Characterization of CMV-specific CD4⁺ T-cell reconstitution following stem cell transplantation through the use of HLA Class II-peptide tetramers identifies patients at high risk of recurrent CMV reactivation

Mohammad Raeiszadeh,² Annette Pachnio,² Jusnara Begum,² Charles Craddock,²,³ Paul Moss,²,³ and Frederick E. Chen¹,²,³

¹NHS Blood and Transplant, Birmingham; ²School of Cancer Sciences, University of Birmingham; and ³Centre for Clinical Haematology, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK

Correspondence: frederick.chen@nhsbt.nhs.uk
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Authors:
Raeiszadeh M., Pachnio A., Begum J., Craddock C., Moss P., Chen F.
Patients, materials and methods

Study population
Peripheral blood samples were cryopreserved from HSCT patients who attended the Queen Elizabeth Hospital between 2003 and 2011. Twenty HLA-DRB1*0701 HSCT patients identified as at high risk of CMV reactivation were enrolled in the study (Table1). Patients were monitored for CMV reactivation twice weekly by quantitative polymerase chain reaction (PCR) from day 0 up to 100 days and weekly up to 6 months. All received acyclovir prophylaxis until day 100. Pre-emptive Ganciclovir was commenced after two positive PCR results. T-cell depletion (TCD) consisted of Alemtuzumab or ATG (Table I). GVHD prophylaxis consisted of Cyclosporin A which is tapered from 3 to 6 months as per protocol.

Ethics
The study was approved by the UK Research Ethics Committee (05/Q2707/175). Patients gave informed consent in accordance with the Declaration of Helsinki.

Materials & Methods
Custom-made phycoerytherin (PE)- conjugated HLA-class II tetramers consisting of HLA-DRB1* 0701 complexed to the CMV glycoprotein B-derived peptide DYSNTHSTRYV (Benaroya Research Institute, WA) were used to identify and enumerate CMV-specific CD4+ T-cells. A range of HLA-class I multimers (Streptamers, IBA GmbH, Germany) was used to study CMV-specific CD8+ T-cells. Phenotypic analysis was performed by using different antibodies for cell surface or intracellular staining and analysed by FACS-Canto (BD-Biosciences) flow cytometry. CMV-specific serum IgG antibody titres were measured using an in-house ELISA.

Statistical Analysis
GraphPad Prism software version 5 (GraphPad Software, La Jolla, USA) was used for preparation of graphs and non-parametric statistical analysis.
Table S1. Characteristics of transplant patients

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<th>UPN</th>
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UPN: Unique patient Number, NHL: Non Hodgkin Lymphoma, AML: Acute Myeloid Lymphoma, MDS: Myelodysplastic Syndrome, CLL: Chronic Lymphocytic Leukaemia
Figure S1. Analysis of reconstitution of CMV-specific T-cells with HLA class II tetramers.

Flow cytometry dot plots of CMV-specific CD4+ T-cells visualised using CMV-specific HLA-DRB1*0701 tetramers complexed with CMV glycoprotein B-derived DYS peptide. HLA-DRB1*0701 tetramers loaded with irrelevant peptides were used as negative control. x-axis, CD4 staining; y-axis, staining with tetramer.
Figure S2. Pattern of reconstitution of CMV-specific CD4⁺ and CD8⁺ and CD4⁺CD57⁺ T-cells after transplantation in relation to episodes of CMV reactivation.
Graphs showing reconstitution of CMV-specific T-cells in (A) a patient with single, (B) a patient with multiple episodes of virema and (C) a patient with a high and stable level of CMV-specific CD4⁺T-cells (15 x 10³/ml) with no CMV reactivation (only total CD8⁺ T-cell level available). Dashed line shows CMV copy number/ml.
Figure S3. Correlation between number of CMV-specific CD4⁺ T-cells and the number of CD4⁺ CD57⁺ T-cells or CMV-specific antibody titre in the post transplant period.

(A) Correlation between the number of DYS-specific CD4⁺ T-cells (x-axis) and CD4⁺CD57⁺ T- cells (y-axis) in patients during the first 9 months following transplantation (r=0.93, p<0.0001). (B) Correlation between the number of DYS-specific CD4⁺ T- cells (x-axis) and the CMV-specific antibody titre during immune reconstitution (r=0.45, p=0.01).
Figure S4. Perforin expression in total CD4⁺ population, CD4⁺CD57⁺ and CD57⁺ DYS-specific T-cells.

Perforin expression in total CD4⁺ population and in CD4⁺ CD57⁺ and CD57⁺ DYS-specific CD4⁺ T-cells. The perforin expression profile of CD4⁺ CD57⁺ cells were very similar to the expression profile of CMV-specific CD4⁺ T-cells.