Journal of Infectious Diseases Advance Access published October 9, 2014



# Influence of IFNL3/4 polymorphisms on the incidence of cytomegalovirus infection after solid-organ transplantation

**Oriol Manuel[1,2,*], Agnieszka Wójtowicz[1,*], Stéphanie Bibert[1], Nicolas J. Mueller[3], Christian van Delden[4], Hans H. Hirsch[5,6], Juerg Steiger[7], Martin Stern[8], Adrian Egli[9], Christian Garzoni[10,11], Isabelle Binet[12], Maja Weisser[5], Christoph Berger[13], Alexia Cusini[11], Pascal Meylan[1,14], Manuel Pascual[2], Pierre-Yves Bochud[1], and the Swiss Transplant Cohort Study (STCS)[‡]**

[1]Infectious Diseases Service, Department of Medicine, University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland

[2] Transplantation Center, Department of Surgery, University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland

[3]Division of Infectious Diseases and Hospital Epidemiology, University Hospital, University of Zurich, Zürich, Switzerland

[4]Service of Transplantation, Department of Surgery, University Hospitals of Geneva, Geneva, Switzerland

[5]Division of Infectious Diseases and Hospital Epidemiology, University Hospital Basel, Basel, Switzerland

[6]Transplantation & Clinical Virology, Department Biomedicine (Haus Petersplatz), University of Basel, Basel, Switzerland

[7]Clinic for Transplantation Immunology and Nephrology, University Hospital Basel, Basel, Switzerland

[8]Immunotherapy Laboratory, Department of Biomedicine, University Hospital Basel, Basel, Switzerland

[9]Clinical Microbiology, University Hospital Basel, Basel, Switzerland

[10]Department of Infectious Diseases, Inselspital, Bern University Hospital and University of Bern, Bern, Switzerland

[11]Clinic of Internal Medicine and Infectious Diseases, Clinica Luganese, Lugano, Switzerland

[12]Division of Nephrology and Transplantation Medicine, Kantonsspital St. Gallen, St. Gallen, Switzerland

[13]Division of Infectious Diseases and Hospital Epidemiology, University Children's Hospital, Zurich, Switzerland

[14]Institute of Microbiology, University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland

[*]Equal contribution

[‡]This study has been conducted in the framework of the Swiss Transplant Cohort Study, supported by the Swiss National Science Foundation and the Swiss University Hospitals (G15) and transplant centers.

Responsible for the Swiss Transplant Cohort Study: I. Binet (SNSF Board), S. De Geest (SNSF Board), C. van Delden (Executive office, SNSF Board), GFK Hofbauer (SNSF Board), U. Huynh-Do (SNSF Board), MT Koller (SNSF Board), C. Lovis (SNSF Board), O. Manuel (SNSF Board), P. Meylan (SNSF Board), NJ Mueller (Chairman of the Scientific Committee, SNSF Board), M. Pascual (Executive office, SNSF Board), S. Schaub (SNSF Board), J. Steiger (Executive office, SNSF Board)

Co-corresponding authors: Oriol Manuel MD, Infectious Diseases Service and Transplantation Center, University Hospital and University of Lausanne. MP14/316, CHUV, Lausanne, Switzerland. Phone: +41 21 314 3020. Fax: +41 21 3141008. E-mail: oriol.manuel@chuv.ch, Pierre-Yves Bochud MD, Infectious Diseases Service, University Hospital and University of Lausanne. BH08 - 658, CHUV, Lausanne, Switzerland. Phone: +41 21 314 4379. Fax: +41 21 314 1033. E-mail: Pierre-Yves.Bochud@chuv.ch





**Abstract**

**Background**: Polymorphisms in the interferon-λ (IFNL) 3/4 region have been associated with reduced hepatitis C virus clearance. We explored the role of such polymorphisms on the incidence of CMV infection in solid-organ transplant (SOT) recipients.

**Methods**: Caucasian patients participating in the Swiss Transplant Cohort Study in 2008-2011 were included. A novel functional TT/-G polymorphism (*rs368234815*) in the CpG region upstream of *IFNL3* was investigated.

**Results**: A total of 840 SOT recipients at risk for CMV were included, among whom 373 (44%) received antiviral prophylaxis. The 12-months cumulative incidence of CMV replication and disease were 0.44 and 0.08, respectively. Patient homozygous for the minor *rs368234815* allele (-G/-G) tended to have a higher cumulative incidence of CMV replication (SHR=1.30 [95%CI 0.97-1.74], P=0.07) compared to other patients (TT/TT or TT/-G). The association was significant among patients followed by a preemptive approach (SHR=1.46 [1.01-2.12], P=0.047), especially in patients receiving an organ from a seropositive donor (D+, SHR=1.92 [95%CI 1.30-2.85], P=0.001), but not among those who received antiviral prophylaxis

(SHR=1.13 [95%CI 0.70-1.83], P=0.6). These associations remained significant in multivariate competing risk regression models.

**Conclusions**: Polymorphisms in the IFNL3/4 region influence susceptibility to CMV replication in SOT recipients, particularly in patients not receiving antiviral prophylaxis.

## Background

Cytomegalovirus (CMV) infection remains one of the most common infectious complications after solid-organ transplantation (SOT) (1). Several risk factors for the development of CMV have been described, the donor (D) and recipient (R) serostatus at the time of transplantation being the main determinant for predicting the risk for subsequent CMV infection (2). Additional risk factors include the type and dose of immunosuppressive drug used and previous occurrence of acute rejection (3). Despite advances in the prevention of CMV replication post transplant, a significant number of recipients may still develop CMV disease, even in the absence of these above mentioned risk factors (4).

While it is widely accepted that the adaptive immunity is essential in the control of CMV replication, particularly the specific CD8+ T cell response against CMV (5), the importance of innate immunity for CMV control has not completely been determined (6). After transplantation, when cellular immunity is impaired due to the effect of immunosuppressive drugs, innate immunity may play a more prominent role in controlling viral replication. For example, some studies have identified that polymorphisms of genes involved in innate immunity, such as toll-like receptors (TLR)-2 (7), TLR-4, and mannose binding lectin (MBL) (8, 9), were associated with an increasing incidence of CMV infection or disease after transplantation.

Type I interferons (IFN) have been long considered to be critical for immune responses to viral infections. However, type III IFNs, also called IFNs-lambda (IFNLs), have recently been described to share many biological functions with type I IFN, and also to have an important role in response to viral infections (10, 11). In particular, genome-wide association studies (GWAS) revealed that polymorphisms in the IFNL3/4 region exert a dramatic influence on the ability to clear the hepatitis C virus (HCV), either spontaneously (12), or in response to antiviral therapy (12-15). A novel TT/-G substitution (*rs368234815*) was recently identified as possibly the most robust or clinically relevant marker predicting HCV clearance (16, 17).

The potential influence of IFNLs polymorphisms on controlling viral infection other than hepatitis C has not been well characterized. In two recent studies in SOT and in hematopoietic stem-cell transplant (HSCT) recipients, the presence of a minor allele of IFNL3 polymorphisms was associated with reduced post transplant CMV replication (18, 19). This was in contrast with a study involving high-risk HIV-infected individuals, where patients harboring the minor allele of *rs368234815* had a higher incidence of CMV retinitis (20). We therefore explored here the role of this novel IFNL3/4 *rs368234815* polymorphism on the incidence of CMV infection and disease in a unique nationwide prospective cohort of SOT recipients, the Swiss Transplant Cohort Study (STCS).

## Methods

### *Study population*

The STCS is a multicenter nationwide cohort study including SOT performed in Switzerland from May 2008 onward (21). The STCS comprises six transplant centers in Switzerland. Kidney transplantation is performed in all centers, liver and heart transplantation in three centers, and lung and pancreas transplantation in two centers. Data on demographic parameters, transplant

type, comorbidities, immunosuppressive treatment, antimicrobial drugs, rejection, infectious and non-infectious events are collected at enrolment, at six months and every 12 months on standardized data forms by local physicians and data managers. Specific data on CMV infection available in the STCS database include the date of the first episode of CMV replication and of each episode of CMV disease, the use of antiviral drugs and the type of CMV event which is classified as asymptomatic replication, viral syndrome, and probable or proven end-organ disease.

For the present study, we included all Caucasian patients transplanted from May 2008 to March 2011 with at least one post transplant follow-up, a positive donor and/or recipient CMV serostatus, DNA available for genotyping and written informed consent for participation in the STCS. Patients who died within 24 hours of transplant were excluded. The STCS protocol has been approved by the Ethics Committees of all participating centers.

*Antiviral and immunosuppressive regimens*

The antiviral preventive strategy per protocol varied among centers and type of transplant (2). Most D+/R- patients received valganciclovir prophylaxis for three to six months, except in two transplant programs, where patients were followed preemptively. Seropositive (R+) patients were managed either by preemptive therapy or antiviral prophylaxis according to the transplant program, except for lung transplant recipients who all received antiviral prophylaxis. In patients receiving antiviral prophylaxis, monitoring of CMV replication by PCR was done after discontinuation of prophylaxis in five out of the six transplant centers every 2-4 weeks for an additional 3-month period, irrespectively of the CMV serostatus. The protocol of the preemptive approach was decided by each center, but basically consisted of screening for CMV DNAemia

by PCR every one to two weeks during the first month post transplant and then every two weeks until three to six months post transplant. Five centers used PCR in plasma and one center used PCR in whole blood. All centers used a home-made PCR, except one center which used a commercial Abbott RealTime CMV Assay. Antiviral therapy in asymptomatic patients with CMV replication was generally started at a cut-off of 2-3 log10 copies/ml of plasma or 3-4 log10 copies/ml in whole blood, but this cut-off varied according to the CMV serostatus, time post transplant, and whether the patient had received lymphocyte-depleting antibodies or not. Only results of the first positive CMV DNAemia (and whether DNAemia was treated or not) were recorded in the STCS database. Immunosuppressive regimens also varied among centers and type of organ transplant.

*IL28B genotyping*

The *rs368234815* polymorphism was genotyped by Competitive Allele Specific PCR (KASP™) system (LGC Genomics, UK), using the ABI 7500 Fast real time thermocycler, according to manufacturer's protocols (http://www.lgcgenomics.com/kaspchallenge). The KASP primers were designed by Kraken™ assay design and workflow management software (LGC Genomics, UK). Automated allele calling was performed using SDS software (Applied Biosystems).

*Clinical definitions*

Antiviral prophylaxis was defined as the use of ganciclovir or valganciclovir started during the first two weeks post transplantation. Patients without such a prophylactic treatment who were at risk for CMV disease (D+/R- and R+ patients) were considered as being managed by the preemptive approach (2). Definition of CMV infection followed international guidelines (22)

defining active CMV infection as the evidence of laboratory confirmation of CMV replication irrespectively of symptoms, and CMV disease as CMV replication with corresponding signs and symptoms.

*Statistical analysis*

The main endpoint of the study was the incidence of CMV replication (thus including asymptomatic CMV infection and CMV disease). The cumulative incidence of CMV replication by genetic variables was calculated by using the stcompet program implemented in Stata (StataCorp LP, College Station, Texas, US). The risk of CMV replication and/or disease for each genetic and demographic variable was assessed by using a semi-parametric regression model (23), also implemented in Stata (stcrreg). Death was considered a competing event. Stepwise multivariate regression model ($P<0.1$) was used to determine the independent risk factors from the predicted variables. We analyzed the incidence of CMV replication in all SOT recipients and in kidney transplant recipients.

**Results**

*Study population*

The characteristics of 840 patients meeting the inclusion criteria are shown in **Table 1**. Overall, 373 (44 %) patients received antiviral prophylaxis, either with valganciclovir upfront or initially with IV ganciclovir. Most patients received induction therapy with either basiliximab (60%) or anti-thymocyte globulins (18%) and a maintenance immunosuppressive regimen including a calcineurin inhibitor, an antimetabolite, and corticosteroids. The 12-months cumulative incidence of CMV infection and disease were 0.44 and 0.08, respectively. Median

time from transplant to CMV replication was longer in patients who received prophylaxis than in patients followed by the preemptive approach (median of 167 days vs. 40 days, P<0.0001). Baseline characteristics in kidney transplant recipients (n=526) were similar as in the whole study population (n=840).

*Impact of the rs368234815 polymorphism on CMV replication according to antiviral strategy*

Overall, 102 (12%) SOT recipients were homozygous for the minor allele of *rs368234815* (-G/-G carriers). The cumulative incidence of CMV replication tended to be higher among -G/-G carriers (0.52) compared to other patients (TT/TT or TT/-G carriers, 0.43, SHR=1.30 [%95 CI 0.97-1.74], P=0.07, **Figure 1 and Table 2**). The association was significant when considering patients followed by the preemptive approach (SHR 1.46 [95% CI 1.01-2.12], P=0.047), but not when considering those who received antiviral prophylaxis (SHR=1.13 [%95 CI 0.72-1.78], P=0.6). The former association was still significant in a multivariate model (SHR 1.57 [95% CI 1.10-2.23], P=0.01) (**Table 3**), after adjustment for other risk factors associated with CMV replication in SOT recipients not receiving prophylaxis, including recipient age, donor/recipient CMV serostatus, transplanted organ type, as well as induction and maintenance immuno-suppressive regimen. In patients with asymptomatic CMV replication, the presence of the minor allele of *rs368234815* had no influence on the rate of patients who required antiviral therapy (p=0.5).

Results were similar when kidney transplant recipients were analyzed separately. Among kidney recipients followed by a pre-emptive approach, -G/-G carriers had a higher cumulative incidence of CMV replication compared to TT/TT and TT/-G carriers (SHR=1.76 [95% CI 1.10-2.84], P=0.02, **Figure 1**). This association was still observed in the multivariate analysis, where -

G/-G carriage was still associated with CMV replication (SHR 1.78 [95% CI 1.18-2.69], P=0.006, not shown). However, no association between *rs368234815* polymorphism and CMV replication was detected among kidney recipients receiving antiviral prophylaxis (SHR 1.11 [95% CI 0.65 -1.89], P=0.7, not shown).

There was no association between the *rs368234815* polymorphism and CMV disease in SOT recipients (-G/-G vs TT/TT and TT/-G, P=0.4) and in kidney transplant recipients (P=1.0, **Supplementary Figure 1**).

*Impact of the rs368234815 polymorphism on CMV replication according to CMV serostatus*

We also analyzed the role of the *rs368234815* separately according to CMV serostatus. Among D+ SOT and kidney transplant recipients who were followed by a preemptive approach, -G/-G carriers had a higher cumulative incidence of CMV replication compared to the other patients (all patients SHR=1.92 [95%CI 1.30-2.85], P=0.001 and kidney recipients SHR=2.28 [95%CI 1.40-3.71], P=0.0009 , **Figure 2**). These association remained significant in the multivariate Cox regression models, after adjustment for relevant covariates (SHR=2.06 [95% CI 1.40-3.01], P<0.0001 and SHR=2.24 [95% CI 1.40-3.58, P=0.001, respectively; Supplementary **Table S1**). Again, no significant associations were observed among patients receiving antiviral prophylaxis (P=0.053 and P=0.2, respectively, Figure 2).

**Discussion**

In this nationwide cohort study of SOT recipients, we assessed the potential influence of polymorphisms in the IFNL3/4 region on the incidence of CMV replication. We found that the TT/-G *rs368234815* substitution, which was recently identified as the best predictor of HCV

clearance (16, 17), was an independent risk factor for developing CMV replication, specifically in the group of patients not receiving antiviral prophylaxis. This was also found for kidney transplant recipients in particular, a more homogeneous group of SOT recipients in terms of immunosuppression and antiviral strategies. We did not observe any difference in the development of CMV disease in patients with or without *rs368234815*, probably due to the overall low incidence of CMV disease in our cohort.

While extensive literature exists on the role of IFNL3/4 polymorphisms in influencing spontaneous or treatment-induced clearance of HCV (12, 13, 15), there are few data assessing a potential association between such polymorphisms and CMV replication, particularly in the transplant setting. In a recent study of 151 HSCT recipients, donor carriage of the minor TT genotype of *IFNL3 rs12979860* SNP (a SNP in strong linkage disequilibrium with *rs368234815*) was associated with a shorter duration of CMV replication in the recipient (18). However, no differences in the overall incidence of CMV infection were observed according to this SNP. In a study of 38 D+R- SOT recipients, the minor G allele of *rs8099917* (which is also in linkage disequilibrium with *rs368234815*) was associated with lower risk of CMV replication after discontinuation of antiviral prophylaxis (19). Finally, in concordance to our data, we found that the same *rs368234815* polymorphism was significantly associated with the occurrence of CMV retinitis in a cohort of 1217 of HIV-infected individuals at risk (HR=2.54, 95% confidence interval 1.20-5.40, P=0.02) (20). Discrepancies between studies may be explained by several factors, including the use of different study populations and/or groups at risk, and the different sample size.

The exact mechanisms by which *rs368234815* may influence susceptibility to CMV replication among SOT recipients receiving no antiviral prophylaxis are not well established.

However, increasing evidence suggests that IFNLs contribute to antiviral responses against viruses other than HCV. The antiviral activity of IFNLs was highlighted in a series of cell culture models, including herpesviruses (24-27). In an intestinal cell model of CMV infection, IFNL1 and IFNL3 were shown to activate STAT1, thereby inducing the production of antiviral proteins and inhibiting the expression of CMV (28). The administration of recombinant IFNLs inhibited replication in mice models of HSV infections (26), thereby confirming the role of these molecules *in vivo*. However, blockade of the IL28 R1 subunit of the IL28B receptor was recently reported to decrease CMV replication in foreskin fibroblast (19). The *rs368234815* polymorphism was associated with reduced HCV clearance in PBMCs stimulated with poly(I:C) (17). It was also associated with the expression of a novel IFNL analogue, named IFNL4, that exerts antiviral activities similar to those of other type I and type III IFNs in vitro (16, 29). Therefore, increased CMV events among SOT recipient carrying the *rs368234815* may be due in part to insufficient IFNL3 expression, or to the expression of IFNL4 itself. Yet, the reason why the expression of IFNL4 would be associated with increased rather than decreased viral replication *in vivo* remains to be elucidated (30). In a mouse model of lymphocytic choriomeningitis virus (LCMV), differences in the IFN-stimulated genes background were observed in acute vs. latent infection, determining the control of LCMV replication (31). Further investigations are needed to understand the exact role of IFNL3 and IFNL4 in antiviral immune responses.

An important finding of our study is that the influence of IFNL3/4 polymorphism on CMV replication disappeared when considering patients receiving CMV prophylaxis. A plausible hypothesis is that, in patients managed by the preemptive approach, CMV replication develops earlier and more frequently after transplant than in patients receiving antiviral

prophylaxis. As cell-mediated immunity is impaired after transplant, the role of innate immunity might be more evident when viral replication occurs early and more often after transplant. This is in concordance with previous studies evaluating the role of NK immunoglobulin-like receptor (KIR) polymorphisms, in which the influence of such polymorphisms in determining CMV replication was significant also during the first three months post transplant, but decreased over time (32). We also found that donor CMV serostatus was more important in determining the influence of the IFNL variants than recipient CMV serostatus. Actually, the wide majority of patients harboring the *rs368234815* polymorphism having received an organ from a seropositive donor developed CMV replication independently whether they were R- or R+. These data suggest that new CMV strains transmitted by the donor (33) may be more difficult to control in recipients harboring the *rs368234815* polymorphism.

Our study has some limitations. First, because only the first episode of asymptomatic CMV replication was collected on the STCS database, we were not able to investigate in the present study whether the IFNL3/4 polymorphisms had an impact on the overall duration of CMV infection and particularly on the response to antiviral therapy. Second, patients who received antiviral therapy were not monitored for CMV replication using the same schedule than patients managed by the preemptive approach just after transplantation; this difference could partially account for the different impact of the IFNL3/4 polymorphisms on CMV replication observed according to the preventive strategy used. Third, the low number of CMV disease events prevented to extract any conclusion about a potential higher risk of progression from asymptomatic viral replication to overt CMV disease in patients with -G/-G carriage. Fourth, the association between CMV replication and IFNL3 polymorphism was observed for the recessive mode of inheritance, while the association observed among HCV infected patients is usually

dominant. This difference may be due to some threshold effect of the amount of IFNL3/4 in response to specific pathogens. Because the recessive model was chosen in a post-hoc analysis, this could also account as a limitation of our study. Finally, the prevention strategies and immunosuppressive regimens were somewhat variable among transplant programs, so that it is possible that some remaining biases specifically related to the transplant center were not corrected by the multivariate analysis. Nevertheless, because of the large number of patients included, the strict and homogeneous definitions used for CMV infection, and the use of a novel polymorphism with predicted functional activity, the data indicate novel evidence of a relationship between IFNL polymorphisms and CMV infection.

In conclusion, in this large cohort of SOT recipients, we found that CMV infection in patients not receiving antiviral prophylaxis was influenced by IFNL genetic variants. This effect was stronger in recipients who received an allograft from a CMV seropositive donor. These results indicate that the INFL *rs368234815* polymorphism might be considered as a novel risk factor for developing CMV complications after organ transplantation. Validation of this association in further clinical studies has the potential to lead a better risk stratification for CMV reactivation and eventually influence future prevention strategies and guidelines, particularly in those patients followed by the preemptive approach.

**Acknowledgements**

We would like to thank all patients who participate in the Swiss Transplant Cohort Study, and acknowledge the work of the central and local data managers and of all the investigators involved in the STCS.

**Funding**:

This project was supported by STCS project No 12 and a grant from the Emma Muschamp Foundation.

P.-Y.B. was supported by the Swiss National Foundation (Grand number 324730-144054). P.-Y.B. is recipient of Mérieux Research Grant (MRG).

The Swiss Transplant Cohort Study is funded by a grant from the Swiss National Research Foundation (Grant number 33CS30_148512).

**Potential conflict of interest:** The authors of this manuscript have no conflicts of interest to disclose.

## References

1. Razonable RR, Humar A, Practice ASTIDCo. Cytomegalovirus in solid organ transplantation. Am J Transplant. 2013;13 Suppl 4:93-106.

2. Manuel O, Kralidis G, Mueller NJ, Hirsch HH, Garzoni C, van Delden C, et al. Impact of antiviral preventive strategies on the incidence and outcomes of cytomegalovirus disease in solid organ transplant recipients. Am J Transplant. 2013;13(9):2402-10.

3. Kotton CN, Kumar D, Caliendo AM, Asberg A, Chou S, Danziger-Isakov L, et al. Updated international consensus guidelines on the management of cytomegalovirus in solid-organ transplantation. Transplantation. 2013;96(4):333-60.

4. Meylan PR, Manuel O. Late-onset cytomegalovirus disease in patients with solid organ transplant. Curr Opin Infect Dis. 2007;20(4):412-8.

5. Crough T, Khanna R. Immunobiology of human cytomegalovirus: from bench to bedside. Clin Microbiol Rev. 2009;22(1):76-98.

6. Egli A, Humar A, Kumar D. State-of-the-art monitoring of cytomegalovirus-specific cell-mediated immunity after organ transplant: a primer for the clinician. Clin Infect Dis. 2012;55(12):1678-89.

7. Kang SH, Abdel-Massih RC, Brown RA, Dierkhising RA, Kremers WK, Razonable RR. Homozygosity for the toll-like receptor 2 R753Q single-nucleotide polymorphism is a risk factor for cytomegalovirus disease after liver transplantation. J Infect Dis. 2012;205(4):639-46.

8. Cervera C, Lozano F, Saval N, Gimferrer I, Ibanez A, Suarez B, et al. The influence of innate immunity gene receptors polymorphisms in renal transplant infections. Transplantation. 2007;83(11):1493-500.

9. Manuel O, Pascual M, Trendelenburg M, Meylan PR. Association between mannose-binding lectin deficiency and cytomegalovirus infection after kidney transplantation. Transplantation. 2007;83(3):359-62.

10. Hayes CN, Imamura M, Aikata H, Chayama K. Genetics of IL28B and HCV--response to infection and treatment. Nat Rev Gastroenterol Hepatol. 2012;9(7):406-17.

11. Kotenko SV. IFN-lambdas. Curr Opin Immunol. 2011;23(5):583-90.

12. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology. 2010;138(4):1338-45, 45 e1-7.

13. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461(7262):399-401.

14. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet. 2009;41(10):1105-9.

15. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet. 2009;41(10):1100-4.

16. Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat Genet. 2013;45(2):164-71.

17. Bibert S, Roger T, Calandra T, Bochud M, Cerny A, Semmo N, et al. IL28B expression depends on a novel TT/-G polymorphism which improves HCV clearance prediction. J Exp Med. 2013;210(6):1109-16.

18. Bravo D, Solano C, Gimenez E, Remigia MJ, Corrales I, Amat P, et al. Effect of the IL28B Rs12979860 C/T polymorphism on the incidence and features of active cytomegalovirus infection in allogeneic stem cell transplant patients. J Med Virol. 2014;86(5):838-44.

19. Egli A, Levin A, Santer DM, Joyce M, O'Shea D, Thomas BS, et al. Immunomodulatory Function of Interleukin 28B During Primary Infection With Cytomegalovirus. J Infect Dis. 2014;210(5):717-27.

20. Bibert S, Wojtowicz A, Taffé P, Manuel O, Bernasconi E, Furrer H, Günthard HF, Hoffmann M, Kaiser L, Osthoff M, Cavassini M, Bochud P-Y. The IFNL3/4 ΔG variant increases susceptibility to cytomegalovirus retinitis among HIV-infected patients. AIDS. 2014;28(13):1885-9.

21. Koller MT, van Delden C, Muller NJ, Baumann P, Lovis C, Marti HP, et al. Design and methodology of the Swiss Transplant Cohort Study (STCS): a comprehensive prospective nationwide long-term follow-up cohort. Eur J Epidemiol. 2013;28(4):347-55.

22. Humar A, Michaels M, Monitoring AIWGoID. American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. Am J Transplant. 2006;6(2):262-74.

23. Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999;94:496–509.

24. Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, et al. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol. 2003;4(1):69-77.

25. Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol. 2003;4(1):63-8.

26. Ank N, West H, Bartholdy C, Eriksson K, Thomsen AR, Paludan SR. Lambda interferon (IFN-lambda), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections in vivo. J Virol. 2006;80(9):4501-9.

27. Robek MD, Boyd BS, Chisari FV. Lambda interferon inhibits hepatitis B and C virus replication. J Virol. 2005;79(6):3851-4.

28. Brand S, Beigel F, Olszak T, Zitzmann K, Eichhorst ST, Otte JM, et al. IL-28A and IL-29 mediate antiproliferative and antiviral signals in intestinal epithelial cells and murine CMV infection increases colonic IL-28A expression. Am J Physiol Gastrointest Liver Physiol. 2005;289(5):G960-8.

29. Hamming OJ, Terczynska-Dyla E, Vieyres G, Dijkman R, Jorgensen SE, Akhtar H, et al. Interferon lambda 4 signals via the IFNlambda receptor to regulate antiviral activity against HCV and coronaviruses. EMBO J. 2013;32(23):3055-65.

30. Booth D, George J. Loss of function of the new interferon IFN-lambda4 may confer protection from hepatitis C. Nat Genet. 2013;45(2):119-20.

31. Srivastava S, Koch MA, Pepper M, Campbell DJ. Type I interferons directly inhibit regulatory T cells to allow optimal antiviral T cell responses during acute LCMV infection. J Exp Med. 2014.

32. Hadaya K, de Rham C, Bandelier C, Bandelier C, Ferrari-Lacraz S, Jendly S, et al. Natural killer cell receptor repertoire and their ligands, and the risk of CMV infection after kidney transplantation. Am J Transplant. 2008;8(12):2674-83.

33. 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(8):1160-6.

## Table 1. Baseline patient's characteristics

| Variable | D+/R- or R+ SOT recipients N=840[1] | D+/R- or R+ kidney transplant recipients N=526 |
|---|---|---|
| **Recipient age (median years; IQR)** | 54 (18) | 54 (20) |
| **Donor age (median years; IQR)** [2] | 53 (22) | 53 (20) |
| **Recipient sex M/F (%)** | 534/306 (64/36) | 336/191 (64/36) |
| **Caucasian ethnicity** | 840 (100) | 526(100) |
| **Transplanted organ (%)** | | |
| **Kidney** | 493 (59) | 493 (94) |
| **Liver** | 163 (19) | - |
| **Lung** | 78 (9) | - |
| **Heart** | 56 (7) | - |
| **Pancreas / islets /small bowel** [3] | 17 (2) | - |
| **Combined** [4] | 33 (4) | 33 (6) |
| **Donor type (%)** | | |
| **Deceased** | 629 (75) | 325 (62) |
| **Living related and unrelated** | 211 (25) | 201 (38) |
| **HLA full mismatch (%)** [5] | 294 (59) | 209 (52) |
| **CMV serostatus (%)** | | |
| **D+/R-** | 218 (26) | 134 (26) |
| **D-/R+** | 263 (31) | 159 (30) |
| **D+/R+** | 359 (43) | 235 (44) |
| **Induction therapy (%)** [6] | | |
| **Basiliximab** | 488 (60) | 308 (62) |
| **Anti-thymocyte globulin** | 143 (18) | 88 (18) |
| **None** | 179 (22) | 103 (21) |
| **Maintenance regimen (%)** [7] | | |
| **Tacrolimus** | 495 (65) | 379 (77) |
| **Ciclosporine** | 205 (27) | 100 (20) |
| **MMF/MPA** | 616 (81) | 429 (87) |
| **Azathioprine** | 22 (3) | 16 (3) |
| **mTOR inhibitors** | 52 (7) | 11 (2) |
| **Corticosteroids** | 517 (68) | 366 (74) |
| **CMV management approach** | | |

| | | |
|---|---|---|
| **prophylaxis (%)** | 373 (44) | 248 (47) |
| **pre-emptive (%)** | 467 (56) | 278 (53) |
| **Acute rejection episode (12-months cumulative incidence)** | 259 (31) | 134 (25) |
| **CMV infection (12-months cumulative incidence)** | 0.44 | 0.47 |
| **CMV disease (12-months cumulative incidence)** | 0.08 | 0.08 |
| **IFNL3/4 -G/-G** | 102 (12) | 61 (12) |

[1] Among 1119 Caucasian individuals enrolled in the STCS genetic study, 279 were excluded because they had both a donor and recipient negative CMV serostatus (D-R-, N=253), had a missing/incomplete CMV serology (N=13), died within 24 hours of transplant (N=7) or had a missing IFNL3/4 genotype (N=6).

[2] Donor age was missing in 163 solid organ transplant recipients and 0 kidney recipients.

[3] Including 12 islet, 4 pancreas and 1 small bowel transplants.

[4] Including 19 kidney-pancreas, 6 kidney-liver, 4 kidney-kidney, 2 kidney-islets, 1 kidney-lung and 1 kidney-kidney-pancreas transplants.

[5] Data were missing in 341 SOT recipients and 124 kidney transplant recipients, respectively

[6] Data were missing in 30 SOT recipients and 27 kidney transplant recipients, respectively

[7] Treatment at 12 months was assessable in 763 SOT recipients and 494 kidney transplant recipients, respectively

**Abbreviations**: CMV: cytomegalovirus; D: donor; IQR: interquartile range; MMF: mycophenolate mofetil; MPA: mycophenolic acid; R: recipient.

**Table 2. Univariate analysis of risk factors for the development of CMV replication in CMV D+/R- or R+ SOT recipients and in patients not receiving antiviral prophylaxis**

| Variable | All patients (N=840) | | Patients not receiving antiviral prophylaxis (N=467) | |
|---|---|---|---|---|
| | SHR (95% CI)[1] | P-value | SHR (95% CI)[1] | P-value |
| **Recipient age (per 10 years)** | 1.12 (1.04-1.21) | **0.003** | 1.11 (1.01-1.22) | **0.02** |
| **Donor age (years)** | 1.03 (0.96-1.11) | 0.4 | 1.05 (0.96-1.14) | 0.3 |
| **Recipient male sex** | 0.97 (0.79-1.20) | 0.8 | 0.99 (0.75-1.31) | 1.0 |
| **Transplanted organ** | | | | |
| **Kidney** | Ref. | | Ref. | |
| **Liver** | 0.79 (0.58-1.07) | 0.13 | 0.58 (0.41-0.82) | **0.002** |
| **Other/combined** | 0.83 (0.64-1.07) | 0.15 | 0.85 (0.58-1.24) | 0.4 |
| **HLA full mismatch** | 1.17 (0.90-1.52) | 0.2 | 1.15 (0.82-1.62) | 0.4 |
| **Rejection episode** | 1.05 (1.01-1.09) | **0.007** | 1.04 (0.98-1.11) | 0.18 |
| **CMV serostatus** | | | | |
| **D+/R-** | Ref. | | Ref. | |
| **D-/R+** | 0.81 (0.61-1.08) | 0.15 | 0.85 (0.54-1.32) | 0.5 |
| **D+/R+** | 1.33 (1.04-1.69) | **0.02** | 1.53 (1.03-2.28) | **0.04** |
| **Induction therapy** | | | | |
| **None** | Ref. | | Ref. | |
| **Basiliximab** | 1.03 (0.78-1.35) | 0.9 | 1.15 (0.83-1.58) | 0.4 |
| **Anti-thymocyte globulin** | 1.19 (0.86-1.65) | 0.3 | 1.99 (1.29-3.08) | **0.002** |
| **Maintenance regimen** | | | | |
| **Tacrolimus** | 0.97 (0.94-1.01) | 0.13 | 0.94 (0.87-1.00) | 0.06 |
| **Ciclosporine** | 1.05 (1.01-1.08) | **0.01** | 1.09 (1.01-1.17) | **0.02** |
| **MMF/MPA** | 1.08 (1.02-1.14) | **0.009** | 1.10 (0.99-1.22) | 0.08 |
| **mTOR inhibitors** | 0.99 (0.90-1.09) | 0.8 | 0.97 (0.83-1.14) | 0.7 |
| **Corticosteroids** | 1.12 (1.04-1.21) | **0.003** | 1.33 (1.01-1.76) | **0.04** |
| **Exposure to antiviral drug**[2] | 0.90 (0.81-0.99) | **0.02** | 0.96 (0.78-1.18) | 0.7 |
| **IFNL3/4 -G/-G**[3] | 1.30 (0.97-1.74) | 0.07 | 1.46 (1.01-2.12) | **0.047** |

[1] SHR stands for the subdistribution hazard ratio (calculated by using semi-parametric regression model of Fine and Gray (23))

[2] Risk of CMV infection during concurrent exposure to valganciclovir or ganciclovir

[3] Genetic association with *rs368234815* is for recessive mode of inheritance (patients homozygous for the rare alleles (-G/-G) are compared to the other [TT/TT and TT/-G])

**Abbreviations:** CI: confidence interval; CMV: cytomegalovirus; SHR: subdistribution hazard ratio; IFNL3/4: interferon lambda 3/4. MMF: mycophenolate mofetil; MPA: mycophenolic acid

**Table 3. Multivariate analysis of risk factors for the development of CMV replication in all CMV D+/R- or R+ SOT recipients and in patients not receiving antiviral prophylaxis**

| | All patients (N=840) | | Patients not receiving antiviral prophylaxis (N=455)[1] | |
|---|---|---|---|---|
| | **SHR (95% CI)[2]** | **P-value** | **SHR (95% CI)[2]** | **P-value** |
| **Recipient age (per 10 years)** | 1.14 (1.06-1.23) | **0.001** | 1.12 (1.02-1.24) | **0.02** |
| **Transplanted organ** | | | | |
| **Kidney** | | | Ref. | |
| **Liver** | | | 0.66 (0.45-0.98) | **0.04** |
| **Other/combined** | | | 0.66 (0.42-1.04) | 0.07 |
| **CMV serostatus** | | | | |
| **D+/R-** | Ref. | | Ref. | |
| **D-/R+** | 0.75 (0.56-1.01) | **0.06** | 0.91 (0.55-1.49) | 0.7 |
| **D+/R+** | 1.29 (0.01-1.70) | **0.04** | 1.60 (1.02-2.60) | **0.04** |
| **Induction therapy** | | | | |
| **None** | | | Ref. | |
| **Basiliximab** | | | 1.14 (0.82-1.58) | 0.5 |
| **Anti-thymocyte globulin** | | | 2.08 (1.21-3.57) | **0.008** |
| **Acute rejection** | 1.05 (1.02-1.09) | **0.005** | | |
| **Maintenance therapy** | | | | |
| **Corticosteroids** | 1.13 (1.04-1.22) | **0.003** | 1.31 (1.01-1.70) | **0.04** |
| **Tacrolimus** | | | 0.91 (0.84-0.99) | **0.02** |
| **MMF/MPA** | 1.07 (1.01-1.13) | **0.02** | | |
| **Cyclosporine** | 1.08 (1.04-1.12) | **<0.0001** | | |
| **Exposure to antiviral drug[3]** | 0.85 (0.77-0.93) | **0.001** | | |
| **IFNL3/4 -G/-G[4]** | 1.32 (0.99-1.75) | 0.06 | 1.57 (1.10-2.23) | **0.01** |

[1] The number of patients in the multivariate analyses (N=455) is slightly lower than the number of patients included in the univariate analysis (N=467) due to missing data for induction therapy in 12 patients.

[2] SHR stands for the subdistribution hazard ratio (calculated by using semi-parametric regression model of Fine and Gray (23)). Covariates with a P value <0.1 were kept in the multivariate analyses.

[3] Risk of CMV infection during concurrent exposure to valganciclovir or ganciclovir.

[4] Genetic association *with rs368234815* is for recessive mode of inheritance (patients homozygous for the rare alleles (-G/-G) are compared to the other (TT/TT and TT/-G)).

**Abbreviations:** CI: confidence interval; CMV: cytomegalovirus; SHR: subdistribution hazard ratio; IFNL3/4: interferon lambda 3/4.

**Legend Figure 1. Cumulative incidence of CMV replication according to prophylaxis and transplanted organ in patients homozygous for the minor allele of *rs368234815* (-G/-G carriers) vs. TT/TT or TT/-G carriers.** A semi-parametric regression model of Fine and Gray (23) was used to evaluate the relative hazards associated with the demographic factors or genetic variants and the endpoints. N indicates the number of patients with CMV infection in each group of patients.

**Legend Figure 2. Cumulative incidence of CMV replication according to antiviral preventive strategy in D+ SOT recipients and in kidney transplant recipients in patients homozygous for the minor allele of *rs368234815* (-G/-G carriers) vs. TT/TT or TT/-G carriers.** A semi-parametric regression model of Fine and Gray (23) was used to evaluate the relative hazards associated with the demographic factors or genetic variants and the endpoints. N indicates the number of patients with CMV infection in each group of patients.

Figure 1

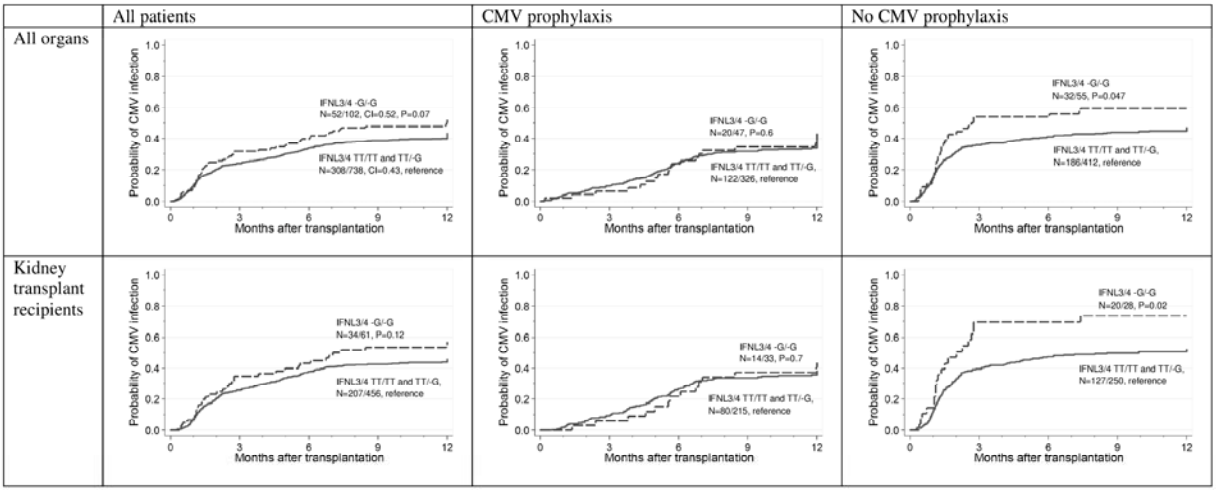

Figure 2