Update on CMV infections

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Infectious complications after Allo-SCT

Fungi
- Candida Spp.
- Aspergillus Spp.

Viruses
- HSV
- CMV, Adeno
- VZV

Bacteria
- Gram-positive
- Gram-negative
- Encapsulated bacteria

Pneumonia
- Bacterial
- Non-bacterial

Days post-SCT
- 0
- 50
- 100
- >365

Based on the Results of the 4th ECIL in Juan les Pins 9/2011 organized by C. Cordonnier
Diagnosis of CMV disease

• The diagnosis of CMV disease must be based on symptoms and signs consistent with CMV disease together with detection of CMV by an appropriate method applied to a specimen from the involved tissue (AII).

• Symptoms of organ involvement together with CMV detection in blood are not enough for diagnosis of CMV disease. There are several possible techniques that can be used for detection of CMV in tissue specimens and each transplant centre should collaborate closely with a good diagnostic virology and histopathological laboratory (AII).

• PCR is usually not appropriate for documentation of CMV disease in tissue specimens since the positive predictive value is too low (BIII).
Treatment of CMV pneumonia

• Antiviral therapy with ganciclovir is recommended (AII).

• Foscarnet might be used in place of ganciclovir (AIII).

• The addition of immune globulin to antiviral therapy should be considered (CII).

• Cidofovir or the combination of foscarnet and ganciclovir can be used as 2nd line therapy (BII).
Treatment of other types of CMV disease

- For other types of CMV disease and in other patient groups either intravenous ganciclovir or foscarnet given without addition of immune globulin is recommended (BII).

- Cidofovir or the combination of intravenous ganciclovir and foscarnet can be used as second line therapy of CMV disease (BII).
CMV-induced interstitial Pneumonia

CMV-disease
no prophylaxis/ no preemptive therapy

Incidence after MUD-BMT: 10 - 25%

Case fatality rate of CMV-IP: 70%!
Prevention of primary CMV infection

- Stem cell transplant patients should be tested before SCT for CMV antibodies (AI)
- Stem cell transplant donors should be tested for CMV antibodies (AI)
- If a patient is found to be seronegative, a CMV seronegative donor should be used if possible (AI)
- CMV seronegative allogeneic stem cell transplant patients with CMV seronegative donors should receive leukocyte depleted (AII) or CMV seronegative blood products (AI) only.
- If leukocyte depleted blood products are used, the products should contain < 5 x 10^6 residual leukocytes / unit (AII)
- Immune globulin for prevention of primary CMV infection is not recommended (BI)
Prevention of CMV disease

Risk Groups: R CMV-sero+ a./o. D CMV-sero+

Antiviral Strategy

Prophylaxis
- Tx
- Engraftment
- GCV/FC

Preemptive Therapy
- Tx
- PCR / Ag +
- GCV/FC
- again +

Day 100

Einsele 1995
Boeckh 1996
Allogeneic SCT patients

- All allogeneic SCT patients, regardless of whether or not they receive CMV prophylaxis, should be monitored for CMV in peripheral blood at least weekly with either the CMV antigenaemia, quantitative PCR or a technique for detection of CMV RNA.

- Cut-off levels for introduction of pre-emptive therapy should be adapted according to the PCR assay and the transplant modality.

- The duration of monitoring should be at least 100 days (BIII).

- Longer monitoring is recommended in patients with acute or chronic GVHD, those having experienced an earlier CMV reactivation, and in patients having undergone mismatched, cord blood, haploidentical or unrelated donor transplantation (BII).
CMV viral load to start preemptive therapy

<table>
<thead>
<tr>
<th>Immuno-suppression</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></td>
<td>High-dose steroids†</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- T cell depletion</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Anti-T cell antibodies</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- CD34 selection</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>Long</td>
<td>Allograft</td>
<td>&gt; 500 copies/mL</td>
<td>1000 copies</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Low dose steroids</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- No T cell depletion</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>or anti T cell antibodies</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Allograft</td>
<td>&gt; 1000 copies/mL</td>
<td>1000 copies if GVHD Other individual assessment based on ↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>after day 100</td>
<td></td>
</tr>
</tbody>
</table>

* 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.

Boeckh M, Ljungman P. Blood 2009;113:5711-5719
Preemptive therapy – Allo SCT

- Pre-emptive antiviral therapy based on detection of CMV antigen or nucleic acid is effective for prevention of CMV disease in allogeneic SCT patients (AI).

- Either intravenous ganciclovir or foscarnet can be used for first line pre-emptive therapy (AI).

- The choice depends on the risk of toxicity and which antiviral drugs have been used previously (BIII).

- Valganciclovir might be used in place of intravenous agents (except in patients with severe intestinal GvHD) especially in low-risk patients (BII), solid data concerning toxicity of the drug in the preemptive setting are still lacking, esp. in patients with low bodyweight or renal dysfunction.
**EBMT Study: Pre-emptive GCV vs ValGCV**

Stratified by center to balance the following possible influencing factors:
The CMV testing method selected, the underlying disease and transplant Modality

Web-based randomization (1:1)

<table>
<thead>
<tr>
<th>Patients</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Valganciclovir</td>
<td>Ganciclovir i.v.</td>
</tr>
</tbody>
</table>

| High Risk for CMV | Low Risk for CMV | High Risk for CMV | Low Risk for CMV |
CMV prophylaxis – Allo SCT

- Intravenous ganciclovir prophylaxis is an effective strategy for prevention of CMV disease and could be used in sub-groups of allogeneic SCT patients at high risk for CMV disease (BI).

- Acyclovir or valacyclovir can be used as prophylaxis against CMV in allogeneic stem cell transplant patients (BI). However, their use must be combined with monitoring and use of pre-emptive therapy (AI).

- Immune globulin has today no role as prophylaxis against CMV infection (AII).
Other recommendations – Allo SCT patients

- All patients with CMV disease before HSCT, should be considered as very high risk patients for CMV disease after SCT. If possible, the transplant should be delayed to allow for appropriate treatment duration before SCT (BIII).

- In patients with CMV disease before SCT, use of secondary anti-CMV prophylaxis during SCT could be considered (BIII)

- Such patients should be closely monitored during the SCT procedure and a low threshold for preemptive treatment used (BIII)

- If a patient is CMV seropositive, to select a graft from a CMV seropositive unrelated or mismatched donor should be considered (BII)
Duration of antiviral chemotherapy and risk of recurrence

Einsele H et al Bone Marrow Transplant 2000

86 patients at risk, first treatment (n = 57), retreatment (n = 19)

No. of patients

Treatment for ≥ 4 weeks:

> 50%
Duration of antiviral therapy >4 weeks as a risk factor for secondary non-viral infections

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Late CMV Disease, Odds Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cGvHD</td>
<td>1100 (1.8 – 8.5 (3.5 – 22.1)</td>
<td>0.0017</td>
</tr>
<tr>
<td>Antiviral Therapy &gt; 4 weeks</td>
<td></td>
<td>0.0073</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Secondary Infections</th>
<th>Odds Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial Infections</td>
<td>53 (35 – 1300)</td>
<td>0.001</td>
</tr>
<tr>
<td>Invasive Fungal Infections</td>
<td>84 (4.1 – 3800)</td>
<td>0.006</td>
</tr>
</tbody>
</table>
Testing for antiviral resistance

• Rising antigenemia or CMV DNA early after initiation (1-2 weeks) is usually not a sign of virological failure.

• Where possible, resistance testing should be performed to allow selection of the correct second line antiviral therapy (BIII).

• If the turn-around time for resistance testing is prolonged, then a change of treatment for a patient with rising viral load or worsening disease in the face of adequate treatment could precede receipt of the test result (BII).
CMV drug resistance

Risk Factors: Delayed T Cell Reconstitution

- Haplo-SCT, CBT, cGvHD
Failure regimens
Second and third line preemptive therapy

- The alternate drug of ganciclovir or foscarnet can be considered for second line pre-emptive therapy (AI)

- Cidofovir can be considered for second line pre-emptive therapy (3-5 mg/kg) but careful monitoring of the renal function is required (BII).

- The combination of ganciclovir and foscarnet might be considered for second line pre-emptive therapy (CII)

- Maribavir (BII), leflunomide (CIII), and artesunate (CIII) are possible third line options for preemptive therapy in patients resistant or intolerant to other antiviral agents.
Patients with hematological malignancies including autologous SCT recipients

- Patients who might receive alemtuzumab or in whom allogeneic SCT can be envisaged should be tested for CMV antibodies (BII).

- CMV seronegative patients receiving T-cell suppressive therapy should receive leukocyte depleted or CMV seronegative blood products only (BIII).

- CMV seronegative autologous SCT patients should receive leukocyte depleted or CMV seronegative blood products only (BIII).

- Immune globulin for prevention of CMV infection or disease is not recommended. (AIII)
Patients receiving alemtuzumab

CMV management strategy must be put in place for patients treated with alemtuzumab (BII)

- Monitoring and antiviral treatment of patients having a positive test for CMV and symptoms compatible with a CMV infection is one management option in patients receiving alemtuzumab (BII).

- In these patients a regular monitoring with antigenemia or PCR is recommended during the period of maximum immunosuppression (during treatment and until 2 months after the end). (BII)

* Treating asymptomatic patients is not obligatory but careful clinical observation of patients with documented CMV reactivation is necessary (BII)

- Withholding alemtuzumab is not considered necessary, unless there are persisting symptoms (BIII).
Other hematology patients

- High-risk autologous SCT patients might potentially benefit from monitoring and the use of preemptive therapy (CII).

- Routine monitoring and preemptive therapy is not considered necessary in other hematology patients (BIII).

- CMV should be considered in patients receiving T-cell suppressive therapy and in CMV seronegative patients who receive stimulated granulocyte transfusions from unscreened donors if they develop symptoms compatible with CMV (unexplained fever, drop in blood counts, lung infiltrates, or gastrointestinal symptoms) (BII).
CMV prophylaxis – patients treated with alemtuzumab

- Valganciclovir prophylaxis is effective and reduces the risk of symptomatic CMV infection in patients treated with alemtuzumab (BI)

- However, the risk/benefit ratio compared to the strategy of treating when a symptomatic CMV infection develops is still undetermined (CII)
CMV prophylaxis patients with other hematological malignancies

• Routine antiviral prophylaxis is not recommended (AIII)
Future developments

- New anti-CMV drugs (CMX 001 and AIC246) and CMV vaccines based on gB or DNA plasmids are in clinical development.
Incidence of CMV-Disease following allo SCT
CMV-sero+ Patients (n=1458)

Risk Factor for Late CMV Disease: delayed Immune Reconstitution

R. Bowden, 2000
Immune Monitoring/Immune Intervention

Immunological monitoring after SCT yields important information for patient management although no standard test exists (BII)

Immunological interventions by infusion of CMV specific lymphocytes or dendritic cell vaccination are interesting options and should undergo controlled prospective clinical trials (CII)
# Reconstitution of the Absolute Numbers of Lymphocytes Subpopulations

<table>
<thead>
<tr>
<th>Subpopulation:</th>
<th>Normalization:</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD56+</td>
<td>30 d</td>
</tr>
<tr>
<td>CD2+</td>
<td>27-30 d</td>
</tr>
<tr>
<td>CD3+</td>
<td>42-48 d</td>
</tr>
<tr>
<td>CD8+</td>
<td>100-120 d</td>
</tr>
<tr>
<td>CD4+</td>
<td>&gt;180 d</td>
</tr>
<tr>
<td>CD4+CD45RA+</td>
<td>&gt;6-24 Mo.</td>
</tr>
</tbody>
</table>
CMV-DNAemia and CMV-peptide specific CD8+ T cells

Detectable CMV DNA load

- 72% of patients with < 5 CMV peptide-spec. CD8+ T cells/µl
- 15% of patients with > 5 CMV peptide-spec. CD8+ T cells/µl

Hebart et al. Blood 2002
Study Design

1. CMV-specific T cell recovery was monitored in 83 CMV-seropositive recipients of an allo-SCT

2. CMV-specific T cell responses were tested every 2 weeks from day + 28 to day 100, then every 2-4 weeks until day 270
Cumulative incidence curves of CMV risk

Gratama JW. et al Blood 2010
Kaplan-Meier survival curve of TRM

Transplant-Related Mortality

Survival probability (%)

Days After Transplant

Rapid Tetramer Recovery

Delayed Tetramer Recovery

Log rank $P = .08$
Conclusions

Quantitation of CMV-specific T-cell Responses

1. If any tetramer result between D28+ and +65 is greater or equal to the threshold of 7 cells/µl. → rapid recovery of CMV-specific T cell immunity → low risk of recurrent CMV infection/disease → stop monitoring d+100

2. In patients with delayed recovery → virological monitoring and pre-emptive strategies beyond d+100 pre-emptive therapy beyond d+100

Gratama JW. et al Blood 2010
T cell reconstitution following SCT

- D
- CD34/selection/ATG
- prothymocytes
- T cells
- Stem cells
- Thymus
- Memory T cells
- 100 days
- Time
Correlation of transferred CMV-specific T cells and number of antiviral treatment courses

Gratama et al. Blood 2001
CMV Infection and aGvHD after allo-SCT
Impact of CD34+ Selection

Hebart / Einsele Blood 2001
Immunotherapy: different strategies

- Donor Lymphocyte Infusion (DLI)
- Allodepleted DLI
- $\gamma\delta$ T cells
- Specific donor CTLs
- Redirected T cells
- DC vaccination
Immunotherapy with unmanipulated Donor-LI

![Graph showing EBV-DNA levels over time. EBV-DNA levels peak at 0.1 mio copies/µg DNA at the onset of symptoms. A line marked "DLI 50,000 CD3+/kg" indicates a dosage of donor leukocyte infusions. A notation "† aGcHD IV" indicates an associated condition.](image-url)
Immunotherapy: different strategies

- Donor Lymphocyte Infusion (DLI)
- Allodepleted DLI
- $\gamma\delta$ T cells
- Specific donor CTLs
- Redirected T cells
- DC vaccination
Lymphocyte-Transfer as therapeutic Option

Effector cells of the adaptive Immune System

- ab T cell (CD4+)
- ab T cell (CD8+)
- APC
- Tumor
- NK cell
- gd T cell

Effector cells of the innate Immune System

- no intrinsic Immune Defect
- no MHC Restriction
- Anti-Tumor/-Infectious Efficacy/ no GvHD
- 1-10 % of T cells (CD3+ CD4- CD8-) in PB
Anti-Tumor Effect of Vγ9Vδ2 T cells in vivo

**preclinical mouse model:** Tumor growth in NOD-SCID mice reconstituted with Vγ9Vδ2 T cells

**clinical Study:** Tumor response in Patients after Activation of Vγ9Vδ2 T cells with bisphosphonate plus IL-2

Kunzmann V et al., *Blood* 2003, 102:200-6
Strategy for depletion of \( \alpha \beta^+ \) T-cells

Chaleff S. et al.: A large scale method for the selective Depletion of \( \alpha \beta \) T-lymphocytes from PBSC for allogeneic Transplantation. Cytotherapy 2007; 9: 746 -754.

- biotin-anti-\( \alpha \)/\( \alpha \) mAb
- + anti-biotin mAb

Graft

<table>
<thead>
<tr>
<th>n = 21</th>
<th>Before</th>
<th>After</th>
<th>Log Depletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>absolute TcR( \alpha \beta )+</td>
<td>1.4 ± 0.54 x10e10</td>
<td>1.1 ± 2.8 x10e6</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>0.77 – 2.54</td>
<td>0.07 – 13.3</td>
<td>3.2 – 5.5</td>
<td></td>
</tr>
</tbody>
</table>
\( \nu \delta^{2} \text{neg} \ \gamma \delta \text{T cell lines are able to lyse CMV infection in patients during the first 24 month post SCT} \)
Immunotherapy: different strategies

- Donor Lymphocyte Infusion (DLI)
- Allodepleted DLI
- Memory B cells
- γδ T cells
- Specific donor CTLs
- Redirected T cells
- DC vaccination
Immunotherapy with spec. Donor-T-Cells

Strategy:

- Depletion of alloreactive T-cells

Sero+-Donor:

   Enrichment of Antigen-spec. T-cells

   a. Repetitive Ag - Stimulation in vitro
   b. Selection by specific TCR-
      MHC/peptide binding
   c. Induction of Ag-spec. Cytokine Secretion
Prophylactic application of Viral Immunity by 4 adoptive Transfers of T cell clones

Riddell SR et al Science 1992
Therapeutic Application:
Viral load upon adoptive transfer of CMV-specific T cell lines

Einsele et al. Blood 2002
Enrichment of virus-specific CD4+ and CD8+ T cells from a donor with a very low precursor frequency

Magnetic Separation

Unspecific Expansion (IL-2 + Feeder cells)

For 10 – 12 days:
Expansion by 2 – 4 log

Prior to enrichment: < 0.1%
After enrichment: 92.1%

Rauser et al. Blood 2004
Einsele et al. Lancet 2003
T-Cell Therapy for CMV-Infection

A
IFN+ T-cells upon pp65 Stimulation [%] pre
post
No. 1
No. 3
No. 5
No. 7
No. 8
No. 9
No. 10
No. 11
No. 13
No. 14
No. 16
No. 18

CMV-specific T-cell response day 0 and day 7-28 post adoptive transfer

B
CMV copies/ml blood
pre
post
No. 4
No. 7
No. 12
No. 13
No. 15
No. 16

CMV-Viremia at day 0 and day 7-28 post adoptive T-cell transfer

Response of CMV viremia post T-cell transfer

No response of CMV viremia post T-cell transfer

No. 1
No. 2
No. 3
No. 4
No. 5
No. 6
No. 7
No. 9
No. 10
No. 11
No. 13
No. 14
No. 15
No. 16
No. 8
No. 12
No. 17
Cytokine-Secretion Assay selected CMV-spec. T cells

<table>
<thead>
<tr>
<th>Age</th>
<th>Dx</th>
<th>Graft</th>
<th>Day post SCT</th>
<th>Purity IFNγ+[%]</th>
<th>CD3/KG</th>
<th>antiviral drugs</th>
<th>CMV infection</th>
<th>Response 4 weeks</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>NHL</td>
<td>MUD</td>
<td>93</td>
<td>42</td>
<td>2677</td>
<td>GCV</td>
<td>Viremia</td>
<td>negative CMV PCR</td>
<td>clearance of CMV</td>
</tr>
<tr>
<td>17</td>
<td>ALL</td>
<td>haplo</td>
<td>50</td>
<td>10</td>
<td>2500</td>
<td>GCV, FC</td>
<td>Viremia</td>
<td>600 CMV copies /ml</td>
<td>clearance of CMV</td>
</tr>
<tr>
<td>0.4</td>
<td>Tay-Sachs</td>
<td>haplo</td>
<td>146</td>
<td>11</td>
<td>2500</td>
<td>GCV, FC</td>
<td>Viremia</td>
<td>2 log decrease</td>
<td>delayed pulmonary toxicity syndrome d 172</td>
</tr>
<tr>
<td>59</td>
<td>AML</td>
<td>haplo</td>
<td>102</td>
<td>95</td>
<td>7529</td>
<td>GCV</td>
<td>Viremia</td>
<td>negative CMV PCR</td>
<td>clearance of CMV</td>
</tr>
<tr>
<td>13</td>
<td>RMS</td>
<td>haplo</td>
<td>60</td>
<td>85</td>
<td>6058</td>
<td>GCV</td>
<td>Viremia</td>
<td>Viremia</td>
<td>sepsis, heart failure d 79 post SCT</td>
</tr>
<tr>
<td>6</td>
<td>ALL</td>
<td>haplo</td>
<td>212</td>
<td>79</td>
<td>4464</td>
<td>GCV</td>
<td>Diarrhoea, Viremia</td>
<td>negative CMV PCR</td>
<td>CMV reactivation after high dose MP</td>
</tr>
</tbody>
</table>

Feuchtinger /Einsele, Blood 2010
Reversible MHC multimers (Streptamers)

- **d-Biotin/Streptactin**
  - $K_D = 10^{-12} M$
  - Higher affinity to Streptactin when compared to Streptatag

- **StreptagIII/Streptactin**
  - $K_D = 10^{-6} M$

**Detachment of MHC multimers from TCR**

Knabel et al. Nature Medicine 2002
Clinical study

No expansion of virus-specific T cells in culture
Protocols for HCMV, ADV, EBV
Patients treated: 8 patients

direct transfer of $10^5$ Streptamer-purified T cells/kg

advantages:
- fast
- transfer to GMP (removal)
- viability, functionality
Antiviral Activity of transferred CMV-spec. CTLs

Zytomegalie-Virus-Titer nach adoptiv Übertragung

Tag nach Übertragung

-1000
0
1000
2000
3000
4000
5000
6000
7000

0 10 20 30 40 50 60 70 80

Virus copies per microliter blood

0.0 %
0.08%
0.38%
0.65%
0.78%
0.99%
0.65%

before
1. week
3. week
4. week
5. week
8. weeks after transfer

CD8 Pacific Blue
Transfer of streptamer-sorted CMVpp65-specific CD8+ T cells

Rapid Reconstitution of adoptively transferred HLA-B7-restricted CMV spec. T cells, but late endogenous reconstitution of HLA A2-restricted CMV-spec. T cells
DC vaccination for infections following allo-SCT
Phase I/II Study in Pts. at high Risk of CMV disease
CMV-seronegative donor, Vaccination between d+21 - 265

Donor

PB (100 ml) → Monocytes

GM-CSF, IL-4, TNFα

CMV-specific Peptides (A1, A2, B7, B8, B24)

Vaccination of Patient
Vaccination: HCMV-Peptide pulsed DCs

- No GvHD induction
- CMV spec. T cell response 10/17
- T cell Response only to epitopes used for vaccination

Grigoleit, *JID* 2007
Cytomegalovirus glycoprotein-B vaccine with MF59 adjuvant in transplant recipients

<table>
<thead>
<tr>
<th>Group</th>
<th>Viraemia &gt;200 genomes per mL</th>
<th>Number treated</th>
<th>Median days of follow-up (range)</th>
<th>Median number of samples (range)</th>
<th>Days PCR positive/total person-days of follow-up (%)</th>
<th>Days treated/total person-days of follow-up (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seronegative donor, seronegative recipient</td>
<td>Placebo (n=10)</td>
<td>0</td>
<td>96 (51–115)</td>
<td>13 (6–25)</td>
<td>0/915 (0%)</td>
<td>0/915 (0%)</td>
</tr>
<tr>
<td></td>
<td>Vaccine (n=12)</td>
<td>0</td>
<td>105 (15–138)</td>
<td>15 (3–26)</td>
<td>0/1204 (0%)</td>
<td>0/1204 (0%)</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>1·00</td>
<td>1·00</td>
</tr>
<tr>
<td>Seronegative donor, seropositive recipient</td>
<td>Placebo (n=7)</td>
<td>2</td>
<td>95 (65–157)</td>
<td>20 (8–25)</td>
<td>2/696 (&lt;1%)</td>
<td>0/696 (0%)</td>
</tr>
<tr>
<td></td>
<td>Vaccine (n=11)</td>
<td>4</td>
<td>93 (89–176)</td>
<td>20 (4–26)</td>
<td>21/1209 (2%)</td>
<td>0/1209 (0%)</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>1·00</td>
<td>0·59</td>
</tr>
<tr>
<td>Seropositive donor, seropositive recipient</td>
<td>Placebo (n=15)</td>
<td>6</td>
<td>95 (45–173)</td>
<td>17 (8–26)</td>
<td>119/1489 (8%)</td>
<td>135/1489 (9%)</td>
</tr>
<tr>
<td></td>
<td>Vaccine (n=7)</td>
<td>4</td>
<td>93 (91–228)</td>
<td>14 (7–21)</td>
<td>6/803 (&lt;1%)</td>
<td>0/803 (0%)</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>0·51</td>
<td>0·22</td>
</tr>
<tr>
<td>Seropositive donor, seronegative recipient</td>
<td>Placebo (n=5)</td>
<td>5</td>
<td>91 (20–278)</td>
<td>19 (5–62)</td>
<td>339/599 (57%)</td>
<td>415/599 (70%)</td>
</tr>
<tr>
<td></td>
<td>Vaccine (n=11)</td>
<td>6</td>
<td>94 (90–122)</td>
<td>19 (10–24)</td>
<td>128/1069 (12%)</td>
<td>142/1069 (13%)</td>
</tr>
<tr>
<td></td>
<td>p value</td>
<td>..</td>
<td>..</td>
<td>..</td>
<td>0·0480</td>
<td>0·0287</td>
</tr>
</tbody>
</table>

N=78                                      | 27                           | 12             |                                  |                                 | 0·480                                               | 0·287                                           |

**Third party T cells:**
Therapy of CMV encephalitis post-CBT

<table>
<thead>
<tr>
<th></th>
<th>day</th>
<th>HLA-A*</th>
<th>HLA-B*</th>
<th>HLA-Cw*</th>
<th>HLA-DRB1</th>
<th>HLA-DQB1</th>
<th>cells/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cord blood 1</td>
<td>0</td>
<td>2601</td>
<td>3004</td>
<td>3801</td>
<td>5802</td>
<td>0602</td>
<td>1301</td>
</tr>
<tr>
<td>cord blood 2 &amp; 3</td>
<td>49</td>
<td>2601</td>
<td>3001</td>
<td>2705</td>
<td>3801</td>
<td>0202</td>
<td>1301</td>
</tr>
<tr>
<td>3rd party CD34</td>
<td>50</td>
<td>0211</td>
<td>3001</td>
<td>1302</td>
<td>3801</td>
<td>0304</td>
<td>0602</td>
</tr>
<tr>
<td>anti CMV T cells</td>
<td>137</td>
<td>01</td>
<td>30</td>
<td>08</td>
<td>57</td>
<td>06</td>
<td>07</td>
</tr>
</tbody>
</table>

Schöttker B. et al., *Nat Clin Practice* 2008
Enrichment of multipathogen-spec. T cells based on activation-dependent CD154-expression and expansion of T-cell lines within 14 days.

Cell numbers are means of at least 2 independent experiments, except for CMV pp65 single lines.

<table>
<thead>
<tr>
<th>antigen</th>
<th>donor ID</th>
<th>cell number before stimulation ($\times 10^6$)</th>
<th>cell number after CD154 separation ($\times 10^6$)</th>
<th>fraction of selected cells (%)</th>
<th>cell number after 14d expansion ($\times 10^6$)</th>
<th>expansion factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV pp65 peptide pool</td>
<td>donor 1, donor 4</td>
<td>52, 55</td>
<td>0.79, 0.60</td>
<td>1.52, 1.10</td>
<td>32, 35</td>
<td>41, 58</td>
</tr>
<tr>
<td>multi-specific T-cell line (CMV, AdV, EBV, C. albicans)</td>
<td>donor 1, donor 4</td>
<td>52, 51.5</td>
<td>0.61, 0.52</td>
<td>1.17, 1.01</td>
<td>35, 36</td>
<td>57, 69.2</td>
</tr>
<tr>
<td>multi-specific T-cell line including A. fumigatus p41</td>
<td>donor 2</td>
<td>42</td>
<td>0.45</td>
<td>1.07</td>
<td>61</td>
<td>136</td>
</tr>
</tbody>
</table>

N. Khanna et al. Blood 2011
Virus-specific T cells in single and multi-pathogen-specific cultures efficiently kill target cells.

N. Khanna et al. Blood 2011
Single and multi-pathogen-specific T-cell lines show nearly abrogated alloreactivity.

Alloreactivity was determined 14 days after expansion with 2 different third-party DC by [³H]-thymidin incorporation.

N. Khanna et al. Blood 2011
Adoptively transferred CTLs migrate to bone marrow but have limited in vivo persistence

Persistence and Migration of $T_{EM}$- and $T_{CM}$- derived T cell clones

Berger C et al., JCI 2008
A human memory T cell subset with stem cell-like properties
Thanks for your attention!

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Fax: 0931/201-640001