® Postepy Hig Med Dosw (online), 2016; 70: 1001-1004
e-ISSN 1732-2693

www.phmd.pl
Review

Received: 2015.07.30
Accepted: 2016.05.26
Published: 2016.09.28

Evidence for the efficacy of immunotherapy in children with high-risk neuroblastoma

Zastosowanie immunoterapii u dzieci z neuroblastomą wysokiego ryzyka

Elwira Szychot[1], Jarosław Peregud-Pogorzelski[2], Paweł Wawryków[2], Andrzej Brodkiewicz[3]

[1] The Institute of Cancer Research, London, UK.
[2] Department of Paediatric Oncology, Pomeranian Medical University, Szczecin, Poland
[3] Department of Paediatrics, Nephrology with Dialysis Therapy and Management of Acute Poisoning, Pomeranian Medical University, Szczecin, Poland

Summary

Neuroblastoma is the most common extra-cranial malignancy of childhood, with the highest incidence in children younger than 4 years. The prognosis depends on many factors, such as age at diagnosis, stage of disease and molecular genetic subtype. More than 50% of children who present with the disease are deemed to have high-risk neuroblastoma. The standard therapy for children with high-risk neuroblastoma consists of intensive chemotherapy, surgery, radiotherapy, myeloablative consolidation with autologous haematopoietic stem cell rescue followed by the treatment of minimal residual disease with *13-cis*-retinoic acid. Unfortunately, more than half of the patients relapse regardless of the treatment intensity.
Combined therapy with monoclonal antibodies (anti-GD2), intravenous interleukin-2 (Il-2), intravenous granulocyte-macrophage colony-stimulating factor (GM-CSF) and oral *13-cis*-retinoic acid have been proved to be effective in some randomised trials. A better understanding of the underlying immunological processes in therapy with anti-GD2 antibodies will allow its success to be evaluated more accurately and direct future endeavours. Nevertheless, the long-term benefit of this treatment approach needs to be established.

Key words: **neuroblastoma • immunotherapy • anti-GD2 monoclonal antibody • interleukin-2**

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=1220380

Word count: 1659
Tables: –
Figures: –
References: 18

Author's address: Elwira Szychot, MD PhD MRCPCH, Paediatric Oncology Grid Trainee (ST7) and NIHR Clinical Lecturer in Paediatric Oncology. The Institute of Cancer Research, 15 Cotswold Road, Sutton, London SM2 5NG; e-mail: szychelv@doctors.org.uk

Introduction

Neuroblastoma is a heterogeneous group of tumours originating from the primordial neural crest cells that form the sympathetic nervous system. It is a rare childhood malignancy accounting for 7% of all cancers in children. Each year it affects 10.5 million children under the age of 15 years [14]. The incidence is highest in children younger than 4 years and the median age at diagnosis is 19 months. It is the most common extra-cranial malignancy of childhood and accounts for 10% of cancer mortality in children [14]. Although there is no racial variation in incidence of this tumour, African Americans are more likely to develop high-risk tumour and a fatal outcome [10]. The prognosis depends on many factors, such as age at diagnosis, stage of disease and molecular genetic subtype. Those features determine whether the tumour will spontaneously regress, metastasise or develop resistance to therapy [5]. More than 50% of children who present with the disease are deemed to have high-risk neuroblastoma. The term high-risk neuroblastoma refers to children of the age of 12 months or above with disseminated disease (stage 4 according to the International Neuroblastoma Staging System (INSS)) or patients with INSS stage 2, 3, 4 or 4s disease with amplification of the *MYCN* proto-oncogene. Amplification of the *MYCN* gene has been shown to be associated with a greater risk of relapse and death from disease progression [7].

The standard therapy for children with high-risk neuroblastoma consists of intensive chemotherapy, surgery, radiotherapy, myeloablative consolidation with autologous haematopoietic stem cell rescue followed by the treatment of minimal residual disease with *13-cis*-retinoic acid [14]. However, management of childhood high-risk neuroblastoma is challenging in view of disease heterogeneity, resistance, and treatment toxicity. More than half of the patients relapse regardless of the intensity of therapy [13]. US data from the high-risk neuroblastoma trial and introduction of immunotherapy with a monoclonal antibody against a tumour-associated antigen, the disialoganglioside GD2, added to the neuroblastoma therapy has revealed promising results [3,9,18]. In the UK immunotherapy, with monoclonal antibodies (anti-GD2) and interleukin-2 (Il-2), has been incorporated into neuroblastoma treatment within a clinical trial. The primary aim of this study is to investigate, in a randomised fashion, the potential of immunotherapy, in addition to 13-*cis*-retinoic acid. [2].

The aim of this essay is to discuss the evidence for the effectiveness of immunotherapy in children with high-risk neuroblastoma.

Immunology and immunotherapy

Neuroblastoma forms a unique immunosuppressive microenvironment preventing the development of efficient T cell immunity. Through downregulation of human leukocyte antigen and adhesion molecules, neuroblastoma cells avoid both T cells and natural killer cells. The malignant cells kill T cells and natural killer cells (NK) by inhibition or release of proteins. The cells can also engage tissue macrophages to deprive them of lymphocytes. High levels of gangliosides and sialic acid comprising sugars and proteins (important for metastasis, adhesion and migration) are carried by neuroblastoma cells. These epitopes are poorly immunogenic. The presence of natural antibodies against neuroblastoma or natural anti-ganglioside antibodies is uncommon [5]. The disialoganglioside GD2 is uniformly expressed by neuroblastomas and some other malignancies [18]. Therefore carbohydrate differentiation antigens such as GD2 seem to be promising targets for antibody-based therapies. GD2 is classified as a distinctive class of T cell-independent carbohydrate antigen. One of its characteristic features is homogeneity within neuroblastoma tumours, and there is seldom evidence of antigen loss. Its expression is limited to neurons, pain nerve fibres and skin melanocytes in normal human tissues [15]. Anti-GD2 antibodies not only passively attach neuroblastoma cells to NK cells, but they also protect NK cells from tumour suppression or inhibition [5]. There are two types of intravenous anti-GD2 IgG antibodies, which have been tested in clinical studies: a chimeric human-murine anti-GD2 monoclonal antibody called ch14.18 and a mouse 3F8. Antibody-dependent cell-mediated cytotoxicity is enhanced by administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-2 (Il-2) [18]. Combined therapy with ch14.18, intravenous interleukin-2 (Il-2), intravenous granulocyte-macrophage colony-stimulating factor (GM-CSF) and oral *13-cis*-retinoic acid proved effective in a randomised trial published by Yu et al. in the New England Journal of Medicine in 2010 [18]. The researchers investigated whether adding ch14.18, GM-CSF, and Il-2 to standard isotretinoin therapy, after intensive multimodal treatment, would improve outcomes in high-risk neuroblastoma. The study group consisted of 226 patients with high-risk neuroblastoma who responded to many different induction regimens followed by high dose chemotherapy. The patients were randomly assigned to receive either a standard treatment with 6 cycles of isotretinoin or immunotherapy with five concomitant cycles of ch14.18, with alternating GM-CSF and Il-2 added to 6 cycles of isotretinoin. The study revealed promising results with up to 20% improvement in 2-year event-free survival [18]. Nevertheless, a long-term benefit of this treatment approach needs to be established. The efficacy of this immunotherapy has only been shown in children with minimal residual disease [6]. It has hardly ever been seen in children with bulky neuroblastoma [5]. The data published by Yu et al. were not confirmed by a retrospective, nonrandomised analysis of 334 children with high-risk neuroblastoma that was conducted by Simon et al. [4,17]. All the patients were treated with initial induction therapy, and 166

patients were administered ch14.18 at doses similar to those used by Yu et al. In contrast to the results of Yu et al., the study revealed no significant improvement in the event-free survival rate or the overall survival rate [17]. However, the updated analysis showed that ch14.18 may prevent late relapse. Recently Kushner et al. showed that long-term progression-free survival is achievable in children with high-risk neuroblastoma who go into a second complete remission or very good partial remission and receive consolidation that involves anti-GD2 immunotherapy and isotretinoin. Long-term progression-free survival is possible in those patients even if these therapies are given prior to relapse [9,16].

Furthermore, based on the US immunotherapy trial, it is impossible to determine whether improved survival is the effect of the antibody or cytokines or both. The aim of the current European first-line trial (High-Risk Neuroblastoma Study 1.5 of SIOP-Europe [SIOPEN]) is to establish in a randomised study whether the results published by Yu at al. can be achieved with anti-GD2 antibodies alone or anti-GD2 antibodies and Il-2. It has been decided to combine only one cytokine Il-2 with anti-GD2 antibodies as clinical and animal data imply that Il-2 rather than GM-CSF may be more effective [12]. The adverse effects of antibody-mediated anti-GD2 immunotherapy given with the concomitant treatment with cytokines, such as GM-CSF and Il-2, are severe. It has been shown that human Il-2 combined with tumour-specific monoclonal antibody increases antibody-dependent cellular toxicity in mice [14]. However, administration of Il-2 is associated with substantial toxicity [11], for example around 23% of patients develop capillary leak [4]. Hypersensitivity reactions are also associated with administration of cytokines [18]. In 2012 Cheung et al. published the results of a retrospective analysis of sequential trials revealing that mouse 3F8 antibodies combined with subcutaneous GM-CSF and oral *13-cis*-retinoic acid without Il-2 are effective in therapy of high-risk stage 4 neuroblastoma treated in first remission. The study group consisted of 169 children with stage 4 neuroblastoma. This single arm study demonstrated a 5-year progression-free survival rate of 62% and overall survival rate of 81%. However, randomised studies are needed to confirm this treatment's efficacy [4].

Regardless of the significant improvement in event-free-survival and overall survival rates with the immunotherapy, further improvement in therapy is still required. The dose of anti-GD2 antibodies has been constrained by pain and other adverse effects of this treatment [1]. Pain is a major limitation of treatment with anti-GD2 antibodies and is considered to be a result of complement activation [5]. Implementation of genetic and molecular biology techniques decreased complement activation and reduced but did not eliminate the pain [5]. Apart from capillary leak, hypersensitivity reactions and pain, the adverse effects observed with the immunotherapy regimen include a risk of developing posterior reversible encephalopathy syndrome (PRES) with potentially critical consequences [8]. The clinical features of the syndrome include hypertension, seizures, headache, visual disturbances and altered higher cognitive processes. Magnetic resonance of the brain shows oedematous changes mainly in the parietal and occipital lobes. Although the underlying pathophysiology is unknown, it is considered that hypertension and injury to the vascular endothelium and the blood-brain barrier play a role in the aetiology of PRES [8]. Hypertension is the most common initial presentation for PRES in children treated for malignancy. In 2013 Krushner et al. reported that 2.3% of patients treated with anti-GD2 antibodies 3F8 were diagnosed with PRES, which can be challenging for the safe implementation of the immunotherapy in children with neuroblastoma [8].

A better understanding of the underlying immunological processes in therapy with anti-GD2 antibodies will enable us to explain its success and direct future research. In 2013 Modak et al. reported that oral barley-derived (1→3),(1→4)-β-D-glucan (BG) synergises with the murine anti-GD2 antibody 3F8 against neuroblastoma. Preclinical studies have shown that β-glucans enhance the clinical activity of antibodies [11]. The group conducted a phase I trial to determine the safety of this treatment regimen in patients with chemo-resistant neuroblastoma. Patients were treated with intravenous 3F8 and concurrent dose-escalating oral BG for 10 days. Although the maximum tolerated dose of BG was not attained, 2 patients developed dose-limiting toxicities: grade 4 thrombocytopenia. The study showed that this therapy was well tolerated and demonstrated antineoplastic activity in chemo-resistant neuroblastoma. The results also showed that plant carbohydrates such as β-glucans have the ability to elicit autoimmune reactions, as observed in the patients who developed thrombocytopenia. Therefore the role of the patient's diet requires consideration when evaluating the results of immunotherapy [11].

Concluding remarks

Although immunotherapy with anti-GD2 has recently been incorporated into treatment of neuroblastoma in the US, its role in relapsed or refractory disease remains questionable. There is a need for confirmatory randomised multicenter trials to verify the role of monoclonal antibodies. Because of the complexity of those trials, progress remains extremely slow. However, the future has never been so promising.

References

[1] Anghelescu D.L., Goldberg J.L., Faughnan L.G., Wu J., Mao S., Furman W.L., Santana V.M., Navid F.: Comparison of pain outcomes between two anti-GD2 antibodies in patients with Neuroblastoma. Pediatr. Blood Cancer, 2015; 62: 224-228

[2] Barone G., Moreno L., Pearson A.D.: CCLG high risk neuroblastoma relapse options and pathway. Children's Cancer and Leukaemia Group; www.cclg.org.uk, 2013; 19: 1-12. http://www.cclg.org.uk/publications/Treatment/Treatment-options-for-relapsed-or-refractory-high-risk-neuroblastoma/NBRELAPS

[3] Cheng M., Ahmed M,, Xu H., Cheung N.K.: Structural design of disialoganglioside GD2 and CD3-bispecific antibodies to redirect T cells for tumor therapy. Int. J. Cancer, 2015; 136: 476-486

[4] Cheung N.K., Cheung I.Y., Kushner B.H., 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; 10; 30: 3264-3270

[5] Cheung N.K., Dyer M.A.: Neuroblastoma: developmental biology, cancer genomics and immunotherapy. Nat. Rev. Cancer, 2013; 13: 397-411

[6] Cheung N.K., Ostrovnaya I., Kuk D., Cheung I.Y.: Bone marrow minimal residual disease was an early response marker and a consistent independent predictor of survival after anti-GD2 immunotherapy. J. Clin. Oncol., 2015; 33: 755-763

[7] Cohn S.L., Pearson A.D., London W.B., Monclair T., Ambros P.F., Brodeur G.M., Faldum A., Hero B., Iehara T., Machin D., Mosseri V., Simon T., Garaventa A., Castel V., Matthay K.K.; INRG Task Force: The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. J. Clin. Oncol. 2009; 27: 289-297

[8] Kushner B.H., Modak S., Basu E.M., Roberts S.S., Kramer K., Cheung N.K.: Posterior reversible encephalopathy syndrome in neuroblastoma patients receiving anti-G_{D2} 3F8 monoclonal antibody. Cancer, 2013; 119: 2789-2795

[9] Kushner B.H., Ostrovnaya I., Cheung I.Y., Kuk D., Kramer K., Modak S., Yataghene K., Cheung N.V.: Prolonged progression-free survival after consolidating second or later remissions of neuroblastoma with Anti-G_{D2} immunotherapy and isotretinoin: a prospective Phase II study. Oncoimmunology, 2015; 4: e1016704

[10] Latorre V., Diskin S.J., Diamond M.A., Zhang H., Hakonarson H., Maris J.M., Devoto M.: Replication of neuroblastoma SNP association at the *BARD1* locus in African-Americans. Cancer Epidemiol. Biomarkers Prev., 2012; 21: 658-663

[11] Modak S., Kushner B.H., Kramer K., Vickers A., Cheung I.Y., Cheung N.K.: Anti-GD2 antibody 3F8 and barley-derived (1→3),(1→4)-β-*D*-glucan: a phase I study in patients with chemoresistant neuroblastoma. Oncoimmunology, 2013; 2: e23402

[12] Moreno L., Barone G., Park J.R., Pearson A.D.: About the benefits of immunotherapy for high-risk Neuroblastoma. J. Clin. Oncol., 2013; 31: 649-650

[13] Morgenstern D.A., Baruchel S., Irwin M.S.: Current and future strategies for relapsed neuroblastoma: challenges on the road to precision therapy. J. Pediatr. Hematol. Oncol., 2013; 35: 337-347

[14] Park J.R., Bagatell R., London W.B., Maris J.M., Cohn S.L., Mattay KM, Hogarty M.: COG Neuroblastoma Committee. Children's Oncology Group's 2013 blueprint for research: Neuroblastoma. Pediatr. Blood Cancer, 2013; 60: 985-993

[15] Schulz G., Cheresh D.A., Varki N.M., Yu A., Staffileno L.K., Reisfeld R.A.: Detection of ganglioside G_{D2} in tumor tissues and sera of neuroblastoma patients. Cancer Res. 1984; 44: 5914-5920

[16] Simon T., Hero B., Faldum A., Handgretinger R., Schrappe M., Berthold F.: No success of anti-GD2 treatment in high risk neuroblastoma? A critical appraisal. Pediatr. Blood Cancer, 2009; 53: 708. Abstract

[17] 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

[18] Yu A.L., Gilman A.L., Ozkaynak M.F., London W.B., Kreissman S.G., Chen H.X., Smith M., Anderson B., Villablanca J.G., Matthay K.K., Shimada H., Grupp S.A., Seeger R., Reynolds C.P., Buxton A., et al.: Children's Oncology Group: Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. N. Engl. J. Med., 2010; 363: 1324-1334

The authors have no potential conflicts of interest to declare.