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Kinetics of Cytomegalovirus (CMV) pp65 and IE-1-Specific IFN γ CD8 $^+$ and CD4 $^+$ T Cells During Episodes of Viral DNAemia in Allogeneic Stem Cell Transplant Recipients: Potential Implications for the Management of Active CMV Infection

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Kinetics of Cytomegalovirus (CMV) pp65 and IE-1-Specific IFN γ CD8 $^+$ and CD4 $^+$ T Cells During Episodes of Viral DNAemia in Allogeneic Stem Cell Transplant Recipients: Potential Implications for the Management of Active CMV Infection

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For Peer Review

REVISED VERSION

Kinetics of Cytomegalovirus (CMV) pp65 and IE-1-Specific IFN γ CD8⁺ and CD4⁺ T Cells During Episodes of Viral DNAemia in Allogeneic Stem Cell Transplant Recipients: Potential Implications for the Management of Active CMV Infection

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Running head: CMV-specific immunity during episodes of CMV DNAemia

ABSTRACT

The dynamics of CMV pp65 and IE-1-specific IFN γ -producing CD8⁺ (IFN γ CD8⁺) and CD4⁺ (IFN γ CD4⁺) T cells and CMV DNAemia were assessed in 19 pre-emptively-treated episodes of active CMV infection. Peripheral counts of IFN γ CD8⁺ and IFN γ CD4⁺ T cells inversely correlated with CMV DNAemia levels ($P=<0.001$ and $P=0.003$, respectively). A threshold value of 1.3 cells/ μ L predicting CMV DNAemia clearance was established for IFN γ CD8⁺ T cells (PPV, 100%; NPV, 93%) and for IFN γ CD4⁺ T cells (PPV, 100%; NPV, 75%). Undetectable T-cell responses were usually observed at the time of initiation of pre-emptive therapy. Either a rapid (within 7 days) or a delayed (median 31 days) expansion of both T-cell populations concomitant with CMV DNAemia clearance was observed in 5 and 8 episodes, respectively. An inconsistent or a lack of expansion of both T-cell subsets was related to a persistent CMV DNAemia. Robust and maintained CMV-specific T-cell responses after CMV DNAemia clearance and cessation of antiviral therapy were associated with a null incidence of relapsing infections at least during the following month. Data obtained in the present study may be helpful in the design of therapeutic strategies for the management of active CMV infections in the allo-SCT recipient.

Key words: Cytomegalovirus, IFN γ CD8⁺ and CD4⁺ T cells, active CMV infection, immunological monitoring, stem cell transplantation.

51 **INTRODUCTION**

52 Pre-emptive antiviral therapy has been adopted by most transplant centers as the first-
53 choice strategy for the prevention of cytomegalovirus (CMV) disease following
54 allogeneic stem cell transplantation (allo-SCT) [Boeckh et al., 2003; Griffiths et al.,
55 2008]. While this strategy has been shown to dramatically reduce the incidence of early
56 CMV disease [Boeckh et al., 2003; Ljungman, 2008], it probably results in over-
57 treatment, as a number of patients who would never progress to CMV disease are
58 treated nevertheless [Ljungman, 2006; Avetisyan et al., 2007]. Hopes have been raised
59 that routine immunological monitoring for CMV-specific T-cell immunity may improve
60 the management of active infection, leading to a more targeted use of antivirals and
61 allowing the identification of patients at high risk for relapsing infections and end-organ
62 disease. Nevertheless, to date, assessment of CMV-specific T-cell immunity has not had
63 a major impact on clinical management of active CMV infection. In order to design
64 potential intervention strategies based on immunological monitoring, characterization of
65 the kinetics of functional CMV-specific T cells during episodes of active CMV
66 infection is required. Studies addressing this issue are, however, scarce [Aubert et al.,
67 2001; Foster et al., 2002; Widmann et al., 2008]. Resolution of episodes of active CMV
68 infection in the allo-SCT setting appears to be ultimately dependent on the expansion of
69 functional CMV-specific T cells in response to CMV replication [Quinnan et al., 1982;
70 Reusser et al., 1991; Riddell et al., 1992]. In this regard, we have previously shown that
71 the lack of prompt expansion of CMV pp65 and IE-1-specific IFN γ -producing CD8⁺
72 (IFN γ CD8⁺) and CD4⁺ (IFN γ CD4⁺) T cells is associated with rising levels of pp65
73 antigenemia and DNAemia during pre-emptive therapy and prolonged duration of
74 antiviral treatment [Tormo et al., 2009]. In the present study, the dynamics of CMV-
75 specific IFN γ CD8⁺ and IFN γ CD4⁺ T cells, which we have previously shown to confer

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76 protection against CMV infection [Solano et al., 2008], and those of CMV DNAemia
77 were assessed in a number of episodes of active CMV infection. Data obtained in the
78 present study are of potential interest for the therapeutic management of active CMV
79 infections in the allo-SCT recipient.

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MATERIAL AND METHODS

Patients

Patients undergoing allo-SCT between December 2008 and May 2009 at the participating hospitals were eligible for inclusion. The study was approved by the Ethics Committees. All patients gave their informed consent to participate in the study. CMV-seronegative patients receiving a graft from a CMV-seronegative donor were not included in the study. Only episodes of active CMV infection treated pre-emptively were included for analysis. The end of the study period was June 22th 2009. Clinical and demographic data of the patients are shown in Table I.

Management of active CMV infection

Virological monitoring of CMV infection was performed by use of the pp65 antigenemia assay (Diagnostics® CMV pp65 Antigenemia Immunofluorescence assay, Chemicon International, Temecula, CA, USA), and/or a plasma real-time PCR assay (CMV real-time PCR, Abbott Molecular, Des Plaines, IL, USA, or LightCycler CMV Quant Kit, Roche, Branchburg, NJ, USA) as previously reported [Solano et al., 2001; Gimeno et al., 2008]. Pre-emptive therapy with oral valganciclovir (900 mg/12 h) or i.v. ganciclovir (5 mg/Kg/12 h) was initiated upon a positive antigenemia result (≥ 1 pp65 positive cells/200,000 cells) or detection of >1000 CMV DNA copies/mL in plasma depending on the participant institution, and discontinued following 2 consecutive negative antigenemia or plasma PCR results (also depending on the participant hospital) obtained 3 to 7 days apart after a minimum of 2 weeks of treatment. Foscarnet (i.v. 60 mg/Kg/12 h) was used instead of ganciclovir in patients with severe neutropenia, and in some patients not responding to ganciclovir therapy after 3 weeks of treatment. For analysis purposes, the duration of a given episode was that comprised between the day of initiation of pre-emptive therapy and the day of the first negative DNAemia result.

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3 126 Diagnosis of CMV disease was achieved as previously reported [Solano et al., 2001;
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5 127 Gimeno et al., 2008]. **When clinically indicated, patients received transfusions of**
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7 **leukocyte-depleted and irradiated packed red cells and platelets.**
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10 **Immunological monitoring**

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12 130 Enumeration of CMV-specific IFN γ CD8⁺ and IFN γ CD4⁺ T lymphocytes was
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15 131 carried out by flow cytometry for ICS (BD Fastimmune, BD-Beckton Dickinson and
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17 132 Company-Biosciences, San Jose, CA, USA) as described previously [Solano et al.,
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19 133 2008]. Briefly, whole blood was simultaneously stimulated with two sets of 15-mer
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21 134 overlapping peptides encompassing the sequence of pp65 and IE-1 CMV proteins (2
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23 135 $\mu\text{g/ml/peptide}$), obtained from JPT peptide Technologies GmbH (Berlin, Germany), in
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25 136 the presence of 1 $\mu\text{g/ml}$ of costimulatory mAbs to CD28 and CD49d for 6 h at 37 °C.
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27 137 Brefeldin A (10 $\mu\text{g/ml}$) was added for the last 4 h of incubation Cells were
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29 138 permeabilized and stained with a combination of labeled moAbs (anti-IFN γ -FITC, anti-
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31 139 CD69-PE, anti-CD4 or CD8-PerCP-Cy5.5 and anti-CD3-APC when the IFN γ CD8⁺ kit
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33 140 was used). Cells were analyzed on a FACSCalibur flow cytometer using CellQuest
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35 141 software (BD Biosciences Immunocytometry Systems). CD4⁺ and CD8⁺ events were
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37 142 gated and then analyzed for the CD69 activation marker and IFN γ production. The total
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39 143 number of CMV-specific IFN γ CD4⁺ and IFN γ CD8⁺ T cells was calculated by
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41 144 multiplying the percentages of CMV-specific T cells producing IFN γ upon stimulation
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43 145 (after background subtraction) by the absolute CD4⁺ and CD8⁺ T cell counts. The
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45 146 specific responses were considered those >0.1% for both CD4⁺ and CD8⁺ T cells.
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53 147 Immunological monitoring was performed once or twice a week during episodes of
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55 148 active CMV infection. For some patients, several blood samples drawn after resolution
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57 149 of the episode of active CMV infection were available for immunological analysis. A
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3 150 total of 146 blood samples from the 18 patients (median 7 samples; range, 2–15 samples)
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5 151 were analyzed.
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152 **Sequence analysis of the UL54 and UL97 genes**

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10 153 CMV DNA extraction from plasma specimens was carried out using the High Pure
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12 154 nucleic acid kit (Roche Diagnostics, GmbH, Mannheim, Germany). The extracted viral
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15 155 DNA was used as a template for amplification of a 975 bp region of the UL97 gene
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17 156 spanning codons 429 to 753, and two regions of the UL54 gene spanning codons 345 to
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19 157 625, and 645 to 1,013, as previously described [Tormo et al., 2009]. The PCR products
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21 158 were purified by the QIAquick purification kit (Qiagen GmbH, Hilden, Germany),
22
23 159 sequenced by the ABI Prism BigDye Terminator Cycle Sequencing Kit v3.1 (PE
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25 160 Applied Biosystems), and analyzed on an ABI 310 automated DNA sequencer.
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29 161 **Statistical analysis**

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31 162 Data were analyzed with the aid of the statistical package SPSS (version 15.0).
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33 163 Comparisons were carried out using the non-parametric Mann-Whitney U-test for
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35 164 unpaired continuous data and the Wilcoxon test for paired continuous data. The
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37 165 Spearman rank test was used for analysis of correlation between continuous variables.
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39 166 For calculation purposes undetectable CMV-specific responses were computed as 0
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41 167 cells/ μ L. A *P* value $<.05$ was considered statistically significant.
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RESULTS

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Features of episodes of active CMV infection

177 Nineteen episodes of active CMV infection (in 18 patients) were included for
178 analysis. The episodes occurred at a median of 56 days post-transplant (range, 14 to 330
179 days). Thirteen out of the 19 episodes (in 12 patients) resolved within the study period
180 (Table II). The remaining 6 episodes (in 6 patients) were still active at the end of the
181 follow up, after a median of 30 days (range 14 to 97 days) of initiation of pre-emptive
182 therapy (Table III). Three out of these 6 patients died during the study period (the cause
183 of death was bacterial sepsis in one patient and severe GvHD in the other 2 patients).

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CMV-specific T cells at the time of initiation of pre-emptive therapy

186 We firstly assessed the CMV-specific T-cell response at the time of initiation of pre-
187 emptive therapy. Blood samples for immunological analysis were available from 14
188 episodes (9 of them developed before day 100 post-transplant). Undetectable IFN γ
189 CD8⁺ and CD4⁺ T-cell responses were observed in 13 out of the 14 episodes. In the
190 remaining episode, which was still active at the end of the follow up, low levels of both
191 CMV-specific T-cell subsets were detected (0.03 IFN γ CD8⁺ T cells/ μ L and 0.26 IFN γ
192 CD4⁺ T cells/ μ L). Nine out of the 14 episodes resolved within the study period, with the
193 time to negative conversion of CMV DNAemia varying widely (3 to 94 days), while no
194 control of CMV replication was achieved in the remaining 5 episodes. These data
195 indicated that peripheral levels of IFN γ CD8⁺ and IFN γ CD4⁺ T cells at the time of
196 initiation of pre-emptive therapy were not predictive of the virological outcome of the
197 episode.

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3 199 **Kinetics of CMV-specific T cells and CMV DNAemia during episodes of**
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5 200 **Active CMV infection**
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8 201 We investigated the dynamics of both CMV-specific T-cell subsets and CMV
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10 202 DNAemia during 19 pre-emptively treated episodes of active CMV infection. Overall,
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12 203 peripheral counts of IFN γ CD8⁺ and IFN γ CD4⁺ T cells inversely correlated with CMV
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14 204 DNAemia levels ($\sigma=-0.806$; $P=<0.001$, and $\sigma=-712$; $P=0.003$, respectively), yet
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16 205 detectable CMV-specific T-cell responses were observed in the presence of CMV
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18 206 DNAemia at some time points, both in unresolved episodes and in episodes which
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20 207 eventually cleared. We found however, that IFN γ CD8⁺ (median, 0.38 cells/ μ L; range,
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22 208 0.03–1.28 cells/ μ L) and IFN γ CD4⁺ (median, 0.32 cells/ μ L; range, 0.06–1.27 cells/ μ L)
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24 209 T-cell levels in the presence of CMV DNAemia were significantly lower ($P=<0.000$ for
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26 210 IFN γ CD8⁺ and $P=<0.003$ for IFN γ CD4⁺ T cells) than IFN γ CD8⁺ (median, 2.46
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28 211 cells/ μ L; range 0.82–15.02 cells/ μ L) and IFN γ CD4⁺ (median 0.56 cells/ μ L; range,
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30 212 0.12–5.2 cells/ μ L) T-cell levels at the time of the first negative PCR result. Taking into
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32 213 consideration the peak value for IFN γ CD8⁺ T cells (1.28 cells/ μ L) found in the
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34 214 presence of CMV DNAemia, a threshold value of 1.3 cells/ μ L predicting CMV
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36 215 DNAemia clearance (negative PCR) was established in our cohort (PPV, 100%; NPV,
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38 216 93%). A certain degree of overlap was found between IFN γ CD4⁺ T cell values in the
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40 217 presence of CMV DNAemia and those at the time of the first negative PCR result. Thus,
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42 218 setting a threshold (1.3 cells/ μ L) above the peak value (1.27 cells/ μ L) found in the
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44 219 presence of CMV DNAemia resulted in a lower NPV (75%). Therefore, the number of
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46 220 IFN γ CD8⁺ T cells was a more reliable marker for predicting CMV DNAemia clearance
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48 221 in our cohort.
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52 222 Three different kinetic patterns of IFN γ CD8⁺ and IFN γ CD4⁺ T cells were observed
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54 223 in relation to CMV DNAemia: (i) A rapid expansion within the first week after
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3 224 initiation of pre-emptive therapy (median 7 days; range 3 to 7 days) concomitant with
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5 225 CMV DNAemia clearance was observed in 5 episodes (see episodes 1 to 5 in Table II).
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8 226 An expansion within the third week (days 15 and 16) was observed in 2 additional
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10 227 episodes (see episodes 6 and 7 in Table II). Overall, median increases of 2.09 IFN γ
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12 228 CD8⁺ T cells/ μ L (range, 0.9-15.13 cells/ μ L) and of 0.67 IFN γ CD4⁺ T cells/ μ L (range,
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15 229 0.12-5.20 cells/ μ L) from baseline were observed in these episodes. None of these
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17 230 patients was under corticosteroid treatment during the episode of active CMV infection.
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19 231 Figure 1A depicts a representative episode of this kinetics pattern (episode 4 in Table II);
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22 232 (ii) An early expansion (median 8 days; range 5 to 21 days) of a lower magnitude
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24 233 (median increase of 0.57 IFN γ CD8⁺ T cells/ μ L-range, 0.21-1.2 cells/ μ L $P=0.02$ -, and
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26 234 of 0.29 IFN γ CD4⁺ T cells/ μ L- range,0.01-0.62; $P=0.063$ -) than that observed in rapidly
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28 235 cleared episodes with delayed resolution of CMV DNAemia (median 31 days, range 20
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30 236 to 94 days), concomitant with a further expansion of both T-cell subsets (median
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32 237 increase of 3.05 IFN γ CD8⁺ T cells/ μ L and of 0.42 IFN γ CD4⁺ T cells/ μ L; $P=0.001$ and
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34 238 $P=0.04$, respectively) was observed in 6 episodes (see episodes 8 to 13 in Table II);
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36 239 Figure 1B illustrates a representative example of this kinetics pattern (data for the
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38 240 episode 8 in Table II). The two longest episodes (episodes 8 and 11) occurred in
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40 241 patients under treatment with corticosteroids for grade III–IV GvHD, and their
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42 242 resolution was coincident with steroid dose tapering; (iii) An inconsistent or a lack of
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44 243 expansion of IFN γ CD8⁺ (median, 0.06 cell/ μ L) and IFN γ CD4⁺ T cells (median, 0.22
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46 244 cell/ μ L; $P=0.001$ and $P=0.03$, respectively, in relation to T-cell increases measured in
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48 245 cleared episodes) with persistent CMV DNAemia that was still detectable at the end of
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50 246 the follow-up period was seen in 6 episodes (Table III). Three out of these 6 episodes
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52 247 developed while patients were under corticosteroid therapy for severe GvHD. Figure 1C
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54 248 shows the data for a representative case (episode 1 in Table III). The latest plasma
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3 249 samples available from the longest unresolved episodes (episodes 1 and 2 in Table III)
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5 250 were screened for the presence of mutations known to confer resistance to ganciclovir
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8 251 or foscarnet. No mutations were found in either sample.
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12 253 **CMV-specific T cells after CMV DNAemia clearance and relapsing**
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15 254 **infections**
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17 255 None of the 12 patients in whom the episode of CMV DNAemia resolved during the
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19 256 study period experienced a relapsing episode at least during the following month (only
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21 257 one of these patients received maintenance valganciclovir therapy after CMV DNAemia
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23 258 clearance). Episodes 2 and 3 in Table II developed in the same patient; However, the
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25 259 relapsing episode occurred 6 months after the resolution of the preceding one. Blood
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27 260 samples obtained at the time of interruption of antiviral therapy (second antigenemia
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29 261 test or PCR giving a negative result) were available for 8 episodes. A median of 2.8
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31 262 IFN γ CD8⁺ T cells/ μ L (range 0.68 to 19.5 cells/ μ L) and of 0.38 IFN γ CD4⁺ T cells/ μ L
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33 263 (range 0.19 to 2.28 cells/ μ L) was found. Follow-up samples obtained after cessation of
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35 264 antiviral therapy (range 3 to 86 days) were available from 4 episodes. Detectable though
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37 265 fluctuating levels of both IFN γ CD8⁺ and IFN γ CD4⁺ T cells were observed over time
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39 266 (see the Figure 1, panel A for a representative example), with a median of 5.1 IFN γ
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41 267 CD8⁺ T cells/ μ L (range 0.57 to 47.7 cells/ μ L) and 0.41 IFN γ CD4⁺ T cells/ μ L (range
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43 268 0.19 to 3.31 cells/ μ L).
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53 270 **CMV-specific T cells in patients with CMV disease**
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55 271 CMV enteritis was diagnosed in 3 patients in the setting of corticosteroid treatment
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57 272 for GvHD (Table II, episode 13, and Table III, episodes 4 and 5). In all cases,
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59 273 undetectable CMV-specific T-cell responses were observed at the onset of the clinical
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3 274 manifestations of the disease. Interestingly, in patient 12 (episode 13 in Table II), CMV
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5 275 DNAemia clearance and a notable improvement in clinical symptoms were related to a
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8 276 marked expansion of IFN γ CD8⁺ T cells, which occurred after steroid dose tapering.
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10 277 The remaining 2 patients failed to expand CMV-specific T cells and had persistent
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12 278 CMV DNAemia. Both patients died during the follow-up period.
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DISCUSSION

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19 281 Several conclusions can be drawn from our data. Firstly, peripheral counts of both
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21 282 IFN γ CD8⁺ and IFN γ CD4⁺ T-cell subsets inversely correlated with CMV DNAemia
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23 283 levels during episodes of active CMV infection. Similar data were obtained in earlier
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25 284 studies using pp65 peptide-tetramers [Aubert et al., 2001] or ICS [Widmann et al., 2008]
26
27 285 to assess the CMV-specific CD8⁺ and CD4⁺ T-cell responses, respectively. In the latter
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29 286 study however, levels of CMV peptide-specific CD8⁺ T cells did not correlate with
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31 287 viremia [Widmann et al., 2008]. In another study [Avetisyan et al., 2006], patients who
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33 288 had detectable CMV-specific IFN γ CD3⁺ T-cell responses at week 4 after allo-SCT
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35 289 displayed lower peak mean viral loads compared with patients who lacked a detectable
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37 290 response. Furthermore, the degree of decline in CMV-specific T cells early after
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39 291 transplant has been associated with the development of CMV viremia [Eid et al., 2009].
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45 292 In our study, the median levels of both CMV-specific functional T-cell subsets were
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47 293 significantly lower in the presence of CMV DNAemia than at the time of the first
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49 294 negative PCR result. A threshold value of 1.3 cells/ μ L for both T-cell populations
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51 295 predicting CMV DNAemia clearance was established in our cohort, the number of IFN γ
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53 296 CD8⁺ T cells being a more reliable marker. These cut-off cell levels are remarkably
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55 297 close to those previously determined by us [Solano et al., 2008] and by other groups
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57 298 [Hebart et al., 2002; Ohnishi et al., 2005; Lilleri et al., 2008; Moins-Teisserenc et al.,
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3 299 2008, Pourgheysari et al., 2009] (different functional T-cell types) providing protection
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6 300 against development of CMV antigenemia or DNAemia.

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8 301 Secondly, in agreement with a previous report by our group [Solano et al., 2008], we
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10 302 found that ultimate control of CMV replication in the course of antiviral therapy
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12 303 depended on a robust and sustained expansion of IFN γ CD8⁺ T cells, and – to a lesser
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14 304 extent – of IFN γ CD4⁺ T cells. A notable expansion of both T-cell subsets concomitant
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16 305 with CMV DNAemia clearance was seen as soon as one week after initiation of pre-
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18 306 emptive therapy in 5 episodes, although, overall, T-cell expansion controlling CMV
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20 307 replication was observed at a median of 15 days after implementation of therapy. In line
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22 308 with our findings, the peak median level of expanding pp65-specific functional CD8⁺ T
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24 309 cells was reported to occur around 20 days after CMV reactivation [Hakki et al., 2003].
25
26 310 In contrast, failure to expand both functional T-cell populations in response to CMV
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28 311 replication resulted in persistent CMV DNAemia despite antiviral treatment and the fact
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30 312 that the emergence of resistant strains was not documented (in the two longest episodes).

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36 313 Three out of the 6 episodes that remained active at the end of the follow up occurred
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38 314 in the setting of corticosteroid therapy for GvHD, supporting the well known fact that
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40 315 corticosteroids impair the reconstitution of the CMV-specific T-cell response in a dose-
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42 316 dependent manner [Hakki et al., 2003; Gratama et al., 2008].

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45 317 Thirdly, the marked expansion of functional CMV-specific T cells and the
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47 318 maintenance of peripheral pools of these T-cell subsets after CMV DNAemia clearance
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49 319 and cessation of antiviral therapy prevented the occurrence of relapsing episodes of
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51 320 active CMV infection at least during the following month. A similar conclusion was
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53 321 reached in earlier studies [Lilleri et al., 2008; Gratama et al., 2008; Moins-Teisserenc et
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55 322 al., 2008].
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3 323 Three patients developed CMV enteritis. In accordance with a previous report
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5 324 [Avetisyan et al., 2006], in all cases clinical onset of disease occurred in the face of
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8 325 undetectable IFN γ CD8⁺ and CD4⁺ T-cell responses. One of these patients recovered
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10 326 from CMV disease, and clinical improvement, as well as CMV DNAemia clearance,
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12 327 was concomitant with a robust expansion of both functional CMV-specific T-cell
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15 328 populations.

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17 329 Our data may have several implications for the therapeutic management of active
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19 330 CMV infection in the allo-SCT recipient. A strategy of deferred antiviral therapy based
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21 331 on the detection of a CMV-specific immune response by ELISPOT at the time of CMV
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23 332 DNAemia detection was safely applied in a number of patients late (around 3 months)
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26 333 after transplant [Avetisyan et al., 2007]. In our cohort, however, no patient stratification
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28 334 could be established according to the magnitude of the CMV-specific T-cell response at
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30 335 the time of initiation of pre-emptive therapy, as all but one patient displayed
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32 336 undetectable responses. The lack of a detectable CMV-specific T-cell response,
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34 337 however, was not predictive of the outcome of CMV infection in terms of the duration
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36 338 of CMV DNAemia. Thus, no patients from our cohort would have benefited from the
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38 339 abovementioned strategy. Our study and that of Avetisyan *et al.* [2007], however, differ
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40 340 in the method employed for enumeration of CMV-specific functional T cells, and most
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42 341 importantly, in the time frame in which active CMV infections occurred (early after
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44 342 transplant in most of our patients and late after transplant in theirs). Further studies are
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46 343 required to determine the clinical usefulness of this therapeutic approach.

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48 344 Early interruption (or dose reduction) of pre-emptive therapy provided that a
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50 345 significant expansion of CMV-specific T cells is documented may be a potential
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52 346 intervention strategy based on immunological monitoring. Our data indicate that
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54 347 peripheral levels of CMV-specific IFN γ CD8⁺ T cells above 1.3 cells/ μ L, which were

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3 348 shown to be associated with CMV DNAemia clearance, might be a reasonable threshold
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5 349 at which to interrupt antiviral therapy. Our data do not allow us to be certain about the
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8 350 virological efficacy and clinical safety of this approach, as the antiviral therapy course
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10 351 was not suspended in our patients until a second negative antigenemia or PCR result.
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12 352 This question can only be answered by means of a controlled clinical trial.
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15 353 Relapsing episodes of active CMV infection develop at an exceedingly high rate
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17 354 following the implementation of short duration pre-emptive treatment regimens
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19 355 [Gimeno et al., 2008]. We have previously shown that their occurrence is related to the
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21 356 presence of low peripheral levels of CMV-specific functional T cells after resolution of
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23 357 the preceding episode [Tormo et al., 2009]. Data obtained in the present study extend
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25 358 our previous observation. In effect, peripheral levels of both T-cell subsets above the
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27 359 abovementioned cut-off at the time of virological clearance and interruption of antiviral
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29 360 therapy consistently prevented the occurrence of relapsing episodes. In these patients a
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31 361 sustained but rather fluctuating CMV-specific T-cell response was observed. On the
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33 362 basis of this finding, maintenance antiviral therapy should be administered after CMV
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35 363 DNAemia clearance provided that protective levels of functional CMV-specific T cell
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37 364 are not reached at the end of the antiviral therapy course.
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43 365 Adoptive transfer of CMV-specific functional T cells is a therapeutic option in
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45 366 episodes of active CMV infections that do not respond to antiviral therapy [Einsele et al.,
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47 367 2008]. According to our data, a lack of consistent expansion of CMV-specific T cells is
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49 368 associated with persistent CMV DNAemia, even in the absence of proven resistance to
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51 369 antivirals. In this sense, it would be reasonable to consider this therapeutic strategy,
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53 370 rather than switching antiviral therapy, in patients failing to respond to antivirals after 4
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55 371 weeks of treatment (time at which expansion of functional CMV-specific T cells
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57 372 eventually controlling CMV replication was documented in most of cleared episodes in
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3 373 our cohort), provided that corticosteroid therapy is not underway, and that emergence of
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5 374 antiviral-resistant strains is ruled out.
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8 375 In summary, our data suggest that routine immunological monitoring during episodes
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10 376 of CMV DNAemia may yield useful information for the therapeutic management of
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12 377 active CMV infection in allo-SCT recipients. Larger studies are nevertheless needed in
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15 378 order to verify this assumption.
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3 517 **FIGURE 1.** Representative patterns of kinetics of CMV pp65 and IE-1-specific IFN γ -
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5 518 producing CD8⁺ (IFN γ CD8⁺ -open circles-) and CD4⁺ (IFN γ CD4⁺) T cells (black
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7 519 diamonds) and CMV DNAemia (triangles) during episodes of active CMV infection. (A)
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9 520 Patient displaying an early expansion of CMV-specific T cells (specially involving the
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11 521 IFN γ CD8⁺ T-cell subset) concomitant with CMV DNAemia resolution. Fluctuating
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13 522 levels of both T-cell populations were observed after interruption of antiviral therapy in
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15 523 the absence of CMV DNAemia; (B) Patient displaying an early expansion of both
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17 524 functional T-cell populations with delayed clearance of CMV DNAemia concomitant
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19 525 with a further expansion of CMV-specific T cells; Fluctuating levels of both T-cell
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21 526 subsets were observed prior to ultimate expansion leading to CMV DNAemia resolution
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23 527 (C) Patient failing to expand either CMV-specific functional T-cell subset and
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25 528 displaying persistent CMV DNAemia. This patient was under antiviral therapy
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27 529 throughout the observational period. The arrow (AT) points out to the time at which
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29 530 antiviral therapy was interrupted.
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TABLE I. Demographic and clinical data of the patients.

Parameter	
Patients	18
Median age, yrs (range)	52.5 (17–67)
Sex, no. male patients/no. female patients	12/6
Diagnosis, n° patients (%)	
Non-Hodgkin`s lymphoma	8 (44)
Acute myeloid leukaemia	4 (22)
Multiple myeloma	3 (16)
Myelodysplastic syndrome	2 (11)
Chronic lymphocytic leukaemia	1 (6)
CMV serostatus, n° patients (%)	
D+/R+	9 (50)
D-/R+	6 (33)
D+/R-	3 (16)
Donor type, n° patients (%)	
HLA-identical sibling	10 (55)
Matched unrelated donor	5 (28)
Mismatched related donor	3 (16)
Conditioning regimen, n° patients (%)	
Non-myeloablative	14 (77)
Myeloablative	4 (22)
Stem cell source	
Peripheral blood	16 (88)
Umbilical cord blood	1 (6)
Bone marrow	1 (6)
Acute or chronic GvHD during episodes of active CMV infection	
Grades 0-I	12 (66)
Grades II-IV	6 (33)

D,donor; R, recipient; +, CMV seropositive; -, CMV seronegative; GvHD,Graft versus host disease.

TABLE II. Virological and immunological data of episodes of active CMV infection, the resolution of which occurred during the study period.

Episode (Day PT)	CMV DNAemia (Copies/mL)			CMV-specific T-cell response IFN γ CD8 ⁺ / IFN γ CD4 ⁺ (cells/ μ L)	
	Initial	Peak	Duration (days)	First detected response (day)	At first negative PCR (day)
1 (232)	1125	1125	7	15.13/0.12 (7)	-*
2 (115)	800	800	3	2.09/1.61 (3)	-
3 (293)	2460	2460	7	15.20/5.20 (7)	-
4 (41)	890	890	7	14.01/1.90 (7)	-
5 (40)	1800	1800	6	1.31/0.43 (6)	-
6 (59)	560	1200	15	0.90/0.38 (15)	-
7 (14)	1100	3200	16	1.45/0.67 (16)	-
8 (30)	1100	6200	94	0.50/0.40 (21)	6.24/0.55 (94)
9 (26)	1980	1980	20	0.65/0.19 (7)	0.82/0.56 (20)
10 (53)	3600	4560	55	0.21/0.62 (21)	2.46/1.23 (55)
11 (63)	1270	3700	61	0.22/0.01 (7)	1.36/0.48 (61)
12 (27)	1200	3100	31	0.67/0.06 (5)	4.34/0.70 (31)
13 (65)	7200	15110	31	1.28/0.46 (9)	10.62/0.35 (31)

PT, post-transplant. Day in the column for the first detected CMV-specific T-cell responses refers to days after initiation of pre-emptive therapy. Episodes 2 and 3 developed in the same patient. *, First detected response concomitant with first negative PCR.

TABLE III. Virological and immunological data of episodes of active CMV infection that were still active at the end of the study period.

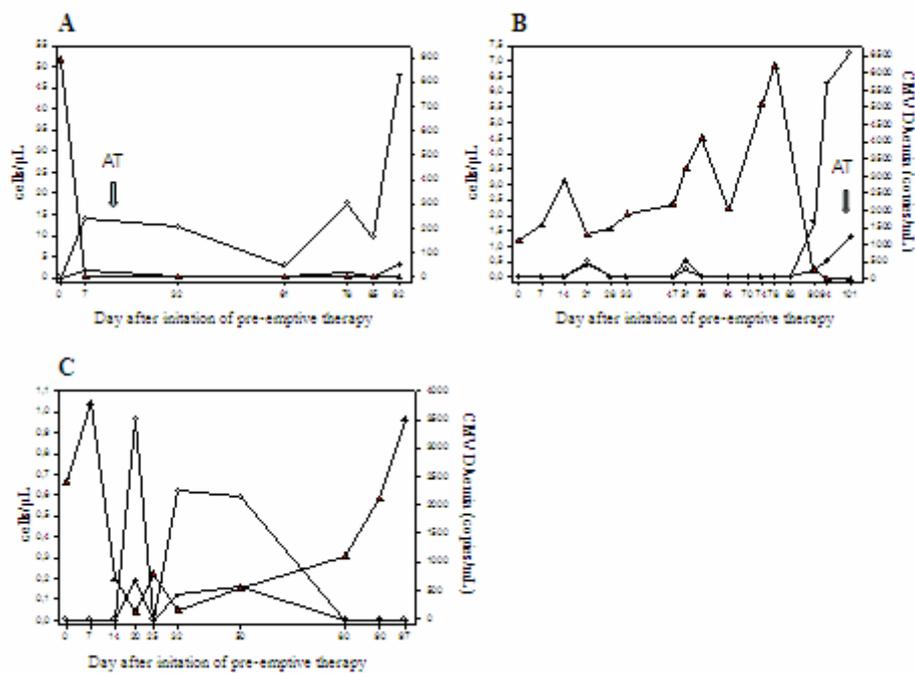
Episode (Day PT)	CMV DNAemia (Copies/mL)		CMV-specific T-cell response IFN γ CD8 $^+$ / IFN γ CD4 $^+$ (cells/ μ L)		Treatment
	Initial	Peak (day)	First detectable response (day)	Peak level (day)	
1 (330)	2390	3790 (7)	0.96/0.19 (20)	- *	FOS/VGCV
2 (62)	2320	35,450 (12)	1.28/1.27 (8)	-	VGCV
3 (160)	1350	10,200 (30)	0.03/0.06 (7)	-	VGCV
4 (42)	5440	5500 (9)	0.09/0.46 (9)	-	FOS
5 (55)	5600	23,450 (19)	ND	ND	VGCV
6 (36)	720	1290 (23)	0.03/0.26 (0)	0.53/0.66 (8)	GAN

FOS, Foscarnet; VGCV, Valganciclovir; GAN, Ganciclovir; ND, Not detectable; PT, Post-transplant. Day in columns refers to days after initiation of pre-emptive therapy.

*-, The peak level was the first detected response.

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For Peer Review



Peer Review