Prevention Strategies to Minimize the Clinical Burden of CMV in Transplant Recipients

Supported by an educational grant from Merck & Co., Inc.
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Recognizing the Burden of CMV and Patient Risk Factors for CMV Post-transplantation

SOT Focus

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Incidence of CMV After SOT

- Without prevention, up to 75% developed active CMV infection (data 1980s–90s)

- Modern era: low rates of CMV infection and end-organ disease
  - With better diagnostics and disease recognition, trend toward more asymptomatic viremia

- Rates vary depending on:
  - Institutional protocols for immunosuppression, CMV prevention
  - Different clinical and virologic thresholds for diagnosis of infection and disease

- Late CMV (after end of prophylaxis)
  - Still “Achilles heel” of CMV prevention
Risk Factors for CMV Disease in SOT Recipients

- Primary infection in recipient without prior immunity: CMV D+/R-
  - CMV D+/R+ 2nd highest risk, then D-/R+
  - CMV D-/R- lowest risk (using filtered/seronegative blood products)

- Overall state of immunosuppression
  - Intensity of immunosuppression
    - Induction/rejection: Lymphocyte-depleting agents (anti-thymocyte globulin)
    - Maintenance immunosuppression: levels of medications used; mTOR inhibitors (everolimus/sirolimus) associated with lower risk CMV

- Host factors that increase risk
  - Advanced age
  - Comorbidities
  - Leukopenia/lymphopenia
  - Genetic factors

- Transplant type
  - Lung, small bowel, composite tissue > heart, liver, kidney transplant
Minireview

Transplant Infectious Diseases: A Review of the Scientific Registry of Transplant Recipients Published Data

C. N. Kotton¹,*, S. Huprikar² and D. Kumar³

NHANES data:* 50.4% adults in USA are CMV sero+

CMV Serology and Replication Remain Associated With Solid Organ Graft Rejection and Graft Loss in the Era of Prophylactic Treatment

Direct Effects of CMV Infection

CMV viral syndrome
- Flu-like syndrome: fever, malaise, myalgias
- Leukopenia, thrombocytopenia

Tissue-invasive disease
- Colitis
- Hepatitis
- Pneumonitis
- Myocarditis
- Nephritis
- Encephalitis
- Retinitis
- Pancreatitis

Indirect Effects of CMV Infection

Indirect Effects

General indirect effects—elevated risks
- Bacterial infection
- Fungal infection
- Viral infection
- PTLD
- Cardiovascular events
- New-onset diabetes mellitus after transplantation
- Immunosenescence
- Acute rejection
- Mortality

Transplant-specific indirect effects
- Chronic allograft nephropathy and/or allograft loss after renal transplant
- Accelerated hepatitis C recurrence after liver transplant
- Hepatic artery thrombosis after liver transplant
- Allograft vasculopathy after cardiac transplant
- Bronchiolitis obliterans after lung transplant

Optimal CMV management may have a major impact on both individual AND programmatic outcomes.

PTLD, posttransplant lymphoproliferative disorder
# Risk Factors for Refractory or Resistant CMV in SOT Recipients

<table>
<thead>
<tr>
<th>Host factors</th>
<th>Viral factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>- CMV-seronegative recipient</td>
<td>- High CMV viral loads at start of therapy</td>
</tr>
<tr>
<td>- Potent immunosuppression</td>
<td>- Ongoing low-level CMV viremia</td>
</tr>
<tr>
<td>- Previous/prolonged antiviral CMV drug exposure</td>
<td>- Failure of CMV viral load to fall despite appropriate treatment</td>
</tr>
<tr>
<td>- Recurrent CMV infection</td>
<td>- Rise in CMV viral load after decline on appropriate therapy</td>
</tr>
<tr>
<td>- Subtherapeutic antiviral level (low dose, poor absorption)</td>
<td>- Poor compliance</td>
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- High CMV viral loads at start of therapy
- Ongoing low-level CMV viremia
- Failure of CMV viral load to fall despite appropriate treatment
- Rise in CMV viral load after decline on appropriate therapy
Suggested Algorithm for Managing Suspected CMV Drug Resistance (strong, low)

Recognizing the Burden of CMV and Patient Risk Factors for CMV Post-transplantation

*HSCT Focus*

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CMV Reactivation and Disease

CMV seropositive status in R or D or both

- CMV reactivation
- CMV disease

Increased risk for poor outcomes


In the seropositive recipient (R+/D- or R+/D-) incidence of CMV infection ranges from 20 to 75%
CMV Infection & Disease in HCT

- CMV infection/reactivation — no end-organ disease and preemptive treatment (PET) required to prevent disease
- CMV disease: viremia + organ dysfunction due to CMV*

- Direct effect of CMV: pneumonitis, hepatitis, GI disease, myocarditis, encephalitis, retinitis, polyradiculopathy, etc.
- Indirect effect of CMV: immunomodulating virus: secondary bacterial and invasive fungal infections (e.g., *Aspergillus*), myelosuppression, graft failure, GVHD

Clinical Case 1: Patient Risk for CMV

- 62-year-old Asian female with acute myelogenous leukemia with high-risk cytogenetics in clinical remission #1 underwent haploidentical stem cell transplantation from her 24-year-old son
- Both patient and her son were CMV IgG+
- Conditioning regimen included single dose of TBI 200 cGy + fludarabine + cyclophosphamide
- Post-HCT at day 3 & 5, received cyclophosphamide
- She engrafted by day 16 with engraftment syndrome that required short course of steroids, and was discharged home by day 28 on tacrolimus and sirolimus
- Discharge medications: acyclovir, TMP-SMX, posaconazole, sirolimus, tacrolimus, pantoprazole

HCT, hematopoietic cell transplant; TBI, total body irradiation
Clinical Case 1 (continued)

- Day +29: CMV reactivation with VL 996 copies/mL – valganciclovir (Val-GCV) 900 mg BID
- Day +36: VL 57,000 c/mL – admitted & started on IV ganciclovir (GCV) 5 mg/kg q12h
- Day +40: VL 135,336 c/mL – hypoxic while on GCV and IVIG QOD for suspected CMV-IP
- Day +40: CT chest – diffuse infiltrates
- Day +42: BAL fluid – cytology and shell vial cultures negative for CMV, BAL CMV VL 11,700 IU/ mL, & HHV6 – 800 c/mL
- Day +46: CMV genotypic resistance assay – no mutations in UL97 or UL54
- Day +48: Pending CMV resistance assay – switched to foscarnet
- Day +57: CMV VL 1623 c/mL
- Day +61: CMV VL <500 c/mL
- Day +75: CMV VL 2109 c/mL
- Day +79: deceased from severe respiratory failure – unable to ventilate

BAL, bronchoalveolar lavage; CMV, cytomegalovirus; GCV, ganciclovir; VL, viral load
Clinical Case 1 (continued)

- WBC at onset of viremia – 1300/mm³ with ALC 208/mm³
- Intermittently pancytopenic on GCV requiring filgrastim
- Developed renal failure requiring CRRT – on foscarnet
- Prolonged ICU stay
- Pulmonary fibrosis as result of ARDS – RotoProne ventilation
- Recurrence of CMV viremia despite being on antivirals

ARDS, acute respiratory distress syndrome; ALC, absolute lymphocyte count; CRRT, continuous renal replacement therapy; GCV, ganciclovir
Polling Question

What are the risk factors for CMV infection and disease?

1. T cell depletion (in-vivo or ex-vivo)
2. Lymphopenia
3. Patient within the first 100 days of HCT
4. Lack of CMV-specific CD8 and CD4 cells
5. All of the above
Risk Factors for CMV Infection/Disease

- CMV serologic status of the recipient and donor
  (Risk based on serology: D-/R+ > D+/R+ > D+/R- > D-/R-)
- T cell depletion: ex-vivo, in-vivo
- Mismatched or unrelated donors
- GVHD: acute and chronic
- High-dose corticosteroids
- Lymphopenia
- Lack of CMV-specific CD8+ CTL or CD4+ immunity
- Transplant type: cord blood, haploidentical

Time to CMV Infection Based on HCT Type

Group I: Allogeneic PBSC

Group II: CD34 selected PBSC

Group III: URD BM with in-vivo T cell depletion


PCR+ patients [%]

Days after Tx
Conditioning and CMV Disease

GVHD and Late CMV Disease

Any GVHD before day 95 (n=119)

No GVHD before day 95 (n=27)

Probability of late CMV disease

Month since transplant

Clinical Burden of CMV

- Large CIBMTR-sponsored study & EBMT database show that CMV+ serology (R/D) is associated with lower overall survival and increased non-relapse mortality (NRM)
- CIBMTR – 9469 Allogeneic HCT 2003–2010 for AML, CML, MDS and ALL
- Median time to reactivation – 41 days with 98% of reactivation within first 100 days of HCT
- CMV reactivation associated with high NRM for all disease groups
- Increased risk of early post-HCT mortality with CMV VL >250 IU/mL

AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphocytic leukemia
Cumulative Incidence of CMV Reactivation vs. Recipient/Donor CMV Serology: CIBMTR Data

Burden of CMV

- Early CMV reactivation (infection) and CMV serostatus increase NRM with resultant decrease in DFS and OS after allogeneic HCT

<table>
<thead>
<tr>
<th>Disease</th>
<th>RR of NRM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>1.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ALL</td>
<td>1.46</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CML</td>
<td>1.49</td>
<td>0.0005</td>
</tr>
<tr>
<td>MDS</td>
<td>1.31</td>
<td>0.03</td>
</tr>
</tbody>
</table>

- There was no protective effect of CMV infection in preventing hematologic disease relapse

DSF, disease-free survival; OS, overall survival; AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphocytic leukemia; NRM, non-relapse mortality

Management

- No approved prophylactic agent available; results from a recent Phase III study of letermovir are promising
  (Marty FM, et al. BMT Tandem Meetings 2017 • Orlando, FL, 26 February 2017)

- Different preventive approaches reported by some institutions in high-risk population of cord HCT

- Currently, preemptive treatment (PET) with antiviral is the standard of care
  - Reduced CMV disease (3–6%) in first 100 days;
  - Late CMV is an issue: risk of 5–18% with mortality of 17% to 46%

- Lack of consensus on CMV VL to initiate PET – variability of practice across transplant centers; although most will treat haploidentical, cord blood, & high-risk patients with any positive viral load
Burden of Preemptive Therapy

**Cost:** Study from NIH: cost differential within 6 months after HCT of $58,000 to $74,000 with CMV preemptive therapy

Median additional inpatient days per patient for antiviral group: 11.5 days
Mean additional inpatient days per patient for antiviral group: 13.9 days

\[ P < 0.017 \]

Burden of Preemptive Therapy

Allogeneic HCT with CMV Infection Gone Wrong?

Pre-Antiviral Era

Antiviral Era: Preemptive Rx

GCV, Ganciclovir; FCN, Foscarnet; IP, interstitial pneumonia

Figure courtesy of Dr. John Zaia, City of Hope National Medical Center, Duarte, CA.
Refractory/Resistant CMV Infection

- Defined as positive CMV PCR for >2 weeks despite appropriate pharmacologic treatment
- Clinical resistance: no mutations conferring resistance: host and viral factors
- Inadequate T cell immune reconstitution
- Antiviral resistance: positive for mutations (UL97, UL54)
- Associated with increased risk of disease development
- Increased risk of developing antiviral resistance
- Prolonged treatment associated with drug toxicities
- Increased risk of treatment-related mortality when it occurs in first 100 days of HCT

Summary

- Being seropositive (R+ or D+) for CMV is a burden to begin with
- Preemptive Treatment - effective in reducing risk of disease but associated with significant morbidity and cost
- Prophylaxis – Currently, no approved drug BUT an agent with least toxicity and most efficacy would be most desirable
- Refractory and resistant CMV is a challenge and requires new approaches to management
  - Prophylaxis
  - Vaccination
  - T cell immunotherapy
  - CMV-specific CAR-T cells
Diagnostic Approaches to Monitor CMV in Transplant Recipients

*HSCT Focus*

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CMV Diagnostics and PET

- Historical perspective on preemptive therapy (PET)
- Tests for CMV monitoring (non-disease situation)
- CMV surveillance post HCT
Coined the term “preemptive therapy”
Targeted therapy preemptively based on biomarkers of risk
Decreased exposure to marrow/renal-toxic antiviral drugs
Platform for application to non-viral diseases

Preemptive Strategy for CMV IP Prevention

The New England Journal of Medicine

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Volume 324 April 11, 1991 Number 15

A RANDOMIZED, CONTROLLED TRIAL OF PROPHYLACTIC GANCICLOVIR FOR CYTOMEGALOVIRUS PULMONARY INFECTION IN RECIPIENTS OF ALLOGENEIC BONE MARROW TRANSPLANTS

Gerhard M. Schmidt, M.D., David A. Horak, M.D., Joyce C. Niland, Ph.D., Steven R. Duncan, M.D., Stephen J. Forman, M.D., John A. Zaia, M.D., and the City of Hope–Stanford–Syntex CMV Study Group*

IP, interstitial pneumonia
CMV Management - Progress

HD = high dose
PET = preemptive therapy
Shell vial = tissue culture diagnosis
Pp65 AG = WBC+ CMV ag diagnosis
PCR = polymerase chain reaction diagnosis

[Diagram showing the timeline of CMV management with key events and interventions from 1986 to 2011.]
Clinical Case 2: Monitoring for CMV

- 26-year-old male with h/o severe aplastic anemia is s/p haploidentical HCT after conditioning with fludarabine, cyclophosphamide and TBI
- Post HCT, he has been screened with CMV PCR starting at day 7 using local lab platform
- At day 36, he had first positive test with VL of 1500 copies/mL
- He was started on intravenous GCV 5 mg/kg q12h
- On 7th day of treatment, viral load was undetectable & treatment changed to maintenance dose (5 mg/kg once daily)
- For unclear reasons, CMV VL was sent out to a reference lab and CMV VL was reported as 100 copies/mL
- Repeat CMV PCR at local lab showed undetectable VL
Polling Question

What would you do next?

1. Go with the outside test result and increase the dose of ganciclovir
2. Go with local lab test and no change needed
3. Check for antiviral resistance by genotypic assay
4. Change to foscarnet due to failure of ganciclovir
Utilizing PCR Assays

- Viral load threshold used to treat CMV varies across transplant centers
- Lack of standardization of the test – conversion to International Units per WHO definitions is a step forward to mitigate this (however, conversion factor varies)
- Decision-making: always use same lab to monitor CMV VL
- Timing to screen for CMV – earlier and earlier…used to be from time of engraftment to day 100 post-HCT
- Early screening may be of benefit in cord blood and haploidentical HCT
Preemptive Treatment

CMV PCR Monitoring

- At City of Hope – in high risk: haploidentical and cord HCT screen from day 7, and others from day 21 onwards through day 100

- Screening beyond day 100 HCT is based on risk factors for CMV infection (Grade 3–4 GVHD, corticosteroid use, etc.)

- Frequency of screening: twice weekly (practice may vary by institution)
PET Approaches

- In patients who develop CMV infection and require PET: monitor CMV VL weekly

- No adjustments in antiviral therapy needed over first 2 weeks based on CMV VL kinetics alone as it is not uncommon for the VL to increase by 1 log in the first 7–10 days of therapy

- Assess for antiviral resistance by genotypic assay in:
  - Those at risk for antiviral resistance, or
  - Those who have persistent viremia despite 2 weeks of appropriate agent, dose and route of administration
Diagnostic Approaches to Monitor CMV in Transplant Recipients

*SOT Focus*

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Updated International Consensus Guidelines on the Management of Cytomegalovirus in Solid-Organ Transplantation

Camille N. Kotton, Deepali Kumar, Angela M. Caliendo, Anders Åsberg, Sunwen Chou, Lara Danziger-Isakov, and Atul Humar, on behalf of The Transplantation Society International CMV Consensus Group

Cytomegalovirus (CMV) continues to be one of the most common infections after solid-organ transplantation, resulting in significant morbidity, graft loss, and adverse outcomes. Management of CMV varies considerably among transplant centers but has become more standardized by publication of consensus guidelines by the Infectious Diseases Section of The Transplantation Society. An international panel of experts was reconvened in October 2012 to revise and expand evidence and expert opinion-based consensus guidelines on CMV management, including diagnostics, immunology, prevention, treatment, drug resistance, and pediatric issues. The following report summarizes the recommendations.

Prophylaxis vs. Preemptive Therapy

**Prophylaxis** period (typically 3–6 months) after transplantation

**Antiviral prophylaxis**

**Preemptive monitoring** period (once weekly for 12–16 weeks);

If CMV is detected (PCR or pp65 Ag), treat until CMV is cleared

More common after SOT

More common after HSCT
Hybrid Strategy for SOT: Preemptive Therapy After Prophylaxis

- Weekly monitoring after end of prophylaxis, for ~12 weeks
- High risk (D+/R-) may be highest yield population (for late disease)
  - Other high-risk groups (potent immunosuppression)
- Guidelines experts use approach, not strongly evidence-based

CMV viral load assay:

- - ++ + + + + - -

Prophylaxis × 3 months

Could have initiated preemptive therapy before disease developed

months
## Polling Question

What test do you most commonly use for monitoring CMV infection/disease in transplant patients?

1. pp65 antigenemia assay (performed at local lab)
2. CMV viral load/PCR, laboratory-developed test (performed at local lab)
3. CMV viral load, commercial assay (performed at local lab)
4. CMV viral load/PCR, sent out to commercial lab
5. CMV viral load/PCR, but I don’t know which one
Current Guidelines for the Diagnosis of Patients at Risk For CMV Infection

- “QNAT* is preferred for diagnosis, decisions regarding preemptive therapy, and monitoring response to therapy due to the ability to harmonize and standardize these tests (strong, moderate).”

- “Either plasma or whole blood is an acceptable specimen for QNAT, with an appreciation of the differences in viral load values and viral kinetics. Specimen type should not be changed when monitoring patients (strong, moderate).”

- “Until harmonization of viral load tests is achieved, it is not possible to establish universal quantitative levels for trigger points of therapy or treatment endpoints. Laboratories must establish their own cutoffs and audit clinical outcomes to verify the trigger points used (strong, moderate).”

*QNAT=quantitative nucleic acid test
Clinical Case 3: Comparing Across Sample Types & Testing Platforms

- 51-year-old heart transplant recipient, Fall 2015
  - CMV D+/R+
- VGCV for CMV prophylaxis × 3 months, with some (irregular) monitoring afterwards
  - “hybrid approach” = prophylaxis then preemptive therapy/monitoring
- Had mycophenolate mofetil dose increased for 1A/1R rejection on biopsy in July 2016
- December 2016: presents with significant fatigue, leukopenia, mild transaminitis
- Outside lab results arrive
Clinical Case 3 (continued)

<table>
<thead>
<tr>
<th>Source</th>
<th>Critical Value Report</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV DNA, QN Real Time PCR</td>
<td>103380 HH</td>
<td>IU/mL</td>
</tr>
<tr>
<td>CMV DNA, QN PCR</td>
<td>5.01 H</td>
<td>log IU/mL</td>
</tr>
</tbody>
</table>

**CRITICAL VALUE REPORT**

**Cytomegalovirus DNA, Quantitative, Real-Time PCR**

**Source**
- CMV DNA, QN Real Time PCR
- CMV DNA, QN PCR

**Results**
- Whole Blood
  - 103380 HH
  - 5.01 H

**Range**
- <200 IU/mL
- <2.30 log IU/mL
Clinical Course

- Given high viral load from external reference lab, she was admitted, started on ganciclovir 5 mg/kg IV q12
  - 103,380 IU/mL on whole blood
- Repeat testing @ MGH lab:
  - 19,000 IU/mL on plasma (different testing platform)
- Switched to oral treatment with VGCV 900 mg PO BID, discharged
## Subsequent Testing & Course

<table>
<thead>
<tr>
<th>Date</th>
<th>CMV viral load plasma (MGH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12/6/16</td>
<td>19,000 IU/mL</td>
</tr>
<tr>
<td>12/12/16</td>
<td>1,070 IU/mL</td>
</tr>
<tr>
<td>12/18/16</td>
<td>“Qual positive”</td>
</tr>
<tr>
<td>12/21/16</td>
<td>&lt;137 IU/mL</td>
</tr>
<tr>
<td>1/4/17</td>
<td>&lt;137 IU/mL</td>
</tr>
<tr>
<td>1/10/17</td>
<td>not detected</td>
</tr>
</tbody>
</table>

“External Reference lab reports that the test was sent as qualitative CMV PCR not quantitative; they said the result is detectable but no value can be given”

Treatment stopped
Polling Question

How often does your program compare CMV QNAT across different testing platforms and/or specimen types?

1. Often
2. Sometimes
3. Rarely
4. Honestly, didn’t know this was an issue
Compared CMV QNAT in plasma on Cobas AmpliPrep/Cobas TaqMan (CAP/CTM) with whole blood on ELITE MGB-CMV in 185 sequential samples, 41 transplant patients

- Plasma was 1 log lower than whole blood
  - They used copies/mL, not IU/mL
- On antiviral therapy, low level of CMV DNA persisted in whole blood, absent in plasma
- “the same biological specimen should be used for a sequential and reliable follow-up of patients at high risk of CMV infection”

Comparison of Two Molecular Assays for Detection of CMV DNA in Whole Blood & Plasma Samples from Transplant Recipients

Panel A  Liver Transplantation on February

Panel B  Onco-hematology patient

Panel C  Bone Marrow Transplantation on April

Panel D  Kidney Transplantation on December

→ Will allow more accurate comparisons across testing platforms
Interlaboratory Comparison of CMV Viral Load Assays

- Samples sent to 33 laboratories in the USA, Canada and Europe
- Variation observed in reported results for individual samples ranged from 2.0 log10 (minimum) to 4.3 log10 (maximum) → 100—10,000 copies/mL
- Variation was greatest at low viral loads

Are We There Yet? Impact of the First International Standard for CMV DNA on the Harmonization of Results Reported on Plasma Samples


Variability of results for individual CMV DNA-positive samples in clinical testing panel. Shaded area highlights results falling within ±0.50 log10 IU/mL of the mean.

Observed vs. expected results for individual samples in the WHO HCMV International Standard panel tested using 10 assays. Solid black line is the line of identity (observed = expected).
Summary: Comparing Across Sample Types & Testing Platforms

- Can’t compare results unless in IU/mL and using same testing platform
- Whole blood results usually higher than plasma
- Minimal utility in qualitative CMV viral load
- Case comments:
  - Resulted in unnecessary admission
  - Trending results across systems/specimen types very challenging
Current Guidelines for the Monitoring of Patients at Risk For CMV Infection

- “Both universal prophylaxis and preemptive strategies are viable approaches for the prevention of CMV disease (strong, high).”

- “For optimal preemptive therapy, there was strong consensus that transplant recipients should be monitored by viral load testing every week for 3 to 4 months after transplantation (strong, moderate). **Meticulous weekly monitoring is needed for preemptive therapy to be effective.**”

- “To mitigate the risk of late CMV, some use a hybrid approach (i.e., **prophylaxis followed by preemptive therapy**), especially for those recipients felt to be at high risk for late CMV disease.”

- “Immunologic monitoring can be used as an adjunct tool to predict risk of viremia and disease in the postprophylaxis and preemptive setting (strong, moderate).”

Optimizing the Preemptive Approach with the Use of Immune Monitoring Assays

*SOT* Focus

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Clinical Case 4: Risk Assessment

- 67-year-old woman undergoes renal transplant, CMV donor seropositive/recipient seropositive (D+/R+)
- She is on a newer immunosuppression regimen with less rejection
- You give her a prescription for valganciclovir for 3 months, as per guidelines, but her copay is high
- She asks you to quantify her personal risk of developing CMV infection
Polling Question

What do you tell her?

1. Likely <10–20% (much higher if D+/R-)
2. Her individual risk can’t really be determined
3. Having active CMV is associated with worse outcomes
4. All of the above
Risk Assessment for CMV after SOT

- Clinical risk factors for CMV:
  - CMV donor seropositive/recipient seronegative (D+/R-)
  - More potent immunosuppression
  - Lung, intestinal, composite tissue transplants

- Prevention: guidelines suggest use of universal prophylaxis or preemptive therapy*
  - For each case prevented, significant effort & cost:
    - Diagnostics, clinical follow-up
    - Antiviral therapy, lab monitoring, use of G-CSF support

Wanted

- Assay highly predictive of CMV risk
  - Prior to transplant (CMV+)
  - After transplant (preemptive therapy)
  - After end of universal prophylaxis
  - After end of treatment of active CMV disease
  - After treatment of rejection
- Rapid turn-around time, easy to send
- Predictive even with potent immunosuppression
- Cost effective
Assessment of CMV-Specific Cell-Mediated Immunity for the Prediction of CMV Disease in High-Risk SOT Transplant Recipients: A Multicenter Cohort Study

- D+/R- on antiviral prophylaxis; Quantiferon CMV assay
- Test done at end of PPX, then at 1, 2 months
- 22% developed CMV disease
- 124 patients:
  - 31 (25%) positive Quantiferon CMV
  - 81 (65.3%) negative
  - 12 (9.7%) indeterminate result (negative mitogen)

CMV-Specific Cell-Mediated Immunity for the Prediction of CMV Disease in High-Risk SOTR

At 12 months, patients with a positive result had a subsequent lower incidence of CMV disease (6.4%) than patients with a negative (22.2%) and an indeterminate result (58.3%, respectively; p<0.001).

Clinical Case 5: Recurrent CMV

- 58-year-old woman underwent heart transplant D+/R-
- 6 months VGCV prophylaxis → 3/2017
- 2 months later, +++CMV VL
- Treated, returned
- Now with GCV-resistant CMV, foscarnet started
- Still CMV IgG negative (Total IgG nl, HIV negative), WBC/lymphs low
- Immunosuppression reduced
- Immunologically, how do we understand her risk of recurrent CMV?
First intervention study to “demonstrate the feasibility and safety of real-time CMV-specific CMI assessment to guide changes to the management of CMV infection”

27 patients (median viral load at onset 10,900 International Units/mL) were treated until viral load negative

At end of treatment:

- 14/27 (51.9%) had a positive CMV-CMI response and had antivirals discontinued; 1 low-level asymptomatic recurrence
- 13/27 (48.1%) patients had a negative CMV-CMI response and received 2 months of secondary antiviral prophylaxis; recurrence was observed in 69.2% (p=0.001)

CMI to Personalize Therapy for CMV Infection

Cytomegalovirus cell-mediated immunity testing showing interferon-γ levels at the end of treatment

Kaplan-Meier curve of cytomegalovirus recurrence ≥ 500 International Units/mL after initial viral load clearance, in patients with either a positive CMV-cell-mediated immunity (blue line) or negative CMI (red line)

CMI, cell-mediated immunity
Conclusions

- Assays of CMV cellular immunity appear promising for predicting risk of disease
- D+/R-: after transplant/after prophylaxis
- Seropositive: before and after transplant
- Additional predictive tool
- May need to include other variables to enhance accuracy
  - Serology, age, organ, HLA, others
- Larger trial data needed
  - Optimal timing, cost effectiveness
Optimizing the Preemptive Approach with the Use of Immune Monitoring Assays

**HSCT Focus**

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Director, Infection Control Section
Director of Clinical Virology
Department of ID/IC/EH
UT MD Anderson Cancer Center
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How to Increase Specificity of Preemptive Therapy Approach in HCT Recipients?

- Combine monitoring of viral load with monitoring of CMV-specific T cell immunity
- This strategy may allow withholding preemptive therapy in patients with low-to-moderate levels of CMV DNA, in presence of CMV-specific T cell responses
- However, protective T cell immunity thresholds need to be determined
Utility of the Enzyme-Linked Immunospot Interferon-γ–Release Assay to Predict the Risk of CMV Infection in HCT Recipients

- Observational prospective study in 63 CMV-recipient positive HCT recipients
  - Low risk: MRD
  - High risk: MUD, haploidentical, CBT, GVHD, prednisone >1 mg/kg
- Blood draws at specific time points from transplantation:
  - HCT—30—60—100 days
- The primary objective: To assess the ability of a T-SPOT®.CMV assay to predict CMV reactivation and/or disease in HCT recipients during the high-risk period

MRD, matched related donor; MUD, matched unrelated donor; CBT, cord blood transplantation
ELISPOT (T-SPOT®.CMV) Technology

Density gradient isolation of mononuclear cells

Quantitation of cells and adjustment of concentration

Incubation with specific antigens on ELISPOT microtiter plate

1. Blood sample is collected. At the lab, PBMCs are separated from the whole blood, washed, counted, and inoculated into 4 separate microtiter wells.

2. PBMCs and specific antigens are added to wells pre-coated with antibodies to IFN-γ and incubated for 16 to 20 hours (37°C, CO₂).

3. IFN-γ is released from activated T cells and captured. Wells are washed and a secondary conjugated antibody is added. Wells are incubated for one hour.

4. Wells are washed. A substrate is added which produces spots where interferon-gamma was secreted by T cells. Spots are counted.

T-SPOT is a registered trademark of Oxford Immunotec Ltd.
## Clinical Characteristics and Outcomes at Day 100

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>CMV reactivation</th>
<th>No CMV reactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>63</td>
<td>23</td>
<td>40</td>
</tr>
<tr>
<td><strong>Age (in years)</strong></td>
<td>56 (21 – 73)</td>
<td>57 (21 – 69)</td>
<td>56 (24 – 73)</td>
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<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>37 (59)</td>
<td>14 (61)</td>
<td>23 (58)</td>
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<tr>
<td>Female</td>
<td>26 (41)</td>
<td>9 (39)</td>
<td>17 (43)</td>
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<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>White</td>
<td>49 (78)</td>
<td>17 (74)</td>
<td>32 (80)</td>
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<tr>
<td>African American</td>
<td>6 (10)</td>
<td>3 (13)</td>
<td>3 (8)</td>
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<tr>
<td>Hispanic</td>
<td>7 (11)</td>
<td>2 (9)</td>
<td>5 (13)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (2)</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Type of Cancer</strong></td>
<td></td>
<td></td>
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<tr>
<td>Acute Leukemia</td>
<td>38 (60)</td>
<td>11 (48)</td>
<td>27 (68)</td>
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<tr>
<td>Chronic Leukemia</td>
<td>8 (13)</td>
<td>3 (13)</td>
<td>5 (13)</td>
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<tr>
<td>Myelodysplastic Syndrome</td>
<td>17 (27)</td>
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<td>8 (20)</td>
</tr>
<tr>
<td><strong>Type of Transplant</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Match Related Donor</td>
<td>23 (37)</td>
<td>5 (22)</td>
<td>18 (45)</td>
</tr>
<tr>
<td>Match Unrelated Donor</td>
<td>35 (56)</td>
<td>15 (65)</td>
<td>20 (50)</td>
</tr>
<tr>
<td>Cord</td>
<td>5 (8)</td>
<td>3 (13)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Corticosteroid use</td>
<td>19 (31)</td>
<td>5 (22)</td>
<td>14 (36)</td>
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<tr>
<td>GVHD</td>
<td>12 (19)</td>
<td>4 (17)</td>
<td>8 (20)</td>
</tr>
<tr>
<td><strong>HCT donor status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV +</td>
<td>41 (65)</td>
<td>13 (57)</td>
<td>28 (70)</td>
</tr>
<tr>
<td>CMV -</td>
<td>22 (35)</td>
<td>10 (43)</td>
<td>12 (30)</td>
</tr>
<tr>
<td>Outcome- All-cause mortality</td>
<td>8 (13)</td>
<td>4 (17)</td>
<td>4 (10)</td>
</tr>
</tbody>
</table>
Scatterplot for CMV Reactivation vs. Number of Spots, Over Different Time Points

Probability of CMV Reactivation
Stratified by High and Low Assay Response

Kaplan-Meier failure estimates

\[ P = 0.009 \]

After the Proof of Concept
A Prospective Observational Study to Evaluate a CMV-Specific Enzyme-Linked Immunospot (ELISPOT) Assay in Allogeneic HCT Recipients: The REACT Study

- Multicenter, prospective, observational study including 239 CMV+ allo-HCT candidates
- T-SPOT®.CMV (ELISPOT) assay was used to assess the production of IFNγ following ex-vivo stimulation with CMV-specific antigens (IE-1 and pp65)
- Serial blood draws (T-SPOT®.CMV and CMV PCR) were done as follows:

Pre-HCT (up to 2 weeks prior) → Total of 14 visits per patient → Week 26 (± 3 days)

Every 2 weeks (± 3 days)

Study Follow-up

Definitions of CMV Infection and Disease in Transplant Patients for Use in Clinical Trials

**CMV Event:** The first episode of significant CMV reactivation, defined as the detection of CMV in blood via the antigenemia assay or the CMV PCR assay, after which anti-CMV therapy was initiated by the treating physician in accordance with institutional guidelines.

**CMV Disease:** The first episode of CMV disease, consisting of “end-organ disease”

## Clinical Characteristics of 239 HCT Recipients

<table>
<thead>
<tr>
<th></th>
<th>CMV Reactivation (n=80)</th>
<th>No CMV Reactivation (n=159)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>37 (46)</td>
<td>100 (63)</td>
</tr>
<tr>
<td>Female</td>
<td>43 (54)</td>
<td>59 (37)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>54 (68)</td>
<td>124 (78)</td>
</tr>
<tr>
<td>African American</td>
<td>7 (9)</td>
<td>7 (4)</td>
</tr>
<tr>
<td>Asian</td>
<td>6 (8)</td>
<td>10 (6)</td>
</tr>
<tr>
<td>Unknown/Other</td>
<td>13 (15)</td>
<td>18 (12)</td>
</tr>
<tr>
<td><strong>Type of Transplant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Match Related Donor</td>
<td>21 (26)</td>
<td>64 (40)</td>
</tr>
<tr>
<td>Matched or Mismatched</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unrelated Donor</td>
<td>45 (56)</td>
<td>68 (43)</td>
</tr>
<tr>
<td>Cord Blood</td>
<td>2 (3)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Haploidentical</td>
<td>11 (14)</td>
<td>24 (15)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (1)</td>
<td>2 (1)</td>
</tr>
<tr>
<td><strong>HCT donor status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV+</td>
<td>43 (54)</td>
<td>87 (55)</td>
</tr>
<tr>
<td>CMV-</td>
<td>35 (44)</td>
<td>62 (39)</td>
</tr>
<tr>
<td>Unknown</td>
<td>2 (2)</td>
<td>10 (6)</td>
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<td><strong>Conditioning Regimen</strong></td>
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</tr>
<tr>
<td>Myeloablative</td>
<td>38 (48)</td>
<td>71 (47)</td>
</tr>
<tr>
<td>Non-Myeloablative</td>
<td>41 (51)</td>
<td>79 (50)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (1)</td>
<td>9 (3)</td>
</tr>
</tbody>
</table>

Maximum Viral Load by Maximum IE-1 (≤150 vs >150)

Test for differences in Viral Load by Maximum IE-1 (≤150 vs >150): IE1 p-value=0.0412; (Non-parametric Wilcoxon-Mann-Whitney test)

Maximum Viral Load based on values occurring after maximum IE-1 count
Box & Whiskers plot: Box is Q1-Q3, Q2 is the median, whiskers extend to min/max

CMV Reactivation in High or Low Response Patients Over Time

What to make out of the low level of CMV reactivation?
Low CMV Viral Load Study: Study Overview

**Study Design:** prospective cohort study

**Target population:** HCT recipients who develop low level CMV-PCR viral load

**Study Size:** 55 patients

**Follow-up:** 60 days

<table>
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<tr>
<th>Sample</th>
<th>Enrollment</th>
<th>Up to day 60 from enrollment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Once</td>
<td>Weekly</td>
</tr>
</tbody>
</table>
Hypothesis

HCT recipients who develop low-level CMV PCR viral load (≤1,000 IU/mL in the absence of active GvHD and/or systemic corticosteroids or ≤500 IU/mL in the presence of active GvHD and/or systemic corticosteroids) and have a positive IGRA, will have a self-limited reactivation and will not progress to high-level CMV-PCR and/or CMV disease, if not treated.

Endpoints

Primary endpoints:

1. Initiation of CMV antiviral therapy due to the combination of:
   - At least a 50% increase in CMV-PCR when compared to baseline value. (e.g. 1000 to 1500 IU/mL)
   - CMV-PCR >1,000 IU/mL in patients without active GvHD and/or systemic corticosteroids within 60 days of enrollment.
   - CMV-PCR >500 IU/mL in patients with active GvHD and/or systemic corticosteroids within 60 days of enrollment
2. New signs and symptoms of CMV disease within 60 days of enrollment

Secondary endpoints:

1. CMV-associated mortality
2. All-cause mortality at 60 days from enrollment

### Characteristics of HCT Recipients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>Progression to High CMV VL</th>
<th>No progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>55</td>
<td>28 (51)</td>
<td>27 (49)</td>
</tr>
<tr>
<td>Age (in years), median (range)</td>
<td>60 (18-73)</td>
<td>60 (18-73)</td>
<td>58 (27-69)</td>
</tr>
<tr>
<td>Sex</td>
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<td></td>
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<tr>
<td>Male</td>
<td>31 (56)</td>
<td>13 (46)</td>
<td>18 (67)</td>
</tr>
<tr>
<td>Female</td>
<td>24 (44)</td>
<td>15 (54)</td>
<td>9 (33)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>38 (69)</td>
<td>20 (71)</td>
<td>18 (67)</td>
</tr>
<tr>
<td>African American</td>
<td>2 (4)</td>
<td>1 (4)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>4 (7)</td>
<td>2 (7)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Other</td>
<td>11 (20)</td>
<td>5 (18)</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Type of Cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td>39 (71)</td>
<td>22 (79)</td>
<td>17 (63)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>8 (15)</td>
<td>2 (7)</td>
<td>6 (22)</td>
</tr>
<tr>
<td>Other</td>
<td>8 (15)</td>
<td>4 (14)</td>
<td>4 (15)</td>
</tr>
<tr>
<td>Type of Transplant - Match Related Donor</td>
<td>15 (27)</td>
<td>2 (7)</td>
<td>13 (48)</td>
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<tr>
<td>Match Unrelated Donor</td>
<td>38 (69)</td>
<td>24 (86)</td>
<td>14 (52)</td>
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<td>Autologous</td>
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<td>0</td>
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<td>Corticosteroid use</td>
<td>25 (45)</td>
<td>15 (54)</td>
<td>10 (37)</td>
</tr>
<tr>
<td>GVHD</td>
<td>11 (20)</td>
<td>6 (21)</td>
<td>5 (19)</td>
</tr>
<tr>
<td>HCT Donor Status</td>
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</tr>
<tr>
<td>CMV-</td>
<td>30 (55)</td>
<td>20 (71)</td>
<td>10 (37)</td>
</tr>
<tr>
<td>CMV+</td>
<td>25 (45)</td>
<td>8 (29)</td>
<td>17 (63)</td>
</tr>
<tr>
<td>Time from HCT to enrollment</td>
<td>22 (2-56)</td>
<td>21 (6-51)</td>
<td>26 (2-56)</td>
</tr>
<tr>
<td>All-cause mortality (at day 60)</td>
<td>4 (7)</td>
<td>2 (7)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>T-SPOT®.CMV</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>High Response</td>
<td>20 (36)</td>
<td>2 (10)</td>
<td>18 (90)</td>
</tr>
<tr>
<td>Low Response</td>
<td>34 (64)</td>
<td>26 (74)</td>
<td>9 (26)</td>
</tr>
</tbody>
</table>

Scatterplots (IE-1): Association Between Spot Count and CMV Load

T-SPOT®.CMV Response and Progression to High CMV Load

Kaplan-Meier failure estimates

Progression to high CMV load (%)

Time from enrollment to CMV progression

- Low Tspot.CMV response
- High Tspot.CMV response

P < 0.001

Conclusions

- Strong association between low CMV-specific T cell responses and progression of CMV infection in HCT recipients

- Serial monitoring of anti-CMV immune response may help stratify allo-HCT recipients at risk of progression from low CMV viral load to significant CMV infection, but further validation is needed

## Future Directions: CMV Immune Monitoring
How We There Yet?

<table>
<thead>
<tr>
<th>Clinical Scenarios</th>
<th>Potential Clinical Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>As part of preemptive strategy</td>
<td>Result may help guide frequency of viral load monitoring and thresholds for initiating antiviral therapy</td>
</tr>
<tr>
<td>Post-therapy for GvHD</td>
<td>For negative assay, viral load monitoring; For positive assay, no further intervention</td>
</tr>
<tr>
<td>Recent completion of therapy for CMV disease or viremia</td>
<td>For negative assay, consider secondary prophylaxis– close monitoring</td>
</tr>
<tr>
<td>(Prediction of recurrence of viremia)</td>
<td>For positive assay, no further therapy</td>
</tr>
<tr>
<td>Risk stratification in patients pre-transplant</td>
<td>For positive assay, assume true positive CMV status</td>
</tr>
</tbody>
</table>
Emerging Strategies in CMV Prophylaxis

Roy F. Chemaly, MD, MPH, FIDSA, FACP
Professor of Medicine
Director, Infection Control Section
Director of Clinical Virology
Department of ID/IC/EH
UT MD Anderson Cancer Center
Houston, TX
Commercially Available Drugs

- Acyclovir
- Ganciclovir
- Foscarnet
- Cidofovir
- Valacyclovir

Shortcomings: Serious toxicities, mainly with high incidence of myelosuppression, and nephrotoxicity in addition to potential resistance.

- Herpes zoster
- Recurrent herpes simplex labialis
- Varicella
- Congenital HSV
- HSV encephalitis

- AIDS - CMV retinitis
- CMV prophylaxis in high risk kidney, heart or pancreas transplant patients
New Drugs on the Horizon
November 9, 2017 – FDA approved letermovir for prophylaxis of CMV infection and disease in adult CMV-seropositive recipients [R+] of an allogeneic hematopoietic stem cell transplant (HSCT).
Brincidofovir (BCV): Broad-Spectrum Antiviral Drug

- Brincidofovir is a nucleotide analog
- Proprietary lipid technology allows oral, twice-weekly dosing that delivers active antiviral (CDV-PP) to intracellular space
- Brincidofovir is the first potential broad-spectrum antiviral agent against DNA viruses
CMX001 (Brincidofovir) to Prevent CMV Disease in HCT, Dose-Ranging Phase 2 Study

Brincidofovir Prevented CMV Reactivation in HCT Recipients

# Brincidofovir Phase II: Toxicity Data

## Serious Adverse Events (in ≥5% of patients in ITT population)

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Acute GvHD %</th>
<th>Diarrhea %</th>
<th>Pneumonia %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>7</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Brincidofovir</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 mg weekly</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>100 mg weekly</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>200 mg weekly</td>
<td>15</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>200 mg twice weekly</td>
<td>40</td>
<td>33</td>
<td>3</td>
</tr>
<tr>
<td>100 mg twice weekly</td>
<td>30</td>
<td>10</td>
<td>8</td>
</tr>
</tbody>
</table>

No evidence of increased myelosuppression or nephrotoxicity!

SUPPRESS Trial
Brincidofovir for Prevention of CMV after Allogeneic HCT in CMV-Seropositive Patients
A Randomized, Double-Blind, Placebo-Controlled, Parallel Group Phase 3 Trial

- **Population:** CMV seropositive allo-HCT recipients
- **Primary endpoint:** CMV infection requiring the initiation of preemptive therapy through Week 24
  - Suppression of CMV viremia
- **Design:** Superiority vs. current standard of care (placebo and monitoring)
- **Power:** >85% power to detect 50% reduction in CMV events vs. placebo
- **Dosing:** Began when patient can swallow tablet; twice-weekly through Week 14

2:1 randomization

<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>14</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 300</td>
<td>Brincidofovir 100 mg BIW</td>
<td>On-study follow-up</td>
<td></td>
</tr>
<tr>
<td>n = 150</td>
<td>Placebo BIW</td>
<td>Increased risk for CMV infection</td>
<td></td>
</tr>
</tbody>
</table>

SUPPRESS: Fewer Subjects Reactivated CMV During On-drug Period

SUPPRESS: More Infections Occurred on BCV During Off-drug Period

First Significant Observation

Reported GvHD events on BCV were predominantly the gut, not skin
  - Suggesting the diagnosis of GvHD was driven by diarrhea

<table>
<thead>
<tr>
<th>N (%)</th>
<th>Brincidofovir (n=303)</th>
<th>Placebo (n=149)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Skin</td>
<td>Liver</td>
</tr>
<tr>
<td>Stage 1</td>
<td>49 (16.2)</td>
<td>3 (1.0)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>42 (13.9)</td>
<td><strong>14 (4.6)</strong></td>
</tr>
<tr>
<td>Stage 3</td>
<td>22 (7.3)</td>
<td>7 (2.3)</td>
</tr>
<tr>
<td>Stage 4</td>
<td>0</td>
<td>6 (2.0)</td>
</tr>
</tbody>
</table>

The median cumulative exposure to corticosteroids was 8-fold higher in subjects on the BCV arm than those on placebo

What’s Next for Brincidofovir?
Intravenous Formulation

- Bypassing the gut appears to avoid local irritation and decrease incidence of diarrhea
- Preliminary data from 28-day preclinical study show that IV BCV has a significantly lower risk of GI effects
  - Maintained body weight during dosing
  - No evidence of injury in preliminary review of the GI tract
**Letermovir (MK-8228)**

- **Letermovir inhibits CMV through a novel mechanism**
  - Inhibits CMV terminase enzyme complex via UL56 binding
    - The terminase complex is important in CMV DNA cleavage and packaging into unit-length genome & packaging into procapsids
- **Potent CMV activity in vitro & in vivo**
- **No cross-resistance with drugs currently used in treatment of CMV**
  - Drug resistance of letermovir mapped to UL56 subunit
  - Resistance of other anti-CMV agents map to UL54 and/or UL97
- **November 9, 2017 – FDA approved letermovir for prophylaxis of CMV infection and disease in adult CMV-seropositive recipients [R+] of an allogeneic hematopoietic stem cell transplant (HSCT).**

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*Figure courtesy of Griffiths PD, Emery VC. N Engl J Med. 2014;370:1844-6.*
Letermovir for CMV Prophylaxis in HCT

A Randomized, Double-Blind, Placebo-Controlled Phase II Trial

- Primary endpoint: to evaluate safety, tolerability and antiviral activity at 12 weeks
- Weekly testing for CMV replication by scheduled visits (PCR and/or antigenemia)
- 132 allo-HCT recipients
- Randomization 1:1:1:1:1 with 33 patients in each letermovir and placebo groups

60 mg:120 mg:240 mg:Placebo

Letermovir Phase II Dose Escalation Efficacy Data

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Letermovir 60 mg</th>
<th>Letermovir 120 mg</th>
<th>Letermovir 240 mg</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of failure of prophylaxis against cytomegalovirus infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All-cause failure %</td>
<td>48</td>
<td>21</td>
<td>12</td>
<td>61</td>
</tr>
<tr>
<td>Virologic failure %</td>
<td>17</td>
<td>8</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Letermovir vs. placebo (odds ratio)</td>
<td>0.60</td>
<td>0.17</td>
<td>0.16</td>
<td>-</td>
</tr>
<tr>
<td>Letermovir vs. placebo (P value)</td>
<td>0.43</td>
<td>0.005</td>
<td>0.003</td>
<td>-</td>
</tr>
</tbody>
</table>

Modified intention-to-treat excluding patients with CMV replication at screening or day 1 detectable by central lab

Primary endpoint: Clinically significant CMV infection through post-HCT Wk 24 (full analysis set†)
- Clinically significant CMV infection defined as CMV disease occurrence or treatment with anti-CMV preemptive therapy
  - Confirmed CMV viremia and CMV disease risk

CMV-seropositive, adult allogeneic HCT recipients with no CMV viremia or acute liver injury and GFR ≥10 mL/min (N=570)

Letermovir 480 mg‡ QD PO or IV (n = 376)

Placebo (n = 194)

Pts assessed through post-HCT Wk 48; preemptive treatment given per study center guidelines

Pts required to begin treatment before post-HCT Day 28.
†Full analysis set included pts with undetectable CMV DNA at baseline.
‡240 mg if concomitantly taking cyclosporine.

CMV, cytomegalovirus; GFR, glomerular filtration rate; HCT, hematopoietic cell transplantation.
Letermovir Phase III – Results

Kaplan-Meier Plot of Time to Onset of Clinically Significant CMV Infection Through Week 24 Post-Transplant FAS Population

Letermovir vs Placebo
Stratified log-rank test, one sided p-value = 0.0005

Number of subjects at risk

<table>
<thead>
<tr>
<th></th>
<th>Letermovir</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>325</td>
<td>170</td>
</tr>
<tr>
<td>Week 2</td>
<td>320</td>
<td>169</td>
</tr>
<tr>
<td>Week 6</td>
<td>299</td>
<td>135</td>
</tr>
<tr>
<td>Week 10</td>
<td>279</td>
<td>96</td>
</tr>
<tr>
<td>Week 14</td>
<td>270</td>
<td>85</td>
</tr>
<tr>
<td>Week 18</td>
<td>254</td>
<td>77</td>
</tr>
<tr>
<td>Week 24</td>
<td>212</td>
<td>70</td>
</tr>
</tbody>
</table>

Source: [P001V01: analysis-adtts]

Letermovir vs Placebo in HCT Recipients: Phase III

All-cause mortality is significantly lower in the letermovir group

Letermovir Phase III: Safety

- GvHD was the most common AE of any severity (39% in both groups)
  - Diarrhea, nausea, fever, and rash also occurred in >20% of pts in both groups with similar frequency

<table>
<thead>
<tr>
<th>Safety Outcome During Treatment Phase, %</th>
<th>Letermovir (n = 373)</th>
<th>Placebo (n = 192)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any AE</td>
<td>97.9</td>
<td>100</td>
</tr>
<tr>
<td>Drug-related AE</td>
<td>16.9</td>
<td>12.0</td>
</tr>
<tr>
<td>Serious AE</td>
<td>44.2</td>
<td>46.9</td>
</tr>
<tr>
<td>- Infection</td>
<td>20.6</td>
<td>18.8</td>
</tr>
<tr>
<td>- GVHD</td>
<td>9.9</td>
<td>10.4</td>
</tr>
<tr>
<td>- Relapse of AML</td>
<td>4.0</td>
<td>4.7</td>
</tr>
<tr>
<td>- Acute kidney injury</td>
<td>1.3</td>
<td>4.7</td>
</tr>
<tr>
<td>- Diarrhea</td>
<td>0.5</td>
<td>2.6</td>
</tr>
<tr>
<td>- Atrial arrhythmia</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Discontinuation due to AE</td>
<td>19.3</td>
<td>51.0</td>
</tr>
<tr>
<td>- CMV treatment</td>
<td>6.2</td>
<td>39.1</td>
</tr>
<tr>
<td>- Other</td>
<td>13.1</td>
<td>12.0</td>
</tr>
</tbody>
</table>

AE, adverse event; AML, acute myeloid leukemia; CMV, cytomegalovirus; GVHD, graft-vs-host disease.
Hematological Analyses

- No evidence of bone marrow suppression
  - Hematological lab parameters similar between letermovir and placebo
  - >60% of subjects had not engrafted at baseline:
    - Incidence of engraftment similar between letermovir (95%) and placebo (91%)
    - Median time to engraftment similar between letermovir (19 days) and placebo (18 days)

---

**Cumulative Rate of Engraftment (%)**

- Week 0
- Week 14
- Week 24

**Stratified log-rank test**
- Two-sided p-value = 0.1047

Chemaly RF, *et al.* Presented at 2017 ECCMID Meeting, April 22-25, 2017; Vienna, Austria.
**Maribavir Prophylaxis for Prevention of CMV Disease in Allogeneic HCT Recipients: A Phase 3, Double-blind, Placebo-controlled, Randomized Trial**

**Screening**
- Stratification: transplant conditioning and CMV serostatus R 90 Centers

**Randomization**
- Maribavir: placebo 454:227

**Study Drug Treatment**
- weekly assessments
  - CMV disease and Safety

**Follow-up**
- Pre-emptive treatment per center standard
- CMV disease confirmed by the endpoint committee

**Primary Endpoint**

---

Maribavir vs. Placebo in HCT Recipients Phase III

"Conclusion: MBV 400–1200 mg BID was effective for treatment of CMV infection resistant/refractory to standard therapy among SCT/SOT recipients. There was no evidence of myelosuppression; data support the safety of MBV administered for up to 24 weeks. Further development of MBV for CMV treatment is warranted."
What about CMV-specific T Cell Therapy and CMV Vaccines?
CMV-specific T cells (CTLs) for Prophylaxis

- Reactivation of CMV interferes with T cell reconstitution after HSCT
- Reconstitution of CD8+ CMV-specific CTLs confers protection against CMV disease following allogeneic HSCT
  - Successful adoptive transfer of donor CMV-specific CTLs confers protection against CMV disease
- Adoptive transfer offers potential for:
  - Acceleration of antigen-specific immune reconstitution
  - Reduced morbidity and mortality of CMV after HSCT
- Concerns limit applications
  - Production logistics/implementation for clinical application (GMP)
    - CliniMACS (Cytokine capture system uses magnetic selection method to obtain virus-specific T cells from peripheral blood)
    - CAR-T cells (Chimeric antigen receptor engineered T cells)
  - Risk for induction of GvHD
  - Questions involving:
    - Antiviral function
    - Migratory capacity
    - Memory and self-renewing potential
    - T cell receptor avidity

CTL, cytotoxic T lymphocytes
CMV-specific T cells (CTLs) for Prophylaxis

- CMV specific CD8⁺ cytotoxic T cell clones isolated from donor blood infused into 14 recipients in 4 infusions 30–40 days post BMT*
  - CMV cellular immunity monitored before, during and 12 weeks after final infusion
  - CMV-CTLs reconstituted in all patients
    - Level of activity against CMV similar to donors
    - Neither CMV viremia nor CMV disease developed in any patient
    - CTL activity declined in patients deficient in CMV-specific CD4+ T cells

- CMV specific cytotoxic T-lymphocytes infused early post HSCT (either incubated or selected)**
  - Massive in-vivo expansion (3–5 log) within days
  - Reduction of viral titers within 5 days
  - Low incidence of CMV reactivation
  - No toxicities observed

- Single-dose donor-derived CMV-CTLs infused in 54 recipients at day 28 post allo-HSCT***
  - CMV reactivated in 26; only 5 post CMV-CTL infusion
  - Ganciclovir or foscarnet therapy in 9; only 1 post CMV-CTL infusion
  - Compared to a contemporaneous control group
    - 17% vs 36% required CMV directed therapy

Cytomegalovirus Vaccines Under Clinical Development

<table>
<thead>
<tr>
<th>Vaccine category</th>
<th>Antigen used</th>
<th>Manufacturer</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA (plasmid)</td>
<td>pp65, gB</td>
<td>Astellas, Vical</td>
<td>1,2 and 3</td>
</tr>
<tr>
<td>Vectored</td>
<td>pp65, gB, UL123/IE1-exon 4, UL122/IE2-exon 5</td>
<td>AlphaVax, Inc (Novartis, GSK) City of Hope, NCI Hookipa Biotech NHLBI</td>
<td>1,2</td>
</tr>
<tr>
<td>Attenuated and DISC</td>
<td>gB, pp65, IE1</td>
<td>Merck UC-SF, Vical CMV Research Foundation</td>
<td>1</td>
</tr>
<tr>
<td>Recombinant/subunit</td>
<td>gB</td>
<td>GSK, NIAID, University College London</td>
<td>1,2</td>
</tr>
<tr>
<td>Recombinant/VLP</td>
<td>gB</td>
<td>VBI vaccines and Canadian center for vaccinology</td>
<td>1</td>
</tr>
<tr>
<td>Peptide</td>
<td>pp65, T cell fused to tetanus epitope or PADRE</td>
<td>City of Hope, NCI</td>
<td>1,2</td>
</tr>
</tbody>
</table>

Summary

- Need for better preventive and therapeutic strategies
  - Reduction in CMV-related morbidity and mortality

- Survival disadvantage persists in some populations

- New antiviral drugs, immune monitoring, and advances in our ability to utilize virus-specific T cells will likely have good impact on patient outcomes in coming years.
Clinical Case 6: CMV Prevention

- 55-year-old, CMV R+ male with AML who underwent MUD HCT from a CMV-negative donor after conditioning with busulfan, fludarabine, and ATG

- Patient engrafted at day +18 and his recent ANC was 1110/mm$^3$ and ALC of 250/mm$^3$

- At day +33, on routine surveillance, his CMV PCR was positive for 470 IU/mL and went up to 790 IU/mL 2 days later

- Patient is completely asymptomatic with no fever, malaise, or other symptoms

ATG, anti-thymocyte globulin
Polling Question

What should you do?

1. Start preemptive therapy with IV ganciclovir or oral valganciclovir
2. Start preemptive therapy with IV foscarnet
3. Start preemptive therapy when CMV viral load reaches at least 1000 IU/mL
4. No need for preemptive therapy because patient is asymptomatic
What should you do?

1. Start preemptive therapy with IV ganciclovir or oral valganciclovir

2. Start preemptive therapy with IV foscarnet

3. Start preemptive therapy when CMV viral load reaches at least 1000 IU/mL

4. No need for preemptive therapy because patient is asymptomatic
Preemptive Treatment

CMV Plasma DNA Level to Start PET at MD Anderson

Any positive but if <500 IU/mL then 2 positives in a row

≥500 IU/mL

≥1000 IU/mL

≥1000 IU/mL

CMV Viral Load and CMV Disease after HCT in the Era of Preemptive Therapy

MV Cox proportional hazards model assessing CMV VL as a time-dependent RF for CMV disease 1 year after HCT, stratified by use of preemptive therapy (n=926)

Cumulative Incidence of Overall Mortality (A) and non-relapse mortality (B) at 1 year after HCT in survivors at day 100 (n=832) stratified by max CMV VL before day 100

Valganciclovir (VGCV) was started at 450 mg twice daily, adjusted for CrCl of 59 mL/min and CMV VL decreased to <137 IU/mL at day 12 of therapy.

He subsequently developed N/V and upper endoscopy was consistent with acute GI GvHD for which he received high-dose corticosteroids (CS).

He was switched to IV GCV at 2.5 mg/kg every 12 h and he received multiple doses of pegfilgrastim for a dip of his ANC below 500/mm³.

At day +62, he had recurrent N/V and his CMV VL increased to 10,320 IU/mL while on oral VGCV for secondary prophylaxis and on systemic CS.
Polling Question

What is your differential diagnosis?

1. Upper GI GvHD
2. CMV gastritis
3. CMV esophagitis
4. All of the above
What is your differential diagnosis?

1. Upper GI GvHD
2. CMV gastritis
3. CMV esophagitis
4. All of the above
Clinical Case 6 (continued)

A repeat UGI showed **CMV disease: CMV gastritis**.

- CMV gastritis with focal areas of inflammation
- CMV infection with gastric ulcerations. IHC for CMV was positive on tissue biopsy

IHC, immunohistochemistry
Clinical Case 6 (continued)

• At that point, foscarnet was started, and CMV genotypic analysis showed the **C592G UL97 mutation**

• Within 2 weeks of starting foscarnet, the patient’s CMV VL was <137 IU/mL but patient developed worsening AKI and foscarnet was held

• At day +110, patient died with worsening/refractory GI GvHD, septic shock from *E. coli*, and MOF

AKI, acute kidney injury; MOF, multiple organ failure
Polling Question

In retrospect, what would you have done differently?

1. Preemptive therapy with foscarnet
2. Hold off on preemptive therapy until the CMV VL is above 1000 IU/mL
3. Prophylaxis with letermovir before engraftment, if commercially available
4. Prophylaxis with letermovir after engraftment, if commercially available
In retrospect, what would you have done differently?

1. Preemptive therapy with foscarnet

2. Hold off on preemptive therapy until the CMV VL is above 1000 IU/mL

3. Prophylaxis with letermovir before engraftment, if commercially available

4. Prophylaxis with letermovir after engraftment, if commercially available
Prevention Strategies to Minimize the Clinical Burden of CMV in Transplant Recipients

Supported by an educational grant from Merck & Co., Inc.