SECTION C:
DISCUSSION, SUMMARIES AND ACKNOWLEDGEMENTS.

The Present and Future of Intestinal Transplantation.

List of abbreviations.

AA	Aminoacid solution.
ACR	Acute cellular rejection.
DBD	Donation after brain death.
IGL-1	Institut Georges Lopez-1 solution.
ITx	Intestinal transplant(ation).
LP	Luminal preservation.
PEG	Polyethylene glycol.
UW	University of Wisconsin solution.

During the past thirty years, intestinal transplantation (ITx) has become a standard treatment option for patients with intestinal failure that suffer from severe complications of parenteral nutrition[167]. The short-term graft and patient survival rates improved due to increased experience, and improvement of immunosuppressive strategies using tacrolimus and induction therapy with leukocyte depleting or anti-IL2 receptor agents[99]. On the other hand, long-term survival rates have not improved, mostly due to rejection and subsequent sepsis[167]. Meanwhile, parenteral nutrition continues to improve, so transplantation rates have dropped from 250 procedures worldwide in 2008 to approximately 175 in 2019 (results presented at the Congress of Intestinal Rehabilitation and Transplantation in Auckland, July 2021).

As described in the previous sections, this decrease is due to the complexity of the procedure and the organ's immune and luminal content, along with the progress made in, and consequent success of, (total) parenteral nutrition. ITx is a life-saving treatment, so this is ultimately not bad news, but also because of this reason it is imperative that the field strides for continuous improvement. The overarching aim of the work described in this thesis is to improve the graft's survival rates and thereby patient quality of life and survival. Two different, but nonetheless connected, parts of the life of the intestinal transplant are herein tackled: preservation (during the transplantation procedure) and management of rejection. The connection between these two aspects has been the use of organ-specific treatments during and after transplantation.

Overview and discussion.

SECTION A dealt with the effect of luminal preservation (LP) during different times of the (multi) organ donor procedure.

An overview of the models that have been set up by different research groups in the field was presented in **Chapter 1**. It was demonstrated that LP delays the onset of moderate to severe mucosal damage. A simple, standardised solution for intestinal preparation for endoscopic procedures (polyethylene glycol 3350, PEG), a standard organ preservation solution such as University of Wisconsin (UW), or a more complex preparation tailored for the intestine (AA), all solutions have shown favourable results of LP in experiments in small and large animal and human models. A few of these studies have also used reperfusion models to show that the protective effect of LP extends beyond the preservation phase. Even though reperfusion of the organ is known to be the most detrimental in ischaemia-reperfusion injury, and thus transplantation in general, it has been a constant struggle in the field of ITx to develop a robust model where these effects can be thoroughly examined. Another aspect lacking on all but human

models is the context of donation after brain death (DBD) an important factor in organ donation, particularly of the intestine.

Chapter 2 focused on the latter issue. With the aid of a rat DBD model, luminal preservation with two different solutions was performed and its effects on preservation injury evaluated. The study delivered three main findings. First, that the level of damage at the beginning of the procurement procedure was already higher in DBD than what was seen in other models without brain-death. This reiterates the quick detrimental effect of brain death on the intestinal mucosa. Second, that the lack of LP left the mucosa in an unreliably fragile state with regards to its histological structure, in that samples taken during the shorter preservation time point were in a worse state than those taken after a longer period of cold storage. Third, LP with either UW or PEG stabilised injury development, which is expected to be time-dependent, but did not show the striking beneficial effects that were present in other published models. Taken together, these findings demonstrate the importance of including brain death as a factor in organ donation models. The effect of LP was probably dampened by the injury initiated by DBD but, added to the evidence found in the literature that was described in Chapter 1, LP is expected to better protect the graft, even after reperfusion. The absence of the reperfusion stage is a shortcoming of this study.

Luminal preservation studies that closely resemble the clinical donation and procurement settings are lacking. **Chapter 3** is the result of the development and implementation of such a clinically relevant model. In this two-centre study, the effects of LP with PEG on donors treated with two different vascular preservation solutions (UW and Institut Georges Lopez-1, IGL-1) were studied after *in situ* administration of a cold PEG-based preservation solution in the donor during the procurement procedure. The model was successfully implemented, and the potential shortcomings addressed during a five-year-long inclusion period by several procurement teams in The Netherlands and Belgium. A protective effect of LP was found at time of procurement, particularly in the jejunum, suggesting that the most sensitive parts of the intestine could already benefit from rapid cooling by early LP, before and during vascular perfusion. Regarding preservation injury, an overall protective effect was seen only in donors who underwent vascular flush with IGL-1. The main problems encountered during the realisation of this project were the availability of donors and several issues which made it impossible to perform a more powerful and complete study including other types of LP solutions and variations on the administration procedures. These issues were mainly of a logistic nature, such as the training of multiple procurement teams, arrange transport to the research centre of the organ, having a big

enough research team to comply with high standards of research whilst attending to the protocol time points. However, the most delaying matter was of legal nature and involved the halt of all studies relying on donor tissue across The Netherlands. While this matter was resolved after sixteen months, the delicate issue of the ethical and legal considerations of organ donation to science as well as to medicine still stand today and should be therefore considered for the following studies.

Chapter 4 closes SECTION A by looking into the effect of luminal preservation in another phase of transplantation other than cold storage. A pig model of complete warm ischaemia was used as proof-of-concept to study the effect of gaseous oxygen (*persufflation*) administered via the lumen during the warm ischaemia phase that occurs during the implantation procedure, right before reperfusion begins. This study showed that *in situ* application of oxygen directly protects the mucosa, but this effect is sensitive to location and direction of gaseous flow. This part of the operation can be time consuming and is prone to procedural delays due to unforeseen complications while the recipient is ready to receive the organ. For this reason, it is important to have a method at hand that can at least maintain the quality of the organ during implantation.

SECTION B focused on the immunity of the intestinal graft by trying to unravel the system's mechanisms and the implementation of a gut-specific treatment in different forms of rejection.

Chapter 5 reviewed the known physiology of immune policing and response after intestinal transplantation. It presented different ways to manipulate these responses to manage rejection, the major cause of low graft survival rates.

Two in-depth case reports followed this information, where the knowledge explained in **Chapter 5** is applied for the first time in human ITx. **Chapter 6**told the story of a patient who has a background of Crohn's disease and underwent a combined intestine and abdominal wall transplantation. The patient suffered during the first year a moderate, unresolved episode of acute cellular rejection (ACR) which ended after the temporary administration of vedolizumab. Vedolizumab is a monoclonal antibody against integrin α4β7 that selectively blocks the migration of gut-homing immune cells back into intestinal mucosa. The case was unique because the skin was not a sentinel marker for rejection (on the contrary), and because it became the first known application of this drug in acute cellular rejection in ITx. Histopathological evidence for the putative mechanism of the drug is absent, but the clinical evidence is clear in both intestine and abdominal wall. Up to the date of submission of this thesis, the patient remains free of rejection (six years).

Chapter 7 presented another original and successful application of vedolizumab in ITx in a patient with a history of mesenteric ischaemia. There were endoscopic signs of chronic rejection fifteen years post-transplantation. After onset of vedolizumab therapy, the inflammatory signs receded almost completely, leaving only a stricture behind. The stricture was surgically removed but the rest of the graft remained functional, and the patient remains free of complaints to date (two years since beginning of treatment). This therapy has therefore benefitted the outcome of chronic rejection which would have otherwise ended in total graft enterotomy. The working mechanisms of the drug were more difficult to understand in this case, but the presence of MAdCAM-1 in deeper blood vessels could indicate that chronic rejection of the intestinal transplant involves not only the gut-homing mechanism in the mucosa but also the larger vessels of the mesentery.

To further understand the working mechanisms of vedolizumab observed in Chapter 6 and Chapter 7, in **Chapter 8**, an exploratory study on the gene expression patterns found after whole-genome RNA sequencing of selected biopsies from both patients was carried out. Patient- and disease-specific patterns were discovered. After onset of therapy, expression of genes related to the homeostasis of zinc were downregulated. Additionally, the expression of vedolizumab target proteins (α4, β7, MAdCAM-1) was not affected by therapy in this context. Signs of activity of anti-inflammatory and anti-apoptotic processes changed after resolution of rejection driven by the therapy, which suggests that vedolizumab acts in different ways as to what was originally proposed, not only by influencing leukocyte migration, but also the activation status of immune cells.

Future perspectives.

The development of research models that closer resemble the clinical situation are needed to reverse the declining trend in intestinal transplantations. Whether it is for further investigation of the effect of luminal preservation or other techniques that can improve the state of the graft prior to pre-transplantation, the broader inclusion of organs from brain death donors and developments of robust reperfusion techniques is important for progress in this field.

Small and large animal brain death models that include preservation and transplantation exist and are being applied in research of ITx. Human models, although slowly becoming more reported in the literature, are still in their infancy and are therefore not completely comparable, particularly regarding the effects of luminal preservation. This thesis presents the first example of a model that was performed in the operation room during real procurement procedures in

LUMINTRAL (SECTION A, Chapter 3). More studies like these are necessary to change the paradigm of intestinal procurement, storage, and implantation procedures. The difficulties surrounding relevant donor inclusions, logistical inconveniences, and patience for study completion must be addressed from the start, including input from multidisciplinary teams and more (inter)national collaborations.

Examples of steps that could be taken in the (near) future are the following.

(1) Further analyses on the tissue gathered during the LUMINTRAL study. An in-depth study of the genetic expression of the available samples can give much insight into the molecular and cellular working mechanisms of preservation injury and the effect of the preservation solutions, both vascular and luminal, even though the metabolic activity of the intestine could be low during cold storage. For that matter, proteomics analyses could be useful[241]. Loss of barrier function is one of the deleterious elements of preservation injury. Equally, the study of the microbiome composition of donors and the assessment of translocation of bacteria into the lamina propria could be useful readouts of this problem. An associative study between both components (donor and microbiome) can be realised as is now being performed for other intestinal diseases[242].

(2) The study of the effects of LP solution administration (a) prior to the commencement of the procurement procedure *vs.* administration during the operation, (b) *in situ* with a jejunal drip (LUMINTRAL), or (c) *ex vivo* at the back table. However appealing due to the theoretical ease of access and less invasion of the procurement procedure, the first method mentioned would relinquish the benefits of rapid cooling of the organ and the possible protection of the rest of the organs that is theoretically driven by the decrease in temperature. The third method could account for certain difficulties seen during some of the procedures from LUMINTRAL, especially the reflux of LP solution to the stomach and the occasional doubt on whether the solution reached the terminal ileum. The question in that case would be whether the benefits demonstrated in this thesis would disappear.

(3) The diversification of the luminal preservation solutions by use of additives that were not considered by the studies reviewed in this thesis. In particular, the combination with gaseous oxygen both at the beginning of cold and warm ischaemia times (the latter showing promising effects in this thesis) and the use of protective agents with antioxidant properties such as hydrogen sulphide[243,244].

(4) The broader exploration of machine perfusion and study of different storage temperatures, following the examples of liver[245,246], kidney[247] and lung[248] donation performed in our centre. This, however, should not be an immediate priority. As the LUMINTRAL study has presented, vascular perfusion alone presents, during normal storage times of about six to seven hours, a sufficient treatment, given the low preservation injury scores observed for both jejunum and ileum. The next step should therefore be to focus on static luminal preservation.

More important, however, is the development of a reliable and complete transplantation model. All studies are essentially incomplete because they lack one or more of the key components (brain death, preservation and/or reperfusion). Rat models could be of use for preservation studies and management of rejection studies, as there are already research groups with substantial experience[249]. The complications of adding DBD to these models are expected, nonetheless not a reason to let this be forgotten. One of the greater challenges is how to apply reperfusion. Admittedly, it is very difficult due to the anatomy of the (small) bowel, to develop a method that can easily be standardised and reproduced. The possibilities of machine perfusion of the intestine are being explored[60]. The effects on preservation of the organ as an endpoint is still debatable, but for testing the effect of different preservation techniques in the laboratory as a reperfusion tool it could still be a very good approach[250].

Although many attempts have been made to develop non-invasive monitoring methods of the intestinal graft, the gold standard remains being the endoscopic biopsy. The advent of high throughput 'omics' techniques (e.g., RNA sequencing) can lead to use of new methods in the clinics once these become widely accessible and applicable. Until then, PCR and ELISA panels for these biomarkers can still be developed at low cost. An additional follow-up biopsy sample could be added to the histopathological processing protocol. Chronic rejection of the intestinal graft is a very insidious disease and the main reason why it evolves into graft failure is due to the lack of early signs in the superficial biopsies and specific clinical management. It is important to study different methods to monitor the graft non-invasively to trace early signs like decreased calibre of the medium sized vessels that are mainly affected in CAE. A study of the calibres and pressure of the mesenteric arterial tree by way of computed tomography, magnetic resonance, or doppler echography during routine endoscopies to see whether an *in vivo* evaluation of the status of these sensitive vessels could be informative as a monitoring tool[251-253]. Another way can be to search for presence of certain ischaemia-driven markers such as the hypoxia-inducible

factor 1-α, which were differentially expressed in the patient presented in Chapter 6 and Chapter 7.

Early detection and adequate treatment of acute and chronic rejection remain the most important challenges after intestinal transplantation. Much can be learned from the expanding treatment options in inflammatory bowel diseases. Infliximab can be used in therapy-resistant memory T-helper type 17-driven rejection[254,255]. The use of vedolizumab as a standard drug for immunosuppression should not be disregarded, aside from the financial considerations. Taken from discussions during the Congress of the Intestinal Rehabilitation and Transplantation Society in Paris, vedolizumab is being considered as part of the induction of immunosuppression in the recipient in preparation for transplantation due to its promising use by blocking the re-entry of inflammatory cells during reperfusion. As this thesis has shown, it could also perform well as a rescue therapy for chronic rejection, particularly against the inflammatory environment within the arterial walls. Other therapies that have been considered and used for different kinds of rejection of the intestinal graft increasingly appear in the literature. Adalimumab was used for first line-resistant acute rejection (following the exemplary cases with infliximab)[256]. mTOR inhibitors (sirolimus) were used in paediatric patients for managing side effects of tacrolimus induction therapy)[257]. Targeting B-cells with rituximab and bortezomib[163,258,259] for cases with (possible) antibody mediated rejection has been successful as well. The ultimate goal, however, is to induce immune tolerance, and this should therefore be the first focus of future improvements in immunosuppression. For this, special focus should be put on the manipulation of the activation of T-helper cells[260] as evidenced from experiences with other organ transplantation[261,262]. Targeting regulatory T-cells seemed also to affect the graft positively as seen in Chapter 6[206].

Conclusion.

The long-term outcome of intestinal transplantation is still lagging in comparison to other solid organ programs. Gradual improvements can be made both in preservation and prevention and treatment of acute and chronic rejection. For the last decade, there has been an understandable increase in the importance of personalised medicine. 'Omics' approaches (high throughput analyses of gene and protein expression) to diseases are of importance for this, and so it is starting to become for transplant medicine[263,264]. The case for personalised medicine in (intestinal) transplantation is therefore to target the organ in question. Because transplantation of the gut is an uncommon procedure, the pre-treatment of the transplant has not taken full

advantage of its unique characteristics, so this aspect was highlighted in this thesis. Application of luminal preservation has been present in the minds of transplant scientists around the world, and our studies in this thesis present the first steps coming closer to a clinical translation of this method. The effects of this preservation technique within a greater context with the first small-animal brain death model, the first fully practical model of administration in humans, and the application of a large-animal model is proof of concept that this method can be successfully applied in many phases of the transplantation procedure. In the transplanted intestine, gut-specific therapy for rejection with vedolizumab is safe, feasible, and shows the potential that organ-targeted immunosuppression can improve the survival rates of the graft in acute and chronic rejection.

If number of intestinal transplantations continues to decrease, the efficiency provided by these kinds of treatments will certainly be welcomed. Further development of pre- and post-transplant treatments as described in this thesis may pave way to increase graft and patient survival.

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Het Heden en de Toekomst van Dunnedarm Transplantatie.

Het heden.

Dunnedarmtransplantatie (DDTx) is de laatste behandelingsoptie voor patiënten die voor hun vocht en voeding afhankelijk zijn van totale parenterale voeding (TPV) maar daarbij ernstige problemen ondervinden.

Ondanks de verbeterde zorg rondom TPV zijn complicaties zoals lijninfectie, trombose of secundair leverschade potentieel ernstig en levensbedreigend. Daarnaast hebben patiënten die langdurig TPV nodig hebben een slechte kwaliteit van leven, vergelijkbaar met die van nierdialyse patiënten. De kosten van TPV-behandeling voor het gezondheidszorgsysteem zijn hoog en uiteindelijk ook hoger dan de kosten van een dunnedarmtransplantatie.

De overlevingskansen na een DDTx zijn lager dan na transplantatie van andere solide organen, zoals lever, long, hart of een nier. De tienjaars overlevingskans van het darm transplantaat is slechts 41% en die van de patiënt 47%. Dit komt omdat de darm een sterk afweersysteem heeft wat voortdurend wordt uitgedaagd door de inhoud van de darm. Ondanks dat deze afweer met medicijnen wordt onderdruk, wordt het slijmvlies van de nieuwe darm toch vaak aangevallen en kan daardoor afgestoten worden. Bij een dergelijke aanval wordt de barrière in de darm beschadigd en treedt er snel een infectie met bacteriën vanuit de darm naar de bloedbaan op. Deze bloedbaaninfectie (sepsis) is de belangrijkste oorzaak van overlijden bij afstoting van een darm.

Het is belangrijk de overlevingskansen na darmtransplantatie te verbeteren. In dit proefschrift worden darm-specifieke maatregelen onderzocht om de darm tijdens de donatie fase beter tegen schade te beschermen. Daarnaast wordt ook het effect onderzocht van een medicijn dat gericht is tegen ontstekingscellen die naar de darm gaan.

Dit proefschrift.

Standaard wordt bij een donor die organen afstaat voor transplantatie een koude bewaarvloeistof (preservatie vloeistof) via de bloedbaan (vasculaire preservatie) aan de donor toegediend om de organen te koelen en op ijs te bewaren en transporteren. In het eerste deel van dit proefschrift (**SECTION A**) wordt onderzocht of het vullen van de darm zelf (het lumen van de darm) met koude vloeistof (luminale preservatie, LP) het slijmvlies van de darm beter tegen schade kan beschermen.

In **Hoofdstuk 1** worden alle tot nu toe gebruikte (dier) modellen voor LP van de darm beschreven. Deze studies laten zien dat LP minder slijmvliesschade geeft dan wanneer alleen

vasculaire preservatie wordt gebruikt. Als preservatievloeistof kan polyethylene glycol 3350 (PEG), een eenvoudige oplossing die ook als darmvoorbereiding voor endoscopieën wordt gebruikt, of een standaard orgaanpreservatie oplossing zoals University of Wisconsin (UW), of een complexere oplossing die speciaal is gemaakt voor de behoeften van de dunne darm (AA) worden gebruikt. Al deze luminaal toegediende vloeistoffen hadden in zowel kleine als grote proefdieren, alsook bij gebruik van menselijke darmen een beter effect op de darm dan wanneer alleen de standaard vasculaire preservatie werd toegepast. Wanneer een darm op ijs bewaard wordt krijgt deze darm geen zuurstof meer (ischemie). Als een darm daarna weer doorbloed wordt (reperfusie) ontstaat er schade. Een aantal studies hebben ook het effect van LP op deze zogenoemde ischemie-reperfusieschade onderzocht en vonden dat dit na LP minder was dan bij standaard vasculaire preservatie. Ook al is reperfusie het meest schadelijke mechanisme tijdens deze eerste fase na de transplantatie, toch is deze ischemie-reperfusieschade nog maar beperkt onderzocht omdat een reperfusie model voor de darm lastig te ontwikkelen is. Bij dunnedarmtransplantatie worden alleen darmen gebruikt van hersendode donoren (DBD, van het Engelse *donation after brain death*). Ook het intreden van hersendood heeft een groot effect op de barrièrefunctie van de darm. Helaas is het effect van deze hersendood in diermodellen voor darmpreservatie nog maar beperkt onderzocht.

Om die reden wordt in **Hoofdstuk 2** een DBD-rattenmodel met LP beschreven. LP werd uitgevoerd met twee verschillende luminale preservatie oplossingen (UW en PEG), en de bijbehorende effecten werden geëvalueerd. Het onderzoek leverde drie belangrijke resultaten op. Ten eerste is bij hersendode ratten er al veel slijmvliesschade op het moment van uitname, hetgeen aangeeft dat hersendood een negatieve invloed heeft op het darmslijmvlies. Ten tweede, de histologische structuur liet na vier en acht uur met LP een aangetaster slijmvlies zien in vergelijking met darmen die geen LP-behandeling hadden ondergaan. Monsters genomen tijdens de eerste evaluatie van de preservatieperiode (vier uur) tonen in deze groep meer schade dan die genomen na een langere periode (acht uur). Ten derde, een gunstig effect van LP werd gezien bij gebruik van zowel PEG als UW. Hierbij vonden we echter niet de opvallende verschillen tussen het gebruikt van PEG of UW als LP, zoals die eerder zijn beschreven in niet-hersendode diermodellen. Samengevat tonen deze bevindingen het belang aan van LP en het opnemen van een fase van hersendood in dunnedarmdonatie en preservatie modellen. Deze modellen zouden nog verder verbeterd kunnen worden door ook reperfusie aan het model toe te voegen, zodat nog beter het effect van LP op ischemie en reperfusie schade onderzocht kan worden.

Luminale preservatiestudies die lijken op de menselijke donatiesituatie en toepasbaar zijn in de klinische praktijk zijn dringend nodig. In **Hoofdstuk 3** worden de ontwikkeling, implementatie en resultaten beschreven van een dergelijk klinische studie met LP in donoren die voldoen aan de criteria voor dunnedarmdonatie. In deze *real life* studie werden de effecten van LP van *in situ* toediening van een ijskoude PEG oplossing tijdens de donorprocedure in twee centra bestudeerd, waarbij tevens preservatie met twee verschillende vasculaire preservatie vloeistoffen (UW in het UMCG en Institut Georges Lopez-1, IGL-1, in Leuven) werden vergeleken. Deze techniek werd in een periode van vijf jaar door verschillende transplantatie teams uit Nederland en België met succes geïmplementeerd. Behandeling met LP bleek de darm al tijdens de uitname te beschermen, met name het jejunum, wat suggereert dat de meest gevoelige delen van de darm baat hebben bij de snelle afkoeling door vroeg gebruik van LP, al vóór het tijdstip van de vasculaire perfusie. Met betrekking tot preservatieschade werd alleen een algemeen beschermend effect gezien bij donoren die met IGL-1 werden behandeld. Deze studie toont aan dat LP toegevoegde waarde heeft bij het beperken van darmschade bij donatie van de darm en ook technisch uitvoerbaar is zonder dat dit nadelige gevolgen heeft voor de algehele donatieprocedure.

Tenslotte beschrijft **Hoofdstuk 4** het effect van LP met extra zuurstof tijdens een andere fase van transplantatie. Een varkensmodel van volledige warme ischemie werd gebruikt als een conceptuele test om het effect van gasvormige zuurstofafgifte (persufflatie) door het lumen te bestuderen tijdens de warme ischemische fase die optreedt tijdens de implantatieprocedure, net voordat de reperfusie begint. Deze studie toont aan dat de *in situ* toediening van zuurstof het slijmvlies direct beschermt, hoewel dit effect afneemt naarmate de afstand tot het zuurstofafgifte punt groter is. Deze methode is echter complex en tijdrovend en kan leiden tot onverwachte complicaties en dus procedurele vertragingen, terwijl de ontvanger klaar is om het orgaan te ontvangen. Verder onderzoek naar een methode om ook zuurstof via het darmlumen toe te kunnen dienen is nodig om dit concept toepasbaar te maken voor de klinische praktijk.

Het tweede deel van dit proefschrift (**SECTION B**) richt zich op de afweerreactie in een darmtransplantaat die kort (acuut) of langere tijd (chronisch) na de transplantatie kan ontstaan. Enerzijds worden de mechanismen onderzocht om een afstotingsreactie te begrijpen. Anderzijds wordt het effect onderzocht van een medicijn wat zich specifiek tegen afweercellen in de darm richt bij zowel een acute als chronische afstotingsreactie.

In **Hoofdstuk 5** worden de tot nu toe bekende mechanismen beschreven die betrokken zijn bij een afstotingsreactie na darmtransplantatie. Tevens worden hierbij de verschillende methoden beschreven om deze afstotingsreacties te voorkomen of te behandelen.

In de volgende twee hoofdstukken worden 2 patiënt casussen (*case reports*) beschreven. **Hoofdstuk 6** beschrijft een patiënt met de ziekte van Crohn die een gecombineerde dunnedarm- en buikwandtransplantatie onderging. Na transplantatie ontwikkelde deze patiënt een acute cellulaire rejectie die niet reageerde op de standaardbehandeling (aanpassingen van voorlopige immunosuppressiva dosering, steroïde booster) maar wel succesvol werd behandeld met de toediening van vedolizumab gedurende enkele maanden. Vedolizumab is een monoklonaal antilichaam tegen het α4β7-integrine dat de migratie van immuuncellen naar het darmslijmvlies blokkeert. Dit betreft een darm-specifieke aanpak, omdat dit antilichaam alleen de afweercellen blokkeert die als postcode "de darm" (*gut homing*) hebben. De casus was uniek omdat dit de eerste succesvolle toepassing van dit medicijn was om acute intestinale cellulaire rejectie te behandelen. Heel belangrijk hierbij was dat de behandeling met vedolizumab ook veilig bleek te zijn. De patiënt raakte een infectie met een astrovirus gewoon kwijt en opvallend genoeg verliep ook een primaire CMV-infectie zonder verdere symptomen. Histopathologisch konden we echter niet een duidelijke vermindering van celmigratie aantonen. Dit darmspecifieke medicijn heeft daarom mogelijk ook nog andere effecten op de afweercellen in de darm. Na de periode van behandeling met vedolizumab is deze patiënt tot het moment van publicatie van dit proefschrift vrij geweest van rejectie episodes (dus al meer dan zes jaar).

In **Hoofdstuk 7** wordt de behandeling met vedolizumab beschreven van een patiënt die chronische rejectie heeft. Dit betrof een patiënt die vijftien jaar geleden een darmtransplantatie had ondergaan wegens een te korte darm na een darminfarct. Deze patiënt ontwikkelde na vijftien jaar een chronische rejectie wat een chronische ontsteking van de bloedvaten van de darm betreft. Deze bloedvaten vernauwen en geven aanleiding tot zweren en vernauwingen waardoor de getransplanteerde darm vaak verwijderd moet worden. Echter, na de start van de behandeling met vedolizumab verdwenen de ontstekingsverschijnselen bijna volledig, waarbij er alleen een kleine vernauwing in het laatste deel van de getransplanteerde darm overbleef. Dit vernauwde deel werd operatief verwijderd, maar met doorbehandeling met vedolizumab bleef de rest van het transplantaat tot op heden (ruim twee en een half jaar geleden) zonder zweren of vernauwingen. Dit is een bijzonder resultaat aangezien een chronische afstotingreactie normaal snel progressief is waarbij de getransplanteerde darm verwijderd moet worden. De farmacofysiologische mechanismen achter het succes van deze casus waren moeilijker te

begrijpen, maar de aanwezigheid van MAdCAM-1 (de α4β7-integrinereceptor) in de bloedvaten in de diepere lagen van het transplantaat zou erop kunnen wijzen dat vedolizumab ook de migratie van afweercellen naar de grotere bloedvaten en daarmee de ontsteking van deze bloedvaten in de darm remt. Het gevonden effect van vedolizumab op chronische rejectie is veelbelovend en zal in meer patiënten onderzocht moeten worden.

Om de werkingsmechanismen van vedolizumab, zoals gevonden in Hoofdstukken 6 en 7, beter te begrijpen, presenteert **Hoofdstuk 8** een verkennend onderzoek naar genoom-brede genexpressiepatronen in geselecteerde darmbiopten van deze patiënten. Er werden specifieke patiënt- en ziekte-gerelateerde patronen ontdekt. Na de start van de behandeling bleek de expressie van genen gerelateerd aan zinkhomeostase verminderd te zijn. Opmerkelijk was dat de expressie van doeleiwitten van vedolizumab (α4, β7, MAdCAM-1) niet worden beïnvloed door therapie. Wel werden er ontstekingsremmende patronen en anti-apoptotische patronen gezien na behandeling van de rejectie episodes. Dit suggereert dat vedolizumab niet alleen de celmigratie van ontstekingscellen naar de darm beïnvloedt, maar ook de activatie van deze cellen.

De toekomst.

Dit proefschrift stelt manieren voor die geschikt kunnen zijn om zowel pre- (LP) als post-transplantatie (vedolizumab) toe te passen om de overleving van een getransplanteerde darm, als ook de ontvangende patiënt te verbeteren. Er is nu ervaring om te gaan experimenteren met verschillende luminale vloeistoffen die kunnen worden aangepast naar de specifieke vasculaire preservatiestof die in elk dunne darm-transplantatiecentrum worden gebruikt, en andere technieken zoals machineperfusie waar het UMCG veel ervaring mee heeft. Er zijn ook meer mogelijkheden om darm-specifieke medicijnen zoals vedolizumab na de darm transplantatie te gebruiken, ook als deel van de standaard immunomodulerende behandeling. Het gebruik van darm-specifieke behandelingen leidt waarschijnlijk tot minder systemische bijwerkingen, dus zowel de kwaliteit van leven als de efficiëntie van de behandeling kunnen hierdoor verbeteren.

~ Sinopsis en castellano ~

El presente y el futuro del trasplante de intestino.

El presente.

El trasplante de intestino (TIx) ha surgido en las últimas tres décadas como tratamiento estándar de última instancia para pacientes con fallo intestinal (crónico), cuyo tratamiento con nutrición parenteral total (NPT) causa serias complicaciones.

A pesar de que la NPT ha mejorado, las complicaciones, entre ellas infecciones de los accesos venosos, trombosis o fallo hepático secundario, son todavía potencialmente severas y atentan con la vida del paciente. Además, estas personas sufren de una empobrecida calidad de vida (comparable con lo que ocurre con pacientes en diálisis). Los costos financieros para los sistemas de salud son además muy altos, incluso mayores que los del trasplante.

El TIx tiene, sin embargo, bajos niveles de supervivencia, particularmente en comparación con otros trasplantes de órganos sólidos. De hecho, la supervivencia del injerto a diez años es de 41% y del paciente de 47%. El contenido del intestino desafía a la mucosa intestinal continuamente, de la misma manera que lo hacen los fuertes regímenes de inmunosupresión actualmente en uso. La presencia de una extrema cantidad de tejido linfoide hace que el órgano sea propenso a intensas reacciones inflamatorias como las que ocurren en los episodios de rechazo. Estos factores pueden causar graves episodios de infección, que, a su vez, puede causar episodios de sepsis, que es la mayor causa de muerte después del rechazo del injerto intestinal.

Es importante mejorar la supervivencia del injerto como del paciente. Esta tesis presenta investigación en la cual se han aplicado tratamientos específicos para el intestino durante la fase de donación y después del comienzo de distintos episodios de rechazo. Primero, tratamientos luminales (es decir, administrados enteralmente), son aplicados durante la fase de preservación, transporte e implante del órgano. Segundo, una droga inmunomoduladora que actúa específicamente en el intestino es usada para tratar episodios de rechazo agudo y crónico.

Esta tésis.

La **SECCIÓN A** trata sobre el efecto de la preservación luminal (LP, del inglés *luminal preservation*) durante diferentes fases del procedimiento de trasplante (múltiple) de órganos. Se define a la preservación luminal a la administración por vía enteral de elementos preservativos que tienen como objetivo mantener la viabilidad de la mucosa y el resto del intestino durante el almacenamiento y transporte.

El **capítulo 1** es una revisión de los modelos preclínicos que han sido preparados por diferentes grupos de investigación en el campo del trasplante intestinal. Los estudios que presentan estos modelos han demostrado que LP retrasa la aparición de daño de la mucosa de nivel moderado a severo. Una simple solución de preparación intestinal para endoscopías (polietilenglicol 3350, PEG), una estandarizada solución de preservación como University of Wisconsin (UW), o una solución más compleja pero preparada exclusivamente para las necesidades del intestino (AA), han mostrado resultados favorables en comparación con el tratamiento estándar de preservación estática sin LP en modelos de animales pequeños y grandes, así también como en modelos con tejido humano. Algunos de estos estudios han incluido técnicas de reperfusión que demuestran que el tratamiento también es beneficioso más allá de la fase de preservación. Incluso sabiendo que la reperfusión es el mecanismo más dañino durante esta primera etapa de la vida del trasplante (injuria por isquemia y reperfusión), pocos estudios han incluido esa porción del proceso dado que el desarrollo de una técnica robusta donde estos efectos puedan ser bien examinados parece ser elusivo. Otro aspecto faltante en estos estudios, con la excepción del modelo con tejido humano, es la inclusión de la donación después de muerte encefálica (DBD, del inglés *donation after brain death*).

El **capítulo 2** se enfoca justamente en ese último asunto. Con la ayuda de un modelo de rata con muerte encefálica, se administró LP con dos soluciones de preservación diferentes, y sus correspondientes efectos fueron evaluados. El estudio entregó tres principales resultados. Primero, que el nivel de daño al comienzo de la ablación es más alto que en los donantes vivos que se usan tradicionalmente. Esto remarca entonces el efecto perjudicial de la muerte encefálica en la mucosa intestinal. Segundo, que la falta de LP deja a la mucosa en un estado frágil y no fidedigno en cuanto a su estructura histológica: las muestras tomadas durante el primer momento de preservación evaluado (cuatro horas) en este grupo sufrieron más daño que aquellas tomadas durante un período prolongado (ocho horas). Tercero, el tratamiento con LP frenó el desarrollo de la injuria en ambos casos (con PEG y UW), pero no mostró los efectos tan llamativos que han sido demostrados en otros modelos anteriormente publicados. En conjunto, estos descubrimientos demuestran la importancia de incluir un modelo de muerte cerebral como un factor influyente en modelos de donación de órganos. El efecto de LP fue probablemente amortiguado por la injuria iniciada por la muerte encefálica, pero, adicionada a la evidencia encontrada en la literatura que fue descrita en el capítulo 1, se espera que este tratamiento beneficie al injerto con mejor protección, incluso después de ser reperfundido en el receptor. La ausencia de un modelo de reperfusión es un defecto del estudio.

Hacen falta estudios de preservación luminal que se asemejen a la situación de donación y ablación clínica. El **capítulo 3** es el resultado del desarrollo e implementación de un modelo clínicamente relevante, en el cual LP fue administrado en donantes que cumplieron con los criterios para donación intestinal. En este estudio realizado en dos centros de trasplante europeos, los efectos de LP con PEG en donantes tratados con dos soluciones de preservación intravascular (UW e Institut Georges Lopez-1, IGL-1) fueron estudiados después de la administración *in situ* de una solución de preservación luminal fría basada en PEG en el donante durante el procedimiento de ablación. El modelo fue implementado exitosamente, y los potenciales defectos fueron corregidos durante un período de inclusión de cinco años por varios grupos de ablación de los Países Bajos y Bélgica. El tratamiento con LP mostró proteger al tejido ya durante el momento de ablación, particularmente en el yeyuno, lo que sugiere que las partes más sensibles del intestino pueden beneficiarse por el enfriamiento rápido proporcionado por el uso temprano de LP, comenzando antes del momento del tratamiento vascular. En cuanto a la injuria por preservación, un efecto protector general fue visto solamente en donantes tratados con IGL-1. Este estudio demuestra que la adición de LP aplaca el daño que se genera durante el procedimiento de donación, y que la técnica es aplicable sin la necesidad de incurrir en situaciones innecesarias o consecuencias adversas al procedimiento de procuración normal.

El **capítulo 4** cierra la Sección A observando el efecto de LP durante otra fase del trasplante. Un modelo porcino de isquemia tibia completa se utilizó como prueba conceptual para estudiar el efecto de la administración de oxígeno gaseoso (*persuflación*) por el lumen durante la fase de isquemia tibia que ocurre durante el procedimiento de implantación, justo antes de que comience la reperfusión. Este estudio demuestra que la aplicación *in situ* de oxígeno protege la mucosa directamente, aunque este efecto es sensible al lugar y la dirección del flujo gaseoso. Esta parte de la operación puede tomar mucho tiempo y es propensa a retrasos procedurales por complicaciones inesperadas, mientras el receptor está listo para recibir el órgano. Por esta razón, es importante tener un método a mano que pueda por lo menos mantener la calidad del órgano al momento de la llegada al centro de trasplante.

La **SECCIÓN B** se enfoca en la inmunidad del injerto intestinal, tratando de entender los mecanismos que ocurren dentro del sistema inmunitario y la implementación de un tratamiento específico para el intestino en diferentes formas de rechazo.

El **capítulo 5** revisa la fisiología conocida del sistema inmune y su respuesta después del trasplante intestinal. Se presentan diferentes formas de manipular estas respuestas para controlar los episodios de rechazo.

Dos reportes a fondo de casos clínicos siguen a dicha información, en los cuales el conocimiento explicado en el capítulo 5 fue aplicado por primera vez en casos de trasplante de intestino humano. El **capítulo 6** cuenta la historia de un paciente con enfermedad de Crohn de base que se sometió a un trasplante combinado de intestino delgado y de pared abdominal. El paciente sufrió de un largo episodio de rechazo agudo celular moderado que resolvió después de la administración temporal de vedolizumab. Vedolizumab es un anticuerpo monoclonal contra la integrina α4β7 que bloquea selectivamente la migración de células inmunes que retornan a la mucosa intestinal (fenómeno de *gut-homing*, o "vuelta al blanco intestinal"). El caso fue único ya que la piel no funcionó como marcador centinela para los episodios de rechazo (al contrario), y porque fue publicado como el primer uso de esta droga para resolver un episodio de rechazo agudo celular de intestino. Evidencia histopatológica para el mecanismo putativo de la droga está ausente, pero la evidencia clínica es clara en ambos injertos de intestino y pared abdominal. Hasta el momento de entrega de esta tesis, el paciente se mantiene libre de episodios de rechazo (más de seis años).

El **capítulo 7** presenta otra aplicación original y exitosa del uso de vedolizumab en trasplante de intestino en un paciente con historia de isquemia mesentérica. Signos endoscópicos de rechazo crónico aparecieron quince años después del trasplante. Después del comienzo de la terapia con vedolizumab, los signos inflamatorios desaparecieron casi completamente, dejando solamente una estenosis en la porción distal del íleon. Esa estenosis fue removida quirúrgicamente, pero el resto del injerto ha permanecido funcional y el paciente se mantiene libre de molestias hasta la fecha (dos años desde el comienzo del tratamiento). Esta terapia ha influenciado beneficiosamente, entonces, el resultado de un episodio de rechazo crónico que hubiese terminado de otra manera en fallo y subsiguiente extracción del injerto. Los mecanismos fármacofisiológicos detrás del éxito de este caso han sido más difíciles de entender, pero la presencia de MAdCAM-1 (el receptor de la integrina α4β7) en los vasos sanguíneos presentes en las capas más profundas del injerto podría indicar que la patofisiología del rechazo crónico no sólo involucra los mecanismos de vuelta al blanco intestinal de la mucosa, sino también de los vasos presentes en el mesenterio.

Para entender más a fondo los métodos de acción de vedolizumab vistos en los capítulos 6 y 7, en el **capítulo 8** se presenta un estudio exploratorio de los patrones de expresión genética encontrados después de un análisis de secuencia de ARN del genoma completo de biopsias seleccionadas de los dos pacientes. Se descubrieron patrones específicos de cada paciente y enfermedad. Después del comienzo del tratamiento, la expresión de genes relacionados con la homeostasis de zinc se encontró disminuida. Adicionalmente, la expresión de proteínas blanco de la droga (α4, β7, MAdCAM-1) no pareció estar afectada por la terapia en este contexto. Los signos de actividad anti-inflamatoria y procesos antiapoptóticos cambiaron después de la resolución de los episodios de rechazo generados por el tratamiento, lo que sugiere que vedoliziumab actúa de maneras diferentes a lo que se había propuesto originalmente, no solamente influyendo la migración leucocitaria, sino también del estado de activación de las células inmunes.

El futuro.

Esta tesis propone maneras que pueden ser apropiadas para aplicar tanto antes (LP) como después (vedolizumab) del trasplante para mejorar la supervivencia del injerto intestinal, así también como la del paciente. Hay ahora suficiente experiencia para experimentar con diferentes líquidos de preservación luminal que puedan ser adaptados a cada solución de preservación vascular que se use en cada centro de trasplante diferente. También se abre la oportunidad para usar otras técnicas como la de perfusión con máquinas, un área donde el centro hospitalario universitario de Groningen tiene mucha experiencia. Hay también más posibilidades para usar medicinas específicas para el intestino, como lo es vedolizumab, en fases tardías del trasplante, como parte del cocktail inmunomodulador estándar. El uso de terapias específicas para el intestino deja probablemente menos lugar para efectos secundarios sistémicos, y deja lugar a mejoras en la calidad de vida y la eficiencia de cada tratamiento.

~ Summary in English ~

The Present and Future of Intestinal Transplantation.

The present.

Intestinal transplantation (ITx) is the standard treatment for patients whose nutritional requirements depend on total parenteral nutrition (TPN) but suffer serious complications from it.

Despite the improvements in TPN, its complications, such as line infections, thrombosis or secondary liver failure are still potentially severe and life-threatening. Patients who require long-term TPN also suffer from a decreased quality of life, comparable to that of patients on kidney dialysis. The costs of TPN treatment for the healthcare system are high, eventually higher than those of ITx.

The chances of survival after an ITx are lower than after transplantation of other solid organs, such as liver, lung, heart, or kidney. The ten-year survival rate of the gut graft is only 41% and that of the patient is 47%. This is because the gut has a strong immune system that is constantly challenged by its contents. Even though the immune system is suppressed and controlled with drugs, the graft's mucosa is often attacked from without (luminal content) and within (recipient). During such attacks, the gut barrier can be damaged, and infections can thus spread quickly through the bloodstream (sepsis). As a result, sepsis is the leading cause of death after intestinal transplant rejection.

It is important to improve the survival rates of both graft and patients. This thesis presents research where intestinal-specific treatments are applied during the donation phase and after episodes of rejection. First, luminal treatments are applied during storage, transport, and implantation of the intestine to protect against preservation damage. Second, a gut-specific immunomodulator is used for treatment episodes of acute and chronic rejection.

This thesis.

A standard donation procedure of the intestine begins by the application of a cold storage fluid (preservation solution) via the bloodstream (vascular preservation) to the donor to cool the abdominal organs. After explantation, the organ is submerged in a bag full of ice-cold preservation solution for storage and transport. **SECTION A** of this thesis presents research into the application of preservation solutions in the lumen of the organ itself (luminal preservation, LP), to understand whether this method can protect the mucosa against preservation injury.

In **Chapter 1**, a review is presented of the (animal) models that have been set up by different research groups in the field of luminal preservation. These studies show that LP protects the

mucosa against preservation injury in comparison to vascular preservation alone, irrespective of the type of study, whether it was performed on small or large animal models, or even on human tissue. A simple, standardised solution for intestinal preparation for endoscopic procedures (polyethylene glycol 3350, PEG), a standard organ preservation solution such as University of Wisconsin (UW), or a more complex preparation tailored for the intestine (AA), all have shown favourable results of LP in experiments in small and large animal and human models. From the moment that the intestinal blood is replaced by the preservation solution, a period without oxygen (ischaemia) begins. The moment when the intestine is engrafted, and blood begins to flow into and through the organ (reperfusion) causes a surge in damage that results in what is known as ischaemia-reperfusion injury (IRI). Some studies have also looked at the effect of LP on IRI and found that the injury was also decreased in presence of this treatment. Even though reperfusion is the most damaging mechanism during the first phase of transplantation, little is known and investigated about the complete process because an intestinal reperfusion model is very difficult to develop and reproduce. In ITx, only organs from donation after brain death (DBD) is being performed. Brain death itself has important effects on the barrier function of the intestinal mucosa. Unfortunately, the effect of this condition is rarely considered in studies analysing the effect of luminal perfusion.

Because of this, in **Chapter 2** a model of DBD with LP is applied. LP was performed by using two different luminal preservation solutions (the standard vascular fluid UW and the standard bowel preparation fluid PEG) and the effects of these treatments are evaluated. This investigation delivered three main findings. First, that the level of damage at the beginning of the procurement procedure was already higher in DBD than what was seen in other models without brain-death. This reiterates the quick detrimental effect of brain death on the intestinal mucosa. Second, that the lack of LP left the mucosa in a fragile state with regards to its histological structure, in that samples taken during the shorter preservation time point were in a worse state than those taken after a longer period of cold storage and were always worse off than when LP was present. Third, LP with either UW or PEG reduced the progression of tissue injury, which is expected to be time-dependent, but did not show the striking beneficial effects that were present in other published models. Taken together, these findings demonstrate the importance of including brain death as a factor in organ donation models. The effect of LP was probably dampened by the injury initiated by DBD but added to the evidence from literature, LP is expected to protect the graft, even after reperfusion, which should be an important addition in the future to reach a better understanding of this injury process.

Human studies on LP that resemble the donation scenario and that are relevant to the clinical practice are necessary. In **Chapter 3**, the development, implementation, and results from such study are described, where LP was administered to donors that fulfilled clinical criteria for intestine donation. The effects of *in situ* implementation of LP with ice-cold PEG during the procurement procedure was studied in two centres, each of which uses a different vascular preservation solution (UW in Groningen or Institut Georges Lopez-1, IGL-1, in Leuven). This technique was successfully performed in experiments during a period of five years by different procurement teams from The Netherlands and Belgium. Treatment with LP seems to protect the organ already at the moment of procurement, particularly the jejunum, which suggests that the most sensitive parts of the intestine could benefit from the rapid cooling brought upon by early use of ice-cold luminal perfusion, even before the beginning of the vascular flush. Regarding preservation injury, an overall protective effect was seen only in donors who underwent vascular flush with IGL-1. This study shows that the addition of LP placates the injury that appears during the donation procedure, and that the technique is applicable without incurring unnecessary or adverse consequences for the normal procurement procedure.

The section ends with a description of the effect of LP during a different phase of transplantation in **Chapter 4**. A pig model of complete warm ischaemia was used as a proof-of-concept to analyse the effect of gaseous oxygen (*persufflation*) administered via the lumen during the warm ischaemia phase of implantation, right before reperfusion begins. This study shows that *in situ* administration of oxygen protects the mucosa, even though this effect is dependent on the location of application and direction of oxygen flow. This part of the operation can be time consuming and is prone to procedural delays due to unforeseen complications while the recipient is ready to receive the organ. More research into a method that can be applied in a clinical setting is needed so that oxygen is also dynamically applicable during implantation procedures.

SECTION B focuses on the immune response targeting the intestinal graft. On one hand, the mechanisms behind rejection are investigated, and on the other hand the effect of a drug that specifically targets immune cells in the intestine is investigated in clinical cases with acute and chronic rejection.

In **Chapter 5**, the hitherto known mechanisms involved in rejection after intestinal transplantation are described including the various methods to prevent or treat these episodes.

In the following two chapters, two case reports are described. **Chapter 6** tells the story of a patient with Crohn's disease who received a combined small intestine and abdominal wall

transplant. After the procedure, she developed an episode of acute cellular rejection that did not respond to standard treatment (dose adjustment of current immunosuppressive treatment, booster with corticosteroids), but successfully resolved after starting treatment with vedolizumab. Vedolizumab is a monoclonal antibody against integrin α4β7, that blocks migration of immune cells into the graft's mucosa. This is a gut-specific treatment because the antibody works only on the immune cells that are programmed to migrate to the gut (so called *gut-homing mechanism*). The case was unique because this was the first successful application of this drug on the treatment of intestinal (cellular) rejection. The treatment appeared to be also safe, as the patient cleared an astrovirus infection asymptomatically and, interestingly, also underwent an asymptomatic primary CMV infection. Clear histopathological signs of reduction in cell migration were not visible. But the clinical picture, given this gut-specific drug's favourable clinical effect, shows that there may also be other effects at play on the immune cells in the gut. Up to the time of publication of this thesis, this patient has been free of rejection episodes after the period of treatment with vedolizumab (more than six years).

In **Chapter 7** the treatment of chronic rejection with vedolizumab is described. This concerned a patient who had undergone an intestinal transplant because of short bowel syndrome after an intestinal infarction. This patient developed chronic rejection after fifteen years due to chronic inflammation of the blood vessels of the intestine. These blood vessels constrict, generating ischaemia, which gives rise to ulcers and strictures that often require removal of the transplanted bowel. However, after initiation of treatment with vedolizumab, the signs of inflammation almost completely disappeared, leaving only a short narrowing in the last part of the transplanted gut. This narrowed portion was surgically removed, but with continued treatment with vedolizumab, the remainder of the graft remained without ulcers or strictures to date (over two and a half years). This is an unusual result as chronic rejection is normally rapidly progressive from the moment that it is diagnosed and requires removal of the transplanted gut. The pharmacophysiological mechanisms behind the success of this case are more difficult to understand, but the presence of MAdCAM-1 (the α4β7 integrin receptor) in the blood vessels in the deeper layers of the graft may indicate that vedolizumab also influences the migration of immune cells to the graft from a different histological layer than what was previously known, where larger blood vessels are present. It might thus inhibit the inflammation of these blood vessels at this level in the gut. The observed effects of vedolizumab on chronic rejection are promising and warrant investigations in more ITx patients.

To better understand the mechanisms of action of vedolizumab discussed in Chapters 6 and 7, **Chapter 8** presents an exploratory study of gene expression patterns found after whole-genome RNA sequence analysis of selected biopsies from the patients discussed in those chapters. Specific patient- and disease-related patterns were discovered. After the start of treatment, the expression of genes related to zinc homeostasis was found to be reduced. In addition, the expression of target proteins of vedolizumab (α4, β7, MAdCAM-1) did not appear to be affected by therapy in this context. However, anti-inflammatory and anti-apoptotic patterns were seen after treatment of the rejection episodes. This suggests that vedolizumab affects not only the cell migration of inflammatory cells to the gut, but also the activation of these cells.

The future.

This thesis proposes ways that may be suitable to apply both pre- (LP) and post-transplantation (vedolizumab) to improve the survival of the transplanted intestine as well as of its recipient. There is now enough expertise to experiment with different luminal fluids that can be tailored to the specific vascular preservation solutions used in each transplant centre, and other techniques such as machine perfusion where the UMCG has much experience. There are also more possibilities to use gut-specific medicines such as vedolizumab in later phases of transplantation, as part of the standard immunomodulatory cocktail. The use of gut-specific therapies probably leaves less room for systemic side effects, so both quality of life and treatment efficiency can be improved.

Acknowledgements.

ACKNOWLEDGEMENTS

Before I start thanking people that have personally supported me, I would like to thank all the donors, their families, and the patients that helped to bring more knowledge into the field of intestinal transplantation. I would also like to thank the animals that have been sacrificed in the name of Science. None did this so willingly, but I hope their giving brings some good to the world.

I believe that this thesis is the culmination of not only the work that began in November 2014, but back in 2012 when I came to the Netherlands. With the benefit of hindsight, when I did not know that "some time abroad" would mean *a lot* of time abroad, I decided to deviate from the marathonic feat that a career in medicine in Buenos Aires meant. Hence, I would like to thank every person that supported me during this period. I should start off in chronological order, thanking the first person I met in Groningen that would stay around me through the past decade, Suruchi: thanks, mainly, for the cultural shocks. Many of them! Georgia, thank you so much for your friendship. I miss you here and I admire you and how hard you work and how sweet and generous you and your family are. You are a topper. Tim, I miss you too, of course. Thanks for your friendship through the years, I just wish you would move closer to Groningen instead of farther each time! You guys and Gabi have been my very important support system through the years, even when I was a bit lost in the canals of Amsterdam. I wish and hope to keep seeing more of you in the future. Rapha, you have been one of the few reasons I would enjoy working in the lab, so I thank you for that and for the good times, the chats about anything other than science, and the chats about science too. I admire you and wish you the best after your PhD, hopefully around!

Naturally, during the years I have spent being a PhD student, I learnt many things. One of the best ways I found out I was able to learn was by teaching what little knowledge I had. I thank the students I had under my supervision, then: Matthijs, Kwaku, Ruben and Sara.

I would also like to thank my friends here in Groningen for the nice times we keep on spending. I should apologise in advance for being too much of myself sometimes, but I am sure that my excellent Scottish accent makes up for it. So, Robert, Pien, Sophia, Stef, Peppe (your knowledge of all things whiskey has surpassed mine, I drink to you, laddie), Hille (you made me sweat more than the whole of the contents of this book together, appreciated), AJ (my blasting comrade), Kirsten (bedankt voor het Nederlands!), thank you so much for these last couple of years. Last, but certainly not least, thanks Dave for all the Guinness. May they keep on coming.

AGRADECIMIENTOS

Como escribí en la sección en inglés, el cierre de este libro implica también el cierre de una etapa que yo considero comenzada a mediados de 2012, con el beneficio de la retrospección, cuando decidí vivir "por un tiempo" en Europa, desviándome del camino maratónico que supone la carrera de Medicina en Buenos Aires. Por eso, esta nota de agradecimiento va a tomar en cuenta a la gente que me ayudó a llegar hasta acá desde ese momento.

Comienzo con mi familia, por supuesto, porque de maneras imaginables e inimaginables (para mí), me han apoyado y siguen bancando de una manera esencial. Los extraño mucho, siempre. A mis padres les agradezco inmensamente haber tenido la audacia de educarnos con la mejor calidad posible en casa, más que nada por habernos abierto la cabeza a los tres, y hacernos crecer entre animales de tan buen corazón como Felipe, Carlín, Julián, John, Juana, Mili, Larva, Mia y Rodri, a quienes extraño mucho y que me enseñaron a querer sin condiciones. A mamá, por haberme enseñado a apreciar la música y el arte que me gustan hoy y que de alguna manera me ayudaron a tener un inglés a la altura de lo necesario para vivir en el exterior. A papá, por ser el nexo entre mi vida personal y profesional, ayudándome a interesarme en el tema de este libro, aunque haya sido de manera indirecta. A ambos por el cariño incondicional que nunca me faltó. A mi hermano Agustín y mi hermanita Marian les agradezco la existencia y el pie a tierra que son siempre en mi vida. Lo que lamento es que no vengan seguido a visitarme, ¡me lo deben! A mi abuela Paca le agradezco el cariño, amor inconmensurable y confianza ciega que siempre tiene en mi. Maña seguramente estará contento por tu apoyo, como lo estoy yo. También le agradezco la habilidad impresionante para estar en contacto conmigo por internet, que tan bien nos hace a los dos cada semana. A mi abuela Julia, a quien extraño muchísimo, le agradezco el apoyo y admiración que siempre me brindó, dándome más crédito que el que merecía en el momento que me elogiaba. A la familia Wilkinson, por el cariño tremendo que me dan y la alegría de poder verlos cada vez que voy. A mi madrina María Elena por su cariño y lindas charlas que tenemos cuando nos vemos en Buenos Aires. A mis tíos y primos Trentadue, gracias por haber puesto a los Países Bajos en el mapa para mí, y a Juan en particular por todo el apoyo durante mis primeros años por acá.

Quiero también agradecerle a mi familia elegida. Creo que he pasado mis mejores momentos acá mientras me escapaba del laburo para pasar unos días de turista con ustedes. Fio, me visitaste una vez, ojalá sean más, porque tenés que mostrarle a Enzo lo que es Europa. Javier, gracias por venir tantas veces y tener tiempo y ganas de pasarlo por acá. También te agradezco

inmensamente la generosidad que tenés compartiendo tu arte conmigo. Espero que este libro le haga justicia a tu foto. Gabi, gracias por el apoyo y la amistad, que estoy seguro empezó ese momento en el Academiegebouw cuando me ignoraste por completo al lado del puesto del TopMaster. Por último, Agustín, muchas gracias por soportar mis intentos de robarme tu sentido del humor. Es que es tan bueno y sólo quiero aprender de los mejores. Pero en serio te agradezco un poco de familiaridad en Grunn y buena onda.

Por el lado profesional quiero empezar agradeciendo a Gabriel que, sin saberlo, ha sido de gran ayuda para conseguir este título. No sólo por razones obvias como *aprobar* la tesis, sino también porque mi contacto durante mis estudios en Favaloro ayudó a que pueda conseguir el trabajo. Aunque la pandemia nos haya impedido trabajar juntos, espero que esto sea el comienzo de una buena colaboración entre ambos hospitales. También quiero agradecer, obviamente, al grupo de La Plata y Favaloro que han trabajado con nosotros. En especial a Meli, Lean y Pablo. Leandro, gracias totales por haber rescatado el proyecto de muerte encefálica con tu esfuerzo y tu cabeza, quedó muy lindo, ¿no? Sos un genio y te felicito por la familia y el laburo que armaste estos años.

Por último, quiero agradecerle a Omar Pignataro, Lucrecia Calvo, Gustavo Giunta y Ricardo Pichel la educación que me dieron dentro de la investigación que me motivó a empezar este trayecto. Ojalá tengan tiempo para ver mi graduación de alguna manera.

Todos ustedes me han bancado a su manera en momentos muy buenos, buenos, malos y muy malos. Me encantaría abrazarlos y poder disfrutar de este momento muy bueno con ustedes la próxima vez que los vea. Gracias a todos por el aguante y espero que me puedan acompañar en la próxima etapa, donde quiera que estén.

Ah, y aguante Boca.

DANKWOORD

Dit boek is uiteraard mede gemaakt door mijn (co)promotores. Het is dus essentieel dat ik begin met het bedanken van mijn "vaders-in-wetenschap", Gerard en Klaas Nico. KN, bedankt voor al je hulp waardoor ik al sinds het begin van mijn wetenschappelijke traject mijn wensen kon uitvoeren, en de ondersteuning sindsdien als mentor tijdens de Masteropleiding. Bedankt, Gerard, voor je ondersteuning en begrip vanaf dag -1, ook tijdens slechte en drukke tijden voor jou. Daarnaast zijn er twee heel sterke co-promotores die enorm hebben geholpen. Dankjewel Jan Willem voor de begeleiding binnen de transplantatie wereld en je altijd snelle en behulpzame commentaren en correcties van het werk binnen en buiten dit manuscript. Tot slot, weliswaar

laatst genoemd maar niet minder waardevol, Gursah, ontzettend bedankt. Je was de meest ondersteunende persoon voor mij binnen het UMCG, zowel op professioneel als persoonlijk vlak. Ik stel al je hulp zeer op prijs en ik hoop dat ik je iets kan teruggeven. Beste (co-) promotores, ik wens jullie een heel succesvolle carrière en ik hoop deze met jullie mee te mogen maken.

Ook in het lab heb ik veel hulp gekregen. Daarvoor wil ik allereerst Tjasso bedanken. Je werkt ontzettend hard en mede daardoor kon ik mijn experimenten goed en tijdig uitvoeren. Het is ook belangrijk voor mij dat ik nu mede dankzij jou in het Nederlands kan schrijven en spreken, omdat je de eerste was die was met mij ging spreken. En bedankt verder voor alle keren dat je mij uitgenodigd hebt om mee te gaan naar de Euroborg. Het is niet La Bombonera maar best wel gezellig. Nu moet FC Groningen alleen nog meer doelpunten gaan scoren, tja. Hopelijk kunnen we er straks weer samen naar toe gaan! Manon, Dianne, Sofie en Jeanette, ook bedankt voor jullie ondersteuning. Ik ben heel erg blij met de groep waarmee ik bij MDL heb gewerkt. Jurya, natuurlijk essentieel voor Gerard en mijzelf, hartstikke bedankt dat je alles onder controle hebt weten te houden. Ook is Han voor mij van belang geweest. Zonder Han geen Groningen voor mij, claro. En geen leuke bands bij Vera. Ik weet het niet, maar ik hoop dat je een belangrijke rol bij mijn promotie zal hebben. Van de Faculteit Medische Wetenschappen wil ik Peter, Dorenda, Tobias en Emilia bedanken voor de leerzame hulp aan het begin van dit promotietraject. Ditzelfde geldt voor de labruimte waar ik enkele darmen kon snijden en toegang kreeg tijdens de hele late uren. Anne Marye, bedankt voor je hulp als mede PhD'er maar ook zeker als TC. Zonder jou zou dit veel langzamer zijn verlopen. Ik hoop dat je snel weer aan het werk kunt, we hebben je nodig! Je hebt een grote last van mijn schouders genomen en dat stel ik erg op prijs. Tijdens mijn opleiding heb ik bij Rob Hennings' lab gewerkt. Rob, je was ontzettend belangrijk tijdens mijn eerste jaar hier in Groningen en ik zal dat nooit vergeten. Verder wil ik de Leuven groep bedanken voor het versterken van onze LUMINTRAL-studie. Mathias, Jacques, Laurens en natuurlijk Emilio voor het starten van de samenwerking, toen in New York.

Jasper, tot slot wil ik jou enorm bedanken voor de hulp, samenwerking en het meedenken tijdens het hele traject. We zullen nooit zo creatief meer zijn als tijdens onze wandeling op 5th Avenue in New York maar hé, als we in de toekomst een klein deel van dit denkvermogen kunnen oproepen dan dingen we zeker nog mee voor een Nobel ofzo.

Post-scriptum

A separate bit for the ones who will stand beside me during that silly-looking ceremony.

Bram, I can't thank you enough for your friendship and your company, so I will not try. I do want to let you know that your help during this time is a bit of a punishment for all the Chuck Norrii (?) that you have ordered for me through the years. My time in the UMCG would have surely been much more boring and much, much less interesting if you were not to have been in the picture, which would mean that this thesis would have been much, much shorter than what it is. You deserve all the Guinii in the world. I also thank Emma, Elsie, Kobus and Kees for keeping this guy sane, of course.

Lynette, here come the diabetes-inducing sentences for you. Thank you for building a home with me! You have made the laatste loodjes of the making of this book much more bearable. You have also taught me a lot about the world, and I thank you for that too. And you have given me the chance to share the care for Zoë and that makes me extremely happy. I also regard in high esteem the photographic work that you have lent for this book. It should be acknowledged and appreciated by everyone that holds a copy in their (digital) hands.

Onwards,

Guido.

Author information.

Guido Trentadue (pictured left) has managed to stay alive since early 1987, when he was born in the Argentinean capital city of Buenos Aires. He does not smoke.

Motivated by societal obligations and a long for a (better shot at a) comfortable financial life (than what a career in music has to offer), he decided to study Medicine in Favaloro University in his birth city, beginning in the year 2006. Almost six years and way too many exams afterwards, he graduated Cum Laude.

Motivated by a curiosity for the scientific method, he joined the TopMasters programme of Medical and Pharmaceutical Drug Innovation of the University of Groningen in 2012. After a learning at Rob Henning's lab in the University of Groningen about working in a laboratory, and in the Tytgat Institute in the Academic Medical Centre in Amsterdam to do a ton of Western Blots, he graduated in July or August 2014 (he does not remember). The promotors of this thesis recruited him for a research position in the Department of Gastroenterology and Hepatology in the University Medical Centre in Groningen, which he started in November 2014. Evidence that he indeed worked during this time is presented within this book.

Almost ten years to the date of moving into The Netherlands, having an unsteady job a bit closer to the clinics, he enjoys a life with a partner, a pet, very late weeknights watching Boca, and the love of family and friends, local and at a distance. He also seems to write in the third person for the sake of appearances.

"If you don't know where you're going, any road will take you there."

George Harrison.