

CMV: An Update

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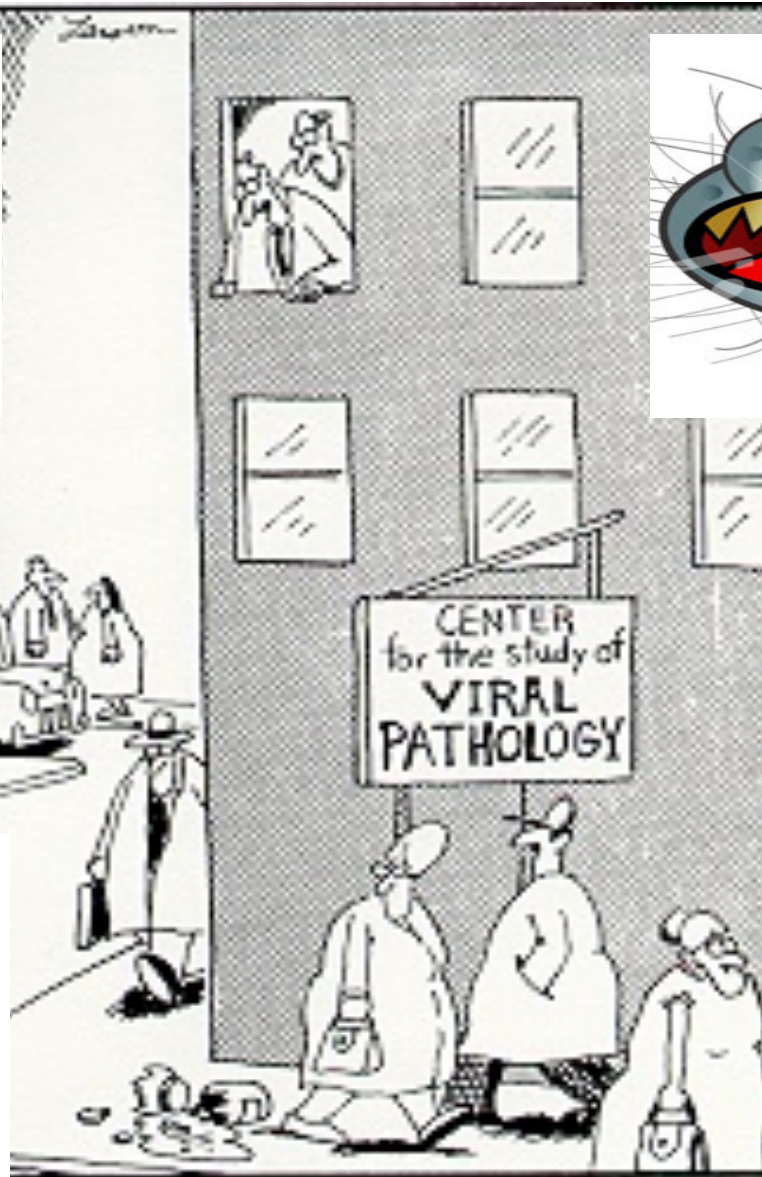
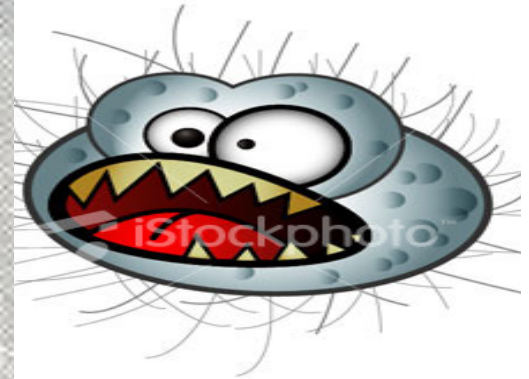


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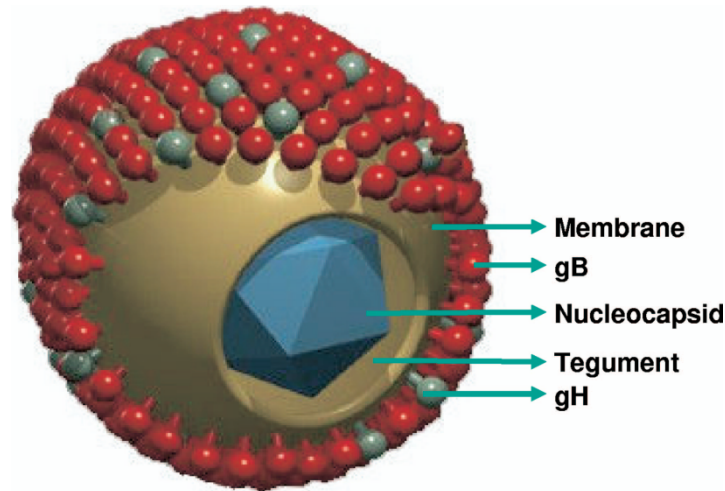
TRANSPLANT CENTER



Viruses may be dangerous



Cytomegalovirus



- *Betaherpesvirinae* subfamily of the *Herpesviridae*
- The structure :
 - Nucleus containing the viral genome (linear double-stranded DNA)
 - Icosahedral protein capsid
 - **>200 genes with significant variation**
 - The tegument protein matrix (e.g., pp65):
 - Proteins with structural roles
 - Proteins which modulates the immune host cell response
 - An outer envelope derived from the host cell nuclear membrane.
 - Glycoprotein gB - involved in cell attachment and penetration
 - Glycoprotein gH- involved in the fusion of the viral envelope with the host cell membrane

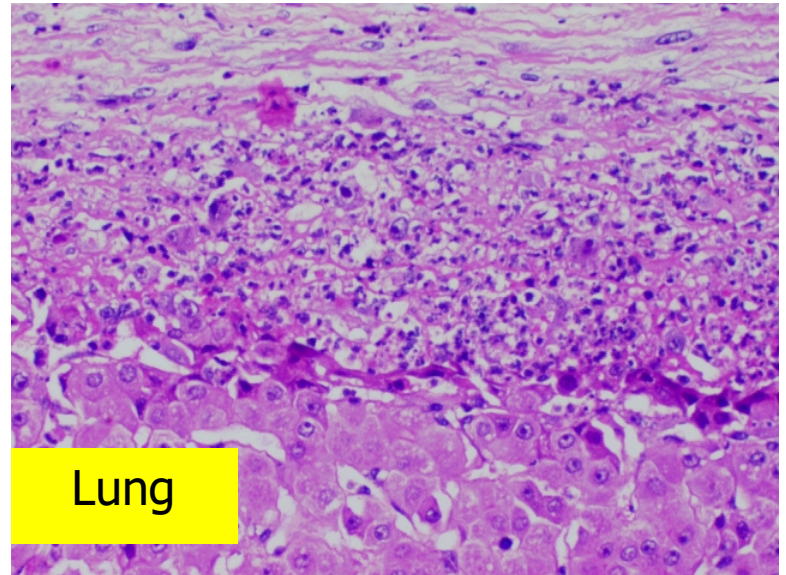
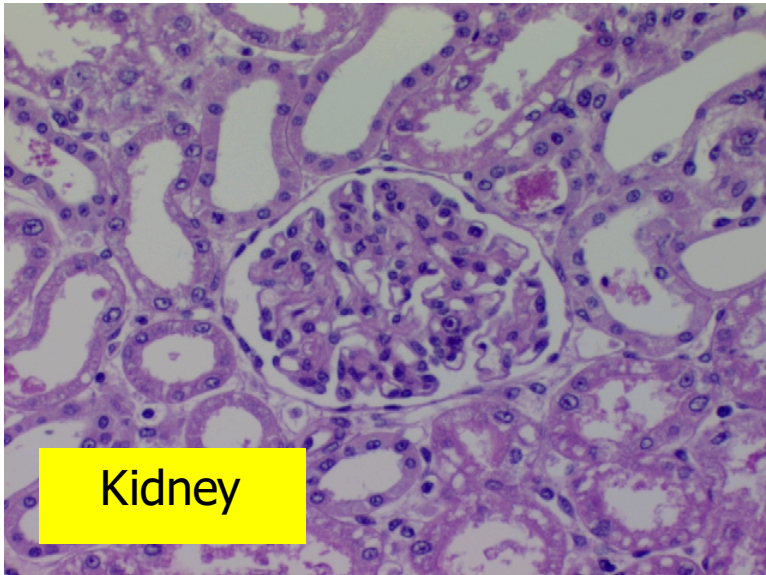
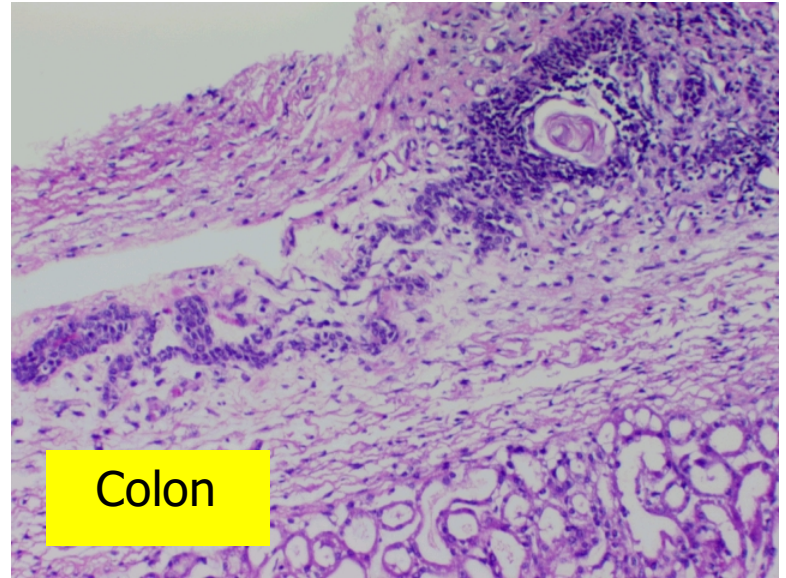
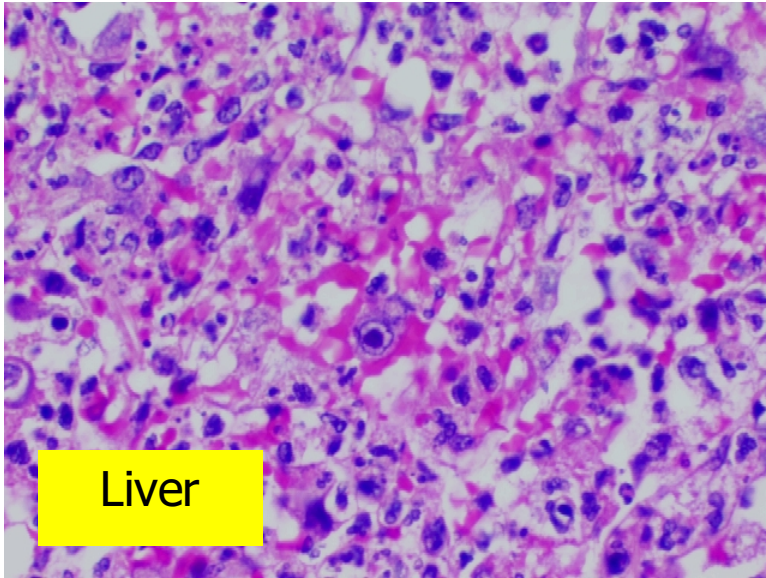
Pérez-Sola, M.J. et al. EIMC2008;26(1):38-47

Crough T et al. Clin Microb Rev, Jan 2009, 76-98

Pathogenic Considerations

- *Longer replication cycle* than other herpesviruses
- *Reactivation* of viral replication is related to host immunity and inflammation
- “CMV Infection” is not uniform
 - **Many genetic variant strains of CMV** with multiple target cells: Monocytes, macrophages, endothelial cells, epithelial cells, parenchymal cells, immature dendritic cells, lymphocytes, CD34+ cells
 - “Latency” in multiple cell types
 - **Biological effects vary by viral strain:**
 - Cellular tropism, penetration and transfer to the cell nucleus
 - **Viral gene expression is cell type-specific**
 - Replication and spread to other cells (virulence)
 - Immune responses to infection by cell type

Disseminated CMV



Risk factors for CMV disease in solid-organ transplant patients

■ **Primary infection (D+/R–)**

- Transplanted organs, cells
- Blood products

■ **Factors favoring CMV reactivation**

- Inflammation/Fever (cytokines)
- Surgery/Trauma
- Intraoperative hypothermia
- Sepsis or severe bacterial infections
- T-cell depletion
- Co-infections with other viruses
 - Herpes virus 6 or 7 (HHV6 or 7)

Factors favoring progression to invasive disease

• **Immunosuppression**

- T-cell depletion
- Mycophenolate, azathioprine
- Methylprednisolone boluses
- Alemtuzumab
- High viral load

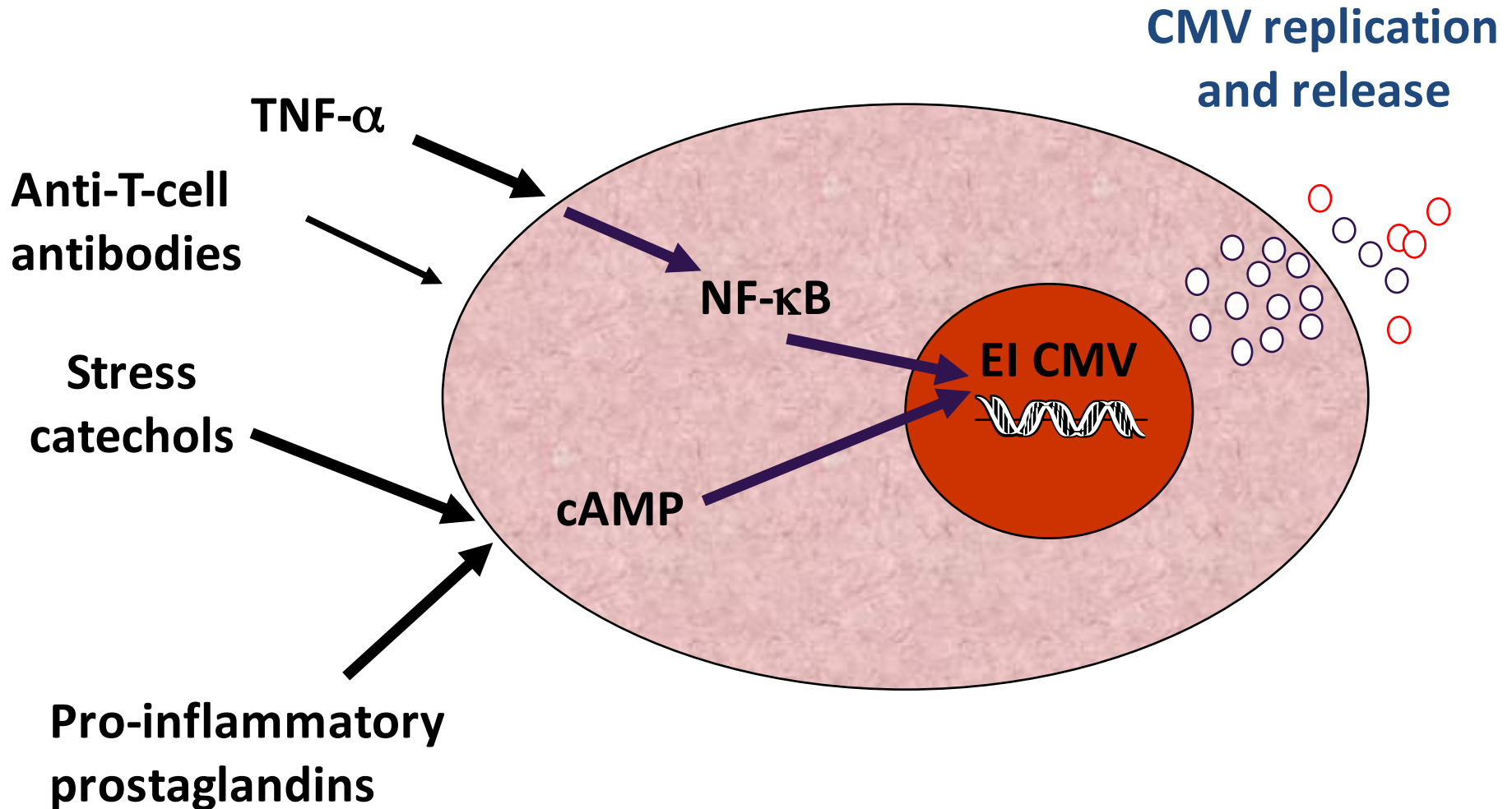
• **Immunomodulation**

- Herpes virus 6 (HHV6) or HHV7

• **Genetic factors**

- Mutations in TLR2 and TLR4 genes
- Deficiency of mannose-binding lectin or genotype associated with low production of MBL

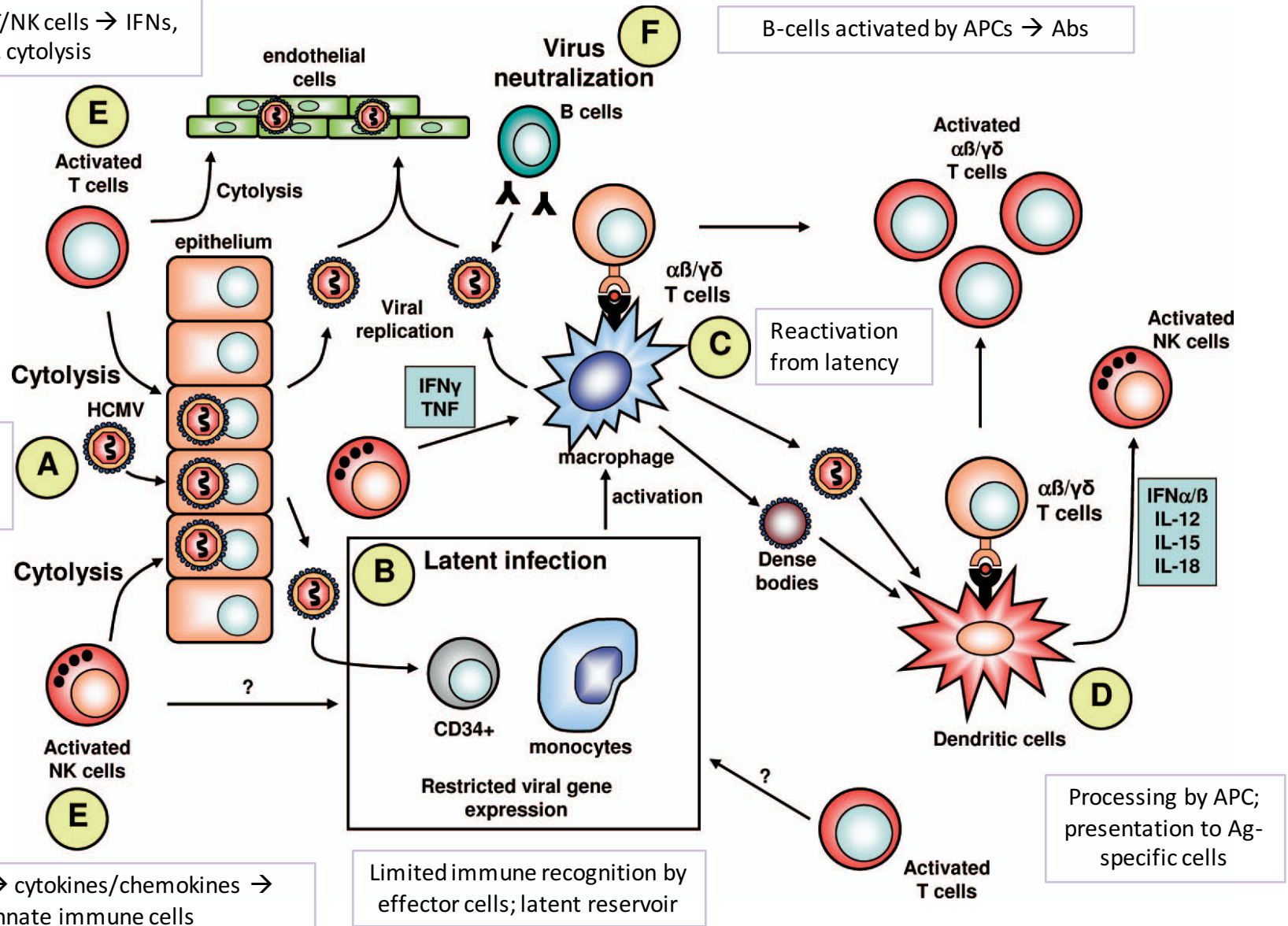
Pathways for CMV reactivation



Immune control of HCMV by innate and adaptative immunity

CMV-infected M ϕ $\rightarrow$ stimulate
Ag-specific T/NK cells $\rightarrow$ IFNs,
TNF, cytotoxicity

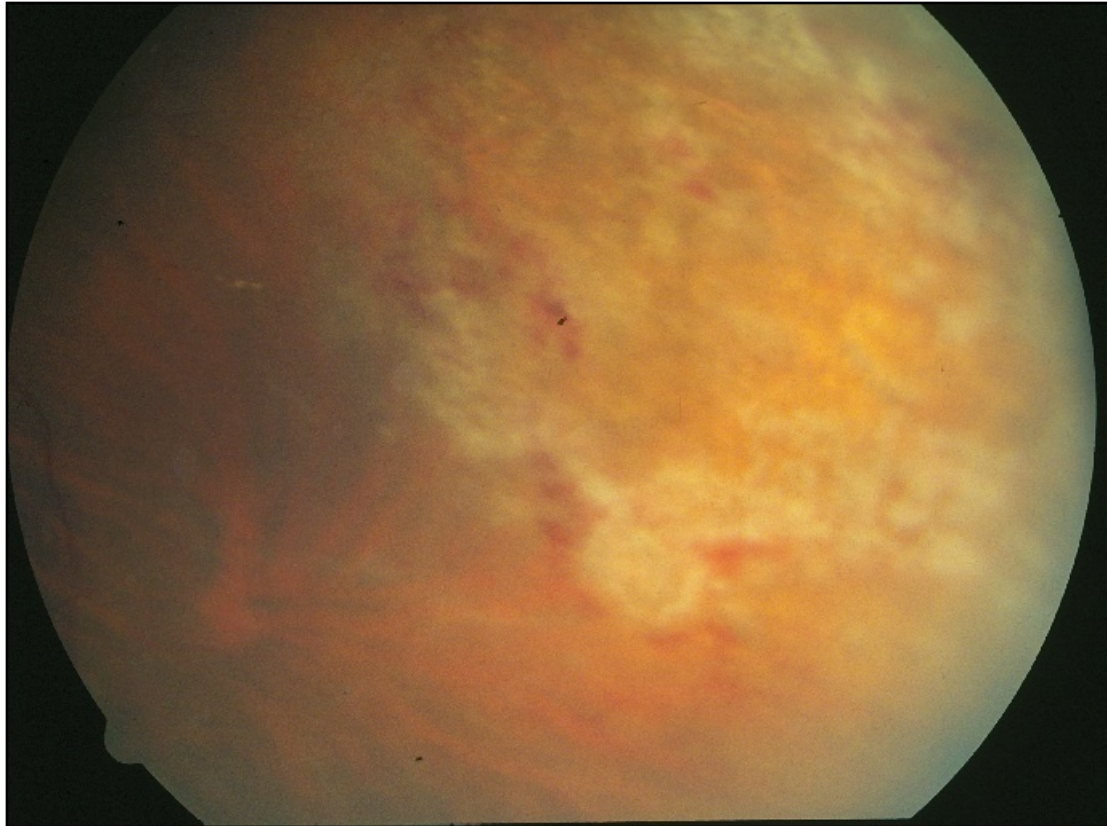
B-cells activated by APCs → Abs



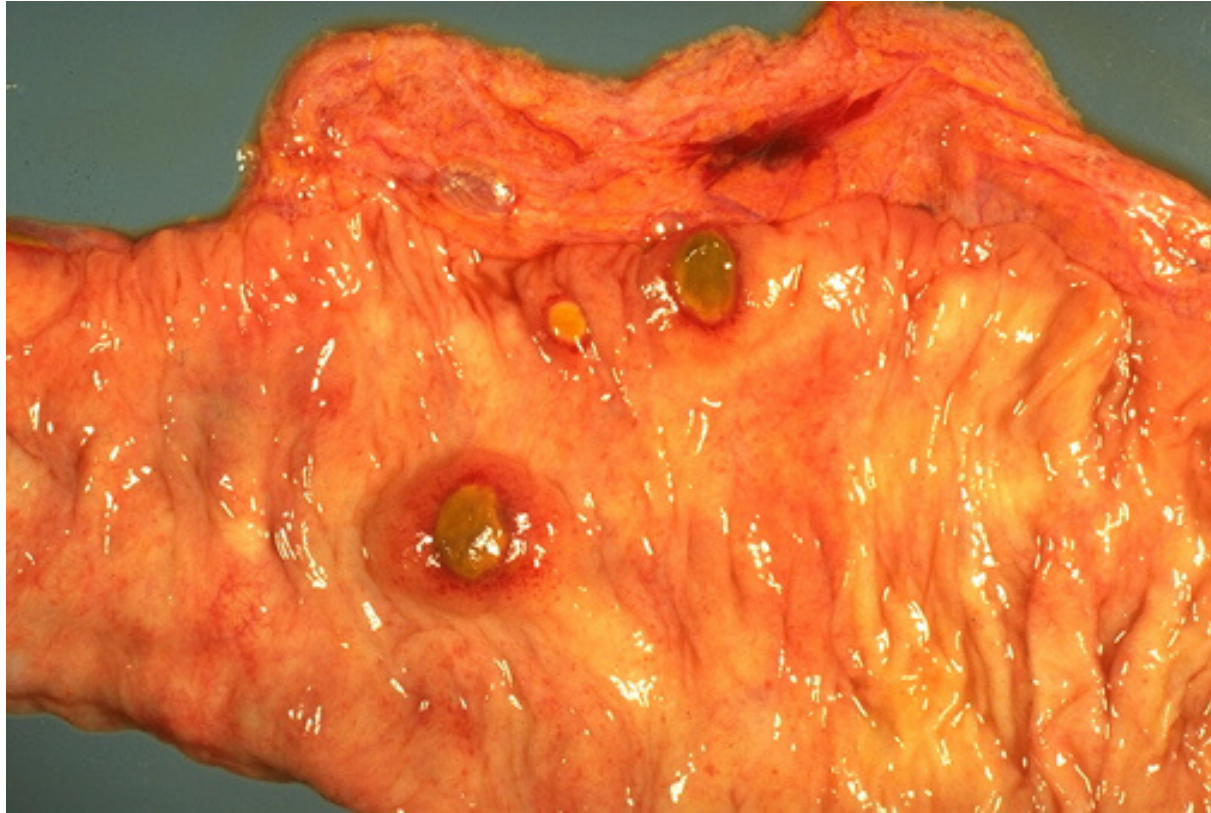
CMV affects many functions of antigen-presenting cells

1. CMV inhibits **differentiation of myeloid antigen-presenting cells** from monocytic precursors (transient block in the cytokine-induced differentiation of monocytes into functionally active CD1a-positive dendritic cells)
2. CMV alters the **immunostimulatory properties** of different subsets of monocytes and DC, rendering them less capable of developing into professional APCs.
3. **Virally encoded IL-10** (cmvIL-10) blunts the antiviral properties of APCs.
4. HCMV hampers antigen-presenting **cell trafficking and phagocytic capacity**.
 - Decreased **APC migration** to sites of inflammation and to draining lymph nodes in response to chemotactic stimuli (RANTES, MIP-1, and MIP-3)
 - HCMV-infected Plasmacytoid DCs have **reduced secretion of cytokines** including IFN- γ , **reduced allostimulation**, decreased CD4 and CD8 T-cells
5. Transitory but **substantial immunosuppression** that inhibits the immune response against the virus and unrelated pathogens, mainly in subjects with primary infection
 - Reactivation of other viral infections during acute HCMV infection
 - Enhances pre-existing immunosuppression in solid-organ transplant and alloSCT recipients, increasing their risk for invasive bacterial and fungal infections

CMV Retinitis: Lung Transplant Recipient

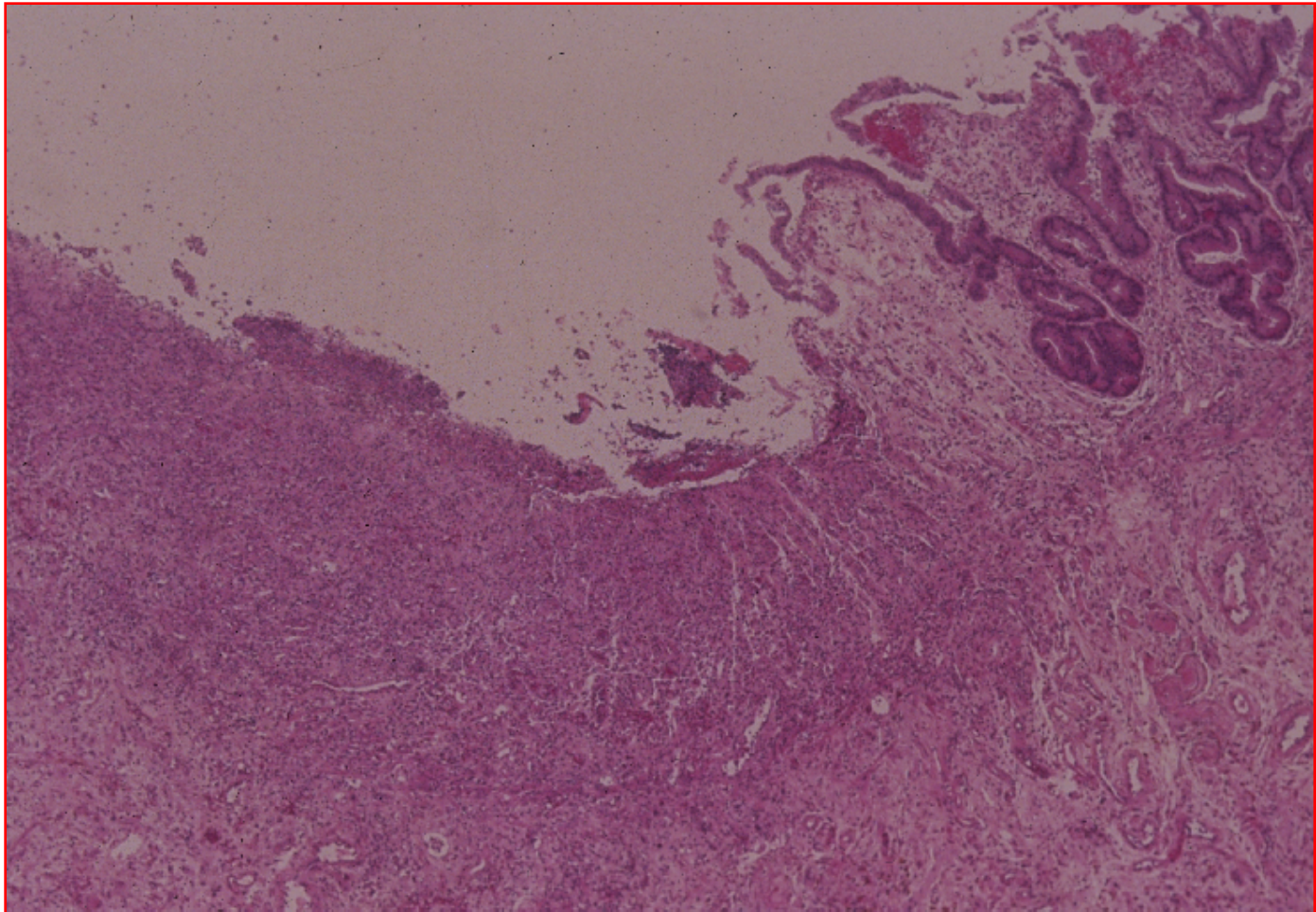


CMV cecal ulceration in patient with negative antigenemia and PCR assays for CMV



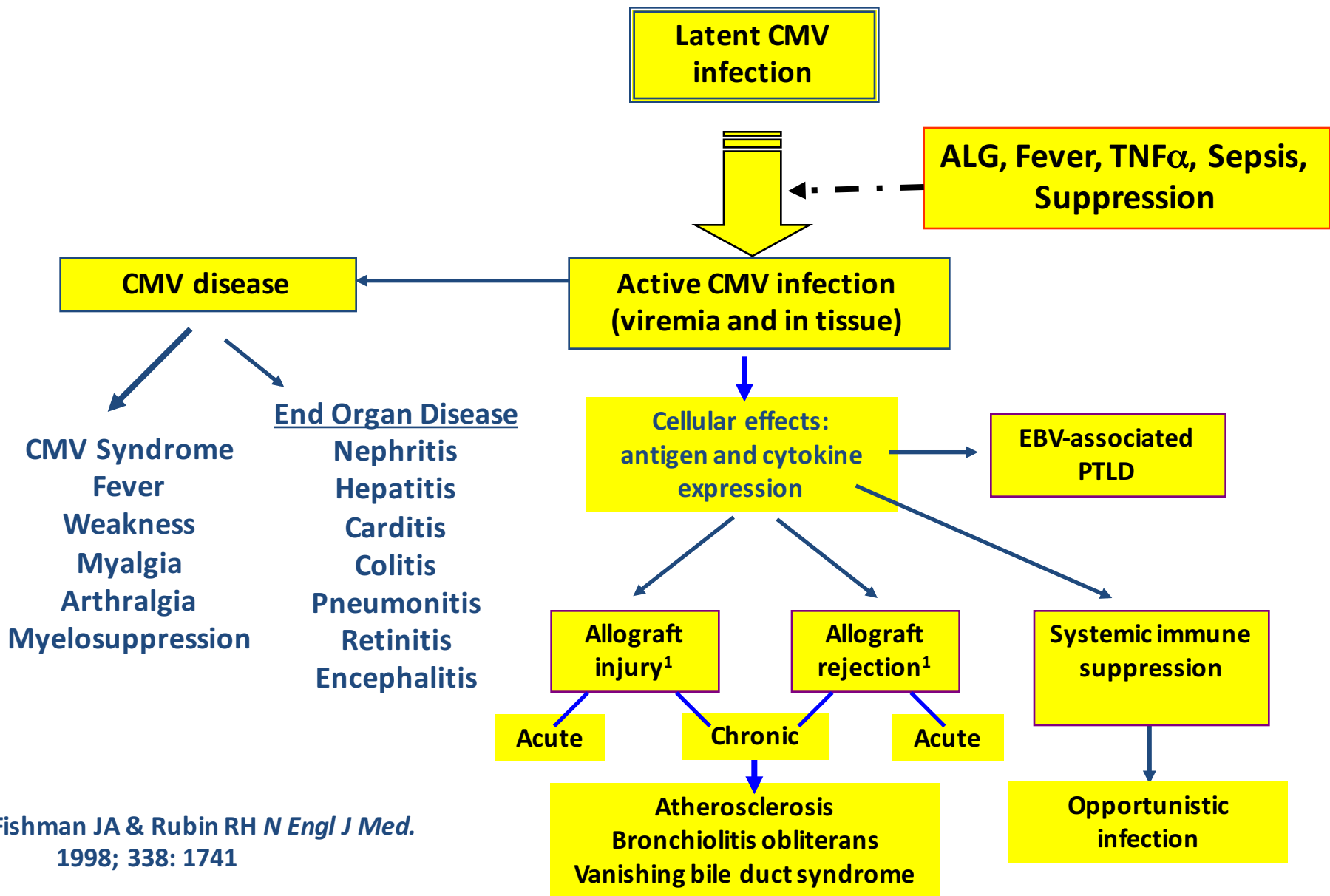
Direct Effects: Invasive Infection by CMV

Invasive Colitis After Liver Transplant



Effects of Viral Infection in Transplantation

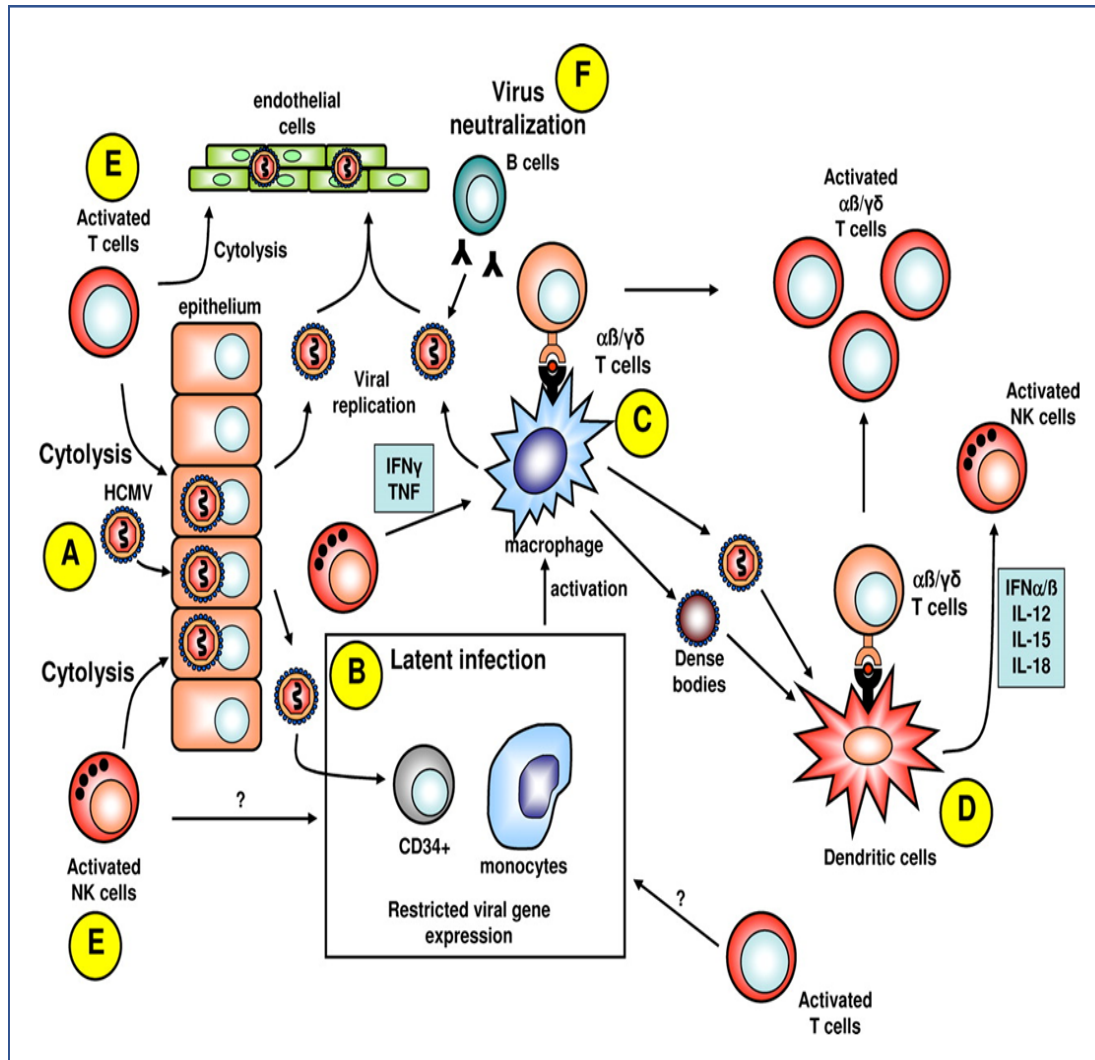
- **“DIRECT EFFECTS” -- CAUSATION OF INFECTIOUS DISEASE SYNDROMES**
 - Fever and neutropenia, hepatitis
 - Colitis, Retinitis, Nephritis, Pancreatitis
- **“INDIRECT” or IMMUNOMODULATORY EFFECTS**
 - Systemic Immune Suppression → OI's
 - Graft Rejection, GVHD
 - Abrogation Of Tolerance
- **Oncogenesis/Cellular Proliferation**
 - Hepatitis B: hepatocellular carcinoma
 - Epstein Barr Virus: B-cell lymphoma (PTLD)
 - Hepatitis C: splenic lymphoma (villous lymphocytes)
 - Papillomavirus: Squamous cell & anogenital cancer
 - HHV8 (KSHV): Kaposi's sarcoma, effusion lymphoma
 - Accelerated atherogenesis, BK-ureteric obstruction



Fishman JA & Rubin RH *N Engl J Med.*
1998; 338: 1741

HCMV Protein	Function
FC Receptor homologue TRL11/IRL11, UL118/119	Blocks antibody-dependent cytotoxicity; binding nonspecific antibody coating against CD8 and NK cells
Pp65 matrix	Phosphorylates IE-1 protein to inhibit MHC class I-restricted antigen presentation
US3,US6, US10, US11	Block generation and export of MHC class I peptides
US3,US6, US10, US11	Reduced expression of MHC class I peptides
US2	Reduced antigen presentation in MHC class II pathway
MHC-I homologue UL40, UL122 miRNA, UL142, UL141	Blocks NK cell activation (also: UL16, pp65)
UL18	MHC class 1 homologue; reduced immune surveillance
UL20	T-cell receptor homologue; reduced antigen presentation
IE86	Inactivates p53; increase smooth muscle proliferation
UL33, UL33, UL78, US27, US28	Transmembrane proteins chemokine receptors; reduced interferon and chemokine effects; reduced inflammation, increased viral dissemination
IL-10 homologue UL111a; IL8 CXC-1 UL146, UL147	Immunosuppression; reduced MHC class I/II expression and lymphocyte proliferation; increased neutrophil chemotaxis; reduced dendritic cell and monocyte chemotaxis and function
UL144	TNF receptor homologue
UL36, UL37	Anti-apoptosis for infected cells

CMV interfaces with innate and adaptive immune systems



Areas of investigation:

- Migration and chemotaxis
- Cellular differentiation
- Cell survival and apoptosis
- Determinants of reactivation and latency
- Pathogen recognition
- Global transcriptional and functional changes

CMV “Indirect Effects”: Possible Mechanisms

- Upregulation of MHC class II antigens and homology between CMV IE antigen and MHC class-I (HLA-DR β)¹⁻²
- Block of CD8+ (MHC class I) recognition
- Blocks CMV antigen processing and display (immediate early Ag modification, poor CTL response)
- Increased ICAM-1, VCAM, cellular *myc* & *fos* (*adhesion*)
- Inversion of CD4/CD8 ratio³⁻⁴
- Increased cytokines: IL-1 β , TNF α , IFN γ , IL-10, IL-4, IL-8, IL-2/IL-2R, C-X-C chemokines, and IL-8⁵⁻⁶
- Increased cytotoxic IgM⁷
- Stimulation of alloimmune response by viral proteins¹⁻²
- Increased PDGF, TGF β ; autoantibodies
- Increased granzyme B CD8+ T-cells, $\gamma\delta$ -T-cells

1. Fujinami RS, et al. *J Virol*. 1988;62:100-105.

2. Beck S. *Nature*. 1988;331:269-272.

3. Schooley RT, et al. *N Engl J Med*. 1983;308:307-313.

4. Fishman JA, et al. *Diagn Immunol*. 1983;1:261-265.

5. Kern F, et al. *J Am Soc Nephrol*. 1996;7:2476-2482.

6. Tong CY, et al. *J Med Virol*. 2001;64:29-34.

7. Baldwin WM 3rd, et al. *Br Med (Clin Res Ed)*. 1983;287:1332-1334.

ICAM-1=Intercellular adhesion molecule-1

PDGF=Platelet-derived growth factor

VCAM=Vascular cell adhesion molecule

Mechanisms – Short Version

- ↑ Adhesion molecules (VCAM, ICAM, LFA-1, VLA-4)
- ↑ Pro-inflammatory cytokines
- ↑ HLA-DR and MHC Class I mimic
- ↑ Anti-endothelial Abs?
- ↓ Antigen presentation (dendritic cell maturation)
- ↓ Leukocyte mobilization

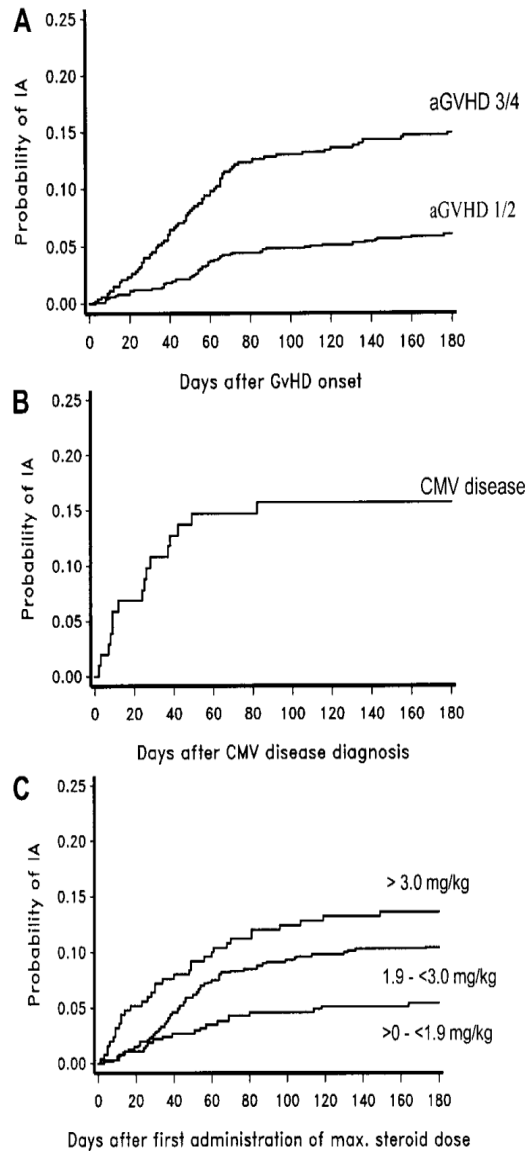
Opportunistic Infections Promoted by CMV Infection in Transplant Patients

- *Pneumocystis carinii*
- Fungal infections (esp. intra-abdominal transplants):
- *Candidemia and intra-abdominal infection* in OLTx; patients with initial poor graft function
- *Aspergillus spp.* Role of CMV in promoting fulminant HCV hepatitis rather than direct effect
- Bacteremia: *Listeria monocytogenes*
- Epstein-Barr virus infection (RC Walker et al, CID, 1995, 20:1346-55), HHV6, HHV8/KSHV?
- HCV: risk for cirrhosis, retransplantation, mortality

Indirect Effects of CMV: Organ-specific

- **Renal:** Decreased early graft function and some chronic dysfunction (increased by HHV6 and HHV7)
 - Acute but possibly not chronic allograft rejection is reduced by CMV prophylaxis
- **Liver:** CMV associated with cirrhosis, graft failure, need for retransplantation & death
 - More aggressive HCV recurrence and fibrosis after OLTx (partially attributed to HHV6)
 - CMV disease is preventable
- **Heart:** cardiac allograft vasculopathy
 - Reduced by ganciclovir +/- CMV Ig
- **Lungs:** CMV and D+/R- associated with Bronchiolitis Obliterans Syndrome, infection, death
 - Reduced by iv ganciclovir +/- CMV Ig
- **Pancreas:** Not studied (no CMV yet in islets)

CMV and HSCT Opportunistic Infections



Cause of death	D ⁺ /R ⁻ (n = 262)	D ⁻ /R ⁻ (n = 628)	P
Sepsis			
Bacterial	18 (6.9)	23 (3.7)	.037
Fungal	7 (2.7)	13 (2.1)	NS
Bacterial + fungal	25 (9.5)	36 (5.7)	.04
Invasive mold infection			
Aspergillosis	21 (8.0)	22 (3.5)	.004
Zygomycosis	2 (0.8)	2 (0.3)	NS
Fusariosis	0	1 (0.2)	NS
All invasive molds	23 (8.8)	25 (4.0)	.004
All bacterial/fungal causes of death	48 (18.3)	61 (9.7)	<.001
All viral causes of death	6 (2.3)	19 (3.0)	NS
Cytomegalovirus	1 (0.4)	1 (0.2)	NS
Other ^a	5 (1.9)	18 (2.9)	NS
Treatment-related mortality	20 (7.6)	48 (7.6)	NS
Veno-occlusive disease	5 (1.9)	10 (1.6)	NS
Diffuse alveolar damage	4 (1.5)	13 (2.1)	NS
Multiple organ failure	3 (1.1)	11 (1.8)	NS
Other ^b	8 (3.1)	14 (2.2)	NS
Severe graft-versus-host disease	12 (4.6)	22 (3.5)	NS
Relapse	34 (13.0)	78 (12.4)	NS
Unknown	5 (1.9)	19 (3.0)	NS
All deaths	125 (48)	247 (39)	.02

Therapeutics - Terminology

- What are you measuring?
 - Antigenemia – measuring CMV specific antigen in PBMCs, for example pp65 assay
 - Viremia – culture-based only (requires replicating virus)
 - **DNAemia** – measuring **CMV DNA by QNAT** (whole blood or plasma) **QNAT calibrated to the WHO standard is preferred** for diagnosis, decisions regarding preemptive antiviral treatment, and monitoring response to therapy.
 - Tissue or BAL – must be normalized to housekeeping gene, urea, unit volume, unit mass.
 - *Viral assays and culture of blood, urine, or oral secretions are discouraged in adults for diagnosis of active infection or disease.*
- Serology
 - Positive IgG is a marker of previous exposure (positive serology does not define “active infection”)

Recommendations: Diagnostics

- *Either plasma or whole blood is an acceptable specimen for QNAT.* Specimen type or assay should not be changed when monitoring patients.
- Despite reporting in IU/ml, viral load values *cannot be directly compared across centers* and/or laboratories unless identical testing reagents and procedures can be assured.
- Changes in viral load exceeding 0.5 log₁₀ IU/ml (3-fold) are considered to represent clinically significant differences in DNAemia

Terminology 2

- **Surveillance** – patient is at risk, but no evidence of an event (infection) or biomarker (NAT)
- **Monitoring** – Patient has event or biomarker (previously known positive assay)
- **Hybrid approach** – universal prophylaxis followed by preemptive therapy (surveillance after prophylaxis)

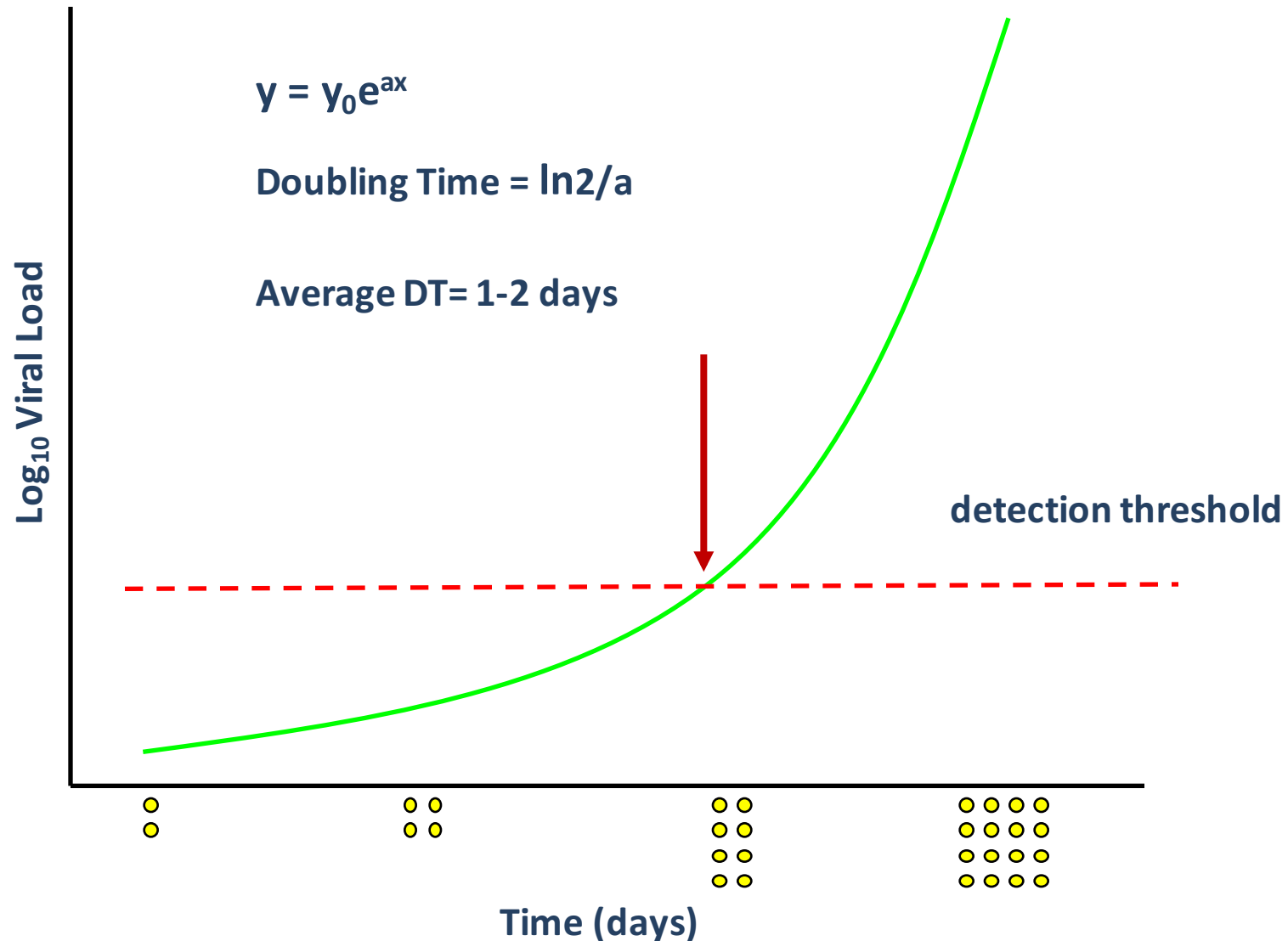
Do we know how to Prevent CMV
Infection?
Universal vs. Pre-emptive therapy

Prophylaxis vs Preemptive Therapy

	Prophylaxis	PreEmptive Therapy
Early CMV DNAemia	Rare	Common
Prevention of CMV disease	Good efficacy	Good efficacy* (*less optimal in high risk populations)
Late CMV (infection/disease)	Common	Rare
Resistance	Uncommon	Uncommon
Ease of implementation	Relatively easy	More difficult
Other herpesviruses	Prevents HSV, VZV	Does not prevent
Other Opportunistic infections	May prevent	Unknown
Cost	Drug costs	Monitoring costs
Safety	Drug side effects	Less drug toxicity
Prevention of rejection	May prevent	Unknown
Graft survival	May improve	May improve

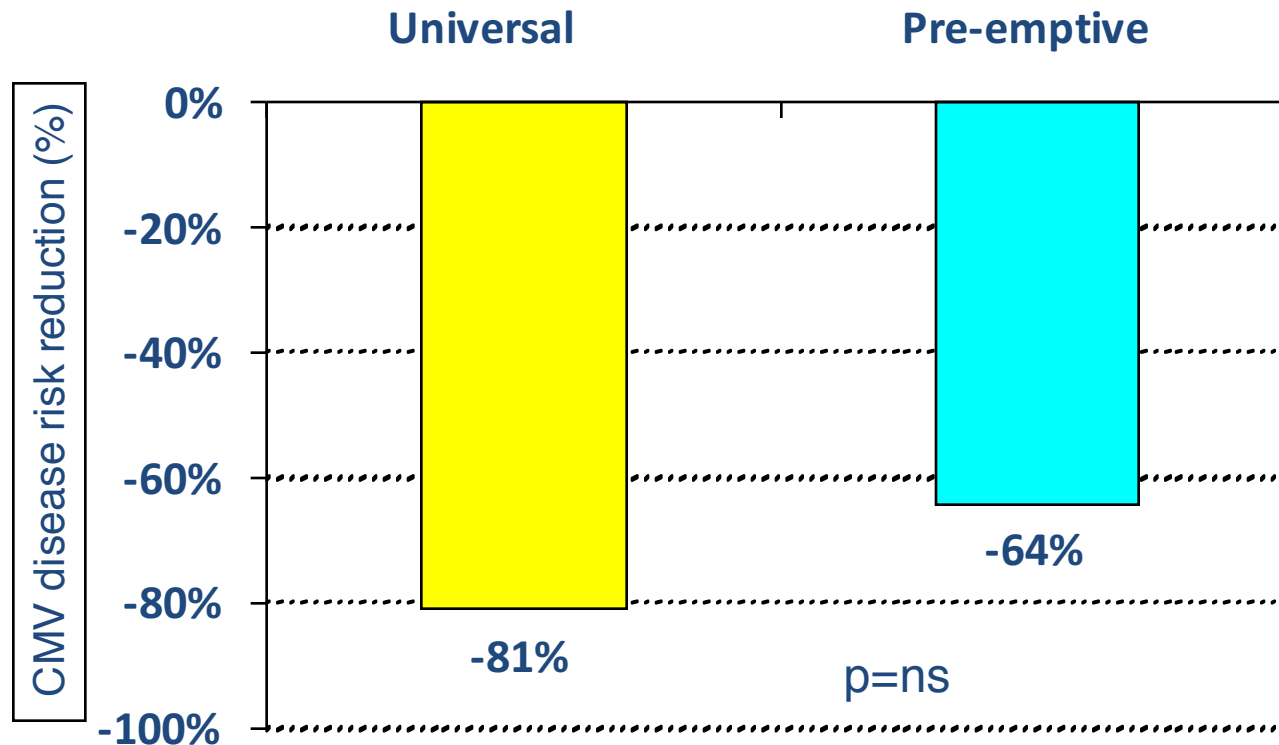
- Prophylaxis may be preferred in D+/R-, with D+ or R+ & antilymphocyte therapy, potent immunosuppression including desensitization (plasmapheresis or immunoadsorption) and ABO incompatible protocols, or with rituximab, bortezomib, eculizumab. Role with belatacept has not been studied.
- Treatment of acute rejection with antilymphocyte antibodies in at-risk recipients should result in reinitiation of prophylaxis or preemptive therapy for 1 to 3 months
- CMV DNAemia is common after cessation of prophylaxis, notably in high risk patients

Preemptive in D+/R-

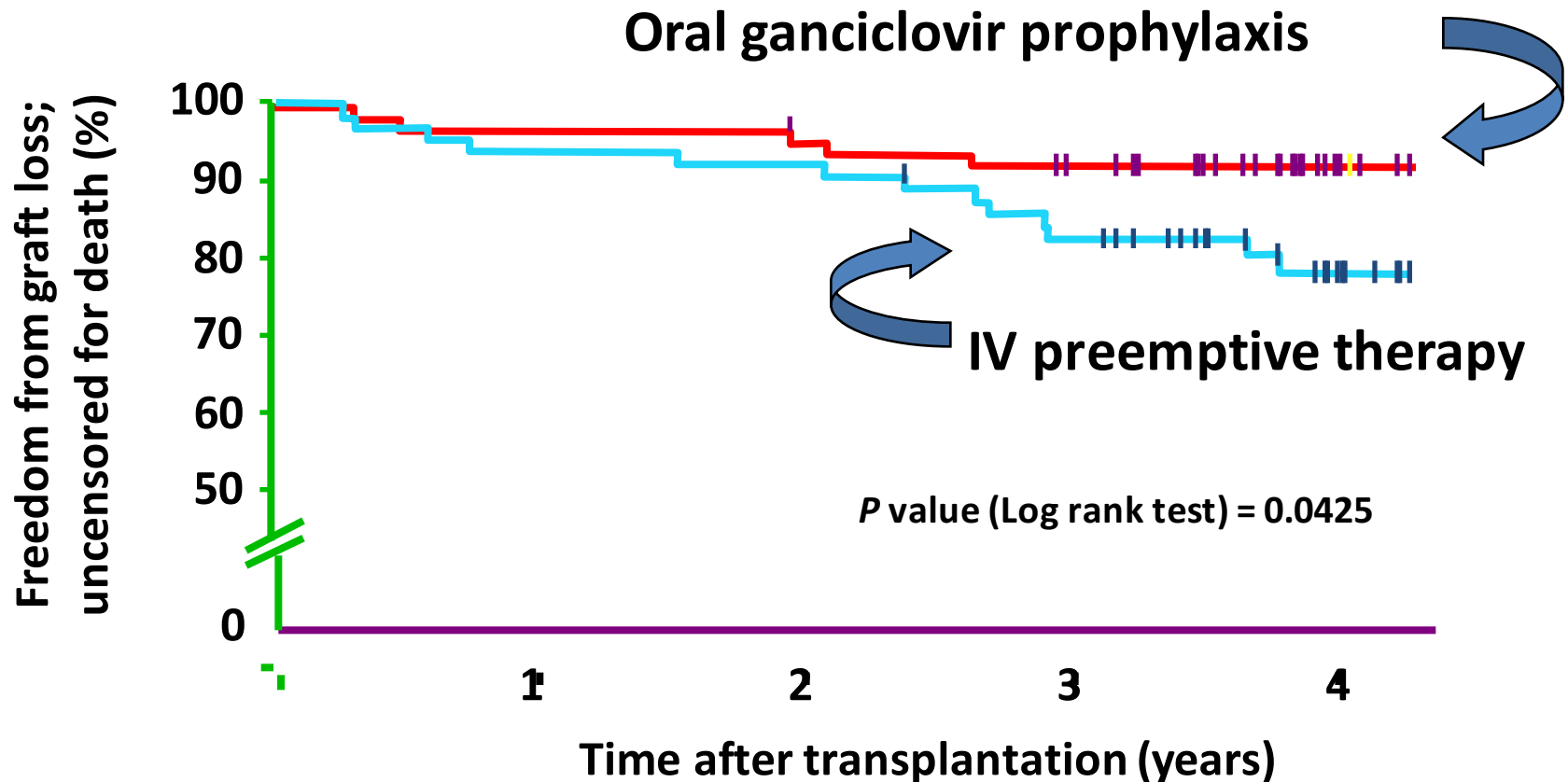


CMV disease in D+/R- renal recipients: Meta-analysis (all agents)

- Universal and Pre-emptive prophylaxis significantly reduce the risk of CMV disease



Anti-CMV Prophylaxis Is Associated With Increased Renal Graft Survival at 4 Years ($P = 0.0425$)



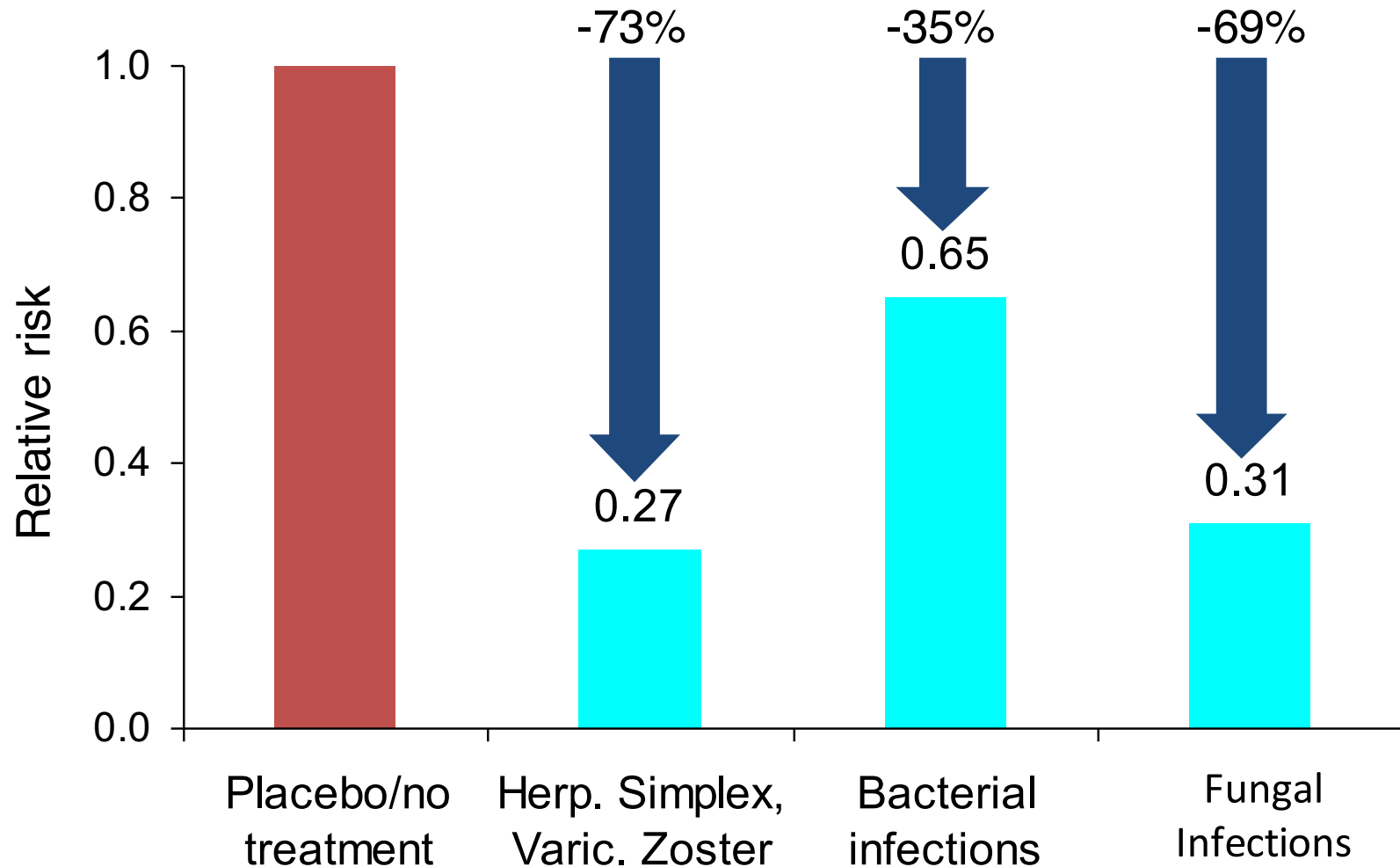
Prophylaxis reduced CMV infection by 65% ($P < 0.0001$)

Kliem V, et al. *Am J Transplant.* 2008;8:975-983. (B)

Khoury JA, et al. *Am J Transplant.* 2006;6:2134-2143. (VGCV) (B)

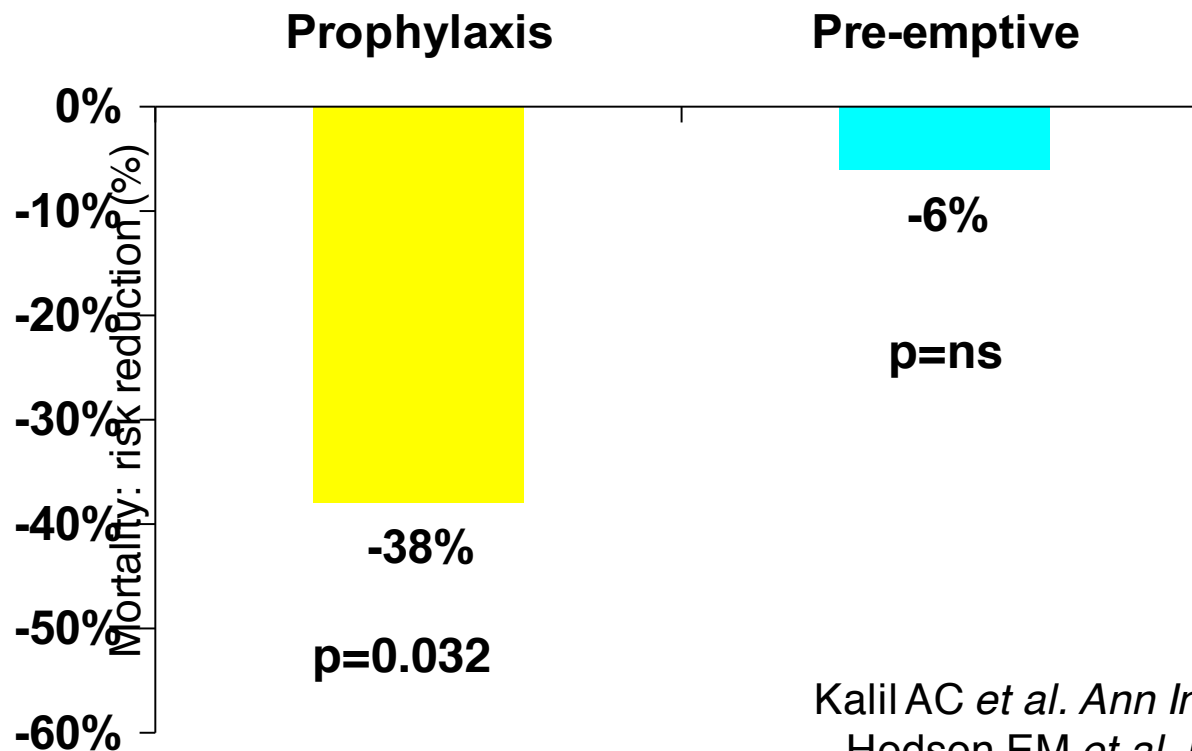
Reischig T, et al. *Am J Transplant.* 2008;8:69-77. (VACV) (B)

Effect of anti-CMV prophylaxis on concomitant infections



Mortality: universal prophylaxis vs. pre-emptive therapy

- Statistically significant risk reduction of mortality with universal prophylaxis (Kalil et al) and all cause mortality (Hodson et al).

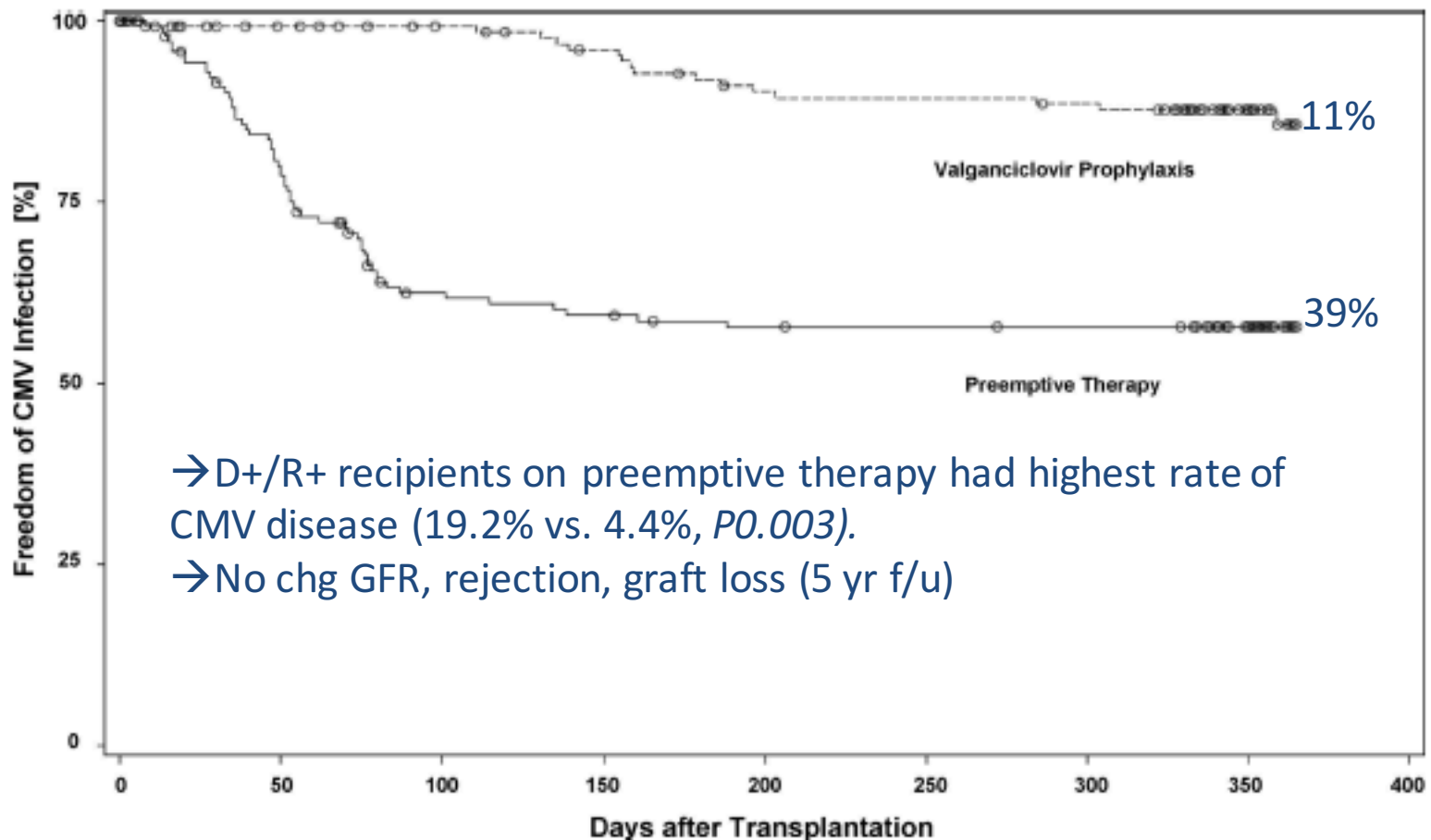


Kalil AC *et al. Ann Intern Med* 2005; 143: 870
Hodson EM *et al. Lancet* 2005; 365: 2105

Valganciclovir Prophylaxis Versus Preemptive Therapy in Cytomegalovirus-Positive Renal Allograft Recipients: 1-Year Results of a Randomized Clinical Trial

Transplantation • Volume 93, Number 1, January 15, 2012

Oliver Witzke,^{1,8} Ingeborg A. Hauser,² Michael Bartels,³ Gunter Wolf,⁴ Heiner Wolters,⁵ and Martin Nitschke;⁶ for the VIPP Study Group⁷



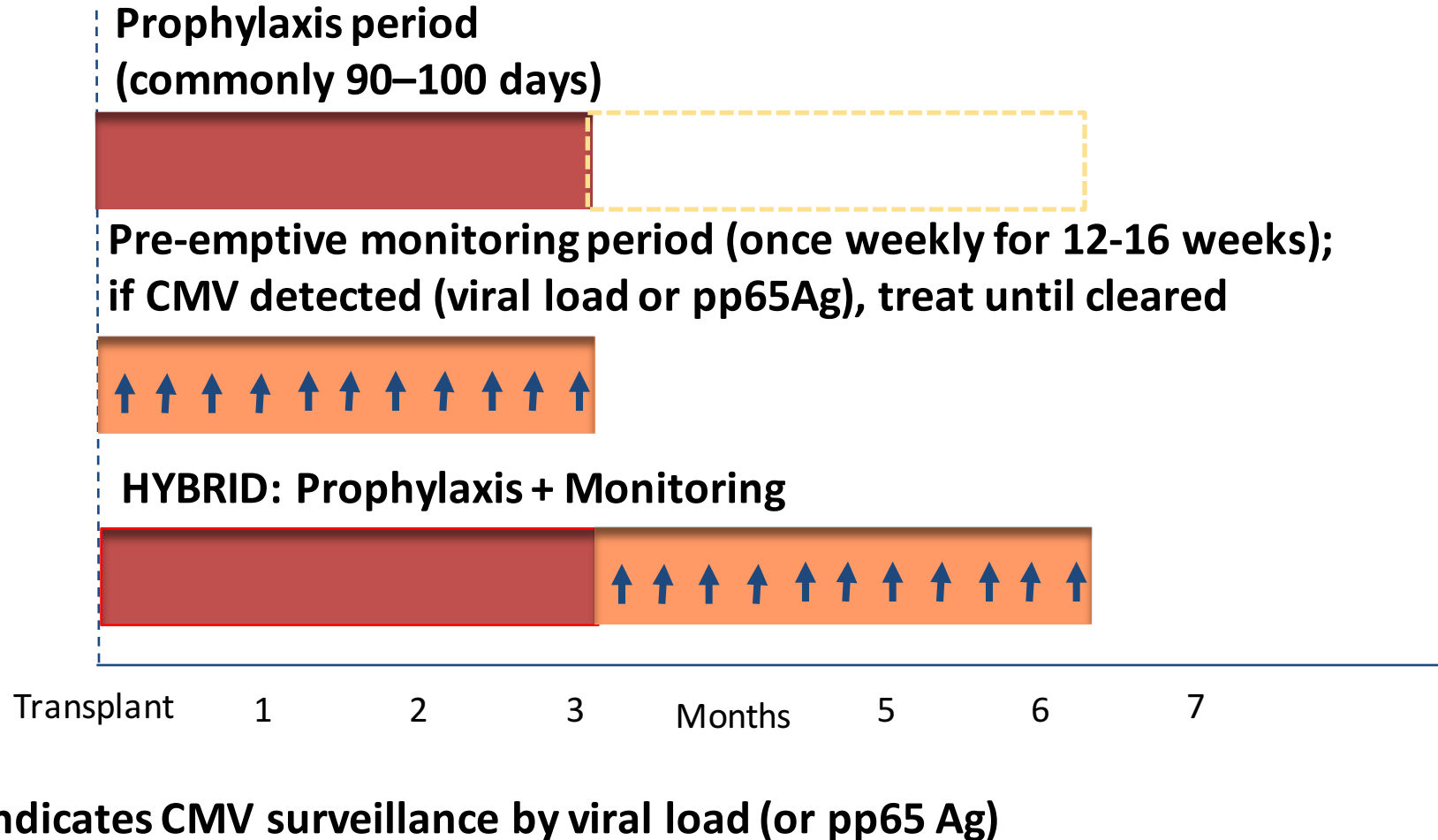
D+R– on Pre-emptive Therapy:

Direct effects of CMV and anti-CMV treatment

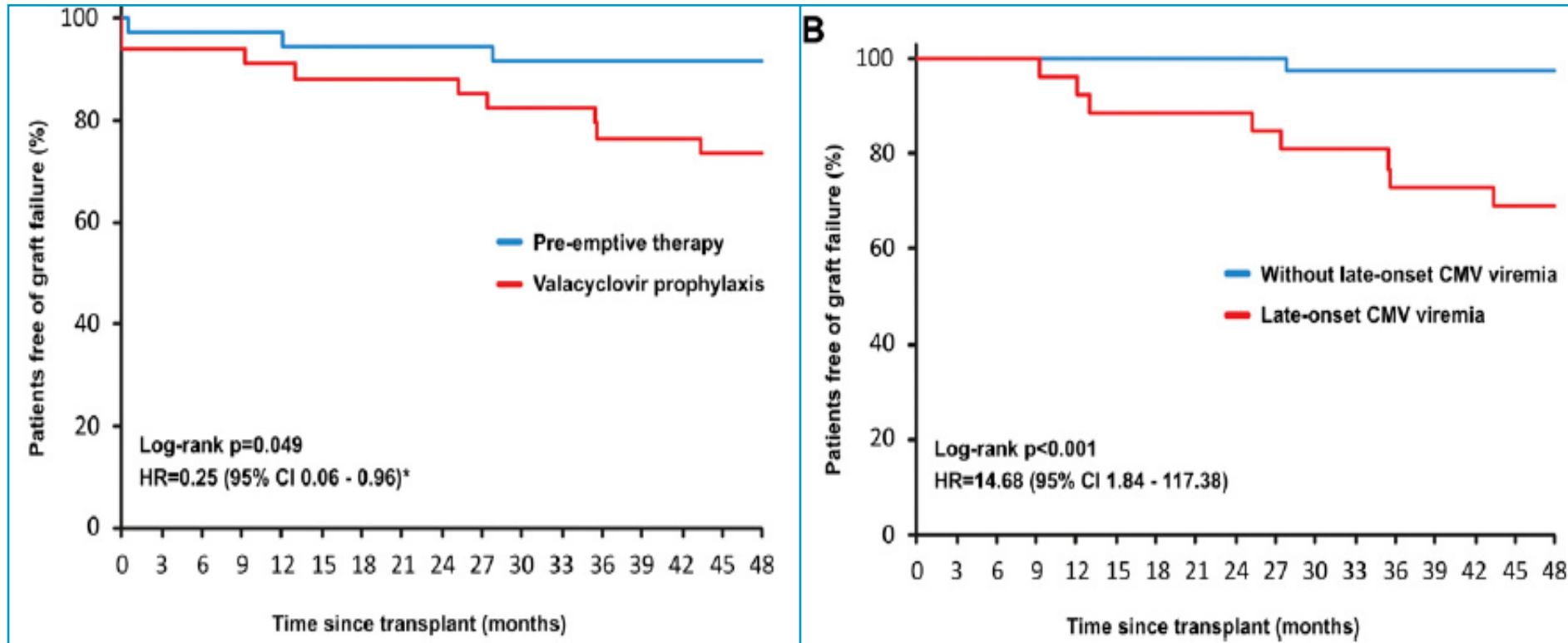
	Prophylactic (n = 32)	Preemptive (n = 80)	P Value
CMV infection	11 (34%)	40 (60%)	0.02
CMV disease	5 (16%)	21 (26%)	0.3
Late-onset infection	9 (28%)	6 (8%)	0.003
Peak viral load (mean, log10 copies/mL)	4.2±1.1	5.0±1.0	0.06
Anti-CMV drug resistance	1 (3%)	13 (16%)	0.05
Recurrent CMV	3 (9%)	18 (23%)	0.1

Couzi et al, High Incidence of Anticytomegalovirus Drug Resistance Among D+R– Kidney Transplant Recipients Receiving Preemptive Therapy, AJT, 2011

Prophylaxis, Pre-emptive Therapy, and Hybrid Care



Long-Term Outcomes of Preemptive Valganciclovir Compared with Valacyclovir Prophylaxis for Prevention of CMV in Renal Transplantation



Prolonged Prophylaxis With Valganciclovir Is Cost Effective in Reducing Posttransplant Cytomegalovirus Disease Within the United States

Emily A. Blumberg,^{1,8} Ingeborg A. Hauser,² Sanja Stanisic,³ Elvira Mueller,³ Karina Berenson,⁴ Christoph G. Gahlemann,⁵ Atul Humar,⁶ and Alan G. Jardine⁷

Transplantation • Volume 90, Number 12, December 27, 2010



Background. Cytomegalovirus (CMV) disease in transplant patients is known to have a substantial clinical and economic burden, and its prevention is expected to have long-term benefits. Evidence from the Improved Protection Against CMV in Transplant trial proved that prolonged prophylaxis of 200 days with valganciclovir compared with 100 days significantly reduces the incidence of CMV in high-risk kidney transplant seropositive donors/seronegative recipients. The aim of this study was to develop a cost-effectiveness model to evaluate prolonged prophylaxis of 200 days with valganciclovir and its long-term economic impact.

Methods. An economic model was designed to simulate long-term costs and outcomes of prolonged prophylaxis with valganciclovir (200 vs. 100 days) in a cohort of 10,000 high-risk renal transplant patients over 5 and 10 years. The first year of the model was based on the results of the Improved Protection Against CMV in Transplant trial and the extension to the long-term periods (5 and 10 years); and quality of life data were based on evidence retrieved through a systematic literature search. This analysis was conducted from the US healthcare payer perspective.

Results. For the 5-year time horizon, the incremental cost-effectiveness ratio of US \$14,859/quality-adjusted life year (QALY) suggests that 200-day valganciclovir prophylaxis is cost effective over the 100-day regimen considering a threshold of US \$50,000/QALY. The 10-year analysis revealed the 200-day prophylaxis as cost saving with a 2380 QALY gain and simultaneously lower cost.

Conclusion. Prolonged prophylaxis with valganciclovir reduces the incidence of events associated with CMV infection in high-risk kidney transplant recipients and is a cost-effective strategy in CMV disease management.



Cooking up a hybrid strategy by
Davide Abate

Hybrid Approaches Vary

- In pre-emptive therapy:
 - Surveillance once weekly for 3 - 4 months
 - NAT-DNAemia at positive threshold → treatment dose +/- reduction in immunosuppression for minimum of 2 weeks of treatment.
 - After resolution, discontinue antiviral and continue weekly surveillance.
 - Specific thresholds for defining optimal PET have not yet been defined
- Use of surveillance after prophylaxis may be considered in patients considered at increased risk for post-prophylaxis CMV disease (e.g., weekly for ~8-12 weeks).
- Special groups:
 - D+/R- patients after liver, heart, and pancreas 3-6 months depending on the degree of immunosuppression, including antilymphocyte antibodies for induction.
 - D+/R- lung transplant recipients: 6 -12 months. R+ lung transplant recipients 6 months prophylaxis while D+/R+ are at higher risk for developing CMV disease.
- Limited reports do not support the routine use of secondary prophylaxis.

Determination, validation and standardization of a CMV DNA cut-off value in plasma for preemptive treatment of CMV infection in solid organ transplant recipients at lower risk for CMV infection

Journal of Clinical Virology 56 (2013) 13–18

C. Martín-Gandul^{a,*,1}, P. Pérez-Romero^{a,1}, M. Sánchez^a, G. Bernal^b, G. Suárez^c, M. Sobrino^d, L. Merino^a, J.M. Cisneros^a, E. Cordero^a, The Spanish Network for Research in Infectious Diseases (REIPI)

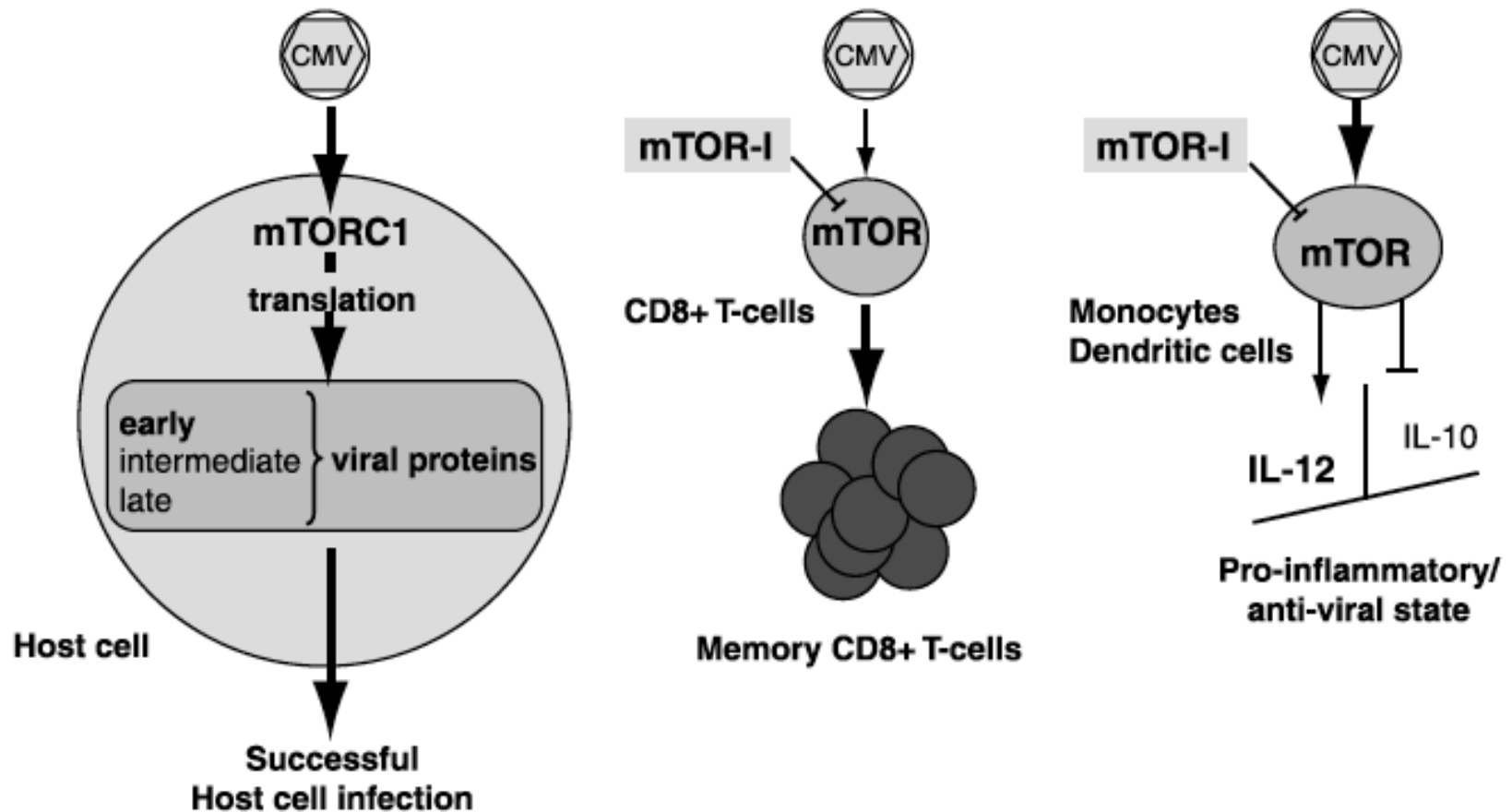
- Prospective cohort study of consecutive CMV+ SOT patients undergoing kidney, liver, liver–kidney) and heart transplantation
- Low risk, R+, no anti-lymphocyte depletion therapy
- Tested every 2 weeks (first 100 days) & every 4 weeks (day 100–180), ~10–11 tests each
- A viral load of **3983 IU/ml*** (2600 copies/ml) was established as the optimal cut-off; 99.6% NPV, sensitivity 90%, specificity 89%
- A majority of patients will not develop CMV disease without specific antiviral therapy (18 of 393 in cohort; 15 before day 100)
- Limitations: can't apply if higher risk, ATG use, different IS regimen

Special Cases

- Transplant recipients on mammalian target of rapamycin (mTOR) inhibitors such as sirolimus and everolimus may have lower rates of CMV; whether this should alter their prevention strategy is unknown. Effect is dose-dependent.
- There are limited data to support the use of CMV immunoglobulin (CMV Ig) for prophylaxis when appropriate antivirals are given.

Review of Cytomegalovirus Infection Findings With Mammalian Target of Rapamycin Inhibitor-Based Immunosuppressive Therapy in De Novo Renal Transplant Recipients

Björn Nashan,^{1,12} Robert Gaston,² Vincent Emery,³ Marcus D. Säemann,⁴ Nicolas J. Mueller,⁵ Lionel Couzi,⁶ Jacques Dantal,⁷ Fuad Shihab,⁸ Shamkant Mulgaonkar,⁹ Yu Seun Kim,¹⁰ and Daniel C. Brennan¹¹



Molecular mechanisms of anti-CMV effects of mTOR inhibitors

Is Cytomegalovirus Prophylaxis Dispensable in Patients Receiving an mTOR Inhibitor–Based Immunosuppression? A Systematic Review and Meta-Analysis

Transplantation • Volume 94, Number 12, December 27, 2012

Joachim Andrassy,^{1,5} Verena S. Hoffmann,² Markus Rentsch,¹ Manfred Stangl,¹ Antje Habicht,³ Bruno Meiser,³ Michael Fischereder,⁴ Karl-Walter Jauch,¹ and Markus Guba¹

- CMV events after solid organ transplantation occurred significantly more often under CNIs (RR=2.27) than with rapamycin.
- mTOR-I + CNI vs. CNI alone in 15 trials of kidney, heart, and liver transplantation → higher CMV incidence when patients received an mTOR-I free immunosuppression (RR=2.45).

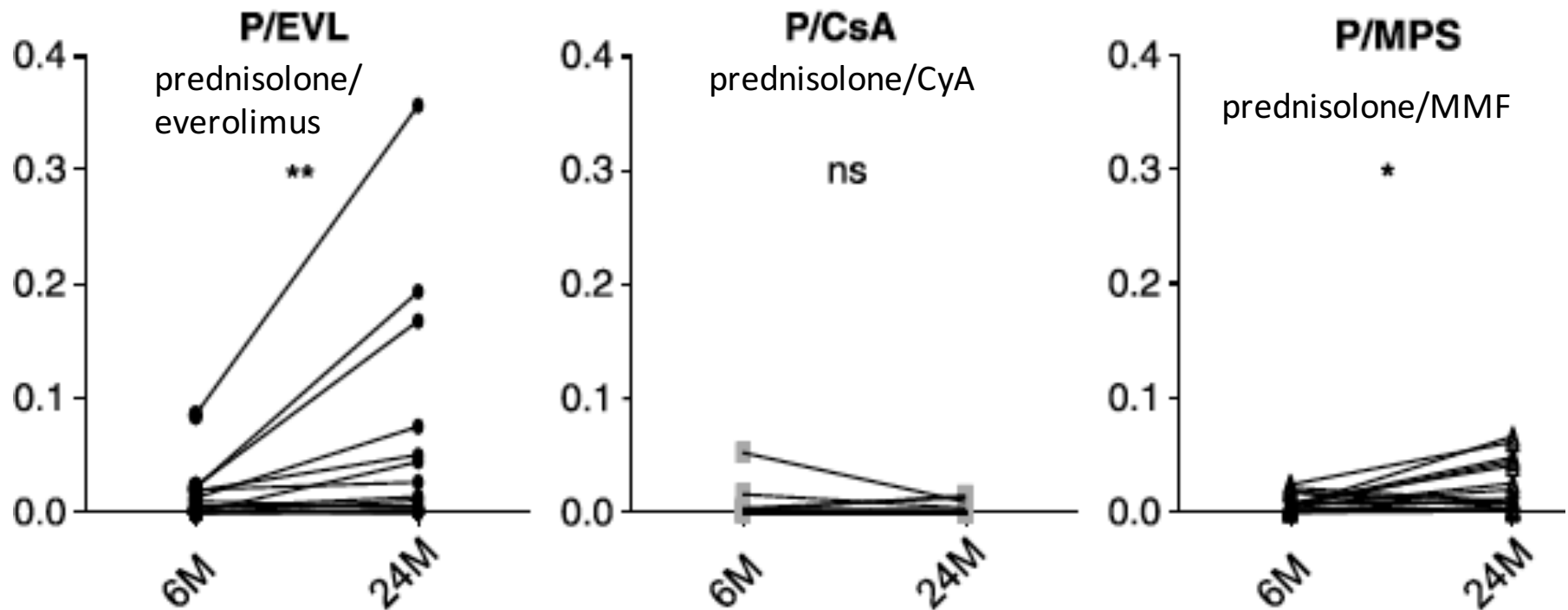
Everolimus-Treated Renal Transplant Recipients Have a More Robust CMV-Specific CD8⁺ T-Cell Response Compared With Cyclosporine- or Mycophenolate-Treated Patients

Transplantation • Volume 95, Number 1, January 15, 2013

Simone H.C. Havenith,^{1,2,4} Si La Yong,^{1,2} Karlijn A.M.I. van Donselaar-van der Pant,¹
René A.W. van Lier,³ Ineke J.M. ten Berge,¹ and Frédérique J. Bemelman¹

CMV-specific CD8⁺ T cells

All CMV IgG+ @ transplant; switched @ 6 mos



mTOR Effect is dose dependent

Brennan et al.

Table 3: Incidence of CMV infection or syndrome by D/R CMV serostatus

Donor/recipient CMV serostatus	Everolimus 1.5 mg/day	Everolimus 3 mg/day	MPA
N	105	105	107
D+/R-, n (%)	17 (16.2%)	19 (18.1%)	27 (25.2%)
N	286	298	276
D+/R+, n (%)	14 (4.9%) [^]	10 (3.4%)*	33 (12.0)
N	101	119	119
D-/R+, n (%)	3 (3.0%)	1 (0.8%)**	8 (6.7%)
N	143	116	139
D-/R-, n (%)	1 (0.7%)	2 (1.7%)	6 (4.3%)

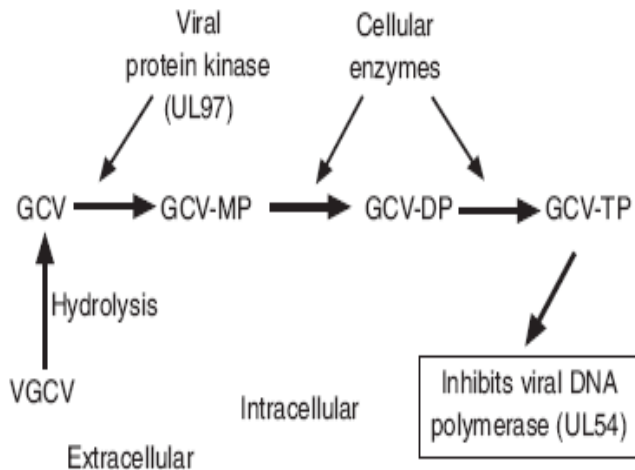
[^]p = 0.0034 versus MPA; *p < 0.001 versus MPA; **p = 0.036 versus MPA.

Cooking Treatment Recommendations



Therapeutic Masters for CMV

Anti-CMV Agents



NEW AGENTS WILL NOT COVER HSV-VZV AS WELL AS VALGANCICLOVIR.

<u>Agent</u>	<u>Route</u>	<u>Target</u>	<u>Toxicity</u>	<u>Availability</u>
Ganciclovir (GCV)	IV>>PO	UL54, DNA polymerase	Marrow	Yes
Valganciclovir (VGCV)	PO	UL54, DNA polymerase	Marrow	Yes
Foscarnet	IV	UL54, DNA polymerase	Renal, metabolic	Yes
Cidofovir (CDV)	IV	UL54, DNA polymerase	Renal	Yes
Brincidofovir (CMX001)	PO	UL54, DNA polymerase	Less renal than CDV	Not for CMV
Letermovir (AIC246)	PO/IV	UL 56, DNA packaging	Limited	Experimental
Maribavir (MBV)	PO	UL97, egress	Limited	Experimental

Therapy

- With the use of **highly sensitive QNAT** (LLOQ<200 IU/ml), consider discontinuing therapy after one result is less than the LLOQ. Confirmatory testing should be done one week after discontinuing therapy.
- If the assay is not highly sensitive then 2 consecutive undetectable (negative) results are needed to discontinue therapy
- Drug resistance should be suspected in patients with clinical treatment failure despite greater than two weeks of antiviral treatment

UL97 Mutations

Genotype frequency	Fold change in ganciclovir EC50 ^a		
	5-15x	2-5x	<2x
Most common	M460V/I, H520Q, A594V, L595S, C603W	C592G	
Less common at codons 460, 590-607	M460T, A594G, 595del ^b , L595F/W, E596Y, 597del2 ^b , 599del, K599T, 600del, 601del, 601del2, C603R, C607Y, del(≥3) ^c	A591V, A594E/T, E596G, C603S, 596del ^b , 600del2, C607F	E596D, N597D, K599E/R, L600I, T601M, D605E ^d
Atypical loci	F342S ^e , K355M ^e , V356G ^e , V466G ^e , C480R ^e , C518Y, P521L ^e	L405P, I610T, A613V	M615V, Y617H, A619V, L634Q, E655K, A674T

(a) Moderate resistance (5-15x), low-grade resistance (2-5x), or insignificant resistance (<2x)

(b) = in frame deletion of codon

(c) In frame deletion of ≥3 codons in the 590-607 range can be assumed to confer moderate ganciclovir resistance (8- to 15-fold). Deletion of less than 3 codons may confer varying degrees of ganciclovir resistance (4- to 10-fold).

(d) D605E is a baseline sequence polymorphism common in east Asia, unrelated to drug resistance

(e) Maribavir cross-resistance documented, all except F342S are markedly growth-inhibited

Algorithm for Suspected CMV Resistance

Genome sequence of UL54 and UL97

Caveats

- Not all genes are sequenced
- Not all mutations are known
- Not all mutations are equal
- Some mutations associated with decreased viral fitness
- Polyclonal infections are common

GCV = ganciclovir; FOS = foscarnet; CDV = cidofovir
 [1] Symptomatic disease or viral load not improving
 [2] Full dose GCV = 5 mg/kg bid i.v.
 High dose GCV = 10 mg/kg bid i.v.
 (adjust doses for renal function)

Kotton CN et al. Updated International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation. *Transplantation*. 2013;96(4):333-360.

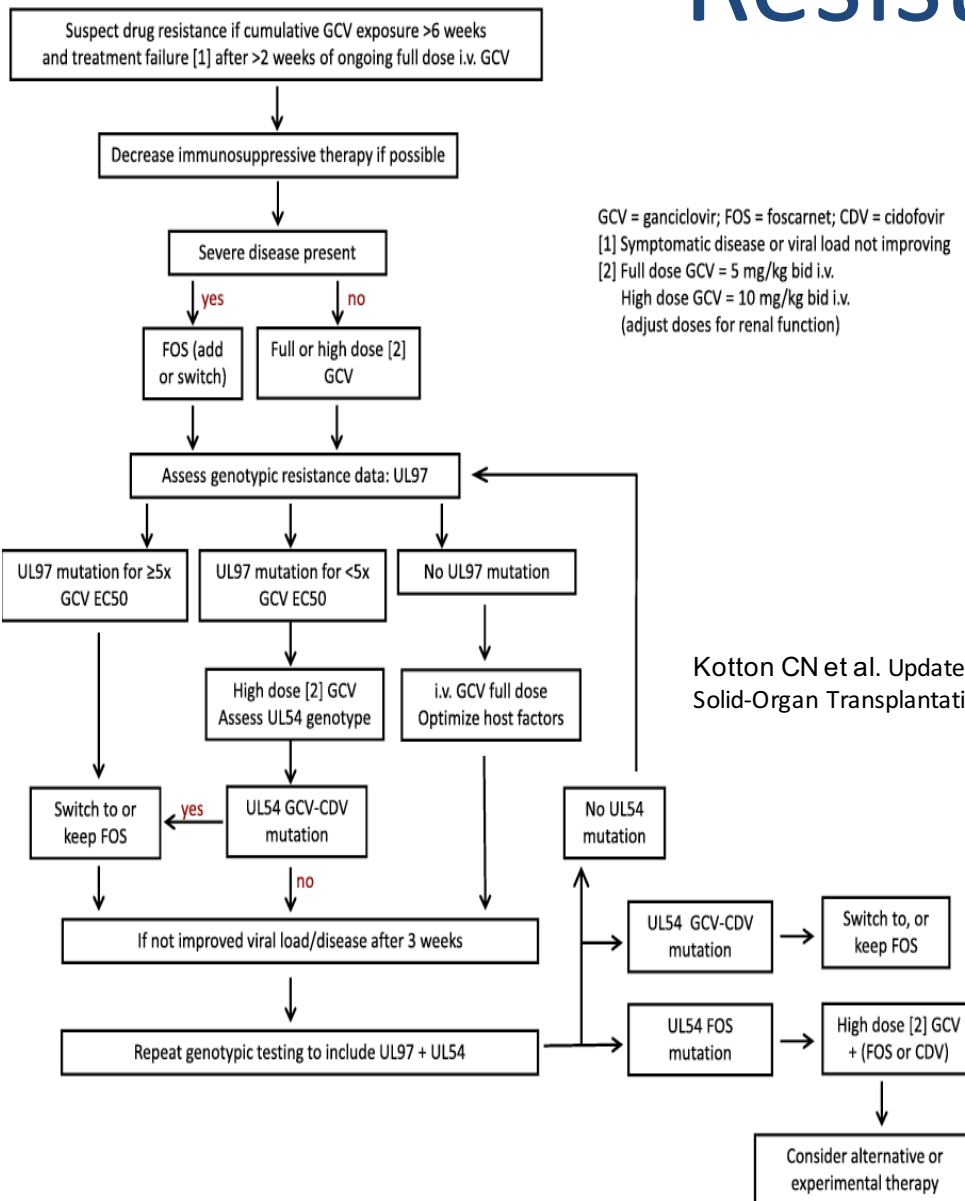
Performed at VIRACOR-IBT LABORATORIES, 1001 NW Technology Drive, Lee's Summit, MO 64086

(NOTE)

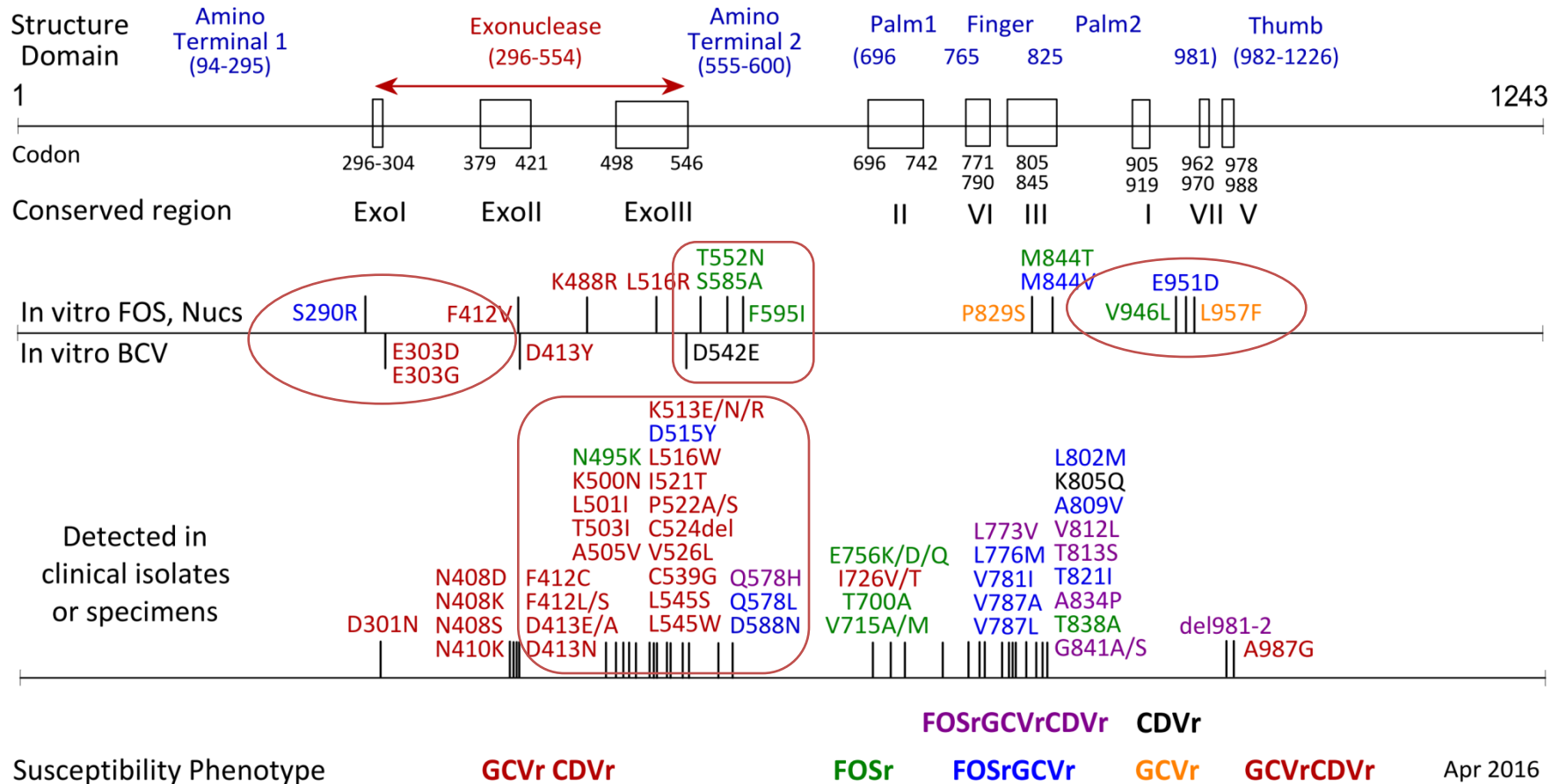
UL97 Gene Target
 Ganciclovir: None Detected.

UL54 Gene Target
 Cidofovir: None Detected.
 Ganciclovir: None Detected.
 Foscarnet: None Detected.

If a mutation has been detected in either the UL97 or UL54 Gene Targets, the mutation site is indicated. A result of "None Detected" indicates that no mutations were detected for that gene target.



CMV UL54 DNA Polymerase Mutations



Apr 2016

**Cross
Resistance**

Exo and region V mutations confer GCV-CDV cross-resistance
FOS-R mutations may confer low-grade GCV±CDV cross-resistance

TABLE 3. UL54 *pol* resistance mutations characterized by marker transfer/recombinant phenotyping

Region	Codon no.	Amino acid(s)/mutation		Ratio ^a		
		Wild type	Mutant	GCV	FOS	CDV
ExoI	301	D	N	2.6	0.5	3
ExoII	408	N	D	4.9	1.3	5.6
	408	N	K	4.2	0.7	21
	410	N	K	2.9	0.8	3
	412	F	C	4.2	1.2	18
	412	“If a typical UL97 mutation that confers a 5- to 10-fold increase in GCV resistance is detected, a switch to FOS is indicated, especially in the presence of ongoing disease or high and increasing viral loads.”				
	413					
IV	495					
ExoIII	501	L	I	6	1.4	9.1
	503	T	I	2.9	0.5	6.1
	513	K	E	5	1.4	9.1
	513	K	N	6	1.1	12.5

Ratio=IC₅₀ of mutant/IC₅₀ of wild type

Immunology

- **Serostatus is useful but reflects highly variable populations in terms of prediction of risk for CMV infection.**
- IgG hypogammaglobulinemia is associated with an increased risk of CMV disease after transplantation. Measurement of total immunoglobulins is suggested in situations where CMV is difficult to control.
- **CMV-specific cellular immune monitoring** has been shown to predict CMV infection in the pre-transplant and post-transplant settings in prospective observational, multi-center studies. Interventional studies to determine precise clinical utility of CMI are ongoing.
- CMV vaccines are in preclinical, phase I and phase II trials. The primary goal of a CMV vaccine should be to prevent or modulate CMV replication and/or CMV disease.
- T-cell therapies should be further evaluated for resistant / refractory CMV.

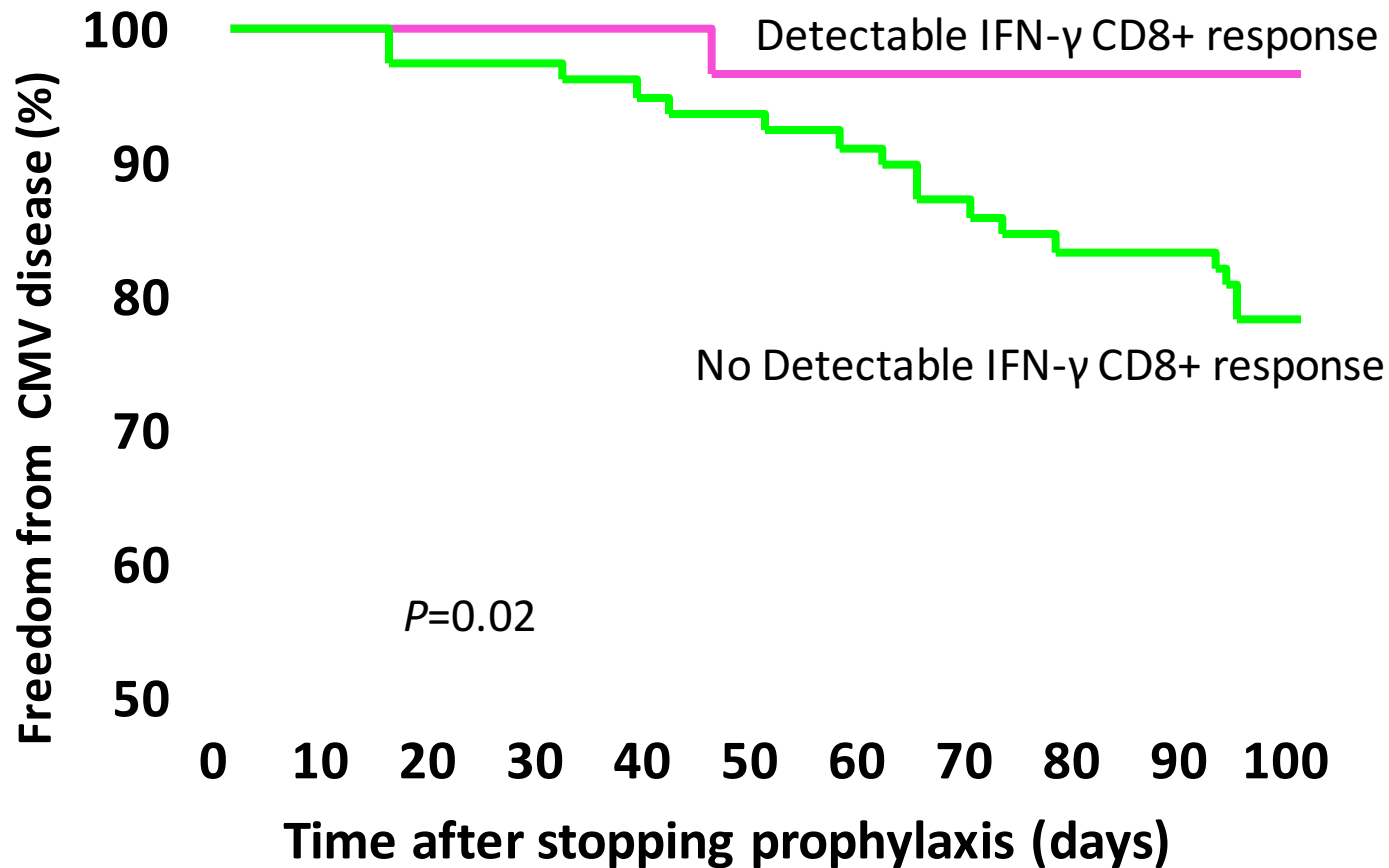
Predictive Markers?

- Serostatus based risk stratification (i.e. D+ /R–, D+ /R+, D–/R+, and D–/R–) hides a substantial heterogeneity of the individual risks for CMV replication and progression to CMV disease.
- The risk of CMV disease among D+ /R– patients in the first year posttransplant range from 6.4 to 58.3% when the organ recipients were regrouped according to CMV-specific cell-mediated immunity testing results at the end of antiviral prophylaxis .
- *Tests and markers capable of individualizing the risk of CMV replication may significantly improve CMV preventative strategies.*

Cell-mediated immunity to predict cytomegalovirus disease in high-risk solid organ transplant recipients: Late onset CMV.

- Utility of testing **CD8+ T-cell response** against CMV as a predictor of late-onset CMV disease after a standard course of antiviral prophylaxis. 108 evaluable patients (D+/R+ n = 39; D-/R+ n = 34; D+/R- n = 35) of whom 18 (16.7%) developed symptomatic CMV disease.
- Testing using the QuantiFERON-CMV assay at baseline, 1, 2 and 3 months posttransplant (21-peptide pool).
- *CMI was detectable in 38/108 (35.2%) patients (cutoff 0.1 IU/mL interferon-gamma).*
- **CMV disease occurred in 2/38 (5.3%) patients with a detectable interferon-gamma response versus 16/70 (22.9%) patients with a negative response; p = 0.038.**
- In the subgroup of D+/R- patients, CMV disease occurred in 1/10 (10.0%) patients with a detectable interferon-gamma response (cutoff 0.1 IU/mL) versus 10/25 (40.0%) patients with a negative CMI, p = 0.12.
- Monitoring of CMI may be useful for predicting late-onset CMV disease

IFN- γ and CMV Disease



Chemokines and Immune Control of Cytomegalovirus in Organ Transplant Recipients.

- CMV viremic organ transplant recipients, chemokine expression—specifically the **chemokine CCL8** (AUC 0.849 95% CI 0.721–0.978; $p = 0.003$) and the **interferon- γ induced chemokine CXCL10** (AUC 0.841, 95% CI 0.707–0.974; $p = 0.004$)—were associated with control of viral replication.
- Homozygous TT polymorphism in the CCL8 promoter (SNP rs3138035) of D+/R- transplant recipients conferred an increased risk of viral replication after discontinuation of antiviral prophylaxis (hazard ratio 3.6; 95% CI 2.077–51.88).
- The primary cell type producing CCL8 in response to CMV peptide stimulation was the monocyte fraction where CCL8 production is associated with spontaneous viral clearance in patients with CMV viremia. There is dose-dependent reduction in CCL8 production with immunosuppression.

Virus-Specific T cell Adoptive Transfer

Restoration of Viral Immunity in Immunodeficient Humans by the Adoptive Transfer of T Cell Clones

Stanley R. Riddell,* Kathe S. Watanabe, James M. Goodrich,
Cheng R. Li, Mounzer E. Agha, Philip D. Greenberg

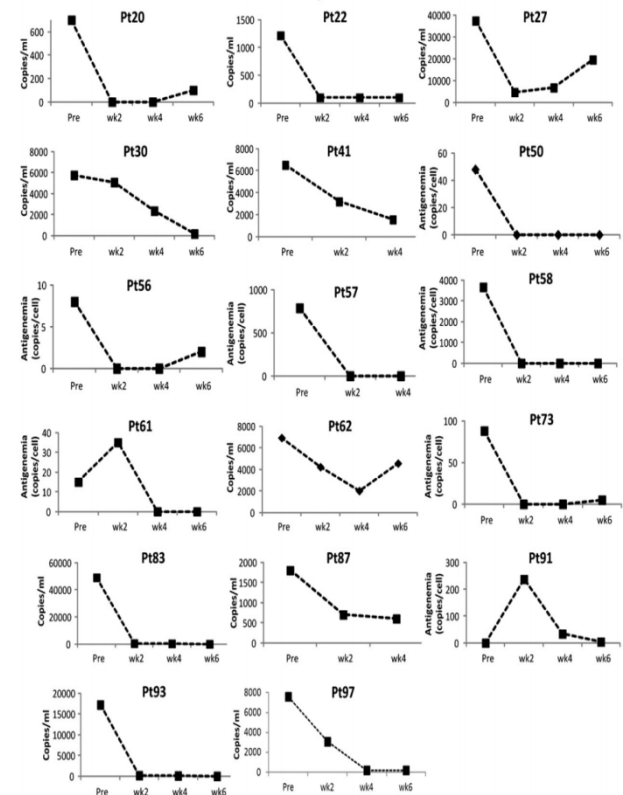
The adoptive transfer of antigen-specific T cells to establish immunity is an effective therapy for viral infections and tumors in animal models. The application of this approach to human disease would require the isolation and in vitro expansion of human antigen-specific T cells and evidence that such T cells persist and function in vivo after transfer. Cytomegalovirus-specific CD8⁺ cytotoxic T cell (CTL) clones could be isolated from bone marrow donors, propagated in vitro, and adoptively transferred to immunodeficient bone marrow transplant recipients. No toxicity developed and the clones provided persistent reconstitution of CD8⁺ cytomegalovirus-specific CTL responses.

Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation

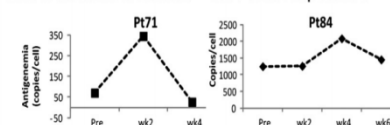
Ann M. Leen,¹ Catherine M. Bollard,¹ Adam M. Mendizabal,² Elizabeth J. Shpall,³ Paul Szabolcs,⁴ Joseph H. Antin,⁵ Neena Kapoor,⁶ Sung-Yun Pai,^{5,7} Scott D. Rowley,⁸ Partow Kebriaei,² Bimalangshu R. Dey,⁹ Bambi J. Grilley,¹ Adrian P. Gee,^{1,10} Malcolm K. Brenner,¹ Cliona M. Rooney,^{1,10} and Helen E. Heslop¹

- PBMCs from HLA homozygous, seropositive donors
- Generated trivalent (CMV, EBV, AdV) CD8 T cells
- Creating a bank of ready to use virus specific CTLs
- Identified 50 patients with refractory viral infections after SCT
- Matched HLA of virus specific T cells with patients
- Adoptive transfer of virus specific T cells weekly
- Subsequently have developed pentavalent T cells (CMV, EBV, HHV6, AdV, BKV)

A Viral load measurements – CMV responders



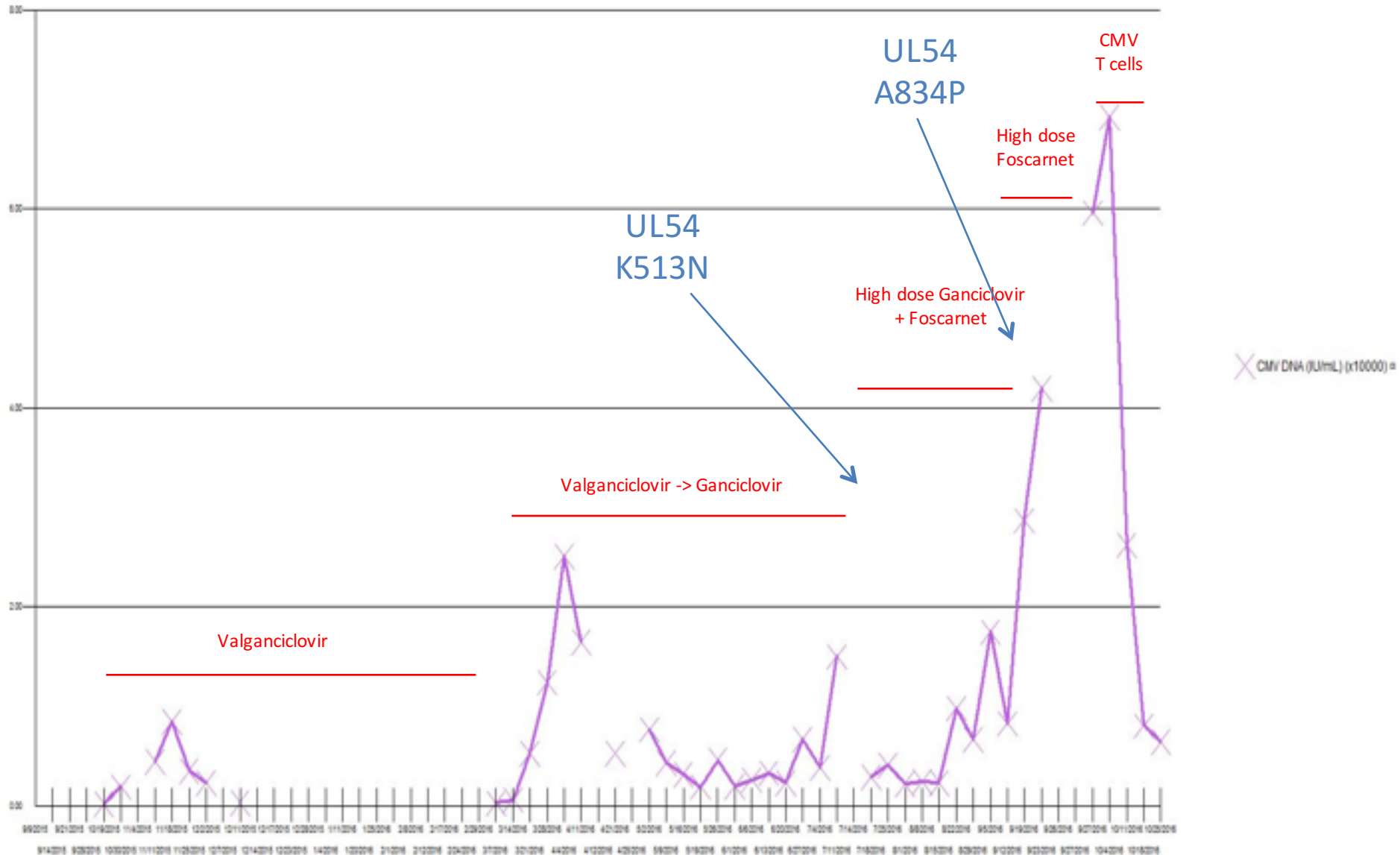
Viral load measurements – CMV non-responders



Leen et al Blood 2013

Papadopoulos et al. Sci Trans Med 2014

CMV disease after SCT



Major Changes

- Biology: Mechanism of CMV effect on macrophage function appears to be via inhibition of inflammatory pathways.
- QNAT: increased sensitivity → positive in GI disease and single negative assay may be sufficient for stopping therapy
- Prophylaxis routine in:
 - All D+R-
 - D+ or R+ heart and lungs.
- Multiple new drugs → soon?
- Use of CMV-specific T-cell therapy might address resistance.