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PROJECT FINAL REPORT

Grant Agreement number: HEALTH-F5-2012-305421

Project acronym: EUROFANCOLEN

Project title: Phase I/II Gene Therapy Trial of Fanconi anemia patients with a new Orphan Drug consisting of a lentiviral vector carrying the FANCA gene: A Coordinated International Action

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4.1. FINAL PUBLISHABLE SUMMARY REPORT

4.1.1. Executive summary (not exceeding 1 page).

4.1.2. Summary description of project context and objectives (not exceeding 4 pages).

4.1.3. Description of the main S&T results/foregrounds (not exceeding 25 pages),

4.1.4. Potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results (not exceeding 10 pages).

4.1.5. Address of the project public website, if applicable as well as relevant contact details. Project logo, diagrams or photographs illustrating and promoting the work of the project (including videos, etc…), as well as the list of all beneficiaries with the corresponding contact names can be submitted without any restriction.

4.2. USE AND DISSEMINATION OF FOREGROUND

- Section A : Dissemination measures, including any scientific publications relating to foreground.

- Section B: Exploitable foreground and plans for exploitation.

4.3. REPORT ON SOCIETAL IMPLICATIONS

4.1. FINAL PUBLISHABLE SUMMARY REPORT

4.1.1. EXECUTIVE SUMMARY (NOT EXCEEDING 1 PAGE).

Fanconi anemia (FA) is a rare inherited syndrome characterized by the early development of bone marrow failure and increasing predisposition to cancer with age. Allogeneic hematopoietic cell transplantation (alloHCT) is the only curative therapy for hematopoietic manifestations of FA, although associated with complications arising from myeloablation, graft versus host disease and increased incidence of squamous cell carcinoma. The main goal of this project was the development of an efficient and safe gene therapy treatment for FA patients of the Complementation group A (FA-A patients) based on two recent innovations: 1) The discovery of potent HSC mobilizers, such as G-CSF combined with plerixafor, not previously used in FA clinical trials, and 2) The development of a new lentiviral vector by members of this Consortium, designed as Orphan Drug by the EC in December 2010.

Throughout the EUROFANCOLEN Project more than 300 FA patients from Spain, France and UK have been studied aiming at characterizing their mutational defects and hematopoietic status, required for the inclusion of FA patients in the proposed clinical trials.

To evaluate the efficacy of the designed strategy to mobilize FA hematopoietic stem cells (HSCs), fifteen patients were recruited in two different mobilization trials. Eleven out of the fifteen patients treated with the mobilizing drugs reached threshold numbers of CD34+ cells in peripheral blood (≥5 CD34+ cells/µL), and more than 300,000 purified CD34+ cells (minimal number required in the protocol for transduction) were collected from all these patients in 1-4 apheresis procedures.

Based on the design of the therapeutic PGK-FANCA.Wpre* lentiviral vector (LV), clinical lots manufactured under GMP conditions were generated, and their efficacy and absence of toxicity tested in preclinical studies. Experiments in FA-A mouse models demonstrated both the efficacy and the absence of toxicity of this LV. In subsequent studies it was possible to demonstrate that the gene correction of $CD34^+$ cells from FA-A patients promoted the *in vivo* repopulating properties of corrected FA HSCs in immunodeficient mice, and mediated an evident *in vivo* proliferative advantage over uncorrected HSCs.

Based on the optimization of FA HSC mobilization and transduction procedures, a gene therapy trial was initiated in 2016 with the objective of demonstrating the safety and efficacy of an implemented approach of gene therapy in FA-A patients. Eight patients were infused with transduced $CD34^+$ cells in the absence of any pre-conditioning regimen. Analyses conducted in the first four patients, now with a relatively long follow-up (2-3 years post-infusion), have shown progressive increases in the proportion of corrected cells in peripheral blood (PB) and (BM), that reached up to 60% in the patient infused with the highest number of corrected cells (250,000 corrected $CD34^+$ cells/Kg and longest follow-up, 30 months post-infusion). Insertion site analyses showed an oligoclonal pattern of hematopoietic reconstitution, and also revealed the engraftment of multipotent gene-corrected HSCs, without evidence of genotoxicity. In patients with higher levels of gene-correction, a significant phenotypic correction in BM HSCs and in PB T cells was also noted, as deduced from the increased resistance of BM progenitors to mitomycin C and decreased chromosomal instability of PB T cells exposed to diepoxybutane. Additionally, a trend of bone marrow failure correction has been observed in patients with highest levels of gene marking.

Taken together, our studies demonstrate for the first time that lentiviral-mediated gene therapy results in the progressive engraftment of corrected HSCs in non-conditioned FA patients, suggesting that the proposed gene therapy may constitute a low-toxicity option for the treatment of BMF in patients with FA.

4.1.2. SUMMARY DESCRIPTION OF PROJECT CONTEXT AND OBJECTIVES (NOT EXCEEDING 4 PAGES).

Fanconi anemia (FA) is a rare inherited disorder mainly associated with bone marrow failure (BMF), and cancer predisposition, principally acute myeloid leukemia (Auerbach and Allen 1991, Butturini, Gale et al. 1994, Kutler, Singh et al. 2003, Rosenberg, Greene et al. 2003, Alter, Giri et al. 2018). Although hematopoietic stem cell transplantation (HSCT) from HLA-identical siblings generally results in good outcomes (Bonfim, de Medeiros et al. 2007, Macmillan and Wagner 2010, Peffault de Latour, Porcher et al. 2013), transplants from alternative donors are associated with higher rates of morbidity and mortality. Additionally, an increased incidence of solid tumors - principally squamous cell carcinomas (SCC) – has been observed in FA transplanted patients, probably due to conditioning regimens and occurrence of graft versus host disease (GVHD) (Rosenberg, Socie et al. 2005, Masserot, Peffault de Latour et al. 2008, Alter, Giri et al. 2018).

Taking into account the efficacy of hematopoietic gene therapy achieved in different monogenic diseases (Cartier, Hacein-Bey-Abina et al. 2009, Aiuti, Biasco et al. 2013, Biffi, Montini et al. 2013, Eichler, Duncan et al. 2017, Thompson, Walters et al. 2018) and based on advances of gene therapy studies in experimental FA models (Tolar, Becker et al. 2012, Adair, Sevilla et al. 2017), new perspectives have been considered for FA gene therapy. Evidence of LV-mediated gene therapy was demonstrated in all tested models of FA, including Fanca$^{-/-}$ (Galimi, Noll et al. 2002, Rio, Segovia et al. 2002, Yamada, Ramezani et al. 2003, Muller, Milsom et al. 2008, Molina-Estevez, Nowrouzi et al. 2015, Fernandez-Garcia, Luisa Lamana et al. 2018). Moreover, evidence of spontaneous *in vivo* proliferative advantage in FA HSCs was demonstrated for the first time in Fancd1$^{-/-}$ mice (Navarro, Meza et al. 2006, Rio, Meza et al. 2008), resembling the behaviour of reverted HSCs in FA mosaic patients(Gregory, Wagner et al. 2001, Gross, Hanenberg et al. 2002, Mankad, Taniguchi et al. 2006). Additional studies in different FA mouse models showed improvements in the engraftment of HSCs that had been transduced for short periods of time (Muller, Milsom et al. 2008, Si, Pulliam et al. 2008, Fernandez-Garcia, Mesa et al. 2017).

Based on the efficacy and safety of LVs, two similar PGK-*FANCA*.Wpre* LVs were developed by members of this Consortium (Gonzalez-Murillo, Lozano et al. 2010) and colleagues from the Fred Hutchinson Cancer Research Center (Becker, Taylor et al. 2010). Both groups reduced the length of the transduction process and protected FA cells from oxidative damage with N-acetylcysteine and a low oxygen atmosphere (Jacome, Navarro et al. 2009, Becker, Taylor et al. 2010, Gonzalez-Murillo, Lozano et al. 2010, Rio, Navarro et al. 2017). Additionally, due to the hypersensitivity of FA HSCs to TNF-α (Dufour, Corcione et al. 2003) the use of a TNF receptor-Fc fusion protein, etanercept, was proposed to prevent cytotoxic effects induced by this growth factor (Rio, Navarro et al. 2017).

To study the functional properties of human FA HSCs, previous studies showed the lower content of HSCs in FA patients in comparison with healthy donor HSCs, and also evidenced homing defects when these cells were transplanted into immunodeficient mice (Zhang, Shang et al. 2008). These observations, together with the limited number of HSCs present in the BM of FA patients (Larghero, Marolleau et al. 2002, Jacome, Navarro et al. 2006, Ceccaldi, Parmar et al. 2012) limited in the past the efficacy of FA gene therapy. These failures were associated to the defects in the engraftment of gene-corrected HSCs, and thus with the absence of clinical efficacy for reverting the BMF of these patients (Liu, Kim et

al. 1999, Walsh, Fu et al. 2001, Kelly, Radtke et al. 2007). Detailed information was provided in two studies (Liu, Kim et al. 1999, Kelly, Radtke et al. 2007). In the first one in FA-C patients (Liu, Kim et al. 1999), purified $CD34^+$ cells were transduced over 3 days with a RV carrying *FANCC*. Up to four infusions of corrected cells were given to these patients at intervals of 3 to 4 months. No evidence of gene-corrected cells was observed after a few months post-infusion. Strikingly, in one patient who received radiation for a gynecological malignancy, cells with the *FANCC* transgene could be detected. In a subsequent trial in FA-A patients (Kelly, Radtke et al. 2007), $CD34^+$ cells were pre-stimulated for 3 days, and followed by two rounds of transduction with a RV encoding for *FANCA*. Neither in this case, the presence of gene marked cells was detected at periods longer than 3 months post-infusion. Potential reasons accounting for the defective engraftment of corrected HSCs in these FA patients were associated to defects in the transduction of the true HSCs after relatively long transductions with RVs, or the infusion of limited numbers of transplanted HSCs in non-conditioned patients.

Aiming at improving HSC collection from FA patients for gene therapy purposes, different drugs were combined with G-CSF. Significantly, plerixafor (AMD3100) was shown to synergize with G-CSF, resulting in a significant mobilization of HSCs in two FA mouse models (Pulliam, Hobson et al. 2008). Based on this and other studies in patients with a poor HSC reserve (To, Levesque et al. 2011), a clinical trial was proposed to demonstrate the efficacy and safety of an HSC mobilization protocol based on the administration of G-CSF and plerixafor in FA patients. The results obtained from clinical studies conducted by the EUROFANCOLEN Consortium showed the safety and the efficacy of this mobilization protocol in young pediatric FA-A patients.

Using mobilized HSCs from FA patients, an improved transduction procedure of these cells was then developed (Rio, Navarro et al. 2017). In that study, mobilized FA $CD34^+$ cells were transduced for a short period of time with the therapeutic PGK-*FANCA*.Wpre* LV (Gonzalez-Murillo, Lozano et al. 2010) and infused into immunodeficient mice. Notably, human myeloid and lymphoid cells were identified in transplanted mice, indicating the engraftment of repopulating cells with multipotent differentiation capacity. Moreover, a marked increase of MMC-resistance in engrafted progenitor cells was observed as compared to data obtained prior to cell infusion, demonstrating for the first time the phenotypic correction and *in vivo* proliferative advantage of corrected FA patient HSCs (Rio, Navarro et al. 2017). These observations suggested that a similar proliferation advantage of corrected FA HSCs should occur after infusion in FA patients, ideally in the absence of conditioning. Based on improvements achieved in the experimental approaches, a phase I/II gene therapy trial in FA-A patients was thus proposed in 2015 with the aim of demonstrating the safety and efficacy of the infusion of gene corrected $CD34^+$ cells in non-conditioned FA-A patients (Clinical trial.gov Id: NCT 03157804). Data obtained by the EUROFANCOLEN Partners in this clinical trial have demonstrated for the first time that lentiviral-mediated gene therapy results in the progressive engraftment of corrected HSCs in non-conditioned FA patients, suggesting that gene therapy should constitute a low-toxicity option for these patients (Rio, P, Navarro et al, under revision).

OBJECTIVES OF THE PROJECT:

The principal objective of this Project was the development of a Phase I/II gene therapy trial for FA-A patients, based on the genetic correction of plerixafor+G-CSF mobilized HSCs with a novel FA lentiviral vector, accompanied by comprehensive and ground-breaking safety and efficacy patient monitoring studies.

To achieve this goal, four scientific working packages were proposed:

WP1: Genetic and hematopoietic studies of FA patients. The objective of this WP was the characterization of the genetic and hematopoietic defects of FA patients to facilitate the recruitment of the patients for the HSC mobilization (WP2) and gene therapy trials (WP4).

WP2: Assessment of the safety and efficacy of plerixafor plus G-CSF–mediated mobilization of CD34+ cells. This WP aimed the implementation of an optimized procedure for the collection of hematopoietic stem cells from FA patients using an efficient HSC mobilization regimen with plerixafor combined with G-CSF.

WP3: Validation of preclinical gene therapy studies with the therapeutic clinical-grade lentiviral vector. WP3 aimed at demonstrating the efficacy and absence of toxicity of the transduction procedure to be used in patients' CD34+ cells with the therapeutic lentiviral vector.

WP4: Assessment of the safety and efficacy of the infusion of gene-corrected CD34+ cells in FA-A patients. The objective of WP4 was to investigate the safety and the efficacy of the infusion of autologous $CD34^+$ cells transduced with the therapeutic lentiviral vector carrying the *FANCA* gene in FA-A patients.

EXPECTED FINAL RESULTS:

The expected final result of EUROFANCOLEN was the demonstration of engraftment and phenotypic correction of HSCs in patients with FA, aiming at preventing the characteristic bone marrow failure syndrome of these patients. The proposed gene therapy approach should constitute a unique therapeutic opportunity for FA patients lacking a suitable donor and a good alternative to current allogeneic transplants.

4.3.1. DESCRIPTION OF THE MAIN S&T RESULTS/FOREGROUNDS (NOT EXCEEDING 25 PAGES)

The main scientific and technological results achieved in EUROFANCOLEN are summarized by Working-packages, as follows:

WP1: Genetic and hematopoietic studies of FA patients.

The objective of this WP was the characterization of the genetic and hematopoietic defects of FA patients to facilitate the recruitment of the patients for the HSC mobilization (WP2) and gene therapy trials (WP4).

Because mutations in any of the 22 different FANC genes may account for the disease of FA patients, EUROFANCOLEN Partners have carried out a very close collaboration to characterize the complementation group of potential candidates to be treated by gene therapy. More than 500 chromosome fragility tests have been performed since the beginning of the project, 58 of which corresponded to newly diagnosed patients.

Year	Total tests	Positive tests	New FA patients
2013	67	39	9
2014	73	42	7
2015	76	40	7
2016	98	52	14
2017	97	37	13
2018	99	52	8
TOTAL	510	262	58

Table 1.1: Summary of chromosome fragility tests and diagnosis of new FA patients performed during the course of EUROFANCOLEN.

Whole exome sequencing was applied to identify pathogenic mutations in 58 newly diagnosed patients, and in 14 additional patients with unknown mutations. Extensive functional genetic studies were required to genetically characterize several patients with missense variants of unknown significance. Chromosome fragility analyses were also performed in all patients recruited into the mobilization trial. Metaphases analysed by GTG bands in patients recruited for the $CD34^+$ selection trial showed in all cases the presence of 46 chromosomes without structural abnormalities. FISH analyses performed to detect numeric or structural alterations in chromosomes 1, 3 and 7 were negative in all instances. Similar results were obtained by SNP array-based high resolution karyotyping. These analyses facilitated to start two clinical trials aiming at improving the collection of hematopoietic stem cells (HSCs) from these patients, as well as their treatment based on a new gene therapy approach with lentiviral vectors (LVs).

Interestingly, we incidentally identified two adult FA patients suffering from the Sertolis Cells Only Syndrome, with pathogenic mutations in *FANCA*. We also excluded *FANCM* as being

a FA gene since several individuals from Spain and France with bi-allelic mutations in *FANCM* and cancer risk did not develop signs of FA disease.

Based on the mutational studies conducted during EUROFANCOLEN, the distribution of FA complementation groups in Spain is shown in **Figure 1.1:**

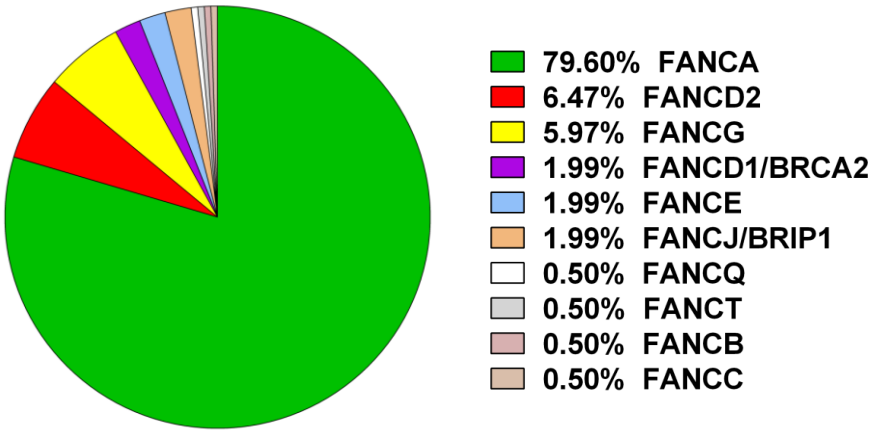


Figure 1.1: Updated distribution of FA subtypes among the 201 genetically characterized FA patients from Spain including the genetic studies carried out in EUROFANCOLEN

Additionally, Partner 8 lead the identification of a novel FA gene (*RFWD3/FANCW*) and participated in the discovery of *FANCW*. Similarly, Partner 7 participated in the discovery of a new FANC gene, *FANCV* during Eurofancolen. It is remarkable that in total, three new FA genes (*FANCQ*, *FANCV* and *FANCW*, were characterized due to the work of Eurofancolen partners, as a co-lateral research aiming the characterization of FA gene mutations.

The average number of new FA patients diagnosed in Spain per year during the course of Eurofancolen was 9-10. Most of them corresponded to the FA-A complementation group, although only a reduced proportion of them finally met the criteria of trials corresponding to WP2 and WP4. We noticed that several teenager FA patients with mild hematology; therefore, putative candidates for these trials, turned out to be mosaics even though a clear non-mosaic diagnosis was reported several years back. This was only possible to detect by repeating the DEB tests years after an initial positive diagnostic DEB test. This was also the case of three FA-A patients and one FA-D1 patient that were diagnostic incidentally at adult age due to azoospermia or early on set breast cancer. Considering that mosaicism is an exclusion criterion in the gene therapy trial and that mosaics are over-represented in the population of adult and teenager FA patients with mild hematology, these results highlight the importance of stringent cytogenetic analyses in PB T cells and deep characterization of inter-strand cross linking resistance in BM progenitors prior to gene therapy.

Finally, we have been able to study the long term follow up of the clinical evolution of a total of 40 mosaic patients as defined by the criterion of having less than 50% of T cells with chromosome breaks, and have found that mosaic patients have a better prognosis, longer life expectancy and lower incidence of BM transplantation as compared to the non-reverted FA patients. When a restrictive criterion of mosaicism was considered (patients with BM

progenitor cells resistant to MMC), none of the true mosaic patients developed leukemia at current follow-up periods. Although the number of patients analyzed is still low, these results strongly suggested that the correction of HSCs by gene therapy would limit the spontaneous development of leukemia in these patients.

To demonstrate that the insertion of the wild-type sequence of FANCA functionally complements FA-A cells defined by genetic screening, in all these patients subtyping studies were performed by retroviral complementation. These analyses were essential for the inclusion of FA-A patients in the gene therapy trial described in WP4. A representative example of a positive complementation is shown in **Figure 1.2.**

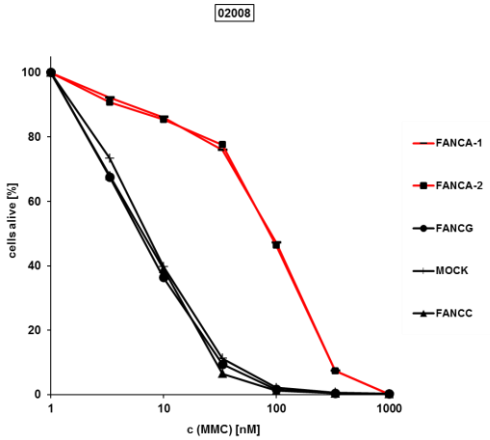


Figure 1.2. Representative complementation assay conducted in a FA-A patient using two different FANCA-retroviral vectors

WP2: Assessment of the safety and efficacy of plerixafor plus G-SCF–mediated mobilization of CD34$^+$ cells.

The main objective of this WP was to determine the safety of a protocol aiming the collection of clinically relevant numbers of CD34$^+$ cells required for a gene therapy trial in FA-A patients. The protocols developed in Spain and France for the mobilization of FA HSCs were based on the administration of filgrastim (G-CSF) and plerixafor for up to 1 week, followed by up to four apheresis procedures, and immunoselection of CD34$^+$ cells.

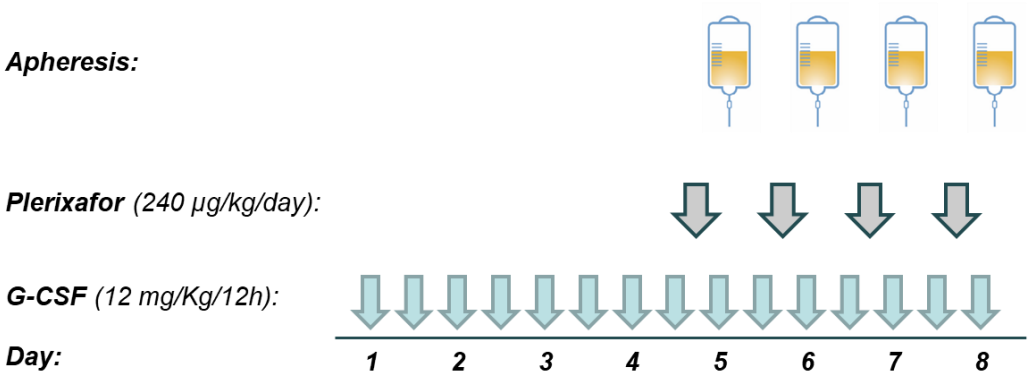


Figure 2.1. Mobilization protocol used for the collection of CD34⁺ cells from FA patients.

In the Spanish trial, 11 patients were recruited. The mobilization of CD34⁺ cells corresponding to these patients showed similar kinetics. Basically, CD34⁺ cell mobilization started after administration of plerixafor. Significantly, all treated patients except the oldest patients with 15 and 16 years of age mobilized threshold numbers of CD34+ cells into PB (≥5 CD34+ cells/µL).

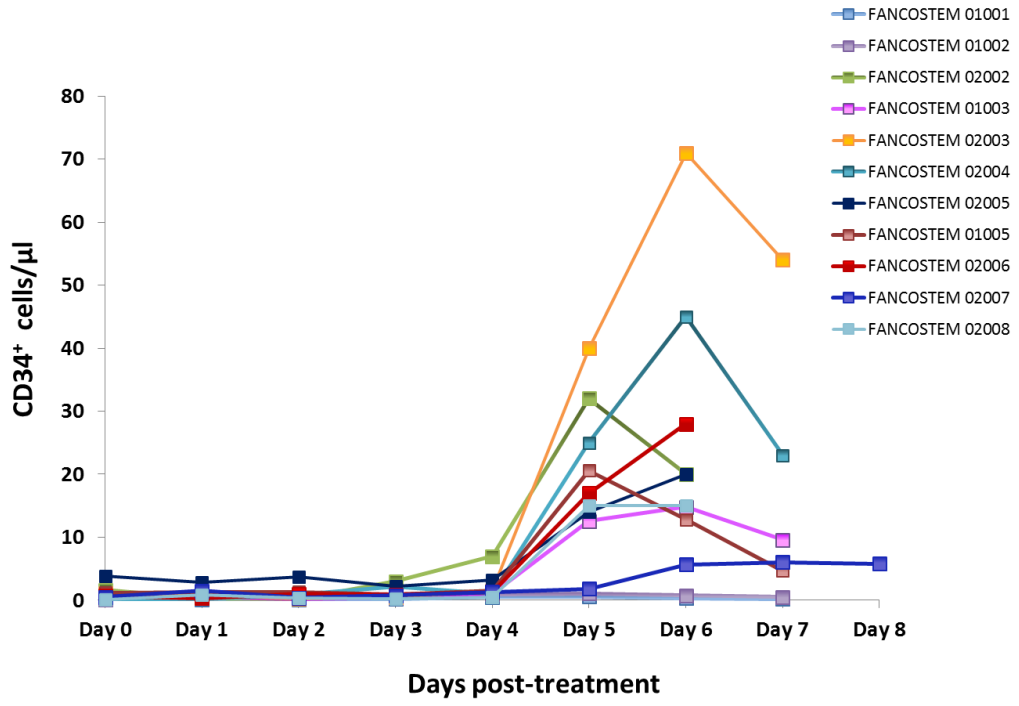


Figure 2.2: Kinetics of CD34⁺ cells mobilization in patients treated with G-CSF and plerixafor. V0 to V7 correspond to the different visits with respect to the initiation of the mobilization treatment (V0).

Prior to immunoselection, an average value of 4.3 million $CD34^+$ cells per Kg of weight was obtained in the whole series. After immunoselection, this value significantly decreased to a mean value of 1.7 million $CD34^+$ cells/Kg. These results showed that a sufficient number of $CD34^+$ cells (>300.000 $CD34^+$ cells/kg, as defined in the exclusion criteria) was collected from every patient that mobilized at least 5 $CD34^+$ cells/µL) in PB.

Seven patients subjected to the mobilization process, and whose $CD34^+$ cells remained cryopreserved until subsequent use for gene therapy, have been followed for at least 1 year (the follow-up period defined in the clinical trial for assessing the safety of the protocol). No severe adverse events associated to the HSC mobilization and collection regimen have been observed in any treated patients, evidencing the safety of the mobilization protocol.

In the French trial, four patients have been recruited. One patient was diagnosed before clinical manifestations thanks to family screening. The collection protocol targeted a quantity of $3x10^6$/Kg $CD34^+$ cells, based on a predicted future weight in 5 years. $CD34^+$ cells and white blood cells (WBC) were monitored tightly along the mobilization protocol. Patients with more than 10 $CD34^+$/µl PB or between 5 and 10 $CD34^+$ cells/µl PB with a clustered aspect detected by flow cytometry after plerixafor injection underwent aphaeresis. Immunoselected $CD34^+$ cells from the aphaeresis product were cryopreserved for further gene therapy manipulation.

$CD34^+$ cells were mobilized into PB in the two patients with 5 and 2-year-old. Both patients underwent aphaeresis procedures. The collection target was not achieved after four days of apheresis in one patient, although it was obtained after the first mobilization day in the other patient. No short-term adverse events were observed. Following $CD34^+$ cell immunoselection, $CD34^+$ cell purity and recovery were poor, although in the normal range described in the literature for FA patients. One month after the collection cell blood counts were unchanged in these two patients.

The results corresponding to this WP demonstrate that the combined use of plerixafor and G-CSF constitutes an efficient and safe approach for the collection of clinically relevant numbers of $CD34^+$ cells from young pediatric FA-A patients.

WP3: Validation of preclinical gene therapy studies with the therapeutic clinical-grade lentiviral vector.

This WP aimed to produce under GMP conditions, the therapeutic vector to be used in FA-A patients, and to assess the efficacy and absence of toxicity of this vector in preclinical models. Additionally, this WP also pursued the development of an optimized transduction protocol to transduce HSCs from FA-A patients, while preserving the repopulating properties of these primitive precursor cells.

Initially, different pre-GMP FANCA-LV lots were generated for evaluating the efficacy of these vectors for transducing mouse and human FA HSPCs. $CD34^+$ cells from healthy donors and FA-A patients showed transduction levels ranging from 40-80%, either in healthy donor or FA-A $CD34^+$ cells, with respective numbers of proviral copies per cell ranging from 0.3 to 0.7. In the case of the FA-A patients, the therapeutic efficacy of the provirus was corroborated by the resistance of FA-A progenitor cells to mitomycin C (MMC).

Biodistribution studies of the therapeutic lentiviral vector were carried out in *Fanca*$^{-/-}$ mice. These studies showed the restricted presence of the therapeutic provirus in the hematopoietic tissues of treated mice, and confirmed the absence of replication competitive lentiviruses following the infusion of transduced mouse HSPCs. Concordantly, ELISA studies in the serum of treated animals could not trace any remaining LV, evidencing the absence of *FANCA*-LV replication.

Because it was unknown whether the safety aspects currently associated to LV-mediated gene therapy could be applicable to DNA repair deficient syndromes such as FA, we investigated this aspect in *Fanca*$^{-/-}$ mice. The aim of this study was to investigate the pattern of LV insertion sites and *in vivo* clonal dynamics of *Fanca*$^{-/-}$ HSCs after gene correction with a GMP-like *FANCA*-LV. These studies demonstrated that transduction of *Fanca*$^{-/-}$ bone marrow precursors with *FANCA*-LV efficiently corrected the phenotype of FA HSCs without any sign of toxicity. A genome-wide screening of LV insertion sites (LIS) in PB and BM from *Fanca*$^{-/-}$ recipients was conducted using LAM-PCR and 454 pyrosequencing. Consistent with previously reported LIS in non-FA models, LIS determined in *Fanca*$^{-/-}$ hematopoietic cells from transplanted recipients were enriched in RefSeq genes but not in regions close to transcription start sites (TSS) (see (Molina-Estevez, Nowrouzi et al. 2015) and **Table 3.1**).

Table 3.1 Summary of insertion sites analyzed by LAM-PCR and NGS. TSS: transcription start site, CpG: cytosine-phosphate-guanine, IS: integration site, BMT: bone marrow transplantation, LV: lentiviral vector, RV: gamma-retroviral vector.

Samples	Mappable reads	Unique IS	RefSeq	RefSeq ±5 Kb	TSS ±5 Kb	CpG island ±5 Kb
FANCA-LV						
Pre-BMT	1,646	321	199 62.0%	207 64.5%	19 5.9%	20 6.2%
Primary (n=31)	156,245	5,451	2915 53.5%	3129 57.4%	311 5.7%	359 6.6%
Secondary (n=24)	76,532	1,019	489 48.0%	526 51.6%	51 5.0%	58 5.7%

The analysis of the dynamics of LV-transduced *Fanca*$^{-/-}$ repopulating clones showed a highly polyclonal repertoire short-term after transplantation, which progressively turned into a less complex repertoire. Nevertheless, a continuous appearance of new clones was observed even in secondary recipients, reflecting a continuous turnover of corrected *Fanca*$^{-/-}$ HSC clones *in vivo.* Additionally, no selection towards specific CIS was observed in transplanted recipients, contrasting with observations made in cells of the same genetic background that were transduced with a genotoxic gamma-retroviral vector (Molina-Estevez, Nowrouzi et al. 2015). Taken together our data showed that FA LV-mediated gene therapy not only efficiently corrects the phenotype of affected HSCs, but also promotes a healthy pattern of clonal turnover in transplanted FA recipient mice.

Subsequent work from Genethon facilitated the generation of GMP-manufactured LVs for the treatment of FA-A patients. All vector lots were conform to specifications. In particular, LVs transduced umbilical cord blood CD34$^+$ cells efficacies of ≥ 0.3 vector copies per cell (VCN) after one transduction cycle. These vectors showed no toxicity in human CD34$^+$ cells up to doses of 4x10^8 IG/ ml, both measured by survival and hematopoietic progenitor content. Transduction efficiencies, measured by qPCR, increased from 0.18 to 0.44 (LV

concentration range: range: 10^8 to $4x10^8$ IG/mL). Since Genethon stopped their activities related to the generation of GMP LVs, a new partner – Yposkesi – was incorporated in EUROFANCOLEN for the manufacturing of the last lot of therapeutic LV required for the treatment of the last FA patients. During the 2017/18 period, this Institution efficiently generated this LV lot, with similar infectivity properties than the previous ones.

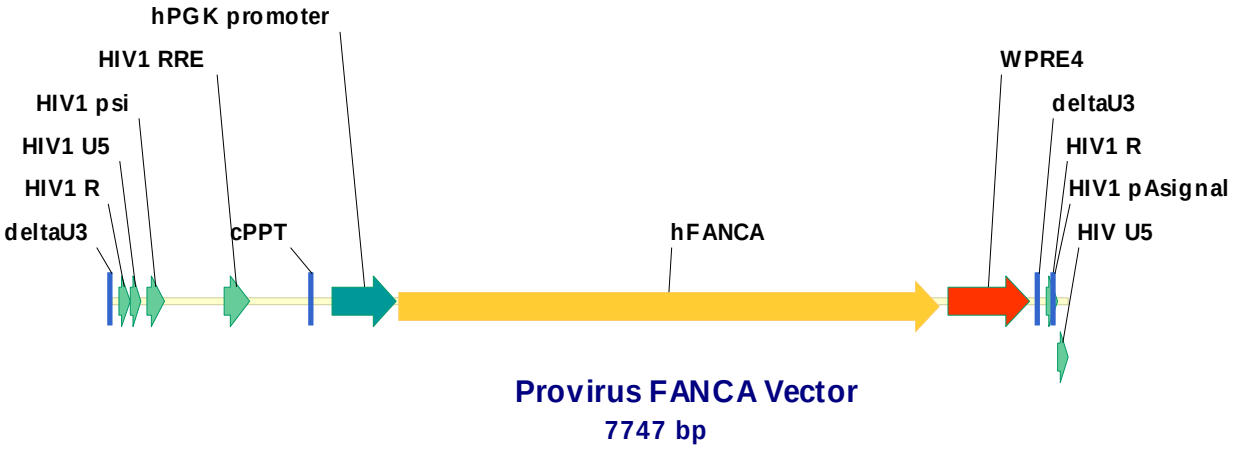


Figure 3.1: Schematic representation of the integrated proviral genome. HIV: human immunodeficiency virus/Psi: incapsidation sequence /LTR: Long Terminal Repeat 5' or 3'/ HIV R: HIV R region/ HIV delta U3: U3 region with a deletion/ RRE: Rev responsive element / cPPT cts: central polypurine tract – central terminal sequence/ hPGK: human phosphoglycerate kinase promoter / hFANCA: human FANCA gene / WPRE4: mutated woodchuck hepatitis virus posttranscriptional regulatory element

To test the efficiency of the transduction protocol in $CD34^+$ cells from FA patients small aliquots of $CD34^+$ cells obtained from patients recruited in the FANCOSTEM trial were transduced with the GMP-FANCA LV, using a short pre-stimulation of 8-12h, followed by a transduction period of only 12-14h. Transduction of mPB-$CD34^+$ cells from four different patients showed a reversion of Mitomycin C (MMC) sensitivity that varied between 20- 41%.

To confirm the engraftment capacity of corrected cells, samples from these patients were transplanted into immunodeficient mice. Engraftment levels ranging from 1.27 to 9.54 % were observed in these studies. Notably, an increase in the proportion of MMC-resistant progenitor cells from 20% prior to transplantation, to 100% at 30 days post-transplantation confirming for the first time the proliferative advantage of gene-corrected progenitor cells from FA patients in an *in vivo* model. (Rio, Navarro et al. 2017).

Taking into account these results, a transduction protocol based in a short pre-stimulation of 8-10h, followed by a transduction period of 12-14h was used for the validation studies with mobilized PB $CD34^+$ cells. These studies showed the presence of 0.39, 0.33 and 0.67 proviral copies per cell, respectively in the three validation runs, fulfilling the specifications required by the Regulatory Agency related to the transduction process. Also specifications relative to the stability and the transport of the product and the aseptic process were achieved. Thus, in March 2015 the GMP certificate for the manufacturing was obtained (ES/055I/15), and also the approval to initiate the FA gene therapy trial (CT: 2011-006100-12)

WP4: Assessment of the safety and efficacy of the infusion of gene-corrected CD34+ cells in FA-A patients.

The objective of this WP was to investigate the safety and the efficacy of the infusion of autologous $CD34^+$ cells transduced with the therapeutic lentiviral vector carrying the *FANCA* gene in FA-A patients.

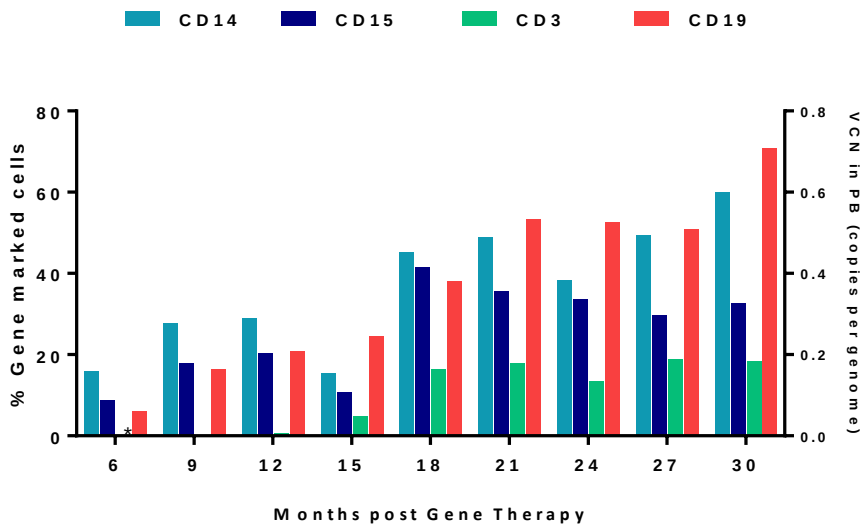
Based on the improved mobilization of $CD34^+$ cells from FA patients utilizing G-CSF and plerixafor, and taking into account the preservation of the repopulating properties associated to the short transduction of these cells with the therapeutic FANCA-lentiviral vectors (Rio, Navarro et al. 2017), in the proposed clinical trial HSCs from FA-A patients were mobilized and transduced as described in the experimental study, and re-infused without any cytotoxic conditioning therapy.

Eight pediatric FA-A patients (ages 3-6 years) fitting the criteria defined in the FANCOLEN-1 trial (NCT 03157804) have been treated. On average, transduction efficacies ranged between 0.17 to 0.78 copies/cell were obtained, suggesting transduction rates ranging from 17% to 78% in $CD34^+$ cells. Based on these results, the estimated total number of corrected $CD34^+$ cells that were infused in the patients ranged between 49,000 to 410,000 transduced $CD34^+$ cells/Kg of weight. In four patients a relatively long follow up has been achieved (2-3 years), and two of these patients have already finished the FANCOLEN-1 follow-up of 3 years. Data corresponding to these four patients will be described.

In two patients, $CD34^+$ cells were collected and then cryopreserved until PB cell counts decreased below values defined in the clinical trial. In two other patients, PB analyses met the criteria required both for the $CD34^+$ cell collection and also the gene therapy trials. Thus, freshly collected $CD34^+$ cells were transduced and infused the day after the last apheresis.

Because of the absence of conditioning and due to the limited number of infused $CD34^+$ cells, none of these patients had significant levels of marked cells in PB or BM before the 4^{th} month post-infusion. Nevertheless, in all these patients progressive increases in the proportion of corrected cells was consistently observed thereafter. The latest analyses (30 months post-infusion) conducted in the patient infused with higher number of corrected cells showed that 55% of total PB cells were gene corrected, and similar values were observed in BM. Also, all these patients showed that gene marking took place in all cell lineages, including the myeloid, B and T cell lineages (See data corresponding to purified PB and BM cell populations from patient 02002 in **Figure 4.1**.

A)



B)

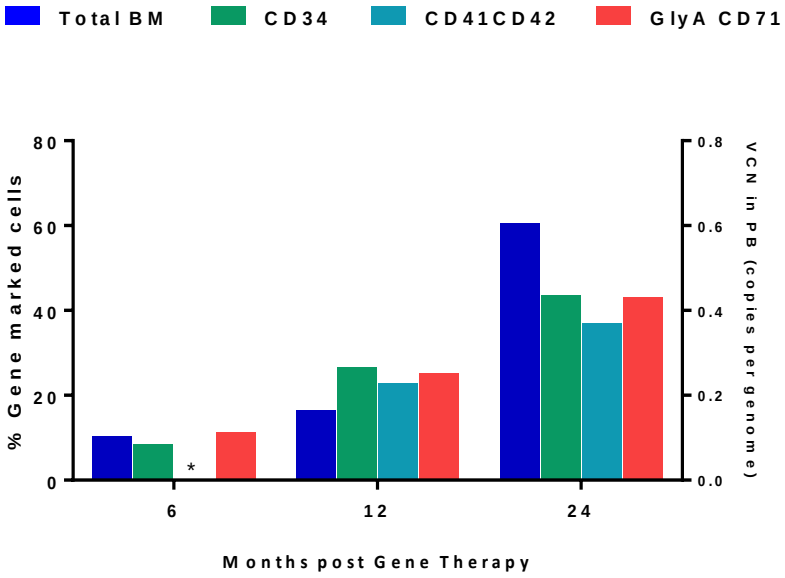


Figure 4.1: Analysis of the proportion of gene corrected cells purified PB cells (Panel A) and BM cells (Panel B), expressed as percentage of corrected cells or VCN/cell at different times post-infusion.

In none of the tested samples evident cytogenetic abnormalities or mutations in a myeloid malignancy 85-gene panel were observed, strongly suggesting that genetic abnormalities potentially indicative of malignancy were not present in any of these patients.

Analyses of unique insertion sites (UIS) showed a similar integration pattern to the one found in previous gene therapy trials from other diseases. Importantly, no preferential integrations were found in close proximity to transcription start sites (TSS), consistent with previous findings observed in our preclinical studies with the PGK-FANCA-Wpre*-LV.

Longitudinal ISA conducted in the two patients with highest levels of gene marking showed higher levels of repopulating clones at the latest follow-up visits, as compared to analyses performed in early times-post-infusion, indicating that increases of gene marking noted in BM and PB were not associated with the dominance of one or a small number of clones with genotoxic insertions. Significantly, in addition to UIS characteristic of one cell lineage, several other UIS were identified in up to four different lineages, demonstrating the engraftment of gene-corrected multipotent HSCs (see representative illustration showing the presence of ISs common to different lympho-hematopoietic lineages in **Figure 4.2**)

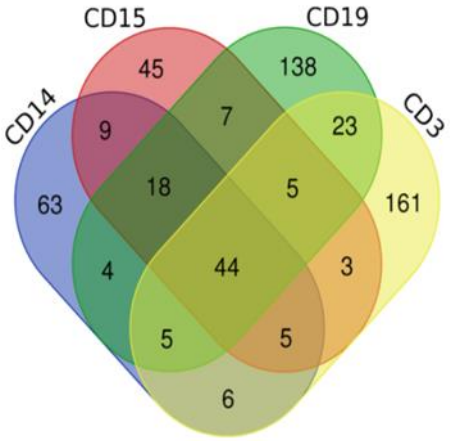


Figure 4.2: Genome-wide Mapping of Vector Unique Integration Sites in purified myeloid and lymphoid populations from a FA patient treated by gene therapy

Taken together, these results demonstrate for the first time that the infusion of gene corrected HSPCs in non-conditioned FA patients is associated with the engraftment of gene-corrected multipotent HSCs without evidence of genotoxic integrations.

To investigate whether increases in the proportion of corrected cells were associated with the reversion of their phenotype, BM samples obtained at periodic intervals after the infusion of corrected cells were exposed to mitomycin C. Progressive increases in the MMC-resistance of the colony forming cells were observed in these treated patients. Similarly, and given that the gold-standard diagnostic test for FA patient is the DEB-induced chromosomal breakage test in PB T cells, this test was carried out in our patients. Progressive decreases in the proportion of aberrant T cells were noted in the patients with highest levels of gene correction, demonstrating that the engraftment of gene corrected hematopoietic cells in FA treated patients is associated with the reversion of their phenotype.

Finally, we investigated whether the progression of BMF was modified by the infusion of corrected cells. The effect associated to the infusion of transduced cells was most evident in the patient with the highest levels of gene correction. In this patient an evident stabilization in the number of leukocytes, neutrophils and hemoglobin values were observed since the 6th month post-infusion. Also, a trend of stabilized neutrophil counts was observed in the three other patients with the longer follow-up times. Concerning the platelet lineage, prophylactic platelet transfusions were given to the patient with the lowest levels of gene

marking. In the three other patients no platelet transfusions were required, and stabilized, though low values of these cells have been observed until the 20-30 months of follow-up.

Taken together, data obtained in the EUROFANCOLEN project has demonstrated for the first time the feasibility of correcting the genetic defect in hematopoietic cells from patients with FA. Importantly, in contrast to allogeneic transplantation, the proposed gene therapy of FA patients should not increase the incidence of solid tumors in these patients since neither conditioning nor graft versus host disease (GVHD) play any role in the proposed FA gene therapy protocol.

The achievements obtained in EUROFANCOLEN strongly indicate that the proposed gene therapy approach will constitute a new and low-toxicity option for the treatment of patients with FA.

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4.3.2. POTENTIAL IMPACT (INCLUDING THE SOCIO-ECONOMIC IMPACT AND THE WIDER SOCIETAL IMPLICATIONS OF THE PROJECT SO FAR) AND THE MAIN DISSEMINATION ACTIVITIES AND EXPLOITATION OF RESULTS (NOT EXCEEDING 10 PAGES).

- **Impact on the health status of FA patients**

The allogeneic transplantation of HSCs from healthy donors currently constitutes the only curative therapy of the BMF characteristic of FA patients (Dufour and Svahn 2008). Although HSCT from HLA-identical siblings generally results in good outcomes (Bonfim, de Medeiros et al. 2007, Macmillan and Wagner 2010, Peffault de Latour, Porcher et al. 2013), transplants from alternative donors are associated with a higher morbidity and mortality. Additionally, an increased incidence of solid tumors - principally squamous cell carcinomas (SCC) – has been observed in FA transplanted patients, probably due to the conditioning regimen and the occurrence of graft versus host disease (GVHD) (Rosenberg, Socie et al. 2005, Masserot, Peffault de Latour et al. 2008, Alter, Giri et al. 2018).

In contrast to allogenic transplantation, the proposed gene therapy approach is based on the transplantation of autologous cells in patients that do not receive any conditioning regimen. This implies that almost no hospitalization is required after the infusion of corrected cells, and also that no risks associated to infections, graft rejection or graft versus host disease (GVHD) will take place as a consequence of the gene therapy in FA patients, contrasting to the main adverse effects of allogeneic transplants.

The proposed gene therapy option would thus constitute a low-toxicity option that may prove particularly impactful for FA patients. The main impact of this new therapy for FA patients can be summarized as follows.

- First, the clinical feasibility of correcting the genetic defect in a very challenging disease that causes BMF and leukemia constitutes a good alternative for a disease with a highly-unmet clinical need.

- Because no cytotoxic conditioning is required, it could be applied in FA patients in early stages of the disease to prevent the BMF and other hematopoietic complications associated to the disease.

- In contrast to allogeneic transplantation, the infusion of autologous cells should not increase the incidence of solid tumors in these patients, since neither conditioning nor GVHD will take place in gene therapy treated patients.

- In the case that solid tumors appear in these patients as a natural evolution of the disease, an improved hematological response to chemotherapy would be expected due to the restored DNA repair pathway in blood and BM cells, thus allowing the administration of more efficient anti-cancer therapies in these patients.

- **Economic impact:**

In addition to the evident impact that our gene therapy strategy should have in the health of FA patients, it will also have a significant impact in economic terms. This is derived from a number of aspects that in comparison to allogenic transplantation can be summarized as follows:

- Minimal hospitalization costs after cell infusion
- No costs associated to eventual infections, graft failure and GVHD, associated to allogenic transplantation
- No costs associated to re-hospitalizations of patients with long-term effects associated with allogenic transplantation; i.e. GVHD or increased incidence of squamous cell carcinomas.

FA gene therapy will have a significant impact on the economic contribution by the patients and their relatives and will avoid expensive adaptations to physical impairment.

The development of gene therapy in FA will foster an entirely new industrial high-quality technology effort in terms of stem cell processing, vector development and production and a medical genetic modification technology.

Costs associated to the production of therapeutic lentiviral vectors and to the manufacturing of gene-corrected cells are decreasing progressively due to the increased number of institutions that can efficiently develop these technologies. Moreover, in the particular case of FA gene therapy, the absence of patient conditioning will imply that this therapeutic approach may constitute one of the most affordable gene therapies for patients with monogenic diseases. This would imply a significant reduction in Europe's health system costs associated with the treatment of a very severe inherited monogenic disease.

Also of significance is the simplicity of efforts that will be required for the gene therapy of FA patients as compared to allogenic transplants, particularly for transplants from alternative donors. Our proposed new therapy is expected to increase the quality of life and autonomy of FA patients, leading to decreased social costs associated with home or hospital care.

- **Employment and Innovation impact:**

The improved medical knowledge and competitiveness of researchers and clinicians involved in the development of FA gene therapy technologies is already having an impact in the achievement of qualified jobs of young scientists.

New guidelines and standards are being developed for the treatment of FA patients that will be followed by the diffusion of standardized and robust protocols and guidelines to optimize the treatment of these patients. Eurofancolen has also allowed the identification of new key elements in gene therapy technology and gene therapeutic medical approaches, which do not only apply to a rare diseases as addressed in this project, but also to other diseases with a higher prevalence and with a direct impact on optimizing the delivery of health care to European citizens.

The scientific leadership and credibility that the Project has created is attracting young talented graduate scientists from many countries outside Europe to come to Europe. Similarly a significant interchange of investigators is taking place among the different laboratories of Eurofancolen. Also the education and mobility activities in the project is facilitating that a number of trained scientists are being contracted by private sectors with expertise in advanced technologies that will finally improve the competitiveness of the European biomedical industry.

- **Patient Associations**

Thanks to the work of Eurofancolen, very active networking activities were initiated with patient Associations, such as the FA associations in Spain, France, Germany, Italy, UK and also with other international Associations grouped by the Fanconi Anemia Research Fund.

In October 2018 all these Associations met in New Port Village (Los Angeles) during the Scientific FARF meeting, where advances in the gene therapy of FA patients were updated to FA families.

- **Innovation and Industrial exploitation:**

After the initial approval of the Orphan Drug designation of the PGK-FANCA.Wpre* LV by the EC in 2010, a new Orphan Drug designation was approved by the FDA in 2016. Thereafter, the Investigation New Drug Dossier (IND) was submitted and approved by the FDA in September 2018.

A license agreement was signed with Rocket Pharma for the commercialization of the PKG.FANCA.Wpre* lentiviral vector. Additionally, an agreement has been signed for the continuation of the clinical studies developed in Eurofancolen, aiming at the Registration of the proposed gene therapy for FA-A patients.

A Scientific Advice Meeting took place with the European Medicine Agency (EMA) in September 5th, 2018, in which the Phase II trial, FANCOLEN-2, now sponsored by Rocket Pharma, was discussed with members of the EMA.

FANCOLEN-2 (EudraCT 2018-002502-31) was approved by the Spanish Regulatory Authority, Agencia Española del Medicamento y Productos Sanitarios in October, 31st 2018, and it is expected that the first patients will be treated during the first months of 2019.

As proposed in Europe, the FANCOLEN-2 trial will also be developed in the USA at the Stanford University, aiming at the Registration of a new treatment of FA-A patients.

Two patents on “Gene therapy for patients with Fanconi anemia (RTWI-002/01US 326219-0000 (62412028)” and on “ Compositions and methods for stem cell transplant (ROPA-008/00US 329592-2018) have been presented and licensed to Rocket Pharma.

- **Main dissemination activities:**

The main dissemination activities performed by members of the EUROFANCOLEN Consortium consisted on the following activities:

- **Presentations in International Scientific Meetings.** A particular emphasis was done to present our results in Meetings of the American Society for Hematology, European and American Societies for Gene and Cell Therapy; European Association for Hematology, National Societies for Hematology and Pediatric Oncology. In several instances these presentations were made in plennary sessions; ie. ASGCT meeting 2018; ESGCT 2017; ASH 2018 (where the results of the FA gene therapy trial were presented in the best of ASH session).

- **Publication of papers in scientific Journals:** The main results achieved in EUROFANCOLEN have been published in international journals of high impact. Currently the preliminary results of the clinical trial are under the second review in Nature Medicine. Since almost all the suggestions from the reviewers have been included in the revised manuscript, we hope that the preliminary results of the clinical trial will be published in this journal.

- **Meetings with Fanconi anemia Associations:** We have promoted meetings with National Associations of Fanconi anemia, to explain affected families the main progresses in the development of the FA clinical trial under development by the EUROFANCOLEN Consortium. A close collaboration has been established with FA Associations from Spain, UK, France, Germany, Italy. A particular collaboration has been promoted with the Fanconi Anemia Research Fund (FARF). This is the strongest international FA Association with specific interests for the development of improved procedures for the diagnosis and treatment of FA patients. This association also groups family Associations from countries of all the world. In 2018 a very important and successful meeting took place in New Port Beach (LA) during the FARRF annual Meeting, in which the EUROFANCOLEN clinical trial was presented to representative FA families and the board of the FARF.

- **Organization and participation in Graduate, Post-graduate and Master courses focused on the development of new therapies for rare diseases.**

- **Publication of flyers and clinical guides on FA:** This has been developed at a National levels, aiming at improving the knowledge of the disease and the follow-up of patients with FA

- **Social activities:** We have actively participated in multiple social activities, including interviews in TV, radio, news-papers … Several social activities have been promoted including theatre performances, concerts, popular marathons, ….

- **Dissemination by the EUROFANCOLEN Webpage, and other Webpages of FA foundations**

- **Main exploitation of results:**

- Thanks to the Orphan Drug dessignation of the tehrapeutic lentiviral vector developed by members of the Consortium, this vactor was licensed to a new Pharmaceutical Company, Rokcet Pharma. In collaboration with this Company, a Meeting with the Scientific Advice Committe of the EMA took place in 2018. Also a meeting took place with the FDA for the presentation of the IND aiming the deelopment of an FA trial in the USA. The IND was approved in 2018.

- Two patents have been presented with Rocket Pharma in the field of FA gene therapy.

- A global gene therapy trial will be developed in Europe by partners of EUROFANCOLEN and also the University of Standford that will be sponsored by Rocket Pharma. Since this trial is already approved both in Europe and the USA, it is expected that it will be opened in the first quarter of 2019. The aim of this trial is the Registration of the LV-mediated gene therapy of FA patients as an alternative tehrapeutic optiion for these patients.

4.1.5. Address of the public web site, if applicable as well as relevant contact details Project logo, diagrams or photographs illustrating and promoting the work of the project (including videos, etc…), as well as the list of all beneficiaries with the corresponding contact names can be submitted without any restriction.

- **EUROFANCOLEN Webpage: www.eurofancolen.org**

- **Contact information:**

  EUROFANCOLEN Coordinator: Dr Juan Bueren: juan.bueren@ciemat.es

  Secretary EUROFANCOLEN: Aurora de la Cal: aurora.delacal@externos.ciemat.es

- **Partners Information:**

  - Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT): Juan Bueren; juan.bueren@ciemat.es
  - Assitance Publique - Hospitaux de Paris (AP-HP): Marina Cavazzana; m.cavazzana@nck.aphp.fr
  - University College London (UCL): Adrian Thrasher; a.thrasher@ucl.ac.uk
  - Servicio Madrileño de Salud (SERMAS): Julian Sevilla; jsevilla.hnjs@salud.madrid.org
  - Institut Catalá de la Salut (ICS-HUVH): Cristina Díaz de Heredia; crdiaz@vhebron.net
  - Association Genethon(GENETHON): Anne Galy; galy@genethon.fr
  - Universite Paris-Diderot 7 (UPD): Jean Soulier; jean.soulier@sls.aphp.fr
  - Universitat Autonoma de Barcelona (AUB): Jordi Surralles; jordi.surralles@uab.cat
  - GATC BIOTECH: Tobias Paprotka; t.paprotka@gatc.biotech.com
  - Deutsches Krebsforschunszentrum (DKFZ/NCT): Manfred Schmidt; manfred.schmidt@nct-heidelberg.de
  - Innovación Desarrollo y Transferencia de Tecnología (IDETRA): Mario Romero; marioromero@idetra.com
  - Yposkesi: Alain Lamproye; alamproye@yposkesi.com

- **Report published in the FARF Newsletter summarizing the news of the International FA Gene Therapy Working Group Meeting held at CIEMAT (October 2016) with an update of the results of the EUROFANCOLEN Trial.**

For example, formaldehyde, the simplest aldehyde, is produced in all of us in our blood from the metabolic process demethylation of RNA. Fortunately, formaldehyde is eliminated by the enzyme ADH5, which we also produce. This enzyme turns formaldehyde into the non-toxic formic acid. Another aldehyde produced by our metabolic processes, acetaldehyde, is removed by ALDH2, a protein that turns the toxic acetaldehyde into the non-toxic acetate.

Dr. Patel's lab studies aldehydes by creating and exploring aldehyde problems in mice. They do this by knocking out ALDH2 and ADH5, the mouse's first line of defense against the toxic effects of formaldehyde (ALDH2) and acetaldehyde (ADH5). The grim results—leukaemia, bone marrow failure, liver cancer, and death—confirm the protective functions of ALDH2 and ADH5. However, many questions remain. Are there other sources of DNA repair problems besides formaldehyde and acetaldehyde? What other aldehydes matter? How much can we extrapolate from mouse models to humans? Dr. Patel's lab has identified at least 20 enzymes in the first tier of protection against aldehydes. The most fundamental and urgent question they are now trying to answer is, how can we help people with FA restore to full functionality their second tier defense against the toxic aldehydes that their products – their ability to repair damaged DNA? Are there other repair pathways that are protective, beyond the FA pathways?

From Cloning to Clinic: A Report from the Gene Therapy International Working Group

Fanconi anemia presents unique challenges to the field of hematopoietic stem cell gene therapy. Patients have both reduced quantity and quality of these cells. Because of this unusual level of complexity, combined with the relative rareness of the disease, the Fanconi Anemia Research Fund (FARF) and Fanconi Hope, United Kingdom, established the Fanconi Anemia Gene Therapy Working Group in 2010. Led by Dr. Jakub Tolar (University of Minnesota), the goal of creating this group was to establish a worldwide platform for gene therapy clinical trials in FA.

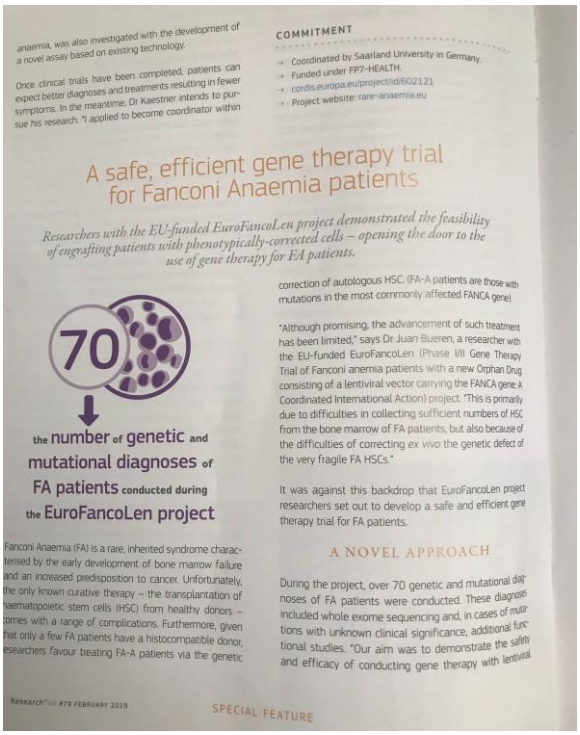
Translational research has shown that DNA repair to restore cellular fitness in FA can be accomplished by either gene addition of the wild-type (normal FA gene) or by editing the genome. Both methods allow autologous cell therapy. This means that the therapy uses the person's own cells, thus avoiding the severities of pre-transplant conditioning and post transplant complications.

The most recent meeting of the gene therapy working group, hosted by Juan Bueren in Madrid last October, showcased the tremendous advance from planning and preclinical research to clinical trials, as well as ongoing research investigations. The group explored a number of technical strategies to increase the efficiency of FA gene therapy. They also discussed the potential of gene therapy for head and neck cancers. The work of the gene therapy group is truly an international effort, with experts representing Spain, Italy, France, Netherlands, Germany and the USA.

This year the group will meet in Heidelberg, Germany, and continue collaboration to generate robust, autologous, and disease-free cells for people with FA.

"The work of the gene therapy group is truly an international effort."

- **Publication in RESEARCH EU MAGAZINE: February 2019**



anaemia, was also investigated with the development of a novel assay based on existing technology.

Once clinical trials have been completed, patients can expect better diagnoses and treatments resulting in fewer symptoms. In the meantime, Dr Kaestner intends to pursue his research. "I applied to become coordinator within

COMMITMENT

- Coordinated by Saarland University in Germany.
- Funded under FP7-HEALTH.
- cordis.europa.eu/project/id/602121
- Project website: rare-anaemia.eu

A safe, efficient gene therapy trial for Fanconi Anaemia patients

Researchers with the EU-funded EuroFancoLen project demonstrated the feasibility of engrafting patients with phenotypically-corrected cells – opening the door to the use of gene therapy for FA patients.

correction of autologous HSC. (FA-A patients are those with mutations in the most commonly affected FANCA gene).

"Although promising, the advancement of such treatment has been limited," says Dr Juan Bueren, a researcher with the EU-funded EuroFancoLen (Phase I/II Gene Therapy Trial of Fanconi anemia patients with a new Orphan Drug consisting of a lentiviral vector carrying the FANCA gene A Coordinated International Action) project. "This is primarily due to difficulties in collecting sufficient numbers of HSC from the bone marrow of FA patients, but also because of the difficulties of correcting *ex vivo* the genetic defect of the very fragile FA HSCs."

It was against this backdrop that EuroFancoLen project researchers set out to develop a safe and efficient gene therapy trial for FA patients.

70

the number of genetic and mutational diagnoses of FA patients conducted during the EuroFancoLen project

Fanconi Anaemia (FA) is a rare, inherited syndrome characterised by the early development of bone marrow failure and an increased predisposition to cancer. Unfortunately, the only known curative therapy – the transplantation of haematopoietic stem cells (HSC) from healthy donors – comes with a range of complications. Furthermore, given that only a few FA patients have a histocompatible donor, researchers favour treating FA-A patients via the genetic

A NOVEL APPROACH

During the project, over 70 genetic and mutational diagnoses of FA patients were conducted. These diagnoses included whole exome sequencing and, in cases of mutations with unknown clinical significance, additional functional studies. "Our aim was to demonstrate the safety and efficacy of conducting gene therapy with lentiviral

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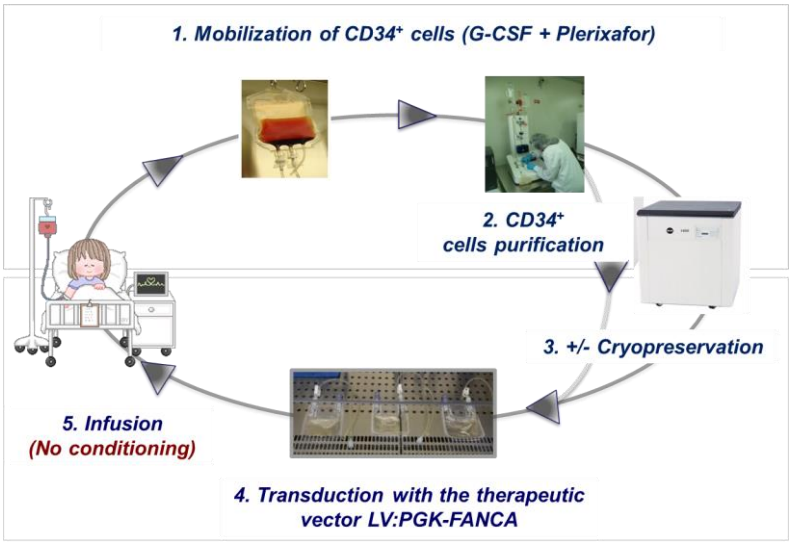
SPECIAL FEATURE

- **Schematic protocol developed for the gene therapy of FA patients:**



- **Presentation of the preliminary conclusions of EUROFANCOLEN in the Plenary Session of the American Society for Gene and Cell Therapy. Chicago, May 2018**

- List of Beneficiaries

FA Foundation/Association	Country	CONTACT	E-MAIL	WEB	FACEBOOK
FANCONI HOPE UK	UK		rad@fanconihope.org	https://www.fanconihope.org/	@fanconihope
The Canadian Fanconi Anemia Research Fund	CANADA		admin@fanconicanada.org	http://www.fanconicanada.org/en/index.php	@Fanconi.Canada
Association Francaise de la Maladie de Fanconi (AFMF)	FRANCE	Marie Pierre Bichet	contact.afmf@gmail.com	https://www.fanconi.com/	@AssociationfrancaisedelamaladiedeFanconi(A.F.M.F)
Deutsche Fanconi-Anämie-Hilfe e.V.	GERMANY	Ralf Dietrich	ralf.dietrich@fanconi.de	https://www.fanconi.de/	@deutsche.fanconi.anaemie.hilfe
FANCONI ANEMIA AUSTRALIA	AUSTRALIA	Wayne Crismani			Fanconi Anaemia Australia/New Zealand
Steven's Association Moonrise	BELGIUM		stevens.assoc.moonrise@gmail.com	es.google.com/site/stevensassociationmoonrisesite/sam	
Fanconi-Anämie Stiftung	GERMANY	Pauline Neigel, Salzgitter	p.neigel@fanconi.info	https://fanconi.eu/?page_id=18	
		Günther Weise	g.weise@fanconi.info		
Fanconi Anaemia Ireland	IRLAND				@FA.ireland.Page
Associazione Italiana Ricerca Sull'anemia di Fanconi (Airfa)	ITALY	Albina Parente	infofanconi@airfa.it	http://www.airfa.it/	@Airfa.Onlus
Associação Portuguesa Para A investigação da Anemia de Fanconi (PFARN)	PORTUGAL				@Fanconi.Portugal
Fanconi Anaemia South Africa	SOUTH AFRICA				
Associazione Svizzera per l'anemia di Fanconi (ASAF)	SUIZERLAND		info@fainfo@fanconi-anemia.ch	http://www.fanconi-anemia.ch/	
The Netherlands Fanconi Anemie (VOKK)	THE NETHERLANDS		bureau@vokk.nl	https://fanconianemie.nl/	@FanconiAnemieNederland

4.2. USE AND DISSEMINATION OF FOREGROUND

A plan for use and dissemination of foreground (including socio-economic impact and target groups for the results of the research) shall be established at the end of the project. It should, where appropriate, be an update of the initial plan in Annex I for use and dissemination of foreground and be consistent with the report on societal implications on the use and dissemination of foreground (section 4.3 – H).

The plan should consist of:

- Section A

This section should describe the dissemination measures, including any scientific publications relating to foreground. **Its content will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

- Section B

This section should specify the exploitable foreground and provide the plans for exploitation. All these data can be public or confidential; the report must clearly mark non-publishable (confidential) parts that will be treated as such by the Commission. Information under Section B that is not marked as confidential **will be made available in the public domain** thus demonstrating the added-value and positive impact of the project on the European Union.

Section A (public)

This section includes two templates

- Template A1: List of all scientific (peer reviewed) publications relating to the foreground of the project.
- Template A2: List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

These tables are cumulative, which means that they should always show all publications and activities from the beginning until after the end of the project. Updates are possible at any time.

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[2] (if available)	Is/Will open access[3] provided?
1	Successful Engraftment of Gene Corrected Hematopoietic Stem Cells in Non-conditioned Fanconi Anemia Patients	Bueren, J	Nature Medicine (under second revision)	Pending						Yes
2	Engraftment and in vivo proliferation advantage of gene corrected mobilized CD34+ cells from Fanconi anemia patients	Sevilla, J Bueren, J	Blood	130			2017	1535-42		Yes
3	Mutations in ERCC4, Encoding the DNA-Repair Endonuclease XPF, Cause Fanconi anemia.	Surrallés, J	Am. J. Hum, Genet				2013	1-7		No

[2] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[3] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

4	Lentiviral-Mediated Gene Therapy in Fanconi Anemia-A Mice Reveals Long-Term Engraftment and Continuous Turnover of Corrected HSCs	Schmidt, M	Current Gene Therapy	15			2015	550-562		No
5	Savior siblings and Fanconi anemia: analysis of success rates from the family's perspective	Surralles, J	Genetics in Medicine	17			2015	935-8		No
6	Evaluation of rare variants in the new Fanconi Anemia gene ERCC4 (FANCQ) as familial breast/ ovarian cancer susceptibility alleles	Surrallés, J	Human Mutation					1615-8		No
7	Advances in Gene Therapy for Fanconi Anemia. Review	Bueren, J	Hum Gene Therapy	29			2018	1114-1123		No
8	Therapeutic gene editing in CD34+ hematopoietic progenitors from Fanconi anemia patients	Río, P Bueren, J	EMBO Molecular Medicine	9			2017	1574-1588		Yes
9	Lessons Learned from Two Decades of Clinical Trial Experience in Gene Therapy for Fanconi Anemia (Review)	Bueren J	Curr Gene Therapy	16			2017	338-348		No
10	Fanconi anemia: A model disease for studies on human genetics and advanced therapeutics	Surralles J	Current Opinion in Genetics & Development	6			2015	32-40		No
11	Improved Hematopoietic Gene Therapy in a Mouse Model of Fanconi Anemia Mediated by Mesenchymal Stromal Cells.	Yañez R Bueren, J	Hum Gene Therapy	29			2018	327-336		No
12	EuroFancolen. 2015. Phase I/II Gene Therapy Trial of Fanconi Anemia Patients with a New Orphan Drug Consisting of a Lentiviral Vector Carrying the FANCA Gene: A Coordinated International Action (EuroFancolen).	Bueren, J	Human Gene Therapy Clinical Development	26			2015	81-82.		Yes
13	Detectable clonal mosaicism in blood as biomarker of cancer risk in Fanconi anemia.	Surrallés, J	Blood, Advances	1			2017	319-329		Yes
14	Biallelic truncating FANCM mutations cause early onset cancer but not Fanconi Anemia.	Soulier, J Surrallés, J	Genet Med.	20			2018	458-463		No
15	'Individuals with FANCM biallelic mutations do no develop Fanconi anemia, but show	Peterlongo P	Genet Med.	20			2018	452-457		No

	risk for breast cancer, chemotherapy sensitivity toxicity and may display chromosome fragility',	Surralles, J								
16	Perspectives of Gene Therapy in Fanconi Anemia.	Bueren, J	Expert Opinion in Orphan Drugs	3			2015	899-910		Yes
17	From exome analysis in idiopathic azoospermia to the identification of a high-risk subgroup for occult Fanconi anemia.	Surralles, J	Genetics in Medicine	21			2019	189-194		No

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES

MORE THAN 200 ACTIVITIES HAVE BEEN CARRIED OUT BY MEMBERS OF EUROFANCOLEN CONSORTIUM BETWEEN 2013-2018.

A SUMMARY OF 10 OF THE MOST REPRESENTATIVE ONES ARE INCLUDED IN THIS TABLE

NO.	Type of activities[4]	Main leader	Title	Date/Period	Place	Type of audience[5]	Size of audience	Countries addressed
1	Conference: 60th Annual Meeting of the American Society for Hematology	Bueren, J	Advances in the Gene Therapy of Patients with Fanconi Anemia.	1-4th Dec, 2018	San Diego. Ca	Scientific Community Industry, Media	5,000	International
2	Conference: 21st Annual Meeting of the American-Society-of-Gene-and-Cell-Therapy (ASGCT). Plennary Session	Bueren, J	Engraftment and Phenotypic Correction of Hematopoietic Stem Cells in Non-Conditioned Fanconi Anemia Patients Treated with Ex Vivo Gene Therapy	16-19th May, 2018	Chicago, IL	Scientific Community Industry, Media	1,500	International
3	Conference: 22nd Congress of the European Hematology Association	Sevilla, J	First evidence demonstrating engraftment and repopulation advantage of gene-corrected hematopoietic repopulating cells in	June 22-25th 2017	Madrid, Spain	Scientific Community Industry,	500	International

[4] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[5] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias, Other ('multiple choices' is possible).

			non-conditioned fanconi anemia patients			Media		
4	Conference: 29th Annual Fanconi Anemia Research Fund Scientific Symposium (FARF).	Surrallés, J	Identification of patients with bi-allelic mutations in FANCM	13-17th Sept 2017	Atlanta	Scientific Community, Families	300	International
5	Conference: 30th Annual Fanconi Anemia Research Fund Scientific Symposium (FARF).	Bueren, J	Lentiviral-mediated gene therapy in non-conditioned FA patients	27-30th Set.18	Newport Beach, Ca	Scientific Community, Families	300	International
6	Organization of the International FA Gene Therapy Working Group Meeting	Schmidt, M	Symposium of the most representative international experts on FA Gene Therapy	16-17th Nov.17	Sevilla. Spain	Scientific Community	50	International
7	Press release. Collaboration with Rocket Pharma in FA gene therapy	Bueren, J	Rocket Pharma, North American Biotechnology Company, is committed to the development of Gene Therapy Drugs generated in Spain	10th Sept, 2016	Spain/USA	Media	--	International
8	Course on Dynamic European Research	Galy, A	Translational research in gene therapy	22nd May, 2018	Paris	Leaders and operatives in public health Institutions	100	International
9	Inaugural Australian and New Zealand Fanconi. Anaemia Family Meeting.	Surrallés, J	Genetics of Fanconi Anemia".	13-14th Oct.2017	Melbourne. Australia	FA families	100	National
10	Organization of the International Congress of the Spanish Research Network on Fanconi Anemia	Bueren, J	Meeting with international experts on FA	29-30th Nov, 2017	Sevilla, Sp	FA experts and families	200	International

Section B (Confidential[6] or public: confidential information to be marked clearly)

Part B1

The applications for patents, trademarks, registered designs, etc. shall be listed according to the template B1 provided hereafter.
The list should, specify at least one unique identifier e.g. European Patent application reference. For patent applications, only if applicable, contributions to standards should be specified. This table is cumulative, which means that it should always show all applications from the beginning until after the end of the project.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[7]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)
Orphan Drug	NO		DRU-2016-5193	Lentiviral vector carrying the Fanconi anaemia-A (FANCA) gene for the treatment of Fanconi anaemia type A	CIEMAT/CIBERER
Patent	NO		PCT/US2017/050837	Gene therapy for patients with Fanconi anemia	CIEMAT/CIBERER
Patent	YES	10/11/2019	US 329592-2018	Compositions and methods for stem cell transplant.	Fundación Investigación Biomédica Hospital Niño Jesús

[6] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[7] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2

Please complete the table thereafter:

Type of Exploitable Foreground[8]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application [9]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
License	Gene correction of hematopoietic stem cells from Fanconi anemia patients	Yes		Gene-corrected hematopoietic stem cells	Biomedicine	Expected commercialization for 2021	Orphan Drug	Owner: CIEMAT/CIBER

In addition to the table, please provide a text to explain the exploitable foreground, in particular:

- **Its purpose**
- **How the foreground might be exploited, when and by whom**
- **IPR exploitable measures taken or intended**
- **Further research necessary, if any**
- **Potential/expected impact (quantify where possible)**

Exploitable Foreground: The licensed Medicinal product consist on gene-corrected autologous $CD34^+$ cells (hematopoietic stem cells) from Fanconi anemia patients. This medicinal product obtained the Orphan Drug designation both by the EMA and the FDA, and has been licensed to Rocket Pharmaceuticals, Inc. who will sponsor the development of a global clinical trial, subsequent to the one developed in Eurofancolen. The aim of this license is the commercialization of a new therapeutic approach for Fanconi anemia patients, that may replace in the future the allogeneic transplantation of these patients. In contrast to allogeneic transplantation, the proposed gene therapy approach is based on the infusion of autologous cells in the patient without any conditioning regimen, thus limiting the hospitalization of the patient to a couple of days post-infusion.

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[9] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html