ALLGEMEINES KRANKENHAUS DER STADT WIEN
Universitätsklinik für Innere Medizin III
Währinger Gürtel 18-20; A-1090 Vienna, AUSTRIA

An den
Fonds zur Förderung der wissenschaftlichen Forschung
Weyringergasse 35
1040 Wien
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Rainer Oberbauer, M.D.
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Vienna 2005-02-15

re: Final report to P-15679 required for a consecutive application
(Ergebnisbereicht für einen Folgeantrag)

Dear Madam, Sir!

Attached pleas find my follow-up research grant proposal to the previously funded application entitled „Prevention of postischemic acute renal allograft failure“. The data obtained during the three years of the FWF financed project have been published in seven full papers. The pdfs of these publications can be downloaded from our website.

1. Perco P, Kainz A, Mayer G, Lukas A, Oberbauer R, Mayer B. A genetic algorithm to derive joint promoter modules in coexpressed genes. Bioinformatics 2005 (in revision)
2. Hauser P, Kainz A, Bergmeister H, Regele HM, Mayer B, Meyer TW, Oberbauer R. Different genome-wide gene expression pattern in the ipsilateral kidney after contralateral nephrectomy or hydronephrosis. Kidney Int 2005 (submitted)
3. Kainz A, Mitterbauer C, Hauser P, Schwarz C, Regele HM, Berlakovich G, Mayer G, Perco P, Mayer B, Meyer TW and Oberbauer R. Alterations in gene expression in cadaveric vs live donor kidneys suggest impaired tubular counterbalance of oxidative stress at implantation. Am J Transplant 2004, 4:1595-1604
4. Hauser P, Schwarz C, Mitterbauer C, Regele MH, Mühlbacher F, Mayer G, Perco P, Mayer B, Meyer TW and Oberbauer R. Genome wide gene expression patterns of donor kidney biopsies distinguish primary allograft function. Lab Invest 2004, 84:353-61
5. Mitterbauer C, Schwarz C, Hauser P, Steininger R, Regele HM, Rosenkranz A, Oberbauer R. Impaired tubulointerstitial expression of ET-1 and NOS isoforms in

donor kidney biopsies with postischemic acute renal failure. Transplantation 2003, 76:715-20

6. Mayer B, Oberbauer R. Mitochondrial regulation of apoptosis. News in Physiol Sci 2003, 18:89-94
7. Schwarz C, Oberbauer R. The influence of organ donor factors on early allograft function. Curr Opin Urol. 2003, 13:99-104

The key finding of our studies is that brain death organ donors exhibit a tremendous upregulation of proinflammatory genes in their kidneys which predispose to a high rate of ARTF. The initial hypothesis about targeting apoptosis regulatory genes in order to reduce ARTF was abolished, because the molecular signatures of overall inflammation and immunoregulatory genes were much stronger than those of the apoptosis cascade.

Aim of the present follow-up proposal is to reduce inflammation in the donor kidney. In a RCT 1g of methylprednisolone or placebo will be infused into the brain death donor hours before organ retrieval and the efficacy of this intervention will be tested by genome-wide gene expression analysis. The clinical end point of the study is the rate and duration of ARTF in the transplant recipient. The present application thus represents the logical next step towards improving long term allograft survival by reducing ARTF. The project can be categorized as translational research - from bench to bedside.

Based on the highly successful performance of the previous project we are convinced that the present application will yield a clear cut answer on the efficacy of organ donor pretreatment – ARTF enigma. ARTF is clinically very important since it represents the main risk factor for reduced long term graft survival.

If we may suggest reviewers, these would be our choices:

International reviewers:

Stuart M. Flechner, M.D. Section of Renal Transplantation, Transplant Center, and Allogen Laboratories, The Cleveland Clinic Foundation, Cleveland, Ohio, USA, E-mail: flechns@ccf.org

2. Yves Vanrentegehem, M.D. Department of Nephrology, University Hospital Gasthuisberg, KU Leuven, Herestraat 47, B3000 Leuven, Belgium. E-mail: yves.vanrenterghem@uz.kuleuven.ac.be
3. Peter Friend, M.D. Nuffield Department of Surgery, University of Oxford, Oxford, OX3 9DU, UK, E-mail: peter.friend@nds.ox.ac.uk
4. Keshwar Baboolal, M.D. Department of Nephrology and Transplantation, University Hospital of Wales, Heath Park, Cardiff, United Kingdom. E-mail: Kesh.Baboolal@CardiffandVale.wales.nhs.uk
5. James W. Scholey, M.D., University of Toronto, 13EN-243, Toronto General Hospital, 200 Elizabeth Street, Ontario M5G 2C4, Canada. E-mail: james.scholey@utoronto.ca

National reviewers:

1. Univ. Prof. Dr. Thomas Wekerle. Department of Surgery, Vienna General Hospital, Waehringer Guertel 18, A 1090 Vienna, Austria. thomas.wekerle@meduniwien.ac.at
2. Univ. Prof. Dr. Rudolf Schweyen. Vienna Biocenter, Institute of Microbiology and Genetics, University of Vienna, Dr.-Bohrgasse 9, A-1030 Vienna, Austria, E-mail: schweyen@gem.univie.ac.at
3. Univ. Prof. Dr. Josef Kovarik. Interna Abteilung 3 des Wilhelminenspital der Stadt Wien, Wien 16; Montleartstraße 37, E-mail: josef.kovarik@wienkav.at
4. Univ. Prof. Dr. Günther Laufer. Klin. Abteilung für Herzchirurgie, Anichstraße 35 A-6020 Innsbruck. Tel. 0512/504-22500 Fax 0512/504-22502. E-Mail: g.laufer@uibk.ac
5. Univ. Prof. Dr. Rudolf Steininger. Univ. Klinik für Transplantchirurgie, Medizinische Universität Wien, Währinger Gürtel 18-20, 1090 Wien, E-mail: rudolf.steininger@meduniwien.ac.at

If I can provide any additional information in the previous or present project, please do not hesitate to contact me any time.

Yours sincerely,

Dr. Rainer Oberbauer

Projekt Nr. P .....-.....
wird vom FWF eingesetzt *(to be completed by the FWF)*

Der Wissenschaftsfonds.

Fonds zur Förderung
der wissenschaftlichen Forschung

A-1040 Wien, Weyringergasse 35

Telefon: +43/1/505 67 40-0
Fax: +43/1/505 67 39
e-mail: office@fwf.ac.at
Internet: http://www.fwf.ac.at

Antrag auf
(Application for)

Förderung des Forschungsprojekts
(funding of the research project)

Deutscher Kurztitel (max. 60 Zeichen inkl. Leerzeichen)
(German running title <max. 60 characters incl. Spaces>)

Prävention des akuten Nierentransplantatversagens - Teil 2

Englischer Kurztitel (max. 60 Zeichen inkl. Leerzeichen)
(English running title <max. 60 characters incl. Spaces>)

Prevention of acute renal allograft failure - part 2

Laufzeit **36** **Monate**
(duration) *(months)*

vorgelegt von
(submitted by)

Rainer Oberbauer

Name der Antragstellerin bzw. des Antragstellers
(name of applicant)

Projekt Nr. P ……-…….
wird vom FWF eingesetzt *(to be completed by the FWF)*

Formeller Teil
(Formal section)

Antragstellerin bzw. Antragsteller *(Applicant)*

Oberbauer Name *(family name)*	**Rainer** Vorname *(first name)*	**Prof. Dr.** Titel/akad. Grad *(title/acad. degree)*	**4225** SV-Nummer *(Soc. Sec. Nr.)*	**13061964** Geb. Datum *(date of birth)*

+43-1-40400-4358 Telefon *(Phone)*	**-43-1-40400-4452** Fax
rainer.oberbauer@meduniwien.ac.at e-mail	**www.meduniwien.ac.at/nephrogene** www site

Forschungsstätte der Antragstellerin bzw. des Antragstellers *(Applicant's research institution)*

Medizinische Universität Wien Universität *(University)*
Univ. Klinik für Innere Medizin 3 Institut/Klinik *(Institute/Clinic)*
Nephrologie und Dialyse Abteilung *(Department)*
o. Univ. Prof. Dr. Werner Waldhäusl LeiterIn der Forschungsstätte (Titel/akad. Grad, Vorname, Name) *(Head of research institution <title/acad.degree, first name, family name>)*

Anschrift der Forschungsstätte *(Address of research institution)*

Währinger Gürtel 18-20 Straße/Gasse/Platz, Nr. *(street/Nr.)*
1090 Wien Postleitzahl/Ort *(zip code/city)*

Anteil der beantragten Mittel in %, die voraussichtlich an der Forschungsstätte verbraucht werden
(Estimated share of applied funds in % spent at the research institution)

90 %

Zustelladresse der Antragstellerin bzw. des Antragstellers,
nur erforderlich, wenn **Projektkorrespondenz nicht an die Anschrift der Forschungsstätte** gerichtet werden soll
(Applicant's postal address <only if project-related correspondence is not to be sent to research institution>)

Straße/Gasse/Platz, Nr. *(street/Nr.)*	
Postleitzahl/Ort *(zip code/city)*	
Telefon *(Phone)*	Fax
e-mail	www site

Projekt Nr. P .....-.....
wird vom FWF eingesetzt *(to be completed by the FWF)*

Wissenschaftsdisziplinen, auf die sich das Projekt bezieht (mindestens eine, nicht mehr als vier Zuordnungen)
***(Scientific disciplines** relevant to the project <at least one, not more than 4 categories>)*

3526	**60 %**	**3614**	**15 %**	**3211**	**10 %**	**3552**	**15 %**

(Angabe der Nummer und der Prozentzahl (Summe muss insgesamt 100 % ergeben); Zuordnung nach dem Code von Statistik Austria, siehe Beilage)
(Please provide code number and percent contribution (sum must equal 100 %); use categories in Statistik Austria code, see enclosure)

☐ **Neuplanung** des abgelehnten Projekts *(resubmission of the rejected project)*

Projektnummer *(project number)*	**Kurztitel** *(running title)*	**beantragte Summe EUR** *(applied sum)*
-		

☒ **Vorprojekt(e)** (Nur, wenn das Projekt die unmittelbare Fortsetzung eines oder mehrerer FWF-Projekte darstellt. In diesem Fall Angabe dieser Projekte sowie Beilage eines **Ergebnisberichtes des letzten dieser Projekte**)
*(**Previous project(s)** <Fill in only when the proposed project is the direct extension of one or more FWF projects. In this case, list these projects and include the summary report of the most recent project>)*

Projektnummer *(project number)*	**Kurztitel** *(running title)*	**bewilligte Summe EUR** *(granted sum)*
P15679 -	**Prevention of postischemic ARTF**	**257,222.10**
-		

☐ **Förderungen von dritter Seite** (Zuwendungen, die im Zusammenhang mit dem vorliegenden Thema bei anderen Förderungsträgern beantragt sind bzw. von anderen Förderungsträgern erhalten werden: z. B. EU, Ministerien etc.)
(Third-party funding <Funds related to the proposed research topic that have been applied for or already awarded by other funding agencies, e.g.: EU, Ministries etc.>)

Förderungsträger *(funding agency)*	**Projektnummer & Kurztitel** *(project number & running title)*	**Summe** *(sum)*	***bw** *(gr)*	***ba** *(ap)*
			☐	☐
			☐	☐

* Zutreffendes bitte ankreuzen: bw = bewilligt (granted)
(tick appropriate box :) ba = beantragt (applied)

Erklärung *(Affirmation)*

Ich werde den FWF informieren, falls bei anderen Stellen um Subventionen im Zusammenhang mit diesem Forschungsprojekt angesucht wird bzw. weitere Förderungen zugesagt werden.
(I shall inform the FWF if I request support for this research project from other organizations or if additional support is granted.)

Ich bestätige mit meiner Unterschrift die Richtigkeit und Vollständigkeit aller Angaben.
(I certify with my signature that the information provided herein is accurate and complete.)

.....
Unterschrift der Antragstellerin/des Antragstellers
(signature of applicant)

Wien , 7.2.2005

Ort, Datum
(place, date)

Erklärung *(Affirmation)*

Ich bin damit einverstanden, dass das auf Seite 1 dieses Projektstammblatts bezeichnete Forschungsvorhaben an der von mir geleiteten Forschungsstätte unter Verwendung ihrer gesamten Infrastruktur durch alle im Projekt involvierten Personen durchgeführt wird.
(I consent to the research proposed on page 1 of this project data form being carried out at the research institution under my direction and declare that the entire infrastructure of the institution will be made available to all project participants.)

Sämtliche Voraussetzungen (baulicher Art, Energieversorgung, Klimatisierung etc.) für die Aufstellung und den Betrieb der im gegenständlichen Förderungsansuchen beantragten Geräte sind an der Forschungsstätte gegeben.
(The research institution fulfills every prerequisite (structural, power supply, air-conditioning etc.) for installing and operating the equipment requested in the present research proposal.)

.....
Unterschrift der Leiterin/des Leiters der Forschungsstätte
(signature of head of research institution)

Wien ,

Ort, Datum, Forschungsstättenstempel
(place, date, stamp of research institution)

Projekt Nr. P ……-…….
wird vom FWF eingesetzt *(to be completed by the FWF)*

NationaleR ForschungspartnerIn *(national research partner)*

Mühlbacher	**Ferdinand**	**o.Prof**		
Name *(family name)*	Vorname *(first name)*	Titel/akad. Grad *(title/acad. degree)*	SV-Nummer *(Soc. Sec. Nr.)*	Geb. Datum *(date of birth)*

01-40400-6897 Telefon *(Phone)*	**01-40400-6898** Fax
ferdinand.muehlbacher@meduniwien.ac.at e-mail	www site

Forschungsstätte der nationalen Forschungspartnerin bzw. des Forschungspartners
(National research partner's research institution)

Meduniwien Universität *(University)*
Univ. Klinik für Chirurgie Institut/Klinik *(Institute/Clinic)*
Transplantationschirurgie Abteilung *(Department)*
o.Prof. Dr. F. Mühlbacher LeiterIn der Forschungsstätte (Titel/akad. Grad, Vorname, Name) *(Head of research institution <title/acad.degree, first name, family name>)*

Anschrift der Forschungsstätte *(Address of research institution)*

Währinger Gürtel 18-20 Straße/Gasse/Platz, Nr. *(street/Nr.)*
1090 Wien Postleitzahl/Ort *(zip code/city)*

Ist die Anstellung von Personal an dieser Forschungsstätte geplant?
(Is the employment of personnel at this research institution foreseen?)
☐ ja *(yes)* ☒ nein *(no)*

Ist die Aufstellung von Geräten an dieser Forschungsstätte geplant?
(Is the installation of equipment (devices) at this research institution foreseen?)
☐ ja *(yes)* ☒ nein *(no)*

Anteil der beantragten Mittel in %, die voraussichtlich an der Forschungsstätte verbraucht werden
(Estimated share of applied funds in % spent at the research institution)

3 %

Erklärung ***(Affirmation)***

Ich werde den FWF informieren, falls bei anderen Stellen um Subventionen im Zusammenhang mit diesem Forschungsprojekt angesucht wird bzw. weitere Förderungen zugesagt werden.
(*I shall inform the FWF if I request support for this research project from other organizations or if additional support is granted.)*

Ich bestätige mit meiner Unterschrift die Richtigkeit und Vollständigkeit aller Angaben.
(I certify with my signature that the information provided herein is accurate and complete.)

.....
Unterschrift der Forschungspartnerin/des Forschungspartners
(signature of research partner)

Wien , Februar 2005

Ort, Datum
(place, date)

Erklärung ***(Affirmation)***

Ich bin damit einverstanden, dass das auf Seite 1 dieses Projektstammblatts bezeichnete Forschungsvorhaben an der von mir geleiteten Forschungsstätte unter Verwendung ihrer gesamten Infrastruktur durch alle im Projekt involvierten Personen durchgeführt wird.
(I consent to the research proposed on page 1 of this project data form being carried out at the research institution under my direction and declare that the entire infrastructure of the institution will be made available to all project participants.)

Sämtliche Voraussetzungen (baulicher Art, Energieversorgung, Klimatisierung etc.) für die Aufstellung und den Betrieb der im gegenständlichen Förderungsansuchen beantragten Geräte sind an der Forschungsstätte gegeben.
(The research institution fulfills every prerequisite (structural, power supply, air-conditioning etc.) for installing and operating the equipment requested in the present research proposal.)

.....
Unterschrift der Leiterin/des Leiters der Forschungsstätte
(signature of head of research institution)

Wien , Februar 2005

Ort, Datum, Forschungsstättenstempel
(place, date, stamp of research institution)

English abstract for FWF public relations work

The specific aim of the proposed RCT is to elucidate whether conditioning of deceased organ donors with 1 g of methylprednisolone or placebo will ameliorate inflammation in the donor kidney and subsequently lead to reduced rates of postischemic acute renal transplant failure (ARTF). ARTF is the main risk factor for shortened allograft survival.

The proposal is based on the data we obtained over the past three years in the FWF project entitled "Prevention of postischemic ARTF". With the financial support from the FWF we determined the genome-wide gene expression pattern of human donor kidneys before transplantation and found that a unique molecular signature of transcripts responsible for inflammation and immune response was present only in kidneys that subsequently developed ARTF. Transcription factor analysis suggested coregulation of these molecular pathways. Phylogenetic footprinting was used to corroborate the in silico findings.

In the present application we will investigate whether the autonomous storm of cytokines and inflammation caused by the brain death syndrome can be diminished by a single shot high dose corticosteroid given hours before the organs are being harvested. This intervention should shut down inflammation in- and reduce immune response to- the donor organ. The efficacy of this conditioning will be evaluated by genome-wide gene expression analysis of transplant kidney wedge biopsies obtained before engraftment. The ultimate clinical study end point is the incidence and duration of ARTF in the transplant recipients. Since the calculated sample size to show a reduction of ARTF from 40 to 20% is roughly 100 donors, a multicenter approach is proposed. The participating transplant centers are Innsbruck, Vienna and Budapest. Based on the numbers transplanted in these three institutions in 2003, sample collection should be completed within one year. The experimental analysis of the gene expression profiles will be completed at the end of the second study year. Bioinformatics work up and transcription factor analysis of the experimentally obtained data will be finalized in the mid third year.

This study is designed to ultimately answer the clinically important question of whether incidence and duration of ARTF can modified. The applicant has set up an infrastructure over the last three years that provides an unique opportunity for the successful management of this research application.

Schlüsselwörter (nicht mehr als sechs)
(Key words <no more than 6>)

transplantation	**acute renal failure**
transcriptome	**bioinformatics**
RCT	

deutschsprachige Kurzfassung für die Öffentlichkeitsarbeit des FWF
(German abstract for FWF public relations work)

Das definitive Ziel der eingereichten RCT ist zu evaluieren, ob die Vorbehandlung des verstorbenen Organspenders mit 1 g Methylprednisolon oder Plazebo vor Organentnahme die massive Inflammation in der Spenderniere unterdrückt und dadurch die Rate des postischemischen akuten Nierentransplantatversagens (ARTF) reduziert. ARTF ist der Hauptrisikofaktor für ein vermindertes Langzeittransplantatüberleben.

Das eingereichte Projekt basiert auf den Daten die wir in den letzten drei Jahren im Zuge des FWF Projektes "Prävention des postischämischen ARTF" erhoben haben. Mit Hilfe der finanziellen Unterstützung des FWF ist es uns gelungen, das genomweite Genexpressionsprofil von humanen Spendernieren vor der Transplantation zu bestimmen. Jene Transplantnieren, die nach der Implantation ein ARTF entwickelten, hatten ein signifikant anderes Genexpressionsprofil als vergleichbare Nieren mit guter Initialfunktion. Die molekulare Signatur dieser ARTF Nieren ist gekennzeichnet durch eine massive Aufregulation von Genen, die Entzündung und Immunresponse steuern. Eine Analyse der Transcriptionsfaktoren dieser Gene deutet darauf hin, daß die meisten dieser Gene durch wenige Promotoren koreguliert werden. Die biologische Bedeutung dieser in silico Analyse wurde durch ein phylogenetische footprinting bestätigt. In diesem Verlängerungsantrag soll nun überprüft werden, ob die durch das Hirntodsyndrom verursachte systemische Entzündung und Immunantwort in den Leichennierenspendern durch eine hohe Dosis eines Kortikosteroides vor der Organentnahme unterdrückt werden kann. Diese Intervention sollte zu einer Hemmung der Entzündung im Spenderorgan und Unterdrückung der Immunantwort auf das Transplantat führen. Die Effektivität dieses Ansatzes wird durch die genomweite Analyse der Genexpression in Transplantatbiopsien evaluiert. Der präzise klinische Endpunkt dieser Studie beinhaltet Inzidenz und Dauer des ARTF im Transplantatempfänger. Da etwa 100 Nierenspender benötigt werden um herauszufinden, ob durch die Intervention die derzeitige Rate an ARTF von 40 auf 20% reduziert werden kann, ist eine Multizenterstudie notwendig. Die beteiligten Zentren sind Innsbruck, Wien und Budapest. Basierend auf den Transplantationszahlen von 2003 in diesen Zentren sollte die Probengewinnung innerhalb eines Jahres beendet werden können. Die experimentelle Aufarbeitung der Genexpressionsprofile wird voraussichtlich am Ende des zweiten Studienjahres fertig sein. Bei überlappendem Beginn sollte die bioinformatische Aufarbeitung der experimentellen Daten inklusive Transkriptionsfaktoranalyse Ende des dritten Quartals im letzten Studienjahr erledigt sein.

Der Studienleiter hat im laufe der letzten drei Jahre eine Infrastruktur geschaffen, die eine einzigartige Chance für die effiziente Durchführung der eingereichten Studie bietet.

Projekt Nr. P ……-…….
wird vom FWF eingesetzt *(to be completed by the FWF)*

Aufstellung der beantragten Kosten in EUR

(Itemization of requested funding)

	Pos. *(item)*	ProjektmitarbeiterIn[1] *(project collaborator [1])*	Art *(status)*	Ausmaß *(contribution)* (%)	1. Jahr *(1st year)*	2. Jahr *(2nd year)*	3. Jahr *(3rd year)*	Summe *(sum)*
Personal *(Personnel)*	1	**Dr. Alexander Kainz**	**DV**	**100**	**50240**	**50240**	**50240**	
	2	**Mag. Paul Perco (Dr. ab Juli 2005)**	**DV**	**100**	**25120**	**50240**	**50240**	
	3							
	4							
		Zwischensumme Personal *(subtotal personnel)*			**75360**	**100480**	**100480**	**276320**

	Pos. *(item)*	Bezeichnung *(designation)*	1. Jahr *(1st year)*	2. Jahr *(2nd year)*	3. Jahr *(3rd year)*	
Geräte *(Equipment)*	1					
	2					
	3					
	4					
		Zwischensumme Geräte *(subtotal equipment)*				

Materialkosten *(Costs for supplies & expendables)*		**Labormaterial & Studienmedikament/Placebo**	**28016**	**1908**		**29924**
Reisekosten *(Travel costs)*		**Am Transplant Congress 2008**			**2000**	**2000**
Werkverträge[2] *(Contract for work & services [2])*	WV1 *(Contract 1)*	**Budapest, Transplantcoordinator**	**2500**			
	WV2 *(Contract 2)*	**Innsbruck & Wien, Transplantcoordinator**	**10000**			
		Zwischensumme Werkverträge *(subtotal contracts)*	**12500**			**12500**
Sonstige Kosten *(Other costs)*		**Publikationskosten 3x500.-**			**1500**	**1500**
		GESAMTSUMME EUR *(TOTAL SUM)*	**115876**	**102388**	**103980**	**322244**

1 Wenn bekannt, Namen einsetzen, ansonsten „N. N.“. Als Beschäftigungsform stehen zur Verfügung: DV = Dienstvertrag; FB = Forschungsbeihilfe; GB = geringfügige Beschäftigung; FS = Forschungssubvention. Bei Bedarf Beiblatt verwenden.

[1] If known, insert names, otherwise „N.N.“. The following employment status may be entered: DV - contract of employment; FB - scholarship for diploma student; GB - employment on an hourly basis; FS - research subsidy. If necessary, use supplementary sheet.)

2 Wenn bekannt, Namen einsetzen, ansonsten Tätigkeit der Werkvertragnehmerin/des Werkvertragnehmers (z. B. ProgrammiererIn, GrabungshelferIn etc.). Bei Bedarf Fortsetzung auf Beiblatt.

[2] If known, insert names, otherwise only type of work or service to be performed (programming, assistance at excavation site etc.). If necessary, use supplementary sheet.)

Table of Contents

A. Specific Aims

This randomized, placebo controlled study seeks to elucidate whether pretreatment of deceased organ donors with corticosteroids before organ retrieval will reduce the rate of postischemic acute renal transplant failure (ARTF) after engraftment. We have shown in recent studies that transplant kidneys from deceased organ donors exhibit a tremendous upregulation of genes belonging to the main functional groups of inflammation and complement activation. This molecular signature was associated with acute renal allograft failure after transplantation. Contrarily, live donor kidneys are distinctly different in their genome-wide gene expression pattern, show no molecular signs of inflammation activation and hardly ever develop delayed allograft function.

In the present proposal we will specifically test whether steroid treatment suppresses transcription of pro-inflammatory genes of deceased donor kidneys leading to a lower rate and shorter duration of ARTF. Gene expression profiling will be performed in the laboratory of the applicant, who has a strong track record in the evaluation of molecular donor kidney factors on early allograft function. The clinical part of this proposal will be performed in the three major renal transplant centers of Vienna, Innsbruck and Budapest with whom a longstanding clinical cooperation exists. Thus the study setting provides an excellent opportunity to unravel the effectiveness of the suggested donor treatment on gene expression and allograft function of the transplant kidney.

A.1. *Specific Aim 1*

This RCT study will specifically answer the question whether treatment of deceased organ donors with steroids will revert transcriptional activation of genes involved in the inflammatory and complement pathways. Brain death triggers an autonomous storm of cytokine activation that is augmented by hemodynamic instability caused by the development of diabetes insipidus and the SIRS of patients in the ICU. The deceased organ donor will be randomized to receive 1000 mg of methylprednisolone infusion or placebo three hours before organ retrieval. Donor kidney biopsies will be obtained before implantation of the organ and genome-wide gene expression analysis will be performed using cDNA microarrays. Thorough bioinformatics work up including promoter and metabolic network analysis will validate biological relevance of the experimentally obtained results.

A.2. *Specific Aim 2.*

The ultimate goal of this proposal is to investigate whether the proposed suppression of inflammation will cause a reduction of the incidence and duration of ARTF in the transplant recipient. ARTF is among the main risk factors of reduced long term graft survival and remained virtually unchanged at a rate of roughly 40% over the last two decades. This is of special notice given the tremendous improvements achieved in short term graft function over that period which however did not project in proportionally improved long term survival.

B. Background and Significance

B.1. *Epidemiology of ESRD and the donor organ shortage (CAD/LIV)*

The continuously rising prevalence and incidence rates of end stage renal failure (ESRD) in Austria and all other European countries represent a global public health problem. Adjusted incidence rates increased by roughly 6 % annually over the last decade (1994-2003) (5, 6) (**Figure 1**). The numbers are expected to rise even faster in the next decade, because life expectancy is constantly increasing. Since no drug treatment exists to revert ESRD patients have to undergo renal replacement therapy (RRT) either by dialysis or kidney transplantation.

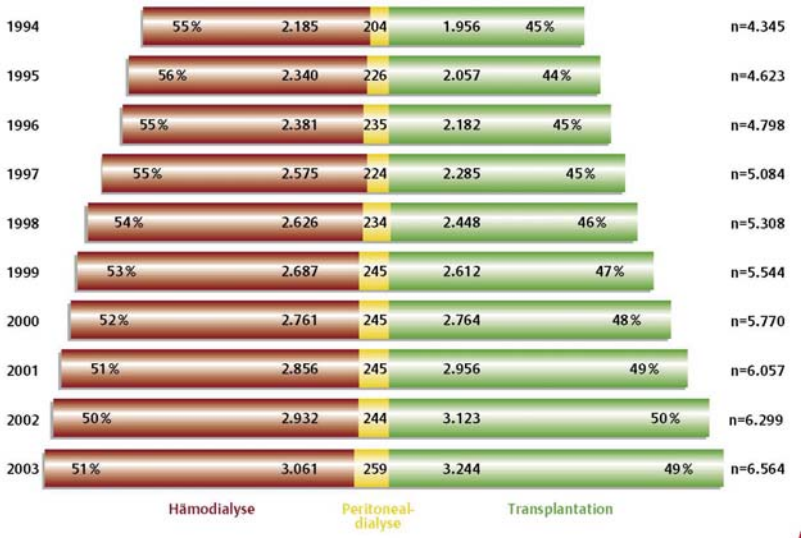


Figure 1. Continuously rising prevalence of renal replacement therapy in Austria over the last decade.

The preferred treatment of ESRD is renal transplantation, because it is considerably cheaper than dialysis and allows for an almost normal life. The major drawback however is the shortage of donor organs. The gap between demand and organ supply is incessantly widening and the average waiting time for a deceased donor organ is currently two years in Austria.

B.2. *Introduction to the specific topic of renal transplant survival*

Substantial success has been achieved in the short term survival of kidney transplants by improved perioperative management and immunosuppressive regimen. Currently the one year graft survival rate averages more than 90% (7). This short term success however did not lead to equally improvement in long term outcome. The median graft half-life for deceased donor organs averaged eight years in 1990 and is currently about nine years (8). Kidney transplants from live donors however exhibit a much longer graft half-life even after adjustment for important confounders such as donor age or cold ischemic time. In Austria however, more than 90 % of all kidney transplants come from deceased donors.

B.3. *ARTF and long-term outcomes*

The fact that transplant kidneys from live donors exhibit longer half-lives is not directly amenable and hard to explain, because the organs from both donor sources worked properly in the donor before explantation. One major difference between the two donor sources is the incidence of ARTF. Roughly one third of deceased donor kidneys are not properly functioning after transplantation so that the recipient has to be treated by hemodialysis until the graft resumes function. ARTF on the other hand is

the rare exception after live kidney transplantation. It has recently been shown in a large cohort study of 122,175 patients that ARTF is highly associated with reduced long term outcome (9). In fact the hazard ratio (HR) for graft failure is almost twice as high in recipients who experienced ARTF compared to those without that problem, adjusted for many covariates. For comparison, the HR of early acute rejection for death censored graft survival was noticeably smaller (**Table 1**) (4). Besides being a key risk factor for reduced long term allograft survival, acute rejection occurs more frequently in grafts with ARTF (9). This interaction was however not tested in the regression analysis of Ojo et al. in 2001.

Variable	HR (95% CI)	*P* Value
Donor Age (Years)		
55 - 64	1.24 (1.08 – 1.42)	0.002
> 65	1.45 (1.31 - 1.80)	<0.001
Donor Hypertension > 10 a	1.17 (1.02 – 1.34)	0.03
Donor DM > 10 a	0.73 (0.45 - 1.20)	0.22
ARTF	**1.99 (1.91 – 2.08)**	**<0.001**
Recipient Age (per Decade)	1.08 (1.06 – 1.10)	<0.001
PRA > 30%	1.21 (1.21 – 1.35)	<0.001
Acute Rej. < 6 months	1.32 (1.26 – 1.39)	<0.001

Table 1. ARTF is the main risk factor for reduced long term graft survival. Data from Ojo et al. 2001 (4).

B.4. *Donor organ factors associated with ARTF*

One main difference between cadaveric and live organ donors is the brain death causing a systemic autonomous storm of inflammation and severe dysregulation of blood pressure homeostasis which is even further aggravated by developing diabetes insipidus (10). The cold ischemic time, which is longer in cadaveric compared to live donors, has been reported to be of only minor relevance for graft survival if below one day (11). The genome-wide gene expression pattern in transplant kidneys does not change during cold ischemia of less than 24 hours (3).

On a morphological basis it is impossible to distinguish donor kidney source, but on the molecular level a discrete set of transcripts is activated in deceased donor organs, depending on the degree of injury. We just showed that the gene expression pattern of donor kidney biopsies which were obtained before transplantation could predict the post-transplant occurrence of ARTF (3). Among the main functional groups distinguishing donor kidneys with subsequent primary function from that with consecutive ARTF were inflammation and complement activation as well as apoptosis induction. A thorough discussion of donor and recipient factors contributing to ARTF was recently published by Schwarz et al. (10).

B.5. *Previous trials on donor pre-treatment*

The current proposal of donor pre-treatment to improve graft survival has been studied in the late 1970s and early 1980s. Of these eight studies in human kidney transplantation, six were performed prospectively and three of these were randomized. The three randomized studies failed to find an effect of steroids (in two studies plus additional cyclophosphamide) on short time graft survival. (12-

14). All other studies reported survival benefits (15-17) . A larger retrospective study showed a graft survival benefit in recipients of pre-treated donor organs at five years after transplantation (18).

Based on these older studies the situation seems to be clear and donor pre-treatment ineffective. However severe problems in the design of all three studies preclude a clear cut answer. First, the primary outcome was short term graft survival of three months or one year. Thus the event rate was so low during that short time period that a classical type 2 error paradox precluded the detection of a difference by the pre-treatment even if the effect would have been huge. Second, the sample sizes of all tree randomized trials were between thirty and forty in each arm and in none of the three papers a rational for that sample size is provided. In the present proposal we calculated a required sample size of 182 kidneys in order to find a 50 % reduction of the rate of ARTF (from 40 to 20%) at an $\alpha < 0.05$ and $\beta = 0.8$. Thus the studies were clearly underpowered and even a detection of a difference in the frequently occurring ARTF (roughly 40 % of cases) was impossible. Thirdly, the inferior immunosuppressive protocols at that time were the main reason for graft loss. After the introduction of cyclosporine into clinical transplantation in 1983 one year graft survival improved from 70% to over 80% immediately and approaches nowadays 95%. Thus short time graft survival is the wrong end point for such a study. As stated above, ARTF has remained unchanged over the last twenty years despite the better immunosuppressive drugs and is considered the main risk factor for long term graft survival. Thus the current proposal is adequately designed to ultimately answer the question whether donor pre-treatment is beneficial for the transplant recipient.

B.6. *Clinical and fiscal significance of the proposal*

It is key for ESRD patients as well as for the society to investigate novel interventions aiming to improve long term graft survival. A well functioning renal transplant offers not only a dramatically better life style but also improves life expectancy of the graft recipient dramatically when compared to wait-listed transplant candidates on dialysis. Even transplantation with marginal donor organs leads to an average increase in life expectancy of five years compared to wait-listed subjects [Ojo, 2001 #191]. Because of the donor organ shortage the society is forced to expand the donor pool and accept these marginal donor organs nowadays almost on a routine base. The postoperative management of these patients is complicated however by a high incidence of delayed graft function.

Besides this adverse effect of ARTF on clinical outcome of patients ARTF it is also associated with a tremendous increase in cost. It has recently been estimated by a German group of health care finance experts that ARTF caused an increment of in hospital cost by roughly € 7,500.- (19). This amount is high, but compared to the even dramatically higher long term cost caused by reduced allograft survival almost negligible. Every year of earlier return to dialysis caused by a preterm failing allograft is associated with cost of roughly € 30,000.- for the Austrian society. Given the fact that the incidence of ARTF remained unchanged at a rate of 30 to 40 % over the last decades it becomes obvious that something has to be done.

The present proposal therefore seeks for an innovative way to reduce the high incidence of ARTF by donor pre-treatment. It is hypothesised that corticosteroid treatment will cause a reduction of transcripts mediating the inflammatory response after brain death in the donor kidney. The effectiveness of this intervention will be evaluated by the reduction in incidence and duration of ARTF in the recipient.

C. Preliminary Studies

The applicant has a longstanding track record in the field of donor factors contributing to ARTF. This expertise ranges from basic experimental studies to clinical trials. A short overview of the published work in that particular area is provided below. All cited papers of our group and additional information are accessible at http://www.meduniwien.ac.at/nephrogene/.

C.1. *Experimental studies*

The molecular and morphological consequences of temporary ischemic injury to a solitary healthy kidney were studied in rats (20). This model was chosen because it closely resembles the clinical situation of human renal transplantation where a solitary kidney undergoes temporary ischemia and exhibits ARTF which is associated with elevated numbers of apoptotic tubule cells (21). The main finding of this study was that lethally injured cells were almost simultaneously replaced by proliferating vital neighboring tubule epithelia. A high proportion of injured cells underwent apoptosis which was regulated by Bcl-2 family members. The role of the Bcl-2 superfamily in renal injury was thoroughly investigated in subsequent papers (22-25). In summary the findings in these papers suggest that donor kidney epithelial cells exhibit an impaired counterbalance of protective Bcl-2 members to proapoptotic stimuli. Reintroduction of Bcl-2 led to reduced rate of tubule apoptosis.

Relevance for the present proposal

The pathophysiology of ARF was intensely investigated by the applicant *in vitro* and in animal models. The results of these investigations provide the pathophysiological rationale to study the effects of donor treatment on ARTF incidence and duration in human kidney transplantation. Especially the observations that sublethally injured tubule cells can be recovered by external intervention strongly support the hypothesis of the current application. Further experimental and clinical evidence for the reasonability of the proposed approach in human studies is provided in the subsequent paragraphs of this proposal.

C.2. *Translational research in human renal transplantation*

It has been shown recently by our group that deceased organ donors exhibit a severe systemic inflammatory response syndrome that causes activation of inflammatory regulators such as adhesion molecules in the donor kidney (**Figure 2**)(2).

Genome-wide gene expression profiling of deceased donor kidneys supported the findings of immunohistochemistry (3). Kidneys from deceased organ donors showed a massive activation of inflammatory genes and activation of the complement cascade (**Table 3**). Compared to live donor organs, genes involved in cellular metabolism and apoptosis counterbalance were significantly suppressed.

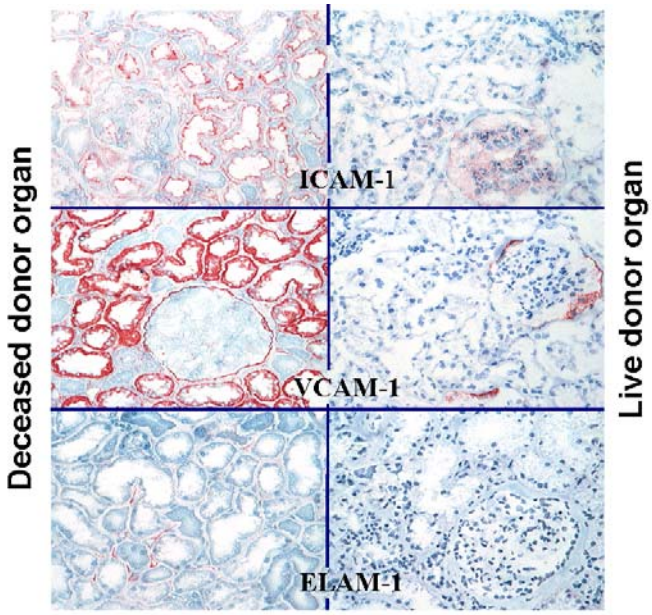


Figure 2. Immunohistochemical staining of donor kidney biopsies. Cadaveric organs exhibit a severe activation of adhesion molecules compared to live donor organs (from (2))

Gene symbol	*Gene name*	*UniGene ID*	*Expression*	
			CAD	*LIV*
Complement system				
BF	B-factor, properdin	Hs.69771	2.57	−0.48
C1R	complement component 1, r subcomponent	Hs.1279	2.23	−0.54
C2	complement component 2	Hs.2253	0.31	−2.28
C1S	complement component 1, s subcomponent	Hs.169756	2.49	0.82
CLU	clusterin	Hs.75106	0.54	−0.91
Immune response				
LTF	lactotransferrin	Hs.105938	3.46	1.16
NK4	natural killer cell transcript 4	Hs.943	2.01	0.11
VCAM1	vascular cell adhesion molecule 1	Hs.109225	5.33	3.65
IL1R1	interleukin 1 receptor, type I	Hs.82112	2.83	1.19
HLA-G	HLA-G histocompatibility antigen, class I, G	Hs.73885	0.34	−1.26
IFITM2	interferon-induced transmembrane protein 2 (1-8D)	Hs.174195	1.02	−0.52
IFNGR2	interferon gamma receptor 2 (interferon gamma transducer 1)	Hs.177559	1.27	−0.27
B2M	beta-2-microglobulin	Hs.48516	3.13	1.81
HLA-DQB1	major histocompatibility complex, class II, DQ beta 1	Hs.73931	1.43	0.14
BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)	Hs.155024	0.71	−0.54

Table 3. Molecular signature of inflammation and complement activation separating cadaveric from live donor transplant kidneys (from Hauser et al. (3))

Furthermore, we identified a unique molecular pattern of regulators of inflammation that predicted subsequent ARTF after engraftment. The main site of molecular dysregulation in deceased donor organs was the tubulointerstitial compartment (1). Glomeruli from deceased donors seem to be less affected than the tubules by the brain death syndrome (**Figure 3**). Additionally impairment of vasoregulatory genes is predominantly seen in the tubulointerstitial compartment of deceased

compared to live donor organs (33). All of these findings might additionally contribute to the higher incidence of ARTF in deceased donor organs.

In order to check whether the identified genes of similar gene ontology are coregulated a transcription factor analysis was performed. Indeed, many of the identified genes exhibit unique binding sites for few transcription factors (**Figure 4**). Phylogenetic footprinting secured the validity of the obtained results. Based on the experimental findings a genetic algorithm for the identification of transcription factor binding sites was designed which was validated in independent experimental data obtained from human myocytes.
Our new algorithm performs superior to most of the few published programs (34).

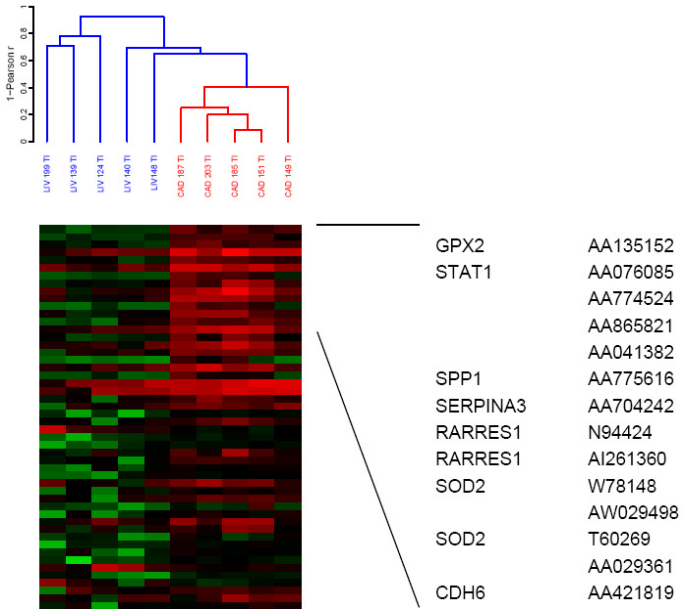


Figure 3. Genes upregulated in the CAD tubulointerstitial tissue compared to LIV specimen (1)

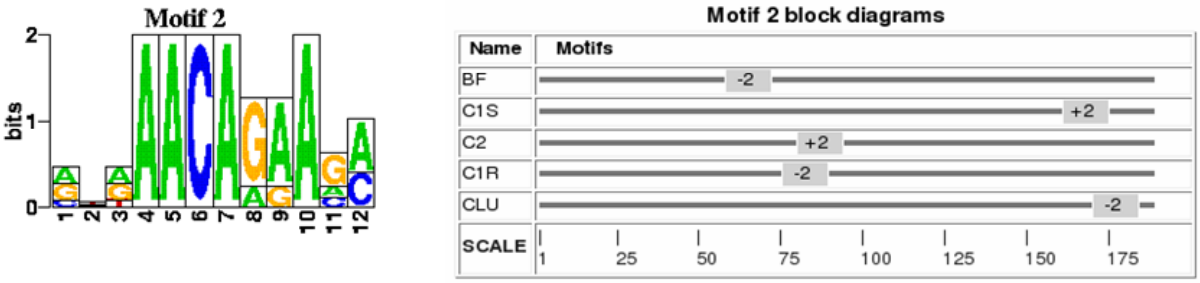


Figure 4. Transcription factor analysis of experimentally derived genes involved in complement activation (see table 3)

Relevance for the present proposal

The work on the response of the donor kidney to systemic inflammation and impaired hemodynamics caused by the brain death syndrome of the donor built the foundation for the current proposal. We could clearly show that activation of many inflammation pathways distinguishes deceased from live donor organs. Furthermore, these signatures were associated with the subsequent development of ARTF. The obvious next step would be the suppression of inflammation in the donor organ before organ retrieval and to test whether these interventions lead to corrected gene expression pattern and subsequently to reduced rates of ARTF.

Thus the current proposal represents the logical next step towards the ultimate goal of reduced ARTF and thus improved long term graft survival.

C.3. *Clinical trials*

The applicant has performed randomized controlled trials as principal investigator in the transplant population. Examples are the investigation of new immunosuppressive drugs on patient and graft survival after renal transplantation or the investigation of bisphosphonate therapy on post-transplant bone disease (35-38).
The very topic of ARTF reduction was addressed in a retrospective study in the applicant's institution. In that paper we were able to show that subjects who were maintained on ACEI or ARB therapy during and after transplantation exhibited a lower rate and duration of ARTF compared to patients without this therapy (39). These findings will eventually lead to a paradigm shift, because so far the perioperative use of these substances was considered contraindicated. Many experts thought that ACEI and ARB may increase the rate and prolong ARTF.

Relevance for the present proposal

The studies cited above serve to demonstrate the ability of the applicant to address the proposed question adequately. In the recent past the authors of this submission managed to create an environment to study the influence of molecular donor kidney factors on short and long term graft survival. The author designed the protocols, performed the studies state of the art and delivered valid results. The results of the current application are directly applicable to the benefit of the continuous growing population of patients with end stage renal disease receiving a kidney allograft.

For a general overview about organ donor factors on early allograft function a recent review by Schwarz et al. may be recommended (10).

D. Research Design and Methods

D.1. *Logistics and management of organ donor pre-treatment*

Three transplant centers, Innsbruck, Vienna and Budapest will participate in this randomized controlled trial. The transplant department at the Medical University of Innsbruck (Director Prof. Dr. R. Margreiter) as well as the Vienna transplant center at the Medical University of Vienna (Director Prof. Dr. F. Mühlbacher) are members of EUROTRANSPLANT (ET) (http://www.eurotransplant.nl/). The transplant department of the Semmelweis clinic in Budapest (Director Prof. Dr. Jenõ Járay) is the leading member of the hungarian transplantation society. The directors of all three participating sites as well as the president of ET, Dr. Yves Vanrenterghem and the director of ETKAC Dr. J. de Fijter (ET kidney advisory committee) will give written permission to the proposed study after the proposal passed the ET ethics committee. The letters of intent of the directors of the participating centers as well as from ET are attached (see attachments). The Hungarian transplantation society held a meeting on February 12, 2005 in which Dr. Robert Langer presented the current proposal to all experts involved in solid organ transplantation in Hungaria. None of the members of this committee had any

objects against the study. The signed protocol of this meeting may be found as attachment to this proposal.

The study was also submitted to the Medical University of Vienna institutional review board on February 14, 2004. The Vienna vote is also valid for Innsbruck. IRB approvals will be forwarded to the funding agency immediately after granting. The Hungarian transplant society does not see the necessity for IRB approval, because the Hungarian IRB guidelines do not apply to deceased organ donors. Similar to ET the hungarian transplant committee has the opinion that the recipient has no legal rights in terms of donor organ selection and thus need not be informed.

Since we propose to immunosuppress the donor systemically after brain death but before organ harvest, the department heads of the other solid organ transplant units (heart, liver, lung and pancreas) participating in the ET and hungarian organ sharing system were informed by a letter from both organizations. None of the chairs of the participating departments in these centers objected the proposal. In fact there are recent data from UNOS, the worldwide largest transplant registry, that donor pre-treatment with steroids, thyroxin and vasopressin is associated with better survival after heart transplantation (40). Furthermore a clinical trial of islet cell transplantation after donor pre-treatment with steroids and glucagons showed improved transplant survival (41).

Each of the three participating centers will receive sufficient amount of vials containing 1000mg of methyprednisolone or placebo before the study start. Methylprednisolone will be purchased from the hospital pharmacy in Vienna. The vials will be number coded by the applicant in Vienna and the information stored in a separate file in the nephrogene kidney transplant database of the applicant (see below, http://www.meduniwien.ac.at/nephrogene/ - trials). Each of the three participating centers will receive its own user and pw which are valid only for their site.

All but non-heart beating deceased donors at the three centers will be eligible for participating in the study. After brain death was declared which is usually at least six hours before explantation, the organ procurement team will access the password protected section of the nephrogene website to receive the randomized number of the vial that needs to be injected. The local study coordinator will send the vial with study medication with the transplant coordinator who removes the lymph node for HLA typing to the donor site (hospital ICU). There the coordinator or the attending doctor in charge will inject the study drug over a period of 15 minutes. To ensure that comparison groups will be of approximately the same size and balanced in each center a block randomization of six will be used (https://www.meduniwien.ac.at/randomizer/).

The transplant coordinator needs to fill out donor and recipient demographics before the vial number can be retrieved from the website. This information will be stored to facilitate recipient follow up. All data will be anonymized to guarantee protection of sensitive data. A test version of the study website has been uploaded and may be accessed at http://www.meduniwien.ac.at/nephrogene/ - trials (user:guest, pw: ARTF; testsite valid for each center, see **Figure 5**).

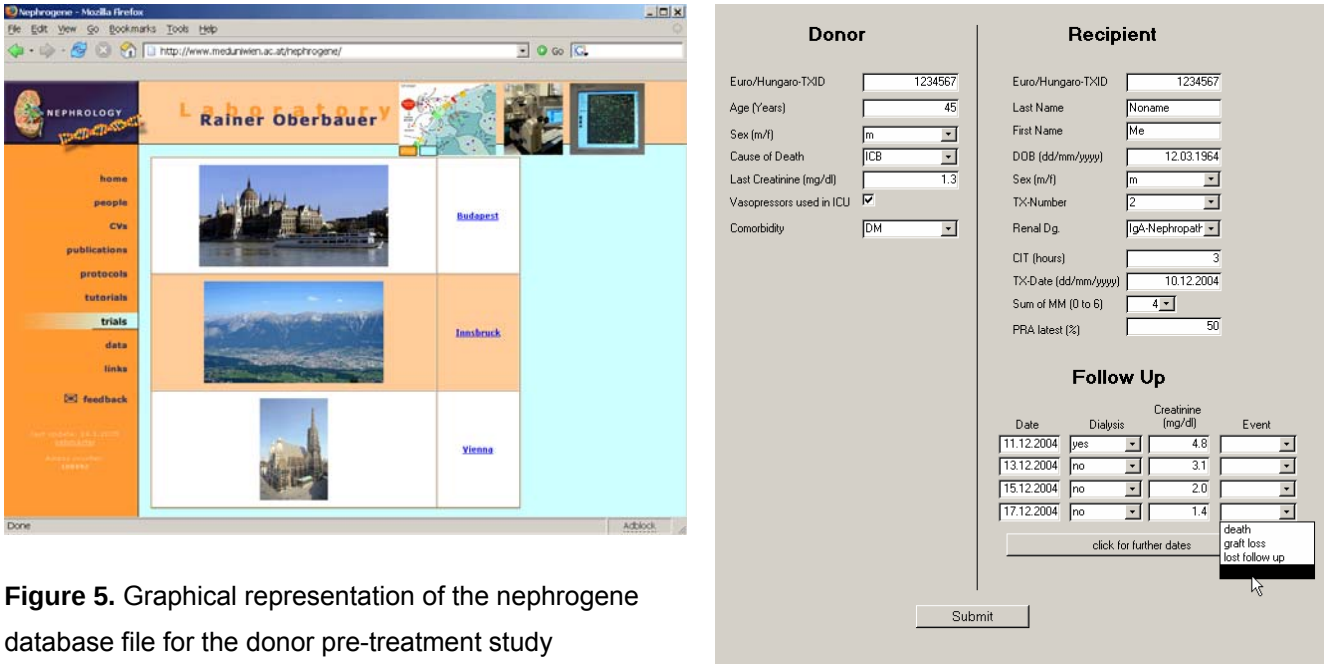


Figure 5. Graphical representation of the nephrogene database file for the donor pre-treatment study

After the donor kidney is retrieved a wedge biopsy will be performed by the organ procurement team and immediately submerged into RNA*later*™ (Ambion, Austin,TX,USA) prefilled 2 ml Eppendorf tubes. The tubes will be labelled with provided barcode stickers containing the randomization number and carried together with the organ to one of the three participating centers in charge. The biopsy specimen will be stored there in a refrigerator at 4°C and samples from Innsbruck and Budapest shipped once a week to the applicant's laboratory by courier mail. The Viennese samples will be collected once a week from the transplant unit upstairs of the applicants lab. We have shown previously that no changes in gene expression occur in the whole transplant kidney during cold ischemia of up to 48 hours and RNAlater prevents RNA degradation in renal biopsy specimen for at least one week (3).

Data management

The applicant established a renal transplant database which includes all patients that were transplanted at the Medical University of Vienna since 1985. The follow up of these patients is almost complete, only 60 out of 2700 patients were lost to follow up due to relocation out of Austria. A screenshot of this relational database can be depicted at http://www.meduniwien.ac.at/nephrogene/ - data link ACEI ARB after TX (user:guest, pw:acei)

A separate file was included into this relational database which is reserved for the current study. As mentioned above, anonymized donor and recipient demographics, as well as clinical follow up data of the recipient after transplantation will be stored. The study coordinators in Vienna, Drs. Christa Mitterbauer and Christoph Schwarz as well as the coordinator in Innsbruck, Hermann Fetz and Dr. Robert Langer in Budapest will ensure data entry during the postoperative in hospital period of whatever center the recipient is taken care of.

D.2. *Statistical Considerations*

The primary clinical study endpoint is the incidence and duration of ARTF. The definition of ARTF is the necessity of more than one dialysis after transplantation irrespectively of reasons or indications by the doctor in charge. The duration of ARTF is calculated as days until the last dialysis after transplantation irrespectively of indication. Cases of primary function who will become dialysis dependent again days after transplantation due to rejection or other causes are not counted as ARTF.

The incidence of ARTF in Vienna averaged 39% over the last year (data from nephrogene). In order to detect an incidence reduction of 50% (from 40 to 20%) given α=0.05 and β=0.8 at a 1:1 randomization a sample size of 91 cases is necessary (**Figure 6**, Fisher's exact test). Since usually both donor kidneys are used for transplantation, the sample size of donors is roughly 100 accounting for some single kidney donors. The sample size is reasonable giving the large transplant numbers in the three centers. In 2003 Innsbruck performed 98, Vienna 143 and Budapest 148 deceased donor kidney transplantations (www.nephro.at and http://www.htp.hu/). These numbers suggest that the clinical part of the study may definitely be completed within one year.

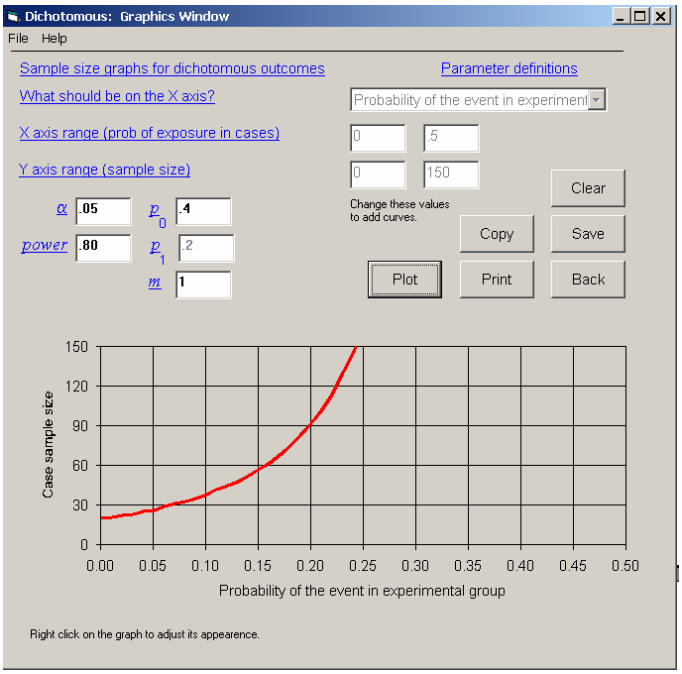


Figure 6. Sample size calculation for an incidence reduction of ARTF from 0.4 to 0.2 by donor pre-treatment.

D.3. *Methods*

D.3.1 General

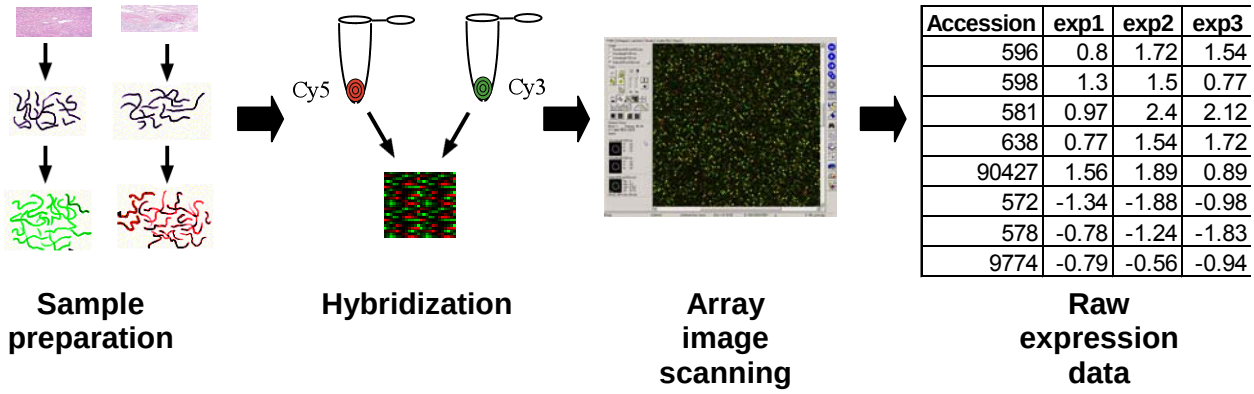
All statistical analyses will be conducted using the SAS for Windows software, version 9.1.3 (The SAS Institute, Inc., Cary, North Carolina). Pearson's chi-square test or Fisher's exact test will be used for the dichotomous outcome of ARTF incidence. Differences in the duration of ARTF will be evaluated as time-to-event analyses using the log-rank test. The analysis will be adjusted for the post-transplant immunosuppressive regimen.

Since the proposed total sample size is 182 kidneys, not all wedge biopsies will be analyzed immediately. We will select twenty cases and equally many controls on a random basis and do the complete experimental and bioinformatics array workup. This strategy was used before because 15 to 20 arrays in well defined samples such as donor kidneys are sufficient to detect clinically relevant differences in expression profiles (3). Besides the relatively low additional information provided by

higher number of experiments per group a considerable amount of effort, time and money can be saved by this approach. If for some reasons intra-group variability of expression profiles is noticeably higher than in our previous studies on the same subject additional ten stored random samples in each group will be analyzed.

D.3.2 DNA microarray technology

As in our previous papers, we will use genome-wide cDNA microarrays from the Stanford Functional Genomics core facility for the proposed identification of molecular signatures. These arrays hold 41,104 features representing 13,385 genes and 3,675 ESTs (expressed sequence tags). 6,523 genes are represented more than once on the array by different cDNA clones. All microarray experiments will be performed as described previously (3). The detailed protocols are available at http://genome-www.stanford.edu/ and http://www.meduniwien.ac.at/nephrogene/protocols. Using a type II experimental set-up, 1 µg of sample and standard Stratagene Universal human reference aRNA will be labeled with CyScribe cDNA post labeling kit (Amersham Pharmacia Biotech, Buckinghamshire, UK) in a two-step procedure (**Figure 7**).



Accession	exp1	exp2	exp3
596	0.8	1.72	1.54
598	1.3	1.5	0.77
581	0.97	2.4	2.12
638	0.77	1.54	1.72
90427	1.56	1.89	0.89
572	-1.34	-1.88	-0.98
578	-0.78	-1.24	-1.83
9774	-0.79	-0.56	-0.94

Figure 7. Flow chart of experimental design.

Sample preparation

Because the RNA of one wedge kidney biopsy is not sufficient for the proposed array experiments, RNA will be amplified to obtain a minimum of 20 µg of total RNA. The amplification will be performed according to a modified version of the Wang protocol using Ambions T 7 MessageAmp aRNA Kit (Ambion; Austin TX, # 1750). The modified version is described in detail at http://cmgm.stanford.edu/pbrown/protocols/ampprotocol_3.html. In brief, the poly (A) tail of the eucaryotic mRNA will be used as a target for priming of the first strand synthesis. The first strand primer is a 57- mer containing poly T (dT15) and the T7 promoter sequence. After second strand synthesis and clean-up, the ds cDNA serves as template for T7 polymerase, which in fact transcribes the cDNA into RNA. After 9 hours at 37°C the amplification via T7 polymerase has reached a range of about three magnitudes. A second cycle is usually performed to gain enough aRNA for the microarray experiments. Quality of the yielded RNA and the efficiency of amplification will be controlled using spectralphotometry, gel- electrophoresis and TaqMan real time PCR.

There are some potential problems however when using the T7 technique. Not all RNAs have the same affinity to the poly-A primer, eventually resulting in non-linear amplification of different RNAs. For our purposes this is not a major obstacle, because we are not looking for absolute numbers of expressed copies per gene, but rather for a difference between kidney samples. Therefore, as long as the samples for comparison are processed pair wise, potential non-linear amplification can be equalized. This can be done reliably as can be seen by verification experiments performed for previous papers (http://www.meduniwien.ac.at/nephrogene/data see Am J Transplant webfigure 1) (1).

Hybridization and washing steps

Amplified samples of good quality will be labeled with the fluorescent dyes Cy 3 and Cy 5 via aminoallyl coupling. The efficiency of the labeling procedure will be controlled using a fluorescencephotometer. To adjust for differences in Cy3 and Cy5 labeling between biopsy specimen and Stratage universal reference RNA, in every experiment both biopsies and control RNA will be labeled in both ways. Afterwards labeled probes will be hybridized to DNA microarrays. For hybridization probes will be dissolved in citrate-buffer and pipetted onto the chip. The active area of the array with the probe will be covered by a cover slide and sealed on the array-surface. Arrays are placed in hybridization chambers and incubated over 24 hours in a 65°C water bath. After incubation the cover slide is removed and the array washed 3 times with citrate-buffer of decreasing concentration. Arrays will be centrifuged to remove all buffer and subjected for scan-analysis quickly to minimize bleaching of the fluorescent signals. The full equipment to conduct the microarray experiments is available in the applicant's laboratory at the Medical University Vienna, AKH-Wien.

Array scanning

The gene chips will be scanned with an Axon scanner in the laboratory of the applicant in Vienna. The read outs are transferred in digital form to a PC with a gene expression analysis software from Axon (GenePix Pro 3.0). With this software it is possible to view the differential expression of about 42,000 genes and ESTs detected in a compact and easy readable form. All array experiments will be performed in Austria as were done in the past four years.

Quality control

Sample aliquots of aRNA will be obtained after each amplification step and subjected to TaqMan PCR for selected housekeeping genes such as beta actin and GAPDH. The sensitivity of this technique is so high, that even RNA amounts of 0.1 ng can be reliably detected. Furthermore, the relative expression levels of the top five expressed genes in the array experiments will be reevaluated by RT-PCR as described previously (1).

D.3.3 Bioinformatics of experimental gene expression profiles

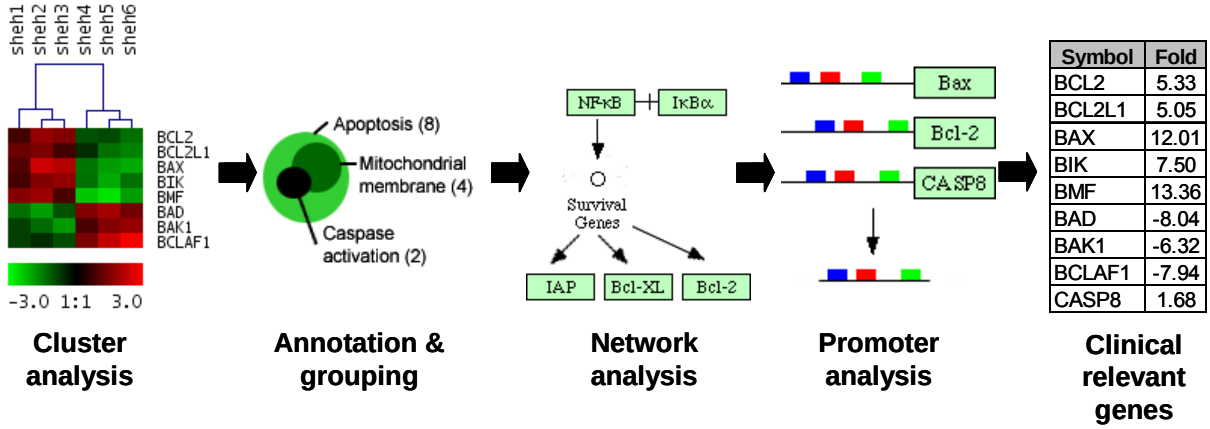
Cluster analysis and statistical framework

The analysis of the large and heterogeneous experimentally obtained microarray data sets will be performed by using different clustering approaches such as hierarchical clustering algorithms and partitioning methods like k-means clustering or self organizing maps (SOMs). The major goal of these methods is to group genes with similar expression patterns, as they are most likely functionally linked in the intracellular regulatory network.

Various statistical methods like SAM (statistical analysis of microarrays) (42), or PAM (prediction analysis for microarrays) (43) along with a two-sample t-test will be applied to the dataset in order to find statistically significant differentially regulated genes between the two patient groups. More important than the statistical significance of these genes will be their annotation on the basis of publicly available information as stored in large biological databases and information resources like the SWISS-Prot database (44), the Pfam database (45), or the LocusLink database (46). Other information resources used in the analysis include the SOURCE system at the Stanford genomics homepage (47), GeneCards (48) as well as the GeneLynx portal (49).

Gene annotation and functional grouping

Functional grouping of proteins under study will be performed using gene ontology terms from the gene ontology consortium (50). Different tools like the GoMiner (51), FatiGO (52), or VennMaster (53) will be used to find statistically overrepresented gene ontology terms in the set of relevant genes (**Figure 8**).



Symbol	Fold
BCL2	5.33
BCL2L1	5.05
BAX	12.01
BIK	7.50
BMF	13.36
BAD	-8.04
BAK1	-6.32
BCLAF1	-7.94
CASP8	1.68

Figure 8. Schematic description of the thorough bioinformatic work up of experimental data.

Pathway analysis

To gain even deeper insight into the metabolic contexts and functions of the genes, various pathway databases and resources will be scanned. Databases holding metabolic pathway information are the KEGG (54), the BioCarta (http://www.biocarta.com/genes/allPathways.asp), and the SPAD database (http://www.grt.kyushu-u.ac.jp/eny-doc/index.html). Simulation software routines, based on the concept of cellular automata, will be applied to the expression dataset to unravel regulatory

interactions, central for an understanding of complex pathways as given along inflammation and apoptosis.

Promoter analysis

Functionally related subsets of genes will be analyzed concerning their regulatory regions (proximal promoter and distal enhancer regions) in order to identify common regulatory mechanisms and transcription factors important for their regulation. With the use of phylogenetic footprinting algorithms and databases holding transcription factor binding sites like JASPAR (55) and TRANSFAC (56) transcription factors will be identified with an important role in the regulation of genes under study. As the combination of single promoter motifs to higher order promoter modules is essential for gene regulation a genetic algorithm approach will be used to find combinations of transcription factors responsible for the regulation of genes under study. Using these identified promoter modules to look for other genes in the dataset with similar regulatory patterns will extend the list of genes under study, thus revealing other clinical relevant genes that did not pass the stringent statistical tests in the initial analysis of the expression raw data.

All of the proposed cutting edge bioinformatics techniques are established in the applicant's laboratory. Some of the software tools were developed by the applicant's nephrogene research group and are investigated on a scientific basis (see website for further information http://www.meduniwien.ac.at/nephrogene/ data - A genetic algorithm to derive joint promoter modules in coexpressed genes; user: perco2004, pw: bio!review) (34).

D.4. *Scientific value and time frame of the proposal*

The proposal will ultimately answer on a genome-wide basis whether donor pre-treatment will abolish inflammation and facilitate cellular metabolism in the transplant kidney. Furthermore we will elucidate for the first time whether donor pre-treatment reduces the incidence and/or duration of ARTF. If the null hypothesis can be rejected and pre-treatment is indeed beneficial, a major step in clinical transplantation would have been achieved. The proposed strategy will likely be utilized in many transplant centers worldwide for the benefits of the individual patient and society.

The applicant assures to perform the proposed study within three years, specifically from Sept 2005 to August 2008. This is a realistic time frame giving the fact that the proposed sample size will be obtained within one year by the multicenter approach. All of the proposed methods and techniques are established in the applicants group of coworkers so that the project could start right after the funding is secured (http://www.meduniwien.ac.at/nephrogene/).

D.4.1 Time table

	Task	Year 1				Year 2				Year 3			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
1	Study setup												
2	Sample collection												
3	Collection of clinical data												
4	Array experiments												
5	Quality control												
6	Array analysis												
7	Bioinformatic workup												
8	Promotor analysis												
9	Statistics of clinical data												
10	Final analysis and manuscript preparation												

The first year of the project will be devoted to study set up and sample collection. It is estimated that the first biopsy specimen will be shipped to Vienna at the end of Q2. The biopsies form Viennese donors will be available as of Q2 in the first year. The biggest junk of year two will be devoted to array experiments and data repository. After mid year two the first bioinformatics workup, at the end of the second year the first bioinformatics analysis will be started, which then will continue until mid year three. The last year is devoted to the statistical analysis, complemented by promoter and metabolic network studies. We expect to have data clearance in Q3 of year 3 and the data ready to be presented in three scientific manuscripts at the end on year 3.

Our track record in the recent past (e.g. FWF P-15679) supports our proposed time frame.
We intend the following milestones of years one through three:

Milestones year 1:

- ✓ Study setup
- ✓ Start with experimental sample processing
- ✓ Start gene array experiments
- ✓ Setup of the bioinformatics routines
- ✓ First gene expression profiles tested for their predictive power
- ✓ Quality control via rt-PCR

Milestones year 2:

- ✓ Complete 20x2 array experiments
- ✓ Start array analysis (scanning gridding, etc…)
- ✓ TaqMan PCR quality control of randomly selected cases
- ✓ Clinical data entry control and follow up
- ✓ Gene ontology categorization start
- ✓ Setup of the bioinformatics routines

Milestones year 3:

- ✓ Promotor and network analysis completed Q3
- ✓ GO-term characterization and in silico analysis finished Q3
- ✓ Bioinformatic analysis finished end Q2
- ✓ Three manuscripts prepared - ready for submission

E. Project relevant costs and time schedule

E.1. *Equipment and appliances costs*

All necessary equipment for the proposed laboratory work is available in the applicant's lab, rooms 6G.11 & 6G.13, 6G.16 in the AKH-Vienna, Währinger Gürtel 18-20. Specifically, a GenePix 4100A array scanner (Axon Instruments, Union City, California) is owned by the applicant and located in the lab. The GenePix Pro 4.1 software, which is necessary for the gridding scanning and submission of raw data to the SMD was purchased from previous grants and is also available . Some of the further bioinformatics work up of the raw data is performed by using the GeneSpring software (Silicon Genetics 2601 Spring Street, Redwood City, CA 94063) which was also purchased by the applicant for previous projects. The proposed array quality control will be performed with TaqMan real-time PCR for selected genes. A Real-time PCR Applied Biosystems cycler and (7700 ABI Prism) Apple control unit is located in room 6G.16 and belongs to the applicant. Furthermore a Zeiss Axiovert 100 microscope and digital imaging device as well as a laser capture microdissection unit (LCM, P.A.L.M. Ebersberg, Germany) are in that room. All necessary common lab equipment such as freezers, centrifuges, 100l liquid nitrogen tanks, PCR hoods and sterile laminar airflow hoods are in the adjacent rooms of 6G.11&13 in the same hallway.

E.2. *Costs of material and supplies*

All materials are calculated for the entire study duration of three years using prices from the 2004 catalogues of the suppliers. As stated in the methods section, we will analyze only 40 to 60 randomly chosen donor kidney biopsies out of 182 samples. All of the material required for the array processing is thus calculated for 60 reactions. The cost of each item is given in Euro (€). Some required items are available in the applicant's lab from previous studies and thus need not be purchased for the proposed trial. Therefore the cost is given as 0.-€.

The proposed number of donors is 100, 50 will be treated with verum, the other 50 with placebo. The cost of 50 samples of 1000mg methylprednisolone (one original package is 5x250mg of Urbason, Aventis Pharma cost € 35.19 UAP) average € 1,408.-. Placebo vials will be prepared by the AKH-Pharmacy (contact Mag. Segel). The cost for 50 placebo vials will be € 500.-.

Materials	Amount	€
Chemicals		
Chloroform	1000ml	99
Isopropanol	1000ml	170
Nuclease free water, GibcoBRL #10977-015	10 l	88
Ethanol 95%, Merck # 1.00983.1011	1x2.5l	169
Formamide	2.5l	562
BSA	500 mg	31
20x SSC Gibco BRL (No.15557-044)	1 L	14
mRNA isolation		
RNeasy mini kit (Qiagen) 50 reactions	3 kits	999
Reverse transcription of mRNA to cDNA		
Sensiscript RT Kit (Qiagen)	3 kit	618
RNAse out Inhibitor	5000 U	84
oligo-dT Primer	25 µg	70
RNA amplification		
RiboAmp RNA Amplification Kit Arcturus, #KIT0201	8x10 reactions = 8kits	4083
RT-PCR quality control		
Primers and probes for two housekeepers and the five top genes on arrays (Applied Biosystems)	2x7 primers & 2x7 probes	2554
Universal Mastermix	4x 5ml	1575
Microarrays		
Produced by the Stanford University microarray facility, laboratory Patrick O. Brown, obtained via Timothy W. Meyer MD	60 arrays (U$115.- = €95.- per array)	5700
Random Primer Gibco BRL (No. 48190-011)	9 units A_{260}	203
Human cot-1 DNA Gibco BRL (No.15279-011)	500 µg	333
Poly-A-RNA Sigma (No. P9403)	25 mg	59
Yeast t-RNA Gibco BRL (No. 15401-011)	25 mg	147
RPN5660 Postlabeling KIT	10 kits	8685
QIAquick PCR Purification KIT	4 x 50	356
Universal Human RNA Referent Stratagene (No. 740000)	2x200 µg	1196
NaOH Plätzchen		15
RNA later	182 samples	206
Total		**28,016.-**

E.3. *Personnel costs and time commitments*

The PI Dr. Rainer Oberbauer is full time employed at the Medical University of Vienna and requests no additional financial reimbursement for the present study. He will oversee the entire project and direct the clinical data acquisition by keeping close contact with the participating persons in each center. Furthermore he will supervise the experimental analysis of the biopsy specimen in his lab. The 'nephrogene group' will conduct regular laboratory meetings in which progress of the project will be reported and the next steps scheduled. In the last year of the project, Dr. Oberbauer will analyze the clinical data and incorporate the findings of the bioinformatics work into the analysis. Finally, the main findings of the study will be reported in scientific manuscripts. Dr. Oberbauer will devote 30% of his protected time to this project.

E.3.1 Logistics and clinical data acquisition

Each of the participating centers will have one or two study coordinators.
In Innsbruck this will be Hermann Fetz and Paul Schobel from the department of transplant surgery. They will be responsible for web-based randomization of donor treatment, making sure that the donor biopsy will be obtained, stored and shipped to Vienna. Furthermore, the in-hospital post-transplant course needs to be entered into the web-based database.

In Vienna the coordinators will be Dr. Christa Mitterbauer and Dr. Christoph Schwarz form the nephrology department and the transplant coordinators Dr. Christopher Burghuber and Dr. Bernhard Edel. No additional salary is projected for Drs. Mitterbauer and Schwarz are full time employed at the Medical University of Vienna. Dr. Christopher Burghuber and Dr. Bernhard Edel however are not employed at the Med. Univ. Vienna and thus will be financially reimburses for their labor. Besides having the same responsibilities as their counterparts in Innsbruck and Budapest, they Vienna coordinators will be responsible for logistic trouble shooting and distribution of information to the other centers.

In Budapest the coordinators will be Dr. Robert Langer and Tímea Feszt, Anikó Maléth. Dr. Langer is employed at the Semmelweis Clinic and requests no additional salary. Tímea Feszt and Anikó Maléth will have the same responsibilities as his counterpart in Innsbruck and thus receive the same reimbursement.

For the coordinator in Innsbruck a financial reimbursement of roughly € 50.- per case will be provided. Based on the 2003 transplant numbers in Innsbruck, a projected number of 60 cases will be randomized in Innsbruck, yielding personnel reimbursement costs of € 3,000.-. Vienna will provide 140 cases yielding € 7,000.-. Budapest will provide 50 cases which yield personnel reimbursement costs of 2,500.-.

E.3.2 Experimental and biostatistics work in Vienna

Dr. Bernd Mayer will oversee the bioinformatics. Dr. Mayer is external professor at the Institute for Theoretical Chemistry and Molecular Structural Biology and heads a bioinformatics group specialized on differential gene expression / promoter / metabolic net analysis. Dr. Mayer will provide the infrastructure and computer routines to perform the bioinformatics analysis proposed in the project. Dr. Mayer requests no financial reimbursement for providing infrastructure and personal time commitment.

Dr. Alexander Kainz has performed the experimental work of the past array papers and part of the bioinformatics of the nephrogene group. In the present proposal he will help with the IT of the study setup and acquisition of clinical data. As of the third quarter in the first year he will devote his entire time to array experiments, quality control and analysis. After all experimental work has been finished Dr. Kainz will use his IT expertise to help in GO-term classification and statistical analysis of the study. Together with the PI he will prepare one or two scientific manuscripts about the main experimental findings of the study. Dr. Kainz is financed until August 2005 via the FWF grant P-15679 of R.O. Dr. Kainz is the key experimentator of this proposal and the entire nephrogene group. A full time Ph.D. salary is requested for Dr. Kainz for the entire three years of study duration.

Mag. Paul Perco will finish his thesis in August 2005 and work as Ph.D. on the bioinformatics of the current application. Additionally Paul Perco will perform the proposed promoter analysis in which he has gained great expertise during his scientific collaboration in Vancouver in 2004 with Dr. Wyeth Wassermann. Dr. Wassermann is the international expert on promoter and transcription factor analysis algorithms. Paul Perco was part time financed from the FWF-project P-15679 to R.O. for his contribution in the analysis of three recent papers from the nephrogene group (1, 3, 34). Paul Perco is key for the proposed project, because no one else in the nephrogene group could do the promoter analysis and metabolic network analysis. A Ph.D. salary form Q3 in the first year onwards to the end of the third year is requested.

E.4. *Travel expenses*

The coordinating center Vienna will invite each of the two other centers once a year for a day of data presentation, discussion and idea exchange to Vienna. Thus the travel expenses of two round trip train tickets from Innsbruck and Budapest to Vienna respectively together with one overnight stay in a medium class hotel will be charged to the current proposal. The amount is roughly € 400.- per year. The PI will present the data in the 2nd and 3rd year of the study at the annual meeting of the American Society of Transplantation. Thus a round trip plan ticket to the U.S. plus three nights of a medium class hotel in year two and three for the study will be requested. The sum of both years is roughly € 2,000.- which includes congress registration fee.

E.5. *Else – publication costs*

We project one paper about the clinical findings of the donor treatment intervention and one about the gene expression changes due to donor treatment. A third paper will be written about the promoter analysis and coregualtion of experimentally derived molecular signatures. The publication cost of three papers in top journals will average € 1,500.-.

E.6. *Total costs for entire study*

Cost	Duration / Amount	€
Study medication & placebo	100 patients	1,908.-
Lab material and supplies	1st & 2nd year	28,016.-
Personnel costs for 2 Ph.D.s	5.5 years	276,320.-
Personnal costs for study coordinators in Innsbruck, Vienna and Budapest	60, 140 and 50 cases of € 50.- each	12,500.-
Travel expenses	once	2,000.-
Publication costs	three papers	1,500.-
Total		**€ 322,244.-**

F. CV of the applicants

All CVs are accessible at the nephrogene website. The user is the family name of each person and the password the first name. The exception is the PI CV where the password is marie_curie (can not be changed because it was provided for ongoing other applications before).

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H. Attachments

Letter of intent Professor Mühlbacher – Vienna

MEDIZINISCHE UNIVERSITÄT WIEN

UNIVERSITÄTSKLINIK FÜR CHIRURGIE
VORSTAND: UNIV. PROF. DR. FERDINAND MÜHLBACHER

To whomsoever it may concern
ETKAC
ET ethical committee
HAT (Hungarotransplant)
Ethikkommission der
Medizinischen Universitäten Wien und Innsbruck
Fonds zur Förderung der wissenschaftlichen Forschung (FWF)

Vienna 2005-02-04

Re: Letter of intent

Dear Madam, Sir,

I am writing this letter to support Dr. Oberbauer's proposed study about the treatment of deceased organ donors with 1 g of methylprednisolon six hours before organ harvest. The requested kidney wedge biopsies will be performed by the procurement team of my department.

Dr. Oberbauer has a longstanding scientific collaboration with the department of transplant surgery and I am confident that the proposed study will yield new molecular and clinical insights into post-transplant acute renal allograft failure.

Sincerely,

Prof. Dr. F. Mühlbacher

Professor of Transplant Surgery

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1/1

Letter of intent Professor Margreiter - Innsbruck

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UNIVERSITÄTSKLINIK FÜR CHIRURGIE
Klinische Abteilung für Allgemein- und Transplantationschirurgie
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07.02.2005

ETKAC
ET ethical committee
HAT (Hungarotransplant)
Ethikkommission der
Medizinischen Universitäten Wien und Innsbruck
Fonds zur Förderung der wissenschaftlichen Forschung (FWF)

Innsbruck, February 2005

Re: Letter of intent

To whom it may concern

Dr. Oberbauer presented a proposal about deceased organ donor treatment to me. It is planned that expired donors will randomly be preconditioned with either 1g of methylprednisolon or placebo about six hours before procurement. A small wedge biopsy will be taken from the harvested kidneys for the analysis of genome-wide gene expression and the clinical follow up of the transplanted organ recorded. The primary study end point is the rate of acute renal transplant failure.

After reading the protocol and having known Dr. Oberbauer for years I am confident that the proposed study will provide valid results on the issue of donor pre-treatment. Thus I strongly support this application.

Sincerely,

Prof. Dr. R. Margreiter

Professor of Surgery

Letter of intent Professor Járay - Budapest

Semmelweis University, Medical School
Department of Transplantation and Surgery
Director: Prof. Dr. Jenő Járay
H-1082 Budapest, Baross u. 23-25.
Tel: +36-1-267-6000, Fax 317-2166

Budapest, February 10th 2005

Letter of intent

To whom it may concern,

Dr. Robert Langer form my department presented this joint study proposal between the transplant centers in Austria and Hungary. The proposed medical intervention in cadaveric organ donors with 1 g of steroids may represent a good choice to reduce inflammation in the graft which may be associated with a lower rate of acute posttransplant renal failure. As the department chief I have no objection against this study. In fact, I strongly support the international collaboration of my transplant team led by Dr. Langer. If there is anything I can further provide to secure granting of this proposal please don not hesitate to contact me any time.

Sincerely,

SEMMELWEIS EGYETEM
ÁLTALÁNOS ORVOSTUDOMÁNYI KAR
Transzplantációs és Sebészeti Klinika

Prof. Dr. Jenő Járay
Professor of Surgery,
Director of the Department of Transplantation and
Surgery of the Semmelweis University Budapest

Letter of intent Professor Vanrenterghem – Eurotransplant

EUROTRANSPLANT

Prof. Dr. Yves Vanrenterghem
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To be presented to
Ethikkommission der Medizinischen Universitäten Wien und Innsbruck
Fonds zur Förderung der wissenschaftlichen Forschung (FWF)

Leuven, 11-02-2005

Regarding: Evaluation of the grant proposal '<u>Prevention of acute renal allograft failure-part 2</u>', submitted by Dr. Rainer Oberbauer

Dear Madam, Sir,

Dr. Oberbauer sent his grant proposal entitled: to me in my function of President of EUROTRANSPLANT.
The study seeks to elucidate whether a randomized trial of 1 g of methylprednisolon or placebo infusion into death organ donors few hours before organ retrieval modifies the genome-wide gene expression in the donor kidney and subsequently lead to a reduction in ARTF. The proposal is elegant and the topic of high priority given the fact that the incidence of ARTF remained virtually unchanged at 20 to 40% over the last decades. Furthermore, ARTF is among the key risk factors for reduced long term graft survival.

Since the proposed systemic donor treatment with 1 g of steroids will potentially affect gene expression profiles in other donor organs and the organs are shared among EUROTRANSPLANT centers a statement from EUROTRANSPLANT is advisable. Similar studies on donor kidney pre-treatment have been performed under the supervision of EUROTRANSPLANT recently after they have been granted by the EC (Ethical committee) and ETKAC (EUROTRANSPLANT kidney advisory committee). The ET briefs from the two most recent granted and directly related studies can be obtained on demand under the application numbers 4096_KAC04 and 4022_EC04.

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Given the past studies I do not see an immediate reason why Dr. Oberbauer's proposal should not receive a positive answer from ET. However the application will be placed on the agenda of the next ETKAC meeting in May 2005 and formally discussed then.

In summary I do not see a major problem with Dr. Oberbauer's application but the definitive approval will be provided by the ETKAC in May 2005.

Yours sincerely,

Y. Vanrenterghem

Letter of intent Hungarian transplant society – meeting in Budapest on February 12, 2005.

HUNGARIAN SOCIETY OF TRANSPLANTATION

PRESIDENT

President:

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Dr. Imre Fehérvári

Budapest, February 12th 2005

Prof. Dr. Rainer Oberbauer
WIEN

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To whom it may concern,

At the last meeting of the Governing board of the Hungarian Society of Transplantation the representatives of the Hungarian centres agreed that the treatment of brain-dead donors with 1 gram of steroids is a normal practice to avoid graft malfunction. No centre had any objection against this treatment.

Sincerely,

MAGYAR TRANSZPLANTÁCIÓS TÁRSASÁG HUNGARICA TRANSPLANTATIONIS SOCIETAS BUDAPEST

Dr. László Szőnyi

President of the Hungarian Society of Transplantation