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This supplement contains the following items:
1. Original protocol, summary of changes.
2. Original statistical analysis plan, final statistical analysis plan, summary of changes.

The DESIGN of the protocol has been published in « Contemporary clinical Trials » (see above pages)

Direction de la Politique Médicale
Département de la Recherche Clinique et du Développement
Délégation Régionale à la Recherche Clinique
1, avenue Claude Vellefaux 75010 Paris

National multicenter prospective study comparing the results of geno-identical allograft to the transfusion program in sickle cell children with cerebral vasculopathy detected by transcranial Doppler

DREPAGREFFE

Promotor code: P 071247
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Investigator coordinator : Dr Françoise BERNAUDIN
Referral Center for Sickle Cell Disease
Pediatrics, CHIC Hospital, Créteil, France

Investigator coordinator: Dr Françoise BERNAUDIN
Address : Pédiatrie, Intercommunal de Créteil, 40 avenue de Verdun, 94010, Créteil
Tel : +331 45 17 53 91 and 92
Fax : +331 41 78 41 19
francoise.bernaudin@chicreteil.fr

Biostatistics : Pr. Sylvie CHEVRET
1 avenue C. Vellefaux, 75010 Paris
Tél : 01 42 49 97 42
Fax : 01 42 49 97 45
Sylvie.chevret@paris7.jussieu.fr

CRA: Riad Baameur
Tel: 01 42 38 53 20
Fax: 01 42 38 53 25
riad.baameur@univ-paris-diderot.fr

Promotor : AP- HP
Département de la Recherche Clinique et du Développement (DRCD)
Assistance Publique – Hôpitaux de Paris (AP-HP)
Hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75010 Paris.

Project Manager
Valérie MILLUL / Karine Seymour
Tél : 01. 44 84 17 51 / 17 42
Fax : 01 44 84 17 99
valerie.millul@sls.aphp.fr

95 **Summary**

Title Code	National multicenter prospective study comparing the results of geno-identical allograft to the transfusion program in sickle cell children with cerebral vasculopathy detected by trancranial Doppler. **DREPAGREFFE** P : 071247
Investigator coordinator	Dr BERNAUDIN
Objectives	**Principal** : Show that the allograft significantly decreases the TCD velocities than the extended transfusion program (TP) **Secondary :** Compare in the 2 groups (transfusion vs transplantation) : - Evolution of cerebral vasculopathy * Percentage of patients who normalized TCD (<170 cm/ sec) * Ischemic lesions on MRI * Possible stenosis on MRA * Serum expression of "angiogenic" molecules * Comparative cognitive performance patient and sibling - Evaluation of transfusion performance, anti-erythroid alloimmunization, and post-transfusion incidents and accidents - Iron overload - Psychological condition and quality of life - Costs
Eligibility criteria	**Inclusion criteria** - Sickle cell anemia (SCA) patients SS/Sb0, - Aged less than 15 years, - With a history of abnormal-TCD (≥ 200 cm/sec) - Having at least one non-SCA sibling of the same parental couple - Of which parents accept HLA typing and transplantation project in the event of the existence of a genotype identical HLA donor in the sibling or the extended transfusion program **Non-Inclusion Criteria** - non SCA-patients (not SS/Sb0) - aged > 15 years - No history of abnormal-TCD (≥ 200 cm/sec) - no non-SCA sibling of the same parental couple - Of which parents refuse HLA typing and transplantation project in the event of the existence of a genotype HLA donor in the siblings or the extended transfusion program.
Sample size	63 patients: 21 SCA-patients in the group “Transplantation” and 42 in the group “Transfusion”
Duration of research	Inclusions over 2 and follow-up over 1 year, ie a study duration of 3years
Methodology	Mendelian-randomized prospective cohort study (allografted) - unexposed (TP), grafted versus transfused, national multicenter. Study of cell therapy.
Exams required	1. The TCD 2. The MRI/MRA 3. Cognitive tests (WIPPSI-R or WISC-4 or WAIS-3) 4. Quality of Life Scale 5. Alloimmunization 6. Expression of phosphatidylserine 7. Expression of angiogenic molecules.

End points	1. Main endpoint Improvement of the cerebral vasculopathy assessed on the measurement of the average at 1 year of the velocities (TAMV) recorded in MCA arteries 2. Secondary endpoints 2.1 Evolution of cerebral vasculopathy - Incidence of ischemic stroke - Survival without ischemic stroke - TCD Velocities - Percentage of patients who normalized velocities at 1 year - Expression of membrane phosphatidyl and angiogenic molecules - Incidence of ischemic lesions in MRI - Incidence of strictures in WARC - Cognitive performance 2.2 Anti-erythrocytic alloimmunization 2.3 Hemolysis and iron overload 2.4 Assessment of Psychological Status and Quality of Life 2.5 Costing

2. BACKGROUND and SUMMARY OF RESEARCH

2.1 Introduction

Sickle cell anemia, in its SS / Sb0 form, is at risk of 11% occurrence of stroke before the age of 20 years (1,2), providers of motor and cognitive sequelae.

Non-invasive transcranial doppler (TCD) allows detection of patients at risk of clinical stroke (3-7): abnormally high velocities> 200 cm / sec predicted a 40% stroke risk within 3 years (4.9) .

The initiation of a transfusion program aimed at maintaining hemoglobin S (HbS) levels below 30% significantly reduces this risk (9) (STOP I study).

However, the STOP II study published very recently (10) shows that this transfusion program cannot be stopped even in patients who have normalized their velocities after 30 months of transfusion program (TP) and who have no obvious stenoses, ARM. Transfusion programs "for life" expose to viral risks that have become minor, but to major ones of alloimmunization as well as those of iron overload. The results obtained with the new oral chelators are very promising (11) but long-term studies remain to be made.

Only the geno-identical allograft makes it possible to stop the transfusion program but, the risks of this procedure are not negligible (vital risk, risk of post-transplant neurological worsening, GVH risks and gonadal risk), although they are very reduced when the transplantation is performed before the age of 15 (12-20). Results have improved dramatically over the past 5 years with a 95.7% recovery hope encouraging transplantation at an earlier stage before complications with sequelae (21).

Its benefits seem important in terms of changes in vasculopathy and quality of life, but this remains to be assessed prospectively.

The possibilities of cryopreservation of the placental blood of the siblings and the techniques of preimplantation diagnosis with double HLA-disease selection will make it possible to increase the percentage of sickle-cell sick children with severe expression of their disease and candidate to allograft (22).

It has therefore become essential to carry out a prospective study comparing the benefits and risks of transfusion programs with allografts (29,30).

Furthermore, there is still room for improvement in the identification of clinical and biological factors predisposing to cerebral vasculopathy, and the study proposed here of membrane phosphatidylserine of red blood cells and blood angiogenic factors may contribute to this.

2.2 Literature review

2.2.1. Prediction of the risk of stroke by transcranial Doppler

The first article reporting the interest of the TCD in sickle cell children was published in 1990. By comparing the velocities of 6 affected children with a history of stroke and intracranial stenosis proved by angiography and those of 115 affected children without neurological history, Adams showed that a velocity of ACM, ACA or terminal IC greater than or equal to 1.90 m / sec strongly evoked a focal stenosis (23).

In a larger study of 34 patients aged 2 to 30 years (mean 12 ± 6) with stroke, explored by TCD and using angiography as the gold standard, he noted a sensitivity of 90% and a specificity of 100% with the following criteria: an averaged velocity higher than or equal to 2m/sec evokes stenosis, absence of flow in the ACM or slow flow less than 0.70 m/sec in ACM is in favor of a thrombosis of the IC and / or ACM, the recorded slow flow being related to a type of augmentative circulation Moya Moya (5).

Adams then demonstrated TCD's ability to predict the occurrence of stroke in asymptomatic sickle cell children (8). One hundred and ninety children were followed for an average of 29 months. Twenty-three had an average velocity greater than 1.70 m / sec. Seven strokes occurred including six of the 23 patients with abnormal TCD. This result was confirmed by a subsequent study including 125 additional children, who found a risk of stroke of 40% within 3 years in children with an average velocity in the terminal CI or ACM higher or equal to 2 meters per second against 2% if the TCD was normal.

Using a Doppler coupled to ultrasounds we verified that the criteria described by Adams in Blind Doppler could be used in Doppler ultrasound. While we had taken as threshold value 1.70 m / sec, vasculopathy suspected by TCD in 9 children on a series of 58 children with sickle cell disease was confirmed by angiography in all but one (6). All the four patients with a velocity greater than 1.90 m / sec had stenosis. Two of them had no neurological history. The confrontation of the TCD data and angiographic data by conventional technique or by magnetic resonance angiography (MRA) shows an acceleration zone in TCD that does not always correspond to an incorporated stenosis. TCD detects localized hemodynamic disturbances preceding the establishment of an organized stenosis but exposing the tissue area downstream ischemia. It is understood that the impact of the therapeutic intervention at this stage of circulatory disorders may be strongly avoiding the evolution towards the stenosis (24).

2.2.2. Blood and Doppler biology

A. Phosphatidylserine

The phosphatidylserine is a membrane phospholipid abnormally externalized on sickle cell red blood cells (59-61). This modifies the adhesion properties of red blood cells and could increase the risk of vasculopathy. It is possible that the red blood cells presenting phosphatidylserine on their surface are capable of altering the wall of the blood vessels, in particular the cerebral vessels, and of participating in the formation of cerebral vasculopathy.

B. **Hypoxia and angiogenic factors**

Angiogenesis is defined as the set of processes leading to the formation of new blood capillaries by the growth or budding of pre-existing vessels. Under normal physiological conditions, this neovascularization is finely controlled and plays a fundamental role in embryonic development and then in adulthood, only in reproductive functions and tissue repair in wounds or ischemia.

Among the molecules involved in the formation of the vessels, there are three main classes of molecules:
- adhesion receptors and matrix proteins;
- enzymes degrading the extracellular matrix;
- angiogenic factors and their receptors: endothelial growth factors (such as Vascular Endothelial Growth Factor, VEGF) as well as remodeling factors (angiopoietin)

It is now recognized that VEGF alone does not act in the control of angiogenesis. Indeed,

VEGF, alone, initiates but does not completely complete the angiogenesis (76). Among the molecules which can act in cooperation with VEGF, mention should be made of angiopoietins. Angiopoietins represent a family of cytokines known to have two main members, Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2), which possess a common receptor called Tie-2 (Tyrosine Kinase with Immunoglobulin Epidermal Growth Factor Homology Domain).

During physiological angiogenesis, angiogenic remodeling involves a preliminary Ang-2 action that destabilizes vascular structures to allow VEGF, if present, to stimulate angiogenesis. In the absence of the latter, vascular regression is observed. Ang-1 intervenes in the late phases of angiogenesis to ensure stabilization of the vascular networks in particular by promoting the recruitment of peri-endothelial cells. This newer discovery of angiopoietins, as synergistic agents of VEGF in the angiogenic balance, offers new perspectives to the study of angiogenesis in a pathological context, particularly in cerebral ischemia (77).

Similarly, EPO can exert distinct effects from its hematopoietic effects on different organs including the brain. By way of example, EPO is angiogenic, neuroprotective and its receptor has been identified at the cerebral level (45, 78)

Numerous factors are at the root of angiogenesis, among which tissue hypoxia is known to play a major role in increasing the synthesis of angiogenic factors in particular via the transcription factor HIF-1 (Hypoxia Inducible Factor-1). Among the angiogenic factors induced by HIF-1 are VEGF, Ang-2 and EPO (46-47, 79)

VEGF, angiopoietins as well as EPO seem particularly interesting in the context of sickle cell anemia. Indeed, the serum level of these molecules and in particular that of Ang-2 revealed a pro-angiogenic state in adult sickle cell (48). It should be noted that in sickle-cell anemia, organic damage is related to vaso-occlusion accidents associated with ischemia-reperfusion. These molecules are known to be involved in post-ischemic and in particular angiogenic processes (46, 80)

Thus, all these studies highlight the relevance of studying the expression of these angiogenic factors at the serum level in sickle cell children with abnormal Doppler velocities and of comparing their transfusion and / or after transplantation.

2.2.3. Contribution of MRI / ARM imaging

1. Description of MRI lesions

MRI is a very sensitive examination in the detection of ischaemia / infarction lesions appearing in iso or hyposignal on the T1-weighted sequences and hypersignal on the T2 and Flair sequences which are the most sensitive. Three types of lesions are described (25): most commonly sylvian cortico-sub-cortical infarction related to severe stenosis or thrombosis of a main artery, anterior junctional infarction in the border area between ACA and ACM, Junctional posterior to the junction of the territories of the ACM and the posterior cerebral artery (ACP), small infarcts of the central gray nuclei, the internal capsule and especially the periventricular white matter and the oval center. Also to evoke the presence of a collateral network type miamoya in the form of a picket in the region of the central gray nuclei, especially since the homolateral IC and / or ACM are not visible

2. Mecanisms

It seems that the first two types are related to macroangiopathy. The third can be isolated or associated with a lesion of large trunks. Indeed, several studies have attempted to classify MRI abnormalities according to the probable causal mechanism: macroangiopathy or pathology of microcirculation and / or embolism. In a study of 25 SS patients with stroke investigated by CT and / or MRI, Adams found that 72% had lesions suggestive of a disease of the large trunks (massive or junctional infarction) whereas 28% had lesions corresponding to Second mechanism (small lesions of the central gray nuclei and of the white substance) (3).

3. Subclinical infarctions: frequency and significance

Systematic MRI scanning of sickle cell children showed that ischemic lesions were found in most patients with a history of stroke but also in a large number of asymptomatic patients with a frequency of 17% to 21.8% in SS patients (25, 26). The risk of clinical stroke is 14 times greater in this population with an incidence of 8.1% in the next 5.2 ± 2.2 years.

4. Magnetic Resonance Angiography (MRA)

This noninvasive vascular imaging has improved considerably in recent years and allows the visualization of arterial thromboses as well as even moderate stenoses (26,27). The time-of-flight sequence in three-dimensional acquisition (3D TOF) is the most used. Injection of contrast medium is not necessary. The very frequent flow artifacts with older equipment in these fast flowing anemic children in the form of a signal vacuum at the two carotid siphons and M1 segments of the middle cerebral arteries have become less troublesome. Console work with careful reconstruction and cutting helps to clear each arterial segment and minimize false positives.

5. Data from our previous multicentre study (PHRC 95)

Between 01/96 and 07/97, 173 sickle cell children between the ages of 5 and 15 years were enrolled in a prospective multicentre study (PHRC 95) for the centers of Créteil, Debré, Rouen, TCD, MRI / MRA and cognitive tests that were also performed in a sister-sister group and also between 5 and 15 years of age. The incidence of pathological TCD (velocities> 200 cm / sec) was 9.6% in the SS / Sb0 population and the incidence of silent stroke 15% (subclinical ischemic lesions). Multivariate analysis showed that lesions with MRI (OR = 2.76, p = 0.047), hematocrit <20% (OR 5.85, p = 0.005) and thrombocytosis> 500x10^9 / l (OR = 3.99, p = 0.004) were significant independent risk factors for cognitive impairment (IQ <75) (28).

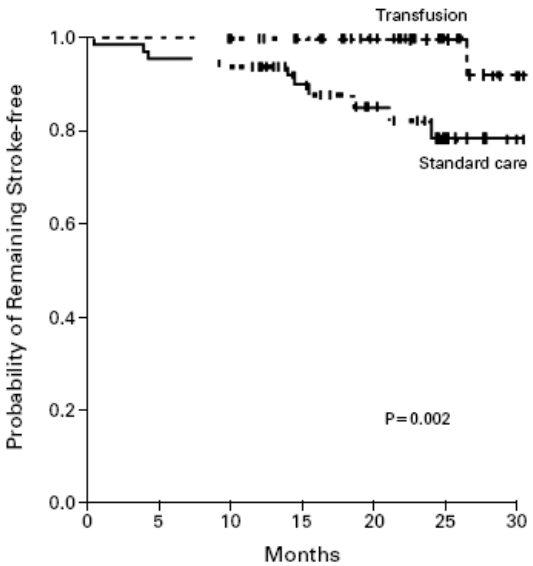


Figure 1. Kaplan–Meier Estimate of the Probability of Not Having a Stroke among Patients Receiving Long-Term Transfusion and Patients Receiving Standard Care.

The P value was calculated by proportional-hazards regression analysis. Tick marks indicate the lengths of observation of patients who did not have a stroke. One patient in the standard-care group who had an intracerebral hematoma was excluded from the analysis.

2.3. PREVENTION OF RISK BY TRANSFUSION PROGRAM

STOP I study

The therapeutic benefit of screening was demonstrated in a randomized, multicenter American study (STOP I) in 130 children with pathologic TCD with a velocity greater than or equal to 2 meters per

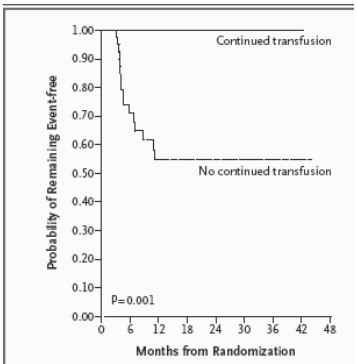


Figure 2. Kaplan–Meier Estimates of the Probability of No End-Point Event among Patients Assigned to Continued Transfusion or No Continued Transfusion.

P values were determined by the log-rank test.

second screened among 1934 children aged 2 years at 16 years old (9). The initiation of a transfusion program aimed at maintaining the hemoglobin S level below 30% has reduced the risk of stroke by 10% per year to 2%. Ten strokes and intracerebral hematoma occurred in 67 patients not transfused to a single stroke in the transfused 63 patients.

The STOP II study (very recently published) (10) shows that it is not safe to stop the transfusion program even in patients who have been transfused for 30 months and have normalized their velocities and having no stenosis at the MRA (occurrence of 2 strokes and 14 recurrences of pathological TCD in the 41 patients whose transfusion program had been stopped).

2.4. Alternative to transfusions

2.4.1 Hydroxyurea

Among the modifiers of erythroid maturation, hydroxyurea (inhibitor of ribonucleotide reductase) is the least hematotoxic.

Hydroxyurea has been proposed since 1992 in France by pediatric teams to children with sickle cell anemia with more than 3 vaso-occlusive attacks (VO) hospitalized per year and / or 2 STA (31,32) and in some cases with severe anemia (36).

The randomized experiment in adults in the USA (33) showed a 50% reduction in the frequency of VOs, acute thoracic syndromes (ATS) and transfusion needs under this treatment.

On the other hand this treatment is not the reference treatment in case of cerebral vasculopathy. Trials conducted by Ware (35) showed a risk of recurrence of stroke of 19% in the 4 months following the initiation of the Hydrea whereas with transfusion subprogram this risk is of 10%.

In Creteil, hydra has been proposed in selected patients with a history of pathological TCD with no MRS stenosis and whose velocities had normalized under the transfusion program with quarterly DTP monitoring: 7 patients were switched from the TP to the Hydrea but 4/7 exhibited an acceleration of the velocities and were then switched to a transfusion program (24).

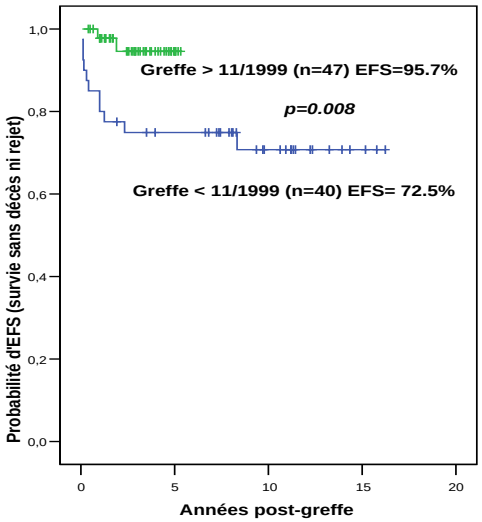
2.4.2. Transplantation of hematopoietic stem cells

Geno-identical transplantation is the only potentially curative treatment and the only safe alternative to stopping the transfusional program of children with cerebral vasculopathy.

About 250 geno-identical allografts have been carried out to date worldwide for 16 years for severe forms of sickle cell anemia (12-21). In this rich marrow disease and competent immune system, the risk of rejection is high and attempts at non-myeloablative conditioning (37) have so far resulted in failures.

The French experiment currently involves 105 patients. Results of the grafts performed between 11/1988 and 12/2004 with a follow-up of more than 2 years and involving 87 patients (aged from 2.6 to 22 years) have just been published (21). These grafts used myeloablative conditioning. With a median follow-up of 6 years overall survival is 93.1% and event-free survival (no death or rejection) of 86.1% (21).

The risk of rejection was significantly reduced by the addition of anti-lymphocyte serum



(ATG) in the packaging, since a risk reduction of 22.6% to 3% (p = 0.003) was obtained (18,21).

GVH (Graft versus Host disease) grade above or equal to II involved 20% of the patients and was responsible for 4/6 of the deaths observed. The risk was significantly influenced by age at age 16 or younger (p = 0.002). Seven patients had non-disabling chronic GVH and none had extensive GVH.

The results of the transplant in this pathology have been very significantly improved during the last 5 years (21):

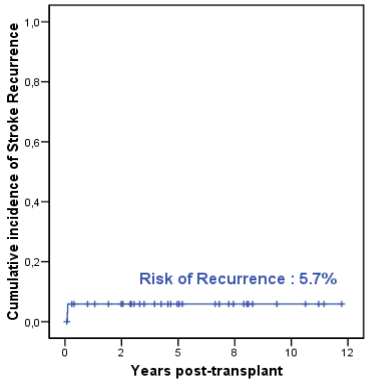
* 40 patients with a mean age of 9.2 ± 4.2 years were transplanted before 11/1999 with the following results: TRM (Transplant Related Mortality) of 15%, Releases 12.5%. The EFS (survival without death or rejection) was 72.5%

* 47 patients with a mean age of 9.7 ± 4.5 years were grafted from 11/99 to 12/2004 and no deaths occurred (TRM = 0%), and the risk of rejection was only 4.2%

Several factors are responsible for this progress: the experience of the grafting centers, the selection of patients who are somewhat less severe and better prepared for transplantation by transfusional leukocyte pellet programs reducing the risk of anti-HLA alloimmunization and thus of rejections

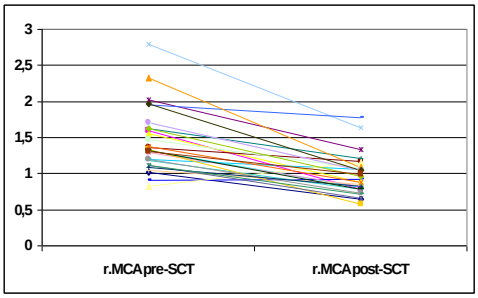
The immediate post-transplant period may lead to an increased risk of convulsions and reversible posterior leukoencephalopathy (18, 21, 38), but the measures adopted over the last few years have considerably reduced this risk (38): maintenance of platelets above 50000, control of magnesemia and BP, replacement of ciclosporin by Cellcept in case of corticosteroid therapy.

In patients with a history of stroke (n = 33), only 2 patients recovered post-transplant stroke: a fatal haemorrhagic stroke due to rupture of Moyamoya at J32 while the patient was well out of aplasia and an ischemic event Transient (TIA) on D10 in the context of hypertension and cyclosporine intolerance (21).



Thus, the risk of post-transplant recurrence was only 5.7% with a median follow-up of 4.6 years. Post-transplantation of imaging showed no new ischemic lesions in all patients with graft intake, but the children retained the sequelae of the old ischemic lesions. The evolution of vascular lesions varied: thrombosed vessels remained, while some stenoses disappeared completely and others continued to evolve, but the underlying absence of sickle cell disease No stroke despite a persistent vasculopathy.

In addition, TCD velocities were significantly reduced (p <0.001) by 138 ± 50 cm / sec before transplantation at 100 ± 34 cm / sec 1 year after transplantation



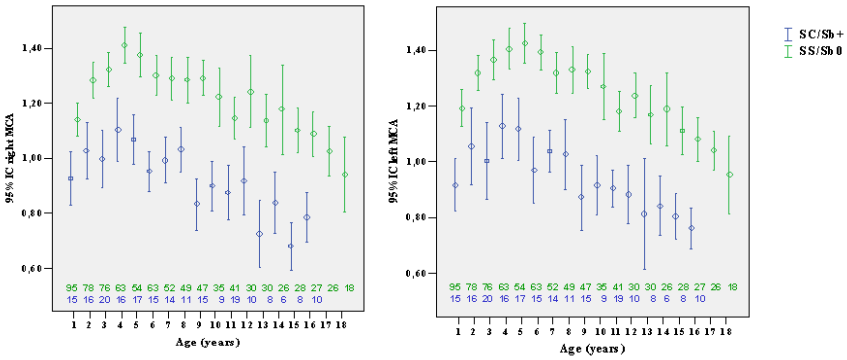
These excellent results in recent years have encouraged the recommendation of transplantation in children with a geno-identical potential donor earlier, before the onset of stroke and their irreversible sequelae (18,21).

2.5. Pilot STUDY AT Créteil ON THE PRE-SCREENING BY TCD

2.5.1. Cohort of neonatal screened children: Early detection of cerebral vasculopathy by TCD from the age of 12-18 months

Since 1992, sickle cell children have been systematically explored by TCD (Dr Suzanne Verlhac) from the age of 12-18 months (24). As of 5/2/06, 289 major sickle cell syndromes (MDS) had been detected and monitored at CHIC. We have early TCD monitoring at 181 patients with SDM including 149 SS/Sb0 and 32 SC/Sb+.

The chart on the right shows the rates observed at the TCD in the annual reports of children with MDS: SS patients have an average velocity> 110 cm / sec between 2 and 14 years while the velocities are always lower in the SC population



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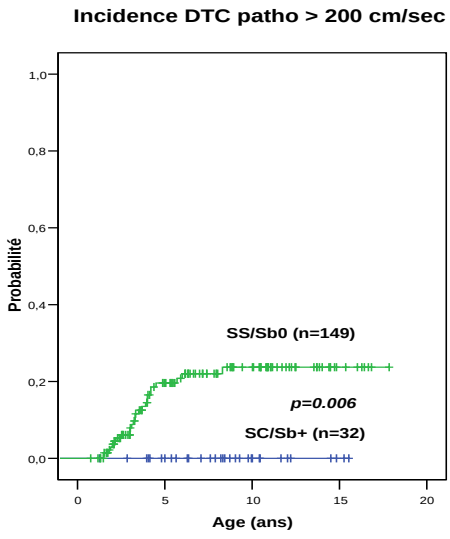


The follow-up mean follow-up with TCD of the cohort of children screened in neonatal in Créteil was 7.0 years ± 4.4 (range 0.8-17.8).

Early detection of vasculopathy by TCD, applied since 1992 in Créteil (39) significantly reduce the risk of stroke reported in the literature as 11% before the age of 20 (1-2)
Indeed only 2 strokes occurred in our cohort of children SS / Sb0 detected in neonatal and monitored by TCD:

- The first occurred in a 1.6-year-old girl in whom the first TCD a month earlier was pathological but the stroke occurred just before

the control of the TCD we were using at the time as recommended by Adams before start of the TP. Since this unfortunate experience, we decided to transfuse as soon as the pathological velocities> 200 cm / sec

- The second stroke involved a 4.4 year old girl in whom the absence of a temporal window did not allow the satisfactory realization of the TCD and the angio-MR had not yet been realized. Since this unfortunate event, we decided to program IRM / ARM as soon as the TCD cannot be realized by unavailability of temporal window as it often translates in our experience the sign of an underlying cerebral suffering (frequently observed in the course of stroke because of the non-expansion of the cerebral side of the stroke).

The graph below shows the (Kaplan-Meier) incidence of pathological TCD as a function of the diagnosis: we observed only pathological TCD (n = 25) in the SS / Sb0 population at the mean age of 3.5 ± 1.6 years (range 1.5-8.3 years) and an estimated incidence (Kaplan-Meier) of 7.9% at 3 years and 19.6% at 5 years whereas no new pathological TCD was observed beyond the age of 8.3 years in our experience.

The experiment of Creteil has now been published (Bernaudin F, Verlhac S, Arnaud C, et al. Impact of early transcranial Doppler screening and intensive therapy on cerebral vasculopathy in a newborn sickle cell anemia cohort. Blood. 2011; 117 (4): 1130-1140; quiz 1436).

2.5.2. LONG-TERM MONITORING OF PATHOLOGICAL TCD

Our experience with children with abnormally high TCD velocities (> 200 cm / sec) and followed between 1992 and 1/2004 was reported in 2005 (24):

- those with a history of stroke (n = 11) with velocities> 200 all retained pathological velocities under TP (8/11 explorable only, 3 lacking a temporal window): 6/8> 170 cm / sec and 2/8> 200 cm / sec

- those without a neurological history (n = 25) evolved as follows:

- 1 girl whose first TCD at the age of 18 months was pathologic(> 200 cm / sec) did a stroke in the following month, just before the TCD control: this unfortunate story made us change our conduct and now, a first transfusion is carried out as soon as the first pathological examination and then the monthly transfusion program is undertaken if the 1st pathological DTP was not related to a transient deglobulation
- 24 patients with no history of stroke but with pathological DTP were placed under TP and regularly monitored

  11/24 kept abnormal velocities> 170 cm / sec

  13/24 normalized their velocities <170 cm / sec within a median time of 0.75 years (0.25-2.3 years)

  4/12 explored by ARM had abnormal ARM (of which 2 normalized under TP
- Thus, 10 patients had standardized TCD and normal or standardized ARM
  3/10 had received TP> 3 years and was stopped
  7/10 switch to Hydréa but 4/7 have recurrence of pathological velocities
  1/10 with desaturation was treated with O2, but due to recurrence of pathological DTP was returned under TP

2.5.3. GRAFTS IN THE CASE OF PATHOLOGICAL TCD

Six children of this series with pathological TCD were allografted:

- 2 with history of stroke:

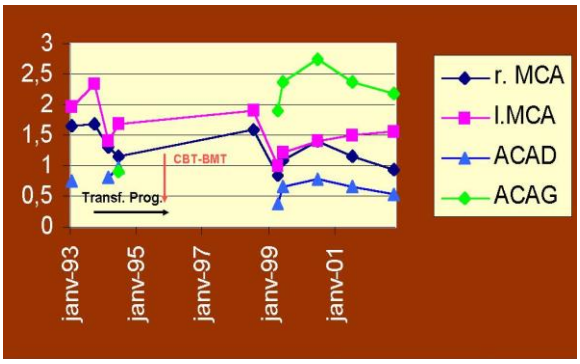
¬ These 2 patients were successfully grafted and are AA and AS as their donors, and have not been retransfused since 1 month post-transplant

¬ The absence of a temporal window did not allow monitoring of the TCD

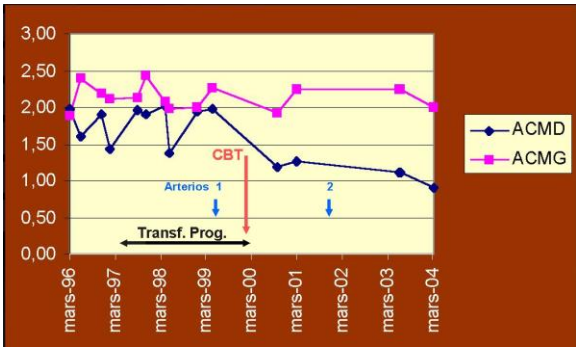
¬ No ischemic damage occurred since the transplant

- 4 without history of stroke

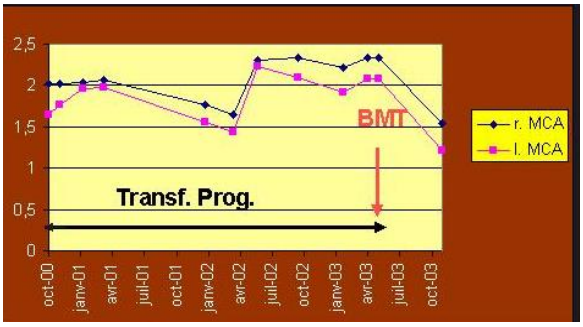
1. This girl was 7 years old when a pathological TCD was highlighted. The birth of an identical HLA sister whose placental blood had been cryopreserved allowed the grafting: unfortunately, there was a graft failure and autologous reconstitution was observed with a high level of HbF of 25% still persisting at 11 years of follow-up post-transplant and responsible for the absence of manifestations of sickle cell disease. On the other hand, the velocities are pathological at level of ACAG and angio-MR confirms persistent arterial disease.



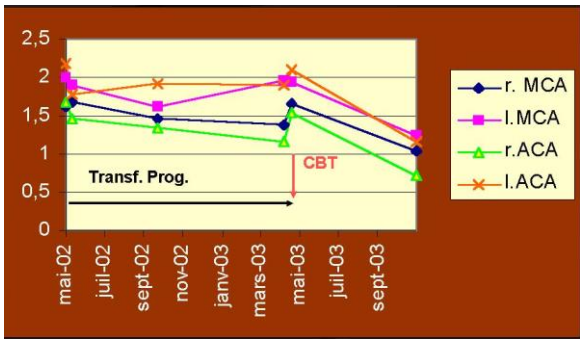
2. This girl was 3.3 years old in 3/96 when the TCD was found pathological. The TP was not undertaken until 1997 after the publication of Adams: the velocities remained pathological despite a TP of 3 years. The birth of an identical HLA brother whose placental blood had been cryopreserved allowed an allograft in 03/2000. At the time of this transplant, a normalization of the velocities on the right was observed while on the left, they remained pathological. This child now aged 12.5 years is perfectly fine, she is AS as her donor, has no stroke and is no longer transfused but maintains pathological TCD on one side and an angioMR arteriopathy without Ischemic lesions MRI.



3. This 3-year-old boy at the time of detection of pathological TCD retained abnormal velocities despite a TP of 12 months whereas very rapidly after the placental blood transplant of his identical HLA sister, a normalization of velocities was observed. It is now AS as its donor and goes perfectly well at 2.7 years of grafting and keeps normal velocities at TCD and a normal MRI / MRI.



4. This boy was 4 years old at the time of the discovery of the pathological TCD and kept abnormal velocities in spite of a prolonged TP of 2.4 years whereas a rapid and definitive normalization was observed in the course of a medullary graft from his brother AS: TCD and MRI / ARM are perfectly normal



We concluded from this monocentric experiment that:

- early detection of cerebral vasculopathy by TCD from the age of 12-18 months is possible

- the initiation of a transfusion program in the case of pathological TCD significantly reduced the risk of stroke from 11% (1.2) to 1.1%

- the incidence of pathological TCD at a given time is 10-12%, but follow-up of patients from birth showed in the Creteil cohort that 19.6% of SS / Sb0 children had pathological TCD before the age of 5 years which is very important and will cause considerable transfusion demand in future years

- the geno-identical graft gives very good results in the child with an expectation of survival without events of about 95%. It alone makes it possible to interrupt a TP in safety and has even allowed in some children a normalization of the velocities at the TCD not obtained by the TP

It is therefore imperative to look for alternatives to extended transfusion programs and to evaluate prospectively and multicentre the benefits / risks of early transplantation

2.6. TOXICITY OF TRANSFUSIONAL PROGRAMS

2.6.1. INFECTIONS

The residual risk of viral infection of transfusion origin is minimal in the case of viruses known and detected in donors since it is estimated at 1 / 3,900,000 for HIV, 1 / 6,000,000 for HCV and 1 /2,400,000 for HBV (81)

Parvovirus B19, which is not detected in donors, can have consequences for sickle cell anemia. Nearly 50% of donors are immunized against Parvovirus B19, but the prevalence of this infection and its consequences with regard to transfusion is not clear (82), so the question remains of the need for negative CGR For Parvovirus B19 in non-immunized sickle-cell anemia.

Transfusion incidents involving bacterial contamination constitute the major infectious risk (1 bacterial infection for 178,000 labile blood products) and can have dramatic consequences in sickle cell patients (83). They are mainly due to commensal skin bacteria or post-prandial bacteremia of the donor at the time of collection.

Finally, the infectious risk also concerns agents not known to date, for which it is not possible to take preventive measures. Patients who are transfused in an iterative manner are potentially at risk.

2.6.2. Allo-Immunisation

Immunological risk remains the major risk of transfusion in sickle cell patients. It is mainly the risk of anti-erythrocytic alloimmunization, since anti-HLA alloimmunization is much less frequent since the systematic deleucocytation of RBCs was introduced in April 1998 (84).

The anti-erythrocytic alloimmunization is relatively frequent in these patients, it can reach 50% according to transfusion studies and transfusion practices. It results mainly from the ethnic polymorphism of antigens of blood groups between donors, mainly of Caucasian origin and recipients of Afro-Caribbean origin.

This polymorphism is found at several levels: at the level of the common antigens, that is to say those classically known to be immunogenic: the antigens RH, FY, JK and MNS. Thus, the C-antigen of the RH system is very frequently expressed in Caucasian donors, whereas it is very rarely expressed in sickle cell recipients.

The same problem arises for the Fya, Jkb and S antigens. Although, in general, alloimmunization can be prevented in the HR system, on the other hand, the resource is insufficient in terms of CGRs from Afro donors And does not prevent alloimmunization from other antigens (85).

The polymorphism of blood group antigens is also part of a number of variants, which are often unknown, causing immuno-hemolytic accidents and lack of transfusion yield. These variants can be found only by the demonstration of associated alleles in molecular biology, a practice not routinely implanted (86-87).

When alloimmunization is established, the risk of immuno-hemolytic accidents and a greater frequency of transfusion-related absences are observed by different mechanisms. The first mechanism is the ignorance of the alloantibody and its ignorance because it has disappeared from the serum before the transfusion and / or the alloimmunization history is not known (88). The antibody reactivated by the transfusion sensitizes the transfused RBCs and induces their destruction, often causing the concomitant destruction of the patient's own GRs probably by the effect of an oxidative stress generated by the hemolysis of the dependent antibody but also by the non-specific binding On sickle cell RBCs of activated complement fractions (89).

A second mechanism is the production of autoantibodies which can lead to serious arrays of transfusional haemolytic anemia (90).

Finally, apart from any obvious alloimmunization, transfused RBCs can be destroyed rapidly (within 10 to 15 days) without exactly understanding the mechanism (91).

When alloimmunization extends to many common or specific antigens of these populations, irreversible transfusional situations can occur, with only the possibility of incompatible transfusions at high risk.

Whatever the situation, these incidents or accidents aggravate the clinical picture which had motivated the transfusion, exacerbates the clinical manifestations and therefore become deleterious for the disease. Transfusion dead ends can no longer find compatible CGR and thus treat the patient.

The prevention of these accidents lies mainly in the knowledge of the extended phenotype of the patient, the phenocompatibility of the CGR and the pre-transfusional compatibility tests in the laboratory.

In France, the phenocompatibility is systematic for RH and KEL systems, the most immunogenic, but cannot be extended to the other systems in first intention due to the shortage of CGR coming from donors of Afro-Caribbean origin. Concerning the extended phenotype, it is carried out systematically, and should be associated with the demonstration of variant antigens more frequent in these populations (D, C, e partial and "public" negative phenotypes).

Finally, compatibility tests should also be systematic as they make it possible to overcome some of the deficiencies of RAI when screening for antibodies directed against low frequency antigens.

2.6.3. Iron overload

Iron overload is unavoidable in long-term transfusion programs. The practice of transfusion exchanges used in sickle cell patients makes it possible to limit it with manual exchanges and even to avoid it with erythrocytapheresis. Iron overload can be responsible for endocrine problems: diabetes, PTH, gonadal and cardiac insufficiency that occur only after very prolonged transfusion programs over 10 years. To avoid these complications, it is necessary to resort to a chelating treatment in principle justified after about twenty transfusions. The overload is assessed on the blood balance by the siderophilin saturation coefficient and the ferritin level. The reference technique for evaluating iron overload was that obtained by PBH, but recent MRI and cardiac MRI techniques made its assessment easier and less dangerous. Reference chelation involves the use of Desferal whose 10-hour nocturnal subcutaneous administration is extremely restrictive, but the appearance on the market of the new oral chelator Exjade should improve the compliance of polytransfused patients.

2.7. ALLOGRAFT TOXICITY

2.7.1. Aplasia

Aplasia during these myeloablative transplants lasts an average of 20 days for medullary graft and 28 days for placental blood grafts and is at risk of infectious complications justifying the use of sterile asepsis measures. Nevertheless, thanks to these measures and the antibiotherapies available to us, the occurrence of aplastic deaths was only one case for 87 transplants.

2.7.2. Graft versus host disease: GVH

In these geno-identical grafts, GVH is related to minor antigenic differences. It is prevented by ciclosporin A administered from D-1 to 6 months-1 year post-transplant. In the first 100 days of the transplant it is called acute GVH and results in skin signs such as pruritic erythematous rash, which can lead to Lyell, diarrhea-vomiting-malabsorption and signs Biological agents. It requires the use of corticosteroids and possibly other immunosuppressants such as Cellcept. Chronic GVH manifests itself after J100 in principle in some patients with acute GVH: it results in mucocutaneous problems such as irregular pigmentation of the skin, labial and jugal lichen, possible scleroderma and / or From digestive problems to malabsorption type. The most serious complication is the occurrence of bronchiolitis obliterans

In the context of geno-identical grafts for Sickle cell anemia (21), acute GVH grade ≥ II was

observed in 20% of patients. The factors significantly associated with GVH risk were age> 15 years and the existence of a mismatch. No GVH ≥ II was observed after placental blood transplantation. Moderate chronic GVH was observed in 11% of patients and extensive in 2.4%. GVH was responsible for 4 deaths in the 87 patients. Nevertheless, the signs of GVH fade over time and none of the 81 living patients is actually disabled by it.

2.7.3. Gonadal risk

1. In girls

The myeloablative conditioning (Endoxan, Busulfex, Thymoglobulin) proposed here for transplantation in sickle cell anemia exposes a risk of prolonged ovarian failure in puberty girls at the time of conditioning and results in a fall in estradiol levels and High levels of FSH and LH requiring the use of estrogen-progestin treatment and raising fears of further infertility. It is not known at this time whether this ovarian failure will be definitive or transient: it is estimated to be at least prolonged for 10 to 15 years. This is why ovarian cryopreservation is proposed: the specimen is taken under caelioscopy and the ovary is frozen in small fragments. This technique made it possible to obtain a pregnancy in a female ex-sickle cell allograft (67-68).

When the graft is performed in the girl, an estrogen-progestin treatment is often necessary for the induction of puberty when bone age reaches 13 years but this is not always necessary and spontaneous and normal puberty with normal rates of d oestradiol, FSH-LH were observed in several young girls grafted (21). Moreover, an induction of puberty has been obtained in Créteil in post-transplantation by reimplantation of ovarian fragment taken from the pre-graft (personal data in the course of publication) published (95).

2. In boys

No gonadal hormone disorder has been observed so far in our French series (21) in boys: testosterone levels are normal as well as FSH-LH levels and in relation to their age and pubertal development which Is always carried out normally in post-graft. On the other hand, no spermatic study has yet been carried out to assess their future fertility. As a precautionary measure, testicular cryopreservation may be proposed to young sickle cell boys.

2.7.4. Other risks

Treatments with total body irradiation are known to increase the carcinological risk but this seems very unlikely with the packaging used in sickle-cell disease that does not use irradiation. Extensive chronic GVH can also be a risk factor. In any case, to date, no secondary cancer has been observed in the wake of sickle cell transplants

All these risks have been detailed in a reply to a letter published in Blood (92)

2.7.5. Psychological aspects and quality of life

The impact of a chronic disease on the emotional development of the child and his / her family is major and needs to be taken into account.

A retrospective study, supported by the French Institution of Transplantation, was carried out in France on 28 sickle cell patients treated with HSC allograft. His goal was to assess their psychological state and to identify, through their narratives of illness and healing, the psychic and cultural stakes of the healing processes (42). Of the 28 patients, nine had psychiatric

disorders of moderate intensity, requiring specialized follow-up. Most had a positive view of the graft and its effects. Interviews showed the importance of individual and family changes. The main issues of healing were donation, the issue of recognition and debt, building identity especially in adolescence and infertility and its transgenerational repercussion.

In addition to cognitive assessment and quality of life, a prospective and comparative study will evaluate the psychological state of sick children and their evolution during two different treatments: transplant or transfusion program.

Studies have reported the cost of transfusion programs in sickle-cell anemia in the USA (41) and geno-identical allograft in France (44). Our study will make it possible to compare the respective costs of these two techniques in a prospective way. The main endpoint is a biological criterion that does not allow a cost effectiveness study to be carried out. We will compare the costs of the allograft transplantation strategy with the objective of providing an additional decision criterion. There is no provision for cost study utility. The costs will be estimated from the point of view of the health care system, for a period of 2.5 years (duration of patient follow-up). The reference is the transfusion program.

2.7.6. Evaluation of costs

Our experience with the GREFIG study (44) indicates that the cost of allograft can be estimated reliably by the length of stay of patients. A study conducted in Créteil comparing the costs of transfusion programs and transplantation showed that the cost of transplantation corresponded to the cost of a 19-month transfusional program involving chelation of iron (93).

3. OBJECTIVES OF RESEARCH

3.1. Hypothesis

The reference therapies for a history of clinical stroke in sickle cell patients are the "lifetime" transfusion program, which reduces the risk of stroke recurrence by 67% to 10% and haematopoietic stem cell transplantation Donor genoid) available to reduce this risk to 6% (18,21).

The primary prevention treatment for the risk of stroke at abnormally high TCD rates is the very prolonged transfusion program (9,10), which reduces the risk of stroke by 40% to 2%.

Allograft has been proposed only to a very small number of patients with no history of stroke but with pathologic TCD (18,21), these results are very encouraging but require to be evaluated in a multicentric and prospective way.

We hypothesize that allograft, despite unquestionable toxicity but limited at this age of life, will allow better prevention or stabilization of cerebral vasculopathy

3.2. Primary objective

Show that the graft significantly decreases the average at 1 year of the velocities (TAMV) of the right and left middle cerebral arteries (MCA) than the extended transfusion program

3.3. Secondary objectives

To contribute to the analysis of the clinical and biological factors influencing the appearance of pathological velocities of the cerebral flow and thus of the cerebral vasculopathy

Compare in both groups (Extended Transfusion Program and Transplantation):

- Evolution of cerebral vasculopathy
O percentage of patients having normalized all cerebral velocities (<170 cm / sec) in both the graft and transfusion arms
O MRI ischemic lesions
O Stenosis at MRA
O serum expression of "angiogenic" molecules
O comparative cognitive performance child sickle cell and siblings
- Assessment of transfusion performance, anti-erythrocytic alloimmunization, and post-transfusion incidents and accidents
- Iron overload
- Psychological condition and quality of life
- Costs

4. CONCEPTION OF RESEARCH

4.1 Main endpoint

Improvement of the cerebral vasculopathy assessed on the measurement of the average at 1 year of the velocities (TAMV) in MCA.

4.2. Secondary endpoints

4.2.1 Evolution of cerebral vasculopathy

- Incidence of ischemic stroke: We will compare the risk of stroke in children with pathological TCD and treated according to the type of treatment applied: prolonged TP or graft

- Survival without ischemic stroke: one will evaluate the time until the first event (stroke or death)

- TCD velocities: all velocities in the different arteries: MCA, ACA, PCA, BT, IC will be collected at baseline and then at 1 year of inclusion in both prolonged TP or graft patients.

- Standardization of velocities to 1 year. The TCD will be considered normalized if all velocities are <170 cm / sec. The percentage of patients who standardized the TCD velocities will be compared between the 2 groups "exposed = allogeneic" and "unexposed = transfused"

- Incidence of ischemic lesions in MRI: we will compare the number and size of the ischemic lesions that have emerged since inclusion in both the prolonged TP or graft groups

- Incidence of stenosis in MRA: the number and extent of stenosis occurring since inclusion will be compared in both prolonged TP or graft groups.

- Expression of membrane phosphatidyl and angiogenic molecules at baseline and at 12 months post-inclusion

- cognitive performance: the delta of cognitive performance (difference between values at the

end of the study -values at inclusion) will be compared in the 2 groups prolonged TP or graft as well as the difference in cognitive performance with the member of the sickle cell brothers

4.2.2. Alloimmunisation anti-érythrocytaire

- Alloimmunizations that have appeared since inclusion will be compared in both groups. In the transfusion group one will also note the necessity of stopping the program if any.

4.2.3. Hemolysis and iron overload

- Hemolysis tests (GR, reticulocytes, erythroblasts, haptoglobin, Bilirubin, LDH) will be compared in both groups: a recent study showed us that there was still a significant degree of TP while this was not observed in Post-transplantation (30).

- Iron overload will be studied on blood sampling (Iron, CTF, ferritin) at baseline, 3, 6 9 and 12 months post-inclusion. Hepatic and cardiac MRI will be performed at baseline and at the end of the study in both groups

4.2.4. Assessment of psychological status and quality of life

The protocol will include a pedopsychiatric evaluation at inclusion and at 1 year post-inclusion. In the group of transplanted children, a pre-transplant and post-transplant evaluation at 2 months will be performed in addition. This assessment will be adapted according to the age of the child. It will be an evaluation in search of the main psychiatric disorders (with a structured diagnostic interview), attachment patterns (stories to be completed) and measuring certain psychological dimensions (anxiety, depression, post-traumatic symptoms and Outsourced). In addition to this quantitative evaluation, there will be a qualitative clinical evaluation of parents / children in pairs with a standardized clinical situation (drawing of the man in relation to the representation of the body, the family, free drawing, play with stickers and animals for children More than three years, free talk with parents and teenagers). For children under 3 years of age, parent-child interactions will be assessed. At 1 year post-transplant, this assessment will be supplemented by a family interview according to the methods used in transcultural psychiatry (43).

Quality of life will be assessed at baseline, 3, 6, 9 and at 12 months post-inclusion. The choice of the quality of life scale will be made with the help of our psychologists and educators, the aim being to evaluate by a self-questionnaire the quality of life of the children as well as the quality of family life (69-75) , As the graft or non-graft may have disrupted or improved the family's experience of the disease.

4.2.5. Evaluation of costs

Our study will make it possible to compare the respective costs of these two techniques in a prospective way. The main endpoint is a biological criterion that does not allow a cost effectiveness study to be carried out. We will compare the costs of the transfusion strategy with those of the allograft, in order to provide an additional decision criterion. There is no provision for cost study utility. The costs will be estimated from the point of view of the health care system, for a duration of 1 year (duration of patient follow-up). The reference is the transfusion program.

The resources used will be documented prospectively in volume for each patient included

from the observation book. These resources include hospitalizations, examinations and ambulatory treatments. The valuation will be carried out on the basis of the national tariffs when they are available (prices of blood products, rate of ambulatory acts.) In terms of hospitalizations, we will use DRG data, supplemented if necessary by accounting data The costs associated with HLA typing, sampling, packaging and, in general, all pre-transplant procedures will also be included in the calculations. The hospitalizations that will follow the allograft will be value

4.3 Description of methods

4.3.1 Methodology

It is a biomedical research of cell therapy, multicentric and carried out in open.

There will be no randomization but the distribution between the two groups will be linked to the chance of having a family geno-identical donor or not.

The study of type exposed (grafted) - unexposed (TP). They will be matched on the age (±1 year), the number at inclusion of possible allo-ACs, the number of stenoses and / or ischemic lesions to the allografted subject. Pair matching will be done during the blind analysis by the DBIM who will not have the notion of the 1 year evolution of cerebral vasculopathy.

- Sample size: 63 patients 21 in the Transplantation and 42 in the Transfusion group

- Expected length of research

Estimated total duration of the research (duration of inclusion and duration of participation) The duration of inclusion is 2 years, the follow-up duration is 1 year (post-transplant or extension of the TP), or a total duration of 3 years.

4.3.2 Unfolding of Research

Recruitment, information and consent.

Sickle cell children (SS/Sb0), aged less than 15 years with a h TCD (velocity (≥ 200 cm / sec) and having a sibling of the same parental couple, will be seen in consultations as part of their pathology, within the sickle cell centers participating in the study.

The investigator will provide appropriate information to parents and patients, including the objectives, benefits, constraints and risks of participating in this research. A reflection period of at least one week will be granted before obtaining the participation agreement for HLA typing and potential transplantation if presence of a genoidentical donor.

 Patients with pathological TCD with 1 or more siblings but no identical HLA will pursue the transfusion program

 Patients with a history of pathologic TCD in whom familial HLA typing will show the existence of a geno-identical donor (HLA identity AB, DR, DQ) will be able to integrate the allograft group

A committee of pediatric experts will receive the child in case the family agrees to the transplant

Informed consent visit

During this visit, the investigator will answer the questions of the parents, the patient and the siblings, and will then collect the informed consent signed by the parents and the patient.

The patient will then:

• an initial clinical assessment
• HLA typing
• a control of its vasculopathy: TCD and MRI/MRA
• cognitive tests (WIPPSI-R and WISC IV)
• a quality of life assessment test
• Two additional blood tubes with a volume of 3 ml each will be recovered during the usual biological check-up in order to study the following biological parameters: hypoxia factors, phosphatidylserine expression, chimerism (for the allograft group).

Follow-up visits

- Vasculopathy, TCD and MRI / ARM checks will be carried out at 12 months.
- the assessment of the quality of life will be carried out every quarter during the 12 months of follow-up
- Two additional blood tubes of 3 ml each will be recovered at 12 months, for the study of biological parameters.

4.5 Description of stopping rules

At any time, the sponsor and / or regulatory bodies may decide to discontinue the trial prematurely for medical and / or administrative reasons. In all cases, the decision shall be taken after mutual consultation and appropriate documentation of the reasons. The investigator will then return the observation books and all documentation related to the study to the proponent.

Rules for discontinuing the study on an individual scale:

- Patients and parents refusing transfusion protocol will not be included.
- Patients with an identical HLA donor and refusing allograft will be analyzed for intention to treat
- Major alloimmunization prohibiting the continuation of the transfusion program

5. SELECTION and EXCLUSION of participants

5.1. Inclusion criteria

- Sickle cell patients SS / Sb0,
- Aged less than 15 years,
- With a history of pathological TCD (≥ 200 cm / sec)
- Having a sibling of the same parental couple
- Of which parents accept HLA typing for the grafting project in the event of the existence of a genotype HLA donor in the sibling or the extended transfusion program.
- Informed and written consent of parents.

5.2. Non-Inclusion Criteria

- Sickle Cell Patients> 15 years
- No history of pathological TCD (≥ 200 cm / sec)
- Not having siblings of the same parental couple
- Of which the parents refuse the HLA typing, for the project of graft in case of existence of HLA donor geno-identical in the sibling or the extended transfusion program.
- No affiliation to a social security scheme (beneficiary or beneficiary)

5.3. Criteria for non-inclusion of donors vis-à-vis communicable agents

Positive HIV Serology
P24 positive antigenic
Positive HCV serology
HBS positive antigen

6. PROCEDURES OF RESEARCH

6.1. Identification of patients

The so-called "pathological" TCD will have been sent by Fax or mail to Dr. Verlhac for control The training of dopplers was monitored and carried out by Dr. Verlhac who created a website in 2006: drepanosite.free.fr and a self-control of Doppléristes will be carried out through the use of an e-learning module assisted by computer.

6.2. HLA family types

After parental agreement, HLA typing of the siblings will have been carried out with that of the parents if necessary.

6.3. STUDY OF PHOSPHATIDYL-SERINE AND VARIANTS RH (DR FRANCE NOIZAT-PIRENNE EFS MONDOR

A determination of the main variants of the RH antigens of transfusional interest will be carried out in molecular biology. The expression of phosphatidylserine on the surface of RBCs will be determined by evaluating annexin V binding to GR in flow cytometry. Variants RH and expression of the phosphatidylserine will be realized on the same tube sent to the EFS Henri Mondor (3ml).

6.4. Etude des facteurs d'hypoxie (Myriam BERNAUDIN, CNRS Caen)

Study of angiogenic molecules (Myriam BERNAUDIN, CNRS, Caen)
Sampling of a tube of 3 mL of blood, the samples will be centralized at the CHI of Créteil.
The blood will then be centrifuged and the Serum will be kept at -80 ° C.
The frozen sera will be transported by the carrier DHL, a quarterly sending of 40 tubes in the dry ice at the CNRS of Caen.

6.5. Transfusion Program

Before each transfusion, NFS, Hemoglobin electrophoresis, RAI and iron balance (Iron, CTF, Ferritin) will be performed

Laboratory compatibility tests will be systematic
Viral serology (HBS, CMV, HIV, HTLV) will be done at baseline and at 1 year post-inclusion

The biological study of hypoxia factors (serum expression of angiogenic molecules: VEGF, EPO, Ang-1 and Ang-2) and membrane phosphatidylserine will be done at inclusion and at 1 year post-inclusion (see Table 4.5.1)

The goal of the transfusion program is to maintain HBS below 30% and Hb between 9 and 11g without increasing hyperviscosity.

The choice between simple transfusions, manual exchange or erythrapheresis will be left to the investigator of the monitoring center

For those performing simple transfusions, the following scheme will be proposed:
Scheduled transfusions every 4 weeks
- 15 ml / kg in the case of Hemoglobin <9 g / dl
- 12 ml / kg in the case of Hemoglobin between 9 and 9.5 g / dl
- 10 ml / kg in the case of Hemoglobin between 9.5 and 10 g / dl
- in case of Hemoglobin > 10g / dl an exchange will be performed or the transfusion delayed one week

6.6. Graft

The transplant will be proposed to patients who have had a pathological TCD and a family geno-identical donor. The donor may be AA, AS or Athal and will be received as for all family transplants involving a minor donor by a committee of pediatric experts. The cellular source of the graft will be medullary or the placental blood possibly cryopreserved.
The minimum cellular dose required for a bone marrow transplant should be ≥ 2x108 CNT / kg and for a placental blood graft ≥ 3x107 CNT / kg.

Graft Protocol (bibliographic reference no. 21)

The recipient and the donor will have
- Control of HLA typing in the graft center
- Extended erythrocyte phenotypic group and RAI
- Search for anti-HLA
- Sampling for the study of chimerism
- G6PD
- Hepatic and Ionogram, Creatinine
- Serology: HIV, HTLV, HBV, HCV, CMV, EBV, Toxo, Syphilis !, Parvo, Measles, VZV, Herpes
- Drop thick if traveling in Africa
- Rx Thorax
- ECG
- Anesthesia consultation

The recipient will have a pre-transplant
- Echo heart with FR and FE and tricuspid regurgitation velocity
- EFR
- Thoracic CT

- Echo abdo dating <3 months
- Cerebral MRI / MRA and TCD will have been performed prior to inclusion

Blood products will be irradiated within 3 months before transplant

- Major donors will have to take RV to the Tribunal de Grande Instance of their Prefecture to deposit their signed consent to the Gift of bone marrow
- Minor donors are received individually and with their parents by a Committee of Pediatric Experts to collect their free consent.
Donor and Receiver are then hospitalized on D-15
- the donor will revert to serology and
- Receiver enters for Central KT installation under AG

Conditioning
- Busulfex (formerly known as Busilvex) from D-10 to -J-7: at 12.8 mg / kg total dose for more than 34 kgs or 15.2 mg / kg for weights of 23-34 kgs, 17.6 mg / kg For weights of 16 to 23 kgs and 19.2 mg / kg for weights of 9 to 16 kgs
- Endoxan (Cyclophosphamide): 200 mg / kg total dose (50 mg / kg IV D-5 to D-2)

- Rabbit anti-lymphocyte serum: Thymoglobulin 20 mg / kg total dose: 5 mg / kg / day D-6 to D-3

Prophylaxis of GVH
Marrow Transplant: CSA-MTX short
Placental blood transplantation: CSA alone

Ciclosporin A will be introduced on D-1 and continued for 6 months to 1 year after transplantation depending on whether or not GVH is present.

Anti-Comitial Prevention
Rivotril will be prescribed throughout the conditioning and as long as ciclosporin A is maintained.
The platelet count will be maintained> 50,000 and magnesemia and BP will be strictly controlled
In the case of acute GVH, to avoid neurological complications related to ciclosporin and corticosteroid therapy, ciclosporin A will be replaced by Cellcept at the introduction of corticosteroids (21)

Post-transplant follow-up
At the end of aplasia, it will be done at the rate of a passage in hospital of day by week until 3 months post-graft then spaced

Anti-toxo or anti-pneumocystis prophylaxis should take into account the donor's G6PD

In the case of post-transplant transfusion needs, these must be compatible with the recipient and the donor and the products will be irradiated

Hemoglobin electrophoresis and chimerism will be monitored regularly at 1, 2, 3, 6, 9 and 12 months post-transplantation

The remainder of the surveillance will be identical to that of the other allograft patients (surveillance of the CMV PCR, IgG assay for example ...)
The anti-pneumococcal prophylaxis by Oracillin will be maintained for 2 years and a vaccination by Prevenar then Pneumo 23 carried out

Recovery will depend on the condition of the child but can usually be done 3 to 4 months after the transplant

Vaccinations by HIB will be done at 2, 3, 4 months post-graft and 3 DTPolio will be done at 1 year post-transplant

6.7. Psychometric tests

Psychometric tests (WIPPSI-R or WISC-IV according to age) will be carried out in the patients participating in the study: those patients who can be allografted (having an identical HLA donor in the siblings) and the others (No identical HLA donor among siblings) will be studied as well as the closest brother or sister of the patient's age. The tests will be repeated at 1 year of inclusion in patients and siblings. Indeed the previous study (PHRC 95) (28) showed us the relevance of testing and "retesting" siblings as well. The tests will be repeated 2 years after the second evaluation.

6.8. Control of cerebral vasculopathy

The following parameters will be checked at baseline and redone at 1 year of inclusion for all allograft and non-grafted patients.
- TCD and MRI / MRA (including cervical incidence)
- Phosphatidylserine and angiogenic molecules

For patients included in the transfusion program, Coombs, RAI and iron balance (Fer, CTF, Ferritin) will be checked at baseline and before each transfusion until follow-up (at 12 months post-inclusion).

For the included patients who will be allografted, Coombs, RAI and iron balance (Fer, CTF, Ferritin) will be controlled at baseline at 3, 6, 9 and 12 months post-inclusion in the DREPAGREFFE section.

7. Description of efficacy parameters

TCD as well as MRI / MRA will be redone at inclusion and then at 12 months post-inclusion (whether the child is under TP or allograft)

Cognitive tests (WIPPSI-R then WISC-IV): will be done at inclusion and redone at 12 months post-inclusion

Scale of quality of life will be evaluated in the child and its relatives by a self-questionnaire (69-75) at the inclusion, 3 months, 6 months, 9 months and 12 months post-inclusion

Alloimmunization: the search for irregular agglutinins will be carried out systematically before each transfusion and in the graft group it will be carried out at the inclusion, at 3 months, 6 months, 9 months and 1 year post-graft. Anti-HLA testing will be performed at baseline and 1 year

Phosphatidylserine and angiogenic molecules will be studied at inclusion and then at 1 year post-inclusion.

8. Ancillary study: biological markers.

This ancillary study proposes to study the biological markers: Phosphatidyl-serine and the factors of hypoxia before initiation of the transfusion program.
It concerns sickle cell patients who have only recently been shown a pathological TCD (velocities ≥ 200 cm / sec) and in whom the last transfusion is more than 3 months old. (See appendix N ° 5)

9. Evaluation of safety

All serious adverse events (SAEs) will be reported within 24 hours by the investigator of the center immediately to the sponsor for reporting to the authorities according to the procedures in force.

9.1. Description des paramètres d'évaluation de la sécurité

- **Adverse event** Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

- **Adverse drug reaction**

Any response to a medicinal product which is noxious and unintended.

- **Serious adverse event**

Any untoward medical occurrence that at any dose results in death, is life-threatening, requires inpatient hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability/incapacity, or is a congenital anomaly/birth defect.

- **Unexpected adverse reaction**

An adverse reaction, the nature, severity or outcome of which is not consistent with the applicable product information: the summary of product characteristics (SmPC) for an authorised product or the investigator's brochure for an unauthorised investigational product.

- **New safety issue**

Any new information regarding safety:
- that could significantly alter the assessment of the benefit-risk ratio for the experimental medication, or for the trial
- or which could lead to the possibility of altering the administration of the experimental medication or altering the conduct of the trial

9.2 Study committees

9.2.1 Steering committee

It will define the general organization and conduct of the research and coordinate the information.
It will initially determine the methodology and will decide in the course of research the conduct to be taken in unforeseen cases, will monitor the conduct of the research particularly in terms of tolerance and adverse events.

It will consist of the clinical initiators of the project, the biostatistician in charge of the project, representatives of the promoter and the URC appointed for this research, in particular: Dr. Françoise Bernaudin (Investig coordinator); Prof. S. Chevret (DBIM); Pr Socié (Head of the Registry Unit at St Louis Hospital); V. MILLUL (DRCD).

9.2.2. Data Safety Monitoring Board

The Data and Safety Monitoring Board (DSMB) can be established by the sponsor. Its primary mission is to serve as a committee for monitoring safety data. It can have other missions, such as monitoring efficacy data (especially if the protocol includes interim analyses).
The DSMB is mentioned in Article L. 1123-7 of the French Public Health Code.

The sponsor is responsible for justifying the creation or absence of a supervisory committee to the Competent Authority (ANSM) and to the CPP.

At the level of tolerance, it will monitor the frequency and imputability of deaths, severe GVH in the graft group, severe alloimmunization in the transfusions group. It may decide to discontinue the study in the event of a difference in mortality or the occurrence of clinical events liable to cause sequelae in one of the groups

In the absence of serious adverse events, the Independent Monitoring Committee will meet once a year to assess the progress of the study, validate possible alloimmunizations and serious GVH.

 The constitution of the members is as follows:

- DR B. BRUNO : CHRU de Lille – Hématologue-Pédiatre
- DR C. PAILLARD : CHRU de Strasbourg – Hematologue –cancérologue –Pédiatre
- PR A FERSTER : HUDERF – Bruxelles – Onco-hématologue- Hématologie générale

The DSMB has agreed to meet once every 6 months in a systematic way for 1 year and then once a year if there is an extension of the follow-up period. Additional meetings or consultations may take place , If requested by the proponent or by one of the members of the CSI if it deems it necessary. Meetings will usually be by conference call unless a physical meeting is deemed necessary by the Committee and the proponent.

9.3 Notification of adverse events (AE)

9.3.1 Non severe AE

Any adverse event - not serious according to the previous definition - observed during the research and its follow-up should be reported in the observation book in the section provided for this purpose.

Only one event must be reported per item. The event may be a significant symptom, diagnosis or examination result. All the clinical or para-clinical elements that make it possible to best describe the corresponding event must be reported.

9.3.2. Severe AE (SAE)

The investigator must report all adverse events that meet one of the seriousness criteria below, except for events listed in section 10.3.3.1 as not requiring notification:
1- Death
2- Life threatening situation
3- Requiring hospitalisation or prolonging hospitalisation
4- Persistent or significant disability or incapacity

5- Congenital abnormality or birth defect
6- Or any other adverse event considered "medically significant"

The investigator must notify the sponsor, **immediately on the day when the sponsor becomes aware**, of all the serious adverse events, except those that are listed in the protocol (see. section 10.3.3.1) or in the investigator's brochure as not requiring immediate notification.

These serious adverse events are recorded in the "adverse event" section of the case report form and the investigator must immediately notify the sponsor's Vigilance division (see 10.3.4).

Any patient with an adverse event should be followed until resolution or stabilization of the patient.

If the event is not serious, the evolution will be noted on the corresponding page of the observation book in the section provided for this purpose.

If the event is serious, follow-up EIG will be sent to the DRCD.

9.5 Grading of SAE

See Appendix 7.

10. STATISTICS

10.1 Description

After monitoring, data processing will be carried out in accordance with the complementary guiding principles of the computer systems of the Guide to Good Clinical Practices of the European Community. More precisely, the data will be entered according to a procedure of double entry, under a manager of databases type ACCESS for Windows. They will be validated after input, before the start of the statistical analysis (from the issuance of requests to the CRAs and clinicians involved in the study).

The statistical analysis will be carried out at the department of biostatistics and medical informatics of the hospital Saint Louis (Paris) on SAS software (SAS Inc, Cary, NC) and R. The main analysis will be carried out in intention to treat, all children in it, whether or not they received the treatment in its entirety or not. For exploratory purposes, a protocol analysis could be carried out secondly, retaining only the children treated according to the therapeutic protocol of their group.

The percentage of patients with normalized velocities will be compared by a non-parametric approach. Only deaths before normalization will be considered a competing risk of the event of interest. A Fine and Gray model will allow to compare the two groups by taking into account potential confounding factors.

The same approach will be applied to secondary endpoints (incidence of ischemic stroke, incidence of ischemic lesions and stenoses, cognitive performance, alloimmunization). The modeling of stroke-free survival will be based on the Kaplan-Meier estimate and the Cox model.
The costs will be compared between the two groups by nonparametric tests to account for the non-normal distribution (skewedness). High cost predictors / explanatory factors will be sought by multivariate analysis.

The significance level will be 5% and all tests will be two-sided.

10.2. Sample size

For an alpha = 5% risk and an 80% potency, 63 children should be included (21 in the Transplantation group and 42 in the Transfusion group) to demonstrate a difference of 1.4 to 1.0 on the mean TAMV recorded in MCA arteries at 1 year (assuming Standard deviation at 0.4, as suggested by previous data).

There will be no randomization but the distribution between the two groups will be linked to the chance of having a family geno-identical donor or not.

The matching makes it possible to take into account the factors of confusion of the comparison of the fates of these two groups. The factors selected are age (± 1 year), alloimmunization level (0, 1 or several allo-AC) and the vasculopathy profile (ie, stenoses already present or not with the MRA, presence or not of Ischemic lesions on MRI).

10.3 Missing data

Any patient included will be taken into account in the analysis until the interruption of his follow-up if necessary. One will seek to document the causes of exit from study.

10.4 MANAGING CHANGES IN THE INITIAL STRATEGY ANALYSIS

Any major modification of the analysis scheme will be submitted to the ethics committee for approval as an amendment to the study.

10.5 Analysis sets

The analysis being defined as intention to treat, all the data of the patients included in the study will be taken into account in the statistical analysis of the study.

11. Data processing and storage of documents and data

AP-HP is the owner of the data, which cannot be used or disclosed to a third party without its prior approval.
Persons with direct access in accordance with the applicable laws and regulations, in particular Articles L.1121-3 and R.5121-13 of the Public Health Code (eg investigators, quality control persons, Monitors, clinical research assistants, auditors and others involved in testing) shall take all necessary precautions to ensure the confidentiality of information relating to investigational medicinal products, tests, appropriate persons and Particularly as regards their identity and the results obtained. The data collected by these persons during the quality control or audits are then made anonymous.

12. Quality

The research will be framed according to the promoter's standard operating procedure.
The conduct of research in the investigating centers and the management of the subjects will be done in accordance with the Helsinki Declaration and the Good Practices in force.

12.1 Monitoring

According to the proponent's procedures, this research is classified in biomedical research, risk D, study in cell therapy.

Therefore all FIUs of all patients will be monitored at 100%.

The ARC representatives of the promoter will visit the investigating centers at the rate corresponding to the protocol of patient follow-up in the protocol, to the inclusions in the different centers.

- Opening visit of each center: before inclusion, for setting up the protocol and acquaintance with the various participants in biomedical research.

- During the following visits, the observation books will be reviewed as the CRA progresses. The principal investigator of each center as well as the other investigators who include or follow up the persons involved in the research undertake to receive the CRAs at regular intervals.
During these on-site visits and in accordance with the Good Clinical Practices, the following elements will be reviewed:
Compliance with protocol and defined procedures for research,
Audit of Informed Patient Consent
Review of source documents and comparison with data reported in the observation booklet as to accuracy, missing data, consistency of data according to the rules laid down by the DRCD procedures.
- Closure visit: retrieval of observation books, assessment at the pharmacy, documents of biomedical research, archiving.

12.2 Transcription of data in the CRF

All information required by the protocol must be provided in the observation booklet and an explanation given by the investigator for each missing data.
Data should be transferred to the observation books as they are obtained, whether clinical or para-clinical. The data must be clearly and legibly copied in black ink (in order to facilitate duplication and computer capture).
The erroneous data recorded on the observation books will be clearly blocked and the new data will be copied onto the notebook with the initials and the date by the member of the team of the investigator who made the correction.
The anonymity of the subjects will be ensured by a code number and the initials of the person who lends itself to research on all the documents necessary for the search or by erasing by appropriate means the personal data on the copies of the source documents, for the documentation of research.
The computerized data on a file will be declared to the CNIL according to the procedure adapted to the case.

13. Ethics and legal considerations

The promoter is defined by the law 2004-806 of 9 August 2004. In this research, AP-HP is the promoter and the Department of Clinical Research and Development (DRCD) ensures the regulatory missions.
Before commencing the research, each investigator will provide the research sponsor's representative with a copy of his / her dated and signed personal resume with his or her registration number to the order of physicians.

13.1 APPLICATION FOR AUTHORIZATION WITH AFSSAPS

In order to start the search, AP-HP as promoter must submit an application for authorization to the competent authority, Afssaps. The competent authority, as defined in Article L. 1123-12, shall take a decision with regard to the safety of persons who lend themselves to biomedical research, taking into account in particular the safety and quality of the products used in the course of the research in accordance with The conditions of use and the security of persons with regard to the acts performed and the methods used as well as the procedures for the follow-up of persons.

13.2 Ethics committee

In accordance with Article L.1123-6 of the Public Health Code, the research protocol must be submitted by the promoter to a Committee for the Protection of Persons The opinion of this Committee shall be notified to the competent authority by the promoter Before starting the search.

13.3 Modifications

The DRCD must be informed of any proposed modification of the protocol by the coordinating investigator.
Amendments should be qualified as substantive or not.

A substantial change is a change that may in one way or another modify the guarantees provided to individuals who lend themselves to biomedical research (modification of an inclusion criterion, extension of an inclusion period, Participation of new centers, etc.).

After the start of the research, any substantial modification of the research on the initiative of the promoter must obtain, before implementation, a favorable opinion from the committee and authorization from the competent authority. In this case, if necessary, the committee ensures that new consent is obtained from the persons participating in the research.

Furthermore, any extension of the research (deep modification of the therapeutic regimen or populations included, prolongation of treatments and / or therapeutic acts not originally foreseen in the protocol) should be considered as a new research.

13.4 Declaration CNIL

The French law provides that the declaration of the computerized file of personal data collected for the search must be made before the actual start of the search.
A reference methodology specific to the processing of personal data carried out in the context of biomedical research defined by Law 2004-806 of 9 August 2004 as falling within the scope of Articles L.1121-1 et seq. Of the Public Health Code has been established By the CNIL in January 2006.
This methodology allows a simplified declaration procedure when the nature of the data collected in the search is compatible with the list provided by the CNIL in its reference document.
When the protocol benefits from a quality control of the data by an ARC representing the promoter and falls within the scope of the simplified CNIL procedure, the DRCD as promoter will ask the person in charge of the computer file to commit In writing on compliance with the MR001 reference methodology.

13.5 Information and Consent

Written consent must be obtained from any person who is suitable for research before performing any act required by biomedical research.

The parents of the patients will be informed by the doctor about the objective, the nature, the constraints and the foreseeable risks of the test. An explanatory note will be given to them along with an informed consent form, which they must date and sign before starting the test. The parents will have a period of reflection of at least one week to give their written consent of participation of their child (ren) to this study.

To ensure medical confidentiality and data protection, written consent forms will be retained by the investigator for a period of fifteen years after completion of the trial. The investigator will attest in the observation note that the consent of the patient was obtained by dating and signing.
The investigator will not begin any test specifically required by the trial until the written consent of the patient's parents has been obtained. Parents of patients will be informed that all test data will be computerized and kept confidential. Since the names of the patients are kept secret, the documentation and evaluation of the data will be identified only by the first three letters of the surname and the first two letters of the first name of the child and by the individual patient number.
The technical protocol and supporting documents will be submitted by the study coordinator to the PPC.

13.6 Final report

The final research report will be written collaboratively by the coordinator and the biostatistician for this research. This report will be submitted to each of the investigators for opinion. Once consensus has been reached, the final version must be endorsed by the signature of each of the investigators and sent to the sponsor as soon as possible after the actual completion of the research. A report drawn up in accordance with the competent authority's reference plan shall be sent to the competent authority and to the PPC within one year after the end of the search, as the last monitoring visit of the competent authority. Last topic included. This period is extended to 90 days if the research is stopped prematurely.

15. Funding ad assurance

For the duration of the research, the Sponsor will take out an insurance policy covering the sponsor's own civil liability as well as the civil liability of all the doctors involved in carrying out the research. The sponsor will also provide full compensation for all harmful consequences of the research for the research subjects and their beneficiaries, unless the sponsor can prove that the harm is not the fault of the sponsor or any agent. The act of a third party or the voluntary withdrawal of the person who initially consented to participate in the research cannot be invoked against said compensation.

Assistance Publique- Hôpitaux de Paris (AP-HP) has taken out insurance from HDI-GERLING through BIOMEDIC-INSURE for the full research period, covering its own civil liability and that of any agent (doctor or research staff), in accordance with Article L.1121-10 of the French Public Health Code.

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DREPAGREFFE trial

National multicenter prospective study comparing the results of genoidentical transplantation to chronic transfusion in sickle cell anemia children with cerebral vasculopathy detected by transcranial Doppler

Substantial amendment No. 1

P071247– IDRCB 209-A012I3-54

Protocol Version N ° 1 of 26 oct 2010	Protocol Version N°2 of the 27 Feb 2013	Justification
<u>Study design</u> Genoidentical transplantation (n=21) Matching 1 transplanted patient with 2 transfused patients (n=42)	Propensity score matching	Suppression of workforce by treated groups and pairing 1-2 Use during analysis (see below) of a match on propensity score to have been allografted
Number of subjects required : a total of 63 patients are required: 21 sickle cell patients in arm « Transplantation » » and 42 in arm « Chronic Transfusion»	the distribution (due to the outline of this unexposed exposed cohort study) has been eliminated	it was found that the transplant could be offered to more children than expected, due to a high frequency of large families in the eligible population

Duration of the research: Inclusions over 2 years and follow-up over 1 year, ie a duration of study of 3 years	Duration of the research: Inclusions over 2 years and 6 months, followed over 3 years, ie a study duration of 5 years 6 months	Extend cohort monitoring for longer term evaluation
Methodology : The study is of type exposed (transplanted) - unexposed (transfused). Each child with donor will be matched to 2 children without donor, age, alloimmunization level and similar vasculopathy profile and treated by prolonged chronic transfusion. Pairing between a patient (with an identical HLA donor) and 2 patients without identical HLA donors will be based on age (+/- 1 year) the number of possible alloantibodies, stenoses and / or ischemic MRI / MRA lesions) present at the time of inclusion. It will be done to the blind analysis by the DBIM which will not have the notion of the evolution to 1 year of the cerebral vasculopathy. The matching makes it possible to take into account the factors of confusion of the comparison of these two groups. The factors selected are age (± 1 year), alloimmunization level (0, 1 or several allo-Antibodies) and the vasculopathy profile (ie, stenoses already present or not with the MRA, presence or not of ischemic lesions on MRI). To increase the potency of the comparison tests,	Methodology : The study is of type exposed (transplanted) - unexposed (transfused). The treatment groups will be compared after pairing with a propensity score including age, alloimmunization level and vasculopathy profile. Pair matching of the propensity score (including age, number of possible alloantibodies, number of stenoses and / or MRI / MRA ischemic lesions present at the time of inclusion) will be done during the blind analysis by the DBIM who will not have the notion of the 1 year evolution of cerebral vasculopathy. The matching makes it possible to take into account the factors of confusion of the comparison of the fates of these two groups.	Precision of the type of study Use of statistical method appropriate to causal inference in the absence of randomization, ie the matching of the propensity to receive the new treatment (here the transplantation)

two unexposed subjects will be matched as much as possible to each exposed subject. The possibility of matching to a single other child is not a factor in the exclusion of the child		
Primary outcome The average at 1 year of the velocities (TAMV) of the right and left middle cerebral arteries	Primary outcome TAMV recorded in the artery with the highest TAMV value at 1 year post-inclusion	
Estimated duration of research Estimated total duration of the research (duration of inclusion and duration of participation) The duration of inclusion is 2 years, the follow-up period is 1 year (post-transplant or prolongation of the PT), or a total duration of 3 years.	Estimated duration of research Estimated total duration of the research (duration of inclusion and duration of participation) The duration of inclusion is 2 years 6 months, the follow-up period is 3 years (post-transplant or prolongation of the PT), or a total duration of 5 years 6 months.	qs
Follow-up visits	Follow-up visits (add) Follow-up after 12 months will be identical to that performed in the course of care, except for cognitive tests .	Usual cohort follow-up (TCD, MRI) but Cognitive tests to be redone
Description of methods Statistics ... A Fine and Gray model will allow comparison of the two groups by taking into account the confounding factors, either by an adjustment (unmatched factors) or by a mixed effect (matched factors, source of intra-cluster	Description of methods Statistics ... A match on the propensity score will make it possible to compare the two groups by taking into account the confounding factors .	Accuracy of analysis due to change of matching method

correlation).		
<u>Expected number of people to be included</u> For an alpha risk of 5% and a potency of 80%, 21 children should be included in the "Transplantation" group and 42 subjects in the "Extended chronic Transfusion" group to demonstrate a difference of 140 to 100 cm/s over the mean of the arterial velocities at 1 year (assuming a standard deviation of 40 cm/s, as suggested by previous data and prevalence of 1/3). To increase the potency of the comparison tests, two unexposed subjects will be matched as much as possible to each exposed subject. The possibility of matching to a single other child is not a factor in the exclusion of the child.	<u>Expected number of people to be included</u> For an alpha = 5% risk and a power of 80%, 63 subjects should be included to demonstrate a difference of 140 to 100 cm/s on the average of the cerebral arteries at 1 year (assuming standard deviation at 40 cm/s, as suggested by previous data and a prevalence of 1/2).	Accuracy of the total workforce alone (qs)

DREPAGREFFE

Title: National multicenter prospective study comparing the results of genoidentical allograft to the transfusion program in sickle cell anemia children with cerebral vasculopathy detected by transcranial Doppler.

Study Design: Non-randomized, multicenter, national prospective trial

Statistical Analysis Plan

Date, 26 OCT 2010

1 DESCRIPTION OF THE STATISTICAL METHODS

After monitoring, data processing will be carried out in accordance with the principles of the Guide to Good Clinical Practices of the European Community. Specifically, the data will be entered using a two-way procedure, under a database manager ACCESS for Windows. They will be validated after input, before the start of the statistical analysis (from the issuing of requests to the CRAs and clinicians involved in the study).

The statistical analysis will be carried out at the department of biostatistics and medical informatics of the Saint Louis hospital (Paris) on SAS software (SAS Inc, Cary, NC) and R (https://www.R-project.org/). The main analysis will be carried out using the intention to treat principle, that is, all children included, whether or not they received the treatment in its entirety, will be considered in the analyses. For exploratory purposes, a per-protocol analysis will be carried out in a second step, with only children treated according to the therapeutic protocol of their group.

The percentage of patients with velocity normalization will be estimated by a non-parametric approach. Deaths before normalization will be considered as a competitive risk of the event of interest. A Fine and Gray model will allow comparison of the two groups by taking into account the confounding factors, either by an adjustment (unmatched factors) or by a mixed effect (matched factors, source of intra-cluster correlation).

The same approach will be applied to secondary endpoints (incidence of ischemic stroke, incidence of ischemic lesions and stenoses, cognitive performance, alloimmunization). The modeling of stroke-free survival will be based on the Kaplan-Meier estimate and the Cox model.

The costs will be compared between the two groups by nonparametric tests to account for the non-normal distribution (skewedness). High cost predictors / explanatory factors will be sought by multivariate analysis.

The significance level will be 5% and all tests will be two-sided.

2 PROPOSED NUMBER OF PERSONS TO BE INCLUDED IN RESEARCH

For an type I error rate of 5% and a statistical power of 80%, 21 children should be included in the "Transplantation" group and 42 subjects in the "Extended Transfusion Program" group to demonstrate a difference of 140 to 100 cm/s on the mean arterial velocities at 1 year (assuming a standard deviation of 40 cm/s, as suggested by previous data).
There will be no randomization but the distribution between the two groups will be linked to the chance of having a family genoidentical donor.

The matching makes it possible to take into account the factors of confusion of the comparison of the two groups. The matching factors selected are age (± 1 year), alloimmunization level (0, 1 or several allo-antibody) and the vasculopathy profile (ie, stenoses already present or not with the MRA, presence or not of ischemic lesions on MRI).

To increase the power of the comparison tests, two unexposed subjects will be matched as much as possible to each exposed subject. The possibility of matching to a single other child is not a factor in the exclusion of the child.

3 METHOD OF TAKING INTO ACCOUNT MISSING, UNUSED OR UNAUTHORIZED DATA

Any patient included will be taken into account in the analysis until the interruption of his follow-up if necessary.
We will try to document the reasons for leaving the study.

4 MANAGING CHANGES IN THE INITIAL STRATEGY ANALYSIS PLAN

Any significant changes to the analysis scheme will be submitted to the ethics committee for approval as an amendment to the study.

5 CHOICE OF PERSONS TO BE INCLUDED IN ANALYZES

Since the analysis is defined as intention to treat, all patient data included in the study will be taken into account in the statistical analysis of the study.

Date, 19 JUN 2013

1. DESCRIPTION OF THE STATISTICAL METHODS

After monitoring, data processing will be carried out in accordance with the principles of the Guide to Good Clinical Practices of the European Community. Specifically, the data will be entered using a two-way procedure, under a database manager ACCESS for Windows. They will be validated after input, before the start of the statistical analysis (from the issuing of requests to the CRAs and clinicians involved in the study).

The statistical analysis will be carried out at the department of biostatistics and medical informatics of the Saint Louis hospital (Paris) on SAS software (SAS Inc, Cary, NC) and R (https://www.R-project.org/). Primary analyses will be done according to the intention-to-treat principle, with group allocation based only on genoidentical donor availability and thus by genetic or “Mendelian” randomization.

For sensitivity analyses, we will use the propensity score-matching methodology to control for residual confounding by indication. Indeed, matching on the propensity score will allow the two groups to be compared, taking into account the confounding factors.
All statistical tests will be two-sided, with p-values of 0.05 or less denoting statistical significance.

2. PROPOSED NUMBER OF PERSONS TO BE INCLUDED IN RESEARCH

For type I error rate of 5% and a statistical power of 80%, 63 children should be included to demonstrate a difference of 1.4 to 1.0 on the relative mean difference in the outcome at 1 year (assuming standard deviation at 0.4, as suggested by previous data, and a prevalence of 1/2).

3. METHOD OF TAKING INTO ACCOUNT MISSING, UNUSED OR UNAUTHORIZED DATA

Any patient included will be taken into account in the analysis until the interruption of his follow-up if necessary. We will try to document the reasons for leaving the study.

4. MANAGEMENT OF AMENDMENTS TO THE INITIAL STRATEGY ANALYSIS PLAN

Any significant changes to the analysis scheme will be submitted to the ethics committee for approval as an amendment to the study.

5. CHOICE OF PERSONS TO BE INCLUDED IN ANALYSIS

Since the analysis is defined as intention to treat, all patient data included in the study will be taken into account in the statistical analysis of the study.

Summary of changes

- The sample size computation has been modified to handle the observed prevalence of genoidentical donors

- The matching on the propensity score of having a genoidentical donor has been considered (rather than individual matching on confounders)

Manuscript

"Prospective Comparison of Matched-Sibling Donor Transplantation vs Standard-Care for Abnormal-Transcranial Doppler in Children with Sickle Cell Anemia"

Subtitle: A Prospective Mendelian Randomized Trial

Summary of registration and first inclusion dates:

6. **Registration :**
   - Study protocol registration by the competent French health authorities (AFSSAPS): 27 July 2010, #EudraCT 2009-A01213-54
   - Study protocol approved by the local independent ethics committee (Comité de Protection des Personnes CPP Ile de France IV, Saint Louis): 18 Jan 2010
   - Clinicaltrials.gov registration: 21 April 2011
7. **Inclusions**
   - Inclusions before study protocol registration to the competent French health authorities : none
   - First inclusion: 31 Dec 2010
   - Inclusions before clinicaltrials.gov registration: 4 patients, included on 31 Dec 2010 (n=2), 23 March 2011 and 20 April 2011, respectively.
8. **Attached documents**
   - Study protocol registration by the competent French health authorities document
   - Study protocol approved by the local independent ethics committee (Comité de Protection des Personnes CPP Ile de France IV, Saint Louis)
   -

Françoise BERNAUDIN, MD on behalf of all authors

+33155873492

RÉPUBLIQUE FRANÇAISE

afssaps
Agence française de sécurité sanitaire des produits de santé

Direction de l'Évaluation des Médicaments et des Produits Biologiques
Département de l'Évaluation des Produits Biologiques
Fax : +33 (0)1 55 87 34 92
Dossier suivi par Sandrine JACOB / 34 95
E-mail : sandrine.jacob@afssaps.sante.fr
Sécurité virale : Perrine NUEZ / 35 74
E-mail : perrine.nuez@afssaps.sante.fr
Assistante : Sabrina LOPES / 3603
Références à rappeler : TC274
GTTC n°87
GTSV n°130

Mme Valérie MILLUL
AP-HP DRCD
Carré historique de l'Hôpital Saint- Louis
1 Avenue Claude Vellefaux
75010 Paris

Saint-Denis, le 27 JUIL. 2010

Lettre recommandée avec demande d'avis de réception

Madame,

Je vous prie de bien vouloir trouver ci-joint la décision d'autorisation de la recherche biomédicale :

« Etude prospective multicentrique nationale comparant les résultats de l'allogreffe génoidentique au programme transfusionnel chez les enfants drépanocytaires avec vasculopathie cérébrale détectée par le Doppler transcrânien. (projet DREPAGREFFE) »

que vous avez sollicitée le 26/04/2010, complétée le 22/06/2010.

Je vous rappelle les conditions liées à cette autorisation, notamment que :

- l'autorisation de la recherche biomédicale est subordonnée au respect des conditions de fabrication et de contrôle décrites dans le dossier de demande d'autorisation ;
- la date effective de commencement de la recherche doit être communiquée à l'Afssaps par le promoteur. Elle correspond à la date de la signature du consentement par la première personne qui se prête à la recherche en France ;
- toute modification substantielle portant sur les éléments du dossier transmis initialement à l'Afssaps, nécessite la délivrance d'une nouvelle autorisation dans les conditions prévues à l'article R.1125-12 ;
- les déclarations de vigilance et le résumé du rapport final doivent respectivement être transmis dans les délais fixés aux articles R.1123-47 et R.1123-60.

Toutefois, en ce qui concerne la préparation des cellules issues du sang placentaire, cette autorisation ne vaut que pour le site de l'unité de thérapie cellulaire de l'Hôpital Saint-Louis. En effet, aucune donnée n'a été fournie sur la préparation des cellules placentaires par le centre de Créteil. Si toutefois, vous envisagez d'inclure cette

Copie : CPP Ile de France IX (Créteil)
Directrice générale de l'ABM, 1 AV DU STADE DE FRANCE, 93212 SAINT-DENIS LA PLAINE CEDEX

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unité, il conviendra de soumettre avec la demande d'autorisation de modification substantielle, les données relatives à la préparation des cellules.

De plus, des remarques sont formulées dans l'annexe ci-jointe. Celles-ci sont non-suspensives car elles ne remettent pas en cause la sécurité des patients inclus dans l'essai. Néanmoins, il est important de les prendre en considération, en effet elles ont pour but d'améliorer le protocole proposé.

Je vous prie d'agréer, Madame, l'expression de mes salutations distinguées.

Pour le Directeur Général
et par délégation
le Directeur de l'Evaluation
des Médicaments et des Produits Biologiques

Pr Philippe LECHAT

ANNEXE

REMARQUES

Les remarques et suggestions proposées ci-dessous ne remettent pas en cause le démarrage de l'essai, néanmoins, il est important de les prendre en considération. En effet, elles ont pour but d'améliorer le protocole proposé.

En l'absence de réponse aux questions notifiées dans l'accusé réception de l'Afssaps du 05/05/2010, les questions suivantes sont maintenues :

SITE DE PREPARATION

La liste des centres chargés de la préparation des cellules souches mérite quelques clarifications, car certaines discordances ont été relevées entre le courrier, le formulaire de demande, et le tableau figurant dans un document joint.
D'après le protocole, il est prévu d'administrer des cellules souches allogéniques issues du sang placentaire ou de la moelle osseuse, ce point doit être clairement mentionné dans le formulaire de demande.

En ce qui concerne la préparation des cellules médullaires allogéniques (à confirmer) celle-ci sera réalisée par les sites suivants :
- Unité de Thérapie Cellulaire, Hôpital Saint Louis
- Département de Biothérapie, Hôpital Necker, 149 rue de Sèvres, 75015Paris
- Banque de tissus et de cellules EFS Hôpital Edouard Herriot 5 place d'Arsonval 69437 Lyon cedex 3
- Centre de Thérapie Cellulaire et Génique. Institut Paoli-Calmettes. 232, bd Ste Marguerite. 13273 Marseille cedex 9
- Unité de Thérapie Cellulaire. Département d'oncologie et d'hématologie clinique. Hôpital de Hautepierre, ,1 avenue Molière. 67000 Strasbourg
- Unité de Thérapie Cellulaire, EFS Normandie site de Bois-Guillaume 609 Chemin de la Bretèque 76230 BOIS-GUILLAUME
- Centre de Biothérapie d'auvergne, CHU Estaing, 1 place Lucie Aubrac, 63003 Clermont-Ferrand Cedex1
- Laboratoire de Thérapie cellulaire Etablissement Français du Sang Aquitaine Limousin site de Bordeaux.

Pour le centre de Clermont Ferrand, le nom du produit cellulaire n'est pas mentionné, celui-ci devrait être précisé. S'il s'agit bien du PPC 47, le produit concerné est autologue.

Pour certains centres il est fait référence au dossier procédé/produit relatif aux cellules souches issues de sang périphérique (CSHP) allogénique (ex PPC48,) voire de CSHP autologues (PPC47, PPC11). Comme mentionnée précédemment, il conviendra de clarifier quelles cellules seront réellement utilisés (sang périphérique ? médullaire ?) et s'il est envisagé d'utiliser des cellules autologues, ce qui qui n'apparait pas dans le protocole. Enfin, il conviendra de mettre à jour le formulaire de demande en conséquence.

SECURITE VIRALE

Les centres ayant déposé une demande d'autorisation procédé/produit à l'Afssaps pour les cellules souches périphériques allogéniques devront fournir une attestation précisant que la qualification des dons est effectuée selon ce même procédé.

Pour les centres n'ayant pas déposé de dossier d'autorisation procédé/produit à l'Afssaps :

Concernant la sélection biologique des donneurs, le demandeur devra préciser les marqueurs recherchés pour chaque pathologie infectieuse, les trousses de détection utilisées pour chaque marqueur ainsi que le laboratoire où sont effectués ces contrôles.

Concernant la sélection clinique des donneurs, les critères d'exclusion au regard des agents transmissibles devront être précisés.

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RÉPUBLIQUE FRANÇAISE

afssaps

Agence française de sécurité sanitaire des produits de santé

Référence : TC274
SJ/SLo

DÉCISION

Du : 27 JUIL. 2010

portant autorisation de la recherche biomédicale

« Etude prospective multicentrique nationale comparant les résultats de l'allogreffe génoidentique au programme transfusionnel chez les enfants drépanocytaires avec vasculopathie cérébrale détectée par le Doppler transcrânien. (projet DREPAGREFFE) »

LE DIRECTEUR GÉNÉRAL
DE L'AGENCE FRANÇAISE DE SÉCURITÉ SANITAIRE DES PRODUITS DE SANTÉ

VU le code de la santé publique, première partie, notamment les articles L.1125-1, L.1125-4, L.1245-4, R.1125-7, R.1125-8, R.1125-9, R.1125-10 et R.1125-11 ;

VU la demande d'autorisation présentée par :

L'AP-HP DRCD
Carré historique de l'Hôpital Saint- Louis
1 Avenue Claude Vellefaux
75010 Paris

le 26/04/2010

pour la recherche biomédicale intitulée :

« Etude prospective multicentrique nationale comparant les résultats de l'allogreffe génoidentique au programme transfusionnel chez les enfants drépanocytaires avec vasculopathie cérébrale détectée par le Doppler transcrânien. (projet DREPAGREFFE) »

VU le courrier de l'Afssaps de demande de complément d'informations en date du 27/05/2010,

VU la réponse apportée par le demandeur au courrier susvisé, le 22/06/2010,

VU l'avis du groupe d'experts mentionné à l'article R.1125-9 du code de la santé publique,

VU l'avis de l'Agence de la biomédecine,

DÉCIDE

Article 1

L'autorisation de mise en œuvre de la recherche biomédicale :

« Etude prospective multicentrique nationale comparant les résultats de l'allogreffe génoidentique au programme transfusionnel chez les enfants drépanocytaires avec vasculopathie cérébrale détectée par le Doppler transcrânien. (projet DREPAGREFFE) »

est octroyée à :

L'AP-HP DRCD
Carré historique de l'Hôpital Saint- Louis
1 Avenue Claude Vellefaux
75010 Paris

Article 2

Cette autorisation de la recherche est valable pour toute la durée de celle-ci, à compter de la date de la présente décision. Toutefois, si dans le délai d'un an suivant la présente décision, la recherche n'a pas débuté, l'autorisation devient caduque, sauf prorogation par décision de l'Agence française de sécurité sanitaire des produits de santé sur justification produite avant l'expiration du délai.

Article 3

L'autorisation de la recherche mentionnée à l'article 1 vaut autorisation, pour cette recherche, des lieux de prélèvement, de conservation, de préparation et d'administration selon les dispositions mentionnées aux articles L.1242-1, L.1243-2 et L.1243-6 du code de la santé publique ainsi qu'autorisation d'importation et d'exportation mentionnée à l'article L.1245-5 du code de la santé publique et autorisation de lieu de recherches selon les dispositions de l'article L.1121-13 du code de la santé publique.

Article 4

Le promoteur doit informer l'Afssaps et justifier toute action engagée pour interrompre temporairement la recherche.

Article 5

Le directeur de la direction de l'évaluation des médicaments et des produits biologiques est chargé de l'exécution de la présente décision.

Pour le Directeur Général
Fait à Saint-Denis, le par délégation
le Directeur de l'Evaluation
des Médicaments et des Produits Biologiques

Pr Philippe LECHAT

Comité de Protection des Personnes - Ile-de-France IX
CHU Henri Mondor 51, avenue du Maréchal de Lattre de Tassigny
94010 Créteil Tél.: 01 49 81 22 61 Fax : 01 49 81 02 81
E mail : cpp.iledefrance9@yahoo.fr
SITE : http://perso.orange.fr@cpp.iledefrance9

N° interne : CPP-IDF IX 09-039
N° ID RCB : 2009-A01213-54

Le Comité a été saisi le 18 novembre 2009 d'une demande d'avis pour le projet de recherche intitulé :

"DREPAGREFFE : Etude prospective multicentrique nationale comparant les résultats de l'allogreffe génoidentique au programme transfusionnel chez les enfants drépanocytaires avec vasculopathie cérébrale détectée par le Doppler transcrânien"

dont le promoteur est :

DIRC Ile de France
Monsieur Christophe MISSE
Carré Historique de l'Hôpital Saint-Louis
Secteur Gris - Porte 23
1, rue Claude Vellefaux
75475 PARIS CEDEX 10

et les investigateur coordonnateur :

Docteur Françoise BERNAUDIN
Service de Pédiatrie
Centre Hospitalier Intercommunal
40, Avenue de Verdun
94010 CRETEIL

Le Comité a examiné les informations relatives à ce projet lors de la séance du :

14 décembre 2009 :

Ont participé aux délibérations :
Collège 1 : Mme BERTRAND (PH SAMU) (T), M. LE BRETON (CCA en Médecine Générale) (S), Mme MACQUIN-MAVIER (PU-PH Pharmacologie Clinique) (T), MM. MAISON(PH – Pharmacologie Clinique - Biostatisticien) (S), MEARY (P.H. Psychiatrie) (S), Mmes ROUDOT-THORAVAL (MCU-PH Santé Publique - Biostatisticienne) (T), SCHULLER (Pneumologue), TIBI (Pharmacien des Hôpitaux) (T), Tran-van-NHIEU (MCU-PH Anatomie et Cytologie Pathologie) (S).
Collège 2 : M. DANESI (Usagers) (T), Mmes ROMANO (Psychologue) (T), TASTET (Ethique) , M. SOULAS (Psychologue) (S).

L'ensemble des membres, ayant participé à la délibération, déclare n'avoir aucun conflit d'intérêts avec le promoteur ou l'investigateur principal.

Après examen de l'ensemble des documents transmis, des questions et des observations ont été formulées. En conséquence, l'avis du comité sera émis après réception des réponses aux questions.

Le 15/01/2010, des éléments de réponses ont été adressés par le promoteur au CPP. Après examen, l'avis a été différé. Le comité avait émis des réserves notamment sur le choix du critère principal de jugement mais aussi les documents d'information et consentement. :

Le comité a réexaminé les informations relatives à ce projet lors de la séance du :

18 janvier 2010 :

Ont participé aux délibérations :
Collège 1 : Mme BERTRAND (PH SAMU) (T), MM. CITTEE (Médecin généraliste) (T), Mme HULIN (Pharmacien des Hôpitaux) (S), M. LE BRETON (CCA en Médecine Générale) (S), Mme MACQUIN-MAVIER (PU-PH Pharmacologie Clinique) (T), M. MAISON(PH – Pharmacologie Clinique - Biostatisticien) (S), Mmes RENVERSADE (Cadre-Infirmière) (T), SCHULLER (Pneumologue).
Collège 2 : Mme BERTHELOT (association de malades), M. GESCHWIND (Ethique Médicale) (S), Mmes ROMANO (Psychologue) (T), TASTET (Ethique) (T), M. SOULAS (Psychologue) (S).

L'ensemble des membres, ayant participé à la délibération, déclare n'avoir aucun conflit d'intérêts avec le promoteur ou l'investigateur principal.

Après délibération et vérification du respect des dispositions de l'article L. 1121-1 et de l'article L.1121-1 du Code de la Santé Publique, et après réception d'une copie de l'autorisation donnée par l'AFSSAPS au démarrage de cette recherche, le CPP estime satisfaisant l'ensemble des réponses fournies à ses questions et aux réserves émises par l'AFSSAPS. En conséquence, l'AVIS FAVORABLE est donné le 27 JUILLET 2010 sur l'ensemble des documents reçu, notamment sur :

- Le protocole, version finale n°1 13/11/2009
- La lettre d'information pour les titulaires de l'autorité parentale sur un enfant mineur participant à une recherche biomédicale, version n° 1.1 du 07/05/2010
- le formulaire de consentement les titulaires de l'autorité parentale sur un enfant mineur participant à une recherche biomédicale, version n° 1.1 du 07/05/2010
- La lettre d'information pour les mineurs donneurs de 7 à 15 ans, participant à une recherche biomédicale, version n° 1.1 du 07/05/2010
- le formulaire de consentement pour les mineurs donneurs de 7 à 15 ans, participant à une recherche biomédicale, version n° 1.1 du 07/05/2010
- La lettre d'information pour les mineurs donneurs de moins de 7 ans, participant à une recherche biomédicale, version n° 1.1 du 07/05/2010
- La lettre d'information pour un enfant de 7 à 15 ans (transfusé non greffé), participant à une recherche biomédicale, version n° 1.1 du 07/05/2010
- le formulaire de consentement pour un enfant de 7 à 15 ans (transfusé non greffé), participant à une recherche biomédicale, version n° 1.1 du 07/05/2010
- La lettre d'information pour un enfant de moins de 7 ans (transfusé non greffé), participant à une recherche biomédicale, version n° 1.1 du 07/05/2010
- La lettre d'information pour les mineurs receveurs de moins de 7 ans, participant à une recherche biomédicale, version n° 1.1 du 07/05/2010
- La lettre d'information pour un enfant de 7 à 15 ans (receveur), participant à une recherche biomédicale, version n° 1.1 du 07/05/2010
- le formulaire de consentement pour un enfant de 7 à 15 ans (receveur), participant à une recherche biomédicale, version n° 1.1 du 07/05/2010

CPP - Ile-de-France IX
Comité de Protection des Personnes
CHU Henri Mondor
51, av. du Maréchal de Lattre de Tassigny
94010 CRETEIL Cedex
Tél. 01 49 81 22 61 - Fax 01 49 81 02 81
e-mail : cpp.iledefrance9@yahoo.fr
http://perso.orange.fr / cpp.iledefrance9

Docteur Françoise ROUDOT-THORAVAL
Présidente du CPP Ile de France IX

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Paris, le 25 juillet 2013

Comité de Protection des Personnes Ile de France IV

Hôpital Saint-Louis
Porte 5 du carré Historique
1 avenue Claude Vellefaux
75475 Paris Cedex 10

Responsable administrative :
Mme I. SCAGLIA
Tél. : 01.42.38.92.88
Fax : 01.42.38.92.98
cpp.iledefrance4@orange.fr

Président :
Dr J.-P. CESARINI
Vice-président :
M. Marc BORAND
Secrétaire générale :
Mme M. ASTRIE-BELLICK
Secrétaires générales adjointes :
Mme B. LEHMANN
Dr S. KLOUCHE
Trésorier :
Pr O. CHASSANY
Trésorière Adjointe :
Mme C. MASCRET

Membres du Comité :
Collège I
Médecins et chercheurs
O. CHASSANY
P. CHAUMET-RIFFAUD
J-P. CESARINI
E. CAROSELLA
S. KLOUCHE
M.-H. DIZIER
B. PAPP
F. ADNET
P. GOLDSCHMIDT
Pharmacien hospitalier
B. LEHMANN
Infirmière
C. DELETOILLE-LANDRE
M. ASTRIE-BELLICK

Collège II
Questions éthiques
J.-C. KRZYWKOWSKI
Psychologue
L. LACOSTE
Travailleur social
M. BORAND
Compétence juridique
C. MASCRET
P. A. DUMAS
P. AUBRY
M. BOUCHER
Associations de maladies et d'usagers
M. BERNARD-HARLAUT
M. TROUGOUBOFF
A. M MASURE

DRCD
Mme Valérie MILLUL
1 av Claude Vellefaux
75010 Paris

Avis complémentaire

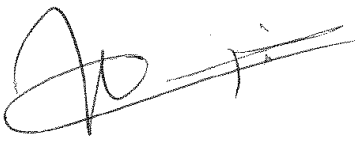
Réf. du CPP : **CPP MONDOR 09-039**	Réf. du Promoteur : **P071247**
Réf. AFSSAPS :	N°EudraCT : **2009-A01213-54**
Promoteur : **AP-HP**	Investigateur : Dr Bernaudin

Le Comité a bien reçu votre courrier du 25 juin 2013 concernant le projet de recherche intitulé :
« Etude prospective, multicentrique nationale comparant les résultats de l'allogreffe génoidentique au programme transfusionnel chez les enfants drépanocytaires avec vasculopathie cérébrale détectée par le Doppler Transcranien.»

Cet amendement porte sur :
- prolongation de la période inclusion
- modification de la répartition des inclus dans les deux groupes de traitement
- modification de l'appariement des deux groupes de traitement
- ajout de précisions sur la constitution et le fonctionnement du Comité de Surveillance indépendant

Le Comité a examiné les informations relatives à ce projet lors de la séance du 25 juillet 2013.
Membres présents : Dr J.-P. Cesarini (I), Pr O. Chassany (I), Mme C. Deletoille (I),Mme L. Lacoste (II), Mme M. Trougouboff (II), Dr E. Carosella (I), Mme AM Masure (II), Mme M. Bernard (II), Mme S. Klouche (I), Mme M. Bouchere (II), Pr F. Adnet (I), J-C Krzywkowski (II). *Le Pr Olivier Chassany a déclaré en début de séance un lien d'intérêt avec le promoteur et en accord avec les membres du Comité, n'a pas participé ni à la délibération, ni au vote du Comité.*

Le Comité émet un avis favorable à l'avis complémentaire.



Dr J-P CESARINI
Président

- ☐ Formulaire de demande de modification substantielle pas datée
- ☐ Votre courrier du 25/06/2013
- ☐ Attestation d'assurance du 25/06/2013
- ☐ Courrier de justification MS du 19/06/2013
- ☐ Tableau comparatif version 2.0 du 21/06/2013
- ☐ Dossier initial suivi au CPP IDF IX

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Comité de Protection des Personnes Ile de France IV

Hôpital Saint-Louis
Porte 5 du carré Historique
1 avenue Claude Vellefaux
75475 Paris Cedex 10

Responsable administrative :
Mme I. SCAGLIA
Tél. : 01.42.38.92.88
Fax : 01.42.38.92.98
cpp.iledefrance4@orange.fr

Président :
Dr Shahnaz KLOUCHE
Vice-président :
M. Marc BORAND
Secrétaire générale :
Mme M. ASTRIE-BELLICK
Secrétaire générale adjointes:
Mme B. LEHMANN
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Pr O. CHASSANY
Trésorière Adjointe :
Mme C. MASCRET

Membres du Comité :
Collège I
Médecins et chercheurs
O. CHASSANY
J-P. CESARINI
E. CAROSELLA
S. KLOUCHE
M.-H. DIZIER
B. PAPP
F. ADNET
P. GOLDSCHMIDT
Pharmacien hospitalier
B. LEHMANN
C. MARITAZ
Infirmière
C. DELETOILLE-LANDRE
M. ASTRIE-BELLICK

Collège II
Questions éthiques
J.-C. KRZYWKOWSKI
Psychologue
L. LACOSTE
A.S VAN DOREN
Travailleur social
M. BORAND
Compétence juridique
C. MASCRET
P. A. DUMAS
P. AUBRY
M. BOUCHER
Associations de maladies et d'usagers
M. BERNARD-HARLAUT
M. TROUGOUBOFF
A. M MASURE

Paris, le 27 mars 2014

DRCD
Mme Valérie MILLUL
1 av Claude Vellefaux
75010 Paris

Avis complémentaire n°2

Réf. du CPP : **CPP MONDOR 09-039**	Réf. du Promoteur : **P071247**
Réf. AFSSAPS :	N°EudraCT : **2009-A01213-54**
Promoteur : **AP-HP**	Investigateur : **Dr Bernaudin**

Le Comité a bien reçu votre courrier du 05 mars 2014 concernant le projet de recherche intitulé :
« Etude prospective, multicentrique nationale comparant les résultats de l'allogreffe génoidentique au programme transfusionnel chez les enfants drépanocytaires avec vasculopathie cérébrale détectée par le Doppler Transcranien.»

Cet amendement porte sur :
- Mise à jour de la liste des centres investigateurs
- Augmentation du nombre de patients à inclure
- Augmentation de 7 mois de la durée totale de l'étude
- Mise à jour de la grille des EIG

Le Comité a examiné les informations relatives à ce projet lors de la séance du 27 mars 2014.
Membres présents : Dr J.-P. Cesarini (I), Mme M. Bernard (II), Mme C. Deletoille (I), Mme B. Lehmann (I), Mme M. Trougouboff (II), Dr S. Klouche (I), Pr O. Chassany (I), Mme M. Astrie (I), M E. Carosella (I), Mme M-H Dizier (I), M P-A Dumas (II), Mme C. Mascret (II), M B. Papp (II).
Le Pr Olivier Chassany a déclaré en début de séance un lien d'intérêt avec le promoteur et en accord avec les membres du Comité, n'a pas participé ni à la délibération, ni au vote du Comité.

Le Comité émet un avis favorable à l'avis complémentaire n°2.

Dr Shahnaz KLOUCHE
Présidente

- ☐ Formulaire de demande de modification substantielle du 05/03/2014
- ☐ Formulaire de demande d'autorisation du 05/03/2014*
- ☐ Votre courrier du 05/03/2014
- ☐ Liste des investigateurs version 3.0 du 25/02/2014
- ☐ Tableau comparatif
- ☐ Résumé du protocole
- ☐ Attestation d'assurance – Lettre dont acte du 14/02/2014
- ☐ Protocole version 3.0 du 25/02/2014

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Contents lists available at ScienceDirect

Contemporary Clinical Trials

journal homepage: www.elsevier.com/locate/conclintrial

Design of the DREPAGREFFE trial: A prospective controlled multicenter study evaluating the benefit of genoidentical hematopoietic stem cell transplantation over chronic transfusion in sickle cell anemia children detected to be at risk of stroke by transcranial Doppler (NCT 01340404)

Sylvie Chevret[a,*], Suzanne Verlhac[b,t], Elisabeth Ducros-Miralles[c], Jean-Hugues Dalle[d], Regis Peffault de Latour[e], Mariane de Montalembert[f], Malika Benkerrou[g], Corinne Pondarré[h,c], Isabelle Thuret[i], Corinne Guitton[j], Emmanuelle Lesprit[k], Maryse Etienne-Julan[l], Gisèle Elana[m], Jean-Pierre Vannier[n], Patrick Lutz[o], Bénédicte Neven[p], Claire Galambrun[i], Catherine Paillard[o], Camille Runel[q], Charlotte Jubert[q], Cécile Arnaud[c], Annie Kamdem[c], Valentine Brousse[f], Florence Missud[g], Marie Petras[l], Lydia Doumdo-Divialle[l], Claire Berger[r], Françoise Fréard[c], Olivier Taieb[s], Elise Drain[s], Monique Elmaleh[t], Manuela Vasile[t], Yacine Khelif[u], Myriam Bernaudin[u], Philippe Chadebech[v], France Pirenne[v], Gérard Socié[e], Françoise Bernaudin[c]

[a] Statistical, Methodology Department, Paris-St-Louis Hospital, Paris-Diderot University, France
[b] Medical Imaging Department, Referral Center for Sickle Cell Disease, Créteil-CHIC Hospital, Créteil, France
[c] Pediatric Department, Referral Center for Sickle Cell Disease, Créteil-CHIC Hospital, Paris-Est University, Créteil, France
[d] Pediatric Hematology Department, Paris-Debré Hospital, Paris-Diderot University, France
[e] Hematology Department, Paris-St-Louis Hospital, Paris-Diderot University, France
[f] Pediatric Department, Referral Center for Sickle Cell Disease, Paris-Necker Hospital, Paris-Descartes University, France
[g] Pediatric Department, Referral Center for Sickle Cell Disease, Paris-Debré Hospital, Paris-Diderot University, France
[h] Pediatric Hematology Department, Lyon Léon Bérard Center, Lyon, France
[i] Pediatric Hematology Department, Marseille-la Timone Hospital, Aix-Marseille University, France
[j] Pediatric Department, Referral Center for Sickle Cell Disease, Bicêtre Hospital, Paris-Sud University, France
[k] Pediatric Department, Referral Center for Sickle Cell Disease, Paris-Trousseau Hospital, Paris-Diderot University, France
[l] Referral Center for Sickle Cell Disease, Pointe à Pitre Hospital, Antilles University, Guadeloupe, France
[m] Pediatric Department, Referral Center for Sickle Cell Disease, Fort de France Hospital, Antilles University, Martinique, France
[n] Pediatric Hematology Department, Rouen-Charles-Nicolle Hospital, Rouen University, France
[o] Pediatric Onco-Hematology Department, Strasbourg-Hautepierre Hospital, Strasbourg University, France
[p] Pediatric Immuno-Hematology Department, Paris-Necker Hospital, Paris-Descartes University, France
[q] Pediatric Hematology Department, Bordeaux-Hospital, Bordeaux University, France
[r] Onco-Hematology Department, St-Etienne Hospital, St-Etienne University, France
[s] Ethno-Psychiatry Department, Avicenne-Hospital, Paris-13 University, France
[t] Pediatric Imaging Department, Robert-Debré Hospital, Paris-Diderot University, France
[u] Normandie Univ, UNICAEN, CEA, CNRS, ISTCT/CERVOxy group, 14000 Caen, France
[v] EFS Ile de France, IMRB INSERM U955 équipe 2 (Créteil), GR-Ex (Paris) and Paris-Est-Créteil University, France

ARTICLE INFO

Keywords:
Sickle cell anemia
Transcranial Doppler

ABSTRACT

Background: Children with sickle cell anemia (SCA) have an 11% risk of stroke by the age of 18. Chronic transfusion applied in patients detected to be at risk by transcranial Doppler allows a significant reduction of stroke risk. However, chronic transfusion exposes to several adverse events, including alloimmunization and iron

* Corresponding author.
E-mail addresses: sylvie.chevret@paris7.jussieu.fr (S. Chevret), suzanne.verlhac@chicreteil.fr (S. Verlhac), ducros.miralles@free.fr (E. Ducros-Miralles), jean-hugues.dalle@aphp.fr (J.-H. Dalle), regis.peffaultdelatour@aphp.fr (R.P. de Latour), mariane.demontal@aphp.fr (M. de Montalembert), malika.benkerrou@aphp.fr (M. Benkerrou), corinne.pondarre@chicreteil.fr (C. Pondarré), Isabelle.THURET@ap-hm.fr (I. Thuret), corinne.guitton@aphp.fr (C. Guitton), emmanuelle.lesprit@aphp.fr (E. Lesprit), maryse.etienne-julan@chu-guadeloupe.fr (M. Etienne-Julan), gisele.elana@chu-fortdefrance.fr (G. Elana), Jean-Pierre.Vannier@chu-rouen.fr (J.-P. Vannier), Patrick.Lutz@chru-strasbourg.fr (P. Lutz), Benedicte.neven@aphp.fr (B. Neven), Claire.GALAMBRUN@ap-hm.fr (C. Galambrun), catherine.paillard@chru-strasbourg.fr (C. Paillard), camille.runel@chu-bordeaux.fr (C. Runel), charlotte.jubert@chu-bordeaux.fr (C. Jubert), cecile.arnaud@chicreteil.fr (C. Arnaud), annie.kamdem@chicreteil.fr (A. Kamdem), valentine.brousse@chicreteil.fr (V. Brousse), florence.missud@aphp.fr (F. Missud), eleonore.petras@chu-guadeloupe.fr (M. Petras), lydia-divialle.doumdo@chu-guadeloupe.fr (L. Doumdo-Divialle), claire.berger@chu-st-etienne.fr (C. Berger), francoise.freard@wanadoo.fr (F. Fréard), olivier.taieb@aphp.fr (O. Taieb), elise.drain@aphp.fr (E. Drain), monique.elmaleh@aphp.fr (M. Elmaleh), Manuela.Vasile@chicreteil.fr (M. Vasile), khelif@cyceron.Fr (Y. Khelif), bernaudin@cyceron.Fr (M. Bernaudin), philippe.chadebech@efs.sante.fr (P. Chadebech), france.pirenne@efs.sante.fr (F. Pirenne), gerard.socie@aphp.fr (G. Socié), Francoise.Bernaudin@chicreteil.fr (F. Bernaudin).

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Cerebral vasculopathy
Chronic transfusion
Hematopoietic stem cell transplantation
Genetic randomization

overload, and is not curative. Hematopoietic stem cell transplantation allows termination of the transfusion program, but its benefit has not been demonstrated.
Design: DREPAGREFFE (NCT01340404) is a multicenter, prospective trial enrolling SCA children younger than 15 years receiving chronic transfusion due to a history of abnormal transcranial Doppler (velocities ≥ 200 cm/s). Only those with at least one non-SCA sibling and parents accepting HLA-typing and transplantation with a genoidentical donor were eligible. Chronic transfusion was pursued in patients with no available donor, whereas others were transplanted. Comparison between the 2 arms (transfusion vs transplantation) was analyzed using both genetic randomization and propensity-score matching as a sensitivity analysis. The primary end-point was the velocity measure at 1 year. Secondary endpoints were the incidence of stroke, silent cerebral infarcts and stenoses, cognitive performance in comparison with siblings, allo-immunization, iron-overload, phosphatidyl-serine, angiogenesis/hypoxia, brain injury-related factor expression, quality of life and cost.
Objectives: To show that genoidentical transplantation decreases velocities significantly more than chronic transfusion in SCA children at risk of stroke.
Discussion: DREPAGREFFE is the first prospective study to evaluate transplantation in SCA children. It compares the outcome of cerebral vasculopathy following genoidentical transplantation versus chronic transfusion using genetic randomization and causal inference methods.

1. Introduction

In sickle cell anemia (SCA), an inherited blood disorder, more severe complications during infancy are overt stroke, a provider of motor and cognitive sequelae. Before the systematic screening of cerebral velocities with transcranial Doppler (TCD), there was 11–12.8% stroke risk by age 20 [1,2]. Non-invasive TCD, introduced in the 1990s, allows the detection of patients at risk of clinical stroke [3–7]. Abnormal TCD defined by a time-averaged mean velocity (TAMV) ≥ 200 cm/s predicts a 40% stroke risk within 3 years, whereas the risk is lower than 2% in patients with normal TCD (TAMV < 170 cm/s) [8]. The randomized STOP-1 study clearly demonstrated that long-term chronic transfusions maintaining HbS% below 30%, allowed a significant reduction of the risk of stroke by 91% in patients with abnormal velocities [9].

Detection of patients at risk of stroke by TCD led to the reduction of the incidence of first stroke in Californian SCA children from 0.88/100 person-years from 1991 to 1998 to 0.17/100 person-years in 2000 [10]. Bernaudin et al. reported in the Créteil-newborn-cohort that early TCD screening and rapid initiation of transfusions in patients detected at risk reduced the risk of stroke by age 18–20 years from 11 to 12.8% as previously reported to only 1.9% [11]. However, we also showed that the cumulative risk of abnormal TCD was high, reaching a plateau of 30% by age 9 and raising concerns about the number of SCA children requiring chronic transfusions [11].

Considering the risk of severe side effects associated with chronic transfusions such as iron overload and erythroid alloimmunization, the STOP-2 study (1998–2005) assessed whether transfusions after at least 30 months could be safely suspended in patients with normalized TCD and no stenosis, showed that stopping transfusions resulted in reversion to abnormal TCD and stroke occurrence and concluded that it was not safe to stop chronic transfusions in patients with a history or abnormal TCD [12].

Otherwise, we have reported an important improvement in the results of geno-identical hematopoietic stem cell transplantation (SCT) offering > 95% chances of cure in patients transplanted since year 2000 [13]. No stroke and no new silent infarcts were observed in patients who were successfully engrafted, and the velocities were significantly reduced. Moreover, we observed rapid post-transplant velocity normalization in 4 children who still had abnormal velocities despite long-term chronic transfusion [13]. These satisfactory results encourage earlier transplantation to avoid neurological and cognitive sequelae.

However, the risks of this procedure are not negligible, including treatment-related mortality, post-transplant neurological worsening, graft-versus-host disease (GvHD) and gonadal dysfunction, although they are quite reduced when transplantation is performed before the age of 15 [13–19].

These results must be evaluated in a multicenter and prospective manner. Thus, we aimed to prospectively evaluate the benefit of genoidentical transplantation over chronic transfusion in SCA children with cerebral vasculopathy. We hypothesize that genoidentical transplantation, despite unquestionable toxicity but limited at this age of life, allows better prevention or stabilization of cerebral vasculopathy in SCA children.

2. Patients and methods

2.1. Study design

DREPAGREFFE is a national French multicenter prospective interventional controlled non-randomized trial but is defined by the random availability of a genoidentical donor.

2.2. Study objectives

The overall hypothesis is that transplantation, despite unquestionable toxicity but limited at this age of life, will allow better prevention or stabilization of cerebral vasculopathy.

Our primary hypothesis is that genoidentical SCT allows significantly decreasing velocities (i.e., TAMV) at 1 year compared with the chronic transfusion program.

Our secondary hypothesis is that TCD will be normalized (TAMV < 170 cm/s) in a greater proportion of transplanted patients than in those receiving chronic transfusion.

Other hypotheses are that following transplantation, the cerebral vasculopathy will be better stabilized than during chronic transfusion as shown by the following:

- A reduction in the number of silent cerebral infarct new occurrence or recurrence
- A reduction in the number of new stenosis and better stabilization or improvement of pre-existing anatomical stenosis

Abbreviations: SCD, Sickle Cell Disease; SCA, Sickle Cell Anemia; HSCT, Hematopoietic Stem Cell Transplantation; BMT, Bone Marrow Transplantation; CBT, Cord Blood Transplantation; TCD, transcranial Doppler; TCDI, transcranial Doppler Imaging; MRI, Magnetic Resonance Imagery; MRA, Magnetic Resonance Angiography; RBCs, Red Blood Cells; MTX, Methotrexate; CSA, Cyclosporine-A; GvHD, Graft-vs-Host-Disease; MCV, Mean Corpuscular Volume; LDH, Lactate Dehydrogenase; G6PD, Glucose 6-Phosphate Dehydrogenase; MCA, Middle Cerebral Artery; ACA, Anterior Cerebral Artery; ICA, Internal Carotid Artery; eICA, Extracranial Internal Carotid Artery; FSIQ, Full Scale Intelligence Quotient; IDMC, Independent Data Monitoring Committee; TAMV, Time-averaged mean of the maximum velocity; AFSSAPS, Agence Française de Sécurité Sanitaire des Produits de Santé; ANSM, Agence Nationale de Sécurité du Médicament

- Neurocognitive performance in SCA children and non-SCA siblings

Moreover, we also test the hypotheses that

- Iron overload will be significantly decreased post-transplant compared with chronic transfusion
- Quality of life will be significantly improved following transplantation

As ancillary studies, we will also perform a comparative study of

- Allo-immunization
- Phosphatidylserine (PS) externalization on red blood cell (RBC) membranes
- Hypoxia/angiogenesis and brain injury-related factor expression
- Costs

2.3. Study organization

The study group for the DREPAGREFFE trial consists of several units: a clinical coordinating center and central imaging center located in Créteil (CHIC hospital), a statistical and data coordinating center in Paris (St. Louis hospital), 13 university teaching hospitals taking care of SCA patients (Créteil, Necker, Robert-Debré, Armand-Trousseau in Paris, Bicêtre, Strasbourg, Rouen, St-Etienne, Lyon, Marseille, Bordeaux, Martinique, Guadeloupe) and 8 stem-cell-transplant units for the realization of transplantations (St. Louis, Robert-Debré, Necker in Paris, Strasbourg, Rouen, Bordeaux, Marseille, Lyon).

An independent external Data Monitoring Committee (DMC) performs periodic, interim safety assessments of the DREPAGREFFE.

2.4. Study population

The eligibility criteria used were as follows:
Inclusion criteria

- SCA children (homozygous SS or sickle/β^0 thalassemia)
- Age < 15 years
- Placed on long-term chronic transfusion due to a history of abnormal TCDI (transcranial Doppler Imaging) (TAMV ≥ 200 cm/s)
- Having at least one non-SCA sibling from the same parental couple who agree to HLA typing and transplantation when available genoidentical donor or extended chronic transfusion for one year in the absence of an available donor
- Informed consent of parents, patients, donors and siblings. Written consent of parents, patients, donors and siblings when aged > 7 years

Exclusion criteria

- Non-SCA disease (not homozygous SS or sickle/β^0 thalassemia)
- Age > 15 years
- No history of abnormal TCDI (TAMV ≥ 200 cm/s)
- Impossibility of receiving transfusions because of alloimmunization
- Lacking non-SCA siblings from the same parental couple
- Parental refusal of HLA typing and transplantation in the case of a genoidentical donor or 1-year extension of chronic transfusion
- No affiliation with a social security scheme

Exclusion criteria for donors

- Positive HIV serology
- P24-positive antigen
- HCV-positive serology
- HBs-positive antigen

2.5. Ethical approvals

Ethical permission was obtained on July 27, 2010 and February 2013 (amendment) from the "Agence Française de Sécurité Sanitaire des Produits de Santé" (AFSSAPS) entitled since 2012 "Agence Nationale de Sécurité du Médicament" (ANSM) and the "Comité de Protection des Personnes" (CPP) Ile de France which is the French IRB in charge of this trial. The trial is monitored by an Independent Data Monitoring Committee (IDMC).

3. Study procedures

3.1. Recruitment and screening

The investigators of each SCA center participating in the DREPAGREFFE trial screened their patient cohorts for those with the criteria required for participation and sent the abnormal TCDI scan to Suzanne Verlhac, who coordinates the TCDI, MRI/MRA study of this trial for control and validation.

The SCA children detected as eligible for the trial are seen in consultations as part of their pathology, and the investigator provides appropriate oral and written information to parents, patients and siblings, including the objectives, benefits, constraints and risks of participating in this research. A reflection period of at least one week is granted before obtaining participation consent.

After parental consent, familial HLA typing is performed (patients, siblings and parents when necessary).

3.2. Inclusion

The enrollment visit takes place once eligibility is confirmed, consent obtained, and the HLA typing results are available.

Patients with no identical HLA enter the chronic transfusion group. Patients in whom familial HLA typing shows the availability of a genoidentical donor (HLA identity AB, DR, DQ) are included in the transplantation group.

3.3. Treatments

3.3.1. Chronic Transfusion group

Before each transfusion, blood screening, including complete blood counts, hemoglobin electrophoresis, screening tests for RBC antibodies and iron balance, are performed. Crossmatches are systematic. Viral serologies (HBS, CMV, HIV, HTLV) are conducted at baseline and at 1 year post-inclusion.

The aim of the chronic transfusion program is to maintain HbS below 30% and hemoglobin between 9 and 11 g/dL without increasing the hyperviscosity. The choice between simple transfusions, manual exchange or erythrocytapheresis is left to the investigator of the SCA center.

For those performing simple transfusions scheduled every 4 weeks, the following scheme is proposed as follows:

- 15 ml/kg in the case of hemoglobin < 9 g/dL
- 12 ml/kg in the case of hemoglobin between 9 and 9.5 g/dL
- 10 ml/kg in the case of hemoglobin between 9.5 and 10 g/dL
- In the case of hemoglobin > 10 g/dL, an exchange will be performed or the transfusion delayed by one week.

In this group, chronic transfusion is maintained for at least 1 year post-inclusion, after which the choice of treatment, transfusion discontinuation or extension or switch to hydroxyurea, is left to the investigator.

To limit the iron overload, oral chelation with deferasirox is administered when the ferritin level is > 1000 µg/L two times within a 1-month interval.

3.3.2. Transplantation group

Transplantation is recommended in patients with a genoidentical donor. The donor may be AA, AS or A-thal. In accordance with French laws, the minor donor is received individually and their parents by an independent committee of pediatric experts to collect their free consent. The stem cell source is bone marrow or cord blood that may have been cryopreserved. The minimum cellular dose required for a bone marrow transplant has to be $\geq 2 \times 10^8$ CNT/kg and for a placental blood graft $\geq 3 \times 10^7$ CNT/kg of recipient. In the recipient, HbS must be lower than 30% for anesthesia (required for central catheter placement and ovarian or testis fragment cryopreservation) and conditioning.

3.3.2.1. Conditioning (Fig. 1).

- Busulfex (Busilvex® Pierre Fabre Médicaments, Boulogne-Billancourt, France) from day −10 to day −7 at a total dose of 12.8 mg/kg for patient weighing > 34 kg or 15.2 mg/kg for weights from 23 to 34 kg, 17.6 mg/kg for weights from 16 to 23 kg and 19.2 mg/kg for weights from 9 to 16 kg.
- Cyclophosphamide: At a total dose of 200 mg/kg (50 mg/kg/day IV from day −5 to day −2).
- Rabbit anti-thymoglobulin: Thymoglobulin® (Genzyme) at a total dose of 20 mg/kg: 5 mg/kg/day from day −6 to day −3.

3.3.2.2. Prophylaxis of GvHD. Cyclosporin-A (CSA) is introduced intravenously on day −1 at 2 mg/kg and adjusted to achieve a whole blood target (100–200 ng/ml). Following engraftment, CSA is given orally with tapering initiated at 6 months post-transplant and stopping at 9 months in the absence of GvHD. In the case of intolerance such as seizures or posterior reversible encephalopathy syndrome (PRES), or in the case of GvHD requiring steroid initiation, which is known to increase the risk of seizure on CSA, it is replaced by mycophenolate-mofetil (MMF) (CellCept®, Roche, Boulogne-Billancourt, France).

Methotrexate (MTX) is given at 15 mg/m^2 at day +1 and then 10 mg/m2 at day +4 and +6.

However, MTX is only given for bone marrow transplantation, whereas only CSA is administered for cord blood transplantation.

Prophylaxis of seizures uses clonazepam during conditioning and cyclosporine-A therapy.

3.4. Risks associated with treatments

3.4.1. Chronic transfusion

3.4.1.1. Infections. The residual risk of viral infection of transfusion origin is minimal in the case of known viruses since it is estimated at 1/3150000 for HIV, 1/10000000 for HCV and 1/640000 for HBV [20].

Parvovirus B19, which is not detected in donors, can have consequences for sickle cell anemia. Nearly 50% of donors are immunized against Parvovirus B19, but the prevalence of this infection and its consequences with regard to transfusion is not clear [21]. Thus, the question remains of the need for negative packed red blood cells for Parvovirus B19 in non-immunized sickle-cell anemia.

Transfusion incidents involving bacterial contamination constitute the major infectious risk (1 bacterial infection for 178,000 labile blood products) and can have dramatic consequences in sickle cell patients [22]. They are mainly due to commensal skin bacteria or post-prandial bacteremia of the donor at the time of collection. Finally, the infectious risk also concerns agents that are not known to date, for which it is not possible to take preventive measures. Patients who are transfused in an iterative manner are potentially at risk.

3.4.1.2. Allo-immunization. Immunological risk remains the major risk of transfusion in sickle cell patients. It is mainly the risk of anti-red blood cell (RBC) alloimmunization. Alloimmunization can lead to life threatening hemolytic transfusion reaction. The anti-RBC alloimmunization is relatively frequent in these patients; it can reach 30–50% according to transfusion studies and transfusion practices and results mainly from ethnic polymorphisms in blood group antigens between donors, mainly of Caucasian origin and recipients of African descent [23]. This polymorphism is found at 3 different levels. (i) Common antigens, which are known to be immunogenic: the RH, FY, JK and MNS antigens. (ii) The variation in common antigens that are incomplete, in which the carrier of a partial antigen can be immunized against the missing epitopes when exposed to complete antigen through transfusion or pregnancy. Approximately 7% of patients carry a partial D antigen [24]. These variants can be found only by identifying the associated alleles in molecular biology, a practice that is not routinely implemented [25,26]. Immunohemolytic accidents and a poor transfusion yield have been associated with the corresponding antibodies. (iii) The rare blood groups characterized by the absence of a high frequency antigen. Specific rare blood groups have been demonstrated in individuals of African descent, requiring identical blood for transfusion. Rare blood units are stored and cryopreserved at the National Rare Blood Bank. The management of transfusion, especially in chronically transfused patients, is a real problem for patients with rare blood types. In general, alloimmunization can be prevented in the RH system; however, the resource is insufficient in terms of packed red blood cells from Afro-Caribbean donors and does not prevent alloimmunization against other antigens [24]. When alloimmunization is established, the risk of immuno-hemolytic accidents and a greater frequency of impaired transfusion performance are observed via different mechanisms. The alloantibody may be unknown because it has disappeared from the serum before transfusion or because the history of alloimmunization is not known [25,26]. The antibody that is reactivated by transfusion sensitizes the transfused RBCs and induces their destruction, often causing concomitant destruction of the patient's own RBCs likely via the effect of oxidative stress generated by the dependent antibody hemolysis but also by non-specific binding to sickle cell RBCs of activated complement fractions [27]. A second mechanism is the production of autoantibodies, which can lead to serious arrays of transfusional hemolytic anemia [28]. Finally, apart from any obvious alloimmunization, transfused RBCs can be destroyed rapidly (within 10 to 15 days) without precisely understanding the mechanism [29]. When alloimmunization extends to many common or specific antigens in these populations, irreversible transfusional impasses can occur, with only the possibility of incompatible transfusions associated with a high risk.

The prevention of these accidents lies mainly in knowledge of the extended phenotype of the patient, matching of the transfused RBCs and pre-transfusional crossmatching in the laboratory. In France, matching for RH and KEL, the most immunogenic blood groups, is mandatory. Matching is extended to the other immunogenic blood groups (FY, JK, LNS) only when patients present a first immunization. It is not possible to extend matching beyond the first intention because of

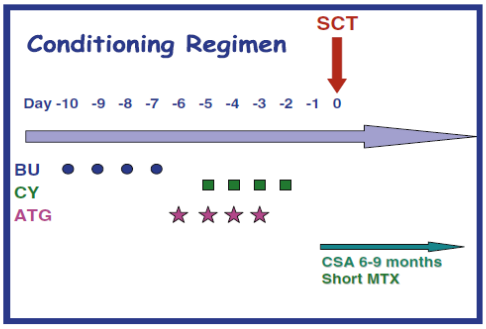

Fig. 1. Conditioning used for the Stem Cell Transplantation.

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the supply shortage of units from Afro-Caribbean donors. Finally, crossmatch tests are systematic because they make it possible to overcome some of the deficiencies of the screening test, especially when antibodies against low frequency antigens are produced.

3.4.1.3. Iron overload. Iron overload is unavoidable in long-term transfusion programs. The practice of transfusion exchanges in sickle cell patients makes it possible to limit the potential of iron overload by manual exchanges and even to avoid it with erythrocytapheresis. Iron overload can be responsible for endocrine problems: diabetes, parathyroid hormones, and gonadal and cardiac insufficiencies that only occur after very prolonged transfusion programs over 10 years. To avoid these complications, it is necessary to resort to a chelating treatment, which is, in principle, requested after approximately twenty transfusions. The overload is assessed based on the blood iron balance by the transferrin saturation coefficient and the ferritin level. The reference technique for evaluating iron overload was that obtained by liver biopsy, but liver and cardiac MRI techniques made its assessment easier and less dangerous. Reference chelation involves the use of deferoxamine, the 10-h nocturnal subcutaneous administration of which is extremely restrictive, but the availability of the oral chelator deferasirox improves the compliance of polytransfused patients [30].

3.4.2. Transplantation (SCT)

3.4.2.1. Aplasia. Aplasia during these myeloablative transplants lasts an average of 20 days for bone marrow transplantations and 28 days for cord blood transplants and has a risk of infectious complications, justifying the use of sterile aseptic measures. Nevertheless, because of these measures and the available antibiotherapies, only one aplastic death occurred among 87 transplants [13].

3.4.2.2. Graft-versus-host disease (GvHD). In these genoidentical transplantations, GvHD is related to minor antigenic differences. It is prevented by cyclosporine A administered from day − 1 to 9 months post-transplant. During the first 100 days of the transplant, it is called acute GvHD and results in skin signs such as pruritic erythematous rash, which can lead to Lyell, diarrhea-vomiting-malabsorption and hepatic biological signs. It requires the use of corticosteroids and possibly other immunosuppressive therapies such as mycophenolate mofetil (MMF, Cellcept®). Chronic GvHD manifests itself after day 100 in principle in some patients with acute GvHD: it results in mucocutaneous problems such as irregular pigmentation of the skin, labial and jugal lichen, possible scleroderma and/or digestive problems with malabsorption. The most serious complication is the occurrence of obliterans bronchiolitis.

In the context of genoidentical transplantation for sickle cell anemia [13], acute GvHD grade ≥ II was observed in 20% of patients. Factors that were significantly associated with the risk of GvHD were age > 15 years and the presence of a mismatch. No GvHD ≥ II was observed after cord blood transplantation. Moderate chronic GvHD was observed in 11% and extensive GvHD in 2.4% of patients. GvHD was responsible for 4 deaths among 87 patients.

In the present trial, all transplanted patients were younger than 15 years, and all sibling donors were strictly genoidentical, thus the risk of GvHD will likely be probably lower. Moreover, the results improved with time, and we have reported that disease-free survival among 121 transplants since 2000 (2000 − 2010) was 96.8% (95% CI: 93.2–100%) at 3 years [31].

3.4.2.3. Gonadal risk

3.4.2.3.1. In girls. The myeloablative conditioning proposed herein for transplantation in sickle cell anemia increases the risk of prolonged ovarian failure in post-pubertal girls at the time of transplant and results in a fall in estradiol levels and high levels of FSH and LH, requiring the use of estroprogestative treatment and raising fears of further infertility. At this time, the proportion of females that will have definitive or only transient ovarian failure is unknown: it is estimated to be prolonged for at least 10 to 15 years. Consequently, ovarian cryopreservation has been proposed: the specimen is collected under caelioscopy before conditioning, and the ovary is frozen in small fragments. This technique makes it possible to obtain two pregnancies with two live babies in one transplanted ex-sickle cell female [32,33].

When the transplant is performed in the prepubertal girl, an estroprogestative treatment is often necessary to induce puberty when the

Table 1
Assessments scheduled in the DREPAGREFFE Trial.

Treatment group	Inclusion	Geno-identical transplantation or Chronic Transfusion											
Visits		M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Signed written consent	X												
Familial HLA typing	X												
Cerebral vasculopathy assessment	X												
	X												X
• TCD													X
• MRI/MRA													
Cognitive Testing (SCA-child + sibling)													X
	X												X
• WIPPSI-R	X												
• WISC IV													
Ancillary study (3 ml of blood)	X												X
- Hypoxia factors													
- Phosphatidylserin													
Quality of life (SCD-child and parents)	X			X			X			X			X
Anti-HLA	X												X
Chimerism (transplantation arm)	X	X	X	X			X			X			X
Irregular agglutinins + direct Coombs (chronic transfusion arm)	X	X	X	X	X	X	X	X	X	X	X	X	X
Irregular agglutinins + direct Coombs (transplantation arm)	X			X			X			X			X
Iron balance: Iron, Transferrin saturation, Ferritin	X			X			X			X			X
Liver and heart MRI	X												X
Hemoglobin electrophoresis (chronic transfusion arm)	X	X	X	X	X	X	X	X	X	X	X	X	X
Hemoglobin electrophoresis (transplantation arm)	X	X	X	X			X			X			X

Monitoring 100% during one year post-inclusion and then, standard cohort follow-up including TCDI and MRI/MRA at 3 years post-inclusion and cognitive testing.

bone age reaches 13 years, but this is not always necessary, and spontaneous normal puberty with normal rates of estradiol and FSH-LH were observed in several girls who were transplanted before puberty [13,34]. Moreover, puberty has been achieved post-transplantation by reimplantation of ovarian fragments obtained before conditioning for transplantation [35].

3.4.2.3.2. In boys. No gonadal hormone disorders have been observed to date in our French series [13,34] in boys: testosterone and FSH-LH levels were normal in relation to their age and pubertal development, which is always assessed post-transplant. However, no semen analysis has yet been performed to assess future fertility. However, the risk of infertility is probably very high despite the apparent possibility of some (very)-long term recovery. As a precautionary measure, testicular cryopreservation is proposed in young sickle cell boys lacking clinical results to date. However, 3 ex-SCA-transplanted males were fathered spontaneously 11, 12 and 20 years post-transplantation.

3.4.2.4. Other risks

3.4.2.4.1. Carcinogenic risk. Treatments with total body irradiation are known to increase the risk of carcinogenesis, but this seems very unlikely with the conditioning used herein without irradiation. Extensive chronic GvHD can also be a risk factor. Regardless, to date, no secondary cancer has been observed in the wake of sickle cell transplantation [34].

3.4.2.4.2. Psychological aspects and quality of life. The impact of a chronic disease on the emotional development of the child and his/her family is major and must be taken into account. The transplantation also increases the risk of stress for the entire family. A retrospective study, supported by the French Institution of Transplantation, was carried out in France on 28 sickle cell patients treated with SCT. The goal was to assess their psychological state and to identify, through their narratives of illness and healing, the psychic and cultural stakes of the healing processes [36]. Of the 28 patients, nine had psychiatric disorders of moderate intensity, requiring specialized follow-up. Most had a positive view of the transplantation and its effects. Interviews showed the importance of individual and family changes. The main issues of healing were donation, the issue of recognition and debt, the building identity especially in adolescence and infertility and its transgenerational repercussions. In addition to cognitive assessment and quality of life, a prospective and comparative study will be performed to evaluate the psychological state of sick children and their evolution during two different treatments: transplantation or chronic transfusion.

3.4.2.4.3. Costs. Studies have reported the cost of chronic transfusion in SCA in the USA [37] and genoidentical transplantation in France [38]. Our study will make it possible to compare the respective costs of these two techniques in a prospective way. The main endpoint is a biological criterion that does not allow a cost effectiveness study to be performed. We will compare the costs of the transplantation strategy with the objective of providing an additional decision criterion. The costs will be estimated in the two groups of treatment (chronic transfusion or transplantation) from the perspective of the health care system for a period of 3 years (duration of patient follow-up) (Table 1).

4. Study assessments

4.1. Clinical and biological history

The electrophoresis of hemoglobin from the patient and his(her) parents in the absence of transfusion or hydroxyurea treatment is recorded or molecular biology analyses performed to determine the presence of homozygous SS or sickle β0-thalassemia. When possible, baseline biological parameters including hemoglobin, reticulocytes, leukocytes, neutrophils, platelets, mean corpuscular volume (MCV), bilirubin, lactate-dehydrogenase (LDH) and glucose-6-phosphate dehydrogenase (G6PD) activity are recorded in the absence of transfusions or molecular biology. Alpha genes and beta-haplotypes are assessed. An extended erythroid phenotype and history of eventual irregular antibodies are recorded.

The clinical history is recorded, including lateralization of the patient, the date and the type of first manifestation related to sickle cell disease, any history of stroke with the date and localization of the neurological deficit, the date of the first transfusion and the number of transfusions performed before inclusion, any splenic sequestration and splenectomy, gallstones and history of cholecystectomy.

At inclusion and at 1 and 3 years post-inclusion, the physical examination includes weight, height, SpO2, systolic and diastolic blood pressure, assessment of splenomegaly or hepatomegaly, puberty scoring, and neurological exam.

4.2. Transcranial Doppler (TCD)

The first article to report interest in TCD in sickle cell children was published in 1990 [39]. By comparing the velocities of 6 affected children with a history of stroke and intracranial stenosis confirmed by angiography and those of 115 affected children without a neurological history, Adams showed that a TAMV in the proximal middle cerebral artery (MCA) or distal internal carotid artery (ICA) greater than or equal to 190 cm/s strongly evoked a focal stenosis [39]. In a larger study of 34 patients aged 2 to 30 years (mean 12 ± 6 years) with stroke, using TCD and angiography as the gold standard, he noted a sensitivity of 90% and a specificity of 100% with the following criteria: a TAMV higher than or equal to 200 cm/s evokes stenosis, absence of flow in the MCA or slow flow < 70 cm/s in MCA favors thrombosis of the ICA and/or MCA, with the recorded slow flow being related to a type of augmentative circulation Moya Moya [5]. Adams then demonstrated the ability of TCD to predict the occurrence of stroke in asymptomatic sickle cell children [8]. One hundred and ninety children were followed for an average of 29 months. Twenty-three had a T-AMV > 170 cm/s. Seven strokes occurred, including six of 23 patients with an abnormal TCD. This result was confirmed by a subsequent study including 125 additional children, which revealed a risk of stroke of 40% within 3 years in children with a TAMV in the terminal ICA or MCA equal to or higher than 200 cm/s compared with 2% if the TCD was normal [8].

Adams used dedicated Doppler (blind or non-imaging TCD) to identify children for treatment. We used a transcranial color Doppler imaging device, combining B-mode imaging with color coding of the Doppler information. TCD and TCDI are similar, provided that TCDI sonographers focused on Doppler signal optimization, including attention to the audible components of the Doppler signal, meticulous tracking of the arterial segments, and careful documentation of the highest velocities [40].

We first verified that the criteria described by Adams in blind transcranial Doppler could be used in our center in TCDI. While we established a threshold value of 170 cm/s, vasculopathy suspected by TCDI in 9 children in a series of 58 children with sickle cell disease was confirmed by angiography in all but one [6]. All four patients with a velocity > 190 cm/s had stenosis. Two of them had no neurological history. Examination of the TCDI data and conventional angiography or magnetic resonance angiography (MRA) revealed that abnormally high velocities do not always correspond to an anatomical stenosis. TCDI detects focal hemodynamic stenosis that precedes the establishment of an organized stenosis but exposes the downstream tissue area to ischemia. It has been demonstrated that the impact of therapeutic intervention at this stage of circulatory disorders may avoid the evolution towards stenosis [41].

In France, color Doppler imaging (TCDI) is used with a low-frequency 2-Mhz transducer, which allows a more accurate identification of the arteries and offers a shorter learning curve for the operator. This

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equipment is widely available in imaging departments, and training has been largely given by the SV who coordinates the assessment of the trial by TCDI and MRI/MRA. All SCA centers receive the training provided in the US [42], and early TCDI screening is recommended [7,43,44]. Since June 2011, assessment of the extracranial part of the ICA via the submandibular approach, was systematically added to the circle of Willis assessment [45]. The velocity is recorded on both sides of each cerebral artery: MCA, anterior cerebral artery (ACA), posterior cerebral artery (PCA), basilar artery (BA), internal carotid artery (ICA) and extracranial internal carotid artery (eICA). Velocities are calculated without angle correction to avoid the risk of flow velocity overestimation, and the flow velocity of ACA and eICA were considered for treatment, in addition to MCA and distal ICA velocities. This parameter is the time-averaged mean of the maximum flow velocity (TAMV), also called the mean velocity, as in the STOP study. Because of the inverse linear relationship between the hematocrit and velocity, it is necessary to control the hemoglobin level on the same day of the TCDI to ensure that the patient does not experience acute anemia that could lead to a misinterpretation of the TCDI result. TCDI data are classified according to the STOP study as either normal (TAMV in every artery < 170 cm/s), conditional (170–199 cm/s), abnormal (≥ 200 cm/s in at least one artery) or inadequate (unavailable temporal windows).

For this trial, TAMV in all cerebral arteries are recorded, but the values entering in the analysis for the primary endpoint are the TAMV values in the artery with the highest TAMV value. TCD is performed at inclusion and at 1 and 3 years post-inclusion.

4.3. Magnetic resonance imaging (MRI)

MRI is a very sensitive tool for the detection of ischemic lesions depicted as hypersignals on the T2 and Flair sequences. Three types of lesions are described [46]. Most commonly, ACM-territory and ACA-territory infarcts related to severe stenosis or thrombosis of these major arteries are observed. Cortical watershed infarcts of the cortex and adjacent subcortical white matter that occur at the border zones between major cerebral arterial territories as a result of hypoperfusion may also be observed, as well as internal watershed infarcts of the deep white matter of the centrum semiovale and corona radiata at the border zone between the lenticulostriate perforators and the deep penetrating cortical branches of the MCA or at the border zone of the deep white matter branches of the MCA and the ACA.

A silent infarct is defined as an MRI signal abnormality measuring at least 3 mm in one dimension that is visible on 2 views on the T2-FLAIR weighted images and detected in a patient with no history of overt stroke and normal neurological exam.

For this trial, the scoring applied for ischemic lesions on MRI is as follow: 3 = territorial, 2 = cortical watershed infarct, 1 = internal watershed infarct, and for atrophia: 0 = absent, 1 = mild, 2 = moderate, 3 = severe.

4.4. Magnetic resonance angiography (MRA)

This noninvasive angiography has improved considerably in recent years and allows the visualization of arterial thromboses as well as even moderate stenoses [47,48]. The time-of-flight sequence in the three-dimensional acquisition (3D TOF) is most commonly used. The injection of contrast medium is not necessary. The very frequent flow artifacts with older equipment in these fast flowing anemic children in the form of a signal vacuum at the two carotid siphons and M1 segments of the MCA have become less troublesome. Console work with careful reconstruction and artery segmentation facilitate the analysis of each arterial segment and minimize false positives. The number and extent of stenoses occurring since inclusion will be compared in both chronic transfusion and transplantation groups. Attention is also paid to the detection of an abnormal basal collateral arterial network resembling "puff of smoke" so-called Moyamoya vessels, especially when the homolateral ICA and/or MCA are not visible.

The MRI/MRA protocol includes FLAIR, T1, T2, diffusion-weighted sequences and 3D time-of-flight angiography of the Circle of Willis. An additional 3D time-of-flight (TOF) multislab MRA (non-contrasted) sequence exploring extracranial ICA and carotid bifurcations are added.

For this trial, the scoring applied for MRA is as follows: 1 = mild stenosis (25–49%), 2 = moderate stenosis (50–74%), 3 = severe stenosis (75–99%), 4 = occlusion for each artery and for Moya: 0 = absent, 1 = mild, 2 = severe.

4.5. Cognitive testing

In a previous national multicenter prospective study, we enrolled 173 sickle cell children aged from 5 to 15 years between January 1996 and July 1997 for assessment with TCDI, MRI/MRA and cognitive tests, which was also performed in non-SCA siblings. The multivariate analysis showed that ischemic lesions on MRI (OR = 2.76, $p = 0.047$), a low hematocrit $< 20\%$ (OR = 5.85, $p = 0.005$) and thrombocytosis $> 500 \times 10^9$/L (OR = 3.99, $p = 0.004$) were significant independent risk factors for cognitive impairment (Full Scale Intelligence Quotient: FSIQ < 75) [49].

In the present study, the Wechsler intelligence scales used in patients and their siblings participating in the study were the "Wechsler Preschool and Primary Scale of Intelligence" (WPPSI-3) for children 3–6 years of age or the "Wechsler Intelligence Scale for Children-4th" (WISC-4) for children 7–16 years of age and the "Wechsler Adult Intelligence Scale" (WAIS-3) for siblings older than 16 years.

4.6. Quality of life and psychological conditions

Health-related quality of life (HRQoL) is a patient-reported outcome (PRO) of how his/her well-being and level of functioning are affected by health or the treatment received. Measurement of HRQoL facilitates our understanding of the burden of disease experienced by patients [50].

In this trial, HRQoL is assessed at inclusion and at 3, 6, 9, 12 and 36 months post-inclusion using the French version of the PedsQL 4.0 generic core scales (physical, emotional, social, school items), which are multidimensional child self-report and parent proxy-report scales with proven reliability and validity in children to distinguish between healthy children and pediatric patients with acute or chronic health conditions [51]. Moreover, the PedsQL™ generic core scales have also been shown to be reliable and valid for use in patients with SCD [52]. Several cross-sectional studies have utilized these scales [53–59] and shown that parents and SCA children report worse HRQoL on all scales compared with healthy black norms, and parents report worse HRQoL than child self-reports [57,59]. Factors associated with a worse overall HRQoL are asthma, pain crises, and missed school [56]. Neurological morbidity in children is associated with worse school HRQoL in child reports and worse overall and psychosocial HRQoL in parent proxy reports [54]. Major pain is associated with worse school functioning in the parent report and with poor physical and psychosocial child self-reports [54].

The aim is to evaluate by self-questionnaire the quality of life of SCA children as well as the quality of family life [60–66], as the transplant or non-transplant may have disrupted or improved the family's experience of the disease.

Psychological conditions are evaluated by psychologists in each center but not measured for research. However, it is scheduled to prospectively interview the parents of the 10 children who undergo transplantation in the present trial, a few weeks before the transplant, a second time three months post-transplant and one year later. Each time, a face-to-face, semi-structured interview is conducted, using interview grids that are specifically designed for each stage of the study. Interviews are conducted by one or two researchers in the pediatric ward in which the medical appointments are usually scheduled.

Researchers are either psychiatrists or psychologists, are all aware of the medical aspects of SCT in SCA, and trained to conduct interviews with specific populations.

4.7. Blood screening

The biological parameters assessed in both arms each month during the 1-year follow-up post-inclusion are described in the table.

Iron overload is studied via blood samples (iron, transferrin saturation, ferritin) collected at baseline and at 3, 6 9, 12 and 36 months post-inclusion in both arms.

In the transplantation arm, donor chimerism is assessed at M1, M2, M3, M6, M9 and M12 and 3 years post-transplant.

4.8. Hepatic and heart MRI

Iron overload may be severe in SCA patients who are highly transfused and must be regularly assessed [67]. Liver iron content (LIC) is a reliable reflection of overall iron content in the organism [68], and MRI has become the reference technique for assessing HIC, replacing liver biopsy because it is noninvasive. Two protocols are used in France that have been validated in comparison to the histological results of iron measurements obtained from liver biopsies: the liver/muscle signal intensity ratio (SIR) developed by Gandon [69] and completed by Rose [70], which requires 5 or 6 sequences and T2* relaxometry with a single multiecho gradient-echo T2 sequence [71]; this sequence is also used to evaluate myocardial iron. Verlhac, who also coordinates this assessment for the trial, reported a study comparing these techniques in 92 MRI performed for highly transfused SCA patients and found a good correlation between them with a Pearson coefficient of 0.89 for measuring HIC of < 16 mg/g [72]. Several studies have shown that there is no correlation between iron overload in the liver and heart, which probably results from different iron accumulation and chelation kinetics in the two organs [73]. This justifies the simultaneous assessment of liver and heart, which is easy with T2* relaxometry.

4.9. Costs

Our trial will allow a comparison of the respective costs of these two techniques in a prospective way. The costs of transplantation are compared to those of chronic transfusion. The costs are estimated from the perspective of the health care system over 3 years (duration of patient follow-up). The resources used are documented prospectively in volume for each patient included in the observation book. These resources include hospitalizations, examinations and ambulatory treatments. The valuation is performed based on national costs when they are available (prices of blood products, rate of ambulatory acts). In terms of hospitalizations, administrative data are used and supplemented if necessary by accounting data. The costs associated with HLA typing, sampling, packaging and, in general, all pre-transplant procedures are also included in the calculations. The hospitalizations occurring post-transplant are also taken into account.

4.10. Biological study

4.10.1. Phosphatidylserine

Phosphatidylserine is a membrane phospholipid that is normally present on the inner side of membranes but is abnormally exposed on sickle-cell RBC membranes [74–76]. Externalization of phosphatidylserine (PS) is one of the key signals for RBC removal and may favor their adherence to endothelial cells. In the context of SCA, this could modify the adhesion properties of RBCs and could increase the risk of vasculopathy. It is possible that RBCs that present PS on their surface are capable of altering blood vessel walls, particularly cerebral vessels, and of participating in the formation of cerebral vasculopathy.

4.10.2. Hypoxia and angiogenic factors

Hypoxia-induced angiogenesis may play an important role in the pathophysiology of SCA. It is well recognized that vascular endothelial growth factors (VEGFs) alone do not play a role in the control of angiogenesis. Indeed, VEGF alone initiates but does not complete angiogenesis [77]. Among the molecules that can function in cooperation with VEGF, angiopoietins should be mentioned. Angiopoietins represent a family of cytokines that are known to have two main members, Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2), which possess a common receptor called Tie-2. During physiological angiogenesis, angiogenic remodeling involves a preliminary Ang-2 action that destabilizes vascular structures to allow VEGF, if present, to stimulate angiogenesis. In the absence of the latter, vascular regression is observed. Ang-1 intervenes in the late phases of angiogenesis to ensure stabilization of the vascular networks in particular by promoting the recruitment of peri-endothelial cells. This discovery of angiopoietins as synergistic agents of VEGF in the angiogenic balance offers new perspectives to the study of angiogenesis in a pathological context, particularly in cerebral ischemia [78]. Similarly, erythropoietin (EPO) can exert distinct effects from its hematopoietic effects on different organs including the brain. For example, EPO is angiogenic, neuroprotective and its receptor has been identified at the cerebral level [79,80].

Numerous factors lie at the root of angiogenesis, among which tissue hypoxia is known to play a major role in increasing the synthesis of angiogenic factors, in particular via the transcription factor HIF-1 (hypoxia inducible factor-1). Among the angiogenic factors induced by HIF-1 are VEGF, Ang-2 and EPO [81–83]. VEGF, angiopoietins, EPO and the placental growth factor (PlGF) seem particularly interesting in the context of sickle cell anemia. Indeed, the serum level of these molecules, and in particular that of Ang-2, revealed a pro-angiogenic state in adult sickle cells [84]. Moreover, recent data demonstrated that hydroxyurea therapy in SCA was associated with a reduction of the raised levels of Ang1, bFGF and VEGF [81].

It should be noted that in sickle-cell anemia, organic damage is related to vaso-occlusion accidents associated with ischemia-reperfusion. These molecules are known to be involved in post-ischemia and in particular angiogenic processes [82,83,85]. Thus, all these studies highlight the relevance of studying the expression of these hypoxic/angiogenic factors at the serum level in sickle cell children with abnormal Doppler velocities and of comparing their expression during chronic transfusion or after transplantation.

4.10.3. Brain injury-related factor expression

SCA is a chronic illness that causes an increased risk of stroke and progressive brain and cognitive dysfunction. Several studies have highlighted the expression of brain injury-related factors such as brain-derived neurotrophic factor (BDNF) and S100B protein as potential peripheral biomarkers of brain damage [86–88]. Therefore, the serum levels of these potential brain-related injury factors will be evaluated at baseline and 12 months post-inclusion in both arms. Moreover, stroke incidence and high TCD velocity have been associated with elevated BDNF in SCA [89]. Plasma BDNF and PDGF-AA levels are associated with high TCD velocity and stroke in children with sickle cell anemia.

Therefore, it will be very interesting to compare the serum level of all these hypoxic/angiogenic and potential brain-related injury factors at baseline and 12 months post-inclusion in both arms.

4.11. Safety assessments

For the patients included in the chronic transfusion program, direct Coombs, irregular agglutinins and iron balance (iron, transferrin saturation, ferritin) will be checked before each transfusion.

For the transplanted patients, the direct Coombs, irregular agglutinins and iron balance (iron, transferrin saturation, ferritin) are performed at 3, 6, 9 and 12 months after inclusion. Anti-HLA testing is

performed at inclusion and 1 year post-inclusion.

An amendment approved by ANSM and CPP in February 2013 allowed extension of the follow-up of patients to 3 years post-inclusion. Thus, blood screening will be assessed again at 3 years as part of the regular follow-up.

4.12. Adverse events

All adverse events (AEs) during the study will be recorded in the case report form, with information about the date of onset and end date (if applicable), severity and seriousness of the AE, investigator's opinion of the relationship to the protocol treatment, treatment for the AE, cause of the event (if known), and information regarding the resolution or outcome. Adverse events classified as serious will be recorded using a serious adverse event reporting tool and reported to the sponsor.

The intensity of an AE is graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE) version 4.03.

5. Outcome measures

5.1. Efficacy evaluation

The primary outcome of the Drepagreffe trial was the average at 1 year of the velocities (TAMV) of the right and left middle cerebral arteries. This was then modified after the amendment by using the following:

- TAMV recorded in the artery with the highest TAMV value at 12 months after inclusion

The secondary outcomes include the following:

- Incidence of stroke
- Survival without stroke
- TCD velocities in all arteries at 1 and 3 years post-inclusion
- Proportion of patients with normalized velocities at 1 and 3 years (TAMV < 170 cm/s in all arteries).
- Incidence of ischemic lesions on MRI.
  - The number of patients with ischemic lesions that appeared since inclusion is compared in both arms
  - The difference in MRI scoring between inclusion and at 1 and 3 years post-inclusion is compared in both arms
- Incidence of stenosis on MRA
  - The number and extent of stenosis occurring since inclusion
  - The difference in MRA scoring between inclusion and 1 and 3 years post-inclusion
  - MRI/MRA performed at inclusion and at 1 and 3 years post-inclusion will be read again in a blinded manner by 2 experts with no knowledge of the patient's history or treatment
- Cognitive performance in patients and siblings
  - Performance at inclusion and at 1 and 3 years post-inclusion
- Quality of life in patients and parents
- Costs in each arm of treatment
- Expression of membrane phosphatidyl, hypoxia, angiogenic and brain injury-derived factors at 12 months in both arms and comparison with values at inclusion

An amendment approved by ANSM and CPP in February 2013 allowed extension of the follow-up of patients to 3 years post-inclusion. Thus, TCDI, MRI/MRA, quality of life scales, and blood screening, will be assessed again at 3 years as part of the regular follow-up. Only cognitive testing is not included in the regular follow-up and has been financed by another grant.

5.2. Safety evaluation

Alloimmunizations that have appeared since inclusion are compared in both groups. In the transfusion group, the program will be terminated if any iron overload is assessed at inclusion and at 12 and 36 months post-inclusion.

5.3. Sample size consideration

For a type I error rate of 5% risk and 80% statistical power, 21 children should be included in the transplantation group and 42 children in the chronic transfusion group to demonstrate a difference from 140 cm/s to 100 cm/s on the TAMV at 12 months assuming a standard deviation of 40 cm/s, as suggested by our previous data [13], and a prevalence of 1/3 donor availability.

The protocol was amended in February 2013 due to a different prevalence of treatment groups. Therefore, the sample size computation was revised accordingly, as follows. For a type I error rate of 5% risk and a 90% statistical power, and assuming a prevalence of 1/2 donor availability, 63 children should be included to demonstrate a difference from 140 cm/s to 100 cm/s on the TAMV at 12 months assuming a standard deviation of 40 cm/s, as suggested by our previous data [13].

5.4. Duration of the research

The inclusion period was scheduled at 24 months and the follow-up for each patient at 12 months. An amendment was approved by ANSM and CPP in February 2013, which allowed extension of the inclusion period by 6 months and of the follow-up by 24 months. Thus, for the patients, the length of the follow-up is 36 months.

5.5. Statistical analysis

The intention-to-treat (ITT) population will be considered for the analysis. Two primary analyses of the main endpoint will be performed based namely on genetic randomization and using propensity score matching.

Genetic randomization considers the availability of the donor as a result of a random experiment; hence, a rough comparison of the main endpoint across treatment groups will be performed for the whole sample.

At the time of protocol amendment, a propensity score matching analysis was also scheduled for the ITT population as a type of sensitivity analysis.

5.6. Description of the stopping rules

The sponsor and/or regulatory bodies may decide at any time to discontinue the trial prematurely for medical and/or administrative reasons. In all cases, the decision shall be determined after mutual consultation and appropriate documentation of the reasons. The investigator will then return the observation books and all documentation related to the study to the proponent.

Rules for discontinuing the study on an individual scale:

- Patients and parents refusing transfusion protocol will not be included.
- Patients with an identical HLA donor who refuse an allograft will be analyzed for the intention-to-treat population.
- Major alloimmunization prohibiting continuation of the transfusion program.

5.7. Trial status

The French DREPAGREFFE Study is a large prospective multicenter interventional study comparing the outcome of cerebral vasculopathy

in SCA children with a history of an abnormal TCDI. The initial protocol, which was approved by "Agence Française de Sécurité Sanitaire des Produits de Santé" (AFSSAPS) and the "Comité de Protection des Personnes" (CPP) on July 27, 2010, scheduled the enrollment of 63 participants and 1-year follow-up.

An amendment, which was approved by "Agence Nationale de Sécurité du Médicament" (ANSM), the new name of AFSSAPS since 2012, and CPP in February 2013, allowed extension of the inclusion period by 6 months and of the follow-up period by 2 years. Thus, the follow-up for each patient is 3 years post-inclusion or transplantation.

Enrollment began on December 31, 2010 and stopped on June 30, 2013. The last transplantation was performed on January 13, 2014 and the clinical follow-up ends on January 2017. The trial is supervised by the Clinical Research Unit in the Paris area.

6. Discussion

This is the first prospective trial to compare transplantation to chronic transfusion in SCA children with cerebral vasculopathy. This multicenter trial enrolled SCA children in long-term chronic transfusion treatment due to a history of abnormal cerebral velocities on TCDI regardless of a history of stroke. The hypothesis is that genoidentical transplantation will facilitate improvement or stabilization of cerebral vasculopathy than chronic transfusion with a favorable balance benefit/risk. The measures of efficacy are velocities on TCDI, silent infarcts and stenoses on MRI/MRA, quality of life and psychological status, iron overload, PS exposure on RBCs, angiogenic factors and costs.

A disadvantage of using observational data for assessing clinical benefit is that treatment groups may differ such that confounding bias can be suspected. Nevertheless, to minimize comparison biases, the availability or not of an HLA-identical sibling donor was considered to be the equivalent of genetic randomization to the allograft or transfusion arm, respectively. Indeed, selection criteria included only patients with at least one non-SCA sibling from the same parental couple who agreed to HLA typing and transplantation when available genoidentical donor. Moreover, we also considered propensity-score matching as a sensitivity analysis to control for potential residual confounding.

The chronic transfusion program applied in patients detected at risk due to abnormal TCD has proven its efficiency for decreasing the risk of stroke by 91% [9], but even after at least 30 months of transfusion in patients with normalized velocities, its discontinuation is not safe and leads to a high risk of stroke or reversion to abnormal TCD [12]. In the Créteil SCA newborn cohort that was screened at an early time point with TCDI, there was a high 29.6% cumulative incidence by age 9 of abnormal TCDI, raising concerns about the number of patients to indefinitely transfuse [11]. To limit these indications of long-term transfusions, a switch to hydroxyurea has been proposed in monocenter [41] and randomized multicenter studies [90]. Hydroxyurea via an increasing hemoglobin level, decreased velocities [91] and inferiority TWITCH trial has shown that hydroxyurea was not inferior to chronic transfusion in SCA children with a history of abnormal TCD who had been previously transfused for at least 1 year [90]. However, as the mean age at enrollment was 9.7 years old and the duration of past transfusions was 4.5 years, we suspect that most of the patients were probably beyond the risk period [92]. Moreover, the short-term follow-up in the TWITCH trial requires caution in determining the safety of this switch [92]. In contrast, in the Créteil SCA cohort, we have extensive experience with the follow-up of 92 patients with a history of abnormal TCDI. A switch to hydroxyurea was prescribed since 1998 to 45 SCA children with normalized velocities and no stenosis. A reversion to abnormal TCDI was observed in 13/45 (28.9%) patients before age 9.5 years, and transfusions were immediately reinitiated to avoid stroke occurrence [93]. Thus, in our opinion, it is cautious to regularly check TCDI after the switch to hydroxyurea [92]. By contrast, in the same cohort, 24 among the 92 patients with a history of abnormal TCDI, were transplanted with a genoidentical sibling, no reversion to abnormal TCD occurred and velocities were normalized in 4 patients who still had abnormal TCDI despite a long-term transfusion program [93]. This monocenter experience encouraged us to propose the prospective multicenter DREPAGREFFE trial.

Silent cerebral infarcts (SCI) are the most common form of neurologic disease in SCA patients [94], and the cumulative risk does not reach a plateau [11] with a cumulative risk of 19.2% by age 8 years, 32.4% by age 14 years, 39.1% by age 18 [95] and 53.3% by age 30 years [96]. In the Créteil cohort [95], the cumulative incidence of SCI in patients with a history of abnormal TCDI was 20/38 (52.6%) vs 44/151 (29.1%) in those with no such history ($p = 0.006$) (personal data). They are associated with the presence of stenosis but also occur in absence of macrovasculopathy [11,95,97]. The risk factors for SCI reported in the literature are baseline [11,95,97,98] and acute [93] severe anemia, stenosis [93,97], a high leukocyte count [99], male sex [98], Senegal β haplotype [99], and relatively high systolic blood pressure [98]. In the STOP-2 study, SCA children with a history of abnormal TCD but normalized velocities and no severe stenosis, 21/79 (27%) had SCI at study entry, and 3/37 (8.1%) developed new MRI lesions in the transfusion-continued group compared with 11/40 (27.5%) in the transfusion-halted group ($p = 0.03$) [100]. The total number of lesions remained essentially unchanged, decreasing from 25 to 24 while increasing from 27 to 45 in transfusion-halted patients [100]. In the SIT trial examining patients with SCI in the absence of abnormal TCD, 6/99 (6%) had a new or enlarged infarct in the 3-year transfusion group compared with 14/97 (14%) in the observation group, corresponding to a significant reduction in incidence: 2.0 vs 4.8 events, $p = 0.04$ [101]. The outcome of stenosis on MRA was evaluated in a subset of patients in the STOP-2 trial. Indeed, MRA studies, were not initially included but were added after initiating the trial. Twenty-six patients had MRA at enrollment and during follow-up. Among the 19 patients with an initial normal MRA, no appearance of stenosis was observed among 10 patients who were randomized to the transfusion-continued arm, whereas one appeared among the 9 patients in the transfusion-halted group [100].

Cognitive performances have been shown to be significantly lower in SCA patients than in non-SCA siblings [49]. SCA patients with a history of stroke exhibit lower performance than those with SCI who themselves have lower performances than those without SCI [49,102–105]. Moreover, we have shown in a previous prospective multicenter study that low performances were associated with the presence of ischemic lesions on MRI, severe anemia and thrombocytosis [49]. To date, no prospective study has evaluated the outcome of cognitive function after SCT compared with chronic transfusion. Only the SIT trial compared chronic transfusion to simple observation in SCA patients with silent infarcts and found no significant difference in cognitive performance at 3 years [101]; however, but only abbreviated scales of intelligence (WASI) were used.

The risk of transplantation is not negligible, including treatment-related mortality, post-transplant neurological worsening, graft-versus-host disease (GvHD) and gonadal dysfunction, although they are quite reduced when transplantation is performed before the age of 15 [13–19]. Important progress have been achieved in the management and results of genoidentical transplantation for SCA patients. In 2007 in France, disease-free survival among 44 SCA patients transplanted since 2000 was 95.3% at 5 years [34]. In 2010, we confirmed that the chances of cure were at least 95% because among 121 patients transplanted since 2000, DFS was 96.8% (95% CI: 93.2–100%) [106]. Moreover, in 2013, we showed that the high ATG dose (20 mg/kg) significantly reduced the incidence of chronic GvHD [107]. The risk of early neurotoxicity (seizures, posterior reversible encephalopathy syndrome) was also reduced by switching CSA to MMF when steroid initiation was required [106]. To preserve the chances of fertility, testis or ovarian fragments were cryopreserved before transplantation.

Health-related quality of life (HRQoL) is a patient-reported outcome (PRO) of how his/her well-being and level functioning are affected by

health or the treatment received. In this trial, HRQoL was assessed with the French version of the PedsQL 4.0 generic core scales because the new PedsQLTM SCD module [108] was not available when this trial was initiated. Measurement of HRQoL facilitates our understanding of the burden of disease experienced by patients [50]. HRQoL has been assessed in the SIT trial comparing chronic transfusions with observation in SCA children with SCI in the absence of abnormal TCD. The investigators used other HRQoL scales, CHQ PF 50, and discovered significantly better scores in the transfused group in terms of physical function, bodily pain and change in health compared with the observational group [109]. This was the first evidence that children with SCA who received regular blood transfusion therapy felt better and had a better overall HRQoL than those who did not receive transfusion therapy. However, a recent study reported very low HRQoL in SCA patients chelated with deferasirox, although the neurological status of SCA patients was not described [110]. Hydroxyurea treatment has been shown to be associated with better overall HRQoL per child report and better parent and child-reported physical HRQoL [58]. A significant improvement in HRQoL was recently reported at 1 year after HSCT for SCA patients [111–113] but there is no trial comparing HRQoL between transfused and transplanted SCA patients.

Thus, DREPAGREFFE trial is the first prospective study to compare the outcome of cerebral vasculopathy in two arms, transfusion vs transplantation, defined by the random availability of having a genoidentical donor. Our hypothesis is that velocities will be significantly more reduced after transplantation and cerebral vasculopathy better stabilized or improved at 1 year, but that it will also be very important to evaluate whether the difference in cognitive performance observed between SCA patients and non-SCA siblings may be reduced after SCT. The other major point of this study will be the evaluation of the benefit/risk balance in terms of safety, quality of life, and cost with the objective of providing an additional decision criterion.

7. Competing risks

We declare that we have no conflict of interests.

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Appendix A. Appendix

Title	National multicenter prospective study comparing the results of geno-identical allograft to the transfusion program in sickle cell children with cerebral vasculopathy detected by transcranial Doppler. DREPAGREFFE
Code	P: 071247
Investigator coordinator	Françoise BERNAUDIN, MD

Objectives	Principal:
	Show that the allograft significantly more decreases the velocities on TCDI than the extended chronic transfusion
	Secondary:
	Compare in the 2 groups (transfusion vs transplantation):
	
	- Outcome of cerebral vasculopathy
	  * Percentage of patients with normalized TCDI (TAMV < 170 cm/s)
	  * Ischemic lesions on MRI
	  * Possible stenosis on MRA
	  * Serum expression of “angiogenic” molecules
	  * Comparative cognitive performance patient and sibling
	- Evaluation of transfusion performance, anti-erythroid alloimmunization, and post-transfusion incidents and accidents
	- Iron overload
	- Psychological condition and quality of life
	- Costs

Eligibility criteria	Inclusion criteria - Sickle cell anemia (SCA) patients (SS/Sb0), - Aged < 15 years, - With a history of abnormal TCDI (≥ 200 cm/s) - with or not a stroke history - Having at least one non-SCA sibling of the same parental couple - Of which parents accept HLA typing and transplantation project in the event of available HLA geno-identical donor among siblings or the extended chronic transfusion Non-Inclusion Criteria - non SCA patients (not SS/Sb0) - aged > 15 years - No history of abnormal TCDI (≥ 200 cm/s) - no non-SCA sibling of the same parental couple - Of which parents refuse HLA typing and transplantation project in the event of the existence of an available HLA geno-identical donor among siblings or the extended chronic transfusion
Sample size	63 patients
Duration of	Inclusions over 2 years and 6 months and follow-up over 3 years, i.e., a study duration of 5 years and 6 months

research	
Methodology	National multicenter non-randomized prospective cohort study but defined by the random availability of having geno-identical donor transplanted (exposed) versus transfused (unexposed). Study of cell therapy.
Exams required	1. The TCDI 2. The MRI/MRA 3. Cognitive tests (WIPPSI-R or WISC-4 or WAIS-3) 4. Quality of Life Scales 5. Alloimmunization 6. Externalization of phosphatidylserine 7. Expression of angiogenic molecules.
End points	1. Main endpoint Improvement of the cerebral vasculopathy assessed on the measurement of velocities (TAMV) at one year 2. Secondary endpoints 2.1 Evolution of cerebral vasculopathy - Incidence of stroke - Survival without stroke - TCD Velocities in all arteries - Percentage of patients with normalized velocities - Expression of membrane phosphatidyl and angiogenic molecules - Incidence of ischemic lesions in MRI - Incidence of stenoses on MRA - Cognitive performance (patient and sibling) 2.2 Anti-erythrocytic alloimmunization 2.3 Hemolysis and iron overload 2.4 Assessment of Psychological Status and Quality of Life 2.5 Costing

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