Management of HCMV infections in haematopoietic stem cell recipients

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Overall Survival and EFS in children with AML given HSCT from a compatible sibling

Survival = 83% (33-92)
EFS = 75% (64-86)
ALL in 2nd CR
DFS by Year of Transplantation

Matched Family Donor

- > 1998 = 62% (43-82)
- < 1998 = 50% (38-64)

Unrelated Donor

- > 1998 = 59% (44-74)
- < 1998 = 25% (8-42)

< 1998: N = 65; E = 31
> 1998: N = 39; E = 11

< 1998: N = 24; E = 18
> 1998: N = 47; E = 16

Survival probability in children with SAA given HSCT from a compatible sibling

Cs-A/MTX: Survival = 94% (87-100)

Cs-A: Survival = 78% (63-93)

Cs-A/MTX: N = 37; Ev = 2
Cs-A: N = 34; Ev = 7

Log-Rank P = 0.05

Bacterial Nonbacterial (Interstitial)

Viral
- HSV
- CMV
- Adeno
- VZV

Fungal
- Candida
- Aspergillus

Bacterial
- Gram Pos
- Gram Neg
- Encapsulated

Risk Factor
- Neutropenia
- Acute GVHD + Rx
- Chronic GVHD

Days after transplant
- 0
- 50
- 100
- 12

Months

Stem cell infusion

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## Frequency of CMV-Disease

<table>
<thead>
<tr>
<th>Transplantation</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney-Tx</td>
<td>8%</td>
</tr>
<tr>
<td>Liver-Tx</td>
<td>29%</td>
</tr>
<tr>
<td>Heart-Liver-Tx</td>
<td>39%</td>
</tr>
<tr>
<td>Allo-HSCT</td>
<td>10% - 50%</td>
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</table>
HCMV in HSCT

- Human cytomegalovirus (HCMV) infection is a well-known cause of morbidity and mortality in hematopoietic stem cell transplantation (HSCT) recipients.
- HCMV-seropositive patients, regardless of the donor status, have a high incidence of HCMV infection and HCMV viremia and antigenemia have been demonstrated to be important predictors of HCMV disease.
Factors influencing the risk of developing HCMV reactivation after HSCT

- Serological status of the patient
- Serological status of the donor
- Source of stem cell employed
- T-cell depletion of the graft
- GVHD prophylaxis
- GVHD occurrence and therapy
- Patient’s immune recovery
Fig. 1. Cumulative probability of overall HCMV infection (antigenaemia) and according to acute GVHD occurrence or steroid treatment.

HCMV in HSCT

• In the 80’s, antiviral treatment was started only upon appearance of clinical symptoms of HCMV infection (deferred or symptomatic therapy), this leading to a high incidence of fatal events.

• Subsequently, pre-emptive (or pre-symptomatic) therapy, i.e. administration of antiviral drugs upon detection of HCMV to treat only patients undergoing viral infection and thus at risk of developing overt disease, was utilized.
Milestones in monitoring and treatment of HCMV infections in transplant recipients at IRCCS Policlinico San Matteo, Pavia

1985
Conventional virus isolation

1990
Development of rapid assays (Antigenemia, Viremia, DNAemia, RNAemia)

1995
Development of assays for HCMV-specific T-cell immunity

2000
Development of automatic DNA extraction and realtime PCR

2005
Clinical evaluation of antigenemia and viremia

2008
Clinical evaluation of RNAemia and DNAemia

Symptomatic treatment
Preemptive treatment
Antigenemia-guided
DNAemia-guided
Fig. 3. Kaplan-Meier estimate of event-free survival (EFS) and transplant-related mortality (TRM) in children with and without HCMV infection (antigenaemia).

Pros and Cons of antigenemia as guiding parameter for preemptive treatment in HSCTR

• **Pros**
  - most widely used assay for guiding preemptive therapy in SOTR and HSCTR
  - simple to perform
  - inexpensive

• **Cons**
  - lack of standardisation, automation and objectivity of test results
  - indirect marker of viral replication;
  - not applicable in HSCTR pts before graft take
Pros and Cons of DNAemia (real-time PCR format)

Pros

- wide dinamic range (>7 log_{10})
- negligible risk for carry-over contamination
- standardisable, semi-automated and objective assay
- direct expression of viral replication
- quantitative
- sensitive

Cons

- need for consensus threshold levels
Human cytomegalovirus immediate early-mRNAemia vs pp65-antigenemia for guiding pre-emptive therapy in children and young adults given HSCT.

180-Day Transplant-Related Mortality

Grade II – IV acute GVHD

Cumulative Incidence (%)

Days after transplantation

Antigenemia vs DNAemia a prospective open-labeled randomized trial: treatment of HCMV infection in the two randomization arms (HSCTR).

**Incidence**
- DNAemia arm (n=17/45; 37.8%)
- Antigenemia arm (n=23/48; 47.9%)

\[ p = 0.416 \]

**Treatment**
- DNAemia arm (n=6/45; 13.3%)
- Antigenemia arm (n=17/48; 33.4%)

\[ p = 0.014 \]

**Cut-offs**
- DNAemia: 10,000 copies/ml blood
- Antigenemia: first confirmed pp65-pos/10^5 PBLs

Lilleri et al, Blood 2008
HCMV viral load to start pre-emptive therapy

<table>
<thead>
<tr>
<th>Immunosuppression</th>
<th>CMV doubling time</th>
<th>Risk Groups</th>
<th>CMV Plasma DNA Level to Start PET at FHCRC*</th>
<th>CMV Whole Blood DNA Level to Start PET at Karolinska Institute**</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>Short</td>
<td>Cord blood</td>
<td>Any level</td>
<td>1000 copies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Allograft</td>
<td>&gt; 100 copies/mL</td>
<td>1000 copies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- High-dose steroids*</td>
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<tr>
<td></td>
<td></td>
<td>- T cell depletion</td>
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<tr>
<td></td>
<td></td>
<td>- Anti-T cell antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- CD34 selection</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Allograft</td>
<td>&gt; 500 copies/mL &gt; or 5-fold ↑†</td>
<td>1000 copies</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Low dose steroids</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- No T cell depletion or anti-T cell antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Allograft</td>
<td>&gt; 1000 copies/mL &gt; or 5-fold ↑†</td>
<td>1000 copies if GVHD Other individual assessment based on ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- after day 100</td>
<td></td>
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* Assays performed weekly or twice weekly (highest risk); limit of detection 25 copies/mL
*† 1 mg per kg of prednisone or higher
† If initial level is less than threshold
** Assays performed weekly, limit of detection 50 copies/mL

Figure 1. CMV viral load to start preemptive therapy (PET) used at the FHCRC in Seattle, WA, and the Karolinska Institute, Stockholm, Sweden.
Standardization of HCMV DNAemia

Quality control: QCMD CMV DNA 2007

- PD in house
- BG in house
- PV in house
- SI in house
- SI Abbott
- Roma Affigene
- PI Affigene
- Roma Artus
- PI Cobas
- BO Nanogen
- CS Nanogen
- MI Nanogen
- MO Nanogen
- NO Nanogen
- Roma2 Nanogen
- TO Nanogen
- UD Nanogen
- Consensus

Quality control: QCMD CMV DNA 2007

- 10^6 - 10^7
- 10^5 - 10^6
- 10^4 - 10^5
- 10^3 - 10^4
- 10^2 - 10^3
- 10^1 - 10^2
- Neg

Copie/ml

Campioni
HCMV in HSCT

- Prophylaxis, i.e. administration of anti-HCMV drugs to all transplanted patients for a median time of 3 months or 100 days after transplantation, has been employed to prevent HCMV disease in HSCT recipients.

- Major drawbacks of such an approach are treatment of a substantial proportion of patients not at risk for disease, drug toxicity, occurrence of late (after discontinuation of prophylaxis) HCMV disease (mainly pneumonia), and low cost-effectiveness.
Incidence of HCMV-disease after alloHSCT

HCMV-sero+ Patients (n=1458)

R. Bowden, „Infectious Diseases“ 2000
CMV-DNAemia and CMV-peptide specific CD8+ T cells

Hebart/Einsele Blood 2002
Simultaneous quantification of HCMV-specific CD4+ and CD8+ effector T cells.

1. Generation and culture of monocyte-derived immature DC

- CD14+ monocytes
- GM-CSF (800 IU/mL)
- IL-4 (500 IU/mL)

5 days

37°C 5% CO₂

Immature DC

CD14-CD1a+ CD83-, CD86low
2. Infection of immature DC with HCMV (VR1814, MOI 10)

24 h

3. Co-culture of infected imDC and PBMC + Brefeldin A

overnight

4. Intracellular flow cytometry analysis of HCMV-specific CD4+ and CD8+ INFγ–producing T cells
Percent of INFγ-producing CD8$^+$ and CD4$^+$ T cells in HCMV-seropositive donor

CD4

CD8

mock-infected DC

HCMV-infected DC

IFNγ-producing T cells

1.05% 2.05%
HCMV-specific CD4+ and CD8+ T cells in HCMV-seronegative and seropositive healthy subjects
HCMV-seropositive HSCT recipients (n=39):

Total and HCMV-specific CD4+ T cell counts

Total CD4+ T cell count increases over time, however remaining lower than that of controls.

HCMV-specific T cell count reaches levels similar to controls from day +60.
HCMV-seropositive HSCT recipients (n=39):

Total and HCMV-specific CD8+ T cell counts

Total CD8+ T cell count reaches levels similar to controls from day +60.

HCMV-specific T cell count reaches levels higher than controls from day +60.
Thus, all seropositive recipients reconstituted HCMV-specific T-cell immunity within 6 months after transplantation.
HCMV-seronegative HSCT recipients from seropositive donors (n=17):

Total and HCMV-specific CD4+ and CD8+ T cell counts

Virus-specific T cell counts are lower than those of HCMV-seropositive patients
Thus, despite all receiving graft from HCMV seropositive donors (100%), as many as 75% seronegative recipients did not show specific T-cell immune response.
No significant difference in HCMV-specific T cell reconstitution during time was found according to:

- Underlying disease (malignant/non-malignant)
- Conditioning regimen (TBI/non-TBI based)
- Donor type (sibling/unrelated/Haploidentical T cell-depleted)
- Donor HCMV serostatus
HCMV T cell immune response 60 days after transplantation and control of viral infection in 39 HCMV seropositive HSCT recipients

**Total CD4+ T cells**

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**HCMV-specific CD4+ T cells**

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Single GCV course: 7 (5-16) days of treatment.
Multiple GCV courses: 49 (31-82) days of treatment.

Significantly lower with respect to other groups.
Sib (DNAemia arm)

- CD4+
- CD8+

T cells/μl

- CD8+ T cells/μl
- CD4+ T cells/μl

HCMV-specific

- CD8+ T cells/μl
- CD4+ T cells/μl

DNA copies/10^5 PBL

- DNA
- pp65-antigenemia

Days post Tx

- 0
- 90
- 180
- 270
- 360

0
0.1
1
10
100
1000

0
1
2
3
4
5
6
7
8
9
10
20
30
40
50
60
70
80
90
100
1000
CONCLUSIONS

A simultaneous quantification of HCMV-specific CD4+ and CD8+ T cells in HSCT patients is achieved in a single test run.

Effective HCMV-specific T cell immunity can promptly develop after HSCT (regardless of donor type, pre-existing immunity or T-cell depletion).

In seropositive recipients, latent virus may be the major antigenic drive for rapid reconstitution of T cell compartment.

Transfer of virus from seropositive donors to seronegative recipient and/or virus-specific memory T-cell expansion seem to be a rare event.

Future studies will be conducted to correlate antiviral intervention to reconstitution of HCMV-specific T cell immune response.