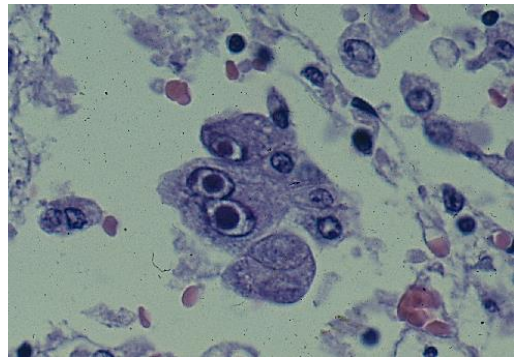


CMV in Transplantation – What do I need to know?

Jay A. Fishman, M.D.

Massachusetts General Hospital and

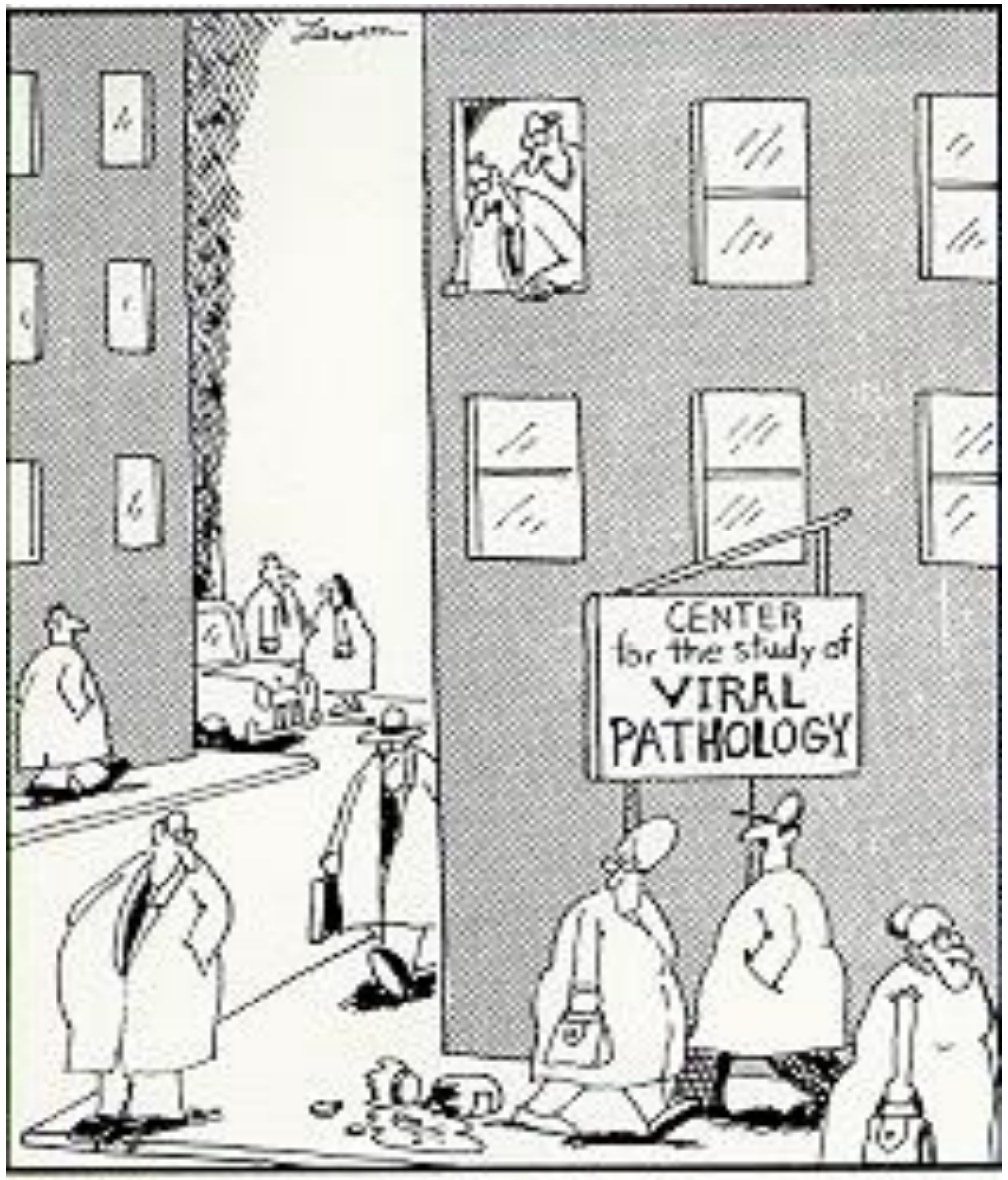
Harvard Medical School

Boston, MA, USA

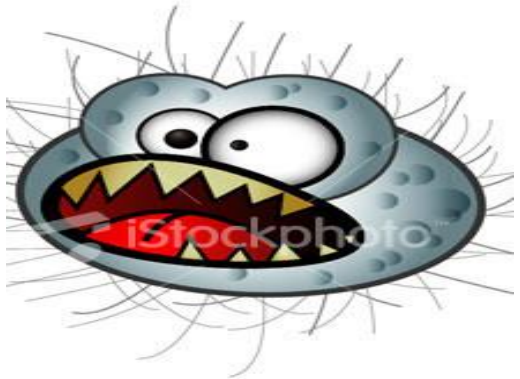
MASSACHUSETTS GENERAL HOSPITAL

TRANSPLANT CENTER

Viruses may be dangerous . . . .



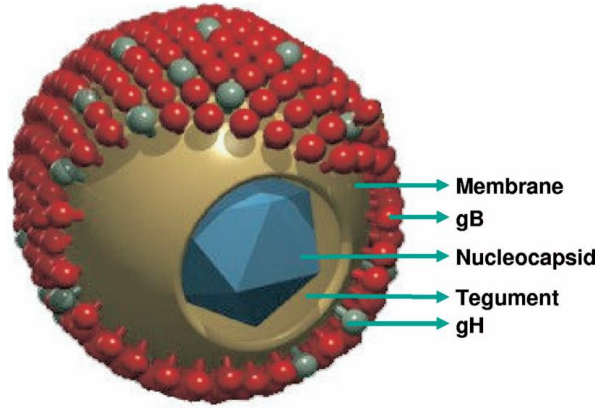
"Uh-oh."



Key Concepts

- **Cytomegalovirus remains a major pathogen in transplantation**
  - **Presents most often with fever and low(er) white blood cell count ("Viral syndrome")**
  - Generally, ignore sputum and urine cultures.
  - Direct and indirect effects of CMV in transplantation
  - Guidelines for management exist
  - Assays standardized to International Units (IU) – use one assay (whole blood, plasma, laboratory) for *each patient*
  - Clinical resistance (notably with belatacept immunosuppression) and molecular antiviral resistance remain challenges
- Prevention (prophylaxis) of infection is linked to risk
  - CMV: D+/R- in SOT; D-/R+ in HSCT
  - Colonization/Leaky immunity
  - Commonly activated in setting of critical illness (ICU) – role in outcomes?
- Yes, we are starting to know how it works! → *Start with biology*

Cytomegalovirus: Some biology

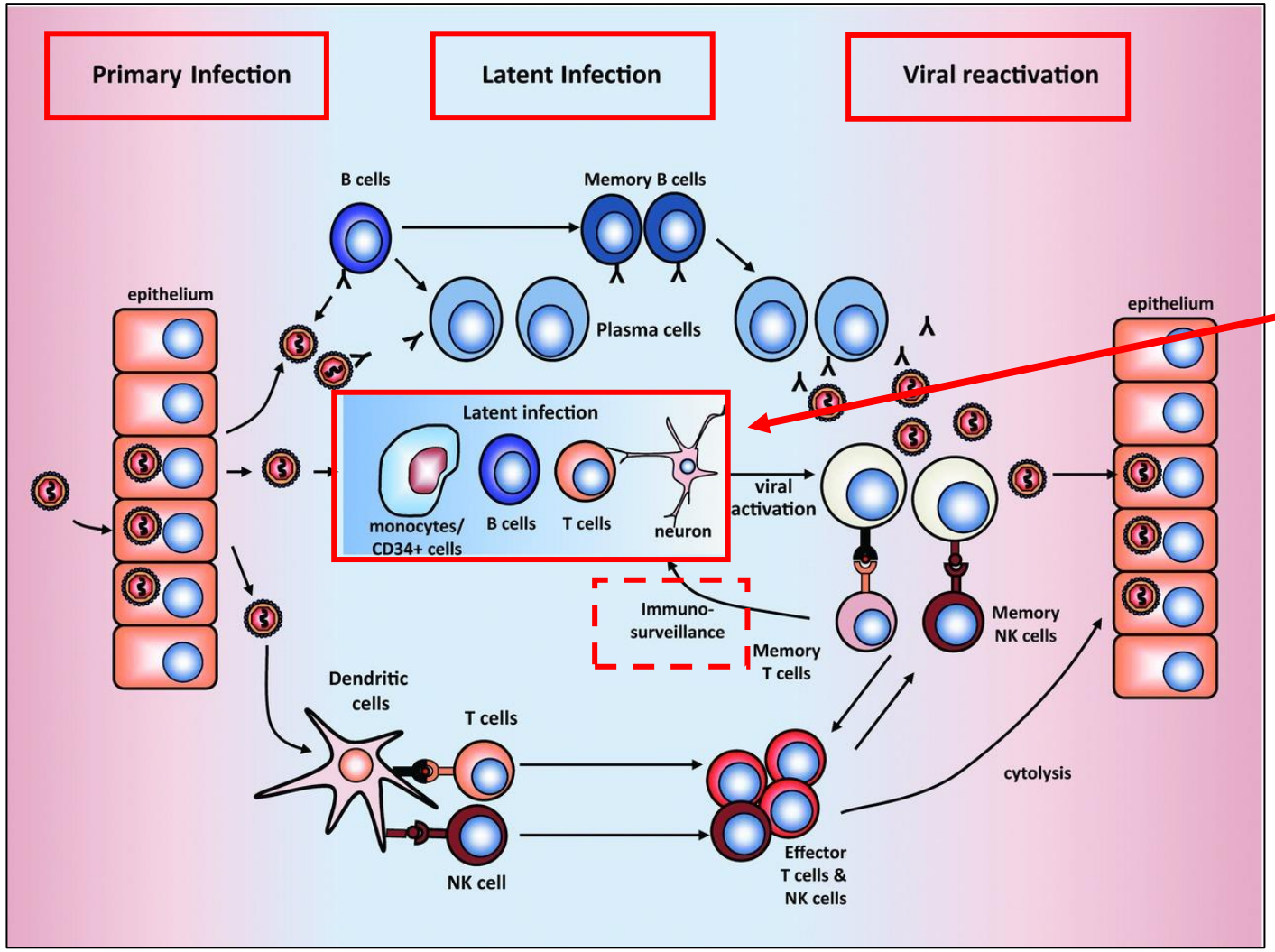


- *Betaherpesvirinae s*ubfamily of the *Herpesviridae*
- The structure :
  - Nucleus containing the viral genome (linear double-stranded DNA)
  - Icosahedral protein capsid
  - **>*200 genes* with significant variation between strains**
  - The tegument protein matrix (e.g., pp65):
    - Proteins with 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 (major vaccination target)
    - 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

CMV Infection, Latency and Surveillance



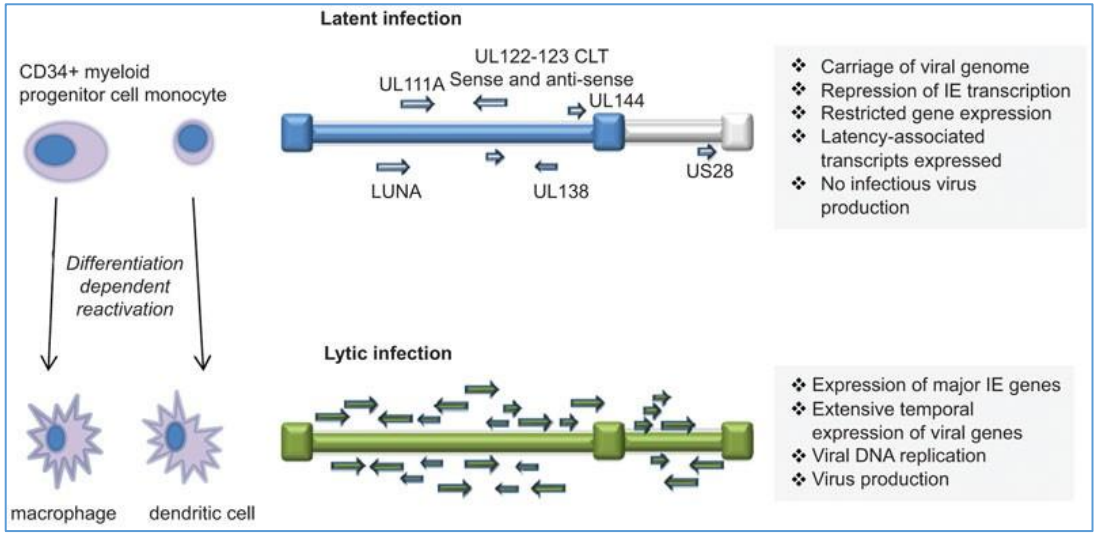
Primary infection via the mucosal epithelium → production of virus particles which disseminate to the sites of latent infection:

- Hematopoietic (monocytes, B cells and T cells), epithelium, endothelium or neuronal cells.
- Induction of a robust humoral (ADCC) and cellular immune response.
- Cellular and humoral immunity with activation via innate immune mediators (NK cells, dendritic cells, invariant T cell populations, and antigen-specific αβ T cells) → lysis of virally infected cells.

Smith and Khanna, Am J Transplant 13, Issue: s3, Pages: 9-23, First published: 24 January 2013, DOI: (10.1111/ajt.12005)

Concepts: Reactivation from Latency

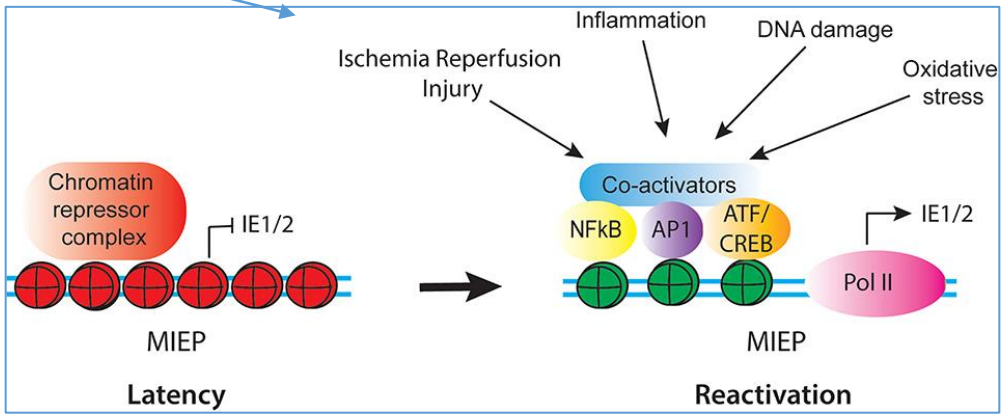
Latency Program: In latently infected CD34⁺ cells and CD14⁺ monocytes, there is a ***targeted suppression of lytic viral gene expression (episomal and viral)*** and ~undetectable levels of major IE proteins & expression of **latency-associated genes** including transcripts from the major **IE (immediate early) region** (UL122–123 CLTs), UL81–82ast (LUNA), UL138, UL111a, UL144 and US28, other mRNAs



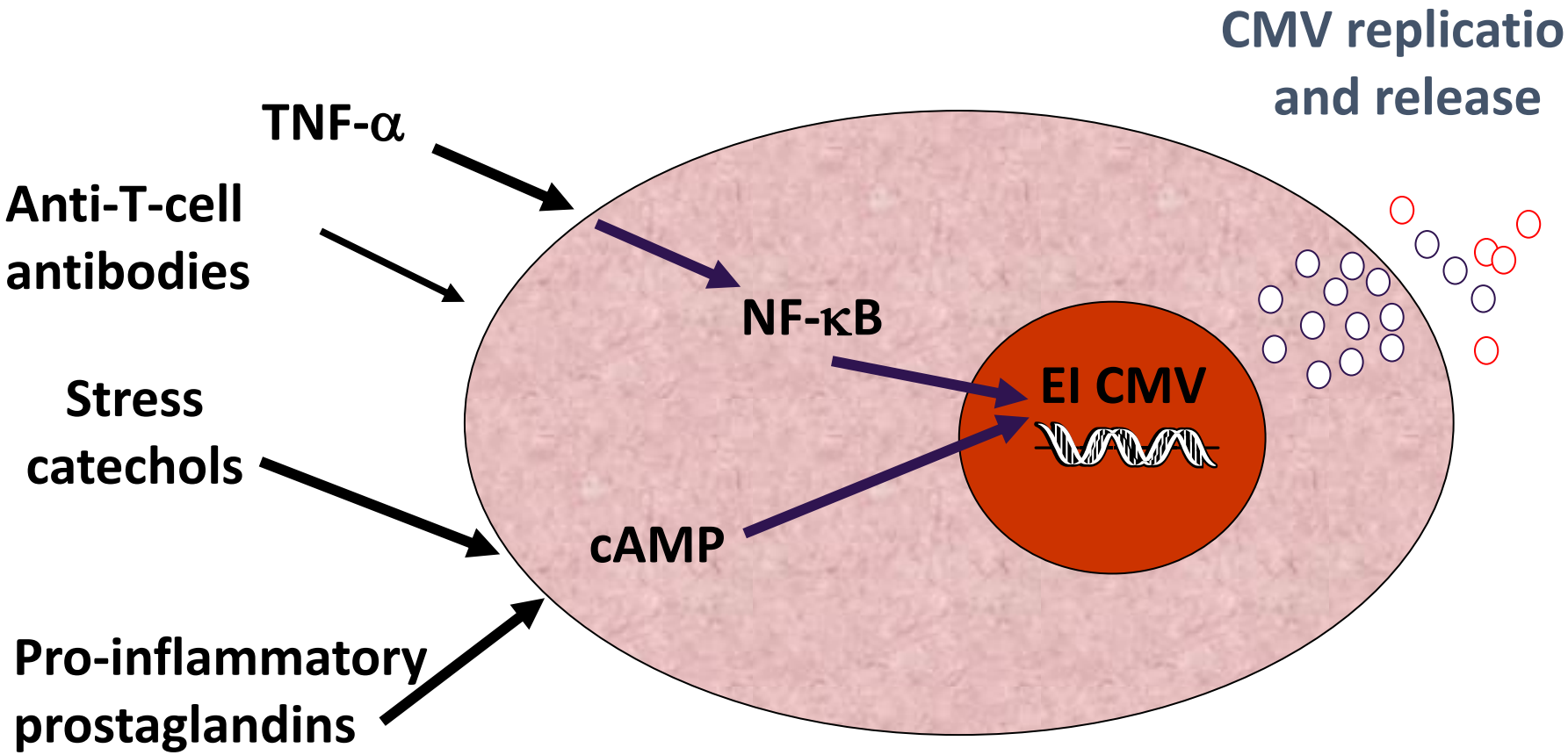
Differentiation* of monocytes to macrophages and mature dendritic cells (mDC)** → de-repression of the major IE proteins and allows ***initiation of the lytic transcription program → viral DNA replication and → de novo virus production.

CMV viremia occurs in up to *36% of normal hosts with sepsis & high disease severity* (ICU care) – role? Mortality is increased in this group (association rather than causal).

HCMV, human cytomegalovirus; mDC, mature dendritic cell; MIEP, major immediate early promoter.

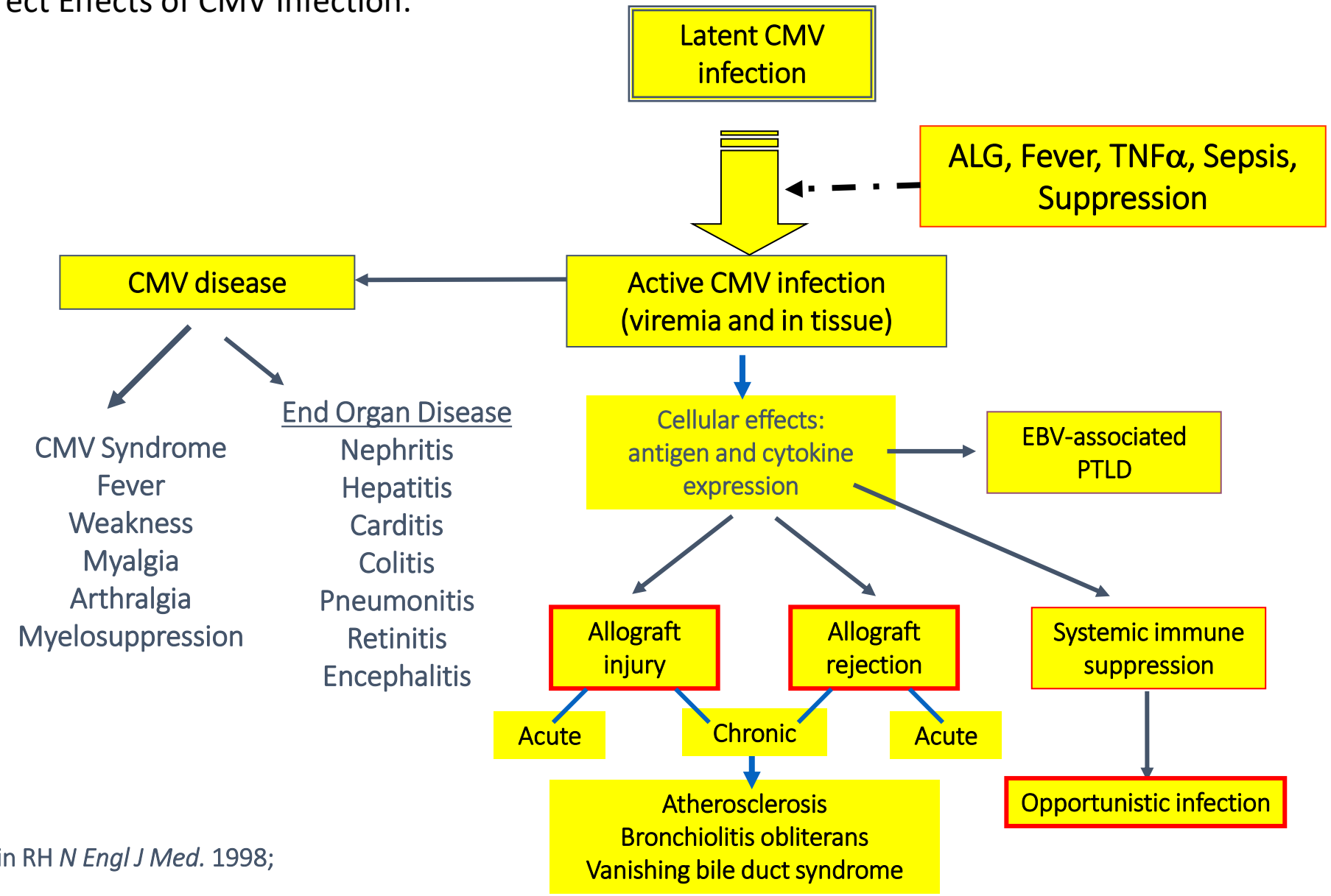


Pathways for CMV reactivation from latency: More late disease with T-cell depletion and fever?



Reinke P et al. *Transplant Infect Dis* 1999; 1:157-64.

Direct and Indirect Effects of CMV Infection: 1998



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

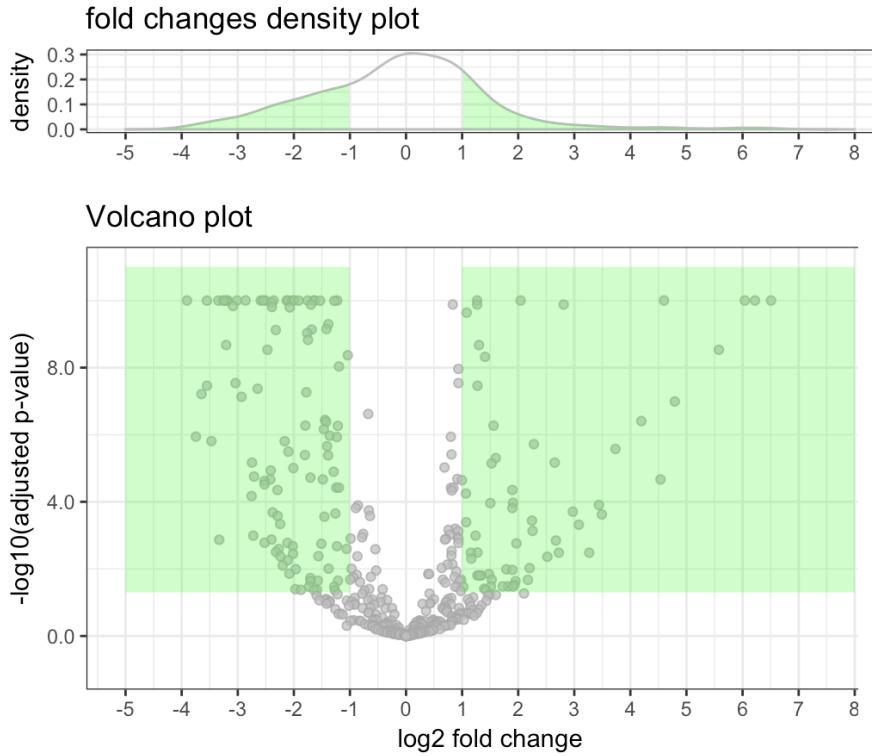
Direct and Indirect Effects of CMV Infection: 2021

Mechanisms?
Hand waving or Magic?

How does CMV predispose to graft rejection and opportunistic infection at the same time?

Volcano plots of genes: log fold change in CMV-infected THP-1 (monocyte) cells

Significance of Changes

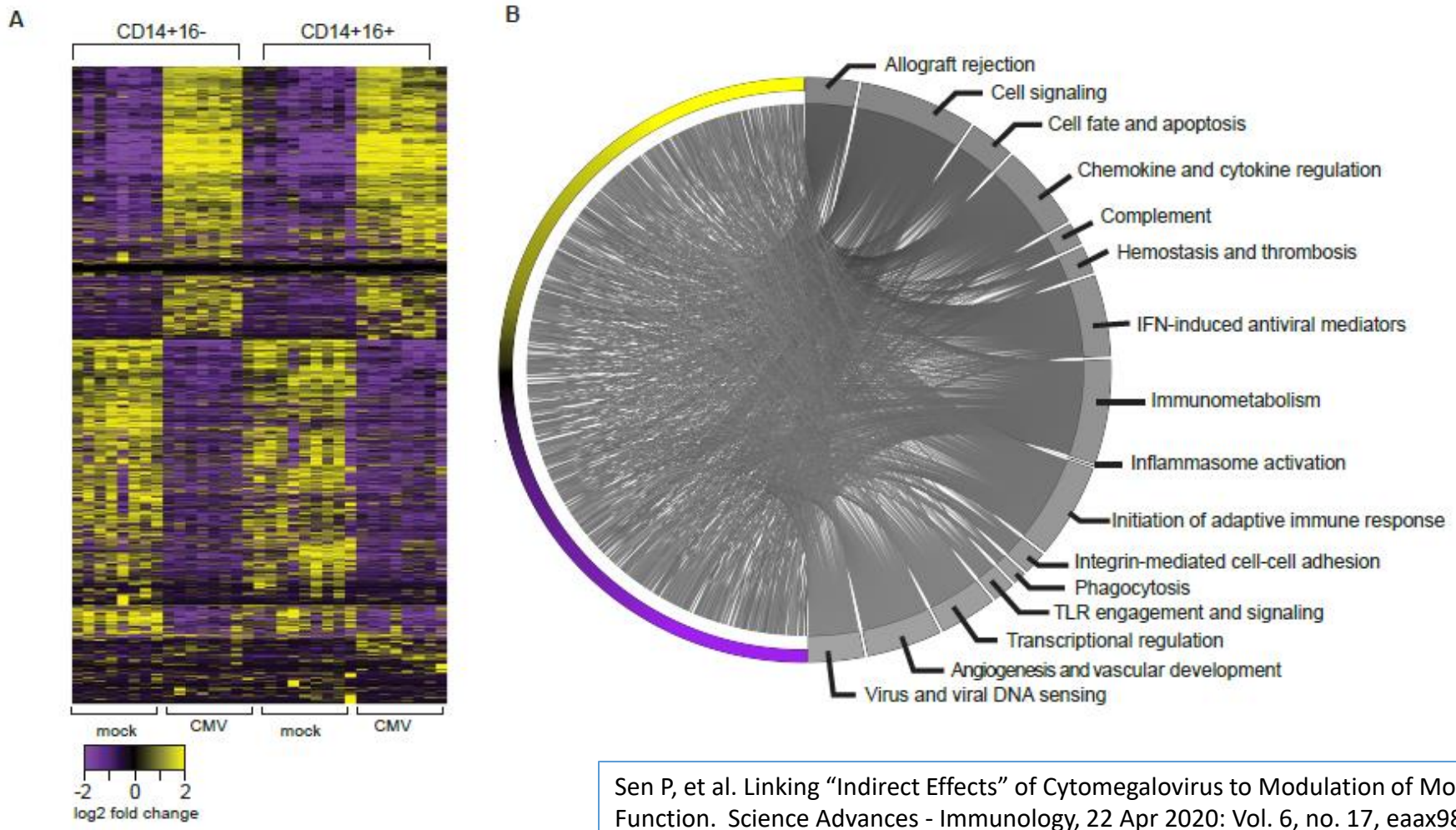


Fold Changes in Gene Expression

Sen, ElKhoury, Fishman

Similar changes seen in primary human monocytes infected in vitro with HCMV.

Pathways altered by CMV



Sen P, et al. Linking "Indirect Effects" of Cytomegalovirus to Modulation of Monocyte Innate Immune Function. Science Advances - Immunology, 22 Apr 2020: Vol. 6, no. 17, eaax9856; DOI: 10.1126/sciadv.aax9856

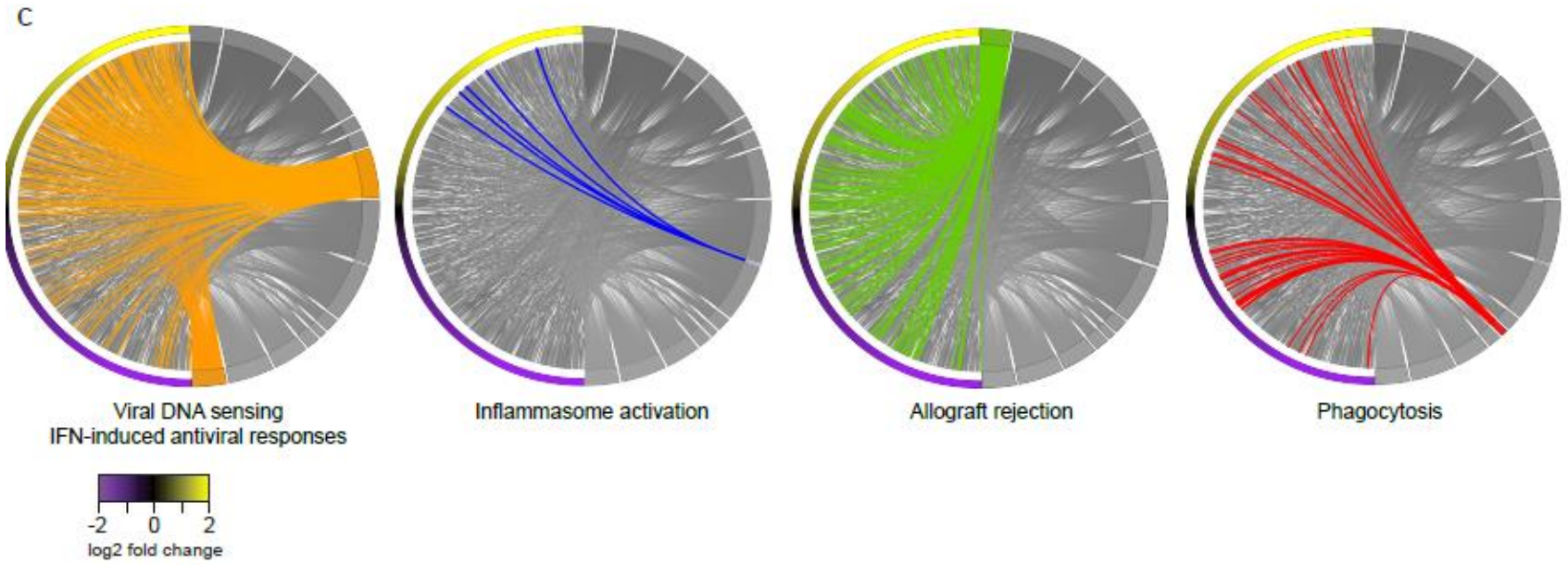
CMV: In vitro infection of monocytes

Poor antiviral responses

Inflammation

Graft Rejection

Decreased Phagocytosis & Scavenger Receptors



Sen P, et al. Linking “Indirect Effects” of Cytomegalovirus to Modulation of Monocyte Innate Immune Function. Science Advances - Immunology, 22 Apr 2020: Vol. 6, no. 17, eaax9856; DOI: 10.1126/sciadv.aax9856

Risk factors for CMV disease in solid-organ transplant patients

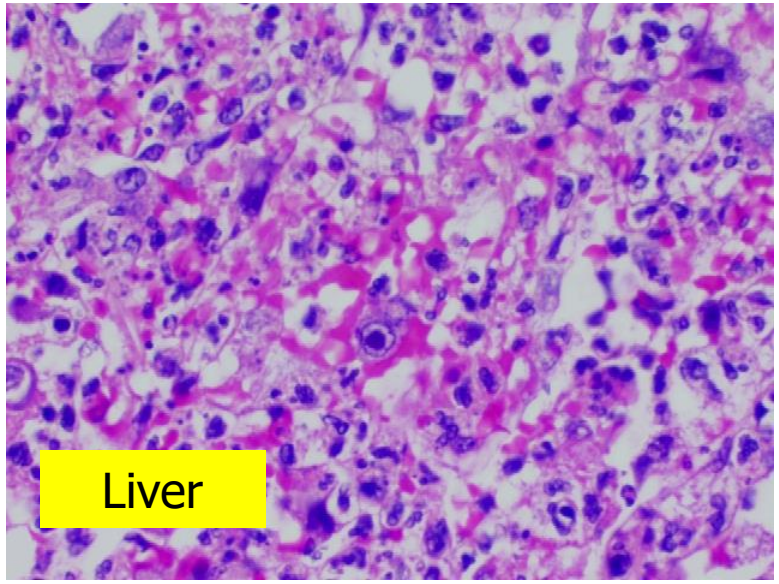
- Primary infection (D+/R–)
  - Transplanted organs, cells
  - Blood products

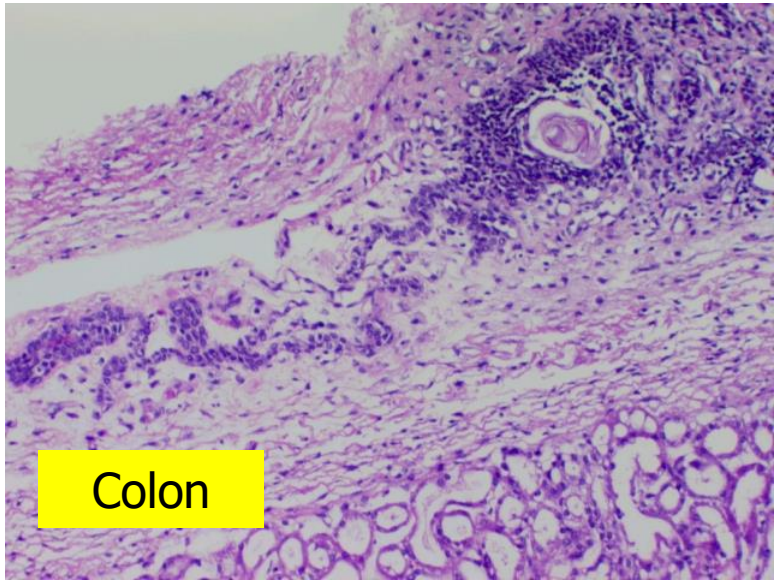
- Factors favoring CMV reactivation
  - Inflammation/Fever (cytokines)
  - Surgery/Trauma
  - Intraoperative hypothermia
  - Sepsis or severe bacterial infections
  - T-cell depletion
  - Co-infections with other viruses
    - Herpes virus 6 or 7 (HHV6 or 7)

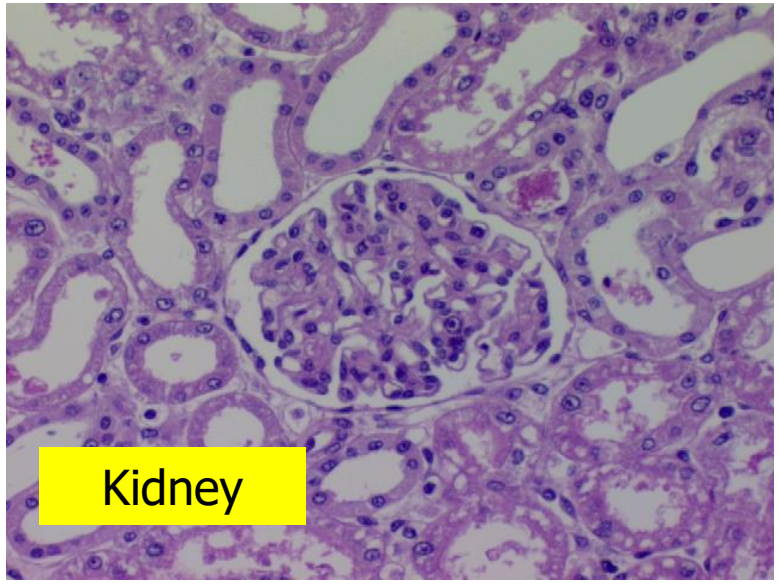
Factors favoring progression to invasive disease

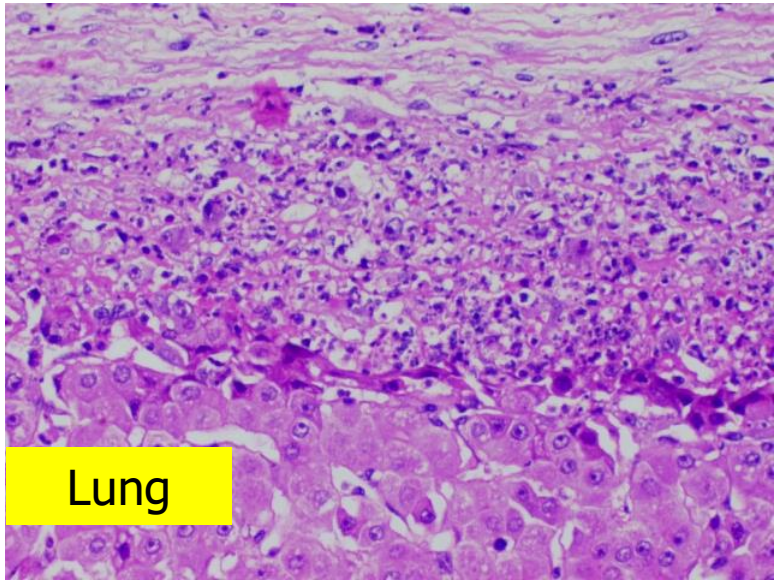
- Immunosuppression
  - **T-cell depletion**
  - Corticosteroid boluses
  - Alemtuzumab
  - High viral load
- Immunomodulation
  - Herpes virus 6 (HHV6) or HHV7
- Genetic factors
  - Mutations in TLR2 and TLR4 genes
  - Deficiency of mannose-binding lectin or genotype associated with low production of MBL

Disseminated CMV

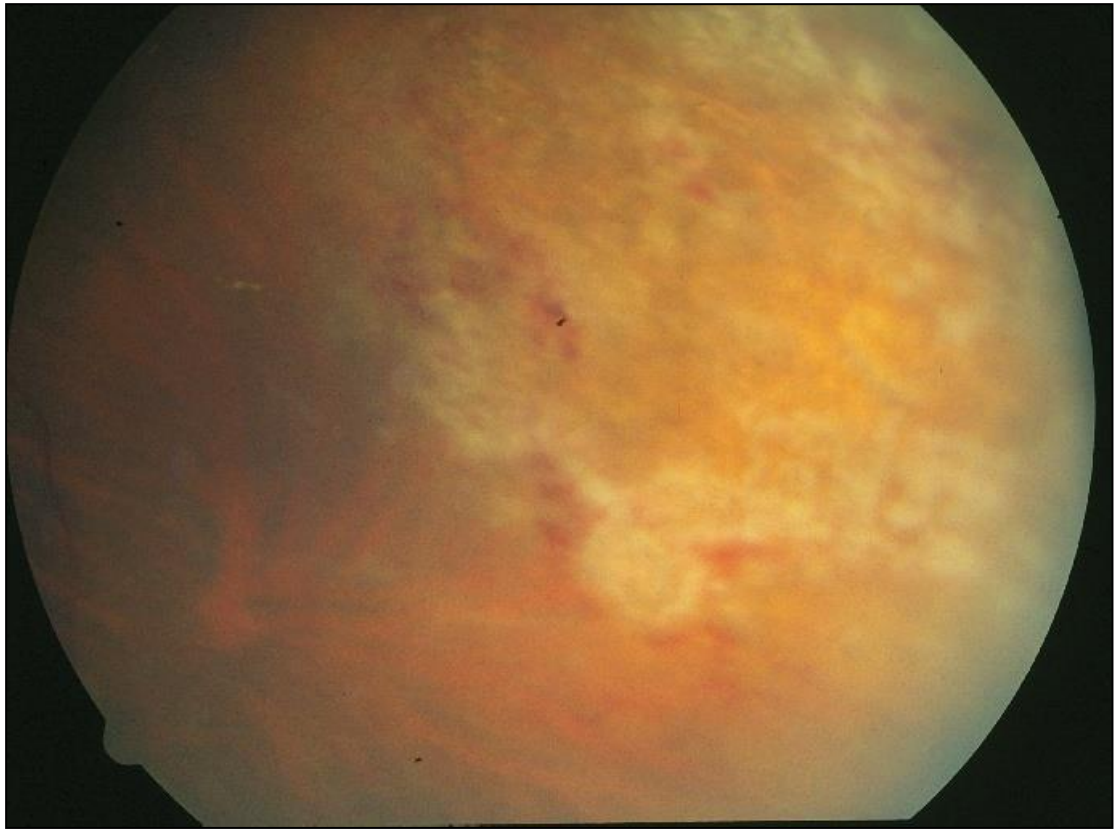




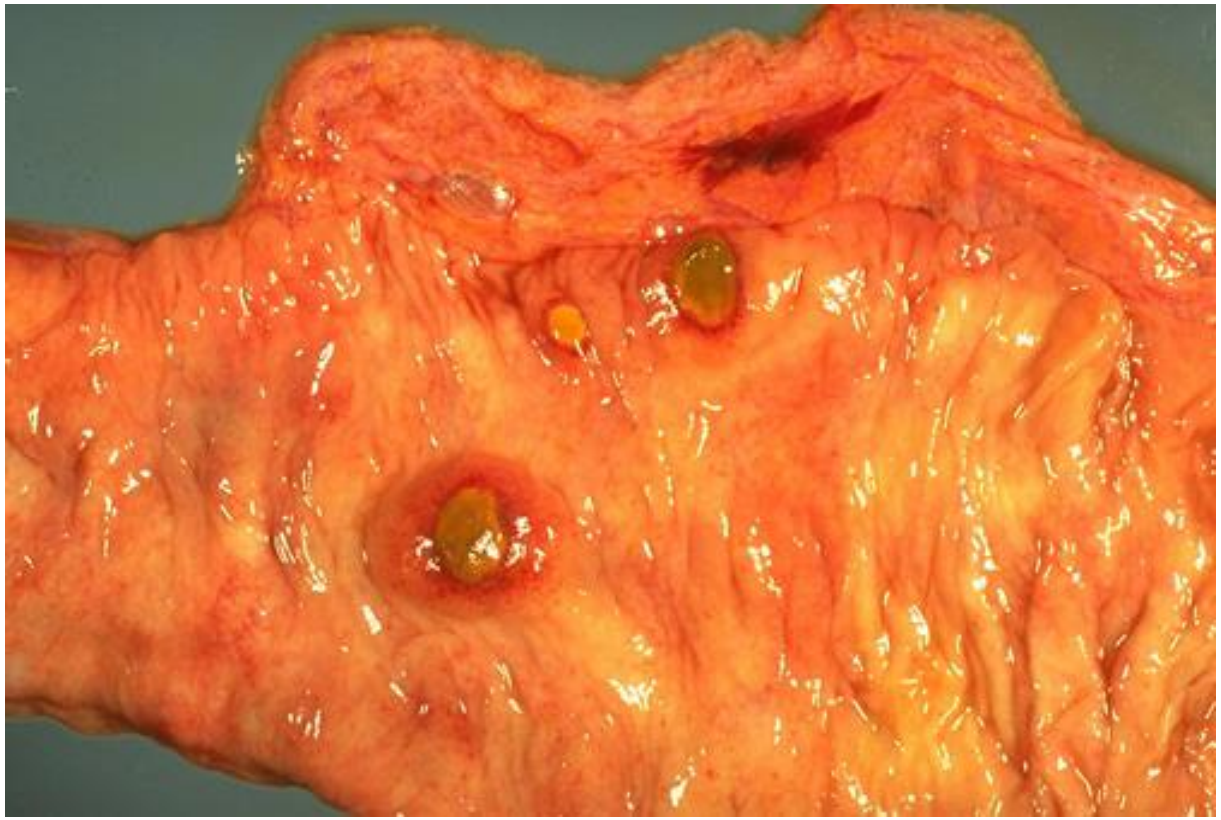




CMV Retinitis: Lung Transplant Recipient



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



Delayed-Onset CMV: CMV Quantitative Nucleic Acid Test (QNAT)

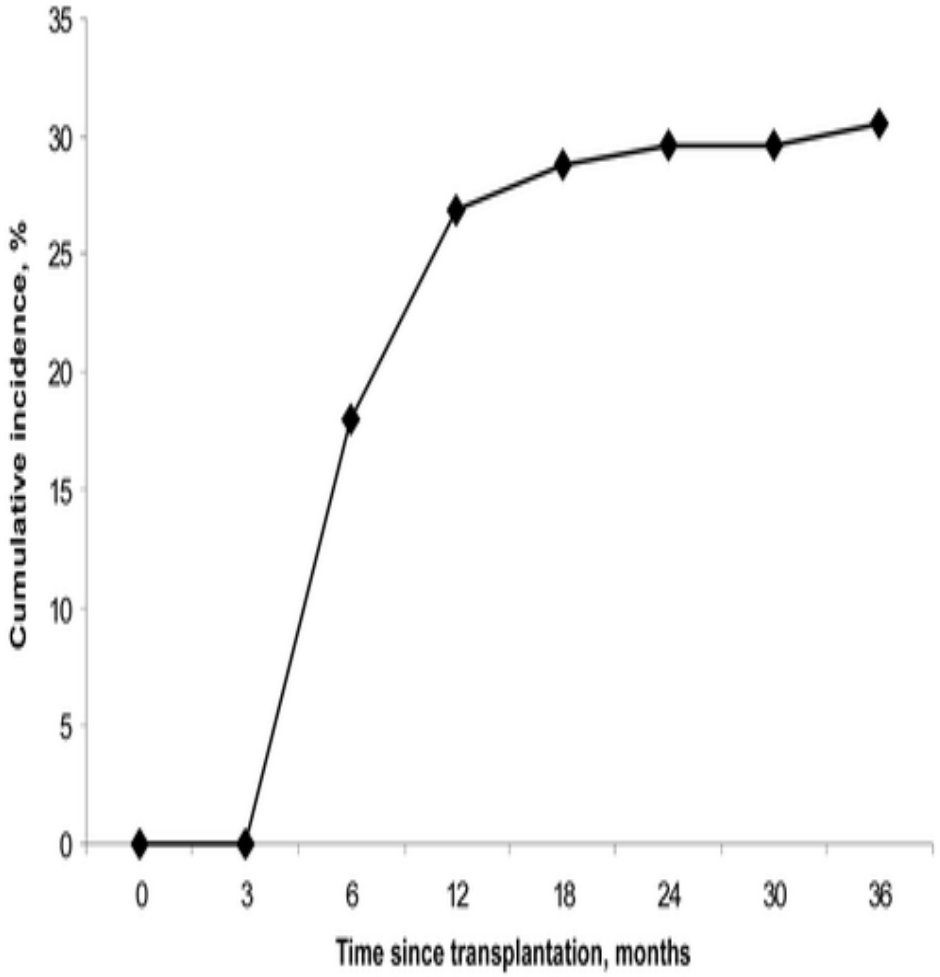


Table 2. Univariate Cox proportional hazard model for risk factors associated with delayed-onset primary cytomegalovirus disease after kidney transplantation.

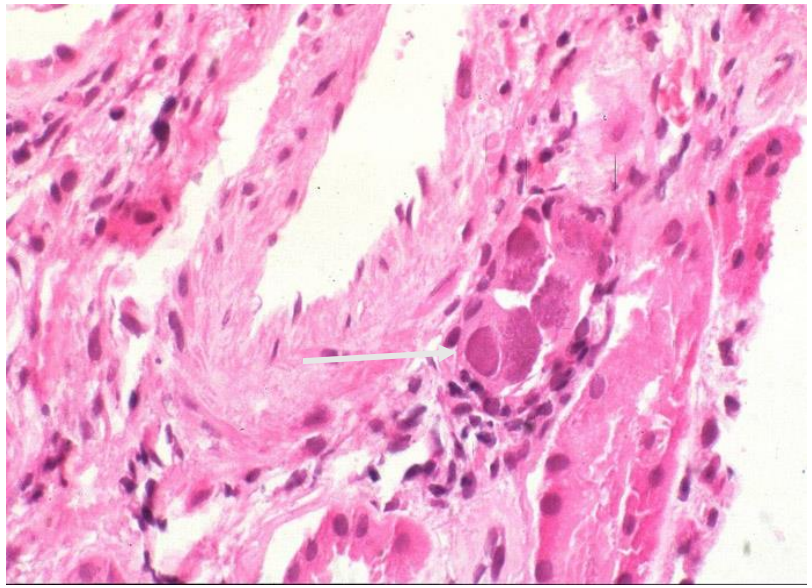
Risk factor	Hazard ratio (95% CI)	*P*
Age at time of transplantation	1.010 (0.989–1.032)	.339
Male sex	0.986 (0.555–1.752)	.963
Charlson comorbidity index (continuous variable)	1.049 (0.900–1.222)	.550
Charlson comorbidity index ⩾3	2.207 (1.155–4.218)	.017
Diabetes mellitus	0.820 (0.462–1.456)	.494
Induction immunosuppressive therapy		
Thymoglobulin	1.398 (0.714–2.734)	.328
Basiliximab	0.587 (0.211–1.634)	.308
Daclizumab	0.532 (0.0734–3.855)	.532
Combination of thymoglobulin, rituximab, intravenous immunoglobulin, and plasmapheresis	0.891 (0.353–2.248)	.808
Maintenance immunosuppressive therapy[a]		
Cyclosporine	0.580 (0.081–4.198)	.554
Sirolimus	0.908 (0.361–2.285)	.835
Tacrolimus	1.026 (0.438–2.406)	.951
Time of onset of bacterial infection after transplantation		
1 month	5.379 (2.386–12.125)	<.001
2 months	3.353 (1.608–6.992)	.001
3 months	1.845 (0.880–3.867)	.104
Time of onset of fungal infection after transplantation		
1 month	8.640 (1.144–65.275)	.034
2 months	3.859 (0.525–28.377)	.185
3 months	2.602 (0.356–19.046)	.346
Acute graft rejection	0.335 (0.120–0.933)	.036
Treated acute graft rejection[b]	0.292 (0.091–0.940)	.039

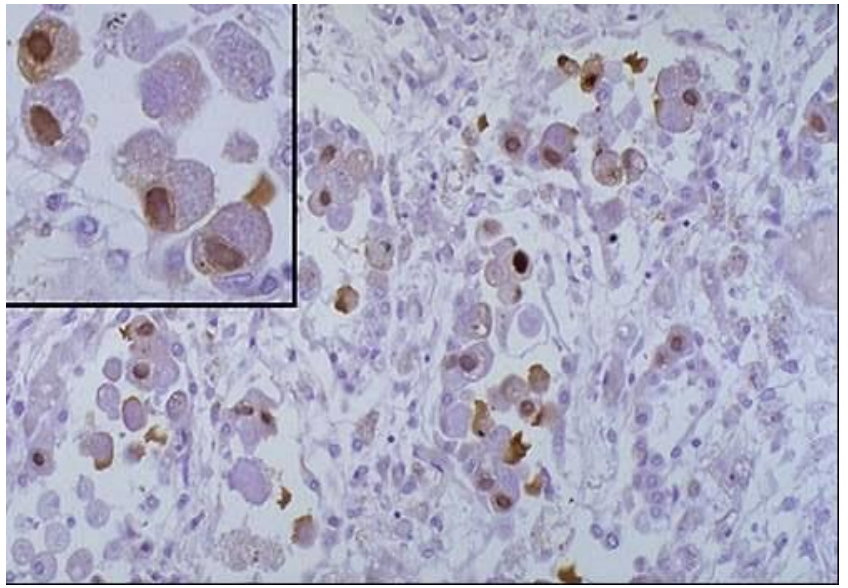
[a] Because almost every study subject was receiving mycophenolate mofetil and prednisone, these were not assessed for their association with delayed-onset primary cytomegalovirus disease.
[b] Treated acute graft rejection followed by 1–3 months of antiviral prophylaxis.

Arthurs *et al*. ***Clin Infect Dis***. 2008; 46: 840-846.

CMV Diagnostics

- Quantitative PCR (IU)
- CMV Antigen (in neutrophils): pp65
- Hybrid capture
  - Detects CMV DNA in leukocytes
  - Amplified signal
- Pathology



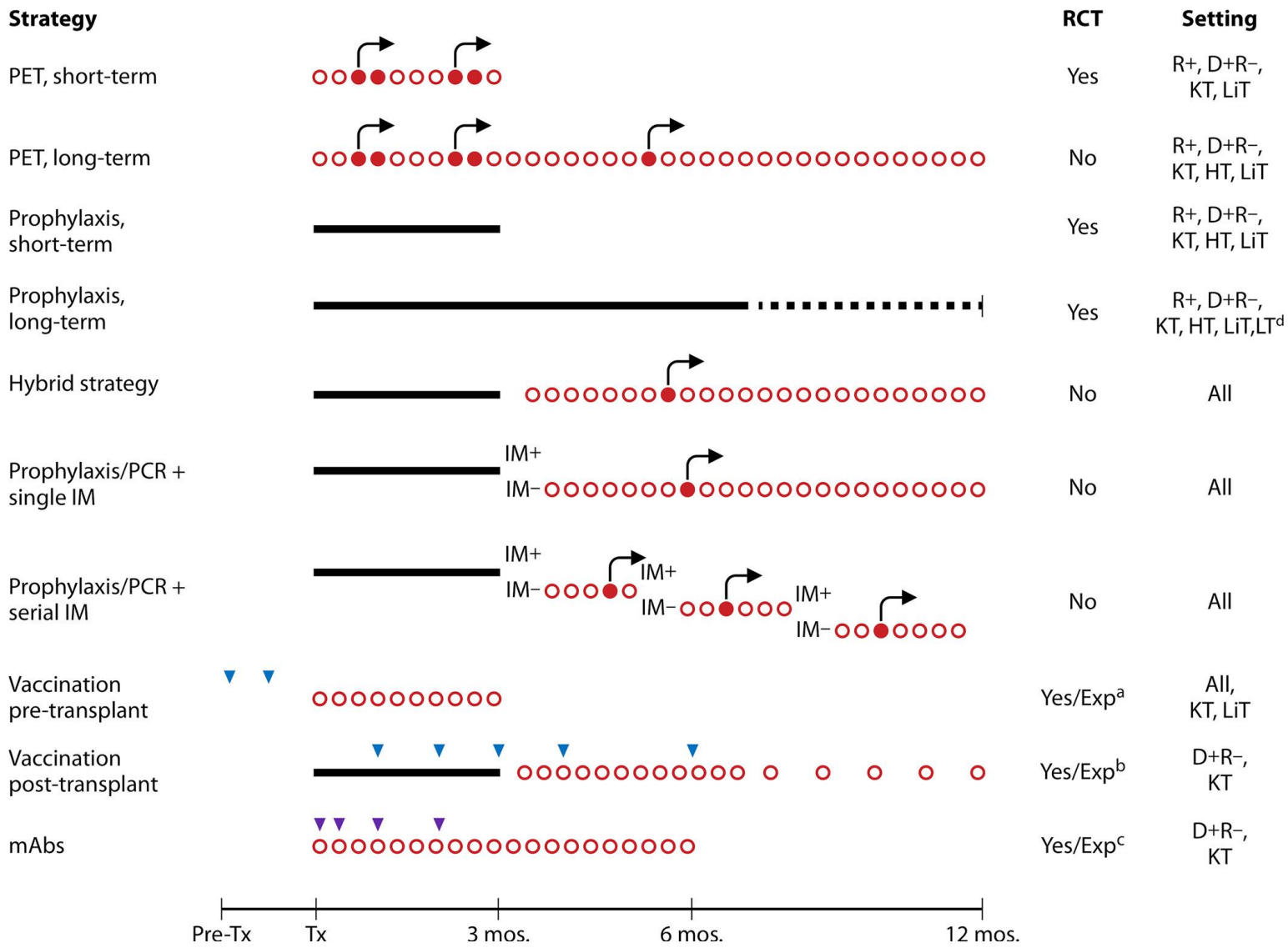


Do we know how to Prevent CMV Infection?
Universal vs. Pre-emptive therapy

CMV Prophylaxis Strategies

- ***Universal prophylaxis***
  - Administration of antiviral agents to all individuals at risk for a fixed duration
  - May increase cost, toxicity, risk of resistance
- ***Preemptive therapy***
  - Administration of antiviral therapy in response to a positive microbiologic assay or clinical scenarios
  - Requires careful monitoring, close patient contact, and use of highly sensitive, quantitative assay
  - 41% missed screening before onset
- ***Hybrid Approach***
  - Limited data to support the use of this approach

Limaye et al Clin Micro Rev 2021: 43

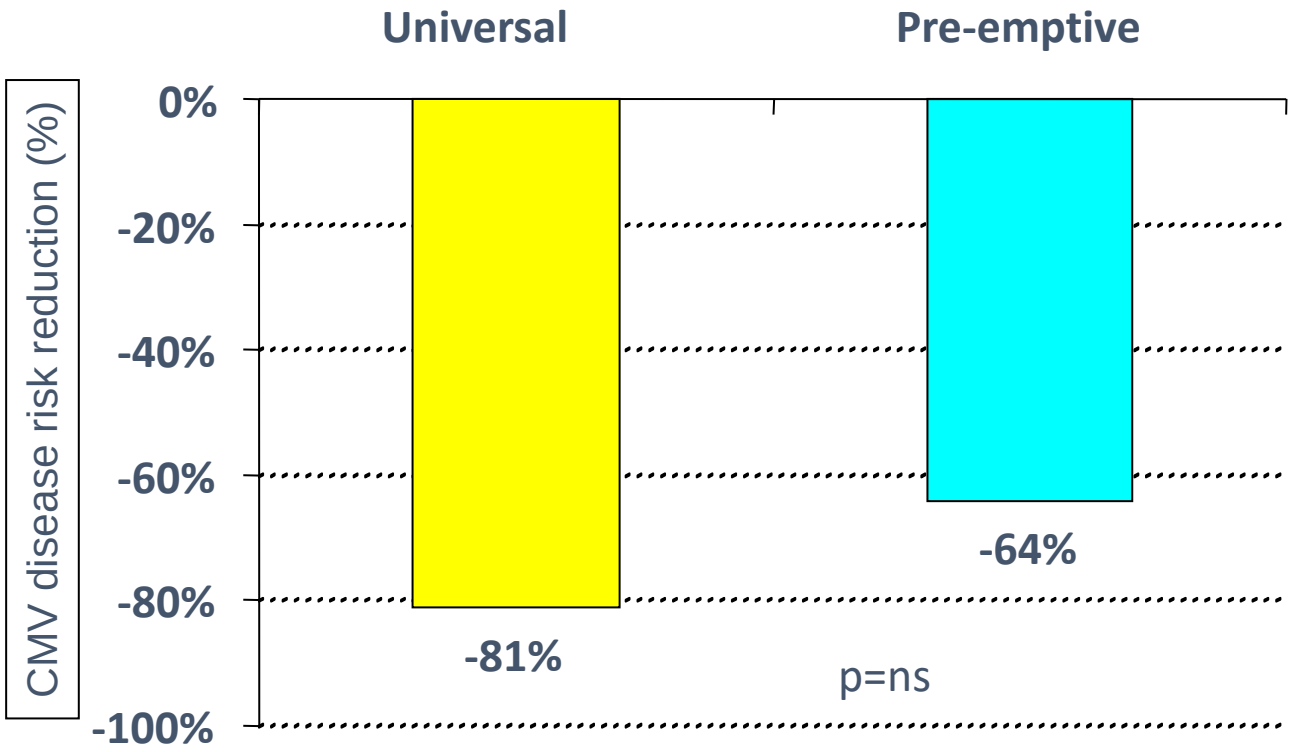


Current Recommendations for Duration of Prophylaxis

	Duration of universal prophylaxis
None	D-/R-, Low risk (any organs) SOTr
3 months	R+ (intermediate-risk) liver, kidney, pancreas
3-6 months	D+/R- (high-risk) Liver, Pancreas and heart SOTr R+ (intermediate-risk) Intestinal/composite tissue SOTr
6 months	D+/R- (High-risk) Kidney and Intestinal, composite tissue SOTs; intermediate-risk lung SOTr (up to 12 months)
6-12 months	D+/R- (High-risk) lung SOTs

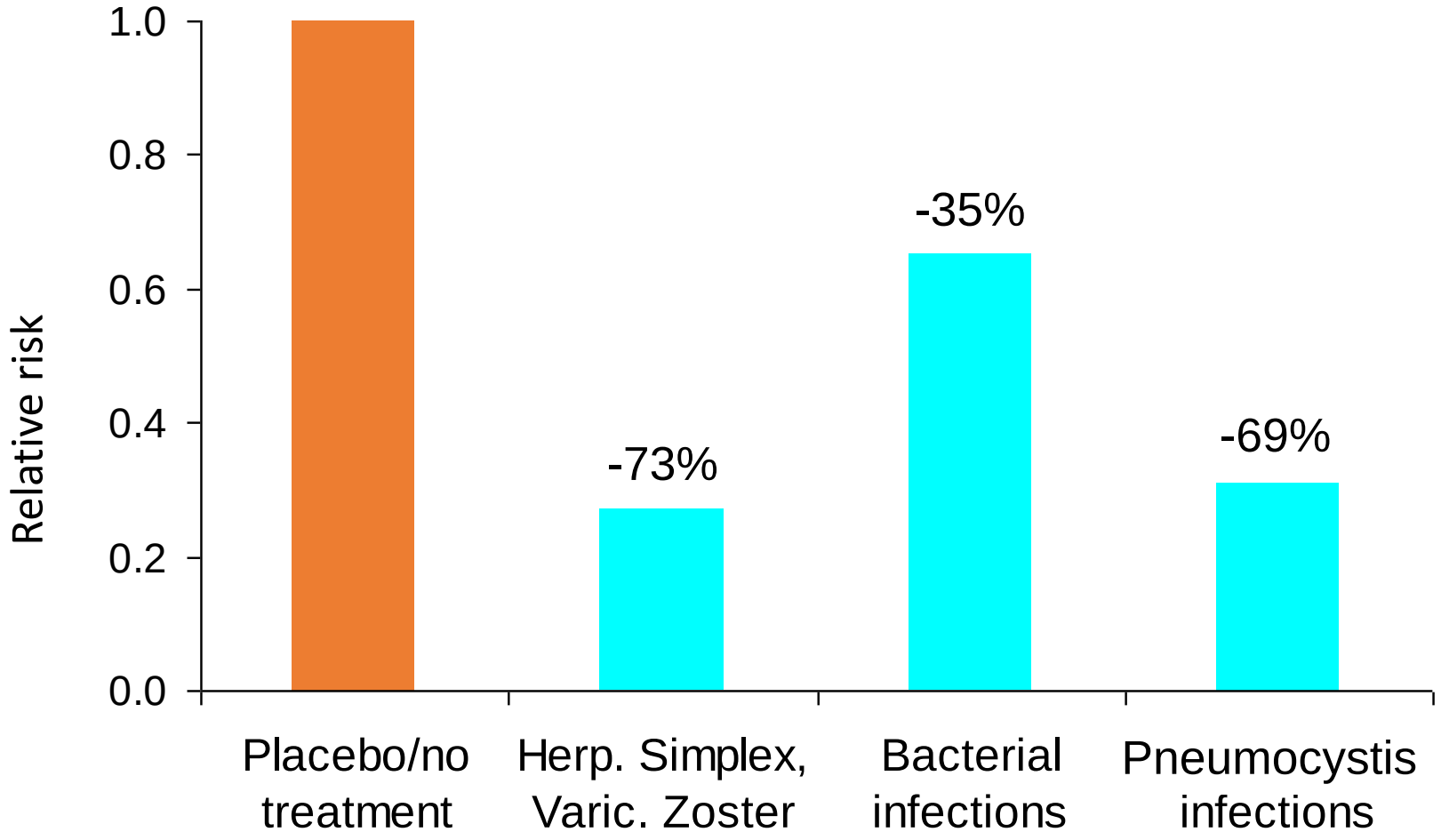
CMV disease in D+/R- renal recipients: Meta-analysis (all agents)

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



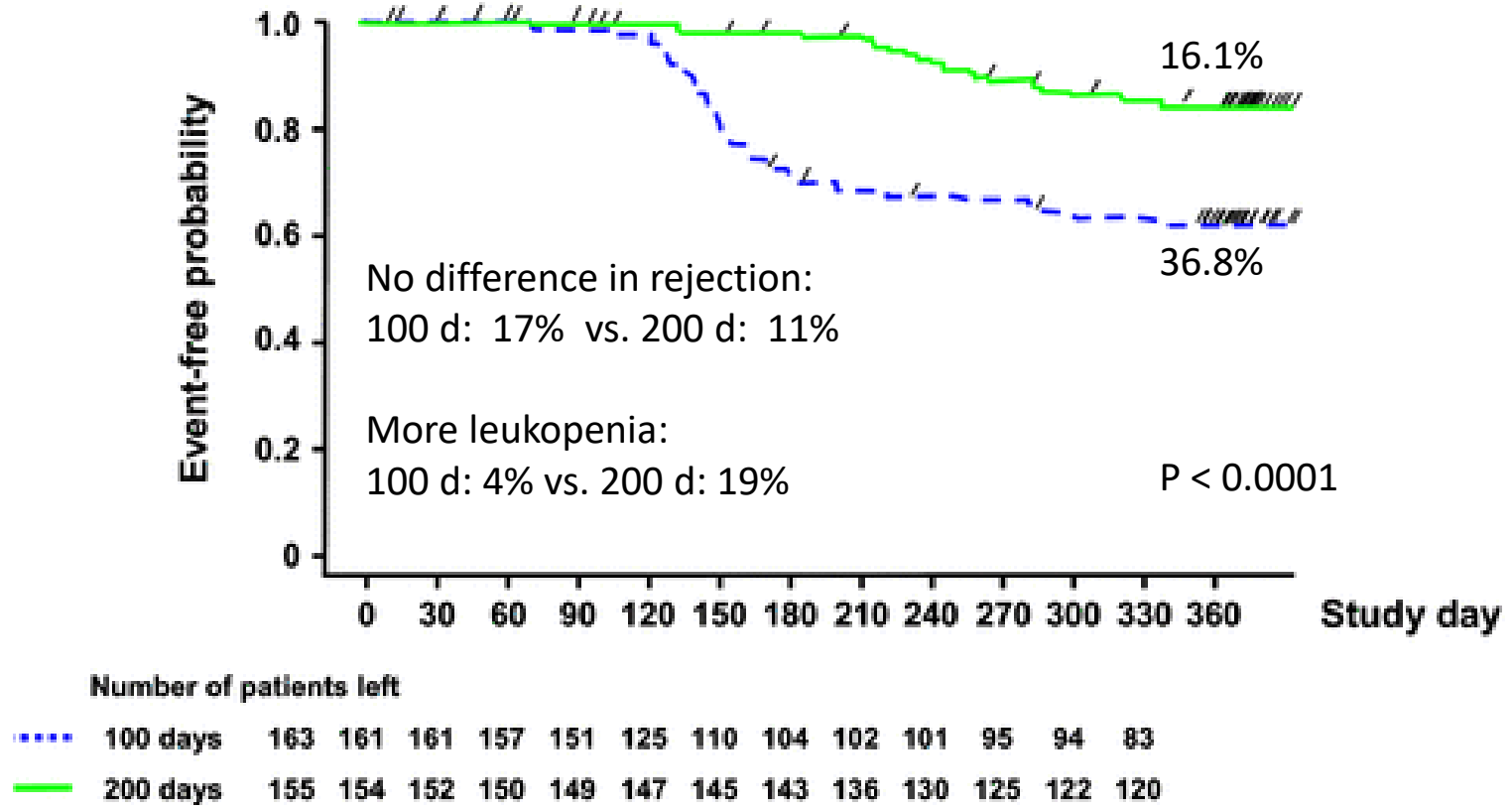
Kalil AC *et al. Ann Intern Med* 2005; 143: 870

Effect of anti-CMV prophylaxis on concomitant infections



Hodson EM *et al. Lancet* 2005; 365: 2105

Prophylaxis: *100 vs. 200 Days of Valganciclovir*

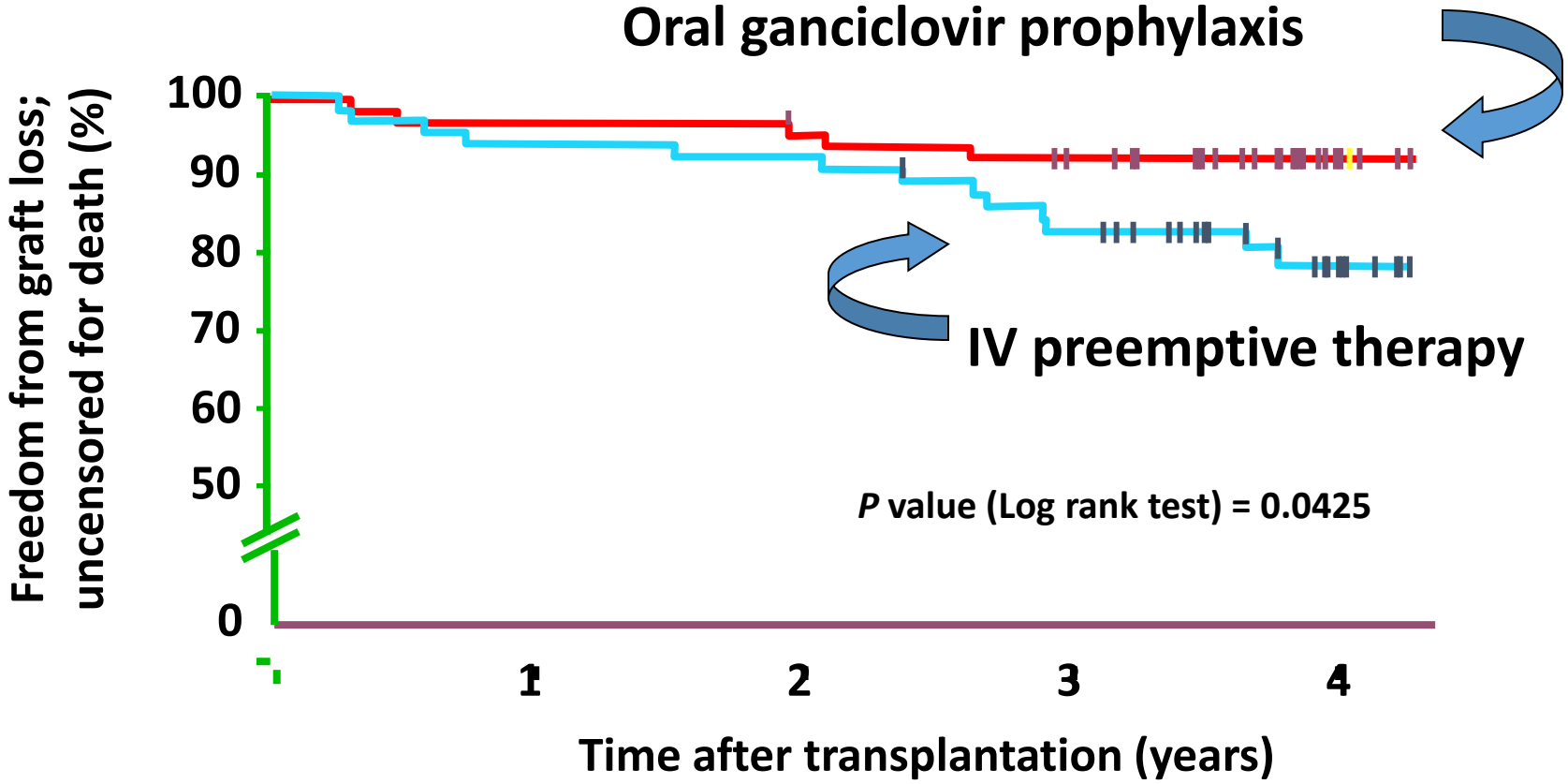


Study: CMV D+/R- Renal Transplant Recipients

Humar *et al*. ***Am J Transplant***. 2010; 10: 1228-1237.

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)

Preemptive therapy in CMV high risk (D+/R-) liver recipients

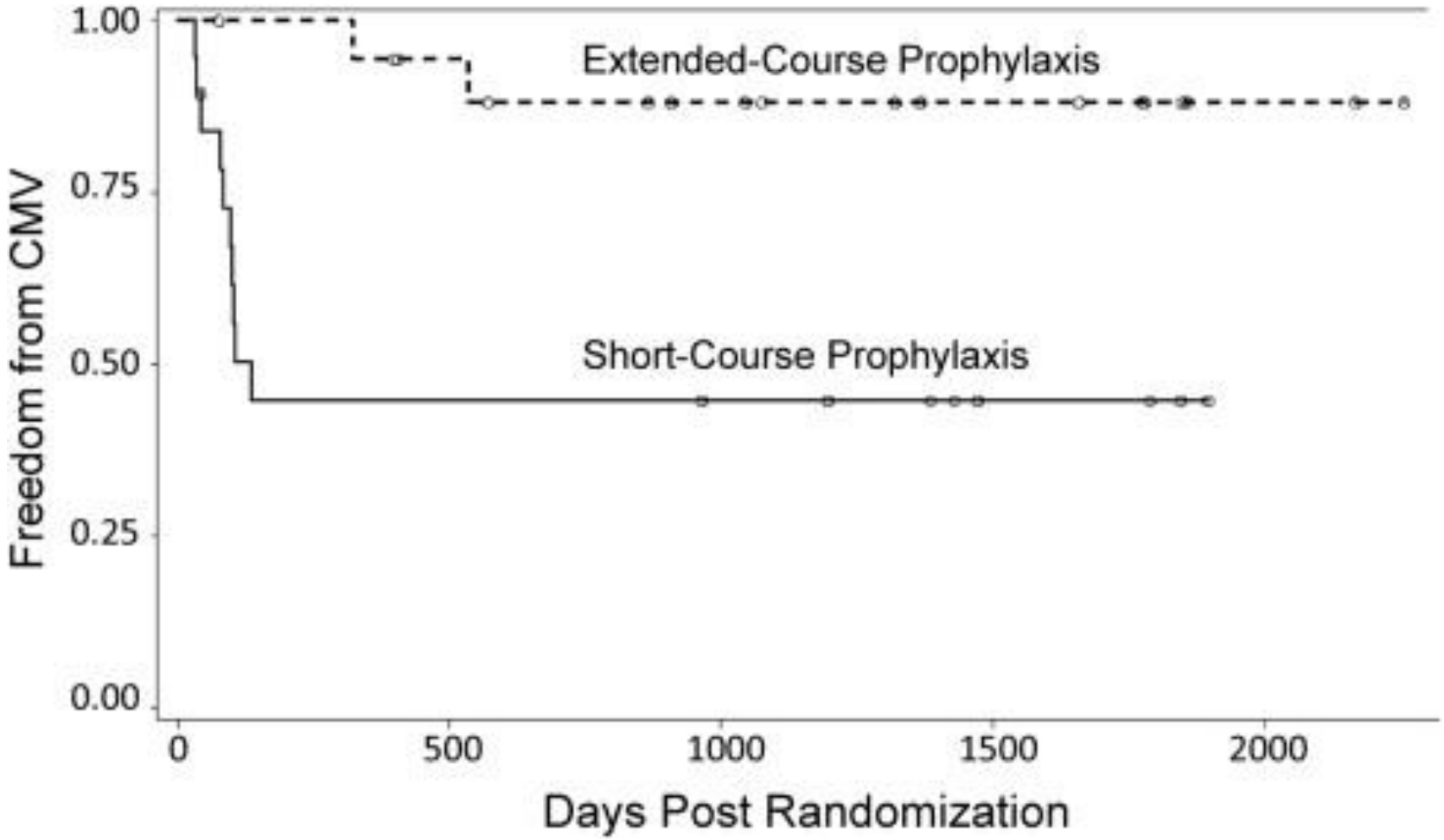
KM Doss et al. Transplant Inf Dis Feb 2023

- Retrospective analysis in 50 liver recipients at single center vs 100 preemptive recipients in CAPSIL study
- The cumulative incidence of CMV disease at 1-year post-transplant was 4/50 (8%) versus 9/100 (9%) in the real-world and CAPSIL cohorts, respectively, p = 1.0.
- The rate of breakthrough CMV disease during the 100-day PET period was low (2/50 [4%]) and similar to the PET cohort from the CAPSIL study (3/100 [3%]).
- All secondary and exploratory outcomes were not significantly different between the real-world and CAPSIL PET cohorts.

Immune Assays to Guide Duration of Prophylaxis?

- **Serology:** Not generally useful in immunosuppressed transplant recipient (and observe seroconversion in R- recipients).
- **CMV-specific T cell assays (Expensive and limited availability; may not cover all HLA types, utility remains uncertain)**
  - **Quantiferon-CMV:** γ-interferon (IFN-γ) produced by CD8+ T-cells (only) stimulated with CMV antigens.
  - **Intracellular cytokine staining (ICS): (**T-cell CD4+ and CD8+) flow cytometry (often unavailable) for surface immunophenotyping and intracellular cytokines
  - **Elispot:** T cell (CD4+ and CD8+) IFN-γ production with CMV antigens (e.g., pp65 and IE-1).
  - **MHC-multimers:** CD8+ T-cell flow cytometric determination.

Prolonged Prophylaxis: *Lung Transplantation*



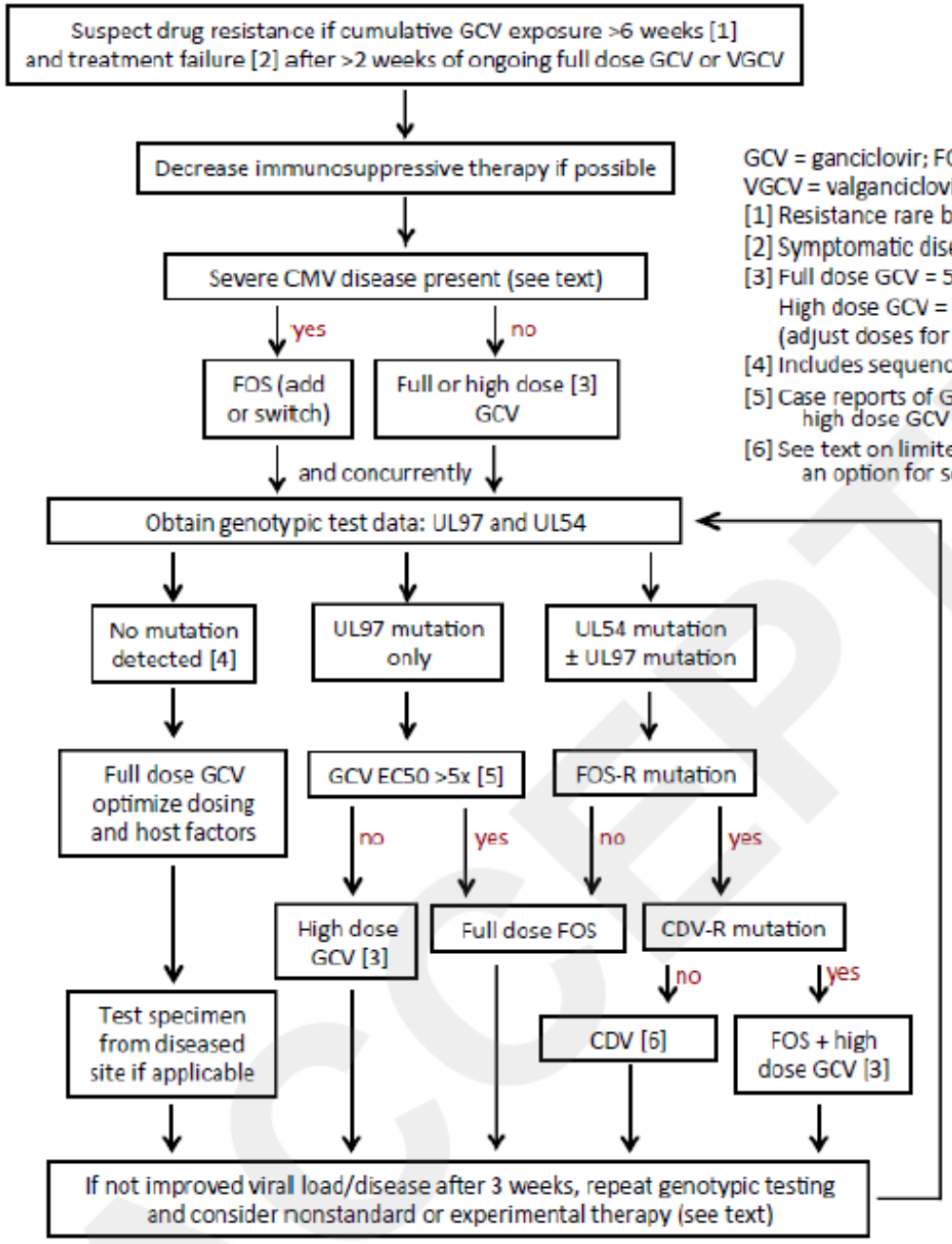
Finlen Copeland *et al*. ***J Heart Lung Transplant***. 2011;30:990-996.

Therapeutic Options

Current Therapeutic Advantages and Limitations: Ganciclovir/Valganciclovir

- **Advantages**
  - Experience
  - Efficacy
    - Prophylaxis/treatment
  - No drug interactions
  - Low pill burden
  - Covers CMV, HSV, varicella, other herpes viruses
  - IV and oral formulations
- **Limitations**
  - Leukopenia
  - Need for renal dosing
  - Cost
  - Reduces/doesn't eliminate risk of disease (after end of prophylaxis)
  - ≈1% SOT patients with CMV infection develop GCV resistance, with ⇧morbidity, mortality

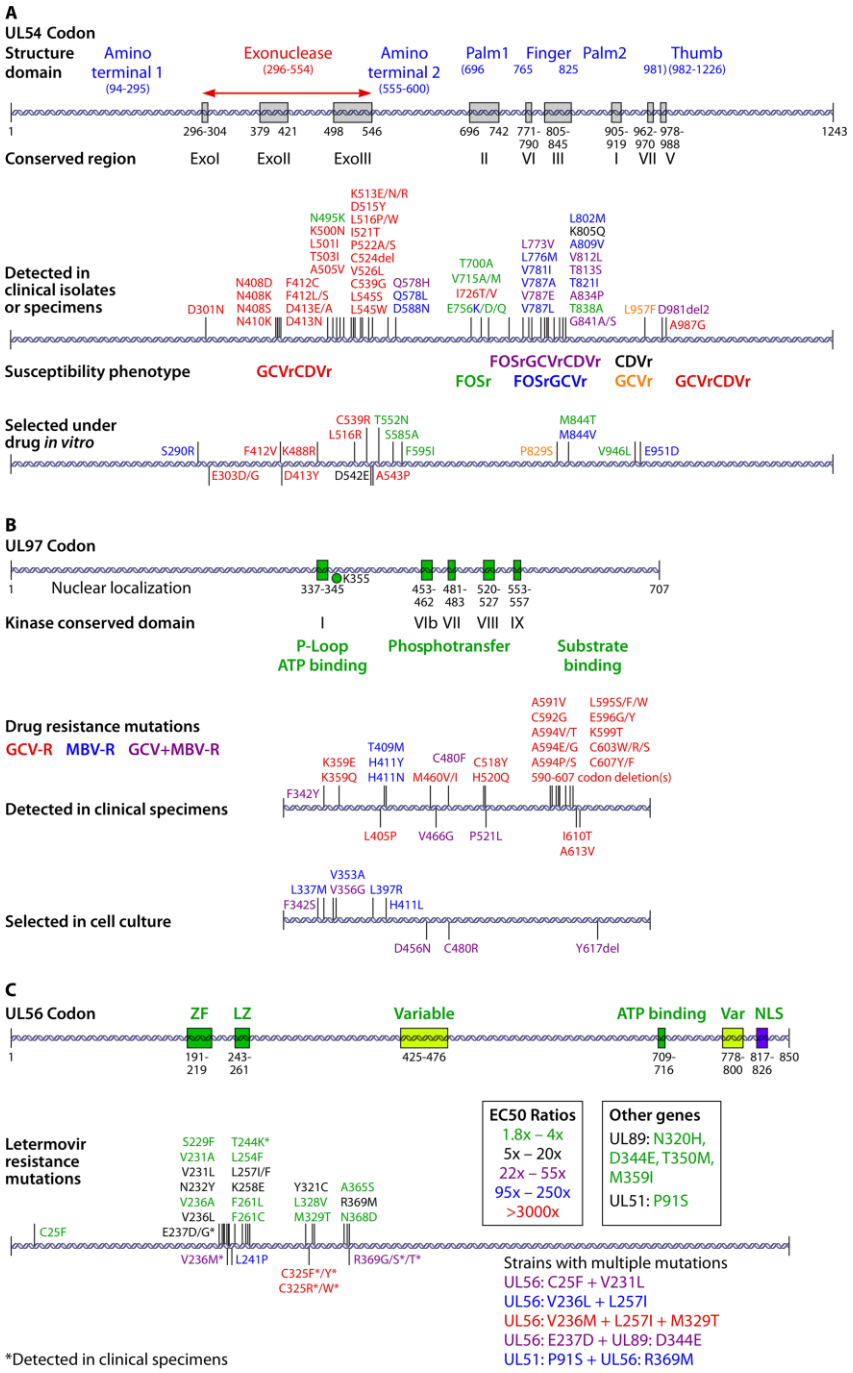
GCV, ganciclovir; IV, intravenous; SOT, solid-organ transplant.



With Clinical CMV Resistance

- UL97 kinase – increase **GCV** (6.5-10mg/kg/day) or **foscarnet** (watch Mg+, seizures, iv only)
- UL54 polymerase –usually include resistance to other drugs → **foscarnet or cidofovir (iv only, renal toxicity)**
- Pan-resistance – new drugs?
- + CMV Immune globulin?
- + Leflunomide (LFTs, Levels)
- Combination (GCV/Fos)
- Atesunate?

Drug resistance-associated mutations in CMV genes



Outcomes in Transplant Recipients Treated With Foscarnet for Ganciclovir-Resistant or Refractory Cytomegalovirus Infection

Robin K. Avery, MD,[1] Ravit Arav-Boger, MD,[2] Kieren A. Marr, MD,[1] Edward Kraus, MD,[3] Shmuel Shoham, MD,[1] Laura Lees, PharmD,[4] Brandon Trollinger, PharmD,[4] Pali Shah, MD,[5] Rich Ambinder, MD,[6] Dionysios Neofytos, MD,[1] Darin Ostrander, PhD,[1] Michael Forman, BS,[7] and Alexandra Valsamakis, MD, PhD[7]

TABLE 4.

Studies published after the year 2000, reporting outcomes of 6 or more transplant recipients treated with foscarnet for established CMV infection

Study	Year/center	Patients	Total, n	Deaths by 1 y	Renal dysfunction end of FOS	Renal dysfunction long term
Current study	2015 Johns Hopkins	FOS-treated R/R SOT + HCT	39 (all FOS)	12/39 (31%)	20/39 (51%)	7/25 (24%) at 6 mo
Pierce et al[21]	2015 Northwestern	FOS-treated R/R SOT	31 (all FOS)	10/31 (32%)	5/21 (24%)	3/21 (14%)
Fisher et al[20]	2014 University of Washington	GCV-R SOT	38 cases, 110 controls	8/38 (21%)	NR	15/37 (41%) at 3 mo
Minces et al[19]	2014 University of Pittsburgh	GCV-R lung transplant	16 (14 FOS)	5/16 (31%)	10/14 (71%)	NR
Myhre et al[17]	2011 Oslo University	GCV-R kidney transplant	27 (10 FOS)	2/10 (20%)	NR	NR
Asakura et al[16]	2010 Nagoya University	FOS-treated HCT	65 CMV disease (all FOS)	45/65 (69%)	3%[a]	NR
Reddy et al[15]	2007 Duke University	GCV-R lung transplant	6 (all FOS)	1/6 (17%)	2/6 (33%)	0/6 (0%)
Isada et al[2]	2002 Cleveland Clinic	GCV-R SOT	13 (10 FOS)	9/10 (90%)	NR	NR[b]

Avery RK, et al. *Transplantation*. 2016;100(10):e74-e80.

CMV Newer Options – the basics

- **Maribavir** (UL97 – viral maturation and egress)
  - Failed in liver SOT and HSCT Prophylaxis (but low dose)
  - Mixed results in therapy - suppressed noninvasive infection SOT, many relapses in GCV-resistance study – approved for unresponsive infection to ganciclovir
    - Effective in small trials at higher doses but **relapse occurred ~37%** (largely while on therapy)
  - **Does not cover HSV/VZV**
  - Unique resistance mutations in UL97 (not cross reactive with GCV) – *antagonistic to GCV*
- **Letermovir** (viral terminase) UL56, oral and intravenous (studied in HSCT)
  - **Prophylaxis only trials**
  - **Does not cover HSV/VZV**
  - Easy resistance in vitro / *Drug interactions* with CyA, tacrolimus, voriconazole, others
  - Activity for treatment is unknown.
- CMX001 (**Brincidofovir**) lipid cidofovir prodrug (oral only), covers herpesviruses
  - **GI toxicity**
  - Iv under development; no longer available
  - Expected UL54 mutations (like cidofovir)

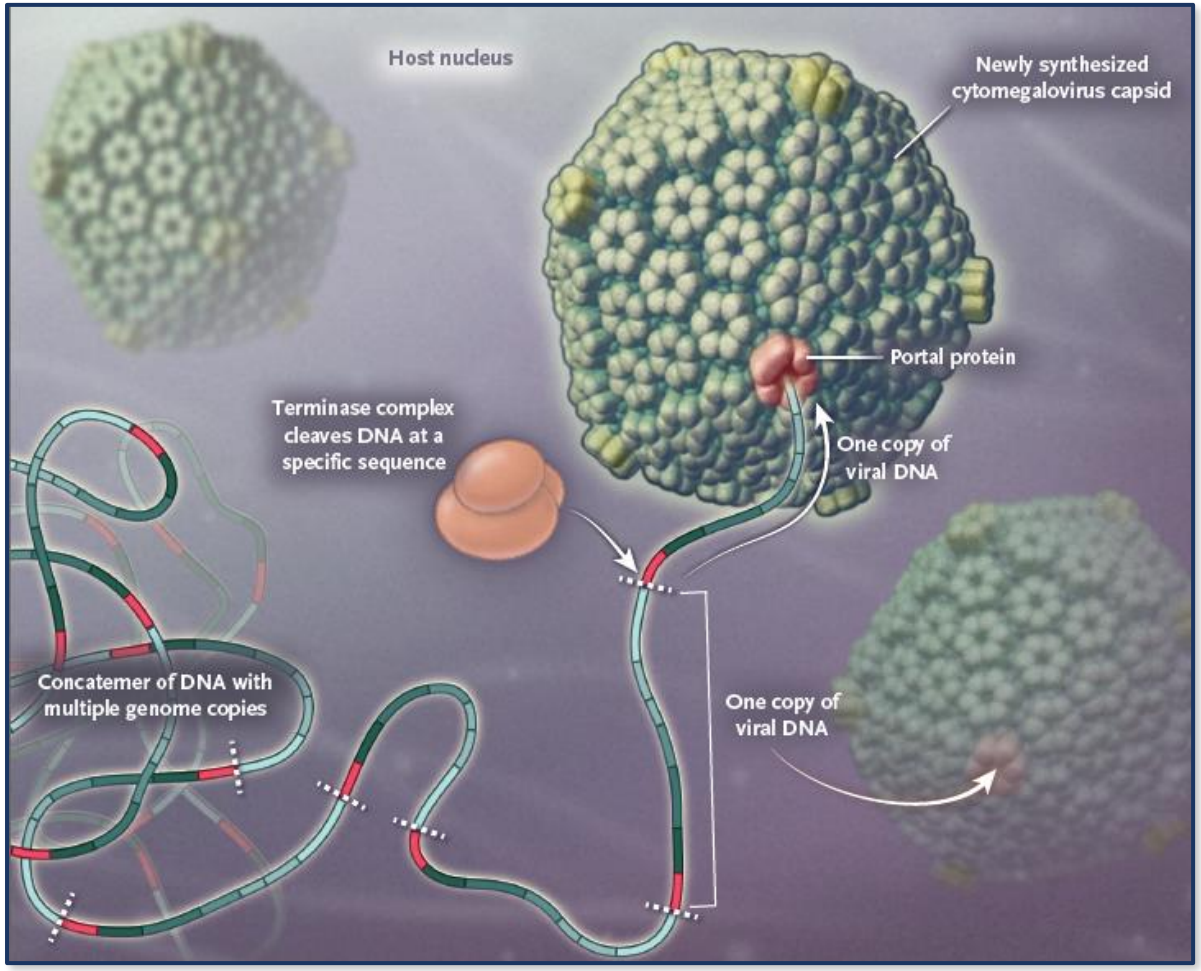
Maribavir (oral)

- UL97 kinase inhibitor of ATP binding to pUL97
  - Elicits a different set of UL97 mutations vs. GCV, clustered around ATP binding site
  - No overlap with ganciclovir-resistant mutations though in same regions
- Mechanistically, pUL97 is required for GCV phosphorylation – **antagonistic to GCV and cannot be coadministered**
- Antiviral in vitro vs. CMV & EBV (but **not** for HSV1/2, VZV, HHV-6, HHV-8 → Acyclovir)
- Possible synergy with foscarnet, cidofovir, letermovir
- Nausea, diarrhea, dysgeusia
- **Increased levels of cyclosporine and tacrolimus**
- **Failed to demonstrate efficacy for CMV prophylaxis in D+/R- liver transplant reciepients**

Piret J et al. Antiviral Res 2019163:91-105; Chou S,. AAC 2015, 59:6588-93; O'Brien MS et al. Antiviral Res 2018, 158:255-63

Letermovir (oral or iv)

- Terminase complex inhibitor
  - Binds at pUL56
- Generally good safety profile
- CMV only activity
  - Does not inhibit HSV1/2, VZV, HHV-6,7,8, EBV
- Mortality benefit (prevention may not persist)
- Low barrier to High-grade resistance in UL56 or UL89 (not polymerase) terminase gene; clinical correlation needed (not UL97/UL54)
- **Increased levels of letermovir with cyclosporine, tacrolimus, sirolimus**
- **Decreases levels of voriconazole (not other azoles)**



Griffiths PD, et al. *N Engl J Med*. 2014;370(19):1844-1846;
RF Chemaly et al, N Engl J Med 2014; 370:1781-1789.

CyA, cyclosporine A; VZV, varicella zoster virus.

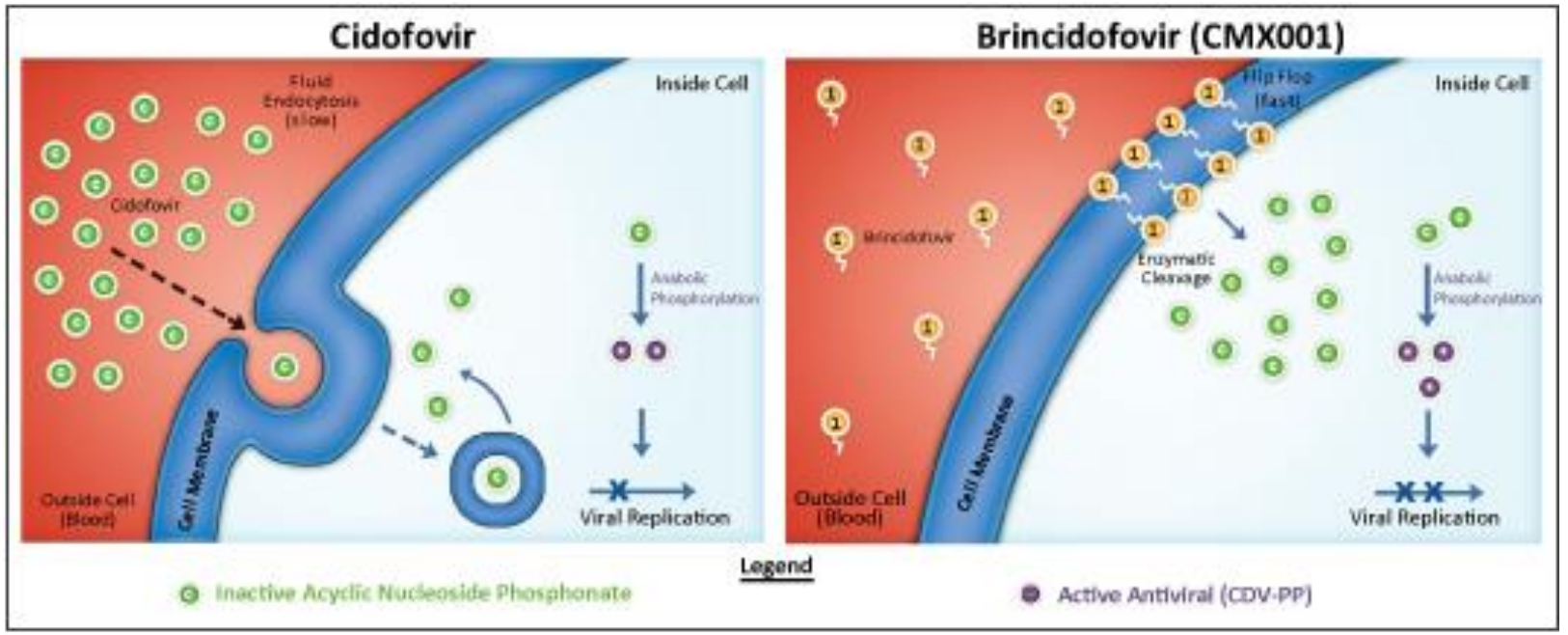
Letermovir vs Valganciclovir – Renal (D+/R-)

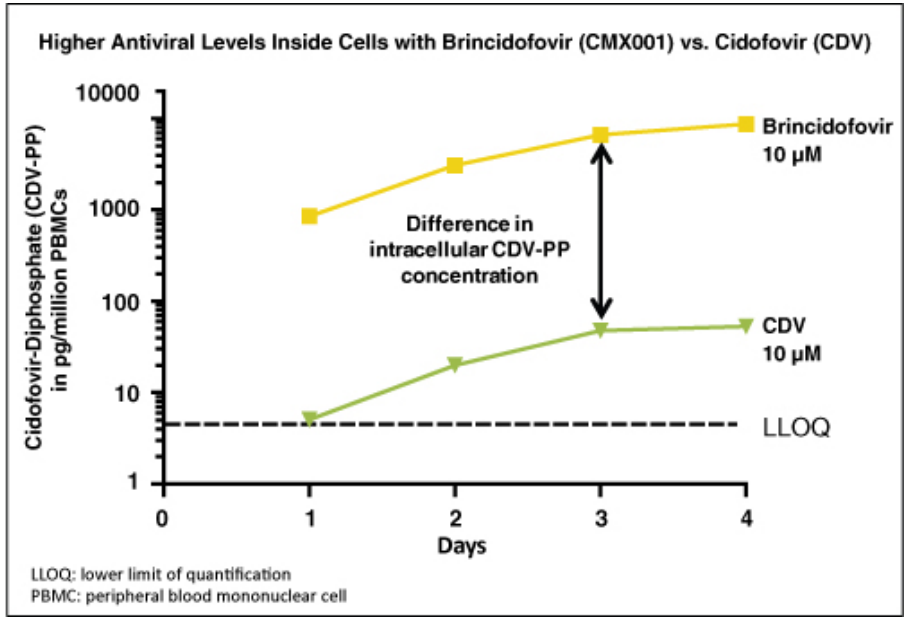
- Prospective, randomized, blinded trial of letermovir + acyclovir (for HSV, VZV) in 601 renal recipients
- Letermovir (n = 289) was noninferior to valganciclovir (n = 297) for prevention of CMV disease through week 52 (10.4% vs 11.8% with CMV disease)
- No participants who received letermovir vs 5 participants (1.7%) who received valganciclovir developed CMV disease through week 28.
- Time to onset of CMV disease was comparable between the groups
- DNAemia was detected in 2.1% of participants in the letermovir group vs 8.8% in the valganciclovir group by week 28. Of these, none (0/52) who received letermovir and 12.1% (8/66) who received valganciclovir had resistance-associated substitutions.
- The rate of leukopenia or neutropenia through week 28 was lower with letermovir vs valganciclovir (26% vs 64%; difference, −37.9% [95% CI, −45.1% to −30.3%]; P < .001)

AP Limaye et al. JAMA 2023. AMA. doi:10.1001/jama.2023.9106
Published online June 6, 2023

Brincidofovir

- Lipid conjugate of cidofovir
- In vitro antiviral activity against all 5 families of dsDNA viruses that cause human disease:
  - Herpesviruses
  - Adenoviruses
  - Polyomaviruses (eg,BK virus)
  - Papillomaviruses
  - Orthopoxviruses

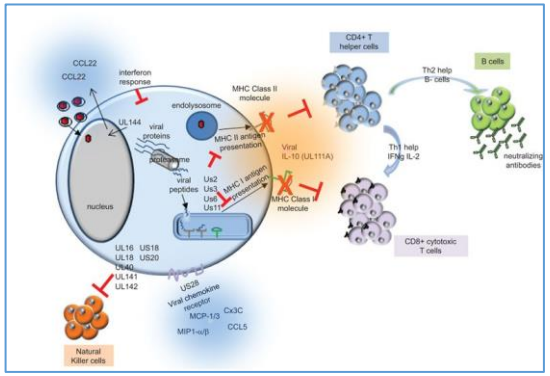




Registration Announcement: Chimerix, Inc. https://www.sec.gov/Archives/edgar/data/1117480/000114420413054339/v356753_s1.htm

Further Challenges and Options

- Reduce immunosuppression (risks rejection as viral load drops)
  - CMV CMI assay, quantify CD4 and CD8 anti-CMV activity
- CMV Ig (hyperimmune globulin) or IgG globulin replacement: ganciclovir intolerant, prolonged leukopenia/neutropenia, refractory disease, hypogammaglobulinemia
  - **CMV-HIG preparations carry higher CMV binding activity and higher neutralizing activity**.
  - **CMV immunoglobulin (CMVIG) preparations have immunomodulatory effects**
- Monoclonal antibodies – some efficacy
- Difficult-to-control CMV with belatacept (see Karadkhele et al , pubmed.ncbi.nlm.nih.gov/32519434; Chavarot et al, pubmed.ncbi.nlm.nih.gov/33283406)
- mTOR inhibitors (sirolimus) in place of CNI
- Combination therapy (limited studies)
  - Foscarnet + ganciclovir (1/2 dose?)
  - Ganciclovir → maribavir
- Cytotoxic T-cell therapy



- *Purging of latent virus* using inhibitors of bromodomains (histone binding domains) → activation of early antigen without viral replication allows targeting of viral epitopes (IJ Groves et al. PNAS 2021 118 (9): e2023025118).

American Journal of Transplantation 2015; 15: 827–832
Wiley Periodicals Inc.

doi: 10.1111/ajt.13023

Case Report

Adoptive T Cell Immunotherapy for Treatment of Ganciclovir-Resistant Cytomegalovirus Disease in a Renal Transplant Recipient

N. Macesic[1], D. Langsford[2], K. Nicholls[2], P. Hughes[2], D. J. Gottlieb[3], L. Clancy[4], E. Blyth[3,5], K. Micklethwaite[4], B. Withers[3], S. Majumdar[1], S. Fleming[6] and J. Sasadeusz[1,*]

Novel autologous T-cell therapy for drug-resistant cytomegalovirus disease after lung transplantation

The Journal of Heart and Lung Transplantation, Vol 35, No 5, May 2016

Paola Pierucci, MD (Hons), MSc,[a]
Monique Malouf, MBBS, FRACP,[a]
Allan R. Glanville, MBBS, MD,[a]
Leone Beagley, BSc (Hons),[b]
Corey Smith, BSc (Hons), PhD,[c] and
Rajiv Khanna, BSc (Med), MSc, PhD[c]

American Journal of Transplantation 2009; 9: 1679–1684
Wiley Periodicals Inc.

doi: 10.1111/j.1600-6143.2009.02672.x

Brief Communication

Adoptive T-Cell Therapy of a Lung Transplanted Patient with Severe CMV Disease and Resistance to Antiviral Therapy

Thank you!!

If I can help:
Fishman.jay@mgh.harvard.edu