NCT Number

NCT02907554

Version : **03-15-2024**

Title

Cyclosporine Pretreatment in Brain-Dead Donors and Kidney Graft Function. The Cis-A-Rein placebo-controlled double-blind randomized controlled clinical trial

Protocol

EFFECT OF ORGAN DONORS PRETREATMENT WITH CICLOSPORINE A ON KIDNEY GRAFT FUNCTION: A DOUBLE-BLIND RANDOMIZED CONTROLLED STUDY

Acronym: Cis-A-Rein study

Sponsor code	ANSM number
RBHP_2014_ICHAI	2014-003544-12

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SUMMARY

Objective of the study: to show that a pretreatment of brain-dead donors with cyclosporine A (CsA) improves renal graft function recovery in kidney recipients after transplantation. The assessment of the primary outcome was the occurrence of delayed graft function defined by the requirement of at least one hemodialysis session within the first 7 days post-transplantation. Secondary parameters were: 1) the occurrence of slow graft function defined by at least one of the following parameters: a 24-hr urine output < 1000 ml at day 1 post-transplantation or a serum creatinine reduction ratio ≥ 30% on day 3 or a serum creatinine reduction ratio ≥ 70% on day 7 post-transplantation; 2) the evolution of the estimated glomerular filtration (eGFR) evaluated by the estimated creatinine clearance (CKD-EPI formula) at day 1, 3, 7, and 3 months after transplantation; 3) the occurrence of a primary graft dysfunction, the occurrence of early acute or delayed graft rejection (at 1-year), the in-hospital length of stay, kidney graft and recipient mortality at 1-year post-transplantation.

Study Design: This trial is a two-arm parallel, randomized, double-blind, controlled multicenter trial.

Number of centers: 15 donor centers and 14 recipient centers.

Study Description: Prospective multicenter, double-blind, randomized controlled trial, over a 36 months period including 324 kidney transplant recipients per group, i.e. a total of 648 recipients.

Problem to resolve: the most frequent complication of kidney transplantation is the delayed graft function (DGF) and the slow graft function (SGF), secondary to renal tubular injuries. The unavoidable ischemia-reperfusion of this procedure represents the main mechanism of such injuries, that are characterized by oxidative stress and reactive oxygen species production. The mitochondrial permeability transition pore (mPTP) opening plays a major role in the development of apoptotic and necrotizing injuries during reperfusion. Harvested organs suffer obligatory from ischemia-reperfusion injuries (duration of cold and warm ischemia). Despite many efforts made to optimize organ perfusion, to improve kidneys conservation and surgical techniques, DGF and SGF still occur in 30 to 50% of kidney grafts. Cyclosporine A inhibits the PTP opening and can reduce ischemia-reperfusion organ damages. These effects are already reported in experimental organ models of ischemia-reperfusion, as well as in liver and heart transplantations performed in animals. Experimental data assessing the effects of CsA pretreatment on kidney graft function remain controversial. A clinical trial has shown that a single CsA bolus administered before coronary percutaneous revascularization reduced significantly the size of myocardial infarction without any deleterious effect caused by CsA. CsA is well known for a long time for its immunosuppressive properties and is routinely administered in kidney graft transplants. On the other hand, a single bolus of CsA administered as a pretreatment in organ donors might improve kidney graft function in kidney graft recipients.

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1. General informations

1.1. Title of the trial

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Version 13 of 20240315

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Concerning study sites, there are more donors than recipient centers. Indeed, usually donor centers are also centers for transplantation (see coupled ICUs and nephrology/urology wards), but some centers are only dedicated for kidney transplantation (see uncoupled/coupled ICUs and nephrology/urology wards).

1.8. Statistical data management

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1.10. Provisional schedule of the study

Favorable authorization from the CPP: April 2015.
Start of the study: December 2017
Duration of the study: 6 years
Recruitment duration: 5 years
Provisional end of the study: December 2023
End of study report: March 2024

2. Scientific rationale of the study

2.1. Last scientific knowledges

Nowadays, kidney transplantation is routine treatment of terminal renal insufficiency with a growing demand. In France, the number of kidney transplantations increased from 40.9 to 44 per millions of residents between 2004 and 2010, either 3000 kidney transplants per year [1]. Besides the rejection and the specific postoperative complications, the delayed and slow kidney graft function remain the most frequent complications [DGF/SGF]. DGF occurs in approximately 30% and SGF in 40 to 60%, with variations of more than 10% according to the risk factors [2-8]. These risk factors are related to the donor's or recipient's characteristics or the quality of organ's preservation. Major risks related to the donors are an age > 55 years, a history of diabetes or arterial hypertension, and hemodynamic instability before the kidney harvesting. Most important recipient's risk factors are a history of diabetes or arterial hypertension, cardiac insufficiency, male, the type of nephropathic disease, the number of kidney graft previously transplanted, dialysis duration before transplantation, and the time spent to wait transplantation. The organ quality is essentially influenced by the cold ischemic time and the conditions of preservation (nature of the storage kidney solution, use of perfusion machine) and intra- and postoperative problems. DGF and SGF are reported to be independent factors of acute early rejection, reduced graft and recipient survivals, and a longer duration of hospitalization [2, 3]. Twenty percent of kidney grafts show a complete dysfunction at 5-years post-transplantation [1].

Ischemia-reperfusion is the main mechanism of DGF/SGF which is clinically characterized by signs of postoperative acute renal failure (ARF). Indeed, the harvested kidney from the brain-dead donor suffers of important and frequent hemodynamic instability; these modifications are unavoidable and intrinsic to the harvesting process. They include numerous warm (during the kidney graft harvesting), and cold ischemic episodes (during the storage of the kidney graft), followed by reperfusion at the time of transplantation. Kidney graft damages are explained by complex and interconnected mechanisms which induce biochemical, structural and functional alterations [2, 3, 5; 7]. Besides the hypoxic-associated injuries, ischemia-reperfusion induces a prolonged reduction of renal blood flow (especially in the external medullary parenchyma), an oxidative stress caused by an increased production of reactive oxygen species (ROS), an activation of pro-inflammatory mediators

which trigger leucocytes adhesion and immune activity. More than ischemia, reperfusion is responsible of the sustainability of these abnormalities which interact and cause at last anatomical damages as cell necrosis and apoptosis, leading to renal dysfunction.

Therefore, numerous preventive pharmacological and non-pharmacological preconditioning strategies still aim to minimize this phenomenon of ischemia-reperfusion to improve kidney graft function post-transplantation. The increasing improvement in organ perfusion from donors, storage graft techniques (storage solution and perfusion machine) and surgical techniques allowed to reduce the risk of DGF/SGF [2, 5, 6, 8]. Nevertheless, none of them enables to reduce early and significantly renal cell death during the kidney harvesting process. Moreover, none of the numerous pharmacological preconditioning strategies (N-Acetylcystein, EGTA, ADP, Mg, inhibitors of the external mitochondrial membrane channels, etc.), has been reported to be efficient in clinical conditions.

Since the end of the 80s, experimental studies have confirmed the key role of the mitochondrial dysfunction in triggering and causing necrotic and apoptotic cell damages secondary to ischemia-reperfusion [9, 11]. This phenomenon is characterized by the opening of a protein channel which is localized on the internal mitochondrial membrane and is called the permeability transition pore (PTP) [12-16]. This PTP channel remains closed during ischemia, but few minutes of reperfusion trigger trigger its opening leading to a decrease in ATP synthesis and consumption, ROS production, mitochondrial NADH and pro-apoptotic protein extrusion, an increase in mitochondrial permeability and volume and finally a burst of the mitochondria. The PTP opening is mainly triggered by the influx of ROS and calcium [17, 18]. This phenomenon induces cell necrosis due to energy failure or apoptosis related to a cytoplasmic release of pro-apoptotic molecules like cytochrome C [12, 19]. The precise nature and structure of the PTP is still a matter of debates. If there is a consensus on its protein nature and its diameter when opened, the nature and the number of proteins involved in the formation of this channel remains unknown. Different proteins have been refuted because PTP persist in mitochondria without these proteins. The only protein accepted consensually is the cyclophilin D (CyP-D). It is not a channel by itself but a protein which regulates the opening of the channel. Indeed, the CyP-D binding on the PTP favors its opening [11]. Experimental studies have confirmed the key role of CyP-D in this phenomenon and in cell death induction [11, 20-25]. Animals with CyP-D gene knock-out reduced ischemia-reperfusion injuries caused by myocardial infarction [22], renal ischemia [23] and focal cerebral ischemia [21].

The inhibition of the PTP opening by various pharmacological agents may improve ischemia-reperfusion damages in different cells and organ models [12, 14, 24, 26-29]. Nevertheless, in animal models, only cyclosporine, a calcineurin inhibitor, well known and universally administered after transplantation to induce immunosuppression, is efficient on the mitochondrial permeability. By releasing CyP-D from the PTP, cyclosporine is a undirect exogenous inhibitor of the PTP opening [9, 13, 29]. In other words, CyP-D represents the molecular target for the anti-apoptotic effect of CsA.

2.2. Latest knowledge on pre-clinical experimental studies

Numerous experimental studies have shown that CsA has protective effects on cell and organ models of ischemia-reperfusion, especially on heart [24, 25, 30-33], brain [24, 26, 34], liver [27, 35] and kidney [28, 36, 37]. Such an effect has been particularly assessed in models of organ transplantation, because all the process of organ harvesting and transplantation reproduces obligatory and perfectly ischemia-reperfusion with a cold- and a warm ischemic time.

The administration of a CsA analog enabled to decrease ischemia-reperfusion damages related to the PTP opening in a rat model of liver transplantation [27]. Similar beneficial effects characterized by a preserved function of the mitochondrial oxidative phosphorylation, of the cell energy status and the prevention of mitochondria swelling have been reported in another model of liver transplantation in rats [35]. CsA administration induces also beneficial effects in experimental models of cardiac

transplantation, which are expressed by an improved myocardial contractility, cell energy reserve, and a decrease in cell and mitochondrial edema after transplantation [33]. Three studies performed in animals have assessed the effects of cyclosporine pretreatment on kidney graft function with conflicting results. In2009, Shihab *et al* [38] have shown that a single injection of 10 mg/kg cyclosporine 1 or 7 days before kidney harvesting improved both kidney graft function from day 3 after transplantation (improved creatininemia and insuline clearance) and histological injuries of the graft. Similar results have been confirmed by the same authors one year later and additional data showed an impact of CsA on the PTP opening [36]. In another model of kidney transplantation, the authors failed to confirm these beneficial effects. These conflicting results may be explained by methodological differences: various criteria to assess renal function (serum creatinine level [39] or creatinine clearance [36, 38]), different cold ischemic times (24 hours [39] and 2 hours) [36, 38].

To our knowledge, only one clinical trial has evaluated the impact of cyclosporine in patients with a myocardial infarction, an ideal clinical condition of ischemia-reperfusion [40]. The authors reported that the administration of a single dose of cyclosporine before percutaneous coronary revascularization decreased the infarct size, without any deleterious effect related to cyclosporine. Nowadays, no clinical trial has assessed the effects of a cyclosporine pretreatment in brain-dead donors on the kidney graft function in recipients.

2.3. Hypothesis and Objectives

Based on the experimental studies which have shown that cyclosporine improves organ functions after ischemia-reperfusion, we hypothesize that a cyclosporine pretreatment in human brain-dead donors, by decreasing the PTP opening, would reduce the DGF/SGF after kidney transplantation, the most frequent postoperative complication. Taking into account the very low risk of toxicity of a single low dose of cyclosporine, we propose to conduct a multicenter, randomized, double-blind, placebo-controlled, superiority trial to assess the effects of a cyclosporine pretreatment of brain-dead donors in kidney graft function.

2.4. Summary of benefits and expected risks for subjects included in clinical research

Benefits:

At individual level

The reduction of DGF/SGF is universally accepted as a beneficial effect for kidney graft recipients. Such an effect is associated with a reduction in the requirement of dialysis sessions in the days and weeks following transplantation with all the possible constraint and complications due this technique. Moreover, the reduction or prevention of DGF is associated with an increased graft survival, a decreased postoperative morbidity and duration of hospitalization, which all may contribute to provide a relevant individual benefice for the patients.

At collective level

At a collective level, kidney graft represented approximately 3000 patients in 2010. A 30% reduction in DGF might prevent a postoperative dialysis for 170 patients in one year in France, resulting in the same time in a substantial financial benefit.

Risks:

Cyclosporine induces nephrotoxicity by 2 processus but precise mechanisms of initiation remain unclear [42-45]. The chronic nephrotoxicity appears with prolonged administration on several months and years. Symptoms are those of renal insufficiency with non-reversible histological damages characterized by tubule-interstitial injuries and glomerular sclerosis leading to the graft rejection. Cyclosporine-induced acute nephropathy appears at least after 1 month of treatment. The nephropathy is reversible without histological abnormalities caused by episodes of renal ischemia.

Cyclosporine induces a disequilibrium between the vasoconstriction and vasodilation of glomerular arteries. Despite the individual variability, cyclosporine nephrotoxicity is all the more important as plasma concentration levels are high (190-272 ng/L) [43]. In clinical practice, the dose administered in kidney transplant is usually of 8 mg/kg/j orally, then adapted to serum levels.

In our trial, we choose to administer a single low dose of cyclosporine to the donor aiming to strongly limit the risk of toxicity and the development of chronic nephropathy. Such a strategy limits also the risk of renal ischemia and acute nephropathy which appears very unlikely with cyclosporine concentration under the toxic threshold as already demonstrated in a clinical trial performed in patients with myocardial infarction [40]. Beside these potential risks and the theoretical risk of anaphylactic reaction, there is no special risk related to this study. The management and medical cares of donors and recipients are not different as routinely performed with respect of good clinical practices [46].

Overall, the balance benefit/risk appears favorable.

2.5. Expected impact

Individual medical benefit: cyclosporine might prevent the occurrence of DGF, a complication well known to increase recipient's morbidity (duration of hospitalization, graft survival, requirement of dialysis, infections, quality of life) and mortality

Financial benefit: cyclosporine might reduce the cost related to transplantation procedure and its long-term management.

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3. Objectives of the study

3.1. Primary outcome

The primary goal is to compare the occurrence of DGF between an interventional group of recipients receiving kidney graft from pretreated brain-dead organ donors with an intravenous infusion of cyclosporine versus a controlled-placebo group of recipients receiving kidney graft from pretreated brain-dead organ donors with a placebo.

3.2. Secondary outcomes

- Compare the occurrence of SGF in recipients assigned to an interventional group of between pretreated brain-dead organ donors with an intravenous infusion of cyclosporine versus a controlled-placebo group of recipients receiving kidney graft from pretreated brain-dead organ donors with a placebo.
- Compare the occurrence of SGF + DGF outcome in recipients assigned to an interventional group of between pretreated brain-dead organ donors with an intravenous infusion of cyclosporine versus a controlled-placebo group of recipients receiving kidney graft from pretreated brain-dead organ donors with a placebo.
- Compare the duration of hospitalization (post-transplantation hospitalization) between the 2 groups of recipients.
- Compare daily urine output at day 1, 3, 7 and 3 months between the 2 groups of recipients.
- Compare serum creatinine level and estimated glomerular filtration rate (eDGF calculated by the CKD-EPI creatinine clearance) at day 1, 3, 7 and 3 months between the 2 groups of recipients.
- Compare the occurrence of primary kidney graft dysfunction at D1 between the 2 groups of recipients.
- Compare in recipients with DGF, the duration between transplantation and the recovery of renal function between the 2 groups of recipients.
- Compare the occurrence of dysfunction of kidney graft at 1-year between the 2 groups of recipients.
- Compare the in-hospital and 1-year survival between the 2 groups of recipients.

4. Trial Method

4.1. Trial design

This clinical trial is a multicenter, two parallel-arm double-blind, randomized-controlled study. According to the participating centers in the study, data reported in 2012 show that 481 brain-dead multiorgan donors were harvested. The number of kidney grafts of these centers was 988. In France, the distribution of kidney for transplantation in the different centers is governed by strict rules of the ""Agence de Biomédecine. Except in case of contra-indication, both kidneys of a donor are harvested (dual kidney donor), one stays *a priori* locally and the other one is distributed in another national center. Only recipients of participating centers will be included in the trial. Therefore, there will be 1 or 2 kidneys from the same donor (single or dual donor)
Ambivalence clause: all recipients in France can receive a pretreated kidney graft with cyclosporine or not, regardless the participating or not center

4.2. Classification of the Research

This trial is a part of research for routine cares.

5 Trial Population

5.1. Inclusion criteria

5.1.1. Brain-dead donor

- Diagnosis of brain death.
- Age between 18 and 80 years.
- Affiliated to the French Social Health Security.

5.1.2. Kidney recipients

- Age between 18 and 80 years.
- Requiring a kidney transplantation.
- Written informed consent for analyzing the data.
- Affiliated to the French Social Health Security

5.2 Non-inclusion criteria

5.2.1. Brain-dead donor

- Contra-indications for multiorgan harvesting (infection, cancer, etc.)
- Pre-existent chronic renal insufficiency.
- Multiorgan harvesting refusal expressed by the donor (documented in the national register of refusal or expressed by the relatives).

5.2.2. Kidney recipient

- Big kidney transplant.
- Multiorgan transplantation.

5.3 Early termination of the procedure

Every recipient can withdraw its consent at any time, without any justification of his decision. Stopping his participation can also be decided for medical reasons by the investigator in charge of the recipient.

5.4 Exclusion period and participation to another clinical trial

No exclusion period due to a participation in other biomedical study is planned.

5.5 Volunteers compensation

No compensation is planned for this clinical trial.

5.6 Modality of recruitment

5.6.1. Brain-dead donor

All brain-dead donors hospitalized in ICU of participating centers will be screened. The inclusion and the randomization will be performed after the legal ascertainment and declaration of brain-death.

5.6.2. Recipients

All recipients receiving a kidney transplantation in a participating center (urology/nephrology units) issued from a donor included in the trial will be screened. Kidney graft transplants will not receive any treatment or care different as those routinely performed. The trial is therefore only observational.

6 Trial Methodology

6.1. Endpoints

6.1.1. Primary endpoint

The primary endpoint is the occurrence of DGF defined by the requirement to initiate at least 1 hemodialysis session within the first 7 days following transplantation [7, 8].

6.1.2. Secondary endpoints

- SGF defined by a 24-hr urine output < 1000 ml at D1 post-transplantation, or a serum creatinine reduction ratio < 30% or <70% at D3 and D7 post-transplantation, respectively [7, 8]
- The composite outcome DGF + SGF as defined previously [7, 8], considering that these 2 complications have exclusive definitions, therefore a recipient cannot have both DGF and SGF.
- The duration of hospitalization defined by the number of days between the recipient admission for transplantation and the hospital discharge.
- The daily urine output collected in recipients at D1, D3 and D7 post-transplantation.
- Serum creatinine level, eGFR calculated by an estimated creatinine clearance based on the CKD-EPI formula at D1, D3 and D7 and 3-month post-transplantation [41].

  CKD-EPI formula: 141 x min (Scr/K,1)a x max(Scr/K.1)-1.209 x 0.993Age x 1.018 (if female), Scr : serum creatinine (µmol/L)
  K: 62 for female and 80 for male
  a: -0.329 for female and -0.411 for male

- The primary graft dysfunction is the lack of early postoperative renal function recovery (in the first 24-hrs after transplantation) defined by a persistent anuria
- In case of DGF, the delay for renal function recovery is defined by the duration (in days) from the first to the last dialysis session after transplantation.
- The kidney graft function is assessed at 1-year by 2 criteria:
  - The occurrence of kidney graft dysfunction is defined by an estimated creatinine clearance < 30 ml/min at 1-year post-transplantation.

- The occurrence of a total graft dysfunction is defined by the requirement to resume totally the hemodialysis treatment at 1-year post-transplantation.
- The mortality rate during the hospitalization and in the first year following transplantation, including all causes of death. Death related to renal origin will be assessed.

6.2 Description of the methodology

This clinical trial is a multicenter, two parallel-arm double-blind, randomized-controlled study and is classified as a part of research for routine cares.

6.3 Methods aiming to reduce or avoid biases

The randomization will be integrated in the eCRF created specifically for the trial. An intensivist not in charge of the donor will performed the randomization. He will connect online with his identification code to give the informations required to proceed for the random allocation of the donor in a group using the module of randomization. The treatment to administer and the identification of the harvested kidneys will be communicated in feedback. The eCRF of the donor will be automatically created and ready for data collection.
The randomization list will be carried out by the principal investigator according to the block's method using the software Nquery© Advisor v 7.0 software.
The randomization will be stratified on the center of the brain-dead donor and on the distribution between 2 and single- kidney graft donors (1 or 2 kidneys from the same donor depending on whether the non-local recipient center is participating or not to the trial).

7. Practical aspects of the protocol

7.1. Description of the practical management (medical appointments / drugs used)

As soon as the clinical diagnosis of brain death is made, the hospital coordination is informed. The diagnosis must be confirmed by cerebral angiography or CT-angioscan or by electroencephalography. When these additional exams confirmed the diagnosis (end of the exam), the certificate of death with the hour is established. Brain death is announced to the relatives during a face-to-face interview. A second interview with the hospital coordinator allows to verify the refusal or the consent for a potential organ harvesting. During this period, the hospital coordination performs the administrative procedures, especially the question in the national register of organ donation refusal. In case of any opposition, the procedure stops. In case of a presumed consent and no information in the national register, the procedure is activated.

The hospital coordination team explains to the family the process for organ donation and procurement (organ harvesting in the operative room, followed by the referral of the dead to the repository as usual for all deads, respect of anonymity for the recipient). No information concerning the treatment relative to the trial will be given to the family of donor. Then, the hospital coordination team verifies that all the required biological samples are performed and declares the presence of a potential brain-dead donor (with its characteristics) to the "Agence de Biomédecine". The latter activates the national system responsible for the distribution of organs in France. The role of the hospital coordination unit is then to manage the organization of organs harvesting in the operative room with all the players (which can come from different regions of the country) involved in this process. In the same time, the anesthesiologist-intensivist manages the donor aiming to optimize the organ perfusions until the transfer of the donor in the operative room.

All parameters which may influence the kidney graft function in the last 24 hours before harvesting will be collected [1, 7]:

- Social and demographic data (age, sex, BMI, cause of death);
- Relevant history and comorbidities (arterial hypertension, diabetes, chronic alcoholism);
- Number and type or organ failure before harvesting based on the SOFA score;
- Biological data and disturbances: hypernatremia, coagulation abnormalities;
- Renal function assessed on serum azotemia and creatinine levels, glomerular filtration rate (DGF) based on the calculated creatinine clearance of the 24 hour measurements before harvesting, pre-existent chronic renal insufficiency;
- Immune and viral data: EBV serology, number of HLA incompatibility.

All parameters concerning the surgery and the organ preservation that may influence the kidney graft function outcome will be collected [1, 7]:

- Preservation data: type of solutions, use of perfusion machine, cold ischemic time;
- Intraoperative data: hemodynamic instability, warm ischemic time, intraoperative complications (anastomosis duration, surgical difficulties, hemorrhage) [1, 7].

The intensivist continues brain-dead donor care until the entry in the operative room. When arriving in the operative room, the brain-dead donor is managed by the anesthesiologist and harvesting surgeons to perform the organs removal in a precise order and procedure (rules of good practice). The distribution and assignment of organs are performed by the "Agence de Biomédecine". At the end of surgery, the brain-dead's body is restored with a total respect of the body integrity. From this time the circuit of the dead subject is similar as for all deads. A soon as removed, the organs are placed in optimum condition of preservation (cold ischemia) in a preservation solution until warming (warm ischemia) at the time of transplantation. From the harvesting to the transplantation, the rules of anonymity and equity will be respected. Only the hospital coordination will know the identity of both donor and recipient.

The randomized allocation for the treatment group of each donor will be performed leading to define 2 groups of treatment:

- one control group (placebo group) of donor treated with a single intravenous infusion of a 5% dextrose placebo (0.5 ml/kg) within 2 hours;
- one group (interventional group) of donor treated with a single cyclosporine intravenous infusion of 2.5 mg/kg within 2 hours.

The randomization will be computerized using a randomization module associated with the electronic Case Report From (eCRF) created for the study (see chapter 6.3). The investigator in charge of the donor, after randomization, may collect the required data of the donor on the eCRF.

In case of agreement, the recipient will be informed before kidney transplantation:

- that he will not know the origin of the kidney graft, that the donor has potentially diseases and that he was possibly included in a clinical trial ;
- of the rules of kidney graft attribution and therefore that he cannot disobey (no possibility to choose the moment and the kidney graft);
- of the risks and the constraints related with surgery and anesthesia during transplantation

The nephrologist which led the indication of kidney transplantation will be informed by the involved hospital coordination unit of organ procurement and transplantation of a possible inclusion of its recipient. He will be responsible to obtain the informed consent of the recipient to participate in this trial, and therefore to connect to the eCRF with the identification already attributed to the donor (précising R for the right kidney and L for the left kidney) and finally initiate the recipient's eCRF. Kidney graft recipients, as well as kidney transplant surgeon and anesthesiologist,

urologists/nephrologists usually in charge of the postoperative period of kidney transplantation will be blinded to randomization of the donor. Recipient's care will not differ between recipients and from that usually performed, as well as criteria of hemodialysis initiation.

Harvested kidney grafts will be transplanted using the usual criteria of immune compatibility, without any modification in the order or in the modality of usual distribution.

All factors which may influence the kidney graft function outcome in the recipient will be collected:

- Demographic data (age, sex, BMI, blood group)
- Significant comorbidities: arterial hypertension, cardiac insufficiency, diabetes, cause of the nephropathy, number of kidney transplants already received, duration of dialysis before transplantation, wait duration for transplantation,
- Recipient viral (C hepatitis) et immune status (donor antibodies, chronic treatment with cyclosporine),
- At day1, 3 and 7 :24 hours urine output (ml) + serum creatinine level and eGFR (CKD-EPI)
- At day 7: requirement of at least one hemodialysis session since transplantation
- At 3 months: serum creatinine level and eGFR (CKD-EPI)
- A 1 year: kidney graft and recipient survival

The follow-up of the kidney transplant goes on depending on the medical evolution of the recipient and the usual recommendations of its nephrologist, without any modification related to the protocol (clinical and biological parameters and frequency of the follow-up. After 1-year following transplantation or in case of death within this period, the recipient completes the trial.

7.2. General logistical organization of the trial

7.2.1. General processes of organ procurement in heart beating bread-dead donors

The organ procurement and transplantation activity are governed by statutory provisions which have been updated in 2004 by the "Agence de Biomédecine" (Law n° 2004-800 of 20240806 related to Bioethic). All legal processes, medical and administrative that are related to these activities have been recently published in the commune agreement of the "Agence de Biomédecine", "Société Française d'Anesthésie-Réanimation" and the "Société de Réanimation de Langue Française" [46]. This full-fledged medical activity is considered as a priority emergent activity and mission of healthcare structures.

Diagnosis of brain-dead

Brain death is defined by a non-reversible destruction of all brain structures caused by a complete cessation of cerebral vascularization. The diagnosis is based on clinical and paraclinical criteria which are precisely defined by the French legislation since 1996 (circulaire DGS n° 96-733 of 19961204). The clinical diagnosis includes the presence of 3 persistent signs:

- A deep hypotonic and are active coma;
- The abolishment of all brainstem reflexes (abolishment of the oculocardiac reflex);
- The absence of spontaneous ventilation attested by a disconnection test.

This clinical diagnosis requires obligatory to be confirmed by additional exams showing with certainty the absence of any intracerebral electrical activity and/or a cerebral circulatory arrest. According to the "circulaire DGS n° 96-733 of 19961204", the confirmation of brain death can be given with 1 of these 3 additional exams:

- Electroencephalogram (EEG) must be isoelectric with a maximal amplitude. The legislation asks 2EEG performed 4 hours apart;

- Intra-arterial cerebral angiography must show a total lack of intracerebral vessels opacification with the injection of the 4 cerebral arteries (carotid et vertebral arteries)
- Cerebral angioscan which must also show a total lack of intracerebral vessels opacification after an intravenous injection. The extracranial arteries must be opacified, demonstrating that the injection of the medium-contrast product has been correctly injected.

In case of hypothermia, intoxication/treatment with barbiturates or alcohol, EEG can show false positive signs. Moreover, EEG must be repeated 4 hours after the first one. Accordingly, cerebral angiography or angioscan are preferred (no false positive).

7.2.2 Structures involved in organ harvesting and transplantation

The "Etablissement Français des Greffes (EFG)" has been created in 1994. He became the new renamed "Agence de Biomédecine" in 2005. Its role is to regulate the activity of organ and tissues harvesting, and to allocate grafts around the clock. To reach this function, there is a regulating and a national support system (SRAN), and several regional systems (SRA).
The hospital coordination represents the healthcare structure and included 1 hospital coordinating doctor which is responsible for the harvesting activity. The doctor is helped by the hospital coordinating nurse.
Each SRA works in cooperation with the hospital coordinator and the SRAN. Therefore, the rule of the kidney allocation prioritizes one kidney regionally (region of the SRAN) and the second one is distributed in another region. The SRAN can interfere in the allocation of grafts between regions in case of a national priority or if the graft is not allocated in the region. No organ can be distributed outside France and the "Agence de Biomédecine" keeps organs only in the French territory. In all cases; the decision for transplantation belongs to the physician in charge of the recipient.
The coordination has several tasks:

- It takes care of the administrative formalities, makes the connection with the SRA and all the physicians involved in the procedure;
- It also has the responsibility to collect the testimony of the family.

The French legislation is based on an official declared opposition or a presumed consent (article L 1221-1 of the law n°2044-800). The first step consists to verify the absence of a formalized refusal notified in the national register created in 1998. In case of no information in the register, an interview with several members of the family and relatives must be realized in an appropriate room in the presence of both the hospital coordinator and the ICU physicians in charge of the donor. The interview with the family aims to research the absence of opposition to organ donation by all the methods (written or oral). According to the French legislation, any form of opposition by the patient must be respected. In case of no opposition, the harvesting is based on the presumed consent of the patient (obtained during the interview with the family). Then, the coordination organizes the harvesting (from the ICU to the operative room). It is also responsible for the restitution of the body of the death (state of integrity) and to support the family after the harvesting, if necessary.

7.2.3 Fate of kidneys attributed to non-participating centers and others organs

This point has been requested from the "Agence de Biomédecine" and it is agreed that the evolution of these grafts will be evaluated *a posteriori* at 6 months post-transplantation (agreement of Pr O. Bastien, medical director of the "Agence de Biomédecine"). To activate this procedure, an authorization will be requested to the medical commission of the agency in order to have the possibility to extract data from the Crystal database which collects all data that concern French transplantations. This procedure is classical as it is routinely realized by the "Agence de Biomédecine".

7.3 Difference with the usual management

7.3.1. For the donor

The protocol does not change care and monitoring of donors which remain identical in the 2 groups. Therefore, all brain-dead donors are orally intubated, and with mechanical ventilation. They are equipped with an indwelling arterial catheter, a central venous catheter and a urinary catheter in order to monitor arterial pressure, central venous pressure, and hourly urinary output, respectively. Daily intravenous water supply consists of 1 ml/kg/h of crystalloids (NaCl 0.9% or Isofundine®). Vascular load is realized with intravenous crystalloids or macromolecules (gelatins), and is adjusted according to the usual service habits (appreciation by the physician in charge of the donor). Taking into account their potential nephrotoxicity, hydroxyethylstarches (Voluven®) are not allowed. SaO2 and $EtCO_2$ (capnograph) are also monitored continuously, as well as the body temperature.

7.3.2. For the recipient

The participation of recipients in the trial will not change at all their care.
Usually, kidney transplant is previously informed that he will not know the origin of the graft and that organ donor may have participate in a clinical research trial. This obeys to the rules of allocation of organs (no possibility to choose its graft organ in the usual transplantation procedure).
Moreover, kidney graft transplants will not receive any different treatment or care as usually prescribed. Therefore, this trial is considered as an observational routine care study.

7.3.3. For the management and delivery of the harvested organs

The participating donors to the trial will not change the process of distribution of organs concerning the patients waiting for kidney transplantation.
Indeed, the hospital coordination declares the presence of a potential organ donor to the "Agence de Biomédecine" who activates the system of national distribution of organs. Therefore, treated organs (in particular here kidneys) will not be exclusively dedicated to the study and could be attributed to other non-participating centers. This trial poses no problem at all concerning a decrease or a lack of grafts availability for non-participating centers.

7.4. Expected duration of recipient's participation and chronology of the trial

Estimated duration of the trial: 6 years
Date of the beginning of the trial (first patient included): December, 2017
Date of the end of the trial (end of the follow-up of the last recipient): December, 2023
Total duration of participation of the recipient: 1 year

The end of the trial will be sent to the ethics committee (CPP) within the 90 following days.
In case of an early termination of the trial, the information will be sent within15 days to the CPP.

8 Drugs, medical devices, others of the trial

8.1. Description of the treatment

Both specific drugs used for the study are 5% dextrose and cyclosporine. Both are packaged in ampoule and sterile packs dedicated to be infused intravenously.

8. 2. Dose, administration modalities and treatment duration

The experimental beneficial effects of cyclosporine have been shown using low dose between 2,5 and 5 mg/kg [34, 40]. On the other hand, cyclosporine induces a rapid effect (from 15 minutes after the injection), which is prolonged for several hours after the administration because of its bound on cyclophilin D [24, 32, 40]. Based on these data and on doses administered in experimental and clinical trials, a single intravenous bolus of 2.5 mg/kg is chosen for this trial aiming to obtain beneficial effect while limiting cyclosporine nephrotoxicity (cf infra). Cyclosporine A (Sandimmum®, ampoule of 250 mg in 5 ml, Novartis Pharma, France) will be diluted 5% dextrose in order to obtain 5 mg/ml. Thus, the injected dose will be 0.5 ml/kg infused with a continuous electric syringe over at least 2 hours in order to avoid anaphylactic reactions. The placebo group will receive 0.5 ml/kg 5% dextrose with an electric syringe over at least 2 hours. The allocation between the 2 groups is attributed by randomization obtained from an intensivist not working in the unit. He will prepare the pretreatment (cyclosporine or placebo). The nurse and the doctor in charge of the donor will be blind for the treatment complete infusion before the arrival of the donor in the operative room. The medical and nurse management of the donor in the ICU and in the operative room is therefore in a blind manner concerning the administration of the trial drug (randomization and preparation for the drug administration by a physician not in charge of the donor).

8. 3. Presentation of drugs

Cyclosporine (Sandimmun®, Novartis France) in conditioned in ampoules of 5 ml injectable solution at 50 mg/ml for intravenous infusion.
Placebo 5% dextrose solution.

8.4. Delivery and compliance

The trial drug, cyclosporine or placebo is attributed by randomization on the eCRF website.

The drug dispensing would be realized according 2 procedures:

- An individual named dispensing using a name prescription signed by the investigator then sent to the center pharmacy.
- A global or semi-global dispensing using an endowment prescription in the ICU followed by a traceability form completed by the investigator then sent to the pharmacy with every use of the drug kit located in the ICU.

Each participating center will choose its own procedure and the modality of stocking: named, global or semi global according to how the pharmacy works.

9 Collected data

The electronic Case Report Form (eCRF)

In order to collect all study data (donor and recipient), a specific eCRF will be created by the principal investigator.
The control of the data quality will be organized on the eCRF by the promoter during the planned monitoring visits by the clinician research assistant of the unit of the principal investigator.

Data base

All data related to the trial will be recorded in the eCRF. The configuration and the implementation of the eCRF for the collected data which include the user training will be under the responsibility of the Clinical Research and Innovation Direction (CRID).

Each participating investigator will be responsible for collecting and recording data directly in the eCRF during the trial. Data will be secured using a specific right access according to the role of each investigator involved in the trial

Control of the data quality

After the final data entry, validity and consistency of them will be performed by the CRID data manager and checked regularly. All along the study, all modifications in the database will be tracked (audit procedure).

At the end of this quality procedure, the database will be freezed and signed by the principal investigator, the data manager and the physician of the CRID. From this moment, no modification of the database will be possible.

The freezed database as well as the data management report will be transferred to the statistician to perform the statistical analysis.

10 Statistical considerations

10.1. Number of subject necessary to include

Based on the available data in the literature, we can hypothesize an occurrence of DGF of 30% in the placebo group [7, 8] and to target a reduction of 30% [47], resulting in an occurrence of 20% of DGF, in the cyclosporine group. Therefore, with an 80% power and an alpha risk at 5%, 294 patients per group would be required (the statistical unit is the recipient with his kidney graft) (nQuery Advisor 7.0). We consider that we will have very few missing data for the analysis of our primary outcomes at 7 days. However, the analysis of several secondary outcomes requires a 1-year follow-up with a negligible missing or not usable data.

We assume a 10% loss to follow-up in this trial, requiring a total inclusion of 648 kidney recipients. In 2012, 746 kidney graft were harvested from 393 brain-dead donors in this participating study centers. We consider that a total of 420 kidney graft would be potentially available for kidney transplantation per year in the participating study centers (conservative hypothesis).

Therefore, if we assume that 30% of kidney grafts well never be included in the trial (organ donation refusal, organ procured but not transplanted, simultaneous clinical trial), we expect to include potentially 294 kidney grafts per year leading to predict a 3-year recruitment.

10.2. Data analysis: general informations

All statistical analysis will be performed using the SPSS software version 17.0.

The study recipient population will be analyzed in the intention-to-treat (ITT) manner, each recipient in its randomized group through its kidney graft donor assignment.

Lost of participant and missing data are very unlikely within the 7 days following kidney transplantation (for example death). Missing data at 7 days will be replaced by a representative value of failure (Worst case scenario).

In this trial, data are not independent because 1 or 2 kidney grafts can be harvested and transplanted from a sole donor (1/1 or 1/2 donor/recipient ratio). To take into account the relationship, a random effect in the donor will be added in the statistical model. In addition to this random effect, adjustments will be performed in the first analysis on the donor center (fixed effect), and considered as a stratification parameter of randomization. In second steps, a sensitivity analysis using additional

adjustments with pre-specified sub-groups will be performed to assess heterogeneity of the treatment effect on DGF. The pre-specified sub-groups were:

- Most well-known risk factors of DGF and kidney graft death (for the donor: age, sex, BMI, treatment of the donor with norepinephrine or systolic arterial pressure <90 mmHg in the day before kidney harvesting, cold ischemic time, history of diabetes and arterial hypertension; for the recipient: age, sex, BMI).
- The number of kidney grafts harvested from expanded criteria donor (ECD) will be also listed in each group. ECD criteria are defined by the United Network for Organ Sharing (UNOS) as follow (48, 49): donors older than 60 years or donors older than 50 years with at least 2 of these following conditions: arterial hypertension, serum creatinine level >136 µmol/l, brain death due to stroke or cerebral hemorrhage.
- In case of a disequilibrium between the 2 groups despite the randomization, analyses would be adjusted on the involved parameters in case of clinical justification.

Before performing each statistical analysis, the appropriateness of the statistical tests will be verified. A $p<0.05$ will be considered to be significant.

10.3. Principal analysis

The statistical analysis would include firstly a descriptive analysis of the study population and of the studied parameters with an assessment of absolute and relative frequencies (and their 95% confident intervals) for categorical variables, and means, medians, standard deviations, and interquartiles for quantitative variables.

As advised by the "Consort" recommendations, major demographic and biological characteristics of donors and recipients will be compared between the 2 groups at inclusion but no statistical test will be performed. The comparability of the 2 arms will be evaluated clinically but not statistically.

A flow-chart with the number of eligible brain-dead donors, of included donors and harvested kidneys, of randomized donors and harvested kidneys, the number of eligible and included kidney recipients will be collected. Characteristics and reasons of lost follow-up recipients will be described.

10.4. Secondary analysis

Secondary analysis will be performed:

- A per-protocol analysis: patient will be analyzed in the group receiving really the treatment and considered if the major outcome was available (DGF at day 7 after transplantation).
- A sensibility analysis for missing data will be performed with the maximum bias strategy using data reflecting failure and successively.
- A per-protocol analysis of the subgroup of ECD (48,49).
- A post hoc analysis after 6 months (from the extraction of the Crystal database of the "Agence de Biomédecine") of the outcome of all organs procured by donors included in the trial.

10.5. Person in charge of the analysis

Pr Pascale Auquier
Dr Karine Baumstarck: methodologist; Mohamed Boucekine: biostatistician
EA3279 Self-Perceived Health Assessment Research Unit and Department of Public Health, Nord University Hospital, APHM, Marseille, France karine.baumstarck@univmed.fr

11. Access rights to source data and documents

11.1. Access to source data

The promoter is in charge of having the consent of all the persons implicated in this research in order to ensure the direct access in the sites of research, of source and document data, and of reports performed to control the quality of the procedure and data of the trial.

The investigators make available documents and individual data strictly required follow-up of the study, of the control of quality and audit of the biomedical research. These documents will be available for all investigators in compliance with the legislative and regulatory provisions participating in the study (articles L.1121-3 and R.5121-13 of public health code).

11.2. Source data

Source documents are defined as any document or original object which allows to prove the existence or the accuracy of data. All data and registered events observed during the study will be kept within 15 years by the investigator and the hospital if data are in the hospital file.

11.3. Privacy of data

In compliance with the provision related to data privacy which are available for persons in charge of the quality control in the biomedical research (article L.1121- 3 public health code), in compliance with the provisions related to the privacy informations of experimental drugs, trials, persons participating and results (article R. 5121-13 public health code), persons with a direct access will take all the required precautions to ensure privacy of all these parameters especially concerning the identity of participating patients and their results. A particular attention will be paid to the respect of the anonymity of the organ donor for the recipient.

These persons, as well as the investigators must obey to the professional confidentiality (in agreement with the conditions defined by the articles 226-13 and 226-14 of the penal code).

During or at the end of the biomedical research, collected data sent to the promoter by the investigators (or anyone involved in the study) will be anonymized.

The name and the address of involved people must not clearly appear.

The only first letter of the family name and of the first name will be registered with a number related specifically to the trial indicating the order of patient inclusions.

The promotor makes sure that each person involved in this trial has given its informed written consent for the access of its individual data and strictly required for the control of quality.

11.4. Inscription in the national register of persons suitable for biomedical research

Not applicable.

12. Quality control and insurance

12.1. Commitment of investigators and promotor

The investigator undertakes to work in agreement with the Helsinki Declaration of the world medical association (Tokyo 2004, revised).

12.2. Insurance and quality

A CRA authorized by the promotor will ensure of the successful completion of the trial, of the collected data issued from written data, of the accuracy, the registration and the report of the data, in agreement with the standard operating procedures implemented in the CHU of Clermont-Ferrand, in accordance with the good practices and the legislative and regulatory provisions.

12.3. Control of quality

The investigator guaranties the authenticity of the collected data related to the trial and accepts the legal provisions allowing the promoter to implement the control procedure of quality.
The coordinating and associated investigators accept to be available for the visits of quality control performed regularly by the CRA. During these visits, the following elements will be checked:

- Informed consent
- Respect of the study protocol and the defined procedures
- Quality of the collected data in the eCRF: accuracy, missing data, consistency of data with the source data (medical files, written meeting date, original biological data, etc.)
- Management of possible products.

12.4. Electronic clinical report form

In order to facilitate and improve data collection, and their analysis during the study, the CRF will be in an electronic format (eCRF).
All informations required by the protocol must be registered in the eCRF by the physician investigator of each participating center, and an explanation must be provided for each missing data. Data must be collected as they are obtained.
False data observed in the eCRF will be clearly corrected and the new data will be copied next to the corrected information with the electronic signature of the investigator, the date, and if possible, the reason given by the investigator or the authorized person who made the correction.
Subject's anonymity will be ensured by a code number and the named initials of the person included in the study in all the required documents (including the electronic documents), or by deleting the individual data in the copy of the source document (designed to the research documentation).
The computerized data in a file will be declared to the "Commission Nationale de l'Informatique et des Libertés" (CNIL) according to the appropriate procedure.

13. Ethical Considerations

The promoter is defined by the law 2004-806 of 20040809.
Before initiating the research, each will give to a representative of the promoter a copy of its Curriculum Vitae with a date and a signature, including its registration number of the Medical Order Council.

13.1. Ethics Committee

The protocol with the informative and consent forms as well as the eCRF of the study will be submitted for the approval of the Ethics committee ("Comité de Protection des Personnes Sud Est VI"). The obtention of a favorable agreement will be sent to the study promoter.

13.2. Information of the patient and written informed consent form

Brain-dead organ donors are dead. Accordingly, there is no reason to give any information or to ask an informed consent for the inclusion in the trial. On the other hand, a testimony of its refusal for organ donation must be traced with the family during the dedicated interview allowing or not a presumed consent. The inclusion in the protocol will be reported in the medical file of the brain-dead donor.

The recipient is placed on the waiting list of transplantation after being informed and having signed the agreement with the rules of kidney graft allocation (no identity of the donor, no choice for the date of transplantation). This signed information clearly stipulates that the recipient can receive a graft that has been included in a clinical trial without any change in the rules of the graft allocation. Nevertheless, the recipients will be informed of the protocol during or after its kidney transplantation.

He will have to give its consent and to sign the authorization for the analysis and the treatment of the collected data related to the transplantation for the study.

All these informations are reported in an informed consent form given to the recipient. This informed written consent will be collected by the investigator. These documents are approved by the dedicated ethics committee and will be used for this trial, except any other document.

Two copies in original will be co-signed by the medical investigator and the recipient, one given to the patient and the second kept in the medical file of the patient.

13.3. Protocol amendments

The modifications of the protocol should be qualified as being substantial or not. According to their nature, they will be subject to receive a new agreement by the ethics committee.

On the other hand, any extension in the research (major modifications of the therapeutic design or of the included populations, renewal of treatments or therapeutic procedures that were unexpected initially in the protocol) should be considered as a new one.

13.4. Management related to the trial

Concerning the brain-dead donor, the only difference with the usual management is the administration of the study treatment.

Concerning the kidney transplant recipient, the only difference with the usual management is the possibility to receive a pretreated kidney graft with the study drug. All the remaining management is similar to the routinely performed one.

14. Data management and conservation of the documents related to the study

14.1. Management related to the trial

Donor: data related to the trial (previously described) will be completed in the eCRF by the intensivist who included the subject.

Recipient: usual data of follow-up for the study are collected in the eCRF.

Data analysis: from these data of the eCRF, an Excel table will be created allowing to perform the statistical analysis by the biostatistician of the trial.

14.2. Declaration to the CNIL

The consulting committee on the treatment of the information in the field of health research (CCTIRS) will be requested to give a favorable opinion concerning the analysis of data required to perform the trial.

After having obtained this agreement, an authorization to the CNIL will be asked concerning the treatment of individual data with the aim of research in routine care.

14.3. Storage

According to the good clinical practices, all the documents related to the protocol must be stored at the end of the trial, and for a duration of 15 years by the principal investigator. All these documents must be located in a locked room which gives enough guarantees for a fire protection, water damage, light or any malicious intent.

15. Communication – Rules for the Authorship

The study will be listed with a number in the database of the Clinical Trial website.
The principal investigator will constitute a steering committee including 4 investigators of the study (Pr Constantin JM, Pr Fontaine E, Pr Ichai C, Pr Riou B) and the statistician methodologist of the study (Dr Baumstarck K).

Rules for the authorship:

1 – Order of authors

- First position, the principal investigator, Pr C. Ichai
- Second position, a study investigator, Pr E. Fontaine
- Third position, the statistician methodologist, Dr K. Baumstarck
- Penultimate position, the clinical research associate and data manager, L Borao
- Last position, a study investigator, JM Constantin
- From the fifth position, a study investigator of each participating center. The order will be determined according to the number of included patients.

2 – Rules for authors

- Only one investigator per center will be an author, usually the intensivist, but possibly the nephrologist/urologist in charge of the kidney transplant recipient or the responsible for the organ donation management. The choice will be defined by the investigators of each center.
- Only centers having recruited more than 30 patients will be listed as authors.
- The other investigators will be considered as co-investigators listed in the appendix list at the end of the manuscript. For each center, there will be no more than 5 co-investigators per center including at least 1 intensivist, 1 nephrologist/urologist according to the center and 1 coordinator or organ procurement and transplantation. Each center will decide the name of the co-investigator and sent it to the principal investigator, le Pr Ichai.

The final manuscript will be written by the steering committee and will be submitted for approval at each author.

Any proposition of post-hoc or additional data analysis will be reviewed by the steering committee.
Some post-hoc studies are already proposed:

- Comparison of the effect of cyclosporine vs placebo on pulmonary graft function issued from the princeps trial

- Comparison of the effect of cyclosporine vs placebo on liver graft function issued from the princeps trial
- Assessment of kidney graft function recovery of all kidney graft transplant recipients (those of the participating centers and those of non-participating centers) using the Crystal database of the Agence de Biomédecine".

16. Feasibility of the study

16.1. Competency of the involved teams

The teams which are involved in the trial are used to care routinely brain-dead organ donors and kidney graft recipients. Indeed, all procedures and management of related to organ donation and graft transplantation are subject to strict criteria driven by administrative authorizations.

16.2. Expected recruitment

The potential number of possible inclusions per year has been determined based on the number of organ donors and kidney graft recipients per year collected by the Agence de Médecine. Accordingly, the predictive calculation of our inclusions seems reasonable to finish the trial in the expected delay.

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