mucosae with gross cervical lymphadenopathy appeared. These new lesions caused upper airway obstruction with respiratory distress that required a tracheotomy and hemiglossectomy. Biopsies of skin and mucosae confirmed Kaposi's sarcoma. Serum immunofluorescence assay showed a high anti-human herpes virus-8 (HHV-8) IgG titer (1:2,560), which demonstrated recent or active HHV-8 infection.[8] Genomic DNA was prepared from sections of paraffin-embedded blocks of the lesion. HHV-8 detection was performed by polymerase chain reaction (PCR) using primers KS1 and KS2.[9] An amplification product of 233 bp was obtained in samples, indicating the presence of HHV-8 virus in neoplastic tissue. Immunosuppressive therapy was discontinued on day + 479, but the lesions did not remit. The patient died from massive lung hemorrhage on day +486. Permission for *post mortem* examination was denied, and the cause of the lung hemorrhage could not be determined.

KS has a negligible incidence in the general population, but has been observed in up to 6% of patients undergoing organ transplantation. Moreover, the incidence of KS is higher in patients treated with CsA and some authors have suggested that this association might reflect a state of overimmunosuppression rather than a specific effect of the molecule.[1-4] Some studies have found DNA sequences of viral agents in tissue samples of KS and suggest the existence of a new herpesvirus, the human herpesvirus-8 or Kaposi's sarcoma-associated herpesvirus.[4,6,9,10] The high anti-HHV-8 IgG titer and the presence of HHV-8 virus in neoplastic tissue of our patient demonstrated recent or active HHV-8 infection.

In our view, prolonged therapy with CsA and other immunosuppressive drugs and the immune deficiency associated with severe chronic GVHD may have predisposed to the development of KS in the case described here.

*Rocío Tamariz-Martel, María Soledad Maldonado, Rosario Carrillo,* Dina Crespo, César Pérez-Caballero, Arturo Muñoz*

*Departments of Pediatrics and *Pathology, Hospital Ramón y Cajal, University of Alcalá, Madrid, Spain*

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***Correspondence***

*Arturo Muñoz Villa, M.D., Servicio de Pediatría, Hospital Ramón y Cajal, Carretera de Colmenar, Km 9.100, 28034 Madrid, Spain. Phone: international +34-91-3368091 – Fax: international +34-91-3368417.*

## References

1. Penn I. The changing pattern of posttransplant malignancies. Transplant Proc 1991; 23:1101-3.
2. Penn I. Sarcomas in organ allograft recipients. Transplantation 1995; 60:1485-91.
3. Shepherd FA, Maher E, Cardella C, et al. Treatment of Kaposi's sarcoma after solid organ transplantation. J Clin Oncol 1997; 15:2371-7.
4. Gotti E, Remuzzi G. Post-transplant Kaposi's sarcoma. J Am Soc Nephrol 1997; 8:130-7.
5. Porta F, Bongiorno M, Locatelli F, et al. Kaposi's sarcoma in a child after autologous bone marrow transplantation for non-Hodgkin's lymphoma. Cancer 1991; 68:1361-4.
6. Gluckman E, Parquet N, Scieux C, et al. KS-associated herpesvirus-like DNA sequences after allogeneic bone-marrow transplantation. Lancet 1995; 346: 1558-9.
7. Erer B, Angelucci E, Muretto P, et al. Kaposi's sarcoma after allogeneic bone marrow transplantation. Bone Marrow Transplant 1997; 19:629-31.
8. Helg C, Adatto M, Salomon D, et al. Kaposi's sarcoma following allogeneic bone marrow transplantation. Bone Marrow Transplant 1994; 14:999-1001.
9. Alkan S, Karcher DS, Ortiz A, Khalil S, Akhtar M, Ali MA. Human herpesvirus-8/Kaposi's sarcoma-associated herpesvirus in organ transplant patients with immunosuppression. Br J Haematol 1997; 96:412-4.
10. O'Neill E, Henson TH, Ghorbani AJ, Land MA, Webber BL, Garcia JV. Herpes virus-like sequences are specifically found in Kaposi sarcoma lesions. J Clin Pathol 1996; 49:306-8.

## Platelet cryopreservation using second-messenger effectors and low-dose (2%) dimethyl sulfoxide. *In vitro* evaluation of post-thawing platelet activity with the platelet function analyzer

**Second-messenger effectors (ThromboSol®), together with low-dose dimethyl sulfoxide (DMSO) (2%), are described as a new cryopreservation solution. The solution allows high yield recovery of cryopreserved platelets with a residual platelet function equal or superior to that of 6% DMSO-cryopreserved platelets. The hemostatic function of cryopreserved platelets was measured by PFA-100™.**

Sir,

At present, 5-6% DMSO is considered the most effective cryopreserving agent in terms of cell yield and residual function of recovered platelets.[1] Recently, second messenger effectors (ThromboSol®) (TC) have been used combined with low-dose (2%) DMSO, to cryopreserve platelet concentrates at –80°C for clinical use.[2-4] The aim of the present work was to compare the yield and function of platelets frozen with either 6% DMSO or TC-2% DMSO.

An intriguing problem is the *in vitro* measurement of objective parameters capable of predicting the *in vivo* hemostatic function of the recovered platelets. Mimicking physiologic platelet-subendothelial interactions, the platelet function analyzer (PFA100™) measures *in vitro* closure time, a surrogate index of *in vivo* bleeding time.[5-8] Having previously developed a procedure to use PFA100™ to assess platelet function in platelet concentrates,[9] we used this procedure to compare residual platelet function in concentrates frozen using the two protocols. Yield, morphology and adhesion capacity were evaluated altogether. Thawed platelets were evaluated prior to and following a single washing procedure to remove about 95% of the TC and DMSO. Platelets were obtained by buffy-coat frac-

tionation of whole blood units collected in CPDA.

ThromboSol® (TC) (LifeCell Corp. Branchburg, NJ, USA) in pure DMSO containing amiloride ($Na^+$ $H^+$ exchange inhibitor), adenosine, and sodium nitroprusside, which are cAMP and cGMP stimulators, respectively. TC was added (1:50v/v) to the platelet suspension. After mixing, the suspension was placed at -80°C. Platelet bags were thawed by immersion into a 38°C water bath, under continuous agitation until complete dissolution of the solid phase. The 6% DMSO frozen platelets were obtained and thawed using standard protocols.

Platelet recovery was assessed by cytometric platelet count (Sysmex K-800, Toa Medical Electronics, Kobe, Japan). Platelet morphology was evaluated using a phase-contrast microscopy equipped with a 40X objective. The primary platelet hemostatic capacity (adhesion phase) was investigated by pipetting 50 µL of platelets ($100x10^9$/L) over the surface of a cleaned 240x240 mm glass coverslip. After incubation in a moist chamber for 30 minutes, non-adherent cells were removed and the adherent cells were counted using a phase-contrast microscope equipped with a 40X objective. Platelet function was measured by PFA100™, following reconstitution of whole blood. Platelet function was defined as closure time (C-ADP or C-epinephrine, accordingly to the cartridge used) or as PFA-Index (PFA-I), calculated using the following formula:

$$\text{PFA-PI} = \frac{[\text{total flow volume } (\mu L) : \text{flow rate } (\mu L/\text{min})] \times \text{closure or elapsed time (sec)}}{100}$$

After thawing and washing, platelet recovery, discoid platelets, and the absolute number (four fields) of glass-adherent cells were measured (Figure 1). The mean results were respectively 71, 69, and 179 for the TC-treated platelets and 57, 54, and 67 for the DMSO-treated platelets. The corresponding *p* values were: < 0.005, <0.001, and < 0.001.

The PFA100™ TM results, evaluated as C-ADP, C-epinephrine, and PFA-PI are reported in Table 1.

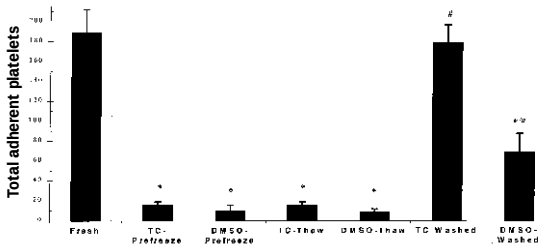


**Figure 1. Glass adhesion capacity of cryopreserved platelets. Platelets were cryopreserved with TC or DMSO as described in the *Design and Methods* section. The platelet samples were analyzed for adherence to glass prior to freezing, following thawing, and following a post-thaw wash step as described in the *Design and Methods* section. The data are expressed as the mean (± standard deviation) total number of platelets which adhered to a constant area of glass surface as described in the *Design and Methods* section. *p<0.001 for the values for cryopreserved samples as compared to the values for fresh platelets. #p<0.05 for the values for cryopreserved samples as compared to the values for fresh platelets. @p<0.001 for the values for TC-treated samples as compared to the values for DMSO-treated samples.**

Following cryopreservation, the platelet function was greatly diminished for both TC- and DMSO-treated platelets in comparison with fresh platelets. Platelet function of the TC-treated cells was partially restored though, after washing. The re-establishment of PFA-100 activity was greater when the ADP cartridge was used. Using the ADP cartridge, the TC-treated platelets displayed a statistically significant improvement in both closure time and PFA-PI as compared to the DMSO-treated washed cells ($p<0.05$). Using the epinephrine system, the DMSO-treated platelets displayed a statistically significant improvement in PFA-PI compared to the TC-treated platelets regardless of the washing step.



**Table 1. PFA100™ measurement of functional activity.**

| | | | *ThromboSol-Treated Platelets* | | | | *DMSO-Treated Platelets* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Thawed* | | | | *Thawed* | | | |
| | *Fresh* | | *Unwashed* | | *Washed* | | *Unwashed* | | *Washed* | |
| *Cartridge* | *ADP* | *epinephrine* | *ADP* | *epinephrine* | *ADP* | *epinephrine* | *ADP* | *epinephrine* | *ADP* | *epinephrine* |
| Closure Time[1] (sec) | 96.7 ±5.2 | 139.9 ±33.8 | 300[3] ±0.0 | 300[9] ±0.0 | 202.0[3] ±45.3 | 268.3[9] ±28.2 | 268.4[3] ±41.9 | 212.0[7] ±80.5 | 253.9[3,5] ±41.2 | 229.1[9] ±59.1 |
| PFA-Index[1] | 2.85 ±0.71 | 3.90 ±1.63 | 14.40[3] ±0.57 | 13.84[9] ±0.67 | 6.96[2] ±3.00 | 10.37[9] ±2.84 | 7.56[3,6] ±2.61 | 6.29[8,11] ±2.74 | 9.53[3,4] ±2.71 | 8.20[8,10] ±3.61 |

*[1]Mean value ± standard deviation, n=8; [2]p<0.005 for the values for cryopreserved samples as compared to the values for fresh samples. [3]p<0.001 for the values for cryopreserved samples as compared to the values for fresh samples. [4]p<0.05 for the values for TC-treated samples as compared to the paired values for DMSO-treated samples. [5]p<0.005 for the values for TC-treated samples as compared to the paired values for DMSO-treated samples. [6]p<0.001 for the values for TC-treated samples as compared to the paired values for DMSO-treated samples.[7]p<0.05 for the values for cryopreserved samples as compared to the values for fresh samples. [8]p<0.005 for the values for cryopreserved samples as compared to the values for fresh samples. [9]p<0.001 for the values for cryopreserved samples as compared to the values for fresh samples. [10]p<0.05 for the values for TC-treated samples as compared to the paired values for DMSO-treated samples. [11]p<0.001 for the values for TC-treated samples as compared to the paired values for DMSO-treated samples. Comparison by the two-tailed paired t-test.*

In conclusion, quantitative, morphologic and functional data, particularly when measured *in vitro* as PFA-PI using an ADP cartridge, showed that TC-cryopreserved platelets are at least as good as, and possibly superior to, DMSO-cryopreserved platelets.

*Piero Borzini, Antonio Lazzaro, Laura Mazzucco, ° Roberta Schiavo,° Jerome Connor,# Salvatore Siena§*

*Servizio di Immunoematologia e di Medicina Trasfusionale, Ospedale S. Spirito, Casale Monferrato, Italy; °Sezione Trapianto Midollo Osseo, Istituto Clinico Humanitas, Rozzano; #LifeCell Corp., NJ, USA, and §Divisione di Oncologia Medica Falck, Dipartimento di Oncologia ed Ematologia, Ospedale Niguarda Ca' Granda, Milan, Italy*

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*Platelet cryopreservation.*

***Correspondence***
*Piero Borzini, Servizio di Immunoematologia e di Medicina Trasfusionale, Ospedale "Santi Antonio e Biagio e Cesare Arrigo", via Venezia, 16, 15100 Alessandria, Italy. Phone: 0131-206230 – Fax: international +39.0131.206859 – E-mail: trasfusionale@ospedale.al.it*

## References

1. Angelini A, Dragani A, Berardi A, Iacone A, Fioritoni G, Tortolano G. Evaluation of four different methods for platelet freezing. In vitro and in vivo studies. Vox Sang 1992; 62:146-51.
2. Currie LM, Livesey SA, Harper JR, Connor J. Cryopreservation of single-donor platelets with a reduced dimethyl sulfoxide concentration by addition of second-messenger effectors: enhanced retention of in vitro activity. Transfusion 1998; 38:160-7.
3. Currie LM, Lichtiger B, Livesey SA, Tansey W, Yang DJ, Connor J. Enhanced circulatory parameters of human platelets cryopreserved with second-messenger effectors: an in vivo study of 16 volunteer platelet donors. Br J Haematol 1999; 105:826-31.
4. Vadhan-Raj S, Currie LA, Bueso-Ramos C, Livesey SA, Connor J. Enhanced retention of in vitro activity of platelets from recombinant human thrombopoietin-treated patients following long-term cryopreservation with platelet-preserving solution (ThromboSol®) and 2% DMSO. Br J Haematol 1999; 104:403-11.
5. Kundu SK, Heilmann EJ, Sio R, Garcia C, Davidson RM, Ostgaard RA. Description of an in vitro platelet function analyzer-PFA100™. Semin Thromb Hemost 1995; 21(Suppl 2):106-12.
6. Favaloro EJ, Facey D, Henniker A. Use of a novel platelet function analyzer (PFA-100™) with high sensitivity to disturbances in von Willebrand factor to screen for von Willebrand's disease and other disorders. Am J Hematol 1999; 62:165-74.
7. Mammen EF, Comp PC, Gosselin R, et al. PFA-100™ system: a new method for assessment of platelet dysfunction. Semin Thromb Hemost 1998; 24:195-202.
8. Rand ML, Carcao MD, Blanchette VS. Use of the PFA-100® in the assessment of primary, platelet-related hemostasis in a pediatric setting. Semin Thromb Hemost 1998; 24:523-9.
9. Borzini P, Lazzaro A, Mazzucco L. Evaluation of the hemostatic function of stored platelet concentrates using the platelet function analyser (PFA-100™). Haematologica 1999; 84:1104-9.

## Anti-D immunoglobulin in children with newly diagnosed immune thrombocytopenic purpura: a pilot study

**Treatment options for childhood immune thrombocytopenic purpura (ITP) include observation, steroids, intravenous gammaglobulin (IVIG) and splenectomy.[1] Recent studies[2-4] have shown that anti-D increases the platelet count in children with ITP but that the time to achieve a platelet count ≥ 20,000/μL is significantly longer than following IVIG.[5] We gave anti-D as a single intravenous dose of 50 μg/kg to 10 consecutive Rh positive children with newly diagnosed ITP.**

Sir,

Newly diagnosed patients aged 6 months to 18 years with a clinical diagnosis of ITP based on history, physical examination and isolated thrombocytopenia with a normal blood smear, were eligible for entry into the study. Initial evaluation consisted of a history and physical examination, complete blood count with differential, blood type and direct Coombs' test. After informed consent had been obtained, all Rh positive, Coombs' negative patients were treated with one dose of intravenous anti-D (Winrho-SD, Univax Biologics, Inc, Rockville, MD, USA) 50 μg/kg over 5 minutes. Complete blood counts were done every 12 hours until the platelet count was ≥ 20,000/μL at which point the patient was discharged home. Response was defined as an increase in platelet count to ≥ 20,000/μL with cessation of bleeding. This study was approved by the institutional review board at Baystate Medical Center, Springfield, Massachusetts.

The patients' characteristics and results of treatment are presented in Table 1. Platelet counts for the first 40 hours post-anti-D are shown in Figure 1. All 10 patients responded to anti-D. The mean time to platelet count ≥ 20,000/μL was 22.3±11.4 hrs (median 16.4 hrs; range 12-39.5 hrs). Median post-anti-D peak platelet count was 253,000/μL occurring at a median of 8 days post-treatment. The average drop in hemoglobin was 1.27 g/dL ±0.7. Three patients were retreated for platelet counts < 20,000/μL. No allergic complications occurred, and no patients required transfusions secondary to anti-D-induced hemolysis. Two patients had mild headache 2-3 hours post-infusion; one also had emesis. All symptoms resolved with acetaminophen and diphenhydramine. Therapeutic options for childhood ITP remain controversial[5-7] and include IVIG, corticosteroids, anti-D or less commonly splenectomy. Anti-D has potential advantages over IVIG: lower cost[8] and fewer side effects (headache, vomiting, aseptic meningitis).[3,4,9] The presumed method of action for anti-D is saturation of Fc receptors in the spleen with antibody-coated red blood cells.

Blanchette *et al.*[5] randomized 146 children with ITP to receive low dose IVIG (0.8 g/kg), high dose IVIG (1 g/kg/day x2), intravenous anti-D (25 μg/kg/day x 2)