ERC Starting Grant 2019

Annex 1 to the Grant Agreement
(Description of the Action)
Part B

Action Acronym: PRE-IMAGE
Action number: 851368
Action Title: Pre-transplant Renal Ex vivo Imaging and Multi-omics for Advanced Graft Evaluation
Principal Investigator: Cyril Moers
Host Institution: University Medical Center Groningen

Section b: ***Curriculum vitae (max. 2 pages)***

PERSONAL INFORMATION

Family name, First name:	**Moers, Cyril**
Researcher unique identifier(s):	0000-0001-8014-8387 (ORCID ID)
Date of birth:	21-03-1979
Nationality:	Dutch
URL for web site:	https://www.rug.nl/staff/c.moers/
Google Scholar profile URL:	https://scholar.google.nl/citations?user=IsEJS4MAAAAJ&hl=nl

EDUCATION

2011 **PhD thesis:** "Preserving organ function of deceased donor kidneys" – **cum laude**, Surgical Research Laboratory, UMCG, University of Groningen, The Netherlands
Name of PhD Supervisor: Prof. Rutger J. Ploeg

2005 **Masters:** Medicine – **cum laude,** UMCG, University of Groningen, The Netherlands

CURRENT POSITION

2017 – today **Transplant surgeon and tenure track researcher,** Department of Surgery – Organ Donation and Transplantation, UMCG, University of Groningen, The Netherlands

PREVIOUS POSITIONS

2015 – 2017 **Fellow**, Transplantation surgery and postdoc researcher, Dept. of Surgery – Organ Donation and Transplantation, UMCG, University of Groningen, The Netherlands

2008 – 2015 **Surgical trainee (registrar),** UMCG, Martini Hospital Groningen, Medical Center Leeuwarden, all in The Netherlands and Oxford University Hospitals, UK

FELLOWSHIPS GRANTS AND AWARDS

2018 **PI,** Research grant (€25,000), received from the Gratama Foundation, The Netherlands
2017 **Co-PI,** Research grant (€108,350 locally), received from the Dutch Kidney Foundation, NL
2017 **PI,** Innovation grant (€100,000), received from the Dutch Kidney Foundation, NL
2015 **PI,** Clinical Fellows personal research grant (€160,000), received from The Netherlands Organisation for Health Research and Development, The Netherlands
2015 **PI,** Kolff postdoc personal research grant (€200,000), Dutch Kidney Foundation, NL
2014 **PI,** Personal research grant (€80,000), Dutch Transplantation Foundation, NL
2013 **ESOT fellowship**, Clinical/research stay Oxford Transplant Centre, Univ. of Oxford, UK
2013 **Gauke Kootstra award,** Best PhD thesis, Dutch Transplantation Society, The Netherlands
2012 **Co-PI,** FP7 consortium research grant (€729,621 locally), received from the EU
2012 **Tekke Huizinga award,** Best PhD thesis in 2011, UMCG, University of Groningen, NL
2010 **Schoemaker award**, Best publication in 2009, The Netherlands Society for Surgery, NL
2010 **Novartis award**, Best publication in the field of organ transplantation in 2009, Dutch Transplantation Society, The Netherlands
2009 **PI,** Research grant (€6,000), Hendrik de Cock Foundation, The Netherlands
2008 **Henk Schippers Young Investigator award,** Eurotransplant International Foundation, NL
2008 **Co-PI,** Research grant (€160,756), Dutch Kidney Foundation, The Netherlands

SUPERVISION

PhD students co-supervised: 4 (Aukje Brat, 2015 – present; Leonie Venema, 2015 – present; Merel Pool, 2017 - present; Rianne Schutter, 2018 – present). **Undergraduate students supervised: 11** (Bram Abou Habaga, 2007; Jeannette Bronkhorst, 2007; Nirvana Kornmann, 2008; Rik Niewzwaag, 2016; Wendy Oost, 2016; Ashline Dijkhoff, 2017; Rob Veenstra, 2017; Otis Varsseveld, 2017; Tim Hamelink, 2017; Liset Wijngaards, 2018; Jaël Vos, 2018). **Masters students supervised: 6** (Merel Pool, 2017; Tim Eertman, 2016; Loes Hartveld, 2017; Maaike Nijhoff, 2018; Otis Varsseveld, 2018; Tim Hamelink, 2018)

TEACHING ACTIVITIES

2015 – today **Teaching medical students, surgical interns and nursing staff**, UMCG, The Netherlands

2008 – 2015 **Teaching surgical interns,** UMCG, Martini Hospital Groningen, Medical Center Leeuwarden, all in The Netherlands and Oxford University Hospitals, UK.

2005 – 2009 **Training and managing a team of 42 medical students** who were responsible for the logistics of an international randomised controlled trial.

ORGANIZATION OF SCIENTIFIC MEETINGS

2018 **Main organizer,** Ex vivo organ perfusion consensus meeting, Cambridge, UK

2017 **Main organizer,** Kidney perfusion scientific workshop, UMCG, NL

INSTITUTIONAL RESPONSIBILITIES

2015 – today **Founding member,** Young Academy of Groningen, Univ. of Groningen, NL
2001 – 2002 **Member** of the University Council, University of Groningen, NL
2001 – 2002 **Member** of the University Committee for Education, University of Groningen, NL

OUTREACH AND POPULARIZATION

2018 **Organizing** a large educational science fair for children
2017 **Publication of an online interview and a YouTube video** on personal research activities intended to popularize medical research among the general public.
2017 **Teaching** groups of patients and societal volunteers on organ donation and transplantation.
2016 – today **Actively managing a renal patient research participation group** to obtain patients' input in planning and conducting experimental research projects.
2015 **Publication of an infographic in Dutch newspaper** De Telegraaf on donor kidney conditioning with mesenchymal stem cells during machine perfusion.
2012 – today **Publication of various videos and interviews** on organ preservation related topics on the website of the University Medical Center Groningen, intended for the general public.

MEMBERSHIPS OF SCIENTIFIC SOCIETIES

2015 – today **Member** of the American Society of Transplant Surgeons
2015 – today **Member** of the Dutch Society for Gastro-Intestinal Surgery
2008 – today **Member** of The Netherlands Society for Surgery
2005 – today **Member** of the Dutch Transplantation Society
2005 – today **Member** of the European Society for Organ Transplantation

PEER REVIEW

New Engl J Med	3 manuscripts	**Kidney Int**	5 manuscripts
Lancet	2 manuscript	**PLoS ONE**	4 manuscripts
Transplantation	>50 manuscripts		
Transpl Int	>20 manuscripts	**Research Councils, UK**	3 research proposals
Am J Transplant	>20 manuscripts	**Catalan Government**	3 research proposals
Neph Dial Transpl	8 manuscripts	**DFG, Germany**	2 research proposals
Clin Transplant	8 manuscripts	**Romanian Government**	1 research proposal

MAJOR COLLABORATIONS

Member of management committee, COST-action PARENCHIMA, a large international network to promote studies on magnetic resonance imaging biomarkers for renal disease. More than 8 EU countries involved.
Member of scientific steering committee, Consortium for Organ Preservation in Europe (COPE), an EU-(FP7)-funded consortium initiating 3 international RCTs and several animal studies aimed at improving organ preservation of deceased donor kidney and liver grafts. Countries involved: The Netherlands, Belgium, United Kingdom, Germany, France, Spain.
Secretary of scientific steering committee, Machine Preservation Trial, a large international randomised controlled trial comparing hypothermic machine perfusion with static cold storage preservation of deceased donor kidneys. Countries involved: The Netherlands, Belgium, Germany.

KEY COLLABORATORS

Prof. Henri Leuvenink, UMCG, topic: ex vivo machine perfusion
Prof. Rainer Bischoff, UMCG, topic: mass spectrometry, proteomics and metabolomics
Prof. Cisca Wijmenga, UMCG, topic: genomics and transcriptomics
Prof. Rutger Ploeg, Univ. of Oxford, topic: ex vivo machine perfusion, stem cells and proteomics
Prof. Bente Jespersen, Univ. of Aarhus, topic: mesenchymal stem cells and ex vivo kidney perfusion
Prof. Kim Mouridsen, Univ. of Aarhus, topic: artificial intelligence and deep learning analysis
Prof. Faikah Gueler, Univ. of Hannover, topic: post-transplant renal MRI
Prof. Heiner Niemann, Univ. of Hannover, topic: xenotransplantation

CAREER BREAKS

Between 01-09-2008 and 10-07-2015, I received my clinical specialist training as a surgical registrar. This amounts to an extension of 3 years, 7 months and 3 days (1311 days in total) counting from my PhD thesis defence on 07-12-2011. During this time, I had full time clinical work, often in non-academic hospitals, with very limited time available after hours to devote to research.

Appendix: All ongoing and submitted grants and funding of the PI (Funding ID)
Mandatory information (not counted towards page limits)

Ongoing Grants (Please indicate "No funding" when applicable):

Project Title	*Funding source*	*Amount (Euros)*	*Period*	*Role of the PI*	*Relation to current ERC proposal*
Transplantation of kidneys from older deceased donors	Dutch Transplantation Foundation	€80,000	2014 – 2019	Project leader	Development of prediction models for kidney transplantation outcome – key experience relevant for constructing models in Objective 3 of ERC project
Developing a novel intervention to repair damaged donor kidneys prior to transplantation: Machine perfusion with mesenchymal stem cells	Dutch Kidney Foundation	€200,000	2015 – 2018	Project leader	No direct relation
Developing a novel intervention to repair damaged donor kidneys prior to transplantation: Machine perfusion with mesenchymal stem cells	The Netherlands Organization for Health Research and Development	€160,000	2015 – 2018	Project leader	No direct relation
Magnetic resonance imaging to assess organ quality during normothermic machine perfusion of deceased donor kidneys	Dutch Kidney Foundation	€100,000	2018 – 2020	Project leader	Development of ex vivo kidney perfusion inside MRI scanner – key methodology which will be used in ERC project
Prolonged ex-vivo normothermic machine perfusion for kidney regeneration	Dutch Kidney Foundation	€108,350	2018 – 2021	Co-PI	Clinical introduction of normothermic ex vivo kidney perfusion – key methodology which will be used in Objective 3 of ERC project
Cryoperfusion of	Gratama	€25,000	2018 – 2020	Project leader	No direct relation

kidneys – A very novel approach towards organ banking	Foundation				

Grant applications (Please indicate "No funding" when applicable):

Project Title	*Funding source*	*Amount (Euros)*	*Period*	*Role of the PI*	*Relation to current ERC proposal*[2]
Artificial intelligence-based analysis of Magnetic Resonance Imaging biomarkers to detect allograft dysfunction after kidney transplantation	Dutch Kidney Foundation	€600,000	2019 – 2023	Co-PI	Artificial intelligence methods will for the first time be used to correlate *post*-transplant renal MRI data with post-transplant complications – no overlap with ERC project (as ERC project utilises such methods *pre*-transplant), but any expertise gained will help both projects
Pre-transplant renal ex vivo imaging and multi-omics for advanced graft evaluation	The Netherlands Organization for Health Research and Development	€800,000	2019 – 2024	Project leader	Full overlap with current ERC proposal (this Dutch application is a downsized version of my ERC proposal). Should I be awarded both grants, I will choose the ERC and drop the Dutch grant

Section c: *Early achievements track-record (max. 2 pages)*

1. RESEARCH ACHIEVEMENTS SUMMARY

My unique research line focuses on pre-transplant organ evaluation and resuscitation.

- In 2009, during my PhD period at the UMCG, I showed that ex vivo machine perfusion leads to better outcome after kidney transplantation (N Engl J Med 2009 and 2012).
- Subsequent to this in 2010 and collaborating with the groups of Prof. Jacques Pirenne in Leuven, Belgium, and Prof. Andreas Paul in Essen, Germany, I discovered that the diagnostic value of conventional parameters during ex vivo organ perfusion is negligible for individual donor kidneys (Transplantation 2010 and Am J Transplant 2011). It is therefore paramount to identify molecular biomarkers which do convey donor kidney quality prior to transplantation.
- From 2014 onwards, my group has perfected the technique of normothermic (37°C) ex vivo machine perfusion. We are now able to precisely control and adapt hemodynamics, temperature, perfusate composition and sample acquisition and can perform stable ex vivo perfusion for prolonged time periods (recent, not yet published experimental perfusion data).
- While developing optimal ex vivo renal perfusion techniques, I have found evidence for distinct molecular patterns which are activated by normothermic machine perfusion (preliminary, not yet published proteomics data shown in B2 section). The next innovative step will be to broaden this omics view and also obtain genomics, transcriptomics and metabolomics data in order to acquire a deep understanding of which molecular processes are active during ex vivo perfusion.
- In the past two years, I have successfully developed a stable ex vivo normothermic perfusion setup for use inside a clinical MRI scanner and so far performed 6 pilot experiments which show that high quality MR images, functional and spectroscopy data can be obtained during such perfusion (not yet published pilot data shown in B1 and B2 sections). Thus, I have the perfect setup in place to also generate radiomics data during ex vivo perfusion.
- Since 2015, I have performed much work to construct innovative prediction models for post-transplant outcome (recent, not yet published data and models). Hence, I have gained broad experience with combining numerous factors into predictive models in kidney transplantation.
- I have now reached a natural point in my career at which I am ready to revolutionize pre-transplant ex vivo kidney evaluation: Analyze human renal tissue and perfusate with a multi-omics approach during ex vivo perfusion, simultaneously perform radiomics of such perfusions inside an MRI scanner and correlate these novel data and conventional parameters with transplant outcome. My experience with constructing multi-factorial predictive models and my collaboration with a group that has a strong track record in artificial intelligence analysis will allow a leap forward in the discovery of innovative pre-transplant diagnostic patterns to accurately indicate transplant outcome.

2. FIVE REPRESENTATIVE PUBLICATIONS

Career total of 29 publications, 3 without PhD supervisor, 11 as lead or corresponding author.

1. **Moers C**, Smits JM, Maathuis M-HJ, Treckmann J, Van Gelder F, Napieralski BP, Van Kasterop-Kutz M, Homan van der Heide JJ, Squifflet J-P, Van Heurn LWE, Kirste GR, Rahmel A, Leuvenink HGD, Paul A, Pirenne J, Ploeg RJ. Machine perfusion or cold storage in geceased-donor kidney transplantation. N Engl J Med. 2009;360(1):7-19. **IF 72.41 – citations: 772**
   In this paper, we present results of a large international randomised controlled trial comparing hypothermic machine perfusion (HMP) with static cold storage of deceased donor kidneys prior to transplantation. We found that HMP was associated with a remarkably better outcome.
2. **Moers C**, Pirenne J, Paul A, Ploeg RJ; Machine Preservation Trial Study Group. Machine perfusion or cold storage in deceased-donor kidney transplantation. N Engl J Med. 2012;366(8):770-1. (peer reviewed letter to the editor on original data, 3 year follow up study of 2009 N Engl J Med publication). **IF 72.41 – citations: 113**
   Here, for this first time, we found that the graft survival benefit of hypothermic machine perfusion over static storage persists even three years post-transplant. As a result of these findings, all deceased donor kidneys in The Netherlands and a growing number of other countries are now preserved by machine perfusion instead of static storage.
3. Groen H, **Moers C**, Smits JM, Treckmann J, Monbaliu D, Rahmel A, Paul A, Pirenne J, Ploeg RJ, Buskens E. Cost-effectiveness of hypothermic machine preservation versus static cold storage in renal transplantation. Am J Transplant. 2012;12(7):1824-30. **IF 6.17 – citations: 51**
   In this study, we showed that machine perfusion preservation leads to major cost savings compared to static cold storage of deceased donor kidneys. These figures have convinced funding authorities to reimburse standard clinical implementation of HMP.

4. De Deken J, Kocabayoglu P, **Moers C**. Hypothermic machine perfusion in kidney transplantation. Curr Opin Organ Transplant. 2016;21(3):294-300. **IF 2.86 – citations: 13**
In this paper, I had the opportunity to sketch my views on near-future developments in the field of renal ex vivo machine perfusion.

5. Kox J, **Moers C**, Monbaliu D, Strelniece A, Treckmann J, Jochmans I, Leuvenink HGD, Van Heurn LWE, Pirenne J, Paul A, Ploeg RJ. The benefits of hypothermic machine preservation and short cold ischemia times in deceased donor kidneys. Transplantation 2018 [Epub ahead of print]. **IF 3.68 – citations: 3**
This very recent paper is important, as it shows that – contrary to widespread belief – shorter periods of ex vivo perfusion are more beneficial to donor kidneys than longer perfusion times.

3. INVITED PRESENTATIONS

2018 **Ex vivo organ repair with stem cells**, *invited speaker* at the European Society for Organ Transplantation's Basic Science in Transplantation Meeting, Rotterdam, The Netherlands

2018 **Machine perfusion for pre-transplant organ evaluation and resuscitation,** *invited speaker* at the Spring Meeting Transregional Collaborative Research Centre 127 of the German Research Society (DFG), Mariensee, Germany

2018 **Machine perfusion for pre-transplant organ evaluation and resuscitation,** *invited speaker* at the Symposium Perspectives in Organ Transplantation, Hannover, Germany

2018 **Preservation of organs,** *invited speaker* at the Astellas Transplantation Symposium, Den Dolder, The Netherlands

2017 **Challenges and innovations in organ donation and transplantation,** *invited speaker* at the Scientific symposium Dutch Kidney Patients Union, Bunnik, The Netherlands

2016 **MSC tracking after ex vivo infusion,** *invited speaker* at the MiSOT Congress, Regensburg, Germany

2016 **Cost effectiveness of hypothermic machine perfusion,** *invited speaker* at the Deutsche Transplantationsgesellschaft, Essen, Germany

2016 **Developing a novel intervention to repair damaged donor kidneys prior to transplantation**, *invited speaker* at the New Kids in Nephrology Symposium, Amsterdam, The Netherlands

2013 **Career development in organ transplantation,** *invited speaker* at the European Society for Organ Transplantation, Vienna, Austria

2013 **Machine preservation of the kidney – past, present and future,** *invited speaker* at the Clinical Review Symposium Dutch Transplantation Society, Utrecht, The Netherlands

2012 **Machine perfusion – evidence for benefits, limitations and future prospects,** *invited speaker* at the Canadian Transplant Forum, Toronto, Canada

2012 **Machine perfusion – expansion of the kidney donor pool?,** *invited speaker* at the Congress of The Transplantation Society (TTS), Berlin, Germany

2008 **Results of the European multicenter trial on kidney preservation,** *invited speaker* at the Association for Organ Procurement Organizations, Phoenix, USA

2008 **The European multicenter trial on kidney preservation,** *invited speaker* at the Israeli Transplant Congress, Caesarea, Israel

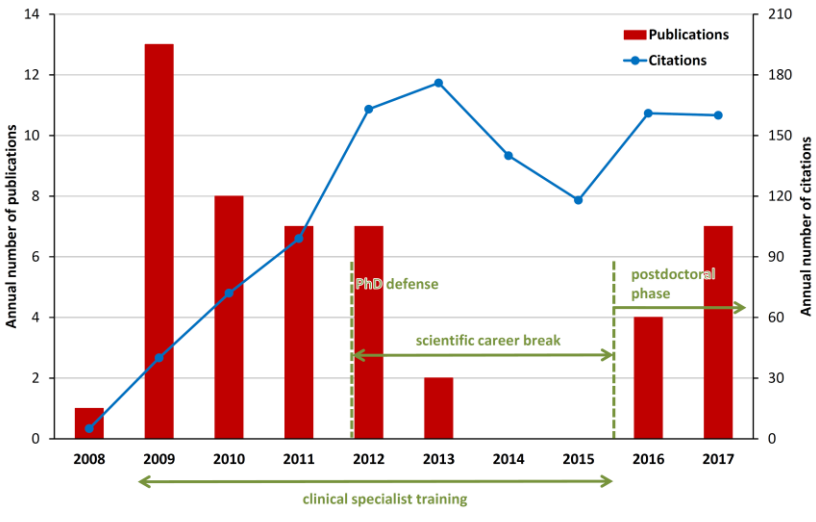
2007 **Results of the Machine Preservation Trial,** *invited speaker* at the International Society for Organ Donation and Procurement, Philadelphia, USA

2007 **The European multicenter trial on kidney preservation,** *invited speaker* at the Eurotransplant Meeting, Noordwijk aan Zee, The Netherlands

2006 **Non-heart beating donation future prospects,** *invited speaker* at the The Transplantation Society New Key Opinion Leader Meeting, Barcelona, Spain

4. IMPACT

Total times cited: **1,827** (Google Scholar)
H-index: **14**
i10-index: **18**



ERC Starting Grant 2019
Research proposal [Part B2] [1]
(not evaluated in Step 1)

Part B2: *The scientific proposal* (max. 15 pages)

Section a. State-of-the-art and objectives

a.1 Context and Overall Aim

- There is a considerable shortage of kidneys recovered from deceased donors, which are essential to save lives of critically ill patients who suffer from renal failure. In order to bridge the gap between organ demand and supply, more kidneys of marginal quality need to be considered for transplantation. Such marginal kidneys require optimized organ preservation and are at risk of inferior post-transplant outcome. **Reliable pre-transplant assessment of organ quality has become a top priority, to prevent transplantation of organs with very poor or even no function.**
- Currently, decisions on organ acceptance or discard rely on the professional opinion the organ recipient's physicians. Their judgment tends to be on the safe side, which results in potentially viable kidneys being discarded on the basis of subjective clinical parameters. Nevertheless, **up to 30% of kidneys that do pass clinical assessment will not show acceptable outcome after all.**
- As a transplant surgeon, it is part of my clinical job to judge whether a kidney from a deceased donor will be suitable for a certain renal patient. On a regular basis, I am confronted with the limitations of this very subjective clinical judgment and I am therefore determined to improve the way we understand and assess the quality of donor organs. Attempts at developing more objective pre-transplant diagnostic tools have failed so far. Biopsies, serum biomarkers, conventional machine perfusion parameters and prediction models based on clinical variables all possess insufficient prognostic strength. **Highly innovative methods need to be explored in order to develop truly and independently predictive pre-transplant assessment tools for donor kidneys.**
- Transplant centers are increasingly interested in utilizing ex vivo organ perfusion to presumably better preserve, improve and assess donor kidneys prior to transplantation. However, the current understanding of the diagnostic potential of this technique is still in its infancy and, frankly, it is not known which markers during ex vivo perfusion are relevant indicators of post-transplant outcome. **To ultimately identify relevant prognostic markers during ex vivo perfusion, it is essential to first obtain a deep understanding of genetic, transcriptional, proteomic and metabolomic mechanisms during perfusion and how these differ from such mechanisms in vivo.**
- Magnetic resonance imaging (MRI) and associated techniques MR spectroscopy and functional MRI are promising non-invasive tools to obtain a wealth of additional tissue-specific information about kidney quality and viability. **So far, innovative MRI techniques have not been applied to image and characterize ex vivo perfused donor kidneys.**
- Currently, it remains unknown which measurements during ex vivo kidney perfusion are relevant predictors of post-transplant outcome. **Hence, there is an urgent need to better understand this relationship and discover the true diagnostic potential of pre-transplant ex vivo perfusion.**

The overall aim of this ERC project is to determine the molecular mechanisms of ex vivo kidney perfusion prior to renal transplantation in order to develop breakthrough pre-transplant perfusion-based diagnostic markers that can indicate kidney transplant outcomes.

This project will **(i)** Transform ex vivo organ perfusion from merely an organ preservation method to a platform for pre-transplant kidney evaluation, which will be adopted by many transplant centers and research groups worldwide; **(ii)** Introduce ex vivo MRI assessment of donor kidneys as a novel method to aid pre-transplant organ evaluation; and **(iii)** Pave the way to ultimately transplant more donor kidneys and thus help more critically ill patients, since potentially marginal organs can prove to be of acceptable quality during ex vivo organ assessment. Conversely, prevent transplantation of non-viable organs and hence reduce serious post-transplant morbidity and mortality.

[1] Instructions for completing Part B2 can be found in the '*Information for Applicants to the Starting and Consolidator Grant 2019 Call'*.

a.2 Scientific Challenge and State-of-the-Art

Kidney transplantation. Transplantation is the optimal treatment for patients who suffer from end stage renal disease.[1,2] The large gap between donor organ availability and clinical demand has resulted in a considerable waiting list for a renal transplant. Presently, the median waiting time is more than 3 years,[3] which results in morbidity and mortality due to the adverse effects of renal failure and prolonged dialysis. In the last decade, transplant centres have been accepting increasing numbers of sub-optimal organs in an attempt to reduce waiting time. Organ function and longevity of such organs is often inferior to organs from ideal donors. **In The Netherlands, for example, the relative share of sub-optimal organ donors in the deceased kidney donor pool has rapidly grown to more than 80%** (Figure 1).[3]

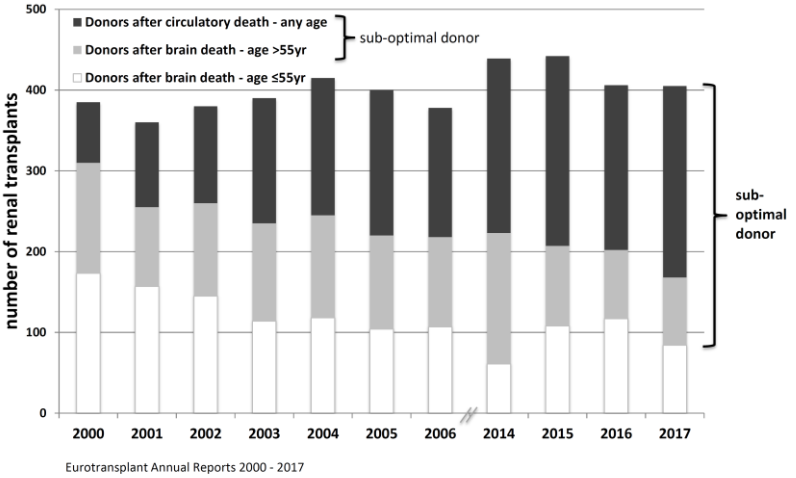


Figure 1: *Number of renal transplants performed per year in The Netherlands stratified by deceased donor type and age.*

Ex vivo organ perfusion. Given the rising numbers of sub-optimal donor kidneys, innovative interventions are needed to better preserve organ quality. In a large international RCT coordinated by our group, we found that ex vivo hypothermic machine perfusion (HMP) of kidneys from deceased donors prior to transplantation is superior to static cold storage preservation at various post-transplant end points.[4–6]
Hypothermia (0-8°C) itself is known to also induce additional organ damage during kidney preservation.[7] *Normo*thermic (18-37°C) ex vivo machine perfusion (NMP) may provide a more physiological environment for an isolated kidney to recover from the initial ischemic event in the donor.[8] So far, evidence suggests superiority of NMP over cold organ preservation.[9,10] NMP also creates a platform for organ quality assessment and for interventions which actively condition the organ prior to transplantation.[11] As a result of these positive findings, and anticipating on its yet to be unravelled diagnostic and resuscitating opportunities, NMP is rapidly becoming a technique that transplant centres worldwide want to have available. Before the beginning of 2019, my own transplant center the UMCG will have clinical grade NMP technology, special ex vivo perfusion rooms and a 24/7 team of perfusion support staff. However, **evidence on which molecular processes are active and which biomarkers during normothermic ex vivo perfusion have a relevant association with transplant outcome is lacking.**

Pre-transplant organ evaluation. Transplant clinicians tend to be very strict in decisions on organ acceptance or discard.[12] Up to 40% of deceased donor kidneys that are offered for transplant are turned down by transplant centers for reasons of presumed inferior organ quality.[13] Current tools to objectively aid such decisions are of only minimal value. The predictive strength of composite clinical nomograms, pre-implantation histology, donor serum and urine biomarkers and conventional machine perfusion parameters is poor and strictly applying these unreliable diagnostic parameters may lead to even more kidney discard.[14]

For clinical transplantation practice, it is important to note that many supposedly "marginal" kidney grafts do show adequate outcome. Finding characteristics that reliably identify only those donor kidneys with unacceptable post-transplant function is the holy grail to obtaining a more objective selection algorithm and reduce unnecessary organ discard. Evidence suggests that endothelial integrity, mitochondrial function and pro-fibrotic pathways are key determinants of renal graft viability and longevity.[15] **However, conventional direct assessment of these parameters just prior to transplantation is not feasible as it requires invasive and time consuming techniques, the duration of which reaches far beyond the maximum ex vivo organ preservation time.**

Multi-omics. In the current era of personalized medicine, increasing effort has been focused on combining genomic, transcriptomic, proteomic and metabolomic data. Wide availability of techniques such as whole genome sequencing, RNA-seq and mass spectrometry have brought multi-omics analysis within reach of many research groups. A multi-omics approach towards finding combinations of biomarkers incorporates different biological layers of information to better describe or predict phenotypic outcome.[16] After a careful quality control and pre-processing steps, data can be analyzed using dimension reduction techniques such as principal components analysis followed by integration methods such as artificial intelligence (AI)

software.[17,18] Various studies have focused on individual tissue, serum, or ex vivo perfusate biomarkers and found these to have poor discriminatory power for post-transplant outcome. **In contrast to single biomarker discovery studies, or even one-dimensional -omics analyses, a multi-omics approach has a tremendously large potential of finding relevant pathways and molecular associations with only limited amounts of sample.**[19] Nevertheless, any complex molecular analyses during ex vivo organ perfusion will require invasive collection of perfusate and/or tissue biopsies. And, most importantly, such analyses may take time to perform. In real clinical transplantation practice, there is only a very limited time available between organ retrieval and transplantation. Therefore, **should relevant predictive multi-omics based biomarkers for post-transplant outcome be found, it will be of paramount importance for actual clinical implementation in the near future that such biomarkers can also be measured with a *non-invasive* and vary rapid method.**

Magnetic resonance imaging (MRI). Recently, several groups have reported that renal micro-perfusion, as well as renal oxygen consumption can be quantified by the functional magnetic resonance imaging (fMRI) techniques arterial spin labeling (ASL) and blood-oxygen-level dependent (BOLD) contrast imaging, respectively. Providing potential surrogate markers for endothelial integrity and mitochondrial function, these imaging sequences have been shown to correlate with kidney graft function, when imaging was performed a few days after transplantation.[20] In addition, coined vessel architectural imaging (VAI), which quantifies changes in vessel architecture and leakage,[21] has high potential to detect micro-vascular damage in the kidney and the technique allows for estimation of tissue oxygen saturation. Focused ("zoomed") high-resolution anatomical MRI facilitates quantification of hypo-perfused areas and high resolution zoomed diffusion weighted imaging (DWI) can detect restricted water diffusion, which may reveal edema and/or inflammation.[22] Furthermore, mapping of T2* relaxation times, including BOLD functional imaging, can provide measures for tissue oxygen delivery.[23] A very novel method to measure mitochondrial function and quantify oxygen consumption utilizes the MRI visible (stable and non-radioactive) oxygen isotope ^{17}O-oxygen, which has a natural abundance of 0.002% but is enriched to above 70% for imaging purposes. By simply blowing the gas through the oxygenator, it is ideally suited for ex-vivo measurements of oxygen consumption in pre-transplant kidney grafts.[24] Another promising approach to assess pre-transplantation graft health is magnetic resonance elastography (MRE), which determines tissue properties such as regional stiffness based on imaging of the propagation of externally applied shear waves. Recent data suggest that hemodynamic parameters modulate kidney tissue stiffness.[25] Finally, MR spectroscopy provides a wealth of possibilities to quantify metabolites in a functioning kidney. Given the above, **MRI is a very promising candidate for non-invasive and quick simultaneous measurement of a large number of relevant organ quality parameters.**

Some of these MRI sequences have already been shown to correlate with post-transplant outcome.[20] However, imaging was always performed several days *after* transplantation, which is obviously too late for imaging data to be considered in decisions on kidney acceptance or discard. Although early recipient management may be fine-tuned based on post-transplant imaging findings, **true organ viability assessment can only be performed with an ex vivo machine perfusion setup that allows acquisition of these data *prior* to transplantation.** Applying MRI techniques to ex vivo perfused kidneys is very challenging due to the extremely strong up to 3 Tesla magnetic field near an MRI scanner. Any component of the perfusion setup which contains ferromagnetic material needs to be placed at a safe distance of at least 5m to prevent it from flying into the scanner. This implies that all pumps, sensors, control electronics and electric wires have to be placed in the electromagnetically shielded control room and only plastic tubing, an organ chamber and the kidney itself can be inside or near the MRI scanner. To my knowledge, **my group is the only research team to have recently successfully developed a stable normothermic ex-vivo kidney perfusion system for use inside an MRI scanner.**

Radiomics. Applying several MRI sequences to a target tissue generates vast amounts of data. A very novel approach to analyzing complex imaging data is radiomics. Powerful software is employed to seek for disease-specific patterns in raw imaging data, thus unveiling independent diagnostic radiological features that are not visible to the human eye. Typically, radiomics are utilized to detect presence or absence of a disease, or to quantify the extent of a certain pathological characteristic (often in malignant tumors). Radiomics can also be used to investigate how complex radiological features of a target tissue correlate with genetic, transcriptional and metabolic information in that tissue, provided that the latter information is known. In tumor biology, radiomics are already utilized to partly replace invasive –omics biomarker measurements.[26] **Hence, radiomics can be applied to find distinct radiological patterns of a tissue which are rapid and non-invasive analogues of multi-omics patterns that need to be measured by means of invasive and time consuming assays.**

a.3 Recent Underpinning Work by the Applicant

Post-transplant outcome predictions. Recently, I have utilized large data sets to construct non-linear prediction models for post-transplant outcome, based on all available clinical donor- and recipient variables (>20). My non-linear (spline-based) models incorporated more than 20 independent variables to predict the risk of a composite end point of either graft failure in the first year after transplantation, or an estimated glomerular filtration rate of 30 ml/min or less at one year post-transplant. C-statistics were generally no higher than 0.65 (indicating modest discrimination) and calibration of these models was also poor. This is in line with findings of other groups that have also attempted to construct meaningful prediction models.[27,28] Earlier, during my PhD, we had already shown that conventional machine perfusion parameters (such as renal intravascular resistance) are unreliable predictors of outcome.[29] **These results showing that clinical variables and conventional machine perfusion parameters are insufficient to reliably predict post-transplant outcome, are highly relevant to the current ERC proposal: The next important step is to discover revolutionary –omics based biomarkers, which do show relevant independent predictive power for post-transplant results.**

Ex vivo perfusion. I have recently perfected normothermic ex vivo kidney perfusion such that it can be meticulously controlled and applied for many hours. My perfusion setup is fully controlled by custom-built software. It can perfuse in a continuous, or a pulsatile fashion, the latter either sinusoid or following a physiologic arterial pressure wave pattern. Pressure, flow, heart rate, temperature and oxygenation can be set and controlled. In addition, I have developed a novel perfusion solution which closely resembles physiologic conditions and, using this medium with the improved setup, succeeded to maintain a stable normothermic renal perfusion for more than 7 hours (Figure 2). **This optimized ex vivo perfusion setup will be the perfect platform to perform multi-omics analyses in renal tissue and perfusate.**

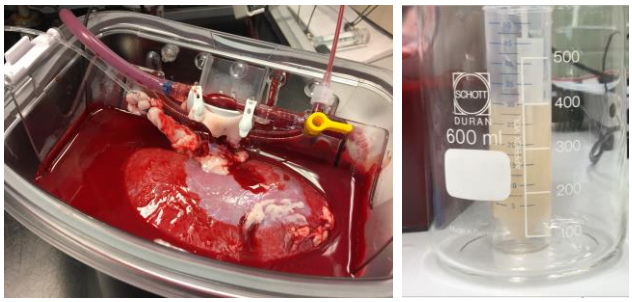


Figure 2: Optimized normothermic ex vivo kidney perfusion setup; *a) Porcine kidney during perfusion at 37°C and 110/70 mmHg; b) Urine is produced during ex vivo perfusion.*

Proteomics. In recent pilot experiments together with the mass spectrometry group of Prof. Benedikt Kessler and Dr. Honglei Huang at the University of Oxford, I have characterized perfusates from ex vivo perfused porcine kidneys and discovered that these kidneys released 567 distinct proteins after 6h normothermic perfusion as compared to baseline, including fatty acid binding protein 3 (FABP3) and amino oxidase (Figure 4). FABP3 has been identified as marker for acute kidney injury.[30] Pig derived TTR was also up-regulated during ex vivo perfusion. TTR is a thyroid hormone-binding protein, to which numerous other small molecules are known to bind in the thyroxine binding sites. Reduced levels of TTR are strongly associated with mortality in renal patients.[31] With this pilot work, I have shown that meaningful proteomics analysis is indeed feasible during ex vivo kidney perfusion, even when conducted in an albumin-rich perfusate. The next important steps will be to also analyze protein profiles in renal tissue, perform repeated longitudinal measurements during ex vivo perfusion and broaden these analyses to also incorporate genomics, transcriptomics and metabolomics. **This multi-omics approach, will allow me to unveil how relevant molecular pathways evolve in time during perfusion, rather than merely identifying protein profiles at a single time point.**

Ex vivo perfusion inside a clinical MRI scanner. I have successfully created the first ever stable ex vivo perfusion setup for use inside clinical MRI scanners (Figure 3). Six recent pilot experiments have all gone very well. I was able to scan the kidney with a broad array of imaging sequences, including anatomical series, functional MRI and MR spectroscopy. **The setup is now ready for use in larger experiments and can also be exported to any other center.**

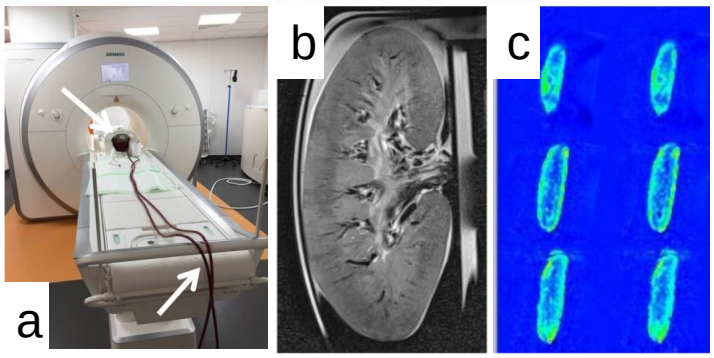


Figure 3: Ex vivo perfusion inside MRI scanner; *a) Setup in scanner, arrows indicate organ chamber and arterial/venous lines; b) T2 high-resolution anatomical image; c) fMRI arterial spin labelling sequence of the same kidney, quantifying capillary micro-perfusion per voxel (six sagittal sections are shown).*

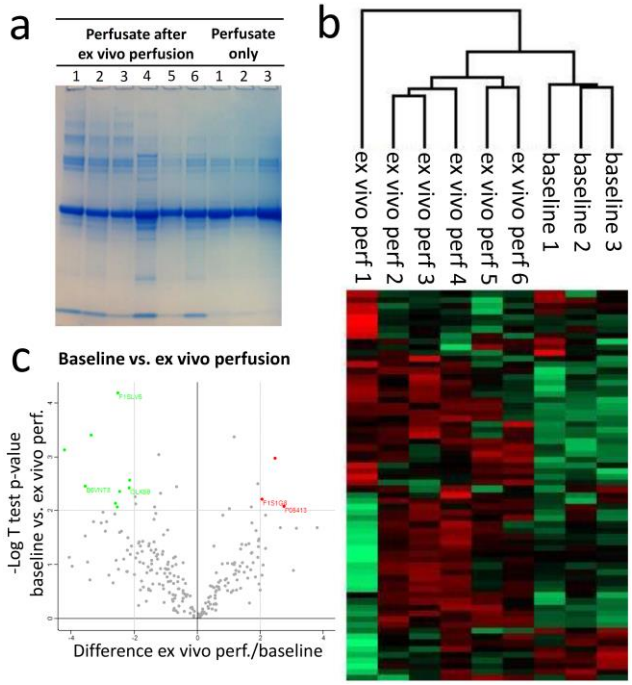


Figure 4: Discovery of novel regenerative markers in kidney perfusates*; a) Proteins secreted into pig kidney perfusates separated by SDS-PAGE and visualised by Coomassie blue staining. Albumin depleted perfusate samples were obtained from baseline (n=3) and after 6h perfusion (n=6); b) Heat map analysis revealed distinct expression profiles (567 unique proteins) between baseline (perfusate alone) and 6h perfusion; c) Volcano plot of secreted proteins. Dots highlighted in the upper right corner (red) indicates up-regulated proteins 6h after perfusion as compared to baseline. Dots highlighted in the upper left corner (green) indicate proteins down-regulated 6h after perfusion as compared to baseline. Elevated proteins P05413 (fatty acid-binding protein), F1S1GB (amine oxidase) are highlighted as red, decreased proteins F1SLV6 (complement C1r), B6VNT8 (actin, alpha cardiac muscle 1), I3LK59 (enolase 1), are highlighted as green.*

a.4 Aims and Objectives

The overall aim of this ERC project is to determine the molecular mechanisms of ex vivo kidney perfusion prior to renal transplantation in order to develop breakthrough pre-transplant perfusion-based diagnostic markers that can indicate kidney transplant outcomes.

To achieve this aim, I have defined 3 specific objectives:

1. To uncover the critical molecular mechanisms that govern ex vivo kidney perfusion and how these differ from a kidney in vivo, utilizing a preclinical porcine model.
2. To translate preclinical findings from Objective 1 into clinically relevant ex vivo pathways and patterns in *human* donor kidneys.
3. To identify innovative perfusion-based diagnostic markers that can help predict human renal transplant outcome, using data to be obtained from a prospective clinical transplantation study.

a.5 Research Plan Overview (see Figure 5 on next page)

In **Objective 1**, a preclinical model will be utilized to identify molecular biomarkers that define the effect of ex vivo normothermic perfusion on porcine kidneys and how ex vivo perfusion differs from a kidney perfused in vivo. One kidney is surgically retrieved and perfused ex vivo for 8 hours. The contralateral kidney remains in vivo for 8 hours while the animal is under anesthesia. From both kidneys, tissue biopsies and circulating perfusate / blood samples will be taken each hour and radiological data are obtained while the anesthetized pig and the ex vivo machine are placed in an MRI scanner. Molecular multi-omics and radiomics analyses will be performed. Ex vivo and in vivo findings will be longitudinally contrasted in order to characterize the typical effect that ex vivo perfusion has on an isolated kidney. In **Objective 2**, discarded human kidneys will be perfused ex vivo for 8 hours and exposed to the same sample and MRI protocol as in **Objective 1**, followed by multi-omics and radiomics analyses. Aim of these experiments is to validate pathways and patterns found in ideal porcine kidneys of **Objective 1** for sub-optimal human donor kidneys. Thus, an array of common molecular and radiological biomarkers that characterize ex vivo perfusion will be identified for kidneys that are close to those that are actually transplanted in humans. **Objective 3**, a prospective clinical transplantation study will be performed to create prediction models for post-transplant outcome, which are based on conventional variables and novel multi-omics markers. Deceased donor kidneys will be perfused ex vivo before transplantation and tissue and perfusate samples are obtained. Recipients of these organs will be followed up for 1 year and outcome data are collected. A supervised artificial intelligence approach will then be utilized to combine conventional and multi-omics variables and create prediction models for post-transplant outcome. Typical ex vivo molecular pathways identified in **Objective 2** will be used to supervise artificial intelligence algorithms in **Objective 3**. In addition, **Objectives 1** and **2** will yield knowledge on how radiomic patterns correlate with molecular multi-omics pathways. Thus, relevant molecular multi-omics predictors of transplant outcome that are found in **Objective**

3 may be translated to a set of non-invasive radiological biomarkers to be tested in near-future studies when human kidneys intended for transplantation will also be perfused ex vivo inside an MRI scanner.

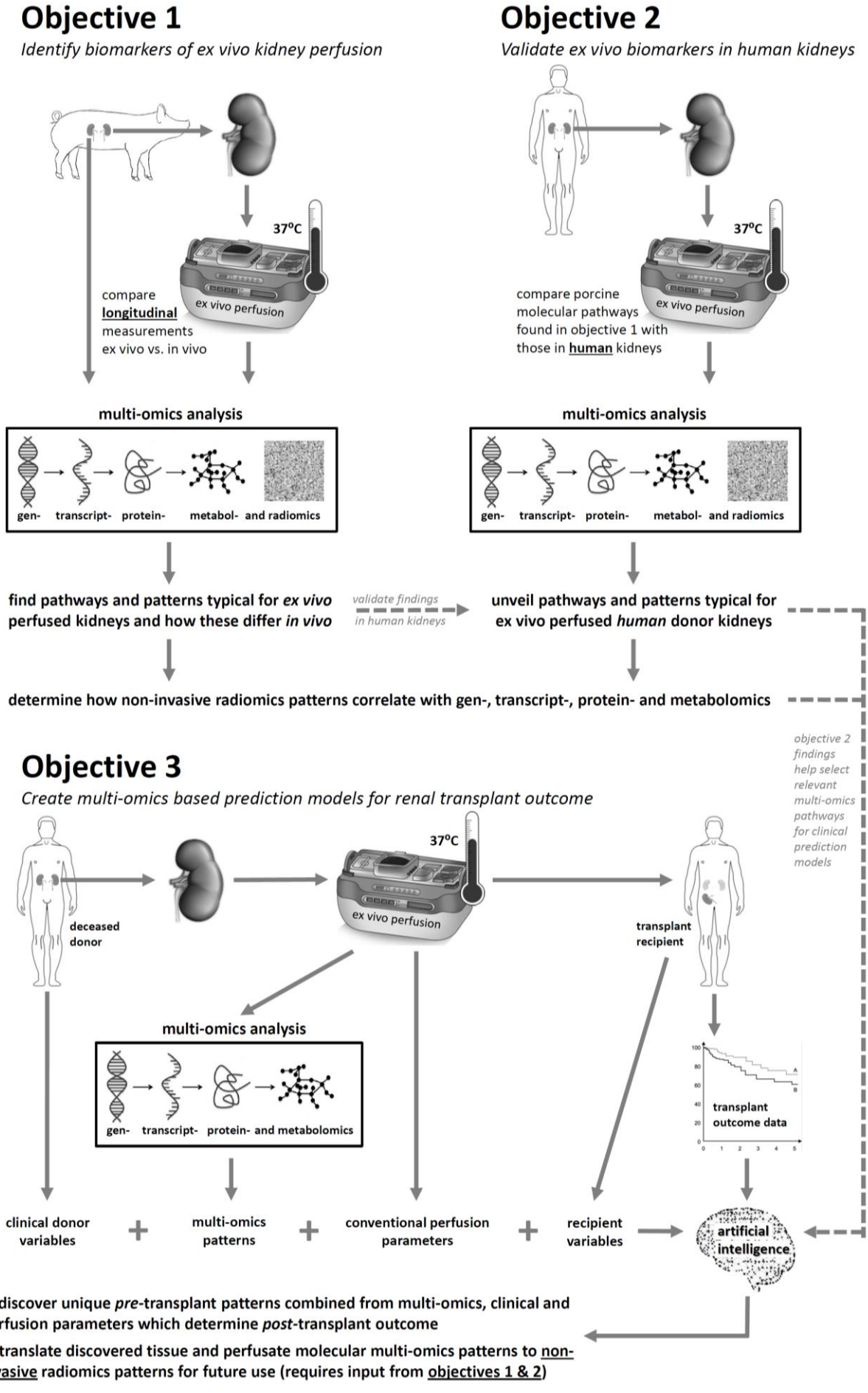


Figure 5: Schematic project overview

a.6 Ground-breaking Nature, Innovative Aspects and Scientific/Societal Impact

The **high gain** of my project will be the identification of molecular mechanisms during ex vivo kidney perfusion and discovery of ground breaking pre-transplant diagnostic patterns which will help to more accurately predict post-transplant outcome.

There will be several ground-breaking and innovative aspects:

- Never before have the mechanisms underlying normothermic ex vivo organ perfusion been studied to such an extent and in such detail.
- This will be the first project to utilize a multi-omics approach with longitudinal data from long term organ perfusions. Such repeated measurements will allow game changing artificial intelligence analyses to find relevant molecular pathways with a relatively low number of experiments.
- My recently developed methodology of performing stable normothermic ex vivo kidney perfusions inside an MRI scanner to obtain high quality pre-transplant radiomics data is ground-breaking and, due to its complexity and reliance on years of perfusion experience, currently only available to my group.
- Combining genomics, transcriptomics, proteomics and metabolomics to dissect molecular mechanisms underlying ex vivo organ perfusion is already very novel. Adding the innovative tool of radiomics to this equation will make the present scientific endeavor unique.
- So far, pre-transplant organ assessment has relied on subjective clinical judgement and inaccurate prediction models based on conventional parameters alone. As a result of the present project and with the help of cutting-edge artificial intelligence technology, I will be able to show how an innovative combination of conventional and molecular pre-transplant factors correlate with relevant post-transplant outcome.

Expected scientific and societal impact of my proposed research:

- This project will transform ex vivo organ perfusion from merely an organ preservation method to a platform for pre-transplant kidney evaluation, which will be adopted by many transplant centers and research groups worldwide.
- It will introduce ex vivo MRI assessment of donor kidneys as a novel method to aid pre-transplant organ evaluation.
- It will pave the way to ultimately transplant more donor kidneys and thus help more critically ill patients, since potentially marginal organs can prove to be of acceptable quality during ex vivo organ assessment. Conversely, novel knowledge generated in this project may prevent transplantation of non-viable organs and hence reduce serious post-transplant morbidity and mortality.

Section b. Methodology

b.1 Scientific Approach/Methodology

Several key approaches are the same for studies in all three objectives. Therefore, these will be discussed first, followed by an overview of methodology that is unique for each individual objective accompanied by a detailed work plan.

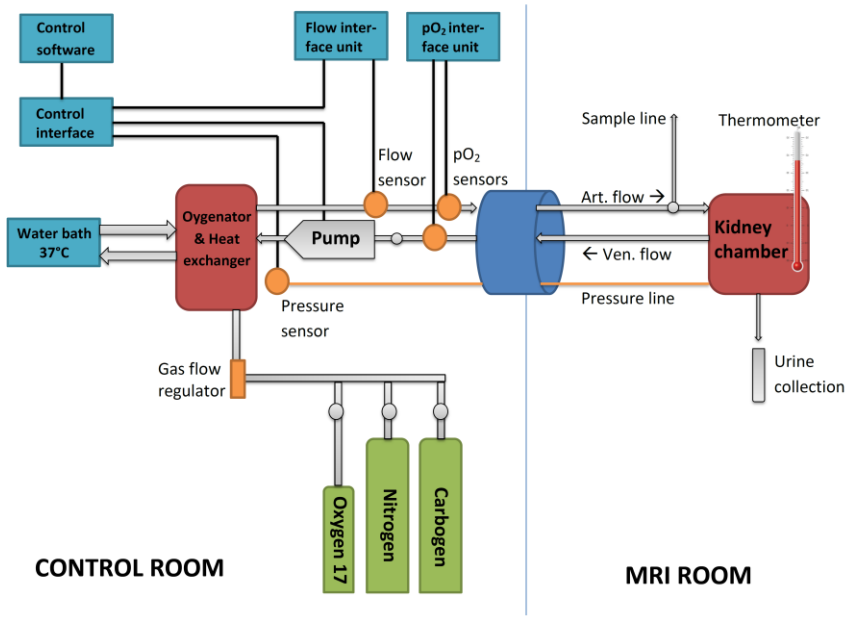


Figure 6: Schematic overview of normothermic ex vivo perfusion setup; *depicting how various components are connected and perfusate recirculates in the circuit. This scheme illustrates the situation when ex vivo perfusion is carried out inside an MRI scanner. Carbogen gas is used for regular oxygenation of the perfusate, nitrogen gas is utilized to quickly de-oxygenate the circuit just prior to administration of ^{17}O-oxygen gas. Ex vivo perfusion is conducted in a pressure controlled fashion (110/70 mmHg) at 37°C.*

Normothermic ex vivo machine perfusion

Perfusion hardware. The perfusion circuit will consist of a LifePort® organ chamber with SealRing cannula (Organ Recovery Systems, Itasca, IL, USA), a magnetic pump head connected to a centrifugal pump unit (Deltastream DP2, Medos Medizintechnik AG, Stolberg, Germany) and an oxygenator with integrated heat exchanger (Hilite 800 LT, Medos Medizintechnik AG, Stolberg, Germany). The perfusate is oxygenated with 0.5 L/min carbogen (95% O_2 / 5% CO_2) and perfusate temperature will be maintained at exactly 37°C. Pressure is measured directly after the SealRing cannula using a clinical grade pressure transducer (TruWave disposable pressure transducer, Edwards Lifesciences, Irvine, CA, USA). The pressure sensor is zero-calibrated to the atmosphere. Flow is monitored using an ultrasonic clamp-on flow probe (Transonic Systems Inc., Ithaca, NY, USA). All components of the NMP setup are connected to a custom-made digital electronic interface, which translates all sensor information to the perfusion software and directs variable power to the perfusion pump as controlled by the software in various continuous feedback loops (Figure 6).

Perfusion control software. LabView® software (National Instruments Netherlands BV, Woerden, Netherlands) will be utilized to control ex vivo perfusion dynamics over the electronic interface. A custom script has been developed which precisely steers perfusion pressure and delivers a physiologic, arterial waveform-like pulsatile perfusion with a systolic pressure of 110 mmHg and a diastolic pressure of 70 mmHg at 60 beats per minute.

Table 1: Normothermic ex vivo perfusion medium composition

Constituent	volume (ml)	Na (mmol/l)	K (mmol/l)	Ca (mmol/l)	Mg (mmol/l)	PO_4 (mmol/l)	HCO_3 (mmol/l)	albumin (g/l)	glucose (mmol/l)
Albuman 200 g/l	110	100						200	
NaCl 0.9%	20	154							
KCl 74.6 mg/ml (1 mmol/ml)	0.6		1000						
$MgSO_4$ 100 mg/ml (0.4 mmol/ml)	0.7				400				
Calciumgluconate (0.225 mmol/ml)	3.3			225					
$NaHCO_3$ 8.4%	11	1000					1000		
Na_3PO_4 3 mmol/ml	0.1	4000				3000			
Glucose 5%	0								278
Water (sterile, for iv injection)	60								
Augmentin 1200 mg/10ml	2	270	100						
RBC preservation solution + SAGM	100	168	5						23.3
Pure RBCs	180								
Final composition:	**488**	**139**	**4.2**	**2.41**	**0.91**	**0.97**	**36**	**71**	**7.6**

Perfusate composition. The perfusion medium is constituted from sterile and clinical grade ingredients, which will be obtained from the UMCG clinical pharmacy. Table 1 shows all constituents and concentrations. Blood type compatible allogeneic red blood cells concentrates (RBCs) will be obtained from donor pigs (porcine model in Objective 1), or human donors through our hospital's blood bank (human studies in Objectives 2 and 3).

Perfusion protocol. Between organ retrieval and start of ex vivo normothermic perfusion, all kidneys (porcine and human) will be placed on a standard clinical oxygenated hypothermic (0-8°C) machine perfusion device (Kidney Assist Transport®, Organ Assist, Groningen, Netherlands) for transport and temporary storage before normothermic perfusion can be commenced. Just prior to normothermic perfusion, the kidney is taken off the hypothermic perfusion device and the renal artery is cannulated with a SealRing cannula, while the kidney is kept on ice. The renal vein is left uncannulated. The ureter is cannulated with a sterile 7 Fr single-J ureteric splint. The kidney is placed in the normothermic perfusion chamber where it will be partially submerged in perfusate. Perfusate from this surrounding reservoir will be continuously pumped into the oxygenator/heat exchanger, enters the renal artery through the SealRing cannula and subsequently drains out of the renal vein back into the reservoir. Thus, perfusion medium is constantly recirculated. Urine produced by the kidney is collected in a sterile fashion in 30 min intervals to record urine volume. After a small urine sample has been taken, all urine is returned to the circulating perfusate half-hourly. This at first sight unconventional approach is chosen to prevent the circuit from quickly losing a relevant amount of its circulating volume. Other protocols, in which urine is not returned to the circulation and volume loss is replaced by some other standardized fluid are sub-optimal as these quickly lead to serious electrolyte disturbances. Since urine produced by an ex vivo perfused isolated kidney contains negligible amounts of waste products, returning urine to the circuit is a safe approach to keep perfusate composition stable.[32] At the start of normothermic ex vivo perfusion, the perfusate will be at room temperature. During the first hour, the kidney will be gradually warmed from room temperature to 37°C and kept at this temperature for the subsequent 7 hours (or 3 hours in Objective 3). As soon as perfusate glucose concentration falls below 5 mmol/l, a continuous infusion of electrolyte-free total parenteral nutrition (Smofkabiven®, Fresenius Kabi BV, Utrecht, Netherlands), and insulin will be commenced, the rate of which will be adjusted to the slope of glucose decline. Also, a continuous infusion of inulin will be started form baseline, in order to estimate glomerular filtration rate during ex vivo perfusion.

Sample protocol during normothermic ex vivo perfusion. At baseline and each subsequent hour, a 2 mL perfusate sample will be taken, centrifuged and supernatant and pellet stored separately at -80°C. Every 30 min, a 0.5 mL urine sample will be taken and also stored at -80°C. Hourly, the minimal circulating volume loss due to perfusate and urine sampling will be replaced by adding 3 mL of sterile perfusion medium without RBCs. At baseline and hourly thereafter, a small hollow needle cortical biopsy will be taken from the kidney and the puncture place secured with a surgical cross-suture to prevent bleeding. One half of the biopsy will be stored in formalin for histology analyses and the other half will be stored in RNAlater® solution (Thermo Fisher Scientific, Waltham, MA, USA) for multi-omics tissue analyses. At the end of the normothermic perfusion period, larger surgical cortical and medullary renal tissue biopsies, as well as a 10 mL perfusate sample are obtained and stored in the aforementioned fashion.

Multi-omics data collection methodology

Genomics. Renal tissue samples will be batch processed and DNA extracted (performed by this project's biotechnician and PhD student, with help from dept. of Human Genetics, UMCG). An Infinium™ Global Screening Array-24 v2.0 BeadChip (Illumina Inc, San Diego, CA, USA) analysis will be employed.

Transcriptomics. In batch processing, RNA will be isolated from renal tissue samples. These samples will be processed for sequencing with an Illumina TruSeq version 2 library preparation kit (performed by this project's biotechnician and PhD student, with help from dept. of Human Genetics, UMCG). Paired end sequencing of 2×50-bp reads will be performed with the Illumina HiSeq 2000 platform. The quality of the raw reads will be checked in FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Read alignment will be performed in STAR 2.3.0[33] and aligned reads are sorted with SAMTools. Quantification of expression at gene level will be done with HTSeq.[34] The raw read counts data will first be normalized and transformed, and subsequently used for differential expression analysis.

Proteomics. Proteomics of perfusate and renal tissue will be performed in the discovery mode using a shotgun approach: The protein-containing sample will be digested with a site-specific protease or a combination of proteases (to increase coverage). The resulting peptides will be analyzed by LC-MS on a high-resolution mass spectrometer (Q-Exactive Plus). For validation, quantitative, targeted LC-MS assays will be developed for the proteins in question using stable-isotope-labelled standards (performed by myself and this project's postdoc, with help from dept. of Analytical Biochemistry, UMCG).

Metabolomics. Circulating metabolites in the perfusate during normothermic ex vivo perfusion will be measured and analyzed with the Nightingale Biomarker Analysis Platform, which is based on nuclear magnetic resonance spectroscopy (Nightingale, Helsinki, Finland). Further to this, profiles of lipids and central carbon cycle metabolites will be measured based on established procedures (performed by this project's postdoc, with help from dept. of Analytical Biochemistry, UMCG).[35–37]

Radiomics. An automated cascade of the following MRI sequences will be performed during each ~60 min ex vivo and in vivo MRI session, generating thousands of images per scan (performed by myself and this project's postdoc, with help from the dept. of Radiology, UMCG and the dept. of Radiology, Aarhus University Hospital):

1. Ultra-high resolution isotropic (0.6mm) zoomed T2 weighted imaging, for detection of ischemic areas.
2. High resolution arterial spin labeling (ASL) based on a flow sensitive alternating inversion recovery-true fast image with steady state precession (FAIR-TrueFISP) method, for measurement of regional blood flow.
3. High resolution zoomed echo planar imaging (EPI) based dual-echo dynamic susceptibility weighted imaging (DSC) for selective assessment of microvascular architecture, leakage and flow.
4. High resolution zoomed intra-voxel incoherent motion (IVIM) based diffusion weighted imaging (DWI) with 11 b-values, for detection of regional tissue (true) water diffusion characteristics, such as diffusion restriction and cellular edema.
5. Fully automated in-line T2* mapping for whole organ pixel-wise T2-relaxation time characterization.
6. BOLD fMRI with varying TE values in order to be able to calculate tissue oxygen delivery.
7. T1ρ mapping before and after administration of 70% enriched ^{17}O-oxygen in order to calculate tissue oxygen consumption per voxel.
8. 2D MR elastography of the kidney graft with in-line post-processing of wave and tissue pressure images, for determination of organ regional stiffness.

An unsupervised multi-layered deep convolutional neural network approach[38] (supplemented with autoencoders[39] and generative adversarial networks[40]), along with relevant clustering and other dimension reduction techniques will be utilized. A searchable database will be populated with descriptive MRI features. Only those features which consistently correlate with distinct measured multi-omics characteristics, or those that discriminate between ex vivo and in vivo perfusion will be extracted (performed by myself and this project's postdoc, with help from the Center for Functionally Integrative Neuroscience, Aarhus University Hospital).

Data interpretation and artificial intelligence methodology

Multi-omics data integration. (Performed by myself and this project's postdoc, with help from the Center for Functionally Integrative Neuroscience, Aarhus University Hospital and the depts. of Human Genetics and Analytical Biochemistry, UMCG). Data will be processed using commercially available as well as in-house developed software and analyzed by comparative, multivariable statistics to obtain models that discriminate situations with lowest cross-validation error. Initially, I will create meta-dimensional models synthetizing data across the biological levels. These models will subsequently serve as input for artificial intelligence models that differentiate outcome such as post-transplant glomerular filtration rate. Challenges to techniques for identifying predictive interactions across different levels of biological regulation include strong robustness to noise as well as ability to combine large volumes of categorical as well as continuous variables from the input as well as the output side. This realistically requires a pipeline of more targeted models, of which parallelized, computationally efficient implementations of random forests such as Random Jungle[41] and the platform-independent Random Forest Generator (ranger)[42] have been demonstrated to be particularly useful in high throughput data for establishing direct (main) effects, as well as interactions as candidate input for subsequent classification algorithms. These filtering procedures will be further complemented in developing a full meta-dimensional model by incorporating known biological functions and interactions. I will pursue identification of complex multi-level interactions using neural networks structures, in particular autoencoders, which have been previously reported to be suitable for synthetizing heterogeneous data.[43] By seeking to reproduce a meta-dimensional input at the output side of a neural network through hidden layers of lower dimensionality, the autoencoder wil utilize a bottleneck approach for extracting the most informative combinations of variables, possibly across scales. The net result will be a low-dimensional representation of heterogeneous multi-scale data, which may be added to the input side of the classification algorithms described in the next section. The biomarker candidates will be prioritized based on fold-change and p-value. By integrating a diverse set of data-DNA variation, protein/metabolite abundance, transcriptome, and radiomics data (Objectives 1 and 2), or transplant outcome data (Objective 3) from the

same experiments/individuals, correlation networks across different layers will be built and genetic determinants for variation in both post-transplant outcome and molecular phenotypes will be identified.

Development of advanced prediction models. (Performed by myself and this project's PhD student, with help from the Center for Functionally Integrative Neuroscience, Aarhus University Hospital and the depts. of Human Genetics and Analytical Biochemistry, UMCG). Extracted multi-omics features, as described above, will be entered into computational models for predicting transplant outcome, primarily defined as estimated glomerular filtration rate at 3 months and 1 year post-transplant. The main considerations in model selection include balancing the volume of input features, number of samples and model flexibility. The number of input features will be controlled in the filtering steps described above. Hence regression-type models may be employed for sparse input to yield relatively simple and interpretable models. With larger volumes of input features interpretable regression type models can still be obtained by utilizing methodology for automatically constraining model size and even automatically selecting variables based on performance in cross-validation with Elastic Nets.[39] I will explore the full predictive power of the combined multi-omics observations using deep feed forward neural networks. I will employ stochastic gradient descent for model optimization and use either genetic algorithms or penalization to avoid over-fitting and identify the most efficient network architectures. Several open source frameworks and libraries are readily available for such analyses. Tensorflow is widely used yet in terms of computational performance the Microsoft Cognitive Toolkit is considered to be preferred. For efficient prototyping I propose to use Keras which runs on top of Tensorflow as well as Microsoft Cognitive Toolkit (and Theano), but features a simplistic and intuitive model-building framework for speedy development.

b.2 Research Plan

Objective 1: Identify biomarkers of ex vivo kidney perfusion

Work plan. During a 2-year period, 60 adult Danish landrace laboratory pigs (~100 kg) will be utilized. Animals will be anaesthetized and midline laparotomy performed. One kidney will be retrieved immediately, while the contralateral kidney remains in situ. The retrieved kidneys will be perfused ex vivo on a normothermic oxygenated machine perfusion device for 8 hours. Hourly, small tissue biopsies and perfusate samples will be taken. The pig will remain under anaesthesia for 8 hours, during which small tissue biopsies and serum samples will be taken hourly. Thus, a comparison can be made between the kidney that is perfused ex vivo and the contralateral kidney, which remains in its anatomical and physiological context. Both the anaesthetized pig and the ex vivo perfused kidney will be brought into a clinical MRI scanner and the above mentioned imaging sequences will be performed. On each tissue and perfusate sample, multi-omics analyses will be conducted.

Deliverables

a. Unique molecular pathways and patterns that characterize an ex vivo perfused kidney and how these differ from such pathways and patterns in vivo.
b. Unique radiomic pathways and patterns that characterize an ex vivo perfused kidney and how these differ from such pathways and patterns in vivo.
c. Relevant non-invasive radiomic correlates with molecular pathways identified in tissue and perfusate.

Objective 2: Validate ex vivo biomarkers in human kidneys

Work plan. Over a 2.5-year period, 25 discarded human kidneys will be ex vivo perfused on a normothermic oxygenated machine perfusion device for 8 hours. Hourly, small tissue biopsies and perfusate samples will be taken. Ex vivo perfused human kidneys will be brought into a clinical MRI scanner and the above mentioned imaging sequences will be performed. On each tissue and perfusate sample, multi-omics analyses will be conducted. Unique molecular and radiomic pathways that were discovered in Objective 1 will be sought for in these kidneys to validate to what extent these also characterize human ex vivo perfusion.

Deliverables

a. Unique molecular pathways and patterns that characterize an ex vivo perfused *human* donor kidney.
b. Unique radiomic pathways and patterns that characterize an ex vivo perfused *human* donor kidney.

Objective 3: Create multi-omics based prediction models for renal transplant outcome

Work plan. A prospective clinical transplantation study will be conducted in the UMCG. During a 3-year period, 120 human kidneys, retrieved from deceased donors aged 50 years and older and accepted for transplantation will be included and ex vivo perfused pre-transplant for a total of 4 hours. Every hour, a small tissue biopsy and a perfusate sample will be taken. Also, an ultrasound guided protocol biopsy will be taken from the transplanted kidney 3 months after transplantation (standard care). Primary end point of this study will be the measured glomerular filtration rate (GFR) at 3 and 12 months post-transplant. In the context of conventional donor, perfusion and recipient parameters, tissue and perfusate multi-omics data will be

associated with GFR at 3 and 12 months, as well as with multi-omics data of the 3 month protocol renal biopsy, by means of artificial intelligence analysis (supervised data integration with input from pathways validated in Objective 2). Thus, advanced prediction models will be created for renal transplant outcome. No ex vivo MRI scans and hence no radiomics analyses will be conducted in Objective 3, as the required perfusion setup is not yet certified for clinical use. Certification and actual clinical implementation of ex vivo perfused MRI in the near future will depend on robustness of results from the present ERC project.

Deliverables

a. Advanced prediction models which integrate conventional clinical and perfusion data with multi-omics data to better predict renal transplant outcome.
b. From those multi-omics biomarkers that are found to be predictive of post-transplant outcome, a radiomic correlate distilled from Objectives 1 and 2, in order to base future clinical studies on, which aim to replace ex vivo pre-transplant invasive and slow tissue/perfusate analyses with non-invasive ex vivo magnetic resonance imaging prior to transplantation.

Sample size calculations. Objective 1: Sample size will be based on the ability to find a relevant correlation between radiomics and multi-omics patterns. A meaningful correlation should in my view be at least r = 0.6. Given the large amount of multiple testing in this pre-clinical exploratory study, I have determined a two-tailed type I error rate (α) of 0.0001. To obtain a power of 0.9 with the above conditions, a minimum of 59 experiments will be required.[44] Objective 2: The same correlation as in Objective 1 will be the basis of sample size calculations. However, since testing in Objective 2 is more focused (patterns found in Objective 1 will be applied to data in Objective 2), I will allow a less stringent two-tailed α of 0.05. To find a correlation of at least r = 0.6 with a power of 0.9, a minimum of 25 experiments will be required.[44] Objective 3: Assuming that 30% of all transplanted kidneys will not reach an acceptable functional level, the main prediction model should be able to detect this outcome with a sensitivity of 90% (95% confidence interval 10%) and a specificity of 90% (95% CI 10%). This will require a minimum of 116 patients in the study.[45]

Ethical considerations. Objective 1: Animal welfare committee approval is required and will be applied for well before the planned start of experiments (also see Figure 7 on p.15). I will comply with EU Directive 2010/63/EU, as well as Dutch and Danish national law on animal experiments and good laboratory animal practice. Objective 2: Under Dutch law, no study-specific written informed consent from deceased donors or donor relatives will be required for scientific research with donor kidneys that have been discarded after retrieval. I will comply with guidelines for utilization of discarded donor organs from the Dutch Transplantation Foundation and the Dutch Ministry of Health. Objective 3: The study will include all kidneys offered and accepted by the surgeon on-call at the time of organ offer. All deceased donor types and donor ages of 50 years or older are eligible for inclusion into the study. Inclusion will take place once an offered and accepted organ has been received, has been inspected and is deemed suitable for transplant. As the study intervention (ex vivo normothermic perfusion) takes place to an isolated donor organ after death of the donor, no informed consent from the donor nor from the donor relatives is required under Dutch law. By consenting to organ donation, donors and relatives automatically consent to organ transplantation-related research with procured and transplanted organs. No study-related interventions, additional samples, or clinical protocol changes will be implemented for patients enrolled in the clinical study. Nevertheless, for obtaining patient-specific follow up data from kidney recipients, medical ethics committee approval *will* be required and requested well before the planned start of the clinical study (also see Figure 7 on p.15). I will comply with the Declaration of Helsinki and Dutch law on medical research with patients. Participation in the clinical study will not affect patients' position on the kidney transplant waiting list or their likelihood of receiving a transplant. Similarly, withdrawal of participants from the study at any point and for any reason will not affect their position on the kidney transplant waiting list or their likelihood of receiving a transplant. Written versions of the Participant Information and Informed Consent will be presented to each potential participant, preferably at the outpatient clinic at the time when a patient is screened for suitability of a kidney transplantation, detailing: the exact nature of the study, what it will involve for the participant, the implications and constraints of the protocol and the known side effects and any risks involved in taking part. It will be clearly stated that participants are free to withdraw from the study at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal. Participants will be allowed as much time as possible to consider the information, and the opportunity to discuss their participation with other independent parties to decide whether they will take part in the study. Written Informed Consent will then be obtained by means of participant dated signature and dated signature of the person who presented and obtained the Informed Consent. The person obtaining the consent (mostly myself) will be suitably qualified and experienced. A copy of the signed Informed Consent will be given to the participant. The original signed form will be retained at the trial site.

Data management and storage. During this research project, all data will be stored on protected servers of the UMCG and the University of Groningen. Every workstation of the UMCG is part of a standard IT-workplace which is backed-up automatically every day on the servers of the UMCG. Data collection and data generation that is not performed on UMCG's workstations will be stored on the UniShare ownCloud Enterprise solution implemented for the University of Groningen. The UniShare account allows users to securely share files with collaborators within and outside the University of Groningen through authorized global access. A signed certificate ensures both privacy and safety of the data. Moreover, a system for version management is in place and deleted files can be restored. Data collected or generated by students and researchers will be added to the UniShare account of the responsible PI. At the end of this research project, all data will be stored for the long-term in a dedicated database on the UMCG cluster storage facility. Approximately 1.5 terabyte of data is expected to be collected for this project over five years. Only the raw data will be archived in a standard format together with the standardized procedure and analysis steps necessary to recreate the analyses. UMCG specific policies and services have been published in full on the web (https://www.umcg.nl/EN/Research/Researchers/General/ResearchCode/Paginas/default.aspx). As far as ethics are concerned the UMCG has a long term policy (since 2007) on research integrity covering rules, regulations and best practices with respect to the use, management and storage of patient data. These will be followed in the present research project.

b.3 Collaboration

University Medical Center Groningen. The Surgical Research Laboratory will provide facilities for porcine ex vivo renal machine perfusion. Our lab has a broad experience with various organ perfusion techniques. The dept. of Surgery possesses a clinical grade organ perfusion facility. The dept. of Human Genetics, led by Prof. C. Wijmenga will provide expertise and facilities for genomics and transcriptomics. The dept. of Analytical Biochemistry, led by Prof. R. Bischoff, provides expertise and facilities for proteomics and metabolomics. The dept. of Radiology, with whom I already have a fruitful collaboration for pilot experiments of ex vivo renal perfusion inside clinical MRI scanners, will provide support, facilities and scan time for experiments in Objective 2.

Aarhus University Hospital, Denmark. I have several successful ongoing collaborative research projects together with the Transplant Nephrology group led by Prof. B. Jespersen. Given their superior facilities for porcine animal experiments, I will conduct experiments of Objective 1 in Aarhus. The Center for Functionally Integrative Neuroscience (CFIN) and MINDLab, led by Prof. K. Mouridsen specializes in artificial intelligence and deep learning data extraction from complex databases. Their patented mathematical models are particularly effective for data sets which are based on relatively small numbers of cases. Our group already has a fruitful collaboration with Prof. Mouridsen in another research project and he will provide advanced training and expertise in artificial intelligence analysis which is paramount to the present project. The dept. of Radiology, with whom I already collaborate in another research project on *post*-transplant renal MRI and in the COST action PARENCHIMA, will provide support, facilities and scan time for experiments in Objective 1.

b4. High-risk and Feasibility

This is a high-risk high-gain project as required by ERC. The key risks are:

1. Not granted animal committee approval to conduct experiments in Aarhus. I may not succeed in implementing my ex vivo MRI setup in Aarhus. Mitigation: I can utilize to the animal experimental facilities of Wageningen University, located in Lelystad, NL, to conduct laboratory animal experiments. Disadvantage will be that there are no MRI facilities on site and I cannot do radiomics in Objective 1. Alternatively, I can switch to porcine kidneys obtained from a slaughterhouse in a controlled fashion and do all ex vivo perfusions and MR imaging in my own institution.
2. Not be able to include sufficient discarded human kidneys. Mitigation: Most of the unsupervised multi-omics discovery analyses can also be performed on human donor kidneys in Objective 3. However, I will then have to drop human kidney radiomics.
3. I may not include sufficient patients in the clinical study, or may not be granted ethics committee approval for the study in its current form. Mitigation: Utilize TransplantLines biobank material (renal biopsies and ex vivo perfusates) and clinical / outcome data from hundreds of kidneys that have already been transplanted in my institution. Although these perfusions were all *hypo*thermic, tissues and perfusates may still convey important multi-omics predictive information.
4. Insufficient sample size in any of the three Objectives to conduct meaningful –omics and artificial intelligence analysis on single time point data during ex vivo perfusion. Mitigation: In all perfusions / experiments, I will have longitudinal data with up to 8 subsequent samples per individual kidney. This will tremendously increase the power to detect relevant patterns and associations.

Section c. Resources (including project costs)

Please indicate the duration of the project in months:[2]	**60**
Please indicate the % of working time the PI dedicates to the project over the period of the grant:	**60%**

Daily work/tasks and scientific focus of project members

I will perform all experimental and many clinical surgical procedures, conduct perfusions, prepare samples for multi-omics analyses, perform data analyses with multi-omics and radiomics experts and include patients in the clinical study and obtain informed consent.
The postdoc (*scientific* focus on Objective 1) will perform most multi-omics analyses, and performs data management and data interpretation. The PhD student (*scientific* focus on Objectives 2 and 3) will prepare perfusions/experiments, collect data and samples and perform clinical data analysis. The biotechnician will help prepare experiments, help with surgical procedures and provides support for sample preparation and analysis.

Narrative description of costs per budget category

Direct personnel costs

These will consist of salary costs for the PI (0.6 fte, 5 years), a postdoc (0.8 fte, 4 years), a PhD student (0.8 fte, 4 years) and a technician (0.4 fte, 3 years). All these staff will be employed by the University Medical Center Groningen (the beneficiary of the present ERC Starting Grant).

Equipment

Equipment required will mainly consist of a perfusion machine to be purchased for use in the clinical study of Objective 3.

Travel

For experiments in Objective 1, travel between Groningen in The Netherlands and Aarhus in Denmark will be regularly required, as these experiments will be conducted at Aarhus University Hospital.

Consumables and other goods and services

These will consist of perfusion disposables and fluids, publication costs and conference costs.

Costs of internally invoiced goods and services

These will consist of costs of MRI scans in Objective 2 (Dept. of Radiology, UMCG), genomics, transcriptomics (Dept. of Human Genetics, UMCG), proteomics and metabolomics (Dept. of Analytical Biochemistry, UMCG) analyses and amount to an estimated total of €153,997.06.

In-kind contributions not used on premises

Animal experiments of Objective 1 will be conducted at the facilities of Aarhus University Hospital in Denmark. Costs incurred there will consist of perfusion disposables, perfusion fluids, animal housing, feeding, handling, surgery and MRI scan fees. The total estimated costs of in-kind contributions not used on premises will be €112,499.75.

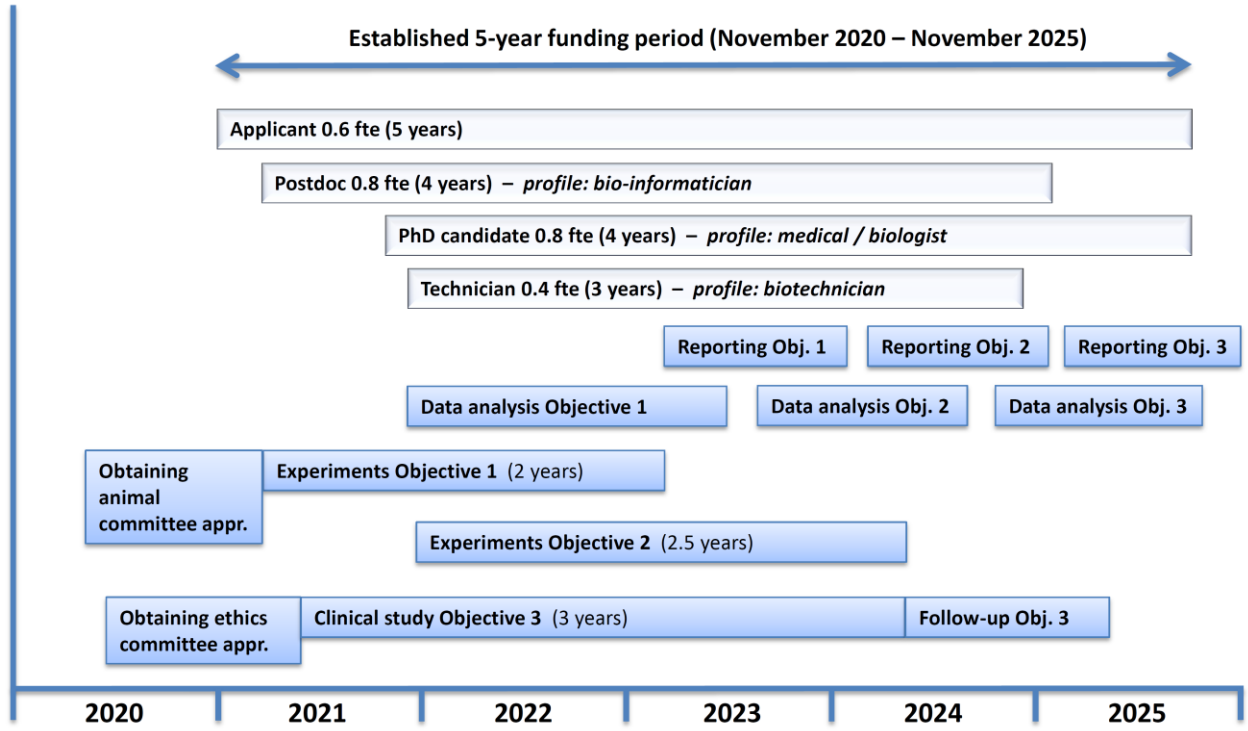


Figure 7: *Practical timeline and work plan for personnel. Obj. denotes objective, appr. denotes approval.*

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