(< 50 at 3 months and < 300 after 3 months) and CMV-specific T-cell immunodeficiency were strong predictors of late CMV disease (18%) and death (46%) after allogeneic HCT.[13]

In summary, the clinical benefit observed in our case with refractory CMV encephalitis, which is almost always a fatal condition,[14] may offer clinicians a potentially effective treatment option if CMV-specific T-cell therapy is not available.

Gorgun Akpek
University of Maryland,
Marlene and Stewart Greenebaum Cancer Center,
Baltimore, MD

Maria Mikulski
University of Maryland,
Marlene and Stewart Greenebaum Cancer Center,
Baltimore, MD

Michael Kleinberg
University of Maryland,
Marlene and Stewart Greenebaum Cancer Center,
Baltimore, MD

Ashraf Badros
University of Maryland,
Marlene and Stewart Greenebaum Cancer Center,
Baltimore, MD

Saul Yanovich
University of Maryland,
Marlene and Stewart Greenebaum Cancer Center,
Baltimore, MD

Aaron P. Rapoport
University of Maryland,
Marlene and Stewart Greenebaum Cancer Center,
Baltimore, MD

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Correspondence: Gorgun Akpek, University of Maryland Greenebaum Cancer Center, 22 South Greene St, N9 E12, Baltimore, MD 21201-1595; e-mail: gakpek@umm.edu.

References

1. Feuchtinger T, Opherk K, Bethge WA, et al. Adoptive transfer of pp65-specific T cells for the treatment of chemorefractory cytomegalovirus disease or reactivation after haploidentical and matched unrelated stem cell transplantation. *Blood.* 2010;116(20):4360-4367.

2. Walter EA, Greenberg PD, Gilbert MJ, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med.* 1995;333(16):1038-1044.

3. Szmania S, Galloway A, Bruorton M, et al. Isolation and expansion of cytomegalovirus-specific cytotoxic T lymphocytes to clinical scale from a single blood draw using dendritic cells and HLA tetramers. *Blood.* 2001;98(3):505-512.

4. Einsele H, Roosnek E, Rufer N, et al. Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. *Blood.* 2002;99(11):3916-3922.

5. Peggs KS, Mackinnon S. Augmentation of virus-specific immunity after hematopoietic stem cell transplantation by adoptive T-cell therapy. *Human Immunol.* 2004;65(5):550-557.

6. Leen AM, Christin A, Myers GD, Liu H. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. *Blood.* 2009;114(19):4283-4292.

7. Ilyas C, Forrest GN, Akpek G. Potential clinical benefit of donor lymphocyte infusion in the treatment of refractory invasive fungal pneumonia. *Bone Marrow Transplant.* 2007;40(6):599-601.

8. Reusser P, Riddell SR, Meyers JD, Greenberg PD. Cytotoxic T-lymphocyte response to cytomegalovirus after human allogeneic bone marrow transplantation: pattern of recovery and correlation with cytomegalovirus infection and disease. *Blood.* 1991;78(5):1373-1380.

9. Hakki M, Riddell SR, Storek J, et al. Immune reconstitution to cytomegalovirus after allogeneic hematopoietic stem cell transplantation: impact of host factors, drug therapy, and subclinical reactivation. *Blood.* 2003;102(8):3060-3067.

10. Chen C, Busson M, Rocha V, et al. Activating KIR genes are associated with CMV reactivation and survival after non-T-cell depleted HLA-identical sibling bone marrow transplantation for malignant disorders. *Bone Marrow Transplant.* 2006;38(6):437-444.

11. Cook M, Briggs D, Craddock C, et al. Donor KIR genotype has a major influence on the rate of cytomegalovirus reactivation following T-cell replete stem cell transplantation. *Blood.* 2006;107(3):1230-1232.

12. Boeckh M, Ljungman P. How I treat cytomegalovirus in hematopoietic cell transplant recipients. *Blood.* 2009;113(23):5711-5719.

13. Boeckh M, Leisenring W, Riddell SR, et al. Late cytomegalovirus disease and mortality in recipients of allogeneic hematopoietic stem cell transplants: importance of viral load and T-cell immunity. *Blood.* 2003;101(2):407-414.

14. Reddy SM, Winston DJ, Territo MC, Schiller GJ. CMV central nervous system disease in stem-cell transplant recipients: an increasing complication of drug-resistant CMV infection and protracted immunodeficiency. *Bone Marrow Transplant.* 2010;45(6):979-984.

To the editor:

CD9 up-regulation on CD34$^+$ cells with ingenol 3,20-dibenzoate does not improve homing in NSG mice

Leung et al demonstrated the potential importance of the tetraspanin CD9[1] in regulating migration, adhesion, and homing of human umbilical cord blood (UCB) CD34$^+$ hematopoietic stem and progenitor cells.[2] Using a neutralizing antibody to CD9 they inhibited marrow and splenic homing of these cells in sublethally irradiated NOD/SCID mice. They also showed inferior homing of CD34$^+$CD9$^-$ cells compared with total CD34$^+$ cells.

Our laboratory has also been interested in defining the role of a tetraspanin-containing polarized membrane domain in the interaction of primitive hematopoietic cells with osteoblasts, critical components of the hematopoietic stem cell niche.[3] We have investigated whether increasing expression of tetraspanins such as CD9 might increase homing in the niche. We and the Leung group have found that exposure of CD34$^+$ cells to a protein kinase C ε agonist, ingenol 3,20-dibenzoate (IDB) can increase CD9 expression. In our study, in contrast to Leung's results, we found that

4 hours' exposure to IDB was insufficient time to increase the fraction of CD34$^+$ cells expressing CD9 expression in either mobilized peripheral blood (MPB) or cord blood (CB) CD34$^+$ cells. There was also no change in CXCR4 expression in the IDB versus control cells at 4 hours.

In 3 experiments, we tested whether longer incubation times with IDB could induce CD9 up-regulation in MPB CD34$^+$ cells. When cells were incubated for 48 hours, the mean fluorescence intensity (MFI) for CD9 increased on the IDB-treated cells compared with the untreated cells as shown in a representative experiment (Figure 1A) and summarized in Figure 1B ($P = .009$; n = 3). An increase in CD9 MFI was also observed on IDB-treated cells after 72 hours in culture and the difference approached significance compared with the untreated group ($P = .094$; n = 3; Figure 1A-B). Expression of other molecules known to be important for adhesion and homing, including VLA-4 and CXCR-4
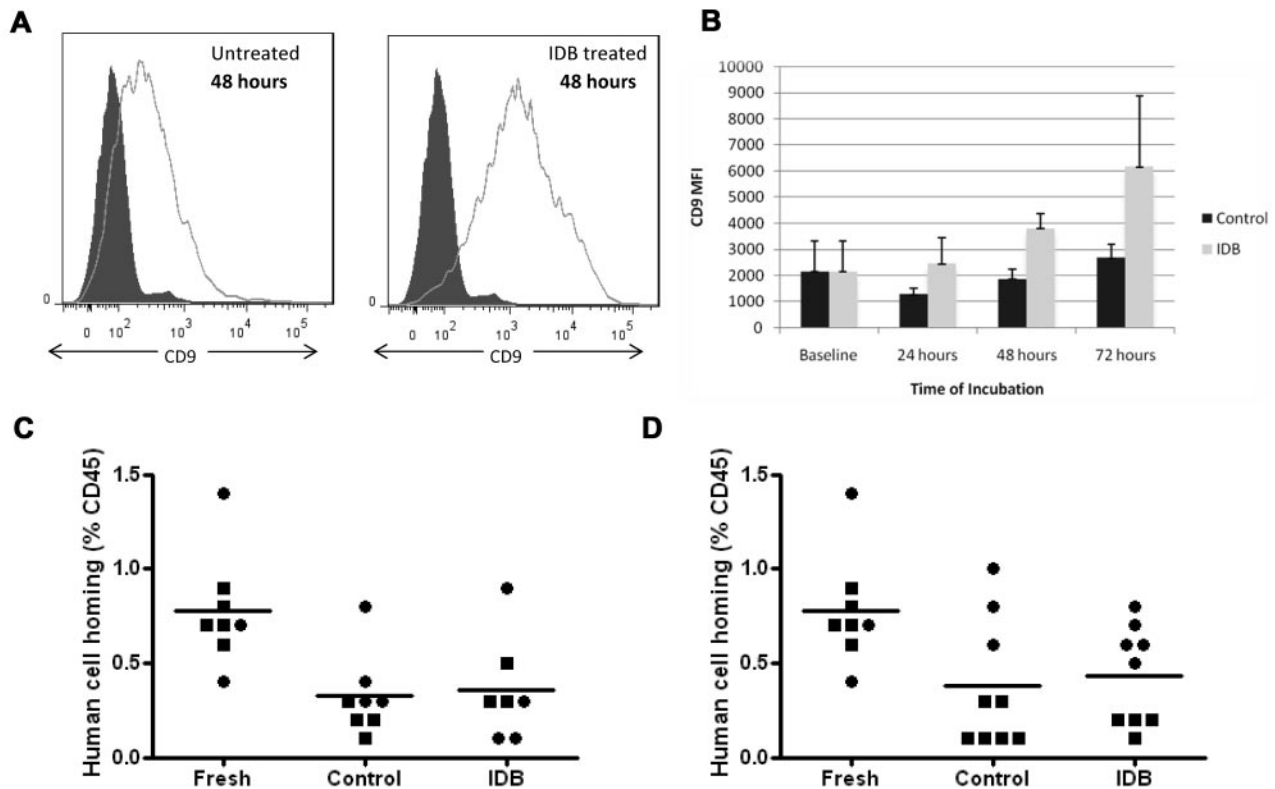
Figure 1. Incubation of human mobilized peripheral blood (MPB) CD34+ cells. Incubation of human mobilized peripheral blood (MPB) CD34+ cells for 48 to 72 hours with IDB up-regulates CD9 expression compared with MPB CD34+ cells incubated without IDB, but homing IDB-treated cells in NSG mice is not improved. (A) Flow cytometric analysis of CD9 expression on human MPB CD34+ cells cultured for 48 hours in the absence (left panel) or presence (right panel) of IDB. (B) Summary of the CD9 MFI of human MPB CD34+ cells at baseline and after culture in the presence or absence of IDB for 24, 48, or 72 hours. (C) Summary of homing studies after 48 hours of incubation with IDB. Human MPB CD34+ cells were exposed to IDB for 48 hours before transplantation into NSG mice; cells in the control group were cultured without IDB. In both groups homing of the human cells to the mouse bone marrow was measured by flow cytometry using human CD45 antibodies 18 hours after transplantation (■, Donor 1; ●, donor 2). (D) Summary of homing studies after 72 hours of incubation with IDB. Human MPB CD34+ cells were exposed to IDB for 72 hours before transplantation into NSG mice; cells in the control group were cultured without IDB. In both groups, homing of the human cells to the mouse bone marrow was measured by flow cytometry using human CD45 antibodies 18 hours after transplantation (■, Donor 1; ●, donor 2).

showed no significant modulation in MFI or fraction of CD34+ cells expressing each molecule after incubation of MPB CD34+ cells for 24 to 72 hours in IDB compared with controls (data not shown).

To determine whether the increase in CD9 expression would affect homing, we transplanted NOD/SCID γ (NSG) mice with cultured MPB CD34+ cells (1×10^6 cells/mouse, n = 3-5 mice/group) incubated for 48 and 72 hours with or without IDB in 2 separate experiments using different donors. NIH Animal Care and Use Committee approval was given. Mice were killed 18 hours after transplantation, and the homing of human cells to the murine bone marrow was analyzed by flow cytometry for human CD45+ cells. Compared with MPB CD34+ cells cultured without IDB, no statistically significant improvement in marrow homing was seen in mice transplanted with cells exposed to IDB for 48 hours ($P = .8$; Figure 1C) or 72 hours ($P = .97$; Figure 1D). In a separate experiment, no transplanted CD45+ cells were detected in blood or splenic tissue (data not shown).

Despite differences in the density of CD9 per cell, exposure to IDB did not result in improved homing in NSG mice. This finding was not because of down-regulation of other surface adhesion receptors essential for hematopoietic stem cell homing. An up-regulation of CD9 did not result in cells lodging in splenic tissue preventing them from reaching the bone marrow. We conclude that while decreasing the percentage of CD34+CD9+ cells inhibits

homing as shown by Leung et al,[1] basal cell–surface CD9 density appears to be sufficient for maximal homing; therefore, CD9 modulation with IDB unfortunately lacks promise for improving homing and engraftment.

Ronan Desmond
Molecular Hematopoiesis Section, Hematology Branch,
National Heart, Lung, and Blood Institute,
Bethesda, MD

Ashley Dunfee
Molecular Hematopoiesis Section, Hematology Branch,
National Heart, Lung, and Blood Institute,
Bethesda, MD

Frederick Racke
Comprehensive Cancer Center, The Ohio State University,
Columbus, OH

Cynthia E. Dunbar
Molecular Hematopoiesis Section, Hematology Branch,
National Heart, Lung, and Blood Institute,
Bethesda, MD

Andre Larochelle
Molecular Hematopoiesis Section, Hematology Branch,
National Heart, Lung, and Blood Institute,
Bethesda, MD

Correspondence: Dr Cynthia E. Dunbar, NHLBI, Bldg 10/4E-5132, 9000 Rockville Pike, Bethesda, MD 20892; e-mail: dunbarc@nhlbi.nih.gov.

References

1. Yáñez-Mó M, Barreiro O, Gordon-Alonso M, et al. Tetraspanin-enriched microdomains: a functional unit in cell plasma membranes. *Trends Cell Biol.* 2009;19(9):434-446.

2. Leung KT, Chan KY, Ng PC, et al. The tetraspanin CD9 regulates migration, adhesion and homing of human cord blood CD34+ hematopoietic stem and progenitor cells. *Blood.* 2011;117(6):1840-1850.

3. Gillette JM, Larochelle A, Dunbar CE, Lippencott-Schwartz J. Intercellular transfer to signalling endosomes regulates an ex vivo bone marrow niche. *Nat Cell Biol.* 2009;11(3):303-311.

To the editor:

Fluorescence in situ hybridization confirms the presence of Merkel cell polyomavirus in chronic lymphocytic leukemia cells

We would like to complement our recently reported data on the detection of the Merkel cell polyomavirus (MCPyV) in chronic lymphocytic leukemia (CLL) cells.[1] We have shown that MCPyV could be identified by PCR in highly purified CLL cells of 19 of 70 (27.1%) CLL patients. In 6 cases, a 246 bp deletion in the helicase region of the large T-antigen (LTAg) of MCPyV was found. Truncating mutations or deletions in the LTAg of MCPyV lead to a loss of the helicase activity and thus prevent viral replication.[2] In addition, in 2 of these CLL cases deleted LTAg was detected concomitant to wild-type MCPyV.

Here we confirm the presence of the MCPyV genome in CLL cells by using fluorescence in situ hybridization (FISH). MCPyV-FISH was performed according to a recently published FISH protocol[3] to detect human papilloma virus (HPV) in formalin-fixed and paraffin-embedded (FFPE) tissue. The MCPyV DNA probe we used was isolated from a Merkel cell carcinoma (MCC) and contained a 4 bp deletion creating a stop codon before the helicase domain. FISH was applied to all 6 FFPE bone marrow trephines (EDTA decalcified) in which we previously detected the 246 bp LTAg MCPyV deletion. Three MCPyV DNA-positive MCCs, as detected by PCR,[4] were used as positive controls showing punctate nuclear signals in MCC indicative for integrated MCPyV in the tumor cells (Figure 1A). Negative controls included omission of the MCPyV probe and several MCPyV-negative tissues, that is, a mantle cell lymphoma and 2 colon carcinomas. None of the negative controls revealed a FISH signal (Figure 1C). Of the 6 CLL cases harboring the 246 bp deletion in the MCPyV-LTAg, 4 revealed punctate signals in the nucleus (Figure 1E,G), comparable to those observed in MCC. The observation that nuclei sometimes show FISH signals differing in intensity (granular staining pattern) might suggest that in addition to MCPyV-DNA integration, transcribed viral RNA is also present.[3,5] FISH analysis on the remaining 2 MCPyV-positive CLLs were inconclusive, most likely because of tissue overfixation and/or poor DNA quality.

In analogy to the punctate nuclear signals observed in anogenital and oropharyngeal lesions by HPV-FISH[3,5] and in MCC by
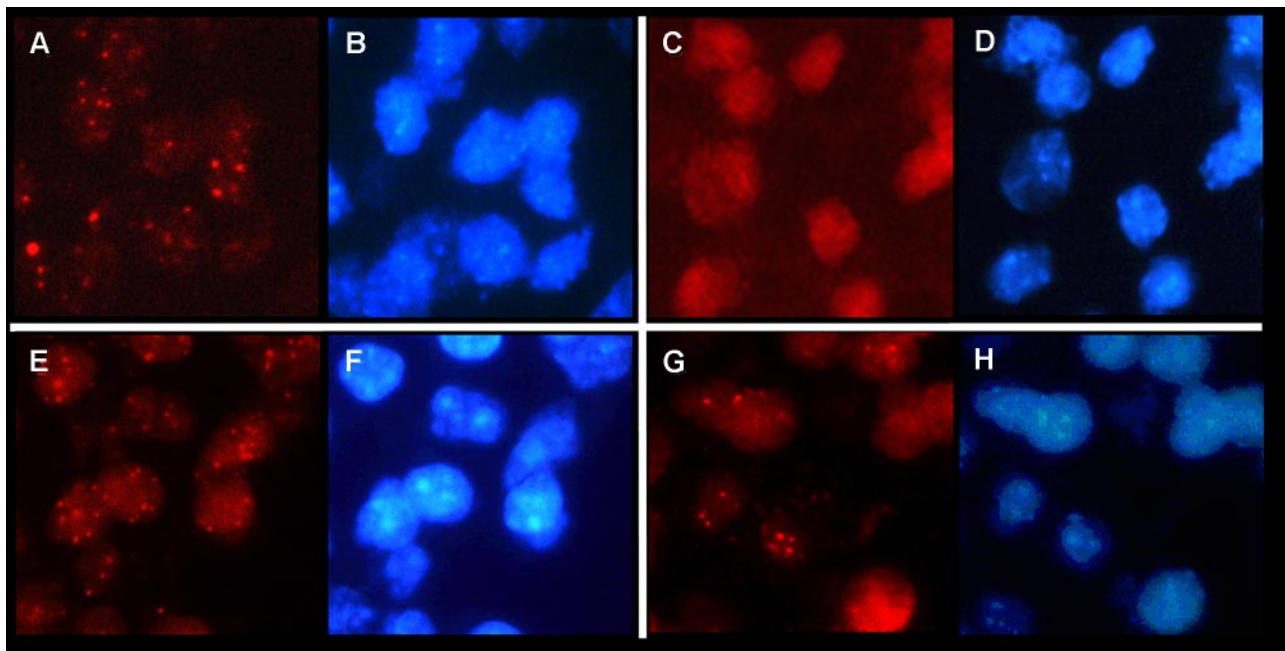

Figure 1. MCPyV detection by FISH in previously reported MCC and CLL cases.[1,4] MCPyV FISH was carried out using tyramide signal amplification (TSA) as described previously.[3,5] Signal evaluation was performed with a Leica DM 5000 B fluorescence microscope (Leica) using 1000× magnification and specific filter sets for Texas red to visualize MCPyV (A,C,E,G) and DAPI showing blue nuclear counterstaining (B,D,F,H). (A-B) MCPyV detection in MCC and corresponding DAPI (B). (C-D) Negative control on MCC tissue without probe and corresponding DAPI (D). (E-F) MCPyV detection in CLL cells of a bone marrow trephine and corresponding DAPI (F). (G-H) MCPyV detection in another CLL case in a bone marrow trephine and corresponding DAPI (H).