

Complete Remission Following Decitabine/Dendritic Cell Vaccine for Relapsed Neuroblastoma

abstract

Patients with relapsed stage 4 neuroblastoma have an extremely poor long-term prognosis, making the investigation of new agents of interest. We report the outcome of the first patient treated in a phase 1 study for relapsed neuroblastoma, using the chemotherapy agent decitabine to upregulate cancer testis antigen expression, followed by a dendritic cell vaccine targeting the cancer testis antigens MAGE-A1, MAGE-A3, and NY-ESO-1. Our patient had persistent tumor in his bone marrow after completion of standard therapy for neuroblastoma, including multiagent chemotherapy, tumor resection, stem cell transplantation, radiation therapy, and anti-GD2 monoclonal antibodies. His marrow disease persisted despite chemotherapy, which was given while the vaccine was being produced. After 3 cycles of decitabine and vaccine, this patient achieved a complete remission and is now 1 year from his last treatment, with no evidence of tumor in his bone marrow or other sites. This patient was noted to have an increase in MAGE-A3-specific T cells. This is the first report combining demethylating chemotherapy to enhance tumor antigen expression followed by a cancer antigen vaccine. *Pediatrics* 2013;131:e336–e341

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KEY WORDS

MAGE-A1, MAGE-A3, NY-ESO-1, cancer-testis antigens, decitabine, neuroblastoma

ABBREVIATIONS

CT—cancer testis

CTL—cytotoxic T lymphocyte

DAC—decitabine

DC—dendritic cell

IL—interleukin

MHC—major histocompatibility complex

Dr Lucas designed and led the study with Dr Krishnadas; Dr Krishnadas was responsible for performing experiments, data analysis, and interpretation; Ms Shapiro coordinated patient visits and vaccination schedule; and Dr Lucas revised the manuscript critically and approved the final version.

This trial has been registered at www.clinicaltrials.gov (identifier NCT01241162).

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Neuroblastoma is the most common extracranial solid tumor in children, and for those older than 1 year with advanced disease, the 3-year progression-free survival rate is only 40%, despite the current use of multiagent chemotherapy, radiation, and autologous stem cell transplantation.^{1,2} Yu et al reported that the addition of anti-GD2 monoclonal antibody therapy to this standard regimen improved 2-year event-free survival from 46% to 66%.³ Unfortunately, half of patients with stage 4 neuroblastoma will eventually have a relapse, and there are currently no options with curative potential when this occurs. Immunotherapy is complicated by the fact that there is varying expression of tumor antigens on this tumor as well as the downregulation of major histocompatibility complex (MHC) class I and II molecules, which are needed for antigen presentation.^{4,5} We have developed a clinical study that combines the administration of a chemotherapy agent to upregulate cancer antigen expression, followed by a vaccine targeting these antigens.

Cancer testis (CT) antigens such as NY-ESO-1, MAGE-A1, and MAGE-A3 have a restricted pattern of expression, generally limited to germ cell and trophoblast tissue, but also expressed on a variety of solid tumors, including neuroblastoma.^{6–8} Decitabine (5-aza-2'-deoxycytidine [DAC]), a potent inhibitor of DNA methylation,^{9–12} has been shown to upregulate the expression of CT antigens in a number of tumor cell lines,^{13,14} potentially making these tumors more susceptible to MAGE-A1-, MAGE-A3-, and NY-ESO-1-mediated killing. At our laboratory, we have recently demonstrated that MAGE-A1, MAGE-A3, and NY-ESO-1 expression is upregulated after exposure to DAC in most neuroblastoma cell lines, including tumors that lacked expression pretreatment.¹⁵

Multiple clinical trials have reported using MAGE-A1, MAGE-A3, and NY-ESO-1 vaccines for adult solid tumors, with clinical and immunologic responses detected postvaccination.^{16–19} There have been no clinical studies targeting these antigens for neuroblastoma. We initiated a phase I CT antigen vaccine trial for patients with relapsed neuroblastoma, first upregulating CT antigen expression by administering DAC, followed by an autologous dendritic cell (DC) – MAGE-A1, MAGE-A3, NY-ESO-1 vaccine. This report describes the outcome of the first patient treated on this trial.

PATIENT PRESENTATION

Our patient is a 6-year-old boy who presented to the hospital at 3 years of age with bone pain and a large posterior mediastinal mass extending from the carina to the lumbar spine, with a 10×12-cm left suprarenal mass. There were clumps of tumor cells on bilateral bone marrow aspirates and multiple bony metastases shown on bone scan. Urine catecholamine measure and meta-iodo-benzyl-guanidine scan were negative. A biopsy of his abdominal mass revealed an undifferentiated, unfavorable-histology neuroblastoma (schwannian stroma–

poor tumor) that was n-myc–nonamplified and of mitosis-karyorrhexis index of 2% to 4%. The patient was approved for treatment in Children's Oncology Group (COG) study ANBL0532; after the initial 2 cycles of chemotherapy, he had a very good response at his primary tumor site and had negative bone marrow aspirates. He was treated with 3 additional cycles of induction chemotherapy and underwent surgical resection of his abdominal tumor, at which time pathologic examination of his tumor was consistent with mature ganglioneuroblastoma. Bilateral bone marrow aspirates and biopsies were negative before stem cell transplantation, and bone examination revealed resolution of the majority of his bony lesions, with persistent metastatic disease within T12 and the sacroiliac joints. He was randomized to receive a single transplant with carboplatin, etoposide, and melphalan and radiation therapy and then underwent treatment in COG ANBL0032 for immunotherapy with anti-GD2 monoclonal antibody ch14.18, interleukin (IL)-2, 13-*cis*-retinoic acid, and granulocyte-macrophage colony-stimulating factor. His end-of-therapy evaluation showed tumor clumps on bilateral bone marrow aspirates and, despite 3 cycles of irinotecan and temozolomide, his marrow disease

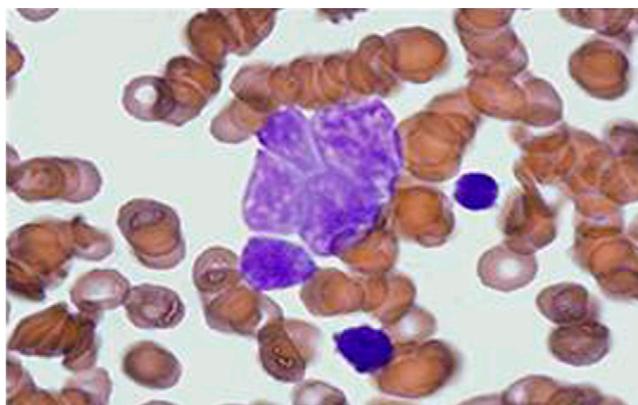


FIGURE 1

Detection of neuroblasts in bone marrow before vaccination. Bone marrow aspirate from patient 1 immediately before the first course of DAC shows a cluster of neuroblasts. There was no detectable tumor on aspirates performed after the third cycle of therapy.

persisted (Fig 1). Peripheral blood mononuclear cells were collected via apheresis for the culture of dendritic cells and the patient began therapy with the DAC/DC vaccine regimen. This study has been approved by the Penn State Hershey Medical Center Institutional Review Board and the US Food and Drug Administration (IND 13973).

Our patient received 3 monthly cycles of DAC 10 mg/m²/d for 5 days, with each cycle followed by 2 weekly vaccines consisting of autologous DC pulsed with overlapping peptides derived from full-length MAGE-A1, MAGE-A3, and

NY-ESO-1 (JPT Peptide Technologies, Berlin, Germany). The Toll-like receptor agonist imiquimod was used at the vaccine site before and after vaccination, to facilitate immune cell infiltration into the vaccine site. After 3 cycles, the patient had no evidence of tumor on bilateral bone marrow aspirates and biopsy samples. During the third treatment cycle, a transient increase in alkaline phosphatase was observed from weeks 3 to 5 (range 1428–5185 IU/L), which fell to 685 IU/L (normal range 80–420 IU/L) by week 8 after receiving DAC. This was initially thought to represent DAC-induced

hepatotoxicity, but serum transaminase and bilirubin levels were normal during this time, and fractionation of the alkaline phosphatase confirmed that it was 70% bone and 30% liver isozyme. C-telopeptide, a sensitive marker of bone reabsorption, was elevated at 2516 pg/mL (range 574–1849 pg/mL). Pretreatment C-telopeptide levels are not available for comparison. This increase in alkaline phosphatase correlated with a decline in the patient's neutrophil and platelet count, which resolved 3 weeks later. There was no evidence of tumor on bilateral bone marrow aspirates and

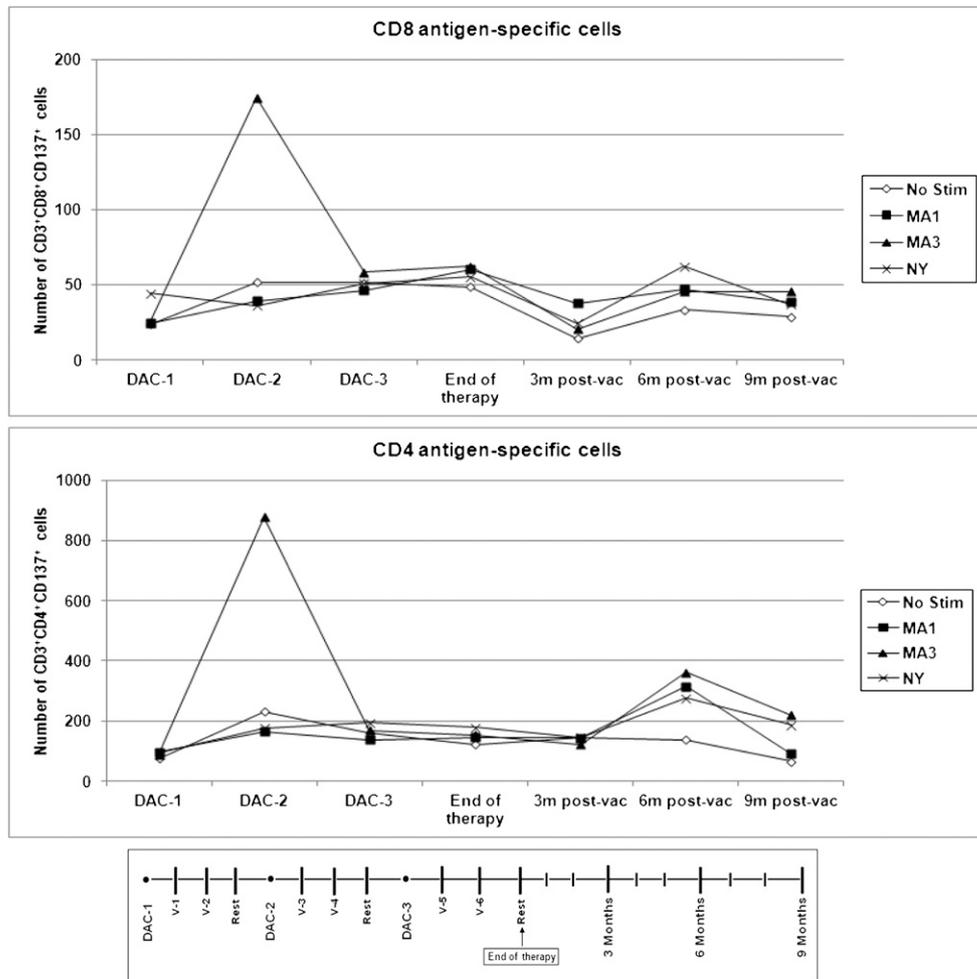


FIGURE 2

Detection of antigen-specific T cells in patient 1 at different time points (before and after vaccination). The patient received 3 cycles of therapy. Each cycle consists of 1 week of DAC (DAC-1: Cycle 1, one week of DAC; DAC-2: Cycle 2, one week of DAC; and DAC-3: Cycle 3, one week of DAC) followed by vaccine 1 (v-1), vaccine 2 (v-2), and a week of rest as indicated in the bottom panel. The numbers of CD3⁺CD8⁺CD137⁺ and CD3⁺CD4⁺CD137⁺ antigen-specific cells per 10⁶ cells in the peripheral blood at intervals postvaccination are represented. T cells were assayed 24 hours poststimulation with MAGE-A1 (MA1), MAGE-A3 (MA3), or NY-ESO-1 (NY) overlapping peptide mixes (JPT Peptide Technologies).

biopsy samples 4 weeks after the start of cycle 3. One year after his last vaccination, the patient's bone marrow remains morphologically free of tumor, including by immunohistochemistry for NB84 (Genzyme Genetics, Los Angeles, CA), and computed tomography scans of the chest, abdomen, and pelvis are normal.

Analysis of T cells postvaccination revealed that the number of MAGE-A3-specific, CD3⁺CD8⁺ CD137⁺ T cells increased from 25 cells/10⁶ to 175 cells/10⁶ at 1 month postvaccination, and these levels declined to 60 cells/10⁶ for the ensuing 2 months (Fig 2). MAGE-A3-specific, CD8⁺ T cells were at pre-treatment baseline levels (range 25–50 cells/10⁶) at 3, 6, and 9 months after the last vaccination. A similar trend

was seen for CD3⁺CD4⁺CD137⁺ T cells responding to MAGE-A3. The percentages of both CD8 and CD4 effector memory T cells (CD3⁺CD8⁺/CD4⁺CD137⁺CCR7⁻CD45RA⁻) increased after the first vaccination (Fig 3), but it is unclear why the most striking increase was seen at this time point and not after subsequent vaccines, and antigen-specific CD4⁺ effector memory T cells increased at 6 and 9 months.

DISCUSSION

The prognosis for patients with relapsed neuroblastoma is extremely poor, considering that these tumors recur after high-dose chemotherapy and radiation. Additional use of multi-agent chemotherapy or radiotherapy generally results in only temporary

control of disease, so novel approaches are needed for these patients. Neuroblastomas have several potential escape mechanisms from immune surveillance, including the down-regulation of MHC molecules and potentially immunogenic cancer antigens.^{4,5,20} To facilitate immune recognition and killing of tumor cells by cytotoxic T lymphocytes (CTLs) that would be expanded after a CT antigen vaccine, we administered low-dose DAC before immunotherapy. Previous studies have shown that MAGE antigen-specific CTLs preferentially kill tumor cells that have had CT antigens upregulated after exposure to DAC, but this has not been tested in a clinical trial.^{15,21} Several studies have been done with MAGE-A1, MAGE-A3, and NY-ESO-1 vaccines for

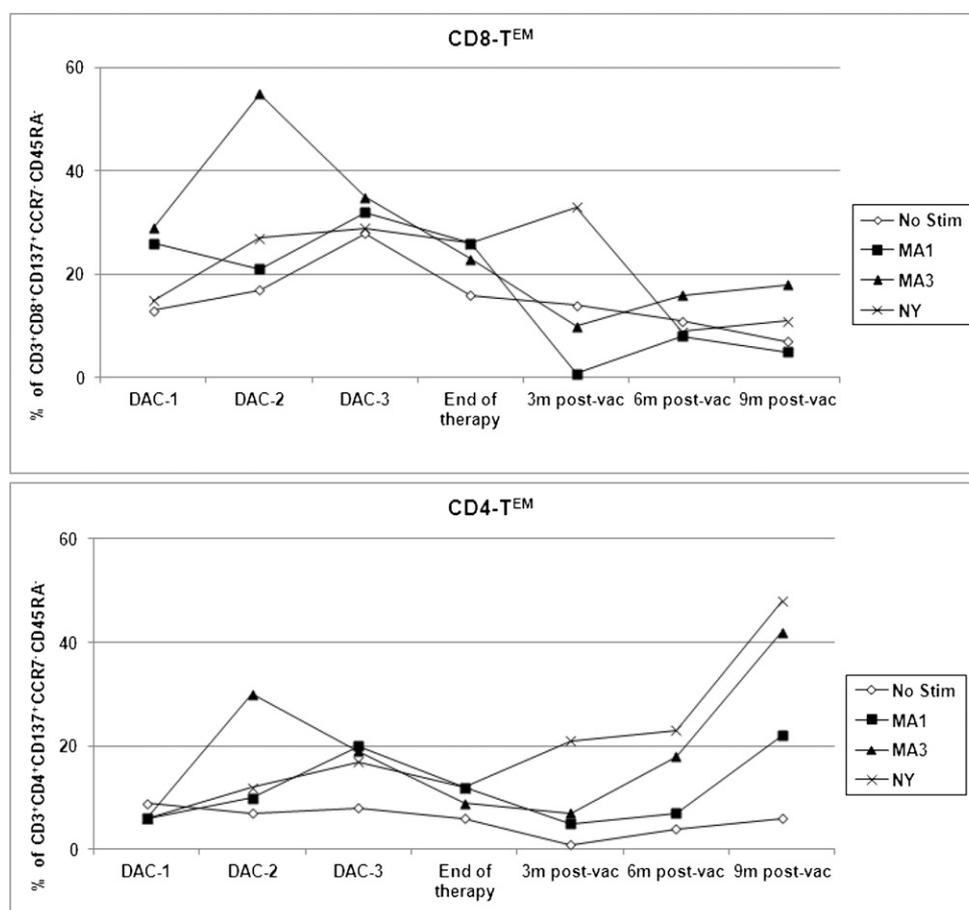


FIGURE 3

Levels of antigen-specific effector memory T cells (TEM) at different time points (before and after vaccination). The percentages of TEM (CD3⁺CD4⁺/CD8⁺CD137⁺CCR7⁻CD45RA⁻) in the peripheral blood that are antigen specific are represented at different time points. MA1, MAGE-A1; MA3, MAGE-A3; NY, NY-ESO-1. (DAC-1: Cycle 1, one week of DAC; DAC-2: Cycle 2, one week of DAC; and DAC-3: Cycle 3, one week of DAC).

adult cancers using either single MHC-restricted epitopes or whole protein, with clinical and immunologic responses seen in some patients.^{16–18} We decided to use a mixture of peptides derived from each full-length antigen, so that patients could be enrolled from diverse HLA backgrounds. With our pre-clinical data on CT antigen upregulation on tumors and well-documented safety of CT antigen vaccines in adults, we initiated the current trial, considering the poor long-term prognosis for these patients and theoretical potential for efficacy.

Immunotherapy studies for neuroblastoma have largely focused on the anticancer effects of monoclonal antibodies directed against GD2, which is expressed on the majority of these tumors.^{22,23} Recently, Louis et al²⁴ reported the use of anti-GD2 chimeric antigen receptor expressing CTLs in patients with neuroblastoma and found that 3 of 11 patients with active disease achieved a complete remission. Caruso et al²⁵ reported the use of an autologous DC–tumor RNA vaccine in 11 children with newly diagnosed neuroblastoma, after the completion of standard therapy, and none of the patients with measurable disease showed an objective antitumor response. Russell and col-

leagues gave patients with neuroblastoma a vaccine consisting of autologous tumor cells that were transduced to express IL-2 or IL-2 and lymphotactin.^{26,27} While there were detectable interferon- γ -producing, autologous tumor-specific T cells in the peripheral blood of some patients postvaccination, the median event-free survival was only 3 months for patients with relapsed disease. These studies show that clinical and immunologic responses are possible with various immunotherapy regimens for neuroblastoma, but new strategies are desperately needed.

Our patient developed an antigen-specific T-cell response postvaccination, most notably against MAGE-A3. It is unclear whether this response was due to chemotherapy, an immune response to the vaccine, or both. Of note is the fact that he developed transient leukopenia and thrombocytopenia at the same time his alkaline phosphatase level became markedly elevated, suggesting that an immune response targeting hematopoietic precursors had been induced. Nevertheless, our patient's clinical response could have been solely the result of the DAC. In addition to facilitating expression of CT antigens on tumor cells, DAC also upregulates the proapoptotic gene caspase-8,

restoring its expression and making neuroblastoma cells more susceptible to apoptosis.²⁸ A phase 1 study for children with relapsed neuroblastoma and other solid tumors used DAC with doxorubicin and cyclophosphamide, with little efficacy.²⁹ There are little data on this drug used alone in patients with isolated marrow disease, although one of the patients for whom the regimen failed as reported by George et al (personal communication) had disease isolated to the bone marrow.

The effects of DAC in facilitating the expression of CT antigens and MHC molecules before vaccination could overcome immune evasion strategies and serve to enhance CT antigen-specific immune responses in patients with neuroblastoma as well as other tumors. Although these results are encouraging, this reports depicts the outcome of a single patient with chemotherapy-resistant, microscopic disease in the marrow. It remains to be seen whether this regimen will result in clinical responses in patients with bulky tumors. Additional research is also needed to determine how DAC-treated tumor cells are being killed and whether there are concomitant humoral responses after this vaccine regimen.

REFERENCES

1. Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. *Nat Rev Cancer*. 2003;3(3):203–216
2. Maris JM. Recent advances in neuroblastoma. *N Engl J Med*. 2010;362(23):2202–2211
3. Yu AL, Gilman AL, Ozkaynak MF, et al; Children's Oncology Group. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. *N Engl J Med*. 2010;363(14):1324–1334
4. Gross N, Beck D, Favre S. In vitro modulation and relationship between N-myc and HLA class I RNA steady-state levels in human neuroblastoma cells. *Cancer Res*. 1990;50(23):7532–7536
5. Ponzoni M, Guarnaccia F, Corrias MV, Cornaglia-Ferraris P. Uncoordinate induction and differential regulation of HLA class-I and class-II expression by gamma-interferon in differentiating human neuroblastoma cells. *Int J Cancer*. 1993;55(5):817–823
6. Chen YT, Scanlan MJ, Sahin U, et al. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc Natl Acad Sci USA*. 1997;94(5):1914–1918
7. Boon T, Cerottini JC, Van den Eynde B, van der Bruggen P, Van Pel A. Tumor antigens recognized by T lymphocytes. *Annu Rev Immunol*. 1994;12:337–365
8. Wölfel M, Jungbluth AA, Garrido F, et al. Expression of MHC class I, MHC class II, and cancer germline antigens in neuroblastoma. *Cancer Immunol Immunother*. 2005;54(4):400–406
9. Marks P, Rifkin RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. *Nat Rev Cancer*. 2001;1(3):194–202
10. Yang H, Hoshino K, Sanchez-Gonzalez B, Kantarjian H, Garcia-Manero G. Anti-leukemia activity of the combination of 5-aza-2'-deoxycytidine with valproic acid. *Leuk Res*. 2005;29(7):739–748
11. Garcia-Manero G, Gore SD. Future directions for the use of hypomethylating

- agents. *Semin Hematol.* 2005;42(3 suppl 2): S50–S59
12. Issa JP, Gharibyan V, Cortes J, et al. Phase II study of low-dose decitabine in patients with chronic myelogenous leukemia resistant to imatinib mesylate. *J Clin Oncol.* 2005;23(17):3948–3956
 13. Adair SJ, Hogan KT. Treatment of ovarian cancer cell lines with 5-aza-2'-deoxycytidine upregulates the expression of cancer-testis antigens and class I major histocompatibility complex-encoded molecules. *Cancer Immunol Immunother.* 2009;58(4): 589–601
 14. Schrupp DS, Fischette MR, Nguyen DM, et al. Phase I study of decitabine-mediated gene expression in patients with cancers involving the lungs, esophagus, or pleura. *Clin Cancer Res.* 2006;12(19):5777–5785
 15. Bao L, Dunham K, Lucas K. MAGE-A1, MAGE-A3, and NY-ESO-1 can be upregulated on neuroblastoma cells to facilitate cytotoxic T lymphocyte-mediated tumor cell killing. *Cancer Immunol Immunother.* 2011;60(9): 1299–1307
 16. Chianese-Bullock KA, Pressley J, Garbee C, et al. MAGE-A1-, MAGE-A10-, and gp100-derived peptides are immunogenic when combined with granulocyte-macrophage colony-stimulating factor and montanide ISA-51 adjuvant and administered as part of a multi-peptide vaccine for melanoma. *J Immunol.* 2005;174(5):3080–3086
 17. Mackensen A, Herbst B, Chen JL, et al. Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34(+) hematopoietic progenitor cells. *Int J Cancer.* 2000;86(3):385–392
 18. Bender A, Karbach J, Neumann A, et al. LUD 00-009: phase 1 study of intensive course immunization with NY-ESO-1 peptides in HLA-A2 positive patients with NY-ESO-1-expressing cancer. *Cancer Immun.* 2007;7:16
 19. Davis ID, Chen W, Jackson H, et al. Recombinant NY-ESO-1 protein with ISCOMATRIX adjuvant induces broad integrated antibody and CD4(+) and CD8(+) T cell responses in humans. *Proc Natl Acad Sci USA.* 2004;101(29):10697–10702
 20. Ploegh HL. Viral strategies of immune evasion. *Science.* 1998;280(5361):248–253
 21. Serrano A, Tanzarella S, Lionello I, et al. Rexpression of HLA class I antigens and restoration of antigen-specific CTL response in melanoma cells following 5-aza-2'-deoxycytidine treatment. *Int J Cancer.* 2001;94(2):243–251
 22. Schulz G, Cheresch DA, Varki NM, Yu A, Staffileno LK, Reisfeld RA. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. *Cancer Res.* 1984;44(12 pt 1):5914–5920
 23. Mujoo K, Cheresch DA, Yang HM, Reisfeld RA. Disialoganglioside GD2 on human neuroblastoma cells: target antigen for monoclonal antibody-mediated cytotoxicity and suppression of tumor growth. *Cancer Res.* 1987;47(4):1098–1104
 24. Louis CU, Savoldo B, Dotti G, et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. *Blood.* 2011;118(23): 6050–6056
 25. Caruso DA, Orme LM, Amor GM, et al. Results of a Phase I study utilizing monocyte-derived dendritic cells pulsed with tumor RNA in children with Stage 4 neuroblastoma. *Cancer.* 2005;103(6):1280–1291
 26. Russell HV, Strother D, Mei Z, et al. A phase 1/2 study of autologous neuroblastoma tumor cells genetically modified to secrete IL-2 in patients with high-risk neuroblastoma. *J Immunother.* 2008;31(9):812–819
 27. Russell HV, Strother D, Mei Z, et al. Phase I trial of vaccination with autologous neuroblastoma tumor cells genetically modified to secrete IL-2 and lymphotactin. *J Immunother.* 2007;30(2):227–233
 28. Teitz T, Wei T, Valentine MB, et al. Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. *Nat Med.* 2000;6(5):529–535
 29. George RE, Lahti JM, Adamson PC, et al. Phase I study of decitabine with doxorubicin and cyclophosphamide in children with neuroblastoma and other solid tumors: a Children's Oncology Group study. *Pediatr Blood Cancer.* 2010;55(4):629–638

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ERRATA

Krishnadas et al. Complete Remission Following Decitabine/Dendritic Cell Vaccine for Relapsed Neuroblastoma. *Pediatrics*. 2013;131(1):e336–e341

An error occurred in this article by Krishnadas et al, titled “Complete Remission Following Decitabine/Dendritic Cell Vaccine for Relapsed Neuroblastoma,” published in the January 2013 issue of *Pediatrics* (2013;131[1]:e336–e341; doi:10.1542/peds.2012-0376). On page e336, under the Funding section, the copy reads: “This work was supported by funds from the Four Diamonds Fund, Hyundai Hope on Wheels and Solving Kids’ Cancer.” This should have read: “This work was supported by funds from the Four Diamonds Fund, Hyundai Hope on Wheels, Solving Kids’ Cancer, The Andrew McDonough B+ Foundation, and the Pierce Phillips Charity.”

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