**Protocol of the Study**

**Scientific Title**

Randomized study comparing QuantiFERON-CMV based versus standard cytomegalovirus (CMV) surveillance protocol in pre-emptive therapy for cytomegalovirus prevention after renal transplantation.

**Public Title**

**CMV-SIPHER:** **CMV-S**pecific **I**mmune monitoring for **P**re-emptive t**HER**apy

**Study site**

Faculty of Medicine in Pilsen, Charles University, and Teaching Hospital

Address: Alej Svobody 80, 304 60 Pilsen, Czech Republic

Department of Internal Medicine I

Department of Surgery

Department of Hemato-oncology

Department of Pathology

Department of Microbiology

Department of Immunology and Allergology

Institute for Clinical and Experimental Medicine (gene expression analyses)

Address: Vídeňská 1958/9, 140 21 Prague, Czech Republic

Transplant Laboratory

**Principal investigator**

Associate Prof. Tomáš Reischig, MD, PhD

Department of Internal Medicine I, Biomedical Centre

Faculty of Medicine in Pilsen, Charles University, and Teaching Hospital

Alej Svobody 80

304 60 Pilsen, Czech Republic

Phone: 00420-37-7103650, Fax: 00420-37-7103506

E-mail: reischig@fnplzen.cz

**Co-investigators**

Pavel Jindra, MD, PhD, Department of Hemato-oncology, Teaching Hospital, Pilsen; Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Pilsen

Associate Prof. Daniel Lysák, MD, PhD, Department of Hemato-oncology, Teaching Hospital, Pilsen; Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Pilsen

Hana Hermanova, MD, Department of Hemato-oncology, Teaching Hospital, Pilsen

Prof. Ondřej Hes, MD, PhD, Department of Pathology, Teaching Hospital, Pilsen; Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Pilsen

Mirko Bouda, MD, Department of Internal Medicine I, Teaching Hospital, Pilsen; Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Pilsen

Martin Kačer, MD, PhD, Department of Internal Medicine I, Teaching Hospital, Pilsen; Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Pilsen

Jana Machová, MD, Department of Internal Medicine I, Teaching Hospital, Pilsen; Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Pilsen

Tomáš Vlas, MSc, Department of Immunology and Allergology, Teaching Hospital, Pilsen; Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Pilsen

Miroslava Švecová, MD, Department of Microbiology, Teaching Hospital, Pilsen

Petra Hrubá, MSc, PhD, Transplant Laboratory, Institute for Clinical and Experimental Medicine, Prague

Prof. Ondřej Viklický, MD, PhD, Department of Nephrology, Institute for Clinical and Experimental Medicine, Prague; Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Pilsen

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**Background**

Cytomegalovirus (CMV) is one of the most common opportunistic pathogen in solid organ transplant recipients.[1](#_ENREF_1),[2](#_ENREF_2) Except for direct effects (CMV disease), there are indirect effects associated not only with CMV disease but, also, with asymptomatic CMV viremia.[1](#_ENREF_1) CMV enhances the immune response to alloantigens, which may result in an increased incidence of acute allograft rejection episodes or interstitial fibrosis and tubular atrophy (IF/TA) after renal transplantation and, eventually, to impaired graft survival.[3-9](#_ENREF_3) Except for low-risk patients, CMV prevention taking the form of prophylaxis or preemptive therapy is recommended.[1](#_ENREF_1) Based on the 2013 International Consensus Guidelines preference of either prophylaxis or pre-emptive therapy could not be recommended even in high-risk (CMV donor positive/recipient negative; D+/R-) kidney and liver transplant recipients.[1](#_ENREF_1)

Pre-emptive therapy is based on prospective monitoring of CMV viral load by sensitive assays such as PCR from whole blood or plasma. Oral valganciclovir or intravenous ganciclovir at therapeutical doses are given if viral load exceeds predefined threshold. Intense CMV surveillance monitoring protocols (weekly for the first 3-4 months) are required to reach good results of pre-emptive therapy approach.[1](#_ENREF_1),[10-13](#_ENREF_10) In contrast, less intense monitoring led to failure in sufficient prevention of CMV disease or long-term indirect effects.[14](#_ENREF_14),[15](#_ENREF_15) Logistics and cost of meticulous CMV monitoring represent the major disadvantages of pre-emptive therapy approach.[1](#_ENREF_1),[16](#_ENREF_16)

Assessment of CMV-specific cell-mediated immunity has the potential to improve management of CMV in solid organ transplantation.[17](#_ENREF_17) There are a variety of T cell assays for CMV. The majority of assays rely on the detection of IFN-γ after stimulation of whole blood or peripheral blood mononuclear cells (PBMC) with CMV specific antigens or overlapping peptides.[1](#_ENREF_1),[17](#_ENREF_17) In solid organ transplantation, QuantiFERON-CMV assay, ELISpot assay or intracellular cytokine staining for IFN-γ using flow cytometry were able to predict the risk of CMV disease if evaluated pre-transplant, identify patients at risk for late-onset CMV disease after stopping CMV prophylaxis and progression of asymptomatic CMV viremia to CMV disease in the pre-emptive therapy setting.[18-23](#_ENREF_18) However, there is the only interventional study based on CMV-specific cell-mediated immunity results. In this non-randomized trial positive result of QuantiFERON-CMV assay at the end of antiviral therapy for significant CMV viremia or CMV disease allowed for safe antiviral drugs discontinuation without recurrence of CMV viremia.[24](#_ENREF_24)

Both ELISpot assay and intracellular cytokine staining are not standardized although studies evaluated ELISpot assay for CMV-specific immunity reported promising results.[18](#_ENREF_18),[25](#_ENREF_25) On the other hand QuantiFERON-CMV assay is currently a commercially available kit (CE marked in Europe) and is an ELISA-based IFN-γ release CD8 assay. The assay has been evaluated in clinical studies of transplant patients at high risk of CMV and shown to be predictive of disease after stopping prophylaxis and of viremia if tested pre-transplant.[20](#_ENREF_20),[21](#_ENREF_21),[26](#_ENREF_26) A cutoff of 0.2 IU/mL of IFN-γ is accepted as a positive result of the assay. Level of <0.2 IU/mL with sufficient response to mitogen control (≥0.5 IU/mL) is used for defining a negative result for CMV-specific immunity. It should be noted a number of patients does not respond to mitogen control (both CMV negative and mitogen negative) reflecting an indeterminate result. Non-response to mitogen may potentially be a marker for global immunosuppression, and has been associated with a subsequent higher incidence of CMV disease.[21](#_ENREF_21) It seems reasonable to classify patients with both CMV negative and indeterminate results as a high-risk group for CMV disease development or CMV viremia progression.

In renal transplant recipients, the inclusion of QuantiFERON-CMV assessed CMV-specific cell immunity to the pre-emptive therapy approach may modify CMV surveillance protocol into a less intensive and avoid valganciclovir therapy for low viral load CMV viremia in the case of detectable CMV-specific immunity. Once efficacy and safety would be proven such an approach will lead to simplification of logistics, less PCR for CMV viremia evaluation and less antiviral drugs use. As a result, pre-emptive therapy would be easier to implement and much cheaper. The presented randomized study will be conducted to evaluate whether pre-emptive therapy using QuantiFERON-CMV based surveillance protocol is non-inferior to standard frequent (weekly) surveillance based on PCR for CMV DNA only. Given the presumed low incidence of CMV disease in both groups and with the aim to maximaze the safety in the experimental (Quantiferon-CMV) group CMV viral load of ≥2000 IU/mL was chosen as a primary efficacy endpoint at 12 months. The use of CMV viral load as a surrogate marker in clinical studies is in accordance with the conclusions of recent CMV Consensus Forum.[27](#_ENREF_27) Moreover, the level of CMV viral load is significant for CMV indirect effects such as impaired long-term graft survival.[9](#_ENREF_9) For a long-term evaluation of CMV indirect effects the incidence of moderate-to-severe interstitial fibrosis and tubular atrophy (IFTA) and/or IFTA with inflammation assessed by protocol biopsy at 3 years after transplantation was selected as a primary endpoint. Both moderate-to-severe IFTA and IFTA with inflammation are strong predictors of poor graft outcome.[28](#_ENREF_28),[29](#_ENREF_29)

**Aim**

The aim of the study is to compare the efficacy, safety and cost of QuantiFERON-CMV guided monitoring protocol for pre-emptive therapy approach for CMV infection prevention with standard protocol with the inclusion of long-term follow up for comparison of CMV indirect effects and alloimmune response.

**Study design**

Prospective, randomized, controlled, open-label, single-center, parallel study

**Interventions**

1. **QuantiFERON-CMV guided pre-emptive therapy**

Patients will be monitored using quantitative real-time PCR from whole blood for CMV DNAemia detection once a week for 4 weeks. In all patients QuantiFERON-CMV will be assessed at 3 weeks. Based on the result of QuantiFERON-CMV:

**1a) Quantiferon-CMV positive patients (CMV stimulation ≥0.2 IU/mL plus mitogen stimulation ≥0.5 IU/mL)**

Quantitative real-time PCR from whole blood for CMV DNAemia will be evaluated at months 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 21, and 24. For safety reasons and for equal probability to detect all episodes of CMV DNAemia, PCR CMV be evaluated at weekly intervals up to Month 4 post-transplantation similarly as in the control group. PCR CMV once a week will be likewise performed during pre-emptive valganciclovir therapy or treatment of CMV disease. In cases of antirejection therapy with methylprednisolone pulses or lymphocyte depleting antibodies (antithymocyte globuline or rituximab) and/or treatment of antibody-medaited rejection (plasmapheresis, immunoadsorption, IVIG, bortezomib) a new Quantiferon-CMV evaluation will be performed 1 week after antirejection therapy initiation. If Quantiferon-CMV positive result persists at least one single PCR CMV test will be performed 1 month after antirejection therapy initiation. In the case of negative or indeterminate QuantiFERON-CMV result weekly PCR CMV monitoring will be started and continued up to Months 4 post-transplantation or for 1 month in later post-transplantation period. Similarly, QuantiFERON-CMV will be reassessed at the end of pre-emptive valganciclovir therapy or therapy of CMV disease. In the case of negative or indeterminate QuantiFERON-CMV result a switch to weekly PCR CMV monitoring will be started and continued up to Month 4 after transplantation. In all QuantiFERON-CMV positive patients with CMV viral load ≥1000 IU/mL detected at sheduled time points, pre-emtive therapy with valganciclovir (Valcyte; Hoffman-La Roche, Grenzach-Wyhlen, Germany) will be instituted at a dose of 900 mg twice daily with food within 7 days at the latest and continued until reaching clearance of CMV DNAmia in 2 consecutive measurements (<50 IU/mL, at least for 14 days). Doses will be tapered based on renal function according to manufacturers’ instructions (Appendix I). During periods without the possibility to administer oral drugs therapy by intravenous ganciclovir (Cymevene; Hoffman-La Roche, Basel, Switzerland) at a dose of 5mg/kg every 12 hours with dose adjustments by renal function (Appendix III) is allowed. If CMV DNAemia will be detected outside of sheduled time points (i.e. during concomitant weekly PCR CMV monitoring), only patients with high-grade (≥10,000 IU/mL) CMV DNAemia or symptomatic CMV disease will receive valganciclovir therapy. QuantiFERON-CMV will be assessed at Month 4 in all patients, however the result will not result in prolonged weekly PCR CMV monitoring beyond Month 4.

**1b) Quantiferon-CMV negative (CMV stimulation <0.2 IU/mL plus mitogen stimulation ≥0.5 IU/mL) and indeterminated (CMV stimulation <0.2 IU/mL plus mitogen stimulation <0.5 IU/mL) patients**

Same weekly PCR CMV monitoring as in the control group including the same schedule of QuantiFERON-CMV assessments. Weekly PCR CMV monitoring up to Month 4 will be maintained irrespective to the change of QuantiFERON-CMV result in later post-transplant period.

1. **Standard protocol guided pre-emptive therapy (=control group)**

Patients will be monitored using quantitative real-time PCR from whole blood for CMV DNAemia detection once a week for 16 weeks and, subsequently, at months 5, 6, 7, 8, 9, 10, 11, 12, 15, 18, 21, and 24. PCR will be likewise performed at least once a week in cases with a positive previous test and during pre-emptive valganciclovir therapy. In cases of antirejection therapy with lymphocyte depleting antibodies (antithymocyte globuline or rituximab) and/or treatment of antibody-medaited rejection (plasmapheresis, immunoadsorption, IVIG, bortezomib) a new weekly PCR monitoring will be started and continued for 1 month. At viral load ≥1000 IU/mL, therapy with valganciclovir (Valcyte; Hoffman-La Roche, Grenzach-Wyhlen, Germany) will be instituted at a dose of 900 mg twice daily with food within 7 days at the latest and continued until reaching clearance of CMV DNAmia in 2 consecutive measurements (<50 IU/mL, at least for 14 days). Doses will be tapered based on renal function according to manufacturers’ instructions (Appendix I). During periods without the possibility to administer oral drugs therapy by intravenous ganciclovir (Cymevene; Hoffman-La Roche, Basel, Switzerland) at a dose of 5mg/kg every 12 hours with dose adjustments by renal function (Appendix III) is allowed. To allow a comparison of CMV-specific immune response in experimental and control group, QuantiFERON-CMV will be assessed at 3 weeks, 4 months and at the end of pre-emptive valganciclovir therapy or therapy of CMV disease. However, the results will not influence clinical management of the patients.

**Treatment of CMV disease (same in both groups)**

Patients developing CMV disease will be treated by valganciclovir at a dose of 900mg twice daily with dose adjustments by renal function (Appendix I). In cases of life-threatening CMV disease or CMV colitis intravenous ganciclovir (Cymevene; Hoffman-La Roche, Basel, Switzerland) at a dose of 5mg/kg every 12 hours with dose adjustments by renal function (Appendix III) will be used as a first line agent. Once the clinical state improves, patients may be switched to valganciclovir. Duration of therapy will be continued until reaching clearance of CMV DNAmia in 2 consecutive measurements (<50 IU/mL, at least for 21 days). PCR for CMV in whole blood will be performed on weekly basis at minimum. Secondary prophylaxis with valganciclovir (900mg once a day with dose reduction depending on renal function) for 1 month will be instituted after each episode of CMV disease.

**Immunosuppressive protocol (same in both groups)**

The standard protocol includes tacrolimus-based combination plus anti-IL2R monoclonal antibody (Basiliximab, Simulect, Novartis; Nürnberg, Germany) induction. Retransplanted patients and/or those with panel reactive antibody of >60% will receive induction rabbit antithymocyte globulin (Thymoglobuline, Genzyme; Marcy I’Eoile, France) and tacrolimus. In the case of preformed donor specific antibodies (DSA) additional pre-transplant and peritransplant plasmapheresis and low-dose intravenous immunoglobuline (IVIG) will be given. Target trough levels of tacrolimus will be 8-12 ng/mL over the first 3 months and subsequently 4-8 ng/mL. Recipients of grafts from highly marginal donors (donation after cardiac death, ≥70 years old, donors with hypertension and significant nephrosclerosis on biopsy, and/or dual kidney transplantation) will be treated with anti-IL2R monoclonal antibody (Basiliximab) and low-dose tacrolimus with target trough levels of 4-8 ng/mL. All groups will be given mycophenolate mofetil at a dose of 2g per day and corticosteroids. Therapeutic drug monitoring for mycophenolic acid (MPA) will be performed with targed MPA AUC of 30-60 mg\*h/L. Acute cellular rejection episodes (biopsy proven, including borderline changes) will be initially treated with high-dose I.V. methylprednisolone. Steroid-resistant rejection or severe (vascular) rejection will be treated by antilymphocyte globuline (Thymoglobuline). Active antibody-mediated rejection early after transplantation will be treated with a combination of plasmapheresis and intravenous immunoglobuline (IVIG) or with bortezomib protocol (bortezomib + rituximab + plasmapheresis) with eculizumab rescue therapy in resistant cases. Late chronic active antibody-mediated rejection will be preferably treated with anti-IL6R monoclonal antibody (Tocilizumab, Roactemra). Patients with subclinical rejection (grade ≥ IA accoring to Banff17) will be given high-dose I.V. methylprednisolone.

**Other prophylactic regimens (same in both groups)**

All patients will receive herpes simplex and varicella zoster virus prophylaxis with valacyclovir (500mg bid) for 1 month plus additional 1 month in the case of antirejection therapy, trimethoprim-sulfamethoxazole for 4 months and oral amphotericin solution for 1 month. Plasma will be tested for polyoma BK virus DNAemia every month during first 6 months and every 3 months until 2 years post-transplantation with pre-emptive immunosuppression reduction at a significant viral load (≥10,000 copies/mL).

**Randomisation and masking**

Before transplantation and after informed consent signature, eligible patients will be randomized, using the random-number table, at a 1:1 ratio with the use of random block sizes of 4, to QuantiFERON-CMV guided or to standard protocol guided pre-emptive therapy. Randomization will be stratified according to donor/recipient (D/R) CMV serostatus before transplantation, i.e. high-risk patients (D+/R- subgroup) will be randomized separatelly. Transplant nephrologist will be responsible for randomization. Sequentially numbered sealed envelopes will be used for allocation concealment. No masking will be provided except for physicians assessing the PCR CMV, QuantiFERON-CMV, CMV-specific cellular immune respose by ELISpot, donor-specific antibody by single antigen beads (Luminex), and renal graft histopathology which will be blinded to the study group of patients.

**Inclusion criteria**

•Adult (>18 years with no upper age limit) renal transplant candidates, male or female.

•Complement-dependent cytotoxicity (CDC) cross-match negative at the time of transplantation.

•Deceased (non-heart-beating donors or dual kidney transplantation are allowed) or living (both related and unrelated, AB0/HLA compatible or incompatible) donors with known CMV serology before transplantation. *Donor CMV serology will be performed in transplant center which procures the donor.*

•D/R CMV serostatus of D+/R-, D+/R+, and D-/R+. *Recipient CMV serology will be regularly (every 3 months) evaluated in all wait-listed patients at the Department of Virology, Universtity Hospital in Pilsen and finally confirmed at the time of transplantation.*

•Ability to sign informed consent.

**Exclusion criteria**

•Unknown pretransplantation CMV serology of the donor or recipient.

•D-/R- CMV serostatus.

•Active systemic viral infection within 2 weeks before transplantation except for active hepatitis B or hepatitis C infection neccesitating antiviral therapy.

•Therapy with systemic antiviral agents within 2 weeks before transplantation except for treatment of hepatitis B or C (lamivudine, adefovir, entecavir, DAAs).

•White blood cell (WBC) count <3.0 x 109/L.

•Platelet count <100 x 109/L.

•Allergy to ganciclovir.

•Inclusion to another clinical trial.

•Inability to provide informed consent.

**Follow-up**

All patients will be followed up to 4 years after transplantation or until death. The same follow up period will be used for patients in whom pre-emptive therapy will be withdrawn due to severe adverse event. After patient inclusion, demographic data, primary renal disease, other co-morbid conditions, duration and type of renal replacement therapy before transplantation, immunological parameters, CMV D/R serology, recipient EBV, HHV6 and HHV8 serology, pretransplant donor specific antibodies (DSA by SAB Luminex assay), initial immunosuppressive therapy, and donor characteristics will be recorded. After transplantation, the patients will be assessed clinically and by laboratory evaluation (Table 1A, 1B). Extra visit may be performed if clinically needed. Together with clinical, virological, and laboratory data, all drugs given during the study including dosages of tacrolimus, mycophenolate mofetil (MPA levels), corticosteroids, antiviral drugs (valganciclovir, I.V. ganciclovir) will be recorded. Levels of tacrolimus, mycophenolate mofetil (MPA levels) will be measured as a part of routine clinical practice. Other laboratory parameters measured, examinations or histological evaluations performed as a part of clinical practice may be used for study purposes.

**Study outcomes**

**Primary end points – short-term follow up at 12 months**

•Cumulative incidence of CMV infection (DNAemia) with viral load of ≥2000 IU/mL defined by positive PCR for CMV DNA in whole blood.

**Primary end point – long-term follow up at 36 months**

•Moderate-to-severe interstitial fibrosis/tubular atrophy (IF/TA) and/or IF/TA associated with inflammation (including i≥1 score, ti≥2 score or i-IFTA≥2 score) in protocol biopsy at 36 months (according to Banff2017 criteria), intrarenal expression of messenger ribonucleic acid (mRNA) cytokines evaluated in each biopsy for severity of IF/TA assessment.

All protocol biopsies will be analysed in a detail for the presence of other histological findings than IF/TA (subclinical rejection, chronic T-cell-mediated rejection, chronic active antibody-mediated rejection or transplant glomerulopathy, trombotic microangiopathy, reccurent glomerulonephritis, calcineurin inhibitor nephrotoxicity, nephrosclerosis, polyomavirus-associated nephropathy).

**Secondary end points – short-term follow up at 12 months**

•Cumulative incidence of CMV disease (defined by clinical symptoms + presence of CMV DNAemia by quantitative PCR CMV DNA test).

All episodes of CMV disease will be analysed in a detail including the severity (syndrome/end-organ disease), diagnostic tests used, the course of therapy, duration of hospitalisation, viral load data, concomitant infections.[30](#_ENREF_30)

(Ljungman P, Boeckh M, Hirsch HH, et al. Definitions of Cytomegalovirus Infection and Disease in Transplant Patients for Use in Clinical Trials. *Clin Infect Dis.* 2017;64(1):87-91.).

•Cumulative incidence of CMV infection (DNAemia) defined by positive PCR for CMV DNA in whole blood.

•Cumulative incidence of CMV infection (DNAemia) with high viral load defined by positive PCR for CMV DNA in whole blood of ≥10,000 IU/mL.

•Incidence of CMV ganciclovir resistant viremia based on UL97 or UL54 mutation.

•Acute rejection diagnosed by renal allograft biopsy (defined according to Banff criteria including both cellular and humoral (antibody-mediated) rejection).

All acute rejection episodes will be analysed in a detail including the severity and type (cellular/humoral, steroid-resistant rejection, subclinical rejection at 3 month protocol biopsy, Banff grades), antirejection therapy (high-dose methylprednisolon, antilymphocyte antibody, plasmapheresis, IVIG, bortezomib, rituximab, eculizumab, tocilizumab).

•Level of CMV-specific T cell immunity assessed by Elispot-Fluorospot at 3 weeks and 4 months.

•Level of CMV-specific T cell immunity assessed by QuantiFERON-CMV at 3 weeks and 4 months.

•Cumulative incidence of donor specific antibodies assessed by single antigen beads (SAB) Luminex.

•Incidence of other infections.

Particular interest will be given to other herpes virus inefction (HSV, VZV, HHV6, HHV7, EBV), other viral infections, polyomavirus viremia/nephropathy, fungal infection (candida, aspergillus, pneumocystis, etc.), sepsis, urinary tract infection, pneumonia.

•Cumulative patient and graft survival (defined as death or graft loss)

•Incidence of adverse events and study drug discontinuation or dose reduction due to adverse event.

Particular interest will be given to heamatological side effects (leuko/neutropenia, trombocytopenia, anemia), psychiatric side effects (hallucinations, confusion), liver enzyme abnormalities, new-onset post transplant diabetes, new-onset malignancy, cardiovascular events.

•Renal function assessed by serum creatinine and estimated glomerular filtration rate calculated by MDRD7 formula

(Levey AS, Bosh JP, Breyer-Lewis J et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999; 130: 877-884).

•Daily urine protein excretion and protein/creatinine ratio.

•Incidence of delayed graft function (defined as a need of dialysis within first week post transplant)

**Secondary end points – long-term follow up at 36 or 48 months**

•Cumulative patient and graft survival (defined as death or graft loss) at 36 and 48 months.

•Renal function assessed by serum creatinine and estimated glomerular filtration rate calculated by MDRD7 formula) at 36 and 48 months.

•Daily urine protein excretion and protein/creatinine ratio at 36 months.

•Late onset CMV disease (defined by clinical symptoms + presence of CMV DNAemia by quantitative PCR CMV DNA test) at 36 months.

•Cumulative incidence of CMV infection (DNAemia) defined by positive PCR for CMV DNA in whole blood at 24 months.

•Level of CMV-specific T cell immunity assessed by Elispot-Fluorospot at 36 months.

•Level of CMV-specific T cell immunity assessed by QuantiFERON-CMV at 36 months.

•Cumulative incidence of donor specific antibodies assessed by SAB Luminex at 36 months.

•Incidence of other infection at 36 months.

Particular interest will be given to other herpes virus inefction (HSV, VZV, HHV6, HHV7, EBV), other viral infections, polyomavirus viremia/nephropathy, fungal infection (candida, aspergillus, pneumocystis, etc.), sepsis, urinary tract infection, pneumonia.

•Incidence of adverse events at 36 months.

Particular interest will be given to new-onset post transplant diabetes, new-onset malignancy, cardiovascular events.

**Pharmacoeconomic analysis**

Actual expenses incurred directly in connection with CMV management will be analyzed during first year afgter transplantation. Calculations include prices of drugs administered for pre-emptive therapy, PCR monitoring, QuantiFERON-CMV, diagnostic procedures in CMV activation (routine laboratory tests, CMV pp65 antigenemia, CMV blood culture, CMV serology), and CMV disease. The cost of hospitalization due to CMV disease will be calculated. All costs will correspond to 2022 prices (=end of short-term study) while considering the effect of inflation. Sensitivity analyses will be calculated assuming low/high drug costs and low/high PCR costs. Patients with graft loss within 2 weeks in conjuction with immunosuppression and CMV preventive regimen withdrawal will be excluded from economic evaluation.

**Definition of CMV DNAemia**

•Positive result of quantitative PCR for CMV DNA in whole blood (lower detection limit = 50 IU/mL)

In all cases of CMV DNAmia, other confirmation tests are recommended such as CMV pp65 antigenemia (positive results = ≥ 1 positive cell per 2x105 evaluated cells), CMV, EBV, HHV6 serology.

**Definition of CMV syndrome**

•Significant CMV DNAemia (≥ 1000 IU/mL) plus 2 or more from the following:

1. Fever ≥38°C for 2 or more days without another explanation.
2. Leucopenia (WBC count <3.5 x 109/L in patients with ≥4.0 x 109/L prior to the development of symptoms, or a >20% decrease if the WBC count prior to the development of symptoms was <4.0 x 109/L) in 2 consecutive samples and/or neutropenia (neutrophil count <1.5 x 109/L in patients with ≥1.5 x 109/L prior to the development of symptoms, or a >20% decrease if the WBC count prior to the development of symptoms was <1.5 x 109/L) in 2 consecutive samples.
3. ≥5% of atypical lymphocytes.
4. Thrombocytopenia <100 x 109/L in patients with ≥115 x 109/L prior to the development of symptoms, or a >20% decrease if the WBC count prior to the development of symptoms was <115 x 109/L) in 2 consecutive samples.
5. Unexplained new or progression of previously stable malaise (toxicity grade 2) or a new or increased fatigue (toxicity grade 3) (National Cancer Institute: Common Terminology Criteria for Adverse Events, version 4.0).
6. Elevation of hepatic aminotransferases (ALT or AST) to 2 times the upper limit of normal.

**Definition of CMV tissue-invasive disease**

•Significant CMV viremia (≥ 1000 IU/mL) plus one or more from the following:

1. Pneumonia (signs and symptoms of pulmonary disease without other cause plus CMV detection lung tissue /=proven diagnosis/ or in BAL /=probable diagnosis/ by viral culture, PCR, antigenemia or detection of CMV in lung tissue by histopathology, IHC analysis or in situ DNA hybridization).
2. Hepatitis (elevation of bilirubin or liver enzymes without other cause plus CMV detection in liver tissue by culture, PCR, IHC analysis or in situ DNA hybridization).
3. Gastrointestinal disease - esophagitis, gastritis, enterocolitis (clinical signs of GIT disease plus macroscopic mucosal lesions by endoscopy plus CMV detection in GIT tissue by histopathology, culture, or in situ DNA hybridization, IHC analysis, with PCR detection is sufficient for probable disease). For probable GI disease macroscopic mucosal lesions are not required.
4. Pancreatitis (clinical plus laboratory plus CT signs of acute pancreatitis plus CMV detection in pancreatic biopsy tissue by histopathology, culture, IHC analysis, or in situ DNA hybridization).
5. Myocarditis (clinical plus ECHO plus ECG signs of myocarditis plus CMV detection in myocardial biopsy tissue by histopathology, culture, IHC analysis, or in situ DNA hybridization).
6. Retinitis (typical lesions confirmed by ophthalmologist).
7. Nephritis (unexplained elevation of s-Cr plus positive biopsy for CMV detection by PCR, IHC analysis, in situ hybridization).
8. CNS disease (clinical signs of CNS disease without other cause plus CMV detection in CSF by culture or CMV detection in CNS tissue by PCR, IHC analysis or in situ hybridization.

**Definition of CMV disease**

•CMV syndrome and/or tissue-invasive CMV disease

**Quantitative real-time PCR DNA CMV from whole blood**

Quantitative real-time PCR will be performed using a commercially available kit (RealStar® CMV PCR kit 1.0, Altona Diagnostics; Germany) according to manufacturers’ instructions on a Rotor-GeneTM 2000/3000 system (Corbett Research; Australia). DNA will be isolated from 200 µL of whole blood using a commercially available kit (QuickGene DNA whole blood kit S (DB-S), Kurabo; Japan) according to manufacturers’ instructions. Elution will be performed in 100 µL CDB buffer, final DNA volume used in calculation of CMV IU/mL (see later) is 100 µL. Thereafter, short part of CMV genome (length between 150 and 200bp) will be amplified by CMV specific primers and amplicon is immediately detected by fluorescent marked (FAM) probes. Heterologenous amplificating system detected by other fluorescent channel (JOE) is used as internal quality control and DNA integrity control (to exclude false negative result due to low quality DNA). Quantification is performed according to calibrating curve generated from concomitantly amplificated quantification standards (IU/uL DNA). By this way, the number of CMV copies per 1 µL DNA is reached. Thereafter, the number of CMV IU/mL of whole blood is calculated from enter sample volume and eluted DNA volume according to following formula:

IU/mL = (IU/µL x eluted volume) / volume of sample for DNA isolation

Analytical sensitivity for Rotor-GeneTM 2000/3000 was not determined. Lower detection limit of the investigation is 50 IU/mL of whole blood. Physicians assessing the PCR results will be blinded to the study group of patients.

**CMV pp65-antigenemia**

In cases of CMV viremia or suspected CMV disease, pp65 antigenemia on isolated polymorphonuclear cells will be additionally examined using a commercially available CINA kit according to manufacturers’ instructions (Argene BIOSOFT; France). One and more positive cells per 200,000 tested will be defined as a positive result.

**CMV ganciclovir resistance**

CMV resistant strains will be detected by genotypic analysis of the UL97 kinase and the UL54 DNA polymerase genes based on known mutations for current anti-CMV drugs. CMV DNA will be extracted from whole blood samples as described in real-time CMV PCR methods. Predefined criteria will be used to identify subjects and samples for genotypic resistance testing: 1) any patient with a positive viral load (>1000 IU/mL) during valganciclovir prophylaxis or after prophylaxis completion, 2) any patient with a positive viral load (>1000 IU/mL) within 21 days of starting pre-emptive valganciclovir therapy, 3) any patient with recurrent viral load (>1000 IU/mL) after previous pre-emptive valganciclovir therapy, 4) any patient with CMV disease with positive viral load (>1000 IU/mL) within 21 days of diagnosis in spite of >2 weeks of full dose i.v. ganciclovir.

**CMV-specific T-cell immune response testing by QuantiFERON-CMV**

CMV-specific T-cell response will be tested in predefined time points different in both groups as described in “Intervention chapter” with fixed common time points for both groups: at 3 weeks, 4 months, and 36 months after transplantation.

Cell-mediated immunity will be determined using the Quantiferon-CMV assay (Cellestis Ltd, a QIAGEN company).[21](#_ENREF_21) One-milliliter aliquots of whole blood will be collected into 3 heparinized tubes. One tube contained a mix of 22 CMVCD8+ T-cell synthetic epitopes (CMV tube); one tube contained phytohemagglutinin (mitogen or positive control); and the third tube contained only heparin (no antigen or negative control). After collection, the 3 tubes will be incubated overnight at 37°C. Following incubation, the tubes will be centrifuged and plasma removed from each tube and placed in a plasma storage container. These containers will be then frozen at −70°C (consistent with the manufacturer’s recommendations regarding storage and processing), and IFN-γ measurement using an enzyme-linked immunosorbent assay (ELISA) was subsequently performed in batch testing in 3 centers (Cleveland, Ohio; Edmonton, Canada; and Leiden, the Netherlands). The assay will be performed in a blinded manner by one technician in each center with experience in the use of the Quantiferon platform. According to the manufacturer, a cutoff of 0.2 IU/mL of IFN-γ is used for defining positivity of the assay. If the level will be <0.1 IU/mL and the mitogen control will be positive (≥0.5 IU/mL), the test is considered to be negative. Technically, if the level of IFN-γ in the CMV antigen tube is <0.1 IU/mL and in the mitogen tube is <0.5 IU/mL, the result is indeterminate. For the purposes of the analysis, negative and indeterminate results were also classified together as being nonreactive.

**CMV-specific T-cell immune response testing by ELISpot/FluoroSpot**

CMV-specific T-cell response will be tested in predefined time points: at 3 weeks, 4 months, and 36 months after transplantation.

As a first step isolation and cryopreservation of mononuclear cells is carried out. In kidney transplant recipients, mononuclear cells are obtained from (donor/recipient`s) peripheral blood (peripheral mononuclear cells - PBMNCs). PBMNCs to be used as both stimulators and responder cells in Flurospot are isolated from cca 40 mililitres of whole blood by Ficoll gradient centrifugation. In cadaver donors splenocyte suspension is obtained from a donor spleen. Mononuclear cells are then isolated by Ficoll gradient centrifugation as well. After centrifugation, a layer of mononuclear cells is obtained. Cells are carefully collected, washed, counted and cryopreserved at -80°C until used in the Fluorospot assay.

Fluorospot assay is a modification of enzyme-linked immunospot (Elispot) assay, which has been adapted for detection of individual primed T cells secreting specific cytokines after antigenic stimulation, in case of Fluorospot utilizing fluorescent-based detection systems, enabling the detection of cells secreting either of two different cytokines, or both, in the same well. An ethanol treated PVDF (polyvinylidene difluoride) 96-well microplate is pre-coated with primary monoclonal antibodies specific for human IFNγ and IL-2. Frozen cells - PBMNCs isolated from kidney transplant recipient - are thawed and rested overnight at 37°C in a 5% CO2, resuspended and pipetted into the wells. In the next step stimuli is added into each well; donor PBMNCs or splenocytes thawed as described above for induction of allogeneic reaction, commercially available CMV peptide pools pp65 and IE-antigen for induction of CMV imunity reaction. Monoclonal antibody anti-CD 3-2 is used as a positive control stimuly. After the antigens for stimulation are added appropriately, the microplate is placed into a 37°C incubator over night. During this incubation period, the immobilized antibodies in the immediate vicinity of the secreting cells bind secreted IFNγ and IL-2. After washing away any cells and unbound substances, biotinylated polyclonal secondary antibodies specific for human IFNγ and IL-2 are added. Following a wash and visualization step a red and green colored precipitates form and appear as spots at the sites of cytokines localizations, with each individual spot representing an individual IFNγ/IL-2 secreting cell. Finally, the dried plate is analyzed in an automated fluorescence plate reader fitted with separate filters for the two fluorophores.

**Monitoring of donor specific antibodies (DSA) by single antigen bead (SAB) testing by Luminex**

Except for clinically indicated cases DSA will be tested in predefined time points. In all patients DSA will be performed pretransplantation, and at 3, 12, and 36 months after transplantation.

Qualitative DSA evaluation will be performed with the use of commercially available kits (LIFECODES LSATM Class I; LIFECODES LSATM Class II; Gen-Probe Transplant Diagnostics, USA). The method is based on incubation of specific microparticules binding HLA glycoproteins of class I or II with a small volume of serum of the patient tested. The specimen is washed several times, thereafter anti-IgG antibody with binded phycoerythrin is added to each specimen. Thereafter, the specimen is incubated, diluted and analyzed using Luminex equipment (Luminex® 100/200TM System, USA). The intensity of signals of each microparticle is analyzed by software and evaluated as positive or negative for antitibody binded. The results are expressed as a mean fluorescence intensity (MFI) for each HLA specificity. For the study pusposes, a fixed cutoff of 1000 normalized MFI will be applied.

**Allograft biopsy – indication and sample processing**

As a rule, suspected acute rejection will be confirmed by core biopsy (16 or 18-gauge needle) using the Banff 2017 classification. Relevant Banff up-dates during the study period may be incorporated. Indications for biopsy include an increase in serum creatinine by more than 20% that can not be explained otherwise, and delayed graft function (DGF). Biopsy will be also undertaken in cases of suboptimal development of graft function (serum creatinine >160 μmol/L). Biopsy-proven acute rejection will be defined as grade ≥IA and/or the presence of antibody-mediated rejection (AMR) with C4d and donor specific antibody positivity, with presumed acute rejection defined by the presence of "borderline changes“. Subclinical rejection will be assessed using protocol biopsy at Month 3 after RTx. Late protocol biopsy will be performed at Month 36 after RTx with the use 18-gauge needle (biopsy gun). A minimum of two cores will be obtained.

Tissues for light microscopy will be fixed in 4% formaldehyde, embedded in paraffin using routine procedure. Sections 3μm thick will be cut from tissue blocks and stained with hematoxylin and eosin, blue trichrome, silver staining, and Congo red staining. Immunohistochemical analysis will be performed in the Ventana automated slide stainer without manual antigen retrieval and is detected using Ventana ultraView universal DAB detection kit (Ventana-Roche, Tucson, AZ) as recommended by manufacturer. The primary antibodies against C4d (Venatana-Roche) and SV40 (Ventana-Roche), p53 (Ventana-Roche), and CMV (Dako, Glostrup, Denmark) will be used. Appropriate positive controls will be employed. In all biopsies, fresh tissue samples will be examined using immunoflorescence staining for C4d (Biomedica Grouppe, Vienna, Austria) and for C3 (Dako) depositions in peritubular capillaries paralelly. All biopsies will be evaluated according to the Banff classification. Small portions of renal tissue from the cortical or juxtamedullary zone will be immediately stored in preserve solution (RNA later, Qiagen, Hilden, Germany) for expression analysis. For RNA isolation and gene expression analysis the renal tissue is homogenized; total RNA is extracted using RNeasy Fibrous Tissue Mini Kit (Qiagene) automated on the QIAcube device and reversely transcribed into complementary DNA (cDNA) using the SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). Complementary DNA samples from each biopsy will be analyzed on TaqMan® Low Density Array cards containing primers-and-probe sets for 95 targets in duplicates for each sample by 7900HT Fast Real Time PCR System (Applied Biosystems, Foster City, CA, USA). The set of targets (chemokines and receptors, interferons, transforming growth factor beta (TGFβ) pathway, genes of extracellular matrix and transcription factors) was chosen on the basis of potential relevance to the study according the existing literature data (for detail see Table 2). Specific gene expression is calculated relative to that of the house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and the calibrator sample (FirstChoice®Human Kidney Total RNA, Ambion, Life Technologies, Carlsbad, CA, USA) by comparative threshold cycle method (2-CT) using RQ Manager 1.2 software (Applied Biosystems, Foster City, CA, USA) for automated data analysis and was expressed as relative quantity (RQ).

**Sample size and statistical analysis**

**For 12-month primary end point**

The anticipated rate of CMV DNAemia ≥2000 IU/mL in the control group is 35% based on our previous study comparing valganciclovir pre-emptive therapy with valacyclovir prophylaxis.[11](#_ENREF_11) The study is designed as a noninferiority trial. QuantiFERON-CMV guided pre-emptive therapy will be considered noninferior if the incidence of CMV DNAemia ≥2000 IU/mL will be 55% or less (accepted range of noninferiory +20%-points absolute). A total of 142 (71 per group) patients will be required to detect statistically significant noninferiory (power = 0.80; alpha = 0.05). Given the anticipated number of patients to be lost to follow-up, a target of 150 patients will be enrolled.

**For 36-month primary end point**

Due to high donor age and frequent use of expanded criteria donors the anticipated incidence of moderate-to-severe (Banff grade II or III) IFTA and/or IFTA with inflammation is 30%. This number correlates with the findings obtained with pre-emptive therapy group in several CMV prophylaxis studies in our centre.[9](#_ENREF_9),[13](#_ENREF_13) QuantiFERON-CMV guided pre-emptive therapy will be considered noninferior if the incidence of moderate-to-severe IFTA and/or IFTA with inflammation will be 50% or less (accepted range of noninferiory +20%-points absolute). A total of 130 (65 per group) patients will be required to detect statistically significant noninferiory (power = 0.80; alpha = 0.05). To meet both short-term and long-term end points a total of 150 subjects should be enrolled given the anticipated number of patients to be lost to follow-up.

**Predefined interim analysis**

For safety reasons after enrollment of 92 participants and completed 12-month follow-up an interim analysis is planned to exclude superiority of control (standard monitoring) group over experimental (QuantiFERON-CMV guided) group. The analysis will compare the incidence of primary end point (CMV DNAemia ≥2000 IU/mL) and CMV disease or high-grade CMV DNAemia (≥10,000 IU/mL). In the case of proven statistically significant superiority of the control group in any of above mentioned outcomes, the study will be stopped.

Quantitative parametric data will be compared between the groups using Student’s t-test and the Mann-Whitney U-test in non-parametric distribution. Qualitative data will be analyzed using Fisher’s exact test or Chi-square test. Patient and graft survival, incidence of CMV disease and DNAmia, and incidence of acute rejection will be calculated using Kaplan-Meier curves, with the log-rank test used for comparison. Cox proportional univariate hazard model will be used to calculate the hazard ratio (HR) and 95% confidence interval (CI) for CMV DNAemia in the QuantiFERON-CMV guided pre-emptive therapy group as compared with control group. Data will be analyzed according to the intention-to-treat principle. Statistical calculations are made using SPSS and Statistica 9.0 software. Values of p<0.05 are considered statistically significant.

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**Table 1A Shedule of visits and lab assessments – CMV-QuantiFERON PREEMPTIVE**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| week/**months** post-Tx | preTx | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 20 | 24 | **7** | **8** | **9** | **10** | **11** | **12** | **15** | **18** | **21** | **24** | **27** | **30** | **33** | **36** | **48** |
| Clinical, CMV disease, adverse events, BP | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| QuantiFERON-CMV | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| PCR CMV (gray-marked for clinical intervention in QFN pos pt) | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| s-Cr | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| high/weight | + | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | + | - | - | - | + | + |
| eGFR (MDRD) | - | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | + | - | - | - | + | + |
| Urine protein/day | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | + | - | - | - | + | + |
| U-Prot/Cr ratio | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | + | - | - | - | + | + |
| s-urea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| s-ALT | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| s-AST | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| s-GMT | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| s-bili | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| s-ALP | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| s-Alb | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| s-CRP | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| K | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Mg | - | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | + | - | - | - | + | - |
| Ca | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| P | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Uric acid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Chol-total | + | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | + | - | - | + | + | + | + | + | + | + | + | + | - |
| LDL chol | - | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | + | - | - | + | + | + | + | + | + | + | + | + | - |
| TG | + | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | + | - | - | + | + | + | + | + | + | + | + | + | - |
| WBC | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Hb | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Platelets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| FK (CyA) | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| MPA AUC | - | + | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| CMV-IgM,IgG | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| HBsAg\* | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| anti HCV Ab | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| PCR HCV  | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| HHV6-IgM,IgG | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| EBV-IgM, IgG | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| HHV8-IgG | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| FluoroSpot CMV | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| Luminex DSA | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | + | - |
| PCR polyoma | - | - | - | - | + | - | - | - | + | - | - | - | + | - | - | - | + | + | + | - | - | + | - | - | + | + | + | + | + | - | - | - | + | - |

**\***HBeAg and PCR HBV will be performed in the case of HBsAg+.

**Table 1B Shedule of visits and lab assessments – Standard monitoring PREEMPTIVE**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| week/**months** post-Tx | preTx | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 20 | 24 | **7** | **8** | **9** | **10** | **11** | **12** | **15** | **18** | **21** | **24** | **27** | **30** | **33** | **36** | **48** |
| Clinical, CMV disease, adverse events, BP | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| QuantiFERON-CMV | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| PCR CMV  | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | - | - | - | - |
| s-Cr | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| high/weight | + | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | + | - | - | - | + | + |
| eGFR (MDRD) | - | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | + | - | - | - | + | + |
| Urine protein/day | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | + | - | - | - | + | + |
| U-Prot/Cr ratio | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | + | - | - | - | + | + |
| s-urea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| s-ALT | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| s-AST | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| s-GMT | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| s-bili | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| s-ALP | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| s-Alb | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| s-CRP | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| K | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Mg | - | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | + | - | - | - | + | - |
| Ca | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| P | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Uric acid | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Chol-total | + | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | + | - | - | + | + | + | + | + | + | + | + | + | - |
| LDL chol | - | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | + | - | - | + | + | + | + | + | + | + | + | + | - |
| TG | + | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | + | - | - | + | + | + | + | + | + | + | + | + | - |
| WBC | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Hb | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| Platelets | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| FK (CyA) | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| MPA AUC | - | + | - | - | + | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| CMV-IgM,IgG | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| HBsAg\* | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| anti HCV Ab | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| PCR HCV  | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| HHV6-IgM,IgG | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| EBV-IgM, IgG | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - |
| HHV8-IgG | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| FluoroSpot CMV | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| Luminex DSA | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | + | - |
| PCR polyoma | - | - | - | - | + | - | - | - | + | - | - | - | + | - | - | - | + | + | + | - | - | + | - | - | + | + | + | + | + | - | - | - | + | - |

**\***HBeAg and PCR HBV will be performed in the case of HBsAg+.

**Table 2: Selected genes for intrarenal mRNA gene expression analysis in protocol biopsy at 36 months after transplantation (extended version, may be corrected based on up to date literature data)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Assay ID** | **Group** | **Gene Symbol** | **Gene name** | **Pathways/function** |
| Hs99999905\_m1 | Dehydrogenase | GAPDH | glyceraldehyde-3-phosphate dehydrogenase |  |
| Hs00171072\_m1 | Chemokine | CCL1 | chemokine (C-C motif) ligand 1 | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Chemokine (C-C motif) Ligands |
| Hs00237013\_m1 | Chemokine | CCL11 | chemokine (C-C motif) ligand 11 | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, TH2 Cytokines, Chemokine (C-C motif) Ligands |
| Hs00234646\_m1 | Chemokine | CCL13 | chemokine (C-C motif) ligand 13 | Chemokine Signaling, Renin-Angiotensin Pathway, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Chemokine (C-C motif) Ligands |
| Hs00171123\_m1 | Chemokine | CCL16 | chemokine (C-C motif) ligand 16 | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Chemokine (C-C motif) Ligands |
| Hs00171074\_m1 | Chemokine | CCL17 | chemokine (C-C motif) ligand 17 | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Chemokine (C-C motif) Ligands |
| Hs00268113\_m1 | Chemokine | CCL18 | chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated) | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Chemokine (C-C motif) Ligands |
| Hs00171149\_m1 | Chemokine | CCL19 | chemokine (C-C motif) ligand 19 | Chemokine Signaling,TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Chemokine (C-C motif) Ligands |
| Hs00234140\_m1 | Chemokine | CCL2 | chemokine (C-C motif) ligand 2 | Chemokine Signaling, Renin-Angiotensin Pathway, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Chemokine (C-C motif) Ligands |
| Hs00171076\_m1 | Chemokine | CCL21 | chemokine (C-C motif) ligand 21 | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Inflammatory Response |
| Hs00171080\_m1 | Chemokine | CCL22 | chemokine (C-C motif) ligand 22 | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Inflammatory Response |
| Hs00270756\_m1 | Chemokine | CCL23 | chemokine (C-C motif) ligand 23 | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Inflammatory Response |
| Hs00171082\_m1 | Chemokine | CCL24 | chemokine (C-C motif) ligand 24 | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Inflammatory Response |
| Hs00234142\_m1 | Chemokine | CCL3 | chemokine (C-C motif) ligand 3 | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Genes Involved in T-cell Polarization, Chemokine (C-C motif) Ligands, Inflammatory Response |
| Hs99999148\_m1 | Miscellaneous function | CCL4 | chemokine (C-C motif) ligand 4 | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, Genes Involved in T-cell Polarization, Chemokine (C-C motif) Ligands, Inflammatory Response |
| Hs00174575\_m1 | Chemokine | CCL5 | chemokine (C-C motif) ligand 5 | Chemokine Signaling, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, TH2 Cytokines , Chemokine (C-C motif) Ligands, Inflammatory Response |
| Hs00171147\_m1 | Chemokine | CCL7 | chemokine (C-C motif) ligand 7 | Chemokine Signaling, Renin-Angiotensin Pathway, TGF-Beta Pathway, Transendothelial Migration of Leukocytes, TH2 Cytokines , Chemokine (C-C motif) Ligands, Inflammatory Response |
| Hs00271615\_m1 | Chemokine | CCL8 | chemokine (C-C motif) ligand 8 | Chemokine Signaling, Renin-Angiotensin Pathway, TGF-Beta Pathway, Transendothelial Migration of Leukocytes , Chemokine (C-C motif) Ligands, Inflammatory Response |
| Hs00236937\_m1 | Chemokine | CXCL1 | chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) | Chemokine Signaling, Transendothelial Migration of Leukocytes , Chemokine (C-X-C motif) Ligands, Inflammatory Response |
| Hs00171042\_m1 | Chemokine | CXCL10 | chemokine (C-X-C motif) ligand 10 | Chemokine Signaling, Chemokine (C-X-C motif) Ligands, Inflammatory Response |
| Hs00171138\_m1 | Cytokine | CXCL11 | chemokine (C-X-C motif) ligand 11 | Chemokine Signaling, Transendothelial Migration of Leukocytes , Chemokine (C-X-C motif) Ligands |
| Hs00171022\_m1 | Growth factor | CXCL12 | chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1) | Cellular Apoptosis Pathway, Chemokine Signaling, Renin-Angiotensin Pathway, Transendothelial Migration of Leukocytes, Chemokine (C-X-C motif) Ligands |
| Hs00757930\_m1 | Miscellaneous function | CXCL13 | chemokine (C-X-C motif) ligand 13 | Chemokine Signaling, Transendothelial Migration of Leukocytes , Chemokine (C-X-C motif) Ligands |
| Hs00601975\_m1 | Chemokine | CXCL2 | chemokine (C-X-C motif) ligand 2 | Chemokine Signaling, Transendothelial Migration of Leukocytes , Chemokine (C-X-C motif) Ligands, Inflammatory Response |
| Hs00171061\_m1 | Chemokine | CXCL3 | chemokine (C-X-C motif) ligand 3 | Chemokine Signaling, Transendothelial Migration of Leukocytes , Chemokine (C-X-C motif) Ligands, Inflammatory Response |
| Hs00605742\_g1 | Chemokine | CXCL6 | chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2) | Chemokine Signaling, Transendothelial Migration of Leukocytes , Chemokine (C-X-C motif) Ligands, Inflammatory Response |
| Hs00171065\_m1 | Cytokine | CXCL9 | chemokine (C-X-C motif) ligand 9 | Chemokine Signaling, Transendothelial Migration of Leukocytes , Chemokine (C-X-C motif) Ligands |
| Hs00174298\_m1 | G-protein coupled receptor | CCR1 | chemokine (C-C motif) receptor 1 | Chemokine Signaling, Transendothelial Migration of Leukocytes, Genes Involved in T-cell Polarization, Inflammatory Response |
| Hs00706455\_s1 | G-protein coupled receptor | CCR10 | chemokine (C-C motif) receptor 10 | Chemokine Signaling, Transendothelial Migration of Leukocytes, Chemokine (C-C motif) Receptors |
| Hs00174150\_m1 | G-protein coupled receptor | CCR2 | chemokine (C-C motif) receptor 2 | Chemokine Signaling, Transendothelial Migration of Leukocytes, Genes Involved in T-cell Polarization, TH2 Cytokines, Inflammatory Response |
| Hs00266213\_s1 | G-protein coupled receptor | CCR3 | chemokine (C-C motif) receptor 3 | Chemokine Signaling, Transendothelial Migration of Leukocytes, Genes Involved in T-cell Polarization, TH2 Cytokines, Inflammatory Response |
| Hs99999919\_m1 | G-protein coupled receptor | CCR4 | chemokine (C-C motif) receptor 4 | Chemokine Signaling, Transendothelial Migration of Leukocytes, Genes Involved in T-cell Polarization, TH2 Cytokines, Inflammatory Response |
| Hs00152917\_m1 | G-protein coupled receptor | CCR5 | chemokine (C-C motif) receptor 5 | Chemokine Signaling, Transendothelial Migration of Leukocytes, Genes Involved in T-cell Polarization, Chemokine (C-C motif) Receptors |
| Hs00171121\_m1 | G-protein coupled receptor | CCR6 | chemokine (C-C motif) receptor 6 | Chemokine Signaling, Transendothelial Migration of Leukocytes, Chemokine (C-C motif) Receptors |
| Hs99999080\_m1 | G-protein coupled receptor | CCR7 | chemokine (C-C motif) receptor 7 | Chemokine Signaling, Transendothelial Migration of Leukocytes, Chemokine (C-C motif) Receptors, Inflammatory Response |
| Hs00171041\_m1 | G-protein coupled receptor | CXCR3 | chemokine (C-X-C motif) receptor 3 | Chemokine Signaling, Transendothelial Migration of Leukocytes, Genes Involved in T-cell Polarization, TH1 Cytokines  |
| Hs00607978\_s1 | G-protein coupled receptor | CXCR4 | chemokine (C-X-C motif) receptor 4 | Chemokine Signaling, Transendothelial Migration of Leukocytes, Genes Involved in T-cell Polarization |
| Hs00174843\_m1 | G-protein coupled receptor | CXCR6 | chemokine (C-X-C motif) receptor 6 | Chemokine Signaling, Transendothelial Migration of Leukocytes |
| Hs01077958\_s1 | Cytokine | IFNB1 | interferon, beta 1, fibroblast | Cytokine Network, Interferon Pathway, B-cell Proliferation, Regulators of Th1 and Th2 Development |
| Hs00174143\_m1 | Cytokine | IFNG | interferon, gamma | Cytokine Network, Interferon Pathway, Regulators of Th1 and Th2 Development |
| Hs00166223\_m1 | Cytokine receptor | IFNGR1 | interferon gamma receptor 1 | Interferon Pathway, Genes Involved in Th1/Th2 Differentiation |
| Hs00194264\_m1 | Cytokine receptor | IFNGR2 | interferon gamma receptor 2 (interferon gamma transducer 1) | Interferon Pathway, Genes Involved in Th1/Th2 Differentiation |
| Hs00241807\_m1 | Cytokine | BMP1 | bone morphogenetic protein 1 | Cellular Apoptosis Pathway, mTOR Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway, Other Extracellular Molecules |
| Hs00154192\_m1 | Cytokine | BMP2 | bone morphogenetic protein 2 | Cellular Apoptosis Pathway, mTOR Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway, Other Extracellular Molecules |
| Hs00370078\_m1 | Cytokine | BMP4 | bone morphogenetic protein 4 | Cellular Apoptosis Pathway, mTOR Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway, Embryonic Development |
| Hs00234930\_m1 | Cytokine | BMP5 | bone morphogenetic protein 5 | Cellular Apoptosis Pathway, mTOR Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway |
| Hs00233470\_m1 | Cytokine | BMP6 | bone morphogenetic protein 6 | Cellular Apoptosis Pathway, mTOR Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway |
| Hs00233477\_m1 | Cytokine | BMP7 | bone morphogenetic protein 7 | Cellular Apoptosis Pathway, mTOR Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway |
| Hs00167060\_m1 | Cytokine | GDF5 | growth differentiation factor 5 | Cellular Apoptosis Pathway, Renin-Angiotensin Pathway |
| Hs00193364\_m1 | Cytokine | GDF9 | growth differentiation factor 9 | Cellular Apoptosis Pathway, Renin-Angiotensin Pathway |
| Hs00153126\_m1 | Growth factor | IGF1 | insulin-like growth factor 1 (somatomedin C) | Cellular Apoptosis Pathway, Renin-Angiotensin Pathway, TGF-b Superfamily Cytokines, Other Extracellular Molecules |
| Hs00171410\_m1 | Protein/peptide hormone | INHA | inhibin, alpha | Macrophage Activation, T-cell Activation, TGF-b Superfamily Cytokines |
| Hs00170103\_m1 | Cytokine | INHBA | inhibin, beta A | Macrophage Activation, T-cell Activation, TGF-b Superfamily Cytokines |
| Hs00764128\_s1 | Cytokine | LEFTY1 | left-right determination factor 1 | Cellular Apoptosis Pathway, Renin-Angiotensin Pathway, TGF-b Superfamily Cytokines |
| Hs00386448\_m1 | Hsp 70 family chaperone | LTBP1 | latent transforming growth factor beta binding protein 1 | Cellular Apoptosis Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway, TGF-b Superfamily Cytokines, Extracellular Matrix Structural Constituents |
| Hs00166367\_m1 | Hsp 70 family chaperone | LTBP2 | latent transforming growth factor beta binding protein 2 | Cellular Apoptosis Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway, TGF-b Superfamily Cytokines, Extracellular Matrix Structural Constituents |
| Hs00186025\_m1 | Hsp 70 family chaperone | LTBP4 | latent transforming growth factor beta binding protein 4 | Cellular Apoptosis Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway, TGF-b Superfamily Cytokines, Extracellular Matrix Structural Constituents |
| Hs00234042\_m1 | Growth factor | PDGFB | platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog) | Cellular Apoptosis Pathway, Renin-Angiotensin Pathway, TGF-b Superfamily Cytokines, Other Extracellular Molecules |
| Hs99999918\_m1 | Cytokine | TGFB1 | transforming growth factor, beta 1 | Cellular Apoptosis Pathway, IL-2 Gene Expression in Activated and Quiescent T-Cells, Renin-Angiotensin Pathway, TGF-Beta Pathway, Genes Involved in T-cell Polarization |
| Hs00234244\_m1 | Cytokine | TGFB2 | transforming growth factor, beta 2 | Cellular Apoptosis Pathway, IL-2 Gene Expression in Activated and Quiescent T-Cells, Renin-Angiotensin Pathway, TGF-Beta Pathway |
| Hs00234245\_m1 | Cytokine | TGFB3 | transforming growth factor, beta 3 | Cellular Apoptosis Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway, CD4+T Cell Marker |
| Hs00170630\_m1 | Other transcription factor | FOS | v-fos FBJ murine osteosarcoma viral oncogene homolog | Chemokine Signaling, Renin-Angiotensin Pathway, TGF-Beta Pathway, TNF Superfamily Pathway, Inflammatory Response, SMAD Target Genes |
| Hs00174131\_m1 | Cytokine | IL6 | interleukin 6 (interferon, beta 2) | Cellular Apoptosis Pathway, Cytokine Network, Interferon Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway, CD4+T Cell Marker |
| Hs00164004\_m1 | Protein kinase | COL1A1 | collagen, type I, alpha 1 | Transendothelial Migration of Leukocytes, SMAD Target Genes |
| Hs00164099\_m1 | Protein kinase | COL1A2 | collagen, type I, alpha 2 | Transendothelial Migration of Leukocytes, SMAD Target Genes |
| Hs00164103\_m1 | Protein kinase | COL3A1 | collagen, type III, alpha 1 | Transendothelial Migration of Leukocytes, SMAD Target Genes |
| Hs01103582\_s1 | Other transcription factor | JUN | jun oncogene | Chemokine Signaling, Interferon Pathway, Renin-Angiotensin Pathway, TGF-Beta Pathway, TNF Superfamily Pathway, SMAD Target Genes |
| Hs00357891\_s1 | Other transcription factor | JUNB | jun B proto-oncogene | Interferon Pathway, SMAD Target Genes, Other Transcription Factors |
| Hs00195432\_m1 | Other transcription factor | SMAD1 | SMAD family member 1 | TGF-Beta Pathway |
| Hs00183425\_m1 | Other transcription factor | SMAD2 | SMAD family member 2 | IL-2 Gene Expression in Activated and Quiescent T-Cells, TGF-Beta Pathway |
| Hs00232222\_m1 | Other transcription factor | SMAD3 | SMAD family member 3 | IL-2 Gene Expression in Activated and Quiescent T-Cells, TGF-Beta Pathway |
| Hs00232068\_m1 | Other transcription factor | SMAD4 | SMAD family member 4 | IL-2 Gene Expression in Activated and Quiescent T-Cells, TGF-Beta Pathway |
| Hs00195437\_m1 | Other transcription factor | SMAD5 | SMAD family member 5 | TGF-Beta Pathway |
| Hs00195441\_m1 | Other transcription factor | SMAD9 | SMAD family member 9 | TGF-Beta Pathway |
| Hs00410929\_m1 | Ubiquitin-protein ligase | SMURF1 | SMAD specific E3 ubiquitin protein ligase 1 | Embryonic Development, Molecules Regulating Signaling of the TGF-β Superfamily |
| Hs00175478\_m1 | Membrane-bound signaling molecule | CD80 | CD80 molecule | CTLA4 Signaling, IL-2 Gene Expression in Activated and Quiescent T-Cells, T-Cell Receptor and CD3 Complex, TGF-Beta Pathway, Antigen Dependent B-cell Activation, Regulators of T-cell Activation  |
| Hs01567025\_m1 | Membrane-bound signaling molecule | CD86 | CD86 molecule | CTLA4 Signaling, Regulators of T-cell Activation |
| Hs00231733\_m1 | Transcription cofactor | CREBBP | CREB binding protein | NF-KappaB Family Pathway, TGF-Beta Pathway, CD4+T Cell Marker |
| Hs00232342\_m1 | Other transcription factor | NFATC1 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 | IL-2 Gene Expression in Activated and Quiescent T-Cells, TH2 Cytokines, VEGF Signaling Pathway |
| Hs00234855\_m1 | Other transcription factor | NFATC2 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2 | IL-2 Gene Expression in Activated and Quiescent T-Cells, TH2 Cytokines, VEGF Signaling Pathway |
| Hs00190046\_m1 | Other transcription factor | NFATC3 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3 | IL-2 Gene Expression in Activated and Quiescent T-Cells, Inflammatory Response, VEGF Signaling Pathway |
| Hs00190037\_m1 | Other transcription factor | NFATC4 | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4 | IL-2 Gene Expression in Activated and Quiescent T-Cells, VEGF Signaling Pathway |
| Hs00765730\_m1 | Other transcription factor | NFKB1 | nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 | Cellular Apoptosis Pathway, Chemokine Signaling, IL-2 Gene Expression in Activated and Quiescent T-Cells, NF-KappaB Family Pathway, Renin-Angiotensin Pathway, Inflammatory Response |
| Hs00374416\_m1 | Other signaling molecule | SOCS2 | suppressor of cytokine signaling 2 | Other Transcription Factors and Regulators |
| Hs00751962\_s1 | Other signaling molecule | SOCS5 | suppressor of cytokine signaling 5 | T-cell Differentiation |
| Hs00959009\_m1 | Cytokine | SPP1 | secreted phosphoprotein 1 | Transendothelial Migration of Leukocytes, T-cell Activation |
| Hs00234829\_m1 | Other transcription factor | STAT1 | signal transducer and activator of transcription 1, 91kDa | Interferon Pathway, Renin-Angiotensin Pathway, TH1 Cytokines  |
| Hs00237139\_m1 | Miscellaneous function | STAT2 | signal transducer and activator of transcription 2, 113kDa | Interferon Pathway, JAK / STAT Signaling Pathway |
| Hs00374280\_m1 | Other transcription factor | STAT3 | signal transducer and activator of transcription 3 (acute-phase response factor) | Renin-Angiotensin Pathway, Transcriptional Regulator  |
| Hs00231372\_m1 | Other transcription factor | STAT4 | signal transducer and activator of transcription 4 | TH1 Cytokines  |
| Hs00559643\_m1 | Other transcription factor | STAT5A | signal transducer and activator of transcription 5A | EGF/PDGF/cytokine mediated Signaling Pathway |
| Hs00273500\_m1 | Other transcription factor | STAT5B | signal transducer and activator of transcription 5B | Chemokine Signaling, Cytokine and Chemokine-mediated Signaling Pathways |
| Hs00598625\_m1 | Other transcription factor | STAT6 | signal transducer and activator of transcription 6, interleukin-4 induced | TH2 Cytokines  |
| Hs00153340\_m1 | Other transcription factor | TP53 | tumor protein p53 | Cellular Apoptosis Pathway, p53 and DNA Damage Response, Wnt Signaling Pathway |
| Hs00231533\_m1 | Zinc finger transcription factor | YY1 | YY1 transcription factor | CD4+T Cell Marker, Other Transcription Factors |

**Appendix I**

**Valganciclovir dosing according to renal function for pre-emptive therapy or treatment of CMV disease.**

|  |  |
| --- | --- |
| **Creatinine clearance [ml/s]** | **Dose** |
| > 1.00 | 900 mg twice daily |
| 0.67 – 0.99 | 450 mg twice daily |
| 0.42 – 0.66 | 450 mg per day |
| 0.17 – 0.41 | 450 mg every 48 h |
| < 0.17 or hemodialysis | 450 mg three times per week |

**Appendix II**

**Valganciclovir dosing according to renal function for secondary prophylaxis.**

|  |  |
| --- | --- |
| **Creatinine clearance [ml/s]** | **Dose** |
| > 1.00 | 900 mg per day |
| 0.67 – 0.99 | 450 mg per day |
| 0.42 – 0.66 | 450 mg every 48 h |
| 0.17 – 0.41 | 450 mg twice weekly |
| < 0.17 or hemodialysis | 450 mg after hemodialysis |

**Appendix III**

**Intravenous ganciclovir dosing according to renal function for pre-emptive therapy or treatment of CMV disease.**

|  |  |
| --- | --- |
| **Creatinine clearance [ml/s]** | **Dose** |
| ≥ 1.17 | 5.0 mg/kg/12 h |
| 0.85 – 1.16 | 5.0 mg/kg/24 h |
| 0.85 – 0.42 | 2.5 mg/kg/24 h |
| 0.41 – 0.17 | 1.25 mg/kg/24 h |
| < 0.17 or hemodialysis | 1.25 mg/kg three times per week |

The value of creatinine clearance will be estimated by Cocroft and Gault equation:

For male CLCr = (140 - age) x body weight / (49 x s-Cr)

For female CLCr = 0,85 x (140 - age) x body weight / (49 x s-Cr)

CLCr - creatinine clearance in mL/s

s-Cr - serum creatinine in µmol/L

age is expressed in years

body weight is expressed in kilograms

**Cocroft D., Gault M.**: Prediction of creatinine clearance from serum creatinine. Nephron 1976, 16:31.

**Appendix IV**

**Summary of major adverse events of valganciclovir**

Nephrologic (renal insuficiency).

Hematologic (anemia, leucopenia, thrombocytopenia).

Gastroenterologic (diarrhea, abdominal pain, dyspepsia, nausea, vomiting, anorexia, liver enzymes abnormalities).

Neurologic (somnolence, insomnia, hypestesia, psychiatric abnormalities, neuropathy).

Sensoric (macular edema, retinal detechment, vitreal abnormalities).

Dermatologic (dermatitis, night sweating, pruritus).

Musculosceletal (backpain).

**Pre-emptive therapy withdrawal due to adverse events:**

In the case of serious adverse event valganciclovir pre-emptive therapy may be withdraw if clinically indicated. However, other reasons of adverse events should be taken into account. In particular, leucopenia or thrombycytopenia are side effects of many other drugs such as mycophenolate mofetil, sirolimus, co-trimoxazole. Also CMV disease has similar laboratory abnormalities as valganciclovir side effects.

Informed consent

Patients with transplanted kidney have to use various drugs which avoid rejection of transplanted kidney and maintain its long-term function. Unfortunately, all of these drugs have adverse events which include weakening immunity of the body against infections. Viral infection caused by cytomegalovirus belongs among the most common and most serious ones. Therefore, we aim to prevent this infection, diagnose and treat it in time.

In pursuance of efforts to avoid such an infection in our patients, we will check the patients more frequently (by clinical evaluation and by laboratory) in the first year after transplantation. All patients will be checked by very sensitive method to detect the virus within blood in a one a week basis. If the virus activity is detected patients will be treated with a drug (VALCYTE TBL) for a minimum of 14 days which suppresses the virus. Sensitive methods used are able to detect virus activity before the onset of infectious symptoms (for instance fever). Therefore, it is assumed such a treatment avoids development of symptomatic infection. In one group of patients, the level of cytomegalovirus specific immunity will be assessed at 3 weeks after transplantation. Patients with detectable immunity against cytomegalovirus will be evaluated for the potential of less frequent monitoring. In recent years we used such a preventive strategy in our center which led to prevention of cytomegalovirus infection in 90% patients. On the other hand, in two thirds of patients without monitoring infection developed. More frequent checkups help us to detect infection earlier before the onset of serious symptoms allowing effective therapy.

The drug Valcyte is commonly used in the Czech Republic as well as in worldwide and is well tolerated. Among the most common side effects of Valcyte belong changes in blood count. Valcyte is indicated for cytomegalovirus prevention after transplantation regardless of your potential enrollment to the study. Every care for the patients enrolled to the study will be the same as in other patients in our transplant center. The results of the study will be used for future treatment of patients after transplantation. In the case of publication, your name will never be released. If you do not wish to enter the study you will be treated by Valcyte for cytomegalovirus prevention according to current protocol in our center. It is your right to withdraw from the study even without any reason declared. Such a behavior will not have any negative consequences for you.

I was informed about study design and all my potential questions have been answered satisfactory. I agree with my participation in the study.

Informing physician: ..................................

Patient: .................................................

Date:

**podepsané poslat s nemocným na chirurgii a kopii nechat pacientovi!**

Informace pro pacienta

Pacienti, kterým byla transplantována ledvina, musí užívat řadu léků, které zabraňují odhojení transplantované ledviny a umožňují její dlouhodobou funkci. Bohužel všechny tyto léky mají i nežádoucí účinky, mezi které patří oslabení obranyschopnosti organismu proti infekcím. Mezi nejčastější a nejzávažnější infekce patří virová infekce způsobená cytomegalovirem. Z tohoto důvodu je snaha této infekci předcházet a včas jí diagnostikovat a léčit.

V rámci snahy zabránit této infekci i u našich pacientů, je budeme v prvním roce po transplantaci kontrolovat (klinicky i laboratorním vyšetřením z krve). U všech pacientů bude v častých intervalech (týdně) zkoumána aktivita viru v krvi. Pokud bude aktivita viru zachycena, pacient bude léčen lékem Valcyte tbl po dobu minimálně 14ti dnů k potlačení virové infekce. Laboratorní vyšetření umožní zachytit aktivitu viru ještě před vznikem symptomů infekce (např. teploty). U jedné skupiny pacientů bude ve 3. týdnu po transplantaci stanovena úroveň obranischopnosti (imunity) proti cytomegalovirus a bude dále zkoumáno, zda pacienti s přítomností obrany proti cytomegaloviru mohou být kontrolováni méně často. Kontrola aktivity viru v krvi a preventivní léčba výrazně sníží pravděpodobnost vzniku cytomegalovirové infekce. V minulých letech jsme tento způsob užívali a dosáhli tím zabránění vzniku cytomegalovirové infekce u více než 90% pacientů. Naopak, u dvou třetin pacientů, kteří pravidelné kontroly na aktivitu viru neměli, tato infekce vznikla. Častější kontroly nám obecně umožní odhalit infekci ještě před vznikem závažných projevů a včas jí léčit.

Lék Valcyte se běžně v České republice i ve světě užívají a jsou pacienty dobře snášeny. Mezi hlavní nežádoucí účinky Valcyte patří změny v krevním obrazu. Léky jsou indikované v prevenci cytomegalovirové nemoci po transplantaci obecně, tj bez ohledu na Vaše zvažované zařazení do studie. Veškerá péče o pacienty zařazené do zmiňované studie bude stejná jako u ostatních pacientů transplantovaných v našem transplantačním centru. Výsledky studie budou využity při léčení nemocných po transplantaci ledviny, případně i jiných orgánů. V případě publikace nebude Vaše jméno nikde uvedeno. Pokud si nebudete přát účastnit se studie, bude Vám předepsán k prevenci cytomegalovirové infekce podle platného protokolu našeho transplantačního centra Valcyte tbl. Je Vaším právem kdykoliv odstoupit ze studie a to i bez udání důvodu, aniž by to mělo jakékoliv důsledky pro Vás.

Byl jsem seznámen s uspořádáním studie, všechny moje případné otázky mi byly uspokojivě zodpovězeny. Souhlasím s účastí ve studii.

Informující lékař: ..................................

Pacient: .................................................

Datum: