**Supplementary appendix**

**THE ASSOCIATION BETWEEN CYTOMEGALOVIRUS INFECTION AND CARDIAC ALLOGRAFT VASCULOPATHY IN THE ERA OF ANTIVIRAL VALGANCICLOVIR PROPHYLAXIS**

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# Table S1. Immunosuppresive regimen and CMV prophylaxis over the years 2000-2018.

|  |  |  |  |
| --- | --- | --- | --- |
| Year | Induction therapy | Maintenance immunosuppression | CMV prophylaxis |
| 2000 | Horse ATG | Tac + MMF + steroids | IG |
| 2003 | Horse ATG | Tac + MMF + steroids | VGCV 3 months |
| 2009 | Rabbit ATG | Tac + MMF + steroids | VGCV 3 months |
| 2013 | Rabbit ATG | Tac + MMF + steroids | VGCV 6 months |

ATG - antithymocyte globulin; IG – anti-CMV immunoglobulin; MMF - mycophenolate mofetil; Tac - tacrolimus; VGCV - valganciclovir

# Text S1. CMV monitoring

Plasma CMV DNAemia was quantified using an internally controlled dual target real-time PCR design, targeting UL54(1) and the highly conserved UL75(2) genes. Total nucleic acids (TNAI) were extracted from 500µl sample spiked with a known concentration of Phocine Herpes Virus (1)) as internal control using the MagNa Pure 96 DNA and Viral NA LV kit (Roche, Almere, the Netherlands) and the Viral NA Universal LV 2.0 protocol. Nucleic acids were resuspended in a final volume of 100µl. Subsequently, 8µl eluate was amplified in a 20µl reaction containing 5µl 4x TaqManTM Fast Advanced Master Mix with UDG (TFA, Life Technologies, Nieuwkerk a/d IJssel, the Netherlands), 0.4µl of UL54, UL75 and PhHV primers and probe mixtures (Table S1); and 5 min 50°C, 20s 95°C, 45 cycles of 3s 95°C and 30s 60°C as thermal profile. Amplification was performed in a LC480 II (Roche Applied Science, Almere, The Netherlands) using Fit point analysis module. Viral load quantification was performed using the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques (code: 09/162, NBISC, Hertfordshire, Great-Britain) as standard, with a lower limit of detection (95% hit rate) of 50 IU/ml. Validation of the procedure was performed according to ISO 15189:2012 guidelines.

# Table S2: Sequences and concentrations of primer and probes used

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Viral pathogen | Oligo name | Sequence 5’-3’ | Conca. | Reference |
| CMV UL54 gene | CMV-fwd | GCCGATCGTAAAGAGATGAAGAC | 60 | Adapted from  (1) |
| CMV-rev | CTCGTGCGTGTGCTACGAGA | 90 |
| CMV-probe | FAM-AGTGCAGCCCCGGCCATCGTTC-BHQ1b | 5 |
| CMV UL75 gene | UL75-fwda | ACGAATACCTCAGCGACCTGTACA | 30 | Adapted from (2) |
| UL75-fwdb | ACGAATACCTCAGCGACCTGTATA | 30 |
| UL75-rev | CGTTCGAGCGAGTGATCG | 30 |
| UL75-probe | FAM-CTGTTCCAGTAGCGGGCGACG-BHQ1b | 5 |
| PhHV  gB-gene | PHHV-1-fwd | GGGCGAATCACAGATTGAATC | 2.5 | Adapted from  (1) |
| PHHV-1-rev | GCGGTTCCAAACGTACCAA | 10 |
| PHHV-1-probe | CY5-TTTTTATGTGTCCGCCACCATCTGGATC-BHQ2c | 5 |

a Conc. = concentration (pmol/50µl reaction)

b BHQ1 = black hole quencher 1

c BHQ2 = black hole quencher 2

# Table S3. CMV breakthrough infections by type of prophylaxis

|  |  |  |
| --- | --- | --- |
| Prophylaxis | CMV breakthrough infection | |
| no | yes |
| Immunoglobulin | 5 (36%) | 9 (64%) |
| VGCV | 42 (74%) | 15 (26%) |

Table presents number of patients with breakthrough infection (percent of prophylactic treatment group). VGCV – valganciclovir.

# Table S4. Multivariable analysis of CAV any grade. Patients with immunoglobulin prophylaxis excluded, N=246.

|  |  |  |
| --- | --- | --- |
| Covariate | CSHR (95% CI) | P value |
| Donor BMI (kg/m2) | 1.03 (0.98 – 1.08) | 0.22 |
| Donor age (years) | 1.01 (0.99 – 1.02) | 0.10 |
| Recipient BMI (kg/m2) | 1.04 (0.99 – 1.09) | 0.11 |
| CMV breakthrough infection | 1.72 (0.78- 3.77) | 0.17 |
| Number of ACR≥2R/AMR episodes | 1.17 (1.03-1.33) | 0.01 |
| Hypertension | 1.51 (1.03-2.22) | 0.03 |
| MMF | 0.68 (0.47-0.98) | 0.04 |
| Total cholesterol at 1 year (mmol/l) | 1.11 (0.97-1.26) | 0.12 |

ACR – acute cellular rejection, AMR – antibody-mediated rejection, BMI – body mass index, CSHR – cause specific hazard ratio, MMF – mycophenolate mofetil

# Figure S1. Temporal trends of complications stratified according to types of prophylaxis in mismatch patients (n = 71).

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CAV – CAV grade 1 or more according to ISHLT, including CAV-related death. Other curves present mortality from the other major causes. CMVIG – anti-CMV immunoglobulin, VGCV - valganciclovir

**References**

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2. Fukushima E, Ishibashi K, Kaneko H, et al.: Identification of a highly conserved region in the human cytomegalovirus glycoprotein H gene and design of molecular diagnostic methods targeting the region. J Virol Methods 2008;151:55-60.