Pediatr Blood Cancer 2015;62:2120–2124

A Phase I Study of the Anti-Idiotype Vaccine Racotumomab in Neuroblastoma and Other Pediatric Refractory Malignancies

Walter Cacciavillano, MD,[1] Claudia Sampor, MD,[1] Cecilia Venier, MSc,[2] Mariano R. Gabri, PhD,[3] Marı́a T.G. de Dávila, MD, PhD,[1] Maria L. Galluzzo, MD,[1] Marcelo D. Guthmann, PhD,[4] Leonardo Fainboim, PhD,[2] Daniel F. Alonso, MD, PhD,[3] and Guillermo L. Chantada, MD, PhD[1]*

Background. Pediatric neuroectodermal malignancies express N-glycolylated gangliosides including N-glycolyl GM3 (NeuGcGM3) as targets for immunotherapy. ***Procedure.*** We evaluated the toxicity and maximum tolerated dose and immunological response of racotumomab, an anti-idiotype vaccine targeting NeuGcGM3 through a Phase I study enrolling children with relapsed or resistant tumors expressing NeuGcGM3. ***Materials and methods.*** Drug dose was escalated to three levels (0.15–0.25–0.4 mg) of racotumomab administered intradermally. Each drug level included three patients receiving a total of three doses, every 14 days. A confirmation cohort was added to the highest dose level. Antibody response was assessed upon study entry and at 4-week intervals for at least three immunological determinations for each patient. ***Results.*** Fourteen patients were enrolled (10 with neuroblastoma, one with retinoblastoma, one with Wilms' tumor, and two with brainstem glioma). Three patients completed the three drug levels and three were enrolled in the confirmation cohort. One patient died of tumor progression before completing the three applications. Racotumomab was well tolerated. The only side effect observed was grade 1–2 toxicity at the injection site. Racotumomab elicited an IgM and/or IgG antibody response directed against NGcGM3 in nine patients and IgM against racotumomab in 11 of 13 evaluable patients. The maximum tolerated dose was not reached and no dose-limiting toxicity was seen. ***Conclusions.*** Racotumomab vaccination has a favorable toxicity profile up to a dose of 0.4 mg, and most patients elicited an immune response. Its activity as immunotherapy for neuroectodermal malignancies will be tested in further clinical trials. Pediatr Blood Cancer 2015;62:2120–2124. © 2015 Wiley Periodicals, Inc.

Key words: immunotherapy; neuroblastoma; pediatric cancer; phase I; racotumomab

INTRODUCTION

Despite aggressive standard multimodality therapy, most patients with high-risk neuroblastoma relapse and die from the tumor and survival remains poor.[1] There is scant information on the outcome of neuroblastoma in middle-income countries, but insufficient availability of diagnostic tools for risk assignment and limitations in delivering high-dose therapy would possibly lead to a worse outcome.[2,3] In the past decade, treatments directed to eradicate minimally disseminated disease such as immunotherapy have become promising options.[1,4] Among these, murine or humanized-murine chimeric monoclonal antibodies directed against GD2, a disialoganglioside have been reported and their use recently gained FDA approval.[5,6] However, anti-GD2 immunotherapy is a complex regimen that needs sophisticated infrastructure which may not be available in most parts of the world.[7,8] Hence, it became necessary for our group to identify other options of effective immunotherapy for neuroblastoma looking for new molecular targets. In order to pursue this aim, we previously reported the expression of N-glycolyl GM3 (NeuGcGM3) in 85% of neuroblastoma cases, including those with MYCN amplification.[9] NeuGcGM3 is an attractive target because it is highly expressed in tumors while being undetectable in healthy human tissues.[10]

Racotumomab, formerly known as 1E10, a murine anti-idiotype vaccine that mimics NeuGcGM3, is in evaluation for adult malignancies[11] and it may be a potentially active drug for immunotherapy of neuroblastoma.[12] Racotumomab was generated from the immunization of BALB/c mice with P3, a monoclonal antibody (mAb) that recognizes N-glycolylated gangliosides and is capable of triggering a specific and strong immune response against these gangliosides.[13,14] In adult patients, racotumomab was not mutagenic and reported toxicity including local side effects at the site of injection.[14,15] We undertook this Phase I clinical trial in pediatric patients with relapsed or resistant neuroblastoma and other refractory pediatric tumors. The aim of this study was to evaluate racotumomab acute toxicity and maximum tolerated dose (MTD) and secondly, to assess the specific immunological response elicited by vaccination with racotumomab.

PATIENTS AND METHODS

Patient Eligibility Criteria

Eligible patients were children with a histological diagnosis of relapsed-refractory neoplasms expressing NeuGcGM3 including

Additional supporting information may be found in the online version of this article.

[1]Hospital de Pediatŕa SAMIC "Prof Dr Juan P Garrahan", Buenos Aires, Argentina; [2]Institute of Immunology, Genetics and Metabolism (INIGEM), University of Buenos Aires, National Council for Scientific and Technological Research (CONICET), Buenos Aires, Argentina; [3]Laboratory of Molecular Oncology, National University of Quilmes, Buenos Aires, Argentina; [4]Elea Laboratories, Buenos Aires, Argentina

Grant sponsor: Instituto Nacional del Cancer (Argentina); Grant sponsor: ANPCyT (Argentina); Grant number: BID-PID129-PAE37.011

Conflict of interest: Marcelo Guthmann is an employee of Elea Laboratories. Claudia Sampor received travel support from Elea laboratories. No other conflict of interest is disclosed by the other authors.

Results of this study were presented at the 46th Congress of the International Society of Pediatric Oncology (SIOP), Toronto, Canada, 2014 and preliminary results from this study were also presented at the Progress in Vaccination Against Cancer (PIVAC 14) meeting, Rome, Italy, September 2014.

*Correspondence to: Guillermo L. Chantada, Hematology–Oncology Service, Hospital JP Garrahan, Combate de los Pozos 1881, C1245AAL Buenos Aires, Argentina. E-mail: gchantada@yahoo.com

Received 30 March 2015; Accepted 21 May 2015

DOI 10.1002/pbc.25631
Published online 7 July 2015 in Wiley Online Library (wileyonlinelibrary.com).

high-risk neuroblastoma, Wilms tumor, or Ewing sarcoma family of tumors, with progressive disease after first-line therapy or with relapse after failing second-line therapy; extraocular or trilateral retinoblastoma with refractory disease after conventional therapy; and brainstem glioma relapsing or progressing after conventional radiation therapy.

A wash-out period of at least 30 days between the last anti-cancer therapy was required for all patients.

Study Design

The institutional review board of the Hospital de Pediatŕa JP Garrahan and the National Regulatory Office (Administración Nacional de Medicamentos, Alimentos y Tecnologá Médica, ANMAT) approved this open-label, non-randomized phase I study protocol (NLM Identifier NCT01598454) in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. Written informed consent from parents or guardians and patient assent when appropriate were obtained before study entry.

The rationale for dosage for this study was based on extrapolation of adult data to the pediatric population. The dose used in adults for clinical trials is 1 mg without modifications for weight or body surface. For a pediatric estimation, this dose, based upon a theoretical body surface of 1.73 m^2 would correspond with 0.58 mg/m^2. So, for a target estimated body surface of children potentially presenting neuroblastoma of 0.55–0.7 m^2, the maximum dose would be 0.4 mg. Hence, the study included three dose levels starting at 0.15 mg, escalating to 0.25 mg, and subsequently to a maximum of 0.4 mg of racotumomab with no adaptations for weight or age. A cohort of three patients was scheduled for each dose level and an additional three were to be included in the final dose level. In case dose limiting toxicity (DLT) was observed at any level, the following patient would be included in the previous dose level with no further escalation. If that occurred, an additional confirmatory cohort of three patients receiving six doses of racotumomab at that dose would be included. In case DLT was observed in the first level, the study would be closed. If no DLT was observed at the 0.4 mg level, no further escalation would be considered and a confirmation cohort would be included with three additional patients receiving six doses of racotumomab every 14 days. In this case, the conclusion would be that the MTD was not reached and that dose would be considered for further clinical testing in children. No further escalation from 0.4 mg was attempted in order to limit the exposure to alum in these young children.

Drug Information

Racotumomab mAb was purified from mouse ascites, and the aluminum hydroxide-precipitated vaccine was produced according to the Good Manufacturing Practice guidelines. The manufacturing process was certified by the Quality Control Department of the Center of Molecular Immunology (CIM), Havana, Cuba. Vials contained 1 mg of racotumomab and 5 mg of alum, in a sodium phosphate-based buffer.

The drug was injected intradermally in one anatomic site (usually the forearm) in the 0.15 and 0.25 mg dose levels and in two separate sites in the 0.4 mg level in order to limit the volume administered to a single site aiming to decrease potential local complications. The drug was administered in an ambulatory setting and children were observed for about 4 hr after the injection and discharged home if no immediate toxicity occurred. The treatment scheme included three doses repeated every 14 days for each initial cohort and six doses every 14 days in the confirmation cohort.

Toxicity Evaluation

Toxicity, including laboratory work-up and clinical examination, was evaluated on each visit for drug administration and at 4 and 8 weeks after the last dose and the common terminology criteria for adverse events (version 3.0) were used for scoring. DLT was defined as any grade 3 or greater toxicity in any system.

Patient Follow-Up and Response Evaluation

Even though tumor response to treatment was not a formal endpoint of this study, disease status after racotumomab therapy was scheduled at 1 and 2 months after completion of protocol therapy. In cases with neuroblastoma with no bone marrow invasion at study entry, determination of minimal residual disease by quantitative real-time PCR for GD2 synthase mRNA[16] was performed from two bone marrow aspirates from the posterior iliac crest and repeated after racotumomab therapy. Response criteria were determined by using the Response Criteria in Solid Tumors (RECIST) 1.0 guidelines.

Immune Response Evaluation

Blood specimens for immune response evaluation were obtained at study entry and monthly thereafter for an additional four specimens.

Antibody titers were determined by enzyme-linked immunosorbent assay (ELISA). Titers were defined as the inverse of the dilution yielding an absorbency of 0.25 and were obtained by interpolation in absorbency versus 1/dilution plots.

Costar 3,590 microtiter plates (Costar, Cambridge, MA) were sensitized with 0.5 mg/well racotumomab (Center of Molecular Immunology). Murine IgG1 (MOPC clone, Sigma Chemical Co., St. Louis, MO) was used as a control for anti-mouse reactivity. Bound IgG antibodies were detected with an alkaline phosphatase-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories, West Grove, PA).

Anti-ganglioside IgM and IgG antibodies were determined using ELISA. Nunc PolySorp microtiter plates (Thermo Scientific, Waltham, MA) were coated with 20 ng per well NeuGcGM3 or N-acetyl-GM3 (Enzo Life Sciences, Farmingdale, NY). Bound antibodies were detected with alkaline phosphatase-conjugated goat anti-human IgM or IgG (Jackson Immuno Research Laboratories).

Tumor Expression of NeuGcGM3

Expression of NeuGcGM3 in biopsy specimens was evaluated at study entry by immunohistochemistry using the 14F7 antibody to NeuGcGM3 as previously reported.[17]

RESULTS

From December 2010 to March 2014, a total of 14 patients with a median age of 8.4 years (range, 1.8–11) were enrolled. Primary diagnoses included metastatic neuroblastoma in 10 cases, diffuse brainstem glioma in two cases, and trilateral retinoblastoma and

anaplastic Wilms' tumor in one case each. All children had grossly disseminated disease that relapsed or progressed under standard therapy. Median time from last chemotherapy to trial enrollment was 63 days (range 34–242). Six patients with neuroblastoma had received consolidation with high-dose chemotherapy and stem-cell rescue at a median of 33 months (range 3–44) before enrollment. Lymphocyte counts were greater than 1,000/mm^3 in all children, but in five cases with neuroblastoma, diffuse bone marrow involvement, thrombocytopenia, anemia, or leukopenia were evident. In all 11 children in whom a biopsy was available, membrane NeuGcGM3 staining by immunohistochemistry was positive. Biopsy specimens were not available in three children; two with non-biopsied diffuse brainstem glioma and one with neuroblastoma in whom the pathology slides available were not adequate for testing.

Three patients were included in the first dose level, four in the second, and three each in the third and confirmation levels. One patient in the 0.25 mg cohort died of tumor progression soon after the second application, so an additional patient had to be included in that cohort. In that child, no assessment of immune response was possible, but toxicity to the first dose could be recorded (Supplementary Table SI).

Racotumomab was well tolerated and its most common local side effects included grade one erythema, induration, and local pain at the injection site which occurred in 10 patients. In all these cases, signs resolved within 5 days. Two patients had local toxicity grade 2, because marked increases in density and firmness on palpation but no retraction were noted. In these children, though these features gradually resolved within days of follow-up, a residual palpable, non-pigmented nodule of less than 0.5 cm persisted. Two patients (one of them receiving only two doses because of early death) did not show any toxicity attributable to racotumomab. No alteration in laboratory values attributable to racotumomab toxicity was detected. No serious adverse effect attributable to racotumomab was seen. Hence, no dose limiting toxicity was found, so the maximum tolerated dose was not reached and 0.4 mg was then considered for further studies in children.

Immune Response

Racotumomab was immunogenic in 85% of the evaluable patients (Fig. 1). Two patients, accrued in the 0.25 and 0.4 mg dose levels, failed to elicit anti-racotumomab antibodies. One of them had brainstem glioma treated with local radiotherapy (5,940 cGy) and the remaining one had stage 4 neuroblastoma. Both patients had absolute lymphocyte counts >1,000/mm^3 and no history of immunodeficiency. High anti-racotumomab titers were observed for the remaining 11 patients, regardless of the dose level. All responses were detectable by week 4 (after receiving the first two racotumomab doses), increased through week 8 (post three or four doses), and remained stable throughout the 8-week follow-up. Interestingly, titers against the isotype-matched control mouse antibody were substantially lower than anti-racotumomab titers, underscoring the immunodominance of the racotumomab isotype (Fig. 1).

Nine of the 11 immunologically responsive patients elicited anti-NeuGc-GM3 antibodies as well. Kinetics of anti-NeuGcGM3 antibodies were heterogeneous (Fig. 1). Whereas the anti-racotumomab response peaked after the second administration and remained stable thereafter, the anti-ganglioside response could require three doses to be detectable.

No patients showed anti-NeuGc-GM3 IgG antibodies in the lowest-dose level. However, three of five patients with an IgM response in the 0.4 mg-dose level presented anti-ganglioside IgG antibodies. Hence, both anti-ganglioside titers and class switch parallel dose levels.

NeuGc-GM3 is the hydroxylated derivative of N-acetyl (NeuAc), which is the most abundant ganglioside in human serum and is present in all normal tissues. Racotumomab induced no detectable anti-NeuAc-GM3 antibodies, even in the patients with the highest anti-NeuGc-GM3 response. The anti-ganglioside antibody response was, therefore, specific to the xenogeneic glycolyl residue of NeuGc-GM3, thereby dismissing the possibility of cross-reactions with self-gangliosides.

Response to Therapy and Outcome

Twelve patients had progressive disease and died at a median of 3 months after study entry (range 1–30). Two patients had stable disease after racotumomab. Both had refractory disease after salvage treatment of relapse or progression. One of them was the only patient with neuroblastoma without bone marrow invasion at study entry. In that child, minimally residual disease was positive at

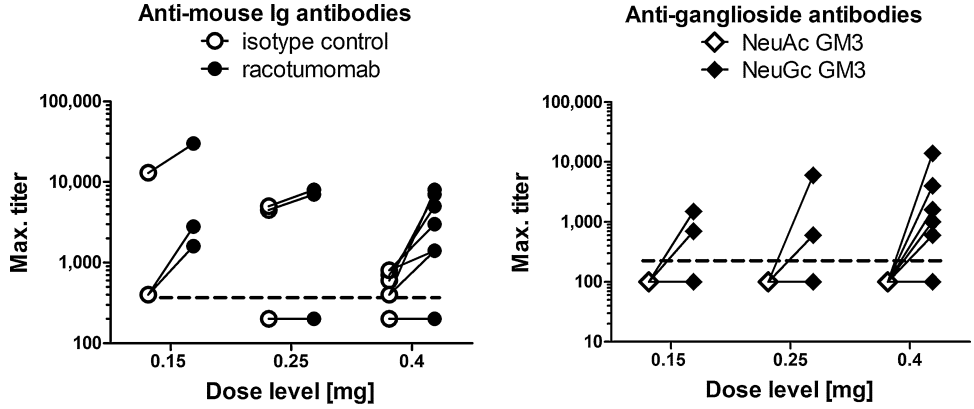

Fig. 1. Maximal titers of anti-racotumomab and anti-NeuGc-GM3 antibody responses for all evaluable patients in each dose level. Specificity controls are shown as empty circles (mouse IgG1 isotype control) and empty lozenges (NeuAc-GM3 ganglioside). Thresholds for positive responses are indicated by the dotted lines.

study entry and negative after completing protocol therapy. However, the child ultimately progressed and died of disseminated disease after 16 months. The remaining child had trilateral retinoblastoma that was persistent after standard chemotherapy. Permission for autologous stem-cell consolidation was declined and the family opted for including the child in this study. In this child, a residual enhancing tumor mass is still seen after 30 months but no progression has occurred despite no further therapy.

DISCUSSION

This first-in-pediatrics, phase I study shows that racotumomab is safe and immunogenic in a population of patients with advanced and refractory pediatric tumors. The MTD was not reached as no DLT occurred and only self-limiting mild to moderate local toxicities were found in our cohort. These results are concordant with the experience in adults receiving racotumomab in whom the drug is also well tolerated as reported in large populations mostly with lung cancer.[14]

Active immunotherapy strategies such as vaccination with racotumomab are promising therapies for neuroblastoma.[18,19] Several previous studies on vaccination for neuroblastoma involved cellular vaccines targeting cellular immunity.[20,21] However, T-cell immunity is severely affected in these children because they are usually given intensive therapy, potentially limiting treatment efficacy. Recently, a trial of a bivalent GD2-GD3 gangliosides vaccine in combination with β-glucan, which has a broad range of immunostimulant effects, has been reported for high-risk neuroblastoma in a second or later remission.[18] This vaccine also showed a mild toxicity profile, triggered specific antibody response in most patients, and revealed promising activity in this high-risk population. Another preclinical study evaluated ganglidiomab, an anti-idiotype vaccine targeting GD2, generating specific humoral response in a mouse model.[19]

Vaccines aim at inducing durable host anti-ganglioside antibodies capable of replicating the antineoplastic effect seen with exogenously administered anti-ganglioside antibodies. However, gangliosides are poorly immunogenic so adjuvants are needed in order to elicit an effective immune response. For racotumomab, aluminum hydroxide is used as adjuvant acting as a depot at the injection site from which the antigen is released slowly, thereby inducing the formation of granulomas that attract immunocompetent cells. As racotumomab is an immunoglobulin vaccine mimicking the NeuGcGM3 ganglioside and acting as a surrogate of the original antigen, it shows a sustained immune response against the ganglioside.[13,15]

As opposed to GD2, which is present in normal melanocytes and peripheral nerves, NeuGcGM3 is not expressed in normal human cells, explaining the low toxicity profile and the lack of autoimmune reactions to racotumomab. Humans have a constitutional 92 bp deletion in the gene encoding the cytidine-mono-phosphate-N-acetylneuraminic acid hydroxylase which catalyzes the conversion of N-acetyl to N-glycolyl sialic acid.[22] Neosynthesis of carbohydrate determinants and expression of N-glycolylated gangliosides were observed in human cancer, possibly by dietary incorporation of nonhuman sialic acid.[23] Tumor cells acquire a glycosylation profile that provides advantages in terms of tumor growth, dissemination, and immune escape. An immunosuppressive capacity of NeuGc-gangliosides is postulated as they exhibit the capacity to downmodulate CD4 molecules on the T lymphocyte surface or to inhibit dendritic cell differentiation and maturation *in vitro*. This tolerance may be altered by active specific immunotherapy.[24]

Despite being highly immunogenic in adults, the immunogenicity of racotumomab has not been tested previously in children. The immune system of children may pose particular challenges for generating an adequate serological response related to age, prior therapy, or other factors. Our group previously reported the induction of an antibody response against NeuGcGM3 in a child receiving racotumomab[12] and in the present study, we confirmed the immunogenicity of this anti-idiotype antibody in a larger pediatric population. Racotumomab was reported to trigger a specific anti-NeuGcGM3 response in adults with melanoma, breast cancer, and lung cancer.[25–27] Interestingly, in our study, 12/14 patients elicited an immune response against racotumomab, despite heavy pre-treatment including stem-cell transplantation in six of them. These results are comparable to those obtained by Kushner et al.[18] with their bivalent GD2–GD3 vaccine that elicited serological response in 12 of 15 patients with neuroblastoma without prior stem-cell transplantation. Nevertheless, adults with lung or breast cancer receiving racotumomab show a higher rate of seroconversion approaching 100%.[25,27] However, these studies used a longer immunization period and immunosuppression of our patients may have been higher due to steroids or intensive chemotherapy.

As previously reported in adults with lung cancer, the antibodies produced were predominantly directed against racotumomab and to a lesser extent against the ganglioside antigen.[13] This is in agreement with previous observations in animal models and adult cancer patients, which also showed the immunodominance of the idiotype of racotumomab over the isotype.[13,27] The latter may be related to both the lack of significant human anti-mouse antibody response (which could have deleterious effects in the wake of continuous re-immunizations) and the ability to generate a ganglioside-specific, Ab1-like response.[13,28]

Racotumomab did not show significant anti-tumor activity in most of our cohort of heavily pre-treated patients with refractory malignancies. Nevertheless, in the only child in whom minimally residual disease was detectable in the bone marrow, it became negative after racotumomab therapy, suggesting some specific anti-neuroblastoma activity. An additional patient with trilateral retinoblastoma has had a non-progressing residual mass for 33 months with no further therapy; however, it is difficult to attribute this to racotumomab as the residual tumor was not biopsied before enrollment in the racotumomab study and thus the possible efficacy of prior chemotherapy cannot be excluded. Nonetheless, survival of children with refractory trilateral retinoblastoma treated with conventional chemotherapy alone, as in this child, is very low.[29] Therefore, a role of racotumomab in this favorable outcome cannot be ruled out. Vaccination strategies are more effective with longer vaccination periods in children with minimally disseminated disease as their only tumor manifestation.[18,30] Therefore, based on our results showing immunogenicity and low toxicity, we are currently evaluating long-term vaccination with racotumomab in children with high-risk neuroblastoma after front-line therapy in a state of minimally disseminated disease.

ACKNOWLEDGMENTS

The authors wish to thank the nursing staff of the Hospital JP Garrahan, Dr. Rodolfo Verna, Silvia Eandi, Graciela Galar and

Daniel Aldrerete for their participation in the care of patients included in this study and Sandra Camarero for her help in immunohistochemistry studies. Roberto Gomez is acknowledged for his logistic support for the study.

REFERENCES

1. Cheung NK, Dyer MA. Neuroblastoma: Developmental biology, cancer genomics and immunotherapy. Nat Rev Cancer 2013;13:397–411.
2. Mehdiabadi GB, Arab E, Rafsanjani KA, Ansari S, Moinzadeh AM. Neuroblastoma in Iran: An experience of 32 years at a referral childrens hospital. Asian Pac J Cancer Prev 2013;14: 2739–2742.
3. Levy D, Aerts I, Michon J, Lumbroso-Le Rouic L, Cellier C, Orbach D. [Childhood cancer: Progress but prognosis still very unequal. Example of retinoblastoma and high-risk neuroblastoma]. Bull Cancer 2014;101:250–257.
4. Park JR, Bagatell R, London WB, Maris JM, Cohn SL, Mattay KK, Hogarty M, C.O.G.N Committee Children's Oncology Group's 2013 blueprint for research: Neuroblastoma. Pedtr Blood Cancer 2013;60:985–993.
5. Schulz G, Cheresh DA, Varki NM, Yu A, Staffileno LK, Reisfeld RA. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. Cancer Res 1984;44:5914–5920.
6. Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, Chen HX, Smith M, Anderson B, Villablanca JG, Matthay KK, Shimada H, Grupp SA, Seeger R, Reynolds CP, Buxton A, Reisfeld RA, Gillies SD, Cohn SL, Maris JM, Sondel PM, G. Children's Oncology. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. N Engl J Med 2010;363:1324–1334.
7. Simon T, Hero B, Faldum A, Handgretinger R, Schrappe M, Niethammer D, Berthold F. Consolidation treatment with chimeric anti-GD2-antibody ch14.18 in children older than 1 year with metastatic neuroblastoma. J Clin Oncol 2004;22:3549–3557.
8. Osenga KL, Hank JA, Albertini MR, Gan J, Sternberg AG, Eickhoff J, Seeger RC, Matthay KK, Reynolds CP, Twist C, Krailo M, Adamson PC, Reisfeld RA, Gillies SD, Sondel PM, G Children's Oncology. A phase I clinical trial of the hu14.18-IL2 (EMD 273063) as a treatment for children with refractory or recurrent neuroblastoma and melanoma: A study of the Children's Oncology Group. Clin Cancer Res 2006;12:1750–1759.
9. Scursoni AM, Galluzzo L, Camarero S, Lopez J, Lubieniecki F, Sampor C, Segatori VI, Gabri MR, Alonso DF, Chantada G, de Davila MT. Detection of N-glycolyl GM3 ganglioside in neuroectodermal tumors by immunohistochemistry: An attractive vaccine target for aggressive pediatric cancer. Clin Dev Immunol 2011;2011:245181.
10. Fernandez LE, Gabri MR, Guthmann MD, Gomez RE, Gold S, Fainboim L, Gomez DE, Alonso DF. NGcGM3 ganglioside: A privileged target for cancer vaccines. Clin Dev Immunol 2010;2010:814397.
11. Gajdosik Z. Racotumomab—A novel anti-idiotype monoclonal antibody vaccine for the treatment of cancer. Drugs Today (Barc) 2014;50:301–307.
12. Sampor C, Guthmann M D, Scursoni A, Cacciavillano W, Torbidoni A, Galluzzo L, Camarero S, Lopez J, de Davila MT, Fainboim L, Chantada GL. Immune response to racotumomab in a child with relapsed neuroblastoma. Front Oncol 2012;2:195.
13. Neninger E, Diaz RM, de la Torre A, Rives R, Diaz A, Saurez G, Gabri MR, Alonso DF, Wilkinson B, Alfonso AM, Combet T, Perez R, Vazquez AM. Active immunotherapy with 1E10 anti-idiotype vaccine in patients with small cell lung cancer: Report of a phase I trial. Cancer Biol Ther 2007;6:145–150.
14. Hernandez AM, Vazquez AM. Racotumomab-alum vaccine for the treatment of non-small-cell lung cancer. Exp Rev Vaccines 2015;14:9–20.
15. Alfonso S, Diaz RM, de la Torre A, Santiesteban E, Aguirre F, Perez K, Rodriguez JL, Barroso Mdel C, Hernandez AM, Toledo D, Gabri MR, Alonso DF, Viada C, Gomez RE, Suarez E, Vazquez AM, Perez R, Macias AE. A randomized, multicenter, placebo-controlled clinical trial of racotumomab-alum vaccine as switch maintenance therapy in advanced non-small cell lung cancer patients. Clin Cancer Res 2014;20:3660–3671.
16. Lo Piccolo MS, Cheung NK, Cheung IY. GD2 synthase: A new molecular marker for detecting neuroblastoma. Cancer 2001;92:924–931.
17. Scursoni AM, Galluzzo L, Camarero S, Pozzo N, Gabri MR, de Acosta CM, Vazquez AM, Alonso DF, de Davila MT. Detection and characterization of N-glycolyated gangliosides in Wilms tumor by immunohistochemistry. Pediatr Dev Pathol 2010;13:18–23.
18. Kushner BH, Cheung IY, Modak S, Kramer K, Ragupathi G, Cheung NK. Phase I trial of a bivalent gangliosides vaccine in combination with beta-glucan for high-risk neuroblastoma in second or later remission. Clin Cancer Res 2014;20:1375–1382.
19. Lode HN, Schmidt M, Seidel D, Huebener N, Brackrock D, Bleeke M, Reker D, Brandt S, Mueller HP, Helm C, Siebert N. Vaccination with anti-idiotype antibody ganglidiomab mediates a GD(2)-specific anti-neuroblastoma immune response. Cancer Immunol Immunother 2013;62:999–1010.
20. Louis CU, Brenner MK. Cellular immunotherapy for neuroblastoma: A review of current vaccine and adoptive T cell therapeutics. Curr Pharm Des 2009;15:424–429.
21. Krishnadas DK, Shapiro T, Lucas K. Complete remission following decitabine/dendritic cell vaccine for relapsed neuroblastoma. Pediatrics 2013;131:e336–e341.
22. Irie A, Koyama S, Kozutsumi Y, Kawasaki T, Suzuki A. The molecular basis for the absence of N-glycolylneuraminic acid in humans. J Biol Chem 1998;273:15866–15871.
23. Yin J, Hashimoto A, Izawa M, Miyazaki K, Chen GY, Takematsu H, Kozutsumi Y, Suzuki A, Furuhata K, Cheng FL, Lin CH, Sato C, Kitajima K, Kannagi R. Hypoxic culture induces expression of sialin, a sialic acid transporter, and cancer-associated gangliosides containing non-human sialic acid on human cancer cells. Cancer Res 2006;66:2937–2945.
24. Boligan KF, Mesa C, Fernandez LE, von Gunten S. Cancer intelligence acquired (CIA): Tumor glycosylation and sialylation codes dismantling antitumor defense. Cell Mol Life Sci 2015;72:1231–1248.
25. Alfonso S, Diaz RM, de la Torre A, Santiesteban E, Aguirre F, Perez K, Rodriguez JL, Barroso Mdel C, Hernandez AM, Toledo D, Gabri MR, Alonso DF, Viada C, Gomez RE, Suarez E, Vazquez AM, Perez R, Macias AE. 1E10 anti-idiotype vaccine in non-small cell lung cancer: Experience in stage IIIb/IV patients. Cancer Biol Ther 2007;6:1847–1852.
26. Carr A, Mazorra Z, Alonso DF, Mesa C, Valiente O, Gomez DE, Perez R, Fernandez LE. A purified GM3 ganglioside conjugated vaccine induces specific, adjuvant-dependent and non-transient antitumour activity against B16 mouse melanoma in vitro and in vivo. Melanoma Res 2001;11:219–227.
27. Diaz A, Alfonso M, Alonso R, Saurez G, Troche M, Catala M, Diaz RM, Perez R, Vazquez AM. Immune responses in breast cancer patients immunized with an anti-idiotype antibody mimicking NeuGc-containing gangliosides. Clin Immunol 2003;107:80–89.
28. Hernandez AM, Rodriguez M, Lopez-Requena A, Beausoleil I, Perez R, Vazquez. AM. Generation of anti-Neu-glycolyl-ganglioside antibodies by immunization with an anti-idiotype monoclonal antibody: A self versus non-self-matter. Immunobiology 2005;210:11–21.
29. de Jong MC, Kors WA, de Graaf P, Castelijns JA, Kivela T, Moll AC. Trilateral retinoblastoma: A systematic review and meta-analysis. Lancet Oncol 2014;15:1157–1167.
30. Cheung NK, Cheung IY, Kushner BH, Ostrovnaya I, Chamberlain E, Kramer K, Modak S. Murine anti-GD2 monoclonal antibody 3F8 combined with granulocyte-macrophage colony-stimulating factor and 13-cis-retinoic acid in high-risk patients with stage 4 neuroblastoma in first remission. J Clin Oncol 2012;30:3264–3270.