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.

This paper was submitted to a another journal from BMJ but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Open. The paper was subsequently accepted for publication at BMJ Open.

(This paper received three reviews from its previous journal but only two reviewers agreed to published their review.)

ARTICLE DETAILS

TITLE (PROVISIONAL)	Rationale and Design of TransplantLines: a Prospective Cohort Study and Biobank of Solid Organ Transplant Recipients
AUTHORS	Eisenga, Michele Freerk; Gomes Neto, Antonio; Van Londen, Marco; Ziengs, Aaltje L; Douwes, Rianne M; Stam, Suzanne P; Osté, Maryse C.J.; Knobbe, Tim J; Hessels, Niek R; Buunk, Anne M; Annema, Coby; Siebelink, Marion J; Racz, Emoke; Spikman, Jacoba M; Bodewes, Frank A.J.A; Pol, RA; Berger, Stefan P; Drost, Gea; Porte, Robert; Leuvenink, Henri G.D.; Damman, Kevin; Verschuuren, Erik A.M.; De Meijer, Vincent E; Blokzijl, Hans; Bakker, Stephan JL

VERSION 1 – REVIEW

REVIEWER	Ali Zarrinpar University of Florida, USA
REVIEW RETURNED	05-Aug-2018

GENERAL COMMENTS	The authors delineate the design of a prospective biobank of solid organ transplant donors and recipients with the goal of allowing multimodal, long term studies in this population. This is an admirable endeavor and a necessary one given the lack of significant recent progress in long term outcomes for transplant recipients. I recommend that the authors address the following in their design and write up. 1. A study of this size and duration will run into difficulties in subject adherence. What do the authors anticipate in rate of adherence to the whole protocol. What is their practice for missed samples? 2. There is no description for what happens if the subjects move or get retransplanted or transplanted with another organ. 3. While they discuss sample and data management, the do not explicitly describe who actually does the sample collection and data entry. How are multiple labs obtained on the same day treated? What about treatment data (e.g. medication list, surgical or other interventional treatments, etc.) 4. With regard to the samples. Who will have access? How will access be prioritized? How will samples be preserved and access logged? What about multiple access to samples? How will the data be stored and accessed? 5. I would recommend some shorter time period samples. There are many after the surgery and for some reason it appears three

	samples prior to transplantation (Inclusion, Screening, Tx) What does at time of “Tx” mean? Prior to incision? During operation? If prior to incision, then how is that different from the early timepoints?

REVIEWER	Simon Knight University of Oxford, UK.
REVIEW RETURNED	06-Aug-2018

GENERAL COMMENTS	This study protocol describes a large, single centre cohort and biobank study for donors and recipients of all solid organ transplants. The data being collected are extensive, which will allow in-depth analysis and relation to transplant outcomes. Major strengths are: - Inclusion of all transplant types in a single study protocol. - The extent of the physical, demographic and quality-of-life data being collected. - Clear description of methods and definition of outcomes Potential weaknesses: - Single-centre, so may lack generalisability - Biobanking is limited (see below) - Burden of number of assessments/questionnaires may limit compliance with follow-up Specific questions/comments: - The infrequent collection of biobank samples may limit utility in detecting biomarkers for routine monitoring of transplant health. Detection of suitable biomarkers may only be possible if sampling happens to fall near the time of a clinical event - No tissue is being collected (pre/post implantation biopsies, protocol biopsies) – these may be useful for proteomics/metabolomics. - In the methods, certain aspects seem to be reserved for a subset of recipients (e.g. physical assessment, cognitive assessment). There is a suggestion that patients will be randomised for these assessments, but the methods or description for this are missing from the protocol? - Recipients/donors with limited language skills and/or poor comprehension are excluded – these patients are exactly those who are at higher risk of poor compliance, high risk social behaviour etc. and would possibly have worse outcomes? I am not sure if there is anything can be done about this, but it may represent a limitation.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Ali Zarrinpar
Institution and Country: University of Florida, USA
Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below
The authors delineate the design of a prospective biobank of solid organ transplant donors and recipients with the goal of allowing multimodal, long term studies in this population. This is an admirable endeavor and a necessary one given the lack of significant recent progress in long term

outcomes for transplant recipients. I recommend that the authors address the following in their design and write up.
1. A study of this size and duration will run into difficulties in subject adherence. What do the authors anticipate in rate of adherence to the whole protocol. What is their practice for missed samples?

Response: We thank the reviewer for the kind words and agree with the reviewer that a study of this size and duration will run into difficulties in subject adherence. As stated in the original version of the manuscript, we expect an overall participation rate of 85% for the TransplantLines study, because our experience is that transplant recipients in general are very willing to participate in studies aiming at improving long-term outcome and quality of life. However, because of the higher frequency of planned study visits for the group undergoing transplantation, we anticipate a lower adherence to the whole protocol in this subgroup. Overall, we anticipate a drop-out percentage of 10% during follow-up in this transplant candidates group. To accommodate the comment of the reviewer, we have added this expectation to the revised version of the manuscript (lines 145-148). Concerning treatment of missing data and inability to generate data from missing samples, we will apply statistical methods using maximum likelihood and multiple imputation, which are now standard for dealing with participant loss and missing data (Christensen H et al. Aging Neuropsychology and Cognition 2004; 11: 169-195). These methods provide more consistent and efficient estimates of population parameters than methods relying on complete cases, mean imputation, last observation carried forward or single-imputation regression methods (Christensen H et al. Aging Neuropsychology and Cognition 2004; 11: 169-195, Woolley SB et al. Parmacotherapy 2009; 29: 1408-1416, Schafer JL et al. Psychological Methods 2002; 7: 147-177, Graham JW et al. Annu Rev Psychol 2009; 60: 549-576). As advised in authoritative reports, these analyses will be complemented with sensitivity analyses to assess robustness of findings (Little JR et al. New England Journal of Medicine 2012; 367: 1355-1360, Lee KJ et al. Respirology 2014; 19: 162-167, Sainani KL. PM R 2015; 7: 990-994). To further accommodate the comment of the reviewer, we have added this text with references as a paragraph on missing data handling to the revised version of the manuscript (lines 581-588).

2. There is no description for what happens if the subjects move or get retransplanted or transplanted with another organ.

Response: We thank the reviewer for noting this. Our study subjects are recipients of a transplanted organ or living organ donors. If it concerns recipients of an transplanted organ, they will always require continued medical care and follow-up by a medical specialist, who will require thorough medical information on the patient and the transplanted organ, to allow for continued dedicated care. So, if a patient moves to another region of the Netherlands or abroad, the medical specialist who will continue care will seek contact for information and it will usually be possible to continue follow-up on long-term outcome and events via this medical specialist. So, follow-up is usually assured and loss to follow-up will be rare. Since study visits are combined with a routine clinical visit, subjects who move out of our region will be excluded from further study visits for the TransplantLines study. Accordingly, we have added this information to the revised version of the manuscript (lines 167-175).
If a subject gets retransplanted with the same kind of organ, this will be classified as graft failure and end of follow-up. Subjects will not be included in the primary database twice. Yet, we will allow for inclusion of subjects retransplanted with the same kind of organ with a new ID in the transplant candidate group, but this will be with the intention to over time build a separate cohort with data and a biobank on retransplantations. When a transplant recipient is later on transplanted with another kind of organ, follow-up will be for the initially transplanted organ. Transplant recipients receiving a combined transplantation, e.g. kidney-pancreas and kidney-liver, will be treated as separate groups, not to be included in overall analyses for the much larger groups of subjects with single transplanted organs. To accommodate the comment of the reviewer, we have added this information to the revised version of the manuscript (lines 158-166).

3. While they discuss sample and data management, the do not explicitly describe who actually does the sample collection and data entry. How are multiple labs obtained on the same day treated? What about treatment data (e.g. medication list, surgical or other interventional treatments, etc.)

Response: We thank the reviewer for this question. Most study visits are at the outpatient clinic, and for these visits blood samples will be taken fasting in the morning. It is unlikely that at these study visits multiple labs will be obtained at the same day, but if they are taken, only the lab obtained at the time of the study visit will be included in the database. In the likely rare case that multiple labs are taken at a day of a study visit, this will likely be a sign of an acute event that occurred after the study visit and it will then later on be linked to the database as the event that occurred (lines 246-252). The actual sample collection at the outpatient clinic (venipuncture, gathering the collected 24-hour urine specimens) will be performed by experienced nurses at our outpatient clinic (lines 210-211). At the visit for transplant surgery, multiple labs will be obtained at the same day. At that day, only the lab results coming available from the samples which are taken at the same time of sampling during surgery, to provide for samples that will be included in the biobank will be linked to the database. These lab results are recognizable by the routine assays that are performed, because they are more extensive and include other routine lab results than the routine lab results coming available from samples taken at other times at the same day (lines 252-258). The retrieved samples will be sent to the research laboratory for processing, analysis and subsequently storage in -80 degrees freezers by trained technicians (lines 214-215, line 218). Data entry is performed by the trained investigators. The trained investigator who performed assessments at the study visit of a subject is responsible for data entry of that subject (lines 547-549). Regarding inclusion of clinical laboratory measurements, we will include the laboratory parameters specifically requested by the physician at the outpatient clinic. Medication list will be provided by the participants and will be verified using the electronic hospital records. Further surgical or other interventional treatments will be extracted along with more medical information from the electronic hospital records upon patient consent as noted in the consent form (lines 261-262).

4. With regard to the samples. Who will have access? How will access be prioritized? How will samples be preserved and access logged? What about multiple access to samples? How will the data be stored and accessed?

Response: We thank the reviewer for bringing up this important point. We have installed a special team consisting of medical doctors of the different fields involved. This team, called Research Team TransplantLines, decides and prioritizes who will get access to the samples of the TransplantLines biobank. The samples can be requested by internal and external researchers against a reasonable fee. All samples are stored at -80°C and access is logged in a linked database. The logging system also provides for registration of multiple access and the number of freeze-thaw cycles that samples have undergone. Multiple access to samples is possible, but for each specific project a new request needs to performed and approved by the Research Team TransplantLines. The data coming available from the assays performed at the provided samples will be linked to the TransplantLines database and be made available to researchers in an anonymized and secured environment for evaluation and statistical analyses. This environment will also monitor and log data handling and store results of analyses. We have added this information to the revised version of the manuscript (lines 567-579).

5. I would recommend some shorter time period samples. There are many after the surgery and for some reason it appears three samples prior to transplantation (Inclusion, Screening, Tx) What does at time of “Tx” mean? Prior to incision? During operation? If prior to incision, then how is that different from the early timepoints?

Response: We agree with the reviewer that it would be interesting to have shorter time period samples available. At the time of applying for approval, this was, however, not allowed by the Medical

Ethical Committee judging on the proposal, largely because it was considered too burdening for participants. At time of transplantation, indeed means during operation prior to incision, and at that timepoint the blood samples are being drawn by the anesthesiologists taking care of the patient. The difference with this sample compared to other samples is that this sample is taken during operation, whereas the other samples are not. For example, a kidney transplant candidate can be screened and included in the TransplantLines study, but may need to wait two years on the waiting list prior to receiving the actual transplantation. Hence, the difference between screening and the moment of transplantation would be two years. We have added this information to the revised version of the manuscript (line 140-145).

Reviewer: 2
Reviewer Name: Simon Knight
Institution and Country: University of Oxford, UK.
Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below
This study protocol describes a large, single centre cohort and biobank study for donors and recipients of all solid organ transplants. The data being collected are extensive, which will allow in-depth analysis and relation to transplant outcomes.

Major strengths are:
- Inclusion of all transplant types in a single study protocol.
- The extent of the physical, demographic and quality-of-life data being collected.
- Clear description of methods and definition of outcomes

Potential weaknesses:
- Single-centre, so may lack generalisability
- Biobanking is limited (see below)
- Burden of number of assessments/questionnaires may limit compliance with follow-up

Response: We would like to thank the reviewer for the time and effort invested in reviewing our manuscript and the constructive questions and comments. Please find below a detailed, itemized response to the questions and comments of the reviewer.

Specific questions/comments:
- The infrequent collection of biobank samples may limit utility in detecting biomarkers for routine monitoring of transplant health. Detection of suitable biomarkers may only be possible if sampling happens to fall near the time of a clinical event

Response: We agree with the reviewer that infrequent collection of biobank samples may limit utility in detecting biomarkers for routine monitoring of transplant health and that detection of suitable biomarkers may only be possible if sampling happens to fall near the time of a clinical event. To accommodate the comment of the reviewer, we have added to the strengths and limitations paragraph of the discussion section of the revised version of the manuscript as a limitation that infrequent collection of biobank samples may limit utility in detecting biomarkers for routine monitoring of transplant health and that detection of suitable biomarkers may only be possible if sampling happens to fall near the time of a clinical event (lines 615-618). To further accommodate the comment of the reviewer, we make also more clear that aside from the fixed time points, biobank samples will also be collected when a biopsy of the transplanted organ is performed. To make this more clear, we rephrased the lines (153-157) in which this was mentioned, added a separate paragraph describing the biobanking (lines 232-237), and we added to the strengths and limitations paragraph of the discussion section of the revised version of the manuscript mentioning that it is a strength of our study

that in addition to taking samples at fixed time points, we also take samples when biopsies are performed (lines 618-622).

- No tissue is being collected (pre/post implantation biopsies, protocol biopsies) – these may be useful for proteomics/metabolomics.

Response: We thank the reviewer for addressing this important point and stand corrected for not mentioning the fact that we are actually performing this quite extensively. During transplantation, pre-transplant biopsies and waste tissue coming available during operation is being biobanked. The waste tissue coming available often includes adipose tissue, skin, ureteral tissue, tracheal tissue, arterial tissue and venous tissue, depending on the kind of transplanted organ. We also collect tissue at the time of protocol biopsies and – like mentioned in the response to the previous comment – at the time of biopsies taken at indication. To accommodate the comment of the reviewer, we have added this information to the revised version of the manuscript (lines 238-240).

- In the methods, certain aspects seem to be reserved for a subset of recipients (e.g. physical assessment, cognitive assessment). There is a suggestion that patients will be randomised for these assessments, but the methods or description for this are missing from the protocol?

Response: We thank the reviewer for pointing out that we have not described the randomization process for the TransplantLines study in our initial manuscript. Indeed, certain aspects are reserved for a subset of recipients. In TransplantLines, at each outpatient clinic study visit, all participating transplant recipients undergo biobanking and a set of standard tests. Because it appeared not feasible to perform all planned additional cognitive and physical tests in each patient, we have decided to randomize transplant recipients to either receive additional physical tests (physical study arm) or additional cognitive tests (cognitive study arm) at their study visit at 12 months post-transplantation or at the first study visit if it concerns transplant recipients with a functioning graft for more than 1 year who were transplanted before the start of TransplantLines. Participants will be randomized on a 1 to 1 ratio and randomization will be performed for each kind of transplant organ (kidney, liver, lung, heart, pancreas, small bowel) separately to ensure balanced randomization of subjects for each type of solid-organ transplant. To accommodate the comment of reviewer, we have now addressed the randomization process in the revised version of the manuscript (section Randomization and additional physical and cognitive tests, lines 355-363).

- Recipients/donors with limited language skills and/or poor comprehension are excluded – these patients are exactly those who are at higher risk of poor compliance, high risk social behaviour etc. and would possibly have worse outcomes? I am not sure if there is anything can be done about this, but it may represent a limitation.

Response: We agree with the reviewer that these patients are at higher risk of poor compliance etc. However, because of Dutch Law and requirements by our Medical Ethical Committee, only transplant recipients that have sufficient understanding of Dutch language to understand the patient information and informed consent are allowed to participate in the study and sign informed consent. Thus, it is unfortunately not possible to do anything about this. To accommodate the comment of the reviewer, we have added this as a limitation to the strengths and limitations paragraph of the discussion section of the revised version of the manuscript (lines 612-615 of the revised manuscript).

VERSION 2 – REVIEW

REVIEWER	Ali Zarrinpar University of Florida
REVIEW RETURNED	21-Sep-2018

GENERAL COMMENTS	Concerns are adequately addressed.