PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Hypothermic oxygenated machine perfusion (HOPE) for orthotopic liver transplantation of human liver allografts from extended criteria donors (ECD) in donation after brain death (DBD); a prospective multicenter randomized controlled trial (HOPE ECD-DBD)
AUTHORS	Czigany, Zoltan; Schöning, Wenzel; Ulmer, Tom Florian; Bednarsch, Jan; Amygdalos, Iakovos; Cramer, Thorsten; Rogiers, Xavier; Popescu, I; Botea, Florin; Froněk, Jiří; Kroy, Daniela; Koch, Alexander; Tacke, Frank; Trautwein, Christian; Tolba, Rene; Hein, Marc; Dejong, Cornelis; Neumann, Ulf Peter; Lurje, Georg

VERSION 1 – REVIEW

REVIEWER	Robert J. Porte University Medical Center Groningen The Netherlands
REVIEW RETURNED	13-May-2017

GENERAL COMMENTS	This protocol describes an investigator initiated, open-label, phase-II, prospective randomized controlled trial with the intention to investigate the effects of hypothermic oxygenated machine perfusion (HOPE) on extended-criteria donor (ECD) allografts in donation after brain death (DBD) orthotopic liver transplantation (HOPE ECD-DBD). Given the increasing use of ECD livers, several strategies are currently being pursued in an attempt to expand the donor pool. According to the investigators of this trial, these ECD-allografts however, exhibit poor tolerance to ischemia-reperfusion (I/R) injury, an important cause of liver damage and thus resulting in the need for improved preservation techniques of these organs. Following promising findings from pre-clinical animal and clinical studies with HOPE, the proposed trial aims to investigate the effects of HOPE on DBD-ECD liver transplantation. The primary end-point of this investigation is stated to be peak alanine aminotransferase-ALT within the first 7 days post-OLT. Additionally, a number of secondary end-points have been listed; including incidence of post-operative complications as assessed by the Clavien-Dindo complication score and the comprehensive complication index (CCI), laboratory parameters such as serum AST, bilirubin, INR, platelet count, albumin, creatinine, urea, glomerular filtration rate using the CKD-EPI equation, to mention but a few. The expected duration of the trial is 3 years and a total of 46 patients are to be included. This study promises to be of potential clinical relevance given the increased utilization of ECD (DBD) allografts for OLT in Germany and the world-over. Moreover, the investigation of the effects of improved preservation techniques of ECD grafts, in this particular case, HOPE,

	may provide valuable insight on the manner in which ECD (DBD) allografts should be optimally preserved in the future. This proposed protocol is well written and incorporates a number of essential aspects, however, there are a number of flaws and pending uncertainties that require revision and clarification. Below are the respective questions to and comments pertaining to them. Major comments; • Albeit describing the promising, beneficial potential of HOPE in ECD allograft preservation and stating that the main purpose of the study is “to test the effects of HOPE compared to conventional cold storage”, the investigators/authors fail to include and precisely state what the hypothesis of this study is albeit agreeing to having done so under item 2b of the CONSORT 2010 checklist of information to include when reporting a randomised trial* (pg 23 of 47 of the protocol). • Additionally, the objective of this study stated as being to “study the effects of HOPE…”is very broad and unspecific. What particular effects exactly do they intend to investigate? What clinical outcome(s)/complication(s) are they presuming the application HOPE may provide benefit to or limit the incidence of? Interestingly, the extended protocol contains a clearer aim of this study (page 37 of 47) however, the authors fail to incorporate it into the condensed manuscript version. • The authors are aiming to apply single portal vein perfusion while they will be using a device that allows dual perfusion of both the portal vein and hepatic artery. Perfusion of the artery may be particularly relevant for preservation of the larger bile ducts and dual perfusion has been shown to be safe and effective. It remains unclear why the investigators will be using single portal perfusion instead of dual perfusion as has been applied by Guarrera et al (AJT 2010) and van Rijn et al (Br J Surg 2017). • Stratified randomization model will be used to ensure balance of prognostic variables between the treatment groups. The only variables included in this stratification process are donor age and expected CIT. However, the most prevalent risk factor classifying livers as high risk or ECD grafts is liver steatosis. In fact, steatosis is also the most important determinant of peak ALT after OLT in the recipient (primary endpoint of this study). Therefore, it is unclear why (and an omission that) graft steatosis is not included in the stratification process. • Definition of ECD includes “donor 50 years and older with cause of death other than trauma”. This would mean that a 52 yo donor who died after a cerebral bleed within 48 hr after admission and with no previous history and normal liver lab and no steatosis is qualified as an ECD liver. This however is clearly not an ECD liver. • Machine perfusion will be applied using the Liver Assist at 4-6 degrees C. This, however, is impossible as the Liver Assist is not able to cool the perfusion fluid lower than 10 degrees C. • Liver are preserved in HTK solution during transportation. Will livers be flushed to remove HTK solution prior to connection to the Liver Assist primed with UW solution? If not, HTK will be mixed with UW during machine perfusion.

	• Peak ALT within the first 7 days post-OLT is stated to be the primary end point of this study. There are a number of limitations to this. Firstly, the authors/investigators provide no clear justification (no referred literature or proven clinical consensus) as to why they solely find ALT the most suitable end-point to provide answers to their primary research question which in itself is broad and unspecific. Secondly, the authors fail to elaborate on what they will consider as clinically elevated ALT levels. Given that all patients undergoing OLT have elevated ALT levels, it seems necessary that the authors establish a valid threshold or cut-off point in order to make reliable conclusions. Finally, and maybe most importantly, due to the well-described wash out effect of machine perfusion there will always be a lower serum ALT in recipients of a machine preserved liver compared to controls. Picking serum ALT as a primary end-point should therefore be considered a self- fulfilling prophecy. • The authors only include a reference to the Clavien-Dindo complication score and the comprehensive complication index (CCI) as one of the secondary outcomes. Could the authors explain why the incidence post-OLT complications are chosen to only just be secondary end-points? Shouldn't this be the primary endpoint as for example is the case in the HOPE study initiated by the Zurich group. Furthermore, given that the authors describe detrimental effect of I/R injury and its effect is rather emphasized in the introduction section, it would be interesting to know why or if the authors had not considered lactate clearance as an important secondary outcome. • Under randomization, the investigators state that "Randomization is performed by the principal investigator with an online randomizing tool for clinical trials (www.randomizer.at) at the time of admission for OLT". Can the investigators elaborate on why they choose for this particular time point? How can they be sure that the potential donor liver will be automatically suitable for transplantation (before macroscopic analysis) after the donor hepatectomy and thus already allocate the recipient to a particular study arm? One important lesson from the COPE Trial organized by the Oxford group has been that randomizing a donor liver before it is actually accepted by the transplant center will introduce a major source of bias as livers that have been allocated to the control group are more difficult to allocate. Therefore, it is strongly advised to randomize livers after they have been formally accepted for transplansplantation (i.e after visual inspection and approval). • Besides a stratification procedure, the investigators do not further elude to the randomization procedure. It is stated that the "principal investigator will perform the randomization", have the investigators taken into consideration illness or other circumstances in which he will not be able to do so? Will there be other authorized (trained) personnel who would be available to do so in such situations or will the study be entirely reliant on the principle investigators presence? • In regards to the blinding, the protocol states that it is open-label for the physicians but what about the patients? Nothing has been stated on what information will be given to the patients i.e. will the patients be made aware of what study arm they belong to? Moreover, will there be an independent assessment committee for assessing the main endpoints (without being informed about the group assignment)?

	• Under inclusion and exclusion criteria (page 7 line 40 – page 8 line 13), the authors fail to list 18 years and above of age as a clear inclusion criteria. Furthermore, the authors do not address patients receiving a combined lung and/or kidney- liver transplantations. Can it be automatically assumed that these patients will be included in the study? Should this be the case, can the investigators elaborate on why they would be included and recipients of, for example, split or living donation would not? • Sample collection: The authors intend to collect liver tissue samples taken upon arrival of the organ (before HOPE or corresponding cold-storage) and at the end of implantation before closure of the abdomen in addition to blood samples are collected as part of the daily routine during the peri- and postoperative course of OLT. Could the investigators elaborate on why no (perfusate) samples will be taken during the machine perfusion period and why they do not find it necessary to do so? Furthermore, parameters during HOPE such as flows, temperature, oxygenation saturation, pO2, pCO2 are not stated to be recorded. Could the authors clarify why they choose not to record and/or evaluate these data? Analysis of these samples could potentially reveal very useful information. • The study duration for both the individual subject as well as the study in general, is not clearly specified. The extended version of the protocol however, does contain this information; which states “The expected duration of the recruitment phase will be 18 months. The study will last 1 year per patient and in total 36 months including evaluation of all data and clinical study report.” This total study duration for the individual patient described is impossible given that post-OLT follow up alone is 12 months (1 year) therefore the time period from approved consent from the patient up until the first month of follow up has not been accounted for. • Table 1. There are several errors in this Table. First, the primary endpoint of trial NCT01317342 is not serum ALT, but major postoperative complications (Clavien Grade ≥III), using the established Clavien classification supported by a recently developed comprehensive complication index (CCI) by Slankamenac et al. The information provided on clinicaltrials.gov is not correct and the authors are advised to contact Dutkowski for the actual version of his protocol which has an adjusted primary endpoint. Moreover, this trial will include 170 patients, not 70. Secondly, the Table is not complete. Trials missing in this Table are NCT03031067, NCT00879268, NCT03098043. • Discussion, Page 13. It is stated that trial NCT02584283 is a multi-center RCT investigating the effects of portal vein perfusion only versus portal- and arterial perfusion during HOPE. This is not correct. This trial is comparing dual HOPE with traditional static cold storage of DCD livers. Minor comments: • Primary endpoint (page 11): Early graft function as assessed by peak alanine aminotransferase-ALT. Please not that serum ALT is not reflecting liver FUNCTION, but rather liver INJURY. • Page 6 (line 24 -25): The investigators state “several strategies have been developed aiming at reconditioning poor quality ECD-

	allografts" but only refer to a single study despite there being over 20 published articles on this topic. More referenced articles would be recommended. • Page 13 (line 21-24): "I/R-injury, depleted energy reserves, and oxidative stress are playing an important role…" is better written as "… oxidative stress play…" • Page 14 (line 12-17): "ECD-allografts exhibit poor tolerance to I/R-injury, a syndrome initiated upon restoration of blood supply after cold and warm ischemia yielding in endothelial and Kupffer cell swelling, vasoconstriction, white blood cell infiltration, and sinusoidal platelet aggregation." No references to (un)published data or literature are given. Investigators are advised to do so. • Page 14 (line 55): "…also including non-ECD organ…" should be "…organs…"

REVIEWER	P. Dutkowski Department of Surgery & Transplantation University Hospital Zurich
REVIEW RETURNED	29-May-2017

GENERAL COMMENTS	The manuscript is on a protocol of a planned randomized trial applying hypothermic oxygenated perfusion (HOPE) in extended criteria DBD liver transplants. The authors intend to compare conventional cold storage against cold storage plus 1 hour HOPE. The primary endpoint is peak ALT. The study is important and timely as ex vivo graft treatment is currently an interesting chance to optimize marginal livers before implantation. The authors may however address several questions: 1. It is repeatedly stated in the manuscript that the study will be the first randomized trial on HOPE in extended criteria DBD livers, which is incorrect. The currently ongoing original multicenter HOPE trial includes many extended criteria livers, and this trial is registered since more than two years (NCT01317342). In facts, most of the included grafts qualify nowadays as extended criteria grafts. 2. The primary endpoint peak ALT is a marker of hepatocyte injury rather than a sign for graft function. This is also the weakness of the study protocol, as ALT appears clinically less relevant. Much more important are biliary complications or graft loss within the first year after transplantation. 3. The primary endpoint of the original HOPE trial is therefore complications, graded by Clavien score >=III within one year, please correct Table 1 in this regard. 4. Please specify better hepatic steatosis for the definition of extended criteria grafts. Will all grafts get a biopsy at procurement to assess the amount of fat? 5. The authors plan to perfuse human livers at 4-5 °C with the Liver Assist device. However, the temperature can currently only be adjusted to 8-10 °C despite adding ice, please correct the protocol in this regard.

	6. The authors indend to pump for 1 hours in the HOPE arm. What happens if recipient hepatectomy is not finisihed after 1h HOPE? Are those livers again exposed to cold storage? I would suggest instead to continue pumping until implantation of the graft.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Robert J. Porte
Institution and Country: University Medical Center Groningen, The Netherlands

Q1: Albeit describing the promising, beneficial potential of HOPE in ECD allograft preservation and stating that the main purpose of the study is “to test the effects of HOPE compared to conventional cold storage”, the investigators/authors fail to include and precisely state what the hypothesis of this study is albeit agreeing to having done so under item 2b of the CONSORT 2010 checklist of information to include when reporting a randomised trial* (pg 23 of 47 of the protocol).

A1: The current version of the protocol manuscript (page 5, para 3, line 3-5) has been updated to provide a clear description of the study hypothesis.

Q2: Additionally, the objective of this study stated as being to “study the effects of HOPE...”is very broad and unspecific. What particular effects exactly do they intend to investigate? What clinical outcome(s)/complication(s) are they presuming the application HOPE may provide benefit to or limit the incidence of? Interestingly, the extended protocol contains a clearer aim of this study (page 37 of 47) however, the authors fail to incorporate it into the condensed manuscript version.

A2: The particular sentence alone, cited by the Reviewer, is indeed vague; however, it is intended to be interpreted in the context of a detailed description of several defined primary and secondary outcomes. The authors have attempted to achieve even more clarity and have adapted the manuscript accordingly (page 5, para 3, line 3-5; and page 10, 11, Study endpoints).

Q3: The authors are aiming to apply single portal vein perfusion while they will be using a device that allows dual perfusion of both the portal vein and hepatic artery. Perfusion of the artery may be particularly relevant for preservation of the larger bile ducts and dual perfusion has been shown to be safe and effective. It remains unclear why the investigators will be using single portal perfusion instead of dual perfusion as has been applied by Guarrera et al (AJT 2010) and van Rijn et al (Br J Surg 2017).

A3: The authors thank the reviewer for raising this very interesting topic. The use of single or dual perfusion in HOPE is still a subject of ongoing debate. The superiority of one over the other has not been confirmed in any clinical trials yet. Therefore, we have decided to use the simple and safe single perfusion design as previously described by Dutkowski et al. in DCD allografts [1]. We believe that single portal vein only cannulation, pending results of HOPE ECD-DBD and the Zurich trial, might receive more support from the community due to pragmatic reasons. Nevertheless, we aim to investigate the effects of single versus dual cannulation in future clinical trials.

Q4: Stratified randomization model will be used to ensure balance of prognostic variables between the treatment groups. The only variables included in this stratification process are donor age and expected CIT. However, the most prevalent risk factor classifying livers as high risk or ECD grafts is liver steatosis. In fact, steatosis is also the most important determinant of peak ALT after OLT in the recipient (primary endpoint of this study). Therefore, it is unclear why (and an omission that) graft steatosis is not included in the stratification process.

A4: When designing our stratified randomization model, we attempted to select relevant prognostic variables which are readily available and easy to assess at the time of randomization. It is well known that with increasing numbers of prognostic variables used for randomization, stratified randomization loses its advantage over simple randomization. Therefore, we decided to use two relevant prognostic variables in our model.
Following the reviewer's valuable comments, the stratification procedure has been adapted accordingly (page 7, Randomization). In the present revision of our study protocol, expected cold ischemia time has been kept as one of the most relevant factors concerning outcome in ECD liver transplantation . After reviewing the literature and our institutional liver transplant database we determined 8 hours CIT as an optimal cut off value for the randomization model (median cold ischemic time of 8 hours is based on our local OLT database (2011-2017) containing more than 320 OLTs). Furthermore, donor age as prognostic variable has been replaced with graft macrosteatosis. For steatosis, we use 30% macrovesicular steatosis as a cut-off value for the stratification process as defined by the NASH Clinical Research Network Scoring System and validated as a prognostic marker in liver transplantation by several authors. Liver histology will be requested and performed for all liver grafts included in the HOPE ECD-DBD trial.

Q5: Definition of ECD includes "donor 50 years and older with cause of death other than trauma". This would mean that a 52 yo donor who died after a cerebral bleed within 48 hr after admission and with no previous history and normal liver lab and no steatosis is qualified as an ECD liver. This however is clearly not an ECD liver.

A5: Based on the Reviewer`s comment, ECD is now more strictly defined according to the official criteria of the German Medical Chamber (page 7, Organ procurement, ECD criteria).

Q6: Machine perfusion will be applied using the Liver Assist at 4-6 degrees C. This, however, is impossible as the Liver Assist is not able to cool the perfusion fluid lower than 10 degrees C.

A6: The above-mentioned erroneous detail has been corrected in the revised version of the protocol (page 8, HOPE versus CCS, para 2).

Q7: Liver are preserved in HTK solution during transportation. Will livers be flushed to remove HTK solution prior to connection to the Liver Assist primed with UW solution? If not, HTK will be mixed with UW during machine perfusion.

A7: Grafts are flushed in all cases with Belzer MPS solution to remove HTK before perfusion. The protocol has been updated with this information (page 8, HOPE versus CCS, para 2).

Q8: Peak ALT within the first 7 days post-OLT is stated to be the primary end point of this study. There are a number of limitations to this. Firstly, the authors/investigators provide no clear justification (no referred literature or proven clinical consensus) as to why they solely find ALT the most suitable end- point to provide answers to their primary research question which in itself is broad and unspecific. Secondly, the authors fail to elaborate on what they will consider as clinically elevated ALT levels. Given that all patients undergoing OLT have elevated ALT levels, it seems necessary that the authors establish a valid threshold or cut-off point in order to make reliable conclusions. Finally, and maybe most importantly, due to the well-described wash out effect of machine perfusion there will always be a lower
serum ALT in recipients of a machine preserved liver compared to controls. Picking serum ALT as a primary end-point should therefore be considered a self- fulfilling prophecy.

A8: The dilemma of choosing primary endpoints in machine perfusion trials in DBD transplantation is apparently a more complex issue than it is in the case of DCD, where increased rate of biliary complications and graft loss represent major clinical challenges. As a matter of fact, we are currently dealing with the first clinical trials in machine perfusion in ECD-DBD transplantation, and therefore it is still unclear on which endpoints and to which extent HOPE will exert its positive effect in various patient groups.
Peak transaminase levels during the early postoperative period, however, is still a very important indicator of graft injury in routine clinical practice. ALT levels are therefore also included as one of the main parameters in the large majority of different scoring systems, which attempt to evaluate early graft injury as well as function and dysfunction. Serum ALT levels were also included within the primary endpoints of the pioneering clinical series of hypothermic machine perfusion by Guarrera et al. in the form of the well-known EAD score. The use of the aforementioned Olthoff´s criteria has also been considered as a primary endpoint within our working group and several extensive discussions have been led on these issues. The EAD score is a dichotomous variable based on arbitrarily set cut off values, which does not allow grading of severity of graft dysfunction. EAD is either present or it is not. The same can be said of any other threshold or cut-off point in similar scores. Scoring systems of graft dysfunction have been introduced and validated several years ago; however, due to their imperfections and statistically low c-indices they cannot be generally recommended to drive clinical decision-making and are still not translated to the everyday clinical practice of most transplant centers. Therefore, in our opinion, the use of EAD scores would not provide a better primary outcome.
Severe complications (Clavien-Dindo grade III and over) might be likewise criticized as a primary endpoint in DBD liver transplantation and machine perfusion. Liver transplantation is a highly invasive procedure often performed in very sick patients with several comorbidities where complications can occur because of many different factors (e.g. surgical technical issues, anaesthesia, intensive care, immunosuppression, comorbidities). A large multicenter analysis of 30-day complications after deceased donor liver transplantation has shown that at least one relevant complication occurs in 79.3% of all patients. At least one severe (Clavien-Dindo grade III and over) complication occurred in 63% (i.e. almost 2/3) of the recipients within the first 30 days. Due to the lack of extensive clinical data regarding HOPE in DBD transplantation, we believe that choosing complications (and even major complications) as a primary endpoint in our trial would be a very questionable decision. Presence of a difference should not be confused with proof of a difference in this regard. Demonstrating a causal relationship between the effect of HOPE and the incidence of many complications considered as major but occurring rather frequently in these complex procedures (e.g. wound dehiscence, relevant postoperative bleeding, bile leak, infected peritoneal fluid collections) is a very difficult task. Our team is probably not the first struggling with these issues when selecting primary endpoints.

The Zurich group initially also intended to use peak serum ALT as a primary outcome in their HOPE trial, which can still be found as up to date information on clinicaltrials.gov on the 3rd of July 2017 (NCT01317342). This has apparently been changed and replaced by the incidence of complications during the study course.
ECD livers from DCD and from other ECD criteria donors in DBD are substantially different. In DCD transplantation, relevant and severe warm ischemia and hepatocellular damage (with transaminase release) occurs already before organ retrieval and cold preservation, resulting in a pre-damaged organ. Organs with other ECD criteria are also pre-damaged, however this is due to steatosis, advanced age of the donor or high serum sodium, not because of a donor-related period of warm ischemia. These ECD-DBD organs are vulnerable for subsequent ischemic-reperfusion injury. The referred “wash out effect” of machine perfusion has been addressed so far mostly in preclinical and ex-vivo studies in DCD transplantation [15, 16] as well as in trials with long machine perfusion periods (3-7 hours). In these settings, utilizing organs from DCD donation with extensive warm ischemic damage at the time point of perfusion, long pumping periods, or limited in vitro reperfusion episodes (2-6 hours), “wash out effect” during cold perfusion might have a relevant role to play.

Nevertheless, it is clearly questionable to what extent very short periods of 1 to maximal 2 hours of cold perfusion could influence the transaminase release and peak ALT during the first 7 days of liver transplantation in DBD transplantation, where the hepatocellular damage and transaminase release mostly occurs after cold storage/perfusion and warm reperfusion. To our understanding, the “ALT wash-out effect” of machine perfusion in ECD-DBD liver transplantation following short periods (1 hour) of cold perfusion can only be considered as “expert opinion” and needs to be explored further in the present and further trials.
To summarize this complex issue, there is no clinical data or expert consensus regarding ECD DBD liver transplantation and HOPE, which would guide decision-making with regards to primary outcomes for machine perfusion trials in DBD liver transplantation. Each outcome used by different groups has its advantages and specific pitfalls. Although, the primary outcome is of major importance in every trial, results must be interpreted in the context of all outcome measures. The main mission of HOPE ECD- DBD and future clinical trials on hypothermic machine perfusion is to explore the clinical effects of HOPE in different patient populations and determine relevant outcome measures influenced the most by the use of this promising method.
Based on these above-mentioned points, we have aimed to adapt our design according to the Reviewers` valued suggestions and have slightly revised our primary and secondary endpoints for the HOPE ECD- DBD trial (page 10-11):

1) As primary endpoint, besides peak ALT, we implemented delta Peak-ALT. To correct for an assumed washout effect of machine perfusion, besides the absolute values, relative changes of serum peak-ALT will be assessed. Peak-ALT will be corrected to the values measured in the routine blood analysis after reperfusion at the time point of admission to the ICU.

2) We introduced the dichotomous EAD score of Olthoff et al. as secondary outcome.

Q9: The authors only include a reference to the Clavien-Dindo complication score and the comprehensive complication index (CCI) as one of the secondary outcomes. Could the authors explain why the incidence post-OLT complications are chosen to only just be secondary end-points? Shouldn’t this be the primary endpoint as for example is the case in the HOPE study initiated by the Zurich group. Furthermore, given that the authors describe detrimental effect of I/R injury and its effect is rather emphasized in the introduction section, it would be interesting to know why or if the authors had not considered lactate clearance as an important secondary outcome.

A9: Regarding the argumentation on the primary endpoints please see A8. Serum lactate levels are routinely measured multiple times daily during the early postoperative case of liver transplantation (first 7 days). According to the suggestions of the Reviewer, lactate has been included as laboratory parameter under the secondary endpoints (page 10, Study endpoints).

Q10: Under randomization, the investigators state that “Randomization is performed by the principal investigator with an online randomizing tool for clinical trials (www.randomizer.at) at the time of admission for OLT”. Can the investigators elaborate on why they choose for this particular time point? How can they be sure that the potential donor liver will be automatically suitable for transplantation (before macroscopic analysis) after the donor hepatectomy and thus already allocate the recipient to a particular study arm? One important lesson from the COPE Trial organized by the Oxford group has been that randomizing a donor liver before it is actually accepted by the transplant center will introduce a major source of bias as livers that have been allocated to the control group are more difficult to allocate. Therefore, it is strongly advised to randomize livers after they have been formally accepted for transplansplantation (i.e after visual inspection and approval).

A10: The authors thank the reviewer for his valuable suggestion and have adapted the randomization process accordingly. Patients are going to be randomized upon acceptance of the organ in the local transplant center. In case of unavailability of the PI, other trained members of the study team will also have access to the randomization tool. The randomization tool registers all updates, therefore the identity of the person who performed randomisation (page 7, Randomization) will be recorded too.

Q11: Besides a stratification procedure, the investigators do not further elude to the randomization procedure. It is stated that the “principal investigator will perform the randomization”, have the investigators taken into consideration illness or other circumstances in which he will not be able to do so? Will there be other authorized (trained) personnel who would be available to do so in such situations or will the study be entirely reliant on the principle investigators presence?

A11: Please see Q and A 10.

Q12: With regards to the blinding, the protocol states that it is open-label for the physicians but what about the patients? Nothing has been stated on what information will be given to the patients i.e. will the patients be made aware of what study arm they belong to? Moreover, will there be an independent assessment committee for assessing the main endpoints (without being informed about the group assignment)?

A12: As stated in the trial design, HOPE-ECD-DBD is an open-label trial which per se means that no blinding is applied (surgical team, patients are not blinded), therefore participants can be informed on which study arm they belong to. This, however, has no pragmatic relevance for the patients, considering that postoperative treatment and follow up are exactly the same for both arms. However, the analysis of endpoints will be performed by an independent committee in a blinded fashion (Institute for Medical Statistics, RWTH Aachen) (page 12, para 1).

Q13: Under inclusion and exclusion criteria (page 7 line 40 – page 8 line 13), the authors fail to list 18 years and above of age as a clear inclusion criteria. Furthermore, the authors do not address patients receiving a combined lung and/or kidney- liver transplantations. Can it be automatically assumed that these patients will be included in the study? Should this be the case, can the investigators elaborate on why they would be included and recipients of, for example, split or living donation would not?

A13: The authors have implemented the valuable suggestions of the Reviewer in the present version of the manuscript and fully agree with these comments. Exclusion of recipients of combined transplantations should be clearly defined due to the unpredictable complex effects in these scenarios (page 6, para 4/page 7, para 1 and 2).

Q14: Sample collection: The authors intend to collect liver tissue samples taken upon arrival of the organ (before HOPE or corresponding cold-storage) and at the end of implantation before closure of the abdomen in addition to blood samples are collected as part of the daily routine during the peri- and postoperative course of OLT. Could the investigators elaborate on why no (perfusate) samples will be taken during the machine perfusion period and why they do not find it necessary to do so? Furthermore, parameters during HOPE such as flows, temperature, oxygenation saturation, pO2, pCO2 are not stated to be recorded. Could the authors clarify why they choose not to record and/or evaluate these data? Analysis of these samples could potentially reveal very useful information.

A14: Collection of perfusate samples has already been mentioned in the previous version of our detailed protocol submitted to the local IRB. The perfusion parameters registered by the Liver Assist are of course exported and evaluated for mathematical patterns. Based on the valuable comment of the Reviewer these details have received more attention in the amended protocol as well as in the revised version of the manuscript (page 10, para 1).

Furthermore, considering the fact that we routinely use side-to-side biliary anastomosis with T-drain based on the technique described by Neuhaus et al. (center 1, University Hospital, RWTH Aachen), it allows us to perform analyses on biliary function and injury using biliary samples.

Q15: The study duration for both the individual subject as well as the study in general, is not clearly specified. The extended version of the protocol however, does contain this information; which states "The expected duration of the recruitment phase will be 18 months. The study will last 1 year per patient and in total 36 months including evaluation of all data and clinical study report." This total study duration for the individual patient described is impossible given that post-OLT follow up alone is 12 months (1 year) therefore the time period from approved consent from the patient up until the first month of follow up has not been accounted for.

A15: Concerning the nature of the intervention, it is difficult to define how much time will pass between the approved consent and the transplantation. The misleading sentence has been changed to provide a clear statement.

Q16: Table 1. There are several errors in this Table. First, the primary endpoint of trial NCT01317342 is not serum ALT, but major postoperative complications (Clavien Grade ≥III), using the established Clavien classification supported by a recently developed comprehensive complication index (CCI) by Slankamenac et al. The information provided on clinicaltrials.gov is not correct and the authors are advised to contact Dutkowski for the actual version of his protocol which has an adjusted primary endpoint. Moreover, this trial will include 170 patients, not 70. Secondly, the Table is not complete. Trials missing in this Table are NCT03031067, NCT00879268, NCT03098043.

A16: The authors would like to kindly thank the Reviewer for his comment on the above-mentioned table. The requested changes have been implemented and marked as data based on personal communication in the revised version of Table 1. Nevertheless, the authors believe that the responsibility lies with the PI to update the trial data on the trial registration site (e.g. clinicaltrials.gov) within a limited period of time, especially in case of major changes in primary endpoints or sample-size to provide maximal transparency.
The authors are well-aware of the trials mentioned by the Reviewer, however as the table legend states "Active RCTs on HOPE in OLT on clinicaltrials.gov", we only attempted to comply running RCTs in Table 1. The mentioned trials are case series and non-randomized trials based on the most actual information available. These trials are mentioned in the discussion section of our manuscript. NCT00879268 is a trial which has already been completed, therefore we have not included this or any other closed or inactive trials in our table, not to mention that the cited work was also a non-randomized study.

Q17: Discussion, Page 13. It is stated that trial NCT02584283 is a multi-center RCT investigating the effects of portal vein perfusion only versus portal- and arterial perfusion during HOPE. This is not correct. This trial is comparing dual HOPE with traditional static cold storage of DCD livers.

A17: The revised version of the manuscript has been updated with the correct information regarding the design of trial NCT02584283.

Q18: Primary endpoint (page 11): Early graft function as assessed by peak alanine aminotransferase-ALT. Please not that serum ALT is not reflecting liver FUNCTION, but rather liver INJURY.

A18: This nomenclature detail has been addressed in the current revision.

Q19: Page 6 (line 24 -25): The investigators state “several strategies have been developed aiming at reconditioning poor quality ECD-allografts” but only refer to a single study despite there being over 20 published articles on this topic. More referenced articles would be recommended.

A19: Further relevant articles have been included in the present revised version of our manuscript.

Q20: Page 13 (line 21-24): “I/R-injury, depleted energy reserves, and oxidative stress are playing an important role...” is better written as “... oxidative stress play...”

A20: Revised accordingly.

Q21: Page 14 (line 12-17): “ECD-allografts exhibit poor tolerance to I/R-injury, a syndrome initiated upon restoration of blood supply after cold and warm ischemia yielding in endothelial and Kupffer cell swelling, vasoconstriction, white blood cell infiltration, and sinusoidal platelet aggregation.” No references to (un)published data or literature are given. Investigators are advised to do so.

A21: Revised accordingly.

Q22: Page 14 (line 55): “...also including non-ECD organ...” should be “...organs...”

A22: Revised accordingly.

Reviewer: 2
Reviewer Name: P. Dutkowski
Institution and Country: Department of Surgery & Transplantation, University Hospital Zurich

Q1: It is repeatedly stated in the manuscript that the study will be the first randomized trial on HOPE in extended criteria DBD livers, which is incorrect. The currently ongoing original multicenter HOPE trial includes many extended criteria livers, and this trial is registered since more than two years (NCT01317342). In facts, most of the included grafts qualify nowadays as extended criteria grafts.

A1: The protocol has been refined according to the suggestion of the reviewer. It should be noted, however, that HOPE ECD-DBD is the first RCT which focuses solely on ECD allografts in DBD transplantation. Nevertheless, the pioneering merits and efforts of well-known working groups in hypothermic oxygenated machine perfusion are mentioned consequently with the greatest respect throughout the whole manuscript.

Q2: The primary endpoint peak ALT is a marker of hepatocyte injury rather than a sign for graft function. This is also the weakness of the study protocol, as ALT appears clinically less relevant. Much more important are biliary complications or graft loss within the first year after transplantation.

A2: The protocol has been revised and peak ALT is referred to as marker of early allograft injury. As it has been extensively discussed in the A8 in the responses to the questions of Professor Porte, we do think there is currently no generally optimal endpoint to use in machine perfusion trials in DBD liver transplantation. In DCD transplantation, biliary complications and graft loss are obvious clinical issues. However, to date we do not have enough clinical data to be able to decide which endpoints are the most optimal in machine perfusion trials in different patient groups within the colourful spectrum of ECD- DBD. It can be assumed that HOPE might have slightly different positive effect for a patient receiving steatotic graft with extended cold ischemia time than for others who receive organs from elderly donors after long intensive care unit stay and high sodium levels. For further critical points please see also A8 above.

Q3: The primary endpoint of the original HOPE trial is therefore complications, graded by Clavien score >=III within one year, please correct Table 1 in this regard.

A3: The primary endpoint has been changed and marked as data based on personal communication in the revised version of Table 1. Please see also A16.

Q4: Please specify better hepatic steatosis for the definition of extended criteria grafts. Will all grafts get a biopsy at procurement to assess the amount of fat?

A4: In all cases considered for inclusion in the HOPE ECD-DBD trial a biopsy will be requested at procurement to assess the extent of hepatic steatosis.

Q5: The authors plan to perfuse human livers at 4-5 °C with the Liver Assist device. However, the temperature can currently only be adjusted to 8-10 °C despite adding ice, please correct the protocol in this regard.

A5: The above-mentioned erroneous details on perfusion parameters has been corrected in the revised version of the protocol.

Q6: The authors indend to pump for 1 hours in the HOPE arm. What happens if recipient hepatectomy is not finisihed after 1h HOPE? Are those livers again exposed to cold storage? I would suggest instead to continue pumping until implantation of the graft

A6: In case of a prolonged and complex recipient hepatectomy, HOPE was intended to be continued until completion of the hepatectomy, to avoid the exposure to cold storage again and the loss of a possible reconditioning effect. The corresponding part of the protocol has been updated to improve clarity.

References:

1. Dutkowski, P., et al., First Comparison of Hypothermic Oxygenated PErfusion Versus Static Cold Storage of Human Donation After Cardiac Death Liver Transplants: An International-matched Case Analysis. Ann Surg, 2015. 262(5): p. 764-70; discussion 770-1.
2. Westerkamp, A.C., et al., Similar outcome after transplantation of moderate macrovesicular steatotic and nonsteatotic livers when the cold ischemia time is kept very short. Transpl Int, 2015. 28(3): p. 319-29.
3. Cameron, A.M., et al., Optimal utilization of donor grafts with extended criteria: a single-center experience in over 1000 liver transplants. Ann Surg, 2006. 243(6): p. 748-53; discussion 753-5.
4. Kleiner, D.E., et al., Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology, 2005. 41(6): p. 1313-21.
5. Chu, M.J., et al., Donor Hepatic Steatosis and Outcome After Liver Transplantation: a Systematic Review. J Gastrointest Surg, 2015. 19(9): p. 1713-24.
6. McCormack, L., et al., Liver transplantation using fatty livers: always feasible? J Hepatol, 2011. 54(5): p. 1055-62.
7. Olthoff, K.M., et al., Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl, 2010. 16(8): p. 943-9.
8. Bruns, H., et al., Early markers of reperfusion injury after liver transplantation: association with primary dysfunction. Hepatobiliary Pancreat Dis Int, 2015. 14(3): p. 246-52.

9. Pareja, E., et al., A score model for the continuous grading of early allograft dysfunction severity. Liver Transpl, 2015. 21(1): p. 38-46.
10.Jochmans, I., et al., "Model for Early Allograft Function" outperforms "Early Allograft Dysfunction" as a predictor of transplant survival. Transplantation, 2017.
11.Guarrera, J.V., et al., Hypothermic machine preservation in human liver transplantation: the first clinical series. Am J Transplant, 2010. 10(2): p. 372-81.
12.Guarrera, J.V., et al., Hypothermic machine preservation facilitates successful transplantation of "orphan" extended criteria donor livers. Am J Transplant, 2015. 15(1): p. 161-9.
13.Parikh, A., et al., A multicenter study of 30 days complications after deceased donor liver transplantation in the model for end-stage liver disease score era. Liver Transpl, 2015. 21(9): p. 1160-8.
14.Firl, D.J., et al., Impact of donor age in liver transplantation from donation after circulatory death donors: A decade of experience at Cleveland Clinic. Liver Transpl, 2015. 21(12): p. 1494-503.
15.Westerkamp, A.C., et al., Oxygenated Hypothermic Machine Perfusion After Static Cold Storage Improves Hepatobiliary Function of Extended Criteria Donor Livers. Transplantation, 2016. 100(4): p. 825-35.
16.Op den Dries, S., et al., Hypothermic oxygenated machine perfusion prevents arteriolonecrosis of the peribiliary plexus in pig livers donated after circulatory death. PLoS One, 2014. 9(2): p. e88521.
17.Westerkamp, A.C., et al., End-ischemic machine perfusion reduces bile duct injury in donation after circulatory death rat donor livers independent of the machine perfusion temperature. Liver Transpl, 2015. 21(10): p. 1300-11.
18.Neuhaus, P., et al., Technique and results of biliary reconstruction using side-to-side choledochocholedochostomy in 300 orthotopic liver transplants. Ann Surg, 1994. 219(4): p. 426-34.

VERSION 2 – REVIEW

REVIEWER	Robert J. Porte University Medical Center Groningen The Netherlands
REVIEW RETURNED	22-Jul-2017

GENERAL COMMENTS	This revised version of the manuscript is of a protocol describing an investigator initiated, open-label, phase-II, multi-centre, prospective randomized controlled trial with the intention to investigate the effects of hypothermic oxygenated machine perfusion (HOPE) on extended-criteria donor (ECD) allografts in donation after brain death (DBD) orthotopic liver transplantation (HOPE ECD-DBD). This revision consists of substantial improvements to the previously submitted manuscript. A clearly defined hypothesis with more concrete primary and secondary end points have been outlined and the various methodological flaws we pointed out to, have been addressed. Despite this, we noticed that this protocol still contains a few flaws. Major comments: - The authors fail to give an explanation in their revised manuscript as to why they explicitly choose to perform HOPE (single/portal perfusion) as opposed to dual HOPE (portal & hepatic artery perfusion) as has been described in literature, as pointed out in the previous review. - In this respect, the authors do not mention or discuss the recent publication on dual HOPE in DCD liver transplantation (van Rijn et al, Br J Surg)

	- The protocol has changed from a single center into a multicenter study without giving an explanation why. Moreover, as far as I know the two centers (Ghent and Bucarest) that have been added to the trial protocol are also participating in the HOPE-DBD trial. It is not clear how patients will be selected for these two overlapping trials. - In the cover letter the authors state “We strongly believe in transparency in clinical trials, which is why we aim to publish our study protocol in a reputed peer-reviewed journal. After all, the improvements in study design stemming from peer- review lead to better scientific outcomes and safeguard patients from deviations in clinical protocols. Unfortunately, this has not been the case for the majority of other running RCTs investigating the effects of hypothermic oxygenated machine perfusion in liver transplantation”. This last remark is a bit awkward since there are only two other RCT’s in the area (as summarized by the authors in Table 1 and their Discussion), so what do the authors mean by “the majority“? Moreover, both other trials have been published at clinicaltrials.gov and the protocol of the DHOPE-DCD trial has been submitted to a peer reviewed journal for publication. - Table 1 doesn’t not include all the current trials involving HMP in liver transplantation (as of 17th July 2017); namely trial NCT03098043 is missing. - Finally, based on the comments of the reviewers the investigators have substantially changed their study protocol (for example the definition on an ECD liver has been changed). Despite this, the investigators have already started recruiting patients into their trial (according to Clinicaltrials.gov). How will the authors deal with enrolled patients and liver grafts that do not meet the adjusted criteria for inclusion? Minor comments: - The manuscript contains a few minor spelling mistakes on page 13 under “Ischemia-reperfusion injury and inflammation”; alcalic phosphatase and hyporthermic conditions should be corrected to “alkaline phosphatase” and “hypothermic conditions”.

REVIEWER	P. Dutkowski Department of Surgery & Transplantation University Hospital Zurich Switzerland
REVIEW RETURNED	24-Jul-2017

GENERAL COMMENTS	This is the revised version of a RCT protocol, aiming to test effects of hypothermic oxygenated perfusion (HOPE) against cold storage in liver transplants. The authors have corrected nicely their manuscript. The only comment from my side is a small correction of Table 1, second row: it looks here, that two RCTs are actively enrolling patients, one with 70 and one with 170 patients. In fact, only the multicenter trial with 170 patients and major complications as endpoint is active, please remove the 70 patients above.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Robert J. Porte
Institution and Country: University Medical Center Groningen, The Netherlands

Q1:
The authors fail to give an explanation in their revised manuscript as to why they explicitly choose to perform HOPE (single/portal perfusion) as opposed to dual HOPE (portal & hepatic artery perfusion) as has been described in literature, as pointed out in the previous review.

A1:
We agree with Dr. Porte that HOPE and D-HOPE are two rivalling methods of hypothermic machine perfusion. However, we would also like to point out that until the superior effects of one or the other approach are not supported with clinical or at least with convincing preclinical evidence in a comparative study, the topic of single- (HOPE) versus dual cannulation (D-HOPE) is rather a matter of comprehensive review papers and scientific discussions but cannot be considered as an objective quality indicator when evaluating our protocol manuscript which aims to investigate the effects of single HOPE in ECD-DBD liver transplantation.

In donation after cardiac death, where biliary complications are presented as a very specific and typical issue, D-HOPE might have several advantages due to the hypothetically better perfusion of the bile duct, which has recently been demonstrated by Dr. Porte´s group (van Rijn et al. Br J Surg 2017). However, there is no clinical data which would confirm the same for ECD-DBD grafts or would attempt to compare the two different methods in patients. As such, we have decided to use the simple and safe single perfusion design as previously described by Dutkowski et al. (Dutkowski et al., Ann Surg 2015). It was demonstrated by the same group in an experimental study that portal vein HOPE is sufficient to completely perfuse the liver in case of rat, pig and discarded human livers (Schlegel et al. J of Hepatology 2016). They also compared pig liver grafts which were perfused either by dual HOPE or by portal vein only HOPE. Angiography showed no difference between the two approaches and both led to complete perfusion of the liver within 30s even under low pressure cold perfusion conditions. The macroscopic and histological evaluation of the distal bile duct in perfused livers (rat, human) demonstrated perfusion of the entire common bile duct via the portal branches within 5 min during single HOPE. Based on these, we and the Zurich group assumed that single cannulation end-ischemic HOPE is the simplest machine perfusion approach from a pragmatic point of view which we believe, pending results of HOPE ECD- DBD and the Zurich trial, might receive more support from the community compared to other protocols. The single HOPE perfusion protocol is efficient, simple, fast, and safe. These are all very important features concerning the clinical survival and general acceptance of a surgical method.

Nevertheless, we think that the clinical comparison of single vs. dual HOPE is of upmost clinical importance and should be addressed in future trials. In fact, we are planning to initiate or participate in such a multi-centre trial for the future.

Q2:
In this respect, the authors do not mention or discuss the recent publication on dual HOPE in DCD liver transplantation (van Rijn et al, Br J Surg)

A2:
The requested interesting and highly relevant paper on dual HOPE and DCD transplantation, published very recently by the group of Dr. Porte is now discussed in the revised version of our manuscript (page 14, line 27-28).

Q3:
The protocol has changed from a single center into a multicenter study without giving an explanation why. Moreover, as far as I know the two centers (Ghent and Bucarest) that have been added to the trial protocol are also participating in the HOPE-DBD trial. It is not clear how patients will be selected for these two overlapping trials.

A3:
For this please see Q and A6.

Q4:
In the cover letter the authors state “We strongly believe in transparency in clinical trials, which is why we aim to publish our study protocol in a reputed peer-reviewed journal. After all, the improvements in study design stemming from peer- review lead to better scientific outcomes and safeguard patients from deviations in clinical protocols. Unfortunately, this has not been the case for the majority of other running RCTs investigating the effects of hypothermic oxygenated machine perfusion in liver transplantation”. This last remark is a bit awkward since there are only two other RCT’s in the area (as summarized by the authors in Table 1 and their Discussion), so what do the authors mean by “the majority“? Moreover, both other trials have been published at clinicaltrials.gov and the protocol of the DHOPE-DCD trial has been submitted to a peer reviewed journal for publication.

A4:
We would like to thank the reviewer for his remark. We are happy to hear that the DHOPE-DCD protocol manuscript has been submitted for peer review and we are looking forward reading the manuscript soon.

Q5:
Table 1 doesn’t not include all the current trials involving HMP in liver transplantation (as of 17th July 2017); namely trial NCT03098043 is missing.

A5:
Here we would like to point out once again that Table 1 is labelled “Active RCTs on HOPE in OLT on clinicaltrials.gov“ and is intended to show active RCTs in the field. NCT03098043 is an inactive non-randomised observation cohort study, thus it does not belong into Table 1.

Q6:
Finally, based on the comments of the reviewers the investigators have substantially changed their study protocol (for example the definition on an ECD liver has been changed). Despite this, the investigators have already started recruiting patients into their trial (according to Clinicaltrials.gov). How will the authors deal with enrolled patients and liver grafts that do not meet the adjusted criteria for inclusion?

A6:
Hereby we would like to clarify the misunderstanding which led to the assumption that we have changed the HOPE-ECD-DBD study protocol while patients’ inclusion had started.

This is not the case. Our design has been updated to include more geographic locations and increase generalizability of the future study results. We would like to emphasize, however, that we have not randomised any patients for the HOPE ECD-DBD trial so far, due to the fact that we were waiting for the response from BMJ Open on the present study protocol manuscript, to avoid the need of major changes in the protocol following a revision. Beside the basic design, however, no major changes have been implemented concerning the primary outcome or study objectives.

ECD criteria have been revised, upon request of the reviewer, according to the national recommendations to allow a better selection of ECD liver grafts and ensure a more homogeneous patient collective. In the meantime, patients have been screened and one patient who has been consented (but has not been transplanted and randomised yet), has already been informed and signed according to the amended protocol and consent form. Information on recruitment data has also been updated on clinicaltrials.gov. The reviewer`s related concern (see Q3) that overlapping studies might be conducted in the additional centres should not affect the publication of our trial design. On the one hand, our study is restricted to ECD organs (where, besides DCD, we believe is the most value of the HOPE technique for the transplant recipients). On the other hand, the study sites need to fulfil the highest technical and logistical standards for participation in the study (e.g., surgical expertise with HOPE), which per se limits the number of European centres for participating in such innovative trials. In this regard, it may also be noted that all participating centres are high volume liver transplantation centres with an annual case load of 50-100 liver transplantations.

Q7:
The manuscript contains a few minor spelling mistakes on page 13 under “Ischemia-reperfusion injury and inflammation”; alcalic phosphatase and hyporthermic conditions should be corrected to “alkaline phosphatase” and “hypothermic conditions”.

A7:
We thank Dr. Porte for identifying above mentioned typographical errors. These were revised accordingly (page 13, paragraph 3-4).

Reviewer: 2
Reviewer Name: P. Dutkowski
Institution and Country: Department of Surgery & Transplantation, University Hospital Zurich

Q1:
The authors have corrected nicely their manuscript. The only comment from my side is a small correction of Table 1, second row: it looks here, that two RCTs are actively enrolling patients, one with 70 and one with 170 patients. In fact, only the multicenter trial with 170 patients and major complications as endpoint is active, please remove the 70 patients above.

A1:
We would like to thank Dr. Dutkowski for his encouraging words and suggestion. Table 1 was revised accordingly (page 21, line 02).