British Transplantation Society Congress 2018
Going viral

Cytomegalovirus: Lessons from the Swiss Transplant Cohort Study

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STCS SWISS TRANSPLANT COHORT STUDY

The Swiss Transplant Cohort Study STCS
www.stcs.ch

Prospectively follows all consenting transplanted patients in Switzerland since 5/2008 (>95%); 6237 patients on March 13th, 2018 (2166 in Zurich, 1/3 of patients)

Collects data based on pre-defined definitions and includes comprehensive patient- and organ-related variables

Sampling for DNA, viable cells and plasma before transplantation, and at 6 and 12 months post-transplant

Infectious diseases endpoints are verified by a transplant ID specialist

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Cytomegalovirus in the STCS

8 papers (out of 44) with cytomegalovirus as a main topic or at least a relevant variable

- Epidemiology (single cohort, intercohort)
- Combination of genetic/immunological findings and their impact on ID endpoints

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Paper I

American Journal of Transplantation 2013; 13: 2402–2410
Wiley Periodicals Inc.

doi: 10.1111/ajt.12388

Impact of Antiviral Preventive Strategies on the Incidence and Outcomes of Cytomegalovirus Disease in Solid Organ Transplant Recipients

Rationale: Effects of CMV infection beyond disease
1239 patients, follow-up 1 year

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CMV disease

(A) High-risk patients (donor positive/recipient negative)

(B) Intermediate-risk patients (recipient positive)

Graft failure-free survival in patients after exclusion of early death or graft failure.

HR 1.63 (95% CI 1.01–2.64)
p = 0.04

All solid organ transplant recipients

HR 2.11 (95% CI 1.21–3.64)
p = 0.007

Kidney and liver transplant recipients

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Key points

- **Association of CMV antiviral strategy with graft survival, but not with CMV disease**
- **The «indirect effect» concept**
- **No causality!**
- **It did not change our local practice**

Paper II

Genes and Immunity (2014) **15,** 495–499

www.nature.com/gene

ORIGINAL ARTICLE

KIR-associated protection from CMV replication requires pre-existing immunity: a prospective study in solid organ transplant recipients

A Gonzalez[1], K Schmitter[1], HH Hirsch[2,3], C Garzoni[4], C van Delden[5], K Boggian[6], NJ Mueller[7], C Berger[8], J Villard[9], O Manuel[10], P Meylan[10], M Stern[1,12] and C Hess[11,12] for the Swiss Transplant Cohort Study

Correlation of Killer cell Immunoglobulin-like Receptor (KIR) genotype and CMV serostatus at the time of transplantation with rates of CMV viremia in a total of 517 (heart (n=57), kidney (n=223), liver (n=165) or lung (n=72) allograft recipients (mix of prevention strategies)

Rationale: Specific KIR genotypes have been associated with protection from CMV

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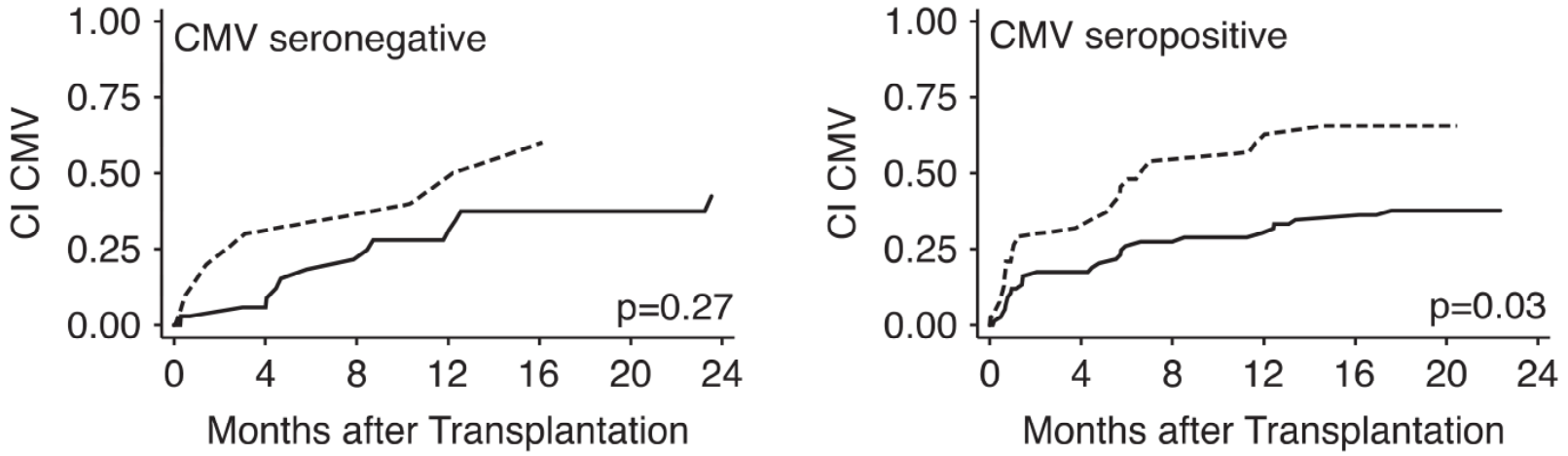
Cumulative incidence of CMV events in patients with intense immunosuppression (heart and lung transplant recipients, and patients receiving ATG induction)



Dashed lines represent patients homozygous for the KIR A haplotype (AA), whereas solid lines represent patients carrying one or two KIR B haplotypes (BX). P-value derived from Gray's test, CI: cumulative indcidence

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Key points

- **KIR genotype (B haplotypes) were associated with protection from CMV replication**
  - **in seropositive individuals**
  - **with intense immunosuppression**
  - **but not sufficiently protective in primary infection**

- **Role of ‘primed’ or ‘memory-like’ NK cells as the cellular correlate for this protective effect**

Paper III

CLINICAL AND TRANSLATIONAL RESEARCH

Transplantation 2014;98: 1013–1018

Cytomegalovirus Serology and Replication Remain Associated With Solid Organ Graft Rejection and Graft Loss in the Era of Prophylactic Treatment

Martin Stern,[1,11] Hans Hirsch,[2,3] Alexia Cusini,[4] Christian van Delden,[5] Oriol Manuel,[6] Pascal Meylan,[7] Katia Boggian,[8] Nicolas J. Mueller,[9] and Michael Dickenmann,[10] and on behalf of all members of the Swiss Transplant Cohort Study

Correlation of CMV infection, biopsy-proven graft rejection, and graft loss in 1,414 patients receiving heart (n=97), kidney (n=917), liver (n=237), or lung (n=163) allografts, 47% of patients with prophylactic strategy

Rationale: Role of CMV beyond disease

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Forest plot of hazard ratios derived from time-dependent Cox models analyzing the impact of CMV replication on the incidence of biopsy proven graft rejection (within 4 weeks)

Heart (n=97), kidney (n=917), liver (n=237), lung (n=163). Cox models included induction and maintenance immunosuppression, number of HLA mismatched between recipient and graft, and incidence of previous rejection episodes as covariables.

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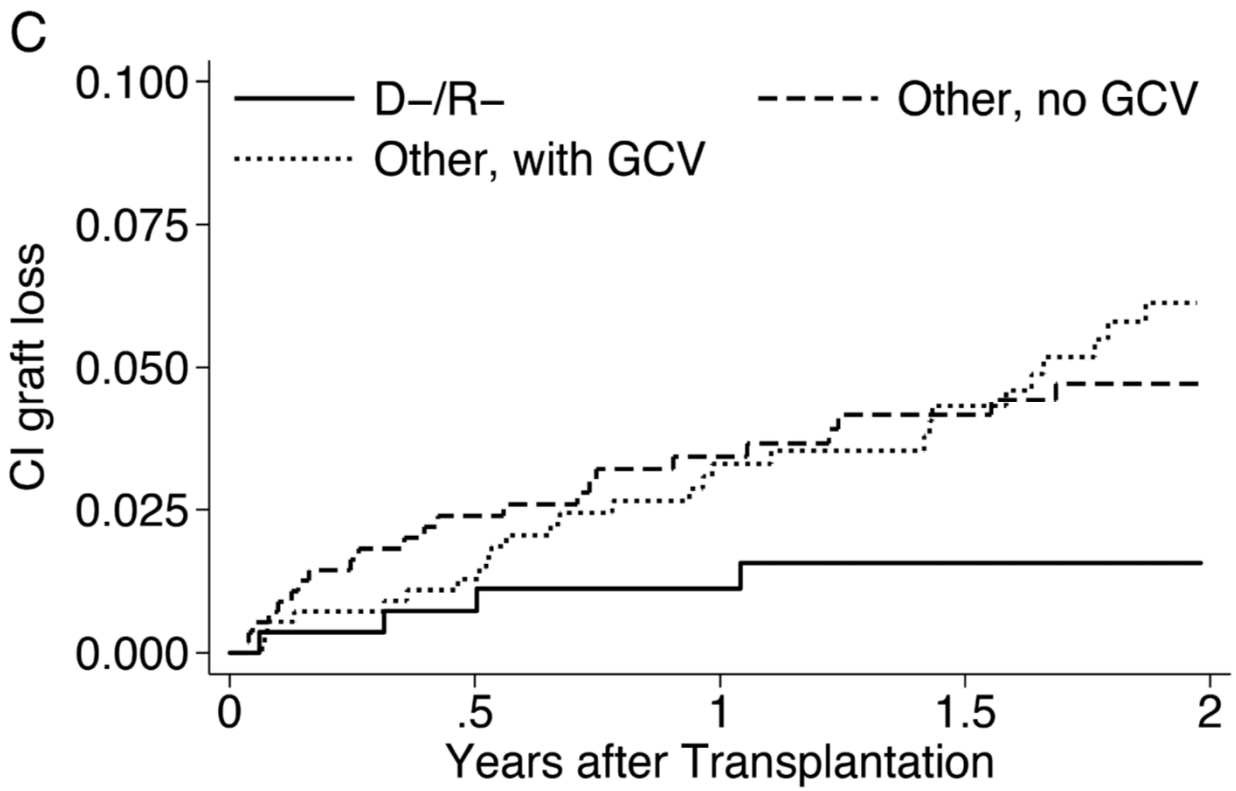
Cumulative incidence of graft loss in D-/R- allografts (solid line, n=278), and in non-D-/R- allografts receiving (dotted line; n=555) or not (dashed line; n=560) (val)ganciclovir prophylaxis



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Key points

- CMV replication (treated or not) was associated with biopsy-proven rejection
- CMV seropositivity (resulting in treatment or not) was associated with graft loss
- CMV seropositivity was associated with biopsy-proven rejection in liver and lung allografts
- Prophylaxis with (val)ganciclovir did not change the rate of graft loss

Paper IV

The Journal of Infectious Diseases 2015;211:906–14

Influence of *IFNL3/4* Polymorphisms on the Incidence of Cytomegalovirus Infection After Solid-Organ Transplantation

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

A total of 840 solid-organ transplant recipients at risk for CMV infection were included, among whom 373 (44%) received antiviral prophylaxis (Donor and/or recipient seropositive)

Rationale: IFNL3/4 polymorphisms are associated with reduced HCV clearance.

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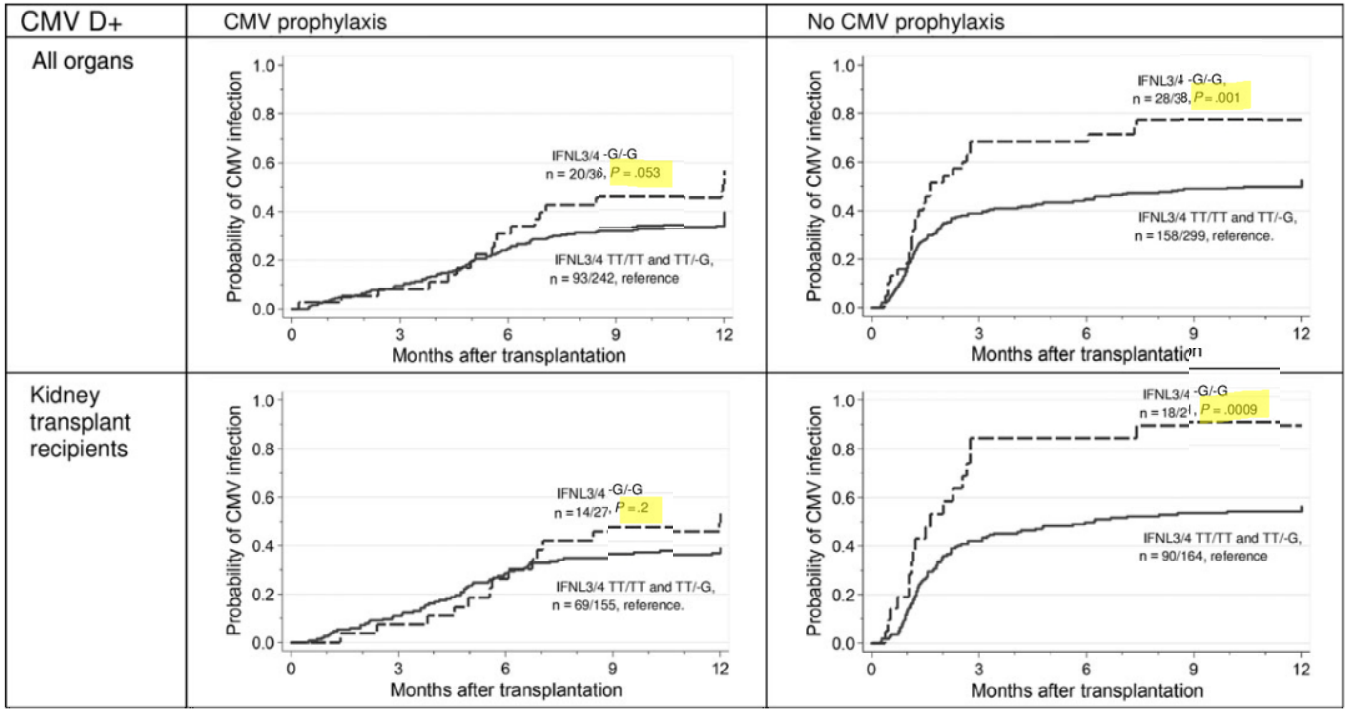
Patients homozygous for the minor allele of rs368234815 (−G/−G carriers) show a higher probability of CMV replication in a CMV donor seropositive constellation



A semiparametric regression model published by Fine and Gray [23] was used to evaluate the relative hazards associated with the demographic factors or genetic variants and the end points. Proportions denote the number of patients with CMV infection/total number of patients in the group.

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Key points

- CMV replication (but not disease) was associated with carriage of the homozygous allele of rs368234815, coding for interferon λ3 and interferon λ4.
- This effect was “masked” if CMV prophylaxis was given
- One of (many) genetic factors involved in control of CMV

Paper V

The Journal of Infectious Diseases® 2017;215:537–46

The Journal of Infectious Diseases

MAJOR ARTICLE

Human MicroRNA Responses Predict Cytomegalovirus Replication Following Solid Organ Transplantation

Sang Hoon Han,[1,2] Deepali Kumar,[1] Victor H. Ferreira,[1] Adrian Egli,[3] Hans H. Hirsch,[4,5] Maja Weisser,[4] Christian Garzoni,[6] Christian van Delden,[7] Pierre-Yves Bochud,[8] Oriol Manuel,[9] Pascal Meylan,[10] Katia Boggian,[11] Shahid Husain,[1] Nicolas J. Mueller,[12,a] and Atul Humar;[1,a] Swiss Transplant Cohort Study[12]

272 SOT HCMV-seropositive recipients who were managed using preemptive therapy. Correlation of type of microRNA with replication of CMV.

Rationale: micro–ribonucleic acid (miRNA)-200b-3p and 200c-3p are predicted to bind to 3' untranslated region of mRNA encoding human cytomegalovirus (HCMV) immediate early protein 2 (IE2).

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- **Pretransplant peripheral blood mononuclear cells were stimulated with HCMV**
- **Collection of RNA 1 day post-stimulation.**
- **Quantification of miRNAs using real-time reverse transcription–polymerase chain reaction.**
- **Correlation with CMV endpoints (disease, asymptomatic viremia)**

- ***In vitro* transfection experiments in foreskin fibroblasts with 200b-3p and 200c-3p and infection with HCMV 1 hour post-transfection. Protein was collected at 3 days postinfection and 7 dpi for immunoblotting for CMV IE2.**

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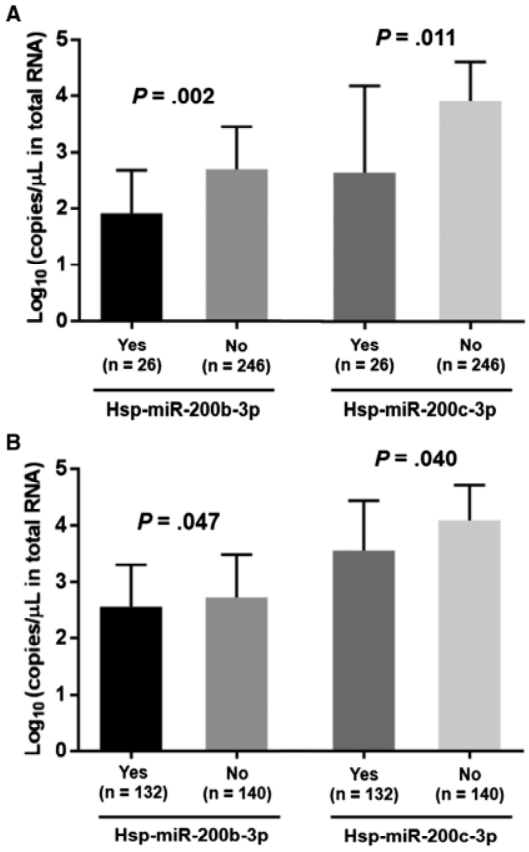
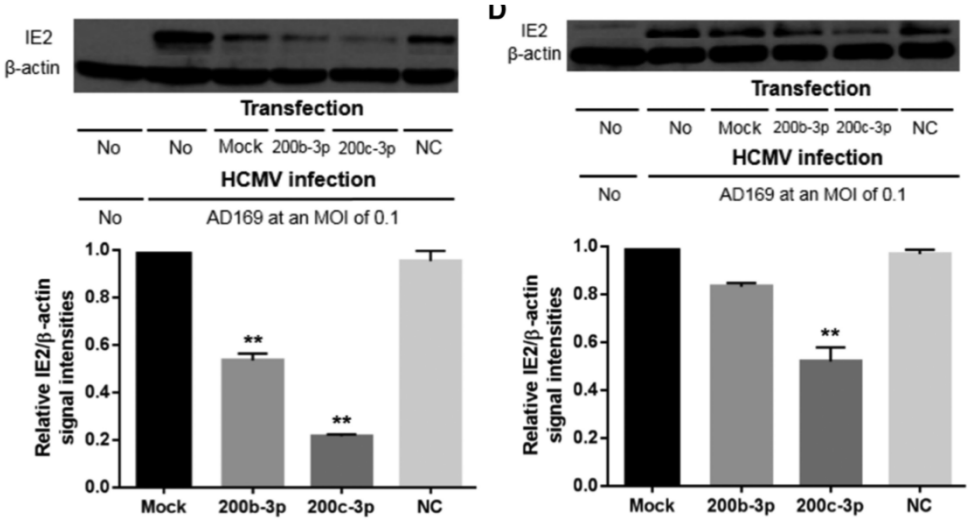


All episodes of HCMV replication (A)

Asymptomatic viremia (B)

Expression levels of hsp-miR-200b-3p and hsp-miR-200c-3p in pre-transplant PBMCs of transplant patients stimulated with HCMV for 24h, and correlation with CVM events



***In vitro* transfection, 1 and 3 days after infection, protein collection after three days**

Key points

- **Potential role for microRNA in the control of CMV replication, *in vivo and in vitro***
- **How strong ?**
- **Only one timepoint pre-transplant was evaluated – what are the dynamics?**

Paper VI

American Journal of Transplantation 2017; 17: 1813–1822
Wiley Periodicals Inc.

doi: 10.1111/ajt.14192

Preventive Strategies Against Cytomegalovirus and Incidence of α-Herpesvirus Infections in Solid Organ Transplant Recipients: A Nationwide Cohort Study

Rationale: Prevention of CMV has an impact on other viral infections, if prophylaxis is given.

A total of 2781 SOT recipients (56% kidney, 20% liver, 10% lung, 7.3% heart, 6.7% others) were included in the study. 1-year follow-up.

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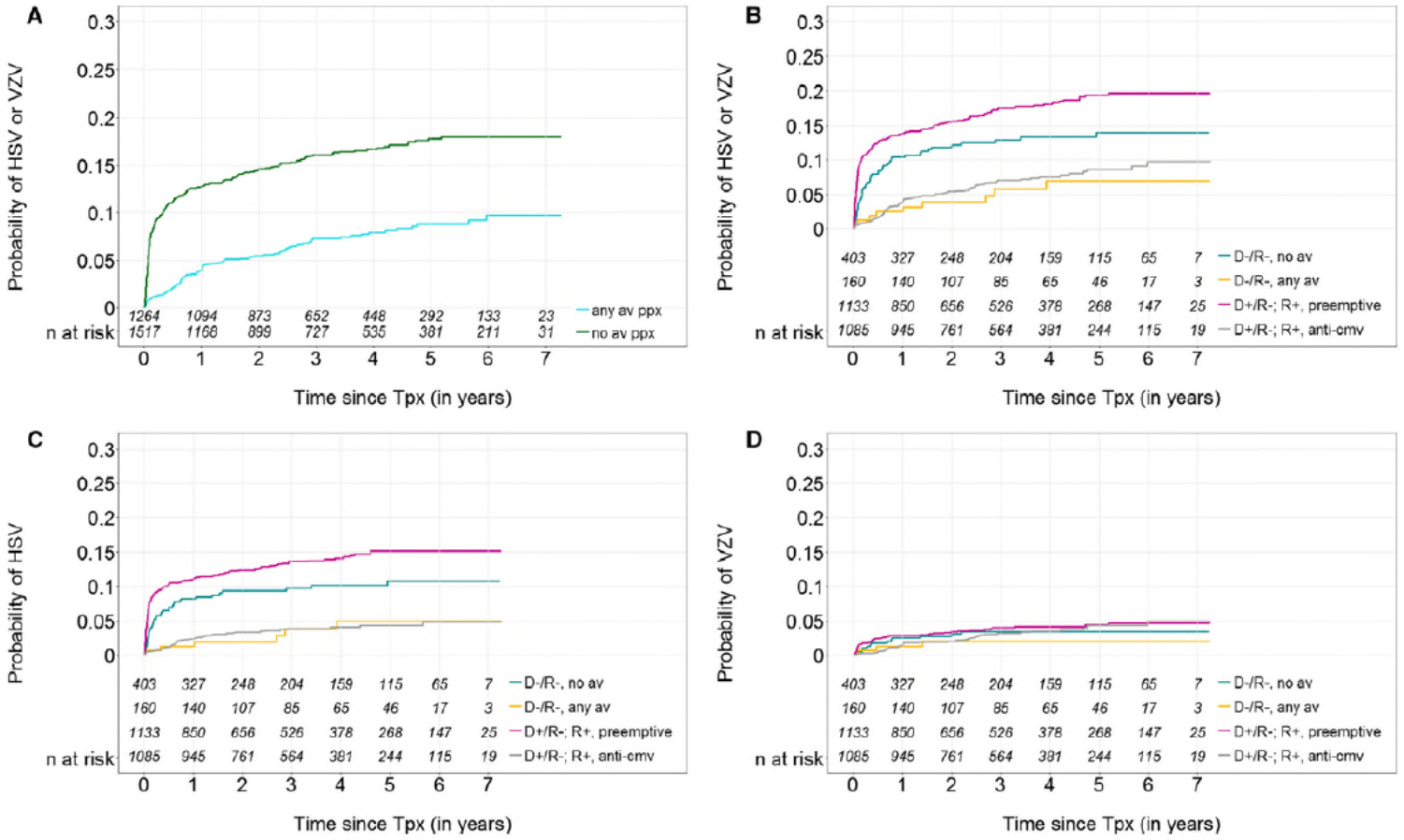
A
Probability of HSV or VZV
0.3
0.25
0.2
0.15
0.1
0.05
0
n at risk
1264 1094 873 652 448 292 133 23
1517 1168 899 727 535 381 211 31
any av ppx
no av ppx
0 1 2 3 4 5 6 7
Time since Tpx (in years)
B
Probability of HSV or VZV
403 327 248 204 159 115 65 7
160 140 107 85 65 46 17 3
1133 850 656 526 378 268 147 25
1085 945 761 564 381 244 115 19
D-/R-, no av
D-/R-, any av
D+/R-; R+, preemptive
D+/R-; R+, anti-cmv
Time since Tpx (in years)
C
Probability of HSV
Time since Tpx (in years)
D
Probability of VZV
Time since Tpx (in years)

Key points

- **Protective role of «CMV» prophylaxis against HSV and VZV is no surprise**

- **The rather high incidence of HSV or VZV infections was (HSV, 8.9% of all patients; VZV, 4.0% of all patients, resulting in 12 and 11 hospitalisations, respectively**

Paper VII

Clinical Microbiology and Infection 24 (2018) 192–198

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Multinational case-control study of risk factors for the development of late invasive pulmonary aspergillosis following kidney transplantation

Rationale: CMV disease as a risk factor for invasive aspergillosis

61 cases of late invasive pulmonary aspergillosis (>180 days after transplantation); out of a total of 112 cases reported with IPA

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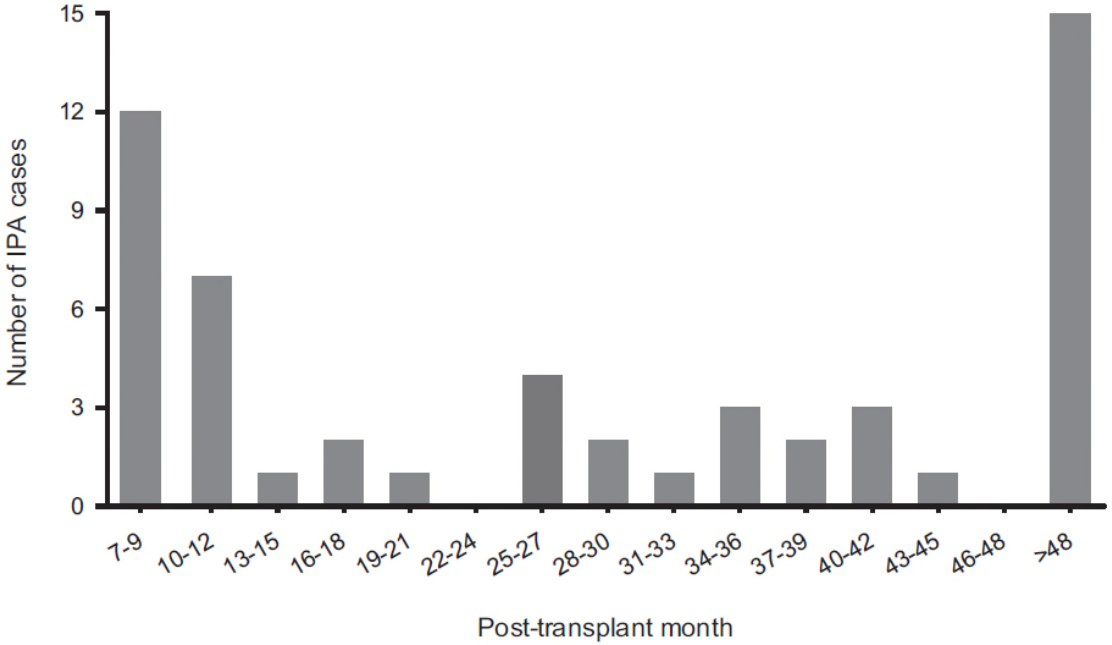
Comparison of donor- and transplant-related factors, post-transplant events, and outcomes

Variable	Late IPA group ($n = 61$)	Control group ($n = 61$)	p[a]
CMV disease	10 (16.4)	1 (1.6)	**0.004**



Key points

- **The association between CMV infection and aspergillosis was confirmed**
- **Immunmodulation? – or mere overimmunosuppression**

Paper VIII

In press, March 12th issue

RESEARCH ARTICLE

The Journal of Clinical Investigation

LILRB1 polymorphisms influence posttransplant HCMV susceptibility and ligand interactions

Kang Yu,[1] Chelsea L. Davidson,[1] Agnieszka Wójtowicz,[2] Luiz Lisboa,[1,3] Ting Wang,[1] Adriana M. Airo,[1] Jean Villard,[4] Jeremie Buratto,[5] Tatyana Sandalova,[5] Adnane Achour,[5] Atul Humar,[6] Katia Boggian,[7] Alexia Cusini,[8] Christian van Delden,[9] Adrian Egli,[10,11] Oriol Manuel,[12] Nicolas Mueller,[13] Pierre-Yves Bochud,[2] Swiss Transplant Cohort Study,[14] and Deborah N. Burshtyn[1]

Rationale: UL18 is a human CMV MHC class I homolog that efficiently inhibits leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1)+ NK cells- resulting in immune evasion

Hypothesis: Low LILRB1 expression on NK cells results in enhanced control of CMV

The role of LILRB1 polymorphisms in the regulatory regions and ligand-binding domains was assessed *in vitro* and *in vivo*

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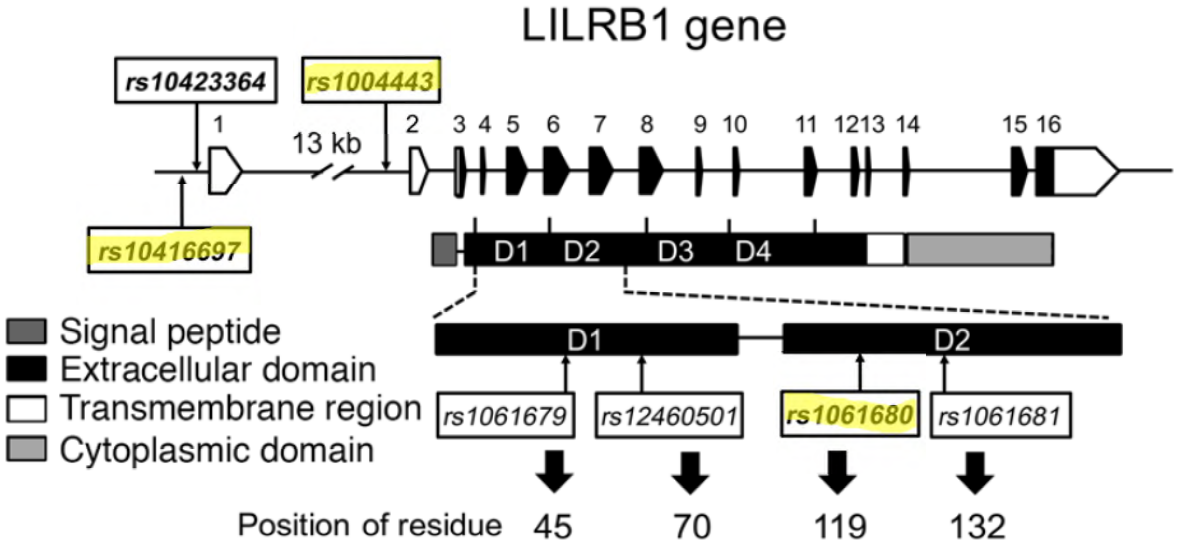

The majority of this polymorphisms result in a higher levels of transcripts and surface expression of leukocyte immunoglobulin-like receptor subfamily B member 1

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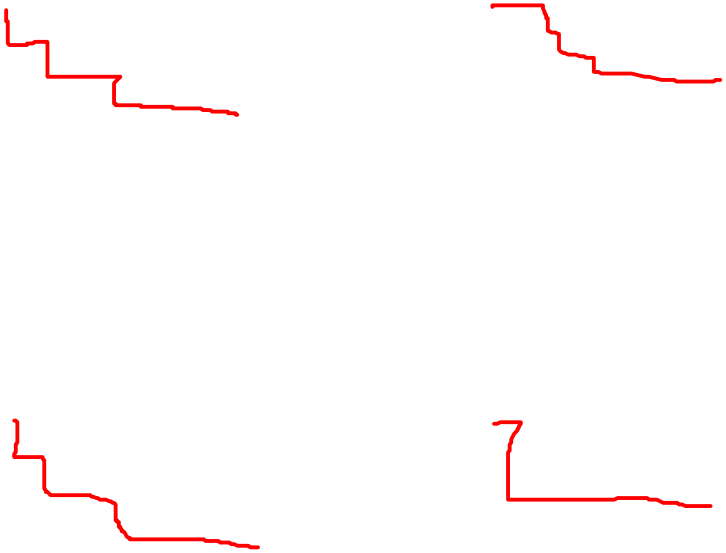

The initial hypothesis that a higher expression and therefore a more likely inhibition by the CMV UL18 homologue would result in worse control of HCMV was wrong!

67 D+/R– Canadian renal transplant patients

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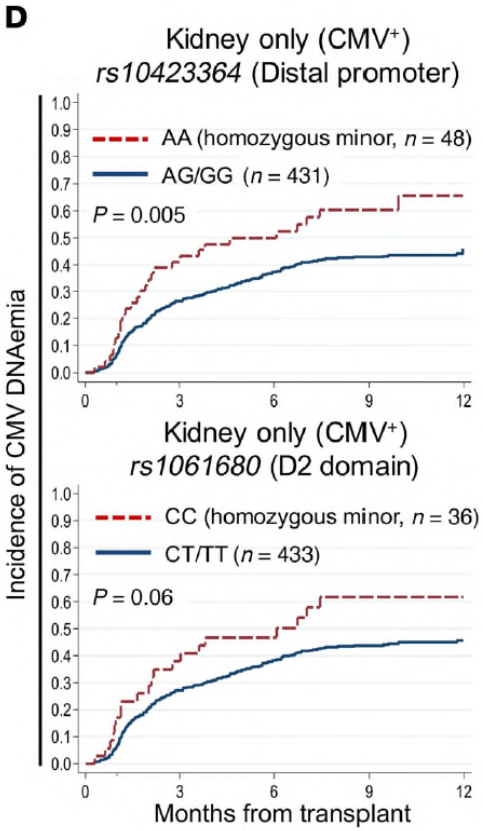

Incidence of posttransplant HCMV DNAemia of D+/R– or R+ in 479 STCS kidney transplant patients for the indicated SNPs

In CMV-positive recipients of a kidney a polymorphism at the distal promoter or the D2 domain resulting in a lower expression of the LILRB1 receptor was associated with better control of viremia – why?

***In vitro* functional differences with CMV UL18 interaction were demonstrated**

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Key points

- **Polymorphisms that predict lower frequency of expression on NK cells were directly associated with poor control of HCMV in transplant patients**
- **LILRB1 protein variant encoded by one of the alleles interacted more strongly with UL18**
- **A small change in affinity between LILRB1 and UL18 can greatly influence control of HCMV after transplantation**
- **Specific LILRB1 alleles that allow for superior immune evasion by HCMV are restricted by mutations that limit LILRB1 expression selectively on NK cells.**

Conclusions

- **Some data support an effect of CMV beyond a direct effect (disease)**
- **The optimal prevention strategy is still unknown**
- **Once many pieces of the HCMV puzzle can be put together, a good model will hopefully at one point allow to better individualize the risk for CMV reactivation and/or disease**

  - with implications for the choice of the strategy

Conclusions

- **The cohort concept inlcuding sampling proved very useful for answering some questions**
  - on an epidemiological level
  - combining immunological findings in the lab with clinical endpoints
  - combing host genetics with clinical endpoints

- **The «real life» versus «randomized trial» discussion is ongoing**

- **It likely needs both!**

Acknowledgments

STCS ID working group: all ist dedicated members

STCS Data centre /IT

Local and national study nurse and data managers

To all involved in the Swiss Transplant Cohort Study

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