Differential decay kinetics of human cytomegalovirus glycoprotein B genotypes following antiviral chemotherapy

Vincent C. Emery\textsuperscript{a,∗}, Oriol Manuel\textsuperscript{b}, Anders Asberg\textsuperscript{c}, Xiaoli Pang\textsuperscript{b}, Deepali Kumar\textsuperscript{b}, Anders Hartmann\textsuperscript{d}, Jutta Preiksaitis\textsuperscript{b}, Mark D. Pescovitz\textsuperscript{e}, Halvor Rollag\textsuperscript{f}, Alan G. Jardine\textsuperscript{g}, Christoph G. Gahlemann\textsuperscript{b}, Atul Humar\textsuperscript{b,∗∗}

\textsuperscript{a} Centre for Virology, Department of Infection, University College Medical School, London, United Kingdom
\textsuperscript{b} Transplant Infectious Diseases, University of Alberta, Edmonton, Canada
\textsuperscript{c} Department of Pharmaceutical Biosciences, School of Pharmacy, University of Oslo, Oslo, Norway
\textsuperscript{d} Department of Medicine, Rikshospitalet-Radiumhospitalet Medical Centre, University of Oslo, Oslo, Norway
\textsuperscript{e} Department of Surgery, Indiana University, Indianapolis, IN, United States
\textsuperscript{f} Institute of Microbiology, University of Oslo, Norway
\textsuperscript{g} Department of Medicine, University of Glasgow, Glasgow, United Kingdom

\textbf{ARTICLE INFO}

\textbf{Article history:}
Received 4 April 2011
Received in revised form 21 November 2011
Accepted 23 January 2012

\textbf{Keywords:}
Viral replication
Ganciclovir
Fitness
Solid organ transplantation

\textbf{A B S T R A C T}

\textbf{Background:} The impact of different cytomegalovirus (HCMV) glycoprotein B (gB) genotypes on pathogenesis remains controversial.

\textbf{Objectives:} To investigate the effect of gB genotypes either as single infections or as part of multiple infections on the early kinetics of response to ganciclovir therapy.

\textbf{Methods:} Patients (n = 239) enrolled in a study of intravenous ganciclovir or valganciclovir for the treatment of HCMV disease were analysed by a gB genotype specific PCR to quantify the amount of each gB genotype present at initiation of therapy (baseline, day 0) and at days 3, 7, 14 and 21 post therapy.

\textbf{Results and conclusions:} In all gB groups (individual gB genotype infections and mixed genotype infections) there was a biphasic decline in viral load after therapy. The first phase half life (days 0–3) was ≤1 day and was followed over the next 18 days by a slower second phase decline with half lives ranging from 3.4 to 4.4 days. The 1st phase rapid decline in viral load was dependent upon gB genotype whereas the ultimate viral load reduction at day 21 was relatively insensitive to gB genotype. A strong correlation between 1st phase decline and extent of viral load reduction at day 21 was observed (r = 0.37; p = 0.002). These data imply that early reductions in HCMV load after therapy may be useful in predicting the duration of drug therapy needed to control HCMV replication.

© 2012 Elsevier B.V. Open access under CC BY license.

1. Introduction

Human cytomegalovirus (HCMV) remains an important infectious complication for the immunocompromised host. A range of direct and indirect effects have been associated with active replication [reviewed in 1, 2]. Viral pathogenesis is directly related to the degree of viral replication with a number of studies showing viral load, and more recently cumulative load experienced during the period of replication, are diagnostic and prognostic markers of recurrent infection and disease [3–5]. Complementary immunological studies indicate that the quality of CD4 and CD8 T-cell responses are critical factors in the control of high level replication [6–12]. HCMV replication in vivo is highly dynamic with doubling times of approximately 1 day with a basic reproductive number in liver transplant recipients experiencing primary infection of approximately 15–17.

At present, antiviral chemotherapeutic control of replication relies upon prophylactic deployment of ganciclovir (VGCV) and treatment of asymptomatic or symptomatic replication with either intravenous ganciclovir (iv GCV) or VGCV (reviewed in 15–17). There remains a paucity of data on the role of different HCMV genotypes in pathogenesis, and their response to immune or antiviral...
mediated control. Although the prototype laboratory adapted AD169 strain was originally sequenced in 1989\textsuperscript{18} and re-sequenced with the Towne strain more recently,\textsuperscript{19} only a limited number of clinical strains have been subject to full genomic sequence analysis.\textsuperscript{20,21} However, various genes have been subjected to more intense sequence analysis at a macro and micro-scale including the surface glycoproteins B and H and UL139, UL144, UL147 and UL148.\textsuperscript{22-28} In the context of gB, four genotypes have been characterized based upon RFLP analysis.\textsuperscript{29} Although gB plays a critical role in HCMV entry and cell-to-cell spread,\textsuperscript{30} the clinical relevance of these gB genotypes remains controversial.\textsuperscript{31-35} At present, the majority of these analyses have taken place in relatively small numbers of patients infected with a single gB genotype. However, we now know that multi-genotype infections are relatively common\textsuperscript{36,37} and we reasoned that genotype specific declines in these mixed infections may provide new insight into the HCMV replication dynamics. The recently completed VICTOR study comparing IV gCV and VgCV for the therapy of HCMV syndrome and disease provided a large database of samples with frequent viral load sampling and a source for gB genotype analysis.\textsuperscript{38} Although we have previously reported on the epidemiology and clinical response rates with gB genotypes\textsuperscript{39} the present study undertakes an in-depth viral kinetics analysis to investigate the potential for differential decay kinetics of different gB genotypes either alone or when in competition with other gB genotypes and to ascertain whether early viral kinetics are associated with ultimate control of replication.

2. Materials and methods

2.1. Patient population and definitions

Solid organ transplant recipients enrolled in a randomized (1:1), open-label, parallel group, active drug-controlled multicentre and non-inferiority trial comparing treatment with oral valganciclovir to intravenous ganciclovir for the treatment of HCMV disease in solid organ transplant recipients (ClinicalTrials.gov NCT00431353) (VICTOR study) were included as previously described.\textsuperscript{38} A total of 321 patients received at least one dose of assigned medication with 164 patients randomized to treatment with 900 mg twice daily valganciclovir and 157 patients to 5 mg/kg twice daily IV ganciclovir included in the intention-to-treat population.\textsuperscript{38} Of these, 259 patients had confirmed HCMV viremia and made up the protocol population. It is this population in which gB genotype analysis was performed. It is important to note that patients in this study must have been diagnosed with HCMV disease prior to enrolment and that initiation of antiviral therapy for HCMV was not based on virologic markers. Both therapeutic drug formulations were administered for an induction period of 21 days, followed by 900 mg daily valganciclovir until day 49. Whole blood samples for viral load monitoring were obtained at the start of therapy (day 0, baseline) and at days 3, 7, 14 and 21 i.e. when patients are receiving full dose medication.

2.2. Glycoprotein B genotyping

Quantitative genotyping of glycoprotein B was performed by quantitative real-time PCR on DNA extracts from whole blood in all patients at days 0, 3, 7, 14, and 21 as described in detail elsewhere.\textsuperscript{40} A mixed infection was defined as HCMV infection with more than one gB genotype in a single sample.

2.3. Kinetics of viral load decline

Given that the results of the VICTOR study showed no differences between the treatment arms, we combined both groups for the analysis of the response of gB genotypes to therapy in either single gB genotype or in the context of mixed gB genotype infections. The kinetics of decline of HCMV load for each genotype within the mixed gB infection population was analysed separately using the mean log HCMV load at days 0, 3, 7, 14, 21. Decline rates were modelled using linear regression analysis and the decline rate constant computed using the formula:

\[
\text{Decline rate} = \frac{\ln(VL(t_1)) - \ln(VL(t_2))}{t_2 - t_1}
\]

where VL is the HCMV gB genotype load at time \(t_1\) or \(t_2\) respectively.

Half lives of decline could then be computed using the following:

\[
T_{1/2} = \frac{\ln 2}{\text{decline rate}}
\]

Comparisons of the different rates of decline were performed using Student’s t-test. The correlation between slope of decline and viral load reductions from baseline to day 21 was assessed using Spearman’s rank correlation test. All \(p\)-values <0.05 were treated as significant.

3. Results

3.1. Patient characteristics and baseline HCMV load in patients with different gB genotypes

The gB genotype was determined for 239/259 of the pre-protocol patients with HCMV disease enrolled in the VICTOR study where patients were randomized to receive either valganciclovir or intravenous ganciclovir at full dose to control their clinical symptoms of HCMV infection. At the initiation of antiviral therapy (day 0, baseline), the frequency each gB genotype was as follows: gB1 (61/239 (26%), gB2 (23/239 (10%), gB3 (24/239 (10%), gB4 (13/239 (6%) and mixed gB genotypes (118/239 (49%) [described in detail in 39]). Within the mixed infection population, the frequencies of the combinations were as follows: gB1/gB2 (n = 19), gB1/gB3 (n = 27), gB1/gB4 (n = 7), gB2/gB3 (n = 11), gB2/gB4 (n = 4), gB3/gB4 (n = 7), a mixture of three gB genotypes (n = 35) and all four genotypes (n = 8). There were no significant differences in age, gender or antiviral treatment received (intravenous gCV or VgCV), type of organ transplanted and HCMV serostatus when stratified according to gB genotype.

Baseline HCMV load i.e. at the initiation of treatment, in whole blood was highest in patients with mixed gB genotype infections (5.37 ± 0.92 log10 genomes/ml) compared to individual gB genotype infections although this was only significant when compared with gB1 and gB2 baseline HCMV loads (4.65 ± 0.93 log10 genomes/ml (p = 0.0001) and 4.69 ± 0.85 log10 genomes/ml (p = 0.002) respectively). In addition, baseline HCMV load for gB1 infections were significantly lower than both gB3 (5.32 ± 1.33 log10 genomes/ml; p = 0.008) and gB4 infections (5.25 ± 0.8 log10 genomes/ml; p = 0.04). Within the mixed gB genotype population, HCMV loads were comparable for gB2, gB3 and gB4 (4.65 ± 1.17 vs 4.64 ± 1.18 vs 4.59 ± 0.89 log10 genomes/ml respectively) but gB1 HCMV loads (4.27 ± 1.17 log10 genomes/ml) were significantly lower (p = 0.05).

3.2. Decay kinetics of gB genotype load in mixed and single infections after initiation of therapy

Initially we investigated the decay kinetics of HCMV in patients with only mixed gB genotype infection (n = 118) for both total HCMV load and for individual gB genotypes within the patients with mixed genotype infections. The decay kinetics for the total HCMV load followed a biphasic decline with an initial phase from days 0 to 3 having a half life of approximately 0.79 days and a
Table 1: Biphasic decline parameters for each gB genotype in patients with mixed gB genotype infections.

| Genotype (n) | Baseline HCMV load (log₁₀ genomes/ml) | 1st phase half life of decline (days) | 2nd phase half life of decline (days) |
|--------------|---------------------------------------|---------------------------------------|---------------------------------------|
| gB1 (n=61)   | 4.65 ± 0.95                           | 1.04 ± 0.81                           | 3.66 ± 2.28                           |
| gB2 (n=23)   | 4.69 ± 0.85                           | 0.65 ± 0.27                           | 4.36 ± 3.31                           |
| gB3 (n=24)   | 5.32 ± 1.33                           | 0.94 ± 1.0                            | 3.46 ± 2.39                           |
| gB4 (n=13)   | 5.25 ± 0.92                           | 0.65 ± 0.35                           | 3.40 ± 1.86                           |

p = 0.013, gB3 (difference 0.38 days (95% CI 0.04–0.71); p = 0.028) and gB4 (difference 0.4 days (95% CI 0.05–0.75); p = 0.025). In contrast, there were no significant differences in the 2nd phase decline between single gB genotype infections and the mixed infection group.

3.3. Correlation between decay kinetics and replicative control at day 21

We next investigated whether the 1st phase decline kinetics of HCMV in whole blood after initiation of therapy was associated with either the 2nd phase decline rate or the absolute reduction in HCMV load by day 21 of therapy. There was no correlation between 1st and 2nd phase decline rates in any groups (mixed gB genotypes or single gB genotype patients). In contrast, there was a significant correlation between 1st phase decline rates and the log reduction in HCMV load between day 0 and day 21 in patients with mixed gB genotype infection (Spearman’s r = 0.37; p = 0.002) or when the single gB genotype infections were combined (Spearman’s r = 0.35; p = 0.0004; Fig. 2). When the individual gB genotypes were analysed separately the 1st phase decline in gB1, gB2 and gB4 infections was significantly correlated with the log decline at day 21 (r = 0.32 (p = 0.02); r = 0.45 (p = 0.04); r = 0.67 (p = 0.02)) respectively whereas the same analysis for gB3 failed to reach statistical significance (r = 0.22; p = 0.28).

4. Discussion

To date there have been relatively few large scale analyses of the in vivo effect of antiviral therapy on different HCMV strains. In the present study we show that subtle differences in the early kinetics of response to antiviral chemotherapy are apparent between different gB genotypes and in patients with mixed gB genotype infections. However, after 21 days of therapy these differences were insignificant i.e. gB genotype appears not to influence the ultimate control of replication after ganciclovir therapy. An important observation in our study was that HCMV load in whole blood appears to follow a biphasic decline with an initial half life of <1 day and a second phase half life of about 4 days. This biphasic decline has been recently been described in a single case report of a stem cell transplant recipient after aresunate therapy. Previous work in HIV-infected HCMV retinitis patients where HCMV load was in a quasi-steady state has shown that HCMV replication is highly dynamic with half life of decline averaging 1 day. Thus, the first phase decline observed in our present study would be consistent with this data.

Table 2: Biphasic decline parameters for single gB genotype infections and cumulatively for patients with mixed gB genotype infections. These decline rates for gB 1–4 should be compared with the gB genotype declines rates in the patients with mixed infections shown in Table 1.

| Genotype       | Baseline HCMV load (log₁₀ genomes/ml) | 1st phase half life of decline (days, number, n) | 2nd phase half life of decline (days, number, n) |
|----------------|---------------------------------------|-----------------------------------------------|-----------------------------------------------|
| gB1            | 4.27 ± 1.17                           | 1.17 ± 1.31 (n=56)                            | 4.27 ± 2.77 (n=47)                            |
| gB2            | 4.65 ± 1.15                           | 0.92 ± 0.78 (n=20)                            | 3.62 ± 2.83 (n=19)                            |
| gB3            | 4.64 ± 1.18                           | 1.17 ± 1.36 (n=23)                            | 5.25 ± 3.96 (n=22)                            |
| gB4            | 4.59 ± 0.89                           | 1.19 ± 1.09 (n=12)                            | 5.08 ± 3.34 (n=10)                            |
| Mixed infection| 5.37 ± 0.92                           | 0.79 ± 0.67 (n=87)                            | 3.84 ± 3.28 (n=81)                            |
we do not think that this has a major influence on response to therapy and in other studies, serostatus has not been associated with different decline rates. However, the interaction between gB genotypes during a mixed infection including competition and relative fitness differences may also contribute to our observations. In order to disentangle this area, whole genome sequencing, or deep sequencing of the HCMV strains present in patients will be informative and should further enhance our knowledge of the genetic fluidity of pathogenic strains of HCMV and allow more sophisticated dynamic models of HCMV replication to be developed.

Conflicts of interest

The following authors have received honoraria from Roche Pharmaceuticals for advisory boards and presentations: Vincent C. Emery, Anders Asberg, Deepali Kumar, Anders Hartmann, Mark D. Pescevitz, Halvor Rollag, Alan G. Jardine and Atul Humar.

References

1. Fishman JA, Emery V, Freeman R, Pascual M, Rostaing L, Schlitt H, et al. Cytomegalovirus in transplantation – challenging the status quo. Clin Transplant 2007;21(3):149–58.
2. Soderberg-Naucler C. HCMV microinfections in inflammatory diseases and cancer. J Clin Virol 2008;41(3):218–23.
3. Emery VC, Sabin CA, Cope AV, Gor D, Hassan-Walker AF, Griffiths PD. Application of viral-load kinetics to identify patients who develop cytomegalovirus disease after transplantation. Lancet 2001;358(June (9220)):2032–6.
4. Schafer P, Tenschert W, Cremaschi L, Schrader M, Zollner B, Laufs R. Area under the viraemia curve versus absolute viral load: utility for predicting symptomatic cytomegalovirus infections in kidney transplant patients. J Med Virol 2001;65(September 1):85–9.
5. Kumar A, Kumar D, Boxin G, Caliendo AM. Cytomegalovirus (CMV) virus load kinetics to predict recurrent disease in solid-organ transplant patients with CMV disease. J Infect Dis 2002;186(Supplement 5):829–33.
6. Gerna G, Lillier D, Fornara C, Comolli G, Lozza L, Campana C, et al. Monitoring of human cytomegalovirus-specific CD4 and CD8 T-cell immunity in patients receiving solid organ transplantation. Am J Transplant 2006;6(October 10):2356–64.
7. Nebbia G, Mattes FM, Smith C, Hainsworth E, Kopycinski J, Burroughs A, et al. Polynuclear cytomegalovirus-specific CD4+ and pp65 CD8+ T cells protect against high-level replication after liver transplantation. Am J Transplant 2008;8(December 12):2590–9.
8. Mattes FM, Vargas A, Kopycinski J, Hainsworth E, Sweny P, Nebbia G, et al. Functional impairment of cytomegalovirus-specific CD8 T cells predicts high-level replication after renal transplantation. Am J Transplant 2008;8(March 5):990–9.
9. La Rosa C, Limaye AP, Krishnan A, Longmate J, Diamond DJ. Longitudinal assessment of cytomegalovirus (CMV)-specific immune responses in liver transplant recipients at high risk for late CMV disease. J Infect Dis 2007;195(March 15):633–44.
10. La RC, Krishnan A, Longmate J, et al. Programmed death-1 expression in liver transplant recipients as a prognostic indicator of cytomegalovirus disease. J Infect Dis 2008;197(January 1):23–33.
11. Egli A, Binet I, Binggeli S, Jager C, Dumoulin A, Schaub S, et al. Cytomegalovirus-specific T-cell responses and viral replication in kidney transplant recipients. J Transl Med 2008;6:29.
12. Sester U, Presser D, Dirks J, Gartner BC, Kohler H, Sester M. PD-1 expression and IL-2 loss of cytomegalovirus-specific T cells correlates with viremia and reversible functional anergy. Am J Transplant 2008;8(7):1486–97.
13. Emery VC, Cope AV, Bowen EF, Gor D, Griffiths PD. The dynamics of human cytomegalovirus replication in vivo. J Exp Med 1999;190(7):177–82.
14. Emery VC, Hassan-Walker AF, Burroughs AK, Griffiths PD. Human cytomegalovirus (HCMV) replication dynamics in HCMV-naive and experienced immunocompromised hosts. J Infect Dis 2002;185(June 12):1713–8.
15. Singh N. Antiviral drugs for cytomegalovirus in transplant recipients: advantages of preemptive therapy. Rev Med Virol 2006;16(5):281–7.
16. Small LN, Lau J, Snyderman DR. Preventing post-organ transplantation cytomegalovirus disease with ganciclovir: a meta-analysis comparing prophylactic and preemptive therapies. Clin Infect Dis 2006;43(October 7):869–80.
17. Snyderman DR. The case for cytomegalovirus prophylaxis in solid organ transplantation. Rev Med Virol 2006;16(August 5):280–95.
18. Chee MS, Bankier AT, Beck S, Bohni R, Brown C, Cerny R, et al. Analysis of the protein-coding content of the sequence of human cytomegalovirus strain AD169. Curr Top Microbiol Immunol 1990;154:125–69.
19. Bradley AJ, Lurain NS, Chazal P, Trivedi U, Cunningham C, Baluchova K, et al. High-throughput sequence analysis of variants of human cytomegalovirus strains Towne and AD169. J Gen Virol 2009;90(October 10):2375–80.
20. Dolan A, Cunningham C, Hector RD, Griffiths PD, Sinzger C, McSharry BF, et al. Genetic content of wild-type human cytomegalovirus. J Gen Virol 2004;85:May (Pt 5):1301–12.

21. Murphy E, Shenk T. Human cytomegalovirus genome. Curr Top Microbiol Immunol 2008;325:1–19.

22. Bradley AJ, Kovacs JJ, Gatherer D, Dangan DJ, Alkhasharh KR, Chan PK, et al. Genotypic analysis of two hypervariable human cytomegalovirus genes. J Med Virol 2008;80:September (9):1615–23.

23. Chou S. Molecular epidemiology of envelope glycoprotein H of human cytomegalovirus. J Infect Dis 1992;166:September (3):604–7.

24. Darlington J, Super M, Patel K, Grundy JE, Griffiths PD, Emery VC. Use of the polymerase chain reaction to analyse sequence variation within a major neutralizing epitope of glycoprotein B (gB58) in clinical isolates of human cytomegalovirus. J Gen Virol 1991;72:August (Pt 8):1985–9.

25. Lurain NS, Fox AM, Lichy HM, Bhorade SM, Ware CF, Huang DD, et al. Analysis of the human cytomegalovirus genomic region from UL146 through UL147A reveals sequence hypervariability, genotypic stability, and overlapping transcripts. Virol J 2006;3:4.

26. Rasmussen L, Geissler A, Cowan C, Chase A, Winters M. The genes encoding the gBII complex of human cytomegalovirus exist in highly diverse combinations in clinical isolates. J Virol 2002;76:November (21):10841–8.

27. Roy DM, Grundy JE, Emery VC. Sequence variation within neutralizing epitopes of the envelope glycoprotein B of human cytomegalovirus: comparison of isolates from renal transplant recipients and AIDS patients. J Gen Virol 1993;74:November (Pt 11):2499–505.

28. Yan H, Koyano S, Inami Y, Yamamoto Y, Suzutani T, Mizuguchi M, et al. Genetic variations in the gB, UL144 and UL149 genes of cytomegalovirus strains collected from congenitally and postnatally infected Japanese children. Arch Virol 2008;153:4:667–74.

29. Chou SW, Dennison KM. Analysis of interstrain variation in cytomegalovirus glycoprotein B sequences encoding neutralization-related epitopes. J Infect Dis 1991;163:June (6):1229–34.

30. Isaacson MK, Compton T. Human cytomegalovirus glycoprotein B is required for virus entry and cell-to-cell spread but not for virion attachment, assembly, or egress. J Virol 2009;83:April (8):3891–903.

31. Humar A, Kumar D, Gilbert C, Boivin G. Cytomegalovirus (CMV) glycoprotein B genotypes and response to antiviral therapy, in solid-organ-transplant recipients with CMV disease. J Infect Dis 2003;188:August (4):581–4.

32. Kouri V, Gonzalez EE, Martinez PA, Capo V, Gonzalez R, Perez I, et al. Distinct genotypic distribution of cytomegalovirus CMV envelope glycoprotein B (gB) in a Cuban cohort of patients with different CMV diseases. Scand J Infect Dis 2007;39:11–12:1038–44.

33. Pequiera E, Ozaki RS, Tomiyama H, Camara NO, Granato CF. Clinical correlations of human cytomegalovirus strains and viral load in kidney transplant recipients. Int Immunopharmacol 2009;9:January (1):26–31.

34. Sarcinella L, Mazzulli T, Willey B, Humar A. Cytomegalovirus glycoprotein B genotype does not correlate with outcomes in liver transplant patients. J Clin Virol 2002;24:February (1–2):99–105.

35. Yu J, Chen H, Horton H, Bansal A, McElrath JM, Reichman R, et al. Influenza-A2 reconstitutes defective human immunodeficiency virus (HIV), and cytomegalovirus (CMV) specific CD8+ T cell proliferation in HIV infection. J Med Virol 2006;78:September (9):1147–57.

36. Coaquette A, Bourgeois A, Dirand C, Varin A, Chen W, Herbein G. Mixed cytomegalovirus glycoprotein B genotypes in immunocompromised patients. Clin Infect Dis 2004;39:July (2):155–61.

37. Novak Z, Ross SA, Patro RK, Pati SK, Kumbala RA, Brice S, et al. Cytomegalovirus strain diversity in seropositive women. J Clin Microbiol 2008;46:March (3):882–6.

38. Asberg A, Hurna A, Rollag H, Jardin AG, Mousas H, Pescovitz MD, et al. Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant 2007;7:September (9):2106–13.

39. Manuel O, Asberg A, Pang X, Rollag H, Emery VC, Preiksaitis JK, et al. Impact of genetic polymorphisms in cytomegalovirus glycoprotein B on outcomes in solid-organ transplant recipients with cytomegalovirus disease. Clin Infect Dis 2009;49:October (8):1160–6.

40. Pang X, Humar A, Preiksaitis JK. Concurrent genotyping and quantitation of cytomegalovirus gB genotypes in solid-organ-transplant recipients by use of a real-time PCR assay. J Clin Microbiol 2008;46:December (12):4004–10.

41. Shapiro MY, Resnick IB, Chou S, Neumann AU, Lurain NS, Stamminger T, et al. Artesunate as a potent antiviral agent in a patient with late drug-resistant cytomegalovirus infection after hematopoietic stem cell transplantation. Clin Infect Dis 2008;46:May (9):1455–7.

42. Neumann AU, Pianko S, Zeuzem S, Yoshida EM, Benhamou Y, Mishan M, et al. Positive and negative prediction of sustained virologic response at weeks 2 and 4 of treatment with alfa-2b or peginterferon alfa-2a in treatment-naive patients with genotype 1, chronic hepatitis C. J Hepatol 2009;51:July (1):115–21.

43. Polis MA, Sidorov IA, Yoder C, Jankelevich S, Metcalf J, Mueller BU, et al. Correlation between reduction in plasma HIV-1 RNA concentration 1 week after start of antiretroviral treatment and longer-term efficacy. Lancet 2001;358:November (9295):1760–5.

44. Rosen HR, Ribeiro RR, Weinberger L, Wolf S, Chung M, Gretch DR, et al. Early hepatitis C viral kinetics correlate with long-term outcome in patients receiving high dose induction followed by combination interferon and ribavirin therapy. J Hepatol 2002;37:July (1):124–30.

45. Hansen SG, Powers CJ, Richards R, Ventura AB, Ford JC, Siess D, et al. Evasion of CD8+ T cells is critical for superinfection by cytomegalovirus. Science 2010;328:April (5974):102–6.

46. Mattes FM, Hansworth EG, Hassan-Walker AF, Burroughs AK, Sweeny P, Griffiths PD, et al. Kinetics of cytomegalovirus load decrease in solid-organ transplant recipients after preemptive therapy with valganciclovir. J Infect Dis 2005;191:January (1):89–92.

47. Goracz I, Guerly C, Traganoski S, Puchhammer-Stockl E. Deep sequencing reveals highly complex dynamics of human cytomegalovirus genotypes in transplant patients over time. J Virol 2010;84:July (14):7195–203.