Amsterdam, 13 June 2025
EMA/CAT/271921/2025
Committee for Advanced Therapies (CAT)
Committee for Medicinal Products for Human Use (CHMP)

Withdrawal assessment report

Fanskya

International non-proprietary name: mozafancogene autotemcel

Procedure no. EMEA/H/C/005537/0000

Note

Assessment report as adopted by the CAT/CHMP with all information of a commercially confidential nature deleted.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
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List of abbreviations

AE	adverse event
AESI	adverse event of special interest
AML	acute myeloid leukaemia
BM	bone marrow
BMF	bone marrow failure
CFU	colony forming unit
CHMP	Committee for Medicinal Products for Human Use
DEB	diepoxybutane
DNA	deoxyribonucleic acid
DP	drug product
ECA	external control arm
EFS	event free survival
EMA	European Medicines Agency
FA	Fanconi anaemia
FA-A	Fanconi anaemia subtype A
FANCA	Fanconi anaemia complementation group A
FAS	full analysis set
FDA	Food and Drug Administration
GvHD	graft vs. host disease
HLA	human leukocyte antigen
HSA	human serum albumin
HSC	haematopoietic stem cell
HSCT	haematopoietic stem cell transplant
IFAR	International Fanconi Anemia Registry
IIT	investigator initiated trial
IMP	investigational medicinal product
ISA	Insertional Site Analysis
LTFU	long term follow-up
LV	lentiviral
MDS	myelodysplastic syndromes

MMC	mitomycin-C
MOI	multiplicity of infection
MSD	matched sibling donor
OR	odds ratio
OS	overall survival
PB	peripheral blood
PIP	Pediatric Investigation Plan
RBC	red blood cells
RCL	replication competent lentivirus
SAE	serious adverse event
SAF	safety analysis population
SCCHN	squamous cell carcinoma of the head and neck
TEAE	treatment-emergent adverse event
VCN	vector copy number

1. CAT recommendation

Based on the review of the data and the applicant's response to the list of questions on quality, safety, efficacy, the application for Fanskya 1-50 × 10^5 cells/mL dispersion for infusion, an orphan medicinal product in the *treatment of paediatric patients with Fanconi Anaemia Type A (FA-A)*

is not approvable since "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time. The details of these major objections are provided in the List of Outstanding issues (see section VII).

In addition, satisfactory answers must be given to the "other concerns" as detailed in the List of Questions.

1.1. Questions to be posed to additional experts

Given the uncertainties on the clinical benefit, the input from a Scientific Advisory Group (SAG) or an Ad Hoc Experts Group (AHEG) is required.

1.2. Inspection issues

1.2.1. GMP inspection(s)

N/A.

1.2.2. GCP inspection(s)

Routine GCP inspections have been conducted for the following clinical studies and sites:

- Study RP-L102-0319, site 1: Stanford University 240 Pasteur Drive Stanford, Palo Alto, California, United States, 94304.
- Study RP-L102-0118, site 2: Hospital Infantil Universitario Niño Jesús Pediatric Oncohematology Service Avenida Menendez Pelayo 65 Madrid, 28009
- Sponsor site: Rocket Pharmaceuticals, Inc. 9, Cedarbrook Drive, Cranbury, New Jersey, USA 08512.

The inspections focused on verification of eligibility and safety data and selected efficacy data reported in the MAA for a sample of trial participants determined by the inspectors; being all (8) of the enrolled trial participants at the two inspected investigator site locations.

The GCP inspection report concludes that while the reported findings, do and did to a certain extent affect the quality of the reported data and claims in the protocol and the MAR, the setup of the trial and especially the 'nature' of the primary, and most relevant secondary, efficacy endpoint/parameters and the way/processes by which these were collected (data from external analyses), is likely to have 'reduced' the impact on these key parameters. Therefore, from the GCP perspective, the (core) trial results could be used in a MAA-procedure.

The assessors agree with the views of the GCP inspections team.

1.3. New active substance status

Based on the review of the data, it is considered that the active substance mozafancogene autotemcel contained in the medicinal product Fanskya is qualified as a new active substance.

1.4. Additional data exclusivity / marketing protection

Not applicable

1.5. Similarity with authorised orphan medicinal products

There are no products with a marketing authorisation with orphan designations in Falconi anaemia Therefore, no similarity assessment is required.

1.6. Derogation(s) from market exclusivity

Not applicable

2. Executive summary

2.1. Problem statement

2.1.1. Disease or condition

The proposed indication for Fanskya is:

"*Fanskya is indicated for the treatment of Fanconi Anaemia Type A (FA-A) in paediatric patients aged 1 to 18 years".*

2.1.2. Epidemiology and risk factors, screening tools/prevention

Fanconi anaemia (FA) is a rare, inherited disorder of DNA repair which leads to genomic instability, disordered cell cycle regulation, and cell death. The incidence is estimated to be 1 in 300,000 live births and the prevalence of 1 to 9 per million (Bagby 2016).

2.1.3. Biologic features, aetiology and pathogenesis

FA is caused by mutations in one of at least 22 different genes, which are described as FANCA to FANCW, of which FANCA, FANCC, and FANCG are most common (80-90%). The most common subtype of FA (60-70%) is FA complementation group A (FANCA) and results from autosomal recessive mutations in the FANCA gene.

As a result of the FANCA mutation, the FA DNA Damage Response Pathway is unable to repair DNA damage caused by interstrand cross-linkages (ICLs), an especially deleterious type of DNA damage in which opposing strands of DNA are abnormally joined. Disordered repair of ICLs leads to genomic instability, aberrant cell cycle regulation, and cell death. (*de Winter 2009; Kottemann 2013; Shimamura 2010; Khincha 2013, UpToDate 2024*). DNA damage can occur during foetal development, which can cause congenital anomalies, and during childhood and adulthood, which results in progressive bone marrow failure (BMF), predisposition to haematologic malignancies and solid tumours.

Mosaicism is a very rare naturally occurring phenomenon in a small population of FA patients. In FA patients with mosaicism, a spontaneous reversion or other compensatory mutation occurs in the FANCA gene of a haematopoietic cell which converts it from an FA haematopoietic cell with a dysfunctional DNA repair mechanism to a haematopoietic cell with a functioning DNA repair mechanism. Since this haematopoietic cell now has a functioning DNA repair mechanism, it has a selective advantage over FA haematopoietic cells and is therefore able to proliferate more successfully. In multilineage mosaicism (<5% of FA patients), the reversion mutation occurs in a long-term

haematopoietic stem and progenitor cell (HSPC) and results in prolonged multilineage haematopoietic normalisation. For this reason, mosaicism has been described in the literature as "natural gene therapy".

2.1.4. PRIME

Fanskya was granted eligibility to PRIME on 12 December 2019 in the following indication: treatment of Fanconi Anemia (FA).

2.1.5. Clinical presentation, diagnosis and stage/prognosis

FA is typically associated with cytopenias, predisposition to malignancy, and congenital and developmental abnormalities characteristic physical abnormalities (eg, short stature, microcephaly, developmental delay, café-au-lait skin lesions). Some individuals have only modest or isolated cytopenias (eg, macrocytic anaemia, thrombocytopenia) for years before they are diagnosed with FA. Congenital malformations are the most common presenting features of FA, but an absence of such findings does not eliminate the possibility of FA. Physical abnormalities including short stature, microcephaly, developmental delay, café-au-lait skin lesions, and malformations belonging to the VACTERL-H association.

Most patients are diagnosed with FA between six to nine years of age (concurrent with the onset of bone marrow failure). However, 9% of individuals are diagnosed after age 16 years, typically, when they present with a malignancy.

The standard clinical diagnostic test for FA is a peripheral blood (PB) T-lymphocyte diepoxybutane (DEB) assay. Cultured in the presence of a DNA-damaging agent like DEB, >60-100% of FA patient cells display chromosomal aberrancies, as opposed to 0-20% of cells in non-FA patients (*Castella 2011; Nicoletti 2020*). If the PB T-lymphocyte DEB test is positive, genetic testing is typically performed to confirm and identify the specific FA-causing variant (*Mehta and Ebens 2021*).

About 80% of patients progress to severe BMF within the first decade of life at a median age of 7 years (*ECA Analysis RP-L102 in FA; Kutler 2003; Sebert 2023*). Moreover, because FA is a DNA repair disorder, FA patients have a reduced and progressively declining number of haematopoietic stem cells (HSCs) and those that remain are fragile. BMF in FA refers to a deficiency or impairment of hematopoietic stem cells (HSCs) that causes bone marrow hypoplasia leading to single or multiple cytopenias and eventual aplasia with pancytopenia. The BMF is caused by premature, selective attrition of CD34+ HSCs, which can be observed prior to the onset of cytopenias. The precise mechanisms of stem cell loss are uncertain. FA is rare, but is the most common inherited bone marrow failure syndrome (IBMFS).

Due to underlying chromosomal instability, FA patients surviving childhood are at an increased risk for haematologic malignancies and solid tumours. Cumulative incidences of myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML) exceed 30% by age 30 (*Kutler 2003*). The risk of solid tumours approaches a 40% incidence by age 40 (*Alter 2018*). Because of the dysfunctional DNA repair mechanism, FA patients poorly tolerate the cytotoxic chemotherapy required for allogeneic HSCT and used in the treatment of haematologic malignancies and solid tumours.

2.1.6. Management

Treatments depend on the severity of bone marrow functioning (UptoDate 2024, Fanconi Anemia: Guidelines for Diagnosis and Management 2014):

Severe BMF (ANC ≤500/microL, platelet count ≤30,000/microL, Hb <8 g/dL)

- Hematopoietic stem cell transplantation (HSCT) from HLA-matched sibling is currently the only therapy available to cure patients with FA of marrow aplasia, prevent progression to MDS or AML, and cure existing MDS or AML
- HSCT from alternate donor sources when a sibling donor is lacking (adult volunteer donors which have been organized in large national registries; umbilical cord blood that is stored in blood banks worldwide; and manipulated stem cells grafts from haploidentical relatives). Success rates on OS are improving over recent years (Mehta et al, Blood, 2017)

Moderate BMF (ANC 500 -1000/microL, platelet count 30,000 - 50,000/microL, Hb 8 - 10 g/dL):

- In those patients whose counts continue to decline, HCT planning is to be started.
- Androgens — Androgen therapy is not curative, but it may be appropriate for patients who lack a closely matched related donor for HCT, or for those for whom HCT is not pursued due to family/caregiver preference or medical eligibility. A retrospective series of 70 patients (1974 to 2014) treated with an androgen reported that two-thirds of patients had an improvement in Hb level, [Paustian, 2016]. The median time to response was 12 to 14 weeks. In most cases, these responses were sufficient to convert the patient from transfusion-dependent to transfusion-independent.
- RBCs – Red blood cell (RBC) transfusion is indicated for any patient with symptomatic anemia. However, chronic RBC transfusions can lead to iron overload, which can lead to significant morbidity and mortality.
- Platelet transfusions in patients with platelet counts <10,000/microL and in any patient with severe bruising, bleeding, or invasive procedures.
- G-CSF - Although G-CSF can raise the neutrophil count in most neutropenic patients with FA, there are concerns that it might increase the risk of MDS or AML in patients with bone marrow failure syndromes

Unmet medical need

Despite advances in care for FA, median overall survival (OS) is 33.5 years, with greater than 30% of patients dying by the age of 20 and more than 65% deceased by the age of 40 (Sebert 2023).

Because of the significant morbidity and mortality associated with allogeneic HSCT in FA (resulting in part from the use of cytotoxic conditioning), allogeneic transplant is reserved as salvage therapy for patients who have progressed to severe BMF (or have developed AML/MDS). Therefore, there is still a high unmet need for an effective treatment to address the haematologic component of FA subtype A (FA-A), ideally one that could prevent BMF and prevent the need for an allogeneic transplant, including the use of cytotoxic conditioning and associated toxicities.

2.2. About the product

Product

Fanskya (mozafancogene autotemcel) (herein referred to as RP-L102) is an ex vivo lentiviral (LV) vector gene therapy consisting of autologous HSCs transduced with an LV (PGK-FANCA-WPRE) that encodes for the FANCA gene.

Mode of action

RP-L102's scientific development rationale is based on a very rare naturally occurring phenomenon known as multilineage mosaicism, estimated to occur in <5% of FA patients (Nicoletti 2020; Ramirez 2021). Multilineage mosaicism results from a spontaneous reversion or other compensatory mutation in the FANCA gene of a long-term HSC, which converts it from an abnormal cell with a dysfunctional DNA repair mechanism to a normal cell with a functioning one. The corrected cell thus has a selective advantage over uncorrected FA cells and can proliferate more successfully. RP-L102 is intended to replicate and expand upon multilineage mosaicism. The introduction of a functional FANCA copy into autologous HSCs enables these gene-corrected HSCs to have a selective advantage over uncorrected HSCs so they can repopulate the bone marrow (BM) and PB without conditioning (with correction first seen in BM and then in PB), leading to haematologic stability (Río 2017).

The proposed indication is:

"Fanskya is indicated for the treatment Fanconi Anaemia Type A (FA-A) in paediatric patients aged 1 to 18 years".

The proposed posology is:

Fanskya must be administered in a qualified treatment centre by a physician experienced in the treatment of Fanconi anaemia and trained in the administration and management of patients treated with Fanskya.

The treating physician should confirm that Fanskya therapy is appropriate for the patient before HSC mobilisation is initiated. Treatment with Fanskya should only be considered in patients who have a baseline bone marrow CD34+ cell concentration of at least 30 CD34+ cells per microlitre (assessed within approximately 3 months prior to initiation of apheresis) and whose bone marrow failure has not yet progressed to severe levels.

Posology

Fanskya is intended for autologous use only (see section 4.4).

Treatment consists of a single dose for infusion containing a dispersion of viable CD34+ cells in one infusion bag.

In clinical studies, doses up to 4.1×10^6 CD34+ cells/kg have been administered. See the accompanying Lot information sheet (LIS) for additional information pertaining to dose.

Mobilisation and apheresis

Patients are required to undergo HSC mobilisation followed by apheresis to obtain CD34+ cells which will be used for medicinal product manufacturing (see section 5.1 for description of the mobilisation regimen used in clinical studies).

The patient's weight at first apheresis collection should be used to calculate the final dose.

Fanskya administration

See Method of administration below and section 6.6 for details on Fanskya administration and handling.

Special populations

Renal impairment

Patients should be assessed for renal impairment to ensure autologous HSC gene therapy administration is appropriate. No dose adjustment is required.

Hepatic impairment

Patients should be assessed for hepatic impairment to ensure autologous HSC gene therapy administration is appropriate. No dose adjustment is required.

Paediatric population

The safety and efficacy of Fanskya in children less than 1 year of age have not been established. No data are available.

Method of administration

Fanskya is for intravenous use only.

2.2.1. Marketing authorisation under exceptional circumstances

The applicant did not request consideration of its application for a Marketing Authorisation under exceptional circumstances in accordance with Article 14(8) of the above mentioned Regulation.

2.3. The development programme/compliance with guidance/scientific advice

Development programme

The clinical development program for Fanskya in patients with Fanconi Anaemia Type A (FA-A) consists of 1 investigator-initiated trial, and 3 Rocket-sponsored parent studies, and 2 long-term follow-up study:

FANCOLEN-I

A Phase 1/2, single arm, multi-site, single dose investigator-initiated trial (IIT) to assess the safety and preliminary efficacy of the IMP infusion in paediatric subjects with FA-A. This study has been completed. The total number of subjects treated was 9. Subjects were to be followed for 3 years; 6 subjects completed the study and 3 subjects discontinued.

RP-L102-0418

A Phase 1, single-arm, single site, single dose Rocket-sponsored clinical study to evaluate the safety and preliminary efficacy of the IMP infusion in paediatric subjects with FA-A. This study has been completed. The total number of subjects treated was 2. Subjects were to be followed for 3 years; 1 subject completed the study and 1 discontinued.

RP-L102-0319

A Phase 2, single-arm, single site, single dose Rocket-sponsored clinical study to evaluate the efficacy of the IMP infusion in paediatric subjects with FA-A. This study is ongoing. The total number of subjects treated is 5. Subjects are to be followed for 3 years. As of 23 October 2024, 3 subjects completed this study as planned and 2 are ongoing.

RP-L102-0118

A Phase 2, single-arm, multi-site, single dose Rocket-sponsored clinical study to evaluate the efficacy of the IMP infusion in paediatric subjects with FA-A. This study is ongoing. The total number of subjects treated is 7. Subjects are to be followed for 3 years. As of 23 October 2024, 4 subjects completed this study as planned, 2 are ongoing and one subject discontinued.

RP-L102-0116-LTFU

A long-term follow-up (LTFU) Rocket-sponsored study to evaluate the long-term safety and efficacy of the IMP infusion for a total of 15 years post-IMP infusion in subjects who were treated in the parent

study FANCOLEN-I. Following the end of participation in FANCOLEN-I, subjects were offered enrolment in this LTFU protocol. As of 11-Sep-2023, 7 of the 9 treated subjects enrolled after completing or discontinuing from FANCOLEN-I. No interventional treatment is administered in this LTFU study.

RP-L102-0221-LTFU

An LTFU Rocket-sponsored study to evaluate the long-term safety and efficacy of the IP infusion for a total of 15 years post-IMP infusion in subjects who were treated in the Rocket-sponsored parent studies. Following the end of participation in RP-L102-0418, RP-L102-0319, or RP-L102-0118, subjects are offered enrolment in this LTFU protocol. As of 23 October 2024, 9 of the subjects had already enrolled after completing or discontinuing from the parent studies. No interventional treatment is administered in this LTFU study.

Compliance with CHMP guideline

The most relevant guidelines applied:

- Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014):): https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-non-clinical-and-clinical-aspects-gene-therapy-medicinal-products_en.pdf
- Guideline on follow-up of patients administered with gene therapy medicinal products (EMEA/CHMP/GTWP/60436/2007): Guideline on Clinical follow-up gene therapy (europa.eu)

Other relevant guidelines/reflection papers:

- Reflection paper on establishing efficacy based on single-arm trials submitted as pivotal evidence in a marketing authorisation (EMA/CHMP/564424/2021)
- Guideline on clinical trials in small populations (CHMP/EWP/83561/2005)
- Points to consider on application with 1. Meta-analyses; 2. One pivotal study. (CPMP/EWP/2330/99).

Scientific advice

The applicant received protocol assistance from the CHMP on the development for the indication from the CHMP on:

Date	Reference	SAWP co-ordinators
20 September 2018	EMEA/H/SA/3899/1/2018/PA/PED/ADT/III	David Brown, Peter Mol
26 January 2023	EMA/SA/0000104998	Peter Mol, Brigitte Schwarzer-Daum

A summary of previous CHMP scientific advice for Fanskya in the proposed indication is described below.

The protocol assistance pertained to the following quality and clinical aspects:

EMEA/H/SA/3899/1/2018/PA/PED/ADT/III - Quality and clinical development

- The proposed nomenclature and definition for drug product, drug substance and starting materials; the testing conducted on the Master Cell Bank used for production of the LV; agreement that PGK-FANCA-WPRE* LV is a raw material and the proposed characterisation tests; the proposed in process and release tests for the drug product(s); the proposed drug product(s) manufacturing

process and characterisation and validation strategy; the proposed design of the cell viability of the fresh drug product(s) and the proposed cryopreservation study for the drug product(s); the proposed drug product(s) characterisation and comparability strategy for a change to commercial manufacturer; the proposed potency assays that measure the function of both the transgene and the cellular component of the final product(s); adequacy of the data to support using transduction enhancers in the proposed clinical study.
- Agreement that the clinical data from the Phase 1/2 study (NCT03157804) can support initiation of an EU Phase 2 registrational study; the proposed Phase 2 trial design including the number of patients, target patient population, dose and primary endpoint; adequacy of the safety database.

EMA/SA/0000104998, January 2023 - Quality and clinical development.

- The approach to support DP comparability assessment across the US and European manufacturing sites; the LV potency assay to support commercial release and stability testing of the lentiviral vector; the proposed comparability assessment across cell processing facilities to support pooling of the US and European pivotal clinical data; the approach to support the analytical method comparability; the proposed DP PPQ strategy to support registration; a 2-step release for DP commercialisation.
- The primary composite endpoint to support conditional approval; the natural history data package to supplement the clinical trial results; the comparability approach of MMC-resistance assays between US and European central laboratory facilities.

The applicant has provided a package about the natural history of the disease. However, several issues have been raised, see efficacy section.

2.4. General comments on compliance with GMP, GLP, GCP

The non-clinical toxicity studies have not been performed conform GLP. An update of study no. 5 and study no. 7 reports with raw data is requested to be able to establish the reliability of the study data and conclusions.

Routine GCP inspections were conducted in 3 sites, concluding that while there were critical findings and they do and did to a certain extent affect the quality of the reported data and claims in the protocol and the MAR, the setup of the trial and especially the 'nature' of the primary, and most relevant secondary, efficacy endpoint/parameters and the way/processes by which these were collected (data from external analyses), is likely to have 'reduced' the impact on these key parameters. Therefore, from the GCP perspective, the (core) trial results could be used in a MAA-procedure. The assessors agree with the views of the GCP inspections team

2.5. Type of application and other comments on the submitted dossier

2.5.1. Legal basis

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application.

2.5.2. Accelerated assessment

The CHMP and CAT did not agree to the applicant's request for an accelerated assessment. While the assessment considered that RP-L102 could be a promising option for FA patients in whom a high unmet medical need exists, it also stated that a conclusion on the benefit risk profile could only be drawn after full assessment of the data in the context of an MAA and precluded the maintenance of an

accelerated timetable. Hence, the CHMP concluded on 09-Nov-2023 to not recommend granting accelerated assessment.

2.5.3. Conditional marketing authorisation

The applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14(7) of Regulation (EC) No 726/2004. The provisions for the granting of such an authorisation are described in Regulation (EC) No 507/2006.

Justification for the Request of a Conditional Marketing Authorisation:

The applicant requests a conditional marketing authorisation for mozafancogene autotemcel, pursuant to Article 14 (7) of Regulation (EC) 726/2004 based on the following grounds:

1. Mozafancogene autotemcel falls under the scope of Article 3 (1) of Regulation (EC) 726/2004 because it is an Advanced Therapy Medicinal Product (as defined in Article 2 of Regulation (EC) No 1394/2007);
2. Mozafancogene autotemcel satisfies categories (1) and (3) of Article 2 of Commission Regulation (EC) 507/2006 respectively. First, mozafancogene autotemcel is a "medicinal product[s] which aim[s] at the treatment, the prevention or the medical diagnosis of seriously debilitating diseases or life-threatening diseases." Specifically, mozafancogene autotemcel is indicated for the treatment of paediatric patients with Fanconi Anaemia Type A (FA-A) which is a serious and life-threatening condition that results in bone marrow failure in 80% of patients in the first decade of life. Second, mozafancogene autotemcel is a "medicinal product[s] designated as orphan medicinal products in accordance with Article 3 of Regulation (EC) No 141/2000." It was designated as an orphan medicinal product on 17 December 2010 for the treatment of Fanconi anaemia type A (EU/3/10/822).
3. Mozafancogene autotemcel fulfills the requirements described in Article (4) of Commission Regulation (EC) 507/2006 as described below.

a) Fulfilment of the requirements described in Article 4 of Commission Regulation (EC) No 507/2006:

Applicant's position

The benefit-risk balance is positive:

Transferring a corrected FANCA gene into sufficient CD34+ cells through autologous stem cell transplantation in patients with Fanconi anaemia (Type A) resulted in an 86% clinical response rate in the applicant's pivotal trials, as measured by bone marrow (BM) MMC-resistance (indicative of the ability of hematopoietic stem cells to repair DNA damage) and hematologic stability. These results were achieved with no antecedent cytotoxic conditioning, and subsequently the safety profile has been exceptionally pristine. With a sustained response, bone marrow failure (BMF) and subsequent need for a highly toxic and potentially fatal allogeneic transplant would be averted. Additionally, these patients avoid the multiplied risk of solid tumors that are almost universally fatal in this population and are linked to prior allogeneic transplant. The benefits far outweigh the risks of treatment, as evidenced by the enormous interest from the patient community to incorporate gene therapy early in a patient's life as a prevention against the fatal manifestations of Fanconi anaemia.

As of the data cut-off for this MAA, 24 subjects were enrolled in the pivotal trials and 23 treated with gene therapy. The risk/benefit of mozafancogene autotemcel was assessed with (1) a safety population that included 23 subjects from FANCOLEN-I and Rocket-sponsored studies; and (2) an efficacy

population that included 14 subjects from Rocket-sponsored studies alone since those subjects all received investigational medicinal product (IMP) manufactured under Process B, i.e., the proposed commercial manufacturing process. All subjects were evaluated under a single statistical analysis.

Because there is a narrow window during which FA patients have sufficient HSC reserves to manufacture RP-L102 and benefit from the therapy before succumbing to severe BMF, the applicant identified the following clinically meaningful biomarker-based primary composite endpoint:

- Phenotypic correction by bone marrow (BM) colony-forming units (CFU) mitomycin-C (MMC) resistance ≥20% (MMC at 10 nM concentration); AND
- Genetic correction by PB vector copy number (VCN) ≥0.1; AND
- Haematologic stability by haemoglobin, neutrophil, and platelet counts remaining at ≥75% of 6-month post-infusion nadir value.

Of the 14 subjects treated in the Rocket-sponsored studies, 11 were evaluable for the primary composite endpoint as of the data cut-off, 11-Sep-2023. Of these 11 subjects, 6 (54.5%) met the primary composite endpoint clinically. This is inclusive of one subject who is considered a clinical therapeutic success, despite not meeting the predefined threshold of the haematologic component of the primary composite endpoint, because one lineage (ANC) was slightly below the 75% threshold at the confirmatory timepoint (i.e., was at 74% of nadir). Five of 11 evaluable subjects (45.45%) fully met the primary composite endpoint, which is statistically significant (p-value=0.0002), with sustained BM MMC ≥20% (phenotypic correction), together with genetic correction (PB VCN ≥0.1) and hematologic stability (Meta-analysis Report-Table 8). Achieving haematological stability as defined in the protocol was found to be clinically meaningful when compared to a real-world external control of non-transplanted FA patients. Specifically, RP-L102-treated subjects had approximately a 4 times higher likelihood of achieving haematologic stability at 18 months post-infusion (i.e., the protocol-stipulated timepoint) compared to the real-world ECA (ECA Analysis RP-L102 in FA).

Six of 7 subjects treated with a cell dose were determined a clinical therapeutic success, having achieved genetic and phenotypic correction and haematologic stability making the response rate for subjects treated with this cell dose 86%. In addition, subjects treated with this cell dose had 17 times higher odds of achieving haematologic stability at 18 months and a lower rate of BMF, allogeneic HSCT, or death compared to the ECA. Specifically, subjects who received the proposed minimum recommended dose demonstrated a 100% event-free survival (EFS) at 3 years (where events are defined as BMF, allogeneic HSCT, or death) compared to 44% EFS in the ECA.

In conjunction with this efficacy profile, mozafancogene autotemcel administration has an extremely favourable safety profile, particularly since it is administered without cytotoxic conditioning. Infusion has been well tolerated, and the safety risks associated with drug product administration are minimal as observed in 23 subjects treated with up to 7 years of follow-up. Most treatment-emergent adverse events (TEAEs) reported across all studies were mild/moderate in severity, non-serious and assessed as not related to RP-L102. One unrelated death occurred in the LTFU study for FANCOLEN-I (RP-L102-0116-LTFU) (Meta-analysis Report-Section 5.3.1).

To date, two serious TEAEs have been assessed as possibly related to mozafancogene autotemcel and include Staphylococcal bacteraemia experienced by a patient enrolled in IIT FANCOLEN-I and infusion-related reaction reported in a subject enrolled in Rocket-sponsored clinical study RP-L102-0118. Both were transient and resolved with no clinical sequelae. Details are provided in Meta-analysis Report-Section 5.3.2.

From a long-term safety perspective, two potential risks that have historically been of concern with ex vivo LV-mediated therapy include (1) the formation of replication competent lentivirus (RCL) and (2)

insertional mutagenesis. Data from RP-L102-treated subjects including up to 7 years of follow-up have shown no evidence of RCL and no evidence of insertional mutagenesis. These assessments will continue as applicable per respective protocols (RP-L102-0319, RP-L102-0118, RP-L102-0116-LTFU and RP-L102-0221-LTFU) to further evaluate the long-term safety of mozafancogene autotemcel.

Hence, the totality of evidence obtained to date supports the favourable benefit/risk profile observed for mozafancogene autotemcel, a treatment option with the prospect of direct benefit for FA patients with minimal risks.

b) It is likely that the applicant will be able to provide comprehensive data.

The applicant has completed enrollment in all interventional studies and continues subject follow-up per protocol in ongoing pivotal studies, RP-L102-0118 and RP-L102-0319 (Table 1) in a representative FA patient population that is compared to a robust natural history external control consisting of 139 FA-A patients globally and demonstrating a positive benefit-risk balance (ECA Analysis RP-L102 in FA). Completion of the ongoing interventional studies up to the stipulated duration of 3-years follow-up will lead to the completion of the full dossier and is proposed as a specific obligation for the conditional marketing authorisation to provide comprehensive clinical data confirming that the medicine's benefits continue to outweigh its risks. In addition, the applicant is conducting long-term follow-up studies: RP-L102-0221-LTFU for subjects enrolled in applicant-sponsored clinical trials and RP-L102-0116-LTFU for subjects enrolled in FANCOLEN-I. In both LTFU-studies, subjects will be evaluated for safety and efficacy for up to 15-years post-mozafancogene autotemcel administration.

In addition to evaluating the primary composite endpoint upon completion of the interventional studies, BMF-free survival and other survival-related assessments as described below will be assessed as secondary endpoints to provide a comprehensive assessment of whether RP-L102 prevents progression to severe BMF:

- overall survival, defined as the number of days from drug-product infusion to the event of interest.
- BMF-free survival: defined as the time in days from drug-product infusion to BMF, or to requirement for BMF salvage therapies, or death from any cause, whichever occurs first.
- MDS/AML-free survival: defined as the time in days from drug-product infusion to any one of the following events, whichever occurs first: diagnosis of MDS, diagnosis of AML, development of progressive cytogenetic and/or molecular abnormalities known to be associated with MDS/AML, initiation of therapeutic treatments for either MDS or AML or death from any cause.
- BMF and MDS/AML-free survival.

Importantly, upon completion of the interventional studies, results from these studies along with interim results from the corresponding LTFU studies, will be compared to a robust ECA to demonstrate clinical meaningfulness of continued hematologic stabilisation and prevention of progression to severe BMF. The ECA utilises the International Fanconi Anemia Registry (IFAR), a large FA registry established and maintained by the Rockefeller University since 1982 which contains clinical and genetic information on over 230 FA-A patients.

The completion of the interventional studies, supplemented with data from the ongoing LTFU studies, allows for a comprehensive assessment of the clinical data because at this juncture, the patients (deemed to have received clinical benefit) will have reached an age in which they would have likely progressed to BMF in the absence of therapy. Specifically, at that time point, the subjects will be approaching or have surpassed the median age of BMF (approximately 7 years). In the context of natural history, and as will be demonstrated through a comparison with the robust ECA, haematologic

stabilisation in gene therapy treated patients at or approaching an age when majority of FA-A patients would have reached severe BMF is highly clinically significant.

Additionally, the applicant will continue assessment on safety and tolerability of mozafancogene autotemcel as defined by incidence, type, severity, and frequency of TEAEs, serious adverse events (SAEs), abnormal clinical laboratory results, vital signs, and new abnormalities in physical or laboratory values for the full duration of the study.

From a long-term safety perspective, RCL and insertional mutagenesis assessments will continue as pre-defined per protocol in each of the ongoing studies (RP-L102-0319, RP-L102-0118, RP-L102-0116-LTFU and RP-L102-0221-LTFU) and further support the long-term safety of mozafancogene autotemcel. Negative assessment for RCL evaluation during the first year post-infusion means subsequent evaluations may be discontinued pending adequate rationale. In addition, the applicant has proposed a specific algorithm for insertion site analysis (ISA) (Meta-analysis Report-Figure 4) that will allow the timely identification of any incidence of a potentially predominant clone as determined by peripheral blood mononuclear cells (PBMC) ISA of ≥10% clonal contribution in the setting of PBMC VCN of ≥0.1 during routine follow-up or enhanced monitoring.

This additional safety and efficacy data is planned to be provided to the Agency on an annual basis to support the renewal of the conditional marketing authorisation. At the proposed time for fulfilment of the specific obligations, with completion of RP-L102-0118 and RP-L102-0319 in May 2026, the applicant will present full data analysis for the registrational studies (RP-L102-0418, RP-L102-0118 and RP-L102-0319) along with an interim safety and efficacy analysis of the RP-L102-0116-LTFU and RP-L102-0221-LTFU data to confirm that the risk-benefit balance remains positive and to ultimately support the conversion of the conditional marketing authorisation into full approval. Together these results are intended to demonstrate sustained blood count stabilization, prevention of progression to BMF, durability of treatment effect, and clinical benefit. These results will be further supported by a comparison with the ECA to confirm the clinical meaningfulness of the treatment effect.

For each ongoing study that is part of a specific obligation, the applicant is providing the corresponding study protocol synopsis (Appendix 1.5.5-1: RP-L102-0118 Protocol Synopsis and Appendix 1.5.5-2: RP-L102-0319 Protocol Synopsis).

- **unmet medical needs of patients will be fulfilled;**

As described above, FA results in rapidly progressive BMF for approximately 80% of patients within the first decade of life (ECA Analysis RP-L102 in FA; Kutler 2003; Sebert 2023) and is universally fatal in the absence of a successful allogeneic HSCT. Because of the significant morbidity and mortality associated with allogeneic HSCT in FA (resulting in part from the use of cytotoxic conditioning), allogeneic transplant is reserved as salvage therapy for patients who have progressed to severe BMF (or have developed AML/MDS). Therefore, there is a high unmet need for an effective treatment to address the haematologic component of FA subtype A (FA-A). Mozafancogene autotemcel has been developed to address this unmet medical need.

Human leukocyte antigen (HLA)-MSD allogeneic HSCT is the only therapy available to potentially alter the course of severe BMF for FA patients with recent series demonstrating 5-year OS rates of 70-90%. Most patients (70-80%) do not have an MSD; historically, alternate donor HSCT outcomes have been poor (long term OS approximately 30%). Despite recent advances for FA HSCT, outcomes remain poor for subgroups (MacMillan 2015), in particular for patients with prior opportunistic infections (3-year OS <20%), prior transfusions (3-year OS <55%), or age >10 years old (OS 63.2%) (Mehta 2017). The risk of developing acute GvHD (aGvHD) in the alternate donor setting is significant and may be up to 30% (Fink 2023). Similarly, the rate of chronic GvHD (cGvHD) following alternate donor transplant is 20% (Fink 2023).

The risk of developing cancer in FA patients who undergo allogeneic HSCT is significantly increased and results from the combined effects of underlying FA DNA repair defect, conditioning chemotherapy, and GvHD. Due to the underlying DNA repair defect, FA patients are already predisposed to developing hematologic malignancies and solid tumors, particularly squamous cell carcinoma of the head and neck (SCCHN). However, transplanted FA patients have a 3- to 4-fold increased risk of solid tumors relative to non-transplanted FA patients (Alter 2018). In addition, transplanted FA patients develop solid organ malignancies earlier compared to non-transplanted patients. The median age of onset of SCCHN in non-transplanted FA patients is approximately 30 years whereas the median age at presentation in transplanted FA patients is even younger, 18-20 years. In one series, the incidence of SCCHN following allogeneic HSCT was increased 4-fold above the risk of SCCHN in FA patients who did not receive a transplant; SCCHNs following allogeneic HSCT occurred on average 16 years earlier relative to those that developed in non-transplanted FA patients (Rosenberg 2005). GvHD further increases the risk of solid tumors, likely secondary to ongoing tissue inflammation and associated stress. In one series, Grade 2–4 aGvHD following allogeneic HSCT was associated with a higher incidence of subsequent head and neck malignancies (Guardiola 2004). In an additional series, all patients with squamous cell carcinoma had previously developed cGvHD (Rosenberg 2005). Because of the significant morbidity associated with allogeneic HSCT, even with an MSD, it is reserved for patients who have progressed to severe BMF (or have developed AML/MDS). Other treatment options for FA-related BMF are limited to supportive care with androgens, transfusions, or growth factors, all of which are associated with transient benefits and significant side effects. Namely, prior androgen administration has also been associated with worse outcomes for subsequent allogeneic HSCT (Guardiola 2004; MacMillan 2015). In the alternate-donor transplant setting, more than 20 packed red blood cell transfusions prior to transplant were associated with increased mortality (Wagner 2007). Haematopoietic growth factors including granulocyte colony-stimulating factor (G-CSF) have also been used as supportive care for FA patients but can be considered only in specific cases, such as if neutropenia is associated with recurrent or serious infections. This treatment option does not represent a definitive therapy and there is a concern that G-CSF therapy may stimulate development or progression of cytogenetic abnormalities (FARF 2020). Therefore, there is still a high unmet need for an effective treatment option to address the hematologic component of FA-A without the toxicities associated with allogeneic HSCT, ideally one that could prevent BMF and obviate the need for an allogeneic transplant, including the use of cytotoxic conditioning, in this devastating disorder. No medicinal products have been approved in the EU to address this unmet need for FA-A. The scientific rationale supporting the mechanism of action of mozafancogene autotemcel is based on a very rare naturally occurring phenomenon in a small population of FA patients known as multilineage mosaicism, estimated to occur in <5% of FA patients (Nicoletti 2020; Ramirez 2021). Multilineage mosaicism results when a spontaneous reversion mutation occurs in the FANCA gene of a haematopoietic cell which converts it from an abnormal cell with a dysfunctional DNA repair mechanism to a normal cell with a functioning DNA repair mechanism. The corrected cell thus has a selective advantage over uncorrected FA cells and can proliferate more successfully. Multilineage mosaicism results when this spontaneous reversion mutation occurs in a long-term hematopoietic stem cell (HSC). As a result, all lineages (lymphoid, myeloid, erythroid, megakaryocyte) are corrected by the reversion mutation; therefore, multilineage mosaicism can lead to sustained blood count normalization over time (Soulier 2005; Mankad 2006; Nicoletti 2020; Ramirez 2021). In addition to hematologic stability, multilineage mosaicism is associated with low incidences of AML/MDS or requirement for allogeneic HSCT (Nicoletti 2020; Ramirez 2021). Multilineage mosaicism provides the natural proof of concept for gene therapy. Mozafancogene autotemcel replicates and expands upon the naturally occurring phenomenon of multilineage mosaicism. By introducing a functional copy of FANCA into autologous haematopoietic stem and progenitor cells (HSPCs) (a subset of the CD34+ cell population), gene corrected HSPCs have a selective advantage over uncorrected HSPCs. Hence, these gene corrected HSPCs can repopulate the BM and PB without conditioning (with

correction first seen in BM and then PB) and lead to hematologic stability. This selective advantage that gene-corrected cells have over uncorrected cells is what enables engraftment in the absence of conditioning. Because multilineage mosaicism is extremely rare (<5% of FA patients), almost no FA patients are expected to spontaneously revert.

Results from the Rocket-sponsored studies clearly demonstrate that mozafancogene autotemcel offers a potentially curative therapy to address this unmet need by preventing progression to severe BMF and avoiding the need for allogeneic HSCT. Significantly, 6 of 7 evaluable subjects (86%) who received the proposed recommended minimum cell dose, achieved clinical therapeutic success and demonstrated sustained phenotypic correction (BM MMC resistance) with concomitant genetic correction (PB VCN) and haematologic stability. Overall, 6 of 11 evaluable subjects were considered clinical therapeutic success. Furthermore, compared to the ECA, subjects treated with the proposed minimum cell dose had a 17-fold higher likelihood of achieving haematologic stability and 100% EFS (events were defined as BMF, allogeneic HSCT, and death). In contrast, non-responders had significant blood count declines in 1 or more lineages.

The safety profile of mozafancogene autotemcel is highly favorable; the risks potentially associated with the investigational product were transient and resolved with no intervention or with supportive care and without clinical sequelae. The safety profile of mozafancogene autotemcel also compares favorably to that of HLA-MSD allogeneic HSCT. In contrast to allogeneic HSCT, the investigational product does not require a suitable donor, is administered without cytotoxic conditioning and attendant risks, and where subjects can expect to be discharged from the hospital within 48 hours following infusion.

As indicated by the clinical safety and efficacy data, mozafancogene autotemcel addresses the considerable unmet medical need for the treatment of FA-A patients and represents a compelling treatment option after diagnosis with FA-A to prevent progression to severe BMF.

- **The benefit to public health of the immediate availability on the market of the medicinal product concerned outweighs the risk inherent in the fact that additional data are still required.**

The immediate availability of mozafancogene autotemcel provides a benefit to the public health interest in that it will enable potentially definitive patient treatment at the earliest possible juncture after diagnosis, prior to the onset of severe BMF, precluding the need for allogeneic HSCT and its multiple associated risks.

Allogeneic HSCT is the only treatment available that can potentially alter the course of FA-associated BMF; however, as described above, it is donor-dependent and associated with significant short- and long-term morbidity and mortality. Moreover, hospitalisation in a specialised transplant unit or intensive care unit setting is required for several weeks after an allogeneic HSCT. For all of these reasons, HSCT is reserved as salvage therapy, and there remains a high unmet medical need with significant interest from the FA patient community for an alternative treatment option, one that does not require conditioning and can avoid allogeneic HSCT. The FA European and US patient advocacy groups, including many FA patients and families, have expressed keen interest in the therapy and their desire to access the therapy as soon as possible. Accordingly, the applicant designed a robust clinical study to allow for efficient and comprehensive assessment of the benefit/risk with a sample size representative of the broader FA patient population. The positive benefit-risk balance seen to date has only further amplified the interest expressed by the patient community to access the therapy.

Specifically, mozafancogene autotemcel allows for prevention of BMF while avoiding the significant risks associated with allogenic HSCT. Specifically, mozafancogene autotemcel relies on the selective advantage of gene-corrected HSCs to enable engraftment and avoids the need for conditioning that is

required for allogeneic HSCT. Administered without conditioning, mozafancogene autotemcel does not require prolonged hospitalisation for intensive monitoring and avoids the risk of potentially life-threatening acute complications. In contrast to allogenic HSCT, following mozafancogene autotemcel infusion, the majority of subjects treated on the Rocket sponsored studies were discharged within 2 days. Also, because no antecedent conditioning is used, mozafancogene autotemcel treatment does not preclude allogeneic HSCT as a salvage therapy for BMF if gene therapy is unsuccessful.

In summary, and as indicated by the clinical safety and efficacy data, mozafancogene autotemcel represents a significant therapeutic advance for the treatment of FA-A patients to prevent progression to severe BMF. It confers minimal toxicity and is associated with an exceptionally high response rate (86%) when administered at cell doses above the proposed recommended minimum dose .

Importantly, as a result of the unrelenting progressive nature of the disease in which every year of life is associated with significant declines in blood counts (10-25% in leukocytes, neutrophils, and platelets) (ECA Analysis RP-L102 in FA), there is a limited window in the first decade of life during which FA patients harbour sufficient HSPCs reserves to enable an HSPC collection robust enough to generate a viable drug product. Delays in availability will result in situations where patients develop BMF such that they are no longer eligible for this highly promising and minimally toxic gene therapy targeting an unmet medical need. Given the very favourable safety profile of mozafancogene autotemcel, particularly since it is administered without cytotoxic conditioning, coupled with the efficacy that has been demonstrated to date, immediate availability of mozafancogene autotemcel represents a benefit to the public health and outweighs any risk inherent in the fact that additional data is continuing to be collected to complete the full dossier and will be provided to the Agency under a conditional marketing authorisation to confirm the positive benefit-risk balance.

2.5.4. Biosimilarity

Not applicable

2.5.5. Additional data exclusivity/ marketing protection

Not applicable

2.5.6. New active substance status

The applicant requested the active substance mozafancogene autotemcel contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union

Assessment of this claim is appended.

2.5.7. Orphan designation

Fanskya was designated as an orphan medicinal product EU/3/10/822 on 17 December 2010 in the following condition: Fanconi anemia (FA).

2.5.8. Similarity with orphan medicinal products

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report, addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

2.5.9. Derogation(s) from orphan market exclusivity

Not applicable

2.5.10. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0002/2024 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP EMEA-C1-002578-PIP01-19-M01 was partially completed.

A Paediatric Investigation Plan (PIP) was submitted on 22-Mar-2019 (EMEA-002578-PIP01-19).

- The Paediatric Committee issued its opinion on 31-Jan-2020 recommending a waiver for paediatric patients from birth to less than 1 year of age be granted (EMA/PDCO/616174/2019). The EMA adopted a Decision on 18-Mar-2020 (P/0114/2020).
- On 21-Aug-2023, the applicant submitted a request for a modification (EMEA-002578-PIP01-19-M01) of the agreed PIP as set out in the above-mentioned Agency's decision. The PDCO Opinion was adopted on 15-Dec-2023 with a final Decision (P/0002/2024) adopted on 03-Jan-2024 granting a deferral for Phase 2 studies completion (RP-L102-0319 and RP-L102-0118).
- The applicant's request for Interim (partial) PIP Compliance check (EMEA-C1-002578-PIP01-19-M01) was submitted on 18-Dec-2023. The PDCO discussed the completed studies 1 (Fancolen-I), 3 (RP-L102-0418) and 5 (RP-L102-0118) and considered that these are compliant with the latest Agency's Decision (P/0002/2024) of 3 January 2024. The PDCO finalised this partially completed compliance procedure on 23 February 2024.
  Scientific discussion PDCO: The PDCO discussed during the February 2024 meeting the partial compliance check for mozafancogene autotemcel for the treatment of Fanconi anaemia subtype A. The subject of the present PIP compliance check were study 1, study 3 and study 5. The deviations found in study 1 were discussed and found not scientifically impacting the interpretation of the results and agreeable if a modification request would have been submitted. Therefore, it was concluded that the studies could be considered compliant.

3. Scientific overview and discussion

3.1. Quality aspects

3.1.1. Introduction

The finished product is presented as a dispersion for infusion containing 1-50 × 10^5 cells/mL CD34+ hematopoietic stem cells transduced with the LV-RP-L102 self-inactivating (SIN) lentiviral (LV) vector encoding human FANCA gene as active substance (mozafancogene autotemcel).

Other ingredients are: Plasma-Lyte A and human albumin

The product is available in an infusion bag.

3.1.2. Lentiviral vector

3.1.2.1. General information

LV-RP-L102 is a VSV-G pseudotyped self-inactivating and replication-incompetent third-generation lentiviral vector carrying the therapeutic FANCA cDNA. The vector is used to transduce and restore the human FANCA gene in CD34+ haematopoietic stem cells.

Lentiviral vector nomenclature has been adequately listed (no INN-name is expected for this starting material) and the structure has been briefly described together with a schematic overview of the vector genome map and orientation of vector components. Annotated sequence is provided and sequencing of the integrated provirus to confirm that the transgene sequence is 100% matched to the reference (predicted) transgene sequence is performed as part of batch release.

General properties of the virus vector (including physical description, size, shape, particle identity, sequence identity, infectivity, and replication deficiency) has been outlined in a tabulated format. Biological activity (restoration of the FA pathway in LCL cells) and potency assay (LCL cells resistance to MMC) descriptions are also included.

In conclusion, the nomenclature, structure, and general properties of the lentiviral vector are deemed to be sufficiently described.

3.1.2.2. Manufacture, process controls and characterisation

Description of manufacturing process and process controls

The sites responsible for the manufacture and testing of the vector and the plasmids used as starting materials for the production of LV-RP-L102 are adequately listed and provided with proof of GMP compliance.

The applicant has provided a flow chart and a narrative summary of each process step. Briefly, WCB vials are thawed and expanded; a transfection reagent is added and incubated for transfection and vector production; the LV is harvested, harvest material is clarified and treated to remove cell debris and residual host cell and plasmid DNA; LV-RP-L102 is purified in several unit operations; concentrated and formulated vector is filtered, filled, frozen and shipped to the long-term storage facility. The descriptions of each step are accompanied by tables with process parameters and operating conditions. The listed parameters and target values are deemed relevant and generally acceptable.

The descriptions for each step are overall considered brief, but in combination with process parameters and operating conditions they are overall deemed sufficiently outlined for an evaluation of the manufacturing process. Some additional details are requested.

The raw material qualification process and incoming material testing has been briefly outlined. In addition to visual controls and check of CoA, identity is confirmed via analytical testing. For medium and high criticality materials, additional tests are also included and performed. Furthermore, for all materials that are considered highly critical, the full CoA is qualified Internally before acceptance. This material qualification system is overall considered adequate for manufacturing of LV-RP-L102. Compendial, non-compendial, and biological raw materials have been listed in tabulated formats. Qualitative descriptions of the media components are provided and a notification process is in place for changes associated with cell culture media and supplements.

Cell banks (MCB and WCB) for the producer cells have been developed. Development is stated to be in compliance with cGMP. Source, history, and manufacturing of MCB at Rocket Pharma has been adequately detailed from RCB to MCB and WCB. Specifications for MCB and WCB have been established

and presented. The information provided is in line with principles laid down in ICH Q5A and Q5D. Genetic stability and productivity have been characterized using three small scale experiments. Furthermore, general stability has been demonstrated by testing and plotting batch viability over time in combination with production of clinical material.

Four plasmids are used to produce LV-RP-L102 by transient transfection of cells and constitute a GMP-grade third-generation self-inactivating LV plasmid system. Source, molecular recombination, transformation of *E. coli*, and generation of RCB cell banks for each final construct at are briefly described. Plasmid maps are provided with sequence and general plasmid information. Manufacturing of *E. coli* MCBs and the final plasmid products is outlined in the dossier. The release and stability test panel for future MCBs complies with current Ph. Eur. requirements. A representative flow chart of the plasmid manufacturing is presented. Plasmid specifications have been provided which are in line with Ph. Eur. 5.14.

The applicant has listed critical process parameters and critical in-process controls with acceptance ranges/criteria. Overall, the information presented in S.2.2 and S.2.4 is considered adequately justified by supporting LV production data and deemed sufficient to control the process.

A process performance qualification (PPQ) study was performed at the commercial site with the manufacture of three consecutive LV-RP-L102 batches at the commercial scale. Results from process parameters, in-process controls, release specification tests, and additional characterization analysis has been gathered and presented. The data show that when operated at set-point conditions, the manufacturing process consistently yields a product that complies with the release specifications. Additional process validation studies (including process yield and recovery, impurity clearance, and vial fill homogeneity testing) support the notion of an adequately validated process for the manufacture of the vector. Process intermediates biochemical hold time studies support the proposed hold times presented in the dossier, and aseptic processing studies are presented and deemed acceptable. For shipping validation, the applicant refers to data and report in S.6[LV].

Manufacturing process development

Process development has been taking place in the form of several optimization phases that are generally supported by small-scale studies as well as full scale verification runs. This includes an evaluation and optimization of the applicant's materials (plasmids and cell banks) produced at Rocket Pharmaceuticals for the use in the production system, studied at small-scale followed by a full-scale validation run; an optimization of sterile filtration to increase process recovery; and three small-scale development studies to evaluate transfection mix preparation, AEX chromatography operations, and SEC operations. The results of the studies were confirmed by at-scale pre-PPQ development batches and a pre-PPQ assessment to confirm the final process. For the confirmation batch a set of improvements were introduced, product attributes were classified as Critical Quality Attributes (CQAs) or Quality Attributes (QAs), and process parameters were defined as critical or non-critical. The quality attribute controlling identity should be classified as critical (**OC**). Analytical data to support the representativeness of the pre-GMP vector batches in relation to the GMP vector batches proposed for commercial use should be provided (**OC**).

Initial manufacturing to support the early phase clinical program of LV-RP-L102 was performed at one manufacturing site, while late phase manufacturing was performed at another site; the vector construct, plasmids, and final formulation buffer remained unchanged. To demonstrate comparability between vector manufactured at the different sites the applicant has provided batch analysis results (analytical comparability), the result of transduction characterization experiments (establishment of MOI needed for the vector), and a head-to-head comparison of drug product quality attributes and

extended characteristics. In addition, a brief summary of the retrospective analysis of the Drug Product release data, and a comparison of clinical efficacy and safety is provided.

Characterisation

Characterization of the lentiviral vector has been performed using the PPQ batches and comprises test for identification of the drug entity by p24, particle characterization by size and shape, DNA sequencing and characterization of the genome size, infectivity of the LV, proof of biologic activity (mRNA/protein expression and MMC-resistance), and replication deficiency. Biological activity has been investigated using orthogonal methods (mRNA/protein expression and MMC-resistance); data show that increase in MOI leads to higher VCN, mRNA and protein expression in K562-FANCA KO cells.

The characterization is overall considered sufficient.

The strategy to ensure replication deficiency involves use of third generation self-inactivating plasmid system and controls established at EoP cells and final formulated LV-RP-L102 starting materials. Overall, the control strategy is deemed suitable.

Impurities

Process related impurities have been evaluated. With the exception of one impurity, for which removal was demonstrated during PPQ, all impurities are included in the specification for LV-RP-L102 and tested at release. This is acceptable.

Defective interfering viral particles, non-infectious particles, free vector envelopes and virus aggregates have been identified as potential product-related impurities.

3.1.2.3. Specification, analytical procedures, reference standards, batch analysis, and container closure

The specification for LV-RP-L102 lentiviral vector is provided with test attributes, acceptance criteria and method identification numbers. Reference is made to Ph. Eur. Monographs for the compendial methods. The specification applies to the release and shelf-life of LV-RP-L102.

Identity is controlled by confirming the presence and correct sequence of the transgene by transducing permissive cells with LV-RP-L102 followed by amplification, extraction and sequencing of a sequence spanning the PGK promoter and the FANCA transgene. The test to confirm integrity of the vector by restriction enzyme mapping is proposed to be removed from the commercial release specification, which is endorsed.

The strength of a batch of LV-RP-L102 is expressed using the infectious viral titer, the physical titer (p24 capsid protein concentration) and potency by MMC resistance bioassay. A test for infectivity (particle to infectivity ratio) is included in the vector specification, however the proposed acceptance criteria are considered too wide and should be tightened or further justified (**OC**).

General tests include clarity, pH, osmolality and visible particles.

Tests for process-related impurities are included in the specification.

The attributes included in the specification to control the safety of the vector preparation are considered appropriate. It is however noted that the LV and drug product is tested for endotoxin using the compendial LAL test based on the Limulus Amoebocyte Lysate (Ph. Eur. 2.6.14). The applicant is informed that the Ph. Eur. recently adopted general text 2.6.32 on recombinant Factor C for Endotoxin control. The applicant has provided plans for transitioning to Ph. Eur. 2.6.32. Detection of replication-

competent lentiviruses (RCL) is performed on vector and on end of production (EOP) cells, and tests include a positive control.

The applicant has utilized data from relevant LV-RP-L102 batches for setting the acceptance criteria for release and stability. The acceptance criteria are generally acceptable

Analytical procedures and reference standards

The applicant has provided brief method descriptions and, for a selection of methods, detailed SOPs. For compendial methods, reference to the respective Ph. Eur. monographs is made. The descriptions for the non-compendial methods are in general sufficient, however the optionality of excluding replicates and removing points in the standard curve should be clarified the frequency with which exclusion of test results have been utilized to fulfil assay criteria should also be detailed and justified (**OC**).

In general, method validation has been performed in line with expectations and all acceptance criteria were met. Method validation summaries and, for non-compendial methods, validation protocols and reports are provided. Overall, the validations are in line with ICH Q2 and deemed suitable for this starting material. As regards the validation for the HCP method, in general the recommendations set out in Ph. Eur. 2.6.34 have been adequately implemented and suitable coverage against the HCP antigens has been demonstrated.

An LV-RP-L102 reference standard (RS) is used during release and stability testing of the vector as reference standard for vector potency testing, and as assay reference control for infectious viral titre and infectivity. A one-tier RS approach is proposed and the protocol for the (re)qualification of existing or new RSs is considered largely adequate, however acceptance criteria for extended characterization tests have been requested (**OC**).

Batch analysis

Batch data are provided for GMP batches which include batches used in development, clinical, process performance qualification and stability studies. The results show reasonable manufacturing process consistency.

Container closure

The vector is stored in a 5 mL polypropylene cryovial with high density polyethylene (HDPE) closure. A representative Certificate of Analysis has been provided. The vials, which are CE marked for storage of diagnostic samples, are single use, non-pyrogenic/pyrogen free (< 0.5 EU/mL per USP < 85>) and meet the United States Pharmacopeia Class VI requirements. Cytotoxicity has also been evaluated using cell lines L929, WI-38 and MRC-5 and vials were found to be non-cytotoxic. The information is approvable.

An extractables and leachable analysis was performed and the presence of volatile, semi-volatile, non-volatile and elemental extractables were evaluated. Considering the overall information on the container, that the LVV is a starting material, and that the cells are washed multiple times after transduction, the applicant’s conclusion that the low levels of extractables pose a very low probability of adverse effects to human health is agreed. Container integrity is considered sufficiently demonstrated. A shipping study was performed to validate the most challenging shipping lane with the longest possible shipment duration from the shipment origin site to the shipment destination site.

3.1.2.4. Stability

Stability studies were conducted using the 5 mL cryovial final container filled with 2 mL of lentiviral vector and testing the storage condition ≤ -65°C. Stability data from multiple batches are presented for timepoints up to 48 months. For three PPQ batches, the accelerated storage conditions -20°C and 2 - 8°C were also evaluated. Furthermore, in a forced degradation study at 36°C for 0, 6, 12, 24 and 48 hours, pH, osmolality, visible particles, clarity, turbidity, infectious titre, physical titre, and infectivity were evaluated. Based on 18 months of real time stability data for the 3 PPQ batches and up to 48 months for supporting batches, a 30-month shelf-life for LV-RP-L102 when stored at ≤ -65°C are proposed. It is agreed that most of the stability indicating attributes remain within acceptance limits at long term stability conditions without any obvious negative trends. Sufficient stability data are available for MMC Resistance (Potency), which is stable throughout long-term storage. The proposed shelf life of 30-months when stored at ≤ -65°C can be endorsed.

A post approval stability protocol and commitment including suitable testing frequency timepoints has been presented. The applicant will place on stability at ≤ -65°C a minimum of 1 batch annually, provided that at least one batch is manufactured. This is acceptable.

3.1.3. Active substance

3.1.3.1. General information

The active substance contained in Fanskya (INN mozafancogene autotemcel) consists of autologous CD34+ haematopoietic stem cells (HSCs) transduced with a self-inactivating lentiviral vector encoding human FANCA gene. The FANCA gene codifies for the FANCA protein which is part of the Fanconi anaemia (FA) pathway core complex. HSCs that contain integrated copies of the functional FANCA gene are expected to restore FA DNA repair pathway to allow normal cell proliferation.

Because of the nature of the active substance – autologous hematopoietic stem cells transduced with a lentiviral vector - neither a structural formula nor a description of general physico-chemical properties is applicable. However, comprehensive general information is provided. Background information relevant for the proposed mechanism of action as well as for the definition of potency and identity measurements is provided.

3.1.3.2. Manufacture, process controls and characterisation

Description of manufacturing process and process controls

The manufacturer of the drug substance is also responsible for in process-testing of the Drug Substance. As the manufacturing process is continuous, no Drug Substance release testing is performed. A MIA covering the proposed activities and product has been provided.

The applicant has provided a flow chart and a narrative summary of each unit operation and of each individual process step of unit operation 1 of the manufacturing process. Briefly, the process consists of pooling of the patients leukapheresis products (only if 2 products have been collected), platelet reduction, CD34+ cell labelling and enrichment, pre-stimulation and transduction, harvest and purification. The Drug Substance manufacturing process results in a single Drug Substance batch which is used immediately for Drug Product formulation. No Drug Substance storage or reprocessing is performed.

In general, the manufacturing process and control is described in sufficient detail and supported by process development studies, but some questions remain to include further detail on the manufacturing procedure (**OC**).

Autologous mobilized peripheral blood collected by leukapheresis, referred to as Hematopoietic Progenitor Cells Apheresis (HPC-A), are the starting material for drug substance manufacturing. The applicant has provided a brief overview of the procedure for mobilization and collection of the autologous cells. A confirmation that collection and testing sites meet the standards of quality and safety regarding donation, procurement and testing of the starting material in accordance with relevant EU legislation, i.e., Directive 2004/23/EC or 2002/98/EC donor selection, is provided The serology testing that is performed prior to mobilization is in line with legal requirement and includes HIV 1/2, HBV, HCV, CMV, HTLV ½ and *Treponema palladium*. The applicant confirmed that CE-marked test kits are used. No acceptance criteria for the outcome of serology testing are defined as a positive serology test result is accepted considering that the product is for autologous use. This could be accepted, provided that it is further justified that the use of apheresis material from a donor who tested positive for an infectious disease marker, will not impact the quality or safety of the product, e.g. by interfering with the transduction step (**OC**).

The specifications defined for HPC-A include number of CD34+ cells/kg body weight with defined limits as well as identity and impurity (performed by flow cytometry), WBC, HCT, Platelets and RBCs with no acceptance criteria established. The absence of specification for these attributes is sufficiently justified. In general, 2 leukapheresis collections are performed (HPC-A-1 and HPC-A-2). HPC-A-1 and HPC-A-2 are stored at 2-8°C until shipment to the Drug Substance manufacturing site. The defined expiry time of 80 hours from the end of collection at the clinical site to the beginning of processing is sufficiently justified. Shipment to the manufacturing site has been appropriately validated.

Tabular overviews are provided of the raw materials that are used in the manufacturing process. The quality control procedures for the raw materials should be further specified (**OC**). The qualitative composition of most of the buffers and media used in the manufacturing is provided, but is still missing for one of the cell culture media (**OC**). For all plasma derived raw materials used in the manufacturing process, it is confirmed that traceability from the donors used for plasma-derived raw materials manufacture to the final product is ensured.

The manufacturing process for RP-L102 is continuous between drug substance to drug product. Details regarding the validation of the process are described in the Drug Product section.

Manufacturing process development

A summary is provided of several development studies that have been performed to establish the manufacturing process.

Process development studies were conducted using HPC-A obtained from healthy donors. It is stated that differences between healthy donor and patient cells are only observed in a small subset of quality attributes, but no additional information is provided. A justification of the use of cells from healthy donors in development studies, ideally based on relevant data has been provided to support the proposed strategy. Studies on CD34+ cell recovery, culture vessel optimization (including small- and large-scale culture containers), cell culture media, transduction process with and without pre-stimulation, transduction enhancers and MOI were performed and relevant quality attributes related to cell functionality, including CFU-VCN, transduction efficiency and potency were investigated.

To establish operational ranges for critical process parameters, the applicant performed two small-scale DoE studies using healthy donor cells in which the impact of the concentration of the different cytokines and additives and transduction enhancers was studied. No significant effects were observed

in the studied range, but the results of the output parameters show considerable variability. Although this could, at least partly, be attributed to donor differences, it also indicates the importance of a consistent manufacturing process to reduce variability as much as possible. Upon request, the applicant tightened the operational limits for the concentration of cytokines, additives and transduction enhancers.

Comparability during clinical development is discussed in the Drug Product section.

Characterisation

The active substance of Fanskya consists of autologous CD34+ cells transduced with the lentiviral vector (LV-RP-L102) encoding human FANCA gene to restore DNA repair function and increase differentiation potential of the cells.

In addition to the characteristics that are controlled at product release (i.e. percentage of viable cells, differentiation potential (CFU), and VCN), the applicant has performed flow cytometry analysis to further characterize the haematopoietic cell populations. Batches from patients and healthy donors were included in this study. Results show considerable batch-to-batch variability, which is not unexpected for an autologous cell-product. The percentage of CD34+ cells is lower in batches from patient cells (<35%) compared to batches from healthy donor cells (>50%) and the percentage of cellular impurities higher.

Upon request, transduction efficiency or VCN in different CD34+ lineages has been characterized. VCN in CFU-GM tends to be a bit lower than in BFU-E, but results are generally in the same range. No correlation with clinical outcome was detected.

Data suggest that cell dose/kg is an important predictor of clinical outcome. As it could not be assessed if cell dose is the only predictor and there were concerns with regard to product comparability after the change in vector manufacturer and the concurrent use of lower MOI for transduction (see Drug Product section), a more in depth evaluation of the data was requested. This issue was raised as a Major Objection.

Potency is controlled by a rapid potency test that quantitates FANCA RNA expression and by a functional activity assay that confirms MMC resistance of the transduced cells. The proposed functional potency assay is reflective of the claimed mode of action of the product. Results of the rapid potency assay are available prior to administration of the product to the patient; results of the functional assay are not available prior to administration. The correlation between the FANCA RNA expression and results of the functional potency assay has been sufficiently evaluated.

Overall, the characterization exercise was considered very limited and is not yet considered sufficient to support the currently proposed control strategy. Further justification was requested that the two-stage control strategy, in which part of the release tests results are not available prior to administration, provides sufficient control of critical quality attributes such as potency, VCN and transduction efficiency and that the release test results that are available at the time of product administration are sufficiently predictive of safety and efficacy. This Major Objection is partly solved. It is agreed that the two-stage release strategy is justified by the short shelf-life of the product. However, due to the limited data-set it is not possible to determine if the acceptance criteria for the release tests results that are available prior to administration are sufficiently predictive for safety and efficacy of the product.

Impurities

The residual amounts of process-related impurities have been justified from a safety point of view. Based on the provided information, it could be agreed that no testing for residual process-related impurities is performed.

A risk evaluation of the presence of nitrosamines has been provided. It is concluded that the materials used in the RP-L102 process contribute no risk of introducing amines, nitrates, nitrosating agents or conditions which could promote N-nitrosamines impurity formation in the final drug product.

Product-related impurities include dead cells, CD34- cells, and non-transduced cells. These impurities are indirectly controlled by the Drug Product specifications for cell viability, % CD34+ cells and VCN.

3.1.3.3. Specification, analytical procedures, reference standards, batch analysis, and container closure

As the RP-L102 manufacturing process is continuous from Drug Substance to Drug Product no Drug Substance release specifications are in place.

3.1.3.4. Stability

As the RP-L102 manufacturing process is continuous from Drug Substance to Drug Product, Drug Substance stability has not been evaluated.

3.1.4. Finished Medicinal Product

3.1.4.1. Description of the product and Pharmaceutical Development

The drug product (DP) is an individually manufactured cell suspension for immediate infusion containing hematopoietic stem cells transduced with the LV-RP-L102 lentiviral vector encoding human *FANCA* gene as drug substance (DS). The DS cells are suspended in Plasma-Lyte A, a non-pyrogenic isotonic solution, that has been supplemented to contain human serum albumin (HSA). There are no preservatives or anti-microbial agents present in the DP. In addition to the composition of the drug product, a detailed composition of excipients, Plasma-Lyte A and human serum albumin is provided.

Each bag is filled with 200 mL (± 0.5 mL) and is intended for single dose intravenous infusion. RP-L102 is provided as a fresh product that is stored and shipped at 2-8℃.

Pharmaceutical development

Manufacturing process development included a change of excipients. A justification of the change is provided including a side-by-side comparability assessment of the qualitative and quantitative characteristics. It can be agreed that the replacement is unlikely to result in significant differences of the DP quality. The choice of excipients is sufficiently justified. The applicant clarified that the HSA used has a marketing authorisation in the EU and is linked to a certified Plasma Master File Information in line with the Guideline on Plasma-derived products has been provided.

Studies to evaluate extractable/leachables and container integrity were performed and do not give rise to questions.

No preservatives or antioxidants are used in the Drug Product formulation. Microbiological safety of the Drug Product is controlled by gram stain and sterility testing. The results of the latter test are only available post-administration, which is justified by the short shelf-life of the product.

A compatibility study was performed using one healthy donor batch and supports the instructions for use and handling of the product.

Manufacturing process development

Process development focuses only on the batches that were used in the pivotal studies. The overview of clinical batches includes 14 batches used in clinical Phase I, early Phase II and late Phase II. These clinical batches were manufactured at different sites.

The clinical data package also includes supportive safety data obtained with batches manufactured using Process A. Information on these batches and comparability remains to be provided to support the use of data obtained with Process A batches in the safety evaluation (**OC**). In addition, information on pre-clinical batches demonstrating that the product tested in the pre-clinical studies is sufficiently representative of commercial Drug Product is missing and should be provided (**OC**).

The comparability evaluation considers above-mentioned clinical batches and engineering runs manufactured using healthy donor cells. Comparability was evaluated retrospectively by comparison of quality attributes of batches manufactured at the different sites. No obvious differences in the tested quality attributes were, however, observed between batches manufactured at the different manufacturing sites, except for VCN-CFU. The applicant indicates that difference in CFU-VCN between sites are due to analytical differences. Information on method development was, however, not found in the dossier. Upon request, the applicant confirmed that no other changes have been made to analytical methods that could have impacted the comparability assessment or hamper a direct comparison of batch analysis results.

To further demonstrate process consistency at the manufacturing sites, the applicant performed 4 split-apheresis runs. The Drug Product release data from paired runs were in general comparable, although in 2 cases CFU-VCN and CFU-VCN per transduced cell showed some differences.

3.1.4.2. Manufacture of the product and process controls

A MIA covering the proposed activities and product has been provided for the finished product manufacturing and testing site.

The RP-L102 drug product is formulated as a single dose cell suspension for intravenous infusion.

The process flow diagram for the DP manufacturing process is has been provided. Briefly, the process consists of final formulation in the Drug Product bag, secondary packaging, visual inspection, and labelling. All steps are conducted aseptically to ensure the sterility of the DP. No intermediates are defined in the DP manufacturing process. The Drug Product manufacturing process is described in sufficient detail.

No reprocessing occurs during DS and DP manufacturing.

Labelling and traceability system is described, particularly the chain of identity. In general, the description is considered sufficient to ensure that the patient receives the right drug product.

A process performance qualification (PPQ) study was performed at the commercial manufacturing site using cells from 2 healthy donors. As the manufacturing process for Fanskya is performed in a continuous flow without interruption, DS and DP process were qualified in the same PPQ runs. The PPQ campaign was performed using two LV-RP-L102 LVV lots produced using the commercial manufacturing process. Data were supplemented with results of CPPs and CQAs from the last clinical batch. The data show that when operated at set-point conditions, the manufacturing process yields a

product that complies with the release specifications. Deviations that occurred during the PPQ were appropriately discussed and do not give rise to further questions.

3.1.4.3. Product specification, analytical procedures, batch analysis

Specifications

RP-L102 is formulated fresh for infusion and cells have a limited shelf-life before they degrade. Therefore, the specification for RP-L102 includes testing both pre- and post-infusion samples.

The Drug Product specification includes a panel of release tests that is performed prior to administration of the product to the patient, and a panel of release tests that is performed for final release. The two two-stage release strategy, in which part of the release tests results are not available prior to administration, could be justified by the short shelf-life of the product. The available data suggest that efficacy can be at least partially ensured by testing CD34+ cells/kg prior infusion but the applicant still needs to appropriately address the risks emerging from the proposed control strategy where VCN levels are available after infusion of the DP into the patient. (**MO**). The actions take in case the results of the final release testing do not comply with the acceptance criteria should be laid down in P.5.1 (**OC**)

The acceptance criteria for the different release tests have been updated following questions raised. Some additional questions are however raised with regard to the acceptance criteria. Due to the limited data-set it is not possible to determine if the acceptance criteria for the release tests that are available prior to administration are sufficiently predictive for safety and efficacy of the product. A **MO** is maintained requesting the applicant to 1) tighten the acceptance criterion for %CD34+ cells, 2) demonstrate that the rapid potency assay and its acceptance criterion can at minimum ensure that the cells have been successfully transduced, and 3) commit to revise the acceptance criteria for the rapid potency assay when additional data are available. In addition, to confirm that clinical efficacy of batches manufactured using the proposed commercial vector is comparable to batches manufactured using vector from a previously used manufacturing site, the applicant should commit to re-evaluate the correlation between cell dose and clinical outcome when data from additional patients are available and compare clinical outcome of batches manufactured with the different vectors that met the cell dose specification.

Analytical procedures and reference standards

The applicant has provided brief method descriptions and, for a selection of methods, detailed SOPs (see Drug Product specifications above – for proprietary methods no SOPs have been provided). The information provided is sufficient.

Method validation has been performed in line with expectations and all acceptance criteria were met.

A reference standard is not used in the testing and release of finished product.

Batch analysis

Batch data are provided for the clinical batches and for batches manufactured using healthy donor cells. The data show considerable variability, which makes it difficult to conclude on manufacturing process consistency. Variability is, however, not unexpected considering the autologous nature of the product.

Container closure

The container closure system for the DP of Fanskya consists of a bag as primary packaging and an aluminium cassette as secondary packaging. Drawings and descriptions of both the primary and the

secondary packaging have been provided. The information provided on the container closure system and secondary packaging is sufficient. It has been confirmed that the used primary packaging is in agreement with the currently valid Ph. Eur. chapters.

A shipping study has been performed demonstrating that the shipper remains intact and is capable to maintain the temperature at 2-8°C for up to 42 hours. Upon request, the applicant also evaluated the potential impact of transport conditions on the cells.

3.1.4.4. Stability of the product

A shelf life of 42-hour when stored at 2-8°C is proposed.

A summary of the batches on stability is provided in Table 4. Stability studies have been conducted for both long-term storage conditions (2-8°C) and forced degradation conditions (heat treatment, freeze/thaw, agitation, and ambient storage).

Stability data are provided for 4 batches manufactured from healthy donor cells and for four clinical batches. Batches were stored in cryovials instead of bags. Data supporting the comparability of product stability in bags and vials is provided in Section 3.2.P.2 and do not give rise to questions.

The batches manufactured from healthy donor cells remain stable up to 52 hours at 2-8°C, although one of the batches shows a decrease in CFUs after 52 hours of storage. Two of the clinical batches show a decrease in CFUs after, respectively, 42 hours and 50 hours. In the other clinical batches, no decrease of CFUs is observed after, respectively, 18 and 32 hours. Overall, the data support a shelf-life of 42 hours.

3.1.4.5. Biosimilarity

Not applicable

3.1.4.6. Post approval change management protocol(s)

Not applicable

3.1.4.7. Adventitious agents

Non-viral adventitious agents

The applicant has briefly discussed the measures taken to ensure microbial safety. Aseptic processing has been verified. The final product is tested for gram stain, endotoxin and mycoplasma (qPCR) pre-administration; the results of the compendial sterility and mycoplasma release tests are available post-administration. This is justified by the short shelf-life of the product. It should, however, be confirmed that appropriate procedures are in place in case the results of the compendial sterility or mycoplasma test demonstrate contamination of the product (**OC**). The microbial control of the lentiviral vector has been described in sufficient detail. The vector is tested for sterility at release. For the raw materials and components used in the Fanskya manufacturing process, it is stated that they are tested against established specifications to ensure purity, identity, and safety. A question is, however, raised as the testing strategy for raw materials and excipients is currently not sufficiently clear (see S.2.3 and P.4). In addition to this, the applicant is asked to indicate if culture media and buffers are sterile filtered before introduction in the Drug Substance and Drug Product manufacturing process, or justify the absence of such a safety measure (**OC**).

A separate discussion of the risk of contamination with TSE is missing. However, as BSE/TSE statements are provided for the starting material (lentiviral vector, cell banks, plasmids) and relevant raw material no further information is requested.

Viral adventitious agents

The applicant has provided a brief discussion of the virus safety of the autologous cells, the raw materials, the lentiviral vector and the HSA that is used as excipients. The safety testing performed for the autologous material is in line with legal requirements. The information on the raw materials of biological origin used in the production of Fanskya is sufficient. The lentiviral vector used as starting material for the manufacture of Fanskya is produced in cells. Virus safety testing of the cells is in line with expectations. The FBS that is used in the manufacturing of the cell banks and in the production process of the LVV is irradiated with at least 30 kGy and complies with Ph. Eur. monograph 2262.

It is agreed that no viral clearance steps are included in the manufacturing process of Fanskya.

3.1.4.8. GMO

The transgene of Fanskya does not contain sequences capable of complementing the non-replicating lentiviral vector, the molecular characterization does not implicate environmental risks and the possibility of RCL formation with a 3rd generation SIN vector system is considered negligible.

Based on the information provided, the molecular characterization of the vectors used is adequate, and the genes, including the transgene, on the plasmids do not code for sequences that will complement the replication deficient character of the lentiviral vector.

Reference is made to section 3.2.6 of this Overview for further discussion on the ERA in relation to this GMO.

3.1.5. Discussion and conclusions on chemical, pharmaceutical and biological aspects

Lentiviral vector

The provided quality documentation for the lentiviral vector (LV) LV-RP-L102, which is used as starting material for the manufacture of the drug substance, is of reasonable quality. However, several other concerns have been raised which should be addressed.

The proposed commercial manufacturing process and its control is in general sufficiently described, but some further details should be included.

A process performance qualification (PPQ) study was performed at the commercial manufacturing site and scale. Data were supplemented with results of (non-)critical process parameters, in-process controls, and extended characterization testing. The data show that when operated at set-point conditions, the manufacturing process yields a product that complies with the release specifications. Manufacturing process development studies generally support the commercial manufacturing process and the control strategy, however additional details are requested.

The provided characterization performed on the virus vector and the discussion of the test results is considered limited and additional information is requested. Further information on process- and product-related impurities should also be provided.

The specification for LV-RP-L102 lentiviral vector is provided with test attributes, acceptance criteria and method identification numbers. Reference is made to Ph. Eur. monographs for the compendial methods. The specification applies to the release and shelf-life of LV-RP-L102. Tests for infectivity

(particle to infectious viral titre ratio) and clarity should be included in the specification for commercial manufacture. Furthermore, the approach for setting the acceptance criteria is generally not endorsed. This approach should be re-evaluated and acceptance criteria set with tighter limits. The option to set tighter acceptance criteria for stability-indicating attributes at release to ensure that the specification is met throughout the shelf-life should be considered. The information provided on analytical procedures and their validation is in general sufficient, but some questions are raised. Analysis data from additional batches is requested to supplement the currently limited available data.

A one-tier reference standard and protocol for (re)qualification is proposed and generally found acceptable, however additional information and acceptance criteria have been requested.

The container closure system is approvable provided that technical drawings of the closure are provided.

LV-RP-L102 is stored at ≤ -65° C with a proposed shelf life of 30 months. This is currently not endorsed; a shorter shelf life should be proposed or additional stability data to support the claimed shelf life should be provided.

Active Substance and Drug Product

The drug product (DP) is a cell suspension for immediate infusion manufactured for an individual patient containing hematopoietic stem cells transduced with the LV-RP-L102 lentiviral vector encoding human *FANCA* gene as drug substance (DS). The final product is formulated in Plasma-Lyte A with HSA.

The manufacturing process is continuous from Drug Substance to Drug Product and therefore no Drug Substance release specifications are in place. The proposed commercial manufacturing process and its control is in general sufficiently described, but some further detail should be included. In addition, several questions are raised with regard to the quality and/or control of the the lentiviral vector starting material (see above).

A process performance qualification (PPQ) study was performed at the commercial manufacturing site using cells from 2 healthy donors. Data were supplemented with results of CPPs and CQAs from the last clinical batch. The data show that when operated at set-point conditions, the manufacturing process yields a product that complies with the release specifications. The residual amounts of process-related impurities have been justified from a safety point of view; it is in general agreed that no release testing for residual process-related impurities is performed. Product-related impurities, including dead cells, CD34- cells, and non-transduced cells, are indirectly controlled by the Drug Product specifications for cell viability, % CD34+ cells and VCN.

Process development focuses on the batches that were used in the pivotal studies. Information on the comparability of the batches used in supportive clinical studies and pre-clinical studies remains to be provided. The batches used in the pivotal studies were manufactured at different sites and using viral vector manufactured at different sites. There are no obvious differences in tested quality attributes between batches manufactured using vectors from different sites. The difference in MOI used for the manufacturing of these batches gives, however, some reason for concern, because the impact of this difference has not been evaluated in depth and the available data are currently insufficient to conclude that efficacy of the clinical batches is comparable. A Major Objection requesting more in depth evaluation of potential predictors of clinical response and further data to support comparability was therefore requested. The data provided in response to this Major Objection did not reveal major differences between batches manufactured using vector from different sites. Overall, the comparability evaluation is, however, hampered by the very limited data set and no definite conclusion can be

drawn. This uncertainty could be accepted considering the potential benefit of the product to the patient, but the observed differences should be further discussed.

The Drug Product specification includes a panel of release tests that is performed prior to administration of the product to the patient, and a panel of release tests that is performed for final release. This two two-stage release strategy, in which part of the release tests results are not available prior to administration, could be justified by the short shelf-life of the product. The available data suggest that efficacy can be at least partially ensured by testing CD34+ cells/kg prior infusion but the applicant still needs to appropriately address the risks emerging from the proposed control strategy where VCN levels are available after infusion of the DP into the patient (**MO**).

Several questions remain with regard to the proposed Drug Product acceptance criteria. A **MO** is raised as, due to the limited data-set, it is not possible to determine if the acceptance criteria for the release tests that are available prior to administration are sufficiently predictive for safety and efficacy of the product. Considering the clinical efficacy and safety of the product this uncertainty could be accepted to some extent but the applicant should 1) tighten the acceptance criterion for %CD34+ cells, 2) demonstrate that the rapid potency assay and its acceptance criterion can at minimum ensure that the cells have been successfully transduced, and 3) commit to revise the acceptance criteria for the rapid potency assay when additional data are available. In addition, to confirm that clinical efficacy of batches manufactured using the proposed commercial vector is comparable to batches manufactured using vector from a previously used manufacturing site, the applicant should commit to re-evaluate the limit for cell dose when data from additional patients are available and compare clinical outcome of batches manufactured with the different vectors that met the cell dose specification.

The Drug Product is supplied in a CE-marked infusion bag. A shelf life of 42-hour when stored at 2-8°C is proposed based on the available stability data from healthy donor and patient cells.

Taken together, the application is currently not acceptable from a quality point of view. A detailed list of questions that should be addressed by the applicant is included below.

3.2. Non-clinical aspects

3.2.1. Introduction

Fanskya (mozafancogene autotemcel) (herein referred to as RP-L102) is an ex vivo lentiviral (LV) vector gene therapy consisting of autologous HSCs transduced with an LV (PGK-FANCA-WPRE) that encodes for the FANCA gene. It is administered non-cryopreserved (fresh) as a single intravenous infusion.

RP-L102 replicates and expands upon the naturally occurring phenomenon of multilineage mosaicism. By introducing a functional copy of FANCA into autologous HSCs, gene corrected HSCs have a selective advantage over uncorrected HSCs. Hence, these gene corrected HSCs can repopulate the bone marrow (BM) and peripheral blood (PB) without conditioning and lead to hematologic stability. This selective advantage of gene-corrected cells (over uncorrected cells) is what enables RP-L102 engraftment in the absence of conditioning.

The non-clinical studies assessed below are aiming to (1) establish proof of concept for the DP, (2) confirm the utility of several transduction enhancers, and (3) demonstrate safety.

3.2.2. Pharmacology

3.2.2.1. Primary pharmacodynamic studies

IN VITRO

Study No. 1: In vitro Optimization of Clinical LV Construct Through Phenotypic Correction of FA-A LCL cells

LCL cells are lymphoblastic immortalised patient cells that recapitulate the major phenotypic hallmarks of FA, including the reduced proliferation capacity and high sensitivity to DNA crosslinking agents. LCL cells are a widely used cellular model of the disease and mitomycin-C (MMC) survival in patient derived LCL cells and were used as basis for LV Potency Assay prior to release of the LV for clinical use.

Several LV constructs expressing FANCA under the control of different promoters (VAV, PGK, CMV and SFFV), and post-transcriptional regulatory elements (WPRE or mutated WPRE* lacking any residual open reading frames) were developed to determine the best clinical lentiviral vector for treatment. Enhanced Fluorescent Green Protein (EGFP)-expressing LVs were utilized as control vectors. FA-A patient-derived lymphoblastoid cell line (LCL) cells were transduced (in pre-coated with 2 µg/cm2 of RetroNectin (Casado 2007)) with these different vectors. Resulting cells were evaluated for FANCA mRNA expression, FANCA protein expression, mono-ubiquitination of FANCD2 (as FANCA protein is crucial for FANCD2-FANCI heterodimer mono-ubiquitination after DNA damage), and phenotypic correction (evaluated by MMC resistance and formation of nuclear FANCD2 foci) and compared to healthy donor (HD) LCL cells.

mRNA and protein expression

FANCA mRNA and protein level in HD LCL cells was set at 1 as reference unit of quantification resulting in a baseline of 0.5 mRNA and protein per copy.

- FA-A LCL cells transduced with VAV-FANCA-WPRE LV and PGK-FANCA LV yielded comparable physiological levels of FANCA mRNA/copy to HD LCLs (i.e., 0.57 ± 0.20 and 0.54 ± 0.19, respectively).
- PGK-FANCA LVs harbouring the WPRE or the mutated WPRE* sequences had increased FANCA mRNA/copy levels (i.e., 1.24 ± 0.60 and 1.43 ± 0.41, respectively).
- FA-A LCL cells transduced with CMV-FANCA and SFFV-FANCA LVs resulted in supra-physiological levels of FANCA mRNA/copy compared to HD LCL cells (i.e., a 3.6-fold and 5.6-fold increase, respectively).

Evaluating FANCA protein level per copy, CMV-FANCA, VAV-FANCA and all PGK-FANCA LVs (with and without WPRE or mutated WPRE*) achieved approximately normal physiological levels of FANCA/copy (i.e., 0.53 to 0.59) like the FANCA/copy in HD-LCL cells (0.50). In contrast, SFFV-FANCA LV yielded supraphysiological levels of protein at 1.7 FANCA/copy.

FANCA mediated FANCD2 mono ubiquitination

FA-A LCL cells transduced with the control vector (EGFP LV) were not able to monoubiquitinate FANCD2. FA-A LCL cells transduced with PGK-FANCA LV or PGK-FANCA-WPRE LV expressed both the non-ubiquitinated as well as the monoubiquitinated forms of FANCD2.

Formation of nuclear FANCD2 foci and MMC resistance

All vectors containing the FANCA gene (coupled to GFP) showed nuclear FANCD2 foci and appeared MMC resistant, which are considered characteristics for phenotypic correction.

IN VIVO

Study No. 2: Transduction and Phenotypic Correction of Fanca-/- HSCs with PGK-FANCA-WPRE LV and Engraftment in Fanca-/- Mice

In experiment No. 2 a homologous model was used in which Fanca -/- mice were transplanted with Lin- bone marrow (BM) cells (also referred to as HSCs) which were transduced with FANCA LV prior to transplantation. In the *in vitro* experiment L1, Lin- BM cells from Fanca-/- mice were transduced with PGK-FANCA-WPRE LV, plated, and incubated for 7 days and compared with Lin- BM cells transduced with EGFP-RV (negative control) or untransduced Lin- BM cells regarding VCN and MMC sensitivity. Average VCN for this clinical LV (in design, as the batch that was used in this experiment was a pre-GMP batch for which no quality data has been provided) was 1.2 LV copies/cell. Notably, Lin- BM cells modified with PGK-FANCA-WPRE LV showed resistance to MMC, in contrast to Lin- BM cells transduced with EGFP-RV and untransduced Lin- BM cells which remained hypersensitive to MMC.

Four *in vivo* experiments (L2-L5) were conducted. Mice were 8-12 weeks of age at start of the experiment. Lin- cells from the Fanca -/- mice were transduced 14 hr with FANCA LV or 48 hr prestimulated and 2x 14 hr transduced (with a 12-hr interval) with eGFP RV. 3-5x10^5 cells/recipient were given to radiated (5Gy) Fanca -/- mice in a sex mismatched fashion and followed for 6 months. Forty-seven (n=47) Fanca-/- mice primary recipients received cells transduced with the clinical vector, and four (n=4) received the EGFP-RV transduced cells. Additionally, two (n=2) animals were transplanted with wild-type cells and three (n=3) received untransduced cells, serving as positive and negative controls, respectively.

In experiment L3-L5, this primary transplantation was followed by a secondary transplantation with a 6-month observation period. 107 BM cells from primary recipients were re-transplanted into secondary female Fanca-/- recipients. Thirty-one (n=31) Fanca-/- secondary recipients were transplanted with BM cells modified with the clinical vector and two (n=2) were transplanted with BM cells transduced with EGFP-RV. Additionally, two (n=2) mice were transplanted with wild-type cells and one (n=1) with untransduced cells serving as positive and negative controls, respectively.

Donor engraftment was above 60% in primary transplant recipients and above 30% in secondary transplant recipients, with VCN ranging from approximately 0.5-4. It has to be noted that engraftment and VCN are different for the three separate experiments for both the primary and the secondary transplantation. The MMC resistance for the FANCA LV treated animals appeared to be similar to wild-type, although at highest tested MMC concentration a slight decrease in MMC resistance in comparison to wild-type cells was observed.

Study No. 3: Phenotypic Correction of FA-A Patient-Derived HSCs with PGK-FANCA-WPRE and Engraftment with in vivo Selective Advantage in NSG Mice

In study No. 3 a heterologous model was used in which gene-modified FA-A human CD34+ HSCs could successfully engraft in NSG mice.

Cells

The FA-A cells were enriched CD34+ cells from FA-A patients recruited in the Phase 1/2 clinical trial FANCOSTEM (NCT02931071) and obtained either from BM aspirates or from small aliquots of mPB (i.e. BM cells mobilized to the blood compartment after G-CSF and plerixafor treatment). The CD34+ HSCs were transduced with PGK-FANCA-WPRE LV and administered to non-obese diabetic severe combined immunodeficiency- Il2rg2/2 or NOD SCID Gamma (NSG) mice.

Optimal vector

This study first tested various LVs on FA-A patient-derived HSCs to confirm PGK-FANCA-WPRE LV as the optimal lentiviral construct for follow-on studies. MMC resistance assays were conducted to show reversion of MMC sensitivity. The percent of CD34+ CFCs resistant to MMC was 51.3% when transduced with PGK-FANCA-WPRE* LV; 56.2% when transduced with PGK-FANCA LV; and 71.0% when transduced with PGK-FANCA-WPRE LV. In comparison, when transduced with the negative control (PGK-EGFP LV), the percent of CFCs resistant to 10 nM MMC was 1.2%. Given the relative comparable results with the different PGK-FANCA vectors, and the potential safety considerations conferred by the mutated WPRE*, the results from this study reaffirmed the use of PGK-FANCA-WPRE* LV (referred to as PGK-FANCA-WPRE in this assessment comment) as the clinical LV.

In vitro

Bone Marrow aspirate CD34+ cells from 3 FA-A patients were transduced with either PGK-FANCA-WPRE LV or a control (PGK-EGFP-WPRE LV) in a single-hit 24-hour transduction sequence. This sequence involved pre-stimulation of FA-A CD34+ cells for 8-10 hours in media enhanced with N-acetylcysteine and Etanercept (anti-TNF antibody) under hypoxic conditions (5% O2) prior to transduction. Following pre-stimulation, cells were transduced for 12-14 hours in the presence of protamine sulphate. The transduced FA-A CD34+ cells were then tested for survival when exposed to 10 nM MMC. Higher CFC survival rates were observed after transduction of FA-A CD34+ cells with the clinical vector (pre-GMP batch) as compared with the control eGFP vector (mean values, 48.37±1.70% vs 9.93±5.34%, respectively; P = 0.018).

G-CSF-Plerixafor mobilized Peripheral blood (G/P mPB) CD34+ cells from 4 additional FA-A patients were subjected to pre-stimulation and transduction as described above. When the gene-modified cells were exposed to 10 nM MMC, the clinical vector (pre-GMP batch) conferred a significant increase in the survival of CFCs as compared with the control vector (PGK-EGFP-WPRE LV) (33.65±5.21% vs 1.03±0.84%, respectively; P = 0.017).

In vivo

Last, to ensure that it was feasible for transduced FA-A HSCs to engraft, demonstrate phenotypic correction, and yield multilineage hematopoietic repopulation, transplantation studies were conducted. Patient derived mPB derived cells from 4 FA-A patients were transduced with FANCA-LV and transplanted into 9 1.5 Gy irradiated NSG mice and qRT-PCR (for vector copy number; VCN), CFC counts and MMC resistance, and immunolabeling assays were conducted on femoral BM cells harvested 4 – 12 weeks after infusion. HSCs from a healthy donor (HD) transplanted into 4 NSG mice served as the control. Two types of administration were used: intravenous and intrabone administration, which gave comparable results. This analysis revealed that NSG mice transplanted with gene-modified FA-A CD34+ cells harboured a moderate but reproducible engraftment of human hematopoietic cells (hCD45+), including differentiation into myeloid, lymphoid, and persistent CD34+ lineages. However, for two mPB donors there were no persistent CD34+ cells found after CFC. In addition, for the other two mPB donors there were no persistent CD34+ cells found with the intrabone administration.

For mPB derived material from 2 patients, it was shown that the number of CFCs present in the CD34+ cells are less resistant to MMC when compared to the amount MMC-resistant progenitors cultured from the BM samples taken 4 weeks post-transplantation. It was concluded by the applicant that when gene-corrected FA-A HSCs are transplanted, they have an *in vivo* selective advantage over uncorrected cells. The applicant argues that due to the presence of a functional DNA repair mechanism, the corrected cells can proliferate more successfully than uncorrected cells and over time lead to

multilineage hematopoietic repopulation. This seems a plausible mechanism but requires efficient transduction of LT-HSC CD34+ cells.

3.2.2.2. Secondary pharmacodynamic studies

Study No. 4: Optimization of Transduction with transduction enhancers

Of note: The applicant submitted this study as part of the secondary pharmacology and also place under this heading in the reports, but it is considered a primary pharmacology study.

The applicant set out to test whether the use of transduction enhancers (TEs) could increase the transduction efficiency and chose several candidates. The selected TE candidates were tested for the most optimal condition (concentration) alone and in combination. To establish the protocol, the applicant used three different vectors, but none of them was the clinical-designed vector. Only for the proof of concept in FA-A patient cells, the clinical designed vector was used.

3.2.2.3. Safety pharmacology programme

Safety pharmacology is not addressed in dedicated studies.

3.2.2.4. Pharmacodynamic drug interactions

Studies addressing pharmacological interactions were not conducted.

3.2.3. Pharmacokinetics

The applicant has not conducted conventional ADME studies. Considering the type of product, this is acceptable.

A combined non-GLP single dose biodistribution and toxicity study was conducted. Wild type (FVB strain) Lin- bone marrow cells were harvested from male mice, pre-stimulated and subsequently transduced with a pre-GMP LV batch (MoI = 200). No transduction enhancers were used in this protocol. Following preconditioning with irradiation, males and females received $2x10^5$ cells intravenously. The control group received $2x10^5$ pre-stimulated, untransduced male Lin- bone marrow cells. Peripheral blood samples were collected at 9 days, 1, 2 and 4 months for cell counts and chemistry. At termination 4 months post-transplantation, several tissues were harvested for macroscopic evaluation (see toxicity study part), VCN determination (i.e. limited to bone marrow, brain and gonads) and FANCA transgene expression (bone marrow only).

The dose used in mice (i.e. $2x10^5$ cells per around 30 gram mouse) would result in a Human Equivalent Dose (HED) of about $6.7x10^6$ cells/kg. Considering that the clinical dose ranged between $4x10^5$ and $4.1x10^6$ cells/kg (according to SmPC section 4.2), the dose used in the non-clinical study exceeded the maximum dose used in clinical studies, as per ICH S12.

In their BD/single-dose toxicity study, the applicant used a qPCR to detect vector DNA in bone marrow, gonads and brain at 4 months post-transplantation. Vector DNA was only detected in bone marrow from both males and females infused with transduced cells (VCN range in males = 0.1-1.1, in females = 0.2-0.8; in comparison, VCNs in patient PBMCs from all clinical trials ranged from 0.022 to 2.823). Using qRT-PCR, mRNA expression of the human FANCA transgene was evaluated in bone marrow (without preselection on specific cell types/lineages) and observed in both males and females from the study group at 4 months post-transplantation, although only relative transgene levels were provided. As such, it remains unclear whether clinically relevant (or potential overexpression) levels of FANCA

were obtained in the bone marrow. FANCA expression was not determined in (other) target and non-target tissue, and long-term/stable FANCA expression in HSPC-derived cells (e.g. in blood) was not evaluated. Clinical improvement of patients, however, could be an indirect sign of sufficient FANCA expression.

There was no clear relationship between vector DNA and transgene mRNA expression in bone marrow. Whether there would be a relationship between vector DNA (VCN) and transgene protein could not be determined, as no data on protein expression was available in this study.

3.2.4. Toxicology

3.2.4.1. Single dose toxicity

A combined non-GLP single dose biodistribution and toxicity study was conducted. Wild type (FVB strain) Lin- bone marrow cells were harvested from male mice, pre-stimulated and subsequently transduced with a pre-GMP LV batch (MoI = 200). No transduction enhancers were used in this protocol. Following preconditioning with irradiation, males and females received $2x10^5$ cells intravenously (with a safety margin of about 2x compared to cell/kg in patients). The control group received $2x10^5$ pre-stimulated, untransduced male Lin- bone marrow cells. Peripheral blood samples were collected at 9 days, 1, 2 and 4 months for cell counts and chemistry. At termination 4 months post-transplantation, several tissues were harvested for macroscopic evaluation, VCN determination and FANCA transgene expression (see biodistribution study part).

No significant treatment-related clinical observations (physical or behavioural) or body weight changes were found. Treatment with the LV-transduced bone marrow cells did not result in relevant changes in ALT, urea, glucose and amylase in serum (no other parameters were evaluated). Peripheral red and white blood cells counts temporarily decreased in both study and control groups to the same degree, as a consequence of the preconditioning regimen. This regimen also resulted in mortality in both groups, although the raw data tables in the study report were inconsistent regarding the number of animals that died prematurely.

3.2.4.2. Repeat dose toxicity

The applicant has not conducted repeat-dose toxicity studies. This can be agreed, as Fanskya is intended as single dose treatment in patients.

3.2.4.3. Genotoxicity

See section 3.2.4.4.

3.2.4.4. Carcinogenicity

The applicant has conducted a non-GLP tumorigenicity study in mice to analyse the integration profile of the vector and any signs of vector-mediated insertional mutagenesis (i.e. malignant clonal populations). $Fanca^{-/-}$ Lin- bone marrow cells were harvested from male mice and transduced with a pre-GMP LV batch (MoI = 20) or transduced with a positive control (gamma-RV with potent SFFV LTR promoter). Only one LV batch was investigated and transduction enhancers were not used in the current transduction protocol. Following preconditioning with irradiation, $Fanca^{-/-}$ females received 3-$5x10^5$ cells intravenously. Six months after transplantation, BM cells were isolated and $1x10^7$ cells were transplanted into irradiated secondary $Fanca^{-/-}$ female recipient mice for another 6 months. Peripheral blood plasma (collected at 1, 3 and 6 months post-transplantation) and 6-months bone marrow from

both primary (n=31) and secondary (n=24) recipients were subjected to genome-wide screening of insertion sites and clonal dynamics analysis. In addition, red and white blood cells counts and the growth of potential solid tumours was monitored.

In primary recipients of LV-transduced cells, the applicant observed 1.60 to 2.24 mean VCN per cell and around 70% engraftment over time in peripheral blood, and 1.79 copies and around 52% engraftment in bone marrow. In secondary recipients, mean VCN per cell was 1.67 to 2.29 copies and > 41% engraftment over time in peripheral blood, and 2.87 copies and around 26% engraftment in bone marrow. Mean VCN values for RV-transduced cells (only available for primary recipients) were generally lower than for LV-transduced cells, while engraftment was comparable or higher with cells transduced with RV. The applicant has not determined VCN in different haematopoietic lineages. Moreover, transgene expression was not evaluated.

All animals survived until scheduled termination. In contrast, several wild type animals in study no. 5 died prematurely due to the preconditioning regimen. Considering that FANCA$^{-/-}$ individuals are more sensitive to the effects of chemotherapy/irradiation, this is a remarkable observation. Whether this is due to differences in the irradiation protocol, transduction protocol or something else is not clear. Moreover, the VCN values in the current study are considerably higher than those observed in the wild type animals (study no. 5). This was not further discussed by the applicant, but this is not considered essential to assess the current study.

Over the 6 months interval, blood cell counts remained fairly stable. There were no signs of product-derived dysplasia or leukaemia in any of the animals (product group and positive control groups). Two solid tumours were observed, but of female origin and negative for the LV pro-virus, and as such not related to the LV-transduced HSPC treatment, as described by the applicant. There were no tumours in mice treated with the positive control retroviral vector. This could be related to the lower number of mice investigated (n=17). Actual (individual) tumorigenicity data and additional testing results of the solid tumours have not been provided in the study report.

Using a more or less standard approach with LAM-PCR and 454 pyrosequencing, the applicant screened for insertion sites in the HSPCs before transplantation and in peripheral blood and bone marrow from the primary and secondary recipients. For the LV (pre-transplant and post-transplant), many insertion sites were found, but only around 5-6% in regions close to transcription start sites, in contrast to the post-transplant insertions related to the gamma-RV (less insertions, but > 27% near transcription start sites). LV-related insertions were not enriched in specific chromosomes, nor accumulated to a large extent in common insertions sites (CIS). The applicant compared genes possibly influenced by insertion in CIS with cancer gene databases, but insertions that could be considered the most dangerous did not reach considerable contributions of the total validated reads, although the relevance of the used databases could not be assessed.

The applicant also investigated the functional categories of the genes in which the LV was inserted nearby (< 50 kb) via ingenuity pathway analysis. The results indicated that LV preferentially integrated in recombination & repair genes and haematopoiesis genes, but this preference was not a consequence of the specific FA pathway defect in Fanca$^{-/-}$ cells.

High clonal diversity was observed with LV-transduced cells over time in both primary and secondary recipients, in agreement with literature on LV integration. There were no dominant clones observed in animals treated with LV-transduced cells. Transduction with gamma-RV also induced clonal diversity, but to a considerably lesser extent than with LV (i.e. lower number of different clones, oligoclonal). Moreover, several clones were observed in both primary and secondary recipients, indicating progression of dominant clones and thus the oncogenic potential of the insertion sites from the gamma-RV, even in a Fanca$^{-/-}$ background.

Thus far, 16 patients have been monitored by ISA up to 84 months post-transplantation (see Clinical section).

3.2.4.5. Reproductive and developmental toxicity

The applicant has not conducted dedicated juvenile or reproductive toxicity studies, nor provided a justification for the absence of these studies. As cells are transduced *ex vivo* with a replication-incompetent LV, the risk for transduction of gametes (and thereby the risk for germline transmission) is likely very limited. This is also stated in the *Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors* (EMEA/273974/2005), indicating that the risk of germline transmission associated with the administration of genetically modified human cells may be considered low and difficult to address in non-clinical germline transmission studies.

3.2.4.6. Toxicokinetic data

Not applicable.

3.2.4.7. Tolerance

No dedicated local tolerance study was conducted. Considering that the product is injected intravenously, the absence of a dedicated local tolerance study can be agreed. It is, however, unfortunate that the applicant has not specifically evaluated the site of injection in the single dose toxicity study.

3.2.4.8. Other toxicity studies

The applicant has conducted a non-GLP compliant RCL detection study in mice. Fanca$^{-/-}$ Lin- bone marrow cells were harvested from male mice and transduced with a pre-GMP LV batch (MoI = 20). Following preconditioning with irradiation, Fanca$^{-/-}$ females received 3-5x10^5 cells intravenously. Six months after transplantation, BM cells were isolated and 1x10^7 cells were transplanted into irradiated secondary Fanca$^{-/-}$ female recipient mice. Following another 6 months, peripheral blood plasma was analysed for RCL by ELISA (p24 HIV protein quantification). Non-transplanted Fanca$^{-/-}$ female mice were used as control. The applicant found an average VCN in bone marrow cells of 3.31 (range = 2.22-7.24) and 58% engraftment (range = 19.14-87.51%) in the secondary recipients. There was no correlation between VCN and engraftment.

Plasma from the secondary recipients (n=8) were all negative for p24 HIV antigen, while the p24 antigen control resulted in a positive outcome in the ELISA. Based on these results, the risk for RCL production in Fanca$^{-/-}$ mice seems to be low. However, it appears that a different transduction protocol (without the addition of transduction enhancers) has been used for the current study when compared to the clinical manufacturing process. As such, the relevance of the study data for the clinic is doubtful. Considering that in the Quality dossier, a validated qPCR method is available to determine the presence (or absence) of RCL in end of production cells and in the final product, the current ELISA data from murine serum are therefore only considered supportive for the absence of RCL. Moreover, clinical RCL testing is has been negative so far (23 subjects).

Dedicated antigenicity and immunotoxicity studies have not been performed, which can be agreed. But the applicant did also not address immunogenicity of the transgene, i.e. whether antibodies against FANCA were detected in FANCA$^{-/-}$ mice following transplantation with the genetically modified HSPCs. Moreover, transgene expression was only analysed in bone marrow for 4 months following transplantation. It is therefore not clear whether stable, long-term expression of the FANCA protein

was present in these animals, which would suggest the absence of a neutralising immune response against the transduced cells and transgene product. Moreover, the applicant did not evaluate the potential induction of antibodies against Gag p24, which would indicate the presence of viral particles on the surface of genetically modified cells. Nevertheless, cellular immunogenicity was tested in 43 PBMC samples (from 11 patients) in the clinical study. No cellular immune responses against the FANCA transgene and LV-associated proteins (VSV-G an HIV-1 Gag p24) were observed, although testing in only 10 samples was reported. In addition, no humoral immunogenicity against the FANCA transgene in 41 serum samples was observed. This is described in the Clinical studies meta-analysis report and assessed in the Clinical AR.

3.2.5. Ecotoxicity/environmental risk assessment

The transgene of Fanskya does not contain sequences capable of complementing the non-replicating lentiviral vector, the molecular characterization does not implicate environmental risks and the possibility of RCL formation with a 3rd generation SIN vector system is considered negligible.

Based on the information provided in the Quality part of the dossier, the molecular characterization of the vectors used is adequate, and the genes, including the transgene, on the plasmids do not code for sequences that will complement the replication deficient character of the lentiviral vector.

3.2.6. Discussion on non-clinical aspects

Pharmacodynamics

In vitro

Study No. 1: In vitro Optimization of Clinical LV Construct Through Phenotypic Correction of FA-A LCL cells

LCL cells are patient derived and recapitulate the major phenotypic hallmarks of FA, including the reduced proliferation capacity and high sensitivity to DNA crosslinking agents. This widely used cellular model of the disease and mitomycin-C (MMC) survival were used as basis for LV Potency Assay prior to release of the LV for clinical use, which is considered acceptable.

PGK-FANCA-WPRE* LV was chosen as clinical vector for efficacy and safety reasons. The vector efficiently transduced FA-A LCL cells, achieving supraphysiological levels of mRNA/ copy, retained FANCD2 mono-ubiquitination capacity and yielded phenotypic correction as measured by reversion of MMC hypersensitivity and restoration of nuclear FANCD2 foci formation. In addition, the mutated WPRE* is preferable for safety reasons. The chosen vector will be referred to as PGK-FANCA-WPRE LV. The approach for selecting the clinical vector can be supported and the choice can be endorsed. However, it should be noted that the transduction protocol was not specified and can therefore not be compared to clinical data.

Study No. 2: Transduction and Phenotypic Correction of Fanca-/- HSCs with PGK-FANCA-WPRE LV and Engraftment in Fanca-/- Mice

Although not developing bone marrow failure (BMF) or malignancy, the Fanca-/- model is considered representative of the FA-A clinical phenotype, as their bone marrow progenitors are sensitive to DNA cross-linking agents, including MMC, and in this respect resemble the hematopoietic phenotype of FA-A patients. This approach can therefore be endorsed.

Experiment L1 showed that transduction of Lin- BM cells from Fanca-/- mice with PGK-FANCA-WPRE LV VCN for this clinical LV (pre GMP batch) resulted in 1.2 LV copies/cell resulting in resistance to MMC. In experiment L2-L5 it was shown that primary transplantation with Fanca -/- Lin- BM cells transduced

with PGK-FANCA-WPRE LV resulted in engraftment of these cells (variable percentage) with a VCN around 1 (variable level).

Donor engraftment was above 60% in primary transplant recipients and above 30% in secondary transplant recipients, with VCN ranging from approximately 0.5-4. The results (% of donor engraftment and VCN) from L2-L5 were separately shown and seem to be different for all experiments, especially when focussing on the development of engraftment and VCN over time (1-, 3- and 6-months post-treatment). These differences are not further explained by the applicant. Moreover, the description of the four in vivo experiments is not sufficiently clear to determine potential differences in the cell transduction protocol and/or transplantation, which may have caused the engraftment and VCN differences. Furthermore, the secondary transplantation seems less effective than the primary transplantation with regard to engraftment (30% and 60% respectively), which could suggest a lower transduction in long-term engrafting cells. The issue of transduction efficiency in long-term engrafting cells is brought up in the comments to study No. 3 below.

The applicant was asked to discuss the data of experiment L2, L3, L4 and L5 and specifically the variability in the development of the donor engraftment percentages and the VCN, also including the variability over time, and the potential explanations for these differences. The applicant explained that observed variability for vector copy number (VCN) and percentage of donor engraftment is considered within the normal variable range for *in vivo* transplant experiments. However, this normal range is not supported with data or literature reference. Furthermore, VCN after primary transplant shows a comparable trend between L3, 4 and 5 (although slightly decreased in L5), while the trend for VCN is different after secondary transplantation for these three experiments. This suggests that the LT-HSCs are transduced with variable efficiency in the three experiments.

Although falling in normal range, the applicant seems to recognize that there is some variability and provided several reasons for that: 1) different animals were utilized in each experiment at a given time to pool Lin- BM cells from male Fanca-/- mice for transduction, 2) cells were transduced at different times (likely different moments in time) L2, L3, L4 and L5 groups yielding different rates of transduction, 3) animals were subjected to sub-myeloablative conditioning for BM depletion to create space for donor cells in the recipient's BM (even if the same amount of irradiation is used for all animals, the effect of irradiation may vary between recipients, which will affect the space for recipient cells to engraft), 4) not in all mice, the engraftment may take place the same way which could be reflected in variety in 1,3, and 6 months according to the applicant, 5) the number of BM cells in PB was poor and the extraction, depending on the animal conditions (such as body weight, size or age) might affect the amount of PB obtained for analysis, which will directly impact the % of engraftment observed and a variability of 20 to 30% difference between animals is usual, considering the detection by PCR (NSR 2), 6) the dose of transduced cells given to the animals were in a range of 3-5x10^5 cells/recipient. Some of the reasons can be understood but none of them were substantiated with data. However, the applicant still wants to convey the message that all of the experiments and timepoints analysed showed stable and consistent donor engraftment, generally above 60% in primary recipients and 30% in secondary recipients, with cells harbouring 0.5-4 copies of the PGK-FANCA-WPRE LV. It is concluded that LV-transduction and engraftment of the total of murine BM cells in recipient mice is shown, but it is not clear to which extent specifically LT-HSCs were efficiently transduced in all three experim

nts, while that is particularly important for this therapy.

As the information on the cell manufacture and transplantation protocol and materials was not specified for each of the four experiments separately, the applicant was also asked to provide all particulars of the four separate experiments. The applicant noted that: *CIEMAT research laboratory*

developed certain experimental techniques, i.e., hematopoietic stem cell (HSC) transplantation in FA animal models (Rio 2002 and Molina-Estevez 2015) that were implemented during the conduct of these experiments. At the time of these studies, CIEMAT did not have a specific written protocol for FANCA-/- mouse BM harvest and transplantation in its Quality management system. Study conduct was documented in laboratory notebooks. Each study report includes a Materials and Methods section describing the methodology and study design, including the test system, animal models, control and test articles, dose administered, procedures for transduction and transplantation, equipment, materials used, and data collection. The question was raised as trends in data were different for experiment L2,3,4 and 5 and the description of the material and methods section was very general and suggest that all experiments have been conducted exactly the same way, but providing varying results, e.g showing different trends. The provided information above does not provide an answer to this question. Furthermore, it is not clear now, whether observed variability might also be due to change in protocols.

Upon requesting details on the protocol for bone marrow harvest and re-transplantation, the applicant explained that for secondary transplants, primary recipients are euthanized 6-months after transplant and BM cells are collected. Then, cells are pooled, counted and transplanted (10E+07 cells/mouse which was initially written as 107 BM) into secondary recipients. Secondary transplantation is a stressful process for long- and short-term hematopoietic stem cells. The provided information on the protocol is fairly limited and it is not clear whether the argument of the stressfulness of the secondary transplantation fully explains the drop of >60% engraftment to >30% engraftment in experiment L3 or whether in that experiment specifically the LT-HSCs were not efficiently transduced / engrafted.

Overall, it is noted that the applicant did not provide new information with regard to the material and methods for experiment L2-L5. The observed differences were explained as in normal ranges, not meaning anything particular. The applicant further explained that potential differences could be due to different doses of the cells and other factors e.g. y-irradiation and engraftment that might both be slightly different per mice. As the dose per mouse has not been provided, a relation could not be determined. Experiments L2-L5 show transduction of Fanca-/- Lin- HSCs with PGK-FANCA-WPRE LV and subsequential engraftment of these cells in Fanca-/- mice, which is more successful upon primary compared to secondary transplantation. However, as details are lacking on material and methods and statistics it can only be concluded that the concept is proven, but efficiency of the process remains unclear. The unclarity about exact LT-HSC transduction efficiency and engraftment will directly influence extent of clinical efficacy and as such, lack of information should be dealt with in the light of the benefit / risk of the treatment and the unmet clinical need. Therefore, the issue is not further pursued from non-clinical perspective.

Study No. 3: Phenotypic Correction of FA-A Patient-Derived HSCs with PGK-FANCA-WPRE and Engraftment with in vivo Selective Advantage in NSG Mice

BM or mPB derived donor cells

The applicant concludes that: transduction efficiency achieved in HSCs collected via peripheral blood mobilization and apheresis is comparable to the transduction efficiency achieved in HSCs collected via bone marrow aspiration. The higher morbidity risk and cost associated with bone marrow aspiration should be taken into consideration. This is not completely agreed as it seems that for the first three donors (BM-CD34+) a lower percentage of CFCs form colonies on the plates, whereas a higher percentage of these cells are MMC resistant. For the other four donors, higher percentage of (mPB-CD34+) cells is forming colonies, whereas a lower percentage of these cells seem MMC resistant. It is not clear whether the source of CD34+ matters for the transduction efficiency in long-term engrafting cells (LT-HSCs) as transduced BM aspirated CD34+ cells and transduced mobilized PB derived CD34+

cells seem to differ in engraftment and MMC resistance. The applicant was asked to explain / provide more information on this difference, also considering the above distinction between early and late progenitors and elaborate on potential clinical consequences. This apparently resulted in a higher average number of colonies obtained in CFC assay using CD34+ cells from mPB, which was significantly higher (77.06 ± 30.87, mean value and standard error of the mean) compared to BM-derived cells (12.22 ± 1.84). Thus, the choice for mPB-derived CD34+ cells as source for the transduction and transplantation is rather based on clinical experience, which can be followed.

Although the applicant agrees that survival percentage in 10 nM MMC seems to be higher in CFC obtained from BM-derived CD34+ cells (48.37 ± 1.70%) compared to the mPB-derived ones (33.65 ± 5.21%), an unpaired t-test analysis of these 2 groups revealed that there are no statistically significant differences between these survival rates and the applicant considered chance on efficacious treatment upon therapy equal for BM- and mPB-derived CD34+ cells, probably due to the high variation in MMC survival of mPB-derived samples. The reason for choosing mPB- above BM-derived CD34+ cells is mainly based on clinical arguments, which can be agreed.

Relevance of the MMC resistance test

The applicant used MMC resistance as efficacy parameter, however, this was considered quite indirect. And as it was applied on CFC cultured cells, it might also have displayed the MMC resistance of only a subset of CD34+ cells. Relationship between MMC resistance and VCN of all cells was depicted in a graph. But VCN was also calculated for CFC positive cells. The applicant was asked to also show the relationship of MMC resistance with VCN of CFC positive cells only and explain the differences taking into c

nsideration the possibility that CFC culturing selects for a certain type of CD34+ cells. In addition, the applicant was asked to justify the use of MMC resistance as main efficacy parameter, and not providing information on VCN and FANCA expression in most of the studies.

In the response, the applicant explained that cell material from FA patients is rather scarce and limits conduct of the number of tests. Preference for the clonogenic assay with the MMC test as read-out for presence of the FANCA protein and its activity, is acknowledged. As in a CFU assay 100-400 cells per plate are seeded, establishment of many different conditions with the appropriate number of replicates is possible. The relation between VCN and MMC resistance was shown and supports the use of the MMC test only for non-clinical purposes. Depicted clinical data suggests that the effect of the therapy (MMC resistance) may start generally late after transplantation and might indicate that only a low number of transduced LT-HSCs are given to patients that will only slowly multiply / engraft due to their advantage in survival compared to the stem cells already present in the BM (as no myeloablation is applied in patients). However, it is regarded important that from the start all /most LT-HSC present in the patient material should be efficiently transduced to give the patient best chances to respond positively to therapy. However, efficacy of the therapy should be based on assessment of the clinical data.

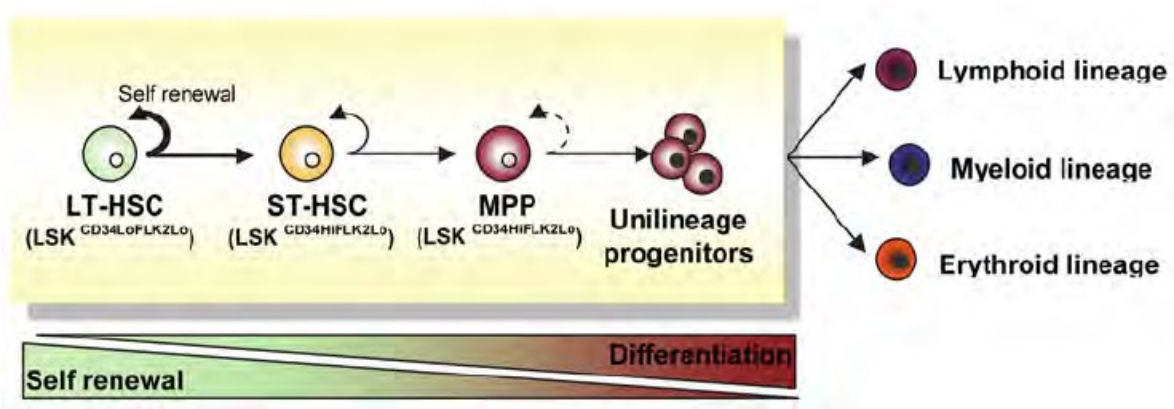
Upon request the applicant provided also the data on correlation between MMC and VCN in CFC positive cells, but strange enough with a lower correlation (lower R), which would suggest that in cells with proliferating capacity in the CFU assay (ST-HSC and may be LT-HSC) the correlation between MMC survival and VCN in positive CFU is not as clear as in CFU overall. However, the VCN of these cells seems higher. The applicant explains these differences as expected and due to the selective pressure of the MMC and comments that: *'presence of MMC only allows for the growth of those colonies with a higher transduction efficacy (gene-corrected cells)'.* This viewpoint is understood.

The applicant claims that this MMC assay can provide information on FANCA transduction/ correction even when material is too limited for mRNA or protein expression levels. Clinical evaluation of the usefulness of the MMC assay can be found in the clinical section.

Long and short term progenitor cells and MPPs.

The CD34+ cell population taken from the patient will likely exist of various types of HSCs. At least a distinction between early and late progenitor cells or otherwise a distinction between Long-Term HSCs (LT-HSC), Short-term HSC (ST-HSC), multipotent progenitor (MPP) cells and uni-lineage progenitors can be made. The LT-HSCs, also referred to as early progenitors, have self-renewal capacity and will provide hematopoietic progeny 16 weeks post transplantation in mice. The second and the third type of HSCs, which could be considered to be the late progenitors) will provide hematopoietic progeny 0-16 weeks post transplantation with the peak between 8 and 16 weeks, while the uni-lineage progenitors will provide hematopoietic progeny the first 8 weeks after transplantation (See figure below). Therefore, the LT-HSCs are regarded the most relevant cells to transduce for this therapy as they will ensure long term repopulation of the bone marrow.

Figure 1. Type of CD34+ cells



Reference: Herrera-Merchan, Antonio, Isabel Hidalgo, Lorena Arranz and Susana González. "Insights Into Stem Cell Aging." (2012).

For mPB derived material from 2 patients, it was shown that the number of CFCs present in the CD34+ cells are less resistant to MMC when compared to the number of MMC-resistant progenitors cultured from the BM samples taken 4 weeks post-transplantation. The study report concludes that PGK-FANCA-WPRE LV induces phenotypic correction of human FA-A HSCs, with the resulting gene-modified cells capable of engraftment, multilineage repopulation, and *in vivo* selective advantage in NSG mice. However, this study was only four weeks long and it is therefore unclear if these results would be valid long term. Recipient mouse was conditioned. It was concluded by the applicant that when gene-corrected FA-A HSCs are transplanted, they have an in vivo selective advantage over uncorrected cells. The applicant argues that due to the presence of a functional DNA repair mechanism, the corrected cells can proliferate more successfully than uncorrected cells and over time lead to multilineage hematopoietic repopulation. This seems a plausible mechanism but requires efficient transduction of LT-HSC CD34+ cells. The applicant showed that transduction of FA-A patient HSPCs with FANCA-LV increased their capability to resist MMC, that this was the case for both BM and G/P mPB retrieved HSPCs and that this resistance capability was increased 1-month post-transplantation in mice. However, it is not clear which CD34+ progenitor cell population was subjected to the analysis and what we can conclude from this experiment. A distinction between early (LT-HSC) and late(r) progenitors (ST-HSC /MPP) in the CD34+ cell population was not made by the applicant in their studies.

Usually LT-HSCs are in vitro cultured via the LTC-IC culturing method (Liu et al., 2013 ***), which takes much longer than the applied CFC culturing of 14 days. It was not clear which CD34+ cell population(s) were selected when using the CFC culturing method of 14 days (named CFC positive cells by the applicant), and how this influenced the interpretation of transduction efficiency, MMC resistance

and the expected endurance of the effect in study No. 2, No. 3, and No. 4. Therefore, the applicant was asked to submit, when available, information on the efficiency of transduction (VCN, FANCA expression levels, MMC resistance) of early progenitors (LT-HSCs) in study No. 2, No. 3, and study No. 4.

The applicant responded that FA-/- BM / mPB derived CD34+ cells are scarce and that it was only possible to analyze MMC resistance could and that this might be indicative for the level of transduction for non-clinical purposes, although absence of information on VCN and FANCA expression is regarded a limitation to the characterization of the cells. This can be understood. It is also recognized that engraftment is complex and needs more than only the LT-HSCs. However, for the success of the therapy, presence of FANCA-LV transduced LT-HSCs in the DP is crucial. The applicant argues that functional assays such as *in vivo* xenograft and *in vitro* colony formation (CFU) assays are the "gold standard" to confirm the existence of LT-HSCs. The *in vivo* test including the secondary transplant was done in NSR2. In this study, a 50% drop in engraftment was observed upon secondary transplant for experiment L3. Whether this can all be attributed to the stressfulness of the procedure for the cell, as suggested by the applicant, remains a question. In addition, several factors have been listed by the applicant that could have resulted in variability in engraftment, raising the question whether an *in vivo* approach is that precise. The *in vitro* method might result in less variable results, for instance when comparing different protocols. However, the *in vitro* assay used by the applicant included a culture of only 14 days, which rather seems to select for ST-HSC and seems too short for detection of the LT-HSCs. Selection of LT-HSC requires longer term culturing. It is thought that colony-forming units detected after > 5 weeks of culturing represent the progeny of LTC-IC that are more primitive (see e.g. Sutherland et al., PNAS, 1990*, Hao et al., 1996** and Liu et al., 2013***). Moreover, most of the (cord blood-derived) cells in the LTC-IC assay are CD34+ CD38- (see e.g. Conneally et al., PNAS, 1997****).

The applicant did not touch upon this aspect of the CFU assay. Furthermore, the applicant assumes, based on the wide tropism of VSVg vectors, that LT-HSCs are as efficiently transduced as other cells in the CD34+ population, however, a reference supporting this information is missing. In the response to a toxicological issue, the applicant refers to publicly available studies in which the mutagenic risk of LV-mediated gene therapy in different HSC populations was investigated. VCNs (and thus transduction efficiencies) would be comparable between short-term and long-term HSCs (phenotyped as CD34+CD38+ and CD34+CD38-, respectively in Zonari et al., 2017*****). However, this has not been confirmed as the 14 days CFC culturing is not regarded fit for purpose. It remains thus unclear how efficient LT-HSCs are transduced in the non-clinical data. Non-clinical data is limited and the effect of the treatment with Fanskya should be primarily based on clinical pharmacology / efficacy data.

Study No. 4: Optimization of Transduction with transduction enhancers

The applicant showed that the selected transduction enhancers were most efficacious to increase transduction efficiency with lentiviral vectors at reasonable vector level, at least in vitro. An in vivo study indicated that the use of this TE combination does not affect lineage reconstitution. Moreover, the transduction enhancers increased the in vivo VCN in NSG mice to >1.5 in the bone marrow (VCN of 0.67 without and 1.53 with TEs). However, all these studies were conducted with other type of vectors than the clinical designed vector.

Only one experiment to test the efficacy of the TEs was conducted with the clinical designed vector in FA-A cells. Transducing FA-A patient cells with FANCA-LV revealed for one donor an approximate 3-fold increase in CFC survival after MMC challenge, relative to transduction in the absence of TEs. Cell recovery appeared to be mildly reduced at the chosen clinical concentrations of TEs. When using cells from another FA-A patient a 2-fold increase in MMC resistance, a slight increase in cell recovery and minimal effect on clonogenic potential was observed. The use of the selected TEs to transduce mPB

CD34+ cells from two different FA–A patients with PGK-FANCA-WPRE LV showed an increase in transduction efficiency as measured by the 2 to 3-fold increase in reversal of MMC sensitivity without compromising CFC cell recovery and lineage distribution.

However, the patient cell data comprised only *in vitro* results after CFC culturing. As reflected in the comments to study No. 3, some unclarities need to be resolved before these conclusions can be agreed. In addition to that, most important parameters for functional improvement would have been VCN and FANCA protein expression determination in early and late progenitor cells. This is missing for this study. There seems to be some support for the use of the TE combination in FA-A, however, definitive proof for the improvement in VCN and FANCA expression in the long-term engrafting cells, is missing.

VCN and FANCA expression data were missing for the study using FA-A cells and the clinical designed vector. The applicant was asked to discuss the clinical relevance of the in vivo PoC studies with the TEs using CD34+ cells transduced with transplanted in NSG mice and comparing the results to the results (VCN) from the clinical study. In addition, if available, the applicant was asked to submit VCN data for the in vitro experiment for efficacy of TEs using FA-A cells and the clinical designed vector.

It can be agreed with the applicant that for non-clinical purposes the MMC resistance test could be regarded a valuable parameter to determine the FANCA activity of the PGK-FANCA-WPRE LV transduced cells. The applicant also explained earlier that FA-A cells are scarce and that the MMC test is most efficient. It is assumed that DP used for study NSR3 and NSR4 are clinical (used) batches, explaining the scarcity of the material, further information on the non-clinical DP used in NSR3 and 4 is requested. When there is not much material left for VCN and mRNA or protein expression determination, the use of MMC test only can be understood.

The applicant was also asked to discuss clinical relevance of the use of the vector. Although the applicant claims that this LV is comprised of a similar backbone as PGK-FANCA-WPRE LV and was associated with comparable efficacy and safety characteristics, this was not supported with data, nor was it discussed why this vector was used for this study and how the results related to the (clinical) data for Fanskya. Again, the data supporting the use of the transduction enhancers are very limited and considered to suggest improvement of the efficiency of transduction relative to the old way. This issue will not be further pursued from non-clinical point of view. However, efficacy of the treatment in patients should be supported by clinical data only.

In study no 4, the applicant attempted to optimize the concentrations of the selected TEs in different test systems. The key experiment transduction efficiency was measured by the resistance to MMC after transduction of mPB CD34+ cells from an FA-A patient with PGK-FANCA-WPRE LV. It is not clear if this experiment has been repeated, there are no error bars or statistical analysis and therefore, no conclusions can be drawn (Figure 6 in the study report/ figure 15 in the NC AR). Different concentrations of TE were tested in a previous experiment (Figure 1 in Study no 4/ Figure 10 NC AR) where it was concluded that none of the evaluated TE concentrations had a significant impact on the viability or colony forming potential. Despite this, the applicant concludes that the results support the use of this combination of TEs to efficiently transduce FA-A CD34+ cells with PGK-FANCA-WPRE LV. From the data presented, Study no 4 seems not robust enough for such a conclusion.

In general, the relevance of the non-clinical data for the clinical situation is doubtful as also pre-GMP vector batches have been used. In addition to that, the transduction protocol used for study 2, 3 and 4 are different from each other and more importantly seem different from the clinical used transduction protocol. The applicant was asked to discuss the relevance of the non-clinical pharmacology studies for clinical practice.

The applicant started with explaining that this product was initiated in academia and that the four experiments presented for pharmacology were subsequent to each other, both in time and in what was investigated. The applicant believes that the conclusions from these early studies can be leveraged for clinical practice, because the clinical vector that was chosen in NSR1 has not been changed genetically afterwards. In NSR3 and NSR4, GMP grade batches have been used. The applicant claims that '*the critical quality attributes relevant to the trial outcomes (e.g., transduction efficiency, expression levels, and safety profiles) are maintained'*. However, the comparability of batch quality is not easily derived from the non-clinical studies as NSR2 is a homologous model (murine cells in the mouse) and NSR3 is a heterologous model (human cells in immunocompromised mouse). Nevertheless, the applicant argues that the manufacturing process was rigorously developed and validated to ensure consistency and reproducibility. The provided information is primarily based on claims from the applicant that are currently not supported with data. As this is considered a quality issue, more data on comparability between pre GMP and GMP grade vector batches is requested in the quality part (see assessment of the response to Q40, D150 Quality JAR).

Regarding the transduction protocol, the applicant argues that it has been stepwise improved to serve the optimization of the transduction efficiency while preserving the integrity of the fragile FA-A HSCs. The combination of cytokines and RetroNectin used in the transduction protocol were established using murine cells (NSR2) and this protocol has been adapted for human cells (NSR3). For the FA-A cells used in NSR3 the transduction protocol was shortened (24h). In study NSR4 the transduction protocol was further optimized by introducing transduction enhancers, which allowed for a further shortening of the duration of transduction (from 24 to 10-16-hours). It can be agreed that this sequence of changes seems rather advantageous for the efficiency and safety of the transduction of the FA-A cells, which will positively contribute to the treatment. However, without details on the DP used for NSR3 and NSR4 it is not possible to conclude on the positive contribution of the changes to the transduction protocol towards the clinical used DP. These details are further requested in the quality part. The issue will not be further pursued from non-clinical perspective. Efficiency of the transduction and engraftment processes should follow from and supported by clinical efficacy data and weighed in the B/R considering also the unmet clinical need.

* Sutherland HJ, Lansdorp PM, Henkelman DH, Eaves AC, Eaves CJ. Functional characterization of individual human hematopoietic stem cells cultured at limiting dilution on supportive marrow stromal layers. Proc Natl Acad Sci U S A. 1990 May;87(9):3584-8. doi: 10.1073/pnas.87.9.3584. PMID: 2333304; PMCID: PMC53946.

** Hao QL, Thiemann FT, Petersen D, Smogorzewska EM, Crooks GM. Extended long-term culture reveals a highly quiescent and primitive human hematopoietic progenitor population. Blood. 1996 Nov 1;88(9):3306-13. PMID: 8896394.

*** The long-term culture initiating cell (LTC-IC) assay, founded on the bone marrow long-term culture (LTC) system, measures primitive hematopoietic stem cells (termed LTC-IC) based on their capacity to produce myeloid progeny for at least 5 weeks. Liu M, Miller CL, Eaves CJ. Human long-term culture initiating cell assay. Methods Mol Biol. 2013; 946:241-56. doi: 10.1007/978-1-62703-128-8_15. PMID: 23179836.

**** Conneally E, Cashman J, Petzer A, Eaves C. Expansion in vitro of transplantable human cord blood stem cells demonstrated using a quantitative assay of their lympho-myeloid repopulating activity in nonobese diabetic-scid/scid mice. Proc Natl Acad Sci U S A. 1997 Sep 2;94(18):9836-41. doi: 10.1073/pnas.94.18.9836. PMID: 9275212; PMCID: PMC23278.

***** Zonari E, Desantis G, Petrillo C, Boccalatte FE, Lidonnici MR, Kajaste-Rudnitski A, Aiuti A, Ferrari G, Naldini L, Gentner B. Efficient Ex Vivo Engineering and Expansion of Highly Purified Human Hematopoietic Stem and Progenitor Cell Populations for Gene Therapy. Stem Cell Reports. 2017 Apr 11;8(4):977-990. doi: 10.1016/j.stemcr.2017.02.010. Epub 2017 Mar 16. PMID: 28330619; PMCID: PMC5390102.

Pharmacokinetics

The suitability of the methods to determine LV VCN and FANCA mRNA expression in various tissues could not be established, as no validation data were provided. Therefore, the applicant was requested to provide the validation data of the assays for LV VCN detection (qPCR and RT-PCR) and for FANCA expression (qRT-PCR) or to show that the methods are at least fit-for-purpose, e.g. by providing a detailed description and performance of the methods. The applicant responded that validation data for these assays are not available nor any clear description of the methods to indicate their fit-for-purpose

could be submitted, as specific protocols were not present. This further underlines the uncertainty about the reliability of the non-clinical data (see non-clinical discussion on Toxicology below).

The applicant stated that the study confirms a normal biodistribution of gene-corrected cells. However, it is not clear what the applicant considers as 'normal'. Biodistribution (and engraftment beyond bone marrow) was only very limitedly evaluated by the applicant. To be able to assess whether the current non-clinical study data are clinically relevant to determine biodistribution of Fanskya cells and to extrapolate study results to patients, the applicant was requested to discuss the design of the study, the comparison between the used pre-GMP and the clinical batch and provide a normal (clinical) biodistribution pattern of gene modified CD34+ based on literature and the potential impact of preconditioning on this distribution. The applicant provided a discussion on the design of study no. 5, with the focus on the absence of use of transduction enhancers in this study, the time of biodistribution analysis and the limited tissue selection for biodistribution. In addition, the applicant provided a brief overview of normal distribution of gene-corrected HSCs following intravenous administration. Considerable distribution/engraftment of cells can be expected in non-haematopoietic tissues, based on literature studies. Although with this discussion and overview not all distribution issues have been solved (e.g. absence of vector DNA determination beyond bone marrow, brain and gonads), taking into account the anticipated wide-spread distribution and the clinical efficacy data, further discussion of the remaining issues is not considered needed.

The applicant is of the opinion that the preconditioning regimen will likely not have an impact on the (wide-spread) engraftment of the transduced cells. This seems to be based on engraftment in haematopoietic tissue, while distribution to (and engraftment in) other tissues was not discussed. It is agreed with the applicant that preconditioning will not be used in patients, but a proper response would provide more insight in the clinical relevance of the data obtained with preconditioned animals. Irradiation preconditioning may impact homing and survival of bone marrow-derived cells in tissues such as the brain (e.g. microglia). However, considering the limited non-clinical biodistribution data for Fanskya in general, no further discussion on the potential impact of preconditioning with irradiation on the normal distribution of LV-transduced CD34+ cells to non-haematopoietic tissues will be requested.

According to the applicant, the LV batch used in the non-clinical PK and toxicity studies is sufficiently representative of batches produced with the clinical manufacturing process. Reference is made to the Quality AR, where this representativeness is further assessed. In this assessment, a follow-up question is raised for to obtain additional batch data.

Toxicology

The non-clinical safety studies (single dose toxicity, analysis of vector insertion sites and mutagenicity, RCL detection) have not been conducted conform the GLP principles. The applicant has submitted a GLP compliance statement as justification for the GLP non-compliance of the studies. According to the applicant, all non-clinical studies were conducted in academia according to a proper study design with minimal bias and performed with robust documentation of data. However, the limited quality of the study reports does not indicate that the applicant has tried to follow GLP principles nor conduct their distribution and safety analysis in line with the current guidance on genetically modified cells. This may have considerable impact on the reliability of the data.

The applicant was asked to justify the absence of GLP-compliance in the conducted non-clinical toxicity studies in the current dossier, justify the discrepancy in and lack of certain individual raw data in the study report from study no. 5 (BD/single-dose toxicity), and discuss the impact of the non-compliance of the toxicity studies on the reliability of the safety data, in accordance with *GLP principles in relation to ATMPs and relevant guidelines*. In their response, the applicant explained that the biodistribution and toxicity studies had been conducted in an academic setting. Although studies conducted in an

academic setting may not have followed all GLP guidance strictly (due to e.g. limited availability of the animal model), but the study reports should provide all information and data that would implicate that the study data can be regarded reliable. According to the applicant, non-clinical studies have been executed with robust documentation of data'. However, the limited quality of the study reports do not indicate that the applicant has tried to follow GLP principles, as the data in the materials and methods section with regard to the read-out assays is very limited and high-level and some individual raw data are missing.

For study no. 5, the data with regard to survival time of individual mice are not in accordance between the different tables and figures. As such, the reliability of this study cannot be established. Considering that the lab notebooks would still be available, the applicant is requested to provide an update of the tables in study report no. 5 to have all animal (survival) data in accordance with each other and with the raw study data from CIEMAT (**OC**).

For study no. 6, it can be agreed with the applicant that the use of the knock-out mouse model prevented a GLP-compliant study design. The materials and methods section sufficiently describe the study setup and which assays have been used to obtain results. VCN and engraftment was not determined for two recipient mice, without justification. Considering that the relevance of the study data for the clinic is doubtful (as a different transduction protocol has been used for the current study when compared to the clinical manufacturing process), the absence of GLP compliance in this study can be accepted.

For study no. 7, the data with regard to LV pro-virus testing of mice in which tumours have developed and raw data related to certain figures were not present or referred to in the study report. As such, the reliability of this study cannot be established. Considering that the lab notebooks would still be available, the applicant is requested to provide the (PCR) data related to the absence of LV pro-virus in the two solid tumours observed in this study. Moreover, raw data for Figure 8 should be submitted as well to complete the study report data. The applicant is further requested to clarify which data in the study report have been used to make Table 12, Figure 4 and Figure 5 or provide the individual raw data when not yet present in the report (**OC**).

Single dose

In the single-dose toxicity study (combined with BD), macroscopic evaluation of the thymus, liver, spleen, brain, lung, kidney and gonads did not reveal abnormalities. Bone marrow was not taken into account in this evaluation. Moreover, microscopic/histopathological analysis was not included in this study, without justification.

In the same study, the applicant did not analyse transgene mRNA or protein expression in peripheral blood. Whether clinically relevant amounts of FANCA were present in the (bone marrow of) treated animals could not be determined (see assessment of biodistribution study part). As such, the potential toxic effects of the FANCA transgene, in case of overexpression, on the function of the genetically modified cells (and their progeny) could not be determined. The applicant was requested to provide a literature-based discussion on the function of the FANCA gene and potential toxicity related to FANCA overexpression (i.e. supraphysiological levels in case of high VCN) in the transduced cells. The applicant mentioned that FANCA overexpression has not been observed thus far. It is not clear how this was evaluated, e.g. in animals or in patients, and what would be the physiological range level of FANCA protein in haematopoietic and non-haematopoietic cells. Nevertheless, according to the study from Lasaga et al. (2023), FANCA mRNA expression is lower in FA-A corrected cells (four donors) compared to healthy donor CD34+ cells, which could be due to the used PGK promoter. This will likely also result in lower FANCA protein, but sufficient to ameliorate the FA-A phenotype. While literature

suggests that high expression levels of can be associated with more cell proliferation and malignant cell behaviour (e.g. Wang et al., 2024, Luo et al., 2025), further discussion on the potential toxicity related to FANCA overexpression is not requested as the chance on this overexpression in FA-A patients treated with Fanskya is considered low and because patients are thoroughly monitored because of their sensitivity to cancer. Apart from the limited quality of the study (report), it appears that no transduction enhancers have been used in the transduction protocol, in contrast to the clinical manufacturing process. As such, the safety of remaining levels of TE as impurities have not been tested non-clinically. Moreover, no quality data regarding the used LV batch (and the similarity compared to clinical batches) is available and transduction conditions are different from the clinical manufacturing process, as mentioned in the assessment of the biodistribution study part.

Taken together, the BD/single-dose tox study design has several limitations and uncertainties related to the transduction protocol, quality of the LV-batch, degree of haematological/clinical chemistry analysis, amount/choice of tissues evaluated and type of tissue analysis (only macroscopically), which have not been appropriately discussed by the applicant. For some parameters no individual data were presented. In addition, there is considerable discrepancy between individual data presented in tables in the study report, which affects the reliability of the data. The interpretation and the translatability of the safety results to patients are thus considerably hampered. A conclusion on general safety of Fanskya cells and transgene expression can therefore not be drawn. Therefore, the conclusion of the applicant that the study indicated that infusion of transduced bone marrow cells did not induce toxic effects in mice following transplantation cannot be shared at the moment as the non-clinical data have several limitations. Safety was only limitedly evaluated non-clinically and in non-conformity with GLP principles. Although the study could provide some support to the concept as a whole (i.e. distribution to target tissue and transgene expression over time), its relevance in predicting human toxicity is considered limited. To be able to assess whether the current non-clinical study data have clinical relevance in determination of general safety of Fanskya cells and in extrapolating these results to patients, the applicant was requested to discuss the study design for toxicity analysis and the impact thereof on the study outcomes with respect to interpretation and translatability to patients. The discussion should at least include including the transduction protocol (e.g. no enhancers, different cytokines used), the limited analysis of clinical observations and blood and tissue analysis. In their response, the applicant primarily discussed the design of the biodistribution part of this study. The applicant has, for example, not justified why body weight (change) and food consumptions were not evaluated (beyond 2 months post-transplantation) in study no. 5, why clinical pathology evaluation was limited to red and white blood cells counts and four plasma chemistry parameters and why no histopathological analysis of the sampled tissues was conducted. In addition, FANCA mRNA expression was not evaluated beyond bone marrow (and only relative transgene levels were provided for this tissue), thus potential toxic effects of the FANCA transgene (e.g. overexpression) on the function of the genetically modified cells (and their progeny) could not be determined. Nevertheless, considering the limited non-clinical toxicity data in general, no further justification of the study design for toxicity analysis (including the limited analysis of clinical observations and blood and tissue analysis) will be requested.

Tumorigenicity

Based on the insertion site analysis data and (poly)clonal pattern of reconstitution, it could be concluded that the LV used in the current product, under the transduction conditions tested in the murine study, integrates with a seemingly random pattern and shows a low insertional mutagenesis potential in Fanca$^{-/-}$ Lin^{-} cells, which is in agreement with literature on the integration profile of other LVs. However, it is not clear whether the integration profile in murine bone marrow cells is predictive for the profile that would be observed in CD34^{+} human cells. Moreover, the transduction of the cells was conducted without the presence of the transduction enhancers and the transduction protocol itself

was different compared to the one used in the clinical manufacturing process. It is not clear whether an increased VCN in the cells due to the use of transduction enhancers would lead to an enhanced mutagenesis risk. Also taking into account the non-GLP compliance of the study, the relevance of the current data for the clinic are unclear. Human ISA data (as discussed in the Clinical section) are therefore considered more relevant, although there is still limited knowledge on how to grade and compare lentiviral integration patterns and how to extrapolate this to the level of cancer risk.

The combination of LAM-PCR and pyrosequencing to obtain possible insertion sites in the mouse is considered adequate. However, it appears that an old reference genome (from February 2006) instead of the standard mm10 from December 2011 was used to align the reads with. The older reference may lack certain annotations. And considering that a restriction to 95% identity is used for the insertion sites, use of an older reference genome may result in missing (relevant) genes/sites. The applicant was asked to explain their reference genome choice, thereby also clarifying whether it has been checked if the genes of interest do match between differences references. The applicant has now justified the use of mm9 instead of mm10 as reference genome for the LAM-PCR and pyrosequencing. Using the most recent version would have been most appropriate, but since clinical trials have been conducted without any safety issue related to LV insertions thus far, it is unlikely that a different reference genome would change that much.

The applicant has only analysed the LV integration profile in whole blood and bone marrow samples, without differentiating between different cell lineages. Especially important for the risk of insertional mutagenesis would be the VCN and integration profile in early progenitors (i.e. long-term repopulation cells) versus late progenitors (i.e. no long-term engrafters). It is anticipated that the applicant did not obtain such data in this study, as reference to early and late progenitors is also missing in the PD part of the non-clinical dossier. Nevertheless, the applicant was asked to discuss the mutagenic risk and consequences of the Fanskya-related LV (or comparable LVs) in early versus late progenitors, using (literature-based) information on the characteristics of these two cell types (e.g. transducability, proliferative capacity, sensitivity to transduction enhancers, etc. during the manufacturing process and *in vivo*). Any available Fanskya (clinical) data on early and late progenitor cell transduction or integration should be included as well. A discussion on LV-related mutagenic risks, based on literature, was provided. It is agreed that the mutagenic risk of the Fanskya-related LV in general will be low, considering the design and production of the vector. In addition, the applicant refers to publicly available studies in which the mutagenic risk of LV-mediated gene therapy in different HSC populations was investigated, although this was limited to transduction efficiency. The applicant did not further discuss potential differences in characteristics between short-term and long-term HSCs.

The applicant refers to the absence of RCL and genotoxic/tumorigenic events in their non-clinical studies for Fanskya. However, as the clinical relevance of these studies (conducted with murine cells) and the reliability of the study data remains unclear, no conclusion on the clinical mutagenic risk for short-term versus long-term HSCs can be drawn. The applicant points out that the number of (transplantable) HSCs for FA patients is limited, which prevents evaluation of the mutagenic risk specifically in human short-term versus long-term HSCs. Taken this clinical limitation into account, general knowledge on the preferred LV-mediated insertions indicates that the mutagenic risk in HSCs subpopulations is likely low, despite potential differences in the characteristics of these subpopulations related to LV infection. The absence of insertional mutagenesis-related safety issues in treated FA patients thus far provides additional support for this conclusion. No further discussion on the LV-mediated mutagenic risk in short-term versus long-term HSCs are requested.

The applicant has evaluated the integration site profile of the LV in mice via LAM-PCR and pyrosequencing. However, the applicant has not performed a functional assessment of the LV insertion profile, e.g. evaluation of vector-induced cellular transformation with an *in vitro* IVIM or SAGA assay or

with an *in vivo* tumour-prone animal model. Considering that murine data may not be fully translatable to humans, no additional non-clinical studies are requested. However, the applicant was asked to provide a literature-based discussion on the risk for cellular transformation following integration of a SIN-LV containing the same or comparable elements (promoter, WPRE, etc.) as the one used in Fanskya, and justify the absence of a functional study for potential cellular transformation in the current (non-clinical/clinical) dossier. As requested, the applicant has provided a literature-based discussion on the risk for cellular transformation following integration of the LV used in Fanskya. This discussion related to the design and development of the LV, i.e. use of four-plasmid production, self-inactivating LTR sequences and the PGK promoter. The applicant refers to *in vitro* assays that could evaluate cellular transformation, but would likely not resemble the *in vivo* situation. *In vivo* assays to determine the risk of LV-related insertional mutagenesis would be considered more appropriate by the applicant. Although this reasoning can be followed, *in vitro* assays may be able to pick up changes in cell growth/functionality earlier than in animals, where only the end result of insertional mutagenesis and subsequent transformation (i.e. malignant cells) would be measurable. It is agreed that signals obtained with *in vitro* assays may not all lead to aberrant cells *in vivo*, but results would at least provide an estimation of the risk for insertional mutagenesis of the LV when compared to relevant (positive) control vectors.

The applicant refers in their response to publicly available studies in which murine Lin- bone marrow cells transduced with a SIN-LV (with the PGK promoter) comparable to the Fanskya-LV were tested in an IVIM assay (Modlich et al., 2009), in tumour-prone mice (Montini et al., 2006) or in gene-corrected mice (Garcia-Gomez et al., 2016). For Fanskya, gene-corrected FA mice were used to access the integration profile of the LV. None of the study results indicated genotoxic issues related to the vector in comparison to control vectors.

In the non-clinical studies submitted with the current dossier, no tumour-prone mouse model was used. Instead, gene-corrected FA mice were used to access the integration profile of the LV, comparing cells before and at different time points after (serial) transplantation. This is acknowledged. This study allowed for evaluation of potential enrichment of insertions towards specific chromosomes, clonal expansion or even tumour formation, but not evaluation of the first steps of potential malignancy (cell transformation). Nevertheless, the available non-clinical and clinical data from CD34+ cell products with comparable lentiviral vectors are sufficient to justify the absence of a Fanskya-LV-specific functional assay for cell transformation.

Reprotoxicity

The applicant did not address the risk of secondary transduction (i.e. bystander cell transduction/carry-over of vector to off-target cells due to the presence of infectious particles on the membrane of injected cells) nor the potential for vector mobilisation. Such off-target cells could also be gametes. To evaluate the risk of transduction of gametes, and in line with the *Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells* (EMA/CAT/GTWP/671639/2008 Rev– 1 - corr), the applicant was asked to provide a (literature-based) discussion on the risk for vector mobilisation and recombination with endogenous viruses and bystander cell transduction with their product, based on the vector design, vector particle removal from the cells during manufacturing and presence of *in vivo* LV inactivating factors. In their response, the applicant has provided a discussion on the number of residual LV particles in the final DP, indicating that the risk of bystander cell transduction *in vivo* is very low. Washing steps are available during the production of the final product and *in vivo*, several inactivating factors would be present. Taking this into account, the applicant provided a theoretical calculation to estimate the residual infectious LV particle number in the final DP. Thereby it is indicated that a minimum of $9.5x10^7$ cells and a maximum of $8.1x10^8$ cells could be transduced in a manufacturing round. With a MoI of 75, a

range of $7.1x10^9$ – $6.1x10^{10}$ particles are present. According to the ERA (see discussion below), a 96000-fold reduction of particles is possible with the dilution and washing steps, leading to a theoretical amount of $7.4x10^4$ – $6.3x10^5$ particles in the final DP. Inactivation of these particles would be possible with human serum, either during the washing steps and *in vivo*. In the human body, a 95-fold reduction would occur according to DePolo et al., 2000. It is not clear on which data this fold reduction has been based, as in this paper survival of retrovirus MLV was decreased from 48.5% to 2.5% in human serum (19-fold reduction) and survival of lentivirus HIV from 81% to 1.2% (68-fold reduction).

When calculating with the 95-fold reduction in human serum *in vivo*, about 781 – 6661 infectious particles could still be present in the blood stream. This would relate to 37 – 317 particles/kg (21 kg FA-A patient), which is extremely lower than LV particles that are infused *in vivo* for other therapies. However, this is not considered a relevant argument, as these infused LV particles are intended to transduce cells *in vivo*, while residual particles in the current product should not. Moreover, the mean weight of 21 kg is not agreed, as for the SAF population, the mean weight was 15.51 kg (range 8.4 – 25.6 kg).

The 781 – 6661 infectious particles could infect around 10 – 89 cells when the *in vitro* MoI of 75 would be *in vivo* as well (NB: this is just an assumption and it may have been more appropriate to use virus concentration instead of MoI). Transfected cells would then most likely be blood cells, as Fanskya is infused intravenously. The applicant does not consider this to be a (clinical) risk. But also the transduction of some individual non-blood cells is considered not to pose a relevant risk according to the applicant.

It is agreed with the applicant that the risk for bystander cell transduction *in vivo* can be considered low. Considering that patients have a mutation in the FANCA gene in all cells (not only hematopoietic cells), bystander transduction and FANCA-A expression in non-CD34+ cells could even have a positive effect and will likely not have an adverse effect on the functioning of the cells, unless LV integrates at undesirable sites that would disturb signalling pathways or cell processes or that could lead to insertional mutagenesis. Since the applicant has only evaluated insertion sites and clonal dynamics of haematopoietic cells (study 7 in D80 AR), the chance for and consequences of LV integration (at undesirable sites) in non-CD34+ cells has not been assessed. Fanconi anaemia patients are already prone to genetic instability, thus any risk of LV integration in non-CD34+ cells should not be neglected.

Taken together, the risk of bystander integration as calculated by the applicant is not yet clear. Therefore, the applicant should i) provide more insight in their assumptions regarding the fold reduction *in vivo* (with human serum), the use of *in vitro* MoI for *in vivo* transfection and the mean weight of patients, or recalculate the number of residual particles using the lower fold *in vivo* reduction (68-fold), a relevant MoI and mean patient weight (15.5 kg), and ii) provide a final conclusion on the risk on bystander cell transduction based on the number of residual infectious particles infused into patients. In addition, the applicant should discuss the chance and consequences of LV integration (at undesirable genomic sites) in non-CD34+ cells of Fanconi anaemia patients in comparison to integration in CD34+ cells (**OC**).

Environmental risk assessment

The conclusion that (ii) negligible amounts of residual infectious particles are present cannot be supported on the provided information, though the applicant shows that a substantial reduction of residual vector particles is achieved. From the provided information it is not clear on what the 95-fold reduction by human serum during washing steps is based.

The applicant should provide more insight in their assumptions resulting in the claim that negligible amounts of residual viral vector particles are present. If the presence of residual viral vector particles cannot be excluded the applicant should justify that negligible risks can be assumed in accordance with Section 3(3) of the Good Practice related to the amount of residual infectious particles in the medicinal product. For this, the applicant should provide a discussion on the possibility of exposure of individuals other than the patient to these residual particles (for example in a worst case situation that involves incidental transfer of blood). This discussion should include the likelihood of such a scenario happening, and -if necessary- the risk minimization measures to be taken. The applicant should provide an overall justification that residual particles do not pose more than a negligible risk to the environment taking into account –as appropriate- any risk minimisation measures (**OC**).

As module 1.6.2. is a "stand alone" document, the applicant **was asked to** complete **description or vector maps of the helper plasmids that are used to produce the lentiviral vector to complete module 1.6.2.** The applicant has provided detailed vector maps **in their response**. A complete description of the helper plasmids is present. The applicant will update module 1.6.2. accordingly. This updated document is awaited to check whether the information on the helper vectors is properly reflected in the document (**OC**).

3.2.7. Conclusion on non-clinical aspects

Pharmacology

Overall, the primary pharmacodynamic program is very limited and consists only of four studies. The studies were initiated between 2006 and 2017, but final reports are dated 2023 which casts doubt on the integrity and quality of the data.

Provided *in vitro* data (study 1) supports the choice for PGK-FANCA-WPRE*-LV as clinical vector. Proof of concept was shown in the Fanca-/- mouse model and in the SCID mouse receiving human transduced CD34+ cells from a FA-A patient. In study 2, However, it is noted that the Fanca-/- mouse model captures some but not all of the clinical manifestations of the disease. The applicant shows in study 2 that transduction of Lin- mice cells with clinical FANCA-LV vector transplanted to FANCA -/- mice results in engraftment of above 60% for primary transplant recipients and above 30% for secondary transplant recipients, with VCN ranging from approximately 0.5-4. Variability in the results were limitedly explained. Patient derived FA-A CD34+ cells transduced with the clinical FANCA-LV vector and cultured *in vitro* in a CFC assay resulted in increased MMC resistance. When transplanted in NSG mice the MMC from BM aspirated cells 1 month post transplantation was higher as compared to transduced cells directly cultured in CFC assay, suggesting that *in vivo* cells with the FANCA gene integrated (successful transduction) will be positively selected. Data and discussion of efficacy in different CD34+ cell populations (early and late progenitors (or LT-HSC, ST-HSC / MPP) as well as a discussion on the clinical relevance of the efficacy parameters was only limitedly provided.

In study 4, the shorter transduction protocol and the transduction enhancers were investigated after the other studies were conducted and hence, not tested in the proof-of-concept studies. In some cases, statistical analyses of the presented data are missing. It seems that the applicant used different transduction protocols in the pharmacology studies and their relevance for the clinical situation was not discussed. Despite the shortcomings of the PD-package, additional studies are not expected to add value at this point. The effect of the addition of combination of transduction enhancers was evaluated in FA-A patient cells using the clinical vector. Cell recovery and lineage distribution were comparable, but MMC resistance was 2-3-fold increase in cells cultured in vitro by CFC culturing method. Clinical relevance of the data using an unrelated vector was not discussed and VCN and FANCA expression data in experiment using the clinical designed vector were not available.

In general, there seems proof of the concept to support the treatment rationale. However, from non-clinical point of view there is only limited information to support efficacious transduction of the LT-HSCs and the successful engraftment. Therefore, the efficacy of the therapy is to be based primarily on clinical data.

Pharmacokinetics

From a pharmacokinetic point of view, the biodistribution study supports the proof-of-concept and provides data that transgene expression in murine bone marrow can be expected for at least 4 months. Nevertheless, the study design has several limitations and uncertainties related to the transduction protocol and quality of the LV-batch. In addition, the suitability of the DNA and mRNA assays has not been established, as no validation data are available. This limits the interpretation and the translatability of the results to patients. A conclusion on distribution of Fanskya cells and the (extent and duration of) expression of FANCA mRNA in different tissues can therefore not be drawn.

Toxicology

The three non-GLP toxicity studies submitted by the applicant have been conducted with a product produced with a different manufacturing (transduction) process than the clinical one. Reliability, interpretation, and translatability of the safety results to patients is further hampered by low quality study design, limited evaluation of specific safety parameters and missing individual animal data. Based on the available non-clinical data, a final conclusion on the safety of Fanskya cells and transgene expression cannot be drawn and the relevance of the data for the clinic remains unclear. Safety assessment of Fanskya should therefore mainly rely on quality and clinical data.

Discussion on safety aspects related to the vector (residual particles/bystander transduction) are partly missing in the current dossier.

3.3. Clinical aspects

- **Tabular overview of clinical studies**

Please see table in section 3.3.4 for the overview of the clinical studies

3.3.1. Clinical pharmacology

3.3.1.1. Methods

PD biomarkers

The PB biomarkers Vector Copy Number and MMC resistance of CFCs derived from Fanconi Anemia patients were assessed by sufficiently validated assays. The MMC resistance assay was also sufficiently cross-validated between two different analysis sites.

Immunogenicity

With respect to immunogenicity, ADAs to FANCA in human serum samples following RP-L102 administration were determined.

Potential antigen-specific immune activation of human peripheral blood mononuclear cells (PBMCs) in response to peptides derived from LVV structural proteins VSVG and Gag p24 amino acid sequences and the FANCA transgene amino acid sequences was assessed.

3.3.1.2. Pharmacokinetics

The investigational medicinal product is a gene therapy. No clinical pharmacology studies were performed. Pharmacokinetic studies are not applicable.

3.3.1.3. Pharmacodynamics

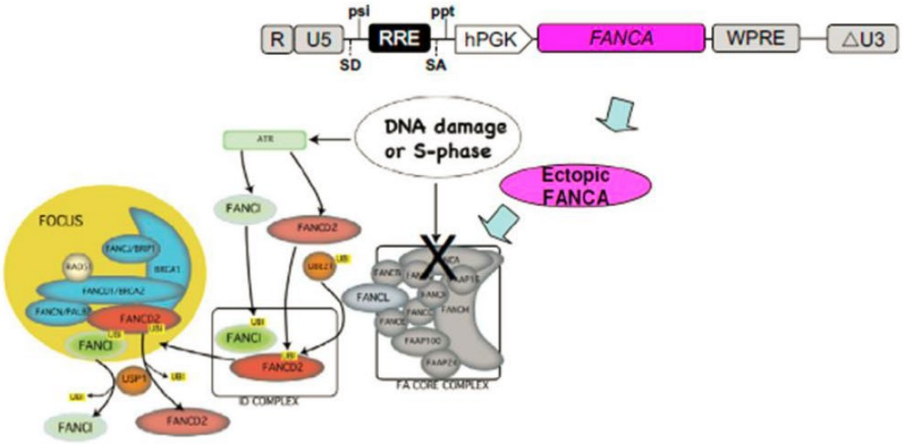
The IMP is a gene therapy. No clinical pharmacodynamic studies were performed although several PD parameters are measured as part of the primary efficacy endpoint (see efficacy section).

Mechanism of action

Fanskya (mozafancogene autotemcel, RP-L102) is an ex vivo lentiviral (LV) vector gene therapy consisting of autologous CD34+ haematopoietic stem cell (HSC). The HSC are collected from mobilised peripheral blood and transduced with an LV (PGK-FANCA-WPRE) that encodes for the FANCA gene, which inserts functional copies of the FANCA gene into patients' HSCs.

The active agent is a self-inactivating LV carrying the therapeutic FANCA gene. The inclusion of a mutation in the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), which lacks residual open reading frames, is used to improve the expression level and stability of the therapeutic gene. After transduction of HSCs from FA-A subjects with the LV, the therapeutic vector integrates into the genome of the cells. Once integrated, the therapeutic FANCA gene is transcribed and translated to produce the therapeutic FANCA protein. The transduced FA cells are then genetically corrected and capable of activating the FA pathway by mono-ubiquitination of FANCD2 and FANCI. These proteins may then migrate to DNA damage sites and promote DNA repair in these cells, as occurs in healthy cells (Figure 5).

Figure 2. Ectopic Expression of FANCA



The scientific rationale supporting the mechanism of action of RP-L102 is based on the naturally occurring phenomenon of multilineage mosaicism (occurring in<5% of FA patients). Multilineage mosaicism results when a spontaneous reversion mutation occurs in the FANCA gene of a long-term HSC which converts it from an abnormal cell with a dysfunctional DNA repair mechanism to a normal cell with a functioning DNA repair mechanism. As a result, all lineages (lymphoid, myeloid, erythroid, megakaryocyte) are corrected by the reversion mutation; therefore, multilineage mosaicism can lead to sustained blood count normalisation or stabilisation over time and is associated with low incidences of AML/MDS or requirement for allogeneic HSCT. RP-L102 replicates and expands upon the naturally occurring phenomenon of multilineage mosaicism.

Primary and Secondary pharmacology

3.3.2. Discussion on clinical pharmacology

Methods

PD biomarkers

The Vector Copy Number, MMC resistance assays, and the T-lymphocyte chromosomal fragility assay are considered sufficiently validated.

Immunogenicity

Potential antigen-specific immune activation of human peripheral blood mononuclear cells (PBMCs) in response to peptides derived from LVV structural proteins VSVG and Gag p24 amino acid sequences and the FANCA transgene amino acid sequences was assessed. A Qualification report of the assay was provided.

The multitiered strategy for the detection of FANCA antibodies is acceptable however no conclusion can be made whether the assays were adequate. Only summary tables of the validation were presented in the bioanalytical report.

No neutralising assay was presented, which is acceptable considering that no patient developed antibodies against FANCA.

Pharmacokinetics

In section 4.2 of the SmPC, no dose adjustment is considered necessary in case of renal or hepatic impairment. Considering the type of product, this is agreed upon from a PK perspective.

In section 4.5 it is stated that '*No interaction studies have been performed. Fanskya is not expected to interact with the hepatic cytochrome P-450 family of enzymes or drug transporters*.' This information is considered acceptable.

In section 5.2, the following is stated: '*Fanskya is an autologous gene therapy medicinal product consisting of autologous cells that have been genetically modified ex vivo. The nature of Fanskya is such that conventional studies on pharmacokinetics, absorption, distribution, metabolism, and elimination are not applicable.'* Patient data analysis showed that phenotypic correction in bone marrow is associated with concomitant bone marrow genetic correction. Further, restoration functional restoration of DNA repair mechanisms in hematopoietic cells was shown. It is agreed that provided data indicate incorporation of the FANCA gene in the bone marrow. For information purposes, the following should be added to section 5.2: '*In preclinical models, vector copy number (VCN) ranged from 0.1-1.1 vector copies per cell in the bone marrow and no vector copies were detected in non-hematopoietic organs such as the brain or gonads.*'

3.3.3. Conclusions on clinical pharmacology

The investigational medicinal product is a gene therapy. No clinical pharmacology studies were performed.

3.3.4. Clinical efficacy

Table 1. Clinical studies

Study ID	Enrolment status Total enrolment/ enrolment/goal	Design Control type	Dose (Mozafancogene autotemcel)	Baseline characteristics
First in human				
FANCOLEN-I*	Completed 9/9	Single-arm, single centre, single dose	No min or max dose specified. Administered dose range 0.0725x10^6 – 1.9100x10^6 CD34+ cells/kg	Median age (min-max): 5.53 (3.2-7.8) Sex: 7/9 males
Pivotal studies (Rocket-sponsored studies)				
RP-L102-0418	Completed 2/2	Single-arm, single centre, single dose	Optimal dose ≥5.0×10^5 CD34+ cells/kg. Median administered dose (min-max) (x10^6 cells/kg): 0.285 (0.20-0.37)	Median age (min-max): 5.85 (5.2-6.5) Sex: 0/2 males
RP-L102-0319	Ongoing, enrolment complete 5/5	Single-arm, single centre, single dose	Optimal dose ≥5.0×10^5 CD34+ cells/kg Median administered dose (min-max) (x10^6 cells/kg): 1.900 (0.30-4.10)	Median age (min-max): 3.34 (1.8-5.9) Sex: 4/5 males
RP-L102-0118	Ongoing, enrolment complete 7/8	Single-arm, multicentre, single dose	Optimal dose ≥5.0×10^5 CD34+ cells/kg Median administered dose (min-max) (x10^6 cells/kg): 1.800 (0.25-3.20)	Median age (min-max): 3.81 (2.2-7.0) Sex: 2/7 males
Long-term follow-up studies				
RP-L102-0116-LTFU*	Ongoing 9/7	Long-term follow up	No intervention	NA
RP-L102-0221-LTFU	Ongoing 14/5	Long-term follow up	No intervention	NA

* Studies FANCOLEN-I and RP-L102-0116-LTFU are not used for the assessment of efficacy due to differences in the population under study and the manufacturing process. These studies are only used as supportive evidence in the safety assessment.

3.3.4.1. Dose-response studies

No formal dose finding studies have been performed. Some insights for the most adequate dose for the pivotal studies were gathered from the FANCOLEN-I study, where the results showed that a higher dose was a potential determinant of efficacy. The doses used in FANCOLEN-I varied from 0.0725x10^6 – 1.9100x10^6 CD34+ cells/kg. For the pivotal studies, a dose of ≥5×10^5 CD34+ cells/kg was considered optimal, and the administered dose ranged from 2.0 ×10^5 to 4.1 ×10^6 cells/kg.

3.3.4.2. Main study(ies)

The efficacy is based on the three Rocket-sponsored studies: Study RP-L102-0418, Study RP-L102-0319, and Study RP-L102-0118 lasting 3 years. Further, there is a long term follow-up study RP-L102-

0221-LTFU enrolling patients from the parent Rocket-sponsored studies lasting 15 years. Data are presented pooled to the parent studies.

As of 23-Oct-2024 cut-off point, 15 patients have been enrolled in the studies, out of which 14 subjects have been treated with RP-L102 in the Rocket-sponsored studies and 13 are evaluable by the cutoff date stated:

- One Rocket sponsored parent trial is completed, the phase 1 Rocket-sponsored trial RP-L102-0418 (n=2), in which 2 patients were treated, 1 patient completed and 1 patient discontinued.
- Two Rocket-sponsored phase 2 trials (RP-L102-0319 and RP-L102-0118) are still ongoing, but enrolment is completed. In RP-L102-0319 (N=5), 5 patients were treated, of whom 3 had completed this study and 2 are ongoing. In study RP-L102-0118 (N=8), 7 patients were treated, of whom 4 had completed this study as planned, 2 are ongoing, and 2 subjects discontinued (1 of them before being treated with RP-L102 due to failed apheresis) .

Rocket-sponsored studies (Study RP-L102-0418, Study RP-L102-0319, and Study RP-L102-0118)

Methods

Study design

The Rocket-sponsored studies are phase 1/2 single-arm, single dose studies to evaluate the safety and efficacy of the IMP infusion in paediatric subjects with FA-A. Subjects were to be followed for 3 years.

Study Participants

The Rocket-sponsored interventional trials incorporated highly similar protocols in which the investigational medicinal product (IMP) was given early in the disease course, before onset of severe cytopenias, with the intent of improving HSC collection. These studies enrolled subjects with limited or negligible BMF by stipulating a minimum BM CD34+ cell concentration at baseline.

Inclusion – exclusion criteria

Main inclusion Criteria (common to the three studies)

Subjects who met all the following criteria were included in the study:

- FA, as diagnosed by chromosomal fragility assay of cultured T-lymphocytes in the presence of DEB or a similar DNA-crosslinking agent.
- Subjects of the complementation group FA-A.
- Minimum age: 1 year and a minimum weight of 8 kg; in addition, RP-L102-0118 also had a maximum age of 17.
- At least 30 CD34+ cells/µL in one BM aspiration within 3 months prior to initiation of CD34+ cell collection.
- 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.

Main exclusion criteria (common to the three studies):

- Subjects with an available and medically eligible HLA-identical sibling donor.
- Evidence of myelodysplastic syndrome or leukemia, or cytogenetic abnormalities predictive of these conditions in BM aspirate analysis. This assessment should be made by valid studies conducted within the 3 months before the subject commences the stem cell mobilization/collection procedures of the clinical trial.
- Subjects with somatic mosaicism associated with stable or improved counts in all PB cell lineages. (If T-lymphocyte chromosomal fragility analysis indicates potential mosaicism, a medically significant decrease in at least one blood lineage over time must be documented to enable eligibility).
- Lansky performance status ≤60%.
- Any concomitant disease or condition that, in the opinion of the Principal Investigator, renders the subject unfit to participate in the study.
- Pre-existing sensory or motor impairment ≥grade 2 according to the criteria of the NCI.
- Hepatic dysfunction as defined by either:
- Bilirubin >3.0 × the upper limit of normal (ULN) or
- Alanine aminotransferase (ALT) >5.0 × ULN or
- Aspartate aminotransferase (AST) >5.0
- Renal dysfunction requiring either hemodialysis or peritoneal dialysis.
- Pulmonary dysfunction as defined by either:

  Need for supplemental oxygen during the prior 2 weeks in absence of acute infection or Oxygen saturation by pulse oximetry <90%.
- Evidence of active metastatic or locoregionally advanced malignancy for which survival is anticipated to be less than 3 years.
- Subject is receiving androgens (i.e. danazol, oxymetholone).

Inclusion criteria specific to each study:

1. Maximum age: NA for study RP-L102-0319; 12 years for study RP-L102-0418; and 17 years for study RP-L102-0118.
2. Study RP-L102-0418:

At least one of the following hematologic parameters below lower limits of normal:

- Hemoglobin
- Absolute neutrophils
- Platelets

3. Study RP-L102-0418:

   If the number of CD34+ 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

Exclusion criteria specific to studies RP-L102-0319 and RP-L102-0118

1. Bilirubin >3.0 × the upper limit of normal (ULN), or Alanine aminotransferase (ALT) >5.0× ULN, or Aspartate aminotransferase (AST) >5.0× ULN. In relation to Hepatic dysfunction: For subjects with bilirubin, ALT or AST above ULN, a workup to identify the aetiology of liver abnormality should be conducted prior to confirmation of eligibility as stipulated in exclusion criterion 5, including evaluation of viral hepatitis, iron overload, drug injury or other causes.
2. Subject is receiving other investigational therapy for treatment/prevention of FA associated bone marrow failure.

Treatments

Screening, mobilisation, transduction, and infusion

The Rocket-sponsored studies were single dose studies. The screening of the patient was carried out within the 60 days prior to mobilisation and collection, through physical examination (including weight and height), PB cell counts, and basic biochemistry. BM aspiration, which will be conducted to determine the CD34+ cell count and to rule out mosaicism and myelodysplasia, and significant clonal alterations may be conducted within 90 days prior to the initiation of mobilisation and collection. The calculation of the dose of G-CSF and Plerixafor will be based on the patient's weight measured on the day of the first administration of G-CSF.

The treatment starts with the mobilisation of CD34+cells. For mobilisation, G-CSF was administered twice daily (12 µg/kg/12 hours) for 11 doses (up to 13 doses). Plerixafor was administered subcutaneously for 2 days at a dose of 240 µg/kg/day, 7–9 hours after the 8th and 10th administration of G-CSF. Apheresis started 4–6 hours after plerixafor administration if ≥5 PB CD34+ cells/µL were measured. Mobilisation with G-CSF and plerixafor continued and a second administration of plerixafor was given on the following day, followed by a second apheresis if the number of CD34+ cells circulating in the PB was again ≥5 cells/µL. If the number of CD34+ cells in the PB sample after the first plerixafor administration was <5 cells/µL, apheresis collection did not start, and continuation of mobilisation with G-CSF (up to 13 doses) with a third administration of plerixafor was considered. Following transport of the apheresis product to the manufacturing facility, CD34+ cells will be immunoselected. Purified CD34+ haematopoietic cells will be transduced ex vivo from the fresh apheresis collections consisting of autologous HSCs with the therapeutic LV (PGK-FANCA-WPRE). Transduction of CD34+ HSPCs will occur under Good Manufacturing Practices (GMP) at the manufacturing facility. Enriched cells will be transduced with the PGK-FANCA-WPRE LV as described in the IMPD, during a processing and manufacturing period of approximately 48 hours (encompassing prestimulation, transduction, harvest and release).

After transduction, product quality control evaluations will be carried out in aliquots of the transduced population. A minimum of 1×10^4 CD34+ cells/kg must be available following lentiviral transduction for infusion or the patient will be withdrawn from the trial (for studies 0319 and 0118). For study RP-L102-0418 there was a cut-off of $\geq2\times10^5$ CD34+ cells/kg to start immunoselection and no specifications for infusion. A cell dose of $\geq5\times10^5$ CD34+ cells/kg subject weight was targeted and considered optimal.

The IMP was manufactured from the fresh apheresis collections and consisted of autologous HSCs transduced with an LV (PGK-FANCA-WPRE) that encodes for the FANCA gene. The IMP was infused as a fresh drug product (not cryopreserved) with no antecedent conditioning. The subject remained hospitalised for at least 48 hours after IMP infusion.

No patients received any prior therapies for BMF including blood transfusions, androgens, growth factors, or other experimental treatments for FA-related BMF. No specific medications were prohibited during the study period, as these therapies are not anticipated to impact the efficacy of gene therapy.

Note: Administration of additional medications/therapies to prevent or correct BMF was considered a treatment failure for the investigational therapy (i.e., androgens, requirement for regularly scheduled red blood cell (RBC) or platelet transfusions, and allogeneic HSCT.)

Objectives

The objective of the Rocket-sponsored studies is to assess the therapeutic efficacy of a hematopoietic gene therapy consisting of autologous CD34+ enriched cells transduced with a LV carrying the FANCA gene in subjects with FA-A.

Outcomes/endpoints

The **primary efficacy endpoint** is a composite endpoint comprised of 3 components evaluating evidence of:

- Phenotypic correction by BM CFU MMC resistance ≥20% (MMC at 10 nM concentration) at Month 12 post-infusion with a confirmatory assessment at either Month 18 or Month 21; AND
- Genetic correction by PB vector copy number (VCN) ≥0.1 at Month 18 post-infusion with a confirmatory assessment at either Month 21 or Month 24. ; AND
- Haematologic stability by haemoglobin, neutrophil, and platelet counts remaining at ≥75% of 6-month post-infusion nadir value at Month 18 post-infusion with a confirmatory assessment at Month 21 or 24. Levels must exceed the following values at the latest documented assessment (≥18 months post-infusion): hemoglobin ≥8 g/dL (NCI-CTCAE v5 Grade <3); neutrophils ≥500/µL (NCI -CTCAE v5 Grade <4); platelets ≥25,000/µL (NCI-CTCAE v5 Grade <4).

The subjects need to achieve the 3 components to meet the primary endpoint.

Justification of components of the primary composite endpoint as provided by the applicant:

- **BM CFU MMC resistance ≥20%:** Determining the level of BM MMC resistance necessary to reasonably predict haematologic stability was based on natural history data and the results from FANCOLEN-I.
  - *Natural history data:* A recent publication from the national Spanish FA registry retrospectively reviewed 223 FA patients and identified 9 patients with FA-A and multilineage mosaicism based on BM MMC resistance (Ramirez 2021). All patients with multilineage mosaicism who had >20% BM CFU MMC resistance (7/7 patients) demonstrated stable haematology for up to 30 years (median follow up: 14.64 years), decades of BMF free survival without requirement for chronic transfusions or allogeneic HSCT, and none developed MDS or AML. As of November 2023, 3 /7 patients are alive and clinically well. The other 4 patients remained haematologically stable until the end of their lives; they developed solid tumours consistent with FA natural history which resulted in death during adulthood. Longitudinal data for BM MMC resistance was available for 3 of the 7 patients with stable haematology, for whom BM MMC resistance remained ≥20% for up to 13 years of follow-up [*added by assessor:* BM MMC resistance varied largely over time, ranging within these 3 patients 21%-140%; 70.9%-266.7%; and 53.8%-92.2%]. These three patients had long-term haematologic stabilisation and MDS/AML-free survival to ages 36, 24 and 34, respectively].

- *FANCOLEN-I data*: Following gene therapy, 2/9 patients developed and maintained BM MMC resistance ≥20% for up to 84 months after therapy (87.2% at 84 months and 37.8% at 72 months post-gene therapy, respectively These patients concurrently developed haematologic stabilization with increases from baseline values seen in haemoglobin and platelets. Patients are now 12.2 and 11.9 years old, respectively, having surpassed the median age at which initial signs of BMF is anticipated (approximately 7 years). [*Added by assessor*: No information was provided for the 7/9 patients].

PB VCN ≥0.1: The threshold of 0.1 was informed by non clinical data and the clinical findings of FANCOLEN-I study.

- *Non-clinical data:* FA knock-out mice (Fanca-/-) transplanted with transduced cells correlated with high levels of engraftment (both in primary and secondary recipients), providing evidence of the repopulation capabilities of gene corrected HSPCs following transplantation.
- *FANCOLEN-I data*: Levels of VCN ≥ 0.1 at 24 months post-infusion in PB or BM were detected in a majority of subjects (5 subjects, 55.6%), indicating successful genetic correction. In subjects in whom PB VCN ≥ 0.1 was identified at month 24, increasing genetic correction was sustained until end of study participation (up to 36 months) and for the majority of these subjects, levels in excess of 0.1 were observed with progressive increases at subsequent timepoints. Importantly, across the cohort, PB genetic correction was associated with concurrent BM genetic correction, demonstrating successful engraftment and proliferation of gene corrected cells in the PB.

- **Haematologic Stability:** Eighty percent of FA patients progress to BMF within the first decade of life. In the absence of definitive therapy, prolonged haematologic stabilisation is highly unlikely and is observed in fewer than 20% of patients over the initial decade of life. Without treatment, most patients die prior to age 10 from severe aplastic anaemia.

  Decline in multiple blood lineages in the absence of definitive treatment was evaluated in 139 FA-A patients (of non-mosaic aetiology) from the International Fanconi Anemia Registry (IFAR). The analysis indicated that during the first 12 years of life, a one-year increase in age of FA-A patients was associated with 19%, 12%, and 5% decreases in platelets, ANC, and haemoglobin, respectively. Based on these findings, patients in the Rocket-sponsored studies are anticipated to experience ongoing haematologic decline consistent with their pre-treatment state, with decreases persisting until RP-L102 engraftment and subsequent repopulation of the BM and PB with gene-corrected cells. Considering the declines in haematologic parameters seen in FA patients in early childhood (including RP-L102 treated patients prior to gene therapy), haematologic stabilisation is considered clinically meaningful and likely predictive of BMF-free survival. During the interval between infusion and RP-L102 engraftment, patients are anticipated to experience ongoing haematologic decline consistent with their pre-treatment state and FA natural history. Therefore, a 6-month post-infusion nadir is expected to most accurately reflect a patient's true baseline blood count. Additionally, mobilisation and collection of HSPCs may transiently exacerbate haemoglobin and platelet declines in patients undergoing gene therapy. The threshold for haematologic stability for each lineage was determined after consultation with experienced international FA clinicians. Because some degree of decrease was anticipated during the 6 to 12 months following therapy and prior to engraftment and proliferation of gene-corrected cells, reductions of >25% from baseline were anticipated as unlikely to be indicative of therapeutic failure during the months subsequent to gene therapy.

  Additionally, as indicated in the haematologic stabilisation component of the primary composite endpoint, haematologic parameters must remain above the following thresholds: haemoglobin ≥8

g/dL (NCI-CTCAE v5 Grade <3); neutrophils ≥500/µL (NCI-CTCAE v5 Grade <4); platelets ≥25,000/µL (NCI-CTCAE v5 Grade <4) to be considered consistent with prevention of severe BMF. The categories used to define BMF in the clinical trial (Table 6) were adapted from the Fanconi Anemia Clinical Care Guidelines, an internationally accepted reference for the clinical care of FA patients developed by FA expert physicians and scientists (FARF 2020). The applicant has defined severe thrombocytopenia as a platelet count <25,000/µL (versus <30,000/µL defined in the FARF BMF categorisation) as this platelet level provides adequate haemostasis in most clinical settings and is above generally accepted transfusion thresholds (Patel and Josephson 2019; Schiffer 2018). In patients with more severe thrombocytopenia, maintenance of a platelet count ≥25,000/µL is clinically meaningful and enables patients to perform activities of daily living without the need for medical intervention. Additionally, this threshold for severe thrombocytopenia is supported by an internationally accepted classification system for aplastic anaemia severity (Davies and Guinan 2007).

Table 2. Classification of Bone Marrow Failure in RP-L102 Clinical Trials

	Mild	Moderate	Severe
Absolute Neutrophil Count	<1,500/µL	<1,000/µL	<500/µL
Platelet Count	150,000–50,000/µL	<50,000/µL	<25,000/µL**
Haemoglobin Level	≥8 g/dL*	<8 g/dL	<8 g/dL

* Less than normal for age, but ≥8 g/dL.

** Severe thrombocytopenia is defined by FARF as a platelet count <30,000/µL.

The key secondary endpoints are:

- Each component of the composite primary endpoint evaluated independently
- In subjects who were not mosaic in PB T-cells prior to gene therapy, level of phenotypic correction in haematopoietic cells determined by a T-lymphocyte chromosomal fragility assay targeting a decrease in PB T-cells with DEB-induced aberrations from pre-infusion levels ≥50% to <50% and a ≥20% decrease from pre-treatment levels
- Overall survival
- BMF-free survival
- MDS/AML-free survival
- BMF and MDS/AML-free survival

The exploratory efficacy endpoints are:

- Genetic correction by BM VCN >0.1 (including CD34+ and lineage-specific progenitors) and PB Subpopulation VCN >0.1 (including lymphoid and non-lymphoid populations) at various timepoints
- Paediatric Quality of Life (QOL) through the Paediatric Quality of Life Inventory and the Paediatric Quality of Life Multidimensional Fatigue Scale.

Sample size

Sample size and power are based on the proportion of subjects who demonstrate reversion of the hypersensitivity of BM CFUs to MMC. The null hypothesis is that fewer than 5% of subjects will have reversion of the hypersensitivity of BM CFUs to MMC based on the prevalence of multilineage

mosaicism. Assuming a historic reversion rate of less than 5% and a true reversion rate of least 36% among subjects receiving the IP, a sample size of 14 subjects will result in approximately 80% power via a one-sample binominal test at a 2-sided 5% significance level. The Sponsor, in alignment with global health authorities (and international expert consensus), considers improvement in BM MMC resistance in at least 5 of 14 subjects to be clinically meaningful (in line with the estimated true reversion rate of 36%).

Randomisation and blinding (masking)

Not applicable, as all studies were single-arm studies.

Statistical methods

Post-hoc, an estimand was defined based on the statistical analysis plan. The clinical question sought to be answered is: What is the responder rate defined as meeting the components BM CFU MMC resistance ≥20%, PB VCN ≥0.1 and haematological stability in eligible FA individuals defined by inclusion and exclusion criteria treated with RP-L201, where myelodysplastic syndrome, acute myeloid leukaemia, progressive cytogenetic and/or molecular abnormalities, and/or discontinuation from study are considered non-response.

The Safety Population (SAF) was to include all subjects from FANCOLEN-I, RP-L102-0418 (US Phase 1), RP-L102-0319 (US Phase 2) and RP-L102-0118 (FANCOLEN-II) who signed an Informed Consent Form and were not screen failures.

The Full Analysis Set (FAS) was to include all subjects from FANCOLEN-I, RP-L102-0418 (US Phase 1), RP-L102-0319 (US Phase 2) and RP-L102-0118 (FANCOLEN-II) who received the infusion of IMP.

The ISE Full Analysis Set (ISE FAS) was to include all subjects who received the single infusion of IMP in the parent studies RP-L102-0418 (US Phase 1), RP-L102-0319 (US Phase 2) and RP-L102-0118 (FANCOLEN-II).

The efficacy analysis will be performed on the ISE FAS population.

Continuous (quantitative) variable summaries will include the number of subjects (n) with non-missing values, mean, standard deviation (SD), median, minimum, and maximum and quartiles. Categorical (qualitative) variable summaries will include the frequency and percentage of subjects.

Point estimates will be reported with corresponding 95% confidence intervals (where estimable), else NE (Not estimable) will be displayed. Unless otherwise indicated, all statistical tests will be conducted at the 0.05 significance level using 2-tailed tests and P values will be reported.

Baseline is defined as the last observation recorded before any mobilization. This will be used as the observation for all calculations of change from baseline (CFB). An exception to the above baseline definition applies to a subset of hematology parameters (Hemoglobin, Absolute Neutrophil counts, and Platelet counts), which are involved in the derivation or description of hematologic stability. To analyse hematologic stability, the baseline is set as the Month 6 post-infusion visit.

In general, there will be no substitutions made to accommodate missing data points.

The primary efficacy estimate is the proportion of subjects who meet the composite endpoint, meaning those who satisfy all three components, among subjects who are evaluable for the primary composite endpoint. This percentage will be tested to a historic rate of 5% using an exact one-sample binomial test against a 2-sided alternative at the 5% significance level. The number and percentage with 95% Wilson Score CI of subjects who meet the composite endpoint will be reported.

A sensitivity analysis will be performed using different thresholds for the hematologic component of the primary composite endpoint, e.g. 70% and 80% instead of the predefined 75%.

A subject who dies, experiences BMF, MDS, AML or cytogenetic abnormalities associated with MDS/AML before having reached 12 months post-infusion will be considered to have not met the endpoint.

Survival time will be expressed as the number of days from the date of IP infusion to the event of interest. Subjects who do not experience the event of interest will be censored at the date of last contact. Survival time will be summarized by the number of events, censored subjects and at-risk subjects (number remaining), product-limit survival estimates with 95% CI where estimable and Nelson-Aaelen cumulative hazard estimates every 6 months post-infusion. A Kaplan-Meier survival plot will be produced.

No subgroup analyses were planned. Post-hoc subgroup analyses were performed for age, sex, manufacturing site, viral vector manufacturer and several critical quality attributes using logistic regression and Fisher's exact tests.

No interim analysis was planned and no multiplicity correction was applied.

Results

Participant flow

Recruitment

As per cutoff date of 23-Oct-24 a total of 26 pooled subjects > 1 year old were screened for eligibility in the three Rocket-sponsored studies, 15 were enrolled and 14 were treated. One subject received mobilisation but failed to meet the protocol threshold of ≥5 CD34+ cells/μL in PB to initiate apheresis and was discontinued from study. The ISE Full Analysis Set (ISE FAS) population consists of 14 subjects (93.3%) who received RP-L102 gene therapy. Disposition of subjects in the Rocket-sponsored studies and the corresponding LTFU is shown in Figure 6 and Table 7.

Figure 3: Disposition of Subjects in Rocket-Sponsored Studies and RP-L102-0221-LTFU (cut-off date 23-Oct-2024)

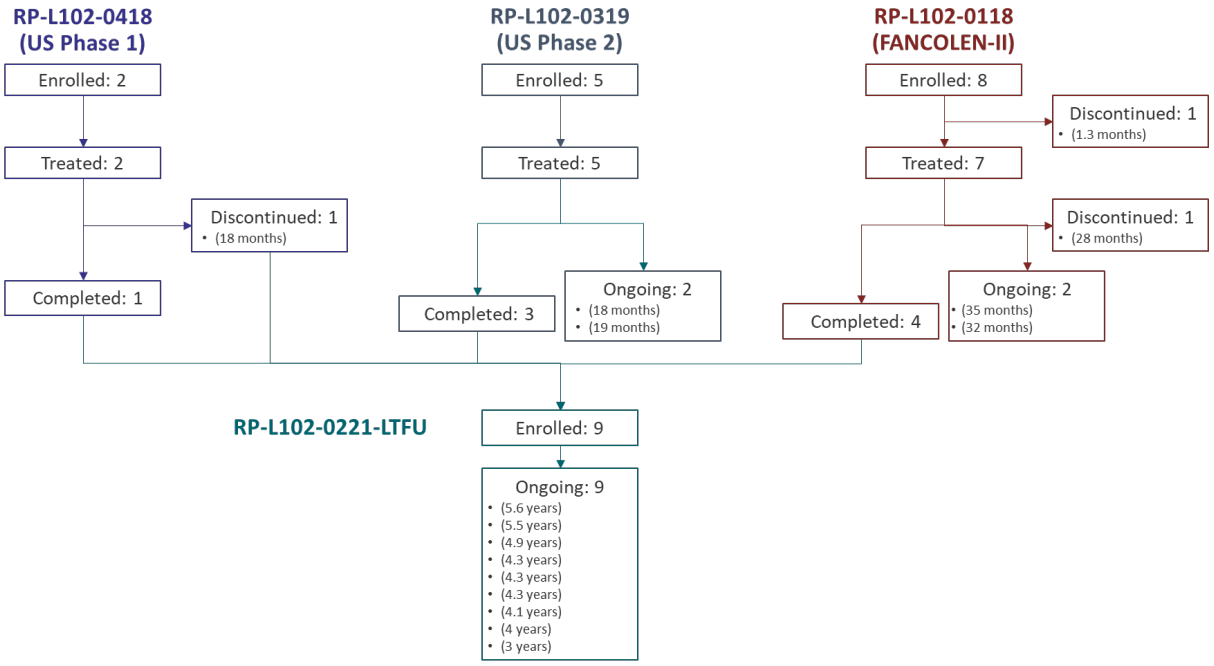


Table 3. Subject Disposition (All Subjects)

Status	RP-L102-0418	RP-L102-0118	RP-L102-0319	RP-L102 Total
Screened for Eligibility	2	16	8	26
Screen Failure	0	8	3	11
Enrolled in Parent Study	2	8	5	15
Subjects with sufficient CD34+ cells in PB to enable apheresis	2	7	5	14
Safety Population [1]	2	8	5	15
ISE FAS Population [2]	2 (100)	7 (87.5)	5 (100)	14 (93.3)
Completed Parent Study	1 (50.0)	2 (25.0)	2 (40.0)	5 (33.3)
Early Termination from Parent Study	1 (50.0)	2 (25.0)	0	3 (20.0)
Request of Primary Care Physician or Investigator	1 (50.0)	0	0	1 (6.7)
Adverse Event	0	1 (12.5)	0	1 (6.7)
Death	0	0	0	0
Other	0	1 (12.5)	0	1 (6.7)
Enrolled in LTFU Study	2 (100)	4 (50.0)	3(60)	9 (60.0)
Completed LTFU Study	0	0	0	0
Early Termination from LTFU Study	0	0	0	0
Duration of Follow-up (years) – n	2	7	5	14
Mean (SD)	4.47 (0.066)	2.49 (0.832)	1.97 (1.427)	2.59 (1.281)
Median	4.47	2.27	2.87	2.91
Min, Max	4.4, 4.5	1.5, 3.8	0.4, 3.2	0.4, 4.5

Abbreviations: FAS=Full Analysis Set; LTFU=Long Term Follow-up; SD=standard deviation.

Status	RP-L102-0418	RP-L102-0118	RP-L102-0319	RP-L102 Total

[1] The Safety population includes all subjects who have signed informed consent and are not screen failures.
[2] The ISE FAS population includes all subjects that have received the investigational product in the parent studies.

Source: RP-L102 ISE–Table 2.1.1

Conduct of the study

The majority of deviations in the studies were due to visits either not performed, performed remotely or performed outside of window due to Covid-19. Others concerned parameters not being measured or collected as per protocol at certain visits.

Baseline data

Table 4. Demographics and Baseline Characteristics (ISE FAS Population)

Statistic or Category	***RP-L102-0418 N=2***	***RP-L102-0118 N=7***	***RP-L102-0319 N=5***	***RP-L102 Total N=14***
Age at Informed Consent (Years) – n	*2*	*7*	*5*	*14*
Mean (SD)	*5.67 (0.867)*	*3.79 (1.749)*	*3.24 (1.588)*	*3.86 (1.703)*
Median	*5.67*	*3.71*	*2.97*	*3.54*
Age at IMP Infusion (Years) – n	*2*	*7*	*5*	*14*
Mean (SD)	*5.85 (0.912)*	*3.97 (1.684)*	*3.44 (1.515)*	*4.05 (1.651)*
Sex				
Male	*0*	*2 (28.6)*	*4 (80.0)*	*6 (42.9)*
Female	*2 (100)*	*5 (71.4)*	*1 (20.0)*	*8 (57.1)*

Abbreviations: *IMP=Investigational Medicinal Product.*

Source: *RP-L102 ISE–Table 2.1.2.1*

The haematologic post-infusion nadir values in the ISE FAS population at Month 6 (or the closest visit to Month 6) are summarised in Table 9.

Table 5. Haematologic Nadir Values (ISE FAS Population)

Statistic or Category	RP-L102-0418 N=2	RP-L102-0118 N=7	RP-L102-0319 N=5	RP-L102 Total N=14
Absolute Neutrophils (x10^3/µL) – n	2	7	3	12
Mean (SD)	1.410 (0.6930)	2.091 (0.9031)	1.550 (0.2946)	1.843 (0.7753)
Median	1.410	1.670	1.710	1.690
Min, Max	0.92, 1.90	1.11, 3.54	1,21, 1.73	0.92, 3.54
Haemoglobin (g/dL) – n	2	7	3	12
Mean (SD)	10.00 (3.253)	11.49 (0.339)	12.90 (0.755)	11.59 (1.436)
Median	10.00	11.40	13.00	11.60

Statistic or Category	RP-L102-0418 N=2	RP-L102-0118 N=7	RP-L102-0319 N=5	RP-L102 Total N=14
Min, Max	7.7, 12.3	11.0, 12.1	12.1, 13.6	7.7, 13.6
Platelets (x10^3/µL) – n	2	7	3	12
Mean (SD)	62.0 (33.94)	145.6 (110.03)	208.7 (89.53)	147.4 (102.56)
Median	62.0	100.0	193.0	114.0
Min, Max	38, 86	35, 318	128, 305	35, 318

Abbreviation: FAS=Full Analysis Set.

Note: The post-infusion nadir haemoglobin, absolute neutrophil count, and platelets is taken from the Month 6 visit. In settings of acute illness, febrile episodes, injury or inflammation where neutrophils and/or platelet counts are demonstrably increased, the post-infusion nadir for neutrophils and/or platelets, is taken as the closest visit prior to Month 6 with a result that is not demonstrably increased and without any documented acute infection, febrile episodes, injury or inflammation.

The post-infusion nadir absolute neutrophil count of Subject is taken from the Month 4 visit.

Source: RP-L102 ISE–Table 2.1.2.2

Characteristics of the medicinal product

Characteristics of the medicinal product, such as final product cell count or cell viability are described in detail in the Quality AR. The CD34+ cell dose ranged from 2.0 ×10^5 to 4.1 ×10^6 cells/kg.

No patients received any prior therapies for BMF including blood transfusions, androgens, growth factors, or other experimental treatments for FA-related BMF.

Numbers analysed

The primary efficacy analysis will be performed on the ISE FAS population. The ISE FAS population includes the 14 subjects who have been treated with RP-L102 in the Rocket-sponsored studies. Per cut off date (23 Oct 2024), 13/14 subjects are evaluable for the primary composite endpoint. See definition of valuable patient in Table 11 footnote. One (1/14) patient is not evaluable because although the patient reached all established endpoints and was respondent for BM MMC and PB VCN, the patients showed a ANC value <75% from month 6 at Month 21 (1.68 ×10^3/µL, 63% of 6-month value). Per protocol, this patient needs an additional confirmatory measure to assess whether it is a failure or a respondent: "a transient decrease to < 75% of 6-month post-infusion nadir value in a lineage or lineages in the absence of any illness, surgery, trauma, or other acute event will not be considered evidence of progressive BMF if progressive increases are identified over at least 2 successive assessments (≥ 1 month apart)". This patient will become evaluable after reaching month 32. Of note, an additional patient was enrolled in the Rocket-sponsored studies (total N=15), but did not received treatment due to failing mobilisation.

Outcomes and estimation

Primary endpoint

The primary endpoint was met by 5/13 evaluable patients Table 11 (cut-off date 23-Oct-2024).

Table 6. Primary Efficacy Composite Endpoint Results (ISE FAS Population)

Statistic or Category	RP-L102-0418 N=2	RP-L102-0118 N=7	RP-L102-0319 N=5	RP-L102 Total N=14
Number (%) of Evaluable Subjects [a]	2 (100)	6 (85.7)	3 (60.0)	13 (92.9)
Number (%) of Subjects who meet success in composite endpoint (95% Wilson Score CI) [1]	0	3 (50.0)	2 (66.7)	5 (38.5) (17.7, 64.5)
Comparison with 5% Historic rate p-value [2]				0.0006
Number (%) of Evaluable Subjects [b]	2 (100)	7 (100)	5 (100.0)	14 (100)
Number (%) of Subjects who achieve phenotypic correction of BM CFUs determined by MMC resistance (95% Wilson Score CI) [3]	0	5 (71.4)	2 (40.0)	7 (50.0) (26.8, 73.2)
Number (%) of Evaluable Subjects [c]	2 (100)	7 (100)	3 (60.0)	12 (85.7)
Number (%) of Subjects who achieve engraftment of gene-corrected hematopoietic cells in PBMC (95% Wilson Score CI [4]	1 (50.0)	5 (71.4)	2 (66.7)	8 (66.7) (39.1, 86.2)
Number (%) of Evaluable Subjects [d]	2 (100)	6 (85.7)	3 (60.0)	11 (78.6%)
Number (%) of Subjects who attain hematologic stability (95% Wilson Score CI) [5]	0	4 (57.1)	2 (66.7)	6 (54.5) (28.0, 78.7)

Abbreviations: BM = bone marrow; CI = confidence interval; MMC = mitomycin-C; PB = peripheral blood; VCN = vector copy number.

Notes:

a. Subjects who received gene therapy and followed for at least 12 months who fail phenotypic correction at M12 or followed for at least 18 months who fail phenotypic correction confirmation or gene engraftment or haematologic stability at M18 and subjects who meet all components of composite endpoint are considered evaluable.

b. Subject is evaluable if BM MMC resistance < 20% at M12, or ≥ 20% at M12 with a non-missing value at M18 or M21, or no data at M12 and < 20% at M18, or no data at M12 and ≥ 20% at M18 with a non-missing value at M21.

c. Subject is evaluable if PB VCN < 0.1 at M18, or ≥ 0.1 at M18 with a non-missing value at M21 or M24, or no data at M18 and < 0.1 at M21, or no data at M18 and ≥ 0.1 at M21 with a non-missing value at M24.

d. Subject is evaluable if incidence of MDS, AML or BMF or incidence of MDS/AML-associated cytogenetic abnormalities at any time, or followed for at least 18 months and does not meet any trilineage haematologic stability criteria at M18, or followed for at least 21 months and does not meet any trilineage haematologic stability criteria confirmation at M21 or followed for at least 24 months and does not meet any trilineage haematologic stability criteria confirmation at M24, or demonstrates trilineage haematologic stability.

[1] Success is defined as all 3 conditions met: phenotypic correction of BM colony-forming units, engraftment of gene-corrected cells in PB and trilineage haematologic stability.

[2] P-value is based on the exact one-sample binomial test.

[3] Phenotypic correction defined as BM CFU MMC-resistance ≥ 20% at M12 with a confirmatory assessment at M18 or M21.

[4] Gene engraftment demonstrated by PB VCN ≥ 0.1 at M18 with a confirmatory assessment at M21 or M24.

[5] Trilineage haematologic stability is demonstrated by Hb levels, ANC and Platelet counts remaining at ≥ 75% of 6-month post-infusion nadir values in the absence of MDS, AML, and/or cytogenetic abnormalities known to be associated with MDS/AML at M18 with a confirmatory assessment at M21 or M24.

Source: Annex Q165–Table 2.3.1

Five patients who met the primary composite endpoint.

A subject did not meet the predefined threshold for haematologic stability because ANC at the 21-month confirmatory timepoint was 74% of nadir, slightly below the protocol-stipulated 75% threshold; however, this subject is still considered a clinical therapeutic success, having otherwise met all other components of the primary composite endpoint. [*Added by clinical assessor:* This patient was censored at month 21 because the patient developed NHL; the patient received chemotherapy, and 2 platelet and 2 RBC transfusions].

Maintenance of response

To test maintenance of response, a sensitivity analysis using a second confirmatory timepoint at month 36 (instead of month 21 or 24, as per protocol) as well as LTFU data in the patients available has been performed. The results show that efficacy is maintained up to month 36 as well as at all available later timepoints (up to 5 years) in patients who initially responded to the treatment.

Results by component of the efficacy endpoint (formally secondary endpoint)

The number of subjects available for analysis per timepoint is variable due to differencing timepoints per component and the definition of evaluable patient (see subsection 'Numbers Analysed')

BM MMC Resistance

Phenotypic correction as demonstrated by an increase in BM MMC resistance to at least 20% at 12 months and confirmed at 18- or 21-months post-infusion was observed in 7/14 subjects with available data.

PB VCN

Genetic correction as demonstrated by PB VCN of at least 0.1 at 18 months and confirmed at either 21- or 24- months post-infusion was observed in 8/12 subjects with available data.

Haematologic Stability

Among the 11 subjects with available data, 7 subjects met the definition of haematologic stabilisation, i.e., haemoglobin levels, ANC, and platelet counts remained at ≥75% of 6-month post-infusion nadir value at 18 months with a confirmatory result at 21 or 24 months. Specifically, 9/11 subjects (81.8%, 95% CI 52.3-94.9) achieved haemoglobin stability, 7/11 (63.6%, 95% CI 35.4-84.8) ANC, and 8/11 (72.7%, 95% CI 43.4-90.3) platelets stable. Further, BMF status over time is considered stable.

Figure 4. Change from Nadir in Haematologic Parameters (ISE FAS Population)

	Param.	Screening	Baseline	M6	M12	M18	M21	M24	M28	M32	M36	Latest	
	Overall	**MILD**	**MILD**	**MILD**	**MILD**	**MODERATE**	**MODERATE**	**MODERATE**	**MODERATE**	**MODERATE**	**SEVERE**		**MODERATE**
	ANC	MILD	MILD	NORMAL	NORMAL	NORMAL	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE	Y5	NORMAL
	Hb	NORMAL	NORMAL	NORMAL	NORMAL	MILD	MILD	MILD	MILD	MILD	SEVERE		MILD
	Plt	MILD	MILD	MILD	MILD	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE	SEVERE		MODERATE
	Overall	**MODERATE**	**MODERATE**	**SEVERE**	**SEVERE**	**SEVERE**	**NA**	**NA**	**NA**	**NA**	**NA**		**NORMAL**
	ANC	MILD	MILD	MODERATE	MODERATE	MODERATE	NA	NA	NA	NA	NA	Y4.5	NORMAL
	Hb	MILD	MILD	SEVERE	MILD	MILD	NA	NA	NA	NA	NA		NORMAL
	Plt	MODERATE	MODERATE	MODERATE	SEVERE	SEVERE	NA	NA	NA	NA	NA		NORMAL
-	**Overall**	**SEVERE**	**SEVERE**	**MODERATE**	**MODERATE**	**MODERATE**	**MODERATE**	**SEVERE**	**MODERATE**	**MODERATE**	**MODERATE**		**MILD**
	ANC	SEVERE	SEVERE	MODERATE	MODERATE	MODERATE	MODERATE	MILD	NORMAL	NORMAL	MODERATE	Y4.5	NORMAL
	Hb	NORMAL	NORMAL	NORMAL	NORMAL	MILD	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL		NORMAL
	Plt	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE	SEVERE	MODERATE	MODERATE	MODERATE		MILD
-	**Overall**	**MILD**	**MILD**	**MILD**	**MILD**	**MILD**	**MILD**	**MILD**	**MILD**	**MILD**	**MILD**		**NORMAL**
	ANC	MILD	MILD	MILD	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	MILD	Y4	NORMAL
	Hb	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL		NORMAL
	Plt	NORMAL	NORMAL	MILD	MILD	MILD	MILD	MILD	MILD	MILD	MILD		NORMAL
	Overall	**MILD**	**MILD**	**MILD**	**MILD**	**MODERATE**	**MODERATE**	**SEVERE**	**NA**	**NA**	**NA**		
	ANC	NORMAL	MILD	NORMAL [a]	MILD	MILD	MILD	SEVERE	NA	NA	NA	M24	
	Hb	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	MILD	NA	NA	NA		
	Plt	MILD	MILD	MILD	MILD	MODERATE	MODERATE	SEVERE	NA	NA	NA		
	Overall	**NORMAL**	**MORMAL**	**MILD**	**MILD**	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**		**NORMAL**		**NORMAL**
-	ANC	NORMAL	NORMAL	MILD	MILD	NORMAL	NORMAL	NORMAL	NOMRAL	ND	NORMAL	Y4	NORMAL
	Hb	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	ND	NORMAL		NORMAL
	Plt	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	ND	NORMAL		NORMAL
	Overall	**MILD**	**MILD**	**MILD**	**MILD**	**MILD**	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**		**NORMAL**
-	ANC	NORMAL	MILD	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	Y4	NORMAL
	Hb	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL		NORMAL
	Plt	MILD	MILD	MILD	MILD	MILD	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL		NORMAL
	Overall	**MILD**	**MILD**	**MILD**	**MILD**	**MILD**	**MODERATE**	**MODERATE**	**MODERATE**	**MODERATE**	**MODERATE**		**MODERATE**
	ANC	MILD	NORMAL	NORMAL	NORMAL	MILD	MODERATE	MODERATE	MODERATE	MODERATE	MODERATE	Y4	MODERATE
	Hb	NORMAL	NORMAL	MILD	MILD	MILD	NORMAL	MILD	NORMAL	NORMAL	MILD		MILD
	Plt	MILD	MILD	MILD	MILD	MILD	MILD	MILD	MILD	MODERATE	MODERATE		MODERATE
	Overall	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**		**MILD**
	ANC	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	Y3.5	MILD
	Hb	NORMAL	NORMAL	NORMAL	HIGH	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL		NORMAL
	Plt	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL		NORMAL
	Overall	**NORMAL**	**NORMAL**	**NORMAL**	**MILD**	**MILD**	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**	**NORMAL**		
-	ANC	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	M36	
	Hb	NORMAL	NORMAL	NORMAL	MILD	MILD	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL		
	Plt	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL		
	Overall	**NORMAL**	**NORMAL**	**MILD**	**NORMAL**	**MODERATE**	**MODERATE**	**MILD**	**NORMAL**	**MODERATE**	**NR**		**MODERATE**
	ANC	NORMAL	NORMAL	NORMAL	NORMAL	MODERATE	MODERATE	MILD	NORMAL	MODERATE	NR	M32	MODERATE
	Hb	NORMAL	NORMAL	MILD	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NR		NORMAL
	Plt	NORMAL	NORMAL	MILD	NORMAL	NORMAL	NORMAL	MILD	NORMAL	NORMAL	NR		NORMAL
	Overall	**NORMAL**	**NORMAL**	**NORMAL**	**MILD**	**MILD**	**NORMAL**	**MILD**	**NORMAL**	**NR**	**NR**		
	ANC	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	MILD	NORMAL	NR	NR	M28	
	Hb	NORMAL	NORMAL	NORMAL	MILD	MILD	NORMAL	NORMAL	NORMAL	NR	NR		
	Plt	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NORMAL	NR	NR		
	Overall	**MILD**	**MILD**	**MILD**	**MODERATE**	**NR**	**NR**	**NR**	**NR**	**NR**	**NR**		
	ANC	NORMAL	MILD	NORMAL	MODERATE	NR	NR	NR	NR	NR	NR	M12	
	Hb	NORMAL	MILD	MILD	MILD	NR	NR	NR	NR	NR	NR		
	Plt	MILD	MILD	MILD	MILD	NR	NR	NR	NR	NR	NR		
	Overall	**MILD**	**MILD**	**MILD**	**MILD**	**MODERATE**	**NR**	**NR**	**NR**	**NR**	**NR**		
	ANC	MILD	MILD	NORMAL	MILD	MILD	NR	NR	NR	NR	NR	M18	
	Hb	NORMAL	MILD	MILD	NORMAL	MILD	NR	NR	NR	NR	NR		
	Plt	MILD	MILD	MILD	MILD	MODERATE	NR	NR	NR	NR	NR		

Abbreviations: ANC=absolute neutrophil count (in $\times 10^3/\mu L$); Hb=haemoglobin level (in g/dL); NA=not applicable; NR=not reached; Plt=platelet count (in $\times 10^3/\mu L$).

Notes: Only scheduled visits are presented. Values for baseline are those at the last visit prior to start of mobilisation. Normal indicates values ≥ LLN.

* Subject RP-L102-0418-001-1002 discontinued from parent study on Day 553 (M18) due to Investigator request and received allogeneic HSCT on Day 567 (M18.5) due to BMF.

† Subject RP-L102-0118-002-2009 discontinued from parent study on Day 830 (M27) due to an AE of NHL and received allogeneic HSCT on Day 972 (M32) due to NHL. The trilineage values at M24 are confounded by chemotherapy administration and transfusion support.

a) Month 6 ANC value was elevated in the context of a fever therefore the most proximate prior visit (Month 4) was used as nadir.

Haematologic Response is colour-coded based on modified CTC AE criteria for bone marrow failure:

Parameter	Mild	Moderate	Severe
Haemoglobin level	< LLN to ≥ 8 g/dL	< 8 g/dL	
Absolute neutrophil count	< 1.5 to ≥ 1 $\times 10^3/\mu L$	< 1 to ≥ 0.5 $\times 10^3/\mu L$	< 0.5 $\times 10^3/\mu L$
Platelet count	< 150 to ≥ 50 $\times 10^3/\mu L$	< 50 to ≥ 30 $\times 10^3/\mu L$	< 30 $\times 10^3/\mu L$

Sensitivity analysis using true baseline values

A sensitivity analysis using the haematologic values before mobilisation to calculate the 75% threshold has also been provided, which allows to account for a possible detrimental effect from mobilisation. In this analysis haematologic stability is achieved in 4 subjects (40.0%); the difference is due to a Subject whose Platelet count at baseline was significantly higher than at Month 6 (222 ×103/µL vs. 100 ×103/µL) thus increasing the 75% threshold value to be achieved. Importantly, the subject's

platelet count was 146 ×103/µL at 36 months, which is minimally below the normal reference range [150 to 400 ×103/µL].

Key secondary endpoints

DEB-induced Aberrations in PB T-Lymphocytes (≥50% pre-infusion to <50% post-infusion and ≥20% decrease from pre-infusion at 18 and 21 or 24 months post-infusion)

PB phenotypic correction as per definition was achieved in 0/11 evaluable subjects. However, because T-lymphocytes are the longest-lived haematopoietic cell population, genetic correction within this compartment is anticipated over a longer timeframe relative to myeloid progenitors and their progeny. DEB-induced aberrations have generally decreased in the third year in 4 of 5 subjects who met the primary composite endpoint.

Overall Survival, BMF-Free Survival, MDS/AML-Free Survival, BMF and MDS/AML-Free Survival

No deaths and no MDS/AML events occurred during the studies. BMF AEs were reported for two subjects; one subject had a qualifying event (Grade 3 Bone marrow failure) on Day 1070 in the LTFU Study; the subject has not required allogeneic HSCT as of the data-cut-off. Another subject had a qualifying event (Grade 3 Bone marrow failure) on Day 509 and subsequently received allogeneic HSCT on Day 567 post-RP-L102 infusion. BMF-free survival at 36 months is therefore 73.3%. [*Added by clinical assessor:* The first patient met the component of PB VCN ≥ 0.1, but none of the subjects were respondents of the primary endpoint.]

Exploratory efficacy results

BM and PB Subpopulation VCN >0.1

BM VCN demonstrates genetic correction in the BM. This genetic correction in the BM correlates with phenotypic correction in the BM (i.e., BM MMC) (r=0.8128).The largest and lowest number of patients reaching the threshold of BMMC VCN ≥0.1 occurs at month 12 (8/12 patients) and months 21, 32, and 48 (0/1 patients).

VCN in PB subpopulations illustrate that genetic correction is multilineage. For PB CD15 the largest and lowest number of patients reaching the threshold of BMMC VCN ≥0.1 are 7/12 (at month 12) and 1/1 (at month 48); for PB CD3, 5/5 (at month 36) and 0/12 (at month 6); and for PB and CD19 6/11 (at month 18) and 1/1 and 1/12 (at month 48 and month 6, respectively).

Paediatric quality of life

PedsQL Generic Core Scale and the PedsQL Multidimensional Fatigue Scale scores did not decrease appreciably from baseline over time.

QOL Parent Total Transformed Score: mean (SD) baseline value was 81.5 (16.17). Largest change from baseline was -5.2 (13.52) at month 24. At the latest timepoint (36 months) the decrease was -2.3 (8.04). QOL Self Total Transformed Score: mean (SD) baseline value was 90.156 (8.86). Largest change from baseline was -17.344 (10.38) (month 6). At the latest timepoint (36 months) the decrease was -13.8 (21.61)

Multifunctional fatigue scale Parent Total Transformed Score: mean (SD) baseline value was 87.4 (17.24). Largest change from baseline was -13.426 (15.48) at month 36 (latest timepoint). Multifunctional fatigue scale Self Total Transformed Score: mean (SD) baseline value was 88.2 (21.80). Largest change from baseline was -12.5 (14.10) at month 36 (also latest timepoint).

Ancillary analyses

No subgroup analyses have been provided by the company.

Study RP-L102-0221-LTFU

Study RP-L102-0221-LTFU is a LTFU Rocket-sponsored study to evaluate the long-term safety and efficacy of the IMP infusion for a total of 15 years post-IMP infusion in subjects who were treated in the Rocket-sponsored parent studies. Following the end of participation in RP-L102-0418, RP-L102-0319, or RP-L102-0118, subjects are offered enrolment in this LTFU protocol. No interventional treatment is administered in this study.

As of 23-Oct-2024, 9 subjects have been enrolled after completing or discontinuing from the parent studies.

The presentation of primary, secondary and exploratory efficacy data pooled all subjects in the ISE FAS population. Subject efficacy data collected in the LTFU study RP-L102-0221-LTFU were merged with the corresponding parent study information and included in all relevant tables.

3.3.4.3. Summary of main efficacy results

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections). The data for the three pivotal studies have been shown through the clinical overview and are also described in the table below together.

Table 7. Summary of efficacy for Rocket-Sponsored studies

Title: Rocket-sponsored studies		
Study identifier	1. RP-L102-0418, NCT03814408 2. RP-L102-0319, NCT04248439 3. RP-L102-0118, NCT04069533, 2018-002502-031	
Design	Phase 1/2, single-arm, single dose, single site (RP-L102-0418 and RP-L102-0319)/multi site (RP-L102-0118).	
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:	3 years as per protocol (studies still ongoing) Not applicable 15 years (in a LTFU study RP-L102-0221-LTFU)
Hypothesis	NA (single arm study)	
Treatments groups	Single arm	Patients with FA-A >1 year old. Treatment: -Mobilisation:G-CSF (12 μg/kg/12 hours for 11 doses-up to 13 doses) and plerixafor(240 μg/kg/day for 2 days) -Gene therapy: Mozafancogene autotemcel, autologous CD34+ cells transduced with a lentiviral vector carrying the FANCA gene; optimal dose ≥5.0×105 CD34+ cells/kg; single intravenous infusion Duration: single infusion Number enrolled: 15; Number treated with gene therapy: 14

Title: Rocket-sponsored studies			
Study identifier	1. RP-L102-0418, NCT03814408 2. RP-L102-0319, NCT04248439 3. RP-L102-0118, NCT04069533, 2018-002502-031		
Endpoints and definitions	Primary composite endpoint	BM MMC, PB VCN, and Hematologic stability	-Phenotypic correction by BM CFU MMC resistance ≥20% (MMC at 10 nM concentration) at Month 12 post-infusion and Month 18 or Month 21; AND -Genetic correction by PB VCN ≥0.1 at Month 18 post-infusion and Month 21 or Month 24. ; AND -Haematologic stability by haemoglobin, neutrophil, and platelet counts remaining at ≥75% of 6-month post-infusion nadir value at Month 18 post-infusion and Month 21 or 24
	Secondary endpoints		-Each component of the primary endpoint -PB T-cells with DEB-induced aberrations from pre-infusion levels ≥50% to <50% and a ≥20% decrease from pre-treatment levels -Overall survival -BMF-free survival -MDS/AML-free survival -BMF and MDS/AML-free survival
	Exploratory Efficacy Endpoints		-Genetic correction by BM VCN and PB Subpopulation VCN -Paediatric QoL
Database lock	Studies are ongoing, cut off date for current analysis 11 Sept 2023		
Results and Analysis			
Analysis description	**Primary Analysis**		
Analysis population and time point description	The efficacy analysis is be performed on the ISE FAS population, which included subjects who received the single infusion of IMP in the Rocket-sponsored studies. Continuous (quantitative) variable summaries will include the number of subjects (n) with non-missing values, mean, standard deviation (SD), median, minimum, and maximum and quartiles. Categorical (qualitative) variable summaries will include the frequency and percentage of subjects. Point estimates will be reported with corresponding 95% confidence intervals where estimable.		
Descriptive statistics and estimate variability	Treatment group		Single arm trial
	Number of subject		14 (13 evaluable by cutoff 23-Oct-24)

Title: Rocket-sponsored studies		
Study identifier	1. RP-L102-0418, NCT03814408 2. RP-L102-0319, NCT04248439 3. RP-L102-0118, NCT04069533, 2018-002502-031	
	Primary composite endpoint (% responders) BM MMC PB VCN Hematologic stability	5/13 (45.5) 6/14 (54.5)* 7/12 (63.6)* 5/11 (45.5)*
	Key secondary endpoints (% events) -PB T-cells DEB-induced aberrations -Death -BMF -MDS/AML -BMF and MDS/AML	 0/11 (0) 0/11 (0) 2/11 (18) 0/11 2/11
Notes	*Number of evaluable patients can be different per component, see definition of valuable patient in the description of main studies	

3.3.4.4. Clinical studies in special populations

Not applicable, agreed from clinical and PK perspective.

3.3.4.5. In vitro biomarker test for patient selection for efficacy

Not applicable

3.3.4.6. Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable, the pivotal studies are already described as pooled data due to the similarities in design.

3.3.4.7. Supportive study(ies)

Several studies are considered supportive studies.

FACOLEN-I and Study RP-L102-0116-LTFU

The initial LV-based FA gene therapy effort was led by the Spanish Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT) and divided into two protocols, an HSPC collection protocol (FANCOSTEM) and an investigational treatment protocol (FANCOLEN-I). FANCOLEN-I is used in the MAA as supportive study.

FANCOLEN-I was a Phase 1/2, single arm, multi-site, single dose, investigator-initiated trial to assess the safety and preliminary efficacy of infusing haematopoietic stem and progenitor cell (HSPCs) collected from the subjects, transduced with the LV, and administered without conditioning in paediatric subjects with FA-A. The total number of subjects treated was 9. Subjects were to be followed for 3 years; the study has been completed. 6 subjects completed the study and 3 discontinued. Because FANCOLEN-I was a first-in-human study and the safety profile of the IMP had not yet been evaluated, therapy could only be administered to subjects in whom BMF had progressed

to a moderate/severe degree to warrant an intervention (such as allogeneic HSCT or investigational gene therapy). Results from FANCOLEN-I established safety and clinical proof of concept for the pivotal studies. Upon completion of FANCOLEN-I, subjects were enrolled in a separate long-term follow-up (LTFU) study, RP-L102-0116-LTFU.

In FANCOLEN-I, CD34+ cells from BM and/or mobilised in PB (fresh and/or cryopreserved) from subjects with FA-A were transduced ex vivo with an LV carrying the FANCA gene. After transduction, subjects received an infusion of genetically corrected stem cells to restore haematopoiesis. Mobilisation was also performed with G-CSF (12 µg/Kg/12 h-8 days) and plerixafor (0,24 mg/kg, max 4 doses).

The main inclusion and exclusion criteria were:

Inclusion Criteria

- Subjects diagnosed with FA-A
- From 1 to 21 years old
- Lansky index >60%.
- Number of cells to transduce: at least 3 $\times 10^5$ purified CD34+ cells/kg.

Exclusion Criteria

Subjects who met any of the following criteria were excluded from the study:

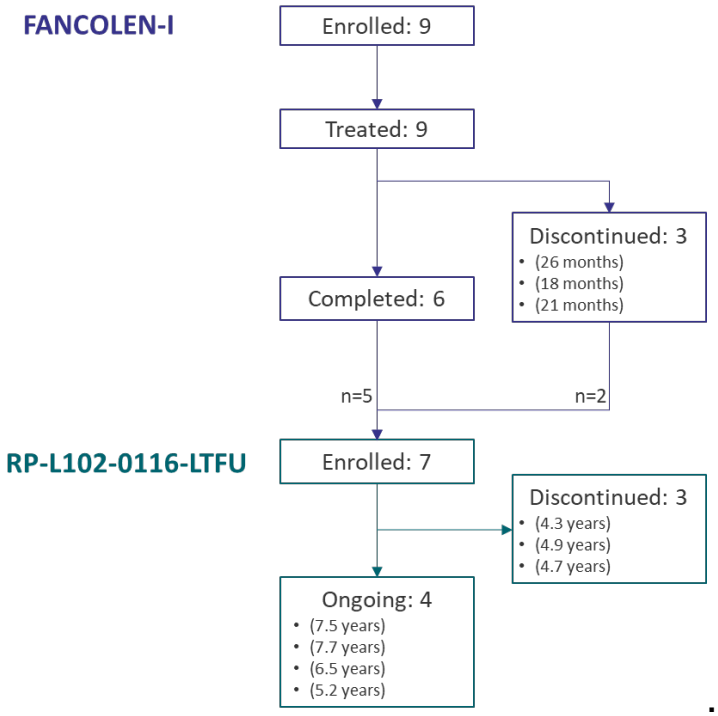
- Subjects with an HLA-identical family donor.
- Evidence of myelodysplastic syndrome or leukaemia, or cytogenetic abnormalities predictive of these conditions in bone marrow aspirate analysis. This assessment should be made by valid studies two months before the subject enters the clinical trial.
- Evidence of somatic mosaicism with improved haematology. (If T-lymphocyte chromosomal fragility analysis indicates potential mosaicism, a medically significant decrease in at least one blood lineage over time must be documented to enable eligibility)
- Any concomitant disease or condition that, in the opinion of the investigator, rendered the subject unfit to participate in the study.
- Pre-existing sensory or motor impairment ≥grade 2 according to the criteria of the National Cancer Institute (NCI).

Primary and secondary efficacy endpoints

The primary endpoint was VCN per Cell in PB and BM: detection of at least 0.1 therapeutic vector copies per nucleated cell of subject BM or PB in the second year after IP infusion (at Month 24).

The secondary endpoint was Clinical Haematologic Response: a significant increase (≥25%) in at least one of 3 haematologic parameters (ANC, hemoglobin, platelet counts) in the second year of IP infusion (at Month 24), without this parameter having decreased significantly over the previous year (Year 2). Of note, this definition is considerably different to the one in the pivotal studies.

Individual Subject Disposition



Demographic characteristics.

Their blood counts at study entry were consistent with moderate/severe BMF.

Statistic or Category	FANCOLEN-I N=9
Age at treatment (Years) – n	9
Mean (SD)	5.63 (1.601)
Median	5.53
Min, Max	3.2, 7.8
Sex	
Male	7 (77.8)
Female	2 (22.2)
Ethnicity	
Hispanic or Latino	6 (66.7)
Not Hispanic or Latino	1 (11.1)
Roma	1 (11.1)
Caucasian	1 (11.1)
Unknown or Not Reported	0
Race	
Asian	0
Black or African American	0
White	7 (77.8)
Unknown or Not Reported	0
More than One Race	0
Missing	2 (22.2)

Haematologic parameters at baseline

Hemoglobin (g/dL) – n	9
Mean (SD)	10.97 (0.786)
Median	10.80
Min, Max	9.9, 12.1
Neutrophils (×103/µL) – n	9
Mean (SD)	4.71 (8.543)
Median	1.010
Min, Max	0.66, 26.19
Platelets (Years) – n	9
Mean (SD)	49 (28.21)
Median	46.0
Min, Max	11,89

Results

Five out of 9 patients met the primary endpoint of VCN in PB or BM >0.1, and 2/9 patient achieved haematologic response (Table 13).

Table 8. Primary and selected secondary efficacy results

Parameter	Statistic Estimate	Overall N=9
Primary efficacy endpoint of VCN per Cell in PB and BM		
Detection of ≥0.1 VCN in either PB or BM at Month 24 Post-Infusion	Number of Subjects (%)	5 (55.6)
	(95% Wilson Score CI)	(26.7% to 81.1%)
Detection of ≥0.1 VCN in BM at Month 24 Post-Infusion	Number of Subjects (%)	5 (55.6)
	(95% Wilson Score CI)	(26.7% to 81.1%)
Detection of ≥0.1 VCN in PB at Month 24 Post-Infusion	Number of Subjects (%)	3 (33.3)
	(95% Wilson Score CI)	(12.1% to 64.6%)

Parameter	Statistic Estimate	Overall N=9
Secondary efficacy endpoint of Clinical Hematologic Response		
Clinical Hematologic Response [a, b]	Number of Subjects (%)	2 (22.2)
	(95% Wilson Score CI)	(6.3% to 54.7%)
Clinical Response in Hemoglobin [c]	Number of Subjects (%)	0
	(95% Wilson Score CI)	(0.0% to 29.9%)
Clinical Response in Absolute Neutrophil Count [c]	Number of Subjects (%)	1 (11.1)
	(95% Wilson Score CI)	(2.0% to 43.5%)
Clinical Response in Platelet Count [c]	Number of Subjects (%)	1 (11.1)
	(95% Wilson Score CI)	(2.0% to 43.5%)

Abbreviations: BMMC = bone marrow mononuclear cells, CI = confidence interval, PBMC = peripheral blood mononuclear cells, VCN = vector copy number.

Note: Any VCN result that was reported as not detected or "No Detectable Amplification (NDA)" was set to 0.

a. A clinical hematologic response is defined as an increase of at least 25% from baseline value at Month 24 in at least one of three hematologic parameters: Hemoglobin, Absolute Neutrophil Count, Platelet Count, without this parameter having decreased significantly in the past year.
b. A significant decrease in the past year means a relative reduction of at least 25% from baseline in at least one protocol stipulated visit that is within 365 days prior to the date of Month 24 post-infusion visit.
c. A clinical response means a 25% increase from baseline at Month 24 without this parameter having decreased significantly in the past year.

Baseline is the last visit before investigational product infusion.

Source: Table 7, Table 8

Since FANCOLEN-I enrolled patients in a more severe state of the disease and uses a different manufacturing process, the findings from FANCOLEN-I are not used to assess the efficacy of the treatment. Further, the definition of efficacy is considerably different from the one in the pivotal studies. Therefore, the findings were used to inform about the dose and manufacturing process for the pivotal studies, and as supportive evidence of the safety profile. See corresponding sections for further information.

Further, Study RP-L102-0116-LTFU is an ongoing long-term follow-up (LFTU) study that includes patients from parent study FANCOLEN-I. The study aims at evaluating long term safety and efficacy from patients enrolled in FANCOLEN-I, it is a non-interventional study, and duration is 15 years. Results are presented pooled to FANCOLEN-I

3.3.5. Discussion on clinical efficacy

The current MAA is for Fanskya, mozafancogene autotemcel, RP-L102, for the indication of the treatment of paediatric patients with Fanconi Anaemia Type A (FA-A). Mozafancogene autotemcel is considered a new active substance and the application is for conditional marketing authorisation.

Dose response studies

No formal dose finding studies have been performed. Some insights for the most adequate dose for the pivotal studies were gathered from the FANCOLEN-I study, where the doses (N=9) varied from 75,000 to 1,910,000 CD34+ cells/kg, and the results showed that a higher dose was potential determinant of efficacy; i.e., two patients who received ≥400,000 CD34+ cells/kg have shown phenotypic correction and haematologic stabilisation for up to 7 years. Further, in the Rocket-sponsored pivotal studies CD34+ cell dose ranged from 2.0 $\times 10^5$ to 4.1 $\times 10^6$ cells/kg body weight. The applicant has provided a correlation analysis for baseline CD34+ concentration and the dose for infusion, where no correlation between the parameters was identified. The applicant also provided an alternative correlation analysis excluding two subjects with manufacturing deviations that were considered likely to have a potential impact on CD34+ cell dose. Still, no statistically significant correlation was identified, albeit a trend towards a correlation could be seen. There may be different explications to this phenomenon, for example an uneven distribution of CD34-cells in the bone marrow affecting the number of cells obtained in screening for BM CD34+ concentration. It is anticipated that it is generally more difficult to achieve a sufficient cell dose in subjects with more severe BMF. Upon request, the applicant has clarified that in case of an available cell dose below the recommended dose, the clinician could choose between still using the available cell dose or starting a new round of mobilisation, which has not been tested in the clinical trials. Therefore, the applicant is asked to discuss potential risks with multiple mobilisations and whether a time frame for potential subsequent mobilisation could be given. It is understood that the cells from the first treatment would then be discarded. The applicant should also discuss whether it is likely that a second mobilisation would yield a larger cell dose than the original treatment **(OC)** This has also been reflected in section 4.4 of the SmPC. While this is appreciated, the proposed wording is considered in need of further updates **(SmPC)**.

Design and conduct of clinical studies

Study design

The application is based on the results of three pivotal studies, referred as Rocket-sponsored studies: Study RP-L102-0418, Study RP-L102-0319, and Study RP-L102-0118 (FANCOLEN-II). The reasons for conducting three separate studies remains unclear, the CSRs for the ongoing studies are not available. These studies are phase 2 single-arm, single dose studies to evaluate the efficacy and safety of mozafancogene autotemcel in paediatric subjects with FA-A. Subjects are to be followed for 3 years and at the end of the studies patients are enrolled in a LTFU study (RP-L102-0221-LTFU). Results of the LTFU study are presented together with the parent studies. Additionally, the study FANCOLEN-I is used as supportive evidence, since it informed about a more adequate disease status for successful HSC extraction, the manufacturing process of the final medicinal product, and the dose for the pivotal studies. Data from the FANCOLEN-I study are not further used for the efficacy assessment because the population was different in terms of disease status and the product was made with a different manufacturing process. Since the Rocket-sponsored studies have similar designs, data are pooled and described together through the efficacy discussion.

The pivotal studies (Rocket-sponsored studies) have enrolled a total population of 15 patients, of which 14 received treatment and 13/14 patients are evaluable at cut-off date of 13 October 2024. The study design was discussed upon during scientific advice (EMEA/H/SA/3899/1/2018/PA/PED/ADT/III), where the limitations about lacking a comparator, short study duration, and the small sample size were mentioned. The lack of a comparator is acceptable since this is a rare disease and no other options for treatment are available in this population. To minimise the uncertainty about the lack of comparator, the applicant provides information about the natural disease progression and a comparison to historical controls. Also, since FA is a heterogeneous disease, the inclusion and exclusion criteria in the pivotal

studies aimed at enrolling patients to some extent similar, i.e. disease status. The main inclusion and exclusion criteria are patients with FA-A from 1 year old, with at least 30 CD34+ cells/µL determined in one BM aspiration, not receiving other therapies for the treatment/prevention of FA BMF, and not having a HLA-identical sibling donor. The inclusion and exclusion criteria are in general considered adequate to reflect a population with FA in an early stage of the disease. Overall, the studies are considered adequate in terms of design, although it is acknowledged that the mentioned limitations challenge the evaluation of the efficacy of the medicinal product. The requested indication, 'treatment of paediatric patients with Fanconi Anaemia Type A (FA-A) in paediatric patients aged 1 to 18 years,' is broader than the population studied, since it does not exclude patients with an available HLA-identical sibling donor. Also, the age range in the indication is broader than the population included in the studies (1.8-7 years old). Nevertheless, the indication is agreed for the following reasons. The treatment is of potential benefit for patients with HLA-identical sibling donors since (1) no conditioning is required (as opposed to HSCT), which is associated to malignancies and solid tumours; (2) the treatment would be used early in the disease, when the availability of donors may still be unknown; (3) treatment with RP-L102 is compatible with a later HSCT if necessary, broadening the treatment options overall. Regarding age, the indication in patients aged 1 to 18 years is agreed. A waiver on the grounds of no significant benefit has been granted for patients <1 year of age, which is understood when balancing the risks of apheresis and the urgency of treatment. Regarding the inclusion of adolescent patients, the proposal can be agreed since available data suggests higher clinical efficacy in patients who were able to mobilised sufficient CD34+ cells. As such, age per se should not be a restricting factor for the indication, but rather disease status and CD34+ availability. Overall, the currently proposed indication is agreed, although an OC regarding the clinical benefit of the treatment in patients with moderate or severe BMF remains.

Treatment

The treatment starts with mobilization, which is conducted with G-CSF and plerixafor. The regimen is common to other mobilisation procedures accepted for gene therapies, where the G-CSF dose (12 µg/kg/12 hours for 11 doses, up to 13 doses) is higher than the posology authorised (10 mcg/kg/day for 5 - 7 consecutive days) for this medicinal product. Also, plerixafor is only authorized in the EU for use in adult and paediatric patients with lymphoma or solid malignant tumours. The proposed regimen has been justified by the applicant by its tolerability and the extended use for mobilisation procedures in adult and paediatric patients for whom mobilisation success is low. While the recommendations could be problematic for general clinical practice, the mobilisation procedure for the manufacturing and administration of RP-L102 will only be performed in specialised centres and by personnel with specific training for the use RP-L102 and experienced in the procedure of mobilisation. This warning is adequately stated in the SmPC. Apheresis would start if ≥5 PB CD34+ cells/µL were measured after mobilisation, the mobilisation and apheresis would occur two times (no cryopreservation is performed). Purified CD34+ haematopoietic cells will be transduced ex vivo with LV PGK-FANCA-WPRE, and the targeted cell dose was $\geq 5 \times 10^5$ CD34+ cells/kg subject weight. The studies were conducted under standardised cell processing methods and already incorporated manufacturing modifications (referred to as "Process B"). However, the applicant mentions differences in the manufacturing process across patients, such as manufacturing sites or virus vector manufacturer. Given the variability in response across patients, the applicant has provided efficacy and safety subgroup analyses considering these factors. Further discussion is available in the Quality AR. The protocol in the different Rocket-sponsored studies varied about the minimum number of cells that should be available for immunoselection or infusion post-transduction. However, these differences across studies are not considered an issue since the CD34+ cell doses available across the studies (ranging from 2.0×10^5 to 4.1×10^6 cells/kg), are well above the thresholds in the protocols.

No specific medications were prohibited during the study, but therapies used for treatment of BMF are considered treatment failure in the study, which is agreed.

Study objectives, primary, and secondary endpoints

The objective of the Rocket-sponsored studies is to assess the therapeutic efficacy of a hematopoietic gene therapy consisting of autologous CD34+ enriched cells transduced with a LV carrying the FANCA gene in subjects with FA-A. For study 0418 the primary aim was initially safety, but preliminary efficacy was also measured. While some differences in the definitions of the primary endpoints were present across the studies, the definitions are harmonised in the dossier and the efficacy results can still be pooled.

The primary composite endpoint consisted of two PD parameters (BM MMC resistance ≥20% at Month 12 and 18/24 post-infusion and PB VCN ≥0.1 at Month 18 and 21/24 post-infusion) and one clinical outcome (haematological stability ≥75% of 6-month post-infusion nadir value at Month 18 and 21/24 post infusion). The three components need to be met to meet the primary endpoint. The importance of the PD parameters to assess engraftment and BM function is acknowledged, although their clinical relevance is not directly obvious. Therefore, haematologic stabilization is considered the most relevant clinical outcome. While the per protocol analysis considered that presenting the data at 12/18 months with confirmatory data at 21/24 months only was sufficient to asses clinical response, the information is considered too limited. As such, a sensitivity analysis at month 36 has been presented. Further, efficacy is evaluated with the totality of the evidence, also considering the long term follow up information available, which is up to 5 years for some patients.

The threshold of BM MMC ≥20% is based on the concept of multilineage mosaicism, on data from 9 FA-A patients with multilineage mosaicism from the national Spanish registry FA, and on data from 2 patients from FANCOLEN-I. Seven out of the 9 patients with mosaicism from the FA registry achieved haematological stability and had BM MMC resistance ≥20%. However, only 1/9 patients had BM MMC close to 20%, and other MMC resistance values range from ~30% to ~80%. The 2 patients from FANCOLEN-I that developed haematologic stability presented BM MMC resistance values of 87.2% at 84 months and 37.8% at 72 months post-gene therapy. It is noticeable that evidence from patients receiving gene therapy is extremely limited, and a difference with the natural event of mosaicism cannot be discarded. From the evidence provided it is seen that BM MMC fluctuates over time (e.g., values in 1 patient varied from ~21% to ~140%). The values of BM MMC were estimated as ($Total\ CFC\ count\ at$ 10 $nM\ MMC/Total\ CFC\ count\ at$ 0 $nM\ MMC$) × 100, using absolute values at each corresponding timepoint, which could reach values >100%. For the component PB VCN, the justification for the threshold ≥0.1 is based on non-clinical data and by expert consensus and the clinical findings in FANCOLEN-I. In FANCOLEN-I those subjects in whom PB VCN ≥ 0.1 was identified at month 24, genetic correction was sustained until end of study participation (up to 36 months) and for the majority of these subjects, levels in excess of 0.1 were observed with progressive increases at subsequent timepoints. PB genetic correction was associated with concurrent BM genetic correction, demonstrating successful engraftment and proliferation of gene corrected cells in the PB. Nevertheless, it is relevant to mention that for none of these PD parameters, BM MMC nor PB VCN, reaching the established thresholds correlated to haematologic response.

For the component haematologic stability, which is considered the most clinically relevant component of the primary endpoint, the protocol definition is to compare values to 6 months post-infusion values only, for which the reasoning is understood, but does not account for the possibility of the treatment causing a decrease in haematologic counts due to mobilisation. Therefore, a sensitivity analysis using values at true baseline (i.e., at screening or just before mobilisation) has been requested (please see result further). Also, the treatment does not achieve haematologic normalisation, but only stabilisation, which only seems relevant if haematologic values are still relatively normal at baseline. Therefore, a

sensitivity analysis showing the number of patients per status of bone marrow failure (mild, moderate, severe) over time (including month 36), with respect to neutrophils, Hb and platelets together and per cell type has been requested (please see results further). Regarding the current definition of hematologic stability, the threshold of ≥75% nadir values is based on the data of the IFAR Natural History Cohort, which shows that platelets, Hb, and ANC decrease about 19%, 5% and 12% per year, respectively. Therefore, the cut-off of ≥75% is considered rather generous. However, since the maintenance of values ≥75% from 6-months post infusion is shown at month 36 as well as later timepoints in those patients available, a clinical benefit may be elicited from the primary endpoint, given that the effects can be isolated and attributable to the treatment.

Post-hoc, an estimand was provided for the primary and secondary endpoints based on the statistical analysis plan. The estimand definition is agreed.

The secondary endpoints of the pivotal studies include decrease in PB T-cells with DEB-induced aberrations, overall survival, BMF-free survival, MDS/AML-free survival, BMF and MDS/AML-free survival. The interest and clinical relevance of these endpoints is fully agreed. However, several of these outcomes, i.e., overall survival, BMF-free survival and MDS/AML-free survival are unlikely to occur during the pivotal studies (three years), especially since the population included in the studies are patients in an early disease status (average age ~4 years), which hampers the assessment of treatment effects on these outcomes.

Exploratory efficacy endpoints are also measured at several timepoints. These endpoints are genetic correction by BM VCN ≥0.1 and PB Subpopulations VCN≥0.1; and Paediatric Quality of Life using the Paediatric Quality of Life Inventory and the Paediatric Quality of Life Multidimensional Fatigue Scale.

Analysis

The three single-arm studies performed by the applicant were pooled in a meta-analysis based on individual subject data. It remains unclear whether this was a pre-planned meta-analysis or if this was decided after the individual studies were initiated or even nearly completed. The meta-analysis statistical analysis plan is dated 17 Jan 2024, indicating this was not the initially planned approach. However, the extremely small sample sizes in the individual studies makes the pooling strategy reasonable, had there been pre-specified primary endpoints and statistical hypotheses.

The sample size calculation is based on a background reversion rate of less than 5%, based on the occurrence of mosaicism, and a clinically relevant treatment effect of 35% based on expert opinion. A treatment effect of 35% is not overly ambitious, but with an acceptable safety profile and considering the severity of the disease could be agreeable. Given the assumptions, the calculation can be followed, however, the sample size calculation in the meta-analysis plan seems to be a construct based on actual recruited numbers and outcomes rather than a planning tool. Furthermore, the definition of primary endpoint and the overall null hypothesis that 5% or fewer of subjects are anticipated to achieve the primary composite endpoint cannot be verified to have been defined prior to knowledge of results from the studies. For the individual studies 0118 and 0319 only the latest version of the protocols has been submitted both dated 06 October 2023. Changes to the previous version include changes in primary endpoint, sample size and statistical methods. Hence, there is no control on type I error in the analysis and the choice of the primary composite endpoint must be regarded as data driven.

The primary population for efficacy analyses (ISE FAS) excludes patients that did not receive an infusion with the investigational product. One patient did not mobilise sufficient CD34+ cells, possibly because this patient had <30 CD34+ cells/μL in BM. A ≥30 cell/μL criterion was subsequently added to the eligibility criteria. Formally this patient was enrolled according to the protocol and is part of the ITT population. However, it can be agreed that analyses are restricted to the modified ITT population of

patients that received IMP. The primary analysis of the primary endpoint and its components was performed using an exact one-sample binomial test, which is considered appropriate for the primary endpoint. Two sensitivity analyses were planned, using different thresholds for haematologic stability (70 and 80%). Secondary time-to-event endpoints were analysed using Nelson-Aaelen cumulative hazard estimates and Kaplan-Meier, which is acceptable as well. Subgroup analyses by age, gender, manufacturing site, viral vector manufacturer and several critical quality attributes was performed using logistic regression. Given the low number of patients, this resulted in estimates with wide confidence intervals, no clear patterns were observable.

Efficacy data and additional analyses

Protocol deviations occurred in 13 of the 14 subjects in the efficacy population, of which 33 were classified as important. The main deviation was assessment not performed due to COVID-19.

During the pivotal studies a total of 26 patients were screened for eligibility, 11/26 patients failed screening, 15/26 were enrolled in the studies, and 14/26 were treated with the medicinal product. Reasons for screening failure were related to the presence of somatic mosaicism and the inclusion criteria of not reaching the minimum number of CD34+ cells/µl in BM. It is noticeable that in the Rocket-sponsored studies the number of screening failures due to somatic mosaicism was rather high (5/26 screened subjects, 5/11 screen failures), compared to the estimations the applicant has provided in other sections of the report (mosaicisms occurring in ~5% of the FA population) (**OC**)**.** There were 3 /15 enrolled patients who discontinued the study; 1 due to failing mobilisation (RP-L102-0118-004-2018 threshold of ≥5 CD34+ cells/µL in PB was not achieved), 1 per request of the clinician/investigator, and 1 due to AEs, the patients developed NHL considered non-related to the treatment. It is acknowledged that the low number of patients screened and enrolled are partly due to the rarity of the disease. However, it remains unclear why patient inclusion to the studies was limited to three countries (US, UK and Spain).

As per cut off date of 23-Oct-24, 13/14 patients are evaluable. Data from the remaining patient is required to be submitted in the next round of assessment. Median age is 3.54 years (min-max 1.5-6.9). Patients did not receive previous therapies for BMF. The median age (3.54 years) is consistent with including patients in an early stage of the disease. Discussion regarding the extrapolation of the results to adolescents has been provided and is agreed. Further, in order to gather more insights about a possible effect of age and gender, a subgroup analysis has been performed. No clear predictor effect for either of the factors can be concluded.

At baseline median ANC was $1.690x10^3$/uL (min-max 0.92-3.54), median Hb was 11.60 g/dL (7.7-13.6) and median platelets was $114.0x10^3$/uL (35-318). While there is certain variation, the median values are in the range of normal/mild severity of BMF according to FA clinical care guidelines. However, the minimum values show that some patients are in a more advance disease status, for whom the clinical benefit of the defined haematologic stability is unclear (**OC**).

The primary composite endpoint was met by 5/13 evaluable patients (38%). Per component of the primary composite endpoint, 7/14 patient reached BM MMC resistance ≥20%, 8/12 met PB VCN ≥0.1, and 7/11 reached haematologic stability defined as ≥75% nadir values at 6 months post infusion (9/11 maintained haemoglobin values, 7/11 ANC, and 8/11 platelets). Further, one non-responder subject exhibited haematological stability with normal bone marrow function for up to M36 despite not showing any sign of engraftment. This may raise the question of whether the haematological stability exhibited by the two responders with normal bone marrow function at baseline could have been reached in the absence of treatment. This needs to be addressed (**OC**)**.**

Sensitivity analysis using an additional confirmatory timepoint at month 36 has been provided, showing consistent results with the analysis per protocol. Additionally, a sensitivity analysis using as reference true baseline values (instead 6-months post infusion values) for the definition of the >75% threshold for haematologic stability has also been provided. In this analysis 4/13 patients responded, 1 less than in the analysis per protocol. This was due to a patient (2008) whose platelets were below the >75% threshold at months 18, 21, and 36 (within the range of mild BMF). The subject's platelets were relatively high at baseline (222 $\times 10^3$/µL), challenging the achievement of >75% maintenance. No transfusion of platelets that may explain the high value at baseline are reported for this patient. This patient had values above >75% from baseline at 4 years follow up. It can be agreed to consider this patient as respondent, as per analysis per protocol.

Further, an analysis per BMF status over time per patient has shown that in all respondent patients, BMF status stayed the same or improved at 36 months or later, compared to baseline values in all patients, resulting in ANC, Hb, and platelets levels in the normal or mild BMF range. For other patients, all non-respondents results show values corresponding to BMF progression, compared to their baseline at one or more timepoints in at least one haematologic parameter, being most often on ANCs.

For the three components of the primary endpoint there is large variation in response (some patients do not show any response) and in time to response across patients; BM MMC ≥20% occurred earliest at month ~6 for two patients and latest at month ~36 (this patient is non-respondent). For PB VCN ≥0.1, the earliest response was observed at month ~9 and the latest at month ~18.

Within patients, fluctuations over time are observed in the three components of the primary endpoint, which is considered reasonable for several reasons; the lack of conditioning means that several colonies are co-existing in BM and PB, and as it naturally occurs, the number of cells per colony fluctuates over time. Over time, the corrected/transduced cells are expected to have advantage due to their ability to repair better than the original patient's cells, and are expected to become the main colony. Also, FA patients are sensitive to infections, which may translate into haematologic fluctuations. Therefore, while fluctuations may be expected and punctual declines could be acceptable, a maintenance on response should be observed in the long term to accept a clinical benefit. Values >75% from month 6 are maintained up to month 36, which is reassuring. It is of interest to note case of a possible very late respondent patient. Subject was clearly a non-responder at the primary analysis when it comes to BM MMC resistance and haematological stability. However, after M36, BM MMC resistance reached 72% at Y5, PB VCN reached 0.994 at Y5, and the subject also exhibited improved haematological stability. The applicant argues that it is highly unlikely that this development is attributable to spontaneous mosaicism, since measures were taken to minimise the risk of enrolling subjects with risk for spontaneous mosaicism. Further, it is pointed out that progressively increasing lentiviral virus [LV] vector copy number were seen, which would not occur with naturally occurring multilineage mosaicism. In addition, according to the applicant, spontaneously occurring multilineage mosaicism is most likely to arise from a reversion mutation in a single long-term haematopoietic stem or progenitor cell and clinical efficacy is therefore more likely to be observed over a protracted interval of multiple years. This is acknowledged. Nevertheless, given large principal relevance of this matter, the applicant is asked to elaborate on the possibility to genetically verify that the development of subject is caused by late engraftment (**OC**)**.** Still, given the fact that the treatment does not achieve haematologic normalisation but only haematologic stability, it seems fundamental to give the treatment as early as possible in the disease, which is reflected in the SmPC.

Additionally, for the long-lasting and successful engraftment of the transduced cells knowledge about the presence of early progenitors / long term engrafting cells in the drug product would be of relevance. However, measuring the presence of long- and short-term progenitors within the drug product does not seem feasible, primarily due to the scarcity of these cells (only 1 in 1000 CD34+ cells

is likely to be a long-term HSPC), natural alterations in the surface marker of the cells in Fanconi anaemia patients, and due to, for instance, *ex vivo* manipulation. From the clinical perspective, the uncertainty regarding the presence of early and late progenitors can be accepted, since maintained response is seen until at least 36 months post infusions in the components of the primary endpoint (BM MMC, PB VCN, and haematologic stability) in patients who initially responded to the treatment. This is considered a clinically relevant duration of response in FA patients, providing reassurance that long-term progenitor cells are present in the drug product. The issue about the presence of short and long-term progenitors in the drug product is not further pursued. Please see also Non-clinical-pharmacology section for further details.

From the results of the secondary endpoints (DEB-induced Aberrations in PB T-Lymphocytes <50% and ≥20% decrease from pre-infusion levels at 18 and 21/24 months, overall survival, BMF-free survival, MDS/AML-free survival, BMF and MDS/AML-free survival) limited information can be elicited. The endpoint DEB-induced aberration in PB T-Lymphocytes was met by 0/11 evaluable patients, although the threshold of <50% is observed in 4 out of the 5 respondent patients at later timepoints. The late response is in line with the fact that T-lymphocytes are the longest-lived haematopoietic cell population and a longer timeframe is needed to see the effect (note that the assessment timepoint for this endpoint was changed from 12 months to 18 months in the open-label study protocol amendment, but it seemed to be still too early to see response). No events of death or MDS/AML occurred during the study time. However, as previously discussed, the lack of effects cannot be translated into a positive effect of the treatment due the population being in an early disease status and short follow up. Two BMF events occurred during the studies, both in patients that did not meet the primary composite endpoint. One patient had BMF at day 1070 (during the LTFU study) and did not require allogenic HSTC, and the second patient had BMF on day 509 and received allogeneic HSCT.

Regarding the exploratory efficacy outcomes (BM/PB subpopulations VCN and Paediatric QoL), no conclusions can be made. In terms of patients reaching the threshold of VCN>0.1 for BM and PB subpopulations, large fluctuations occur over time, which is in line with the fluctuations discussed for the primary endpoint. However, the interpretation of the results is hampered since the denominator is very variable over time, and at several timepoints only 1 subject has been measured. For the Paediatric QoL, two scales are used. For none of the scales a minimal clinically important difference is mentioned by the applicant. The scores tend to decrease over time, with the highest change from baseline being -17.344 (SD 10.38). Whether this is a relevant change in the context of FA is unknown. Further, available data are also limited with data from only 2 patients at some timepoints. The interpretation of the results is not possible with the data available. The issue is not pursued.

Additionally, the clinical outcomes of i) hematologic stability and ii) time to BMF, allogeneic HSCT, or death are compared to an external control (real-world cohort International FA Registry-IFAR), which is considered valuable given the lack of control in the pivotal studies. The value of a comparison with an external control and the importance of describing the natural history of the disease was discussed during scientific advices. To some extent, the applicant has followed the advice provided.

Finally, the applicant aims at applying for a conditional MA, which means that additional comprehensive clinical data leading to a full dossier should be generated. The applicant has submitted a justification in support of fulfilment of the requirements described in Article 4 of Commission Regulation (EC) No 507/2006. Please, see following section for further information (**MO**).

Additional expert consultation

Given the uncertainties on the clinical benefit, the input from a SAG/AHEG is required.

Engagement with healthcare professionals and patients was sought through written questions from the regulatory agencies to these organisations.

The questions posed to the organisations included general and specific questions related to disease progression, current treatments and unmet medical needs, acceptable endpoints, fluctuations in disease manifestations, and expected benefits and tolerable adverse events of new medicines for FA. The perspective of the healthcare professionals and patients is overall is in line with the one of regulators, highlighting the relevance of having alternatives to HSCT, the importance of administering gene therapy early in the disease, the importance of maintaining a positive and/or stable trend in hematologic values over time, and in a close to normal range of cell counts, the benefits of not performing conditioning to administer gene therapy, and the major benefit of reducing the risks of tumours in FA patients, given that this is eventually proven.

Therefore, the responses to the questions support the approval of a broader indication (without mentioning the availability of the HLA-matched sibling donor) in order to allow a feasible treatment algorithm in clinical practice. Further, the responses endorse the relevance of the clinical outcome of haematologic stability only if cytopenia's are mild and stability is maintained long term. This is acceptable given that relevant clinical outcomes, such as BMF, cannot be assessed within a reasonable timeframe. Overall, the totality of the data rather than specific endpoints or timepoints should be considered.

Assessment of paediatric data on clinical efficacy

Only paediatric subjects were included in the studies with Fanskya.

Additional efficacy data needed in the context of a conditional MA

The applicant is applying for a conditional MA. The applicant has provided a separate document to substantiate fulfilment of the requirements described in Article 4 of Commission Regulation (EC) No 507/2006. In this justification it is indicated that: Completion of the ongoing interventional studies up to the stipulated duration of 3-years follow-up will lead to the completion of the full dossier and is proposed as a specific obligation for the conditional marketing authorisation to provide comprehensive clinical data confirming that the medicine's benefits continue to outweigh its risks. In addition, the applicant is conducting long-term follow-up studies: RP-L102-0221-LTFU for subjects enrolled in applicant-sponsored clinical trials and RP-L102-0116-LTFU for subjects enrolled in FANCOLEN-I. In both LTFU-studies, subjects will be evaluated for safety and efficacy for up to 15-years post-mozafancogene autotemcel administration. This additional safety and efficacy data is planned to be provided to the Agency on an annual basis to support the renewal of the conditional marketing authorisation. Further, the applicant aims at setting up a FA Registry Study that aims at enrolling approximately 20 patients during an estimated 5-year recruitment period. The registry will collect efficacy and safety information for a period of at least 15 years, which is considered adequate and necessary to address the uncertainty of the long-term effects of the product. However, the feasibility of including 20 patients in the registry in 5 years is questioned. See further discussion in section '5.7.3. Additional considerations on the benefit-risk balance'

Upon completion of the full dossier, the remaining subject that it is not yet available for evaluation of the primary composite endpoint (due to the need of a second confirmatory timepoint to prove transitional low ANCs) will become evaluable. Completion of the 3-year follow-up (marking the end of the study, defined as last patient, last visit as applicable), for each of the remaining subjects at time of MAA, will be May 2026. Though the presented substantiation for CMA with respect to additional efficacy data to be collected within the time frame of CMA is acknowledged, the provided arguments that these

14 patients, in addition to the potential data collected in the registry study, would render the data comprehensive are not agreed. The efficacy and safety information that will be generated from the pivotal studies, LTFU studies and FA registry study is still considered too limited to agree that a fully comprehensive data will be available in a reasonable period of time. This is primarily due to the rarity of the disease and the limited amount of patients enrolled in the pivotal studies. As such, and unless new measures are proposed by the applicant, the applicant may envisage a marketing authorisation under exceptional circumstances (**MO**). Additionally, a more detailed study protocol is warranted for the FA Registry study, following the EMA Guideline on Registry-based studies.

3.3.6. Conclusions on clinical efficacy

Although the efficacy outcomes of the Rocket-sponsored studies show a positive result in 5/13 patients and it is reassuring that efficacy was maintained up to 4-5 years after treatment, the overall interpretation and comprehensiveness of the efficacy of the treatment is hampered. This is, primarily, due to the lack of comparator and inadequate comparison to the external control, the low number of patients receiving the treatment (13/14 evaluable patients), questions about the sample size calculation and type I error protection, and the lack of possible predictors of response. With these limitations, it is challenging to determine whether a responder rate of approximately 40% could be deemed sufficient to consider efficacy established. (**MO**). Further, it is unclear how haematologic stability may be relevant to patients in a moderate or severe BMF status at treatment initiation. Other questions regarding the need of providing the latest available data, insights about possible response without engraftment, and the implications of multiple mobilisation procedures are also raised. The applicant is requested to discuss these issues (OC). Additionally, currently proposed additional measures associated to the conditional MA to mitigate the limitations of the current efficacy data are not considered sufficient and the applicant may envisage the application of a marketing authorization under exceptional circumstances (**MO**).

3.3.7. Clinical safety

3.3.7.1. Patient exposure

Safety data Pooling Strategy

Safety data was pooled for all subjects from Rocket-sponsored Studies RP-L102-0418, RP-L102-0118, RP-L102-0319, and the investigator-initiated study FANCOLEN-1. Subject safety data collected in the two long-term follow-up studies (RP-L102-0221-LTFU and RP-L102-0116-LTFU) were merged with the corresponding parent study information and included in all relevant tables.

Of note, Rocket's license agreement did not grant access to the subject level data for FANCOSTEM. For this reason, mobilisation and apheresis data for FANCOLEN-I subjects are not included in the dataset.

Populations Used in Safety Evaluation

The analysis populations relevant to safety were defined as follows:

- Safety Population (SAF): The SAF population included all subjects from FANCOLEN-I, RP-L102-0418 (US Phase 1), RP-L102-0319 (US Phase 2) and RP-L102-0118 (FANCOLEN-II) who signed an Informed Consent Form and were not screen failures.

- Full Analysis Set (FAS): The FAS population included all subjects from FANCOLEN-I, RP-L102-0418 (US Phase 1), RP-L102-0319 (US Phase 2) and RP-L102-0118 (FANCOLEN-II) who received the infusion of IMP.

Exposure to Investigational Product

A summary of subject exposure to IMP in the FAS population is provided in Table 14, in which the infusion of the IMP per study is presented. The IMP was infused via a single intravenous infusion without any prior conditioning.

The mean of final product cell count in the pooled set of subjects was 6.334 $\times 10^5$ cells/mL. The corresponding mean for CD34+ cell dose/kg in the pooled population was 1.681 $\times 10^6$ cells/kg with a range of 0.073 to 4.100 $\times 10^6$ cells/kg. The total colony forming units (CFUs) showed a wide variation with a minimum and maximum between 105 and 6255.6 per 10^5 cells. Mean CFU VCN was calculated as 1.375. Mean MMC resistance at 10 nm MMC was 46% in the pooled population.

In the 14 subjects who have been treated with RP-L102 in the Rocket-sponsored studies, the dose range of exposure (CD34+ Cells/kg) was <4x105 CD34+ cells/kg in 5 patients (33.3); 4 to <10x105 CD34+ cells/kg in 2 patients (13.3) and ≥10x105 CD34+ cells/kg in 7 patients (46.7) and Missing 1 (6.7) in one patient. For subjects enrolled in FANCOLEN-I, the estimated cell doses and mean VCN in CFUs varied considerably. For subjects enrolled in Rocket-sponsored studies, cell doses and other product characteristics are comparable or improved relative to FANCOLEN-I. The CD34+ cell dose administered in the Rocket-sponsored studies ranged from 2.0 $\times 10^5$ to 4.1 $\times 10^6$ cells/kg.

Table 9. Infusion of Investigational Medicinal Product (FAS Population)

Parameter	RP-L102-0418 N=2	RP-L102-0118 N=7	RP-L102-0319 N=5	RP-L102 Total N=14	FANCOLEN-1 N=9	Pooled N=23
Final Product Cell Count ($\times 10^5$ cells/mL) – n	2	7	5	14	9	23
Mean (SD)	4.195 (2.8496)	5.766 (2.5835)	6.720 (0.9338)	5.882 (2.1654)	7.036 (9.7384)	6.334 (6.1310)
Median	4.195	4.900	7.000	6.205	2.870	5.400
Min, Max	2.18, 6.21	2.34, 9.42	5.40, 7.80	2.18, 9.42	0.68, 30.60	0.68, 30.60
Final Product Cell Viability (%) – n	2	7	5	14	9	23
Mean (SD)	85.95 (4.313)	95.36 (2.432)	88.10 (3.872)	91.42 (5.095)	89.01 (6.190)	90.48 (5.543)
Median	85.95	95.40	86.30	92.25	90.70	90.80
Min, Max	82.9, 89.0	92.0, 98.6	84.7, 94.0	82.9, 98.6	81.5, 97.4	81.5, 98.6
CD34+ Cell Dose ($\times 10^6$ cells/kg) – n	2	7	5	14	9	23
Mean (SD)	0.285 (0.1202)	1.496 (1.1034)	1.890 (1.6288)	1.464 (1.2898)	0.699 (0.5999)	1.681 (2.770)
Median	0.285	1.800	1.900	1.170	0.540	0.540
Min, Max	0.200, 0.370	0.250, 3.200	0.300, 4.100	0.200, 4.100	0.073, 1.910	0.073, 4.100
Total CFUs ($/10^5$ cells) – n	2	7	3	12	8	20
Mean (SD)	1380.0 (1018.23)	2179.9 (1272.33)	840.0 (504.78)	1711.6 (1179.11)	2680.7 (2419.97)	2099.2 (1788.78)
Median	1380.0	1913.3	700.0	1411.8	1977.8	1411.8
Min, Max	660.0, 2100.0	840.0, 4400.0	420.0, 1400.0	420.0, 4400.0	105.0, 6255.6	105.0, 6255.6
VCN in Liquid Culture – n	2	7	5	14	9	23
Mean (SD)	2.15 (0.092)	2.02 (0.758)	1.76 (1.060)	1.94 (0.796)	1.474 (1.297)	1.779 (1.003)
Median	2.15	1.70	1.46	1.70	0.91	1.65
Min, Max	2.08, 2.21	1.55, 3.68	0.62, 3.45	0.62, 3.68	0.530, 3.940	0.530, 3.940
Mean CFU VCN – n	2	7	3	12	0	12
Mean (SD)	0.770 (0.2121)	1.574 (0.5937)	1.313 (1.0308)	1.375 (0.6946)	NE	1.375 (0.6946)
Median	0.770	1.910	0.800	1.240	NE	1.240
Min, Max	0.62, 0.92	0.73, 2.16	0.64, 2.50	0.62, 2.50	NE	0.62, 2.50
Transduction Efficiency (%) – n	2	4	3	9	0	9
Mean (SD)	69.60 (3.677)	85.00 (4.690)	84.83 (8.808)	81.52 (8.662)	NE	81.52 (8.662)
Median	69.60	87.00	87.50	87.00	NE	87.00
Min, Max	67.0, 72.2	78.0, 88.0	75.0, 92.0	67.0, 92.0	NE	67.0, 92.0
CD34+ Cell Identity (%) – n	0	4	5	9	9	18
Mean (SD)	NE	19.25 (9.71)	21.98 (17.20)	20.77 (13.61)	49.85 (36.73)	35.31 (30.75)
Median	NE	19.50	23.00	23.00	70.60	27.00
Min, Max	NE	8.0, 30.0	4.0, 40.9	4.0, 40.9	1.3, 90.6	1.3, 90.6
MMC Resistance at 10 nM (%) – n	2	7	3	12	8	20
Mean (SD)	40.00 (9.899)	55.03 (20.382)	59.33 (7.234)	53.60 (16.998)	35.50 (24.680)	46.36 (21.782)
Median	40.00	50.00	63.00	50.50	30.10	45.90
Min, Max	33.0, 47.0	32.0, 93.0	51.0, 64.0	32.0, 93.0	6.3, 76.5	6.3, 93.0

Abbreviations: CFU=colony forming unit, MMC=Mitomycin C, SD=standard deviation, VCN=vector copy number.

Notes: Percentages are n/Number of subjects in the FAS Population*100. Vector copy number in bulk liquid culture of investigational medicinal product is reported as 'Copies/cell'.

Following database lock, errors in the final investigational product metrics were identified for products administered Numbers above reflect corrected values. Values in FANCOLEN-I column reflect corrected metrics based on FANCOLEN-I CSR–Table 4.

Source: RP-L102 ISS–Table 1.1.4

Subject Disposition

Subject disposition is provided in Table 15. A total of 35 pooled subjects were screened for eligibility in the studies. Of these, 11 subjects were ineligible ("screen failures", being the reasons the presence of somatic mosaicism or not meeting the inclusion criteria related to the minimum number of CD34+ cells in BM at baseline) and 24 were enrolled in their respective parent study (the SAF population). One subject received mobilisation but failed to meet the protocol threshold of ≥5 CD34+ cells/µL in peripheral blood (PB) to initiate apheresis and was discontinued from the study. The FAS population consisted of 23 subjects who received infusion of the IMP.

Table 10. Subject Disposition (All Subjects)

Parameter	RP-L102-0418	RP-L102-0118	RP-L102-0319	RP-L102 Total	FANCOLEN-1	Overall
Screened for Eligibility	2	16	8	26	9	35
Screen Failure	0	8	3	11	0	11
Enrolled in Parent Study	2	8	5	15	9	24
Sufficient PB CD34+ cells to enable apheresis	2	7	5	14	NA	14
Subject who failed mobilization	0	1	0	1	NA	1
SAF Population [a]	2	8	5	15	9	24
FAS Population [b]	2	7	5	14	9	23
ISE FAS Population	2	7	5	14	-	-
Enrolled in Parent Study	2	8	5	15	9	24
Ongoing in Parent Study	0	4 (50.0)	3 (60.0)	7 (46.7)	0	7 (29.2)
Completed Parent Study	1 (50.0)	2 (25.0)	2 (40.0)	5 (33.3)	6 (66.7)	11 (45.8)
Early Termination from Parent Study	1 (50.0)	2 (25.0)	0	3 (20.0)	3 (33.3)	6 (25.0)
Adverse Event	0	1 (12.5)		1 (6.7)	0	1 (4.2)
Investigator Request	1 (50.0)	0		**1 (6.7)**	0	1 (4.2)
Mobilization Failure	0	1 (12.5)		**1 (6.7)**	0	1 (4.2)
Ineffective Treatment	0	0		0	3 (33.3)	3 (12.2)
Enrolled in LTFU Study	2 (100)	2 (25.0)	1 (20.0)	5 (33.3)	7 (77.8)	12 (50.0)
Completed LTFU Study	0	0	0	0	0	0
Early Termination from LTFU Study	0	0	0	0	1	1 (4.2)
Duration of follow-up (years)	n=2	n=7	n=5	n=14	n=9	n=23
Mean (SD)	4.47 (0.066)	2.49 (0.832)	1.97 (1.427)	2.59 (1.281)	5.11 (1.871)	3.57 (1.958)
Median	4.47	2.27	2.87	2.90	4.92	3.15
Min, Max	4.4, 4.5	1.5, 3.8	0.4, 3.2	0.4, 4.5	2.2, 7.7	0.4, 7.7

Abbreviations: FAS=full analysis set; LTFU=long term follow-up; NA=not applicable; SAF=safety analysis population; SD=standard deviation.

a. The SAF population includes all subjects who have signed informed consent and are not screen failures.

b. The FAS population includes all subjects who received the investigational product.

c. The ISE FAS population includes all subjects who received the investigational product in parent studies RP-L102-0418, RP-L102-0319 and RP-L102-0118.

Source: Meta-Analysis–Table 3

As of 11-Sep-2023, 23 subjects have been treated with RP-L102. To date, 11 subjects (45.8%) have completed the parent studies lasting 3 years, and 5 subjects discontinued after having received the IMP infusion. As of 11-Sep-2023, 12 out of 16 eligible subjects (75.0%) have enrolled in the LTFU studies. The mean duration of follow-up is 3.57 years, up to a maximum of 7.7 years.

Demographic and background characteristics of pooled safety set

The mean age of patients at IMP infusion was 4.67 years with an age range of 1.8 to 7.8 years. The proportion of male (54.2%) and female (45.8%) subjects was similar. Most subjects were white (62.5%). Mean weight was 15.51 kg with a range between 8.4 and 25.6 kg.

Prior Therapies

No subject received any prior (i.e., from screening to the day before infusion) therapies for BMF including blood transfusions, androgens, growth factors, or other experimental treatments for FA-related BMF.

Mobilization regimen

As part of the study stipulated mobilisation regimen, all subjects received G-CSF and plerixafor.

Concomitant Therapies

No specific medications were prohibited during the study period as these therapies are not anticipated to impact the efficacy of gene therapy. Administration of additional medications/therapies to prevent or correct BMF were considered a treatment failure for the IMP. These therapies include androgens (i.e. danazol), a requirement for regularly scheduled RBC or platelet transfusions, and allogeneic HSCT.

Table 11. Patient exposure (cut off 11-SEPT-2023)

	Patients enrolled	Patients exposed*	Patients exposed to the proposed dose range	Patients with long term** safety data
Blinded studies (placebo-controlled)				
Blinded studies (active -controlled)				
Open studies	FANCOLEN-I: 9 (IMP, manufactured by Process A)	9	N/A	9
Open studies	Rocket-Sponsored: 15 (IMP, manufactured by Process B)	14	Not all patients received the recommended minimum dose, see LoQ	12
Post marketing	N/A			
Compassionate use	N/A			

* Received 1 dose of active treatment
** Minimum of 12 months follow-up after infusion.

3.3.7.2. Adverse events

Adverse Events

Safety and toxicity of RP-L102 were evaluated through an analysis of incidence, type, severity, and frequency of AEs, treatment-emergent AEs (TEAE), serious AEs (SAE), abnormal clinical laboratory results, vital signs, and newly occurring abnormalities in laboratory values for the duration of the study. The potential risks associated with the IMP include those associated with mobilisation procedures, HSC collection procedures, and infusion of the IMP.

Evaluation of the safety and tolerability of the infusion was defined by incidence and frequency of adverse events (AE), serious AEs (SAE), their severity and relationship to IMP or mobilisation/apheresis procedures, AEs of special interest (AESI), defined as any new treatment-emergent AE of haematologic malignancy, non-haematologic (solid organ) malignancy, severe BMF requiring the administration of chronic platelet or red blood cell (RBC) transfusions, or severe BMF requiring an allogeneic or other HSCT, and cytogenetic abnormalities.

All AEs, TEAEs, and SAEs were coded using the MedDRA dictionary v26.0. A treatment-emergent AE (TEAE) was defined as any AE which begins on or after the date of infusion. The number and percent of subjects reporting AEs and the number of events, grouped by MedDRA system organ class (SOC) and MedDRA preferred term (PT) were tabulated by maximum severity, seriousness, relationship to IMP, relationship to granulocyte colony-stimulating factor (G-CSF), relationship to plerixafor, and relationship to apheresis.

Pre-Infusion Adverse Events

In order to produce the IMP, haematopoietic stem cell mobilisation (treatment with G-CSF and plerixafor) followed by apheresis to obtain CD34+ cells, is required. Apheresis collections started in subjects who continued mobilisation if there were ≥5 CD34+ cells/μL circulating in the peripheral blood (PB) after the second and third administrations of plerixafor. The IMP was manufactured from the fresh

apheresis collections and consisted of autologous haematopoietic stem cells (HSCs) transduced with a lentivirus (LV) (PGK-FANCA-WPRE) that encodes for the FANCA gene.

The 9 subjects enrolled in the IIT FANCOLEN-I study are not included in the analysis of pre-infusion AEs as Rocket's license agreement did not grant access to the full mobilisation/apheresis data set of this study. However, Rocket was made aware of one subject who experienced non-serious Grade 2 pre-infusion AEs of Bronchitis and Asthma. Both resolved without sequelae.

A summary of pre-infusion AEs reported in the safety population of Rocket-sponsored studies is provided in Table 17. Pre-infusion AEs are defined as AEs that started prior to RP-L102 administration.

Overall percentage of pre-infusion AEs

All 15 subjects reported at least 1 pre-infusion AE (100%). Grade 3 pre-infusion AEs were reported in 10 subjects (66.7%), and Grade 4 pre-infusion AEs in 4 subjects (26.7%).

Regarding severity of EAs, two subjects experienced 3 Grade 3 pre-infusion SAEs of Hypersensitivity, Bronchiolitis and Respiratory distress (13.3%). None were assessed as related to mobilisation/apheresis procedures. All 15 subjects experienced pre-infusion AEs assessed as at least possibly related to mobilisation and/or apheresis procedures.

Table 12. Summary of Pre-Infusion Adverse Events (SAF Population)

AE Category	RP-L102-0418 N=2	RP-L102-0118 N=8	RP-L102-0319 N=5	RP-L102 Total N=15
Subjects with at least 1 AE	**2 (100)**	**8 (100)**	**5 (100)**	**15 (100)**
Maximum AE Severity [1]				
Grade 1 (Mild)	0	0	0	0
Grade 2 (Moderate)	0	1 (12.5)	0	1 (6.7)
Grade 3 (Severe)	0	6 (75.0)	4 (80.0)	10 (66.7)
Grade 4 (Life-threat)	2 (100)	1 (12.5)	1 (20.0)	4 (26.7)
Grade 5 (Death)	0	0	0	0
Subjects with SAE	0	1 (12.5)	1 (20.0)	2 (13.3)
Subjects with AE leading to study discontinuation	0	0	0	0
Subjects with AE leading to drug discontinuation	0	0	0	0
Subjects with AE related to any pre-infusion treatment or procedure	2 (100)	8 (100)	5 (100)	15 (100)
Subjects with AE related to G-CSF	1 (50.0)	4 (50.0)	3 (60.0)	8 (53.3)
Subjects with AE related to plerixafor [2]	2 (100)	2 (25.0)	3 (60.0)	7 (46.7)
Subjects with AE related to apheresis procedure [2]	2 (100)	7 (87.5)	5 (100)	14 (93.3)

Abbreviations: AE = adverse event, G-CSF = granulocyte colony-stimulating factor, IMP = investigational medicinal product, SAE = serious adverse event.

Notes: Results are n (n/Number of subjects in the safety population within each time period*100). Pre-infusion AEs are defined as AEs that started prior to RP-L102 administration. AEs were coded using MedDRA version 26.0.

[1] Subjects are counted only once at worst severity. If a severity designation is missing, the AE will be considered severe.

[2] AEs with a relationship of possible, probable, definite, or missing are considered related.

Source: RP-L102 ISS–Table 1.2.1

Most commonly reported (severe) pre-infusion AEs

Most subjects had at least one severe pre-infusion AE. The most commonly reported (severe) pre-infusion AEs (at least 2 Subjects Overall) were Anaemia, Platelet count decreased, and Thrombocytopenia.

Pre-infusion related adverse events

Most subjects experienced pre-infusion AEs that were assessed as related to more than one study procedure. The safety profile is consistent with the known manifestations of the underlying disease

and/or risks associated with G-CSF, plerixafor, and/or apheresis procedures according to the applicant. A summary of pre-infusion related AEs is presented in Table 18.

Table 13. Summary of Pre-Infusion related Adverse Events (SAF Population)

	RP-L102 Total N=15						
Parameter	Any n (%)	G-CSF n (%)	Plerixafor n (%)	Apheresis n (%)	G-CSF & Plerixafor n (%)	G-CSF & Apheresis n (%)	Plerixafor & Apheresis n (%)
Subjects with at least 1 Adverse Event related to Mobilisation/Apheresis Procedure	**15 (100)**	**8 (53.3)**	**7 (46.7)**	**14 (93.3)**	**9 (60.0)**	**15 (100)**	**14 (93.3)**
Anaemia	5 (33.3)	0	1 (6.7)	5 (33.3)	1 (6.7)	5 (33.3)	5 (33.3)
Platelet count decreased	8 (53.3)		1 (6.7)	8 (53.3)	1 (6.7)	8 (53.3)	8 (53.3)
Thrombocytopenia	4 (26.7)	1 (6.7)	2 (13.3)	3 (20.0)	3 (20.0)	4 (26.7)	3 (20.0)
Vomiting	3 (20.0)	0	2 (13.3)	1 (6.7)	2 (13.3)	1 (6.7)	3 (20.0)
Alanine aminotransferase increased	2 (13.3)	2 (13.3)	2 (13.3)	0	2 (13.3)	2 (13.3)	2 (13.3)
Aspartate aminotransferase increased	2 (13.3)	2 (13.3)	2 (13.3)	0	2 (13.3)	2 (13.3)	2 (13.3)
Bone pain	2 (13.3)	2 (13.3)	2 (13.3)	1 (6.7)	2 (13.3)	2 (13.3)	2 (13.3)
Hypoalbuminaemia	2 (13.3)	0	0	2 (13.3)	0	2 (13.3)	2 (13.3)
Leukopenia	2 (13.3)	2 (13.3)	2 (13.3)	2 (13.3)	2 (13.3)	2 (13.3)	2 (13.3)
Lymphocyte count decreased	2 (13.3)	0	0	2 (13.3)	0	2 (13.3)	2 (13.3)
Nausea	2 (13.3)	1 (6.7)	1 (6.7)	2 (13.3)	1 (6.7)	2 (13.3)	2 (13.3)
Tachycardia	2 (13.3)	1 (6.7)	1 (6.7)	2 (13.3)	1 (6.7)	2 (13.3)	2 (13.3)
Abdominal pain	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)
Anxiety	1 (6.7)	0	0	1 (6.7)	0	1 (6.7)	1 (6.7)
Blood alkaline phosphatase increased	1 (6.7)	1 (6.7)	1 (6.7)		1 (6.7)	1 (6.7)	1 (6.7)
Catheter site pain	1 (6.7)	0	0	1 (6.7)	0	1 (6.7)	1 (6.7)
Decreased appetite	1 (6.7)	0	1 (6.7)	0	1 (6.7)	0	1 (6.7)
Diarrhoea	1 (6.7)	0	1 (6.7)	0	1 (6.7)	0	1 (6.7)
Fatigue	1 (6.7)	0	0	1 (6.7)	0	1 (6.7)	1 (6.7)
Gamma-glutamyltransferase increased	1 (6.7)	1 (6.7)	1 (6.7)		1 (6.7)	1 (6.7)	1 (6.7)
Headache	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)
Hyperhidrosis	1 (6.7)	0	0	1 (6.7)	0	1 (6.7)	1 (6.7)
Hypocalcaemia	1 (6.7)	0	0	1 (6.7)	0	1 (6.7)	1 (6.7)
Hypokalaemia	1 (6.7)	0	0	1 (6.7)	0	1 (6.7)	1 (6.7)
Joint range of motion decreased	1 (6.7)	0	0	1 (6.7)	0	1 (6.7)	1 (6.7)
Myalgia	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)
Neck pain	1 (6.7)	0	0	1 (6.7)	0	1 (6.7)	1 (6.7)
Neutrophil count decreased	1 (6.7)	0	0	1 (6.7)	0	1 (6.7)	1 (6.7)
Pallor	1 (6.7)	0	0	1 (6.7)	0	1 (6.7)	1 (6.7)
Pruritus	1 (6.7)	1 (6.7)	0	0	1 (6.7)	1 (6.7)	0
Pyrexia	1 (6.7)	1 (6.7)	0	0	1 (6.7)	1 (6.7)	0

Abbreviations: G-CSF = granulocyte colony-stimulating factor.

Source: RP-L102 ISS–Table 1.2.3.3

Treatment-emergent Adverse Events <u>after infusion of IMP RP-L102</u>

A summary of TEAE after infusion of RP-L102 reported in this population (FANCOLEN-I and Rocket-sponsored studies) is provided in Table 19.

With regard to frequency of TEAEs, all 23 subjects reported at least one TEAE.

Most TEAEs reported across all studies were mild/moderate in severity, non-serious, and assessed as not related to RP-L102.

To date, two *serious TEAEs* have been assessed as possibly related to RP-L102 and include Staphylococcal bacteraemia and a transient infusion-related reaction. Both recovered without clinical sequalae, see details below. One unrelated death occurred in RP-L102-0116-LTFU and is further described below.

Table 14. Summary of TEAEs after infusion of IMP (FAS)

Category	RP-L102-0418 N=2	RP-L102-0118 N=7	RP-L102-0319 N=5	RP-L102 Total N=14	FANCOLEN-1 N=9	Overall N=23
Subjects with at least 1 AE	**2 (100)**	**7 (100)**	**5 (100)**	**14 (100)**	**9 (100)**	**23 (100)**
Maximum AE Severity [1]						
Grade 1 (Mild)	0	1 (14.3)	0	1 (7.1)	1 (11.1)	2 (8.7)
Grade 2 (Moderate)	0	2 (28.6)	3 (60.0)	5 (35.7)	0	5 (21.7)
Grade 3 (Severe)	0	3 (42.9)	2 (40.0)	5 (35.7)	2 (22.2)	7 (30.4)
Grade 4 (Life-threatening)	2 (100)	1 (14.3)	0	3 (21.4)	5 (55.6)	8 (34.8)
Grade 5 (Death)	0	0	0	0	1 (11.1)	1 (4.3)
Subjects with SAE	2 (100)	1 (14.3)	2 (40.0)	5 (35.7)	6 (66.7)	11 (47.8)
Subjects with AE leading to study discontinuation	1 (50.0)	1 (14.3)	0	2 (14.3)	0	2 (8.7)
Subjects with AE leading to drug discontinuation	0	0	0	0	0	0
Subjects with AE related to IMP [2]	0	1 (14.3)	0	1 (7.1)	3 (33.3)	4 (17.4)
Subjects with AE related to any pre-infusion treatment or procedure	0	3 (42.9)	5 (100)	8 (57.1)	NA	8 (34.8)
Subjects with AE related to G-CSF		2 (28.6)	2 (40.0)	4 (28.6)	NA	4 (17.4)
Subjects with AE related to plerixafor [2]		2 (28.6)	2 (40.0)	4 (28.6)	NA	4 (17.4)
Subjects with AE related to apheresis procedure [2]		1 (14.3)	4 (80.0)	5 (35.7)	NA	5 (21.7)

Abbreviations: AE = adverse event, G-CSF = granulocyte colony-stimulating factor, IMP = investigational medicinal product, SAE = serious adverse event.
Note: Results are n (n/Number of subjects in the safety population within each time period*100). Post-infusion AEs include all treatment-emergent AEs. AEs were coded using MedDRA version 26.0.
[1] Subjects are counted only once at worst severity. If a severity designation is missing, the AE will be considered severe.
[2] AEs with a relationship of possible, probable, definite, or missing are considered related.
Source: RP-L102 ISS–Table 1.2.1

Treatment-emergent Adverse Events by System Organ Class (SOC) after infusion of IMP

The incidence of TEAEs by SOC after infusion of IMP is provided in Table 20. The most commonly reported TEAEs by SOC belonged to the SOCs of Infections and infestations (20 subjects, 87.0%), General disorders and administration site conditions (15 subjects, 65.2%), Blood and lymphatic system disorders (13 subjects, 56.5%), Gastrointestinal disorders and Respiratory, thoracic, mediastinal disorders (12 subjects each, 52.2%).

Table 15. Incidence of TEAEs by primary system organ class (FAS)

System Organ Class	RP-L102-0418 N=2	RP-L102-0118 N=7	RP-L102-0319 N=5	RP-L102 Total N=14	FANCOLEN-1 N=9	Overall N=23
Subjects with at least 1 AE	**2 (100)**	**7 (100)**	**5 (100)**	**14 (100)**	**9 (100)**	**23 (100)**
Infections and infestations	2 (100)	7 (100)	2 (40.0)	11 (78.6)	9 (100)	20 (87.0)
General disorders and administration site conditions	1 (50.0)	6 (85.7)	1 (20.0)	8 (57.1)	7 (77.8)	15 (65.2)
Blood & lymphatic system disorders	2 (100)	4 (57.1)	2 (40.0)	8 (57.1)	5 (55.6)	13 (56.5)
Gastrointestinal disorders	0	4 (57.1)	2 (40.0)	6 (42.9)	6 (66.7)	12 (52.2)
Respiratory, thoracic, mediastinal disorders	1 (50.0)	3 (42.9)	2 (40.0)	6 (42.9)	6 (66.7)	12 (52.2)
Injury, poisoning, and procedural complications	1 (50.0)	3 (42.9)	2 (40.0)	6 (42.9)	3 (33.3)	9 (39.1)
Investigations	2 (100)	3 (42.9)	4 (80.0)	9 (64.3)	0	9 (39.1)
Musculoskeletal and connective tissue disorders	1 (50.0)	2 (28.6)	1 (20.0)	4 (28.6)	2 (22.2)	6 (26.1)
Metabolism and nutrition disorders	0	1 (14.3)	2 (40.0)	3 (21.4)	2 (22.2)	5 (21.7)
Skin & subcutaneous tissue disorders	1 (50.0)	2 (28.6)	1 (20.0)	4 (28.6)	1 (11.1)	5 (21.7)
Eye disorders	0	1 (14.3)	1 (20.0)	2 (14.3)	1 (11.1)	3 (13.0)
Congenital, familial and genetic disorders	0	1 (14.3)	0	1 (7.1)	1 (11.1)	2 (8.7)
Immune system disorders	0	1 (14.3)	0	1 (7.1)	1 (11.1)	2 (8.7)
Cardiac disorders	1 (50.0)	0	0	1 (7.1)	0	1 (4.3)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0	1 (14.3)	0	1 (7.1)	0	1 (4.3)
Nervous system disorders	0	1 (14.3)	0	1 (7.1)	0	1 (4.3)
Psychiatric disorders	0	0	1 (20.0)	1 (7.1)	0	1 (4.3)
Hepatobiliary disorders	0	0	0	0	1 (11.1)	1 (4.3)
Reproductive system and breast disorders	0	0	0	0	1 (11.1)	1 (4.3)
Surgical and medical procedures	0	0	0	0	1 (11.1)	1 (4.3)
Vascular disorders	0	0	0	0	1 (11.1)	1 (4.3)

Source: RP-L201 ISS–Table 1.2.2.1

Treatment-emergent Adverse Events after infusion of IMP by Preferred Term

The incidence of TEAEs by Preferred Term (PT) after infusion is provided in Table 21. The most commonly reported PTs were Pyrexia (14 subjects, 60.9%), Anaemia (10 subjects, 43.5%), Thrombocytopenia (10 subjects, 43.5.%), Upper respiratory tract infection (9 subjects, 39.1%), and Neutropenia (8 subjects, 34.8%).

Table 16. Incidence of Frequent (at Least 10% Overall) TEAEs after infusion of IMP by Preferred Term (FAS)

Preferred Term	RP-L102-0418 N=2	RP-L102-0118 N=7	RP-L102-0319 N=5	RP-L102 Total N=14	FANCOLEN-1 N=9	Overall N=23
Subjects with at least 1 AE	**2 (100)**	**7 (100)**	**5 (100)**	**14 (100)**	**9 (100)**	**23 (100)**
Pyrexia	1 (50.0)	6 (85.7)	0	7 (50.0)	7 (77.8)	14 (60.9)
Anaemia	2 (100)	2 (28.6)	2 (40.0)	6 (42.9)	4 (44.4)	10 (43.5)
Thrombocytopenia	2 (100)	3 (42.9)	1 (20.0)	6 (42.9)	4 (44.4)	10 (43.5)
Upper respiratory tract infections	0	1 (14.3)	1 (20.0)	2 (14.3)	7 (77.8)	9 (39.1)
Neutropenia	1 (50.0)	2 (28.6)	0	3 (21.4)	5 (55.6)	8 (34.8)
Vomiting	0	3 (42,9)	1 (20.0)	4 (28.6)	3 (33.3)	7 (30.4)
COVID-19	0	4 (57.1)	1 (20.0)	5 (35.7)	1 (11.1)	6 (26.1)
Nasopharyngitis	0	3 (42.9)	0	3 (21.4)	3 (33.3)	6 (26.1)
Neutrophil count decreased	1 (50.0)	2 (28.6)	2 (40.0	5 (35.7)	0	5 (21.7)
Diarrhoea	0	1 (14.3)	2 (40.0)	3 (21.4)	2 (22.2)	5 (21.7)
Alanine aminotransferase increased	0	2 (28.6)	2 (40.0)	4 (28.6	0	4 (17.4)
Cough	1 (50.0)	3 (42.9)	0	4 (28.6)	0	4 (17.4)
Platelet count decreased	0	0	4 (80.0)	4 (28.6)	0	4 (17.4)
Respiratory tract infection	0	1 (14.3)	0	1 (7.1)	3 (33.3)	4 (17.4)
Aspartate aminotransferase increased	0	2 (28.6)	1 (20.0)	3 (21.4)	0	3 (13.0)
Leukopenia	2 (100)	1 (14.3)	0	3 (21.4)	0	3 (13.0)
Pain in extremity	1 (50.0)	2 (28.6)	0	3 (21.4)	0	3 (13.0)
Skin infection	1 (50.0)	2 (28.6)	0	3 (21.4)	0	3 (13.0)
Viral infection	0	1 (14.3)	1 (20.0)	2 (14.3)	1 (11.1)	3 (13.0)
Epistaxis	0	0	1 (20.0)	1 (7.1)	2 (22.2)	3 (13.0)
Gastroenteritis	0	0	1 (20.0)	1 (7.1)	2 (22.2)	3 (13.0)
Varicella	0	1 (14.3)	0	1 (7.1)	2 (22.2)	3 (13.0)
Laryngitis	0	0	0	0	3 (33.3)	3 (13.0)

Source: RP-L102 ISS–Table 1.2.3.1

Severity of Treatment-emergent Adverse Events after infusion of IMP

With regard to severity of TEAE after infusion of IMP, the incidence of Grade ≥3 TEAEs by PT is provided. Most TEAEs were mild/moderate in severity, non-serious, and assessed as not related to RP-L102.

In Rocket-sponsored studies, 8 of 14 subjects experienced Grade ≥3 TEAEs (57.1%). In the FANCOLEN-I trial 8 of 9 subjects experienced Grade ≥3 TEAEs (88.9%). Overall, in all studies 16 of 23 subjects experienced Grade ≥3 TEAEs (69.6%).

The most frequently reported Grade ≥3 TEAEs in all studies were Neutropenia (8 subjects, 34.8%), Thrombocytopenia (7 subjects, 30.4%), Anaemia (6 subjects, 26.1%), Neutrophil count decreased (5 subjects, 21.7%), and Pyrexia (3 subjects, 13.0%). All other Grade ≥3 TEAEs were reported in ≤2 subjects each. Four subjects experienced severe TEAEs assessed as related to RP-L102, including Neutropenia reported in 2 subjects, as well as Pyrexia and Staphylococcal bacteraemia in one subject each; all resolved without sequelae.

A subject enrolled on RP-L102-0116-LTFU experienced an unrelated fatal SAE of Respiratory failure (Grade 5) secondary to persistent fungal and concomitant cytomegalovirus infections following allogeneic HSCT.

- ***Other Significant Treatment-emergent Adverse Events after infusion of IMP*** Treatment-emergent Adverse Events Requiring Transfusion (platelet or RBC) after IMP

A total of 3 subjects (13%) reported at least 1 TEAE requiring a transfusion (Table 22).

- A subject received platelet transfusions for Thrombocytopenia and for Fall (one each) and 4 RBC transfusions for BMF. The injury due to this Fall was assessed as not related to RP-L102.
- A subject received one platelet transfusion for Platelet count decreased, 11 RBC transfusions for anaemia and one for BMF.
- A subject received 2 platelet and 2 RBC transfusions, all for NHL (RP-L102 ISS–Listing 1.10.1).

Table 17. Incidence of TEAEs Requiring Transfusion by Preferred Term (FAS)

Preferred Term	RP-L102-0418 N=2	RP-L102-0118 N=7	RP-L102-0319 N=5	RP-L102 Total N=14	FANCOLEN-1 N=9	Pooled N=23
Subjects with at least 1 AE requiring Transfusion	**2 (100)**	**1 (14.3)**	**0**	**3 (21.4)**	**0**	**3 (13.0)**
Bone marrow failure	2 (100)	0		2 (14.3)		2 (8.7)
Anaemia	1 (50.0)	0		1 (7.1)		1 (4.3)
Fall	1 (50.0)	0		1 (7.1)		1 (4.3)
Non-Hodgkin's lymphoma	0	1 (14.3)		1 (7.1)		1 (4.3)
Thrombocytopenia	1 (50.0)	0		1 (7.1)		1 (4.3)

Source: RP-L102 ISS–Table 1.2.4.4

- *Treatment-emergent Adverse Events Related to Pre-Infusion Study Procedures or treatment*

The 9 subjects enrolled in FANCOLEN-I are not included in the analysis of TEAEs related to pre-infusion treatment and procedures as Rocket's license agreement did not grant access to mobilisation/apheresis data.

In the Rocket-sponsored studies, 8 subjects (57.1%) treated with RP-L102 experienced TEAEs assessed as at least possibly related to mobilisation and/or apheresis procedures as summarised in Table 23. The most frequent TEAEs related to mobilisation and/or apheresis procedures were in the SOC of Investigations, with the only PTs reported in more than a single subject being Platelet count decreased (3 subjects, 21.4%) and Neutrophil count decreased (2 subjects, 14.3%). Most related TEAEs were mild/moderate in severity and resolved without sequelae. According to the applicant, the safety profile is consistent with the known manifestations of the underlying disease and/or risks associated with G-CSF, plerixafor, and/or apheresis procedures.

Table 18. Incidence of TEAEs Related to Any Pre-infusion Procedure or Treatment by Preferred Term (FAS)

Preferred Term	RP-L102-0418 N=2	RP-L102-0118 N=7	RP-L102-0319 N=5	RP-L102 Total N=14
Subjects with at least 1 AE related to Mobilization/Apheresis Procedure [1]	**0**	**3 (42.9)**	**5 (100)**	**8 (57.1)**
Platelet count decreased		0	3 (60.0)	3 (21.4)
Neutrophil count decreased		1 (14.3)	1 (20.0)	2 (14.3)
Alanine aminotransferase increased		0	1 (20.0)	1 (7.1)
Neutropenia		1 (14.3)	0	1 (7.1)
Pain in extremity		1 (14.3)	0	1 (7.1)
Thrombocytopenia		0	1 (20.0)	1 (7.1)
Vomiting		0	1 (20.0)	1 (7.1)
White blood cell count decreased		0	1 (20.0)	1 (7.1)
Subjects with at least 1 AE related to G-CSF		**2 (28.6)**	**2 (40.0)**	**4 (28.6)**
Alanine aminotransferase increased		0	1 (20.0)	1 (7.1)
Neutropenia		1 (14.3)	0	1 (7.1)
Pain in extremity		1 (14.3)	0	1 (7.1)
Platelet count decreased		0	1 (20.0)	1 (7.1)
Subjects with at least 1 AE related to plerixafor		**2 (28.6)**	**2 (40.0)**	**4 (28.6)**
Alanine aminotransferase increased		0	1 (20.0)	1 (7.1)
Neutropenia		1 (14.3)	0	1 (7.1)
Pain in extremity		1 (14.3)	0	1 (7.1)
Platelet count decreased		0	1 (20.0)	1 (7.1)
Subjects with at least 1 AE related to apheresis		**1 (14.3)**	**4 (80.0)**	**5 (35.7)**
Platelet count decreased		0	3 (60.0)	3 (21.4)
Neutrophil count decreased		1 (14.3)	1 (20.0)	2 (14.3)
Thrombocytopenia		0	1 (20.0)	1 (7.1)
Vomiting		0	1 (20.0)	1 (7.1)
White blood cell count decreased		0	1 (20.0)	1 (7.1)

Abbreviations: AE = adverse event, G-CSF = granulocyte colony-stimulating factor.
[1] Related = Probable, possible, definite or missing. Not related = Unrelated or unlikely related.
Source: RP-L102 ISS–Table 1.2.3.3

Treatment-emergent Adverse Events Related to IMP RP-L102

The incidence of TEAEs related to IMP by Preferred Term in the FAS population is summarised in Table 24. A total of 4 subjects reported TEAEs assessed as possibly related to RP-L102, including 1 subject from Rocket-sponsored studies and 3 subjects enrolled in FANCOLEN-I.

A subject experienced a transient serious TEAE of Infusion related reaction (Grade 2) deemed related to RP-L102. The event resolved within 2 hours with supportive care and without any clinical sequelae.

The 3 subjects in FANCOLEN-I included a subject who experienced non-serious AEs of Pyrexia (Grade 3), which resolved within 1 day, and Neutropenia (Grade 4), which resolved within 25 days. A subject experienced a serious TEAE of Staphylococcal bacteraemia (Grade 3) possibly related to RP-L102. The subject recovered within 14 days with antibiotic treatment and no clinical sequelae. Additionally, a subject experienced non-serious AEs of Anaemia (Grade 3) and Neutropenia (Grade 4) and was eventually discontinued due to ineffective treatment.

Table 19. Incidence of TEAEs Related to IMP by Preferred Term (FAS)

Preferred Term	RP-L102-0418 N=2	RP-L102-0118 N=7	RP-L102-0319 N=5	RP-L102 Total N=14	FANCOLEN-1 N=9	Overall N=23
Subjects with at least 1 AE related to IMP [1]	**0**	**1 (14.3)**	**0**	**1 (7.1)**	**3 (33.3)**	**4 (17.4)**
Neutropenia	0	0	0	0	2 (22.2)	2 (8.7)
Anaemia	0	0	0	0	1 (11.1)	1 (4.3)
Pyrexia	0	0	0	0	1 (11.1)	1 (4.3)
Staphylococcal bacteraemia	0	0	0	0	1 (11.1)	1 (4.3)
Infusion related reaction	0	1 (14.3)	0	1 (7.1)	0	1 (4.3)

Abbreviations: AE = adverse event, G-CSF = granulocyte colony-stimulating factor; IMP=investigational medicinal product.
[1] Related = Probable, possible, definite or missing. Not related = Unrelated or unlikely related.
Source: RP-L102 ISS–Table 1.2.3.2

Adverse drug reactions

The applicant has not provided a separate analysis of ADRs.

3.3.7.3. Serious adverse events, deaths, and other significant events

Adverse Events of Special Interest (AESI)

AEs of special interest (AESI), defined as any new treatment-emergent AE of haematologic malignancy, non-haematologic (solid organ) malignancy, severe BMF requiring the administration of chronic platelet or red blood cell (RBC) transfusions, or severe BMF requiring an allogeneic or other HSCT, and cytogenetic abnormalities.

The AESIs selected were as follows:

- Any new haematologic malignancy: Haematologic malignant tumours (MedDRA SMQ)
- Any new non-haematologic (solid organ) malignancy: Non-haematologic tumours of unspecified malignancy (MedDRA SMQ)
- Severe BMF requiring the administration of chronic platelet or red blood cell transfusions: Transfusion CRF with reason = MedDRA PT of Bone marrow failure + Severity grade ≥3
- Severe BMF requiring an allogeneic or other HSCT: Transplant CRF with reason = MedDRA PT of Bone marrow failure + Severity grade ≥3
- Cytogenic abnormalities

Rationale for the AESI selected:

- Lentivirus (LV) vectors are retroviruses which integrate into the host genome to enable long-term transgene expression (Bulcha 2021). Although insertional mutagenesis was reported in several older gene studies employing gamma-retroviral vectors, this has not been observed in any of the LV-based haematopoietic cell targeted studies other than the elivaldogene autotemcel cerebral adrenoleukodystrophy (CALD) program (Long-Boyle 2023). LVs are notable for preferential transgene insertion into less oncogenic portions of the host cell genome relative to other vectors, including gamma-retroviral vectors, which were associated with leukemogenic events in earlier gene therapy studies. LV-mediated insertion occurs predominantly in transcriptionally active genes, and not in proximity to promotor sites or growth-regulatory genes (Schroder 2002; Mitchell 2004; Wu 2003). Third-generation self-inactivating LVs additionally were designed with additional safety-

focused features, including absence of enhancer sequences or other components that could upregulate expression of genes other than the transgene (Schambach 2013).

- To date, 3 of 67 CALD patients developed myelodysplastic syndrome (MDS) following treatment with elivaldogene autotemcel (two cases of MDS with single lineage dysplasia and one case of MDS with excess blasts), which were assessed as lentiviral mediated. Of note, this vector utilises a ubiquitous viral MND promotor, which has been associated with in vitro transactivation activity and is unique relative to the promotors used in the RP-L102 and other LV-based programs (Koldej 2013; Astrakhan 2012; Schwarzer 2021; Eichler 2017).
- Fanconi anaemia (FA) is a cancer predisposition syndrome. Due to underlying chromosomal instability, FA patients surviving childhood are at increased risk for haematologic malignancies and solid tumours with cumulative incidences of MDS and acute myeloid leukemia exceeding 20% by age 20 and 30% by age 30 (Kutler 2003). Incidences of solid organ malignancies (including but not limited to squamous cell carcinoma of the head and neck) increase steadily during the second and third decades of life and approach 40% by age 40 (Alter 2018).
- Fanconi anaemia is a rare deoxyribonucleic acid-repair disorder that results in bone marrow failure (BMF). BMF is a frequent, early, and devastating component of FA. Most FA patients (approximately 80%) develop BMF during the initial decade of life (Kutler 2003). Human leukocyte antigen-matched sibling or alternate donor allogeneic haematopoietic stem cell transplantation represent the only therapy available to potentially and sustainably alter the course of severe FA-mediated BMF (MacMillan 2015; Mehta 2017).

AESI of Any new Haematologic Malignancies

One subject experienced Grade 4 SAE of non-Hodgkin lymphoma (NHL) at 22 months post-infusion unrelated to RP-L102 and attributed to underlying disease. Assessment of the surgical tumour biopsy sample demonstrated no appreciable LV integration. According to the narratives a mean vector copy number (VCN) of 0.00314 was demonstrated in the surgical tumour biopsy sample. In contrast, PB and BM VCN were 0.2573 and 0.4227, respectively, at the time of diagnosis. These PB and BM VCN values were approximately 80- to 130-fold higher than the tumour sample VCN and indicated that there was negligible evidence of LV genetic markings within the tumour, and that the malignancy was extremely unlikely to be related to gene therapy, according to the applicant. Genetic testing via whole exome sequencing performed on the subject and his parents did not reveal any concurrent genetic syndromes that may have further predisposed this subject to developing lymphoma. The subject underwent induction chemotherapy followed by successful allogeneic HSCT on 14-Feb-2023. The SAE is considered recovered/resolved.

AESI of any Non-Haematologic Malignancies

No new non-haematologic (solid organ) malignancy was reported in the time period.

AESI of Any Severe Bone Marrow Failure Requiring Transfusion

Two subjects treated in Rocket-sponsored studies experienced severe BMF:

- A subject presented with Grade 3 BMF starting on Day 1070 (approximately 3 years post-infusion) which was unrelated to the IMP and required transfusion support; the event is considered ongoing.
- A subject presented with Grade 3 BMF starting on Day 509 which was unrelated to the IMP and required transfusion support; the subject received an allogeneic HSCT on Day 567 and the event is considered resolved.

BMF was also experienced by 4 subjects enrolled in FANCOLEN-I per review of the Bone Marrow Failure CRF; however, they are not included in the AESI tables as the BMFs were not reported as AEs. Instead, these subjects experienced BMF at study entry since FANCOLEN-I as a first-in-human IIT initially limited enrolment to subjects whose BMF had progressed to a point to warrant an intervention such as allogeneic HSCT or investigational gene therapy. Three of these subjects were withdrawn from the study and subsequently received allogeneic HSCTs due to RBC and platelet transfusion dependence and BMF, respectively. The other subject completed the study and received an allogeneic HSCT for life-threatening neutropenia and anaemia.

AESI of Any Severe BMF Requiring an Allogeneic or Other HSCT

One of the two subjects in Rocket study who reported an AESI of severe BMF required a HSCT.

Based on information reported in the "Bone Marrow Failure" CRF from FANCOLEN-I, 4 additional subjects experienced worsening of underlying severe BMF that required allogeneic HSCT.

Cytogenetic Abnormalities

A subject experienced a Grade 1 SAE of Cytogenetic abnormality, identified as dup(1) (q21.1q44), del(11) (q22.3q25), in Study RP-L102-0116-LTFU. The start date (study day) of the cytogenetic abnormality was 27-Jun-2022 (Day 2357), 6.5 years post-infusion. The event was assessed as unlikely related to RP-L102; underlying FA was reported as the other alternative event cause. 1q duplication is a common cytogenetic abnormality in subjects with FA. Assessments indicated that the 1q abnormality was present in uncorrected but not in gene-corrected hematopoietic progenitor cells.

Gene Therapy Specific Laboratory Assessments

Immunogenicity

Immune testing to detect potential antigen-specific, cell-mediated immune activation was performed on 43 available PBMC samples from 11 subjects.

Testing for cell-mediated immune responses against the FANCA transgene protein and the LV-associated proteins vesicular stomatitis virus glycoprotein (VSV-G) and human immunodeficiency virus type 1 (HIV-1) Gag p24 was reportable in 10 of the 43 samples. All reportable samples tested were negative for response to the target proteins, including those from subjects whose results either met or did not meet RP-L102 response criteria. Additionally, to assess for a humoral response against the FANCA transgene protein, testing was performed in 41 available serum samples. There was no evidence of clinically significant cell-mediated or humoral immune responses in any of the patients tested.

Insertional Site Analysis (ISA)

LV-gene modified blood cells were also evaluated at selected timepoints for clonality and insertion site genomic location (also known as integration site analysis [ISA]). ISA was only performed when vector copy number (VCN) exceeded 0.02 for a given population (results are not meaningful nor reproducible in settings of limited gene marking).

Rationale of performing ISA only when VCN exceeds 0.02
As the purpose of ISA is not limited to locating the sites of lentiviral insertion into genomic DNA, but also monitoring emergence of any dominant clone (e.g., subsequent to malignant transformation). If the vector copy number (VCN) is very low, the possibility of clonal dominance can reasonably be excluded. Conversely, if a clone increases in abundance, the corresponding VCN will also increase, thus enabling analysis by ISA and subsequent detection of the clone. The possibility of a clonal dominance at very low VCN is not precluded but this hypothetical event is not expected to have a significant impact. In case of suspicious blood counts or other diagnostic parameters suggesting a potential

vector-induced malignancy, ISA could be attempted even at lower VCN levels. The required threshold value of 0.02 in VCN for ISA is empirical, however, it is substantiated by data from the applicants testing facility. The threshold is implemented to ensure that the reported data are both meaningful and reliable. Based on exploratory studies at the testing facility, samples with VCNs as low as 0.02 can be analysed but it is extremely difficult to quantify the contributions of individual integration sites due to the low sonic abundance values observed. Furthermore, to ensure reliable quantification of insertion site contribution, a threshold of 95 nanograms has been established on input genomic DNA for ISA. Assuming 6.6 picograms of genomic DNA per diploid cell, this translates to the analysis of approximately 14,394 cells containing the insertion of interest when VCN equals 1. In the case of VCN=0.02, the theoretical number of cells that have the insertion drops to below 300, a level at which it is technically challenging to produce meaningful results.

In the absence of conditioning, gene corrected cells only comprise a portion of the peripheral blood (PB); hence, VCN was included in the calculation to determine the contribution of a particular integration relative to all unique integration sites (UIS).

Figure 5. Calculation of PB mononuclear cells Integration Site Contribution

$$When\ VCN < 0.1:$$

$$RelFreq = [\%\ contribution\ to\ all\ UIS] \times [VCN]$$

$$\text{OR}$$

$$When\ VCN\ \geq 0.1:$$

$$RelFreq = [\%\ contribution\ to\ all\ UIS] \times [1.0]$$

The identification of "potentially predominant oligoclonality" in the PB, defined as clonal contribution ≥10% with a PBMC VCN ≥0.1, will warrant additional monitoring, as indicated in Figure 12. More specifically, if "*potentially predominant oligoclonality*" is determined in total blood cells (or PB mononuclear cells [PBMC]), ISA (with parallel VCN analysis) will be expedited and complete blood count (CBC) with differential will be performed within 3 months of the previous PBMC ISA sample collection, if feasible, and no later than within 30 days of the previous ISA result being reported. The target turnaround time for results from repeat ISA and VCN analyses will be within 30 days of sample collection. If the repeat PBMC ISA result indicates "*potentially predominant oligoclonality*" (≥10% clonal contribution) and PBMC VCN ≥0.1, a report will be made to the health authorities of "potentially predominant oligoclonality" within 30 days of the repeat ISA result. Additionally, the subject will undergo a BM evaluation including BM ISA testing within 30 days of the ISA result. Furthermore, enhanced PB monitoring including CBC with differential, ISA, and VCN analysis will be performed every 3 months.
As part of the current protocol, subjects undergo regular BM evaluations, as would be the case in standard-of-care (routine) management of FA patients of similar age. To avoid additional unnecessary invasive BM testing:

- If most recent BM was performed within 6 months and indicated no cytogenetic, morphologic, or other abnormalities consistent with MDS or malignancy, and no new or worsening cytopenias are evident, BM may be performed according to protocol-stipulated schedule.
- If most recent BM (performed after identification of potentially predominant oligoclonality) indicated no cytogenetic, morphologic, or other abnormalities consistent with MDS or malignancy,

and no new or worsening cytopenias are evident, CBC with differential and ISA/VCN may be performed every 6 months.

- If two consecutive BM assessments (performed after identification of potentially predominant oligoclonality) indicated no cytogenetic, morphologic, or other abnormalities consistent with MDS or malignancy, and no new or worsening cytopenias are evident, CBC with differential and ISA/VCN may be performed every 12 months.

Evaluations, including gene expression studies, will be performed if the IS indicative of potentially predominant oligoclonality is in an oncogene or tumour suppressor gene. Furthermore, if multiple insertion sites are identified within the same clone, single cell sequencing or genome sequencing approaches will be used to perform further evaluations in PB and/or BM, which may warrant additional BM procedures to assess BM CFUs.

Data from RP-L102-treated subjects with up to 7 years of follow-up have shown no evidence of insertional mutagenesis. The use of the PGK-FANCA-WPRE* LV in the RP-L102 studies is itself a validated mechanism for mitigation of insertional mutagenesis. LVs are notable for preferential transgene insertion into less oncogenic portions of the host cell genome relative to other vectors, including gamma-retroviral vectors, which were associated with leukemogenic events in earlier gene therapy studies. LV-mediated insertion occurs predominantly in transcriptionally active genes, and not in proximity to promotor sites or growth-regulatory genes (Schroder 2002; Mitchell 2004; Wu 2003).

Overall Clonality and Diversity of Gene-modified Cells

One of the safety measures in the RP-L102 clinical program is any incidence of a potentially predominant clone as determined by PBMC ISA of ≥10% clonal contribution in the setting of PBMC VCN of ≥0.1. Table 25 summarises the mean clonality and diversity scores for all PBMC samples. Figure 13 shows a graphical representation of the clonality and diversity by combining Shannon, Gini and UC50.

The Shannon index is used to describe the overall diversity of the integration site pool. The higher the Shannon index (Chao & Shen, 2003), the more polyclonal the integration site repertoire in the individual sample. Low Shannon values ≤ 2.1 indicate oligoclonal insertion patterns with low complexity and more prominant clones. We further discriminate polyclonal Shannon values (2.1 – 5.0) and highly polyclonal insertion patterns≥ 5.0.

The Gini index runs from 0 to 1 and describes how equally clones contribute to the integration site pool (Gini, 1912). A low index suggests a polyclonal insertion pattern with even sequence abundance distribution, and a high index describes the unequal contribution of more dominant sequences to the overall sequence pool.

The UC50 value describes how many integrations in a sample contribute to 50% of all sequences (Berry et al., 2017). When the UC50 is below ten, only a few sequences dominate the overall integration site pool. The higher the UC50 value, the more polyclonal the integration site pool.

The cut-off values for oligoclonality, polyclonality, or highly polyclonal samples were derived from a meta-analysis of Rocket gene therapy programs (Meta-Analysis–Section 5.9). Only PBMC samples with a VCN of at least 0.02 are included in analysis; results are not meaningful or reproducible in settings of limited gene markings.

When analysing samples from all FA trials, the mean VCN of 0.37 (range 0.022 – 2.823) demonstrates an overall low and variable gene marking in individual subjects.

A correlation analysis showed significantly higher VCNs at later time points after gene therapy (Spearman r=0.5289; p-value <0.001). The elevated mean Gini of 0.67 and the low mean Shannon

index of 3.68, together with low UC50 values and the overall low number of unique integrations describe an oligo- to polyclonal insertion pattern in the samples (n=108).

When comparing FANCOLEN-I and the Rocket-sponsored studies, which utilise Process B manufacturing optimisations, an overall 1.3-fold higher mean VCN, 1.9-fold higher number of unique integrations and consequently a 1.4-fold lower Gini and 1.5-fold higher Shannon index were observed for the Rocket-sponsored studies, indicating that subjects in these studies had a higher polyclonality and diversity of gene-modified cells. More diverse integration patterns, both at any given timepoint and over multiple timepoints, indicate that a larger group of long-term hematopoietic cells are contributing to the BM and PB haematopoietic milieu; such findings provide reassurance that no dominant and/or potentially malignant clone has arisen.

Table 20. Clonality and Diversity Parameters Aggregated for all Subject Samples

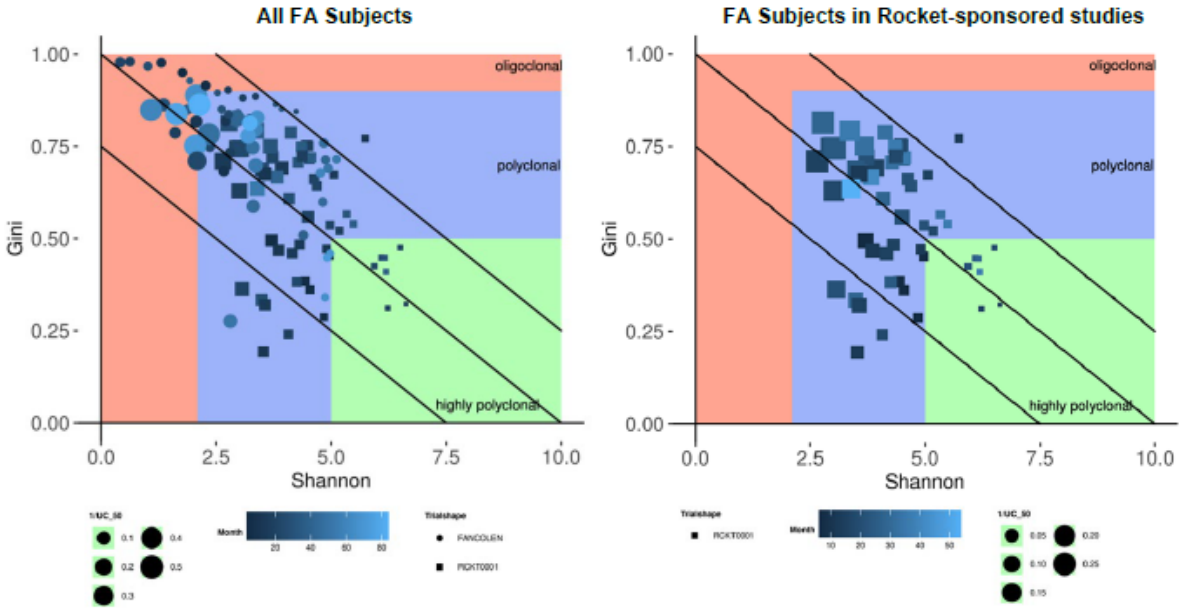
	Gini	Shannon	UC50	VCN	UIS	Samples
Trials(s)	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	n
RP-L102 and FANCOLEN-I	0.67 ± 0.19	3.68 ± 1.33	33 ± 44	0.37 ± 0.35	205 ± 225	108
RP-L102 only	0.57 ± 0.17	4.41 ± 0.99	36 ± 50	0.42 ± 0.42	269 ± 267	55
FANCOLEN-I only	0.78 ± 0.15	2.93 ± 1.23	29 ± 37	0.31 ± 0.25	138 ± 145	53

Abbreviations: SD=standard deviation; UIS=unique integration site; VCN=vector copy number.

Notes: RP-L102 are the subjects in Rocket-Sponsored Studies RP-L102-0418, RP-L102-0319, RP-L102-0118, and associated RP-L102-0221-LTFU. FANCOLEN-I are the subjects in FANCOLEN-I and associated RP-L102-0116-LTFU. UC50 value describes how many integrations in a sample contribute to 50% of all sequences.

Source: RP-L102 CISA Report–Table 5

Figure 6. Combined Shannon, Gini, and UC50 Representation



Notes: Samples with a high Gini and low Shannon index (cutoff 2.1 – orange area) are oligoclonal. Samples with a medium Gini and Shannon index (2.1 - 5.0 – light blue area) are polyclonal and samples with very low Gini and high Shannon indice (5.0 - 10.0 – light green area) are highly polyclonal. The black lines indicate the mean and confidence intervals of the distributions. Dots/Squares outside the confidence intervals might represent sequencing artefacts. The size of the dot/square represents the reciprocal UC50 value. The bigger the dot/square, the lower the UC50, the more dominant the contribution of individual integrations. The colour of each dot/square represents the time interval between treatment and the assessment; the darker blue represents earlier assessments, and the lighter blue longer-term assessments (i.e. 20 month or greater).
Source: RP-L102 CISA Report–Figure 1, RP-L102 CISA Report–Figure 2

Abundance of Integrations over Time in Rocket-sponsored Studies

Figure 14 shows stacked linear plots representing the relative frequency of unique integrations sites (UIS) over time for these subjects. As of 11-Sep-2023, no subject in Rocket-sponsored studies has presented with persistent predominant oligoclonality defined as an IS with ≥10% relative frequency at an initial ISA with confirmation of ≥10% relative frequency in the subsequent ISA.

All integration sites of individual samples were visualised in a stacked area graph per patient over time. If an integration was present within the Top 10 integrations at any time, it was represented as a coloured stratum in the graph. The grey-coloured region represents all other integrations. Abundance values were VCN-normalised.

Abundance of Integrations over Time in FANCOLEN-I

The ISA results for 6 subjects treated in FANCOLEN-I with appreciable PB VCN (such that ISA may be evaluated) and more than one ISA performed are detailed in the dossier. In contrast to ISA results seen in the Rocket-Sponsored studies, ISAs across most evaluable FANCOLEN-I subjects indicated overall oligoclonal to polyclonal patterns, reflecting the relatively low CD34+ cell doses and more modest DP VCNs. Long-term data up to 7 years demonstrate no evidence of RP-L102 related oncogenesis.

Replication-competent Lentivirus

Blood cells were collected at specified time points and evaluated for replication-competent lentivirus (RCL) during follow up. If all assessments during the first year were negative, subsequent evaluations were collected and stored in the absence of a clinical concern to warrant testing. The samples were tested in any subject for whom RCL was documented in serum or blood cells at any time point through Year 1 post-study treatment administration in the parent RP-L102 studies.

Data from RP-L102-treated subjects including up to 7 years of follow-up have shown no evidence of RCL when assessed at protocol stipulated timepoints in accordance with Agency guidance, and there have been no clinical events to warrant additional testing. Samples will be archived and tested if there are clinical concerns.

Serious Treatment-emergent Adverse Events after infusion of IMP

The incidence of serious treatment-emergent AEs by Preferred Term is provided in Table 26. Most SAEs were reported in FANCOLEN-I. All SAEs were reported in one subject each except for Pyrexia which was reported in 3 subjects. Most SAEs were assessed as not related to RP-L102.

Two SAEs were assessed as at least possibly related to RP-L102:

- A subject experienced a Grade 2 Infusion-related reaction. The AE was considered related to RP-L102 and was categorised as serious because the protocol-stipulated hospitalisation following RP-L102 infusion was extended for an additional day of monitoring. The SAE was transient; vital signs stabilised within 2 hours of event onset and the event resolved with supportive care and without any clinical sequelae.
- A subject experienced a Grade 3 SAE of Staphylococcal bacteraemia related to RP-L102 administration. The subject developed fever within 15 minutes of the start of the infusion and blood cultures were positive for S. warneri. Initial empiric intravenous antibiotic therapy including meropenem and vancomycin was initiated for a 6-day course and then changed to oral amoxicillin therapy to complete the antibiotic course for a total of 15 days. The subject recovered from the bacteraemia with initiation of antibiotics; there were no clinical sequelae.

Table 21. Incidence of serious TEAEs by Preferred Term (FAS)

Preferred Term	RP-L102-0418 N=2	RP-L102-0118 N=7	RP-L102-0319 N=5	RP-L102 Total N=14	FANCOLEN-1 N=9	Pooled N=23
Subjects with at least 1 SAE	**2 (100)**	**1 (14.3)**	**2 (40.0)**	**5 (35.7)**	**6 (66.7)**	**11 (47.8)**
Pyrexia	0	0	0	0	3 (33.3)	3 (13.0)
Anaemia	1 (50.0)	0	0	1 (7.1)	0	1 (4.3)
Fall	1 (50.0)	0	0	1 (7.1)	0	1 (4.3)
Influenza	1 (50.0)	0	0	1 (7.1)	0	1 (4.3)
Non-Hodgkin's lymphoma	0	1 (14.3)	0	1 (7.1)	0	1 (4.3)
Infusion related reaction	0	1 (14.3)	0	1 (7.1)	0	1 (4.3)
Gait disturbance	0	0	1 (20.0)	1 (7.1)	0	1 (4.3)
Viral infection	0	0	1 (20.0)	1 (7.1)	0	1 (4.3)
Vomiting	0	0	1 (20.0)	1 (7.1)	0	1 (4.3)
COVID-19	0	0	0	0	1 (11.1)	1 (4.3)
Campylobacter gastroenteritis	0	0	0	0	1 (11.1)	1 (4.3)
Osteomyelitis acute	0	0	0	0	1 (11.1)	1 (4.3)
Staphylococcal bacteraemia	0	0	0	0	1 (11.1)	1 (4.3)
Tonsilitis	0	0	0	0	1 (11.1)	1 (4.3)
Upper respiratory tract infection	0	0	0	0	1 (11.1)	1 (4.3)
Diarrhoea	0	0	0	0	1 (11.1)	1 (4.3)
Oesophageal food impaction	0	0	0	0	1 (11.1)	1 (4.3)
Rectal haemorrhage	0	0	0	0	1 (11.1)	1 (4.3)
Febrile neutropenia	0	0	0	0	1 (11.1)	1 (4.3)
Asthmatic crisis	0	0	0	0	1 (11.1)	1 (4.3)
Respiratory failure	0	0	0	0	1 (11.1)	1 (4.3)
Cytogenetic abnormality	0	0	0	0	1 (11.1)	1 (4.3)
Myositis	0	0	0	0	1 (11.1)	1 (4.3)

Source: RP-L102 ISS–Table 1.2.4.1

Deaths

A subject enrolled on RP-L102-0116-LTFU experienced a fatal SAE of Respiratory failure secondary to persistent fungal and concomitant cytomegalovirus infections following allogeneic HSCT. This SAE was assessed by both the Principal Investigator and the applicant as not related to RP-L102. The subject was a 12-year-old male at the time of death who received RP-L102 on 26-Oct-2016 and required a subsequent allogeneic HSCT from a matched unrelated donor for progressive BMF 4 years later (October 2020). The subject's post-allogeneic HSCT course was complicated by primary graft failure, for which he received a haploidentical PB stem cell transplant in December 2020 and a CD34+ boost in February 2021. The subject had several subsequent complications, including the lung infection and graft failure.

ADRs of special interest, serious ADRs and deaths causally related to the medicinal product.

No data provided.

3.3.7.4. Laboratory findings

Clinical Laboratory Evaluations

Haematology

According to the applicant, the evaluation of haematology parameters did not indicate any notable trends or safety concerns.

Clinical Chemistry

According to the applicant, the evaluation of clinical chemistry parameters did not indicate any notable trends or safety concerns.

Vital Signs, Physical findings, and Other Observations Related to Safety

Vital Signs

There were no notable trends or safety concerns in vital signs according to the applicant.

3.3.7.5. In vitro biomarker test for patient selection for safety

Not applicable.

3.3.7.6. Safety in special populations

Subgroup Analyses

Not applicable.

Intrinsic Factors

No intrinsic factors such as patients with hepatic impairment or patients with renal impairment were evaluated.

Extrinsic Factors

No extrinsic factors were evaluated.

Use in Pregnancy and Lactation

There have been no studies in pregnant or lactating animals. It is not known whether the IMP would affect foetal or neonatal development in animals or whether the IMP is present in breast milk. Furthermore, there is no data regarding germline transmission or foetal effects from gene transfer therapy.

RP-L102 was administered to paediatric patients. Effects on future reproduction and pregnancy are unknown.

Pregnant women were excluded from the clinical studies and all participants were required to take adequate measures to prevent conception for the duration of the parent studies. Due to the nature of gene therapy products in general, patients will be advised to consider the risks associated with mobilisation on pregnancy and fertility in the RP-L102 prescribing information.

3.3.7.7. Immunological events

See section 3.3.7.3. (Serious adverse events, deaths, and other significant events) for a paragraph on immunogenicity.

3.3.7.8. Safety related to drug-drug interactions and other interactions

Drug Interactions

According to the applicant, drug interactions are not of concern with a gene therapy product. No formal drug interactions studies have been performed with RP-L102. RP-L102 is not expected to interact with the hepatic cytochrome P-450 family of enzymes or drug transporters.

Due to the absence of data and uncertainties regarding live vaccine administration, the prescribing information will include the following advice for physicians: Follow institutional guidelines for vaccine

administration. The safety and effectiveness of immunisation with live viral vaccines during or following RP-L102 treatment has not been studied.

3.3.7.9. Discontinuation due to adverse events

Treatment-emergent Adverse Events Leading to Discontinuation

Two subjects experienced a TEAE leading to study discontinuation:

- Study RP-L102-0418: A subject experienced a Grade 3 Bone marrow failure on Study Day 509 which led to discontinuation from the study.
- Study RP-L102-0118: A subject experienced a Grade 4 SAE of Non-Hodgkin's lymphoma (NHL) on Study Day 664 which led to discontinuation from the study (RP-L102 ISS–Listing 1.7.4).

3.3.7.10. Post marketing experience

Not applicable. The product is not authorized.

3.3.8. Discussion on clinical safety

Data collection and exposure

The safety data of RP-L102 is based on the pooled data of one investigator-initiated parent study Fancolen-I and three phase 1/2 Rocket sponsored parent studies (Study RP-L102-0418, Study RP-L102-0319, and Study RP-L102-0118), each lasting 3 years and two long-term follow-up studies to evaluate long-term safety (and efficacy) of RP-L102 for up to 15 years post-IMP infusion for subjects who completed the Rocket parent studies (RP-L102-0221-LTFU) and subjects who completed the Fancolen-I parent study LTFU study (RP-L102-0116-LTFU). LTFU study data were merged with the corresponding parent study information. Patients enrolled in FANCOLEN-1 had a more severe disease in need for an intervention such as HSCT whereas the subsequent studies enrolled younger patients with limited or negligible BMF, thus, the safety database is heterogeneous in terms of disease severity.

All 3 parent Rocket studies were single-arm, single dose studies to evaluate safety (and efficacy) of the IMP infusion in paediatric subjects aged >1 year with FA-A early in the disease course prior to the onset of severe cytopenias, with the intent of improving HSC collection. However, in the FANCOLEN-I study subjects with advanced BMF warranting an intervention were included, who received cell doses and mean vector copy number (VCN) in colony forming units (CFUs) in the drug product that varied considerably, of which also the manufacturing process was different.

In total 15 patients have been enrolled in the in the Rocket-sponsored studies, out of which 14 subjects have been treated with RP-L102. The safety data collected in subjects from the pivotal Rocket-sponsored trials (reported as RP-L102 Total or ISE FAS population) consists of 14 patients exposed, i.e. 6 male patients aged ≤5 years, 3 female patients aged ≤5 years, and 5 aged >5 years. For 2 patients, the duration of Follow-up (at least) is 1 to <12 months (13.3%), for 3 patients follow-up is 18 to <24 months 3 (20%), and for 9 patients follow-up is ≥24 months (60.0%).

At cut-off date of initial submission (11-Sep-2023), the FANCOLEN-I study and the Phase 1 Rocket-sponsored study RP-L102-0418 are completed, but the other Rocket-sponsored studies are still ongoing. At the new cut-off date of 23 October 2024, 13 additional months of follow-up have been provided.

All AEs, TEAEs, and SAEs were coded using the MedDRA dictionary v26.0. TEAEs were defined as any AE which begins on or after the date of infusion of the IMP rP-L102. AEs were categorized by system

organ class and PT. The term ADR has not been used in the dossier, but instead the applicant refers to TEAE related to study intervention determined by the investigator. All treatment-emergent AEs (TEAE) determined by investigators and assessed by the applicant as at least possibly related to filgrastim, plerixafor, and/or apheresis procedures or to Fanskya were considered ADRs and selected for inclusion in section 4.8. However, the exact method used to define ADRs is unclear. Therefore, the applicant is requested to clarify the process of TEAE related to study treatment determined by the investigator as being considered ADRs and how events were selected for inclusion in section 4.8 of the SmPC (**OC**). The adverse event pattern is described separately for the pre-infusion procedure (mobilisation agents and apheresis) and for the treatment with mozafancogene autotemcel, which is acceptable. However, no data of pre-infusion period the IIT FANCOLEN-I study are available due to no data access, but considering that this study is supportive only, this is accepted.

In total, 24 subjects were enrolled in their respective parent study (the SAF population). Of the 15 patients enrolled in the Rocket-sponsored studies, one subject received mobilisation but failed to meet the protocol threshold of ≥5 CD34+ cells/μL in peripheral blood (PB) to initiate apheresis and was discontinued from the study. The FAS population therefore consisted of 23 subjects who received the IMP, which is considered low but acceptable considering the rarity of the disease. As of the cut-off date for this MAA submission (September 11, 2023), 23 patients have been treated with the product in single-arm studies (9 patients in FANCOLEN-1 and 14 patients in the subsequent applicant-sponsored studies). At cut-off date, 11 subjects (45%) have completed, and 7 patients are still in the parent studies. whereas 12 out of 16 eligible subjects (75.0%) have enrolled in the LTFU studies, indicating that several patients who discontinued in the parent studies re-entered in the LTFP studies. Five subjects discontinued after having received the IMP infusion. Three early terminations from the parent studies are reported, which were due to Adverse event, Investigator Request, and Mobilization Failure. An update of these data presented in the initial Meta-analysis, based on the new cut-off date of 24 October 2024 is requested (**OC**).

The mean duration of follow-up after infusion after submission of an additional 13 months is 3.57 years, up to a maximum of 7.7 years. The median follow-up for the entire safety database is 3.15 years. The mean cell count of the final product in the pooled safety data set of all subjects was 6.334 $\times 10^5$ cells/mL. The corresponding mean for CD34+ cell dose/kg in the pooled population was 1.681 $\times 10^6$ cells/kg with a range of 0.073 to 4.100 $\times 10^6$ cells/kg. The total colony forming units (CFUs) showed a wide variation with a minimum and maximum between 105 and 6255.6 per 10^5 cells. Mean CFU VCN was calculated as 1.375. Mean MMC resistance at 10 nm MMC was 46% in the pooled population. In the 14 subjects who have been treated with RP-L102 in the Rocket-sponsored studies, the dose range of exposure (CD34+ Cells/kg) was <4x105 CD34+ cells/kg in 5 patients (33.3); 4 to <10x105 CD34+ cells/kg in 2 patients (13.3) and ≥10x105 CD34+ cells/kg in 7 patients (46.7) and Missing 1 (6.7) in one patient.

Adverse events

In order to produce the IMP, haematopoietic stem cell mobilisation (with G-CSF and plerixafor) followed by apheresis to obtain CD34+ cells, is required. The IMP used in the pivotal studies was manufactured from the fresh apheresis collections and consisted of autologous HSCs transduced with an LV (PGK-FANCA-WPRE) that encodes for the FANCA gene. Both the pre-infusion procedure (mobilisation and apheresis) and the treatment with the IMP (RP-L102) have their own safety profile and described separately, which is acceptable.

AEs reported during the Pre-infusion procedure (mobilisation and/or apheresis)

Note: no safety data of pre-infusion period the IIT FANCOLEN-I study are available due to no data access (9 patients).

All 15 subjects (100%) participating in the Rocket studies reported at least 1 pre-infusion AE, defined as AEs that started prior to RP-L102 administration. Grade 3 pre-infusion AEs were reported in 10 subjects (66.7%), and Grade 4 pre-infusion AEs in 4 subjects (26.7%), and most commonly reported (severe) pre-infusion AEs were Anaemia, Platelet count decreased, and Thrombocytopenia. Pre-infusion SAEs were reported in 2 subjects (13.3%), which were assessed as not related to mobilisation/apheresis procedures. All 15 subjects experienced pre-infusion AEs assessed as at least possibly related to mobilisation and/or apheresis procedures. Most of these AEs were laboratory findings, were Grade 1-2 and resolved without sequelae. The majority of AEs ≥ 3 resolved within one week. There were no serious AEs (SAE) related to any mobilisation/apheresis procedure. Consistent with the known side effects of mobilisation/apheresis procedure, the most common associated AEs were Platelet count decreased, Anaemia and Thrombocytopenia. The majority of events resolved within 1 month. 13 subjects required transfusion support; 5 received red blood cell transfusions only, 4 received platelet transfusions only and 4 required both red blood cell and platelet transfusions. Two subjects developed AEs that were attributed to G-CSF and plerixafor consisting of ALT/AST increased which resolved with one week. It is agreed with the applicant, that the safety profile appears consistent with the known manifestations of the underlying disease and/or adverse event pattern associated with G-CSF, plerixafor, and/or apheresis procedures. However, the adverse events reported after apheresis and mobilisation, could not be completely compared to G-CSF and plerixafor, considering the specific indication for mobilisation/apheresis as part of the current gene therapy and the underlying disease. Further, different preferred terms (PTs) for several blood-related analyses in the dossier and SmPC are used such as, "*neutropenia*" vs "*neutrophil count decreased*", "*thrombocytopenia*" vs "*platelet count decreased*" and "*white blood cell count decreased*" vs "*leukopenia*", depending on the reported verbatim information, the MedDRA lowest LLT selected may be for a medical condition vs. an investigation result (e.g., Neutropenia vs. Neutrophil count decreased, Thrombocytopenia vs. Platelet count decreased). Nevertheless, the adverse event pattern associated with the pre-infusion procedure or treatment is considered to be manageable, and supporting that the tolerability of this procedure in the Fanconi anaemia population is not worsened by the pre-treatment.

Treatment-emergent adverse events

Treatment-emergent Adverse Events after infusion of RP-L102

In total 23 subjects were treated with an infusion of RP-L102; 14 subjects in the Rocket-sponsored studies and 9 subjects in the FANCOLEN-I study. All 23 subjects reported at least one TEAE (100%). Most TEAEs reported across all studies were mild/moderate in severity, non-serious, and assessed as not related to RP-L102. The most commonly reported preferred terms were Pyrexia (14 subjects, 60.9%), Anaemia (10 subjects, 43.5%), Thrombocytopenia (10 subjects, 43.5.%), Upper respiratory tract infection (9 subjects, 39.1%), Neutropenia (8 subjects, 34.8%), Vomiting (30.4%), COVID-19 (26.1%) and Nasopharyngitis (26.1%). Of note, some of these TEAEs may be related to the underlying disease and to characteristics of a young population. With regard to severe TEAEs post-infusion of IMP, 16 of 23 subjects experienced Grade ≥3 TEAEs (69.6%), of which Neutropenia (8 subjects, 34.8%), Thrombocytopenia (7 subjects, 30.4%), Anaemia (6 subjects, 26.1%), Neutrophil count decreased (5 subjects, 21.7%), and Pyrexia (3 subjects, 13.0%) were reported most frequently. Four subjects experienced severe TEAEs assessed as related to RP-L102 by the investigator, including Neutropenia reported in 2 subjects, as well as Pyrexia and Staphylococcal bacteraemia in one subject each; all resolved without sequelae. The very low number of TEAEs assessed as related to RP-L102 is remarkable. A total of 3 subjects (13%) reported at least 1 TEAE requiring a RBC or platelet transfusion. A total of 4 subjects (17.4%) reported TEAEs assessed as possibly related to RP-L102, including 1 subject from Rocket-sponsored studies with a serious Infusion-related reaction and 3 subjects from the FANCOLEN-I study, and 3 subjects in FANCOLEN-I of whom one patient experienced

Pyrexia (Grade 3) and Neutropenia (Grade 4), one subject experienced Staphylococcal bacteraemia (Grade 3), and one subject experienced Anaemia (Grade 3) and Neutropenia (Grade 4).In the Rocket-sponsored studies, 8 of the 14 subjects (57.1%) treated with RP-L102 experienced TEAEs (defined as any AE which begins on or after the date of IMP infusion) assessed as at least possibly related to the pre-infusion mobilization and/or apheresis procedures starting in the post-infusion period. The most frequent TEAEs related to mobilization and/or apheresis procedures were Platelet count decreased (3 subjects, 21.4%) and Neutrophil count decreased (2 subjects, 14.3%).

AEs of special Interest (AESIs)

The following AESIs were defined: 1. *Any new haematologic malignancy, Haematologic malignant tumours (MedDRA SMQ); 2. Any new non-haematologic (solid organ) malignancy: Non-haematologic tumours of unspecified malignancy (MedDRA SMQ); 3. Severe BMF requiring the administration of chronic platelet or RBC transfusions: Transfusion CRF with reason = MedDRA PT of Bone marrow failure + Severity grade ≥3; 4. Severe BMF requiring an allogeneic or other HSCT: Transplant CRF with reason = MedDRA PT of Bone marrow failure + Severity grade ≥3; 5. Cytogenic abnormalities.*

One 3-year old subject experienced a non-Hodgkin lymphoma (NHL) at 22 months post-infusion. Since the surgical tumour biopsy sample in the subject experiencing NHL at 22 months post-infusion showed "no appreciable" LV integration, the lymphoma was assessed as unrelated to Fanskya and attributed to underlying disease. The current Integration Site Analysis (ISA) method defines a sample requirement of a minimum VCN of 0.02. The negligible VCN in the tumour biopsy (VCN=0.00314) is such that reliable quantification of LV clonal contributions via ISA analysis is not possible, and therefore was not performed. No new non-haematologic (solid organ) malignancy was reported in the time period.

With regard to severe BMF, two subjects treated in Rocket-sponsored studies experienced grade 3 severe BMF (14.3%). One patient developed severe BMF at 3 years post infusion and required transfusion support which event is still ongoing. The other patient developed severe BMF at 567 days post infusion, and received an allogenic HSCT which resolved the BMF. Patient discontinued study per request of physician. BMF was also experienced by 4 subjects enrolled in FANCOLEN-I per review of the Bone Marrow Failure CRF, but these cases were not reported as AEs. Instead, these subjects experienced BMF at study entry since FANCOLEN-I as a first-in-human IIT initially limited enrolment to subjects whose BMF had progressed to a point to warrant an intervention such as allogeneic HSCT or investigational gene therapy. Subsequently, 3 of them discontinued the parent study (after infusion) and received allogenic HSCT. The 4th patient completed the study and received an allogeneic HSCT for life-threatening neutropenia and anaemia. As discussed above, two of the patients in the applicant-sponsored studies experienced BMF during the study (on day 505 and 1070, respectively). Given that the study enrolled patients with negligible BMF at baseline it is a possibility that Fanskya may have accelerated their progression to BMF. An Interrupted time series analysis was performed that did not reveal any significant evidence of either improvement or detoriation in the patient's condition. However, as the study design is intended for evaluating interventions at a population level, not for individuals, it can not be concluded that RP-L102 administration did not contribute to an accelerated BMF progression. To support the results shown in the graph the applicant is asked to compare the results with the expected natural disease progression (**OC**).

A total of 3 subjects (13%) reported at least 1 TEAE requiring a transfusion of RBC and/or platelets, i.e. platelet transfusions for Thrombocytopenia, Fall with subsequent injury, and NHL, and RBC transfusions for BMF, anaemia and for NHL. In one subject in the FANCOLEN-I study a cytogenic abnormality was reported 6.5 years post-infusion, which was thoroughly reviewed and assessed as unlikely to be related to Fanskya, but most likely secondary to the underlying disease. In view of the assessments indicating that the 1q abnormality was present in uncorrected but not in gene-corrected hematopoietic progenitor cells, this conclusion of unlikely relatedness is considered acceptable.

Insertional mutagenesis and malignant transformation are reflected in the RMP and will be followed in the proposed non-interventional registry of patients treated with Fanskya.

Overall, it can be supported that Fanskya is not associated with the AESIs selected, but for the one case of NHL a clarification is needed whether an integration site analysis has been performed.

Since Fanskya is a gene therapy product, specific Gene Therapy Specific Laboratory Assessments were performed including immunogenicity testing, insertional site analysis (ISA), and replication competent lentivirus (RCL). With regard to *Immunogenicity*, cell-mediated immune activation using a qualified enzyme-linked immunosorbent spot (ELISpot) assay was performed, as well as a validated electro-chemiluminescent assay for detection of anti-FANCA antibodies to assess for a humoral response. The outcome was assessed as showing no evidence of cell-mediated or humoral immune responses.

As to *Insertional Site Analysis (ISA)*, throughout the clinical studies, the incidence of a potentially predominant clone was assessed by determining PBMC ISA of ≥10% clonal contribution in the setting of PBMC VCN of ≥0.1. Data from 14 patients is available. In general, subjects in the more recent Rocket-sponsored sponsored studies displayed an increased degree of polyclonality compared to FANCOLEN-1, which may be due to the modified manufacturing method and possibly the higher administered HSC dose. Data from RP-L102-treated subjects with up to 7 years of follow-up have shown no evidence of insertional mutagenesis. Even in subjects in the FANCOLEN-I trial, which showed a lower polyclonality and diversity of gene-modified gene, also no evidence of insertional mutagenesis was observed, which is reassuring. Comparing results from FANCOLEN-I and Rocket-sponsored studies seems to indicate that the current Process B manufacturing is indeed optimized in this respect. Of note, ISA was only performed when vector copy number (VCN) exceeded 0.02 for a given population, because results would not meaningful nor reproducible in settings of limited gene markingIn the case of VCN=0.02, the theoretical number of cells that have the insertion drops to below 300, a level at which it is technically challenging to produce meaningful results.

In FANCOLEN-1, two subjects displayed predominant oligoclonality. One subject displayed sustained oligoclonality up to month 84 but no indication of AML/MDS. Further data up to month 107 showed continued oligoclonality but stable blood counts and no evidence of AML/MDS. Another subject displayed a predominant clone at month 48. No further information is available, the patient underwent HSCT and died of transplant related complications (case has been further discussed under the SAE section).

According to the applicant, no subjects developed a dominant clone in the applicant-sponsored studies. It is however noted that the first subject displayed a top contributing integration of 14.86% and the second subject displayed a top contributing integration of 10.41%. These values were obtained at the last available measurement (54 and 41 months, respectively) and were according to the assessor's understanding not fulfilling the criteria of sustained increase. In a subject, there was a possible trend for development of oligoclonality; in the last available measurement (month 21) two clones contributed to 8.56% and 7.98%, respectively. Further data was provided. Subject had a varying pattern in the table on Integration site analysis with insertions in proximity to different genes at different times and fluctuating values. New data was available from months 60, 61, and 67.

Subject had a predominant clone above the threshold for an integration in proximity of the GTF2I-gene. The contribution fluctuated over time but was slightly over the threshold at all times (months 41, 48, 54 and 60).

A newly identified subject had a threshold above 10% for an integration in proximity to the MIR4302-gene at months 28 and 32. At month 36 it was reduced to 8.68%.

All three subjects have a remaining polyclonal Insertion site pattern at all time-points.

The added data show that there are predominant clones over time, but the overall pattern is polyclonal and there are no signs of haematological malignancies at this stage. However, it is important to continue to follow the patients. Insertional mutagenesis and malignant transformation have been included as an important potential risk in the RMP and there are planned activities to follow up.

Furthermore, subject is possibly having a delayed engraftment displayed as increasing PB VCN and BM MMC resistance.

According to the currently proposed SmPC, the minimum recommended dose of Fanskya As discussed in the efficacy section, it is not clear how to proceed if the available CD34+ dose is below this amount. From the data presented, oligoclonality was mainly occurring in patients receiving a CD34+ dose in the lower range, i.e. 2 patients from the FANCOLEN-1 study (a subject receiving $5.4x10^5$/kg; and another subject receiving $7.08x10^5$/kg), furthermore, as discussed above, subject (receiving $2x10^5$/kg) and another subject (receiving $4,8x10^5$/kg) from the applicant-sponsored studies displayed a top contributing integration >10%. No clear association can be seen with dose and predominant clones. It is still plausible that lower cell doses, leading to more limited engrafment may predispose to clonal predominance and this will continue to be evaluated.

Regarding *Replication competent lentivirus (RCL),* the applicant reported no evidence of replication-competent lentivirus RCL has been shown up to 7 years of follow-up. In addition, in other clinical studies employing LVs no cases of RCL have been reported. As reported by the applicant, RP-L102 utilises a third-generation self-inactivating LV, which includes modifications that render these vectors incapable of forming viral particles that can replicate. The evaluation of the potential risk of RCL is assessed as low and considered acceptable. In this respect, it is reassuring that the applicant intends to continue RCL testing of all RP-L102 lentiviral vector commercial lots prior to use in the RP-L102 drug product manufacturing process, both at end of production and final vector as part of batch release.

Serious TEAEs and deaths, and discontinuations after infusion of IMP

In all studies, 11 of 23 subjects experienced serious TEAEs (47.8%). Most SAEs were reported in FANCOLEN-I. All SAEs were reported in one subject each except for Pyrexia which was reported in 3 subjects (13%). Most cases of fever are likely a result of childhood- and FA-related infections rather than the product. This is supported by the long time to first onset, with a mean time of 342.6 days. Only two SAEs were assessed as at least possibly related to RP-L102: the first case concerned a Grade 2 Infusion-related reaction that resolved without any clinical sequelae. The other case concerned a Grade 3 SAE of Staphylococcal bacteraemia related to the infusion and was confirmed by blood culture. This case was confirmed to be caused by contamination. Preventive measures were applied and no more cases of contamination were identified. One death due to fatal SAE Respiratory failure of a patient who received RP-L102 in FANCOLEN-I on day 1588 after allogeneic HSCT from a matched unrelated donor for progressive BMF 4 years later followed by several subsequent complications, including lung infection and graft failure. As previously discussed, subjects in this study had a more severe disease at baseline than in the subsequent studies and the SAE was not considered by the investigator as related to RP-L102 which is acknowledged. As to Discontinuations due to adverse events, two patients discontinued in the Rocket-sponsored trials due to a TEAE. Subject RP-L102-0418-001-1002 experienced a Grade 3 BMF which was followed by a HCST and discontinued study per request of physician, and Subject RP-L102-0118-002-2009 experienced a Grade 4 SAE of Non-Hodgkin's lymphoma (NHL). In the parent FANCOLEN-I study in total 3 subjects discontinued due to an AE (FA-1003, FA-2008 and FA-2013). These patients experienced worsening of underlying severe BMF that required allogeneic HSCT. None of the AEs that led to discontinuation were assessed as related to the IMP, but for the case of NHL more information is requested, before a final conclusion can be drawn that also this case was not related to the IMP.

Other safety aspects

As to Laboratory findings, ALT/AST elevations as an adverse event were reported in 6 patients, which in 2 patients was considered possibly related to Plerixafor, which medication was discontinued in one patient, In all other cases, no action was taken. Hyperglycaemia as an AE was reported in 2 patients. There were no AEs of bilirubin elevation. In the Rocket-sponsored studies 27% (4 of 15 enrolled subjects) had elevated ALT and 47% (7 of 15 enrolled subjects) had elevated AST at screening. 4 subjects had transient AEs of elevated transaminases which resolved without sequelae; one additional subject has had ongoing Grade 3 ALT and AST elevations which began at day 1084 post-infusion and which have not been associated with any clinical symptoms or evidence of hepatic synthetic or other hepatobiliary dysfunction.

As to safety related to drug-drug interactions and other interactions, drug interactions were assessed of no concern with a gene therapy product, which is acceptable. However, in section 4.5 of the SmPC it is mentioned that patients should not take anti-retroviral medicinal products from at least one month prior to mobilisation until at least apheresis is completed. Anti-retroviral medications may interfere with Fanskya manufacturing. A justification is currently lacking (**OC**).

Safety in special populations

No data is available. This is acceptable, considering the rarity of the disease to be treated and the limited number of available patients.

Additional expert consultation

Please see clinical efficacy discussion with respect to the consultations.

Assessment of paediatric data on clinical safety

Only paediatric subjects were included in the studies with Fanskya.

Additional safety data needed in the context of a conditional MA

The applicant is applying for a conditional MA. The applicant has provided a separate document to substantiate fulfilment of the requirements described in Article 4 of Commission Regulation (EC) No 507/2006. In this justification it is indicated that: Completion of the ongoing interventional studies up to the stipulated duration of 3-years follow-up will lead to the completion of the full dossier and is proposed as a specific obligation for the conditional marketing authorisation to provide comprehensive clinical data confirming that the medicine's benefits continue to outweigh its risks. In addition, the applicant is conducting long-term follow-up studies: RP-L102-0221-LTFU for subjects enrolled in applicant-sponsored clinical trials and RP-L102-0116-LTFU for subjects enrolled in FANCOLEN-I. In both LTFU-studies, subjects will be evaluated for safety and efficacy for up to 15-years post-mozafancogene autotemcel administration. This additional safety and efficacy data is planned to be provided to the Agency on an annual basis to support the renewal of the conditional marketing authorisation. Further, the applicant aims at setting up a FA Registry Study that aims at enrolling approximately 20 patients during an estimated 5-year recruitment period. The registry will collect efficacy and safety information for a period of at least 15 years, which is considered adequate and necessary to address the uncertainty of the long-term effects of the product. However, the feasibility of including 20 patients in the registry in 5 years is questioned. See further discussion in section ‘5.7.3. Additional considerations on the benefit-risk balance’

Upon completion of the full dossier, data of the remaining subject who has not yet met the 36-month timepoint for evaluation will become evaluable. Completion of the 3-year follow-up (marking the end of the study, defined as last patient, last visit as applicable), for each of the remaining subjects at time of MAA, will be May 2026. Though the presented substantiation for CMA with respect to additional data to be collected within the time frame of CMA is acknowledged, the provided arguments that the total of 14 patients, in addition to the potential data collected in the registry study, would render the data comprehensive are not agreed. The efficacy and safety information that will be generated from the pivotal studies, LTFU studies and FA registry study is still considered too limited to agree that a fully comprehensive data will be available in a reasonable period of time. This is primarily due to the rarity of the disease and the limited amount of patients enrolled in the pivotal studies. As such, and unless new measures are proposed by the applicant, the applicant may envisage a marketing authorisation under exceptional circumstances (**MO**). Additionally, a more detailed study protocol is warranted for the FA Registry study, following the EMA Guideline on Registry-based studies.

3.3.9. Conclusions on clinical safety

The single dose infusion with Fanskya was generally well-tolerated and had a manageable safety profile in paediatric participants with Fanconi anaemia (FA). Treatment with Fanskya was associated with Neutropenia, Anaemia, Pyrexia, Staphylococcal bacteraemia and Infusion related reaction. The safety pattern observed in the preceding mobilization and apheresis, that is required to prepare the product, appears consistent with the known manifestations of the underlying disease and/or adverse event pattern associated with G-CSF, plerixafor, and/or apheresis procedures. However, the safety data are currently merged with data of the FANCOLEN-I study, in which unlike in the Rocket-sponsored studies, subjects with advanced BMF warranting an intervention were included, and who received cell doses and mean VCN in CFUs in the drug product that varied considerably, and had a different manufacturing process. Therefore, the safety data collected only in subjects from the pivotal Rocket-sponsored trials who received the product to be marketed should be provided and discussed separately, but it is expected that with the deletion of the safety data collected in the Fancolen-I study, the safety profile of the IMP to be marketed will be even milder than currently presented.

No major objections have been raised regarding the observed safety profile. However, several OCsremain, which should be addressed before definite conclusions on the safety of Fanskya can be drawn.

Further, currently proposed additional measures associated to the conditional MA to mitigate the limitations of the current safety data are considered not sufficient and the applicant may envisage the application of a marketing authorization under exceptional circumstances (**MO**).

3.4. Risk management plan

3.4.1. Safety specification

Summary of safety concerns

The applicant proposed the following summary of safety concerns in the RMP:

Table 22. Part SVIII.1: Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	Insertional mutagenesis and malignant transformation
Missing information	Long term safety

3.4.1.1. Discussion on safety specification

Important identified risks

The applicant assessed no important identified risk that should be included in the list of safety concerns in the RMP. Specifically, the applicant did not include cytopenias, infections or infusion-related reaction as a safety concern in the RMP. This is considered acceptable.

Important potential risk

The risk of Insertional mutagenesis and malignant transformation has been included as an important potential risk. This is acceptable and in line with current guideline. Since no cases have been identified, this risk remains potential.

The applicant did not include immunogenicity or safety concerns associated with the mobilisation and HSPC collection procedures as a safety concern in the RMP. This is considered acceptable.

Missing Information

There are limited long-term safety data from patients that were included in the clinical trials. It is considered appropriate to consider the long-term safety as missing information.

It is acknowledged that there are no or limited long term efficacy data, but that should not be included in the RMP. The applicant removed "long term efficacy" from the missing information. This is considered acceptable.

3.4.1.2. Conclusions on the safety specification

Having considered the data in the safety specification, the rapporteur agrees that the safety concerns listed by the applicant are appropriate.

3.4.2. Pharmacovigilance plan

Routine pharmacovigilance activities

Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:

- **Specific adverse reaction follow-up questionnaires:**

  Questionnaire for insertional mutagenesis and malignant transformation follow-up

- **Lentiviral Insertion Testing as Applicable**

  Testing for transgene will be conducted where tissue is available. A SAE data collection template will be employed to characterise all new malignancy reports including ISA on tumour samples

PRAC Rapporteur's assessment comment

The applicant proposed a specific adverse reaction follow-up questionnaire for insertional mutagenesis and malignant transformation to facilitate reporting and evaluation. This is considered reasonable as spontaneous post-marketing reports can differ considerably with regard to the content, which may impede case evaluation. Specific information is necessary to evaluate cases of suspected insertional mutagenesis and/or malignant transformation and a clearly structured questionnaire can be helpful in obtaining key details. The form may contain a reference to the Lentiviral Insertion Testing (detailed in the additional pharmacovigilance activity section). The applicant is asked to submit the questionnaire as part of RMP annex 4 (**OC**).

The applicant proposed and justified inclusion of Lentiviral Insertion Testing as a routine pharmacovigilance activity, which is considered relevant to evaluate pharmacovigilance data and the safety profile of the product post approval. However, considering the company support necessary for Lentiviral Insertion Testing in order to facilitate the evaluation of adverse reaction reports, this is therefore considered classifying as an additional pharmacovigilance (cf. comment in section Additional pharmacovigilance activities).

Additional pharmacovigilance activities

Table 23: Part III.3.1: On-going and planned additional pharmacovigilance activities

Study, status	**Summary of objectives**	**Safety concerns addressed**	Milestones	**Due dates**
Category 1 – Imposed mandatory additional pharmacovigilance activities which are conditions of the marketing authorisation				
None				
Category 2 – Imposed mandatory additional pharmacovigilance activities which are Specific Obligations in the context of a conditional marketing authorisation or a marketing authorisation under exceptional circumstances				
None				
Category 3 – Required additional pharmacovigilance activities				
Non-interventional post authorisation, registry study *planned*	To assess overall survival and characterise the incidence and severity of ADRs experienced by patients treated with Fanskya in the post authorisation setting for up to 15 years.	Insertional mutagenesis and malignant transformation Long term safety	Final Registry Protocol Submission to EMA Interim report Final Report	Dec-2026 Dec-2036 Jun-2046
Long-term follow-up study for Rocket-sponsored parent studies (RP-L102-0221- LTFU) *ongoing*	• To evaluate long-term safety • To determine long-term persistence of the therapeutic LV (provirus) in haematopoietic cells in BM and blood • To determine long-term clonality patterns beyond the 3-year follow-up in parent studies • To evaluate, when relevant, RCL in	Insertional mutagenesis and malignant transformation Long term safety	Interim report 1 Interim report 2 Last subject last visit Final database lock Final report	Sep-2023 Sep-2028 April-2038 July-2038 Oct-2038

Study, status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
	serum and peripheral blood (PB) cells • To determine the long-term stability and normalisation of blood counts. • To determine the phenotypic correction of BM and PB cells • To enable preliminary assessment of the incidence of haematologic malignancies			
Long-term follow-up study for IIT FANCOLEN-I (RP-L102-0116- LTFU) *ongoing*	• To evaluate long-term safety. • To determine long-term persistence of the therapeutic LV (provirus) • To evaluate potential correlations between provirus/transgene persistence and haematologic stability. • To determine long-term clonality patterns beyond the 3-year follow-up in the parent study. • To evaluate, when relevant, RCL in serum and PB cells	Insertional mutagenesis and malignant transformation Long term safety	Interim Report 1 Interim Report 2 Last subject last visit Final database lock Final report	Sep-2023 Sep-2028 Dec-2033 Mar-2034 Jun-2034

Planned activities not included in the summary table of additional PhV activities

"Lentiviral Insertion Testing as Applicable" to facilitate the evaluation of transgene presence and any relationship to gene therapy in the event of a spontaneous AE of haematologic malignancy. The applicant proposes to present results of the lentiviral insertion testing service in PSURs.

PRAC Rapporteur's assessment comment

The applicant included two ongoing LTFU studies and one planned study in the Pharmacovigilance plan and proposed all of them as category 3 studies. Overall, the proposed additional pharmacovigilance (aPhV) activities for the currently presented safety concerns are not acceptable in view of long-term efficacy and safety.

For the ongoing **Long-term follow-up (LTFU) studies**, safety data will be included in PSURs submitted in accordance with the EURD list and final study reports (in 2034 and 2038, respectively) are proposed. Key elements outlined for the LTFU studies appear reasonable. However, the applicant proposed interim reports, of which some have due dates in the past, which needs to be reviewed and verified (**OC**). Descriptions of the studies (Tables 16, 17) should be aligned accordingly.

For the LTFU study it is proposed that if a patient develops a hematologic malignancy, a blood or other sample will be obtained to enable determination of whether the malignant clone developed from a

gene-corrected lineage or an uncorrected FA haematopoietic population. Testing will be guided by clinical context, feasibility, and medical judgment at the time and may include ISA and other gene expression analysis as means to assess any potential relationship to the gene therapy. Testing will be considered as routine pharmacovigilance. While the description in the RMP is supported, the applicant did not submit a revised protocol and it is strongly recommended to update the respective description in the LTFU protocols at the next opportunity for the sake of completeness and clarity.

For follow-up in the post-marketing setting, a **non-interventional post authorisation study** is planned. Details were outlined in a protocol synopsis. The applicant proposes a study based on a company-led data collection system (i.e. a registry) allowing for timely, patient-level data access. The applicant stated that existing patient registries were reviewed and considered not suitable without revealing details. However, the applicant is again requested to carefully explore existing data sources – treatment centres and disease registries – as data collection within this framework is to begenerally preferred (**OC**). According to the applicant, the planned study size is 20 patients during an estimated 5-year recruitment period and all patients treated with Fanskya are to be invited to participate in the post authorisation study, which is supported. The study's primary objective is long-term safety of treatment with fanca-cel, i.e. characterisation of the incidence and severity of selected ADRs and to monitor for potential clinically important events that have not yet been identified; secondary objectives comprise long-term efficacy, durability of treatment response, and evaluation of potential insertional oncogenesis; exploratory objectives include impact on hematologic stabilization, and evaluation of the incidence and nature of any subsequent malignancies. Data collection will also include Bone Marrow Assessments and Laboratory Assessments (e.g., blood count) if this information is available from standard clinical practice of the treating clinicians. Overall, the study objectives are considered relevant and are supported.

It is noted that agreements with Treatment Centres supposed to submit data to the registry as well as support and/or encouragement to participate may be relevant to a successful conduct of the study and hence should be considered when preparing the study protocol.

The applicant changed the proposed due date for the Protocol Submission to EMA in RMP Table 18 from 3 months post EC decision to Dec-2026 without justification. This newly proposed due date appears to be late considering that the PASS should allow for a timely enrolment of fanca-cel treated patients; for this a due data three- or four-months post approval seems more appropriate unless reasonably justified (**OC**). The reporting of safety data in PSURs should remain listed as a milestone in RMP Table 18 (**OC**).

In view of the limited clinical experience with the product, an imposed study is recommended to further characterise the product's safety profile and its long-term safety (**OC**).

The proposed additional pharmacovigilance activity "Lentiviral Insertion Testing as Applicable" is considered important to address the safety concern of Insertional mutagenesis and malignant transformation. It is considered important that the applicant establishes a framework to facilitate sample testing of haematological malignancies taking into account local regulations. The applicant proposes to consider Lentiviral Insertion Testing as a component of routine pharmacovigilance activities and included it as such in the updated RMP. The applicant highlighted that tumour (or bone marrow) biopsy is an essential standard-of-care clinical practice for workup when malignancy is suspected and patient consent is obtained; the SmPC provides relevant and the HCP guide also includes the requirement to inform patients concerning this matter prior to treatment. However, with the need of company support, testing for transgene is considered to be best represented as an additional pharmacovigilance activity. The applicant is therefore asked to add a category 3 pharmacovigilance activity for the risk of insertional mutagenesis and malignant transformation. This activity should cover an appropriate framework and process guidance to support and facilitate

collection and testing of existing samples from patients who have developed malignancies in the post-marketing setting. The overall process of tumour sample collection, should be well defined to provide detailed instructions on standardised procedures (sample request, sample collection, provision of sampling kits, analyses) and responsibilities in managing follow-up of reports of malignancies (**OC**). Overall, the reasoning of the applicant is considered reasonable and the classification as routine pharmacovigilance is supported at this point in time.

The set-up of the summary table of additional pharmacovigilance activities (RMP Table 18) was revised with the update to RMP v0.2 but table formatting still needs to be reviewed (milestones, due dates). The due date for the milestone "Safety data reported in PSURs" could be phrased: Submitted in accordance with the EURD list. (**OC**)**.**

In view of the limited information on efficacy and safety available until now and depending on the discussion in the CAT, the categorisation of proposed studies may be subject to change. Please note that modified or additional safety concerns may need to be considered in the pharmacovigilance plan depending on the CAT proposal.

3.4.2.1 Overall conclusions on the PhV Plan

The PRAC, having considered the data submitted, is of the opinion that the proposed post-authorisation PhV development plan could be sufficient to identify and characterise the risks of the product provided satisfactory responses to the unresolved issues.

3.4.3. Plans for post-authorisation efficacy studies

There are no plans for post authorisation efficacy studies.

3.4.4. Risk minimisation measures

3.4.4.1. Routine risk minimisation measures

Table 24 Part V.1: Description of routine risk minimisation measures by safety concern

Safety concern	**Routine risk minimisation activities**
Important Potential Risk: Insertional mutagenesis and malignant transformation	**Routine risk communication:** SmPC: Section 4.3: Patients with haematologic malignancies Section 4.4: Special warnings and precautions for use PL: Section 2: What you need to know before you are given Fanskya Section 4: Possible side effects **Routine risk minimisation activities recommending specific clinical measures to address the risk**: SmPC: Section 4.4: Special warnings and precautions for use PL: Section 2: What you need to know before you are given Fanskya **Other routine risk minimisation measures beyond the Product Information**: Legal status: Prescription only medicine.

	Use restricted to physicians at qualified treatment centres experienced in the treatment of Fanconi anaemia and trained for administration and management of patients treated with Fanskya.
Missing Information: Long term safety	**Routine risk communication:** SmPC: Section 4.4: Special warnings and precautions for use PL: Section 2: What you need to know before you are given Fanskya **Routine risk minimisation activities recommending specific clinical measures to address the risk:** None. **Other routine risk minimisation measures beyond the Product Information:** Legal status: Prescription only medicine. Use restricted to physicians at qualified treatment centres experienced in the treatment of Fanconi anaemia and trained for administration and management of patients treated with Fanskya.

PRAC Rapporteur's assessment comment

The applicant plans to restrict use to physicians at qualified treatment centres experienced in the treatment of Fanconi anaemia, which seems reasonable in light of the rarity of the disease.

The applicant proposed educational/training material for HCP as additional risk minimisation measure and summarized key content in the RMP annex 6. The applicant is asked to clarify this point (**OC**). Restriction of use to qualified centres in form of a controlled access programme is considered an additional risk minimisation measure that aims to minimise an important risk with significant public health or individual patient impact (EMA/204715/2012 Rev 2), which seems not to apply to this case.

Description of routine risk minimisation measures by safety concern may need revision to include further or modified safety concerns as proposed by the CAT Rapporteurs.

3.4.4.2. Summary of additional risk *minimisation measures*

The following additional risk minimisation measures were proposed by the applicant that address the safety concerns of Insertional mutagenesis and malignant transformation (important potential risk) and Long-term safety (missing information):

Guide for Healthcare Professionals (HCPs).

Key content:

- adequate monitoring of insertional mutagenesis and malignant transformation
- identification and management of long term serious adverse reactions
- provision of all relevant information to patients
- contact details for testing after development of a malignancy
- inform patients about the importance to enrol in a registry for follow up of long-term safety

Guide for Patients/Caregivers

Key content:

- the risks of insertional mutagenesis and malignant transformation and serious adverse reactions associated with Fanskya
- the need to report any symptoms or concerns to their treating doctor immediately
- the need to carry the patient card at all times
- a reminder to patients to show the patient card to all HCPs, including in conditions of emergency, and a message for HCPs that the patient received treatment with Fanskya
- fields to record contact details of the prescriber and batch number.
- The importance to participate in the patient's registry for long-term safety

Educational/Safety advice tool (Patient Card)

Key content:

- Patients who receive Fanksya shall be provided with a patient card, which will capture information regarding the treatment (e.g. medical product batch number, date of treatment and contact details of the prescriber).

PRAC Rapporteur's assessment comment

The applicant intends to restrict Fanskya use to clinicians trained for its use and thus proposes to include an HCP guide as an additional risk minimization measure and provided details in the updated RMP v0.2. The addition of a HCP guide is supported but some questions regarding the scope and content remain (**OC**).

In addition, educational materials and patients (parents) and a patient card were proposed to address the safety concerns of Insertional mutagenesis and malignant transformation and the missing information on long term safety. Key points of their content are outlined in RMP annex 6. In addition to the limited data basis, a peculiarity of the product appears to lie in the delayed clinical effect, which could be a challenge for HCP and patients, or their parents, in light of high patient expectations. It may be important that healthcare professionals, patients and parents are aware of this special aspect of treatment with mosafancogene autotemcel.

3.4.5 Overall conclusions on risk minimisation measures

The PRAC having considered the data submitted was of the opinion that:

the proposed risk minimisation measures could be sufficient to minimise the risks of the product provided satisfactory responses to the unresolved issues. Supplementary risk minimisation measures may be required relating to the updated safety specification.

Protected Personal Data (PPD) and Commercially Confidential Information (CCI) considerations for the RMP

The rapporteur did not identify PPD/CCI in the RMP parts other than Safety Specification.

3.4.6. Conclusion on the RMP

The CHMP and PRAC considered that the risk management plan version 0.2 could be acceptable if the applicant implements the changes to the RMP as detailed in the endorsed Rapporteur assessment report and in the list of outstanding issues in section 7.

The applicant is reminded that in case of a positive opinion, the body of the RMP and Annexes 4 and 6 (as applicable) will be published on the EMA website at the time of the EPAR publication, so considerations should be given on the retention/removal of personal data (PD) and identification of commercially confidential information (CCI) in any updated RMP submitted throughout this procedure.

3.4.7. PRAC outcome

PRAC endorsed the PRAC Rapporteur's RMP assessment and added the following points for consideration.

Pharmacovigilance Plan

The roles and responsibilities of the applicant with regards to Lentiviral Insertion Testing activity would be better described as part of an additional pharmacovigilance activity. While the testing of samples following a diagnosis of a malignancy might be considered routine clinical practice for these patients, the support that the applicant could provide for testing and the reporting to EMA should be formalised in a research protocol. This protocol should cover an appropriate framework and process guidance to support and facilitate collection and testing of existing samples from patients who have developed malignancies in the post-marketing setting. The overall process of tumour sample collection should be well defined to provide detailed instructions on standardized procedures (sample request, sample collection, provision of sampling kits, analyses) and responsibilities in managing follow-up of reports of malignancies. Milestones for protocol submission post-approval, and interim reports submission should be proposed in the RMP. A study synopsis should be included in the RMP with the responses.

The applicant's proposal to use a company-led single-arm cohort study including only patients treated with Fanskya instead of using established (EU) registry data sources is not considered sufficiently explained. The applicant should re-discuss the feasibility of using data from existing registries including patients with Fanconi anemia. EMA could facilitate a discussion with registry owners, to clarify the regulatory requirements and facilitate a collaboration that would benefit the safety follow-up of patients treated with Fanskya.

3.5. Pharmacovigilance

3.5.1. Pharmacovigilance system

It is considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

3.5.2. Periodic Safety Update Reports submission requirements

The active substance is not included in the EURD list and a new entry will be required. The new EURD list entry uses the {EBD} or {IBD} to determine the forthcoming Data Lock Points. The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion.

The applicant should indicate if they wish to align the PSUR cycle with the international birth date (IBD).

The first periodic safety update report should cover the six-month period following the initial scientific opinion for this product on <date of initial scientific opinion>.

Subsequently, the scientific opinion holder shall submit periodic safety update reports for this product every six months until otherwise agreed.

Non-conformity with agreed paediatric investigation plan

Not applicable.

5. Benefit risk assessment

5.1. Therapeutic context

5.1.1. Disease or condition

Fanconi anaemia (FA) is a rare, inherited disorder of DNA repair. The incidence is estimated to be 1 in 300,000 live births and the prevalence of 1 to 9 per million (Bagby 2016). FA is caused by mutations in one of ≥22 different genes, which are described as FANCA to FANCW, of which FANCA, FANCC, and FANCG are most common (80-90%). The most common subtype of FA (60-70%) is FA complementation group A (FANCA) and results from autosomal recessive mutations in the FANCA gene. Inability to repair DNA results in progressive bone marrow failure (BMF) occurring within the first decade of life at median age of 7 years (ECA Analysis RP-L102 in FA; Kutler 2003; Sebert 2023). Individuals diagnosed after 16 years of age typically present with a malignancy. Median overall survival (OS) is 33.5 years, with greater than 30% of patients dying by the age of 20 and more than 65% deceased by the age of 40 (Sebert 2023). Due to disordered DNA repair, patients are also at an increased risk for haematologic malignancies with cumulative incidences of MDS and AML exceeding 30% by age 30, and solid tumours with a 40% incidence by age 40, and congenital malformations (eg, short stature, microcephaly, developmental delay, café-au-lait skin lesions).

The proposed indication for Fanskya is:

"*Fanskya is indicated for the treatment of Fanconi Anaemia Type A (FA-A) in paediatric patients aged 1 to 18 years.".*

The product Fanskya (mozafancogene autotemcel) (referred to as RP-L102) is an ex vivo lentiviral (LV) vector gene therapy consisting of autologous HSCs transduced with an LV (PGK-FANCA-WPRE) that encodes for the FANCA gene. It is administered non-cryopreserved (fresh) as a single intravenous infusion.

RP-L102's scientific development rationale is based on a very rare naturally occurring phenomenon known as multilineage mosaicism, estimated to occur in <5% of FA patients (Nicoletti 2020; Ramirez 2021). Multilineage mosaicism results from a spontaneous reversion or other compensatory mutation in the FANCA gene of a long-term HSC, which converts it from an abnormal cell with a dysfunctional DNA repair mechanism to a normal cell with a functioning one. Since corrected cells have proliferative advantage compared to uncorrected cells, patients with mosaicism often reach haematologic stability. Due to this reason, no myeloablative conditioning is required for treatment with Fanskya, and it is expected that transplanted gene modified cells would also have proliferative advantage in the bone marrow.

5.1.2. Available therapies and unmet medical need

Treatments depend on the severity of bone marrow functioning (*UptoDate 2024*, *Fanconi Anemia: Guidelines for Diagnosis and Management 2014, ASH Education Program, Darfour et al, 2022*):

Severe BMF (ANC ≤500/microL, platelet count ≤30,000/microL, Hb <8 g/dL)

- Hematopoietic stem cell transplantation (HSCT) from HLA-matched sibling is currently the only curative treatment for patients with FA of BMF, prevent progression to MDS or AML, and cure existing MDS or AML
- HSCT from alternate donor sources when a sibling donor is lacking

Moderate BMF (ANC 500 -1000/microL, platelet count 30,000 - 50,000/microL, Hb 8 - 10 g/dL):

- In those patients whose counts continue to decline, HSCT planning is to be started.
- Androgens — Androgen therapy is not curative, but it may be appropriate for patients who lack a closely matched related donor for HSCT, or for those for whom HSCT is not pursued
- RBC transfusion – indicated for any patient with symptomatic anaemia. However, chronic RBC transfusions can lead to iron overload.
- Platelet transfusions in patients with platelet counts <10,000/microL and in any patient with severe bruising, bleeding, or invasive procedures.
- G-CSF - Although G-CSF can raise the neutrophil count in most neutropenic patients with FA, there are concerns that it might increase the risk of MDS or AML in patients with bone marrow failure syndromes.

Unmet medical need

Despite advances in care for FA, median overall survival (OS) is 33.5 years, with greater than 30% of patients dying by the age of 20 and more than 65% deceased by the age of 40 (Sebert 2023). Because of the significant morbidity and mortality associated with allogeneic HSCT in FA (resulting in part from the use of cytotoxic conditioning), allogeneic transplant is reserved as salvage therapy for patients who have progressed to severe BMF (or have developed AML/MDS). Therefore, there is still a high unmet need for an effective treatment to address the haematologic component of FA subtype A (FA-A), ideally one that could prevent BMF and prevent the need for an allogeneic transplant, including the use of cytotoxic conditioning and associated toxicities.

5.1.3. Main clinical studies

The benefit risk is primarily based on data from the three phase 1/2 Rocket sponsored parent studies Study RP-L102-0418, Study RP-L102-0319, and Study RP-L102-0118, each lasting 3 years and its long-term follow-up (RP-L102-0221-LTFU), to evaluate long-term safety and efficacy of RP-L102 for up to 15 years post-IMP infusion for subjects who completed the Rocket parent studies. At cut-off point of 23-Oct-2024, the phase 1 Rocket-sponsored trial RP-L102-0418 is completed (n=2), in which 1 patient completed and 1 patient discontinued. The other two phase 2 Rocket parent studies are still ongoing: in RP-L102-0319, 5 patients were treated, of whom 2 are ongoing and 3 have completed this study; in study RP-L102-0118, 7 patients were treated, of whom 2 are ongoing, 4 completed and 1 subject discontinued. At cut-off, 9 patients were enrolled in the ongoing RP-L102-0221-LTFU study.

All 3 studies were single-arm, single dose studies to evaluate the efficacy of the IMP infusion in paediatric subjects with FA-A early in the disease course prior to the onset of severe cytopenias, with the intent of improving HSC collection. They were aged >1 year with minimum weight of 8 kg, and had

to have at least 30 CD34+ cells/μL blood cell count within 3 months prior to initiation of CD34+ cell collection. Subjects with an available and medically eligible HLA-identical sibling donor, myelodysplastic syndrome or leukaemia, subjects with somatic mosaicism associated with stable or improved counts were the main exclusion criteria. No myeloablative conditioning was required. Prior to infusion of RP-L102, patients are required to undergo HSC mobilisation followed by apheresis to obtain CD34+ cells which will be used for medicinal product manufacturing.

The primary efficacy endpoint is a composite endpoint comprised of 3 components evaluating evidence of: (1) phenotypic correction by BM CFU MMC resistance ≥20% (MMC at 10 nM concentration) at Month 12 post-infusion with a confirmatory assessment at either Month 18 or Month 21; AND (2) genetic correction by PB vector copy number (VCN) ≥0.1 at Month 18 post-infusion with a confirmatory assessment at either Month 21 or Month 24. ; AND (3) haematologic stability by haemoglobin, neutrophil, and platelet counts remaining at ≥75% of 6-month post-infusion nadir value at Month 18 post-infusion with a confirmatory assessment at Month 21 or 24. Levels must exceed the following values at the latest documented assessment (≥18 months post-infusion): hemoglobin ≥8 g/dL (NCI-CTCAE v5 Grade <3); neutrophils ≥500/μL (NCI -CTCAE v5 Grade <4); platelets ≥25,000/μL (NCI-CTCAE v5 Grade <4). The subjects need to achieve the 3 components to meet the primary endpoint.

Since the Rocket-sponsored studies have similar designs, efficacy data are pooled, also including data from RP-L102-0221-LTFU. The Rocket parent studies have enrolled a total population of 15 patients, of which 14 received RP-L102 and 13 are evaluable at cut-off date of 23 October 2024. Note: also the safety data are pooled, but also with inclusion of the supportive FANCOLEN-I Phase 1 parent study and its LTFU study RP-L102-0116-LTFU.

Supportive studies consisted of the completed investigator initiated FANCOLEN -I study lasting 3 years in subjects with advanced BMF warranting an intervention, and who received cell doses and mean vector copy number (VCN) in colony forming units (CFUs) in the drug product that varied considerably. The product used in this Phase 1 study was also produced by a different manufacturing process compared to the pivotal studies. FANCOLEN-I included 9 subjects, of whom 6 patients completed the study and 3 patients discontinued. Subjects who completed parent study FANCOLEN-I could enrol in the long-term follow-up study RP-L102-0116-LTFU of 15 years. At cut-off date, 7 patients were enrolled in this ongoing follow-up study.

5.2. Favourable effects

The number of evaluable patients by cut-off date 23 October 2024 in the pivotal Rocket sponsored studies is 13 patients, and 5/13 (38%) evaluable patients met the **primary composite endpoint**. Of note, the total number of patients enrolled was 15, and 14 patients have been treated with the medicinal product (thus, 5/15 (33%) and 5/14 (36%) met the primary endpoint). Per component of the primary composite endpoint, 7/14 patient reached BM MMC resistance ≥20%, 8/12 met PB VCN ≥0.1, and 7/11 maintained all haematologic values at ≥75% of 6 months post infusion nadir values (9/11 maintained haemoglobin values, 7/11 ANC, and 8/11 platelets). Efficacy is shown to be maintained at least up to 36 months in all respondent patients. For patients with data available, response is maintained up to 4-5 years. Additionally, a sensitivity analysis using haematologic values at true baseline to define the >75% threshold for haematologic stability endpoint showed consistent results with the analysis per protocol (using data at 6-months post-infusion). Also, an analysis showing the number of patients per bone marrow status (mild, moderate, severe) over time showed that patients generally maintained or improve their BMF status.

Regarding the **key secondary endpoints**, no events of death or MDS/AML have occurred during the Rocket-sponsored studies nor the following LTFU study (RP-L102-0221-LTFU). Two events of BMF occurred to two patients who were considered non-responders based on the primary endpoint analysis.

The treatment with the medicinal product requires **mobilisation** but does not require **conditioning**.

5.3. Uncertainties and limitations about favourable effects

The batches used in the pivotal studies were manufactured at different sites and using viral vector manufactured at different sites. There are no obvious differences in tested quality attributes between batches manufactured using vectors from different sites. The difference in MOI used for the manufacturing of these batches gives, however, some reason for concern, because the impact of this difference has not been evaluated in depth and the available data are currently insufficient to conclude that efficacy of the clinical batches is comparable. A Major Objection requesting more in depth evaluation of potential predictors of clinical response and further data to support comparability was therefore requested. The data provided in response to this Major Objection did not reveal major differences between batches manufactured using vector from different sites. Overall, the comparability evaluation is, however, hampered by the very limited data set and no definite conclusion can be drawn. This uncertainty could be accepted considering the potential benefit of the product to the patient, but the observed differences should be further discussed. To confirm that clinical efficacy of batches manufactured using the proposed commercial vector is comparable to batches manufactured using vector from a previously used manufacturing site, the applicant should commit to re-evaluate the limit for cell dose when data from additional patients are available and compare clinical outcome of batches manufactured with the different vectors that met the cell dose specification.

Due to the two-stage release strategy, part of the release test results, including potency, are not available prior to administration. This strategy could be justified by the short shelf-life of the product. However, due to the limited data-set it is not possible to determine if the acceptance criteria for the release tests results that are available prior to administration are sufficiently predictive for safety and efficacy of the product.

The available data suggest that efficacy can be at least partially ensured by testing CD34+ cells/kg prior infusion but the applicant still needs to appropriately address the risks emerging from the proposed control strategy where VCN levels are available after infusion of the DP into the patient (**MO**). In addition, the applicant should demonstrate that, at minimum, it is sufficiently ensured that cells have been successfully transduced and commit to re-evaluate the acceptance criteria related to potency/purity when additional data are available (**MO**).

The reasoning for the recommended $CD34^+$ dose is understood and accepted. Of note, all subjects receiving more than 20 x 10^5 cells/kg were responders, indicating that an even higher dose could be favourable from an efficacy point of view, but the available cell dose is pending the outcome of mobilisation and leukapheresis. This is reflected in the SmPC. Upon request, the applicant has clarified that in case of an available cell dose below the recommended dose, the clinician could choose between still using the available cell dose or starting a new round of mobilisation, which has not been tested in the clinical trials. Therefore, the applicant is asked to discuss potential risks with multiple mobilisations and discuss whether it is likely that a second mobilisation would yield a larger cell dose than the original treatment (**OC**)

The pivotal studies are **single arm studies** and the **sample size** is small (15 patients enrolled, of which 14 have been treated, and 13 evaluable by the current cutoff). The studies have been pooled for analysis, but it remains unclear whether this was a pre-planned meta-analysis (the meta-analysis statistical analysis plan is dated 17 Jan 2024, which is after study initiation). No valid (external)

comparator is available, and responder rate is ~40%. It is questioned whether the sample size calculation is data driven and the type I error protected. Furthermore, no information about possible predictive factors for clinical response across patients is available (i.e., patients characteristics or characteristics of the medicinal product) other than the general observation that no subject receiving less than a certain dose was a responder. With these uncertainties it is currently challenging establish clinical efficacy (**MO**). Two patients who were non-responders continued to progress to BMF. Further, most patients included in the studies (12/14) had hematologic values corresponding to normal or mild BMF at baseline, and therefore, the benefit of the treatment for patients in more advance BMF status is unclear (**OC**).

Time to response varies largely across patients, with no rationale provided for such variability. Additionally, intra-patients fluctuations are also wide. Nevertheless, patients who are respondents according to the primary endpoint timepoint maintain their response up to at least 36 months.

The **secondary endpoints** are considered the most clinically relevant outcomes (specifically overall survival, BMF-free survival, and MDS/AML-free survival). However, these events are likely to occur in the long term, rather than during the short study duration. Therefore, while limited events occurred during the studies (2 BMF events in 2 patients who were non-responders), the duration of the studies is too short to draw conclusion regarding these events.

An analysis using an external control is performed. However, this analysis is associated with possible bias and cannot be used other than in a descriptive manner; data about CD34+ concentration was not available, and relevant aspects for the disease course, such as age or disease status are not (adequately) matched or the number of patients included is too limited.

5.4. Unfavourable effects

Note: The adverse event patterns observed during the pre-infusion procedure (haematologic stem cell mobilisation with treatment with G-CSF and plerixafor and apheresis to obtain CD34+ cells) and the treatment with the IMP (RP-L102) are provided separately.

Pre-infusion

Based on the data of the Rocket studies only, most commonly reported (severe) pre-infusion AEs were Anaemia, Platelet count decreased, and Thrombocytopenia. Two subjects experienced severe pre-infusion SAEs of Hypersensitivity, Bronchiolitis and Respiratory distress (13.3%), assessed as not related to mobilisation/apheresis procedures. All 15 subjects experienced pre-infusion AEs assessed as at least possibly related to mobilisation and/or apheresis procedures, which included anaemia, platelet count decreased, thrombocytopenia, vomiting, ALT/AST increased, bone pain, leukopenia, lymphocytopenia. Based on TEAEs, 8 of the 14 subjects (57.1%) treated with RP-L102 experienced TEAEs assessed as at least possibly related to pre-infusion (mobilization and/or apheresis) of which Platelet count decreased (3 subjects, 21.4%) and Neutrophil count decreased (2 subjects, 14.3%) were reported most frequently.

Post-infusion of IMP

Most commonly reported TEAEs were Pyrexia (14 subjects, 60.9%), Anaemia (10 subjects, 43.5%), Thrombocytopenia (10 subjects, 43.5.%), Upper respiratory tract infection (9 subjects, 39.1%), and Neutropenia (8 subjects, 34.8%). Severe TEAEs were reported in 69.6% of the subjects of which Neutropenia (8 subjects, 34.8%), Thrombocytopenia (7 subjects, 30.4%), Anaemia (6 subjects, 26.1%), Neutrophil count decreased (5 subjects, 21.7%), and Pyrexia (3 subjects, 13.0%) were reported most frequently. One death due to respiratory failure secondary to persistent fungal and concomitant cytomegalovirus infections following allogeneic HSCT was reported in a subject enrolled in

the long-term follow-up in the FIH study FANCOLEN-I, assessed as unrelated to RP-L102. In the Rocket-sponsored studies, 2 subjects discontinued due to a TEAE; one due to BMF followed by a HCST, and one due to development of Non-Hodgkin's lymphoma (NHL). In total 11 of 23 subjects experienced serious TEAEs (47.8%), which all were reported in one subject each, except for Pyrexia which was reported in 3 subjects. Two SAEs were assessed as at least possibly related to RP-L102: a Grade 2 Infusion-related reaction and a Grade 3 SAE of Staphylococcal bacteraemia.

A total of 4 subjects (17.4%) reported TEAEs assessed as possibly related by the investigator to RP-L102, of which 2 were assessed as serious, i.e. Infusion-related reaction, and Staphylococcal bacteraemia and 2 as non-serious Pyrexia (Grade 3) with Neutropenia (Grade 4), and non-serious Anaemia (Grade 3) with Neutropenia (Grade 4).

With respect to AE of special interest, one subject experienced a non-Hodgkin lymphoma (NHL) at 22 months post-infusion. A surgical tumour biopsy sample was assessed for the presence of the FANCA transgene. The results showed that the surgical tumour biopsy sample demonstrated no appreciable lentiviral virus (LV) integration with a mean vector copy number (VCN) of 0.00314. In contrast, peripheral blood (PB) and bone marrow (BM) VCN were 0.2573 and 0.4227, respectively, at the time of diagnosis, i.e. an approximately 80- to 130-fold higher than the tumour sample VCN, indicating that the diagnosed malignancy is very unlikely related to the gene therapy. In this same tumour sample, molecular abnormalities characteristic of T-cell lymphoblastic lymphoma, including *NOTCH1* mutation, were observed. The very low VCNs identified in the tumour biopsy sample are very likely the result of small numbers of circulating blood cells within the lymphoma. The VCN in the tumour biopsy (VCN=0.00314) is considered too low that reliable quantification of LV clonal contributions via ISA analysis is not possible, and therefore was not performed. Based on above outcome, the lymphoma was assessed as unrelated to Fanskya and attributed to underlying disease.

With regard to severe BMF, two subjects treated in Rocket-sponsored studies experienced grade 3 severe BMF, of whom one developed severe BMF at 3 years post infusion requiring transfusion support which event is still ongoing. The other patient developed severe BMF at 567 days post infusion, and received an allogenic HSCT. BMF was also experienced by 4 subjects enrolled in FANCOLEN-I at study entry, which were not reported as AEs.

A total of 3 subjects (13%) reported at least 1 TEAE requiring a RBC or platelet transfusion due to BMF, anaemia, injury due to a fall, and due to NHL, which cases were assessed as unrelated to IMP.

In one subject in the FANCOLEN-I study a cytogenic abnormality was reported. Assessments indicated that the 1q abnormality was present in uncorrected, but not in gene-corrected hematopoietic progenitor cells, and therefore considered as most likely secondary to the underlying disease.

Regarding immunogenicity, insertional site analysis (ISA), and replication competent lentivirus (RCL), no safety issues have been described so far.

5.5. Uncertainties and limitations about unfavourable effects

Long-term safety data is limited for Fanskya; both the number of (treated) subjects in the safety database is very limited, as well as the current follow-up time (max. 7.7 years in total, with median follow-up of 2.9 years in the Rocket-sponsored studies), though these figures need to be updated in the Meta-analysis with data up to the new cut-off date of 23 October 2024 (**OC**). The very limited number of patients in the safety data base is an important uncertainty. The known important theoretical risks as well as other adverse events may have been missed if they have a frequency off less than 1/23, which is in the common range.

Fanconi anaemia is related to an increased risk for malignancies. Due to the limited data set, it is not possible to assess whether Fanskya could be related to an increased cancer risk. Further, safety uncertainties such as the risks of administrating the product for infusion before the results of VCN numbers in the product is available remain. Insertional mutagenesis and malignant transformation are reflected in the RMP and will be followed in the proposed noninterventional registry of patients treated with Fanskya.

Regarding oligoclonality, a FANCOLEN-1 subject displayed sustained oligoclonality up to month 84 but no indication of AML/MDS. No subjects developed a dominant clone in the applicant-sponsored studies. In two subjects a possible trend for development of oligoclonality was noted, additional data on these patients showed that there are predominant clones over time, which occurred after the genetic and phenotypic corrections, when improvement in haematological parameters has started, but the overall pattern is polyclonal and there are no signs of haematological malignancies at this stage. However, it is important to continue to follow the patients.

From the data presented, oligoclonality was mainly occurring in patients receiving a CD34+ dose in the lower range. The applicant is requested to discuss whether a low dose may predispose development of oligoclonality and reflect on the proposed recommended minimum dose related to this possible risk (**OC**).

Two of the patients in the applicant-sponsored studies experienced BMF during the study. Given that the study enrolled patients with negligible BMF at baseline. Further data did not reveal any significant evidence of either improvement or deterioration in the patient's condition. However, as the study design is intended for evaluating interventions at a population level, not for individuals, it cannot be concluded that RP-L102 administration did not contribute to an accelerated BMF progression. To support these results the applicant should compare the results with the expected natural disease progression (**OC**).

5.6. Effects table

Table 25. Effects table for Fanskya, indicated for the treatment of paediatric patients with Fanconi Anaemia Type A (FA-A) (data cut-off: 23 October 2024).

Effect	Unit	Treatment	Uncertainties/ Strength of evidence
Favourable Effects[a] [1]			
		N = 13	
Defined by the three component below	n (%)	5 (38)	**Primary composite endpoint** **Unc**: • No comparator arm • Small sample size • BM MMC and PB VCN are PD parameters, their clinical relevance is not directly obvious •
BM MMC resistance (≥20% at Month 12 and Month 18 or 21)	n/N (%)	7/14 (50)	
PBMC VCN (≥0.1 at Month 18 and Month 21 or 24)	n/N (%)	8/12 (67)	
Hematologic stability (Hb levels, ANC and Platelet counts remaining at ≥75% of 6-month post-infusion nadir values at month 18 and month 21 or 24)	n/N (%)	5/11 (46)	
			Key secondary endpoints
Deaths	n (%)	0	**Unc**: • Short follow up to elicit any effects on these outcomes • No comparator arm • Small sample size
BMF	n (%)	2	
MDS/AML	n (%)	0	
Unfavourable Effects [2]			
TEAE related to infusion IMP		N = 23	**Unc**: • exact frequency is uncertain- • reported in Fancolen-I study only
Neutropenia	N (%)	2 (8.7)	
Anaemia	N (%)	1 (4.3)	
Pyrexia	N (%)	1 (4.3)	
Staphylococcal bacteraemia	N (%)	1 (4.3)	
Infusion related reaction	N (%)	1 (4.3)	
AESI			
non-Hodgkin lymphoma (NHL)	N	1	**Unc:** • relatedness to Fanskya uncertain
immunogenicity	x	x	**Unc:** • assessment currently not possible

Abbreviations: NA = not applicable; BM=Bone Marrow; PB=Peripheral Blood; MMC=Mitomycin-C; PBMC=Peripheral Blood Mononuclear Cells; DEB=diepoxybutane; BMF=bone marrow failure; MDS=myelodysplastic syndromes; AML=acute myeloid leukaemia.
Notes: [a]The favourable effects are pooled data from the pivotal studies (Rocket-sponsored studies: RP-L102-0418, Study RP-L102-0319, and Study RP-L102-0118)
[1] Rocket-sponsored studies [2] Fancolen-I + Rocket studies

5.7. Benefit-risk assessment and discussion

5.7.1. Importance of favourable and unfavourable effects

Mozafancogene autotemcel is a new active substance for the treatment of FA-A, and it is an *ex vivo* lentiviral (LV) vector gene therapy consisting of autologous HSCs transduced with an LV (PGK-FANCA-WPRE) that encodes for the FANCA gene. FA is an inherited DNA repair disorder that results in in progressive bone marrow failure (BMF) at median age of 7 years. Individuals diagnosed after 16 years

of age typically present with a malignancy. Despite advances in care for FA, FA is a devastating disease, with greater than 30% of patients dying by the age of 20 and more than 65% deceased by the age of 40 (Sebert 2023). There is high unmet need for an effective treatment. The requested indication is '*Treatment of Fanconi Anaemia Type A (FA-A) in paediatric patients aged 1 to 18 years.'*

Rationale for the development is based on the naturally occurring phenomenon of multilineage mosaicism, by which a compensatory mutation in the FANCA gene of a long-term HSC converts an abnormal cell with a dysfunctional DNA repair mechanism to a normal cell with a functioning FANCA gene. The corrected cells have a proliferative advantage over uncorrected FA cells and repopulate the bone marrow and peripheral blood, leading to haematologic normalisation or stability overtime. The treatment with mozafancogene autotemcel requires the extraction of CD34+ and their transduction with LV encoding functional FANCA gene, but does not use conditioning, as it is expected that gene modified cells would have proliferative advantage in bone marrow, similar to the case of mosaicism. The treatment is administered fresh as a single intravenous infusion.

The current application is mainly based on the preliminary results of three pivotal Rocket-sponsored studies (RP-L102-0418, Study RP-L102-0319, and Study RP-L102-0118) and their LTFU study. All 3 main studies have similar designs, therefore, results are presented pooled, which is supported. The number of patients included in the studies is limited (14). The cut-off date is 23 Oct 24, and additional data from the not yet evaluable patient and longer follow up data for all patients should be available by now. By the cut-off date, 9/14 treated patients have completed the three-year follow-up period and have been enrolled in the LTFU studies.

The patient population enrolled in the studies is considered to be representative for the target population in clinical practice with respect to disease severity. The Rocket-sponsored studies were conducted in paediatric subjects with FA-A early in the disease course prior to the onset of severe cytopenias, with the intent of improving HSC collection and chance of treatment success. This is supported, as the choice is based on the results from the FIH study in more severe patients, where efficacy of the medicine was shown to be suboptimal. Importantly, suggestions in the SmPC are made to reflect this point adequately. The applicant aims for a broad indication, targeting paediatric patients aged 1 to 18 years of age. Although the patients included in the pivotal clinical studies ranged from 1.8 to 7 years of age, the broader indication is agreed since it is acknowledged that in clinical practice the possibility to successfully mobilise and extract the CD34+ cells is essential for treatment success, and this is not purely affected by age, but rather by disease status. The pivotal studies also excluded patients with HLA-identical sibling donors, who are also currently included in the indication. This is agreed since the exclusion of this patients from the studies was based on ethical reasons (unknown efficacy/safety profile of the drug), and not on possible differences in efficacy or safety. Moreover, treatment with Fanskya does not preclude HSCT if necessary. Therefore, these patients can also benefit from an additional treatment option before reaching the last one, - HSCT.

Results of the pivotal studies show response in the primary composite endpoint in 5/13 evaluable patients. Of the 14 treated patients in the pivotal studies, 9 patients have received the recommended cell dose. All 5 respondent patients were treated with the proposed recommended dose or higher. Although the clinical relevance of the PD components of the primary endpoint (BM MMC resistance and PB VCN) is not directly obvious, haematologic stability is a clinically relevant parameter, although not the ultimate clinical goal, which is to prevent or delay BMF. This and other clinically relevant outcomes are included as secondary endpoints. This is acceptable because long follow up data would be necessary to elicit these effects, given that the included patients are young children with a sufficient number of bone marrow CD34+ cells for Fanskya production who are still relatively haematologically stable at the time of treatment initiation. From this point of view, the benefit of the treatment for patients in more advanced BMF status is unclear. A treatment success rate of 38% (5/13 patients)

might not be extremely large. However, it can be a clinically relevant achievement if the response can be ultimately attributed to the product, considering the (sustained) efficacy (i.e., consistent results when using true baseline values as reference for the >75% threshold of haematologic stability, and maintenance of haematologic stability and maintained BMF status up to 36 months), a seemingly very acceptable mild safety profile of the treatment, and the severity of the disease. The key secondary endpoints of overall survival, BMF and MDS/AML are considered the most clinically relevant outcomes. Two events of BMF occurred during the study in two non-responder patients. While no conclusions can yet be drawn, these results may be indicative of delay or prevention of BMF in respondent patients. However, due to the limited follow up available at this point, it is not possible to draw firm conclusions on these outcomes. Two patients who failed treatment underwent HSCT since this treatment option remains available after the use of Fanskya. Theoretically, this gene therapy will not be able to reduce cancer risk (especially solid tumours), but poses a benefit over HSCT due to not needing HLA matched donors and no prior cytotoxic conditioning. The latter increases risk of solid tumours due to the underlying DNA repair defect and further complications in patients with Fanconi anaemia. This benefit over HSCT, however, remains theoretical, and at this stage still needs to be demonstrated.

The absence of a comparator arm makes it difficult to put the observed effects into context. To mitigate the limitation of lacking a comparator, a comparison analysis to real-world data is performed. However, the several limitations of the data impair to draw firm conclusions.

A rationale for the lack of response in 8/13 is not available, despite the provided subgroup analyses, other than a correlation analysis showing that patients receiving a lower dose of the medicinal product had a lower response to BM MMC and PB VCN.

The safety profile of the treatment is considered to be manageable and rather favourable. Specifically, the absence of a toxic myeloablative conditioning is a great advantage, considering high toxicity risks in patients with Fanconi anaemia. Overall, 23 patients have been exposed to the medicinal product, which is considered a very low number but could be acceptable considering the rarity of the disease. The limited number of subjects in the safety data base means that known theoretical risks as well as other adverse events could have been missed. With the new cut-off date of October 2024, the data on long term safety is increased. The limited number of patients needs to be taken into consideration in the benefit/risk balance.

The pooled dataset is heterogenous, since it contains both patients with a more severe disease state (FANCOLEN-I) and less severe disease state (Rocket-sponsored studies). The adverse event pattern is described separately for the pre-infusion procedure (mobilisation and apheresis) and for the treatment with mozafancogene autotemcel. The safety profile is overall in line with the known safety aspects of mobilisation and apheresis and the disease progression. Most of the reported AEs were mild and moderate in severity and not considered to be related to the study treatment. The analysis of AEs per study suggests a slightly better adverse event pattern in the Rocket-sponsored studies than noted in the pooled safety data set, which is likely inherent to the less severe disease state in the Rocket-sponsored studies, compared to FANCOLEN-I. Further, the potential risk of insertional oncogenesis was studied by insertional site analysis (ISA), and the results show no evidence of insertional mutagenesis, which is reassuring. Due to the limited data set, it is not possible to assess whether Fanskya could be related to an increased cancer risk. This potential risk is mentioned in RMP and will be further monitored post-marketing for at least 15 years, which is agreed. Development of oligoclonality which could lead to AML/MDS is a potential risk with the treatment and warrants additional information for some subjects. There are no indication of AML/MDS in the data so far.

Two patients, with negligible BMF at baseline, experienced BMF during the study. The applicant should compare these patients with the expected natural disease progression and discuss whether Fanskya may have accelerated their progression to BMF (**OC**).

The labelling regarding the ADRs is not yet justified and requires further discussion. Further, as more data are gathered at the new cut of date of October 2024, an update of the initially submitted meta-analysis of safety and efficacy is needed **(OC)**.

5.7.2 Balance of benefits and risks

In terms of benefits, mozafancogene autotemcel seems to result in haematologic stability for at least 36 months in a number of patients (~40%) exposed to the medicinal product. Most of the exposed subjects were relatively haematologically stable at treatment initiation. Further data are needed to support an effect on preventing or postponing bone marrow failure. These data may eventually be gathered in the LTFU studies currently ongoing (follow up for 15 years). Further, since most patients included in the study had haematologic values in the normal or mild BMF ranges, the benefit of the treatment for patients in more advance BMF status is unclear. Also, in order to fulfil the postmarketing requirements, a larger dataset and longer follow-up time to demonstrate the effect of the treatment and its durability, and to gather data on uncertainties such as the baseline CD34+ values and available dosing for infusion is necessary. If no data from additional patients can be provided, a marketing authorisation under exceptional circumstances may be envisaged. The use of mozafancogene autotemcel appeared to be well tolerated and has an acceptable safety profile, with safety issues in line with the known safety profile of the pre-infusion procedure and the disease progression. Nevertheless, safety uncertainties such as the risks of administering the product for infusion before the results of VCN numbers in the product is available remain. Overall, while the response rate (~40%) may not seem extremely large, the product could be considered of clinical benefit for a part of the FA population for the following reasons, given that the response can be ultimately attributable to the product.

- The unmet medical need in a life-threatening disease (median overall survival ~33.5 years)
- The maintained efficacy in terms of haematologic stability for at least 36 months in all initially responding patients
- No need of conditioning, which is an advantage vs HSCT as conditioning is associated with increased risk of solid tumours in FA patients
- A safety profile manageable and rather favourable
- The possibility of all other treatments in the future, i.e., treatment with RP-L102 does not limit preclude future HSCT if necessary, and therefore, it is an additional treatment option that does not restrict other currently available treatments.

However, the benefit/risk balance cannot be ascertained at this point, since MOs regarding the release acceptance criteria of the final drug product, the efficacy of the product, and the type of marketing authorisation are raised. Specifically, a treatment effect is still not considered convincingly shown. Due to the rarity of the disease, the Fanskya development programme has been conducted in an open-label uncontrolled setting in a low number of patients without a valid external comparator. Most patients enrolled in the studies had normal peripheral blood values at baseline, and have not yet reached the age where the clinically relevant effects of prevention or avoidance of BMF and the need of allogeneic HSCT could be fully evaluated. Thus, it is still not clear how the shown efficacy has been isolated and can be attributable to the product. The use of mozafancogene autotemcel appeared to be well tolerated and have an acceptable safety profile so far, provided the limited long-term follow-up. However, at the moment it cannot be deducted that the shown benefit outweighs the potential risks of the treatment, in view of the risks for insertional mutagenesis. In this context, the potential safety risks of releasing the product for infusion before the results of VCN numbers in the product is available is also of

importance (please refer also to MO on quality). The Benefit/risk ratio is thus still not established. The applicant is requested to comment on the issues raised.

The B/R of the medicinal product is currently negative.

5.7.3. Additional considerations on the benefit-risk balance

Conditional marketing authorisation

The comprehensiveness of the data package will be discussed based on a total of 9 criteria.

1. **Quality of evidence**. The applicant claims the use of a clinically relevant endpoint, which is agreed for the haematologic stability component. Further, the applicant refers to the use of the commercial product in the pivotal studies, ensuring the quality of the product (see discussion in the quality section), and the minimisation of selection bias in relation to the target population by identifying patients with sufficient bone marrow preserved. However, it should be highlighted that only 9/14 patients in the pivotal studies received the intended commercial dose of the medicinal product. Further, different manufacturing processes have been used to develop the products during the pivotal studies (see Quality assessment). The use of a single-arm open-label trial is justified in this rare disease. However, the absence of a compactor arm and contextualisation of the data with an external, retrospectively collected natural history control inherently leads to bias. The applicant used an external comparator to contextualise the disease (International FA Registry-IFAR), which is appreciated. Nevertheless, the inherent limitations of such comparator and the limited amount of matched patients in the external comparator impairs the use of such analysis to draw robust conclusions (see Q165 part C). Despite the efforts of the applicant, the quality of evidence is still not considered high.

2. **The precision of effect size**. As discussed previously, while a treatment effect has been demonstrated on the primary efficacy endpoint, based on the small sample size its precision is inevitably low. Further, only 9/14 patients received the intended commercial dose of the medicinal product in the pivotal studies. It is acknowledged that the rarity of the disease complicates the inclusion of more patients, but this does not change the fact of having low precision.

3. **Clinical meaningfulness of the endpoint.** Not all endpoints are considered to be equally clinically meaningful. In this rather young patient population, i.e. the young children with a sufficient number of bone marrow CD34+ cells for Fanskya production who are still relatively haematologically stable at treatment, the most relevant endpoint is preservation of haematological stability long-term. In the second round, 3-year data and LTFU data have been provided for 10 patients. It can be agreed that for those patients who met the primary composite endpoint at the first timepoint (month 18), efficacy was maintained up to 3-years. Data to draw conclusions about the prevention, or postponing, of bone marrow failure compared to natural history of Fanconi anaemia patients, however, are still limited.

4. **Efficacy: duration of efficacy.** The maintenance of efficacy defined as BM MMC, PB VCN, and haematologic stability has been shown to at least up to 3 years in those patients that responded at the first timepoint (5/13 patients at month 18). Response for 3 years is considered clinically relevant. Efficacy in terms of prevention of BMF is not proven yet. Follow-up data is necessary to assess longer efficacy, which the applicant aims to gather in the ongoing LTFU studies and with the set-up of a FA registry study post-marketing. Discussion about the presence of long-term engrafters in the drug product has been provided in question 152. .

5. **Safety: exposure.** It is agreed that Fanskya seems to be well tolerated, and that it poses a safety advantage compared to conventional HSCT, since no prior conditioning is needed. However, the safety database will remain very limited due to the rarity of the disease, specially when restricted to the target population and patients' exposed to the intended commercial dose (N=9).

6. **Safety: length of follow-up.** The safety follow-up duration is considered quite extensive to characterise short-term safety risks, however, long term safety follow up is needed to sufficiently address the uncertainties. The applicant aims at gathering such information in the ongoing LTFU studies over a period of 15 years and with the set-up of a FA registry study post-marketing.

7. **Target population versus the study population.** The proposed indication is broader than the study population, as the study population was limited with regard to age in the pivotal studies. It can be agreed with the applicant that the study only included patients up to 7 years old due to the natural progressive course of disease and the need of enrolling patients in an early disease status. As such, age should not be the reason to restrict the indication of the product. Nevertheless, given than the product maintains haematologic values, and does not normalise them, it is still unclear how patients in a moderate or severe BMF status may benefit from the treatment.

8. **Pharmacological rationale.** The mechanism of action is clear since it is based on the naturally occurring event of mosaicism. However, it is not demonstrated that the medicinal product will behave and result in the same effects that natural mosaicism has. The applicant claims that 'Multilineage mosaicism, whether spontaneously occurring or following gene therapy, is most effectively demonstrated with a BM colony forming unit (CFU) MMC resistance assay'. However, as shown in question 165 Part A, the analysis of the different components of the primary endpoint, BM MMC resistance does not always translate in stabilised haematologic values. Further, in question 165 Part E, no clear correlation between BM MMC resistance and haematologic stability is shown.

9. **Natural history/ course of the disease.** The natural history of the disease has been relatively well described.

Given the above discussion, it is agreed with the applicant that the current data set submitted is not considered comprehensive. The applicant therefore requests a conditional MA (CMA)**.** The applicant has provided a justification for all of the requirements for a CMA as set out in Article 4 of the Commission Regulation (EC) No 507/2006 and guidance EMEA/509951/2006, including proposals for generating comprehensive datasets in the authorised indication. Completion of the ongoing interventional studies up to the stipulated duration of 3-years follow-up will lead to the completion of the full dossier and is proposed as a specific obligation for the CMA to provide comprehensive clinical data confirming that the medicine's benefits continue to outweigh its risks. In addition, the applicant is conducting long-term follow-up studies: RP-L102-0221-LTFU for subjects enrolled in applicant-sponsored clinical trials and RP-L102-0116-LTFU for subjects enrolled in FANCOLEN-I. In both LTFU-studies, subjects will be evaluated for safety and efficacy for up to 15-years post-mozafancogene autotemcel administration. Additionally, the applicant will set the FA Registry Study, which aims at enrolling approximately 20 patients during an estimated 5-year recruitment period, and patients will be followed for at least 15 years. Available data will be provided annually. The proposal of including approximately 20 patients in the registry study is deemed limited but understandable, given the rarity of the disease. However, the 5-year recruitment goal is considered optimistic, given the recruitment difficulties faced in the Rocket-sponsored studies. The registry will collect key safety and efficacy parameters, which are considered adequate. No methodological details on the conduct of the registry study were provided. Overall, the efforts of the applicant to build a comprehensive dossier are appreciated. However, the efficacy and safety information that will be generated from the pivotal studies, LTFU studies and FA registry study is considered too limited to agree that a fully comprehensive data will be available in a reasonable period of time. This is primarily due to the rarity of the disease and the limited number of patients enrolled in the pivotal studies. As such, and unless new measures are proposed by the applicant, the applicant may envisage a marketing authorisation under exceptional circumstances (**MO**)**.**

5.8. Conclusions

The overall benefit /risk balance of mozafancogene autotemcel is currently negative.

6. Biosimilarity assessment

Not applicable.

7. Recommended conditions for marketing authorisation and product information in case of a positive opinion

In view of the major objections it is premature to recommend any conditions for marketing authorisation.

7.1 Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004 (REG), Fanskya (mozafancogene autotemcel) should be included in the additional monitoring list for the following reasons: new active substance, biological medicine, conditional approval or approved under exceptional circumstances, requirement to carry out additional studies.