10

General discussion and future perspectives

Introduction

With acute rejection rates lowered to 10-20% but limited progress with regard to long-term survival a new challenge lies ahead in optimizing immunosuppression in renal transplantation [1]. Individualizing and fine-tuning current immunosuppressive regimens is now the most promising strategy to improve long term graft survival for renal transplant recipients. The current maintenance immunosuppressive drugs, calcineurin inhibitors (CNIs), are known for their efficacy but also for their toxicity such as new onset diabetes mellitus, neurotoxicity and renal toxicity especially in the higher dose ranges [2]. Efficacy of CNI minimizing or even CNI free strategies shortly after transplantation are currently widely investigated [3–5]. Less nephrotoxic regimens including mTOR inhibitors have been developed during the last decade, but on the other hand new serious side effects, relative high discontinuation rates and/or intolerability postpone wide implementation [4,6]. Although strict therapeutic drug monitoring is implemented some patients remain at risk for serious side effects and rejection. Identifying these patients before initiation of therapy could help prevent therapy failure. The main challenge is to find the right immunosuppressive regimen and exposure at the right time for individual patients. This thesis is constructed out of a number of different analyses to further optimize maintenance immunosuppressive therapy for renal transplant recipients to prolong long-term graft survival, starting with a comparison of the most used analytical methods for therapeutic drug monitoring of everolimus, followed by evaluations of potential predictive biomarkers for everolimus pharmacokinetics and pharmacodynamics and finally also potential predictive biomarkers for calcineurin inhibitor pharmacokinetics and dynamics are explored.

Therapeutic drug monitoring techniques

Because of its highly variable pharmacokinetics and narrow therapeutic window everolimus therapeutic drug monitoring (TDM) is essential for preventing serious side effects and rejection [7]. Currently a variety of analytical methods to perform TDM are available [8–10], and methods may differ in accuracy and specificity. Whether these differences are clinically relevant is an important question. Because of high protein binding and to distribution into erythrocytes whole blood is the matrix of choice for everolimus

TDM [11]. The two widely used analytical techniques for everolimus blood concentration measurement, fluorescence polarization immuno assay (FPIA) and liquid chromatography tandem mass spectrometry (LC-MS/MS), were compared in chapter 3 of this thesis. The findings showed that these two methods are not in agreement. Everolimus concentrations determined by FPIA are, on average, 23% higher than LC-MS/MS. However, the variability found between FPIA and LC-MS/MS could be twofold for concentrations lower than 15 μg/L or AUC_{0-12h}. This suggests a relatively large effect on variability of FPIA versus LC-MS/MS when monitoring everolimus trough concentrations. The large variability in concentrations determined with FPIA can lead to clinically relevant differences in dosing advice compared with LC-MS/MS despite using a correction factor of 23%. The within-patient variability for trough concentrations appeared to be higher using the FPIA method [12], most likely caused by nonspecific binding of the antibodies [13] and crossreactivity of metabolites, which are actually present in relatively high concentrations before the next dose [14,15]. The variability in differences in dosage advice showed that the risk of suboptimal dosage advice is present and clinically relevant. In general LC-MS/MS is a more specific, more stable, and more accurate method for everolimus TDM compared to FPIA and is able to simultaneously measure several immunosuppressive drugs in a single run. However the most important limitations for broad introduction of LC-MS/MS for everolimus TDM are the need for a high initial capital investment and highly trained technicians for operation and maintenance. Centralization of sample measurements in combination with dried blood spot methodology might be a solution to this problem. While pharmacodynamic monitoring instead of pharmacokinetic monitoring in theory should give a more accurate insight on the mTOR inhibition and clinical effects a suitable method has not yet been found and implemented. Other innovative methods of measuring concentrations at the site of action like PBMCs could potentially give a more precise view at the level of immunosuppression but are currently under development and not yet accepted in clinical practice. Therefore TDM of everolimus whole blood concentrations using LC-MS/MS currently is still the method of choice.

Variability in pharmacokinetics of everolimus

Everolimus is metabolized by CYP3A4, CYP3A5, CYP2C8 and is a substrate for P-glycoprotein (P-gp), and is characterized by its high inter patient variability. The nuclear

pregnane X receptor (PXR) mediates expression of CYP3A4 and multi drug resistance proteins (MDR1 and MDR2) and could therefore potentially influence everolimus pharmacokinetics [16–18]. Monitoring area under the blood concentration versus time curve (AUC) instead of trough concentration is often more informative. However, AUC monitoring when using trapezoidal calculations remains laborious for both patients and the clinic. Limiting sampling strategies based on Bayesian estimation could be solution to this problem. A limitation of TDM is that during the critical period of the first days after transplantation or conversion to another immunosuppressive regimen the exposure cannot be influenced. Getting the initial dose right is therefore very important. Especially drugs with a long elimination half-life such as everolimus are at risk of under or overexposure because correcting them takes more time. Reaching target exposure is as soon as possible after drug initiation is essential, however currently no factors for everolimus initial dose differentiation have been identified. Pharmacogenetics, when looking at polymorphisms coding for metabolizing enzymes which lead to altered drug metabolism could be a potential factor as previously shown for tacrolimus [19]. These factors could potentially shorten the time to reach target exposure. To address the above mentioned problems the research described in chapter 4 was performed. Pharmacometrics, which uses mathematical models based on physiology, pharmacology and disease for quantitative analysis of interaction between drugs and patients was used to build a population pharmacokinetic model, a limited sampling model and evaluate potential factors influencing pharmacokinetics (covariates). The pharmacokinetics of everolimus of (primarily Caucasian) renal transplant patients using everolimus and prednisolone was best described by a two-compartmental model with first order absorption and lag time. Everolimus pharmacokinetics was not significantly influenced by genetic polymorphisms in coding genes for the metabolizing enzymes CYP3A5, CYP2C8, ABCB1 and PXR or drug transporter ABCB1. Therefore, the currently known single nucleotide polymorphisms (SNPs) are not able to predict everolimus systemic exposure to a clinically relevant extent and shorten the time to reach target exposure. In addition, demographic covariates such as total body weight, age, sex, hematocrit, albumin, length, body mass index, body surface area, lean body weight, underlying disease, co-medication and ethnicity did not significantly influence everolimus pharmacokinetics [20]. Ideal body weight did significantly correlate with the variability in apparent distribution volume of the central compartment and can be physiologically explained by the fact that everolimus is for more than 75% partitioned into red blood cells and 75% of the plasma fraction is

bound to plasma proteins since length and sex are incorporated in the ideal body weight formula [11,21]. In conclusion, no factors for initial dose differentiation of everolimus were identified. Weak CYP3A inhibitors such as statins, nifedipine and sulfamethoxazole/trimethoprim did not have a clinically relevant effects on pharmacokinetics, which was in accordance with previous findings [22] although strong CYP3A inhibitors and inducers are known to strongly influence everolimus pharmacokinetics [23]. Monitoring everolimus during initiation and discontinuation of such drugs is therefore essential.

Therapeutic drug monitoring of everolimus

The most common way to perform everolimus therapeutic drug monitoring is monitoring based on trough concentrations. However, besides the higher impact of assay variability [12] when using one marker to predict everolimus systemic exposure, the correlation between C_{trough} and AUC is not optimal and could in theory lead to therapy failure and side effects [24] . Worse predictive performance of a TDM marker can lead to incorrect dose adjustments resulting in exposure outside the target range. The developed limited sampling model (Chapter 4) is an improvement in terms of inconvenience for patient and clinic and predictive performance. C_{trough} and C_2 monitoring based on the population pharmacokinetic model resulted in an improved predictive performance compared to C_{trough} monitoring. Whether TDM based on trough or AUC_{0-12h} does lead to differences the occurrence of hazardous side effects and clinical benefit in long term warrants to be investigated more thoroughly before clinicians can be convinced to use AUC monitoring instead of trough monitoring. Since the majority of research suggests that tacrolimus does not influence everolimus pharmacokinetics, the applicability of the developed model might include on tacrolimus + everolimus regimens. Since CNI minimizing and CNI free strategies are being actively investigated worldwide [4,5,25–28] there could be an increasing interest for implementation of the developed model in clinical practice.

Pharmacodynamics: side effects and everolimus discontinuation

Despite its proven efficacy and close TDM, everolimus is also known for some serious side effects with relative high discontinuation rates [6,29]. Leukopenia, thrombocytopenia,

hypertriglyceridemia and hypercholesterolemia are the most common side effects of mTOR inhibitors [4] and can often be managed with counteracting medication or dose reduction [6,30]. Although less common but a potentially life threatening side effect of everolimus is non-infectious interstitial pneumonitis. It typically presents itself within 2 to 6 months after start of therapy [31,32]. The exact mechanism of mTOR inhibitor-induced pneumonitis is still unknown, but direct damage to alveolar structures, formation of immunogenic molecules that react with specific antibodies, and direct immunologic drug responses are suggested as possible mechanisms [33]. A dose relationship may be present and is supported findings of by higher incidence in oncology where higher daily doses are prescribed [34,35]. Moreover a higher incidence was found in males on sirolimus therapy compared to females [36]. Infectious diseases are an important cause of death in renal transplant recipients [37,38] and strongly related to excessive and/or long-term clinical immunosuppression [39]. Everolimus is associated with a relatively low incidence of viral infections as compared to other immunosuppressive groups [40–42]. Everolimus is also associated with an increased incidence of new onset diabetes mellitus (NODM) which subsequently is associated with increased graft failure and mortality due to cardiovascular events [43]. NODM is therefore a serious complication of immunosuppressive therapy in transplant recipients which shortens long term survival [44]. Finding risk factors for everolimus discontinuation and the mentioned severe side effect could help further improve individualized immunosuppressive therapy by excluding patients at high risk from everolimus therapy or monitor them more intensively. In chapter 5 and 6 risk factors were explored for everolimus-discontinuation and serious side effects in renal transplant recipients on dual therapy.

In the case-cohort study (Chapter 5) no clear predisposing factors were identified for non-infectious interstitial pneumonitis. Pulmonary CT scans revealed an organizing or non-specific interstitial pneumonitis-like pattern. The course seems benign with disappearance of symptoms within one year after discontinuation of the drug. The incidence (12.7%) reported was higher than previously reported in renal transplant recipients on mTOR-inhibitors, varying between 4 and 6.8% [45–47]. In patients treated with everolimus for renal cell carcinoma the incidence of non-infectious interstitial pneumonitis has been reported to be around 25% [34,35]. This high incidence of non-infectious interstitial pneumonitis has been attributed to higher dosage of everolimus in these patients in combination with a higher detection level of pneumonitis due to routinely performed pulmonary CT scans. In the case cohort study, drug exposure was relatively high with an AUC around 170 μg*h/L

and trough levels around 10 μg/ml since everolimus was prescribed as part of a double immunosuppressive regimen. However, average everolimus exposure was not higher in the cases compared to controls. All patients subjectively recovered within one year, however long-term outcome after non-infectious pneumonitis remains unclear since at least theoretically non-infectious pneumonitis may result in pulmonary fibrosis. Since the presentation of non-infectious pneumonitis can be insidious or even asymptomatic, performing radiographic imaging of the lungs when patients present with dyspnea, cough or fever while on treatment with this drug according to the algorithm shown in Chapter 5 is recommended.

A more sophisticated time to event analysis was used to investigate risk factors for everolimus discontinuation and the serious side effects non-infectious interstitial pneumonitis, infection and NODM (Chapter 6). Such an approach has advantages compared to non-parametric and semi parametric analyses, because it enables inclusion of time-varying covariates and allows simulation based on the final model. Results showed that excess exposure during the study period and older age were risk factors for everolimus-discontinuation. Since the majority of discontinuation was side effect related this is in line with earlier finding that certain side effects have previously shown to be dependent on exposure [48,49]. As can be concluded from our results, clinicians should prevent renal transplant recipients from reaching excess everolimus exposure (i.e. AUC_{12}> 120 -150 μg*h/L), therefore close TDM remains warranted. Looking at the high discontinuation rates and low rejection risk we can extrapolate an initial target trough level between 6 μg/L and 8 μg/L from this study and an initial dose of 2 mg b.i.d. This initial dose might lower the rate of overexposure compared to 3 mg which was used in the study. Higher age resulted in a higher risk of everolimus-discontinuation probably due to fact that often patients with higher age have more comorbidities and senescence of their immune system with changes in T-cell function [50] where the immunosuppressive effect of the same immunosuppression exposure might be higher. Older patients with more comorbidities also have more difficulty to cope or accept additional side effects compared to young patients with no comorbidities.

The risk of experiencing non-infectious pneumonitis was increased by prolonged excess exposure. Furthermore renal transplant recipients with a PXR (NR1|2)(-24113G>A): AA genotype had a higher risk of developing pneumonitis compared to those carrying the AG or GG genotype although the effect seemed to be limited. The increase in risk of patient with that was found for patients with PXR (NR1|2) (-24113G>A) AA genotype might be related to an increased accumulation of everolimus in the lungs. In experimental animals high affinity for lungs and kidney were found for everolimus [51] and could this could

be the case in humans. PXR is a nuclear receptor whose primary function is to sense the presence of foreign toxic substances and in response up regulate the expression of proteins involved in the detoxification and clearance of these substances from the body. PXR polymorphism could subsequently have an effect on drug transporter activity since PXR is able to influence enzyme activity and multi drug transporter proteins [16–18]. Infections continue to be an important feature in the first year following both renal and heart transplant and occur in around 50% of patients [37]. The incidence of (opportunistic) infections is related to the intensity and type of immunosuppression [38]. No significant relationships for infection could be identified in the current analysis, but in general differences are more pronounced when two different immunosuppressive regimens are compared [39,52].

Although known from literature, important risk factor for the development of NODM include African ethnicity, increased age, obesity, increased number of transplants, donor type, a family history of diabetes and the use of prednisolone [53], but none of these relationships were strong enough to be detected in this patient cohort. The analysis for NODM had some specific limitations; the dataset lacked a significant number of patients from African ethnicity, Family history of diabetes was not available in the dataset en could therefore not be included in the covariate analysis. Exposure did not seem to affect the occurrence of NODM which was in accordance with previous studies [49]. In conclusion, the current findings can be used to further optimize everolimus based immunosuppressive therapy by preventing excessive drug exposure by strict therapeutic drug monitoring and restrict the initial dosing to a maximum of 2 mg b.i.d.

Influence of the most promising single nucleotide polymorphisms on maintenance immunosuppressant pharmacokinetics

Pharmacogenetics has only been adopted to a small extent in clinical practice for renal transplant recipients. In chapter 7 the influence of the most promising single nucleotide polymorphism: *CYP3A4*22*, *CYP3A5*3* variant alleles and its combined clusters on the pharmacokinetics of the three main kidney transplant immunosuppressive drugs cyclosporine, everolimus and tacrolimus was investigated. Cyclosporine, everolimus and tacrolimus are primarily eliminated by CYP3A enzymes [7,54–56] and as shown before in in-vitro and in-vivo studies, CYP3A4 is involved in their pharmacokinetics [55,57,58].

CYP3A4 is most likely predominant in cyclosporine and everolimus metabolic clearance and CYP3A5 contributes more significantly to tacrolimus metabolic clearance compared with CYP3A4 [55,56]. In contrast to CYP3A5, CYP3A4 lacked a reliable genetic marker for prediction of CYP3A4 expression which was suitable for dosing adjustments [59,60], however *CYP3A4*22* was previously marked as a potential reliable marker [61,62]. The results presented in chapter 7 demonstrated that carriership of the *CYP3A4*22* allele is significantly associated with a decreased cyclosporine clearance. Carriers of the *CYP3A4*22* allele showed 15% lower cyclosporine clearance as compared to non-carriers. In clinical practice this effect is not high enough to justify dose modification based on *CYP3A4*22*, since only an effect of at least 20% on clearance would lead to dose adjustments due to considerable degree of intra-individual variability in pharmacokinetics. Combining individual SNPs in theory would increase the predictive power of the single polymorphisms. However using *CYP3A* combined genotype of *CYP3A4* and *CYP3A5* as a predictor for cyclosporine, everolimus or tacrolimus clearance does not seem to be an improvement compared to the individual polymorphisms. Finally it was also demonstrated that patients carrying at least one *CYP3A5*1* allele have on average 53% higher tacrolimus clearance compared to non-carriers. The difference in tacrolimus clearance between *CYP3A5*1* carriers and non-carriers found was similar to what was published previously [19,63]. Dosing adjustments based on *CYP3A5*3* could be indicated to quickly reach target exposure, however the variability explained by *CYP3A5*3* is limited and the variability within the *CYP3A5* genotype groups remains significant and therefore close TDM remains essential. The absence of a clinically relevant influence of *CYP3A5*3* on cyclosporine and everolimus pharmacokinetics was in line with previous studies [60,64,65]. In conclusion, *CYP3A4*22* does not influence cyclosporine, everolimus or tacrolimus pharmacokinetics to a clinically relevant extent. Therefore the newly discovered *CYP3A4*22* or *CYP3A* combined genotypes are not indicative to be used for dose adjustments in clinical practice to further improve immunosuppressive therapy of cyclosporine, tacrolimus or everolimus in the investigated patient population. Hepatic microsomal P450 enzymes require P450 oxidoreductase (POR). Polymorphisms in the gene encoding POR have been linked to altered CYP activity [66]. In an additional analysis for everolimus (Chapter 8) the effect of *POR*28*, *CYP3A5*3* and their combined genotypes were explored. In contrast to what was previously found for tacrolimus [67,68] and in accordance to what was found for sirolimus [69] *POR*28*, or the combination of combination of *POR*28* & *CYP3A5*3* appeared not to be suitable as a biomarker to improve prediction of everolimus exposure.

Risk factors for delayed graft function, acute rejection and sub clinical rejection in a CNI based regimen

Over the past decades acute rejection (AR) rates have decreased dramatically, mainly due to calcineurin inhibitor (CNI) based immunosuppressive regimens. One of the dominant risk factors , previously identified for AR is delayed graft function (DGF) which is highly related to transplant related factors such as vulnerability of the allograft and/or prolonged preservation times [70]. Clinical episodes of AR have previously been identified as a risk factor for subclinical rejection (SCR) [71]. SCR is by definition histologically defined acute rejection and, as such, has been associated with subsequent interstitial fibrosis and tubular atrophy and with time progressive deterioration of renal function and inferior graft survival. Despite low acute rejection rates in the first year after transplantation with current standards for immunosuppressive therapy, long-term outcome after renal transplantation has not improved accordingly [72]. In chapter 9, a relatively large homogenous group of standard to low risk transplant recipients participating in the run-in phase of a multicenter, randomized clinical trial on quadruple therapy with basiliximab, prednisolone, mycophenolate sodium and CsA with controlled systemic drug exposure was analysed, aimed to identify pharmacological risk factors for DGF, AR and SCR 6 months after renal transplantation. Especially, the variability in CsA exposure and/or genetic variability in genes encoding calcineurin, P-glycoprotein and CYP3A5 were of interest. The incidence of AR and prevalence of SCR with controlled and early reduced systemic CsA-exposure at 6 months was found to be 14 and 18%, respectively. In this context pharmacological factors, including exposure and genetic variability in the selected genes, were not found to be related to the risk for DGF, AR or SCR. Receiving a kidney from a deceased donor was the dominant risk factor for DGF, with DGF being the primary risk factor for time to first AR. For SCR the most important risk factors were previous acute rejection, and being recipient of a deceased donor kidney. These factors were also associated with a lower 6-months protocol biopsy rate (overall reduction of 24%). Other factors related to "dropping-out" were female sex and carrying a copy of the *ABCB1* TTT-haplotype. The incidence of biopsy "drop-out" was the lowest for patients without a copy of the *ABCB1* haplotype. Finally a significant relationship ($P<0.05$) was found between rejection treatment including ATG and a lower subsequent prevalence of SCR. Three isoforms for calcineurin have been described: alpha, beta en gamma. Genetic variability in two genes coding for calcineurin, the target protein of CNIs were determined. The

gene coding for calcineurin beta (*PPP3CB)* could be primarily of relevance since this gene principally encodes the calcineurin present in cells of the immune system, whereas the gene coding for calcineurin alfa (*PPP3CA)* is thought to be more relevant in other tissues including renal tubular epithelial cells. Variability in the *PPP3CA* gene within kidney donors would be more relevant for renal toxicity and perhaps DGF. To investigate these theoretical genetic risk factors we determined haploblocks in both genes, but in the current cohort genetic variability in *PPP3CB* was not related to time to first AR, DGF or the prevalence of SCR. The selected haplotype combination reflects the overall variability in the calcineurin gene, but may not specifically represent variability in the structure of the actual calmodulin and calcineurin binding parts, responsible for the susceptibility for CsA as previously hypothesized [73]. In addition, expression of this protein may be regulated by other (nuclear) factors. No relationship could be identified between any of the selected genes in drug transport (*ABCB1*), metabolism (*CYP3A5, CYP3A4, CYP2C8*) and the regulation of these genes (PXR - *NR1I2*). Carrying at least one copy of the *ABCB1* TTT-haplotype, however, was related to an almost 2-fold higher "drop-out" rate for a 6-month protocol biopsy. At least theoretically, these patients may be prone to a higher frequency of adverse events, since the TTT-haplotype is associated with lower P-glycoprotein activity. This is independent from kidney survival, where the *ABCB1* genotype of the donor may be of higher relevance [74,75]. A combined donor-recipient homozygosity for the C3435T variant in *ABCB1* was associated with chronic allograft damage [76]. In accordance with our results no relation has been found between tacrolimus, carrying the CYP3A5*1 allele and AR or SCR [77,78].

The findings of the sub analysis of rejection treatment on the prevalence of subsequent SCR confirms the previously reported low prevalence observed with induction therapy with depleting antibodies in patients cohort dominated by living donor kidney transplant recipients. Early minimization of CsA or tacrolimus is increasingly applied an attempt to reduce toxicity and to improve long term outcome [3,79,80]. While there is still debate whether SCR should be treated as acute rejection episode, it is generally accepted that persistent or recurrent SCR constitutes a potential threat to (functional) survival of the transplanted kidney [81–84]. To safely taper CNI therapy within the immunosuppressive regimen after renal transplantation the risk of acute rejection should be minimized. It is generally assumed that CNI minimization or withdrawal is safest if a protocol biopsy shows no subclinical rejection [79,82,85] and exposure to the remaining drug(s) is individualized and adequate.

The integrated approach used in this last chapter combining demographic characteristics, transplant-related factors together with detailed drug-exposure and variability in genetic parameters in genes related to pharmacokinetics as well as pharmacodynamics, is very powerful to detect relationships with clinical events and identified DGF as a risk factor for early acute rejection. Moreover, a history of acute rejection recipients of kidneys from a deceased donor were identified as the dominant risk factors for inflammation in 6-month protocol biopsies despite controlled systemic drug exposure. Although, effects of exposure and genetics could not be identified in this analysis, likely this approach can be successful in identifying risks of late acute (cellular or humoral) rejection and calcineurin toxicity, in transplant recipients when using genetic information of the donors. Kidneys from donors carrying the *ABCB1* variant haplotype 1236T/2677T/3435T have previously been associated with inferior graft survival and renal function [75], while donors carrying the 3435TT genotype were associated with nephrotoxicity [74]. Such a conclusive analysis should include genetic variability in the genes *ABCB1, CYP3A5, PPP3CA* of the donor.

Future research perspectives

The balance between high efficacy and a minimum of side effects of immunosuppressive treatment is fragile, especially in transplantation were the main immunosuppressive drugs have a low bioavailability, a narrow therapeutic index and high inter-patient variability. Finding the right immunosuppressive regimen and exposure for the right patient at the right time is the main challenge for the future. This thesis aimed to fulfill a part of this challenge, however, only small steps forward were made and much more research is needed to find the optimal immunosuppressive treatment for the individual patient. Finding factors that are predictive for short term (clinical and subclinical rejection) and subsequently long term outcome (graft survival) are essential to achieve an increase in survival for renal transplant recipients. Transplant characteristics such as donor type, HLA-DR mismatch, cold ischemic time and donor age are currently still the most predictive factors for the initial immunological risk. Although strict therapeutic drug monitoring is performed for most drugs still some patients are at risk for rejection or toxicity, therefore better biomarkers are needed to guide adequate clinical immunosuppression. Ideally a biological marker reflecting the immunological status of an individual should be used for monitoring immunosuppressive treatment. Unfortunately pharmacodynamic

markers are still not suitable for clinical practice or not available. Currently attempts are made to measure immunosuppressive drug concentrations at the site of action like PBMCs [86] but research is still in its early stages. Especially drugs that are substrates of P-glycoprotein (P-gp) like CNIs and mTOR inhibitors are of interest since P-gp could potentially have a large impact on drug disposition in PBMC resulting in differences in immunosuppressive effect. On the other hand less invasive biomarkers for early prediction of acute rejection as free circulating DNA and donor-specific antibodies are currently also under investigation [87,88]. Also promising biomarkers for nephrotoxiticy are under development [89]. Combining such biomarkers with a pharmacokinetic model might help to find an individual's unique target concentration range. Pharmacogenetics on pharmacokinetic parameters has been of great interest during the past decade in the field of renal transplantation, however only a few suitable pharmacogenetic markers predicting exposure have been found. Furthermore the additional value of initial dosing based on pharmacogenetic markers with respect to long term outcome has not yet been established. The focus of pharmacogenetics should be expanded to pharmacodynamics parameters like polymorphisms in the mTOR gene or calcineurin gene to identify patients at risk for certain side effects or under immunosuppression. All efforts should be pointed at finding the optimal immunosuppressive treatment for the individual patient. To make this possible more effort should be made for collaboration between research groups. Especially in Europe the need for collaboration between clinicians and scientists is essential to gather and analyze large datasets needed to evaluate the effect of future biomarkers on patient outcome in large patient populations. Subsequently a systems pharmacology approach should be used incorporating the most important sources of variability in terms of pharmacokinetics and pharmacodynamics.

Conclusions

mTOR inhibitors form a promising new class of immunosuppressive drugs for maintenance immunosuppression in renal transplantation. The available evidence demonstrates that IL-2RA induction with an mTOR inhibitor can successfully reduce CNI exposure by at least half without a penalty in terms of rejection in low- or moderate-risk de novo transplant recipients and may offer renal and antiviral benefits [90]. Besides these advantages, high drug-discontinuation rates and some serious side effects have been limiting for broad

introduction of mTOR inhibitors into the field of kidney transplantation. Therapy should be further optimized be means of finding the right exposure at the right time. With this in mind AUC monitoring can become more and more important, especially in the Netherlands where active patient participation and at home monitoring with dried blood spot technology are important aspects of how transplantation care will be organized in the future. This can only be made possible with wide adaptation of pharmacometric tools to assure the optimal balance between minimal patient inconvenience and accurate monitoring of immunosuppressive therapy. Few single nucleotide polymorphisms have been identified to predict exposure of maintenance immunosuppressive drug, with CYP3A5*1 allele as the only undisputed and widely adopted predictive marker for tacrolimus clearance. Although an increasing amount of transplantation centers currently use this marker for initial dose differentiation, long term benefit has not yet been established. Therapeutic drug monitoring of immunosuppressive whole blood concentrations is still common practice, although more advanced variants such as monitoring intracellular (PBMC) drug concentrations slowly emerge, while pharmacodynamic monitoring is still not possible but promising new biomarkers are emerging. Pharmacometrics is the ideal tool to correlate clinical events to possible predictive factors as shown in chapter six and nine of this thesis. These types of analyses should become more widely adapted to the transplantation field. Combining available data in the renal transplantation research society and searching collaboration with pharmacometricians can assure optimal use of the available research data and will increase the chance of improvement of long term outcome of the renal transplant recipient population.

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