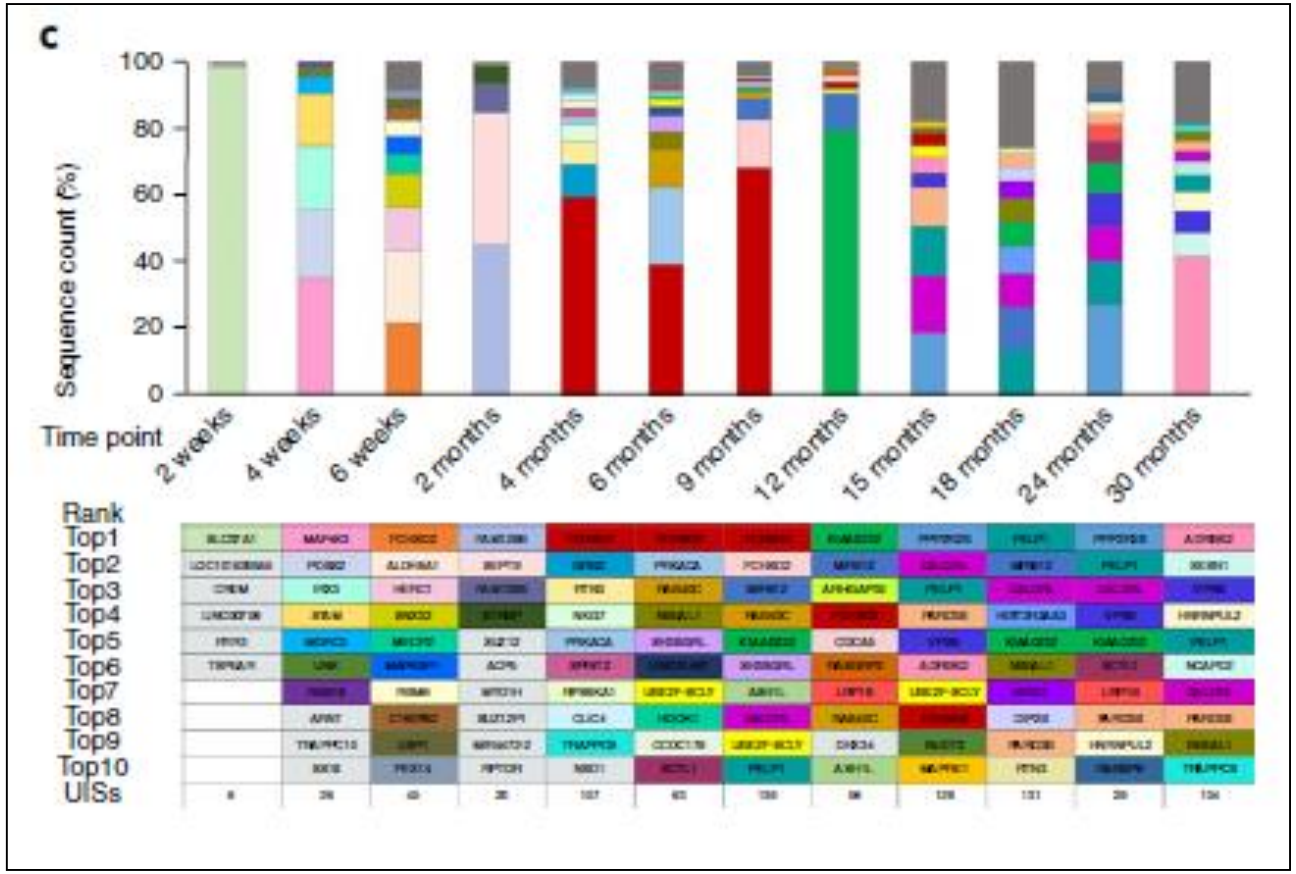
Fig. 4 | PB cell counts in patients with FA before and after gene therapy. a, Evolution of neutrophils (left; blue), hemoglobin levels (middle; red) and platelets (right; green) in PB of patients with FA before and after gene therapy. Green arrows indicate the administration of prophylactic platelet transfusions. **b**, Kinetics of uncorrected (red) and corrected leukocytes (green) in the PB of patients with FA before and after gene therapy.

Intégration sites

No preferential site

++ for oncogenes like *LMO2, CCND2 & MECOM*

Oligoclonal profile & no clonal dominance



Fancolen-1: Conclusion

Preliminary results in only 4 patients but proof of concept

Time is needed for engraftment: at best no progression to BMF

Safe (but not so friendly for the child) procedure; no side effects

How to do better?

- **HSC to be collected as early as possible: OK for multiplex families but…**
- **At best use fresh cell (no congelation)**

FANCOLEN-I: LTFU evaluation

New report on FANCOLEN-I with more pts (7 in the long-term FU) and more follow-up.

Question raised: is eltrombopag may be useful?

ELT is not active in FA

But ELT is efficient in pts with AA: so it may be also usefull in this seting and active on CORRECTED FA cells….

Rio & al, accepted in The Lancet (2024)

FANCOLEN-II

PROTOCOL FANCOLEN-II

Protocol Title:	A Phase II Clinical Trial to Evaluate the Efficacy of the Infusion of Autologous CD34+ Cells Transduced with a Lentiviral Vector Carrying the FANCA Gene (Orphan Drug) in Patients with Fanconi Anemia Subtype A
Protocol Name:	FANCOLEN-II
EudraCT Number:	2018-002502-31

An open-label, global registrational clinical trial of RP-L102 for the treatment of FA has completed enrollment: 14 pts included (Spain, UK & USA). The Marketing Authorization Application (MAA) has been accepted for review by the European Medicines Agency (EMA) based on the positive efficacy and safety data from the Phase 1/2 study of RP-L102. The Biologics License Application (BLA) for FA remains on track for submission to the U.S. Food and Drug Administration (FDA) in the first half of 2024.

FANCOLEN-II

4.1 Inclusion Criteria

Patients will only be able to enter this trial if they met all the following criteria:

1. Fanconi anemia as diagnosed by chromosomal fragility assay of cultured lymphocytes in the presence of DEB or a similar DNA-crosslinking agent
2. Patients of the complementation group FA-A
3. Minimum age: 1 year and a minimum weight of 8 kg.
4. Maximum age: 12 years
5. At least one of the following hematologic parameters below lower limits of normal of
   - Hemoglobin
   - Absolute neutrophils
   - Platelets
6. At least 30 CD34+ cells/µL are determined in one BM aspiration within 3 months prior to CD34+ cell collection
7. If the number of C34+ cells/ µL in BM is in the range of 10-29, PB parameters should meet two of the three following criteria:
   - Hemoglobin: ≥11g/dL
   - Neutrophils: ≥900 cells/µL
   - Platelets: ≥60,000 cells/µL
8. Provide informed consent in accordance with current legislation
9. Women of childbearing age must have a negative urine pregnancy test at the baseline visit, and accept the use of an effective contraception method during participation in the trial

1 to 12 years

FANCOLEN-II

Figure 1 Schematic Diagram of the Trial Design

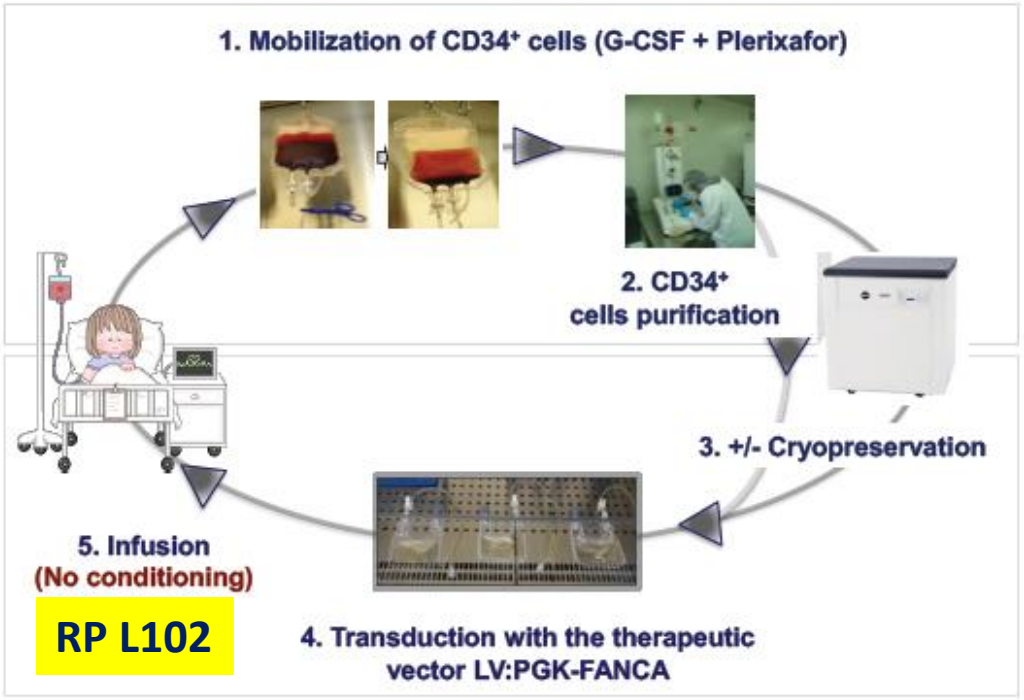
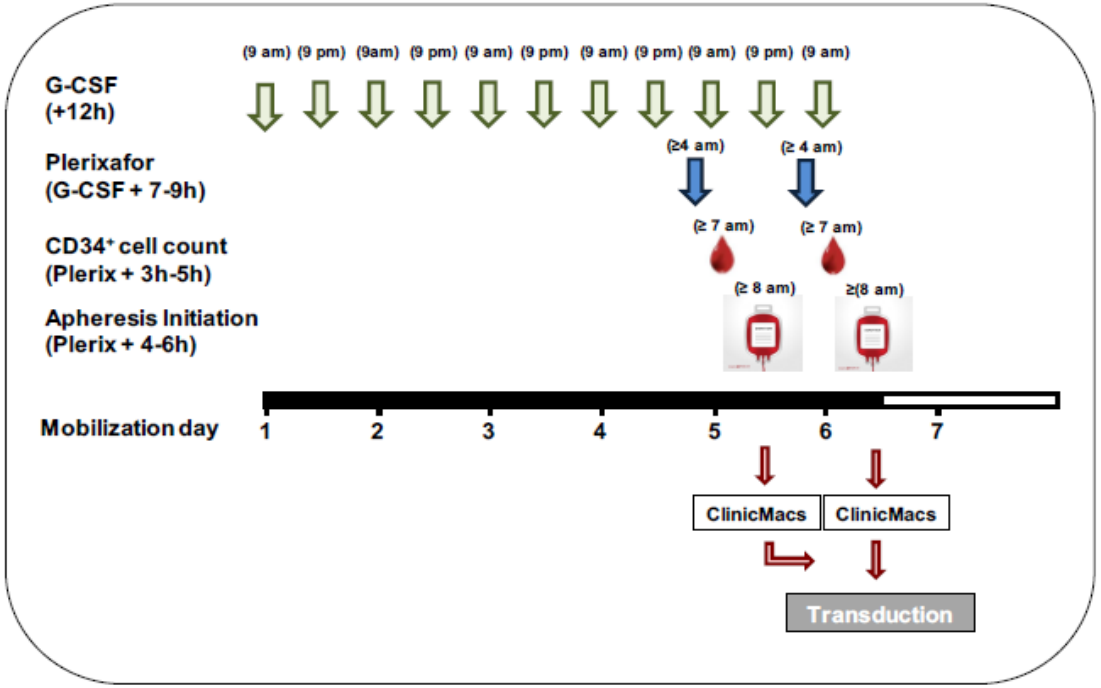


Figure 2 Illustration of the CD34+ mobilization protocol with G-CSF (Neupogen) and Plerixafor (Mozobil)



Close for inclusions. 14 children treated
No clinical information at the moment

Gene therapy in FA: to open the discussion

PRO	CON
Positive knowledge from pts with somatic mosaicisim <u>No conditioning +++:</u> - Limited toxicity - HSCT still possible - Risk of cancer is not augmented by the procedure (no CR, no cGVH)	Limited to *FANCA* Collection of HSCH is challenging especially over 3 years. <u>Required:</u> > 240,000 cCD34+ cells/kg Time needed to improve BCC with no pt to date in hematological CR Still experimental What about clonal evolution?

In practice: GT in FA

For many FA patients TG can not actually been discussed:

- **Too old**
- **Too severe cytopenias**

At best clinical availability in 2025 or 2026?

Currently, whe have to plan HSCT and to go to transplant when required

FARF 2024

Gene therapy for the oral mucosa of FA

Craig Dorrell[1], *Deepak Sahel*[2], *Gaurav Sahay*[2], *Markus Grompe*[1]

[1]*Department of Pediatrics, Papé Family Pediatric Research Institute, Pediatric Blood & Cancer Biology Program, Stem Cell Center, Oregon Health & Science University, Portland OR; ?2Department of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, Portland OR.*

Objective: Oral squamous cell carcinoma (SCC) is a major cause of morbidity and mortality in Fanconi Anemia (FA). Although gene replacement or repair strategies are promising approaches for the treatment of many genetic diseases, this strategy has not been generally seen as suitable for cancer-causing syndromes like FA SCC because of the very high efficiency required for a clinically useful outcome. To date, no publications on oral mucosa gene therapy exist. If, however, a useful degree of gene correction in the basal epithelium can be achieved and corrected cells expand to replace their mutant neighbors, by natural or induced selection, a substantial reduction in tumor incidence could be achieved.

Diamond Blackfan Anemia syndrome

Very heterogeneous disease

Therapeutic independence is not rare: 20% of pts at a given time

SGR events are claimed to be rare (published cases < 10) but maybe actually more frequent (Italian & Saint-Jude studies)

Why gene therapy in DBAS may be a more easy approach than in FA?

BM is rich and it is likely that collection of HSC will be much more easy

GT may be an option in adult pts for whom HSCT is not an option

Patients are not in an emergent situation: we have time to plan this and to wait for significant results (the patient is on transfusion)

Partial result will be clinically relevant: any reduction in the burden of transfusion will be associated with a clinical benefit (iron overload ++)

European consortium: DBA Gene cure lead by J. Bueren team

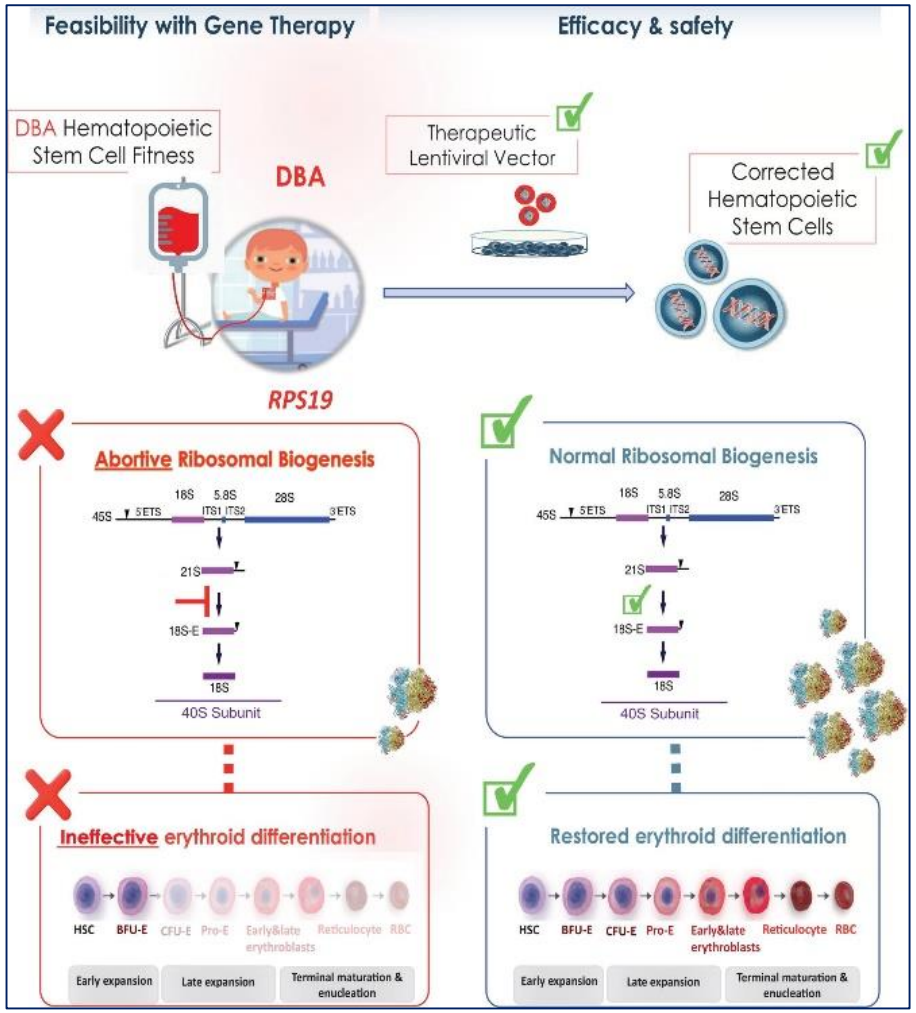
Goal: to achieve all pre-clinical studies required to gene therapy in DBAS patients

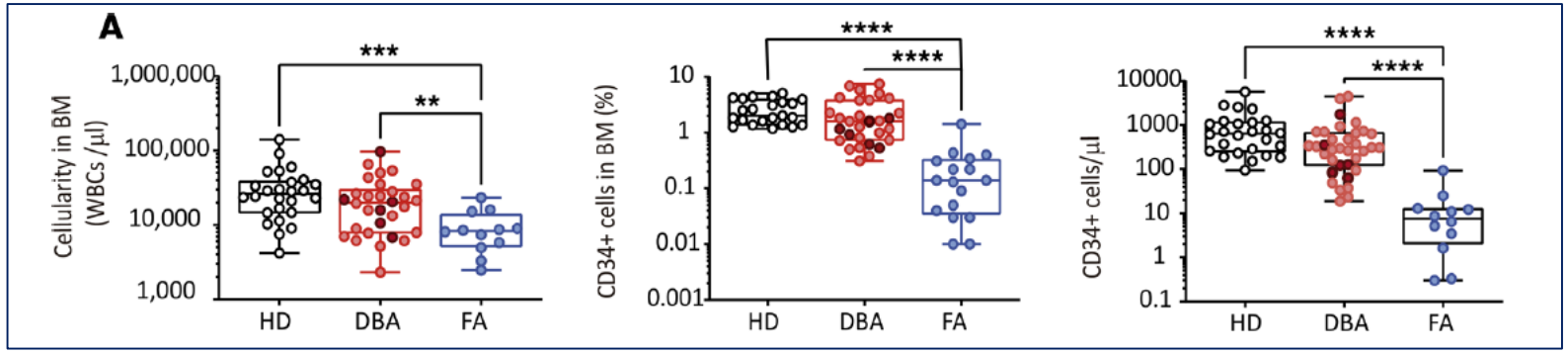
EUROPEAN JOINT PROGRAMME RARE DISEASES

Lentivirus-mediated gene therapy corrects ribosomal biogenesis and shows promise for Diamond Blackfan anemia

Yari Giménez,[1,2,3] Manuel Palacios,[1,2,3] Rebeca Sánchez-Domínguez,[1,2,3] Christiane Zorbas,[4] Jorge Peral,[1,2,3] Alexander Puzik,[5] Laura Ugalde,[1,2,3] Omaira Alberquilla,[1,2,3] Mariela Villanueva,[1,2,3] Paula Río,[1,2,3] Eva Gálvez,[6] Lydie Da Costa,[7,8] Marion Strullu,[9] Albert Catala,[10] Anna Ruiz-Llobet,[10] Jose Carlos Segovia,[1,2,3] Julián Sevilla,[6] Brigitte Strahm,[5] Charlotte M. Niemeyer,[5] Cristina Beléndez,[2,11,12] Thierry Leblanc,[9] Denis L.J. Lafontaine,[4] Juan Bueren,[1,2,3] and Susana Navarro[1,2,3]

J Clinical invest, 2024





The HSC reservoir in DBA pts is not significantly reduced

☞ Collection of HSC will not be a restriction

Construction of 2 vectors. One (*PGK.CoRPS10 LV)* restores erythroid differenciation and demonstrated the long-term repopulation properties of corrected DBA CD34+ cells. Concomitantly, long-term restoration od ribosomal biogenesis was verified.

Do he have specific difficulties in DBAS?

Gene frequency: current studies focus on *RPS19*: 25% of patients but we have 22 DBA genes and some are involved in < 1% of pts…

DBAS is an autosomic dominant disease with haplo-insufficiency: you have to restore an adequate level of RPS19: enough but not too much

BM is rich: a conditioning regimen will be required (like in GT for other red-cell diseases…); moreover: no evidence of repopulating advantage of corrected cells

☞ **Potential risk for liver toxicity in iron-overloaded pts**

In practice: GT in DBA

GT is moving in DBA and at least 3 clinical trials are planned to begin in 2025 for pts with *RPS19* variants

In EU the trial will be open only in Spain (and for Spanish pts only)

At least you can give some hope to a few of your patients especially those with *RPS19* variants…

NB: the Spanish team has also an ongoing pre-clinical study for *RPL5* and one US group works on *GATA1 (presented at ASH 2023)*

Merci pour votre attention

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APLASIE MEDULLAIRE centre de référence

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- SLS: Flore SICRE de FONTBRUNE & Régis PEFFAULT DE LATOUR

- BCT: Laboratoire: Lydie Da COSTA

- Isabelle Marie, Isabelle BRINDEL & Amélie MAROUANE

Patients associations

Reconnue par le Ministère de la Santé

MaRIH

Filière de santé Maladies Rares Immuno-Hématologiques