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
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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.
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

CMV Disease in Transplant Recipients: Strategies, Challenges and Opportunities
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, 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.
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.

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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.
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


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

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


Infection in Transplant Patients and Normal Hosts

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

Tumor necrosis factor.

Other Pathways for Reactivating CMV

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

Other Pathways for Reactivating CMV

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

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.

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

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)
**CMV Infection, Immunosuppression, Clinical Disease**

<table>
<thead>
<tr>
<th>Type</th>
<th>Donor, Recipient Status</th>
<th>Immunosuppression</th>
<th>Symptomatic Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>D+, R-</td>
<td>“Any”</td>
<td>50%</td>
</tr>
<tr>
<td>Reactivation</td>
<td>D-, R+</td>
<td>No ATG</td>
<td>10%-15%</td>
</tr>
<tr>
<td>Reactivation</td>
<td>D+, R+</td>
<td>No ATG</td>
<td>25%</td>
</tr>
<tr>
<td>“Cytokine storm”</td>
<td>D+, R+</td>
<td>ATG</td>
<td>&gt; 50%</td>
</tr>
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</table>

*3-6 weeks after cytokine storm, 56%+ symptomatic disease.
ATG=Antithymocyte globulin.

**Antimicrobial Therapy in the Organ Transplant Patient**

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

Prevention of Direct Manifestations of CMV

- Prophylaxis
- Preemptive
- Therapeutic

“Viremia = Truth”


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 post transplant 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.

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.

Future Issues

1. Diagnosis of indirect syndromes
2. Importance of human herpesvirus-6
3. How to best treat or prevent virus
4. Optimal immunosuppression
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.
Managing CMV in Hematopoietic Cell Transplant Recipients: Challenges and Opportunities

Michael Boeckh, MD
Fred Hutchinson Cancer Research Center
University of Washington
Seattle, WA

Current Prevention Strategies CMV

- Prophylaxis
- Pre-emptive Treatment
- Antigenemia
- DNA/RNA

Days after Transplantation

1

1

2

2
CMV Prevention in HCT Recipients

Why Not Prophylaxis?

- It works but...
  - Toxicity
  - Overtreatment
  - Delayed immune reconstitution
  - Lack of improvement in overall survival with presently available drugs (except acyclovir)
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

Source: Boehr & Nichols Blood 2004

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
- Foscarnet (Reusser et al. Blood 2002)
  - Equivalent to IV GCV for CMV disease-free survival
  - Less neutropenia
- Cidofovir: no randomized trials
Control of CMV
Future Strategies

- Novel anti-CMV drugs
  - Maribavir

- T cell therapy

- Vaccination strategies

Anti-CMV Drugs: Mechanism of Action

Maribavir

UL97 (CMV) DNA elongation DNA packaging Capsid egress

Protein kinase

Ganciclovir

Alternate substrate
Incorporation into growing DNA

Chain termination
**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

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

From Laezari et al. AAC. 2002;48: 2969-2976; with permission.
### Maribavir

#### Safety in Phase II HCT Study

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

* P < 0.05

Winston et al. ASH 2006 abstract

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### Maribavir

#### D/C due to Related Adverse Events

<table>
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<th>AEs</th>
<th>Placebo</th>
<th>100 mg BID</th>
<th>400 mg QD</th>
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<td>N</td>
<td>28</td>
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<td>26</td>
</tr>
<tr>
<td>Taste dist.</td>
<td>0</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>Nausea</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>3 (12%)</td>
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<tr>
<td>Vomiting</td>
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<td>1 (4%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
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<tr>
<td>Dysphagia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (4%)</td>
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<tr>
<td>GERD</td>
<td>1 (4%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rash</td>
<td>1 (4%)</td>
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<td>0</td>
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</table>

Total 3 (11%) 4 (14%) 3 (11%) 9 (35%)

Winston et al. ASH 2006 abstract
## 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*

### Maribavir Safety in Phase II HCT Study

<table>
<thead>
<tr>
<th></th>
<th>Plac</th>
<th>100 BID</th>
<th>Maribavir</th>
<th>400 QD</th>
<th>400 BID</th>
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<tr>
<td>Neutropenia on study drug</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ANC &lt; 1000</td>
<td>14%</td>
<td>21%</td>
<td>18%</td>
<td>15%</td>
<td></td>
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<tr>
<td>ANC &lt; 750</td>
<td>14%</td>
<td>14%</td>
<td>11%</td>
<td>12%</td>
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<tr>
<td>ANC &lt; 500</td>
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<td>11%</td>
<td>7%</td>
<td>4%</td>
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<tr>
<td>Neutropenia until day 100</td>
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<td></td>
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<tr>
<td>ANC &lt; 1000</td>
<td>39%</td>
<td>25%</td>
<td>21%</td>
<td>35%</td>
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<tr>
<td>ANC &lt; 750</td>
<td>39%</td>
<td>14%</td>
<td>18%</td>
<td>27%</td>
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<td>21%</td>
<td>11%</td>
<td>11%</td>
<td>12%</td>
<td></td>
</tr>
</tbody>
</table>

*Winston et al. ASH 2006 abstract*
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

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
Engraftment (14-30 days)
Maribavir
2:1 GCV/FSC for PCR/AG+
Placibo
Post-study follow-up

CMV Disease in Transplant Recipients: Strategies, Challenges and Opportunities
Imune 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

Current Vaccine Candidates

Summary of current status of CMV vaccines

- Live, attenuated vaccines
- DNA vaccines
- Recombinant vaccines
- Peptide vaccines
- Live-virus vaccines

From Schleiss M, Herpes. 2005;12:66-75; with permission.
CMV DNA Vaccine
Phase II: HCT Recipient +/- Donor Vaccination

- Related donor: sero - or +
- Recipients: sero +
- Vaccine: gB and pp65 plasmid (Vical Inc.)

Transplantation

Preemptive Rx

Related Donor Vaccination

Recipient Vaccination

Days
-7 -4 -2
Months
0 1 2 3 4 5 6 7 8

Endpoints
- Viral load
- Need for PET
- Immunogenicity
- Safety

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
<table>
<thead>
<tr>
<th>Maribavir co-Investigators</th>
<th>Lab and Clinical Studies</th>
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<tr>
<td>D Winston</td>
<td>T Stevens-Ayers</td>
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<td>J van Burik</td>
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<tr>
<td>G Papanicolaou</td>
<td>C Varley</td>
</tr>
<tr>
<td>R Vij</td>
<td>S Chatterton Kirchmeier</td>
</tr>
<tr>
<td>E Vance</td>
<td>J Ferrenberg</td>
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<td>G Alangaden</td>
<td>E Minrich</td>
</tr>
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<td>R Chemaly</td>
<td>G Jolly</td>
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<td>F Peterson</td>
<td>J Heugel</td>
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<td>N Choudhary</td>
<td>C Dahigren</td>
</tr>
<tr>
<td>R Klein</td>
<td>J Huggler</td>
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<tr>
<td>M Spague</td>
<td></td>
</tr>
<tr>
<td>S Villano</td>
<td></td>
</tr>
</tbody>
</table>

Adoptee T cell studies

S Riddell
P Greenberg
T Manley
K Kirby
Cytomegalovirus Disease in Solid Organ Transplant Recipients

Raymund R. Razonable, MD
Division of Infectious Diseases
Mayo Clinic College of Medicine
Rochester, Minnesota


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
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.


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


- 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.

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

- No Prophylaxis
- Prophylaxis

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, Young LS. Clinical Approaches to Infection in the Compromised Host.

Risk Factors for CMV Disease in Solid Organ Transplantation
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


CMV-Specific T-Cell Responses Following Alemtuzumab Induction

**CMV and Mannose-Binding Lectin (MBL)**

- **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.

---

**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)

**Reduced Risk**

- CMV D-/R+
- Antiviral prophylaxis
- Preemptive therapy

---

**ALG=anti-human lymphocyte globulin; ATG=anti-thymocyte globulin; HHV=human herpes virus; OKT3=anti-CD3 monoclonal antibody.**
Prevention of CMV Disease After SOT

- 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

Preemptive Therapy (1 of 2)

45 SOT patients

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median time to negative PCR (P=0.9)</th>
<th>Half-life of viral decline (P=0.7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV GCV 5 mg/kg BID (n=23)</td>
<td>14 days</td>
<td>1.73 days</td>
</tr>
<tr>
<td>VGCV 900 mg PO BID (n=22)</td>
<td>15.2 days</td>
<td>2.16 days</td>
</tr>
</tbody>
</table>

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,

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

Anti-CMV Prophylaxis: Meta-analyses

<table>
<thead>
<tr>
<th>Study Author</th>
<th>CMV Disease (Relative Risk)</th>
<th>All-Cause Mortality (Relative Risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hodson</td>
<td>0.42 (0.34–0.52)</td>
<td>0.63 (0.43–0.92)</td>
</tr>
<tr>
<td>Kalil</td>
<td>0.20 (0.13–0.31)</td>
<td>0.62 (0.40–0.96)</td>
</tr>
<tr>
<td>Small</td>
<td>0.34 (0.24–0.48)</td>
<td>0.99 (0.68–1.43)</td>
</tr>
</tbody>
</table>


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

'Risk Factors for Delayed CMV Disease

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


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

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


Drug-Resistant CMV in the Era of VGCV Prophylaxis

225 CMV D+/R- SOT Patients

- No CMV disease
- CMV disease
- GCV-resistant CMV disease

GCV-Resistant CMV: Clinical Features

<table>
<thead>
<tr>
<th>Late-onset CMV disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue-invasive CMV disease</td>
</tr>
<tr>
<td>Recurrent CMV disease</td>
</tr>
<tr>
<td>Decreased allograft survival</td>
</tr>
<tr>
<td>High mortality</td>
</tr>
</tbody>
</table>

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


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
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
Current CMV Antivirals
(Viral DNA Polymerase Inhibitors)

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

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
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

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
**CMV UL97 Kinase Mutations**

- **Codon**
  - Nuclear localization signal
  - Kinase subdomain
- **Putative function**
  - ATP-binding
  - P-Transfer
- **Strain Variation**

<table>
<thead>
<tr>
<th>Codon</th>
<th>GCVr mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>345</td>
</tr>
<tr>
<td>2</td>
<td>520</td>
</tr>
<tr>
<td>3</td>
<td>590-607</td>
</tr>
</tbody>
</table>

- **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
  - Oral GCV recipients

- **ATP binding:** ATPadenosine triphosphate.

**CMV DNA Polymerase Mutations and Associated Phenotypes**

- **Functional domains**
  - 3'-5' Exonuclease
  - Catalytic

- **Codon range (orf regions)**
  - 379-421
  - 492-586
  - 698-711
  - 905-962

- **Mutations in drug-resistant isolates**
  - GCVR
  - CDVR
  - FOSR
  - GCVr CDVr

- **Phenotype**
  - All listed mutations have been found in clinical isolates and validated by marker transfer

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

Frequency of GCV Resistance

- AIDS/retinitis
  - 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
    - 3%–6% oral ganciclovir, 0% valganciclovir; non-lung
    - 5% of 80 heart, 4/32 with disease
  - Higher incidence in lung transplant recipients
    - 16% of 120
    - 3/11 with 1 death
  - Median onset of resistance 5-6 months post-transplant

---

Treatment of GCV-resistant 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

Resistance – Lung Transplant

[Diagram showing lung transplant with resistance and treatment timelines]
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
  - GCV 5 mg/kg bid vs GCV 5 mg/kg qd + FOS 90 mg/kg id
  - As initial preemptive 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/no controls
  - Main problem is toxicity; half-dose treatment unproven


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 mM; A77-1726 (leflunomide) = 8 µM
    - ~50% risk ratio CMV disease with sirolimus vs. azathioprine/mycophenolate
- Other unapproved medications (anecdotal use)
  - Leflunomide +/- FOS^{1-3}
    - Watch for hepatotoxicity
  - Artesunate

1. Avery SK. Clin Infect Dis. 2007;45:488-489
**Experimental Drug: Maribavir**

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
  - Orally bioavailable, low toxicity (taste disturbance)
  - Reduced viral shedding ~3 log in 4-week trial
- Phase II trial in stem cell transplants
  - 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

2. VirPharma, unpublished data.

---

**Maribavir – Antiviral Properties**

- Selective and potent inhibition of UL97 kinase
- Cellular factors affect antiviral activity
  - 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
  - Antagonizes GCV (UL97 phosphorylation)
  - Likely additive with others
- Resistance – being explored in cell culture
  - UL97 mutations (635, 397, 409, 411) confer medium to very high level resistance
  - UL27 mutations (various): low-level 2x – 5x resistance
  - No cross-resistance with GCV/CDV/FOS

CMV UL97 Kinase Mutations

<table>
<thead>
<tr>
<th>Codon</th>
<th>Nuclear localization signal</th>
<th>Kinase subdomain</th>
<th>Putative function</th>
<th>ATP-binding</th>
<th>P-transfer</th>
<th>Substrate binding</th>
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<tr>
<td>337</td>
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<td>Vb</td>
<td></td>
<td></td>
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<tr>
<td>345</td>
<td></td>
<td>Vi</td>
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<tr>
<td>397</td>
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<td>Vii</td>
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<tr>
<td>507</td>
<td></td>
<td>Viii</td>
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</tbody>
</table>

Strain Variation

<table>
<thead>
<tr>
<th>UL79 Mutation</th>
<th>MBV Resistance</th>
<th>GCV Resistance</th>
</tr>
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<tbody>
<tr>
<td>V352A</td>
<td>15x</td>
<td>1x</td>
</tr>
<tr>
<td>L397R</td>
<td>&gt;200x</td>
<td>1.5x</td>
</tr>
<tr>
<td>T409M</td>
<td>80x</td>
<td>1x</td>
</tr>
<tr>
<td>H411Y</td>
<td>12x</td>
<td>0.5x</td>
</tr>
<tr>
<td>H411Y+V353A</td>
<td>160x</td>
<td>0.6x</td>
</tr>
<tr>
<td>M460V</td>
<td>0.4x</td>
<td>8x</td>
</tr>
</tbody>
</table>

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)
Dr. Robert H. Rubin

Dr. Michael J. Boeckh


Dr. Raymund R. Razonable


REFERENCES

Dr. Raymund R. Razonable (continued)


Dr. Sunwen Chou


Dr. Sunwen Chou (continued)


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