matched unrelated donor and died of respiratory distress syndrome. The second patient exhibited hematologic recovery on day +16 and the BM aspirate performed on day +17 confirmed the CR. Moreover, after the consolidation, a molecular response consisting of a 1-log reduction of the BCR-ABL transcript (Figure 1B) and absence of the T315I-ABL mutation was also evident. The patient was unsuitable for an HSCT and imatinib (600 mg/day) was re-started as maintenance therapy, taking into account both the disappearance of the ABL point mutation and the possibility of utilizing a second-generation TKI as further salvage therapy. After five months from clofarabine-cyclophosphamide treatment, she is still in CR with a low number of BCR-ABL transcript copies (Figure 1B) and absence of ABL mutations. The clofarabine-cyclophosphamide combination was very well tolerated. No patient suffered from nausea, vomiting or dose-limiting toxicities. Possibly in view of the rapid hematologic recovery and strict antimicrobial prophylaxis, neither patient developed infections.

Clofarabine followed by cyclophosphamide seems to be a very promising salvage treatment for adult patients with advanced ALL, even in the presence of adverse prognostic factors like the BCR-ABL rearrangement, relapse after TKI administration and presence of the T315I mutation. Clinical trials on large series of patients will conclusively clarify the efficacy and safety of this combination.

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References

1. Thomas DA. Philadelphia chromosome-positive acute lymphocytic leukemia: A new era of challenges. Hematology Am Soc Hematol Educ Program 2007;435-43.
2. Vignetti M, Fazi P, Cimino G, Martinelli G, Di Raimondo F, Ferrara F, et al. Imatinib plus steroids induces complete remissions and prolonged survival in elderly Philadelphia chromosome-positive patients with acute lymphoblastic leukemia without additional chemotherapy: results of the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) LAL0201-B protocol. Blood 2007;109:3676-8.
3. Foà R, Vitale A, Guarini A, De Propris MS, Elia L, Cimino G, et al. First line treatment of adult Ph+ acute lymphoblastic leukemia (ALL) patients. Final results of the GIMEMA LAL1205 study. Blood 2008;112:305[Abstract].
4. Ravandi F, Thomas D, Kantarijan H, Faderl S, Koller C, Dara S, et al. Phase II study of combination of hyperCVAD with dasatinib in frontline therapy of patients with Philadelphia chromosome (Ph) positive acute lymphoblastic leukemia (ALL). Blood 2008;112: 2921[Abstract].
5. Kantarjian HM, Jeha S, Gandhi V, Wess M, Faderl S. Clofarabine: Past, present and future. Leuk Lymphoma 2007;48:1922-30.
6. Jeha S, Gaynon PS, Razzouk BI, Franklin J, Kadota R, Shen V, et al. Phase II study of clofarabine in pediatric patients with refractory or relapsed acute lymphoblastic leukemia. J Clin Oncol 2006;24:1917-23.
7. Faderl S, Thomas DA, Ghandi V, Borthakur G, Huang X, Plunkett W, et al. Phase I/II study of clofarabine plus cyclophosphamide in adults with relapsed and refractory acute lymphoblastic leukemia (ALL). Blood 2007;110: 4335[Abstract].
8. Karp JE, Ricklis RM, Balakrishnan K, Briel J, Greer J, Gore SD, et al. A Phase 1 clinical-laboratory study of clofarabine followed by cyclophosphamide for adults with refractory acute leukemias. Blood 2007;110:1762-9.
9. Meloni G, Iacobelli S, Fazi P, Vignetti M, Di Raimondo F, Ferrara F, et al. A new method to evaluate the prognostic value of response to prednisone pre-treatment in adult (15–60 yrs) patients with acute lymphoblastic leukemia: Results of the GIMEMA LAL 2000 Study. Blood 2005; 106:1829[Abstract].
10. Annino L, Vegna ML, Camera A, Specchia G, Visani G, Fioritoni G, et al. Treatment of adult acute lymphoblastic leukemia (ALL): long-term follow-up of the GIMEMA ALL 0288 randomized study. Blood 2002;99:863-71.
11. Vitale A, Guarini A, Ariola C, Meloni G, Perbellini O, Pizzuti M, et al. Absence of prognostic impact of CD13 and/or CD33 antigen expression in adult acute lymphoblastic leukemia. Results of the GIMEMA ALL 0496 trial. Haematol J 2007;92:342-8.
12. Soverini S, Martinelli G, Amabile M, Poerio A, Bianchini M, Rosti G, et al. Denaturing-HPLC-based assay for detection of ABL mutations in chronic myeloid leukemia patients resistant to Imatinib. Clin Chem 2004;50:1205-13.

Transient loss of consciousness in pediatric recipients of dimethylsulfoxide (DMSO)-cryopreserved peripheral blood stem cells independent of morphine co-medication

Toxicity related to the infusion of dimethylsulfoxide-cryopreserved peripheral blood stem cells (DMSO-PBSC) manifests mostly as cardiovascular side effects. Neurotoxicity[1] including transient global amnesia,[2,3] seizures,[4,5] and stroke[6,7] has been reported as a rare complication primarily in adults. In children, data[8] are sparse. In light of a recent report implicating morphine co-medication as a major contributing factor,[8] we evaluated retrospectively our own data base, including all infusions of DMSO-PBSC applied in our pediatric center between January 1st 2002 and December 31st 2008. We report on 2 incidences of transient loss of consciousness following 131 infusions of DMSO-PBSC.

Case 1. A 17-year old female suffering from recurrent medulloblastoma was admitted for prolonged pancytopenia due to topotecan maintenance therapy. Throughout her medical history, this patient had received intensive therapy, including high-dose chemotherapy and autologous peripheral blood stem cell transplantation, craniospinal irradiation, as well as CNS-directed therapy, namely intrathecal chemotherapy and radioimmunotherapy (Table 1). Thirty minutes after receiving a cryopreserved autologous stem cell boost, the patient initially complained of bilateral loss of vision. Fifteen minutes later, she became unconscious (Glasgow coma scale 3/15). The patient's cardiorespiratory condition was stable. During transport to emergency CT, she developed one short episode of clonic seizure restricted to both arms, which

Table 1. Patients' characteristics, medical history, clinical presentation and outcome.

	Case 1	Case 2
Age, gender	17 years, female	7 mos, male
Weight	60 kg	6.8 kg
Malignancy	Medulloblastoma, spinal fluid positive+ (M+)	Fibrosarcoma
Prior medical therapy	Carboplatin-based chemotherapy (2 cycles), HDCH and ASCT Intrathecal etoposide (10 cycles) Intrathecal liposomal cytarabine (6 cycles) Intrathecal DOTATOC radioimmunotherapy (2 cycles) Maintenance therapy (oral temozolomide, topotecan)	Doxorubine, vincristine, cyclophosphamide chemotherapy (4 cycles), vincristine, actinomycine D, cyclophosphamide chemotherapy (3 cycles)
Prior irradiation	Craniospinal irradiation	None
Conditioning regimen	None	Fludarabine, thiotepa, melphalan, OKT-3
Source of stem cells	Autologous peripheral blood stem cells	CD3/CD19-depleted haploidentical stem cells
Volume of stem cell product	2×40 mL (=1.3 ml/kg)	50 mL (= 7.4 mL/kg)
Viability (7-AAD$^-$ events) post-thaw	78%	74%
Amount of DMSO	146 mg/kg	814 mg/kg
Cell dose	2.1×10^8 NC / kg	7.7×10^8 NC / kg
Co-medication within 24 h before onset	Thyroxine substitution; no morphine co-medication	Acyclovir, fluconazole, metronidazole, methyl-prednisolone, OKT-3, phenobarbitone (per protocol); furosemide; no morphine co-medication
Time from stem cell infusion to onset of symptoms	30 min	40 min
Type of symptoms	Loss of vision Loss of consciousness Seizure	Loss of consciousness
Diagnostics	Emergency CT: no changes EEG: moderate dysrhythmia no hypersynchrone activity	Cranial ultrasound: no changes EEG: normal
Outcome	Recovery within 3 h No neurological sequelae MRT after one month: no changes, SD	Recovery after 2 h No neurological sequelae Cranial ultrasound after 3 /11 mos: no changes
Status	Death from PD 7 mos later	Alive and well, in CR

ASCT: autologous stem cell transplantation; CR: complete remission; DOTATOC: Yttrium 90 –Somatostatin-Receptor directed radioimmunotherapy; HDCH: high-dose chemotherapy; M+: spinal fluid tested positive for medulloblastoma cells; NC: nucleated cells; OKT3: Tcell antibody as rejection prophylaxis used in pediatric haploidentical transplants; PD: progressive disease; SD: stable disease.

responded promptly to dexamethasone. Her CT scan revealed no signs of ischemia or bleeding, her EEG displayed moderate dysrhythmia. Cerebrospinal fluid analysis revealed normal lactate, protein and glucose, and no leukocytes or malignant cells. Likewise, serum electrolytes, glucose, LDH were within normal ranges. The patient remained unconscious for approximately *two hours*. When she woke up, she was disoriented for an additional hour. Three hours after the incident, she had completely recovered, but showed retrograde amnesia for the complete episode. She was discharged the following day without any neurological symptoms. Following the stem cell boost, she recovered from neutropenia and thrombocytopenia after 14 days. Follow-up MRT one month later showed no specific findings.

Case 2. A 7-month old male infant diagnosed with fibrosarcoma of his lower back who had responded well to preceding chemotherapy, but still had non-operable residual disease extending into his spinal canal was considered eligible for haploidentical stem cell transplant as consolidation therapy after extensive consultation and written parental consent. Following the standard conditioning regimen of fludarabin, thiotepa and melphalan, he was transplanted with a cryopreserved CD3/CD19-depleted haploidentical stem cell graft from his father. Forty minutes following infusion, he suddenly lost consciousness. His cardiorespiratory condition was stable. Serum electrolytes and blood gases were within normal ranges. Emergency cranial ultrasound revealed no pathology, EEG was normal. In addition, he showed no clinical signs of seizure. One hour after the onset of symptoms, the infant gradually regained consciousness. Two hours after the onset, he had completely recovered.

DMSO is currently the only sufficiently validated reagent for cryopreservation of hematopoietic stem cells; standard practice in most centers is cryoconservation with 10% DMSO.[9] However, stem cell washing[10] before infusion or cryopreservation with DMSO mixed with hydroxyethyl starch[11] may represent alternative approaches. In children, few data on neurotoxicity following infusion of DMSO-PBSC are available.[8] The incidence in our series of consecutive stem cell infusions was found to be 2/131 (1.5 %). The only other report in children[8] thus far had implicated morphine co-medication as contributing factor which can be ruled out in our 2 patients. Furthermore, post-thaw viability of both products was within the expected range (78% and 74%, respectively) excluding the assumption that an inadequately high number of dead or apoptotic cells might have contributed to neurotoxicity. Other triggers such as disturbances of electrolytes, glucose or blood gases, intravascular activation of coagulation could be excluded

in both patients. The time to onset was within the first 60 min after infusion, in accordance with the majority of reports in adults.[1,2,3,5]

Toxicology analysis of DMSO in rodents indicates a dose-dependent increase in neurotoxicity.[12] The first patient who had received only a relatively small amount of DMSO (146 mg/kg), may have been more sensitive to the neurotoxic effects due to her brain tumor and to prior intensive CNS-directed therapy. The second patient, an infant without prior CNS history, developed symptoms at a dose level of 814 mg/kg, which is at the higher end of the applicable dose range.[2] Certainly, children with a body weight of 10kg and below are at risk for receiving higher dosage of DMSO. As a consequence of the observed toxicities, our institutional policy has changed to keep the maximum volume applied at 4 mL/kg cryopreserved stem cells containing 10% DMSO and to recommend splitting doses on subsequent infusion days if necessary.

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References

1. Mueller LP, Theurich S, Christopeit M, Grothe W, Muetherig A, Weber T, et al. Neurotoxicity upon infusion of dimethylsulfoxide-cryopreserved peripheral blood stem cells in patients with and without pre-existing cerebral disease. Eur J Haematol 2007;78:527-31.
2. Junior AM, Arrais CA, Saboya R, Velasques RD, Junqueira PL, Dulley FL. Neurotoxicity associated with dimethylsulfoxide-preserved hematopoietic progenitor cell infusion. Bone Marrow Transplant 2008;41:95-6.
3. Otrock ZK, Beydoun A, Barada WM, Masroujeh R, Hourani R, Bazarbachi A. Transient global amnesia associated with the infusion of DMSO-cryopreserved autologous blood stem cells. Haematologica 2008;93:e36-7.
4. Bauwens D, Hantson P, Laterre PF, Michaux L, Latinne D, De Tourtchninoff M, et al. Recurrent seizure and sustained encephalopathy associated with dimethylsulfoxide-preserved stem cell infusion. Leuk Lymphoma 2005;46:1671-4.
5. Hequet O, Dumontet C, El Jaafari-Corbin A, Salles G, Espinouse D, Arnaud P, et al. Epileptic seizures after autologous peripheral blood progenitor infusion in a patient treated with high-dose chemotherapy for myeloma. Bone Marrow Transplant 2002;29:544.
6. Windrum P, Morris TCM. Severe neurotoxicity because of dimethyl sulphoxide following peripheral blood stem cell transplantation. Bone Marrow Transplant 2003;31:315.
7. Hoyt R, Szer J, Grigg A. Neurological events associated with the infusion of cryopreserved bone marrow and/or peripheral blood progenitor cells. Bone Marrow Transplant 2000;25:1285-7.
8. Caselli D, Tintori V, Messeri A, Frenos S, Bambi F, Arico M. Respiratory depression and somnolence in children receiving dimethylsulfoxide and morphine during hematopoietic stem cell transplantation. Haematologica 2009;94:152-3.
9. Windrum P, Morris TC, Drake MB, Niederwieser D, Ruutu T. EBMT Chronic Leukaemia Working Party Complications Subcommittee. Variation in dimethyl sulfoxide use in stem cell transplantation: a survey of EBMT centres. Bone Marrow Transplant 2005;36:601-3.
10. Syme R, Bewick M, Stewart D, Porter K, Chadderton T, Glück S. The role of depletion of dimethyl sulfoxide before autografting: on hematologic recovery, side effects, and toxicity. Biol Blood Marrow Transplant 2004;10:135-41.
11. Rowley SD, Feng Z, Chen L, Holmberg L, Heimfeld S, MacLeod B, Bensinger WI. A randomized phase III clinical trial of autologous blood stem cell transplantation comparing cryopreservation using dimethylsulfoxide vs dimethylsulfoxide with hydroyethylstarch. Bone Marrow Transplant 2003;31:1043-51.
12. Cavaletti G, Oggioni N, Sala F, Pezzoni G, Cavalletti E, Marmiroli P, et al. Effect on the peripheral nervous system of systemically administered dimethysulfoxide in the rat: a neurophysiological and pathological study. Toxicology Lett 2000;118:103-7.