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PREVENTION OF ACUTE RENAL ALLOGRAFT INJURY

PhD thesis

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The list of abbreviations

DGF	delayed graft function
DSA	donor specific antibodies
ARF	acute renal failure
ICU	intensive care unit
OR	odds ratio
MI	myocardial infarction
ReOp	reoperation
BUN	blood urea nitrogen
DOI	distant organ injury
GFR	glomerular filtration rate
CFR	chronic renal failure
ADH	antidiuretic hormone
RCT	randomized controlled trial
LCM	laser capture microdissection
QC	quality control
OEDTR	Österreichisches dialysis and transplant registry
POC	proof of concept
BCAR	biopsy confirmed acute rejection
GEO	gene expression omnibus
SNPs	single nucleotide polymorphism
SIRS	systemic inflammatory response syndrome
ITT	Intention to treat (analysis)

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ET	Eurotransplant
SAM	significance analysis of microarrays
FDR	false discovery rate
NaPi2a	natrium(sodium) phosphate cotransporter
HRPTEC	human renal proximal tubule epithelial cells
MM	mismatch
ASO	antisense oligonucleotide
IRI	ischemia reperfusion injury
PCR	polymerase chain reaction
HCV	hepatitis C virus

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1 Introduction

Kidney transplantation is clearly the preferred treatment of end stage kidney disease since it allows for a nearly normal life quality and is in the long run considerably cheaper than maintenance dialysis (24).

The incidence rates of patients undergoing kidney transplantation vary considerably among countries and regions reflecting different national regulations and public opinion on organ donation and transplantation in general. The incidence as well as the prevalence of kidney transplantations per million population is in strong correlation with the socioeconomic situation and the Human Development Index (HDI) and income level of countries. Industrialized regions in the world such as USA, Australia and Europe with high HDIs of well above 0.6 show a high incidence (> 30 per million population) of patients undergoing kidney allografting but with a considerable regional variability.

Accordingly, there is also a marked regional and global disparity in the prevalence of kidney transplant patients. The prevalence rates also correlate tight with the socioeconomic status and range from 50 to 300 per million population where the poorest regions have the lowest rates. In countries with a HDI below 0.3 there is virtually no renal transplantation performed. In addition, sound data in these regions are not available and thus an informed discussion on transplantation in these regions is not possible. Lastly, the medical insurance type, religious believes and philosophy are key determinants for access to transplantation.

Given the expenses of this high-end medical procedure requiring the tight collaboration between specialists from different areas such as surgery, immunology, pathology, radiology, tissue typing, infectious disease and nephrology render it intuitive that only countries with working medical infrastructure can offer such regimen. In addition, usually only patients with medical insurance covering such initially expensive

procedures can undergo this form of treatment. Lastly, the recipients of a kidney allograft need to take life-long immunosuppressive drugs, which are expensive and only affordable to patients with health insurance or to very wealthy patients. The introduction of these modern immunosuppressants in the mid ninety eighties prolonged graft survival dramatically. National and international data unambiguously show that great success have been achieved over the last years in prolonging patient and graft survival after kidney transplantation (25).

However; most of the improvement that was achieved can be attributed to the increased one year graft function not only due to better immunosuppressive drugs but also due to better pre-transplant risk evaluation (figure 1).

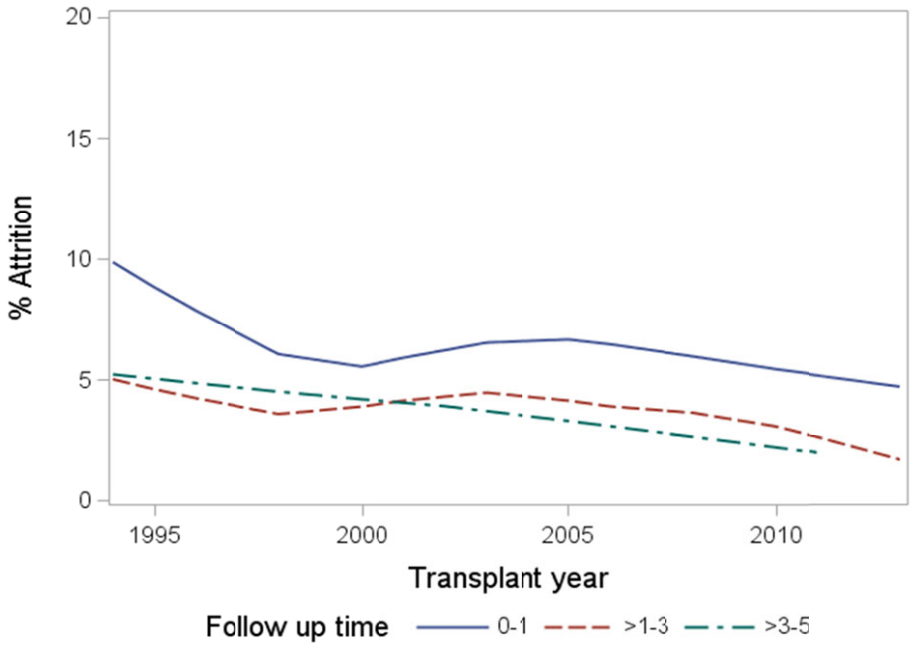

Figure 1. Annual attrition of renal allografts per period of transplantation (personal data from the Vienna Transplant Center).

Nowadays, modern transplant medicine requires the determination of preformed anti-donor HLA antibodies (DSAs or donor specific antibodies), technical planning of the surgical procedure in live donor transplantation, appropriate postoperative care. Despite the main achievements in mid-term graft patency, the rate of not immediately

functioning transplants from deceased organ donors remained unchanged at a relatively high level.

The incidence of post-transplantation acute renal failure (ARF) is the leading singular early clinical condition with the strongest negative impact on survival. ARF eventually resolves after some days but delayed allograft function (DGF) affects roughly 25% of all recipients of deceased donor transplants but virtually never in live donor transplants (figure 2) (26).

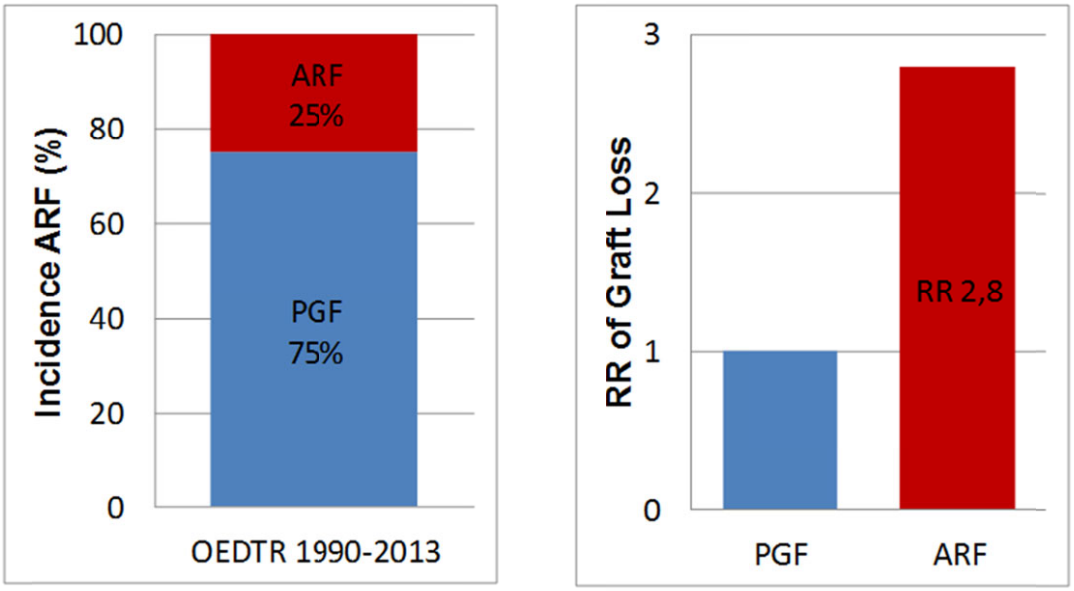

Figure 2. The incidence rates of ARF/DGF in the applicant's home institution over the last one and a half decade remained stable at 25% with a strong association of graft survival. The relative risk of graft loss more than doubled in those transplants with DGF/ARF compared to primary functioning grafts (PF).

This highly prevalent condition in deceased donor transplantation represents the main risk factor for a reduced allograft survival. Studies showed that kidney transplants with DGF have a more than doubled risk for graft loss compared to matched transplants with primary or immediate graft function (1, 27). DGF eventually resolves over the first weeks but recent data suggest that these allografts are prone to a more accelerated rate

of fibrosis, specifically under the current standard immunosuppression of cyclosporine or tacrolimus. These facts clearly show that there is an urgent need for improvement.

1.1 Background and significance

1.1.1 Delayed Graft Function

DGF has been identified as the key target for intervention and great efforts have been undertaken to study the underlying pathophysiology and identify risk factors for ARF as main cause of DGF. There is no uniform definition of post-transplant DGF but most authors use the need of post-transplant dialysis as dichotomous outcome. However, on a more fine-granular basis, DGF/ARF is actually a continuum which ranges from one post-transplant dialysis due to volume management or hyperkalemia to the most extreme other end of the spectrum called primary non-function. The rate of slow or incomplete function is clearly associated with reduced long term patency of the graft and mortality. Longer DGF is also associated with a more pronounced immune response and inflammation in the grafts as evidenced by studies that utilized early protocol biopsies. It is still unclear whether treatment of these early inflammation in these management biopsies with high doses of corticosteroids for example will lead to less fibrosis in the long run and increased graft longevity. Therefore it is of utmost importance to study the signal cascade of DGF/ARF on a molecular level.

It is likely that post-transplant ARF shares some common features of ARF in the native kidney occurring in roughly one out of four to six patients admitted to the medical ICU (2). If the ARF is so severe that a form of renal replacement therapy is required such as hemofiltration or hemodialysis, the risk of mortality increases dramatically. Studies in patients after cardiac surgery for example showed, that ARF is the second strongest predictor of death after cardiac arrest and ranks even before such devastating conditions as perioperative myocardial infraction or prolonged mechanical ventilation (3) (table 1).

Table 1. Risk of mortality expressed as odds ratio (OR) in patients after cardiac surgery developing dialysis dependent ARF compared to equally sick patients without ARF (adapted from Chertow GM et al. (3)).

	OR (95% CI)
Cardiac Arrest	23 (19 – 28)
ARF pre-post adjusted OR	8 (6 – 10)
Perioperative MI	5 (4 – 6)
C. Insult or Coma	4 (3 – 6)
Low output failure	4 (3.3 – 4.3)
ReOp - Hemorrhage	2.7 (2.3 – 3.2)
Prolong. mechan. Ventilation	1.3 (1.1 – 1.5)

These results seem initially not intuitive given the fact that renal failure can be treated by hemofiltration, a rather routinely used technique without procedural risk. So why is the risk of dying still so high in patients with ARF?

The likely explanation is that hemofiltration can be used efficiently to remove fluid and low molecular weight ‘toxins’ such as the renal markers creatinine or BUN but cannot normalize the many other malfunctions that are associated and/or caused by ARF. These adverse entities have been recently coined as ‘distant organ injury’ or DOI (4). DOI includes cardiac problems (high output failure, cardiomyopathy, pericarditis), pulmonary comorbidities (edema, alveolitis, pneumonia, hemorrhage), gastrointestinal disease (erosions, ulcerations, hemorrhage, pancreatitis, colitis), neuromuscular pathologies (neuropathy, myopathy, encephalopathy), hematologic/immunologic deficiencies (anemia, thrombopathy, hemorrhage and impaired humoral and cellular host defense) and protein catabolism.

These simple epidemiological analyses which highlight the devastating and essentially poorly treatable condition of the ARF syndrome makes it intuitively clear that prevention of ARF of native kidneys in the ICU and after transplantation is of paramount importance. In order to prevent this condition more insight is required from the initiating and early processes of developing ARF up to the full blown and eventually

resolving ARF. Since it is not possible to perform sequential renal biopsies on a daily basis experimental animal studies have been used to study postischemic ARF.

1.1.2 Experimental ARF

One of the most representative experimental models for the human situation is the unilateral nephrectomy and clamping of the contralateral renal vascular pedicles in rats for 40 minutes at normothermia. Dialysis dependent ARF in humans is usually multifactorial and occurs predominantly in kidneys with some form of pathology such as atherosclerosis and reduced GFR. Therefore the single kidney ARF of the rat clamp model is also very representative for the human renal allograft setting.

Within one hour after reperfusion the tubular epithelial cell lining is already damaged and one or the other cell became detached from the basement membrane, the tubules are dilated and the creatinine increased twofold (figure 3). One day after reperfusion a full blown ARF developed on a morphological as well as functional level. Most of the tubuli are filled with detritus and fibrin; epithelial cells are predominantly necrotic as evidenced by pycnotic nuclei, and some apoptotic cells. No open lumen is visible within the tubular cross sections and the serum creatinine increased tenfold.

One week after reperfusion the creatinine has returned to normal but the morphology is by far not normal suggesting that creatinine is not a good kidney injury marker because it underestimates the severity of damage dramatically. Morphology improves over time but the late consequences of the ARF are cystic dilatation of some tubuli and higher propensity of chronic renal failure (CRF) as also overserved in patients with initially resolved ICU-ARF. Reemphasizing again however that no morphological data in humans exist because sequential biopsies are not feasible.

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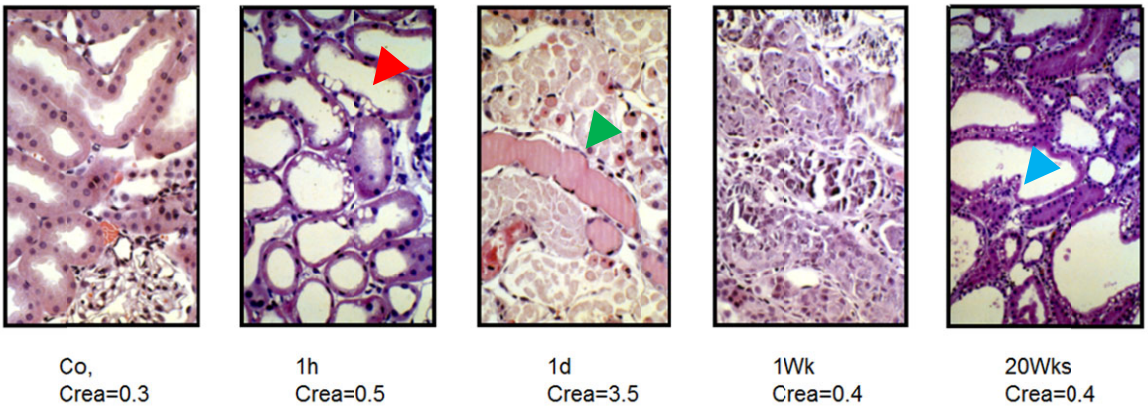

Figure 3. Morphology of developing ARF in a rat kidney ischemia reperfusion model. Time on the x-axis refer to 1hour, 1 day, 1 week and 20 weeks after reperfusion (print magnification 400x, adapted from Hauser P, Oberbauer R (28)). Already one hour after reperfusion, tubular dilation can be seen with occasional loss of epithelia cells (red arrow). One day after reperfusion all avital cell have been shedded into the lumen. Cell nuclei are pyknotic and adjacent to the cell membrane. Fibrinous detritus is obstruction the remaining tubule lumen (green arrow). At this stage the serum creatinine concentration has increased to its maximum of 3.5mg/dl. After one week, the morphology is still severely impaired but the serum creatinine has almost returned towards its baseline value suggesting that it is not a good biomarker to assess the degree of renal injury. The late consequences of ARF can be seen at 20 weeks after the insult. Cystic dilated tubuli are making up most of the renal parenchyma (blue arrow).

Drilling from the morphological level deeper into the events and processes that occur on a molecular level shows surprising some choreographed processes in the ongoing chaos. This is especially true in the reparation phase of ARF as has been shown in post-transplant ARF studies.

1.1.3 Donor Factors

The allogenic transplantation of organs from one to another organism is inevitably accompanied with a period of hypoxia over hours leading to a series of molecular and cellular alterations of the tissue of the donor organ. These changes mainly include tissue damage by reactive oxygen species, nitric oxide or peroxinitrite as one of the strongest

oxidants that develop in response to hypoxia (5). In addition, a considerable share of the injury observed in the deceased donor kidney is derived from processes occurring in the brain dead donor.

Brain death induces an autonomous storm of cytokines and catecholamines which trigger inflammation followed by profound hypotension after an initial hypertensive crisis and subsequent tissue hypoxemia. This event is perpetuated by the absence of ADH release from the death brain and culminated in the kidneys inability to reabsorb the huge amounts of filtered water eventually culminating in full blown diabetes insipidus. Reactive renal vasoconstriction perpetuates the ischemia/hypoxia of renal tubule epithelial cells as the main target of injury. White and colleagues recently showed that heterotrimeric G-proteins are among the key regulators of renal epithelial cell function during homeostasis and in response to ischemic injury (6). The authors hypothesized that G-protein βγ subunit (Gβγ) dimer activity may be a novel therapeutic target since it is strongly activated during IRI. Eventually, iatrogenic vasopressor use and developing hypernatremia perpetuate these catastrophic processes. A recent study showed that desmopressin use in the donor can ameliorate hyponatremia and was associated with higher propensities of immediate graft function (7).

Ischemic damage of the tissue is further on continued by subsequent reperfusion and flushing with oxygen. Once again, highly reactive and cytotoxic species like the hydroxyl radical lead to further partially irreversible alterations and destruction of the tissue. Even more important, reperfusion of blood into previously damaged tissue is accompanied with a strong immune response resulting in the infiltration of monocytes and neutrophils through the activated endothelium. Taken together, ischemic injury with reperfusion associated inflammatory responses seems to be important factors for the development of ARF.

Additionally, there are certainly post-donor factors such as the duration of cold and likely more important the warm ischemic period that contribute considerably to ARF in

the human renal allograft setting. Studies showed that cold static ischemia above twenty hours gradually increase the risk of ARF (27). In recent years machine perfusion techniques have been developed that showed that the risk of ARF, especially in older donors, could be reduced (8). However the reported benefit may even increase by normothermic perfusion of the donor organs (9).

1.1.4 Recipient Factors

Accordingly, the duration of the suture of the vascular anastomosis takes a skilled surgeon in patients with normal anatomy between 30 and 40 minutes. Longer anastomosis times however increase the risk of ARF unproportionally (10). How much the alloimmune response contributes to ARF is not unambiguously determined. Cellular rejection can be clearly differentiated from DGF but the contribution of preformed HLA and non-HLA antibodies is not so easily discernible and no studies investigated morphological and serological features of antibody mediated rejection in first week management or protocol biopsies. However most of the transplant centres nowadays screen patients on the waiting list for the presence of donor specific HLA-antibodies and thus the incidence is likely very low. The contribution of non-HLA antibodies to DGF has not been evaluated yet. In fact there is plausible evidence coming from clinical observations that non-HLA alloimmunity is contributing to ARF/DGF. Patients with Alport syndrome exhibit a full loss of function of the alpha five chain of the type four collagen. If these recipients receive a donor kidney with an intact collagen structure, recipients will make antibodies and a rapid Goodpasture like syndrome develops in roughly 5% leading to early graft loss after prolonged DGF. It is not clear yet why only a minority of cases develop this devastating rapid immune response and why some of the patients develop anti-collagen antibodies but exhibit early and mid-term graft patency. Recent global consortia are currently evaluation the impact of these genetic mismatches between donor and recipient in large genetic associational studies such as the International Genetics & Translational Research in Transplantation Network (www.igenetrain.org).

1.2 Related Work of other Investigators

Since ARF/DGF represents one of the main risk factors for premature graft loss and mortality and occurs in roughly one in four transplant recipients, the clinical need for prevention and therapy is evident. Thus many research groups tackled this pathology and investigated adjacent processes from the molecular level up to clinical intervention trials. Recent reviews nicely summarized the current understanding and clinical state of DGF/ARF research and prophylaxis/therapy (11-13, 29).

The transfer of organs from one to another organism inevitably causes some decree of hypoxia which leads to a cascade of molecular and cellular choreographed but also anarchic events and in the donor organ. Ischemic damage of the tissue is aggravated by subsequent reperfusion of the kidney with oxygen containing blood and the alloimmune response of the recipient. Even more important, reperfusion of blood into previously damaged tissue is accompanied with a strong immune response resulting in the infiltration of monocytes and neutrophils through the activated endothelium (30). In summary, ischemic injury with reperfusion associated inflammatory responses seems to be important factors for the development of ARF. There is even evidence that severe brain injury might potentiate the immunologic responses to the graft as a variety of cytokines have been demonstrated to be released in deceased donors (14, 15).

Given the overriding risk of ARF for reduced long-term graft survival and the identified risk factor, several studies investigated preventive measures or strategies. Among the modifiable factors are an optimal donor management and a minimum duration of cold and especially warm ischemic times. Counterbalance of the systemic inflammation of the deceased donor by dopamine has been reported to be associated with a reduced risk of ARF (16). The same authors subsequently showed in a RCT that dopamine donor treatment reduced the need for dialysis after transplantation. However, most of the donors will require vasopressor use anyway and thus we were interested in any additional benefit of an anti-inflammatory intervention. Studies in the late 1970s and early 1980s tackled this enigma but none was randomized and all were of small sample

size (17-20). Furthermore, that endpoint was graft loss in this pre-cyclosporine era. Thus these old studies are not amenable to nowadays clinical practice.

In contrast, three small randomized studies found that corticosteroids plus additional cyclophosphamide in 2 studies did not effect on graft survival within the first year (21-23). The primary outcome in these trials was short-term graft survival, and their ability to detect important effects of treatment was limited by the low incidence of early graft failure.

The only one adequately powered state RCT on the effect of inhibition of systemic donor inflammation of the incidence and duration of ARF has been conducted by the applicant and will be discussed in detail below (31).

Once ARF has developed in native as well as post-transplant kidneys no causal therapy exists. It is advisable however that those parameters known to contribute to ARF such as arterial hypotension and renal hypoperfusion, infection and inflammation as well as dehydration should be corrected. A scientific proof that these activities actually led to a reduced duration of DGF remains elusive. Nevertheless, the overall ARF rate remains still at a constant high level so that novel approaches for the prevention and treatment of ARF are urgently needed.

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2 Objectives

The overall aim of this thesis was to elucidate the molecular pathophysiology and discover prognostic factors for posttransplant ARF. The objective was not only to explain the underlying pathophysiology but rather to invent potential preventive and therapeutic strategies to overcome this unwanted phenomenon. By amelioration of the high DGF rates we speculate that the mid- and long term graft survival will increase and shortage of the scare donor organs will be alleviated due to longer lasting grafts. This utilitarian approach has been introduced also in large transplant networks such as in the United States (UNOS) where the donor organ quality is matched with the expected survival of the recipients to avoid futility because currently most of the allograft recipients die with a functioning graft.

In fact based on the findings of our studies a precise goal was to test in vitro and in vivo strategies in experimental models of ARF. Based on these proofs of concept experiments a series of large animal studies will be ultimately performed to test for the applicability of our findings in the setting of human renal transplantation.

Objectives: The molecular regulation of ARF is highly complex and not all parts of the kidney are equally affected. Most of the injury occurs in the tubular epithelial cells and therefore we set out to elucidate the sequential molecular events in microdissected tubules of deceased donor human kidneys. Live donor kidney biopsy specimens were used as healthy controls. Genome wide studies were utilized as tool to derive candidate features of regulation. Since it was expected that several dozens of regulators will be found and that it will be unlikely to find a single therapeutic intervention that captures all of these mediators, miRNA profiles were obtained as well. The specifically designed bioinformatics suite miRway was applied to search for the candidate miRNA that regulated most of these candidates. The causal inference was tested by designing ASOs (antisense oligonucleotides against the target miRNA) to be used for in vivo experiments of ARF.

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2.1 Work plan

A carefully designed work plan has been set up and followed over the last decade and a half in order to thoroughly study the epidemiology, pathophysiology and clinical consequences and potential prophylactic and therapeutic interventions. A schematic overview of this work plan is illustrated in figure 4.

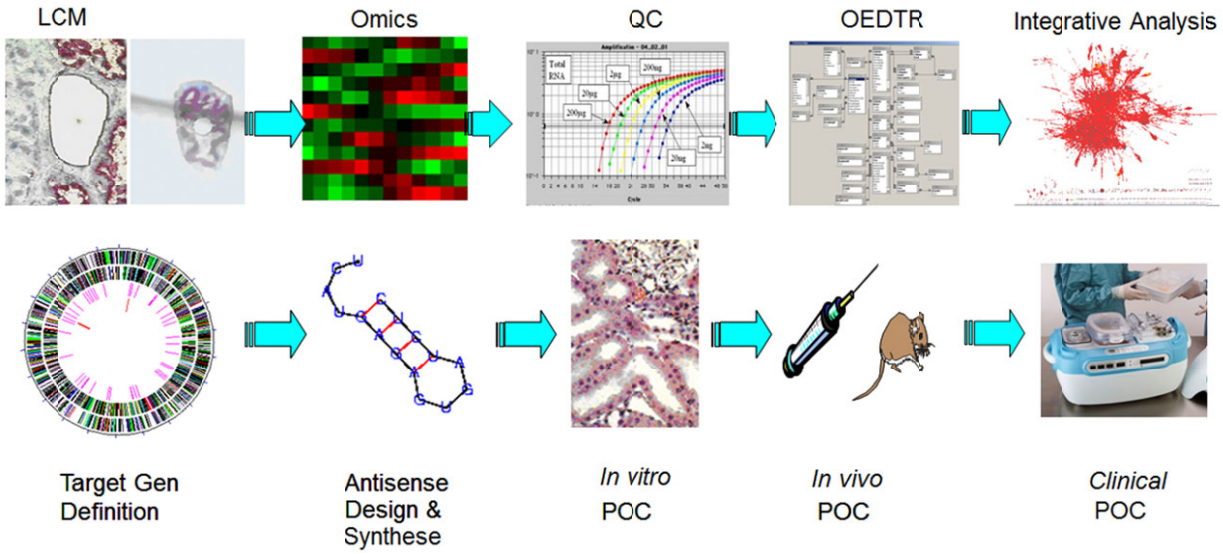

Figure 4. Work plan for the study of ARF experimentally and in the human post-transplant setting (LCM … laser capture microdissection; QC … quality control; OEDTR … OEsterreichisches Dialyse and Transplant Registry; POC … proof of concept)

To assess the associations of morphologic and molecular features in the human deceased donor kidneys, pre-implantation biopsies were obtained in large amounts in order to have a sufficient number of outcomes, i.e. cases of ARF, which occur in roughly 25% of deceased donor kidney transplants. A collaboration with the Semmelweis University Clinic in Budapest was initiated since in this excellent transplant center roughly 200 kidney transplants are performed annually. Together with the Medical University of Vienna and the Elisabethinen hospital in Linz, we had access to over 400 renal transplants per year. The post-transplant course was monitored and recorded into a web-based database (figure 5).

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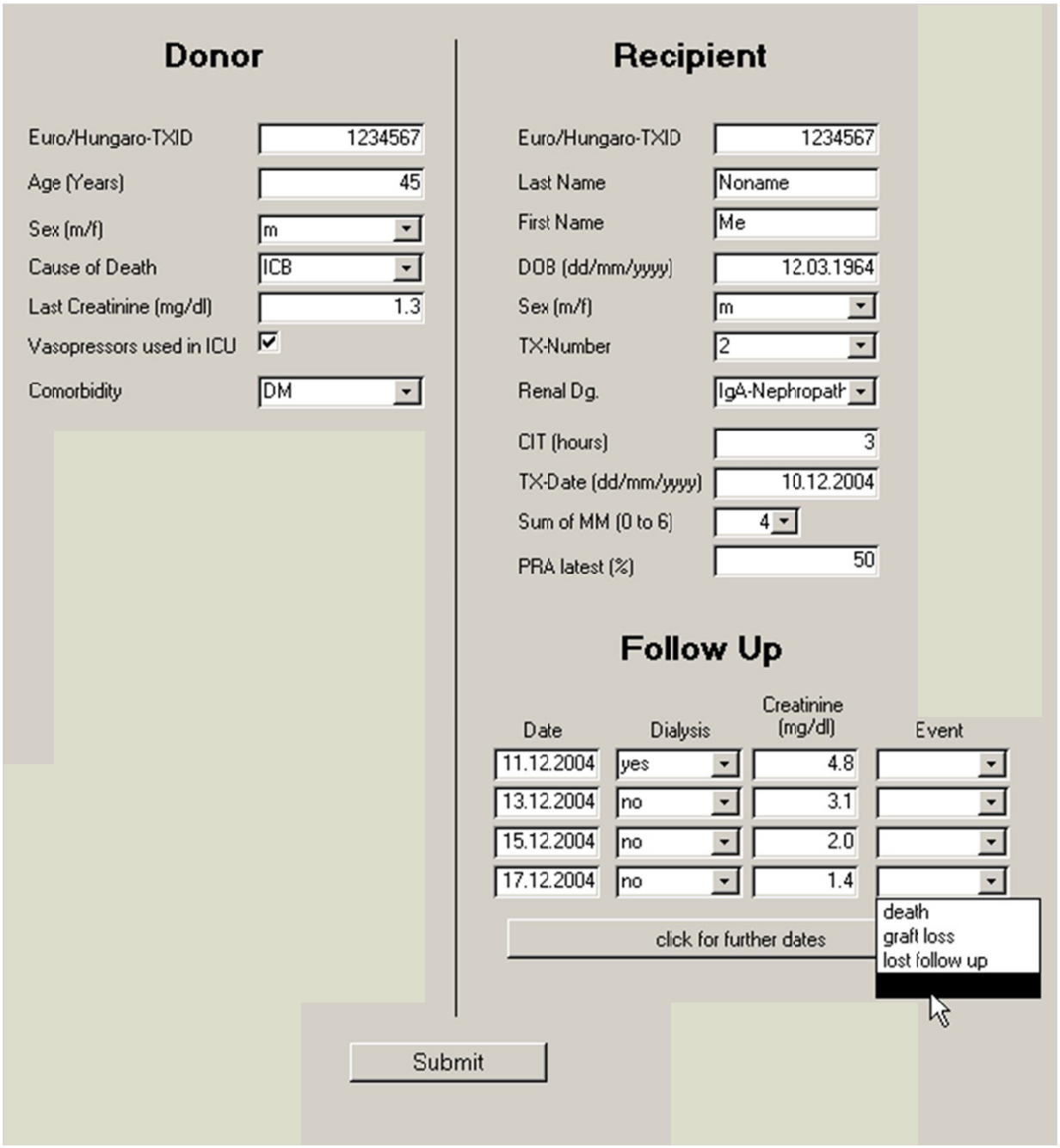

Figure 5. Screenshot of the web-frontend of the ARF database

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3 Methods

A detailed description of all methodological utilized in this research may be found at the applicants website http://www.meduniwien.ac.at/nephrogene under 'protocols'.

In brief, the following techniques were used in the research projects:

- Affymetrix mRNA and miRNA arrays
- cDNA Arrays
- Cell culture
- ELISA
- Immunohistochemistry
- Peptide Arrays
- Protein concentration
- qRT - PCR
- Western blotting

A detailed description of the animal experiments may be found at the respective sections of this paper.

3.1 Histogenomics

To account for the fact that the kidney does not consist of homogenous tissue but is rather structured into the functional units of nephrons, many of the biopsy specimens obtained were subjected to microdissection. The genome wide gene expression profile was analysed in donor kidney biopsies which developed ARF/DGF after transplantation and compered to signatures in kidneys with primary graft function. The Venn diagram in figure 6 shows the genomic features that discriminated ARF from the primary functioning grafts by tissue compartments.

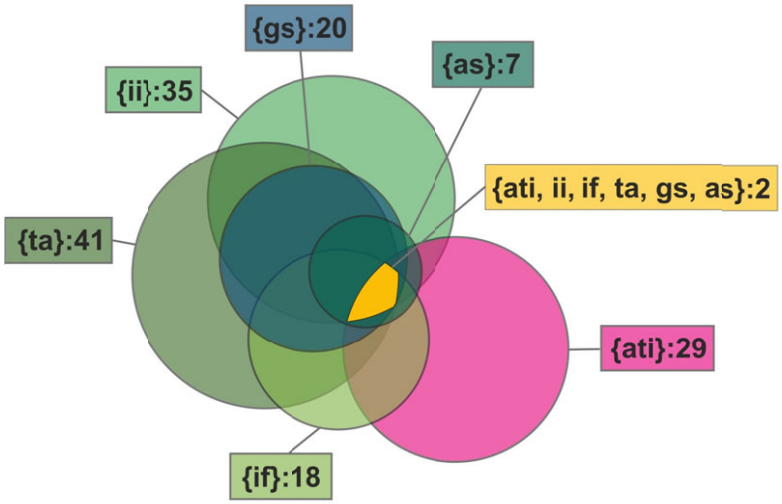

Figure 6. Histogenomics of donor kidneys (n=82) (adapted from Perco P et al. (32)). Abbreviations refer to histological findings in the kidney compartments: gs … glomerulosclerosis, as … arteriolosclerosis, ati … acute tubular injury, if … interstitial fibrosis, ta … tubular atrophy, ii … interstitial inflammation.

The detailed protocol of kidney gene expression profiling may be found on my lab-website http://www.meduniwien.ac.at/nephrogene/index.php/protocol/whole-genome-arrays-2

Since most of the gene expression profiles follow a highly choreographed patter and given the highly complex changes during initiation, establishment and resolution of ARF, we used an integrative analytical approach to derive a sound understanding and clear results of these part of the studies.

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3.2 Systems Biology

The integrative analyses using a systems biology approach was carried out in collaboration with Bernd Mayer and coworkers from emergentec (www.emergenetc.com). Emergentec has developed an in silico network of molecular processes and interactions between protein coding features derived from various omics tracks and published literature that mirror the physiological dependencies (figure 7).

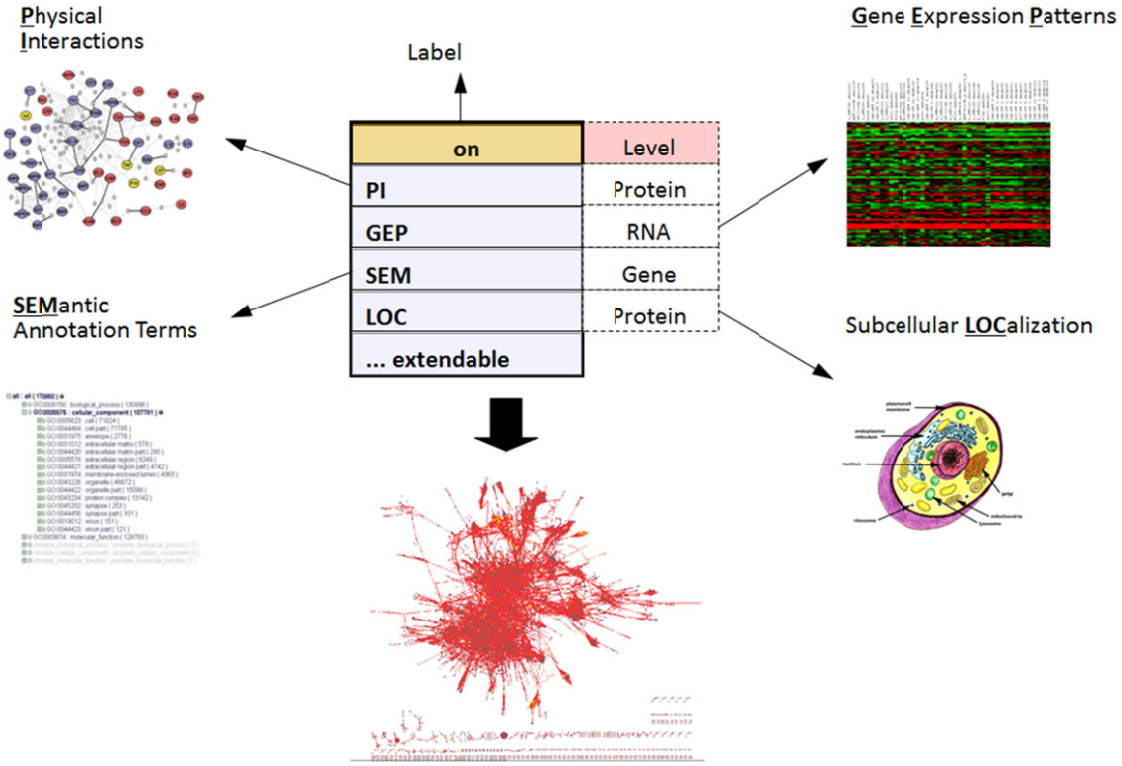

Figure 7. Integrated Analyses of a Systems Biology derived omicsNET that covers 13,000 vertices and 800,000 edges and allows for the integrated analyses of complex biological data (33).

The donor kidney profiles are superimposed on the annotated relations network (figure 8A and 8B).

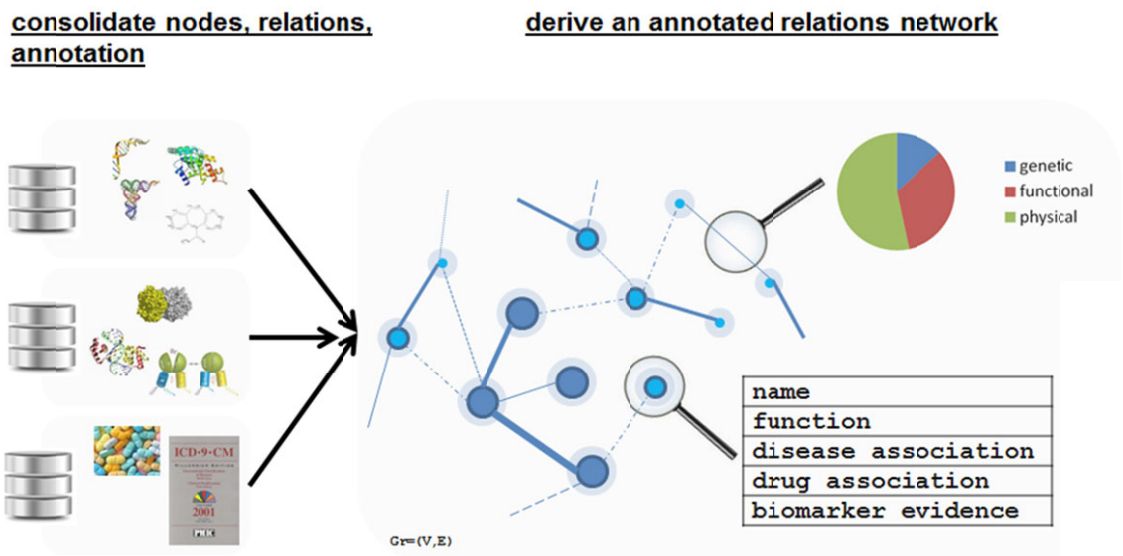

Figure 8A. Schematic representation of the molecular network 'omicsNET'

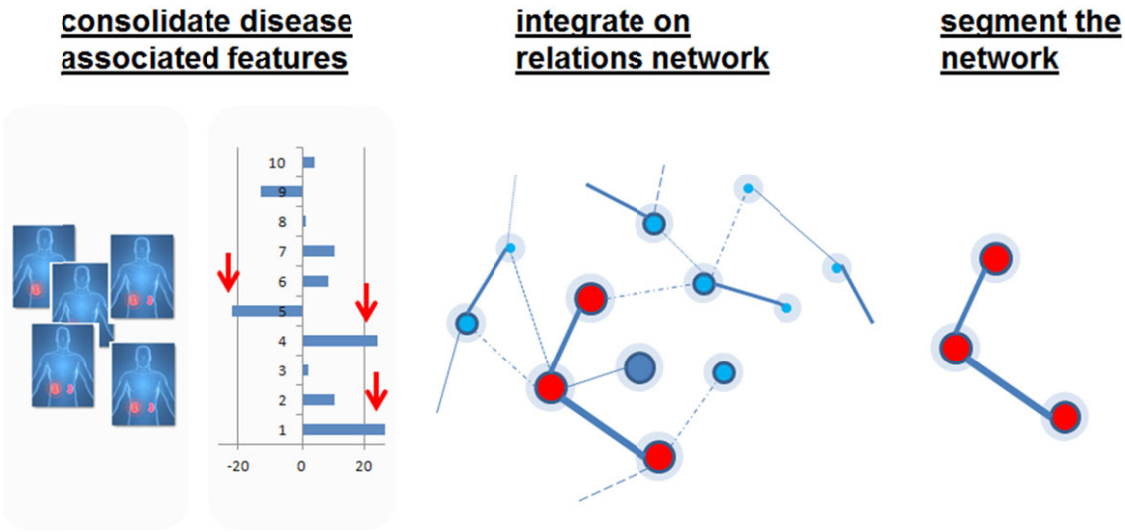

Figure 8B. Scheme of the disease specific segmented subgraphs and functional units. The ARF specific segmented network is shown in figures 10A and 10B of the results and interpretation section.

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3.3 Discovery and Validation Studies

After the transciptomics studies, cDNA and miRNA arrays were conducted on the same biopsies in a novel prospective cohort of renal allograft recipients with the aim to uncover the complex molecular regulation of ARF in further depth. A CONSORT type of flow chart is depicted in figure 9. The sample size of 122 donors were chosen to obtain roughly the same representative numbers of ARF and BCAR follow up biopsies in the first week and a half after transplantation. Ten protocol biopsies without ARF and BACR were taken in the same period and served as control tissue.

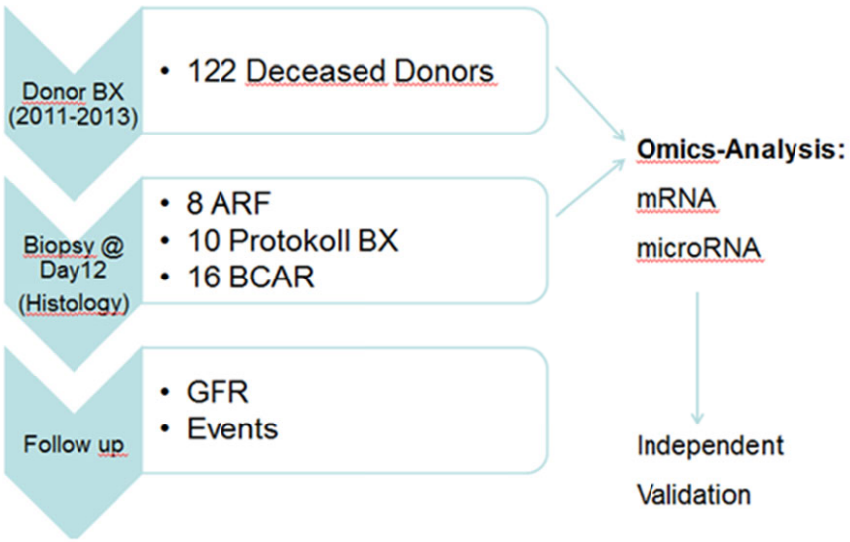

Figure 9. Work flow of the simultaneous determination of mRNA and miRNA expression in the same biopsies in a prospective cohort of renal allograft recipients. Independent validation of observed findings in the discovery cohort was performed by in silico analysis and statistical testing compared to public domain data (GEO).

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4 Results

4.1 ARF Specific Molecular Network

Based on the available omics profiles (figure 10A), an ARF specific network was defined and subsequently superimposed on the omicsNET and segmented. The significant features and dependencies/interactions between the nodes are displayed in figure 10B. Most of the differentially regulated features in the ARF kidneys belong to the gene ontologies of inflammation, immunity and host defence, some were transcription factors.

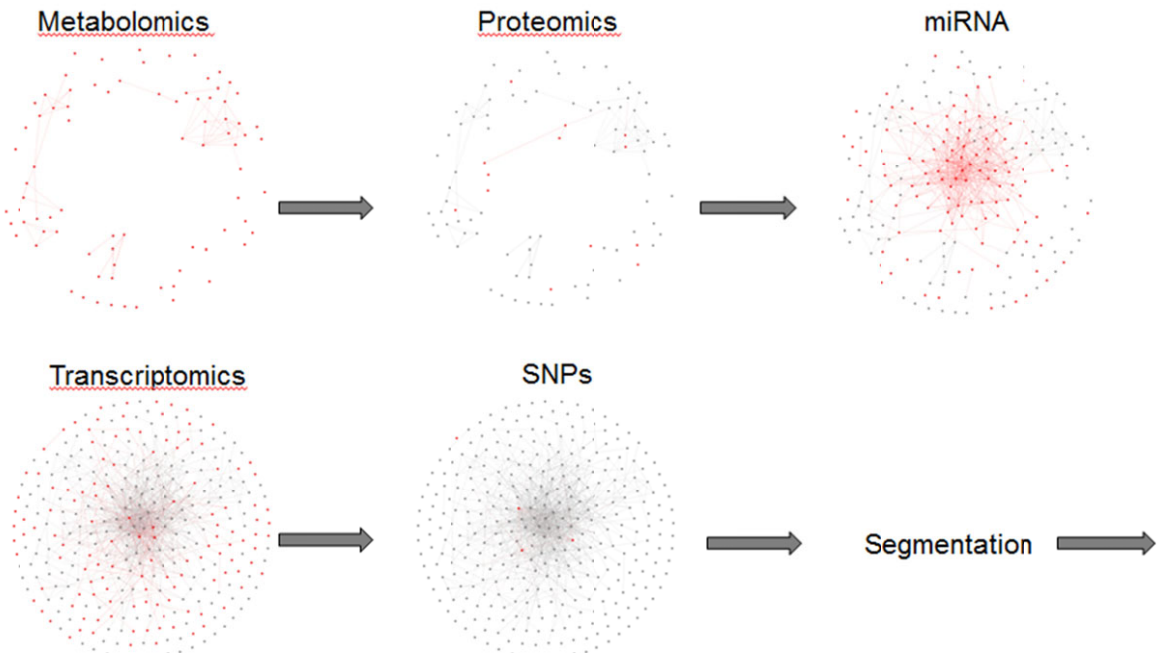

Figure 10A. ARF specific molecular network of the various omics categories

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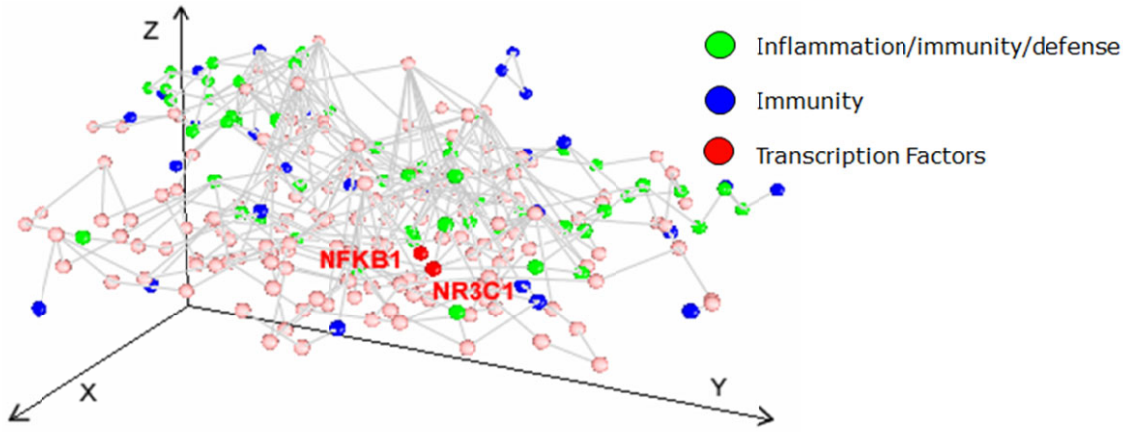

Figure 10B. Inflammation network in donor kidneys which subsequently developed post-transplant ARF. The colour code refers to the gene ontologies of the individual features (34).

4.2 The Inflammatory Response

The interpretation of the transciptomics findings is unambiguously that inflammation in the kidneys occurs in the deceased donor before the organs are harvested. This fits very well to the above described events that occur during the stay of the donor in the ICU such as the systemic inflammatory response syndrome (SIRS), renal hypoperfusion due to arterial hypotension, diabetes insipidus and vasopressor use. The observed renal inflammation can not only be seen on the RNA level but is also transcribed in the expression of proteins that are the main effectors of the inflammation cascade such as endothelial adhesion molecules. Immunohistochemical staining of the three main representants ICAM-1, VCAM-1 and ELAM-1 showed a strong signal in donor kidneys that subsequently developed post-transplant ARF but was virtually absent in donor kidneys that exhibited subsequently immediate graft function (figure 11).

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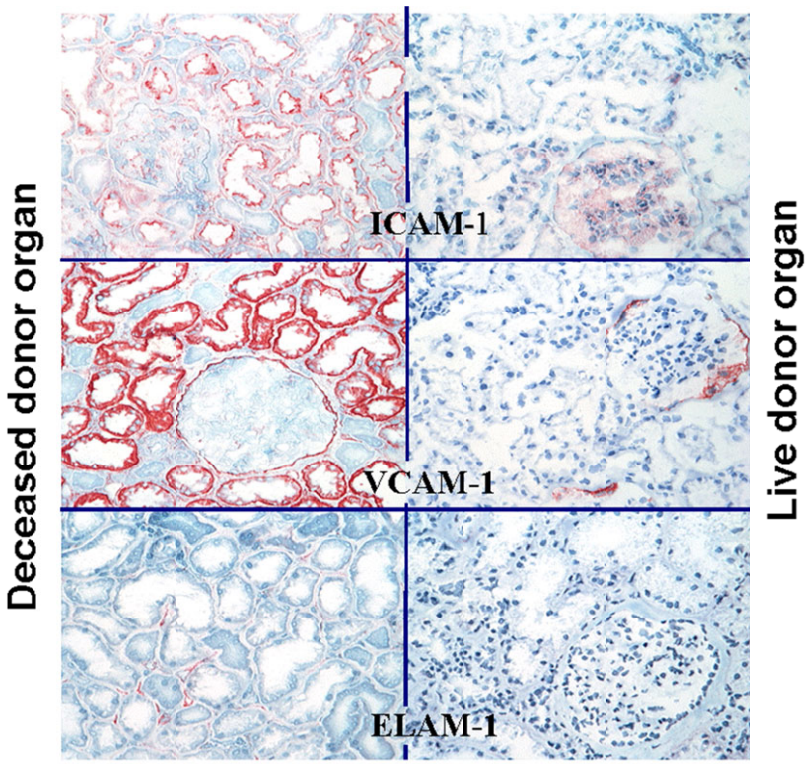

Figure 11. Inflammatory proteins (adhesion molecules) are highly abundantly expressed in kidneys with subsequent development of post-transplant ARF (left panels) but are not detectable in kidneys with subsequent primary allograft function (right panels). (adapted from Schwarz et al (30)). These immunohistochemical stainings show that systemic inflammation in the brain death donor causes upregulation of adhesion molecules in the donor kidneys. It is of notice that the observed association is not necessary causally related to the subsequent risk of ARF and thus the subsequent RCT was designed and conducted (see figure 9 in the next paragraph on RCT).

The observed inflammatory signals in the donor kidneys with post-transplant ARF however were derived from observational studies and thus can only be considered as associational finding. The proof of causality of inflammation as determinant of ARF thus can only be conducted in an interventional study. Therefore, we designed and conducted a RCT to test whether the treatment of inflammation in the donor causes a reduction of the incidence of post-transplant ARF/DGF. DGF is the primary endpoint was defined as the need for more than one post-transplant dialysis. A finer resolution of

the potential effect was the duration of DGF and the number of dialysis session required.

4.3 The RCT – a Logistic Challenge

In this blinded multicentre trial 207 deceased donors were randomized to 1000mg of methylprednislon or placebo single shot injection three to six hours before the organ retrieval. Preimplantation biopsies were obtained to test whether the randomization, timing and dose of the intervention (1000mg methylprednislon) was appropriately chosen to reduce the inflammation cascade on a genome wide level in the kidneys. The biopsies were subjected to microarray testing and the post-transplant course was monitored. A web base case record form (CRF) was used to facilitate the administration of the study and allow for a state of the art online monitoring of the participating centers.

The trial was quite challenging since it is unforeseen where a potential donor will become available and if one is registered, the blinded study medication needed to be carried by the transplant coordinator to the donor site and the verum/placebo injected in the predefined time frame before organ harvest.

The ethical committees of the Medical University of Vienna as well as Eurotransplant approved the study (Ethical Committee of the Medical University of Vienna, Vienna, Austria [study protocol EK-067/2005; to be found at http://ohrp.cit.nih.gov/search]) and the Eurotransplant kidney advisory committee (study protocol 6021KAC06)). A more in depth presentation of the methods and details about the study protocol may be found in the methods section of the publication.

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The trial was powered to detect a 50% reduction in the incidence of post-transplant DGF from a historical 25% of all deceased donor transplants to 12.5% (see power calculation in the screenshot of figure 12).

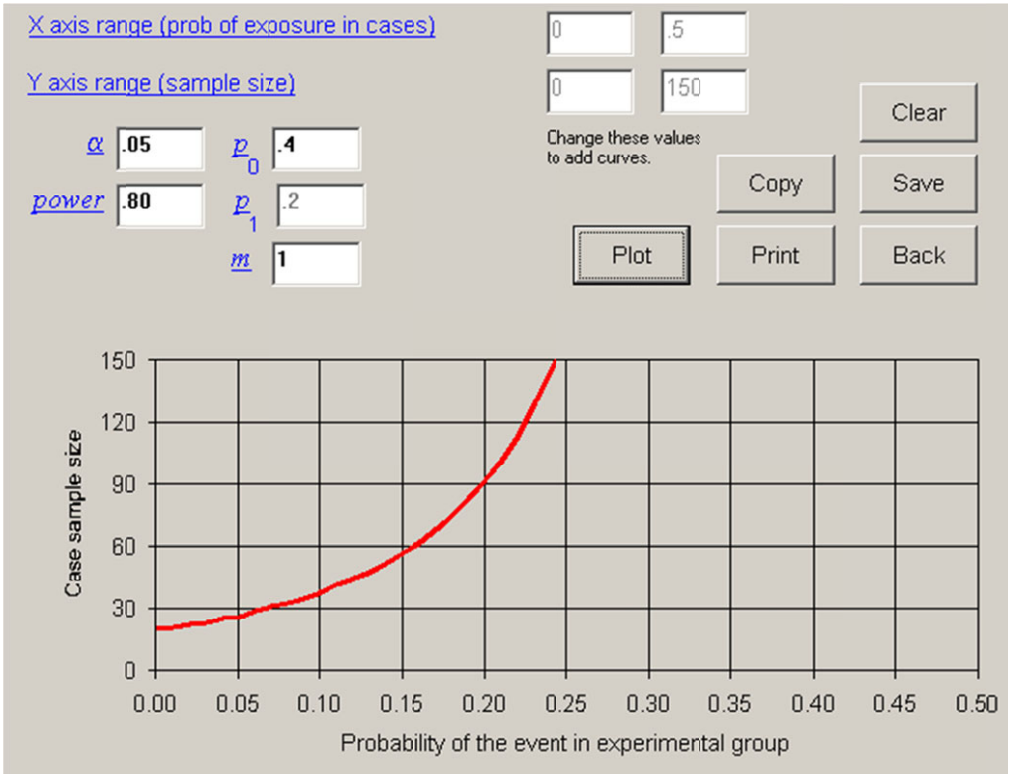

Figure 12. Power calculation of the RCT on the prevention of DGF after deceased donor kidney transplantation. Event rate in the control group was set at 24%, the computed sample size to half event rate was 176. The adjustment of the donor sample size for 8% lost to follow up and non-transplanted (NU … non-used) organ planning for an ITT analysis increased the sample size to 207 [N*=N(1/1-LFU/NU)2=176(1/.92)2=207]

The number of 207 donors needed to be increased to 274 to account for various predefined exclusion criteria including kidneys shipped in the ET region to non-participating centers. The CONSORT flow chart (http://www.consort-statement.org/) of the conducted study is illustrated in figure 13.

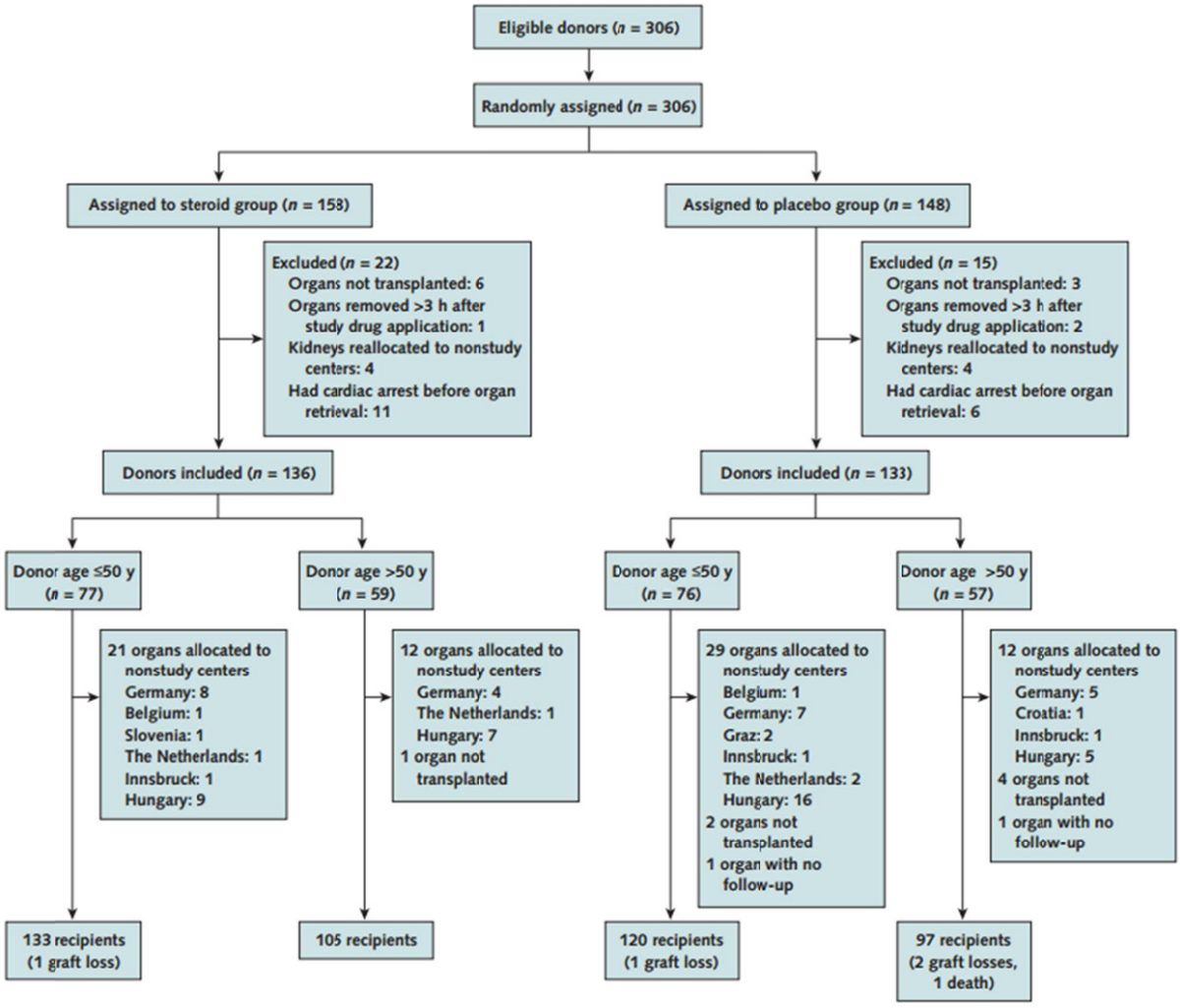

Figure 13. CONSORT flow chart of the steroid donor study (adapted from Kainz A et al. (31)).

Details on the demographic parameters of the study population is provided in table 2.

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Table 2. Demographic characteristics of donors and recipients by treatment assignment

Characteristic	Steroid	Placebo	*P* Value
Donors, *n*	136	133	–
Mean donor age (SD), *y*	47.1 (15.0)	48.5 (14.0)	0.45
Donor sex, *n*			0.65
Female	62	57	
Male	74	76	
Last mean creatinine level of donor (SD)			0.84
µmol/L	78.6 (25.6)	79.5 (34.4)	
mg/dL	0.88 (0.29)	0.90 (0.39)	
Vasopressors used, *n*			0.08
No	22	12	
Yes	114	121	
Multiorgan donor, *n*			0.17
No	106	94	
Yes	30	39	
Cause of death, *n**			0.89†
Trauma	42	36	
Intracranial hemorrhage	87	91	
Cardiac arrest	5	4	
Other	12	11	
Recipients, *n*	238	217	–
Mean recipient age (SD), *y*	49.6 (14.4)	49.2 (14.0)	0.72
Recipient sex, *n*			0.42
Female	76	77	
Male	162	140	
Number of transplants, *n*			0.51†
1	204	193	
2	23	20	
3	8	4	
4	2	0	
5	1	0	
Mean cold ischemic time (SD), *h*	16.9 (13.4)	16.9 (15.3)	1.00
Latest mean panel reactive antibody (SD), %	6 (17)	4 (12)	0.18
Mean HLA mismatches (SD), *n*	3 (1)	3 (1)	0.35

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The transcriptomics experiments on randomly selected 20 biopsies, 10 of each intervention group (steroid/placebo) were conducted on cDNA microarrays holding 41,421 features (batch SHEO) from the Stanford University Functional Genomics core facility. Data analysis was performed by unsupervised hierarchical clustering of biopsies by gene expression (figure 14). The y-axis shows the grouped gene signatures by ontologies. The colour code below the x-axis indicated the relative up- (red) or downregulation (green) of features compared to a set of reference transcripts (Agilent). As clearly indicated, the steroid pre-treatment of the donors led to a reduction of many features of the inflammation cascades (top lines) and activation of metabolism genes (bottom lines). Based on these data we were confident that the coordination, timing, dosing and intervention of this blinded and logistically challenging worked fine.

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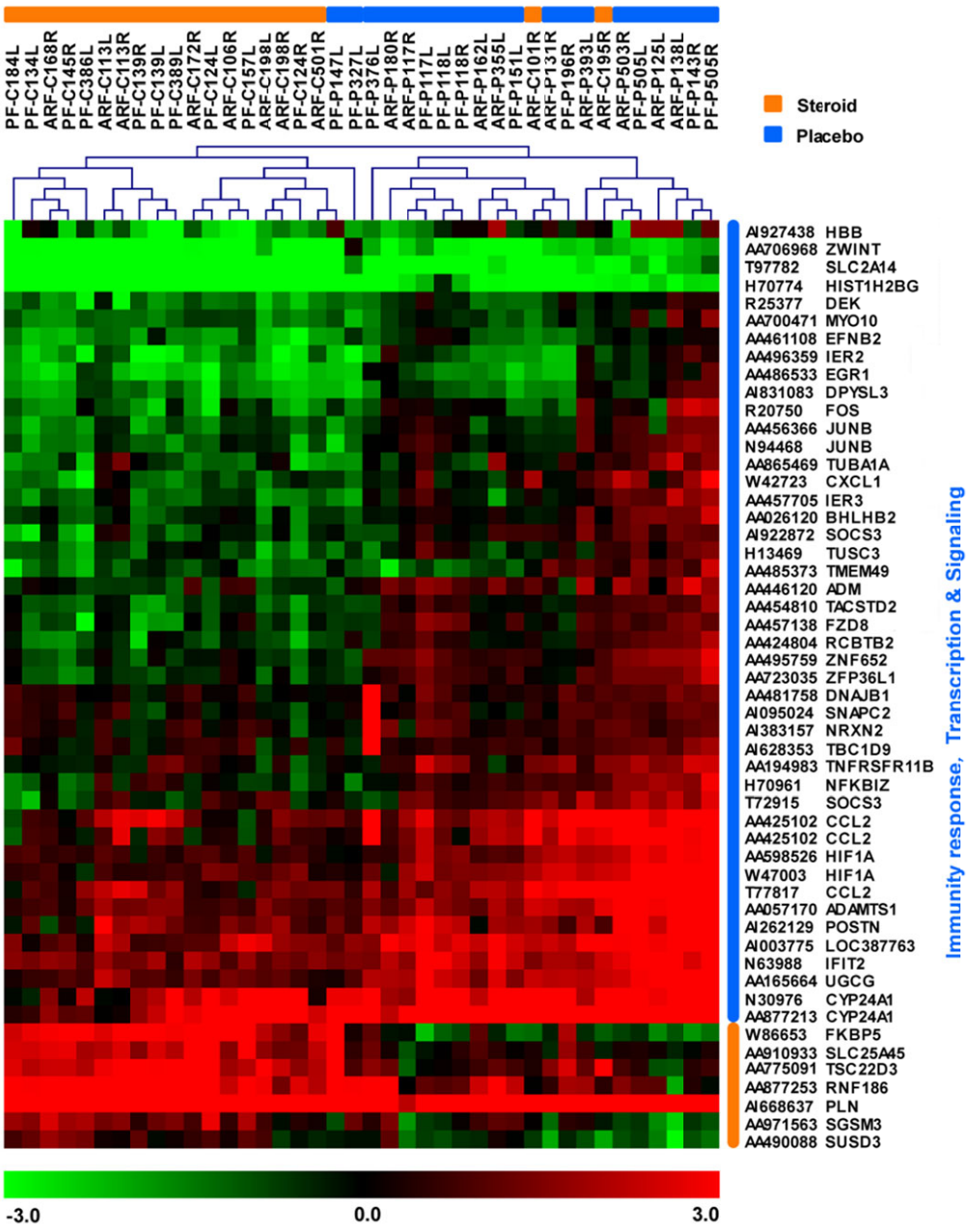

Figure 14. Unsupervised hierarchical clustering of biopsies by gene expression in the two treatment groups. The gene ontologies of differentially regulated genes are indicated on the right side of the figure. The colour code at the bottom shows the x-fold differential regulation of features vs a standard Stratagene Universal human reference amplified RNA (Agilent).

4.4 Results on DGF in the RCT

Despite the global suppression of inflammation in the donor organs by steroid treatment, the incidence of DGF was not different between groups (table 3).

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Table 3. The incidence of post-transplant DGF/ARF in the steroid group was 22% and 25% in the placebo group respectively. Also the number of required post-transplant dialysis was not different between the two groups.

	Steroids	Placebo	p-value
% Pts requiring dialysis during the first 7 days (0/1/>1)	65/13/22	63/12/25	0.700
Number of dialysis during the first 7 days (0/1/2/3/4/5)	154/32/18/28/2/4	137/27/27/18/8/2	0.115

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Accordingly, the resolution of DGF as indicated by creatinine trajectories remained unchanged after donor steroid treatment (figure 15).

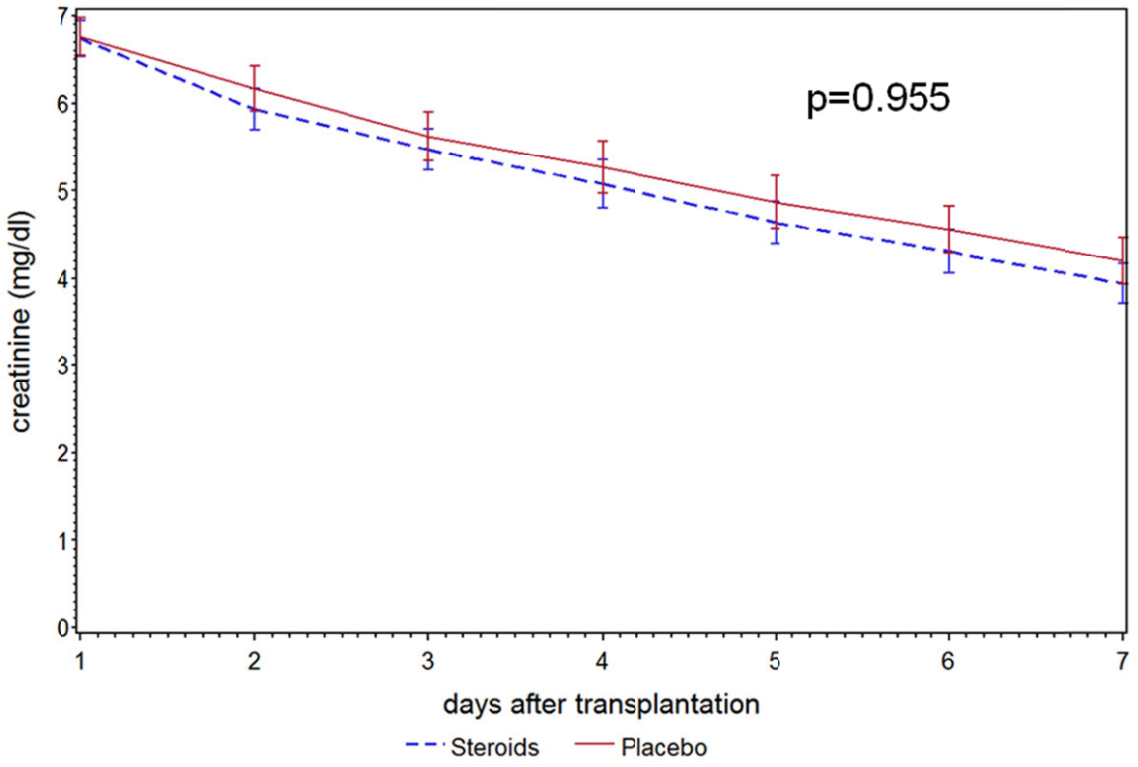

Figure 15. Trajectories of post-transplant creatinine by randomization group. The p-value refers to the F-test in the mixed linear model for longitudinal data.

As secondary endpoint of this labour, time and cost intensive academic study, the initial graft function of other transplant organs of the enrolled deceased donors have been evaluated. Amatschek et al. showed that steroid pre-treatment did not reduce that degree of injury and graft patency in liver allografts (figure 16) (35).

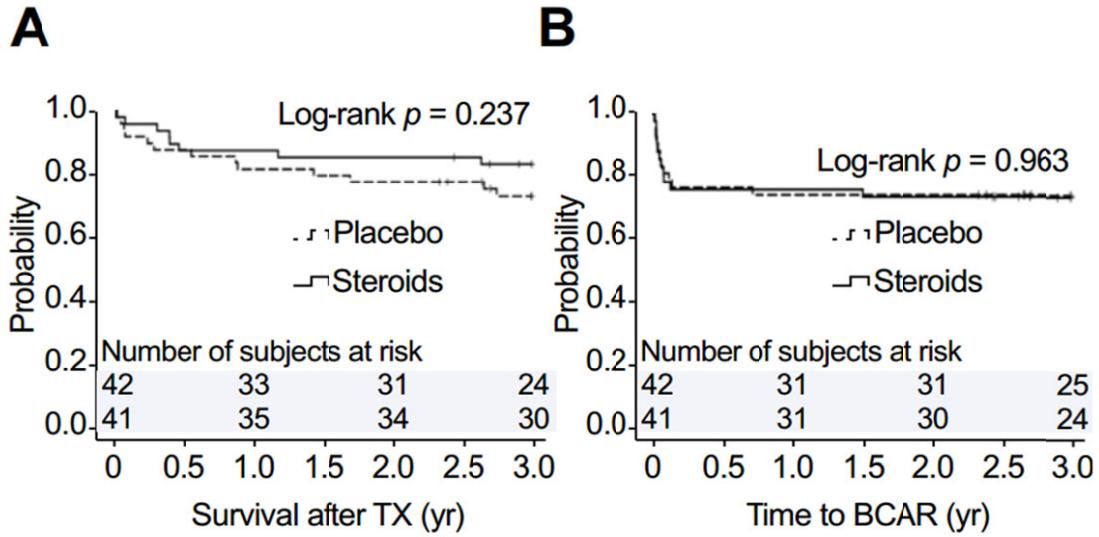

Figure 16. Kaplan–Meier estimates of (A) survival (p = 0.24) and (B) biopsy confirmed rejection (log-rank p = 0.96) risks are shown for steroid pre-treated livers and placebo. The number of subjects at risk is provided above the x-axis. Given p values were derived from log-rank tests. BCAR, biopsy-confirmed rejection; TX, transplantation. (adapted from Amatschek A et al. (35)).

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4.5 Effect of Steroid Donor Treatment on other Transplanted Organs

Steroid pre-treatment did not effect on the incidence of rejections or mortality. Furthermore, the trajectories of the transaminases showed no impact in the degree of peri-transplant liver injury by steroid pre-treatment. Post hoc analysis of interactions between treatment and baseline risk factors, i.e. donor characteristics is illustrated in the forest plot in figure 17.

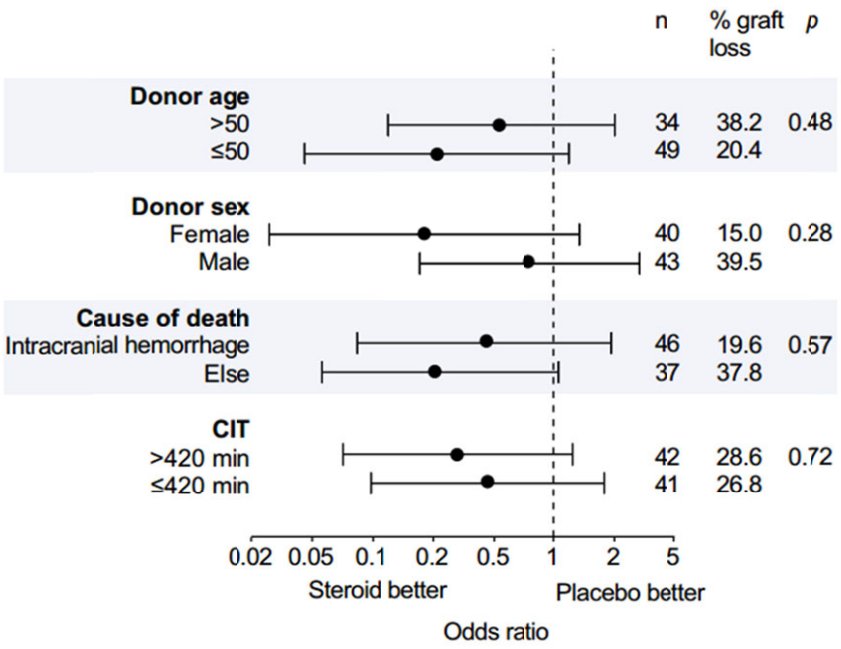

Figure 17. Interaction analysis of graft loss by various donor characteristics and treatment group. No effect modification by the steroid intervention could be observed (from Amatschek A et al. (35)).

4.6 The miRNA Discoveries

Since it became clear that the suppression of inflammation in the donor organs was not sufficient to change the early outcomes ARF/DGF after engraftment, novel approaches were investigated assuming that other contributors than inflammation were causally responsible for initiation and maintenance of ARF leading to DGF after transplantation. In order to cover a wider range of regulatory target pathways than steroid treatment

could accomplish, the microRNA (miRNA) expression in the organs at risk of ARF were determined.

MicroRNAs (miRNAs) are a class of small non-coding 18 to 24 nucleotide-long RNAs that have been implicated recently in diverse cellular functions. miRNAs interfere in translation of mRNAs for a wide variety of proteins regulating cell proliferation, cell death, cellular morphogenesis and differentiation. Production and function of miRNAs requires a set of proteins summarized in the term miRNA machinery (figure 18). A recent summary of the genesis of miRNA, their contribution to pathologies in the transplant kidney as well as potential therapeutic applications may be found in the review by J. Wilflingseder et al. (36).

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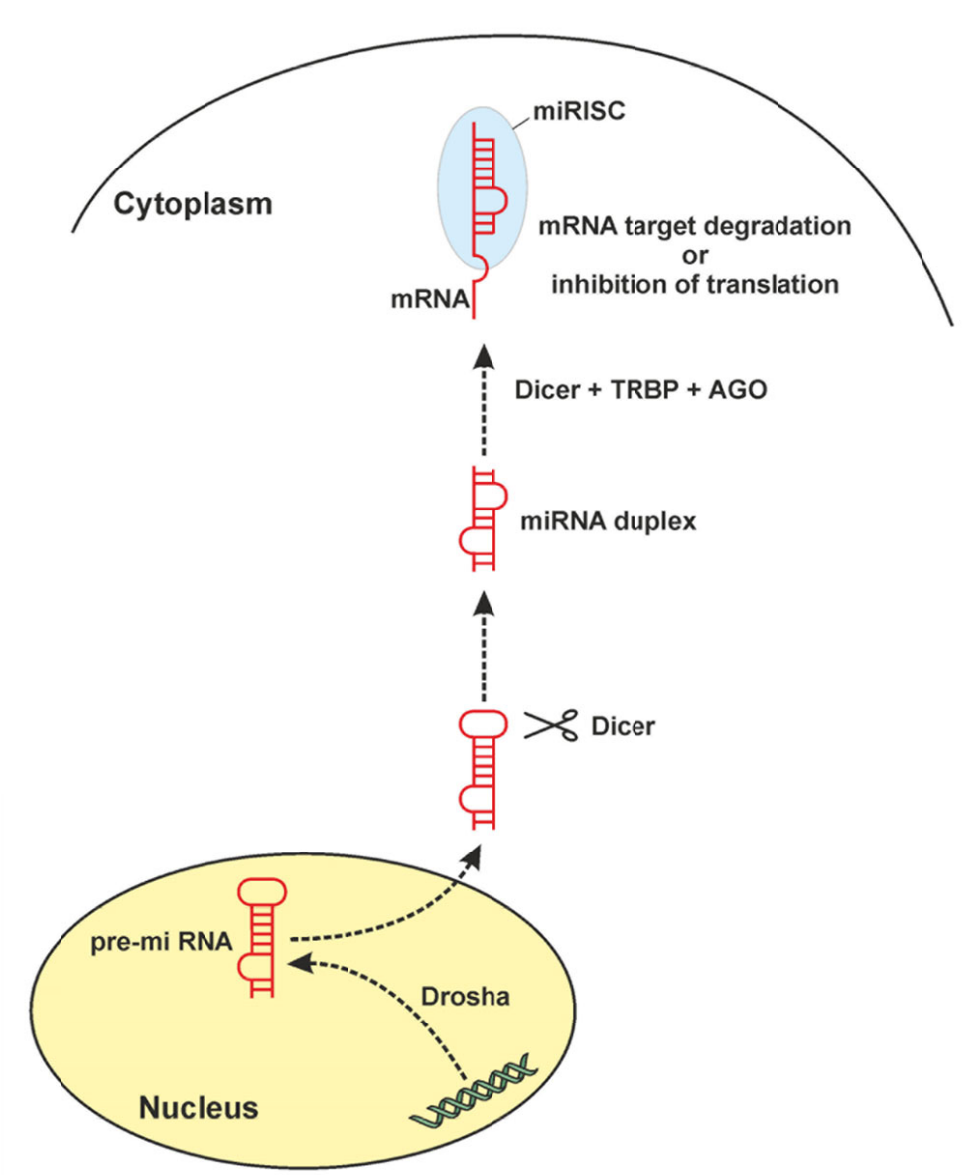

Figure 18. Scheme of miRNA processing and its effects on transcription and translation

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The simultaneous analysis of mRNA and miRNA transcripts in the ARF compared to protocol biopsies yielded several features that remained statistically significant after adjustment for multiple testing using the SAM false discovery rate <10% penalization. The significant features of both analysis in aggregate form are displayed in figure 19 (37).

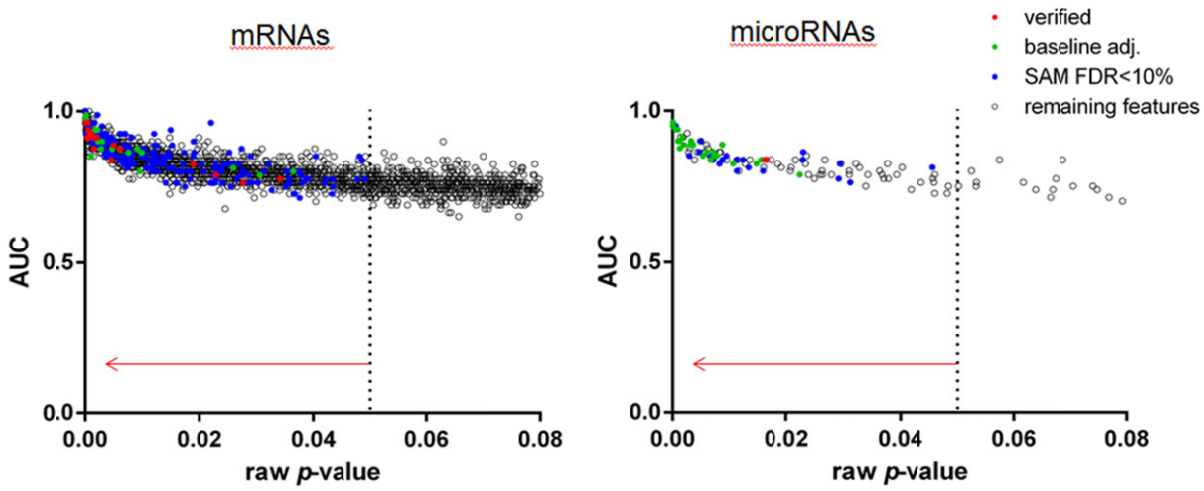

Figure 19. Molecular features discriminating DGF from primary graft kidney biopsies according to their p-value of significance.

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4.7 miRNA Target Prediction

The prediction of miRNA targets is currently mainly based on computational prediction and only few target proteins have been experimentally validated. Since the available prediction routines yield rather inhomogeneous results, we designed a web-based computational algorithm that allows the integration of all available routines and individual adjustment of the significance threshold (figure 20). This algorithm coined miRway is accessible on my lab-website at http://mirway.nephrogene.at/MirWayFrontend/faces/start.xhtml

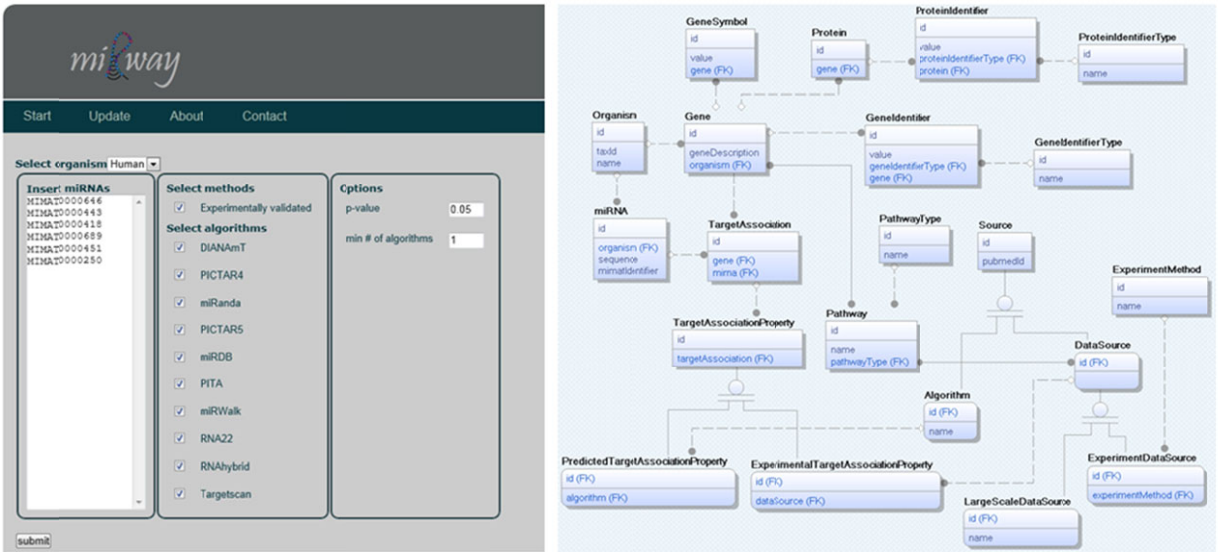

Figure 20. Screenshot of the miRNA target prediction frontend MIRWAY (left panel) and the underlying database (right panel) (Ivcevic S, Stütz C et al. 2014 personal communication).

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We identified miR-182-5p as the best discriminator between DGF and primary patency (figure 21).

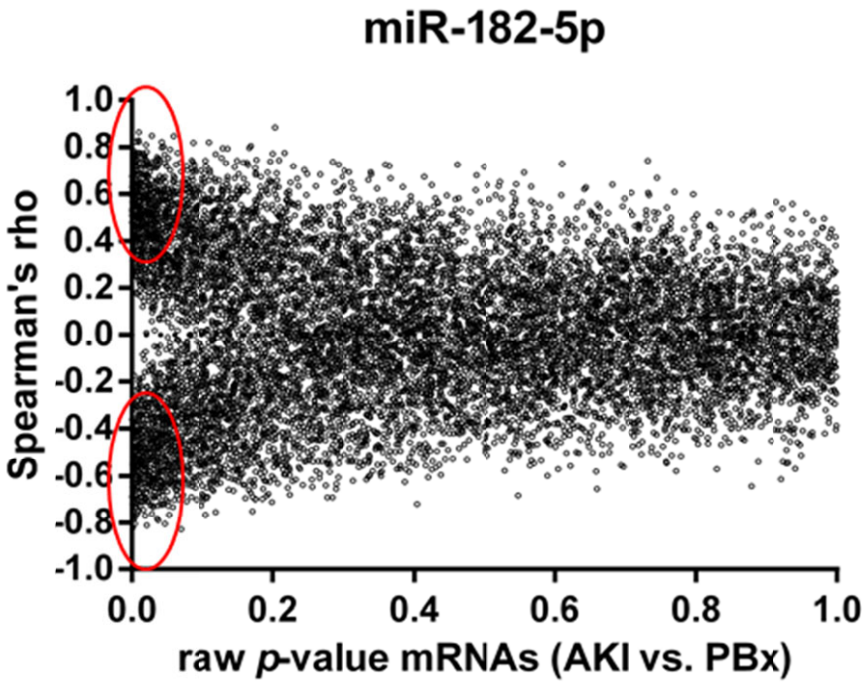

Figure 21. Statistically significant discriminator mRNA leads of miR-182-5p targets for ARF vs PF.

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Target candidate proteins were identified and showed many proteins that have been presented to be involved in ARF regulation by cell cycle regulation and repair such as cycle regulators such as PCNA, cdc2, cdk4 or clock and transcription factors such as FOXO3 (figure 22).

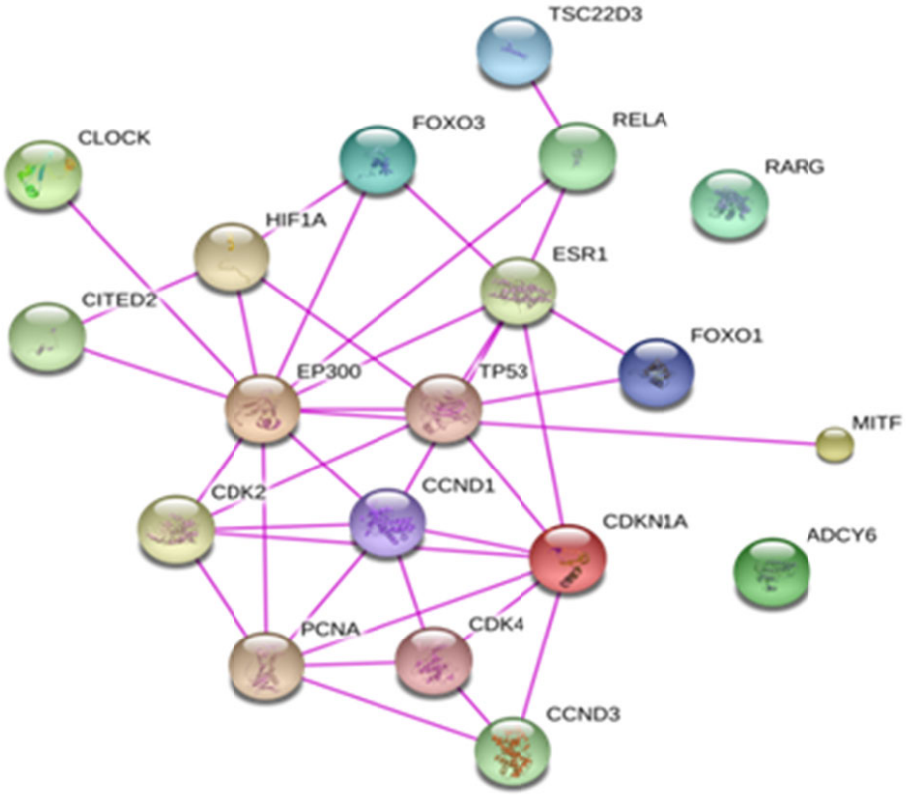

Figure 22. Network regulation of predicted miR-182 target proteins. Several plausible cell cycle regulators such as PCNA, cdc2, cdk4 or clock and transcription factors such as FOXO3 were identified as candidates.

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Validation of the array experiments for the lead miRNA-182 was performed by qPCR and showed similar regulation (figure 23).

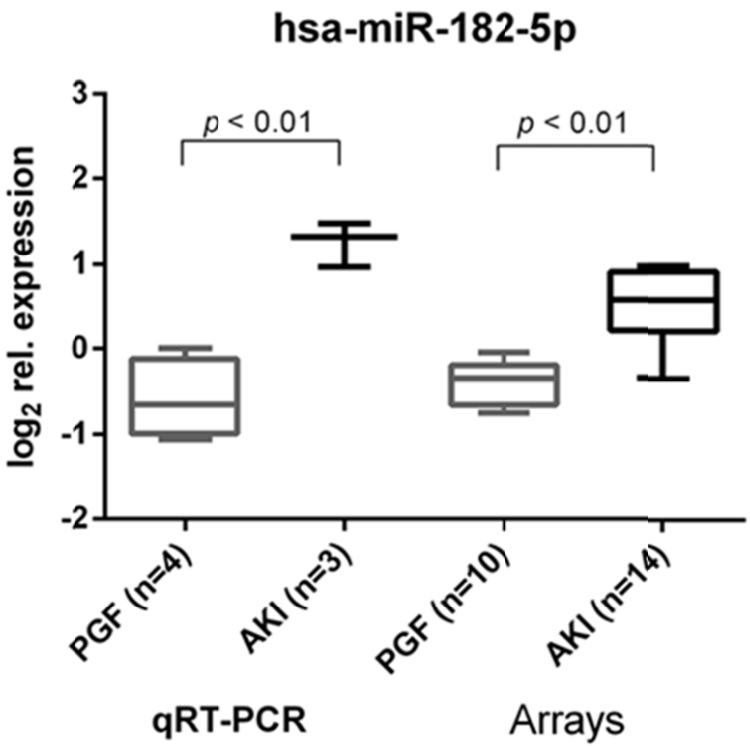

Figure 23. Validation of miR-182 array data by qPCR for grafts with **p**rimary function (PGF) vs those that developed acute k**i**dney injury (AKI).

4.8 In vivo Kinetics and Efficacy of Antisense Oligonucleotides

In experimental rat studies we found longer time ago, that most of systemically administered short (18-22mers) and synthetically derived oligonucleotides (antisense DNA) end up in the kidney. Specifically, about 50% of the injected dose is reabsorbed in the proximal tubule epithelial cells of the kidneys and remain stable inside the tubule cells for at least four days (figure 24) (27).

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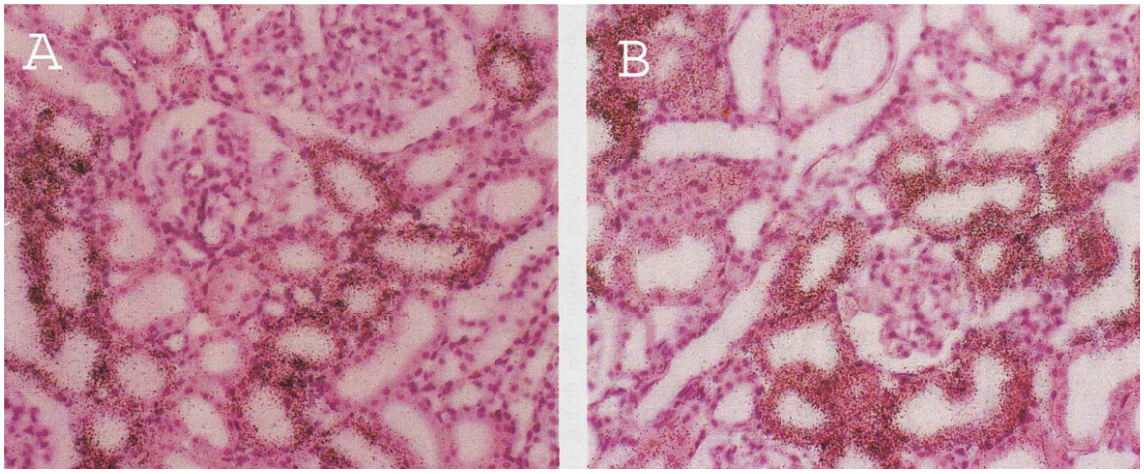

Figure 24. Uptake of the antisense oligonucleotides in the proximal tubule epithelial cells four hours (A) and four days (B) respectively.

In urine, degraded products appear as early as few hours after injection. Electron microscopic studies showed that the oligonucleotides are not trapped in endo-lysosomes and thus may be suitable for antisense inhibition of target mRNAs and miRNAs.

This hypothesis was tested in vivo by inhibiting the mRNA of the sodium-phosphate cotransporter NaPi2a. NaPi-2a was chosen because this transporter is located in the proximal tubule and is responsible for more than 90% of the tubular phosphate reabsorption. We showed that a specific inhibition of NaPi2a by antisense oligonucleotides was possible and caused a decrease in phosphate transport across the apical membrane of the proximal tubule cell (25). The kinetics of the phosphate reabsorption in isolated brush border membrane vesicles is displayed in figure 25.

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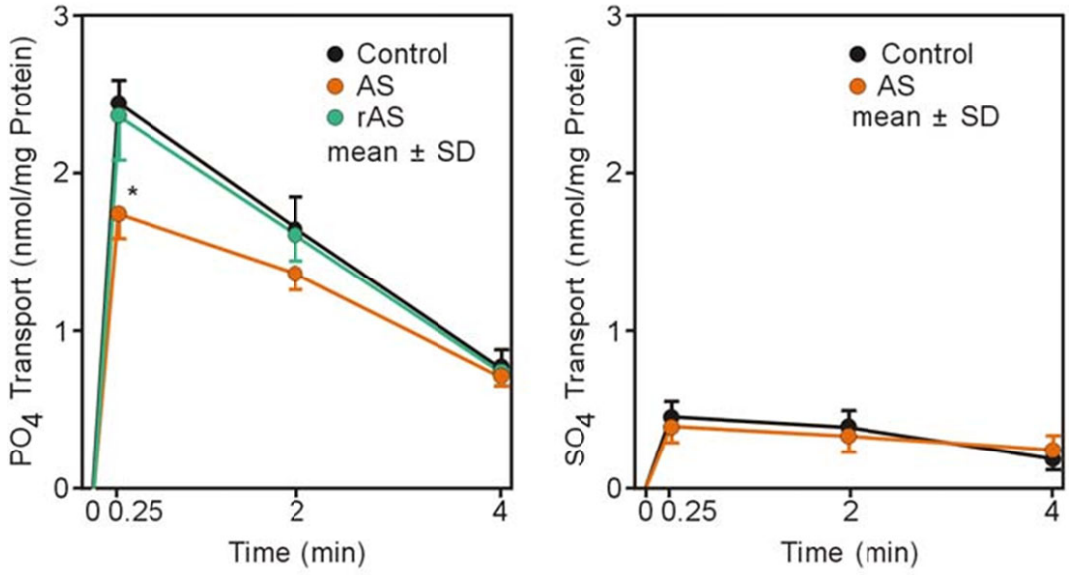

Figure 25. Selective inhibition of the renal sodium phosphate cotransporter NaPi2a by antisense oligonucleotides (AS). Reversed sequences of the AS (rAS) and scrambled controls and the unaffected sodium sulfate transporter were used as reference (adapted from Oberbauer R et al. (38)).

4.9 Clinical Applicability and Utility

4.9.1 In vitro Data

Having identified miR-182 as candidate interventional lead, we designed antisense oligonucleotides (ASO, anatgomiRs) against miR-182 and evaluated their selectivity and efficacy of miR-182 inhibition in cell culture of human renal proximal tubule epithelial cells (HRPTECs).

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Studies showed a dose dependent inhibition of the target miRNA in vitro (figure 26).

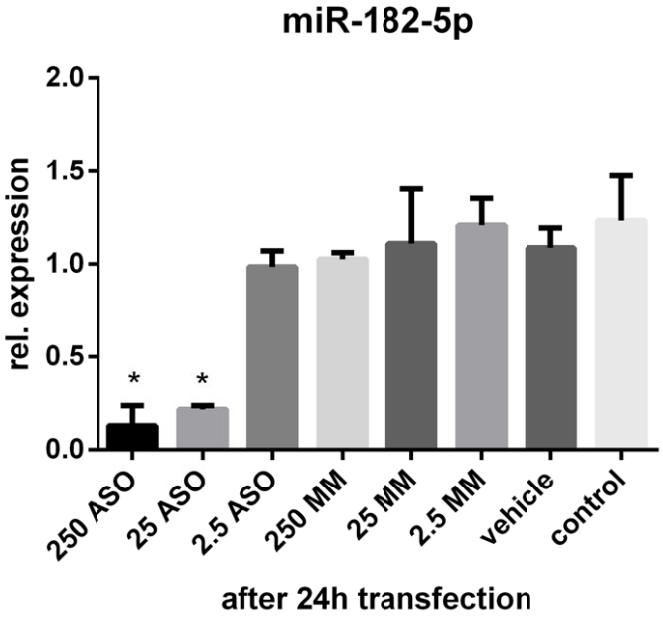

Figure 26. AntagomiR inhibition of miR-182 in cultured HRPTEC in vitro. Doses of 25mM antagomiR-182 (ASO) blocked the target miRNA, mismatched controls (MM) as well as vehicle and saline control did not affect miRNA abundance in the PCR experiments.

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4.9.2 In vivo data

Acute renal failure was induced in SD rats by right uninephrectomy and clamping of the left renal artery for 40 minutes (figure 27).

Figure 27. Rat model of ARF. Animals were subjected to sided uninephrectomy and cross clamping of the left renal artery for 40 minutes.

Rats were pretreated 12 hours before insult with either anatgomiR-182 (ASO 2.5 and 25mM) or equal concentrations of mismatched antisense oligonucleotides. Intrarenal expression of miR-182 remained selectively reduced in the anatagomiR treated rats (figure 28).

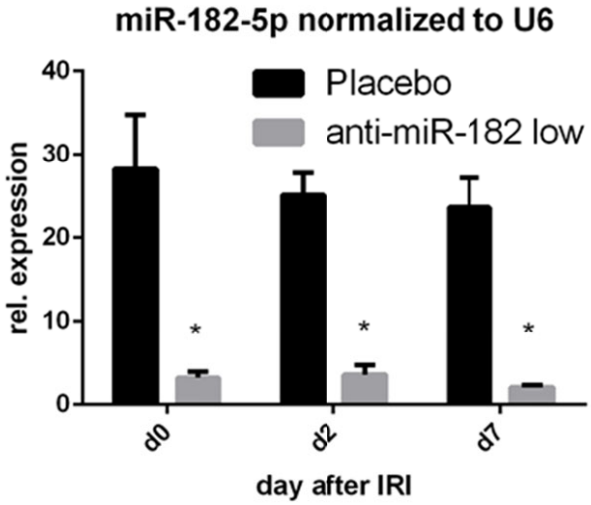

Figure 28. Intrarenal expression of miR-182 at various time points after ischemia reperfusion. Rats were pretreated with either antagomiR (ASO) or mismatched antisense 12 hours before induction of injury.

The trajectories of serum creatinine were determined daily for seven days after the injury. Histological evaluation of kidney was performed on days two and seven after insult. AntagomiR-182 (ASO) treatment led to an amelioration of the functional (creatinine and BUN) and morphological insult as evidence in figures 29, 30 and 31 respectively.

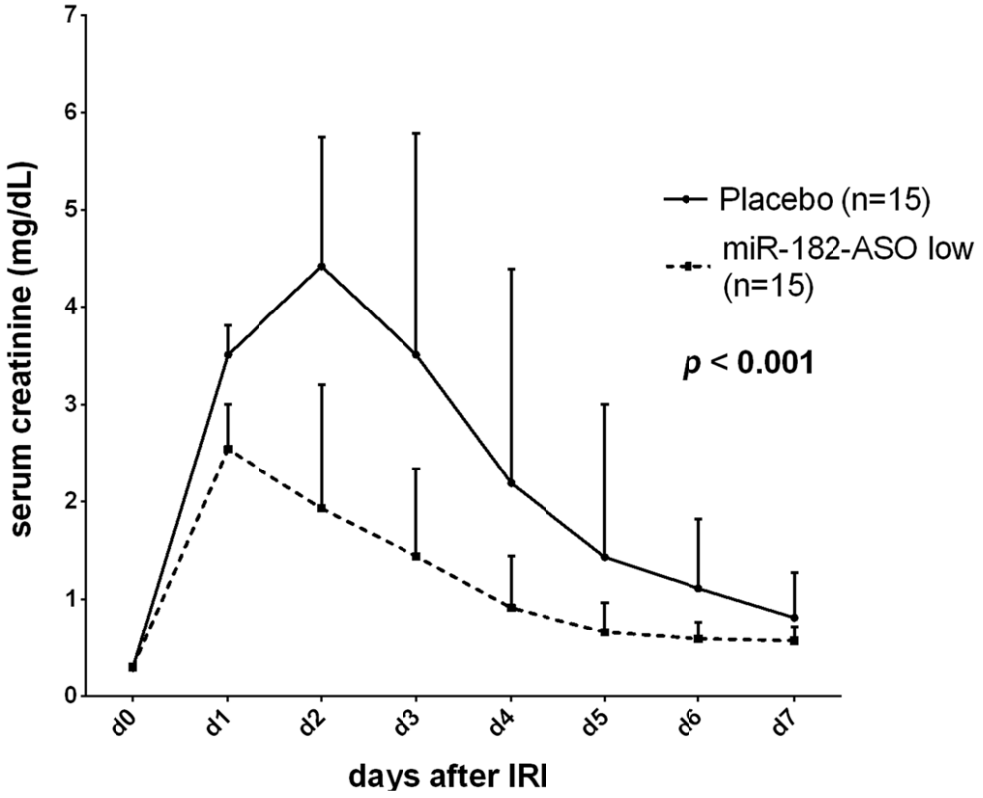

Figure 29. Trajectories of serum creatinine over the first seven days after ischemia reperfusion injury in rats. ASO low refers to an antimiR concentration of 2.5mM or equal concentrations of mismatched control.

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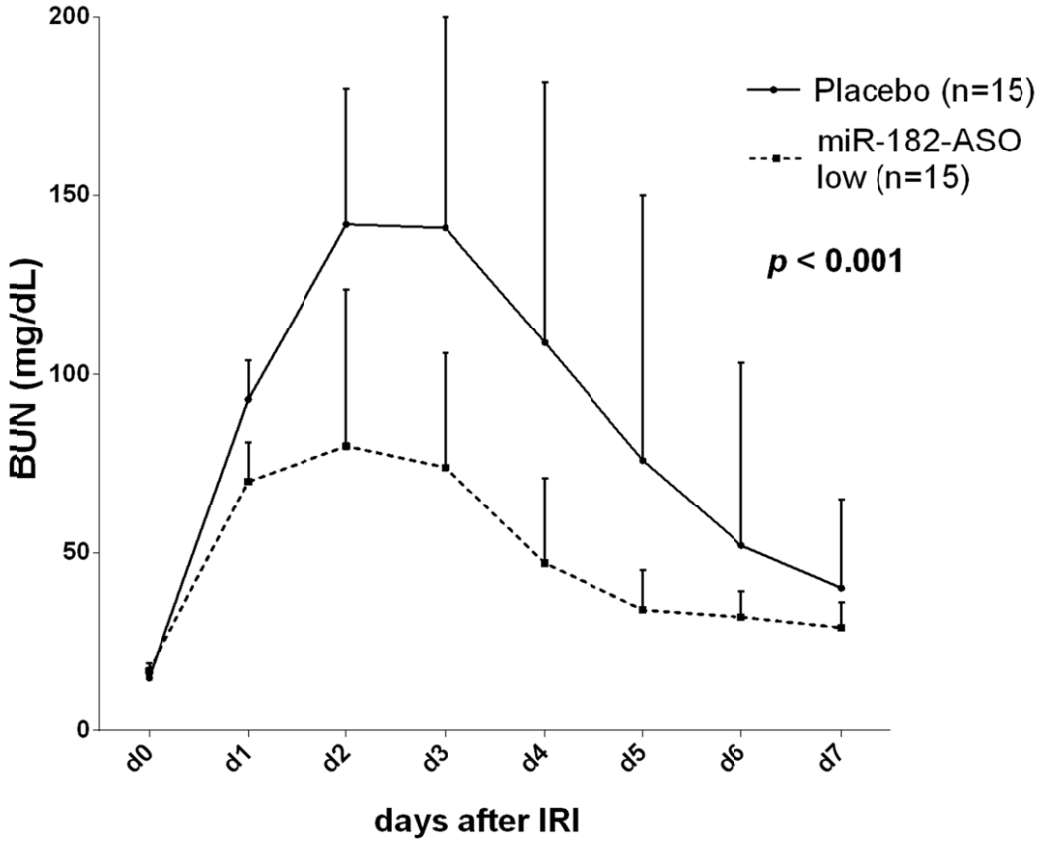

Figure 30. Trajectories of BUN over the first seven days after ischemia reperfusion injury in rats. ASO low refers to an antimiR concentration of 2.5mM or equal concentrations of mismatched control.

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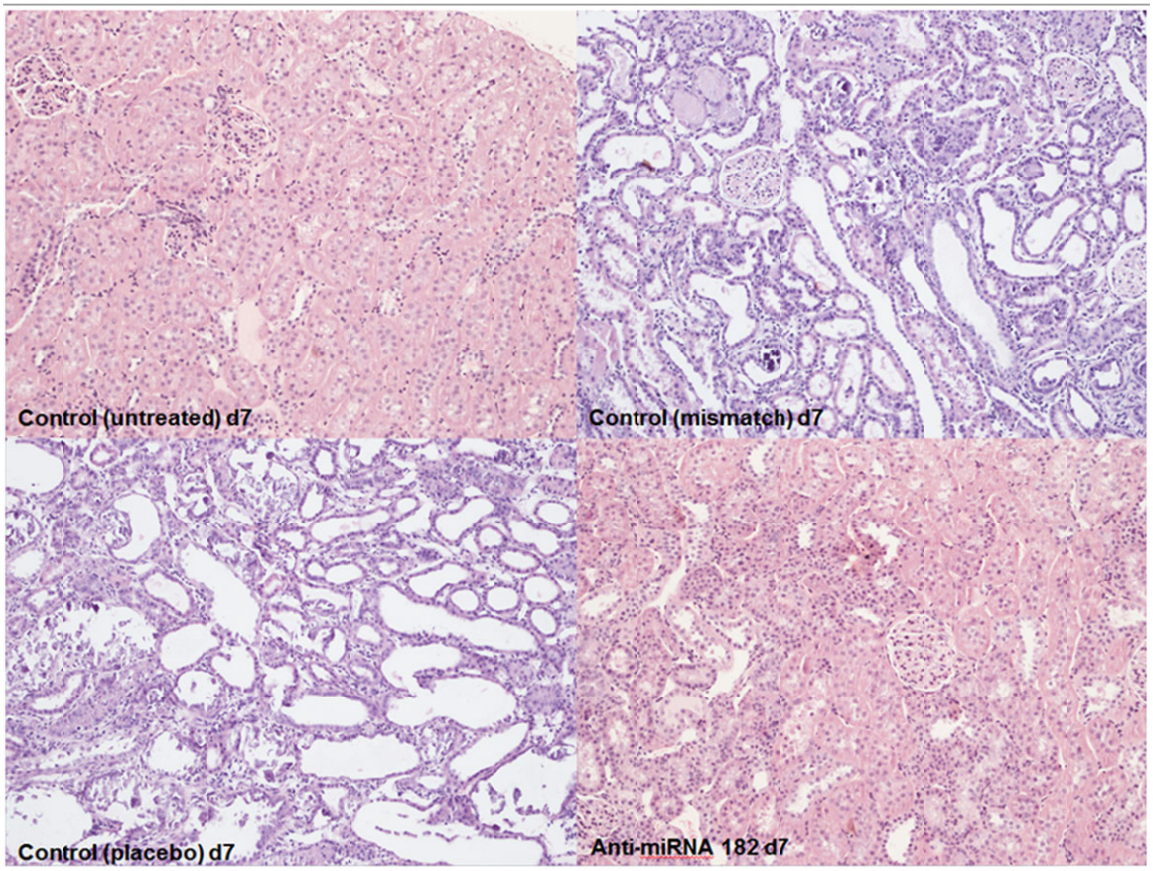

Figure 31. Histomorphology of representative rat kidneys of anti-miR and mismatch treated as well as untreated controls. Damage could be dramatically reduced by the antimiR pretreatment as indirectly also evidence by intact brush border alkaline phosphatase production and thus different colour intensities are corresponding to the degree of injury. The treatment group and time after injury in days is indicated in the left lower corner. ASO (lower right) treated rats showed dramatically less injury compared to the control (placebo and ASO-mismatch) groups.

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Morphological evaluation of the various treatment groups was performed by a blinded pathologist. Injury severity was scored semiquantitatively from 0 to 3. The number of evaluated kidneys per groups and time after ischemia reperfusion as well as histopathology scores are provided in figure 32.

Group	day	Code	kidney	Overall Injury
anti-miR-high	7	R84L	L	1
anti-miR-high	7	R86L	L	1
anti-miR-high	7	R98L	L	1
anti-miR-low	2	R102L	L	1
anti-miR-low	7	R61L	L	1
anti-miR-low	7	R65L	L	1
anti-miR-low	7	R68L	L	2
anti-miR-low	2	R95L	L	1
MM-high	7	R47L	L	3
MM-high	7	R90L	L	3
MM-high	7	R92L	L	3
Placebo	2	R101L	L	1
Placebo	7	R37L	L	3
Placebo	7	R38L	L	3
Placebo	7	R76L	L	3
Placebo	2	R93L	L	2
Placebo	2	R96L	L	3
anti-miR-high		R86R	R	0
anti-miR-high		R98R	R	1

Figure 32. Semiquantiative scoring of the injury severity by treatment class and time after IRI. Anti-miR (ASO), MM (mismatched ASO), low refers to a dose of 2.5mmol, high to 25mmol.

The long-term effects of the ASO intervention can certainly not be evaluated in these seven day experiments but it is interesting that already at seven days, genes belonging to the ontologies of fibrosis were markedly suppressed in ASO treated rats vs control (figure 33).

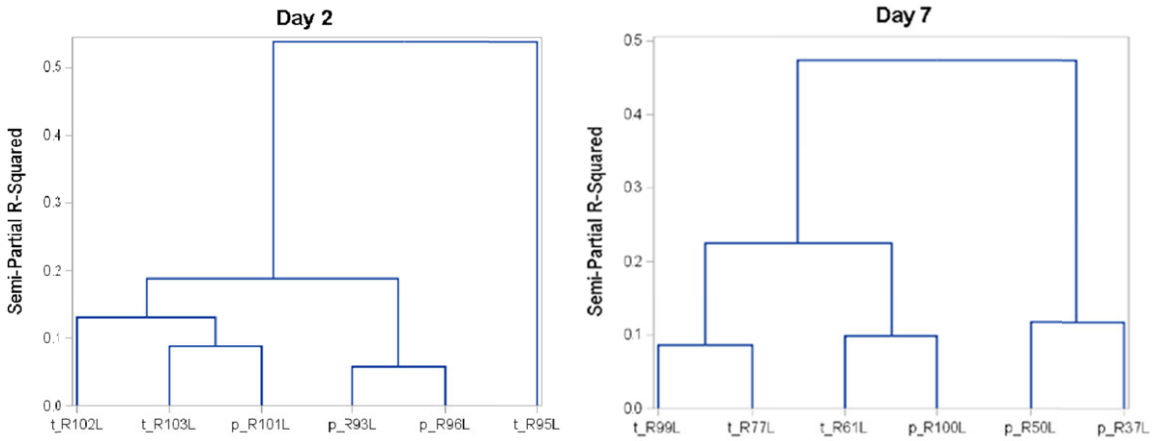

Figure 33. Unsupervised clustering of gene ontologies of fibrosis pathways in randomly selected three ASO and three control treated rat kidneys two and seven days after injury. ASO (antimiR-182) treated rats (indicated as t_) and placebo (indicated as p_) did not discriminate at day two but already at day seven suggesting also potential of long term efficacy of ASO (antimiR-182) in fibrosis prevention.

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The following 20 genes were selected on the basis of public domain databases such as GEO (table 4).

Table 4. Fibrosis and connective tissue markers represented on the Affymetrix platforms. These ontologies were specifically analysed in order to elucidate whether ASO (antiMir-182) could act on prevention of fibrosis as the long-term consequence of ARF.

Fibrosis marker	Collagens
AngII	COL4A1
AT1	COL4A2
AT2	COL4A3
AGE	COL4A4
RAGE	COL4A5
TGFB1	COL4A3bp
TGFBR2	
TGFBRI	
IL1B	
TNFA	
SMAD2	
SMAD3	
SMAD4	
SMAD7	
IKBA	
P50	
P65	
ERK	
P38	

4.9.3 Abundance of miR-182 target proteins in injured kidneys

There are likely many effector proteins of the observed effect of anatgomiR-182 (ASO) treatment given the fact that miRNAs regulate a whole network of proteins (see also figure 18). Our custom mir target prediction tool mirway, to be found at http://mirway.nephrogene.at/MirWayFrontend/faces/start.xhtml predicted several cell cycle and cell viability/apoptosis regulatory proteins and adjacent pathways (figure 34).

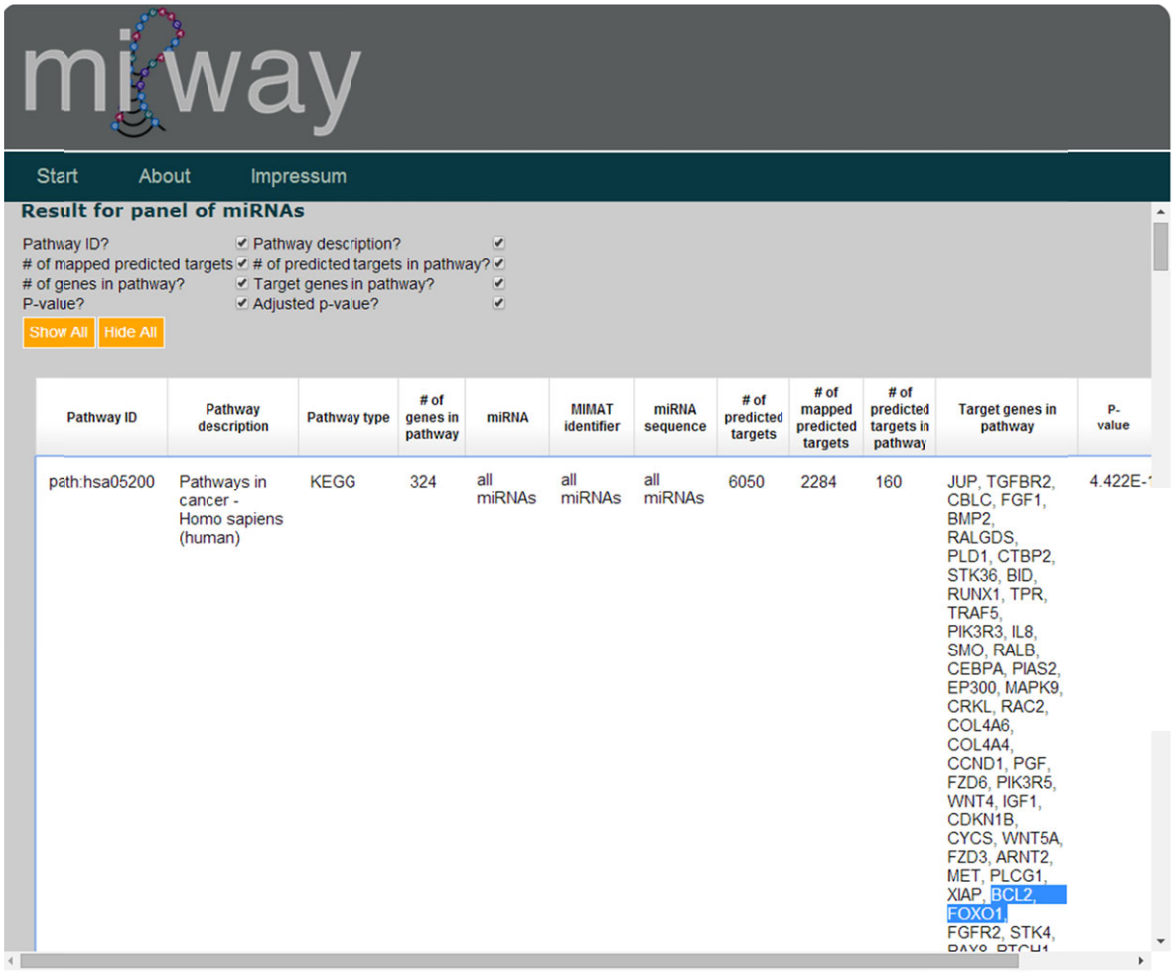

Figure 34. miR-182 target features predicted by our custom mirway bioinformatics tool (© nephrogene).

So far only few of the predicted target proteins have been experimentally validated. However, many targets belong to protein networks of cell cycle regulation and apoptosis. Thus it is of note that suppression of miRs that inhibit cell cycle and

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antiapoptosis would lead to a more efficient regeneration of injured tissue. To test this hypothesis, mice were injected with ASO or placebo control and kidneys harvested after 48 and 96 hours respectively. Western blots of the candidate proteins Bcl.2 and FOXO1 were performed and the results are displayed in figure 35.

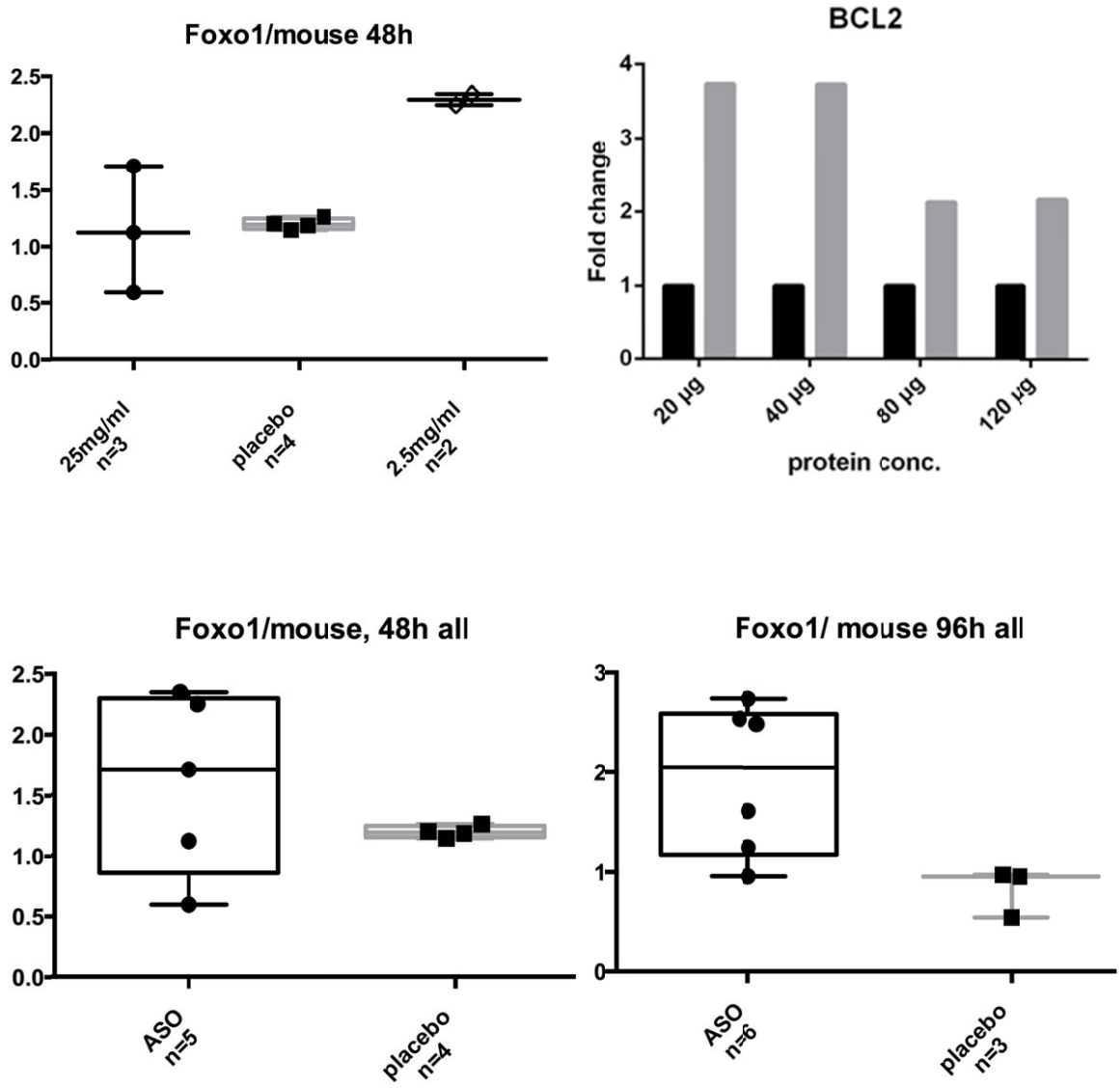

Figure 35. Bcl-2 and Foxo-1 protein are activated (inhibition of inhibitory miRNA) by anti-miR-182 (ASO) versus placebo (MM-ASO). Experiments were performed in mice injected with ASO or placebo.

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After investigations from cell culture to whole animal experiments showed that anti-miR-182 selectively inhibited miRNA-182 activity and activation of target proteins as well as amelioration of IRI in the rat models, we proceeded with studies of anti-miR-182 in ex vivo perfused pig kidneys. Freshly harvested pig kidneys were subjected to ex vivo normotheric machine perfusion with UW solution (University of Wisconsin) solution at a pressure of 80mmHg. The setup of the experiment is illustrated in figure 36.

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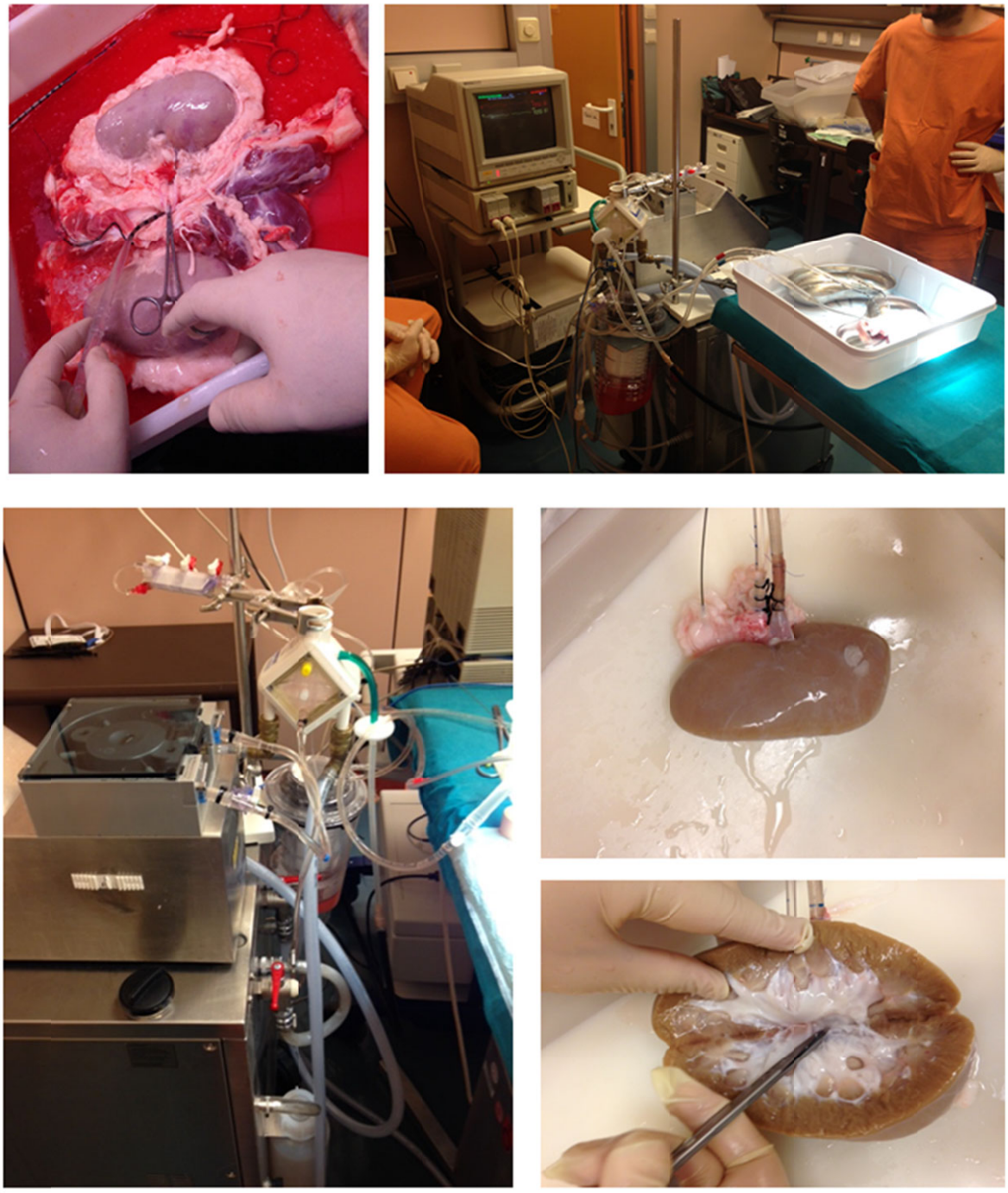

Figure 36. Normothermic machine perfusion of a pig kidney with UW solution at 80mmHg.

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The experimental set up is provided as:

- Run for 6.5 hours
- Antisense oligonucleotide was administered after 30 minutes of perfusion and circulated for 6 hours
- Pressure: 75-90 mmHg (depending on position of the cannulas)
- Flow: 0.14-0.188 L/min (increased in the first 2 hours then remained constant)
- O2 consumption 68% after 30 minutes of perfusion, 48% after 3h of perfusion
- Biopsies were taken regularly (every 2 hours)
  For RNA extraction
  For protein extraction
  For histology
- Urine samples were taken regularly
- Urine produced: 20-40ml/30min, increased slowly

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Quantitative PCR of the biopsies from the pig kidney showed a selective inhibition of miR-182 over the perfusion time of six hours (figure 37). Target proteins were not determined since the half-life of these proteins is considerably longer and the kinetics in isolated perfused organs largely unknown.

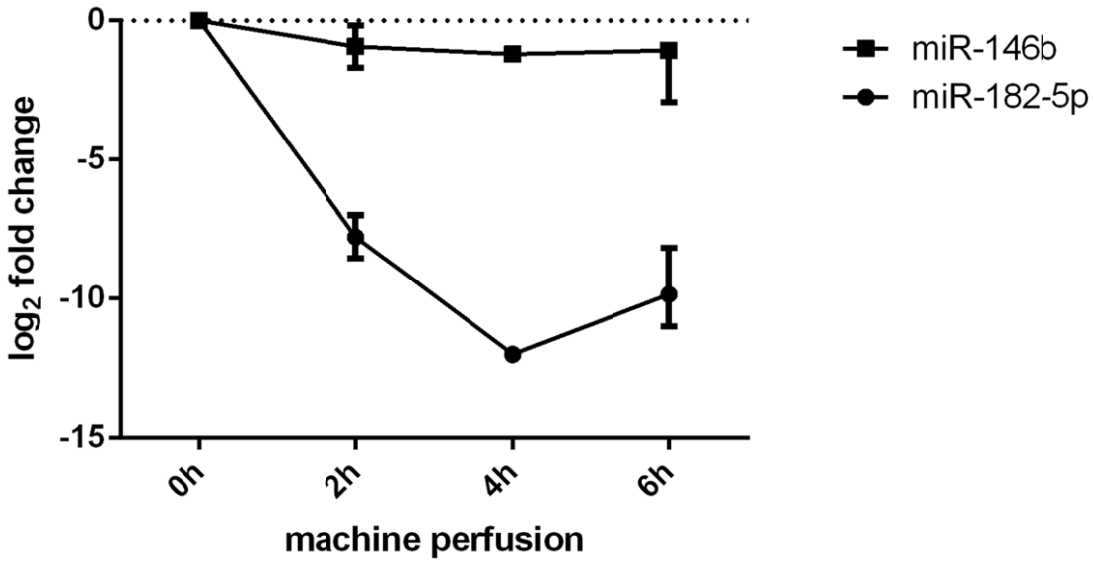

Figure 37. Inhibition kinetics of miR-182 by antisense oligonucleotide in sequential biopsies of normothermic ex vivo perfused pig kidneys. miR-146b was used as control miRNA to test the selectivity of the antisense construct.

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5 Discussion

The endeavour of studying the whole spectrum of acute renal allograft failure from basic molecular levels up to the patient level took one and a half decade and involved many skilled and determined coworkers, who are listed on the nephrogene lab website. When starting in the mid 1990ies the omics revolution just has started and I had the privilege to conduct my research fellowship at Stanford University, a location on the forefront of these ground-breaking discoveries. Stanford built a DNA microarray facility that spotted their own chips on glass slides and as faculty I had the privilege to could obtain these arrays at an affordable price.

Needless to say that many of the appearing technical challenges such as batch variability, incomplete hybridisation, and standardization were not solved then. Accordingly, the bioinformatics tool required to give the ten-thousands of gene expression data a biological meaning were just to be designed and programmed. Nevertheless, it was a great example and excellent exercise one can only gain if involved in the early phases of amazing technologies. The early data suffered from poor external validity and humble reproducibility. The problem was thought to be rectifiable by PCR validation of candidates which however is a different technique with optimized conditions for each gene assayed. Thus results were quantitatively often not directly comparable but in general the qualitative direction of regulation could be confirmed by these experiments. An example may be found in as early studies as Hauser P et al. (38).

Later when technologies became more standardized and custom provider sold their expertise to commercial providers, omics technologies became more standardized and technically sound. We also switched from the custom arrays to commercial provider platforms. It remains still scientifically necessary however to display also the raw data of experiments on public data repositories such as GEO (gene expression omnibus). All the raw data of our omics experiments are there publicly available.

The steroid donor study was a very ambitious project and initially hard to fund. However, the logistically challenging study was conducted as carefully and precise as possible for an academic study with limited funds. The results were sound and a clear recommendation could be given based on our findings, i.e. steroid donor pretreatment does not reduce the rate of post-transplant ARF.

Clearly more thorough investigations could have been done in the human kidney biopsy specimen to tackle also the ARF regulation on the protein level. However, peptide arrays are not yet advanced to a state of reproducibility that is required for such studies. Furthermore, longer follow up of recipients would have been required to uncover potential later effects of steroid pretreatment on clinical entities such as cellular and humoral rejections or the pace of graft fibrosis. It is of note however, that the clinical trial was specifically powered for the primary endpoint of ARF-incidence. In fact, we are currently working on the retrieval of data from study participates to evaluate these outcomes.

The target prediction of mRNA and miRNA leads was conducted in collaboration with experienced bioinformaticians. Given the several redundant but also the many unknown relations and pathways of molecular features, a margin of uncertainty still remains of whether the chosen leads represent the best choice. Further interventional studies with anti-miR-182 however showed that the resource intense in silico work was done properly. We showed that a selective inhibition of miR targets could be achieved ex – and in vivo. Accordingly, the timing and concentrations of anatgomiR treatment were determined by carefully kinetics study but results may not be directly amenable into the human setting, i.e. clinical medicine. In summary several obstacles were observed in this almost two decades of IRI research but most could be overcome by strategic planning and innovative thinking. Thus I may conclude that presented findings are technically and statistically sound and our research helped to advance the field of acute renal failure not only in the transplant setting but also in native kidneys.

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5.1 Further Work

Given the results of the work of the last decade and the stimulating results of the ex vivo perfused pig kidneys, we will test the antisense effect of our construct first in human donor kidneys which are not used for transplantation in a similar ex vivo perfusion. If the results are similar to the pig kidney data, a clinical study will be designed with the ultimate goal to conduct a RCT on the effect of our construct on the rate of DFG in deceased donor kidney transplantation. Supporting data that antisense against target miRNAs can be injected safely in human comes from a recent study in HCV patients (NEJM 2013). MicroRNA-122 Treatment of Hepatitis C - GT1 was safe and caused a sustained viral clearance in patients with previous treatment failure to standard therapy.

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6 Conclusions

In my thesis I showed that the clinically enigma of posttransplant ARF is tightly regulated by a series of molecular processes. On an omics wide basis several predictors belonging to the ontologies of inflammation, immunity and cell death were studied. The main finding was that most of these cofactors are regulated by a few miRNAs which could be used as promising prophylactic and therapeutic intervention. Specifically, miR-182 was identified as key player and inhibition of this miRNA by antisense oligonucleotide in vivo ameliorated the course of ARF. Ex vivo perfusion of pig kidneys with this molecular construct showed suppression of miR-182 as early as two hours which makes this approach potentially useful for the clinical setting of deceased donor kidney transplantation.

In the future I will continue my work and extend the findings into the human setting. Initially I will make use of discarded organs not usable for transplantation to test the kinetics of perfused antisense constructs. Human kidney transplantation is the ideal setting since the organ is ex vivo for several hours and perfusion of donor kidneys has become standard in some countries. Injection of the construct into the perfusion fluid is easy and systemical exposure to the recipient thus can be avoided. I am truly confident that my research has the potential to reduce the high rate of postischemic ARF which is one of the key risk factors for DGF and subsequent reduced long term allograft survival. As of now there is no prophylaxis and treatment available and thus there is a clear clinical need to tackle this devastating condition.

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7 Summary

The work of the last decade focused on the molecular pathways of ischemia reperfusion injury in human kidney transplantation. The work plan was carefully designed and experiment meticulously planned. The projects were continuously supported since 1996 by Austrian research grants (FWF) and EU-grants. In summary my research group nephrogene showed that inflammation in the donor organ is highly predictive of DGF after transplantation. Suppression of this inflammatory process in the donor ameliorated inflammation on a genome wide level but did not change the incidence of DGF. Thus new avenues were chosen to tackle this important enigma. Antisense oligonucleotide constructs against miRNAs were investigated by systems biology approaches and lead identified also experimentally. The top candidate – antagomiR-182 – was able to suppress the target miR selectively also ex - and in vivo and led to inhibition of the suppression of miR-182 target proteins such as Bcl-2 or FOXO-1. This molecular intervention led functionally to a reduced severity of IRI as evidenced by creatinine trajectories in rat models and improved histopathology of the IRI kidneys.

We are confident that the application of our approach to human kidney transplantation is feasible and will lead to a clinical breakthrough in the treatment of acute renal failure in the transplant but eventually also in native kidneys of patients suffering from acute renal failure in the ICU.

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7 Összefoglalás

Az elmúlt évtized munkája a vesetranszplantáció folyamán kialakult ischémia/reperfúziós károsodás (IRI) molekuláris útvonalaival foglalkozott. A projekteket 1996 óta folyamatosan az osztrák kutatási alapprogram (Fonds zur Förderung der wissenschaftlichen Forschung - FWF) és EU-támogatások finanszírozzák.

Összefoglalva a kutatócsoportom igazolta, hogy a donor szervgyulladása megjósolja a transzplantáció utáni késleltetett vesefunkciót (DGF). A gyulladási folyamat csökkentése a donorban javította a gyulladást genomszinten, de a DGF előfordulásán nem változtatott. Ezért új utakat választottunk e fontos talány megoldására. A folyamat alatt deregulált miRNA-kat kutattuk rendszerbiológiai megközelítéssel majd szerepüket kísérletesen is bizonyítottuk, antiszensz oligonukleotidok használatával. A fő oligonukleotid jelölt –az antagomiR-182- szelektíven gátolta a cél miRNA-t ex- és in vivo is, valamint ez a gátlás a miR-182 (Bcl-2, FOXO-1) célfehérjéire is kihatott. Patkány modelleken vizsgálva ez a molekuláris intervenció a kreatinin értékek alapján lecsökkentette az IR súlyosságát és javította az IRI vesék hisztopatológiáját.

Biztosak vagyunk benne, hogy a mi megközelítésünk a jövőben beépíthető a humán vesetranszplantáció menetébe, és klinikai áttöréshez vezet az akut veseelégtelenség kezelésében nem csak a transzplantáció során, hanem az intenzív osztályon ápolt, akut veseelégtelenségtől szenvedő betegek kezelésében is.

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