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Summary
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Renal transplantation is currently the best option for patients with end stage renal disease. Despite the low acute rejection rates and successful treatment in the first year after renal transplantation, long term outcome after renal transplantation remains poor. An important factor influencing survival is optimal immunosuppressive treatment. The work presented in thesis aimed at optimizing immunosuppressive therapy in renal transplant recipients and especially therapy consisting of the mTOR inhibitor everolimus by identifying pharmacological and pharmacogenetic factors influencing pharmacokinetics, and pharmacodynamics such as side effects and patient outcome.

The mTOR inhibitors are a relatively new therapeutic group in renal transplantation and have shown their efficacy in recent trials. Their main advantage compared to calcineurin inhibitors cyclosporine and tacrolimus are their relative lack of nephrotoxicity. In Chapter 2, a systematic review describes the knowledge of clinical pharmacokinetics and pharmacodynamics of mTOR inhibitors in renal transplantation at the start of this PhD project. The narrow therapeutic window of mTOR inhibitors, together with high variability in pharmacokinetics, makes therapeutic drug monitoring essential for individualizing the dose and thereby preventing toxicity or rejection. For these reasons it is very important to use a reliable and accurate bioanalytical assay. In Chapter 3 the differences between the mostly used analytical assays of measuring everolimus in whole blood and its effect on dosing advice are investigated. Results showed that the analytical methods Fluorescent Polarization Immuno Assay (FPIA) and Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) are not in agreement and everolimus blood concentration measurement using FPIA results in systematically higher (23% on average) everolimus concentrations compared with LC-MS/MS. Furthermore the use of FPIA can lead to clinically relevant differences in everolimus dosage advice and higher intra-patient variability. Therefore LC-MS/MS outperforms FPIA for clinical monitoring and intervention of everolimus therapy in adult renal transplant recipients on dual therapy with prednisolone. Therapeutic drug monitoring (TDM) of everolimus is performed based on either trough or AUC monitoring and pharmacogenetics might be a valuable addition to TDM in order to reach the target drug concentration as soon as possible by individualizing the initial dose. Especially drugs with a long elimination half-life such as everolimus are at risk of under or overexposure because it takes more time to reach steady state target concentration. Polymorphisms in genes coding for metabolizing enzymes...
might therefore be of interest for optimizing immunosuppressive therapy. In Chapter 4, a population pharmacokinetic model of everolimus in a calcineurin free regimen was developed and predictive factors for pharmacokinetics such as pharmacogenetics were explored. Everolimus pharmacokinetics was not significantly influenced by genetic polymorphisms in coding genes for the metabolizing enzymes CYP3A5, CYP2C8, PXR or drug transporter ABCB1 (also known as P-glycoprotein) and these polymorphisms are therefore not suitable as a marker for initial dose individualization. Finally a limited sampling model was developed which enables physicians to accurately predict everolimus exposure with limited patient discomfort and burden. Using $C_{\text{trough}}$ and $C_{2}$ as limited sampling markers resulted in an improved predictive performance compared to $C_{\text{trough}}$ monitoring.

Despite its proven efficacy and close TDM, everolimus is also known for some serious side effects with relative high discontinuation rates. In Chapter 5 potential risk factors such as demographics, underlying disease, transplant related factors, renal function and average everolimus exposure for the most severe side effect of mTOR inhibitors, interstitial pneumonitis, were evaluated in a case control study. No risk factors could be identified. In a more sophisticated time to event analysis (Chapter 6), risk factors for discontinuation and the side effects interstitial pneumonitis, infection and new onset diabetes mellitus were explored in a population of renal transplant patients on a regimen of everolimus and prednisolone dual therapy. Risk factors of everolimus discontinuation of renal transplant recipients on a regimen of everolimus and prednisolone dual therapy were constant too high everolimus exposure and high age. The initial dose of 3 mg b.i.d used in this study might be too high given the high initial exposure, the high discontinuation rate and low acute rejection rate. Furthermore, risk factor for the hazardous side effect non-infectious interstitial pneumonitis were a too high everolimus exposure and PXR (NR1|2) (-24113G>A): AA genotype. For infection and new onset diabetes mellitus no significant covariates were detected. Lower initial dosing and prevention of too high everolimus exposure by strict TDM might decrease the high everolimus discontinuation rates and the incidence of interstitial pneumonitis. Pharmacogenetics has only been adopted in clinical practice to a small extent for renal transplant recipients. In Chapter 7 the most promising polymorphisms ($CYP3A5^{*}3$ and $CYP3A4^{*}22$) in renal transplantation were evaluated for influence on the pharmacokinetics on the maintenance immunosuppressive drugs cyclosporine, everolimus and tacrolimus. Results showed that $CYP3A4^{*}22$ does not influence cyclosporine, everolimus or tacrolimus pharmacokinetics to a clinically
relevant extent. Furthermore this study confirmed that CYP3A5*3 is only suitable as a predictive marker for tacrolimus clearance but close TDM remains essential due to the remaining variability between patients with the same genotype. The CYP3A4 and CYP3A5 combined genotypes do not further improve the predictive performance compared to the predictive performance of the polymorphisms alone. Based on our study the newly discovered CYP3A4*22 or CYP3A combined genotypes cannot be advised to be used for dose adjustments in clinical practice to further improve immunosuppressive therapy of cyclosporine, tacrolimus or everolimus. In addition Chapter 8 reports the findings of the effect of peroxide reductase (POR) and CYP3A5 polymorphisms and their combination on everolimus pharmacokinetics. In contrast to what was found for tacrolimus but in accordance with the findings for sirolimus POR*28 polymorphism or the combination with the CYP3A5 polymorphism did not have a significant and clinical relevant impact on everolimus pharmacokinetics. 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. Acute rejection has been previously found to be a risk factor for subclinical rejection. Subclinical rejection (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. In Chapter 9 risk factor were identified for delayed graft function, acute rejection and subclinical rejection in patients on a cyclosporine based immunosuppressive regimen. The incidence of acute rejection (AR) and prevalence of SCR with controlled and early reduced systemic cyclosporine exposure within 6 months was found to be 14% and 18%, respectively. Pharmacological factors, including exposure and genetic variability in the genes coding for relevant pharmacokinetic and pharmacodynamics proteins, were not found to be related to the risk for delayed graft function (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 AR. For SCR the most important risk factors were a previous acute rejection episode, and being recipient of a deceased donor kidney. Finally a significant relationship was identified between rejection treatment including ATG and a lower subsequent prevalence of SCR. Finally in Chapter 10 the results from the performed research are discussed and future perspectives are presented. MTOR inhibitors form a promising new class of immunosuppressive drugs for maintenance immunosuppression in the field of kidney
transplantation and may offer renal and antiviral benefits without increasing the risk of acute rejection. Despite these advantages and TDM, mTOR inhibitors are also known for high discontinuation rates and some serious side effects. Even with all current options of immunosuppression long term outcome for renal transplant recipients is still poor. Immunosuppressive therapy should therefore be further optimized by means of finding the amount of immunosuppression at the right time. Finding new biomarkers for early detection of (subclinical) rejection and toxicity are therefore essential. Pharmacometrics is the ideal science for reaching this goal. Research collaborations of pharmacometricians and nephrologists should be formed to assure optimal use of the available clinical data to eventually improve long term outcome of renal transplant recipients.