

# BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email [editorial.bmjopen@bmj.com](mailto:editorial.bmjopen@bmj.com)

## Rationale and Design of TransplantLines: a Prospective Cohort Study and Biobank of Solid Organ Transplant Recipients

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-024502
Article Type:	Protocol
Date Submitted by the Author:	29-May-2018
Complete List of Authors:	<p>Eisenga, Michele Freerk; Universitair Medisch Centrum Groningen, Nephrology  Gomes Neto, Antonio; Universitair Medisch Centrum Groningen, Nephrology  Van Londen, Marco ; Universitair Medisch Centrum Groningen, Nephrology  Ziengs, Aaltje; Universitair Medisch Centrum Groningen, Nephrology  Douwes, Rianne; Universitair Medisch Centrum Groningen, Nephrology  Stam, Suzanne; Universitair Medisch Centrum Groningen, Nephrology  Osté, Maryse; Universitair Medisch Centrum Groningen, Nephrology  Knobbe, Tim; Universitair Medisch Centrum Groningen, Nephrology  Hessels, Niek; Universitair Medisch Centrum Groningen, Nephrology  Buunk, Anne; Universitair Medisch Centrum Groningen, Neuropsychology  Annema, Coby; Univ Groningen  Siebelink, Marion; Universitair Medisch Centrum Groningen, Groningen Transplant Center  Racz, Eموke; Universitair Medisch Centrum Groningen, Dermatology  Spikman, Joke; Universitair Medisch Centrum Groningen, Neuropsychology  Bodewes, Frank; Universitair Medisch Centrum Groningen, Pediatrics  Pol, RA; University Medical Center Groningen,,  Berger, Stefan; Universitair Medisch Centrum Groningen, Nephrology  Drost, Gea; Universitair Medisch Centrum Groningen, Neurology  Porte, Robert; Universitair Medisch Centrum Groningen, Hepato-pancreatico-biliaire Chirurgie en Levertransplantatie  Leuvenink, Henri ; Universitair Medisch Centrum Groningen, Surgery  Damman, Kevin; Universitair Medisch Centrum Groningen, Cardiology  Verschuuren, Erik; Universitair Medisch Centrum Groningen, Pulmonary Disease and Tuberculosis  De Meijer, Vincent; Universitair Medisch Centrum Groningen, Surgery  Blokzijl, Hans; Universitair Medisch Centrum Groningen, Gastroenterology and Hepatology  Bakker, Stephan; University of Groningen, University Medical Center Groningen, Internal Medicine</p>
Keywords:	Cohort study, TRANSPLANT MEDICINE, Survival

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

SCHOLARONE™  
Manuscripts

For peer review only

## Rationale and Design of TransplantLines: a Prospective Cohort Study and Biobank of Solid Organ Transplant Recipients

**Authors.** Michele F. Eisenga<sup>1\*</sup>, M.D., Antonio W. Gomes Neto<sup>1\*</sup>, M.D., Marco van Londen<sup>1\*</sup>, M.D., Aaltje L. Ziengs<sup>1,2</sup>, MSc, Rianne M. Douwes<sup>1</sup>, M.D., Suzanne P. Stam<sup>1</sup>, BSc, Maryse C.J. Osté, BSc, Tim J. Knobbe<sup>1</sup>, Bsc, Niek R. Hessels<sup>1</sup>, BSc, Anne M. Buunk<sup>2</sup>, MSc, Coby Annema<sup>3</sup>, PhD, Marion J. Siebelink<sup>3</sup>, PhD, Emoke Rácz<sup>4</sup>, M.D., PhD, Jacoba M. Spikman<sup>2</sup>, PhD, Frank A.J.A. Bodewes<sup>5</sup>, M.D., PhD, Robert A. Pol<sup>6</sup>, M.D., PhD, Stefan P. Berger<sup>1</sup>, M.D., PhD, Gea Drost<sup>7</sup>, M.D., PhD, Robert J. Porte<sup>6</sup>, M.D., PhD, Henri G.D. Leuvenink<sup>6</sup>, M.D., PhD, Kevin Damman<sup>8</sup>, M.D., PhD, Erik A.M. Verschuuren<sup>9</sup>, M.D., PhD, Vincent E. de Meijer<sup>6</sup>, M.D., PhD, Hans Blokzijl<sup>10</sup>, M.D., PhD, and Stephan J.L. Bakker<sup>1</sup>, M.D., PhD.

**Collaborators.** Martin H. De Borst<sup>1</sup>, M.D., PhD, Margriet F.C. De Jong<sup>1</sup>, M.D., PhD, Jan Stephan F. Sanders<sup>1</sup>, M.D., PhD, Gerjan Navis<sup>1</sup>, M.D., PhD, Jan Willem J. Elting<sup>7</sup>, M.D., PhD, Marina A.J. Tijssen<sup>7</sup>, M.D., PhD, Marieke T. de Boer<sup>6</sup>, M.D., PhD, Adelita V. Ranchor<sup>11</sup>, PhD, Ilja M. Nolte<sup>12</sup>, PhD, Rob J. Bieringa<sup>12</sup>, Paul Koenes<sup>12</sup>, Wim van der Bij<sup>9</sup>, M.D., PhD.

<sup>1</sup>Division of Nephrology, Department of Internal Medicine; <sup>2</sup>Department of Neuropsychology;

<sup>3</sup>Groningen Transplant Center, <sup>4</sup>Department of Dermatology; <sup>5</sup>Department of Pediatrics;

<sup>6</sup>Department of Surgery; <sup>7</sup>Department of Neurology; <sup>8</sup>Department of Cardiology; <sup>9</sup>Department of Pulmonary Diseases and Tuberculosis; <sup>10</sup>Department of Gastroenterology and Hepatology;

<sup>11</sup>Department of Health Psychology; <sup>12</sup>Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

\*Denotes equal contribution of these authors

1  
2  
3 **Running title:** TransplantLines Biobank and Cohort Study

4 **Keywords:** TransplantLines; cohort study; transplant recipients; survival

5 **Abstract:** 274 words

6  
7 **Text:** 5420 words

8  
9 **Tables:** 3

10  
11 **Figures:** 3

12  
13 **ClinicalTrials.gov identifier:** NCT03272841

14  
15  
16 **Email addresses authors:** MF Eisenga, [m.f.eisenga@umcg.nl](mailto:m.f.eisenga@umcg.nl); AW Gomes Neto,  
17 [a.w.gomes.neto@umcg.nl](mailto:a.w.gomes.neto@umcg.nl); M van Londen, [m.van.londen@umcg.nl](mailto:m.van.londen@umcg.nl); AL Ziegns,  
18 [a.l.ziengs@umcg.nl](mailto:a.l.ziengs@umcg.nl); RM Douwes, [r.m.douwes@umcg.nl](mailto:r.m.douwes@umcg.nl); SP Stam, [s.p.stam@umcg.nl](mailto:s.p.stam@umcg.nl); MCJ  
19 Osté, [m.c.j.oste@umcg.nl](mailto:m.c.j.oste@umcg.nl); TJ Knobbe, [t.j.knobbe@umcg.nl](mailto:t.j.knobbe@umcg.nl); NR Hessels,  
20 [n.r.hessels@umcg.nl](mailto:n.r.hessels@umcg.nl); AM Buunk, [a.m.buunk@umcg.nl](mailto:a.m.buunk@umcg.nl); MJ Siebelink, C Annema, E Rácz,  
21 JM Spikman, [j.m.spikman@umcg.nl](mailto:j.m.spikman@umcg.nl); FAJA Bodewes, [f.a.j.a.bodewes@umcg.nl](mailto:f.a.j.a.bodewes@umcg.nl); RA Pol,  
22 [r.pol@umcg.nl](mailto:r.pol@umcg.nl); SP Berger, [s.p.berger@umcg.nl](mailto:s.p.berger@umcg.nl); G Drost, [g.drost@umcg.nl](mailto:g.drost@umcg.nl); RJ Porte,  
23 [r.j.porte@umcg.nl](mailto:r.j.porte@umcg.nl); HGD Leuvenink, [h.g.d.leuvenink@umcg.nl](mailto:h.g.d.leuvenink@umcg.nl); K Damman,  
24 [k.damman@umcg.nl](mailto:k.damman@umcg.nl); EAM Verschuuren, [e.a.m.verschuuren@umcg.nl](mailto:e.a.m.verschuuren@umcg.nl); VE de Meijer,  
25 [v.e.de.meijer@umcg.nl](mailto:v.e.de.meijer@umcg.nl); H Blokzijl, [h.blokzijl@umcg.nl](mailto:h.blokzijl@umcg.nl); and SJL Bakker,  
26 [s.j.l.bakker@umcg.nl](mailto:s.j.l.bakker@umcg.nl)

27  
28  
29 **Corresponding author:**

30 Michele F. Eisenga, M.D.

31 Department of Internal Medicine, Division of Nephrology

32 University Medical Center Groningen

33 P.O. Box 30.001, 9700 RB Groningen, the Netherlands

34 Phone: 0031 050 361 61 61 Email: [m.f.eisenga@umcg.nl](mailto:m.f.eisenga@umcg.nl)

## Abstract

### Introduction

In the past decades, short-term results after solid organ transplantation have markedly improved. Disappointingly, this has not been accompanied by parallel improvements in long-term outcomes after transplantation. To improve graft and recipient outcomes, identification of potentially modifiable risk factors and development of biomarkers is required. We provide the rationale and design of a large prospective cohort study of solid organ transplant recipients (TransplantLines).

### Methods and analysis

TransplantLines is designed as a single center prospective cohort study and biobank including all different types of solid organ transplant recipients, as well as living organ donors. Data will be collected from transplant candidates before transplantation, during transplantation, at 3 months, 6 months, 1 year, 2 years, 5 years, and subsequently every 5 years after transplantation. Data from living organ donors will be collected before donation, during donation, at 3 months, 1 year, and 5 years after donation and subsequently every 5 years.

Primary outcomes are mortality and graft failure. Secondary outcomes will be cause-specific mortality, cause-specific graft failure and rejection. Tertiary outcomes will be other health problems, including diabetes, obesity, hypertension, hypercholesterolemia, and cardiovascular disease, and disturbances that relate to quality of life, i.e. physical and psychological functioning, including quality of sleep, and neurological problems such as tremor and polyneuropathy.

### Ethics and dissemination

Ethical approval has been obtained from the relevant local ethics committee. The TransplantLines cohort study is designed to deliver pioneering insights in transplantation and donation outcomes. The study design allows comprehensive data collection on perioperative

1  
2  
3 care, nutrition, social- and psychological functioning and biochemical parameters. This may  
4  
5 provide a rationale for future intervention strategies to more individualized, patient-centered  
6  
7 transplant care and individualization of treatment.  
8  
9

### 10 11 **Strength and limitations**

- 12  
13 - Large biobank and cohort study with extensive data collection on a myriad topics  
14  
15 related to transplantation and/or donation
- 16  
17 - Inclusion of all types of solid organ transplant recipients
- 18  
19 - Long follow-up to assess many relevant clinical outcomes
- 20  
21 - Single center study
- 22  
23 - Residual confounding cannot be excluded due to observational design  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Background

Solid organ transplantation is the preferred treatment for end-stage organ failure. During the past decades, advances in immunosuppressant medications, treatment of infections, perioperative medical care, and surgical techniques (including living donation) have led to important improvements in early post-transplant graft and patient survival.<sup>1</sup> However, on the long-term, graft failure is a major cause of patient mortality and morbidity in all types of transplantation.<sup>2-4</sup> For example, in renal transplant recipients, half of the cadaveric renal allografts fail within a timeframe of 10 years.<sup>5</sup> Apart from reduced survival, transplant recipients often develop health problems that greatly reduce their perceived quality of life (Figure 1).<sup>6,7,8</sup>

The multitude of health problems that recipients experience after transplantation include amongst others obesity, diabetes, hypertension, heart failure, and malignancies.<sup>9-11</sup> These are likely the consequence of a combination of factors, including (1) continuous exposure to treatment with immunosuppressive drugs necessary for prevention of rejection of the transplanted organ, (2) damage induced by pre-existing exposure to end-stage organ failure, and (3) adverse life-style and environmental factors, all potentially expressed against (4) a background of increased (epi)genetic susceptibility. Among these, immunosuppressive treatment, adverse life-style, and environmental factors are good candidates for modification to decrease the load of post-transplant health problems. It should be realized that immunosuppressive treatment is currently mainly “one-size fits all”. Hence, improvement can be achieved by development of biomarkers that can allow for recognition of transplant recipients in which immunosuppressive load can be safely reduced or in which certain drugs can better be avoided, and for biomarkers which can guide such individualized immunosuppressive treatment. To improve long-term transplant outcomes, it is imperative to identify modifiable risk factors, especially among those recipients who are at increased risk.



1  
2  
3 To date, it is largely unknown in which transplant recipients immunosuppressive  
4 medication can be safely reduced to prevent the development of health problems.  
5  
6 Furthermore, in terms of healthcare costs, it is important to prevent recurrent hospital  
7 admissions, re-transplantations or – in case of kidney transplantation – return to dialysis,  
8 which are all associated with very high expenses.<sup>12</sup> To effectively develop interventions to  
9 reduce mortality and morbidity after transplantation, more research is necessary on clinical  
10 and biochemical risk factors present in transplant recipients. Also, the use of living donors for  
11 kidney and liver transplantation requires a living donor program with good long-term  
12 outcomes for the donor and recipient. Living kidney donors, for example, have an increased  
13 risk for end-stage renal disease (ESRD)<sup>13,14</sup>, while only registry data exist on the effect of  
14 living donor characteristics on recipient outcomes.<sup>15 16</sup>

15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
Until now, many registries and large cohort studies focus on one type of solid organ  
transplantation, limiting comparability between different transplant populations. As a result,  
studies investigating biomarkers, quality of life, and development of health problems and  
adverse outcomes across different solid organ transplant populations are scarce. Despite the  
differences which exists in patient characteristics and treatment after different solid organ  
transplantations, there are many similarities in health problems that occur among subtypes of  
transplantation. The objective of TransplantLines study is to identify risk factors for  
development of long-term health problems after transplantation and to develop new  
interventions to improve outcome, both combined for all solid organ transplant recipients as  
well as specific for each subtype of transplantation.

## Methods/Design

### Study design and setting

1  
2  
3 The TransplantLines study is a unique, novel prospective biobank and cohort study, which  
4 aims to provide a better understanding of causes of disease- and ageing-related outcomes and  
5 health problems, both physical and psychological, in solid organ transplant recipients and  
6 donors (ClinicalTrials.gov Identifier: NCT03272841). The University Medical Center  
7 Groningen (UMCG) is the largest transplantation center in the Netherlands, and the only  
8 Dutch center that covers all types of solid organ transplantation, as well as living kidney and  
9 liver donation programs. The study protocol has been approved by the Institutional Review  
10 Board (METc 2014/077), adheres to the UMCG Biobank Regulation, and is in accordance  
11 with the WMA declaration of Helsinki and the declaration of Istanbul. All participants will  
12 give written informed consent upon enrollment. Follow-up and prospective events will be  
13 recorded over time. An overall participation rate of 85% is expected across the different  
14 transplant populations and a total number of 3000 participants is aimed.

### 30 **Transplant patients**

31  
32 The study population comprises all solid organ transplant recipients, i.e. heart-, lung-, kidney-  
33 , liver-, and small bowel transplant recipients. Both new transplant candidates as well as  
34 transplant recipients are eligible to participate in the study. Participants of all ages will be  
35 included in TransplantLines. Children (age <18 years) will be eligible for participation upon  
36 consent by a legal representative (<12 years) or a shared consent of both the child and legal  
37 representatives ( $\geq 12$  years). The study will also include candidates for re-transplantation.  
38 Exclusion criteria for participation in the TransplantLines study will be no mastery of the  
39 Dutch language or no capability to intellectually comprehend questionnaires or physical tests.  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

### 51 **Living donors**

1  
2  
3 Living kidney and liver donors will also be included in the study. The goal of including  
4 donors is to study the effects of donation, improve living donor safety and donors will serve  
5 as controls for their recipients, allowing for matched longitudinal analyses. Prospective living  
6 kidney and liver donor candidates ( $\geq 18$  years old) will be eligible to participate in the study,  
7 as well as living organ donors who have donated an organ prior to the start of the  
8 TransplantLines study. Exclusion criteria will be no mastery of the Dutch language or no  
9 capability to intellectually comprehend questionnaires or physical tests.  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19

### 20 **Transplant recipients timeline**

21 All participants of the TransplantLines study will be examined at fixed time points as shown  
22 in **Figure 2**. Transplant candidates will be first seen at pre-transplant screening. Prior to  
23 transplantation, all transplant candidates undergo a routine clinical screening. Generally,  
24 transplant candidates will be transplanted if surgery risks and transplant benefit are optimized,  
25 based on an individualized multidisciplinary clinical decision. Further study visits will be  
26 performed at time of transplantation, at 3 months, 6 months, 12 months, and 2 years after  
27 transplantation, and hereafter follow-up will be performed every 5 years.  
28  
29  
30  
31  
32  
33  
34  
35  
36

37 Transplant recipients with a functional graft for at least 1 year post-transplantation and  
38 who received a solid organ transplant prior to the start of the TransplantLines study will be  
39 included at the next outpatient clinic visit. Henceforth, patients will be examined every five  
40 years and follow-up samples will be collected. Aside from the fixed time points, biobank  
41 samples of transplant recipients will be collected if a biopsy of the transplanted solid organ is  
42 necessary, on clinical indication.  
43  
44  
45  
46  
47  
48  
49  
50  
51

### 52 **Living donors timeline**

1  
2  
3 All donors of the TransplantLines study will be examined at fixed time points as shown in  
4 **Figure 2**. The first study visit of donor candidates will occur at pre-donation screening. Prior  
5 to donation, all candidates undergo a routine clinical screening. Generally, donors will be  
6 accepted if surgery risks and transplant benefit are optimized, based on an individualized  
7 multidisciplinary clinical decision taking national and international guidelines into  
8 account.<sup>17,18</sup> Subsequently, study visits will be performed at time of nephrectomy, and at 3  
9 months post-donation. At 12 months post-donation, donors will fill in a questionnaire and at 5  
10 and 10 years post-donation there will be another study visit. Hereafter follow-up will be  
11 performed every five years. Living organ donors who have donated an organ prior to the start  
12 of the TransplantLines study, will be included at their next donor follow-up visit to their  
13 outpatient clinic.  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28

## 29 **Data collection**

### 30 ***Biobank***

31 Blood, 24-hour urine, feces, nails, and hair will be collected of participants at each  
32 TransplantLines visit. Participants will be instructed to collect a 24-hour urine sample  
33 according to strict protocol at the day before their visit to the outpatient clinic, i.e. discard  
34 their morning urine specimen, collect all subsequent urine throughout the next 24 hour and  
35 include the next morning's first specimen of the day of the visit to the outpatient clinic. Blood  
36 will be drawn after an 8-12 hour overnight fasting period in the morning after completion of  
37 the 24-hour urine collection.  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47

48 As blood samples, 1 serum tube of 10 mL, 2 EDTA samples of 10 mL, 1 citrate tube  
49 of 6 mL, 1 lithium-heparin tube of 10 mL, and 1 PAXgene tube of 10 mL will be collected of  
50 each participant at each TransplantLines visit. Subsequently, tubes will be centrifuged at  
51 1300g for 10 minutes, except the citrate tube which is centrifuged at 2500g for 10 minutes. Of  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 the 24-hour urine collection, three urine tubes will be collected of which one tube will be  
4 partially acidified. All blood- and urine samples will be subsequently aliquoted and shipped to  
5 the core laboratory for storage in -80°C (-112 °F) freezers (Panasonic, 's-Hertogenbosch, the  
6 Netherlands) (**Table 1**). Blood- and urine samples will be analyzed in the following years for  
7 multiple research questions that will arise.  
8  
9  
10  
11  
12

13  
14 Participants will be asked to collect a feces sample the day prior to the  
15 TransplantLines visit. A FecesCatcher (TAG Hemi VOF, Zeijen, the Netherlands) will be sent  
16 at the patients' home, and feces sample will be collected in appropriate tubes and frozen  
17 immediately after collection. The participant will transport the feces sample in cold storage  
18 (with ice cubes or in a cooler) to the TransplantLines visit the following day. Subsequently,  
19 the feces sample will be immediately stored at -80°C (-112 °F). Feces samples will be  
20 primarily used for microbiome analyses. Solid organ transplant recipients have a shift in the  
21 gut microbiome with a decrease in predominant organisms, a loss of bacterial diversity, and  
22 emergence of new dominant population. This may result in increased risk of infection,  
23 rejection, and mortality. Therefore, we would like to examine the gut microbiome in relation  
24 to the development of health problems after transplantation.  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40

### ***Clinical and laboratory characteristics***

41  
42 Clinical laboratory measurements requested by the physician will be included in the study  
43 database upon patient consent. Demographic characteristics along with data on medication  
44 use will be provided by the participants and will be verified using the electronic hospital  
45 records. Medical information including donor and recipient information at time of  
46 transplantation, underlying disease, hospital admissions, complications after transplantation,  
47 co-morbidities, graft failure, and mortality will be extracted from the electronic hospital  
48 records.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

### *Questionnaires*

Biobank data will be expanded with an extensive set of questionnaires to collect data on physical, psychological, and social impact of undergoing a transplantation (**Figure 3**). Transplant candidates will be asked to fill out a comprehensive questionnaire during screening prior to transplantation and at 1 year post-transplantation. Transplant recipients with a functional graft for more than 1 year post-transplantation and who received the solid organ prior to start of the TransplantLines study will be asked to complete the same questionnaire. A subset of questionnaires will be provided at the other predefined time-points, i.e. at 3, at 6 months, and at 2 years after transplantation. Topics addressed by questionnaires include among others nutritional intake and diet, health-related quality of life, life-style factors such as physical activity, sleep quality, and smoking behavior, psychological impact such as anxiety, depression, coping, and well-being, and social impact such as employment and family relationships. Specification of all the different questionnaires with related subject is shown in **Table 2**. Questionnaires will be send digitally or by mail, as requested. During study visits, all questionnaires will be checked by a trained investigator for completeness and validity.

### *Standard assessments*

Blood pressure (mmHg) will be measured according to a standard clinical protocol using an automatic device (Philips Suresign VS2<sup>+</sup>, Andover, MA, USA). To prevent a white-coat effect, participants will be seated during which blood pressure and heart rate will be measured four times, with an interval of three minutes between measurements. Hereafter, participants will be asked to stand up straight for one minute, after which blood pressure and heart rate measurements will be repeated once in standing position. Measurements will be performed

1  
2  
3 with participants being on their regular medication, including anti-hypertensive drugs at  
4  
5 trough.

6  
7 Anthropometry measurements will include body weight, body length, and waist- and  
8  
9 hip circumference. Body weight (kg) will be measured in light weight clothing without shoes  
10  
11 using a calibrated digital measuring scale (SECA 877, Seca GMBH, Hamburg, Germany).  
12  
13 Height (cm) will be measured using a wall-secured stadiometer (SECA 222). Waist- and hip  
14  
15 circumference (cm) will be calculated using a measuring tape roll with standardized retraction  
16  
17 mechanism (SECA 201). Waist circumference will be measured midway between the lowest  
18  
19 rib and the iliac crest with the participant in standing position. Hip circumference will be  
20  
21 determined at the maximum circumference over the trochanter major. All anthropometry  
22  
23 measurements will be assessed twice, with inclusion of a third measurement contingent upon  
24  
25 a difference of more than half a kilogram in weight or more than one centimeter in length.  
26  
27

28  
29 Handgrip strength will be assessed with the Jamar Hydrolic Hand Dynamometer  
30  
31 (Patterson Medical JAMAR 5030J1, Warrentville, Canada).<sup>19</sup> Participants will be instructed to  
32  
33 sit in a chair with their shoulders in adduction, their arms rotated into neutral position, their  
34  
35 elbows flexed to 90°, and forearms and wrists held in neutral position. Hereafter, participants  
36  
37 will be instructed to perform a maximal isometric contraction. Handgrip strength will be  
38  
39 tested three times with an interval of 30 seconds rest for recovery between each attempt. The  
40  
41 dominant hand will be stated in all measurements. Furthermore, to create uniformity among  
42  
43 assessments, the second handle position of the hand dynamometer will be utilized which has  
44  
45 been shown to be the most accurate position.<sup>20</sup>  
46  
47

48  
49 Lung function will be measured by means of an Asma-1 handheld spirometer  
50  
51 (Vitalograph, Buckingham, United Kingdom).<sup>21</sup> Of all participants, the Forced Expiratory  
52  
53 Volume (FEV1), as marker of lung function, will be recorded.  
54  
55  
56  
57  
58  
59  
60



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Body composition will be determined using a multifrequency bio-electrical impedance device (BIA, Quadscan 4000, Bodystat Ltd, Douglas, British Isles) at 5, 50, 100, and 200 Hz, which allows to distinct between lean body mass and fat body mass taking into account differences in volume status.<sup>22</sup> Main outcome variables from the BIA are estimated fat mass, fat free mass, and body fat percentage. In brief, the BIA measurement will be performed with the participant in supine position with arms and legs abducted from the body. Sensor electrodes will be placed on the dorsum of the right hand and feet, with a minimal distance of five centimeters between the electrodes. Measurement will not be executed if the participant has a temperature exceeding 37.9°C/100.2°F or has a functioning ICD/pacemaker.

Advanced glycation endproducts (AGEs) will be determined using an AGE reader SU (DiagnOptics Technologies, Groningen, The Netherlands).<sup>23</sup> The AGE reader SU measures skin autofluorescence (AF) by using the characteristic fluorescent properties of certain AGEs to estimate the level of AGEs accumulation in the skin. AGEs have been implicated in the pathogenesis of vascular damage and cardiovascular disorders and aid to characterize the cardiovascular risk profile of transplant recipients.<sup>24</sup>

Transplant recipients are known to be at increased risk for cutaneous malignancies, mainly related to long-term use of immunosuppressive medication.<sup>25</sup> To identify which transplant recipients are especially prone to develop dermatological health problems, a detailed dermatological history with emphasis on malignancies and subsequent treatment will be obtained. Next, a standardized dermatological examination will be performed by the trained investigator. The dermatological examination includes the determination of eye color, natural hair color at adolescence and skin type according to the classification of Fitzpatrick.<sup>26</sup> In addition, the presence and quantity of lentigines, moles, freckles, and warts are examined.

To assess frailty, the Clinical Frailty Scale (CFS) will be scored at study visits by the trained investigator. The CFS is a validated frailty measurement and frailty is scored based on



1  
2  
3 clinical judgment on a continuous scale from 1 (very fit) to 9 (terminally ill). A CFS-score of  
4  
5  $\geq 5$  is generally considered to be frail.<sup>27</sup>  
6

7 To assess nutritional status, a Patient-Generated Subjective Global Assessment (PG-  
8 SGA, PT-Global, Philadelphia, USA) will be scored.<sup>27,28</sup> The PG-SGA is an patient-centered  
9 adaptation of the original Subjective Global Assessment (SGA). The different domains  
10 assessed by the PG-SGA are: 1) changes in body weight, 2) changes in nutritional intake, 3)  
11 symptoms which negatively influence intake, absorption, and utilization of nutrients, 4) level  
12 of activities and function, 5) conditions that increase nutritional risk or requirements, 6)  
13 metabolic stress, and 7) physical examination. Based on the PG-SGA score, subjects can be  
14 classified as well-nourished, moderately malnourished or severely malnourished.  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25

### 26 **Physical protocol**

27  
28 In addition to standard assessments, participants in the “physical” arm of the protocol will be  
29 asked to accomplish a standing balance test, a 2-Minute Walk Test (2MWT), a 4-Meter Walk  
30 Test (4MWT), a dexterity test, a Five Time Sit To Stand test (FTSTS), Timed Up and Go test  
31 (TUG), a rigorous neurological examination, and a breath analysis. With inclusion of the first  
32 four tests together with the handgrip strength, the five physical components of the National  
33 Health Institute Toolbox for motor assessment are being assessed.<sup>29</sup>  
34  
35  
36  
37  
38  
39  
40

41 The *standing balance test* will be performed with an accelerometer (Axivity,  
42 Newcastle, United Kingdom), attached to the lower back. The standing balance test has been  
43 described in detail previously.<sup>29</sup> Balance will be evaluated in 5 different positions, i.e. 1) feet  
44 together on hard surface, eyes open; 2) feet together on hard surface, eyes closed; 3) feet  
45 together on foam surface (Balance Pad Elite; Airex Specialty Foams, Aargau, Switzerland),  
46 eyes open; 4) feet together on foam surface, eyes closed, and 5) feet in tandem stance, eyes  
47 open. Participants will be asked to have arms crossed on their chest and each position will be  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 tested for 50 seconds. Upon failure, with recording of time to failure, a second attempt will be  
4 performed. In case of non-success at the second attempt, the test will be discontinued.

5  
6  
7 Endurance will be tested with a *2MWT*.<sup>30</sup> The 2MWT has been shown to be highly  
8 correlated, without compromising validity and reliability, with the 6-minute walking test, an  
9 important submaximal exercise test.<sup>31,32</sup> To calculate distance covered by subjects on the  
10 2MWT, two pylons are set apart 15 meters and subjects are instructed to walk as fast as  
11 possible without running, until the investigator commands to stop. Participants are updated on  
12 the remaining time after 1.00 and 1.45 minutes, and the final five seconds are indicated by a  
13 countdown. The total walking distance in 2 minutes is recorded in total meters covered with  
14 remaining scored in centimeters.

15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
Locomotion, measured as gait speed, will be tested with a *4MWT*. Gait speed is a  
simple measure to summarize the overall disease burden and disability.<sup>33,34</sup> In brief, two  
pylons will be set apart 4 meters and instructed to walk at usual pace. Seconds from start to  
end of the 4 meters will be recorded. The 4MWT is measured twice after first a trial round.

Manual dexterity will be measured in all transplant recipients using the *9-Hole Peg Test* (9-HPT, Sammons Preston Rolyan, Chicago, IL). The 9-HPT requires participants to repeatedly place and remove nine pegs into nine holes, one at a time, as quickly as possible, and is considered to be the gold standard metric for manual dexterity.

Functional mobility will be tested in participants using the *FTSTS* and *TUG*. The FTSTS is a functional performance measure of leg strength or the force-generating capacity of muscle by using the body's weight for resistance during functional activities.<sup>35</sup> The FTSTS will be executed three times after a first trial round. Participants will be instructed to stand up five times as fast as possible, from sitting position with their feet flat on the floor and arms folded across the chest. Measurements start upon command, and subsequently the time

1  
2  
3 required to stand up and return sitting is recorded. Time is measured in seconds and this task  
4  
5 is repeated five times.<sup>36</sup>

6  
7 The TUG is a basic test for functional mobility and is based on strength, coordination,  
8  
9 and balance.<sup>37</sup> For the test, a pylon and chair will be put apart 3 meters. The test will be  
10  
11 performed four times, with the first round being a trial. Participants are instructed to stand up  
12  
13 from the chair without support of the arms, subsequently walk with their normal gait speed  
14  
15 around the pylon, and go back to the chair to sit down again. In case, participants use a  
16  
17 walking aid in normal day life, the test will be performed with the use of a walking aid. The  
18  
19 TUG is measured in seconds, from the moment the participant is instructed to get up until the  
20  
21 moment the participant sits down again.  
22  
23

24  
25 Transplant recipients have an increased susceptibility to develop peripheral  
26  
27 neuropathy and tremor, mainly due to the continuous use of immunosuppressive medication,  
28  
29 especially calcineurin inhibitors.<sup>38,39</sup> Therefore, an extensive neurological examination will be  
30  
31 performed and will consist of strength testing, classifying polyneuropathy, and tremor  
32  
33 quantification. Detailed strength testing of different muscle groups (feet flexion/extension, hip  
34  
35 flexion, biceps flexion and wrist extension) will be performed with a digital dynamometer  
36  
37 (C.I.T. Technics, Haren, the Netherlands).<sup>40</sup> Hereafter, sensibility tests will be performed  
38  
39 using a pin-prick and monofilament pen (Novo Norisk BV, Alphen aan de Rijn, the  
40  
41 Netherlands) on bare skin five times per measurement at the dorsal side of the 1<sup>st</sup> phalange of  
42  
43 both feet with the subject closing their eyes. Upon failure of sensibility, the dorsal side of the  
44  
45 foot and lower limb will be tested. Proprioception will be measured by moving the 1<sup>st</sup>  
46  
47 phalange of both feet in dorsal flexion and plantar flexion five times with the participants  
48  
49 closing their eyes. Upon failure, the dorsal side of the foot and index finger will be measured.  
50  
51

52  
53 Vibration sense will be measured using a handheld biothesiometer (Bio Medical  
54  
55 Instrument Co, Ohio, USA).<sup>41</sup> The biothesiometer has a rubber tractor that vibrates at 100 Hz  
56  
57  
58  
59

1  
2  
3 when operating from 50 Hz mains. In brief, participants will be measured in a supine position  
4 on a bed barefooted. The vibrating tractor will be applied bi-laterally to four different  
5 measurement points of the participants: top of the hallux, forefoot, lateral malleoli, and wrist.  
6  
7 Before applying the vibrating tractor to the points to be tested, the amplitude of the vibrating  
8 tractor is increased from zero to the point where the vibration is perceptible and beyond the  
9 threshold to the highest amplitude possible to familiarize participants with the sensation. For  
10 the measurement, the participants will be asked to concentrate on the test and report the first  
11 sensation of the vibration by saying “Stop”. Each measurement point is tested twice. If the  
12 difference between the first two measurements is greater than 20%, the measurement point is  
13 tested a third time.  
14  
15

16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Prior to tremor quantification, participants will be asked to complete part C of the  
Fahn-Tolosa-Marin tremor rating scale that involves tremor-related functional disability.<sup>42</sup>  
The questionnaire involves speaking, bringing liquids to the mouth, eating, hygienic care,  
dressing, writing, work and household related tasks. The questionnaire uses a 5-point scale,  
with ‘0= no functional ability’, and 4= ‘severe disability, the task cannot be executed’.

To quantify tremor, two accelerometers (University Medical Center Groningen,  
Groningen, the Netherlands) will be attached to the dorsal side of both hands. The  
accelerometers will record movement in the coronal, transversal and sagittal planes as well as  
linear acceleration and deceleration in both hands continuously during the measurements.  
Amplitudes and frequency of these measurements will be recorded on a stand-alone computer.  
Participants will be asked to assume seven different positions while seated, which are  
measured for 30 seconds each: arms down, wrists extended; arms forward, wrists and fingers  
relaxed; arms forward, wrists and fingers in 0 position; index fingers pointed towards each  
other; bilateral finger-nose task; weighted arms down with wrists expanded; weighted arms  
forward with wrists and fingers extended.

1  
2  
3 Finally, participants will be asked for collection of a breath sample in which hydrogen  
4 and methane will be measured with the Quintron BreathTracker (Milwaukee, Wisconsin,  
5 USA).<sup>43</sup> Both hydrogen and methane are exclusively formed by anaerobic fermentation in the  
6 gut, and therefore can be utilized as markers for metanogenic microflora in transplant  
7 recipients.<sup>44</sup>  
8  
9  
10  
11  
12

### 13 14 15 **Cognitive protocol**

16 Participants randomized into the “cognitive” arm of the study protocol undergo a series of  
17 neuropsychological tests performed by a trained neuropsychologist or masterstudent  
18 neuropsychology under supervision of a trained neuropsychologist. The tests are administered  
19 in a quiet room with no disturbances. For timed tests, a digital clock is used. The tests are  
20 performed in a fixed order and no feedback regarding the results is given to the participant  
21 during administration. An overview of the neuropsychological tests is specified in **Table 3**.  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32

### 33 **Neuropsychological tests**

34 The *Cognitive Screening Test (CST)* is a Dutch screening test for dementia, measuring  
35 orientation in time and place, and memory for common facts.<sup>45</sup> The questionnaire consists of  
36 20 items (e.g. date of birth, name of the reigning monarch, season) and the score is calculated  
37 as the total of questions answered correctly with a maximum of 20.  
38  
39  
40  
41  
42  
43

44 *Nederlandse Leestest voor Volwassenen (NLV)*, Dutch version of the National Adult  
45 Reading Test. The participant has to read aloud a list of 50 irregularly spelled words. The total  
46 score on the test is converted into an estimation of the premorbid intelligence quotient.<sup>46</sup>  
47  
48  
49

50 The *Clock Drawing Test (CDT)* is a cognitive screening instrument.<sup>47</sup> Participants are  
51 asked to draw a clock and set the time to ‘a quarter to two’. A maximum total score of 14 can  
52 be achieved.  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 The *15 Words Test* (Dutch version of the Rey Auditory Verbal Learning Test  
4 (RAVLT), measures verbal memory.<sup>48</sup> In this task a set of 15 unrelated words is presented to  
5 the participant, consecutively over five trials. Participants are asked to recall as many words  
6 as possible immediately after each trial (Immediate Recall). The score is the total words  
7 recalled in 5 trials, with a maximum of 75. After 20 minutes, participants are asked to recall  
8 as many of the 15 words as possible (Delayed Recall). Additionally, a recognition task will be  
9 performed. Participants are presented with a list of 30 words and are asked which words they  
10 recognize from the list they have been presented before.  
11  
12  
13  
14  
15  
16  
17  
18  
19

20 *Digit Span*, subtest of the Wechsler Adult Intelligence Scale (WAIS-IV).<sup>49</sup> This  
21 subtest consists of two tasks, the Digit Span Forward and the Digit Span Backward. The Digit  
22 Span Forward is a task for immediate auditory memory span. In this task, participants are  
23 asked to repeat a series of numbers in the same order as the examiner did. The Digit Span  
24 Backward measures working memory. Participants have to repeat the presented numbers in  
25 reversed order. The score is the total strings repeated, with a maximum of 32.  
26  
27  
28  
29  
30  
31  
32

33 The *Word Fluency*, subtest of the Groninger Intelligentie Test (GIT-2), is a verbal task  
34 measuring semantic memory.<sup>50</sup> Participants are asked to name as many words within a certain  
35 category within one minute. Total score per category (respectively animals and professions)  
36 were calculated.  
37  
38  
39  
40  
41

42 The *Controlled Oral Word Association Test (COWAT)* is a verbal task measuring  
43 executive control.<sup>51</sup> Participants have to name as many words as possible that start with a  
44 specific letter within one minute. In the meantime, participants have to comply to several  
45 rules that are given on beforehand. Total scores from three different starting letters (D-A-T)  
46 were calculated.  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 The *Symbol Digit Modalities Test (SDMT)* measures psychomotor speed.<sup>52</sup> The test  
4 consists of matching symbols and numbers as fast as possible in 90 seconds. The total score of  
5 correct matches is calculated.  
6  
7

8  
9 *Trail Making Test (TMT)*. This test consists of two parts: Trail Making Test – A  
10 (TMT-A) and Trail Making Test – B (TMT-B). Part A is a measure of attention and  
11 information processing speed. This task involves connecting 25 numbers in ascending order,  
12 as quickly as possible. The TMT-B is a measure of divided attention and cognitive flexibility.  
13 In this condition, numbers as well as letters have to be connected in ascending order,  
14 alternating between numbers and letters (1-A-2-B- etc.). Both parts of the test are timed to  
15 completion (number of seconds).  
16  
17  
18  
19  
20  
21  
22  
23

24 The *Key Search Test* is a subtest of the Behavioral Assessment of the Dysexecutive  
25 Syndrome (BADS) and assesses the ability to plan and monitor progress. Participants are  
26 presented with a square which represents a field in which ‘keys have been lost’. Participants  
27 must show how they would search the field to find the keys. Searching strategy is scored by  
28 means of functionality and maximum total score of 16 can be achieved.  
29  
30  
31  
32  
33  
34  
35  
36

### 37 **Outcomes**

38 The primary outcomes of the TransplantLines study are all-cause mortality and graft failure,  
39 which is defined as death due to failure of the transplanted organ, return to organ replacement  
40 therapy or re-transplantation. Secondary outcomes will be cause-specific mortality, cause-  
41 specific graft failure and rejection. Tertiary outcomes will be other health problems, including  
42 diabetes, obesity, hypertension, hypercholesterolemia, and cardiovascular disease, and  
43 disturbances that relate to quality of life, e.g. physical and psychological functioning, quality  
44 of sleep, and neurological problems such as tremor and polyneuropathy.  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 The TransplantLines biobank study aims to identify risk factors for health problems  
4 and patient-centered outcomes (e.g. adverse drug events, lifestyle, quality of life, social  
5 participation, physical and cognitive functioning). Due to the nature of the biobank, not all  
6 research questions are predefined and will arise during the course of inclusion. In contrast to  
7 many other studies, TransplantLines also aims to identify and ameliorate complaints  
8 experienced by transplant recipients, such as tremors and diarrhea, which to date have largely  
9 been overlooked by clinicians.  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19

### 20 **Data management and analysis**

21 Data will be recorded digitally in an electronic case report form (eCRF) in a certified  
22 Electronic Data Capture and Clinical Data Management System (Utopia Data Management  
23 System version 1.13.6, Research Data Support, University Medical Center Groningen). All  
24 data are checked by trained researchers and are subsequently stored anonymously in a secured  
25 electronic environment. The TransplantLines database will be linked to registries and  
26 databases of the Dutch Health Database (DHD), Netherlands Comprehensive Cancer  
27 Organisation (IKNL), Central Bureau of Statistics (CBS), InterAction Database (IADB),  
28 Dutch Nephrology Registration/Registration Renal Replacement Therapy (Nefrovisie,  
29 Renine), Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands  
30 (PALGA), National Organ Transplant Registry (NOTR), PHARMO Institute for Drug  
31 Outcome Research (PHARMO), Routine Outcome Monitoring (RoQua) and the Dutch  
32 Institute of Clinical Auditing database (DICA) through a generic layer. A data management  
33 board will be formed to maintain data infrastructure, construct Material Transfer Agreements  
34 (MTA) and to govern use of the TransplantLines biobank and database. Extractions from  
35 TransplantLines database will be performed using a retrieval suite in Utopia software package  
36 only after approval of the data management board. Data will always be extracted  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 anonymously. SPSS statistics version 23 (IBM, Armonk, NY), R version 3.2.3 (CRAN,  
4 Vienna, Austria), STATA 14.1 (STATA Corp., College Station, TX) or a similar statistical  
5 package will be used for analysis. Data collection and management is performed in  
6  
7 accordance with the Handbook for Adequate Natural Data Stewardship (Netherlands  
8  
9 Federation of University Medical Centers, 2017).  
10  
11  
12  
13  
14

## 15 **Discussion**

16  
17 The TransplantLines prospective cohort study seeks to identify risk factors for the  
18 development of long-term health problems after transplantation and ultimately to develop new  
19 and innovative interventions to improve graft survival, patient survival, and quality of life  
20 after transplantation. The TransplantLines biobank will encompass all solid organ  
21 transplantations and living organ donors. It will consist of follow-up data from all fields that  
22 are involved in organ transplantation; internal medicine, surgery, gastroenterology,  
23 hepatology, pulmonology, cardiology, dermatology, neurology, occupational medicine,  
24 children's medicine, (neuro)psychology, physiotherapy, and social work.  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34

35 Although short-term transplant outcomes have improved in the last decades, graft and  
36 recipient life expectancy remains limited. In the TransplantLines study, data and samples will  
37 be collected before, during, and after transplantation to gather further insight in the impact of  
38 transplantation on transplant recipients. In addition, we aim to preemptively detect those  
39 transplant recipients who are at increased risk to develop graft failure or health problems. By  
40 investigating a wide range of clinical, social/psychological and biochemical parameters, this  
41 study aims to contribute to increased transplant survival, patient survival, but also to an  
42 increased quality of life and a more patient-centered approach to transplant care.  
43  
44  
45  
46  
47  
48  
49  
50  
51

52 Our study has strengths and limitations. The major strengths of this study are the  
53 collection of extensive data on a myriad topics related to transplantation, the inclusion of all  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 types of solid organ transplant recipients and living organ donors, and a study with a long  
4 follow-up to assess many relevant clinical outcomes. Limitations of the current study are that  
5 it comprises a single center study and that residual confounding cannot be excluded in  
6 analyses in the TransplantLines study due to its observational design.  
7  
8  
9

10  
11 TransplantLines may serve as a basis for hypothesis-generating studies that yield  
12 insights in a wide range of clinical, social/psychological and biochemical parameters in solid  
13 organ transplant recipients, as well as living donors. Biomarkers may be identified to develop  
14 more individualized immunosuppressive treatment. This will lead to novel clinical trials in  
15 transplantation and patient-tailored approaches for new treatment options. Furthermore, the  
16 results of TransplantLines may serve to identify new modifiable risk factors and lifestyle  
17 factors in transplantation. Ultimately, this information will likely contribute to a more  
18 individualized treatment for transplant patients and improved living donor screening and  
19 follow-up. Thereby we aim to qualitatively and quantitatively improve outcomes after  
20 transplantation.  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34

### 35 **Study status**

36 Data collection is in progress.  
37  
38  
39  
40

### 41 **List of abbreviations used**

42 METc – Medical Ethical Committee  
43

44 UMCG – University Medical Center Groningen  
45

46 EDTA - Ethylenediaminetetraacetic acid  
47

48 SOP – Standard Operating Procedure  
49

50 BIA – Bio-Impedance Analysis  
51

52 ICD – Implantable Cardioverter-Defibrillator  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 SAF – Skin Auto-Fluorescence

4 AGE – Advanced Glycation End-product

5  
6  
7 PG-SGA - Scored Patient-Generated Subjective Global Assessment

8  
9 2MWT – 2-Minute Walk Test

10  
11 FTSTS – Five Time Sit To Stand Test

12  
13 TUG – Timed Up and Go

14  
15 CRF – Clinical Research Form

16  
17  
18 PI – Principal Investigator

19  
20  
21  
22 **Author contributions**

23  
24 M.F.E. designed the study, conducts the study for transplant recipients, wrote the manuscript,  
25 and is responsible for final content of the manuscript; A.W.G.N. conducts the study for  
26 transplant recipients and revised the manuscript; M.vL. conducts the study for living donors  
27 and wrote the part of living donors in the manuscript; A.L.Z. conducts the study for  
28 transplant recipients, living donors and wrote the cognitive protocol; R.M.D., S.P.S, T.J.K.,  
29 and M.C.J.O. conduct the study for transplant recipients; N.R.H. conducts the study for living  
30 donors; M.J.S., C.A., E.R., J.M.S., R.A.P., G.D., R.J.P., H.G.D.L., K.D., E.A.M.V., V.E.dM  
31 gave input from their field of knowledge when designing the study and revised the  
32 manuscript; R.A.P. is responsible for kidney biobank sampling and logistics; H.B. conducts  
33 the study and revised the manuscript; S.J.L.B. designed the study, is principal investigator and  
34 project leader, and takes full responsibility for the whole study. All collaborators have revised  
35 the manuscript and approved the final version of the manuscript.  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

51  
52 **Acknowledgements**  
53  
54  
55  
56  
57  
58  
59  
60

1  
2 We thank prof. John Mathers and dr. José Lara from Newcastle University for aid in setting  
3  
4 up a test package for the TransplantLines study based on the Healthy Ageing Phenotype  
5  
6 measurements.  
7  
8  
9

### 10 11 **Funding information**

12 This work was supported by a grant from Astellas BV  
13  
14  
15

### 16 17 **Competing interests**

18 None  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## References

1. Kirk R, Dipchand AI, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dobbles F, Rahmel AO, Stehlik J, Hertz MI, International Society for Heart and Lung Transplantation. The Registry of the International Society for Heart and Lung Transplantation: fifteenth pediatric heart transplantation report--2012. *J Heart Lung Transplant*. 2012; 31: 1065-1072.
2. Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: a critical reappraisal. *Am J Transplant*. 2011; 11: 450-462.
3. Lodhi SA, Lamb KE, Meier-Kriesche HU. Solid organ allograft survival improvement in the United States: the long-term does not mirror the dramatic short-term success. *Am J Transplant*. 2011; 11: 1226-1235.
4. Dharnidharka VR, Lamb KE, Zheng J, Schechtman KB, Meier-Kriesche HU. Lack of significant improvements in long-term allograft survival in pediatric solid organ transplantation: A US national registry analysis. *Pediatr Transplant*. 2015; 19: 477-483.
5. Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med*. 2000; 342: 605-612.
6. Aasebo W, Homb-Vesteraas NA, Hartmann A, Stavem K. Life situation and quality of life in young adult kidney transplant recipients. *Nephrol Dial Transplant*. 2009; 24: 304-308.
7. Kugler C, Fischer S, Gottlieb J, Tegtbur U, Welte T, Goerler H, Simon A, Haverich A, Strueber M. Symptom experience after lung transplantation: impact on quality of life and adherence. *Clin Transplant*. 2007; 21: 590-596.

- 1  
2  
3 8. Matas AJ, McHugh L, Payne WD, Wrenshall LE, Dunn DL, Gruessner RW, Sutherland  
4 DE, Najarian JS. Long-term quality of life after kidney and simultaneous pancreas-kidney  
5 transplantation. *Clin Transplant*. 1998; 12: 233-242.  
6  
7  
8  
9  
10 9. Morath C, Mueller M, Goldschmidt H, Schwenger V, Opelz G, Zeier M. Malignancy in  
11 renal transplantation. *J Am Soc Nephrol*. 2004; 15: 1582-1588.  
12  
13  
14  
15 10. Rodrigo E, Fernandez-Fresnedo G, Valero R, Ruiz JC, Pinera C, Palomar R, Gonzalez-  
16 Cotorruelo J, Gomez-Alamillo C, Arias M. New-onset diabetes after kidney transplantation:  
17 risk factors. *J Am Soc Nephrol*. 2006; 17: S291-5.  
18  
19  
20  
21  
22 11. Jezior D, Krajewska M, Madziarska K, Kurc-Darak B, Janczak D, Patrzalek D,  
23 Boryslawski K, Klinger M. Posttransplant overweight and obesity: myth or reality?  
24 *Transplant Proc*. 2007; 39: 2772-2775.  
25  
26  
27  
28  
29 12. Held PJ, McCormick F, Ojo A, Roberts JP. A Cost-Benefit Analysis of Government  
30 Compensation of Kidney Donors. *Am J Transplant*. 2016; 16: 877-885.  
31  
32  
33  
34 13. Mjoen G, Hallan S, Hartmann A, Foss A, Midtvedt K, Oyen O, Reisaeter A, Pfeffer P,  
35 Jenssen T, Leivestad T, Line PD, Ovrehus M, Dale DO, Pihlstrom H, Holme I, Dekker FW,  
36 Holdaas H. Long-term risks for kidney donors. *Kidney Int*. 2014; 86: 162-167.  
37  
38  
39  
40  
41 14. Muzaale AD, Massie AB, Wang MC, Montgomery RA, McBride MA, Wainright JL,  
42 Segev DL. Risk of end-stage renal disease following live kidney donation. *JAMA*. 2014; 311:  
43 579-586.  
44  
45  
46  
47  
48 15. Reese PP, Boudville N, Garg AX. Living kidney donation: outcomes, ethics, and  
49 uncertainty. *Lancet*. 2015; 385: 2003-2013.  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 16. Massie AB, Leanza J, Fahmy LM, Chow EK, Desai NM, Luo X, King EA, Bowring MG,  
4 Segev DL. A Risk Index for Living Donor Kidney Transplantation. *Am J Transplant*. 2016;  
5 16: 2077-2084.  
6  
7  
8  
9  
10 17. Miller CM, Durand F, Heimbach JK, Kim-Schluger L, Lee SG, Lerut J, Lo CM, Quintini  
11 C, Pomfret EA. The International Liver Transplant Society Guideline on Living Liver  
12 Donation. *Transplantation*. 2016; 100: 1238-1243.  
13  
14  
15  
16  
17 18. Trotter JF. Selection of donors and recipients for living donor liver transplantation. *Liver*  
18 *Transpl*. 2000; 6: S52-8.  
19  
20  
21  
22  
23 19. Angst F, Drerup S, Werle S, Herren DB, Simmen BR, Goldhahn J. Prediction of grip and  
24 key pinch strength in 978 healthy subjects. *BMC Musculoskelet Disord*. 2010; 11: 94-2474-  
25 11-94.  
26  
27  
28  
29  
30 20. Trampisch US, Franke J, Jedamzik N, Hinrichs T, Platen P. Optimal Jamar dynamometer  
31 handle position to assess maximal isometric hand grip strength in epidemiological studies. *J*  
32 *Hand Surg Am*. 2012; 37: 2368-2373.  
33  
34  
35  
36  
37 21. Drew CD, Hughes DT. Characteristics of the Vitalograph spirometer. *Thorax*. 1969; 24:  
38 703-706.  
39  
40  
41  
42  
43 22. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gomez JM, Heitmann BL,  
44 Kent-Smith L, Melchior JC, Pirlich M, Scharfetter H, Schols AM, Pichard C, Composition of  
45 the ESPEN Working Group. Bioelectrical impedance analysis--part I: review of principles  
46 and methods. *Clin Nutr*. 2004; 23: 1226-1243.  
47  
48  
49  
50  
51 23. Mulder DJ, Water TV, Lutgers HL, Graaff R, Gans RO, Zijlstra F, Smit AJ. Skin  
52 autofluorescence, a novel marker for glycemic and oxidative stress-derived advanced  
53  
54  
55  
56  
57  
58  
59  
60

glycation endproducts: an overview of current clinical studies, evidence, and limitations.

*Diabetes Technol Ther.* 2006; 8: 523-535.

24. McIntyre NJ, Fluck RJ, McIntyre CW, Taal MW. Skin autofluorescence and the association with renal and cardiovascular risk factors in chronic kidney disease stage 3. *Clin J Am Soc Nephrol.* 2011; 6: 2356-2363.

25. Zwald FO, Brown M. Skin cancer in solid organ transplant recipients: advances in therapy and management: part I. Epidemiology of skin cancer in solid organ transplant recipients. *J Am Acad Dermatol.* 2011; 65: 253-61; quiz 262.

26. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol.* 1988; 124: 869-871.

27. Rockwood K, Song X, MacKnight C, Bergman H, Hogan DB, McDowell I, Mitnitski A. A global clinical measure of fitness and frailty in elderly people. *CMAJ.* 2005; 173: 489-495.

28. Ottery FD. Definition of standardized nutritional assessment and interventional pathways in oncology. *Nutrition.* 1996; 12: S15-9.

29. Reuben DB, Magasi S, McCreath HE, Bohannon RW, Wang YC, Bubela DJ, Rymer WZ, Beaumont J, Rine RM, Lai JS, Gershon RC. Motor assessment using the NIH Toolbox. *Neurology.* 2013; 80: S65-75.

30. Bohannon RW, Wang YC, Gershon RC. Two-minute walk test performance by adults 18 to 85 years: normative values, reliability, and responsiveness. *Arch Phys Med Rehabil.* 2015; 96: 472-477.



- 1  
2  
3 31. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories.  
4  
5 ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med.* 2002;  
6  
7 166: 111-117.  
8  
9  
10 32. Butland RJ, Pang J, Gross ER, Woodcock AA, Geddes DM. Two-, six-, and 12-minute  
11  
12 walking tests in respiratory disease. *Br Med J (Clin Res Ed).* 1982; 284: 1607-1608.  
13  
14  
15 33. Studenski S, Perera S, Patel K, Rosano C, Faulkner K, Inzitari M, Brach J, Chandler J,  
16  
17 Cawthon P, Connor EB, Nevitt M, Visser M, Kritchevsky S, Badinelli S, Harris T, Newman  
18  
19 AB, Cauley J, Ferrucci L, Guralnik J. Gait speed and survival in older adults. *JAMA.* 2011;  
20  
21 305: 50-58.  
22  
23  
24  
25 34. Perera S, Patel KV, Rosano C, Rubin SM, Satterfield S, Harris T, Ensrud K, Orwoll E,  
26  
27 Lee CG, Chandler JM, Newman AB, Cauley JA, Guralnik JM, Ferrucci L, Studenski SA. Gait  
28  
29 Speed Predicts Incident Disability: A Pooled Analysis. *J Gerontol A Biol Sci Med Sci.* 2016;  
30  
31 71: 63-71.  
32  
33  
34  
35 35. Bohannon RW, Barreca SR, Shove ME, Lambert C, Masters LM, Sigouin CS.  
36  
37 Documentation of daily sit-to-stands performed by community-dwelling adults. *Physiother*  
38  
39 *Theory Pract.* 2008; 24: 437-442.  
40  
41  
42  
43 36. Bohannon RW. Test-retest reliability of the five-repetition sit-to-stand test: a systematic  
44  
45 review of the literature involving adults. *J Strength Cond Res.* 2011; 25: 3205-3207.  
46  
47  
48 37. Kwan MM, Lin SI, Chen CH, Close JC, Lord SR. Sensorimotor function, balance abilities  
49  
50 and pain influence Timed Up and Go performance in older community-living people. *Aging*  
51  
52 *Clin Exp Res.* 2011; 23: 196-201.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 38. Arnold R, Pussell BA, Pianta TJ, Lin CS, Kiernan MC, Krishnan AV. Association  
4 between calcineurin inhibitor treatment and peripheral nerve dysfunction in renal transplant  
5 recipients. *Am J Transplant*. 2013; 13: 2426-2432.  
6  
7  
8  
9  
10 39. Langone A, Steinberg SM, Gedaly R, Chan LK, Shah T, Sethi KD, Nigro V, Morgan JC,  
11 STRATO Investigators. Switching STudy of Kidney TRansplant PATients with Tremor to  
12 LCP-TacrO (STRATO): an open-label, multicenter, prospective phase 3b study. *Clin*  
13 *Transplant*. 2015; 29: 796-805.  
14  
15  
16  
17  
18 40. van der Ploeg RJ, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. *J*  
19 *Neurol Neurosurg Psychiatry*. 1991; 54: 244-247.  
20  
21  
22  
23  
24 41. Frenette B, Mergler D, Ferraris J. Measurement precision of a portable instrument to  
25 assess vibrotactile perception threshold. *Eur J Appl Physiol Occup Physiol*. 1990; 61: 386-  
26  
27  
28  
29 391.  
30  
31  
32  
33 42. Elble R, Bain P, Forjaz MJ, Haubenberger D, Testa C, Goetz CG, Leentjens AF,  
34 Martinez-Martin P, Pavy-Le Traon A, Post B, Sampaio C, Stebbins GT, Weintraub D, Schrag  
35 A. Task force report: scales for screening and evaluating tremor: critique and  
36  
37  
38  
39  
40  
41  
42  
43 43. Tap J, Derrien M, Tornblom H, Brazeilles R, Cools-Portier S, Dore J, Storsrud S, Le Neve  
44 B, Ohman L, Simren M. Identification of an Intestinal Microbiota Signature Associated With  
45  
46  
47  
48  
49  
50  
51  
52  
53 44. Sahakian AB, Jee SR, Pimentel M. Methane and the gastrointestinal tract. *Dig Dis Sci*.  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 45. Graaf Ad, Deelman BG. Cognitieve Screening Test. Handleiding. Lisse: Swets and  
4  
5 Zeitlinger; 1991.  
6  
7  
8 46. Schmand B, Lindeboom J, van Harskamp F. Nederlandse leestest voor volwassenen:  
9  
10 handleiding (NLV). Amsterdam: Pearson Assessment and Information B.V.; 1992.  
11  
12  
13 47. Shulman KI, Shedletsky R, Silver IL. The challenge of time: Clock drawing and  
14  
15 cognitive function in the elderly. *Int J Geriatr Psychiatry*. 1986; 1: 135-140.  
16  
17  
18 48. Saan R, Deelman B. De 15-Woorden Tests A en B. (Een voorlopige handleiding).  
19  
20 Groningen: University Medical Center Groningen, department of Neuropsychology; 1986.  
21  
22  
23 49. Wechsler D. WAIS-IV-NL: Wechsler Adult Intelligence Scale - Fourth Edition -  
24  
25 Nederlandstalige bewerking: Technische handleiding en Afname en scoringshandleiding.  
26  
27 Amsterdam: Pearson Assessment and Information B.V.; 2012.  
28  
29  
30 50. Luteijn F, Barelds D. GIT2: Groninger Intelligentie Test 2. Amsterdam: Pearson  
31  
32 Assessment and Information B.V.; 2004.  
33  
34  
35 51. Schmand B, Groenink SC, den Dungen M. Letterfluency: psychometrische eigenschappen  
36  
37 en Nederlandse normen. *Tijdschr Gerontol Geriatr*. 2008; 39: 64-74.  
38  
39  
40 52. Smith A. Symbol Digits Modalities Test Los Angeles: Western Psychological Services.  
41  
42 Los Angeles: Western Psychological Services; 1968.  
43  
44  
45 53. Hoeymans N, van Lindert H, Westert GP. The health status of the Dutch population as  
46  
47 assessed by the EQ-6D. *Qual Life Res*. 2005; 14: 655-663.  
48  
49  
50 54. Reips UD, Funke F. Interval-level measurement with visual analogue scales in Internet-  
51  
52 based research: VAS Generator. *Behav Res Methods*. 2008; 40: 699-704.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 55. Ware JE, Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I.  
4  
5 Conceptual framework and item selection. *Med Care*. 1992; 30: 473-483.  
6  
7  
8 56. Wendel-Vos GC, Schuit AJ, Saris WH, Kromhout D. Reproducibility and relative validity  
9  
10 of the short questionnaire to assess health-enhancing physical activity. *J Clin Epidemiol*.  
11  
12 2003; 56: 1163-1169.  
13  
14  
15 57. Schafer-Keller P, Steiger J, Bock A, Denhaerynck K, De Geest S. Diagnostic accuracy of  
16  
17 measurement methods to assess non-adherence to immunosuppressive drugs in kidney  
18  
19 transplant recipients. *Am J Transplant*. 2008; 8: 616-626.  
20  
21  
22  
23 58. Dobbels F, Moons P, Abraham I, Larsen CP, Dupont L, De Geest S. Measuring symptom  
24  
25 experience of side-effects of immunosuppressive drugs: the Modified Transplant Symptom  
26  
27 Occurrence and Distress Scale. *Transpl Int*. 2008; 21: 764-773.  
28  
29  
30  
31 59. Beurskens AJ, Bultmann U, Kant I, Vercoulen JH, Bleijenberg G, Swaen GM. Fatigue  
32  
33 among working people: validity of a questionnaire measure. *Occup Environ Med*. 2000; 57:  
34  
35 353-357.  
36  
37  
38 60. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep  
39  
40 Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res*. 1989;  
41  
42 28: 193-213.  
43  
44  
45  
46 61. Marteau TM, Bekker H. The development of a six-item short-form of the state scale of the  
47  
48 Spielberger State-Trait Anxiety Inventory (STAI). *Br J Clin Psychol*. 1992; 31 ( Pt 3): 301-  
49  
50 306.  
51  
52  
53  
54 62. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity  
55  
56 measure. *J Gen Intern Med*. 2001; 16: 606-613.  
57  
58  
59

- 1  
2  
3 63. Gillanders DT, Bolderston H, Bond FW, Dempster M, Flaxman PE, Campbell L, Kerr S,  
4 Tansey L, Noel P, Ferenbach C, Masley S, Roach L, Lloyd J, May L, Clarke S, Remington B.  
5 The development and initial validation of the cognitive fusion questionnaire. *Behav Ther.*  
6  
7 2014; 45: 83-101.  
8  
9  
10  
11  
12 64. Topp CW, Ostergaard SD, Sondergaard S, Bech P. The WHO-5 Well-Being Index: a  
13 systematic review of the literature. *Psychother Psychosom.* 2015; 84: 167-176.  
14  
15  
16  
17 65. Ziegelmann JP, Griva K, Hankins M, Harrison M, Davenport A, Thompson D, Newman  
18 SP. The Transplant Effects Questionnaire (TxEQ): The development of a questionnaire for  
19 assessing the multidimensional outcome of organ transplantation - example of end stage renal  
20 disease (ESRD). *Br J Health Psychol.* 2002; 7: 393-408.  
21  
22  
23  
24  
25  
26  
27 66. Pearlin LI, Schooler C. The structure of coping. *J Health Soc Behav.* 1978; 19: 2-21.  
28  
29  
30  
31 67. Abma FI, Amick BC, 3rd, Brouwer S, van der Klink JJ, Bultmann U. The cross-cultural  
32 adaptation of the work role functioning questionnaire to Dutch. *Work.* 2012; 43: 203-210.  
33  
34  
35  
36 68. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC.  
37 Functional bowel disorders. *Gastroenterology.* 2006; 130: 1480-1491.  
38  
39  
40  
41 69. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH,  
42 Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire.  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Table 1.** Overview stored samples per participant in the TransplantLines Biobank

Sample	Color code	Tube size	Number	Temperature
Serum	Red	1500 µL	4	-80°C /-112°F
EDTA plasma	Purple	1500 µL	6	-80°C /-112°F
Buffy coat	Purple	N/A	1	-80°C /-112°F
Blood with RBC	Purple	1500 µL	2	-80°C /-112°F
Lithium-heparin	Green	1500 µL	4	-80°C /-112°F
Citrate	Blue	500 µL	4	-80°C /-112°F
PAXgene	Transparent	2.5 mL	1	-80°C /-112°F
24 hour urine	Yellow	1500 µL	6	-80°C /-112°F
Acidified 24 hour urine	Yellow	2000 µL	2	-80°C /-112°F
Feces	Black	20 mL	1	-80°C /-112°F
Nails	Purple	0.5 µL	1	-80°C /-112°F
Hair	Purple	2000 µL	1	-80°C /-112°F

**Table 2.** List of questionnaires in the TransplantLines study

<b>Questionnaires</b>	<b>Related subject</b>
EQ6D <sup>53</sup>	EuroQoL 6 dimensions
VAS scale <sup>54</sup>	Visual Analogue Scale
SF36 <sup>55</sup>	Short Form-36 Health Survey
SQUASH <sup>56</sup>	Short Questionnaire to Assess Health - Enhancing Physical Activity
BAASIS <sup>57</sup>	Adherence to Immunosuppressive Drugs
MTSOSDS-R59 <sup>58</sup>	Modified Transplant Symptom Occurrence and Symptom Distress scale
CIS <sup>59</sup>	Checklist Individual Strength (Fatigue)
PSQI <sup>60</sup>	Pittsburgh Sleep Quality Index
STAI6 <sup>61</sup>	Short form State Trait Anxiety Inventory
PHQ9 <sup>62</sup>	Patient Health Questionnaire (Depression)
CFQ <sup>63</sup>	Cognitive Functioning Questionnaire
WHO-5 <sup>64</sup>	World Health Organization-5 (Well-Being Index)
TxEQ <sup>65</sup>	Transplant Effects Questionnaire
Mastery scale <sup>66</sup>	Pearlin Mastery Scale
UCL-47	Utrecht Coping List-47
USER-P	Utrecht Scale for Evaluation of Revalidation - Participation
Work	Participation in Labor
WRFQ <sup>67</sup>	Work Role Functioning Questionnaire
FAD	Family Assessment Device
ABO	Active Engagement, Protective Buffering and Overprotection Questionnaire
Social support	Social Support Questionnaire
DAG/BHQ <sup>68</sup>	Bowel Health Questionnaire
FFQ <sup>69</sup>	Food Frequency Questionnaire
Self-efficacy movement	LIVAS-scale for Physical Self-Efficacy
Sedentary behavior	OBiN Sedentary Behavior Questionnaire
Smoking behavior	Smoking behavior questionnaire
Alcohol use <sup>a</sup>	The Alcohol Use Disorders Identification Test

<sup>a</sup>The AUDIT questionnaire will only be gathered from liver transplant recipients

**Table 3.** Overview of the different tests performed in TransplantLines study per study protocol

**General (all protocols)**

Parameter/test	Details
General parameters	Collection of Biobank material and evaluation of questionnaires, check quality of data
Blood Pressure	Using an automatic or semi-automatic device
Weight	Using digital measuring scale
Length	Using measuring tape fixed to wall
Waist- and hip size	Using measuring tape roll
BIA	Bio-Impedance Analysis (Quadscan 4000)
SAF	Skin Auto-Fluorescence (AGE reader SU)
Dermatological questionnaire	After physical examination by student researcher
Clinical Frailty Scale	After physical examination by student researcher
PG-SGA	Scored Patient-Generated Subjective Global Assessment
Long function	Using spirometry (Vitalograph Asma 1)
Breath analysis	Using Quintron Breath Tracker

**Physical protocol**

Parameter/test	Details
Balance Test	Using Axivity Accelerometer
Hand grip	Using Hydrolic Hand-held Dynamometer
Physical Strength	Multiple muscle groups, using Digital Dynamometer
Sensibility Tests	Using pin-prick, monofilament and biothesiometer
Tremor analysis	Using Tetras scale and Axivity Accelerometers
Manual dexterity	Using Dexterity PEG-Board

**Cognitive protocol**

Nederlandse Leestest voor	Dutch version of the National Adult Reading Test
Volwassenen	(NART)
Digit Span	Subtest of the Wechsler Adult Intelligence Scale
	(WAIS-IV)
15 Wordstest	Dutch version of Ray Auditory Verbal Learning Test
	(RAVLT)



1		
2		
3	Cognitive Screening Test	Cognitive Screening Test (20)
4	Trail Making Test	
5	Clock-drawing Test	
6	Symbol Digit Modalities Test	
7	Letter Fluency Test	Dutch version of the Controlled Word Association
8		Test (COWAT)
9	Word Fluency Test	Subtest of the Groningen Intelligence Test (GIT)
10	Key Search Test	Subtest of the Behavioral Assessment of the
11		Dysexecutive Syndrome (BADs)
12	Nederlandse Leestest voor	Dutch version of the National Adult Reading Test
13	Volwassenen	(NART)
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		
36		
37		
38		
39		
40		
41		
42		
43		
44		
45		
46		
47		
48		
49		
50		
51		
52		
53		
54		
55		
56		
57		
58		
59		
60		

## Figure legends

**Figure 1.** Overview of different health problems that arise on the long term after transplantation, both physical, psychological, and social.

**Figure 2.** Flowchart of the different visits in the TransplantLines study. At every study visit biobank, general tests, and questionnaires will be performed. Specifically addition at each timepoints; at transplantation perioperative residual material will be collected. At 3 months after transplantation cognitive protocol will be performed. At 6 months physical protocol will be carried out. At 12 months randomization to physical or cognitive protocol will occur. At 2 years after transplantation, a limited set of tests will be executed. Follow-up will be performed each 5 years.

**Figure 3.** Overview of the three main pillars of the TransplantLines study, i.e. questionnaires, biobank, and tests. The collection of data in these pillars at multiple time points will allow to investigate whether biomarkers at baseline can better predict occurrence of adverse outcomes and whether correction could possibly result in an improved survival.

# Patient and Graft survival (e.g. poor long-term outcomes)

**Physical Problems**  
(e.g. accelerated ageing, side effects)



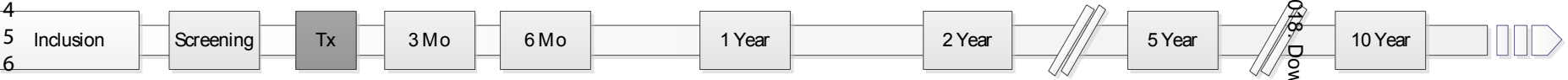
**Mental Problems**  
(e.g. impaired cognition, quality of life)

**Social Problems**  
(e.g. participation loss, poor coping)

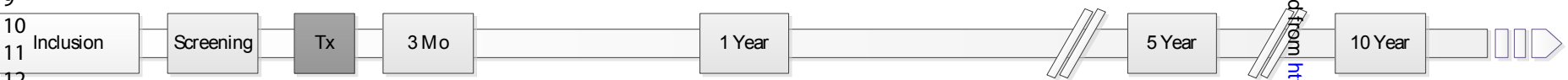
1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41

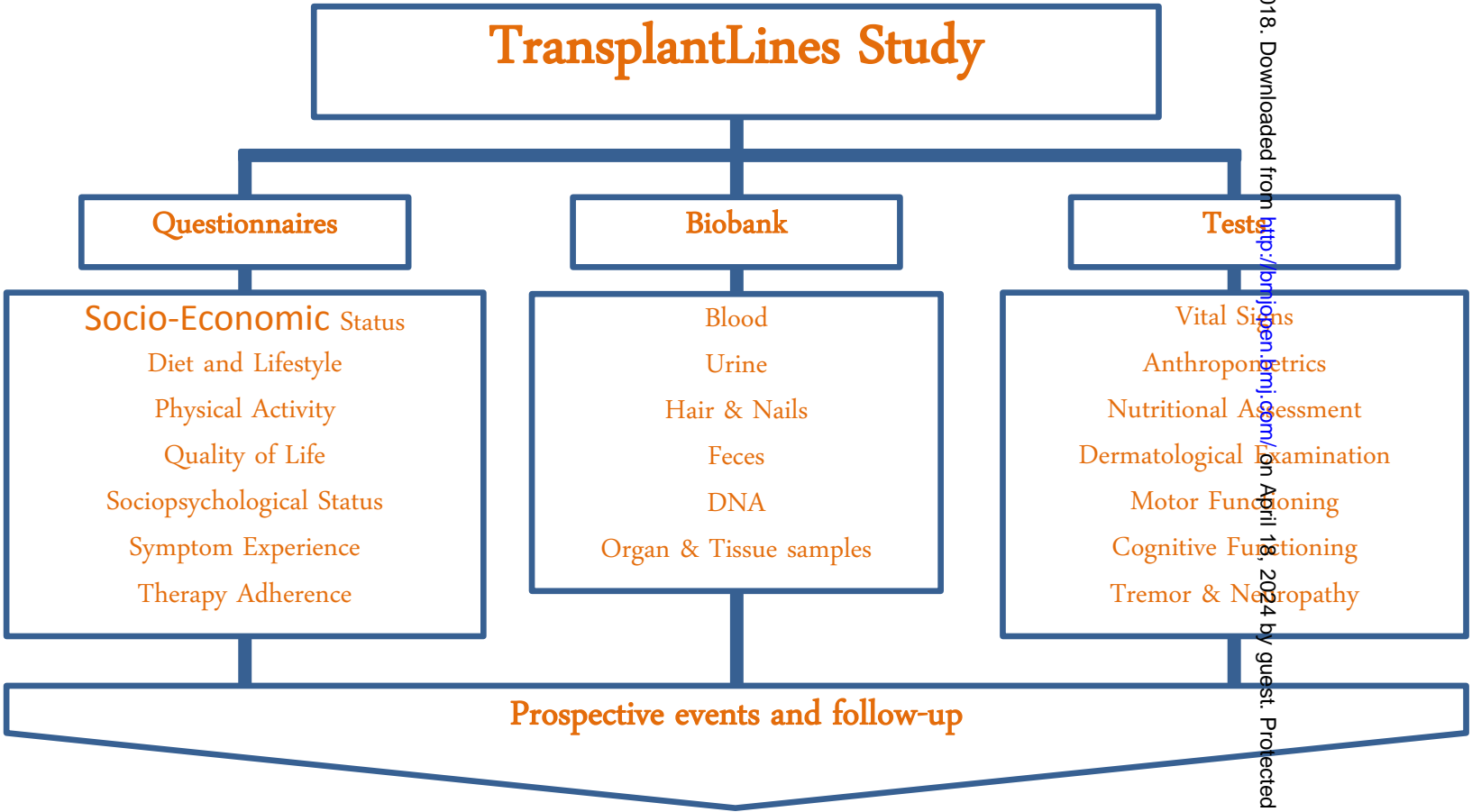
Transplant Recipients



Living Donors



Downloaded from <http://bmjopen.bmj.com/> on April 18, 2024 by guest. Protected by copyright.



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41

# BMJ Open

## Rationale and Design of TransplantLines: a Prospective Cohort Study and Biobank of Solid Organ Transplant Recipients

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-024502.R1
Article Type:	Protocol
Date Submitted by the Author:	19-Sep-2018
Complete List of Authors:	Eisenga, Michele Freerk; Universitair Medisch Centrum Groningen, Nephrology Gomes Neto, Antonio; Universitair Medisch Centrum Groningen, Nephrology Van Londen, Marco ; Universitair Medisch Centrum Groningen, Nephrology Ziengs, Aaltje; Universitair Medisch Centrum Groningen, Nephrology Douwes, Rianne; Universitair Medisch Centrum Groningen, Nephrology Stam, Suzanne; Universitair Medisch Centrum Groningen, Nephrology Osté, Maryse; Universitair Medisch Centrum Groningen, Nephrology Knobbe, Tim; Universitair Medisch Centrum Groningen, Nephrology Hessels, Niek; Universitair Medisch Centrum Groningen, Nephrology Buunk, Anne; Universitair Medisch Centrum Groningen, Neuropsychology Annema, Coby; Univ Groningen Siebelink, Marion; Universitair Medisch Centrum Groningen, Groningen Transplant Center Racz, Eموke; Universitair Medisch Centrum Groningen, Dermatology Spikman, Jacoba; Universitair Medisch Centrum Groningen, Neuropsychology Bodewes, Frank; Universitair Medisch Centrum Groningen, Pediatrics Pol, RA; University Medical Center Groningen,, Berger, Stefan; Universitair Medisch Centrum Groningen, Nephrology Drost, Gea; Universitair Medisch Centrum Groningen, Neurology Porte, Robert; Universitair Medisch Centrum Groningen, Hepato-pancreatico-biliaire Chirurgie en Levertransplantatie Leuvenink, Henri ; Universitair Medisch Centrum Groningen, Surgery Damman, Kevin; Universitair Medisch Centrum Groningen, Cardiology Verschuuren, Erik; Universitair Medisch Centrum Groningen, Pulmonary Disease and Tuberculosis De Meijer, Vincent; Universitair Medisch Centrum Groningen, Surgery Blokzijl, Hans; Universitair Medisch Centrum Groningen, Gastroenterology and Hepatology Bakker, Stephan; University of Groningen, University Medical Center Groningen, Internal Medicine
<b>Primary Subject Heading</b>:	Surgery
Secondary Subject Heading:	Renal medicine, Respiratory medicine, Gastroenterology and hepatology,

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

	Cardiovascular medicine
Keywords:	Cohort study, TRANSPLANT MEDICINE, Survival



## Rationale and Design of TransplantLines: a Prospective Cohort Study and Biobank of Solid Organ Transplant Recipients

**Authors.** Michele F. Eisenga<sup>1\*</sup>, M.D., Antonio W. Gomes-Neto<sup>1\*</sup>, M.D., Marco van Londen<sup>1\*</sup>, M.D., Aaltje L. Ziengs<sup>1,2</sup>, MSc, Rianne M. Douwes<sup>1</sup>, M.D., Suzanne P. Stam<sup>1</sup>, BSc, Maryse C.J. Osté, BSc, Tim J. Knobbe<sup>1</sup>, Bsc, Niek R. Hessels<sup>1</sup>, BSc, Anne M. Buunk<sup>2</sup>, MSc, Coby Annema<sup>3</sup>, PhD, Marion J. Siebelink<sup>3</sup>, PhD, Emoke Rácz<sup>4</sup>, M.D., PhD, Jacoba M. Spikman<sup>2</sup>, PhD, Frank A.J.A. Bodewes<sup>5</sup>, M.D., PhD, Robert A. Pol<sup>6</sup>, M.D., PhD, Stefan P. Berger<sup>1</sup>, M.D., PhD, Gea Drost<sup>7</sup>, M.D., PhD, Robert J. Porte<sup>6</sup>, M.D., PhD, Henri G.D. Leuvenink<sup>6</sup>, M.D., PhD, Kevin Damman<sup>8</sup>, M.D., PhD, Erik A.M. Verschuuren<sup>9</sup>, M.D., PhD, Vincent E. de Meijer<sup>6</sup>, M.D., PhD, Hans Blokzijl<sup>10</sup>, M.D., PhD, and Stephan J.L. Bakker<sup>1</sup>, M.D., PhD.

**Collaborators.** Martin H. De Borst<sup>1</sup>, M.D., PhD, Margriet F.C. De Jong<sup>1</sup>, M.D., PhD, Jan Stephan F. Sanders<sup>1</sup>, M.D., PhD, Gerjan Navis<sup>1</sup>, M.D., PhD, Jan Willem J. Elting<sup>7</sup>, M.D., PhD, Marina A.J. Tijssen<sup>7</sup>, M.D., PhD, Marieke T. de Boer<sup>6</sup>, M.D., PhD, Adelita V. Ranchor<sup>11</sup>, PhD, Ilja M. Nolte<sup>12</sup>, PhD, Rob J. Bieringa<sup>12</sup>, Paul Koenes<sup>12</sup>, Wim van der Bij<sup>9</sup>, M.D., PhD.

<sup>1</sup>Division of Nephrology, Department of Internal Medicine; <sup>2</sup>Department of Neuropsychology;

<sup>3</sup>Groningen Transplant Center, <sup>4</sup>Department of Dermatology; <sup>5</sup>Department of Pediatrics;

<sup>6</sup>Department of Surgery; <sup>7</sup>Department of Neurology; <sup>8</sup>Department of Cardiology; <sup>9</sup>Department of Pulmonary Diseases and Tuberculosis; <sup>10</sup>Department of Gastroenterology and Hepatology;

<sup>11</sup>Department of Health Psychology; <sup>12</sup>Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

\*Denotes equal contribution of these authors



**Running title:** TransplantLines Biobank and Cohort Study

**Keywords:** TransplantLines; cohort study; transplant recipients; survival

**Abstract:** 274 words

**Text:** 5420 words

**Tables:** 3

**Figures:** 3

**ClinicalTrials.gov identifier:** NCT03272841

**Email addresses authors:** MF Eisenga, [m.f.eisenga@umcg.nl](mailto:m.f.eisenga@umcg.nl); AW Gomes Neto, [a.w.gomes.neto@umcg.nl](mailto:a.w.gomes.neto@umcg.nl); M van Londen, [m.van.londen@umcg.nl](mailto:m.van.londen@umcg.nl); AL Ziengs, [a.l.ziengs@umcg.nl](mailto:a.l.ziengs@umcg.nl); RM Douwes, [r.m.douwes@umcg.nl](mailto:r.m.douwes@umcg.nl); SP Stam, [s.p.stam@umcg.nl](mailto:s.p.stam@umcg.nl); MCJ Osté, [m.c.j.oste@umcg.nl](mailto:m.c.j.oste@umcg.nl); TJ Knobbe, [t.j.knobbe@umcg.nl](mailto:t.j.knobbe@umcg.nl); NR Hessels, [n.r.hessels@umcg.nl](mailto:n.r.hessels@umcg.nl); AM Buunk, [a.m.buunk@umcg.nl](mailto:a.m.buunk@umcg.nl); MJ Siebelink, [m.j.siebelink@umcg.nl](mailto:m.j.siebelink@umcg.nl); C Annema, [j.h.annema@umcg.nl](mailto:j.h.annema@umcg.nl); E Rácz, [e.racz@umcg.nl](mailto:e.racz@umcg.nl); JM Spikman, [j.m.spikman@umcg.nl](mailto:j.m.spikman@umcg.nl); FAJA Bodewes, [f.a.j.a.bodewes@umcg.nl](mailto:f.a.j.a.bodewes@umcg.nl); RA Pol, [r.pol@umcg.nl](mailto:r.pol@umcg.nl); SP Berger, [s.p.berger@umcg.nl](mailto:s.p.berger@umcg.nl); G Drost, [g.drost@umcg.nl](mailto:g.drost@umcg.nl); RJ Porte, [r.j.porte@umcg.nl](mailto:r.j.porte@umcg.nl); HGD Leuvenink, [h.g.d.leuvenink@umcg.nl](mailto:h.g.d.leuvenink@umcg.nl); K Damman, [k.damman@umcg.nl](mailto:k.damman@umcg.nl); EAM Verschuuren, [e.a.m.verschuuren@umcg.nl](mailto:e.a.m.verschuuren@umcg.nl); VE de Meijer, [v.e.de.meijer@umcg.nl](mailto:v.e.de.meijer@umcg.nl); H Blokzijl, [h.blokzijl@umcg.nl](mailto:h.blokzijl@umcg.nl); and SJL Bakker, [s.j.l.bakker@umcg.nl](mailto:s.j.l.bakker@umcg.nl)

**Corresponding author:**

Michele F. Eisenga, M.D.

Department of Internal Medicine, Division of Nephrology

University Medical Center Groningen

P.O. Box 30.001, 9700 RB Groningen, the Netherlands

Phone: 0031 050 361 61 61 Email: [m.f.eisenga@umcg.nl](mailto:m.f.eisenga@umcg.nl)

## Abstract

### Introduction

In the past decades, short-term results after solid organ transplantation have markedly improved. Disappointingly, this has not been accompanied by parallel improvements in long-term outcomes after transplantation. To improve graft and recipient outcomes, identification of potentially modifiable risk factors and development of biomarkers is required. We provide the rationale and design of a large prospective cohort study of solid organ transplant recipients (TransplantLines).

### Methods and analysis

TransplantLines is designed as a single center prospective cohort study and biobank including all different types of solid organ transplant recipients, as well as living organ donors. Data will be collected from transplant candidates before transplantation, during transplantation, at 3 months, 6 months, 1 year, 2 years, 5 years, and subsequently every 5 years after transplantation. Data from living organ donors will be collected before donation, during donation, at 3 months, 1 year, and 5 years after donation and subsequently every 5 years.

Primary outcomes are mortality and graft failure. Secondary outcomes will be cause-specific mortality, cause-specific graft failure and rejection. Tertiary outcomes will be other health problems, including diabetes, obesity, hypertension, hypercholesterolemia, and cardiovascular disease, and disturbances that relate to quality of life, i.e. physical and psychological functioning, including quality of sleep, and neurological problems such as tremor and polyneuropathy.

### Ethics and dissemination

Ethical approval has been obtained from the relevant local ethics committee. The TransplantLines cohort study is designed to deliver pioneering insights in transplantation and donation outcomes. The study design allows comprehensive data collection on perioperative

1  
2  
3 care, nutrition, social- and psychological functioning and biochemical parameters. This may  
4  
5 provide a rationale for future intervention strategies to more individualized, patient-centered  
6  
7 transplant care and individualization of treatment.  
8  
9

### 10 11 **Strength and limitations**

- 12 - Large biobank and cohort study with extensive data collection on a myriad topics  
13  
14 related to transplantation and/or donation
- 15 - Inclusion of all types of solid organ transplant recipients
- 16 - Long follow-up to assess many relevant clinical outcomes
- 17 - Single center study
- 18 - Residual confounding cannot be excluded due to observational design
- 19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Background

Solid organ transplantation is the preferred treatment for end-stage organ failure. During the past decades, advances in immunosuppressant medications, treatment of infections, perioperative medical care, and surgical techniques (including living donation) have led to important improvements in early post-transplant graft and patient survival.[1] However, on the long-term, graft failure is a major cause of patient mortality and morbidity in all types of transplantation.[2-4] For example, in renal transplant recipients, half of the cadaveric renal allografts fail within a timeframe of 10 years.[5] Apart from reduced survival, transplant recipients often develop health problems that greatly reduce their perceived quality of life (Figure 1).[6-8]

The multitude of health problems that recipients experience after transplantation include amongst others obesity, diabetes, hypertension, heart failure, and malignancies.[9-11] These are likely the consequence of a combination of factors, including (1) continuous exposure to treatment with immunosuppressive drugs necessary for prevention of rejection of the transplanted organ, (2) damage induced by pre-existing exposure to end-stage organ failure, and (3) adverse life-style and environmental factors, all potentially expressed against (4) a background of increased (epi)genetic susceptibility. Among these, immunosuppressive treatment, adverse life-style, and environmental factors are good candidates for modification to decrease the load of post-transplant health problems. It should be realized that immunosuppressive treatment is currently mainly “one-size fits all”. Hence, improvement can be achieved by development of biomarkers that can allow for recognition of transplant recipients in which immunosuppressive load can be safely reduced or in which certain drugs can better be avoided, and for biomarkers which can guide such individualized immunosuppressive treatment. To improve long-term transplant outcomes, it is imperative to identify modifiable risk factors, especially among those recipients who are at increased risk.

1  
2  
3 To date, it is largely unknown in which transplant recipients immunosuppressive  
4 medication can be safely reduced to prevent the development of health problems.  
5  
6 Furthermore, in terms of healthcare costs, it is important to prevent recurrent hospital  
7 admissions, re-transplantations or – in case of kidney transplantation – return to dialysis,  
8 which are all associated with very high expenses.[12] To effectively develop interventions to  
9 reduce mortality and morbidity after transplantation, more research is necessary on clinical  
10 and biochemical risk factors present in transplant recipients. Also, the use of living donors for  
11 kidney and liver transplantation requires a living donor program with good long-term  
12 outcomes for the donor and recipient. Living kidney donors, for example, have an increased  
13 risk for end-stage renal disease (ESRD),[13, 14] while only registry data exist on the effect of  
14 living donor characteristics on recipient outcomes.[15, 16]  
15

16  
17 Until now, many registries and large cohort studies focus on one type of solid organ  
18 transplantation, limiting comparability between different transplant populations. As a result,  
19 studies investigating biomarkers, quality of life, and development of health problems and  
20 adverse outcomes across different solid organ transplant populations are scarce. Despite the  
21 differences which exists in patient characteristics and treatment after different solid organ  
22 transplantations, there are many similarities in health problems that occur among subtypes of  
23 transplantation. The objective of TransplantLines study is to identify risk factors for  
24 development of long-term health problems after transplantation and to develop new  
25 interventions to improve outcome, both combined for all solid organ transplant recipients as  
26 well as specific for each subtype of transplantation.  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49

## 50 **Methods/Design**

### 51 **Study design and setting**

1  
2  
3 The TransplantLines study is a unique, novel prospective biobank and cohort study, which  
4 aims to provide a better understanding of causes of disease- and ageing-related outcomes and  
5 health problems, both physical and psychological, in solid organ transplant recipients and  
6 donors (ClinicalTrials.gov Identifier: NCT03272841). The University Medical Center  
7 Groningen (UMCG) is the largest transplantation center in the Netherlands, and the only  
8 Dutch center that covers all types of solid organ transplantation, as well as living kidney and  
9 liver donation programs. The study protocol has been approved by the Institutional Review  
10 Board (METc 2014/077), adheres to the UMCG Biobank Regulation, and is in accordance  
11 with the WMA declaration of Helsinki and the declaration of Istanbul. All participants will  
12 give written informed consent upon enrollment. Follow-up and prospective events will be  
13 recorded over time. An overall participation rate of 85% is expected across the different  
14 transplant populations and a total number of 3000 participants is aimed.

### 30 **Transplant patients**

31  
32 The study population comprises all solid organ transplant recipients, i.e. heart-, lung-, kidney-  
33 , liver-, and small bowel transplant recipients. Both new transplant candidates as well as  
34 transplant recipients are eligible to participate in the study. Participants of all ages will be  
35 included in TransplantLines. Children (age <18 years) will be eligible for participation upon  
36 consent by a legal representative (<12 years) or a shared consent of both the child and legal  
37 representatives ( $\geq 12$  years). The study will also include candidates for re-transplantation.  
38  
39 Exclusion criteria for participation in the TransplantLines study will be no mastery of the  
40 Dutch language or no capability to intellectually comprehend questionnaires or physical tests.  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

### 51 **Living donors**

1  
2  
3 Living kidney and liver donors will also be included in the study. The goal of including  
4 donors is to study the effects of donation, improve living donor safety and donors will serve  
5 as controls for their recipients, allowing for matched longitudinal analyses. Prospective living  
6 kidney and liver donor candidates ( $\geq 18$  years old) will be eligible to participate in the study,  
7 as well as living organ donors who have donated an organ prior to the start of the  
8 TransplantLines study. Exclusion criteria will be no mastery of the Dutch language or no  
9 capability to intellectually comprehend questionnaires or physical tests.  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19

### 20 **Transplant recipients timeline**

21 All participants of the TransplantLines study will be examined at fixed time points as shown  
22 in **Figure 2**. Transplant candidates will be first seen at pre-transplant screening. Prior to  
23 transplantation, all transplant candidates undergo a routine clinical screening. Generally,  
24 transplant candidates will be transplanted if surgery risks and transplant benefit are optimized,  
25 based on an individualized multidisciplinary clinical decision. Further study visits will be  
26 performed at time of transplantation, at 3 months, 6 months, 12 months, and 2 years after  
27 transplantation, and hereafter follow-up will be performed at 5 years after transplantation and  
28 every consecutive 5 years. At time of transplantation means during operation prior to incision,  
29 and at that timepoint the blood samples are being drawn by the anesthesiologists taking care  
30 of the patient. The difference with this sample compared to other previous samples is that this  
31 sample is taken during operation, whereas the other samples are not. For example, a kidney  
32 transplant candidate can be screened and included in the TransplantLines study, but may need  
33 to wait two years on the waiting list prior to receiving the actual transplantation. Since we  
34 realize that a study of this size and duration combined with the frequency of study visits will  
35 result in lower subject adherence, we estimate a 10% dropout overall in follow-up in this  
36 transplant candidates group.  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 Transplant recipients with a functional graft for at least 1 year post-transplantation and  
4  
5 who received a solid organ transplant prior to the start of the TransplantLines study will be  
6  
7 included at the next outpatient clinic visit. Henceforth, patients will be examined every five  
8  
9 years and follow-up samples will be collected. Aside from the fixed time points, biobank  
10  
11 samples of transplant recipients will be collected at times of protocol biopsies that are  
12  
13 performed in the kidney transplant program (6 months after transplantation) and the heart  
14  
15 transplant program (repeatedly during the first year after transplantation) and if a biopsy is  
16  
17 taken, on clinical indication, usually because of worsening of transplant function, with  
18  
19 suspicion of acute or chronic rejection.  
20  
21

22 If a subject gets retransplanted with the same kind of organ, this will be classified as  
23  
24 graft failure and end of follow-up. Subjects will not be included in the primary database twice.  
25  
26 Yet, we will allow for inclusion of subjects retransplanted with the same kind of organ with a  
27  
28 new ID in the transplant candidate group, but this will be with the intention to build over time  
29  
30 a separate cohort with data and a biobank on retransplantations. When a transplant recipient is  
31  
32 later on transplanted with another kind of organ, follow-up will be for the initially  
33  
34 transplanted organ. Transplant recipients receiving a combined transplantation, e.g. kidney-  
35  
36 pancreas and kidney-liver, will be treated as separate groups, not to be included in overall  
37  
38 analyses for the much larger groups of subjects with single transplanted organs.  
39  
40

41 In case that a transplant recipient moves to another region of the Netherlands or  
42  
43 abroad, the transplant recipient will always require continued medical care and follow-up by a  
44  
45 medical specialist, who will require thorough medical information on the patient and the  
46  
47 transplanted organ, to allow for continued dedicated care. Therefore, the medical specialist  
48  
49 who will continue care will seek contact for information and it will usually be possible to  
50  
51 continue follow-up on long-term outcome and events via this medical specialist. So, follow-  
52  
53 up is usually assured and loss to follow-up will be rare. Since study visits are combined with a  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 routine clinical visit, subjects who move out of our region will be excluded from further study  
4  
5 visits for the TransplantLines study.  
6  
7

### 8 9 **Living donors timeline**

10 All donors of the TransplantLines study will be examined at fixed time points as shown in  
11  
12 **Figure 2.** The first study visit of donor candidates will occur at pre-donation screening. Prior  
13  
14 to donation, all candidates undergo a routine clinical screening. Generally, donors will be  
15  
16 accepted if surgery risks and transplant benefit are optimized, based on an individualized  
17  
18 multidisciplinary clinical decision taking national and international guidelines into  
19  
20 account.[17, 18] Subsequently, study visits will be performed at time of nephrectomy, and at  
21  
22 3 months post-donation. At 12 months post-donation, donors will fill in a questionnaire and at  
23  
24 5 and 10 years post-donation there will be another study visit. Hereafter follow-up will be  
25  
26 performed every five years. Living organ donors who have donated an organ prior to the start  
27  
28 of the TransplantLines study, will be included at their next donor follow-up visit to their  
29  
30 outpatient clinic.  
31  
32  
33  
34  
35  
36

### 37 **Patient and Public Involvement**

38 The aim of the TransplantLines study is to provide a better understanding of the causes of  
39  
40 disease- and ageing-related outcomes and health problems. This aim was derived from patient  
41  
42 surveys and parts of the collected data are co-designed by patients and healthcare  
43  
44 professionals. Because of its scope, patients will play a role in the organization of the study,  
45  
46 helping with recruitment and conduct of the study. Also, students from a broad range of  
47  
48 studies will play a role in the organization of the study, e.g. master students from medicine,  
49  
50 biomedical sciences, neuropsychology, psychology, physical therapy, communication  
51  
52 sciences, dietician students, and laboratory technician students. Patients and collaborators will  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 be informed of major study results by press releases from the University Medical Center  
4 Groningen.  
5  
6  
7

## 8 9 **Data collection**

### 10 ***Biobank***

11  
12 Blood, 24-hour urine, feces, nails, and hair will be collected of participants at each  
13 TransplantLines visit. Participants will be instructed to collect a 24-hour urine sample  
14 according to strict protocol at the day before their visit to the outpatient clinic, i.e. discard  
15 their morning urine specimen, collect all subsequent urine throughout the next 24 hour and  
16 include the next morning's first specimen of the day of the visit to the outpatient clinic. Blood  
17 will be drawn after an 8-12 hour overnight fasting period in the morning after completion of  
18 the 24-hour urine collection. Blood drawing and receipt of the collected 24 hour urine samples  
19 is performed by experienced nurses at our outpatient clinic.  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29

30  
31 As blood samples, 1 serum tube of 10 mL, 2 EDTA samples of 10 mL, 1 citrate tube  
32 of 6 mL, 1 lithium-heparin tube of 10 mL, and 1 PAXgene tube of 10 mL will be collected of  
33 each participant at each TransplantLines visit. Subsequently, tubes will be centrifuged by  
34 technicians at 1300g for 10 minutes, except the citrate tube which is centrifuged at 2500g for  
35 10 minutes. Of the 24-hour urine collection, three urine tubes will be collected of which one  
36 tube will be partially acidified. All blood- and urine samples will be subsequently aliquoted  
37 by technicians and shipped to the core laboratory for storage in -80°C (-112 °F) freezers  
38 (Panasonic, 's-Hertogenbosch, the Netherlands) (**Table 1**). Blood- and urine samples will be  
39 analyzed in the following years for multiple research questions that will arise.  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49

50 Participants will be asked to collect a feces sample the day prior to the  
51 TransplantLines visit. A FecesCatcher (TAG Hemi VOF, Zeijen, the Netherlands) will be sent  
52 at the patients' home, and feces sample will be collected in appropriate tubes and frozen  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 immediately after collection. The participant will transport the feces sample in cold storage  
4 (with ice cubes or in a cooler) to the TransplantLines visit the following day. Subsequently,  
5 the feces sample will be immediately stored at -80°C (-112 °F). Feces samples will be  
6 primarily used for microbiome analyses. Solid organ transplant recipients have a shift in the  
7 gut microbiome with a decrease in predominant organisms, a loss of bacterial diversity, and  
8 emergence of new dominant population. This may result in increased risk of infection,  
9 rejection, and mortality. Therefore, we would like to examine the gut microbiome in relation  
10 to the development of health problems after transplantation.  
11  
12  
13  
14  
15  
16  
17  
18  
19

20 Additional blood and urine samples will also be collected in the event of worsening  
21 graft function and an organ-transplant biopsy is indicated. Prior to the biopsy, 1 serum tube of  
22 10 mL, 2 EDTA samples of 10 mL, 1 citrate tube of 6 mL, 1 lithium-heparin tube of 10 mL,  
23 and 1 PAXgene tube of 10 mL will be collected. At the same time, 1 serum tube of 10 mL and  
24 1 EDTA sample of 10 mL and 1 spot urine sample of 10 mL will be collected and directly  
25 stored on ice to prevent (ongoing) in vitro complement activation.  
26  
27  
28  
29  
30  
31  
32

33 Furthermore, during transplant surgery and transplant biopsies, tissue samples will be  
34 collected of the transplanted organ and surrounding tissues, including fat-, skin-, ureter-,  
35 tracheal-, biliary-, arterial and venous tissue, that have been discarded as pathological waste.  
36  
37  
38  
39  
40  
41  
42

43 **Table 1.** Overview stored samples per participant in the TransplantLines Biobank

44 Sample	45 Color code	46 Tube size	47 Number	48 Temperature
49 Serum	50 Red	51 1500 µL	52 4	53 -80°C /-112°F
54 EDTA plasma	55 Purple	56 1500 µL	57 6	58 -80°C /-112°F
59 Buffy coat	60 Purple	N/A	1	-80°C /-112°F
Blood with RBC	Purple	1500 µL	2	-80°C /-112°F
Lithium-heparin	Green	1500 µL	4	-80°C /-112°F

Citrate	Blue	500 µL	4	-80°C /-112°F
PAXgene	Transparent	2.5 mL	1	-80°C /-112°F
24 hour urine	Yellow	1500 µL	6	-80°C /-112°F
Acidified 24 hour urine	Yellow	2000 µL	2	-80°C /-112°F
Feces	Black	20 mL	1	-80°C /-112°F
Nails	Purple	0.5 µL	1	-80°C /-112°F
Hair	Purple	2000 µL	1	-80°C /-112°F

### ***Clinical and laboratory characteristics***

Clinical laboratory measurements requested by the physician will be included in the study database upon patient consent. 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. 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. Demographic characteristics along with data on medication use will be provided by the participants and will be verified using the electronic hospital records. Medical information including donor and recipient information at time of transplantation, underlying disease,

hospital admissions, complications after transplantation, further surgical or other interventional treatments, co-morbidities, graft failure, and mortality will be extracted from the electronic hospital records.

### **Questionnaires**

Biobank data will be expanded with an extensive set of questionnaires to collect data on physical, psychological, and social impact of undergoing a transplantation (**Figure 3**).

Transplant candidates will be asked to fill out a comprehensive questionnaire during screening prior to transplantation and at 1 year post-transplantation. Transplant recipients with a functional graft for more than 1 year post-transplantation and who received the solid organ prior to start of the TransplantLines study will be asked to complete the same questionnaire. A subset of questionnaires will be provided at the other predefined time-points, i.e. at 3, at 6 months, and at 2 years after transplantation. Topics addressed by questionnaires include among others nutritional intake and diet, health-related quality of life, life-style factors such as physical activity, sleep quality, and smoking behavior, psychological impact such as anxiety, depression, coping, and well-being, and social impact such as employment and family relationships. Specification of all the different questionnaires with related subject is shown in **Table 2**. Questionnaires will be send digitally or by mail, as requested. During study visits, all questionnaires will be checked by a trained investigator for completeness and validity.

**Table 2.** List of questionnaires in the TransplantLines study

<b>Questionnaires</b>	<b>Related subject</b>
EQ6D[19]	EuroQoL 6 dimensions
VAS scale[20]	Visual Analogue Scale
SF36[21]	Short Form-36 Health Survey

1		
2		
3	SQUASH[22]	Short Questionnaire to Assess Health - Enhancing Physical Activity
4	BAASIS[23]	Adherence to Immunosuppressive Drugs
5		
6	MTSOSDS-R59[24]	Modified Transplant Symptom Occurrence and Symptom Distress
7		scale
8		
9	CIS[25]	Checklist Individual Strength (Fatigue)
10		
11	PSQI[26]	Pittsburgh Sleep Quality Index
12		
13	STAI6[27]	Short form State Trait Anxiety Inventory
14	PHQ9[28]	Patient Health Questionnaire (Depression)
15		
16	CFQ[29]	Cognitive Functioning Questionnaire
17	WHO-5[30]	World Health Organization-5 (Well-Being Index)
18		
19	TxEQ[31]	Transplant Effects Questionnaire
20		
21	Mastery scale[32]	Pearlin Mastery Scale
22		
23	UCL-47	Utrecht Coping List-47
24	USER-P	Utrecht Scale for Evaluation of Revalidation - Participation
25	Work	Participation in Labor
26		
27	WRFQ[33]	Work Role Functioning Questionnaire
28		
29	FAD	Family Assessment Device
30	ABO	Active Engagement, Protective Buffering and Overprotection
31		Questionnaire
32		
33	Social support	Social Support Questionnaire
34		
35	DAG/BHQ[34]	Bowel Health Questionnaire
36		
37	FFQ[35]	Food Frequency Questionnaire
38		
39	Self-efficacy movement	LIVAS-scale for Physical Self-Efficacy
40	Sedentary behavior	OBiN Sedentary Behavior Questionnaire
41		
42	Smoking behavior	Smoking behavior questionnaire
43		
44	Alcohol use <sup>a</sup>	The Alcohol Use Disorders Identification Test

---

<sup>a</sup>The AUDIT questionnaire will only be gathered from liver transplant recipients

### ***Standard assessments***

Blood pressure (mmHg) will be measured according to a standard clinical protocol using an automatic device (Philips Suresign VS2<sup>+</sup>, Andover, MA, USA). To prevent a white-coat effect, participants will be seated during which blood pressure and heart rate will be measured

1  
2  
3 four times, with an interval of three minutes between measurements. Hereafter, participants  
4 will be asked to stand up straight for one minute, after which blood pressure and heart rate  
5 measurements will be repeated once in standing position. Measurements will be performed  
6  
7 with participants being on their regular medication, including anti-hypertensive drugs at  
8  
9  
10  
11  
12 trough.

13  
14 Anthropometry measurements will include body weight, body length, and waist- and  
15 hip circumference. Body weight (kg) will be measured in light weight clothing without shoes  
16 using a calibrated digital measuring scale (SECA 877, Seca GMBH, Hamburg, Germany).  
17  
18 Height (cm) will be measured using a wall-secured stadiometer (SECA 222). Waist- and hip  
19  
20 circumference (cm) will be calculated using a measuring tape roll with standardized retraction  
21  
22 mechanism (SECA 201). Waist circumference will be measured midway between the lowest  
23  
24 rib and the iliac crest with the participant in standing position. Hip circumference will be  
25  
26 determined at the maximum circumference over the trochanter major. All anthropometry  
27  
28 measurements will be assessed twice, with inclusion of a third measurement contingent upon  
29  
30 a difference of more than half a kilogram in weight or more than one centimeter in length.  
31  
32  
33  
34

35 Handgrip strength will be assessed with the Jamar Hydrolic Hand Dynamometer  
36  
37 (Patterson Medical JAMAR 5030J1, Warrentville, Canada).[36] Participants will be instructed  
38  
39 to sit in a chair with their shoulders in adduction, their arms rotated into neutral position, their  
40  
41 elbows flexed to 90°, and forearms and wrists held in neutral position. Hereafter, participants  
42  
43 will be instructed to perform a maximal isometric contraction. Handgrip strength will be  
44  
45 tested three times with an interval of 30 seconds rest for recovery between each attempt. The  
46  
47 dominant hand will be stated in all measurements. Furthermore, to create uniformity among  
48  
49 assessments, the second handle position of the hand dynamometer will be utilized which has  
50  
51 been shown to be the most accurate position.[37]  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 Lung function will be measured by means of an Asma-1 handheld spirometer  
4 (Vitalograph, Buckingham, United Kingdom).[38] Of all participants, the Forced Expiratory  
5 Volume (FEV1), as marker of lung function, will be recorded.  
6  
7

8  
9 Body composition will be determined using a multifrequency bio-electrical impedance  
10 device (BIA, Quadscan 4000, Bodystat Ltd, Douglas, British Isles) at 5, 50, 100, and 200 Hz,  
11 which allows to distinct between lean body mass and fat body mass taking into account  
12 differences in volume status.[39] Main outcome variables from the BIA are estimated fat  
13 mass, fat free mass, and body fat percentage. In brief, the BIA measurement will be  
14 performed with the participant in supine position with arms and legs abducted from the body.  
15 Sensor electrodes will be placed on the dorsum of the right hand and feet, with a minimal  
16 distance of five centimeters between the electrodes. Measurement will not be executed if the  
17 participant has a temperature exceeding 37.9°C/100.2°F or has a functioning ICD/pacemaker.  
18  
19

20  
21 Advanced glycation endproducts (AGEs) will be determined using an AGE reader SU  
22 (DiagnOptics Technologies, Groningen, The Netherlands).[40] The AGE reader SU measures  
23 skin autofluorescence (AF) by using the characteristic fluorescent properties of certain AGEs  
24 to estimate the level of AGEs accumulation in the skin. AGEs have been implicated in the  
25 pathogenesis of vascular damage and cardiovascular disorders and aid to characterize the  
26 cardiovascular risk profile of transplant recipients.[41]  
27  
28

29  
30 Transplant recipients are known to be at increased risk for cutaneous malignancies,  
31 mainly related to long-term use of immunosuppressive medication.[42] To identify which  
32 transplant recipients are especially prone to develop dermatological health problems, a  
33 detailed dermatological history with emphasis on malignancies and subsequent treatment will  
34 be obtained. Next, a standardized dermatological examination will be performed by the  
35 trained investigator. The dermatological examination includes the determination of eye color,  
36 natural hair color at adolescence and skin type according to the classification of  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 Fitzpatrick.[43] In addition, the presence and quantity of lentigines, moles, freckles, and warts  
4  
5 are examined.

6  
7 To assess frailty, the Clinical Frailty Scale (CFS) will be scored at study visits by the  
8  
9 trained investigator. The CFS is a validated frailty measurement and frailty is scored based on  
10  
11 clinical judgment on a continuous scale from 1 (very fit) to 9 (terminally ill). A CFS-score of  
12  
13  $\geq 5$  is generally considered to be frail.[44]

14  
15 To assess nutritional status, a Patient-Generated Subjective Global Assessment (PG-  
16  
17 SGA, PT-Global, Philadelphia, USA) will be scored.[44, 45] The PG-SGA is an patient-  
18  
19 centered adaptation of the original Subjective Global Assessment (SGA). The different  
20  
21 domains assessed by the PG-SGA are: 1) changes in body weight, 2) changes in nutritional  
22  
23 intake, 3) symptoms which negatively influence intake, absorption, and utilization of  
24  
25 nutrients, 4) level of activities and function, 5) conditions that increase nutritional risk or  
26  
27 requirements, 6) metabolic stress, and 7) physical examination. Based on the PG-SGA score,  
28  
29 subjects can be classified as well-nourished, moderately malnourished or severely  
30  
31 malnourished.  
32  
33

### 34 35 36 37 **Randomization and additional physical and cognitive tests**

38  
39 In addition to standard assessments, participants will receive additional physical tests or  
40  
41 cognitive tests at their study visit at 12 months post-transplantation or at the first study visit if  
42  
43 it concerns transplant recipients with a functioning graft for more than 1 year who were  
44  
45 transplanted before the start of TransplantLines. Participants will be randomized (1:1 ratio)  
46  
47 into either the “physical” arm or the “cognitive” arm of the study. Randomization will be  
48  
49 performed for each transplant-program separately to ensure balanced randomization of  
50  
51 subjects for each type of solid-organ transplant. Participants randomized into the “physical”  
52  
53 arm of the study protocol will be asked to accomplish a standing balance test, a 2-Minute  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 Walk Test (2MWT), a 4-Meter Walk Test (4MWT), a dexterity test, a Five Time Sit To Stand  
4  
5 test (FTSTS), Timed Up and Go test (TUG), a rigorous neurological examination, and a  
6  
7 breath analysis. With inclusion of the first four tests together with the handgrip strength, the  
8  
9 five physical components of the National Health Institute Toolbox for motor assessment are  
10  
11 being assessed.[46] A subset of these tests will also be performed at the 6 months post-  
12  
13 transplantation study visit in all solid-organ transplant recipients. Participants randomized into  
14  
15 the “cognitive” arm of the study protocol undergo a series of neuropsychological tests  
16  
17 performed by a trained neuropsychologist or master student neuropsychology under  
18  
19 supervision of a trained neuropsychologist. The tests are administered in a quiet room with no  
20  
21 disturbances. For timed tests, a digital clock is used. The tests are performed in a fixed order  
22  
23 and no feedback regarding the results is given to the participant during administration. An  
24  
25 overview of the neuropsychological tests is specified in **Table 3**. A subset of these test will  
26  
27 also be performed at 3 months post-transplantation in all solid-organ transplant recipients.  
28  
29  
30  
31  
32

33 **Table 3.** Overview of the different tests performed in TransplantLines study per study  
34 protocol  
35

36  
37 **General (all protocols)**

38 <b>Parameter/test</b>	39 <b>Details</b>
40 General parameters	41 Collection of Biobank material and evaluation of 42 questionnaires, check quality of data
43 Blood Pressure	44 Using an automatic or semi-automatic device
45 Weight	46 Using digital measuring scale
47 Length	48 Using measuring tape fixed to wall
49 Waist- and hip size	50 Using measuring tape roll
51 BIA	52 Bio-Impedance Analysis (Quadscan 4000)
53 SAF	54 Skin Auto-Fluorescence (AGE reader SU)
55 Dermatological questionnaire	56 After physical examination by student researcher
57 Clinical Frailty Scale	58 After physical examination by student researcher

1		
2		
3	PG-SGA	Scored Patient-Generated Subjective Global
4		Assessment
5		
6	Long function	Using spirometry (Vitalograph Asma 1)
7	Breath analysis	Using Quintron Breath Tracker
8		
9	<b>Physical protocol</b>	
10		
11	<b>Parameter/test</b>	<b>Details</b>
12	Balance Test	Using Axivity Accelerometer
13	Hand grip	Using Hydrolic Hand-held Dynamometer
14	Physical Strength	Multiple muscle groups, using Digital Dynamometer
15	Sensibility Tests	Using pin-prick, monofilament and biothesiometer
16	Tremor analysis	Using Tetras scale and Axivity Accelerometers
17	Manual dexterity	Using Dexterity PEG-Board
18		
19	<b>Cognitive protocol</b>	
20	Nederlandse Leestest voor	Dutch version of the National Adult Reading Test
21	Volwassenen	(NART)
22	Digit Span	Subtest of the Wechsler Adult Intelligence Scale
23		(WAIS-IV)
24	15 Wordstest	Dutch version of Ray Auditory Verbal Learning Test
25		(RAVLT)
26	Cognitive Screening Test	Cognitive Screening Test (20)
27	Trail Making Test	
28	Clock-drawing Test	
29	Symbol Digit Modalities Test	
30	Letter Fluency Test	Dutch version of the Controlled Word Association
31		Test (COWAT)
32	Word Fluency Test	Subtest of the Groningen Intelligence Test (GIT)
33	Key Search Test	Subtest of the Behavioral Assessment of the
34		Dysexecutive Syndrome (BADS)
35		

### Physical Protocol Measurements and Tests

The *standing balance test* will be performed with an accelerometer (Axivity, Newcastle, United Kingdom), attached to the lower back. The standing balance test has been described in

1  
2  
3 detail previously.[46] Balance will be evaluated in 5 different positions, i.e. 1) feet together  
4 on hard surface, eyes open; 2) feet together on hard surface, eyes closed; 3) feet together on  
5 foam surface (Balance Pad Elite; Airex Specialty Foams, Aargau, Switzerland), eyes open; 4)  
6 feet together on foam surface, eyes closed, and 5) feet in tandem stance, eyes open.  
7  
8  
9  
10  
11 Participants will be asked to have arms crossed on their chest and each position will be tested  
12 for 50 seconds. Upon failure, with recording of time to failure, a second attempt will be  
13 performed. In case of non-success at the second attempt, the test will be discontinued.  
14  
15  
16

17  
18 Endurance will be tested with a *2MWT*. [47] The *2MWT* has been shown to be highly  
19 correlated, without compromising validity and reliability, with the 6-minute walking test, an  
20 important submaximal exercise test. [48, 49] To calculate distance covered by subjects on the  
21 *2MWT*, two pylons are set apart 15 meters and subjects are instructed to walk as fast as  
22 possible without running, until the investigator commands to stop. Participants are updated on  
23 the remaining time after 1.00 and 1.45 minutes, and the final five seconds are indicated by a  
24 countdown. The total walking distance in 2 minutes is recorded in total meters covered with  
25 remaining scored in centimeters.  
26  
27  
28  
29  
30  
31  
32  
33  
34

35 Locomotion, measured as gait speed, will be tested with a *4MWT*. Gait speed is a  
36 simple measure to summarize the overall disease burden and disability. [50, 51] In brief, two  
37 pylons will be set apart 4 meters and instructed to walk at usual pace. Seconds from start to  
38 end of the 4 meters will be recorded. The *4MWT* is measured twice after first a trial round.  
39  
40  
41  
42  
43

44 Manual dexterity will be measured in all transplant recipients using the *9-Hole Peg*  
45 *Test* (9-HPT, Sammons Preston Rolyan, Chicago, IL). The 9-HPT requires participants to  
46 repeatedly place and remove nine pegs into nine holes, one at a time, as quickly as possible,  
47 and is considered to be the gold standard metric for manual dexterity.  
48  
49  
50  
51

52 Functional mobility will be tested in participants using the *FTSTS* and *TUG*. The  
53 *FTSTS* is a functional performance measure of leg strength or the force-generating capacity of  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 muscle by using the body's weight for resistance during functional activities.[52] The FTSTS  
4 will be executed three times after a first trial round. Participants will be instructed to stand up  
5  
6 five times as fast as possible, from sitting position with their feet flat on the floor and arms  
7  
8 folded across the chest. Measurements start upon command, and subsequently the time  
9  
10 required to stand up and return sitting is recorded. Time is measured in seconds and this task  
11  
12 is repeated five times.[53]  
13  
14

15  
16 The TUG is a basic test for functional mobility and is based on strength, coordination,  
17  
18 and balance.[54] For the test, a pylon and chair will be put apart 3 meters. The test will be  
19  
20 performed four times, with the first round being a trial. Participants are instructed to stand up  
21  
22 from the chair without support of the arms, subsequently walk with their normal gait speed  
23  
24 around the pylon, and go back to the chair to sit down again. In case, participants use a  
25  
26 walking aid in normal day life, the test will be performed with the use of a walking aid. The  
27  
28 TUG is measured in seconds, from the moment the participant is instructed to get up until the  
29  
30 moment the participant sits down again.  
31  
32

33 Transplant recipients have an increased susceptibility to develop peripheral  
34  
35 neuropathy and tremor, mainly due to the continuous use of immunosuppressive medication,  
36  
37 especially calcineurin inhibitors.[55, 56] Therefore, an extensive neurological examination  
38  
39 will be performed and will consist of strength testing, classifying polyneuropathy, and tremor  
40  
41 quantification. Detailed strength testing of different muscle groups (feet flexion/extension, hip  
42  
43 flexion, biceps flexion and wrist extension) will be performed with a digital dynamometer  
44  
45 (C.I.T. Technics, Haren, the Netherlands).[57] Hereafter, sensibility tests will be performed  
46  
47 using a pin-prick and monofilament pen (Novo Norisk BV, Alphen aan de Rijn, the  
48  
49 Netherlands) on bare skin five times per measurement at the dorsal side of the 1<sup>st</sup> phalange of  
50  
51 both feet with the subject closing their eyes. Upon failure of sensibility, the dorsal side of the  
52  
53 foot and lower limb will be tested. Proprioception will be measured by moving the 1<sup>st</sup>  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 phalange of both feet in dorsal flexion and plantar flexion five times with the participants  
4 closing their eyes. Upon failure, the dorsal side of the foot and index finger will be measured.  
5

6  
7 Vibration sense will be measured using a handheld biothesiometer (Bio Medical  
8 Instrument Co, Ohio, USA).[58] The biothesiometer has a rubber tractor that vibrates at 100  
9 Hz when operating from 50 Hz mains. In brief, participants will be measured in a supine  
10 position on a bed barefooted. The vibrating tractor will be applied bi-laterally to four different  
11 measurement points of the participants: top of the hallux, forefoot, lateral malleoli, and wrist.  
12 Before applying the vibrating tractor to the points to be tested, the amplitude of the vibrating  
13 tractor is increased from zero to the point where the vibration is perceptible and beyond the  
14 threshold to the highest amplitude possible to familiarize participants with the sensation. For  
15 the measurement, the participants will be asked to concentrate on the test and report the first  
16 sensation of the vibration by saying “Stop”. Each measurement point is tested twice. If the  
17 difference between the first two measurements is greater than 20%, the measurement point is  
18 tested a third time.  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32

33 Prior to tremor quantification, participants will be asked to complete part C of the  
34 Fahn-Tolosa-Marin tremor rating scale that involves tremor-related functional disability.[59]  
35 The questionnaire involves speaking, bringing liquids to the mouth, eating, hygienic care,  
36 dressing, writing, work and household related tasks. The questionnaire uses a 5-point scale,  
37 with ‘0= no functional ability’, and 4= ‘severe disability, the task cannot be executed’.  
38  
39  
40  
41  
42  
43

44 To quantify tremor, two accelerometers (University Medical Center Groningen,  
45 Groningen, the Netherlands) will be attached to the dorsal side of both hands. The  
46 accelerometers will record movement in the coronal, transversal and sagittal planes as well as  
47 linear acceleration and deceleration in both hands continuously during the measurements.  
48 Amplitudes and frequency of these measurements will be recorded on a stand-alone computer.  
49 Participants will be asked to assume seven different positions while seated, which are  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 measured for 30 seconds each: arms down, wrists extended; arms forward, wrists and fingers  
4 relaxed; arms forward, wrists and fingers in 0 position; index fingers pointed towards each  
5 other; bilateral finger-nose task; weighted arms down with wrists expanded; weighted arms  
6 forward with wrists and fingers extended.  
7  
8  
9

10  
11 Finally, participants will be asked for collection of a breath sample in which hydrogen  
12 and methane will be measured with the Quintron BreathTracker (Milwaukee, Wisconsin,  
13 USA).[60] Both hydrogen and methane are exclusively formed by anaerobic fermentation in  
14 the gut, and therefore can be utilized as markers for metanogenic microflora in transplant  
15 recipients.[61]  
16  
17  
18  
19  
20  
21  
22  
23

#### 24 **Cognitive Protocol Measurements and Tests**

25  
26 The *Cognitive Screening Test (CST)* is a Dutch screening test for dementia, measuring  
27 orientation in time and place, and memory for common facts.[62] The questionnaire consists  
28 of 20 items (e.g. date of birth, name of the reigning monarch, season) and the score is  
29 calculated as the total of questions answered correctly with a maximum of 20.  
30  
31  
32  
33

34  
35 *Nederlandse Leestest voor Volwassenen (NLV)*, Dutch version of the National Adult  
36 Reading Test. The participant has to read aloud a list of 50 irregularly spelled words. The total  
37 score on the test is converted into an estimation of the premorbid intelligence quotient.[63]  
38  
39  
40

41  
42 The *Clock Drawing Test (CDT)* is a cognitive screening instrument.[64] Participants  
43 are asked to draw a clock and set the time to 'a quarter to two'. A maximum total score of 14  
44 can be achieved.  
45  
46  
47

48  
49 The *15 Words Test* (Dutch version of the Rey Auditory Verbal Learning Test  
50 (RAVLT), measures verbal memory.[65] In this task a set of 15 unrelated words is presented  
51 to the participant, consecutively over five trials. Participants are asked to recall as many  
52 words as possible immediately after each trial (Immediate Recall). The score is the total  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 words recalled in 5 trials, with a maximum of 75. After 20 minutes, participants are asked to  
4 recall as many of the 15 words as possible (Delayed Recall). Additionally, a recognition task  
5 will be performed. Participants are presented with a list of 30 words and are asked which  
6 words they recognize from the list they have been presented before.  
7  
8  
9

10  
11 *Digit Span*, subtest of the Wechsler Adult Intelligence Scale (WAIS-IV).[66] This  
12 subtest consists of two tasks, the Digit Span Forward and the Digit Span Backward. The Digit  
13 Span Forward is a task for immediate auditory memory span. In this task, participants are  
14 asked to repeat a series of numbers in the same order as the examiner did. The Digit Span  
15 Backward measures working memory. Participants have to repeat the presented numbers in  
16 reversed order. The score is the total strings repeated, with a maximum of 32.  
17  
18  
19  
20  
21  
22  
23

24 The *Word Fluency*, subtest of the Groninger Intelligentie Test (GIT-2), is a verbal task  
25 measuring semantic memory.[67] Participants are asked to name as many words within a  
26 certain category within one minute. Total score per category (respectively animals and  
27 professions) were calculated.  
28  
29  
30  
31  
32

33 The *Controlled Oral Word Association Test (COWAT)* is a verbal task measuring  
34 executive control.[68] Participants have to name as many words as possible that start with a  
35 specific letter within one minute. In the meantime, participants have to comply to several  
36 rules that are given on beforehand. Total scores from three different starting letters (D-A-T)  
37 were calculated.  
38  
39  
40  
41  
42  
43

44 The *Symbol Digit Modalities Test (SDMT)* measures psychomotor speed.[69] The test  
45 consists of matching symbols and numbers as fast as possible in 90 seconds. The total score  
46 of correct matches is calculated.  
47  
48  
49

50 *Trail Making Test (TMT)*. This test consists of two parts: Trail Making Test – A  
51 (TMT-A) and Trail Making Test – B (TMT-B). Part A is a measure of attention and  
52 information processing speed. This task involves connecting 25 numbers in ascending order,  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 as quickly as possible. The TMT-B is a measure of divided attention and cognitive flexibility.  
4  
5 In this condition, numbers as well as letters have to be connected in ascending order,  
6  
7 alternating between numbers and letters (1-A-2-B- etc.). Both parts of the test are timed to  
8  
9 completion (number of seconds).  
10

11       The *Key Search Test* is a subtest of the Behavioral Assessment of the Dysexecutive  
12  
13 Syndrome (BADS) and assesses the ability to plan and monitor progress. Participants are  
14  
15 presented with a square which represents a field in which ‘keys have been lost’. Participants  
16  
17 must show how they would search the field to find the keys. Searching strategy is scored by  
18  
19 means of functionality and maximum total score of 16 can be achieved.  
20  
21

## 22 23 24 **Outcomes**

25  
26 The primary outcomes of the TransplantLines study are all-cause mortality and graft failure,  
27  
28 which is defined as death due to failure of the transplanted organ, return to organ replacement  
29  
30 therapy or re-transplantation. Secondary outcomes will be cause-specific mortality, cause-  
31  
32 specific graft failure and rejection. Tertiary outcomes will be other health problems, including  
33  
34 diabetes, obesity, hypertension, hypercholesterolemia, and cardiovascular disease, and  
35  
36 disturbances that relate to quality of life, e.g. physical and psychological functioning, quality  
37  
38 of sleep, and neurological problems such as tremor and polyneuropathy.  
39  
40

41  
42       The TransplantLines biobank study aims to identify risk factors for health problems  
43  
44 and patient-centered outcomes (e.g. adverse drug events, lifestyle, quality of life, social  
45  
46 participation, physical and cognitive functioning). Due to the nature of the biobank, not all  
47  
48 research questions are predefined and will arise during the course of inclusion. In contrast to  
49  
50 many other studies, TransplantLines also aims to identify and ameliorate complaints  
51  
52 experienced by transplant recipients, such as tremors and diarrhea, which to date have largely  
53  
54 been overlooked by clinicians.  
55  
56  
57  
58  
59  
60

### **Data management, analysis, and access to data and samples**

Data will be recorded digitally in an electronic case report form (eCRF) in a certified Electronic Data Capture and Clinical Data Management System (Utopia Data Management System version 1.13.6, Research Data Support, University Medical Center Groningen). Data entry is performed by the trained investigators. The trained investigator who performed assessments at the study visit of a participant is responsible for data entry of that participant. All data are later checked again by the trained investigators and are subsequently stored anonymously in a secured electronic environment. The TransplantLines database will be linked to registries and databases of the Dutch Health Database (DHD), Netherlands Comprehensive Cancer Organisation (IKNL), Central Bureau of Statistics (CBS), InterAction Database (IADB), Dutch Nephrology Registration/Registration Renal Replacement Therapy (Nefrovisie, Renine), Nationwide Network and Registry of Histo- and Cytopathology in the Netherlands (PALGA), National Organ Transplant Registry (NOTR), PHARMO Institute for Drug Outcome Research (PHARMO), Routine Outcome Monitoring (RoQua) and the Dutch Institute of Clinical Auditing database (DICA) through a generic layer. A data management board will be formed to maintain data infrastructure, construct Material Transfer Agreements (MTA) and to govern use of the TransplantLines biobank and database. Extractions from TransplantLines database will be performed using a retrieval suite in Utopia software package only after approval of the data management board. Data will always be extracted anonymously. SPSS statistics version 23 (IBM, Armonk, NY), R version 3.2.3 (CRAN, Vienna, Austria), STATA 14.1 (STATA Corp., College Station, TX) or a similar statistical package will be used for analysis. Data collection and management is performed in accordance with the Handbook for Adequate Natural Data Stewardship (Netherlands Federation of University Medical Centers, 2017). A team consisting of medical doctors of the

1  
2  
3 different fields involved, called Research Team TransplantLines, is installed to decide and  
4  
5 prioritize who will get access to the samples and data of the TransplantLines biobank and  
6  
7 cohort study. Use of samples and data can be requested by internal and external researchers  
8  
9 against a reasonable fee. All samples are stored at -80°C and access is logged in a linked  
10  
11 database. The logging system also provides for registration of multiple access and the number  
12  
13 of freeze-thaw cycles that samples have undergone. Multiple access to samples is possible,  
14  
15 but for each specific project a new request needs to be performed and approved by the Research  
16  
17 Team TransplantLines. The data coming available from the assays performed at the provided  
18  
19 samples will be linked to the TransplantLines database and be made available to researchers  
20  
21 in the certified Electronic Data Capture and Clinical Data Management System which will  
22  
23 allow for evaluation and statistical analyses. This environment will also monitor and log data  
24  
25 handling and store results of analyses.  
26  
27  
28  
29  
30

### 31 Missing data handling

32  
33 Concerning treatment of missing data and inability to generate data from missing samples, we  
34  
35 will apply statistical methods using maximum likelihood and multiple imputation, which are  
36  
37 now standard for dealing with participant loss and missing data.[70] These methods provide  
38  
39 more consistent and efficient estimates of population parameters than methods relying on  
40  
41 complete cases, mean imputation, last observation carried forward or single-imputation  
42  
43 regression methods.[70-73] As advised in authoritative reports, these analyses will be  
44  
45 complemented with sensitivity analyses to assess robustness of findings.[74-76]  
46  
47  
48  
49

### 50 Discussion

51  
52 The TransplantLines prospective cohort study seeks to identify risk factors for the  
53  
54 development of long-term health problems after transplantation and ultimately to develop new  
55  
56  
57  
58  
59

1  
2  
3 and innovative interventions to improve graft survival, patient survival, and quality of life  
4 after transplantation. The TransplantLines biobank will encompass all solid organ  
5 transplantations and living organ donors. It will consist of follow-up data from all fields that  
6 are involved in organ transplantation; internal medicine, surgery, gastroenterology,  
7 hepatology, pulmonology, cardiology, dermatology, neurology, occupational medicine,  
8 children's medicine, (neuro)psychology, physiotherapy, and social work.  
9  
10  
11  
12  
13  
14

15  
16 Although short-term transplant outcomes have improved in the last decades, graft and  
17 recipient life expectancy remains limited. In the TransplantLines study, data and samples will  
18 be collected before, during, and after transplantation to gather further insight in the impact of  
19 transplantation on transplant recipients. In addition, we aim to preemptively detect those  
20 transplant recipients who are at increased risk to develop graft failure or health problems. By  
21 investigating a wide range of clinical, social/psychological and biochemical parameters, this  
22 study aims to contribute to increased transplant survival, patient survival, but also to an  
23 increased quality of life and a more patient-centered approach to transplant care.  
24  
25  
26  
27  
28  
29  
30  
31  
32

33 Our study has strengths and limitations. The major strengths of this study are the  
34 collection of extensive data on a myriad topics related to transplantation, the inclusion of all  
35 types of solid organ transplant recipients and living organ donors, and a study with a long  
36 follow-up to assess many relevant clinical outcomes. Limitations of the current study are that  
37 it comprises a single center study and that residual confounding cannot be excluded in  
38 analyses in the TransplantLines study due to its observational design. It is also a limitation  
39 that transplant recipients with limited language skills and/or poor comprehension are  
40 excluded, because these patients are likely those who are at higher risk of poor compliance  
41 and high risk social behavior, which would possibly have worse outcomes. A further  
42 limitation is that our infrequent collection of biobank samples may limit utility in detecting  
43 biomarkers for routine monitoring of transplant health and detection of suitable biomarkers  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 may only be possible if sampling happens to fall near the time of a clinical event. It may be  
4  
5 considered a strength that in addition to taking samples at fixed time points, we also take  
6  
7 samples when biopsies are performed, both at times of protocol biopsies and at times of  
8  
9 biopsies taken at clinical indication, usually because of worsening of transplant function, with  
10  
11 suspicion of acute or chronic rejection.  
12

13  
14 TransplantLines may serve as a basis for hypothesis-generating studies that yield  
15  
16 insights in a wide range of clinical, social/psychological and biochemical parameters in solid  
17  
18 organ transplant recipients, as well as living donors. Biomarkers may be identified to develop  
19  
20 more individualized immunosuppressive treatment. This will lead to novel clinical trials in  
21  
22 transplantation and patient-tailored approaches for new treatment options. Furthermore, the  
23  
24 results of TransplantLines may serve to identify new modifiable risk factors and lifestyle  
25  
26 factors in transplantation. Ultimately, this information will likely contribute to a more  
27  
28 individualized treatment for transplant patients and improved living donor screening and  
29  
30 follow-up. Thereby we aim to qualitatively and quantitatively improve outcomes after  
31  
32 transplantation.  
33

### 34 35 36 37 **Study status**

38  
39 Data collection is in progress.  
40  
41  
42

### 43 44 **List of abbreviations used**

45  
46 METc – Medical Ethical Committee

47  
48 UMCG – University Medical Center Groningen

49  
50 EDTA - Ethylenediaminetetraacetic acid

51  
52 SOP – Standard Operating Procedure

53  
54 BIA – Bio-Impedance Analysis  
55  
56  
57  
58  
59  
60

1  
2  
3 ICD – Implantable Cardioverter-Defibrillator

4 SAF – Skin Auto-Fluorescence

5  
6  
7 AGE – Advanced Glycation End-product

8  
9 PG-SGA - Scored Patient-Generated Subjective Global Assessment

10  
11 2MWT – 2-Minute Walk Test

12  
13 FTSTS – Five Time Sit To Stand Test

14  
15 TUG – Timed Up and Go

16  
17 CRF – Clinical Research Form

18  
19 PI – Principal Investigator

## 20 21 22 23 24 **Author contributions**

25  
26 M.F.E. designed the study, conducts the study for transplant recipients, wrote the manuscript,  
27 and is responsible for final content of the manuscript; A.W.G.N. conducts the study for  
28 transplant recipients and revised the manuscript; M.vL. conducts the study for living donors  
29 and wrote the part of living donors in the manuscript; A.L.Z. conducts the study for  
30 transplant recipients, living donors and wrote the cognitive protocol; R.M.D., S.P.S, T.J.K.,  
31 and M.C.J.O. conduct the study for transplant recipients; N.R.H. conducts the study for living  
32 donors; A.M.B., M.J.S., C.A., E.R., J.M.S., F.A.J.A.B, R.A.P., S.P.B., G.D., R.J.P., H.G.D.L.,  
33 K.D., E.A.M.V., V.E.dM gave substantial input from their field of knowledge when designing  
34 the study and revised the manuscript; R.A.P. is responsible for kidney biobank sampling and  
35 logistics; H.B. conducts the study and revised the manuscript; S.J.L.B. designed the study, is  
36 principal investigator and project leader, and takes full responsibility for the whole study. All  
37 authors approved the final version of the manuscript and agree to be accountable for all  
38 aspects of the submitted work. All collaborators have revised the manuscript and approved the  
39 final version of the manuscript.

## Acknowledgements

We thank prof. John Mathers and dr. José Lara from Newcastle University for aid in setting up a test package for the TransplantLines study based on the Healthy Ageing Phenotype measurements.

## Funding information

This work was supported by a grant from Astellas BV

## Competing interests

None

## References

- 1 Kirk R, Dipchand AI, Edwards LB, et al. The Registry of the International Society for Heart and Lung Transplantation: fifteenth pediatric heart transplantation report--2012. *J Heart Lung Transplant* 2012;31:1065-72 doi:10.1016/j.healun.2012.08.001 [doi].
- 2 Lamb KE, Lodhi S, Meier-Kriesche HU. Long-term renal allograft survival in the United States: a critical reappraisal. *Am J Transplant* 2011;11:450-62 doi:10.1111/j.1600-6143.2010.03283.x [doi].
- 3 Lodhi SA, Lamb KE, Meier-Kriesche HU. Solid organ allograft survival improvement in the United States: the long-term does not mirror the dramatic short-term success. *Am J Transplant* 2011;11:1226-35 doi:10.1111/j.1600-6143.2011.03539.x [doi].
- 4 Dharnidharka VR, Lamb KE, Zheng J, et al. Lack of significant improvements in long-term allograft survival in pediatric solid organ transplantation: A US national registry analysis. *Pediatr Transplant* 2015;19:477-83 doi:10.1111/petr.12465 [doi].
- 5 Hariharan S, Johnson CP, Bresnahan BA, et al. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med* 2000;342:605-12 doi:10.1056/NEJM0101063420605 [pii].
- 6 Aasebo W, Homb-Vesteraas NA, Hartmann A, et al. Life situation and quality of life in young adult kidney transplant recipients. *Nephrol Dial Transplant* 2009;24:304-8 doi:10.1093/ndt/gfn537 [doi].
- 7 Kugler C, Fischer S, Gottlieb J, et al. Symptom experience after lung transplantation: impact on quality of life and adherence. *Clin Transplant* 2007;21:590-6 doi:10.1111/j.1399-3113.2007.00693.x [pii].



- 1  
2  
3 8 Matas AJ, McHugh L, Payne WD, et al. Long-term quality of life after kidney and  
4 simultaneous pancreas-kidney transplantation. *Clin Transplant* 1998;12:233-42.  
5  
6  
7  
8 9 Morath C, Mueller M, Goldschmidt H, et al. Malignancy in renal transplantation. *J Am Soc*  
9  
10 *Nephrol* 2004;15:1582-8.  
11  
12  
13 10 Rodrigo E, Fernandez-Fresnedo G, Valero R, et al. New-onset diabetes after kidney  
14 transplantation: risk factors. *J Am Soc Nephrol* 2006;17:S291-5 doi:17/12\_suppl\_3/S291 [pii].  
15  
16  
17 11 Jezior D, Krajewska M, Madziarska K, et al. Posttransplant overweight and obesity: myth  
18 or reality?. *Transplant Proc* 2007;39:2772-5 doi:S0041-1345(07)01095-0 [pii].  
19  
20  
21  
22 12 Held PJ, McCormick F, Ojo A, et al. A Cost-Benefit Analysis of Government  
23 Compensation of Kidney Donors. *Am J Transplant* 2016;16:877-85 doi:10.1111/ajt.13490  
24  
25  
26  
27  
28 [doi].  
29  
30  
31 13 Mjoen G, Hallan S, Hartmann A, et al. Long-term risks for kidney donors. *Kidney Int*  
32 2014;86:162-7 doi:10.1038/ki.2013.460 [doi].  
33  
34  
35  
36 14 Muzaale AD, Massie AB, Wang MC, et al. Risk of end-stage renal disease following live  
37 kidney donation. *JAMA* 2014;311:579-86 doi:10.1001/jama.2013.285141 [doi].  
38  
39  
40  
41 15 Massie AB, Leanza J, Fahmy LM, et al. A Risk Index for Living Donor Kidney  
42 Transplantation. *Am J Transplant* 2016;16:2077-84 doi:10.1111/ajt.13709 [doi].  
43  
44  
45  
46 16 Reese PP, Boudville N, Garg AX. Living kidney donation: outcomes, ethics, and  
47 uncertainty. *Lancet* 2015;385:2003-13 doi:10.1016/S0140-6736(14)62484-3 [doi].  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 17 Miller CM, Durand F, Heimbach JK, et al. The International Liver Transplant Society  
4  
5 Guideline on Living Liver Donation. *Transplantation* 2016;100:1238-43  
6  
7 doi:10.1097/TP.0000000000001247 [doi].  
8  
9  
10 18 Trotter JF. Selection of donors and recipients for living donor liver transplantation. *Liver*  
11  
12 *Transpl* 2000;6:S52-8 doi:10.1053/jlts.2000.18685 [doi].  
13  
14  
15 19 Hoeymans N, van Lindert H, Westert GP. The health status of the Dutch population as  
16  
17 assessed by the EQ-6D. *Qual Life Res* 2005;14:655-63.  
18  
19  
20 20 Reips UD, Funke F. Interval-level measurement with visual analogue scales in Internet-  
21  
22 based research: VAS Generator. *Behav Res Methods* 2008;40:699-704.  
23  
24  
25 21 Ware JE, Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I.  
26  
27 Conceptual framework and item selection. *Med Care* 1992;30:473-83.  
28  
29  
30 22 Wendel-Vos GC, Schuit AJ, Saris WH, et al. Reproducibility and relative validity of the  
31  
32 short questionnaire to assess health-enhancing physical activity. *J Clin Epidemiol*  
33  
34 2003;56:1163-9 doi:S0895435603002208 [pii].  
35  
36  
37 23 Schafer-Keller P, Steiger J, Bock A, et al. Diagnostic accuracy of measurement methods to  
38  
39 assess non-adherence to immunosuppressive drugs in kidney transplant recipients. *Am J*  
40  
41 *Transplant* 2008;8:616-26 doi:10.1111/j.1600-6143.2007.02127.x [doi].  
42  
43  
44 24 Dobbels F, Moons P, Abraham I, et al. Measuring symptom experience of side-effects of  
45  
46 immunosuppressive drugs: the Modified Transplant Symptom Occurrence and Distress Scale.  
47  
48 *Transpl Int* 2008;21:764-73 doi:10.1111/j.1432-2277.2008.00674.x [doi].  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 25 Beurskens AJ, Bultmann U, Kant I, et al. Fatigue among working people: validity of a  
4  
5 questionnaire measure. *Occup Environ Med* 2000;57:353-7.  
6  
7  
8 26 Buysse DJ, Reynolds CF,3rd, Monk TH, et al. The Pittsburgh Sleep Quality Index: a new  
9  
10 instrument for psychiatric practice and research. *Psychiatry Res* 1989;28:193-213 doi:0165-  
11  
12 1781(89)90047-4 [pii].  
13  
14  
15 27 Marteau TM, Bekker H. The development of a six-item short-form of the state scale of the  
16  
17 Spielberger State-Trait Anxiety Inventory (STAI). *Br J Clin Psychol* 1992;31 ( Pt 3):301-6.  
18  
19  
20 28 Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity  
21  
22 measure. *J Gen Intern Med* 2001;16:606-13 doi:jgi01114 [pii].  
23  
24  
25 29 Gillanders DT, Bolderston H, Bond FW, et al. The development and initial validation of  
26  
27 the cognitive fusion questionnaire. *Behav Ther* 2014;45:83-101  
28  
29 doi:10.1016/j.beth.2013.09.001 [doi].  
30  
31  
32 30 Topp CW, Ostergaard SD, Sondergaard S, et al. The WHO-5 Well-Being Index: a  
33  
34 systematic review of the literature. *Psychother Psychosom* 2015;84:167-76  
35  
36 doi:10.1159/000376585 [doi].  
37  
38  
39 31 Ziegelmann JP, Griva K, Hankins M, et al. The Transplant Effects Questionnaire (TxEQ):  
40  
41 The development of a questionnaire for assessing the multidimensional outcome of organ  
42  
43 transplantation - example of end stage renal disease (ESRD). *Br J Health Psychol*  
44  
45 2002;7:393-408 doi:10.1348/135910702320645381 [doi].  
46  
47  
48 32 Pearlin LI, Schooler C. The structure of coping. *J Health Soc Behav* 1978;19:2-21.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 33 Abma FI, Amick BC,3rd, Brouwer S, et al. The cross-cultural adaptation of the work role  
4 functioning questionnaire to Dutch. *Work* 2012;43:203-10 doi:L60354360J658155 [pii].  
5  
6

7  
8 34 Longstreth GF, Thompson WG, Chey WD, et al. Functional bowel disorders.  
9  
10 *Gastroenterology* 2006;130:1480-91 doi:S0016-5085(06)00512-9 [pii].  
11  
12

13 35 Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a  
14 semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985;122:51-65.  
15  
16

17  
18 36 Angst F, Drerup S, Werle S, et al. Prediction of grip and key pinch strength in 978 healthy  
19 subjects. *BMC Musculoskelet Disord* 2010;11:94,2474-11-94 doi:10.1186/1471-2474-11-94  
20  
21 [doi].  
22  
23

24  
25 37 Trampisch US, Franke J, Jedamzik N, et al. Optimal Jamar dynamometer handle position  
26 to assess maximal isometric hand grip strength in epidemiological studies. *J Hand Surg Am*  
27  
28 2012;37:2368-73 doi:10.1016/j.jhsa.2012.08.014 [doi].  
29  
30

31  
32 38 Drew CD, Hughes DT. Characteristics of the Vitalograph spirometer. *Thorax*  
33  
34 1969;24:703-6.  
35  
36

37  
38 39 Kyle UG, Bosaeus I, De Lorenzo AD, et al. Bioelectrical impedance analysis--part I:  
39 review of principles and methods. *Clin Nutr* 2004;23:1226-43 doi:10.1016/j.clnu.2004.06.004  
40  
41 [doi].  
42  
43

44  
45 40 Mulder DJ, Water TV, Lutgers HL, et al. Skin autofluorescence, a novel marker for  
46 glycemic and oxidative stress-derived advanced glycation endproducts: an overview of  
47 current clinical studies, evidence, and limitations. *Diabetes Technol Ther* 2006;8:523-35  
48  
49 doi:10.1089/dia.2006.8.523 [doi].  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 41 McIntyre NJ, Fluck RJ, McIntyre CW, et al. Skin autofluorescence and the association  
4 with renal and cardiovascular risk factors in chronic kidney disease stage 3. *Clin J Am Soc*  
5 *Nephrol* 2011;6:2356-63 doi:10.2215/CJN.02420311 [doi].  
6  
7  
8  
9  
10 42 Zwald FO, Brown M. Skin cancer in solid organ transplant recipients: advances in therapy  
11 and management: part I. Epidemiology of skin cancer in solid organ transplant recipients. *J*  
12 *Am Acad Dermatol* 2011;65:253,61; quiz 262 doi:10.1016/j.jaad.2010.11.062 [doi].  
13  
14  
15  
16  
17 43 Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. *Arch*  
18 *Dermatol* 1988;124:869-71.  
19  
20  
21  
22  
23 44 Rockwood K, Song X, MacKnight C, et al. A global clinical measure of fitness and frailty  
24 in elderly people. *CMAJ* 2005;173:489-95 doi:173/5/489 [pii].  
25  
26  
27  
28 45 Ottery FD. Definition of standardized nutritional assessment and interventional pathways  
29 in oncology. *Nutrition* 1996;12:S15-9.  
30  
31  
32  
33 46 Reuben DB, Magasi S, McCreath HE, et al. Motor assessment using the NIH Toolbox.  
34 *Neurology* 2013;80:S65-75 doi:10.1212/WNL.0b013e3182872e01 [doi].  
35  
36  
37  
38 47 Bohannon RW, Wang YC, Gershon RC. Two-minute walk test performance by adults 18  
39 to 85 years: normative values, reliability, and responsiveness. *Arch Phys Med Rehabil*  
40 2015;96:472-7 doi:10.1016/j.apmr.2014.10.006 [doi].  
41  
42  
43  
44 48 ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories.  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 49 Butland RJ, Pang J, Gross ER, et al. Two-, six-, and 12-minute walking tests in respiratory  
4 disease. *Br Med J (Clin Res Ed)* 1982;284:1607-8.

5  
6  
7  
8 50 Studenski S, Perera S, Patel K, et al. Gait speed and survival in older adults. *JAMA*  
9 2011;305:50-8 doi:10.1001/jama.2010.1923 [doi].

10  
11  
12  
13 51 Perera S, Patel KV, Rosano C, et al. Gait Speed Predicts Incident Disability: A Pooled  
14 Analysis. *J Gerontol A Biol Sci Med Sci* 2016;71:63-71 doi:10.1093/gerona/glv126 [doi].

15  
16  
17  
18 52 Bohannon RW, Barreca SR, Shove ME, et al. Documentation of daily sit-to-stands  
19 performed by community-dwelling adults. *Physiother Theory Pract* 2008;24:437-42  
20 doi:10.1080/09593980802511813 [doi].

21  
22  
23  
24 53 Bohannon RW. Test-retest reliability of the five-repetition sit-to-stand test: a systematic  
25 review of the literature involving adults. *J Strength Cond Res* 2011;25:3205-7  
26 doi:10.1519/JSC.0b013e318234e59f [doi].

27  
28  
29  
30 54 Kwan MM, Lin SI, Chen CH, et al. Sensorimotor function, balance abilities and pain  
31 influence Timed Up and Go performance in older community-living people. *Aging Clin Exp*  
32 *Res* 2011;23:196-201 doi:8027 [pii].

33  
34  
35  
36 55 Arnold R, Pussell BA, Pianta TJ, et al. Association between calcineurin inhibitor treatment  
37 and peripheral nerve dysfunction in renal transplant recipients. *Am J Transplant*  
38 2013;13:2426-32 doi:10.1111/ajt.12324 [doi].

39  
40  
41  
42 56 Langone A, Steinberg SM, Gedaly R, et al. Switching STudy of Kidney TRansplant  
43 PATients with Tremor to LCP-TacrO (STRATO): an open-label, multicenter, prospective  
44 phase 3b study. *Clin Transplant* 2015;29:796-805 doi:10.1111/ctr.12581 [doi].  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 57 van der Ploeg RJ, Fidler V, Oosterhuis HJ. Hand-held myometry: reference values. *J*  
4  
5 *Neurol Neurosurg Psychiatry* 1991;54:244-7.

6  
7  
8 58 Frenette B, Mergler D, Ferraris J. Measurement precision of a portable instrument to  
9  
10 assess vibrotactile perception threshold. *Eur J Appl Physiol Occup Physiol* 1990;61:386-91.

11  
12  
13 59 Elble R, Bain P, Forjaz MJ, et al. Task force report: scales for screening and evaluating  
14  
15 tremor: critique and recommendations. *Mov Disord* 2013;28:1793-800  
16  
17 doi:10.1002/mds.25648 [doi].

18  
19  
20  
21 60 Tap J, Derrien M, Tornblom H, et al. Identification of an Intestinal Microbiota Signature  
22  
23 Associated With Severity of Irritable Bowel Syndrome. *Gastroenterology*  
24  
25 2017;152:111,123.e8 doi:S0016-5085(16)35174-5 [pii].

26  
27  
28 61 Sahakian AB, Jee SR, Pimentel M. Methane and the gastrointestinal tract. *Dig Dis Sci*  
29  
30 2010;55:2135-43 doi:10.1007/s10620-009-1012-0 [doi].

31  
32  
33  
34 62 Graaf Ad, Deelman BG. Cognitieve Screening Test. Handleiding. Lisse: Swets and  
35  
36 Zeitlinger 1991.

37  
38  
39 63 Schmand B, Lindeboom J, van Harskamp F. Nederlandse leestest voor volwassenen:  
40  
41 handleiding (NLV). Amsterdam: Pearson Assessment and Information B.V. 1992.

42  
43  
44 64 Shulman KI, Shedletsky R, Silver IL. The challenge of time: Clock drawing and  
45  
46 cognitive function in the elderly. *Int J Geriatr Psychiatry* 1986;1:135-40.

47  
48  
49  
50 65 Saan R, Deelman B. De 15-Woorden Tests A en B. (Een voorlopige handleiding).  
51  
52 Groningen: University Medical Center Groningen, department of Neuropsychology 1986.

- 1  
2  
3 66 Wechsler D. WAIS-IV-NL: Wechsler Adult Intelligence Scale - Fourth Edition -  
4  
5 Nederlandstalige bewerking: Technische handleiding en Afname en scoringshandleiding.  
6  
7 Amsterdam: Pearson Assessment and Information B.V. 2012.  
8  
9  
10 67 Luteijn F, Barelds D. GIT2: Groninger Intelligentie Test 2. Amsterdam: Pearson  
11  
12 Assessment and Information B.V. 2004.  
13  
14  
15 68 Schmand B, Groenink SC, den Dungen M. Letterfluency: psychometrische eigenschappen  
16  
17 en Nederlandse normen. *Tijdschr Gerontol Geriatr* 2008;39:64-74.  
18  
19  
20 69 Smith A. Symbol Digits Modalities Test Los Angeles: Western Psychological Services.  
21  
22 Los Angeles: Western Psychological Services 1968:83-91.  
23  
24  
25  
26 70 Christensen H, Mackinnon A, Jorm AF, et al. The Canberra longitudinal study: Design,  
27  
28 aims, methodology, outcomes and recent empirical investigations. *Aging, Neuropsychology,*  
29  
30 *and Cognition* 2004;11:169-95 doi:10.1080/13825580490511053.  
31  
32  
33  
34 71 Woolley SB, Cardoni AA, Goethe JW. Last-observation-carried-forward imputation  
35  
36 method in clinical efficacy trials: review of 352 antidepressant studies. *Pharmacotherapy*  
37  
38 2009;29:1408-16 doi:10.1592/phco.29.12.1408 [doi].  
39  
40  
41  
42 72 Schafer JL, Graham JW. Missing data: our view of the state of the art. *Psychol Methods*  
43  
44 2002;7:147-77.  
45  
46  
47 73 Graham JW. Missing data analysis: making it work in the real world. *Annu Rev Psychol*  
48  
49 2009;60:549-76 doi:10.1146/annurev.psych.58.110405.085530 [doi].  
50  
51  
52  
53 74 Little RJ, D'Agostino R, Cohen ML, et al. The prevention and treatment of missing data in  
54  
55 clinical trials. *N Engl J Med* 2012;367:1355-60 doi:10.1056/NEJMs1203730 [doi].  
56  
57  
58  
59



1  
2  
3 75 Lee KJ, Simpson JA. Introduction to multiple imputation for dealing with missing data.

4  
5 *Respirology* 2014;19:162-7 doi:10.1111/resp.12226 [doi].  
6  
7

8 76 Sainani KL. Dealing With Missing Data. *PM R* 2015;7:990-4  
9

10 doi:10.1016/j.pmrj.2015.07.011 [doi].  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

## Figure legends

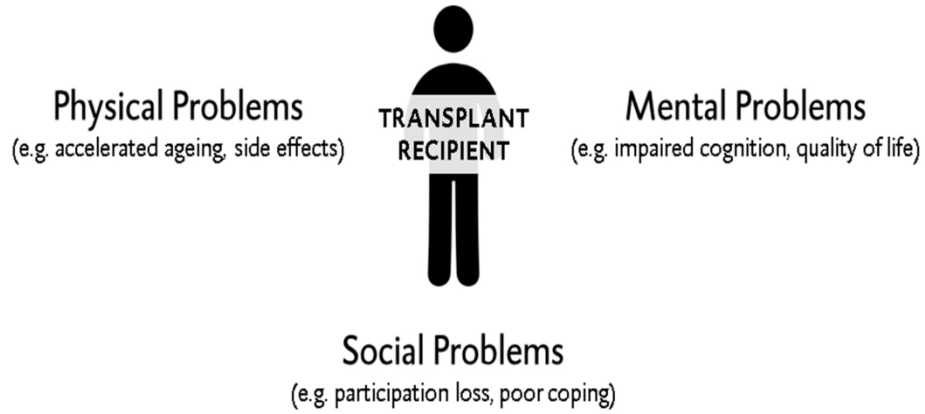
**Figure 1.** Overview of different health problems that arise on the long term after transplantation, both physical, psychological, and social.

**Figure 2.** Flowchart of the different visits in the TransplantLines study. At every study visit biobank, general tests, and questionnaires will be performed. Specifically addition at each timepoints; at transplantation perioperative residual material will be collected. At 3 months after transplantation cognitive protocol will be performed. At 6 months physical protocol will be carried out. At 12 months randomization to physical or cognitive protocol will occur. At 2 years after transplantation, a limited set of tests will be executed. Follow-up will be performed each 5 years.

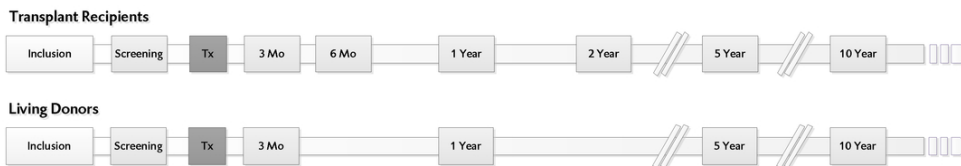
**Figure 3.** Overview of the three main pillars of the TransplantLines study, i.e. questionnaires, biobank, and tests. The collection of data in these pillars at multiple time points will allow to investigate whether biomarkers at baseline can better predict occurrence of adverse outcomes and whether correction could possibly result in an improved survival.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

**Patient and Graft survival**  
(e.g. poor long-term outcomes)



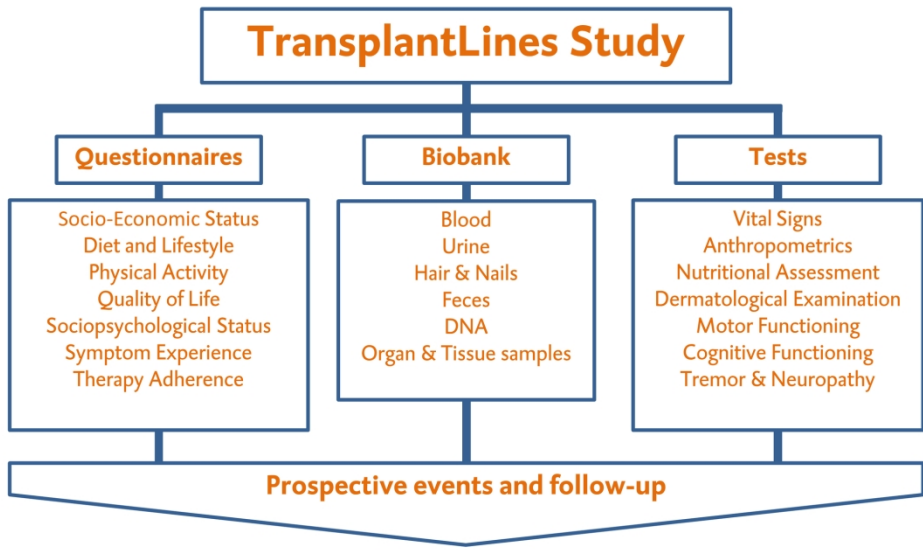
190x142mm (300 x 300 DPI)



93x34mm (300 x 300 DPI)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



190x142mm (300 x 300 DPI)