24th ECCMID Barcelona, Spain 10 – 13 May 2014
ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

EW073
Saturday, May 10th 2014
11:00-13:00

Session 17: How to prevent the consequences of EBV infection in transplant recipients?
arranged with the ESCMID Study Group for Infections in Compromised Hosts (ESGICH)

Is there any role of antivirals and rituximab in the pre-emptive management of PTLD?

Hans H Hirsch

Transplantation & Clinical Virology
Department Biomedicine (Haus Petersplatz)

Infectious Diseases & Hospital Epidemiology
University Hospital Basel
Basel
Switzerland

Outline

- What do we observe clinically ?
- What is the role of Epstein-Barr virus ?
- Is there a rationale for antivirals ?
- What is the clinical evidence ?
- What should we do now ?
- What is on the horizon ?

- What do we observe clinically?

EBV-associated PTLD

- Incidence posttransplant <1% - 25%
- Heterogeneous clinical presentations
  - Asymptomatic, fatigue, fever, leukopenia, lymphadenopathy,
  - Lymph nodes, organs, transplants, mucocutaneous sites
  - Imaging studies (PET-CT)
- Definitive diagnosis by histology
  - Clonality, EBV markers
- Mortality 20% - 90%
- Therapy
  - Reduce immunosuppression, anti-CD20, chemotherapy, adoptive T-cell transfer
- Preemptive mode guided by EBV load

Post-Transplant Lymphoproliferative Disease

- Solid organ transplantation (SOT)
  - Incidence <1% to 25%
  - Mortality 20%-80%
- Hematopoietic stem cell transplantation (HSCT)
  - Incidence <1% to 13%
  - Mortality 20%-80%
- EBV-*positive* PTLD *earlier* (often <1 yr posttransplant)
- EBV-*negative* PTLD *later* (often >1 yr posttransplant)

Lundgren et al. (2009) Blood 113: 4992
Gulley & Tang (2010) Clin Micro Rev 23: 350
Green & Michaels (2013) Am J Transplant 13: 41
Mynarek et al. (2013) Clin Devel Immunol: e814973

Progression through PTLD Stages

- Early lesions (50%)
  - Polyclonal B-cell hyperplasia (*mononucleosis*-like)
  - Polyclonal plasma cells
  - Intact tissue architecture (A)
- Polymorphic (30%)
  - Small and large lymphocytes (B)
  - Polyclonal or monoclonal with early malignant transformation
- Monomorphic (15%)
  - Monoclonal disease (C)
  - “diffuse large B-cell lymphoma”
  - Nodal, allograft, extranodal
- Other
  - Hodgkin, NK, T-cell lymphoma

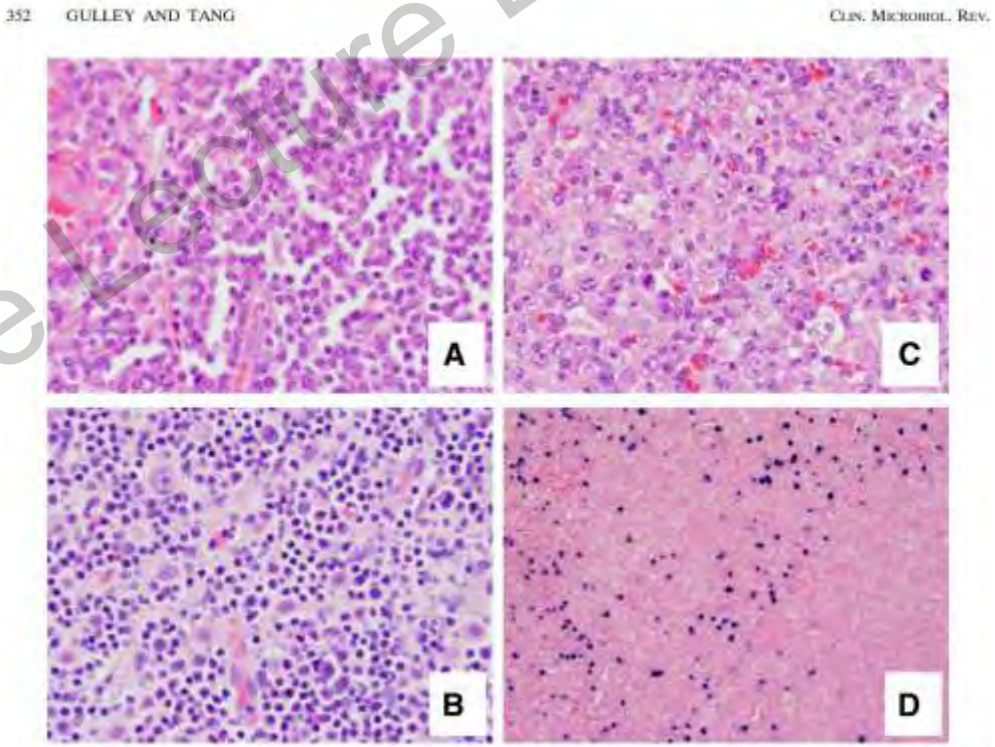


FIG. 1. Histopathologic features reflect clinical subtypes of PTLD. (A) An early lesion (plasmacytic hyperplasia) contains mature polyclonal plasma cells that expand but do not replace tissue architecture. (B) In polymorphic PTLD there is a mixture of small and large lymphocytes. (C) In monomorphic PTLD there are sheets of large lymphoid cells reminiscent of diffuse large B cell lymphoma. (D) EBER *in situ* hybridization reveals a purple EBER signal localized to the nuclei of tumor cells. (Photomicrographs courtesy of Yuri Fedoriw, University of North Carolina at Chapel Hill; reproduced with permission.)

Gulley & Tang (2010) Clin Micro Rev 23: 350

Rate of PTLD after SOT

- Organ: Immunosuppression, lymphoid tissues
- Age: 4-fold to 10-fold higher in children than in adults
- Time: 3-phase dynamic in the first 2 years posttransplant

Table 1: Cumulative 1- and 5-year incidence of PTLD in pediatric and adult SOT recipients by transplanted organ as reported in the 2010 OPTN/SRTR Annual Report (97)*

	Pediatric		Adult	
Organ	1 year	5 years	1 year	5 years
Lung/heart–lung	4.0	16%	1.0	1.5%
Liver	2.1%	4.7%	0.25%	1.1%
Pancreas (isolated)	N/A	N/A	2.3%	2.3%
Heart	1.6%	5.7%	0.3%	0.7%
Kidney	1.3%	2.4%	<0.2%	0.6%

*Data for intestinal transplant recipients not broken down by pediatric versus adult and therefore not included.

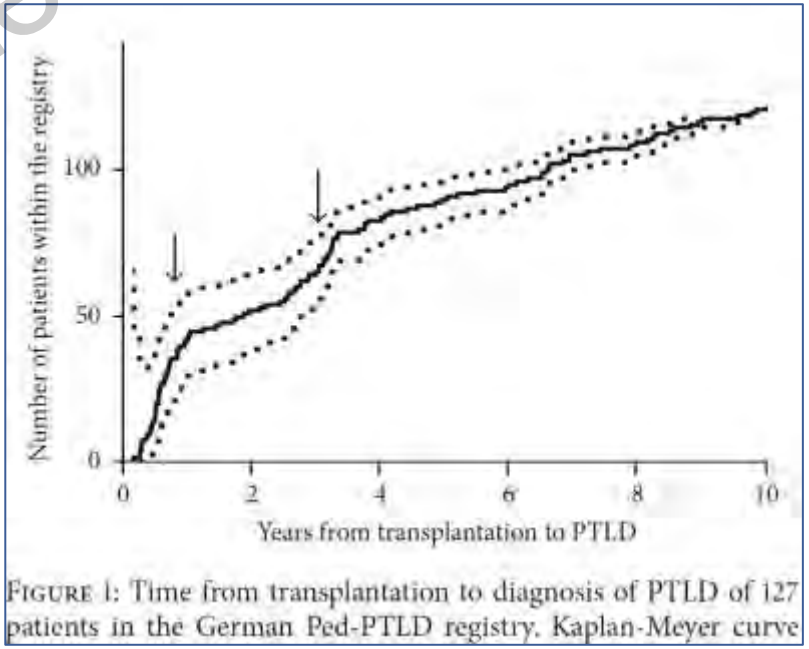


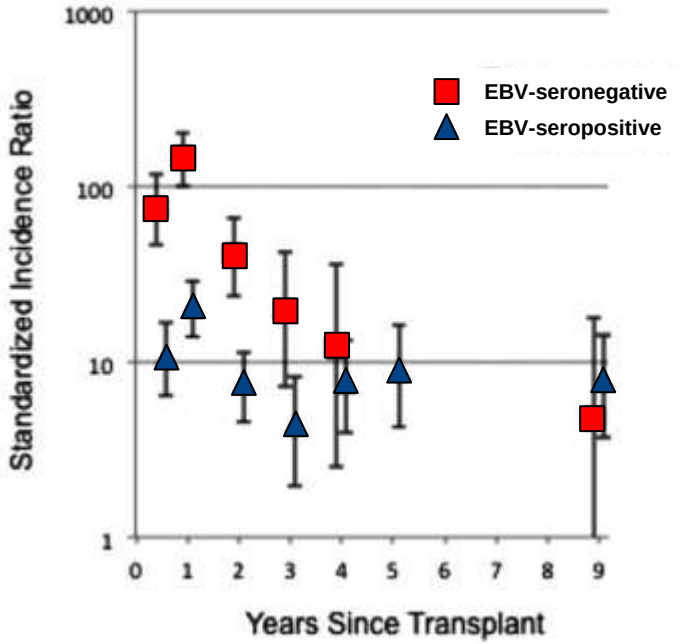
FIGURE 1: Time from transplantation to diagnosis of PTLD of 127 patients in the German Ped-PTLD registry. Kaplan-Meyer curve

Green & Michaels (2013) Am J Transplant 13: 41
Mynarek et al. (2013) Clin Devel Immunol: e814973

PTLD in SOT

- Diffuse large B-cell lymphoma (DLBCL) USA 2000/08
- Standardized incidence ratios (SIR)
  - N observed in 96'615 SOT / N expected in general population

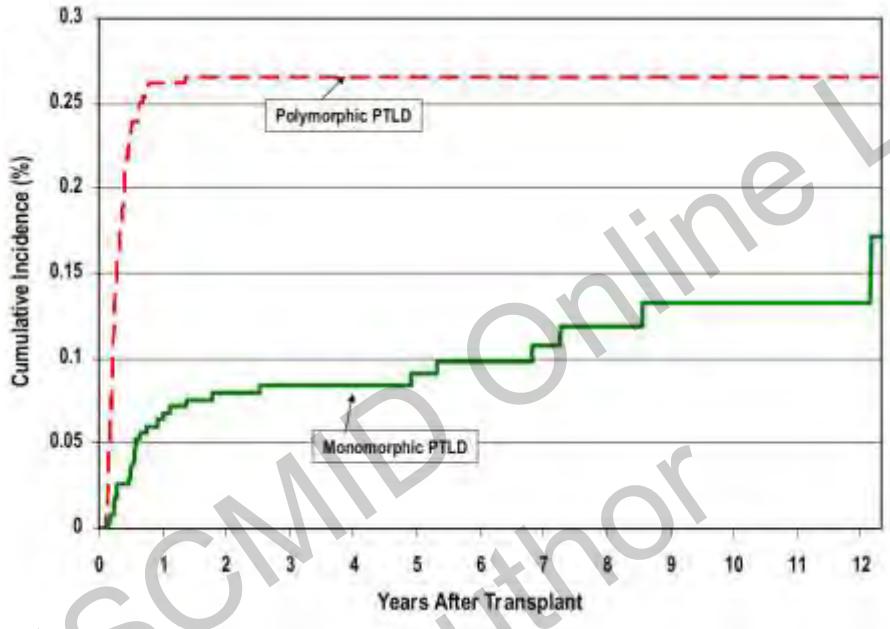
	EBV-negative recipients			
	DLBCL Cases	SIR	RR[a]	95% CI
All transplants	70	78.7		
Age at transplantation				
0–19	22	2,123	39.0	20.3–77.0
20–39	16	294	5.6	2.7–11.4
40–49	7	59.2	1.3	0.5–3.0
50–59	15	52.8	1.0	Reference
≥60	10	23.7	0.4	0.2–0.9
Transplanted organ				
Kidney	35	77.0	1.0	Reference
Liver	8	34.4	0.6	0.2–1.2
Heart	9	89.4	1.5	0.7–2.9
Lung	16	249	3.8	2.1–6.8
Polyclonal antibodies				
No	45	61.9	1.0	Reference
Yes	25	154	2.9	1.6–5.0
IL2 receptor antagonists				
No	50	79.8	1.0	Reference
Yes	20	76.1	1.0	0.6–1.8

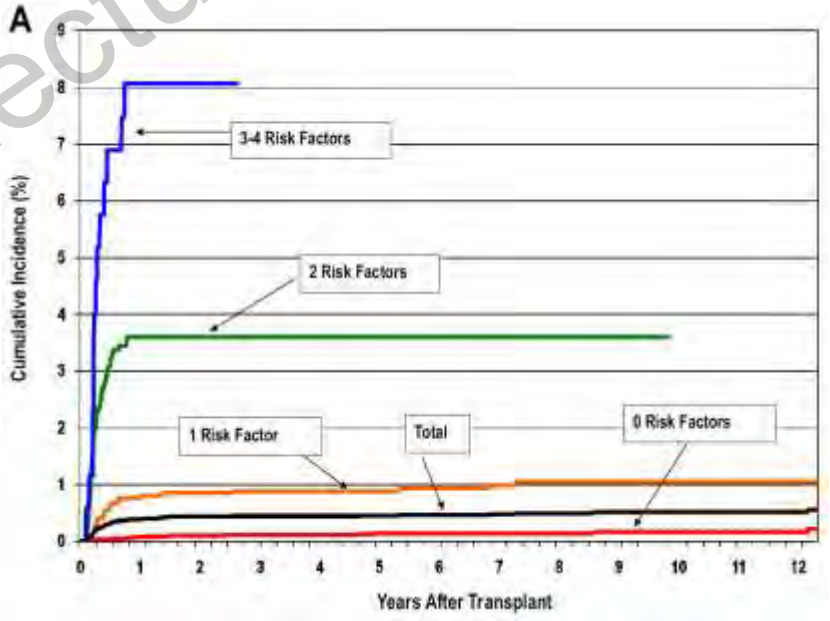


Gibson et al (2014) Am J Hematol 13: 41

PTLD after allogeneic HSCT

- Allogeneic HSCT in 271 centers worldwide (N=26‘901)
- PTLD (N=127), 83% in 1 yr posttransplant





Lundgren et al. (2009) Blood 113: 4992

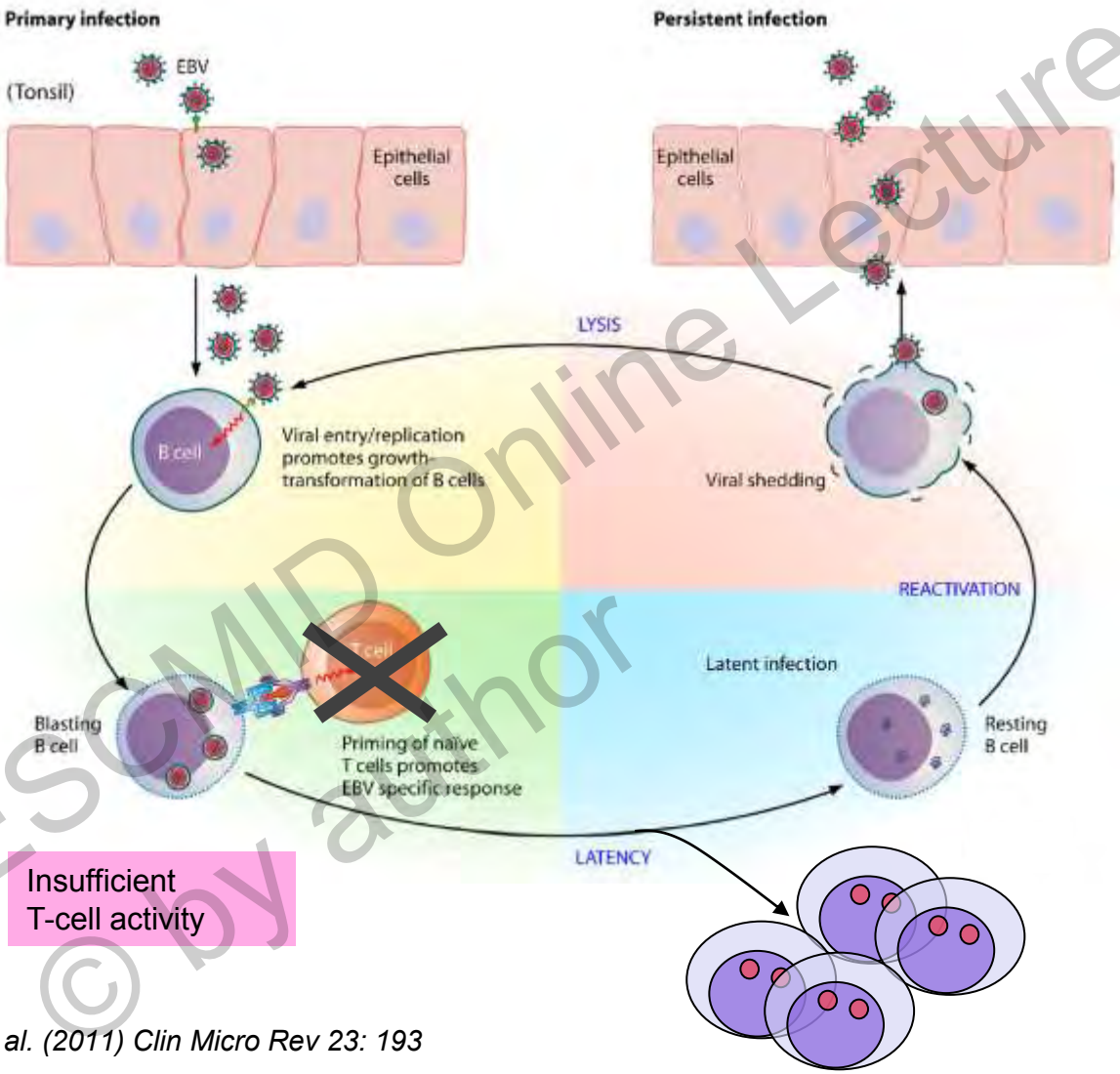
EBV-PTLD

Transplant	Percentage
Kidney	1.0
Liver	2.2
Heart	3.4
Lung	1 – 8
Heart-lung	9.4
Intestinal	7 – 11
Multivisceral	13 – 33
HSCT	1 - 24

Preiksaitis et al. 2004 Clin Inf Dis 39: 1016
Lundgren et al. (2009) Blood 113: 4992
Gulley & Tang (2010) Clin Micro Rev 23: 350
Green & Michaels (2013) Am J Transplant 13: 41
Mynarek et al. (2013) Clin Devel Immunol: e814973

- What is the role of Epstein-Barr virus ?

EBV Infection Cycle



Insufficient T-cell activity

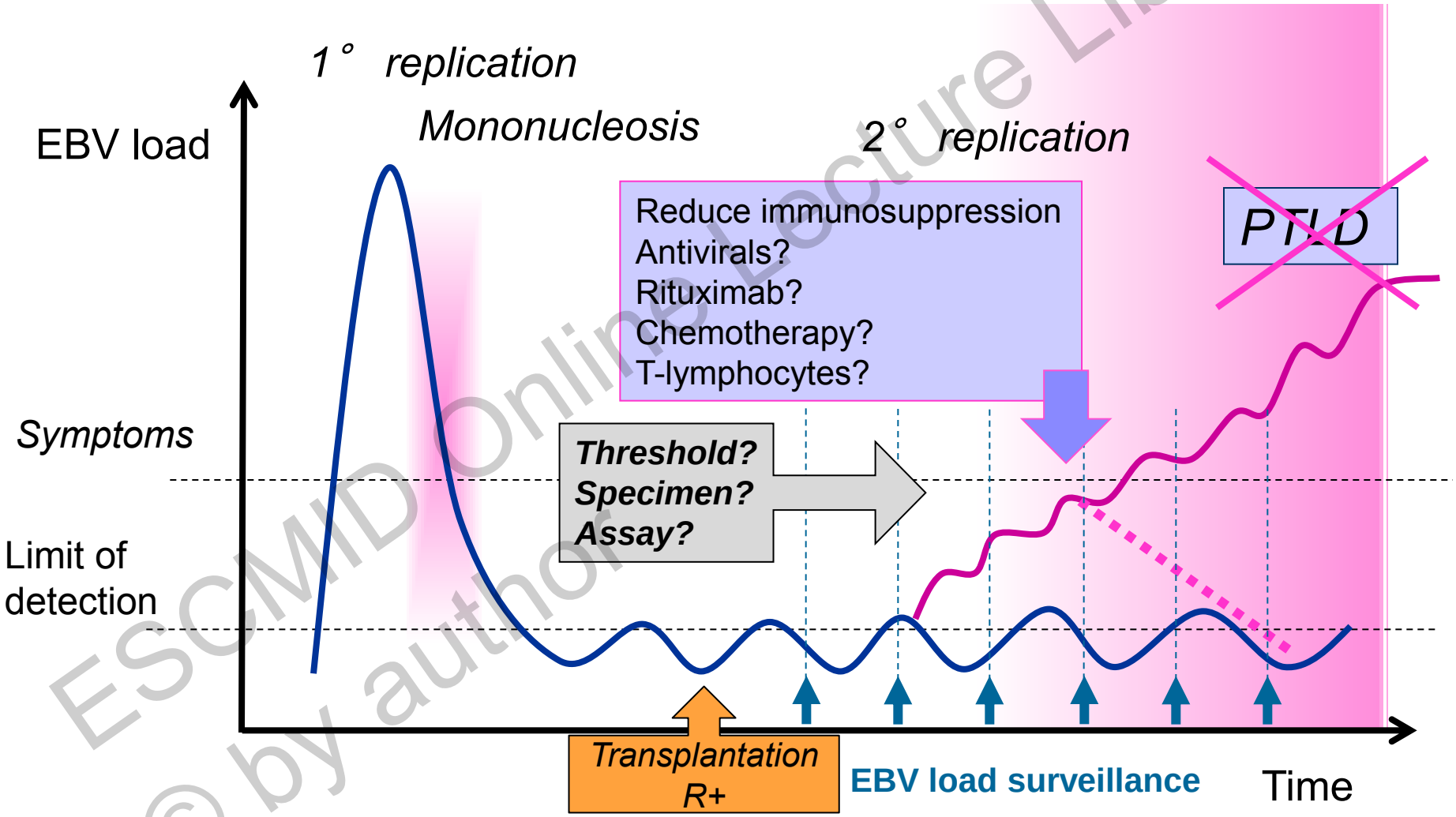
Plasma viral load
- Virion
- free DNA from lysed virions
- free DNA from lysed infected cells

Lysis from
- Lytic replication
- Killer T-cells
- Tumor growth

Cell-associated viral load
- Latent B-cells
- Latent tumor cells
- Phagocytes with debris

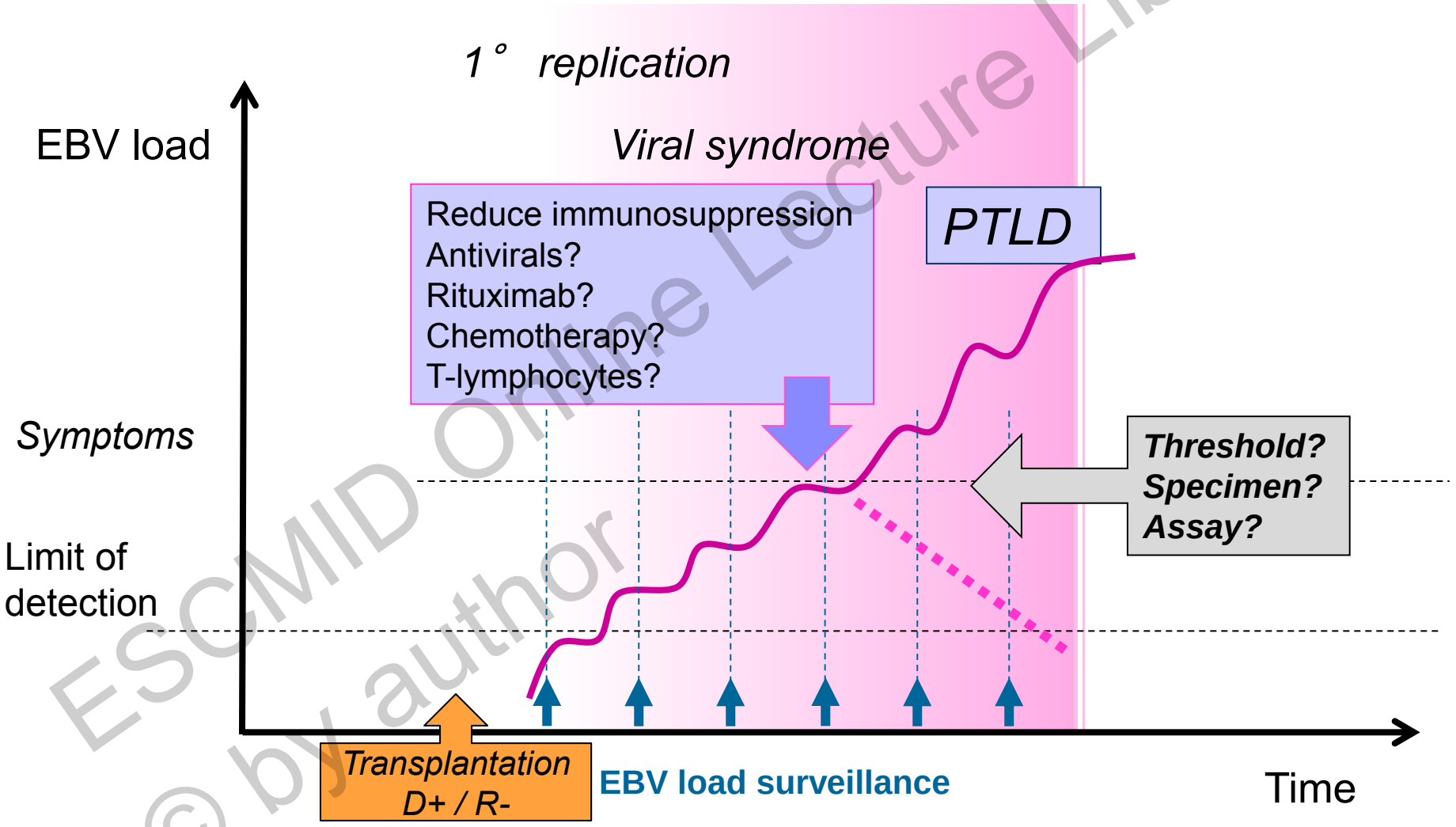
Oumade et al. (2011) Clin Micro Rev 23: 193

EBV load and PTLD in R+ Patients



Steady-state EBV load of an immunocompetent healthy EBV-seropositive individual (about 50 copies per µg DNA)

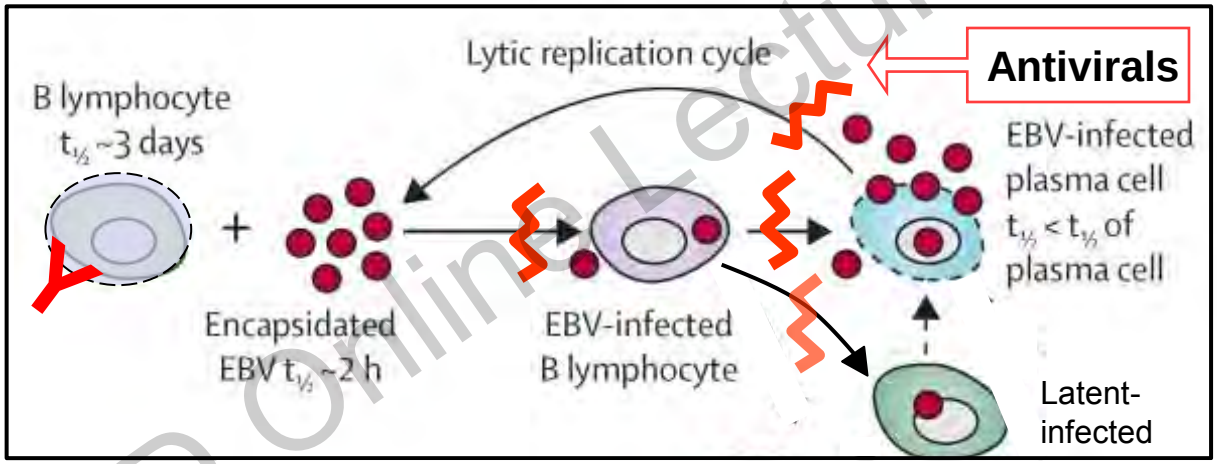
EBV High-Risk D+ / R- Transplantation

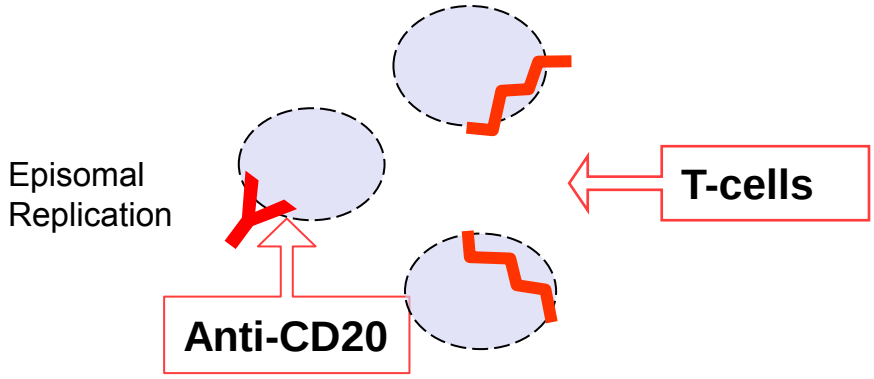


- Is there a rationale for antivirals ?

EBV Infection and Replication

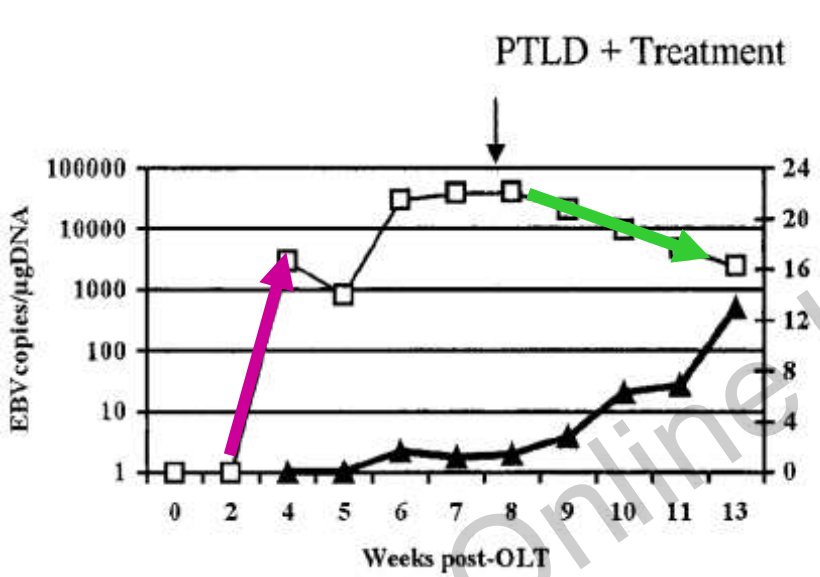
- Lytic replication (virus release, and recruiting new cells)
- Episomal replication (cell division by stimulation/transformation)

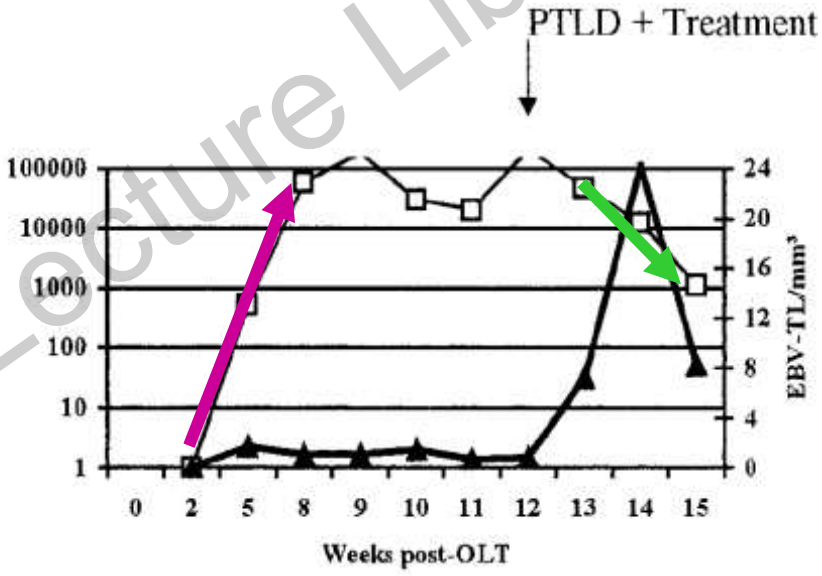




Funk, Gosert, Hirsch (2007) Lancet Inf Dis 7: 460

EBV Load Kinetics in Liver Transplants





	Doubling time	Sampling interval	Half-life	Sampling interval
Smets et al (2002),[27] liver transplanted children				
Patient 1	Approximately 2 days	Week 2–4	7 days	Week 8–10
Patient 2	≤4 days	Week 2–8	3 days	Week 12–15

Funk, Gosert, Hirsch (2007) Lancet Inf Dis7: 460

Antivirals reduce EBV-infected B-cells

- Number of EBV-infected B-cells decreases over time of valacyclovir treatment ($T_{1/2}$ 11 months; P=0.02)
  - Recruitment of host cells declines, infected B-cells turn-over
- EBV copy number per infected B-cell did not change

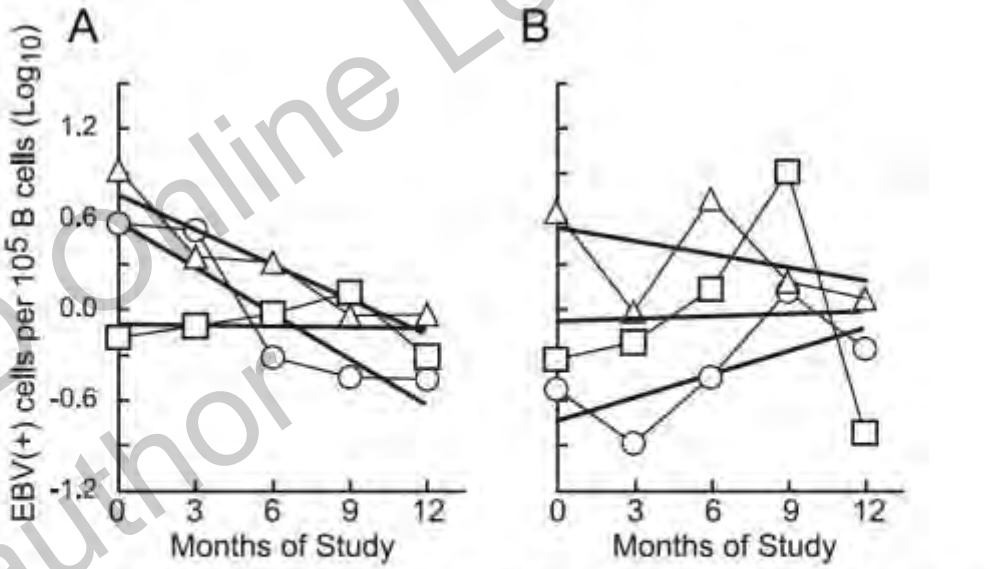
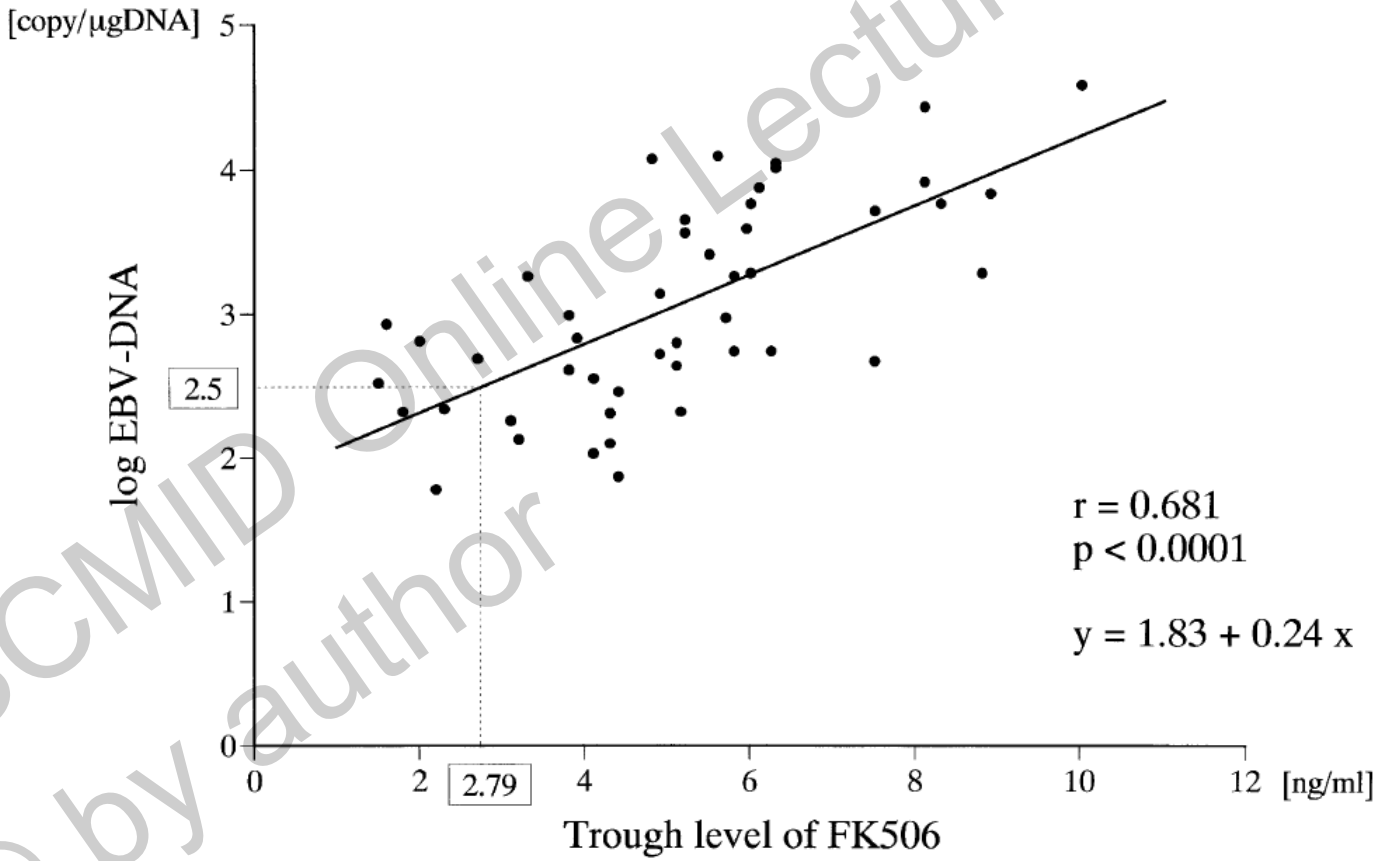


FIG. 1. EBV-positive cells per 10^5 B cells over time and linear regression lines for the first three subjects from the valacyclovir (A) and control (B) groups. Each symbol connected with a broken line represents data for one subject. Solid lines associated with symbols indicate linear regression lines of the data for each subject.

Hoshino et al (2009) J Virol 83: 11857

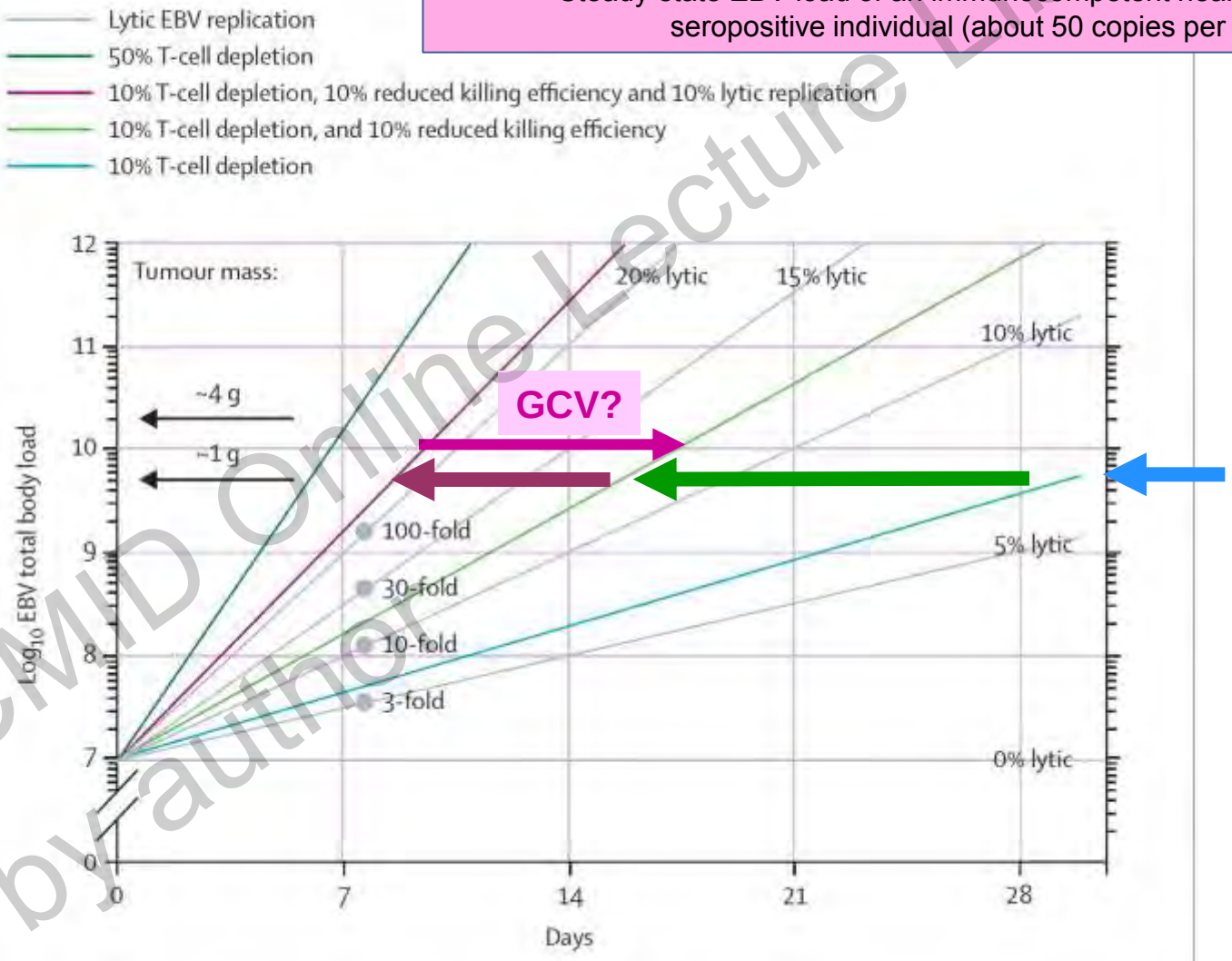
Tacrolimus increases EBV load in PBMC



Orii et al. 2002 Transpl Int 15: 529

EBV Replication and Tumor Size

Steady-state EBV load of an immunocompetent healthy EBV-seropositive individual (about 50 copies per µg DNA)



Funk, Gosert, Hirsch (2007) Lancet Inf Dis 7: 460

Rationale

- Rationale for antivirals
  - Reduce lytic EBV replication
  - Reducing recruitment of novel host cells
  - Prophylaxis in high-risk patients can reduce EBV events
  - *Antivirals cannot affect episomal replication*
- Rationale for depleting B cells (anti-CD20)
  - Removing EBV-recruitable host B-cells
  - Removing EBV-transformed B-cells
  - *Anti-CD20 cannot affect plasma cells*

- What is the clinical evidence ?

Ganciclovir is associated with reduced PTLD

- Case-control study (100 PTLD cases vs. 375 controls)
- Matching for immunosuppression, rejection, demographics

Table 4: Multivariate conditional logistic regression model: association between prophylactic anti-viral use and post-transplant lymphoproliferative disorder

Variable	Odds ratio	95% CI
Prophylactic anti-viral (none)	1.00	—
With acyclovir	0.46	0.14–1.52
With ganciclovir	0.17	0.05–0.56
With both acyclovir and ganciclovir	0.18	0.04–0.80
EBV status pre-transplant (positive)	1.00	—
Pre-transplant EBV unknown	1.82	0.54–6.10
Pre-transplant EBV negative	12.58	3.51–45.06
CMV status pre-transplant (positive)	1.00	—
Pre-transplant CMV unknown	3.77	0.73–19.38
Pre-transplant CMV negative	0.89	0.44–1.79

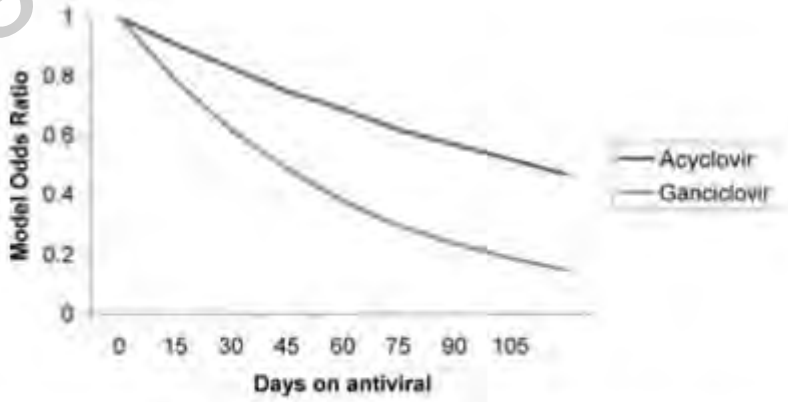


Figure 1: Risk of post-transplant lymphoproliferative disorder with days on anti-viral therapy during the first year following transplant.

Funch et al. (2005) Am J Transplant 5: 2894

Ganciclovir (+ Immunoglobulin) for EBV D+/R- SOT

- Prospective, open-label RCT (N=34; 25 children; 9 adults)
  - Kidney (n=12), liver (n=13); lung (n=8)
  - GCV+placebo vs. GCV+IVIG for 3 months, unblinded until 12 months
- PTLD rate not significantly different (N=3; 8.8%)
- EBV load

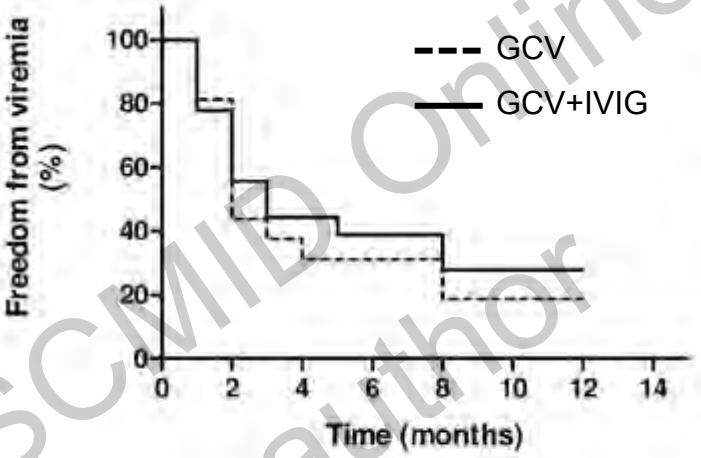


FIGURE 2. Kaplan-Meier curve showing time to EBV viremia in the two arms of the study. Solid line is ganciclovir plus intravenous immune globulin and dotted line is ganciclovir alone. Viremia is defined as any detectable viral load. $P=0.41$ log rank statistic.

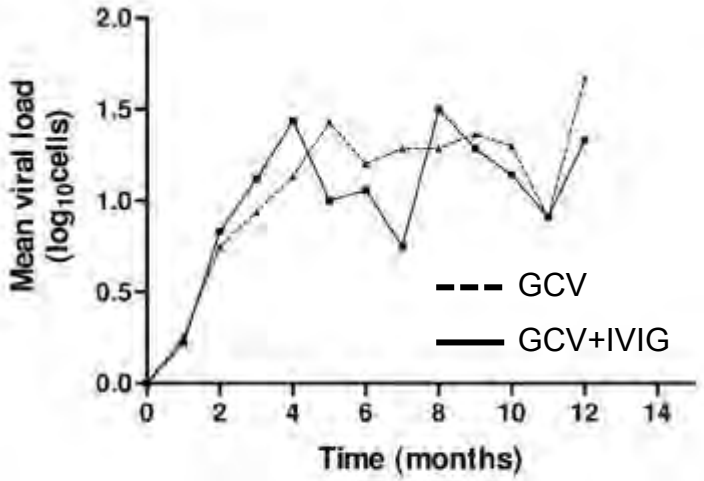
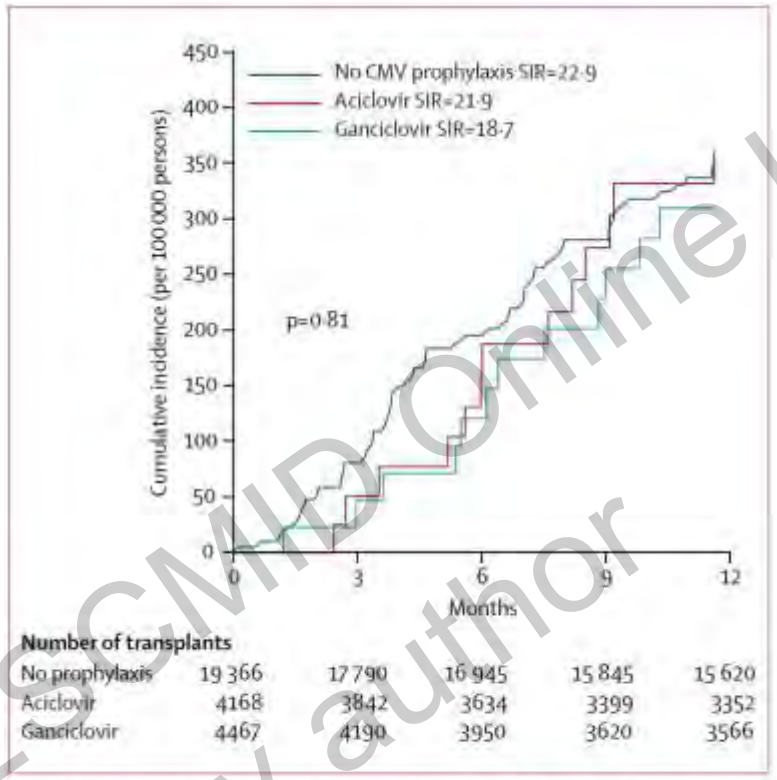


FIGURE 4. Mean EBV viral loads at monthly time points in the two arms of the study. Solid line is ganciclovir plus intravenous immune globulin and dotted line is ganciclovir alone. $P=0.80$ by repeated measures ANOVA.

Humar et al. (2006) Transplanation 81: 856

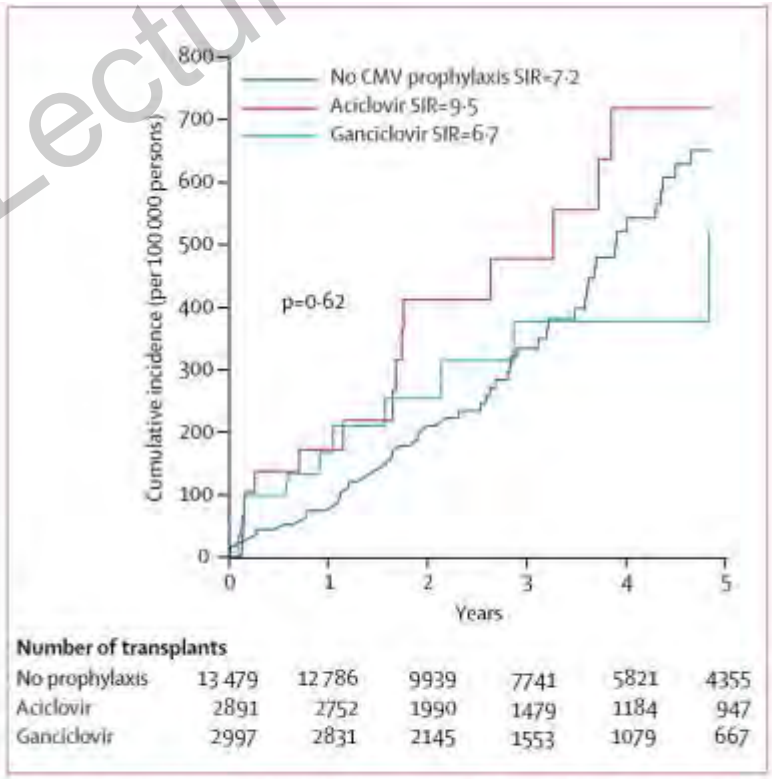
Effect of CMV Prophylaxis on PTLD

- Retrospective multicenter registry analysis (CTS)
- Deceased donor kidney transplants (N=44’838)



Number of transplants					
No prophylaxis	19 366	17 790	16 945	15 845	15 620
Aciclovir	4168	3842	3634	3399	3352
Ganciclovir	4467	4190	3950	3620	3566

Figure 3: Incidence of non-Hodgkin lymphoma in the first post-transplantation year in recipients receiving aciclovir, ganciclovir, or no CMV prophylaxis
Transplants done in 1995–2004 included in analysis.



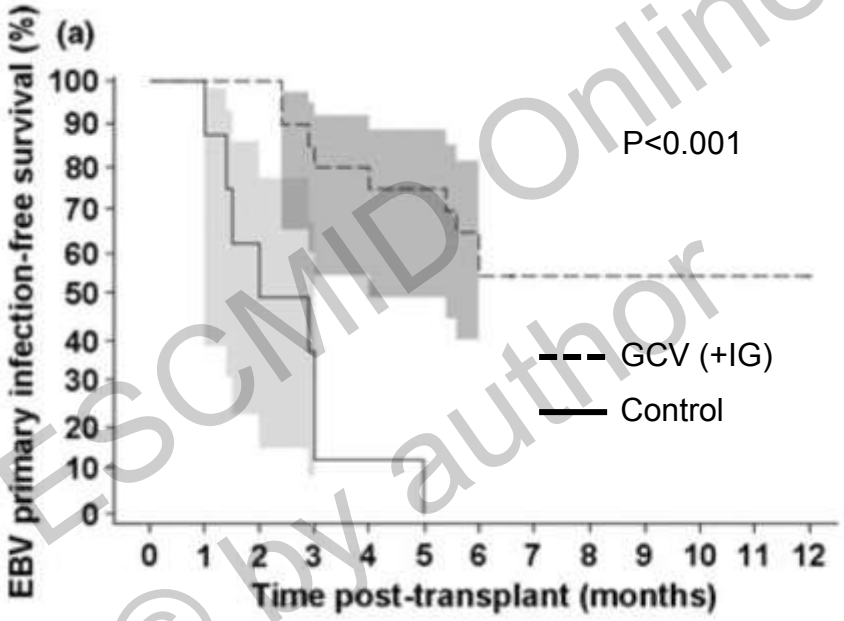
Number of transplants						
No prophylaxis	13 479	12 786	9939	7741	5821	4355
Aciclovir	2891	2752	1990	1479	1184	947
Ganciclovir	2997	2831	2145	1553	1079	667

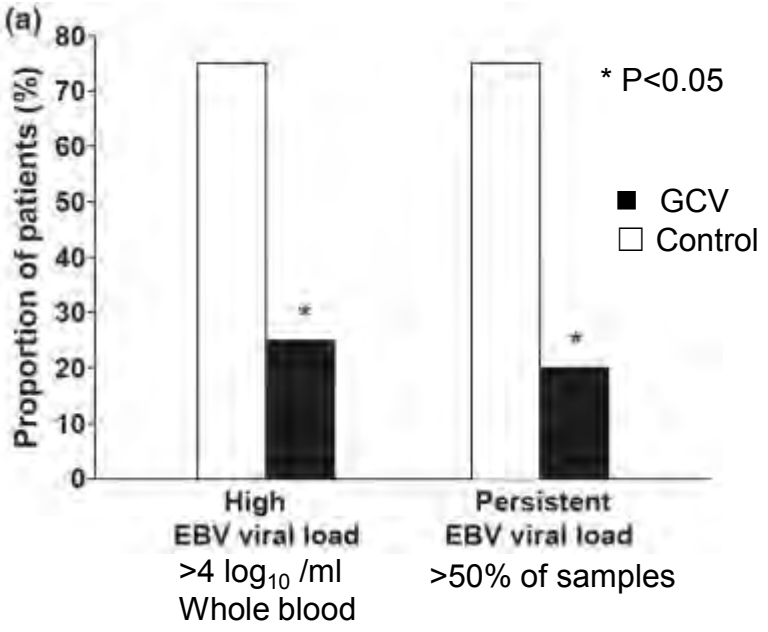
Figure 4: Incidence of non-Hodgkin lymphoma in 5-year follow-up after first post-transplantation year in recipients receiving aciclovir, ganciclovir, or no CMV prophylaxis

Opelz et al. (2007) Lancet Oncol 8: 212

(Val-)Ganciclovir for EBV D+/R- Kidney Transplantation

- Prospective pediatric cohort study of 28 from 114 patients
  - (Val-)GCV prophylaxis (N=20) vs. controls (n=8)
- Immunosuppression evaluated by Vasudev unit score
- EBV DNAemia-free survival at 5 months: 55% *vs.* 0%
- EBV-related symptoms reduced at 1 yr 25% vs. 62.5%

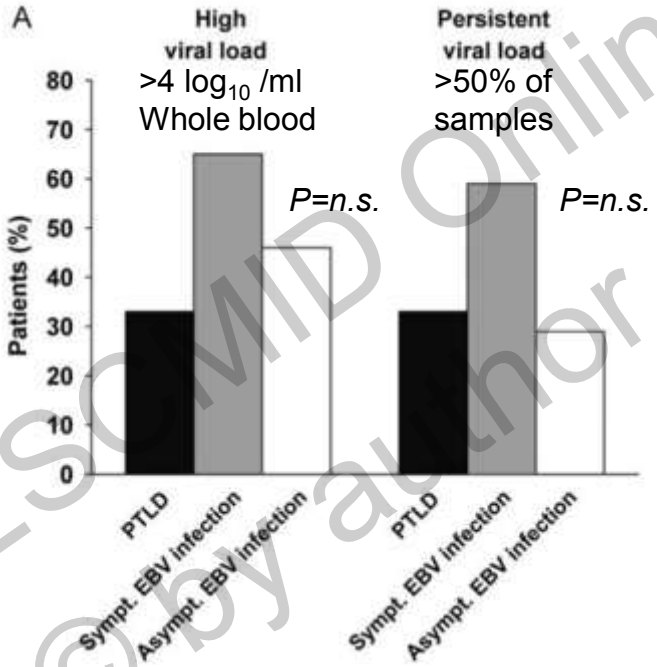


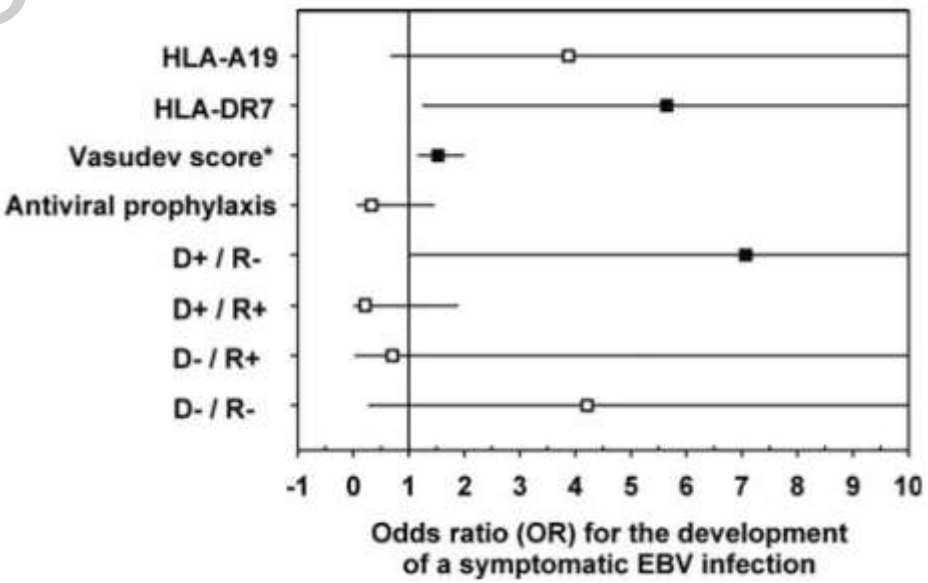


Höcker et al. (2012) Transpl Internat 25: 723

EBV Replication and Disease after Pediatric Kidney Transplantation

- Prospective cohort of 106 patients (11.4±6 yrs)
- EBV DNAemia, EBV-related symptoms (*flu* or *IM*), and PTLD
- Primary EBV infection seen in 23 (63%) of 43 R- patients
- No relationship between viral load and morbidity





Höcker et al. (2013) Clin Infect Dis 56: 84

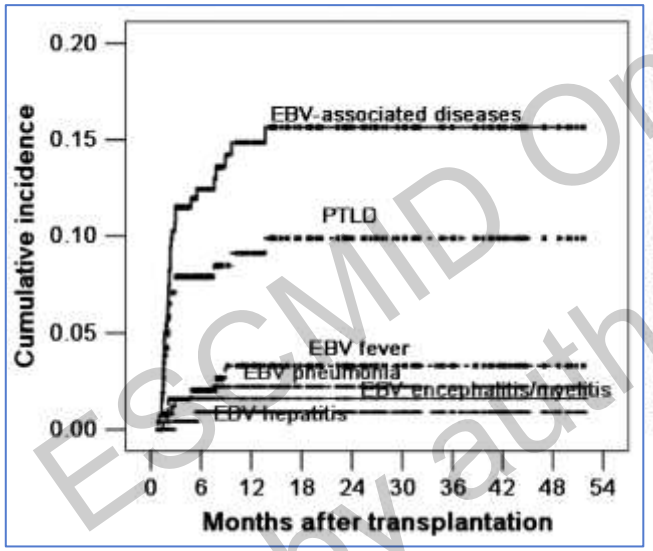
EBV Load Monitoring and Rituximab

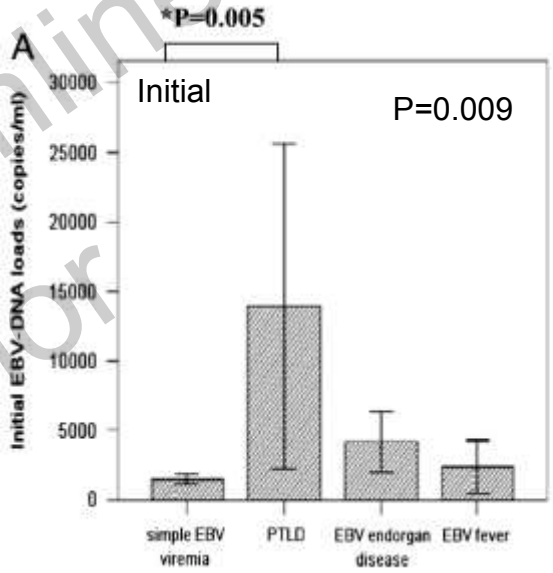
- Prospective monitoring of EBV D+/R- kidney transplant recipients
  - 34 (7.8%) of 437 patients in 2007 – 2009 (ATG induction in 97%)
  - CsA + Sir (77%); SIR + MYC (11%)
  - (Val-)Ganciclovir prophylaxis for 3 months
  - EBV load monitoring bi-weekly for 6 months
- Viremia in 20 (61%) at 124 days posttx (27 – 346)
  - In 11 (55%) patients under GCV prophylaxis
  - Symptoms in 10 (30%)
- Reduction of immunosuppression + preemptive Rituximab
  - Clearance of EBV viremia in 5/6 patients after 1x dose
  - No recurrence, no PTLD
- No rituximab
  - No clearance of viremia in 14 patients; 4 cases of PTLD

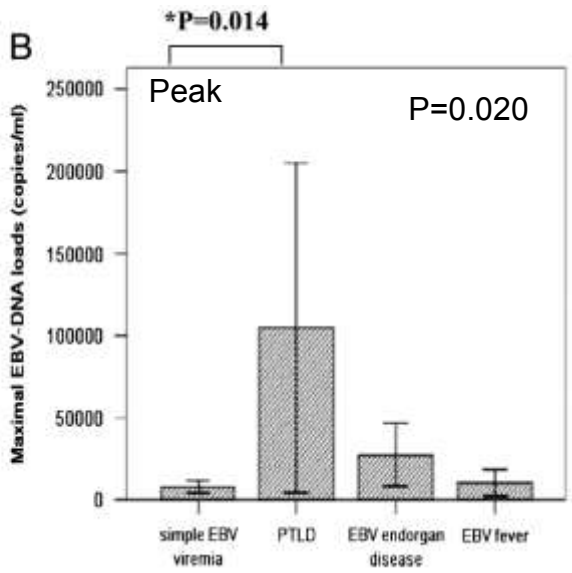
Martin et al. (2011) Am J Transplant 11: 1058

EBV Replication-associated Disease after allo-HSCT

- Prospective cohort of 263 patients (26 yrs, range 11-63)
  - Acute leukemia (N=197); CML (N=44); lymphoma (N=10)
- EBV viremia (N=77); EBV disease (N=36)
- ATG is an independent risk factor for PTLD and EBV disease







Xuan et al. (2013) Transplantation 96: 560

Treatment and Outcome of EBV Diseases in HSCT

- Prospective cohort of 263 patients (26 yrs, range 11-63)
  - Acute leukemia (N=197); CML (N=44); lymphoma (N=10)
- Persistent EBV viremia treatment
  - GCV 10mg/kg/day, reduced immunosuppression (RI)
  - Rituximab (R), R-COP

TABLE 3. Treatment and outcome in patients with EBV-associated diseases (excluding the 6 patients without use of rituximab)

	Total (cases)	RI	R	R-based chemotherapy[a]	Therapeutic DLI/CTL	Overall response	Infections arising after R treatment within 6 months	Outcome
PTLD	17	10	5	12	3 DLI, 2 CTL	16 CR, 1 NR	3 viral, 1 bacterial, and 1 tuberculosis infections	10 survived, 7 died (2 died of PTLD,[b] 3 infection, 1 aGVHD, and 1 leukemia relapse)
PTLD accompanied by EBV pneumonia	1	1	1	0	1 DLI	1 NR	1 mixed infection (bacteria and fungi)	1 died of EBV-associated disease
EBV-associated other diseases	12	6	12	0	0	10 CR, 2 NR	1 mixed (bacteria and fungi) and 1 viral infection	7 survived, 5 died (2 died of EBV-associated diseases, 2 aGVHD, and 1 infection)
EBV fever	7	3	7	0	0	7 CR	0	7 survived
EBV end-organ diseases	5	3	5	0	0	3 CR, 2 NR	1 mixed (bacteria and fungi) and 1 viral infection	5 died (2 died of EBV-associated diseases, 2 aGVHD, and 1 infection)

[a] Included 7 patients with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) and 5 with R-COP (rituximab, cyclophosphamide, vincristine, and prednisone).
[b] Included one patient who died of PTLD relapse.
R, rituximab.

Xuan et al. (2013) Transplantation 96: 560

- What should we do now ?

Guidelines

- Use of antivirals for prophylaxis or preemptive treatment of PTLD is not recommended (*A II* or *BIII*)
- Use of rituximab for prophylaxis or preemptive treatment of PTLD is not recommended (*AII* or *BIII*)

- *Some experts* would consider the use of these antivirals as an adjunct to reducing immunosuppression in order to reduce *de novo* infection and recruitment of B-cells into PTLD
- Unlike reducing immunosuppression, a partial response to antivirals should be observable with 1-2 weeks, and their use be abandoned if that is not observed.

Green & Michaels (2013) Am J Transplant 13: 41
Allen & Preiksaitis AST IDCOP (2013) AmJ Transplant 13: 107
SanJuan et al. & ESGICH (2014) Clin Microbiol Infect: (Epub)
Styczynski et al. ECIL-4 Guidelines (2014) http://www.ebmt.org/Contents/Resources/Library/ECIL/Pages/ECIL.aspx

Antivirals and Rituximab for Prophylaxis of PTLD?

Prophylaxis

- No quality *Level I* evidence from proper RCT
- *Level II* cohort and observational studies have different confounders
- For high-risk patients, (Val-)ganciclovir prophylaxis for 3 – 6 months should be considered
  - EBV D+/R- SOT (especially pediatric patients)
  - Profound T-cell depletion, lung and intestinal transplants
  - *Integrate information on CMV status, rejection, toxicity*
- Surveillance of EBV load in blood with decreasing frequency posttransplant
  - Bi-weekly until month 3; monthly until month 6 – 12
  - Patients with flu-like or mononucelosis-like symptoms

Antivirals and Rituximab for Pre-emptive management of PTLD?

Preemptive management

- For patients with high-risk *and* suggestive EBV load markers
- EBV D+/R- SOT patients
  - *De novo* detection of EBV DNAemia
  - EBV load kinetics by twice weekly monitoring
  - No clinical symptoms or signs
- ➢ Reduce immunosuppression
- ➢ Consider adding (Val-)ganciclovir
- ➢ No role of rituximab

Failure of reducing immunosuppression and valganciclovir

- Virological: No effect on EBV load with 3 weeks
- Clinical: Search for or clinical symptoms and signs
- ➢ *Consider radiological work-up and "preemptive" rituximab*

- What is on the horizon ?

New Strategies for Purging Latent EBV ?

Table 3: Combination therapy approaches in the treatment of EBV malignancies.

Lytic replication inducer	Drug	Target cells	*In vivo*	Reference
DNA methylase transferase inhibitors				
5-Azacytidine	GCV and 5-bromodeoxyuridine	EBV+ and EBV- BL cells	None	Moore et al. [75]
HDAC inhibitors				
Arg-Butyrate	GCV	LCL from lung transplant recipient	Single human patient	Mentzer et al. [70]
Arg-Butyrate	GCV		10 human patients	Mentzer et al. [76]
Arg-Butyrate	GCV		15 human patients	Perrine et al. [74]
Valproic Acid*	Cisplatin, 5-FU, Gemcitabine, Doxorubicin	LCL, gastric carcinoma-EBV, NPC	SCID mice	Feng et al. [77]
Na-Butyrate	GCV	P3HR1	None	Ghosh et al. [78]
Radiation				
γ-Radiation + Na-butyrate	GCV & AZT	LCL and BL cell lines	SCID mice	Westphal et al. [71]
γ-Radiation	AZT+GCV	LCL-4A1A	Nude rats	Roychowdhury et al. [79]
B-cell receptor blockade				
Rituximab+Dexamethasone	GCV	AKATA	Nude mice	Daibata et al. [80]
Proteasome inhibitor				
Bortezomib	^{131}I-FIAU	BL cell line	SCID xenograft	Fu et al. [81]
Other				
Cis-platinum, 5-fluorouracil, Taxol	GCV	Gastric carcinoma, NPC	Nude mice	Feng et al. [82]
Gemcitabine and Doxorubicin	GCV	LCL, and BL cell lines	SCID mice	Feng et al. [83]

Ghosh, Perrine, Pfaller (2012) Adv Virol 509296

New Strategies for EBV ?

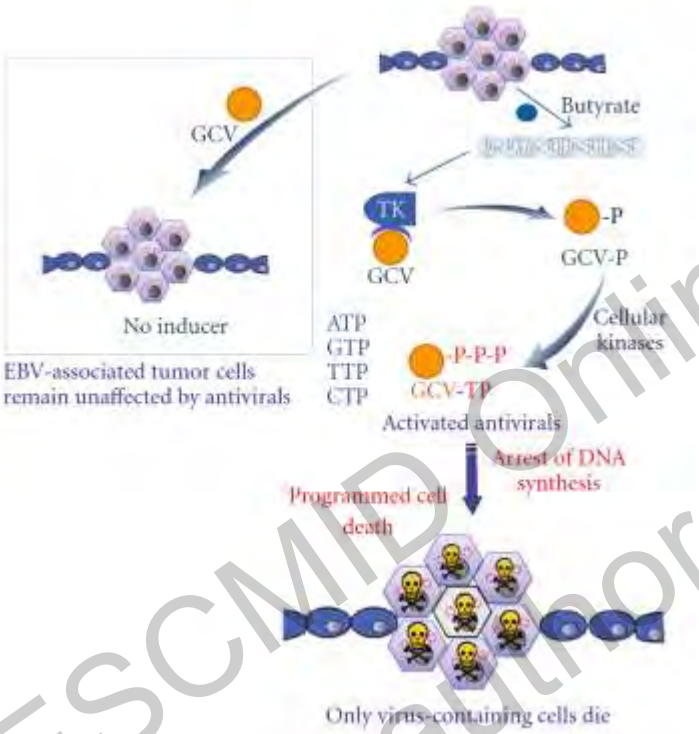


Table 2: Virus-directed novel approaches.

Classes	Comments	Reference
Targeting EBV episome	Low dose hydroxyurea treatment	Chodosh et al. [50], Slobod et al. [51]
Inhibition of EBV transforming protein	Antisense RNA against LMP-1 oncoprotein	Kenney et al. [52]
EBV-dependent expression of cellular toxins	Expression of detrimental cellular proteins through OriP dependent expression vector	Hirai et al. [53], Kenney et al. [54]
Combination therapy	Induction of EBV lytic replication + cytotoxic drugs	Numerous, Listed in Table 3

Ghosh, Perrine, Pfaller (2012) Adv Virol 509296

Thank you !

Transplantation & Clinical Virology
Tobias Bethge
Vroni DelZenero
Michela Cioni
Andrea Glaser
Rainer Gosert
Celine Leboeuf
Julia Manzetti
Min-Ji Lu
Nicole Pina
Jacqueline Samarides
Gunhild Unterstab
Fabian Weissbach
Marion Wernli
Ksenia Yakhontova

Past members
Alexis Dumoulin
Adrian Egli
Georg Funk
Helen Hachemi
Nina Khanna
Sabrina Köhli
Denise Kranz
David Leuenberger

DBM
Giulio Spagnoli
Paul Zaja
Pascal Lorentz

Swiss Transplant Cohort Study
Biocenter Proteomics Facility Timo Glatter

Nephrology USB
Jürg Steiger
Stefan Schaub
Michael Dickenmann
Michael Mayr

Pathology USB
Michael Mihatsch
Helmut Hopfer

University Hospital North Norway
Christine H. Rinaldo

University of Zürich
Walter Schaffner

Istituto Gaslini Genua, Italy
Fabrizio Ginevri

San Matteo Pavia, Italy
Patrizia Comoli

UMTC Baltimore, Md, USA
Cinthia Drachenberg
Emilio Ramos

University of Helsinki
Irmeli Lautenschlager
Klaus Hedmann

Karolinska Institutet Stockholm
Tina Dalianis

EBV Replication after Adult Kidney Transplantation

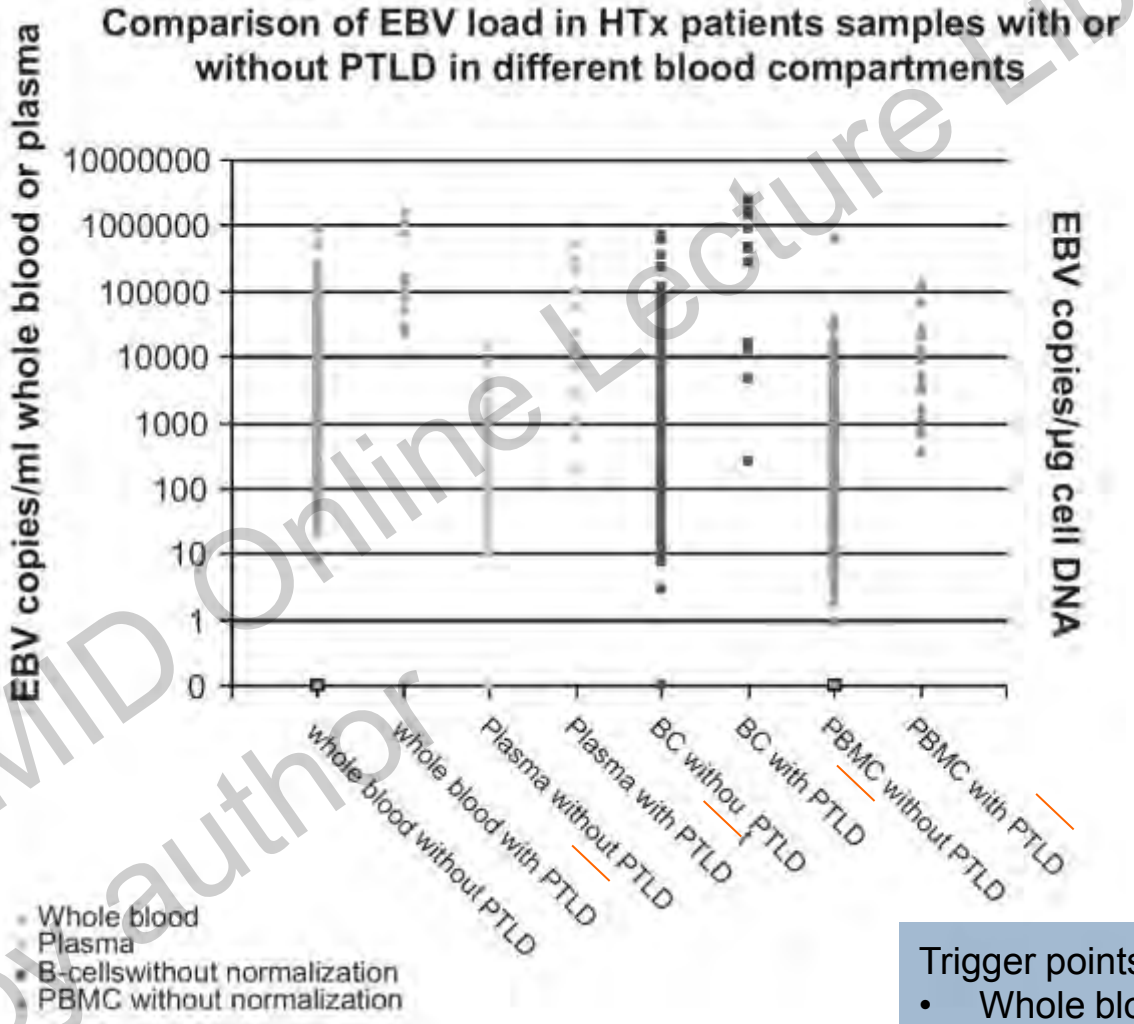
- Retrospective cohort of 383 patients (49±16 yrs)
- All on oral GCV or Val-GCV (except CMV D-/R-)
- EBV (D+/R-) developed wb-viremia 12/18 (67%) compared to R+ patients 143/365 (39%) P=0.02
- EBV replication significantly associated with graft loss

Table 4: Cox model: hazard ratio (HR) of death-censored graft loss and 95% confidence intervals (CI)

		HR	CI 95%	P
EBV infection	No	1	–	–
	Yes	1.54	1.05–5.44	0.043
Acute rejection	No	1	–	–
	Yes	1.94	1.16–3.59	0.018
Delayed graft function	No	1	–	–
	Yes	2.61	1.011–5.98	0.021
One year posttransplant creatinine clearance	≥ 50 mL/min	1	–	–
	< 50 mL/min	4.77	1.76–12.91	0.001

Bamoulid et al. (2013) Am J transplant 13: 656

EBV load in Heart Transplants



Trigger points suggested
- Whole blood >20'000 cp/mL
- Plasma >1000 cp/ml
- Serial VL Increase 20x – 50x

Ruf et al. 2012 J Clin Virol 53: 186