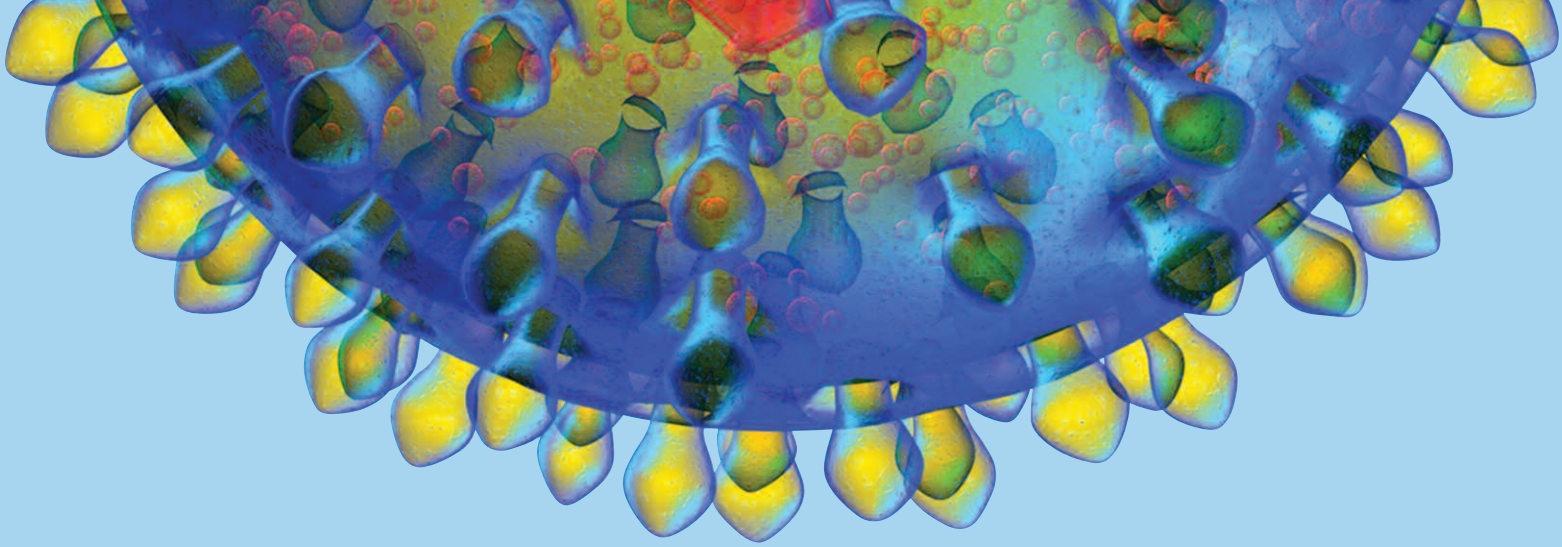


A CONTINUING MEDICAL EDUCATION ACTIVITY

CMV Disease in Transplant Recipients:

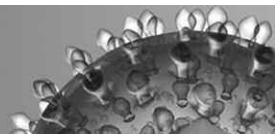
Strategies, Challenges and Opportunities

Jointly sponsored by Robert Michael Educational Institute LLC and Postgraduate Institute for Medicine

Supported by an educational grant from ViroPharma Incorporated

CONTENTS

WELCOME



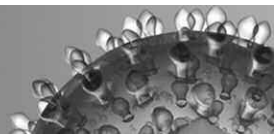
Thank you for joining us for **CMV Disease in Transplant Recipients: Strategies, Challenges and Opportunities**, a continuing medical education symposium presented during the 47th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy.

We would also like to thank our esteemed speakers for sharing their time and expertise. Through this program, they will address the risk factors for cytomegalovirus (CMV) in hematopoietic stem cell transplant (HSCT) recipients, the clinical features of transplant patients with CMV, and challenges and therapeutic strategies for managing CMV infection and drug resistance.

This workbook includes the presenters' slides to help guide you through the program. If you would like to receive 2.5 continuing education contact hours, please complete the Evaluation form.

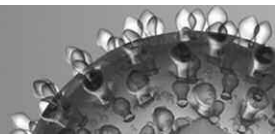
We hope that you will find this program rewarding and informative.

PROGRAM AGENDA



5:00 PM to 5:30 PM	Registration and Dinner Buffet
5:30 PM to 5:35 PM	Welcome *Robert M. Colleluori* *President, CEO* Robert Michael Educational Institute LLC
5:35 PM to 6:05 PM	Overview of CMV Infection in Transplant Recipients *Robert H. Rubin, MD, FACP, FCCP*
6:05 PM to 6:35 PM	Managing CMV in Hematopoietic Cell Transplant Recipients: Challenges and Opportunities *Michael J. Boeckh, MD*
6:35 PM to 7:05 PM	Cytomegalovirus Disease in Solid Organ Transplant Recipients *Raymund R. Razonable, MD*
7:05 PM to 7:35 PM	CMV Drug Resistance: Clinical Impact and Potential Strategies *Sunwen Chou, MD*
7:35 PM to 8:00 PM	Panel Question-and-Answer Session

SYMPOSIUM OVERVIEW



Target Audience

This activity has been designed to meet the educational needs of physicians and clinical pharmacists involved in the care of patients who are at risk for cytomegalovirus (CMV) infection.

Activity Purpose

This symposium is intended to assist clinicians and pharmacists in understanding how to prevent and manage CMV infection in hematopoietic stem cell transplant (HSCT) recipients and solid organ transplant (SOT) recipients.

Statement of Need

Cytomegalovirus (CMV) infection is an important cause of morbidity and mortality in recipients of hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT). Immunosuppression following transplantation is an important risk factor for the development of CMV infection. In turn, CMV disease is associated with an increased risk of graft loss, development of bacterial or fungal opportunistic infections, and increased mortality in this patient population.

Several strategies exist to prevent CMV infection and disease in transplant recipients. Because each strategy has inherent advantages and limitations, controversy exists regarding the best method for CMV prevention. Despite significant progress in elucidating the pathophysiology of CMV infection and the spectrum of disease in transplant recipients, diagnostic and therapeutic challenges remain. Thus, a clear need exists for additional research into and improved therapies for patients who have this persistently ominous pathogen.

Educational Objectives

After completing this activity, the participant should be better able to:

- List risk factors for cytomegalovirus (CMV) infection in hematopoietic stem cell transplant (HSCT) recipients and solid organ transplant (SOT) recipients
- Describe clinical features of CMV disease in transplant recipients
- Explain therapeutic strategies for the management of CMV infection in transplant recipients
- Identify challenges in managing CMV infection in transplant recipients, including potential strategies to optimize patient outcomes
- Cite the mechanisms and clinical implications of drug resistance in CMV

Statement of Support

This program is jointly sponsored by Robert Michael Educational Institute LLC and Postgraduate Institute for Medicine.

Robert H. Rubin, MD, FACP, FCCP

Osborne Professor of Health Sciences and Technology
Professor of Medicine, Harvard Medical School
Associate Director, Division of Infectious Disease
Brigham and Women's Hospital
Director, Center for Experimental Pharmacology and Therapeutics
Harvard—MIT Division of Health Sciences and Technology
Boston, MA

Robert H. Rubin, MD, is Osborne Professor of Health Sciences and Technology and Professor of Medicine at Harvard Medical School, Associate Director of the Division of Infectious Disease at Brigham and Women's Hospital, and Director of the Center for Experimental Pharmacology and Therapeutics in the Harvard–MIT Division of Health Sciences and Technology, Boston, Massachusetts.

After receiving a Bachelor of Arts degree *magnum cum laude* and *Phi Beta Kappa* from Williams College, Dr. Rubin earned a medical degree *cum laude* from Harvard Medical School. He served his internship and residency at the Peter Bent Brigham Hospital and his infectious diseases training at Massachusetts General Hospital. Dr. Rubin also is a graduate of the Epidemic Intelligence Service of the Centers for Disease Control and Prevention.

Dr. Rubin's clinical and research interests include infection in the immunocompromised host, experimental pharmacology and drug development, and clinical research. He directs the Clinical Investigator Training Program (CITP), which is a 2-year program leading to a Master of Science degree from Harvard Medical School.

Dr. Rubin was the first chairman of the Infectious Disease Section of the American Society of Transplantation and is currently Chairman of that section for the Transplantation Society. He is the founding editor of the journal *Transplant Infectious Disease* and is a member of multiple editorial boards. Dr. Rubin has published more than 400 articles, seven books, and multiple teaching modules on the Internet for distance learning.

Michael J. Boeckh, MD

Associate Member, Program of Infectious Diseases
Fred Hutchinson Cancer Research Center
Associate Professor, University of Washington School of Medicine
Seattle, WA

Michael J. Boeckh, MD, is an associate member of the Program of Infectious Diseases at the Fred Hutchinson Cancer Research Center and Associate Professor at the University of Washington School of Medicine in Seattle, Washington. After training in internal medicine in Berlin, Germany, he came to Seattle in 1990, where he completed a fellowship in infectious diseases at the Fred Hutchinson Cancer Research Center, University of Washington School of Medicine. He stayed on as a faculty member.

Dr. Boeckh's major clinical research interest is the epidemiology, immune response, transmission, and prevention of cytomegalovirus (CMV) in immunocompromised patients. Another focus of his work is the pathogenesis and management of respiratory viruses in stem cell transplant recipients. Dr. Boeckh has published numerous articles on CMV and respiratory viral infections in transplant recipients and is the author of several overview articles and book chapters on the management of viral infections in immunocompromised patients.

Raymund R. Razonable, MD

Assistant Professor of Medicine
Mayo Clinic College of Medicine
Rochester, MN

Raymund R. Razonable, MD, is currently a consultant in the Division of Infectious Diseases at the Mayo Clinic and Assistant Professor of Medicine at the Mayo Clinic College of Medicine in Rochester, Minnesota. After graduating with honors as Doctor of Medicine, Dr. Razonable pursued training in internal medicine at the Beth Israel Hospital in New York and later in infectious diseases at the Mayo Graduate School of Medicine. During his training, he received awards of distinction, including the Alexander Award as the Most Outstanding Medical Resident and the Geraci Award for the Most Outstanding Infectious Disease Fellow.

Dr. Razonable's clinical and research interests are centered primarily on transplant infections. He has published more than 75 original and review articles, book chapters, and other manuscripts in the field of infectious diseases. The vast majority of his work has revolved around the epidemiology, risk factors, treatment, and outcomes of cytomegalovirus (CMV) disease after solid organ transplantation. He is currently working on the interaction between virus and the immune system in an effort to understand the pathogenesis of CMV disease.

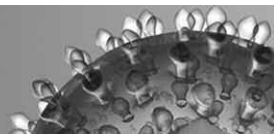
Dr. Razonable has served as a reviewer for more than 20 medical journals and is currently a member of the Editorial Advisory Board of the *Journal of Infectious Diseases*. He is a member of the American Society for Microbiology, Infectious Diseases Society of America, and American Society of Transplantation.

Sunwen Chou, MD

Professor of Medicine
Oregon Health & Science University
Portland, OR

Sunwen Chou, MD, is Professor of Medicine at Oregon Health & Science University and its affiliated VA hospital in Portland. In recent years his long-standing program of cytomegalovirus research has focused on antiviral drug resistance, with emphasis on the associated clinical situations, genetic mechanisms, and molecular diagnostic considerations. This work has helped to define the drug resistance properties conferred by viral mutations observed in treated patients. Dr. Chou is currently exploring the role of experimental drugs with different antiviral mechanisms as a means of avoiding cross-resistance.

ACCREDITATION & CREDIT



Physician Continuing Education

Accreditation Statement

This activity has been planned and implemented in accordance with the Essential Areas and Policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of Postgraduate Institute for Medicine (PIM) and Robert Michael Educational Institute LLC (RMEI). PIM is accredited by the ACCME to provide continuing medical education for physicians.

Credit Designation

Postgraduate Institute for Medicine designates this educational activity for a maximum of *2.5 AMA PRA Category 1 Credits*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Pharmacist Continuing Education

Accreditation Statement

Postgraduate Institute for Medicine is accredited by the Accreditation Council for Pharmacy Education as a provider of continuing pharmacy education.

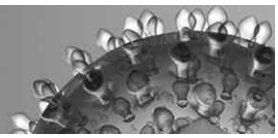
Credit Designation

Postgraduate Institute for Medicine designates this continuing education activity for 2.5 contact hours (0.25 CEUs) of the Accreditation Council for Pharmacy Education. (Universal Program Number 809-999-07-080-L01)

A statement of credit will be issued only upon receipt of a completed activity evaluation form and will be mailed to you in 4 to 6 weeks.

Fee Information

There is no fee for this educational activity.



Disclosure of Conflicts of Interest

Postgraduate Institute for Medicine (PIM) assesses conflict of interest with its instructors, planners, managers and other individuals who are in a position to control the content of CME activities. All relevant conflicts of interest that are identified are thoroughly vetted by PIM for fair balance, scientific objectivity of studies utilized in this activity, and patient care recommendations. PIM is committed to providing its learners with high-quality CME activities and related materials that promote improvements or quality in healthcare and not a specific proprietary business interest of a commercial interest.

The following faculty reported a real or apparent conflict of interest:

- **Dr. Robert H. Rubin** has asked that we advise participants in this activity that he has an affiliation with Pfizer, Merck & Co., Inc., and Amgen Inc. *(Research* and *Educational Support)*.
- **Dr. Michael J. Boeckh** has asked that we advise participants in this activity that he has an affiliation with Roche Labs, Vical, Inc., ViroPharma Incorporated, and Novartis Pharmaceuticals *(Contracted Research)* and AiCuris, ViroPharma Incorporated, and Nektar *(Consulting Fees)*.
- **Dr. Raymund R. Razonable** has asked that we advise participants in this activity that he has an affiliation with Roche *(Consulting Fees* and *Contracted Research)*.
- **Dr. Sunwen Chou** has no affiliations with commercial interests to disclose.

The following planners and managers have the following to disclose:

Robert Michael Educational Institute LLC

- **Robert M. Colleluori** has no affiliations with commercial interests to disclose.
- **Sherri Kramer, MD,** has no affiliations with commercial interests to disclose.
- **Patricia C. Walter** has no affiliations with commercial interests to disclose.

Postgraduate Institute for Medicine

- **Jan Hixon, RN, BSN, MS,** has no affiliations with commercial interests to disclose.
- **Linda Graham, RN,** has no affiliations with commercial interests to disclose.
- **Trace Hutchison, PharmD,** has no affiliations with commercial interests to disclose.

Disclosure of Unlabeled Use

This educational activity may contain discussion of published and/or investigational uses of agents that are not indicated by the FDA. Postgraduate Institute for Medicine (PIM), Robert Michael Educational Institute LLC and ViroPharma Incorporated do not recommend the use of any agent outside of the labeled indications.

The opinions expressed in the educational activity are those of the faculty and do not necessarily represent the views of PIM, Robert Michael Educational Institute LLC and ViroPharma Incorporated. Please refer to the official prescribing information for each product for discussion of approved indications, contraindications and warnings.

Disclaimer

Participants have an implied responsibility to use the newly acquired information to enhance patient outcomes and their own professional development. The information presented in this activity is not meant to serve as a guideline for patient management. Any procedures, medications or other courses of diagnosis or treatment discussed or suggested in this activity should not be used by clinicians without evaluation of their patient's conditions and possible contraindications on dangers in use, review of any applicable manufacturer's product information and comparison with recommendations of other authorities.


CMV Disease in
Transplant Recipients:
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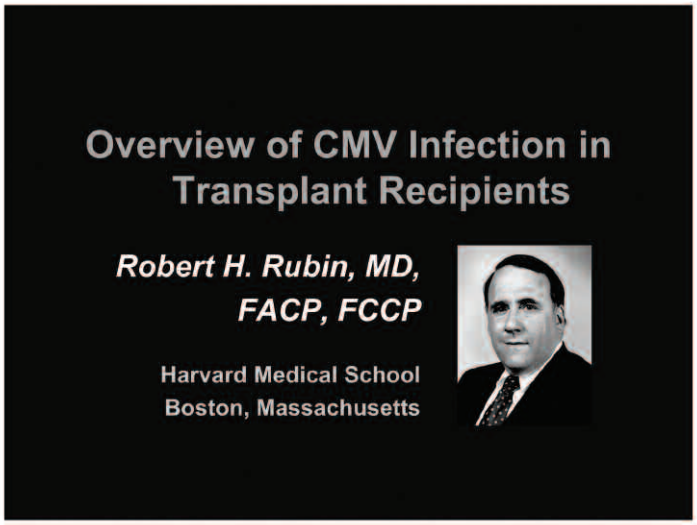
Overview of CMV Infection in
Transplant Recipients
Robert H. Rubin, MD,
FACP, FCCP
Harvard Medical School
Boston, Massachusetts

2

Transformation of Organ Transplantation

From: Interesting experiment in human immunobiology

To: Most practical means of rehabilitating patients with end-stage organ dysfunction of diverse etiology

Result: 90%+ one-year survival of allograft
- Heart
- Kidney
- Liver
- Lung (75%)

Evidence of infection >50% in first year

Rubin RH, Young LS. *Clinical Approach to Infection in the Compromised Host.* New York: Kluwer Academic/Plenum; 2002.

3

General Principles of Infectious Diseases (ID) in Transplant Recipients

- Prevention of infection is the goal
  - Early diagnosis of infection is key to survival
  - Impaired inflammatory response attenuates severity and symptoms; early diagnosis made difficult
  - Aggressive biopsy, advanced imaging
- Microbial burden is key prognostic factor

Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

4

CMV Infection in the Organ Transplant Patient

- Most important single pathogen
  - Also important as a model of the effects of possible virus
- Beta herpesvirus
  - Direct effect
    - Classic ID syndromes (mononucleosis, pneumonia, fever of undetermined origin, colitis, etc)
  - Indirect effects
    - Oncogenesis
    - Contributes to net state of immunosuppression
    - Allograft injury

Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

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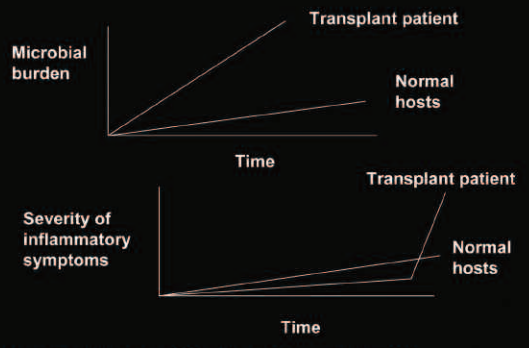
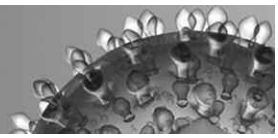
Infection in Transplant Patients and Normal Hosts



Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

6



Pathogenesis of CMV in the Transplant Patient

- TNF → TNF receptors – initiates reactivation from latency on latently infected cells
  - Activation of protein kinase C and nuclear factor – κB
  - Results in formation of activated p65/p50 nuclear factor – κB heterodimer
  - Translocates into nucleus
  - Binds to CMV immediate early enhancer region → initiation of CMV replication

TNF=tumor necrosis factor.

Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

7

Other Pathways for Reactivating CMV

- Stress catecholamines → increased cyclic adenosine monophosphate (cAMP) → stimulation of the reactivation process
- Proinflammatory prostaglandins → CMV activates through cAMP

Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

8

Other Pathways for Reactivating CMV

- CMV activation linked with inflammation, infection, and stress
- Amplification and dissemination
  - The "Second Wave"

Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

9

Pathogenesis of the Direct Effects of CMV

- Key host defense: MHC-restricted, virus-restricted, cytotoxic T-cell response
- Initial site of invasion, replication
  - Vascular endothelial cells → lytic infection
  - Result: "viral vasculitis"
- Antigenemia assay = after endothelial cell recapture → phagocytosis of products of lysis → antigenemia
- Hypothesis: vascular injury → future atherosclerosis; vasculopathy of transplanted organ

MHC=major histocompatibility complex.
Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

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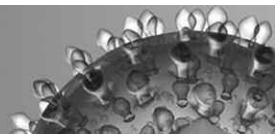

Characteristics of CMV Tissue Invasion

- Fewer lytically infected cells
- Increased number of activated leukocytes
- Proposed mechanism: a few CMV-infected cells → interleukin-1, which greatly increases activated leukocytes, which injure tissue

Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

11

Epidemiology and Consequences of CMV Infection

- Acquisition: transplant, transfusion, intimate contact
- Seropositive = latent virus capable of being reactivated
- Reactivation = inflammation and proinflammatory cytokines (eg, TNF)

Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

12

CMV Infection, Immunosuppression, Clinical Disease

Type	Donor, Recipient Status	Immuno-suppression	Symptomatic Disease
Primary	D+, R-	"Any"	50%
Reactivation	D-, R+	No ATG	10%–15%
Reactivation	D+, R+	No ATG	25%
"Cytokine storm"	D±, R±	ATG	> 50%

*3–6 weeks after cytokine storm, 50%+ symptomatic disease.
ATG=Antithymocyte globulin.
Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

13

Antimicrobial Therapy in the Organ Transplant Patient

- Antiviral drugs
  - Ganciclovir
    - IV — Primary resistance NO!
    - Oral valganciclovir
    - Oral ganciclovir (+/- efficacy)
    - IV foscarnet
- Use of antiviral drugs
  - How long to treat?
    - "Long enough!"
  - Prophylaxis
  - Preemptive
  - Therapeutic

Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

14

Prevention of Direct Manifestations of CMV

- Prophylaxis
- Preemptive
- Therapeutic

"Viremia = Truth"

Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

15

Timetable of Infection Post Organ Transplant

- Use: predictive value of + or – very high
  - Guide to risk of infection
  - Diagnosis in the face of difficult symptoms (eg, colitis, pneumonia)
  - Opportunistic infection – and HCV burden
- Time posttransplant for symptomatic disease
  - 1st month: No opportunistic infections; "surgical complications"
  - 1–6 months: Virus +/- opportunistic infection
  - 6 months: 80% good result = respiratory virus (flu), asymptomatic nodules
    10% chronic hepatitis
    10% "ne'er do wells"

HCV=hepatitis C virus.

Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

16

Clinical Syndrome with CMV and Other Infections

- Increase in other viruses
  - HCV
  - HBV
  - EBV
- 90%+ of opportunistic infections, in setting of viral infection
- EBV-induced PTLD → 7- to 10-fold increased incidence of PTLD

EBV=Epstein-Barr virus; HBV=hepatitis B virus; PTLD=posttransplantation lymphoproliferative disorder.

Rubin RH, Young LS. New York: Kluwer Academic/Plenum; 2002.

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Future Issues

1. Diagnosis of indirect syndromes
2. Importance of human herpesvirus-6
3. How to best treat or prevent virus
4. Optimal immunosuppression

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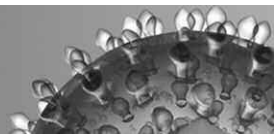
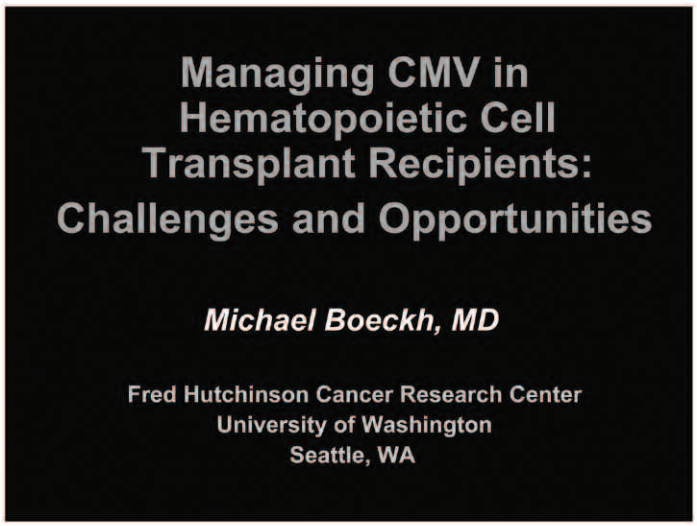


Picabia: *Our heads are round so that our thinking can change directions.*

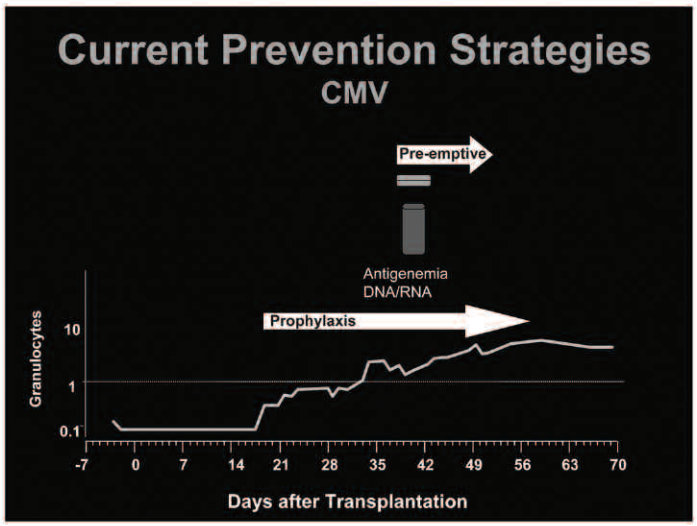
Voltaire: *Medical skill involves keeping the patient amused while Nature cures.*

Holmes: *I firmly believe that if the whole materia medica could be sunk to the bottom of the sea, it would be all the better for mankind and all the worse for the fishes.*

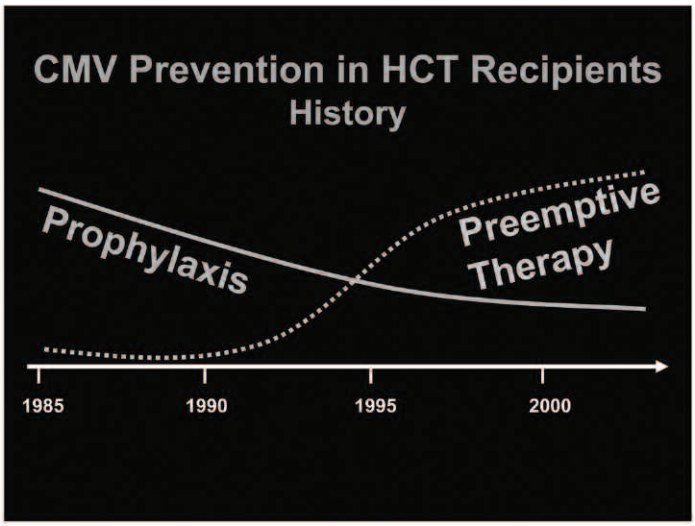
19


Managing CMV in Hematopoietic Cell Transplant Recipients: Challenges and Opportunities
Michael Boeckh, MD
Fred Hutchinson Cancer Research Center
University of Washington
Seattle, WA

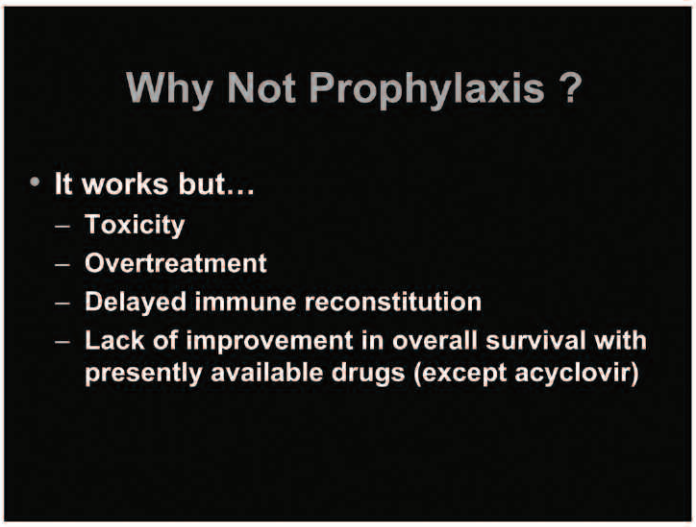
1


Current Prevention Strategies
CMV
Pre-emptive
Antigenemia
DNA/RNA
Prophylaxis
Granulocytes
10
1
0.1
-7
0
7
14
21
28
35
42
49
56
63
70
Days after Transplantation

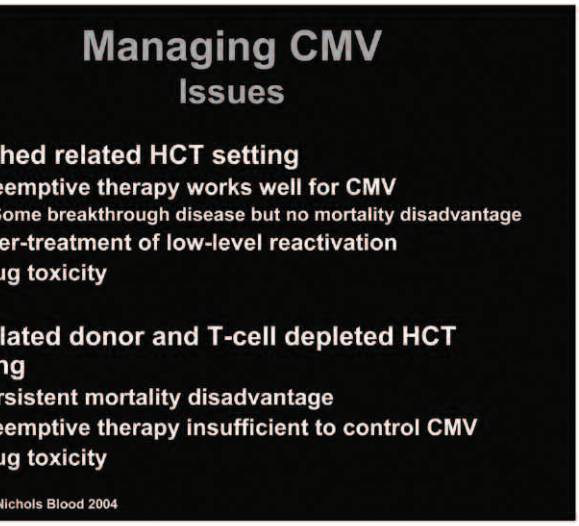
2


CMV Prevention in HCT Recipients
History
Prophylaxis
Preemptive Therapy
1985
1990
1995
2000

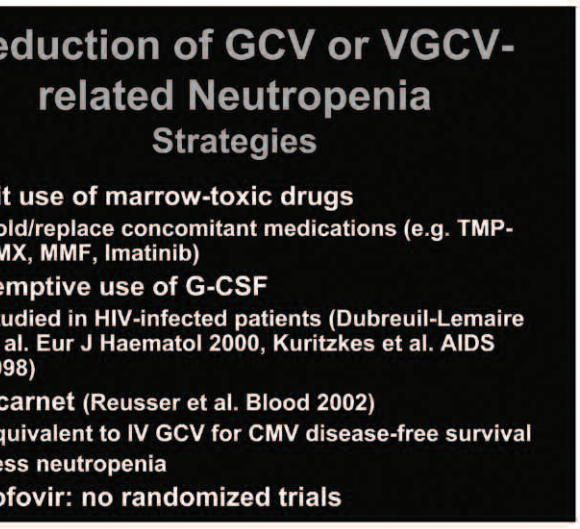
3


Why Not Prophylaxis ?
• It works but…
– Toxicity
– Overtreatment
– Delayed immune reconstitution
– Lack of improvement in overall survival with presently available drugs (except acyclovir)

4


Managing CMV
Issues
• Matched related HCT setting
– Preemptive therapy works well for CMV
• Some breakthrough disease but no mortality disadvantage
– Over-treatment of low-level reactivation
– Drug toxicity
• Unrelated donor and T-cell depleted HCT setting
– Persistent mortality disadvantage
– Preemptive therapy insufficient to control CMV
– Drug toxicity
Boeckh & Nichols Blood 2004

5


Reduction of GCV or VGCV-related Neutropenia
Strategies
• Limit use of marrow-toxic drugs
– Hold/replace concomitant medications (e.g. TMP-SMX, MMF, Imatinib)
• Preemptive use of G-CSF
– Studied in HIV-infected patients (Dubreuil-Lemaire et al. Eur J Haematol 2000, Kuritzkes et al. AIDS 1998)
• Foscarnet (Reusser et al. Blood 2002)
– Equivalent to IV GCV for CMV disease-free survival
– Less neutropenia
• Cidofovir: no randomized trials

6

Control of CMV
Future Strategies

- Novel anti-CMV drugs
  - Maribavir
- T cell therapy
- Vaccination strategies

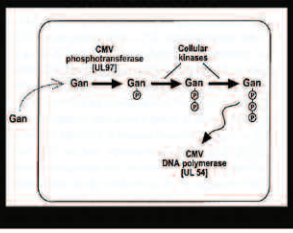
7

Anti-CMV Drugs: Mechanism of Action

Maribavir

UL97 (CMV) → DNA elongation
Protein kinase DNA packaging
Capsid egress

Ganciclovir



Alternate substrate
Incorporation into growing DNA
→ Chain termination

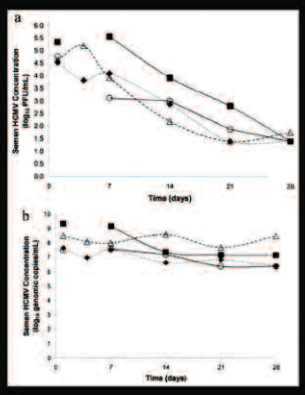
8

Maribavir
Specificity

- Maribavir has been shown to inhibit replication of EBV *in vitro*
- Active against ganciclovir-resistant strains *in vitro*
- Maribavir does not have significant activity against:
  - HSV-1,HSV-2
  - VZV
  - murine CMV
  - HHV-6 or HHV-7
  - HBV
  - HIV

9

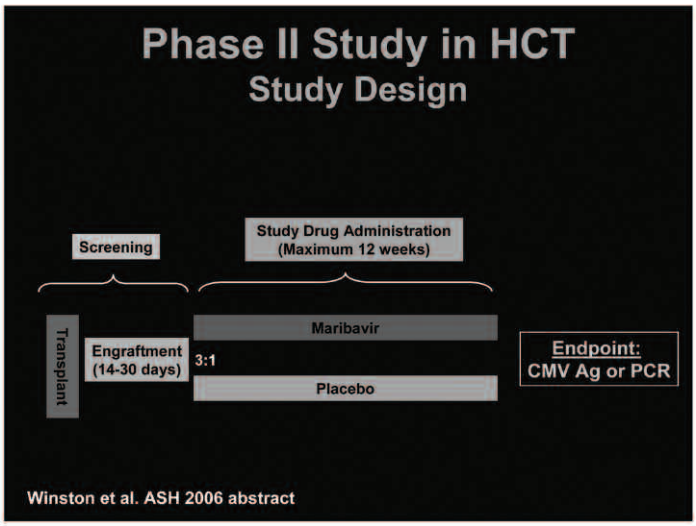
Phase I Dose Escalation Trial of 1263W94 (Maribavir) in HIV-Infected Men with Asymptomatic HCMV Shedding



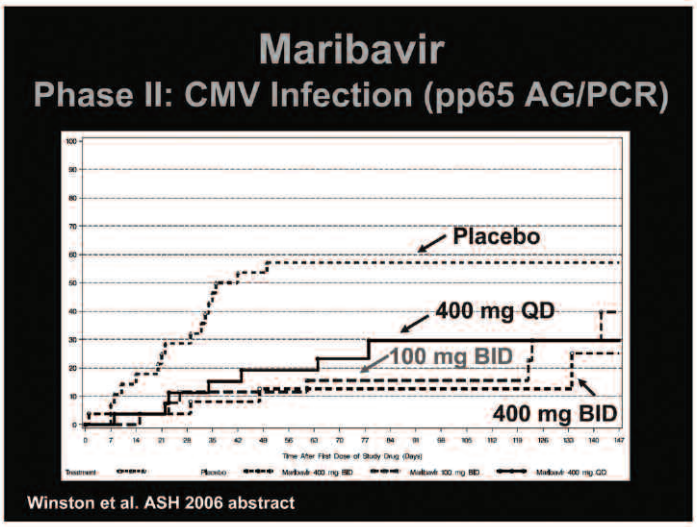
(a) Median concentration-time profiles of HCMV in semen, measured in PFU per milliliter by using plaque assays. Symbols: , 100 mg t.i.d. (n = 7); , 200 mg t.i.d. (n = 5); , 400 mg t.i.d. (n = 6); , 600 mg b.i.d. (n = 6). (b) Median concentration-time profiles of HCMV DNA in semen, measured in log_{10} copies per milliliter by using PCR analysis. Symbols: , 100 mg t.i.d. (n = 7); , 200 mg t.i.d. (n = 4); , 400 mg t.i.d. (n = 5); , 600 mg b.i.d. (n = 2).

From Lalezari et al. *AAC*. 2002;46: 2969-2976; with permission.

10


Phase II Study in HCT
Study Design
Screening
Study Drug Administration
(Maximum 12 weeks)
Transplant
Engraftment
(14-30 days)
3:1
Maribavir
Placebo
Endpoint:
CMV Ag or PCR
Winston et al. ASH 2006 abstract

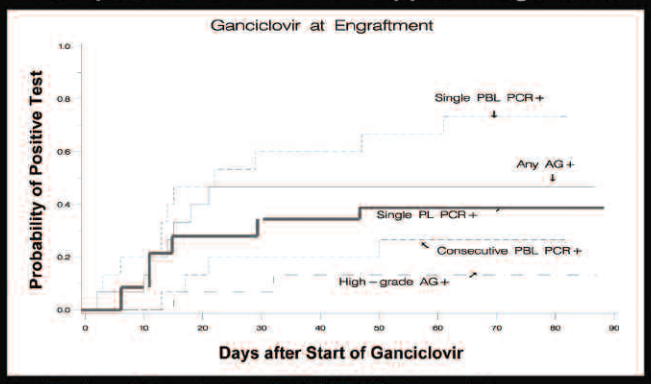
11


Maribavir
Phase II: CMV Infection (pp65 AG/PCR)
Placebo
400 mg QD
100 mg BID
400 mg BID
Winston et al. ASH 2006 abstract

12

Ganciclovir Prophylaxis

PCR-plasma *vs* PCR-PBL *vs* pp65 Antigenemia

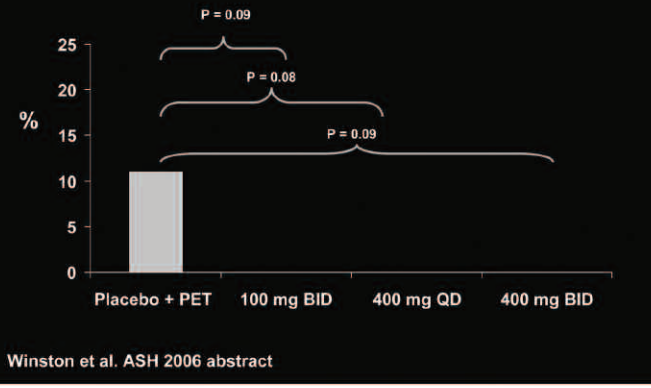


From Boeckh et al. *Transplantation.* 1997;64:108-113; with permission.

13

Maribavir

Phase II: CMV Disease



Winston et al. ASH 2006 abstract

14

Maribavir
Safety in Phase II HCT Study

Related AEs	Placebo	100 mg BID	400 mg QD	400 mg BID
N	28	28	28	26
GVHD, 2-4	13 (46%)	4 (14%)	8 (29%)	6 (23%)
Taste dist.	0	6 (21%)*	5 (18%)*	8 (31%)*
Nausea	0	2 (7%)	4 (14%)	4 (15%)
Diarrhea	0	1 (4%)	1 (4%)	0
Vomiting	1 (4%)	3 (11%)	3 (11%)	1 (4%)
Dry mouth	0	1 (4%)	0	1 (4%)
Rash	1 (4%)	2 (7%)	1 (4%)	0

* $P < 0.05$

Winston et al. ASH 2006 abstract

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Maribavir
D/C due to Related Adverse Events

AEs	Placebo	100 mg BID	400 mg QD	400 mg BID
N	28	28	28	26
Taste dist.	0	1 (4%)	1 (4%)	4 (15%)
Nausea	1 (4%)	1 (4%)	1 (4%)	3 (12%)
Vomiting	0	1 (4%)	1 (4%)	1 (4%)
Dysphagia	0	0	0	1 (4%)
GERD	1 (4%)	0	0	0
Rash	1 (4%)	1 (4%)	0	0
Total	3 (11%)	4 (14%)	3 (11%)	9 (35%)

Winston et al. ASH 2006 abstract

16

Maribavir
Safety in Phase II HCT Study

- No significant differences in
  - Viral signs
  - ECG parameters
  - Liver function tests
  - Renal function
  - Platelet counts
  - Red blood cell counts

Winston et al. ASH 2006 abstract

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Maribavir
Safety in Phase II HCT Study

	Plac	100 BID	Maribavir 400 QD	400 BID
Neutropenia on study drug				
ANC < 1000	14%	21%	18%	15%
ANC < 750	14%	14%	11%	12%
ANC < 500	7%	11%	7%	4%
Neutropenia until day 100				
ANC < 1000	39%	25%	21%	35%
ANC < 750	39%	14%	18%	27%
ANC < 500	21%	11%	11%	12%

Winston et al. ASH 2006 abstract

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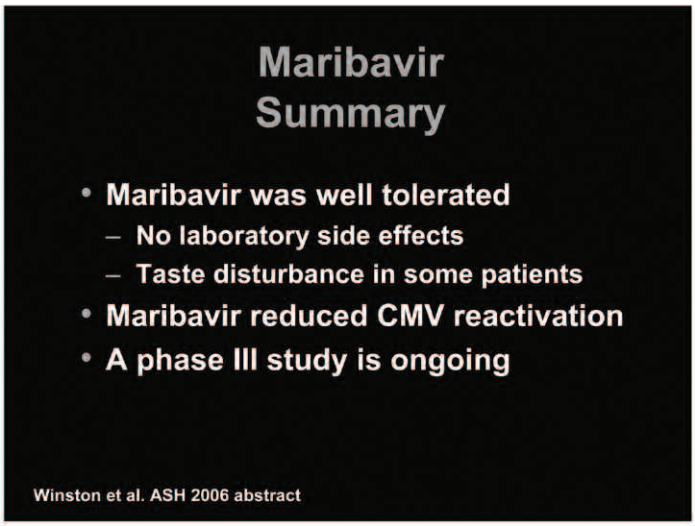
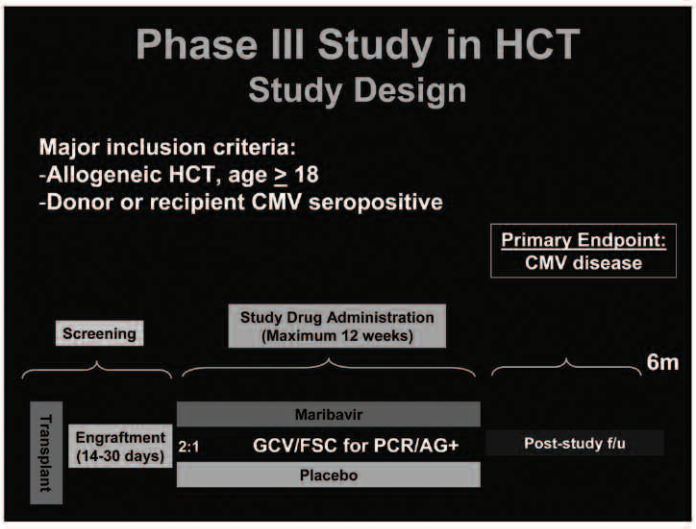

Maribavir
Summary
• Maribavir was well tolerated
– No laboratory side effects
– Taste disturbance in some patients
• Maribavir reduced CMV reactivation
• A phase III study is ongoing
Winston et al. ASH 2006 abstract

19


Phase III Study in HCT
Study Design
Major inclusion criteria:
-Allogeneic HCT, age ≥ 18
-Donor or recipient CMV seropositive
Primary Endpoint:
CMV disease
Screening
Study Drug Administration
(Maximum 12 weeks)
6m
Transplant
Engraftment
(14-30 days)
2:1
Maribavir
GCV/FSC for PCR/AG+
Placebo
Post-study f/u

20

Immune Augmentation Strategies

- CMV-specific T cell therapy
  - Specific clones
  - Lines
  - Rapid expansion/selection
- CMV vaccination
  - Donor + recipient
  - Combination with T cell therapy
- Non-specific enhancement
  - Keratinocyte growth factor
  - IL-7
  - T cell precursors

21

Current Vaccine Candidates

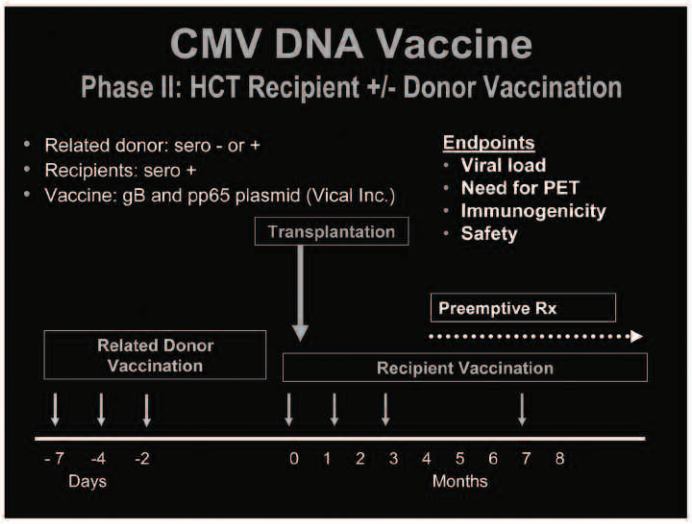
Summary of current status of CMV vaccines

Vaccine	Comments/status
Live, attenuated vaccines	
• AD169 vaccine	• Reactogenic; this vaccine is no longer in clinical trials
• Towne vaccine	• Limited efficacy in renal transplant recipients; studies still ongoing. Prime-boost effect when administered with recombinant gB
• Towne/Toledo chimeras	• Phase I study in CMV-seropositives; vaccine was safe, well tolerated
gB protein subunit vaccine	• Safe, well tolerated, immunogenic • Efficacy studies ongoing
ALVAC vaccines	• gB and pp65 (*UL83*) ALVAC vaccines evaluated in Phase I clinical trials • Immunogenic, well tolerated • No 'prime-boost' effect with ALVAC-gB and purified recombinant gB
DNA vaccines	• Phase I studies in healthy volunteers • Immunogenic, well tolerated
Vaccines only evaluated in preclinical models	
VEE- and pox-vectored vaccines	• Preclinical testing only • Immunogenic in animal models
Peptide vaccines	• Preclinical testing only • Potential for ex vivo expansion of CMV-specific T-cells for adoptive transfer
Dense-body vaccines	• Preclinical testing only • Immunogenic in animal models • Enriched for pp65 (*UL83*); noninfectious

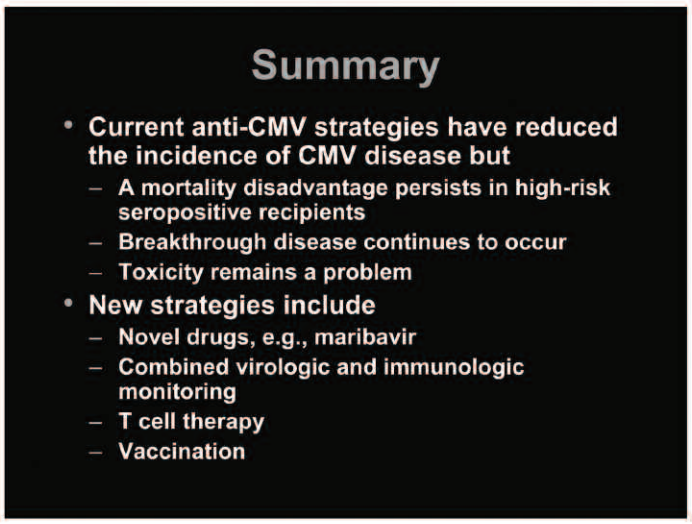
VEE, Venezuelan equine encephalitis; gB, a glycoprotein; pp65, human cytomegalovirus phosphorylated matrix protein.

From Schleiss M, *Herpes*. 2005;12:66-75; with permission.

22


CMV DNA Vaccine
Phase II: HCT Recipient +/- Donor Vaccination
Related donor: sero - or +
Recipients: sero +
Vaccine: gB and pp65 plasmid (Vical Inc.)
Endpoints
Viral load
Need for PET
Immunogenicity
Safety
Transplantation
Preemptive Rx
Related Donor Vaccination
Recipient Vaccination
-7 -4 -2
Days
0 1 2 3 4 5 6 7 8
Months

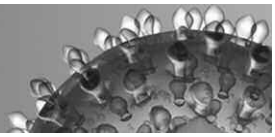
23


Summary
Current anti-CMV strategies have reduced the incidence of CMV disease but
A mortality disadvantage persists in high-risk seropositive recipients
Breakthrough disease continues to occur
Toxicity remains a problem
New strategies include
Novel drugs, e.g., maribavir
Combined virologic and immunologic monitoring
T cell therapy
Vaccination

24


Maribavir co-Investigators
D Winston
J van Burik
V Pullarkat
G Papanicoloau
R Vij
E Vance
G Alangaden
R Chemaly
F Peterson
N Chao
J Klein
K Sprague
S Villano
Lab and Clinical Studies
T Stevens-Ayers
K White
J Smith
C Varley
S Chatterton Kirchmeier
J Ferrenberg
E Minrich
G Jolly
J Heugel
C Dahlgren
J Huggler
Adoptive T cell studies
S Riddell
P Greenberg
T Manley
K Kirby

25



Cytomegalovirus Disease in Solid Organ Transplant Recipients

Raymund R. Razonable, MD

Division of Infectious Diseases
Mayo Clinic College of Medicine
Rochester, Minnesota

Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC). Chicago, IL. September 16, 2007.

1

Objectives

- Impact of CMV on transplant outcomes
- Risk factors for CMV in solid organ transplant (SOT)
- Prevention and treatment of CMV in SOT
- Emerging syndromes
  - Delayed-onset CMV disease
  - Ganciclovir (GCV)-resistant CMV
  - Compartmentalized CMV disease

2

Clinical Case No. 1

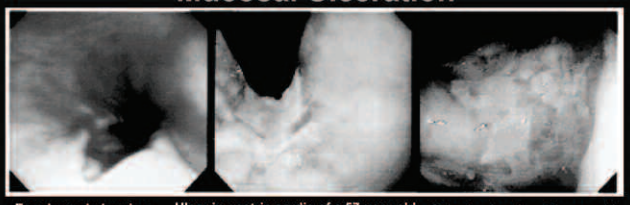
- 64-year-old woman with chronic glomerulonephritis (GN)
- LUDKT/thymoglobulin/tac-MMF-pred
- 6th week – acute rejection/corticosteroids
- CMV D+/R- → VGCV x 3 months
- 4th month: fever, vomiting, diarrhea
- CMV PCR: 474,000 copies/mL blood

tac-MMF-pred=tacrolimus-mycophenolate mofetil-prednisone; LUDKT=living unrelated donor kidney transplant; PCR=polymerase chain reaction; VGCV=valganciclovir.

Eid AJ, et al. *Clin Transplant*. 2007; in press.

3

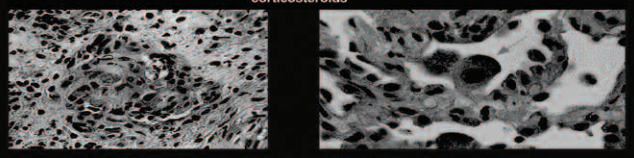
Gastrointestinal CMV Disease Causing Mucosal Ulceration



Esophageal ulcer in a 25-year-old patient with AIDS

Ulcer in gastric cardia of a 57-year-old patient with severe chronic obstructive lung disease taking corticosteroids

Colonic ulcer in a 54-year-old kidney transplant patient



Top: From Goodgame RW. *Ann Intern Med*. 1993;119:924-935; with permission.

4

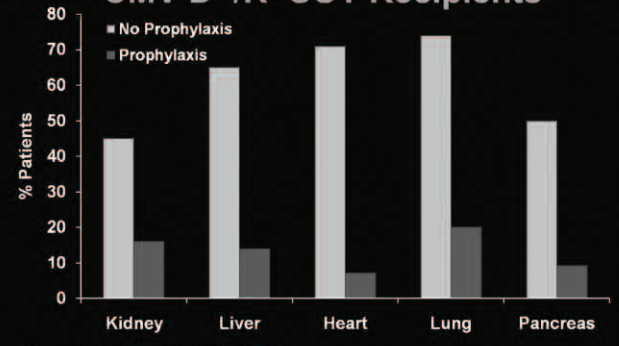
Direct Effects of CMV (2000–2006)

- CMV syndrome — 50%-60%
  - Fever with myelosuppression
- Invasive CMV disease — 40%-50%
  - Hepatitis
  - Pneumonia
  - Gastrointestinal disease — (90%)
  - Retinitis
  - Encephalitis
  - Others

Eid AJ, Razonable RR. *Curr Opin Organ Transplant.* 2007 December; in press.

5

Current Burden of CMV Disease in CMV D+/R- SOT Recipients



Gane E, et al. *Lancet.* 1997;350:1729-1733.
Humar A, et al. *Am J Transplant.* 2005;1462-1468.
Lowance D, et al. *N Engl J Med.* 1999;340:1462-1470.
Paya C, et al. *Am J Transplant.* 2004;4:611-620.
Macdonald PS, et al. *J Heart Lung Transplant.* 1995;14(1 Pt 1):32-38.

6

Indirect Effects of CMV

- Acute rejection
- Chronic rejection
  - Accelerated transplant vasculopathy
  - Bronchiolitis obliterans
- Opportunistic infections
  - Epstein-Barr virus (EBV)–related posttransplantation lymphoproliferative disorder (PTLD)
  - Fungal superinfections
- Viral interactions: herpes and other viruses
- Mortality

Rubin RH. *JAMA*. 1989;261:3607-3609.
Rubin RH, Young LS. *Clinical Approaches to Infection in the Compromised Host*. New York: Springer; 2000:573-579.

7

Risk Factors for CMV Disease in Solid Organ Transplantation

8

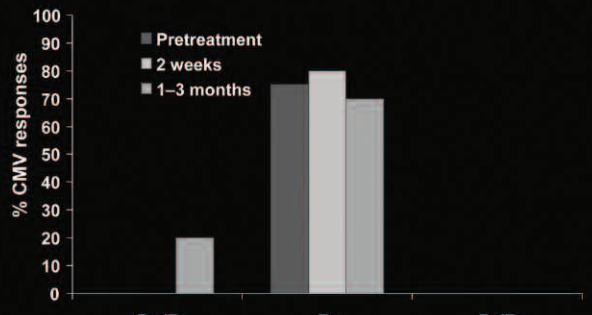
Clinical Case No. 1

- 64-year-old woman with chronic GN
- LUDKT/thymoglobulin/tac-MMF-pred
- 6th week – acute rejection/corticosteroids
- CMV D+/R- → VGCV x 3 months
- 4th month: fever, vomiting, diarrhea
- CMV PCR: 474,000 copies/mL blood

Eid AJ, et al. *Clin Transplant.* 2007; in press.

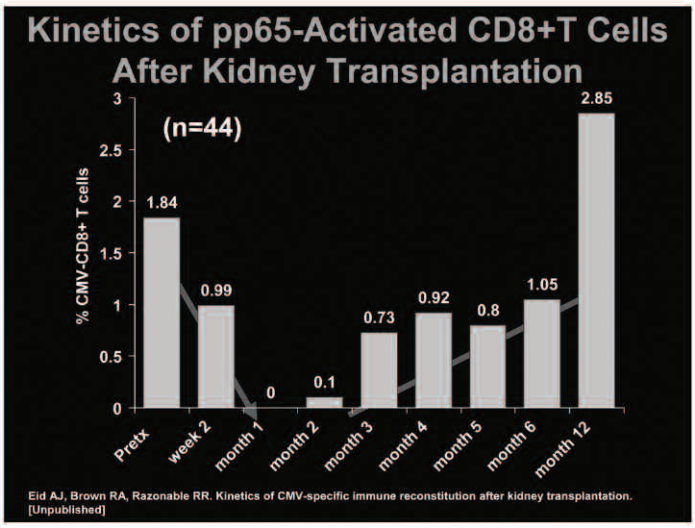
9

CMV-Specific T-Cell Responses Following Alemtuzumab Induction

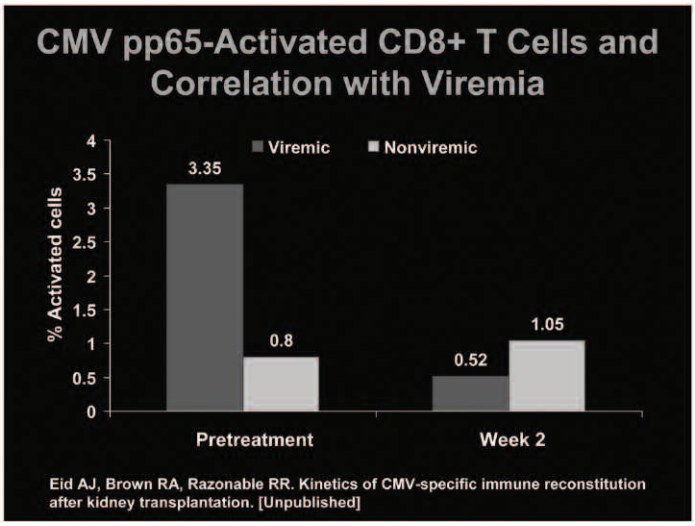


From Zeevi A, et al. *Am J Transplant.* 2007;7:471-475; with permission.

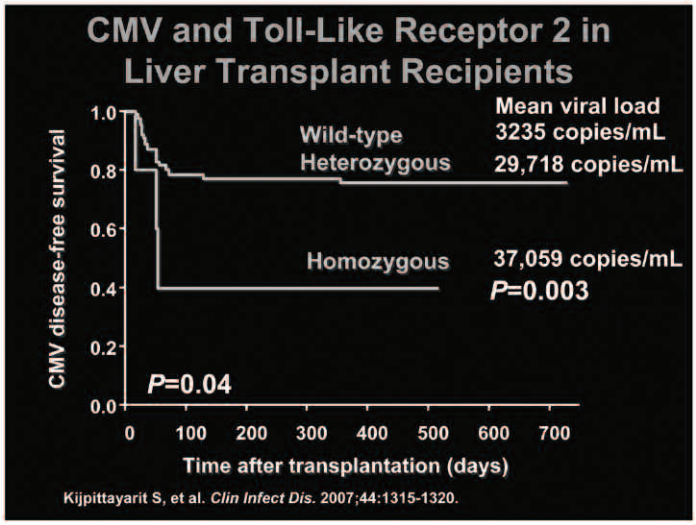
10


Kinetics of pp65-Activated CD8+T Cells After Kidney Transplantation
(n=44)
% CMV-CD8+ T cells
3
2.5
2
1.5
1
0.5
0
1.84
0.99
0
0.1
0.73
0.92
0.8
1.05
2.85
Pretx
week 2
month 1
month 2
month 3
month 4
month 5
month 6
month 12
Eid AJ, Brown RA, Razonable RR. Kinetics of CMV-specific immune reconstitution after kidney transplantation. [Unpublished]

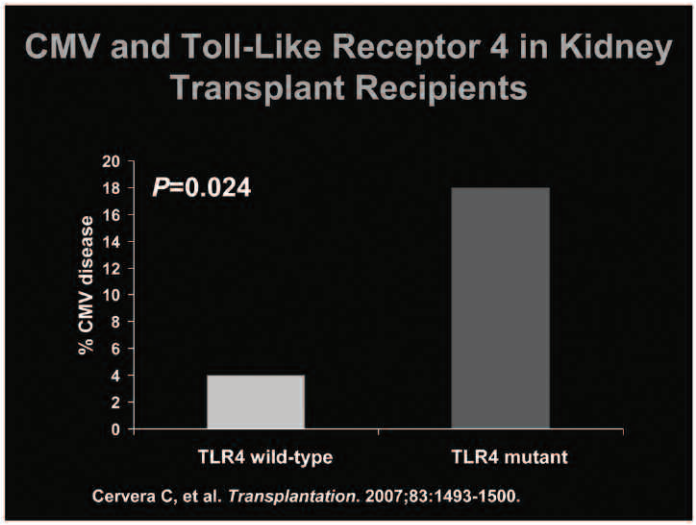
11


CMV pp65-Activated CD8+ T Cells and Correlation with Viremia
Viremic
Nonviremic
% Activated cells
4
3.5
3
2.5
2
1.5
1
0.5
0
3.35
0.8
0.52
1.05
Pretreatment
Week 2
Eid AJ, Brown RA, Razonable RR. Kinetics of CMV-specific immune reconstitution after kidney transplantation. [Unpublished]

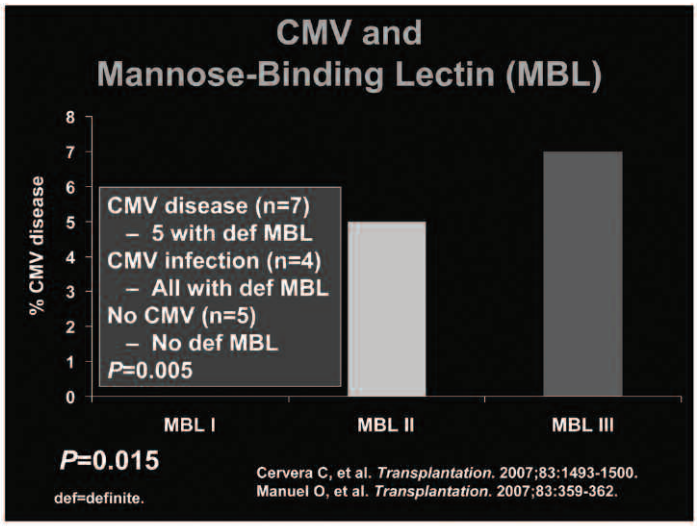
12


CMV and Toll-Like Receptor 2 in Liver Transplant Recipients
Mean viral load
Wild-type
3235 copies/mL
Heterozygous
29,718 copies/mL
Homozygous
37,059 copies/mL
P=0.003
P=0.04
CMV disease-free survival
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
600
700
Time after transplantation (days)
Kijpittayarit S, et al. Clin Infect Dis. 2007;44:1315-1320.

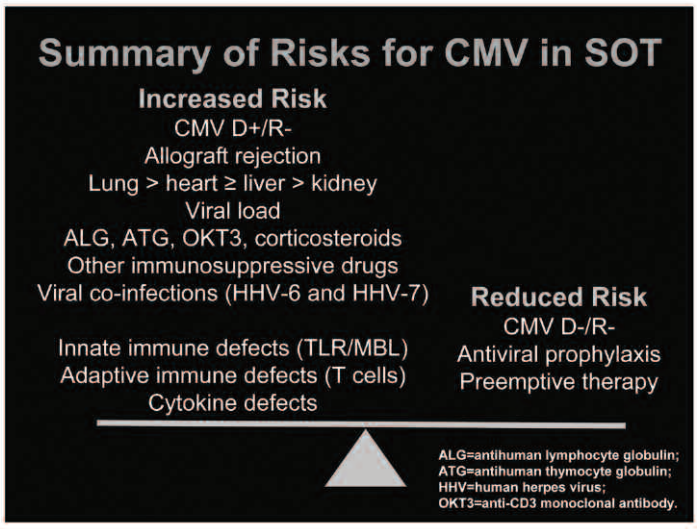
13


CMV and Toll-Like Receptor 4 in Kidney Transplant Recipients
P=0.024
% CMV disease
20
18
16
14
12
10
8
6
4
2
0
TLR4 wild-type
TLR4 mutant
Cervera C, et al. Transplantation. 2007;83:1493-1500.

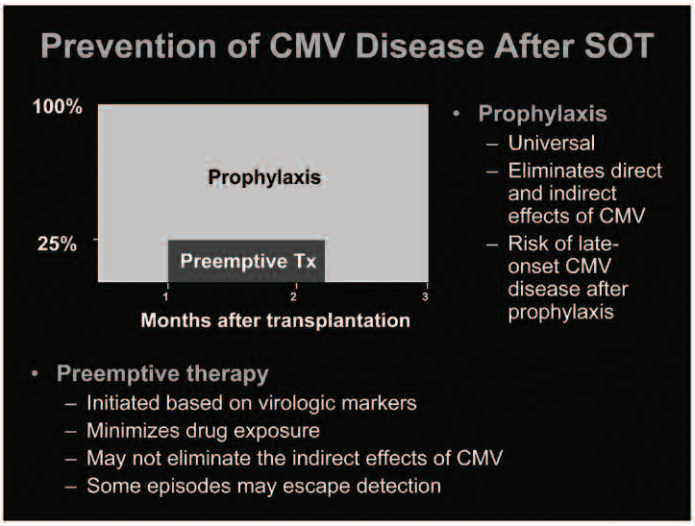
14


CMV and
Mannose-Binding Lectin (MBL)
% CMV disease
8
7
6
5
4
3
2
1
0
CMV disease (n=7)
– 5 with def MBL
CMV infection (n=4)
– All with def MBL
No CMV (n=5)
– No def MBL
P=0.005
MBL I
MBL II
MBL III
P=0.015
def=definite.
Cervera C, et al. Transplantation. 2007;83:1493-1500.
Manuel O, et al. Transplantation. 2007;83:359-362.

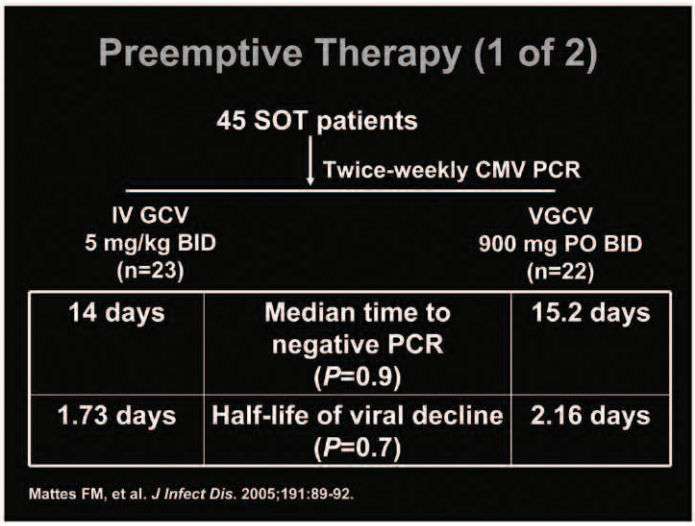
15


Summary of Risks for CMV in SOT
Increased Risk
CMV D+/R-
Allograft rejection
Lung > heart ≥ liver > kidney
Viral load
ALG, ATG, OKT3, corticosteroids
Other immunosuppressive drugs
Viral co-infections (HHV-6 and HHV-7)
Innate immune defects (TLR/MBL)
Adaptive immune defects (T cells)
Cytokine defects
Reduced Risk
CMV D-/R-
Antiviral prophylaxis
Preemptive therapy
ALG=antihuman lymphocyte globulin;
ATG=antihuman thymocyte globulin;
HHV=human herpes virus;
OKT3=anti-CD3 monoclonal antibody.

16


Prevention of CMV Disease After SOT
100%
25%
Prophylaxis
Preemptive Tx
1
2
3
Months after transplantation
• Prophylaxis
– Universal
– Eliminates direct and indirect effects of CMV
– Risk of late-onset CMV disease after prophylaxis
• Preemptive therapy
– Initiated based on virologic markers
– Minimizes drug exposure
– May not eliminate the indirect effects of CMV
– Some episodes may escape detection

17


Preemptive Therapy (1 of 2)
45 SOT patients
Twice-weekly CMV PCR
IV GCV
5 mg/kg BID
(n=23)
VGCV
900 mg PO BID
(n=22)
14 days
Median time to negative PCR
(P=0.9)
15.2 days
1.73 days
Half-life of viral decline
(P=0.7)
2.16 days
Mattes FM, et al. J Infect Dis. 2005;191:89-92.

18

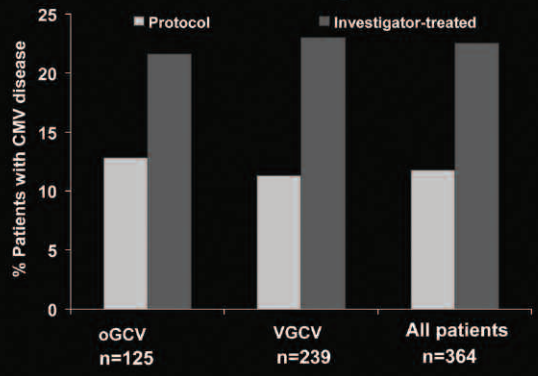
Preemptive Therapy (2 of 2)

- **Compliance**
  - 7 of 17 (41%) patients who developed CMV disease missed at least 1 CMV PCR prior to diagnosis of CMV disease
- **Rapid replication in CMV D+/R-**
  - 25% of CMV D+/R- had negative CMV PCR during the week prior to the onset of clinical disease

Walker JK, et al. *Transplantation.* 2007;83:874-882.
Razonable RR, et al. *J Infect Dis.* 2003;187:1807-1808.

19

Oral GCV (oGCV) vs VGCV Prophylaxis in CMV D+/R- Non-Lung SOT Patients



Paya CV, et al. *Am J Transplant.* 2004;4:611-620.

20

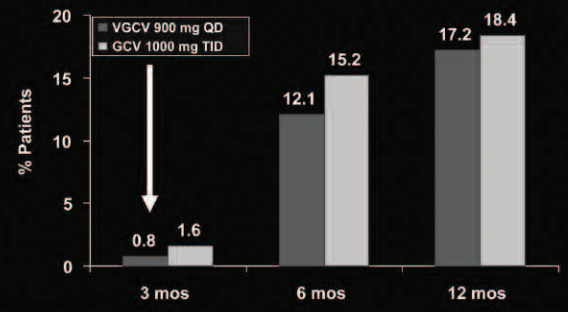
Anti-CMV Prophylaxis: Meta-analyses

Study Author	CMV Disease (Relative Risk)	All-Cause Mortality (Relative Risk)
Hodson	0.42 (0.34–0.52)	0.63 (0.43–0.92)
Kalil	0.20 (0.13–0.31)	0.62 (0.40–0.96)
Small	0.34 (0.24–0.48)	0.99 (0.68–1.43)

Eid AJ, Razonable RR. *Curr Opin Organ Transplant.* 2007 December; in press.

21

Delayed-Onset Primary CMV Disease in D+/R- SOT Patients



Paya CV, et al. *Am J Transplant.* 2004;4:611-620.

22

Risk Factors for Delayed CMV Disease

Variable	Hazard Ratio
CMV D+/R-	11.00
Allograft rejection	6.60
Female gender	2.19
Blood group A	2.36
Low creatinine clearance	4.28
MMF use at end of prophylaxis	1.99
Prednisone use at end of prophylaxis	2.70

Arthurs SK, et al. *J Heart Lung Transplant.* 2007. In press.
Arthurs SK, et al. *Liver Transplant.* 2007. In press.
Freeman RB, et al. *Transplantation.* 2004;78:1765-1773.
Limaye AP, et al. *Transplantation.* 2004;78:1390-1396.
Razonable RR, et al. *J Infect Dis.* 2001;184:1461-1464.

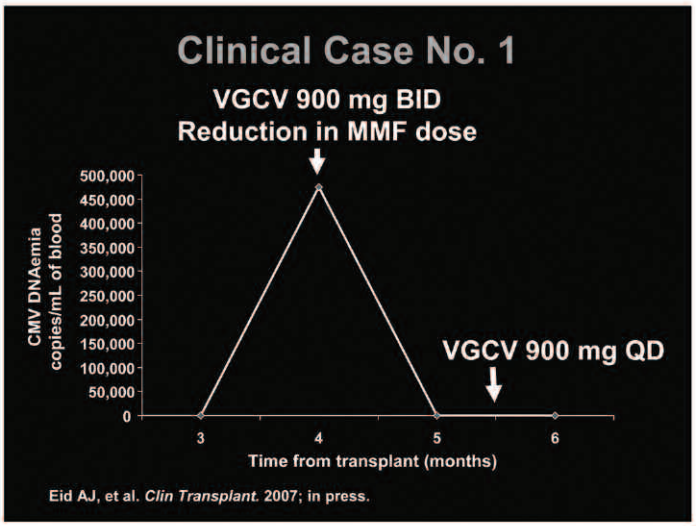
23

Treatment of CMV Disease

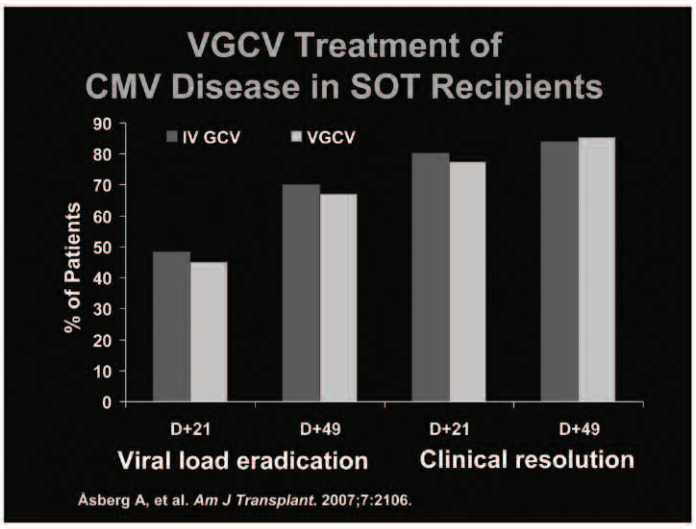
- IV GCV is the preferred drug for treating CMV disease in SOT recipients
- Typically, treat CMV disease for 2 to 4 weeks
- However, duration of treatment must be guided by molecular methods
  - Challenge: compartmentalized CMV diseases

Cytomegalovirus. *Am J Transplant.* 2004;4(Suppl 10):51-58.

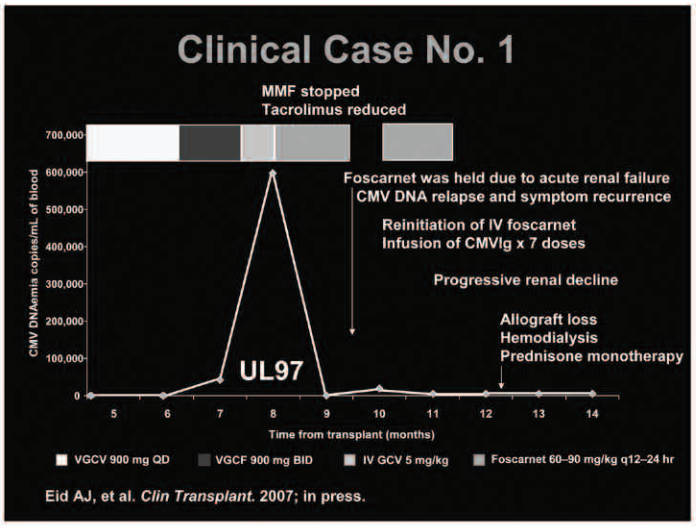
24


Clinical Case No. 1
VGCV 900 mg BID
Reduction in MMF dose
CMV DNAemia copies/mL of blood
500,000
450,000
400,000
350,000
300,000
250,000
200,000
150,000
100,000
50,000
0
3
4
5
6
Time from transplant (months)
VGCV 900 mg QD
Eid AJ, et al. Clin Transplant. 2007; in press.

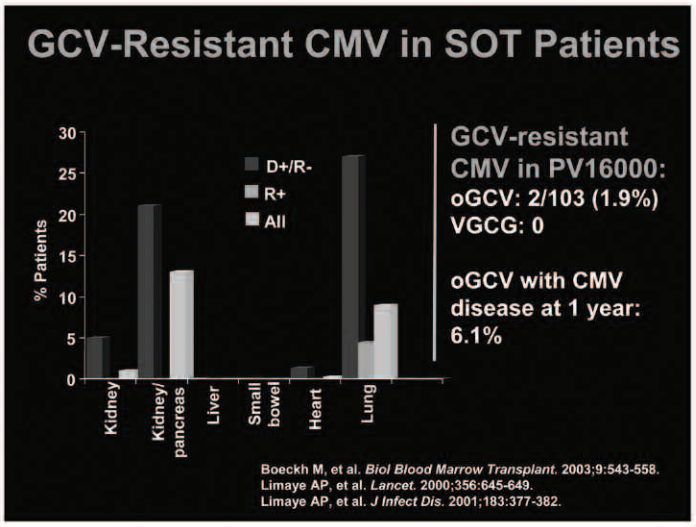
25


VGCV Treatment of
CMV Disease in SOT Recipients
IV GCV
VGCV
% of Patients
90
80
70
60
50
40
30
20
10
0
D+21
D+49
D+21
D+49
Viral load eradication
Clinical resolution
Åsberg A, et al. Am J Transplant. 2007;7:2106.

26


Clinical Case No. 1
MMF stopped
Tacrolimus reduced
CMV DNAemia copies/mL of blood
700,000
600,000
500,000
400,000
300,000
200,000
100,000
0
Foscarnet was held due to acute renal failure
CMV DNA relapse and symptom recurrence
Reinitiation of IV foscarnet
Infusion of CMVIg x 7 doses
Progressive renal decline
Allograft loss
Hemodialysis
Prednisone monotherapy
UL97
5
6
7
8
9
10
11
12
13
14
Time from transplant (months)
VGCV 900 mg QD
VGCF 900 mg BID
IV GCV 5 mg/kg
Foscarnet 60–90 mg/kg q12–24 hr
Eid AJ, et al. Clin Transplant. 2007; in press.

27


GCV-Resistant CMV in SOT Patients
% Patients
30
25
20
15
10
5
0
D+/R-
R+
All
Kidney
Kidney/ pancreas
Liver
Small bowel
Heart
Lung
GCV-resistant CMV in PV16000:
oGCV: 2/103 (1.9%)
VGCG: 0
oGCV with CMV disease at 1 year: 6.1%
Boeckh M, et al. Biol Blood Marrow Transplant. 2003;9:543-558.
Limaye AP, et al. Lancet. 2000;356:645-649.
Limaye AP, et al. J Infect Dis. 2001;183:377-382.

28

Antiviral Drug Resistance: Risk Factors

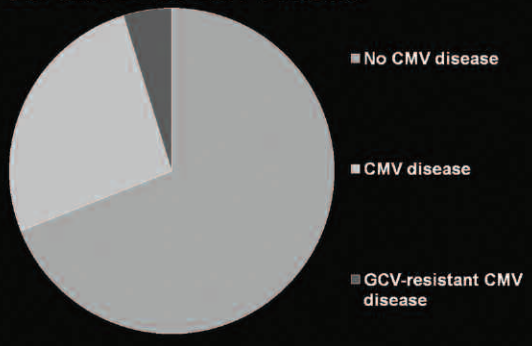
- Lack of CMV-specific immunity (D+/R-)
- High viral replication
- Multiple episodes of CMV disease
- Potent immunosuppression
- Lung and kidney–pancreas transplant recipients
- Prolonged antiviral drug administration
- Suboptimal tissue–plasma drug concentration

Razonable RR, Paya CV. *Herpes.* 2003;10:60-65.

29

Drug-Resistant CMV in the Era of VGCV Prophylaxis

225 CMV D+/R- SOT Patients



Eid AJ, et al. *Clin Transplant.* 2007; in press.

30

GCV-Resistant CMV: Clinical Features

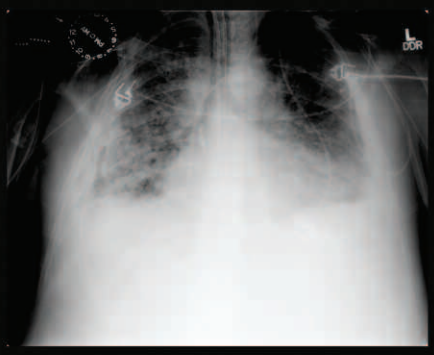
Late-onset CMV disease
Tissue-invasive CMV disease
Recurrent CMV disease
Decreased allograft survival
High mortality

- Alternative drugs: foscarnet (FOS), cidofovir, FOS-GCV
- Investigational drugs: leflunomide, maribavir

Bhorade SM, et al. *J Heart Lung Transplant.* 2002;21:1274-1282.
Eid AJ, et al. *Clin Transplant.* 2007; in press.
Isada CM, et al. *Transpl Infect Dis.* 2002;4:189-194.
Limaye AP, et al. *Lancet.* 2000;356:645-649.

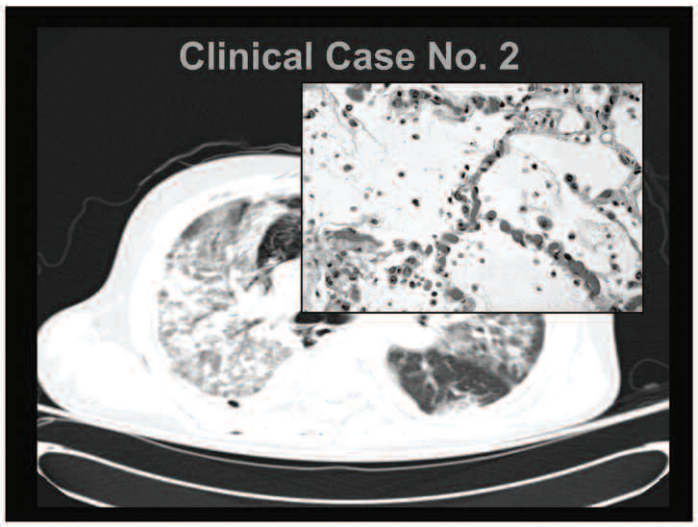
31

Clinical Case No. 2

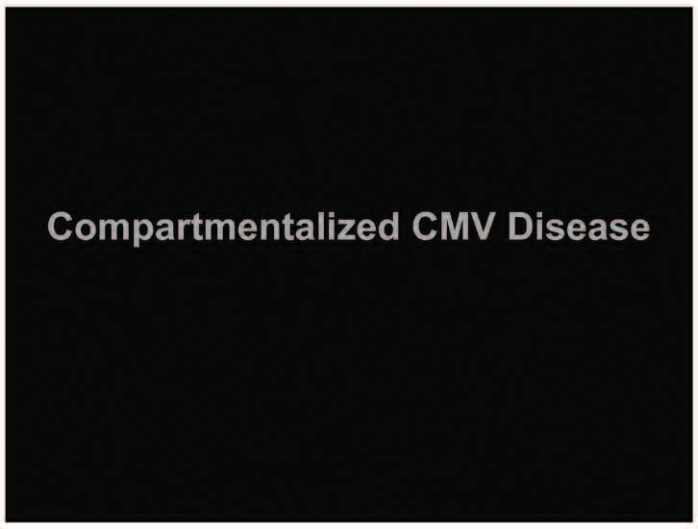


GCV-induced neutropenia in a 37-year-old CMV D+/R- liver transplant recipient with a history of CMV disease
Negative CMV PCR results in blood

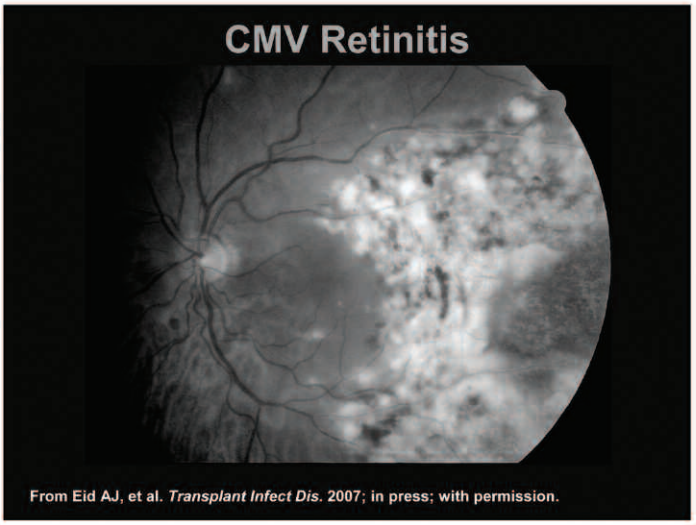
32



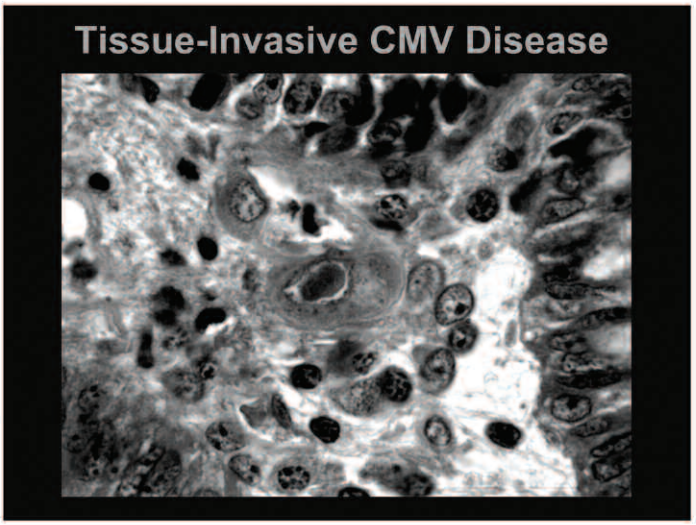
33



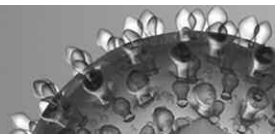
34


CMV Retinitis
From Eid AJ, et al. *Transplant Infect Dis.* 2007; in press; with permission.

35


Tissue-Invasive CMV Disease

36



Conclusions

- CMV remains an important pathogen in SOT
  - Direct and indirect effects
- Benefits of preventive measures to decrease the incidence of CMV and its indirect effects
  - Delays disease onset in a subset of patients
- Current challenges: delayed-onset CMV disease, GCV-resistant CMV, and compartmentalized disease
- Improved strategies for management are needed

37

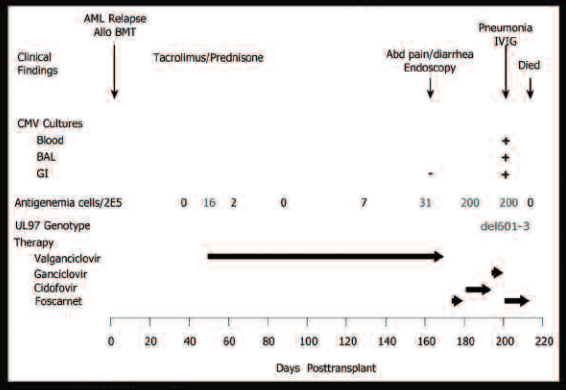
CMV Drug Resistance: Clinical Impact and Potential Strategies

Sunwen Chou, MD

Oregon Health & Science University
Portland, Oregon

1

CMV Resistance – Typical Setting

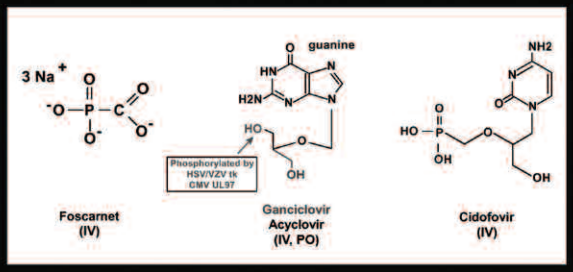


Abd=abdominal; Allo BMT=allogeneic bone marrow transplant; AML=acute myelogenous leukemia; BAL=bronchoalveolar lavage; GI=gastrointestinal.

Marfori JE, et al. *J Clin Virol.* 2007;38:120-125.

2

Current CMV Antivirals
(Viral DNA Polymerase Inhibitors)



Main adverse effects (may be dose-limiting)
- Ganciclovir (GCV)/valganciclovir: marrow suppression
- Foscarnet (FOS), cidofovir (CDV): nephrotoxicity

HSV=herpes simplex virus; VZV=varicella zoster virus.

3

Risk Factors for CMV Drug Resistance

- Prolonged drug exposure (usually months)
- Host immunodeficiency
  - Transplant, HIV, medications, cancer, etc
  - Primary infection (eg, D+R- transplant)
  - Specific transplant organs (eg, lung, pancreas)
- Suboptimal antiviral drug activity
  - Missed doses because of toxicity, etc
  - Oral bioavailability/adherence
- Increasing circulating CMV load or disease while on therapy: may or may not be drug resistance

4

CMV Resistance – Phenotypic Assays

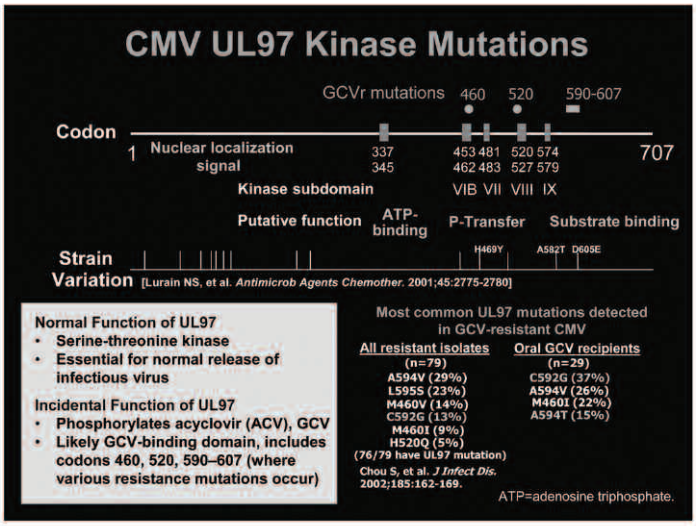
- Drug vs. viral isolate in cell culture
- IC_{50}: drug concentration that inhibits virus by 50%
- Difficult to standardize
  - Slow-growing virus, often not available to test
  - Calibrated inoculum required, may take weeks
  - Quantitation assays inefficient
  - Growth affected by cell culture condition
- Not fast enough to guide clinical decisions
- Most resistant isolates have 2x – 10x increased IC_{50}; can be higher if multiple viral mutations

5

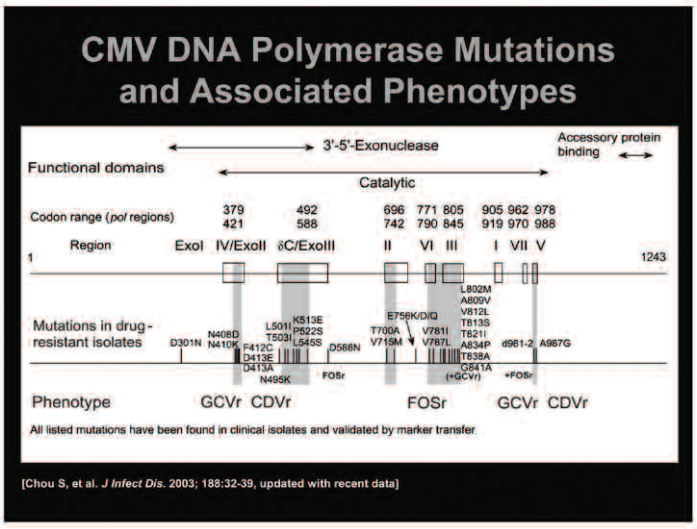
CMV Resistance – Genotypic Assays

- Amplify UL97 and *pol* sequences from isolate or direct from clinical specimen; check for mutations
- UL97 codons: 460, 520, 590–607 affect GCV only
  - Detect mutations by sequencing, restriction enzyme digestion, etc
- *pol* codons: 300–1000 may affect all current drugs
- Check amino acid changes against known database of mutations conferring resistance
- Detection threshold ~20% mutant population
- Turnaround time of <1 week may improve clinical decision-making

6


CMV UL97 Kinase Mutations
GCVr mutations 460 520 590-607
Codon
1
Nuclear localization signal
337
345
453 481
462 483
520 574
527 579
707
Kinase subdomain
VIB VII VIII IX
Putative function
ATP-binding
P-Transfer
Substrate binding
Strain Variation
H469Y
A582T D605E
[Lurain NS, et al. Antimicrob Agents Chemother. 2001;45:2775-2780]
Normal Function of UL97
• Serine-threonine kinase
• Essential for normal release of infectious virus
Incidental Function of UL97
• Phosphorylates acyclovir (ACV), GCV
• Likely GCV-binding domain, includes codons 460, 520, 590–607 (where various resistance mutations occur)
Most common UL97 mutations detected in GCV-resistant CMV
All resistant isolates (n=79)
A594V (29%)
L595S (23%)
M460V (14%)
C592G (13%)
M460I (9%)
H520Q (5%)
(76/79 have UL97 mutation)
Oral GCV recipients (n=29)
C592G (37%)
A594V (26%)
M460I (22%)
A594T (15%)
Chou S, et al. J Infect Dis. 2002;185:162-169.
ATP=adenosine triphosphate.

7


CMV DNA Polymerase Mutations and Associated Phenotypes
3'-5'-Exonuclease
Accessory protein binding
Functional domains
Catalytic
Codon range (pol regions)
379 421
492 588
696 742
771 790
805 845
905 919
962 970
978 988
Region
ExoI IV/ExoII δC/ExoIII II VI III I VII V
1
1243
Mutations in drug-resistant isolates
D301N
N408D N410K
L501I T503I
K513E P522S L545S
F412C D413E D413A N495K
FOSr
D588N
T700A V715M
E756K/D/Q
V781I V787L
L802M A809V V812L T813S T821I A834P T838A G841A
(+GCVr)
d981-2
A987G
+FOSr
Phenotype
GCVr CDVr
FOSr
GCVr CDVr
All listed mutations have been found in clinical isolates and validated by marker transfer.
[Chou S, et al. J Infect Dis. 2003; 188:32-39, updated with recent data]

8

Evolution of Resistance Mutations

- After initial GCV exposure (weeks–months)
  - UL97 mutations are seen first (>90% of CMVr)
  - Later, *pol* mutations add on to cause high-grade GCV resistance (~30x) and CDV cross-resistance
- After FOS exposure: other *pol* mutations, usually with limited or no GCV-CDV cross-resistance
- Therefore, FOS is the usual second-line drug after GCV resistance develops; however,
- Single or multiple *pol* mutations are known that confer multi-drug resistance, because all current drugs target the CMV DNA polymerase

9

Frequency of GCV Resistance

- AIDS/retinitis[1]
  - 20%–5% after 1 year depending on HAART
- Transplant setting (solid organ)
  - Almost always in primary infection (D+R-)
  - 5%–10% of (D+R-) recipients overall[2]
    - 3%–6% oral ganciclovir, 0% valganciclovir; non-lung[3]
    - 5% of 80 heart, 4/32 with disease[4]
  - Higher incidence in lung transplant recipients
    - 16% of 120[5]
    - 3/11 with 1 death[6]
  - Median onset of resistance 5–6 months post-transplant

1. Martin BK, et al. *Clin Infect Dis*. 2007;44:1001-1008.
2. Gilbert C, Boivin G. *Antimicrob Agents Chemother*. 2005;49:873-883.
3. Boivin G, et al. *Transpl Infect Dis*. 2005;7:166-170.
4. Li F, et al. *Clin Infect Dis*. 2007;45:439-447.
5. Lurain NS, et al. *J Infect Dis*. 2002;186:760-768.
6. Limaye AP, et al. *J Infect Dis*. 2002;186:724-725.

HAART=highly active antiretroviral therapy.

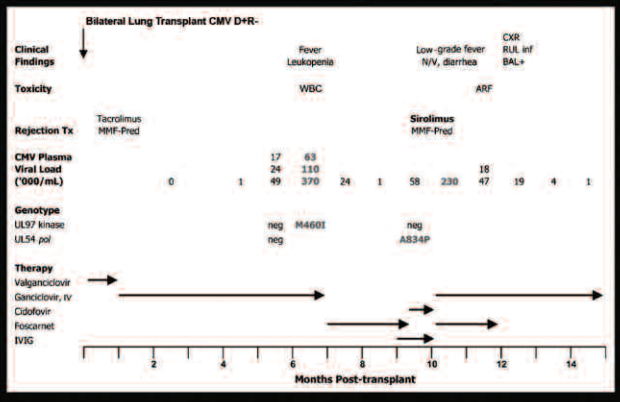
10

Treatment of GCVr CMV

- Foscarnet (the standard alternative treatment)
  - Renal toxicity in setting of other transplant medications
  - Fluid/electrolyte management problems
  - Some suggest combining with GCV
- Cidofovir (doubtful, toxicity often limiting)
  - Best to have *pol* genotypic data
- Immunomodulators with anti-CMV activity
  - mTor inhibitors: sirolimus, everolimus
  - Other (leflunomide, FK778, antibodies, etc)
  - May have adjunctive role, not FDA-approved
- Experimental anti-CMV drugs
  - For example, maribavir, in Phase III clinical trials

11

Resistance – Lung Transplant



ARF = acute renal failure; N/V=nausea/vomiting; BAL=bronchoalveolar lavage; CXR=chest x-ray; MMF-Pred=mycophenolate mofetil-prednisone; RUL inf=right upper lobe infiltrate; Tx=treatment; WBC=white blood cell.

12

GCV-FOS Combination Treatment

- ***In vitro* GCV-FOS synergy?**
  - Published data conflicting (methods/criteria)
  - Not observed in my laboratory (additive/not antagonistic)
- **Clinical experience**
  - Prospective study in stem cell transplant (STC)/ solid organ transplant (SOT) recipients[1]
    - GCV 5 mg/kg bid vs GCV 5 mg/kg/d + FOS 90 mg/kg/d
    - As initial pre-emptive treatment — resistance not suspected
    - Monitored by clearing of CMV DNA in blood by polymerase chain reaction (PCR)
    - Result: combination trending worse as initial therapy
  - In setting of possible GCV resistance
    - GCV + FOS useful in some cases not responding to GCV
    - Case reports/small series[2]/no controls
    - Review: Drew WL, *J Clin Virol.* 2006;35:485-488
  - Main problem is toxicity; half-dose treatment unproven

1. Mattes FN, et al. *J Infect Dis.* 2004;189:1355-1361.
2. Mylonakis E, et al. *Clin Infect Dis.* 2002;34:1337-1341.

13

Host Factor Treatment

- Reduce overall immunosuppression if possible
- Cellular kinase inhibitors (not FDA-approved for CMV)
  - Roscovitine, sirolimus, etc
  - Measurable *in vitro* anti-CMV effect
    - IC_{50}: sirolimus = 0.14 nM; A77-1726 (leflunomide) = 8 µM
  - ~50% risk ratio CMV disease with sirolimus vs. azathioprine/mycophenolate
    - Review: Webster AC, et al. *Transplantation.* 2006;81:1234-1248
- Other unapproved medications (anecdotal use)
  - Leflunomide +/- FOS[1-3]
    - Watch for hepatotoxicity
  - Artesunate

1. Avery RK. *Clin Infect Dis.* 2007;45:448-449.
2. Avery RK, et al. *Bone Marrow Transplant.* 2004;34:1071-1075.
3. Battiwalla M, et al. *Transpl Infect Dis.* 2007;9:28-32.

14

Experimental Drug: Maribavir

Benzimidazole L-riboside

UL97 kinase inhibitor: a new antiviral mechanism

- UL97 kinase required for normal CMV assembly
- Distinct from incidental role in phosphorylating GCV
- Maribavir has no activity against HSV, VZV (unlike GCV)

Clinical experience to date

- Phase I trial in AIDS[1]
  - Orally bioavailable, low toxicity (taste disturbance)
  - Reduced viral shedding ~3 log in 4-week trial
- Phase II trial in stem cell transplants[2]
  - Posttransplant prophylaxis for up to 12 weeks
  - Well tolerated, reduced viral reactivation ~50%–75%
- Phase III prophylaxis trials ongoing (stem cell)
- Phase III trials starting (liver transplant)
- No data on treatment of invasive disease

1. Lalezari JP, et al. *Antimicrob Agents Chemother.* 2002;46:2969-2976.
2. ViroPharma, unpublished data.

15

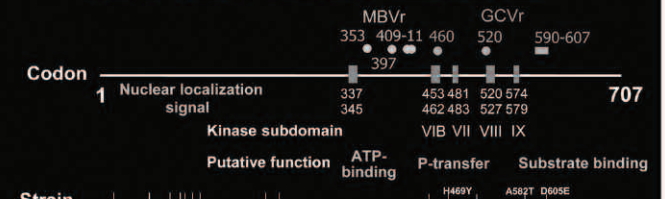
Maribavir – Antiviral Properties

- Selective and potent inhibition of UL97 kinase[1]
- Cellular factors affect antiviral activity[2]
  - Cell type, state of activation
  - Some cellular kinase inhibitors enhance maribavir activity
- Viral factors – strain differences (little information so far)
- Relationship to existing drugs – GCV/CDV/FOS[3]
  - Antagonizes GCV (UL97 phosphorylation)
  - Likely additive with others
- Resistance – being explored in cell culture
  - UL97 mutations (c353, 397, 409, 411) confer medium to very high level resistance[4]
  - UL27 mutations (various): low-level 2x – 5x resistance[5]
  - No cross-resistance with GCV/CDV/FOS[6]

1. Biron KK, et al. *Antimicrob Agents Chemother.* 2002;46:2365-2372.
2. Chou S, et al. *Antimicrob Agents Chemother.* 2006;50:2557-2559.
3. Chou S, Marousek GI. *Antimicrob Agents Chemother.* 2006;50:3470-3472.
4. Chou S, et al. *J Infect Dis.* 2007;196:91-94.
5. Chou S, et al. *J Virol.* 2004;78:7124-7130.
6. Drew WL et al. *J Clin Virol.* 2006; 37:124-127

16

CMV UL97 Kinase Mutations



UL97 Mutation	MBV Resistance	GCV Resistance
V353A	15x	1x
L397R	>200x	1.6x
T409M	80x	1x
H411Y	12x	0.5x
H411Y+V353A	160x	0.6x
M460V	0.4x	8x

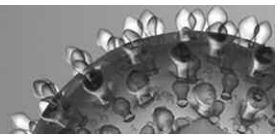
17

CMV Resistance – Summary

- Risk factors (D+R-, lung, treatment duration, etc)
- If increasing viral load during prolonged treatment, confirm with genotypic testing if possible
- Based on known mutation patterns,
  FOS is usual alternative for GCV
  GCV+FOS combination: possible but may be toxic
- Optimize immunomodulation
- New drug: maribavir (anti-UL97 Phase III)
  - Low toxicity, no cross-resistance noted to date
  - Antagonizes GCV but may be synergistic with cellular kinase inhibitors
  - Encourage clinical trial participation (currently as preventive treatment post transplant)

18

REFERENCES



Dr. Robert H. Rubin

Rubin RH, Young LS. *Clinical Approach to Infection in the Compromised Host*. New York: Kluwer Academic/Plenum; 2002.

Dr. Michael J. Boeckh

Boeckh M, Gallez-Hawkins GM, Myerson D, Zaia JA, Bowden RA. Plasma polymerase chain reaction for cytomegalovirus DNA after allogeneic marrow transplantation: comparison with polymerase chain reaction using peripheral blood leukocytes, pp65 antigenemia, and viral culture. *Transplantation*. 1997;64:108-113.

Boeckh M, Nichols WG. The impact of cytomegalovirus serostatus of donor and recipient before hematopoietic stem cell transplantation in the era of antiviral prophylaxis and preemptive therapy. *Blood*. 2004;103:2003-2008.

Lalezari JP, Aberg JA, Wang LH, Wire MB, Miner R, Snowden W, Talarico CL, Shaw S, Jacobson MA, Drew WL. Phase I dose escalation trial evaluating the pharmacokinetics, anti-human cytomegalovirus (HCMV) activity, and safety of 1263W94 in human immunodeficiency virus-infected men with asymptomatic HCMV shedding. *Antimicrob Agents Chemother*. 2002; 46:2969-2976.

Schleiss M. Progress in cytomegalovirus vaccine development. *Herpes*. 2005;12:66-75.

Winston D, van Burik J-A, Pullarkat V, Papanicolaou G, Vij R, Vance E, Alangaden G, Chemaly R, Petersen F, Chao N, Klein J, Sprague K, Dougherty C, Villano S, Boeckh M. Prophylaxis against cytomegalovirus infections with oral maribavir in allogeneic stem cell transplant recipients: results of a randomized, double-blind, placebo-controlled trial. *Blood* (ASH Annual Meeting Abstracts). 2006;108:Abstract 593.

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Dr. Raymund R. Razonable

Arthurs SK, Eid AJ, Kremers WK, Pedersen RA, Dierkhising R, Patel R, Razonable RR. Clinical features and outcomes of delayed-onset primary cytomegalovirus disease in cardiac transplant recipients. *J Heart Lung Transplant*. 2007; in press.

Arthurs SK, Eid AJ, Pedersen R, Dierkhising R, Kremers WK, Patel R, Razonable RR. Delayed-onset primary cytomegalovirus disease after liver transplantation. *Liver Transplant*. 2007; in press.

Asberg A, Humar A, Rollag H, Jardine AG, Mouas H, Pescovitz MD, Sgarabotto D, Tuncer M, Noronha IL, Hartman A, on behalf of the VICTOR Study Group. Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients. *Am J Transplant*. 2007;7:2106.

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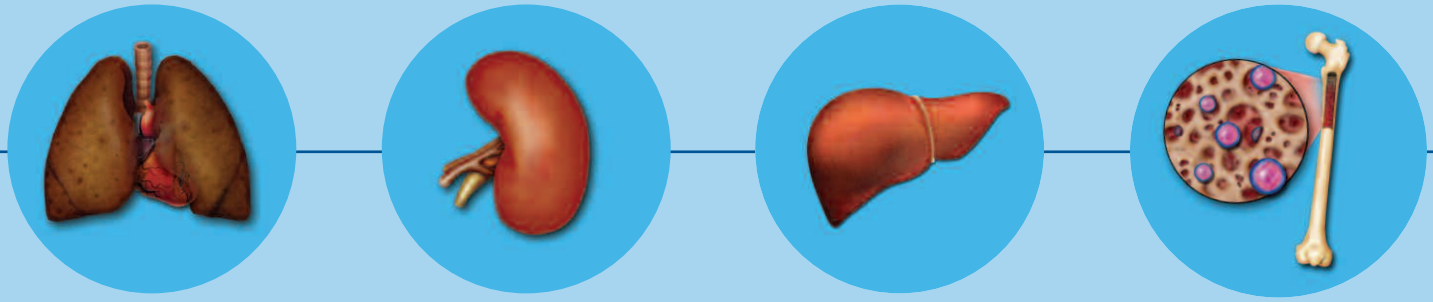

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