Published in final edited form as:

J Pediatr Hematol Oncol. 2019 April ; 41(3): 163–169. doi:10.1097/MPH.0000000000001369.

Advances in Anti-GD2 Immunotherapy for Treatment of High-Risk Neuroblastoma

Julie Voeller, M.D.¹ and Paul M. Sondel, M.D., Ph.D.^{1,2}

¹Department of Pediatrics, Division of Hematology, Oncology and Bone Marrow Transplant, University of Wisconsin, Madison, WI

²Departments of Human Oncology and Genetics and Carbone Cancer Center, University of Wisconsin, Madison, WI

Abstract

Neuroblastoma (NBL) is the most common extracranial solid tumor in pediatrics, yet overall survival is poor for high-risk cases. Immunotherapy regimens using a tumor-selective anti-disialoganglioside (anti-GD2) monoclonal antibody (mAb) have been studied for several decades now, but have only recently been incorporated into standard of care treatment for patients with high-risk NBL with clear benefit. Here we review a brief history of anti-GD2-based immunotherapy, current areas of neuroblastoma research targeting GD2, and potential diagnostic and therapeutic uses targeting GD2.

Keywords

anti-disialoganglioside; anti-GD2; monoclonal antibody; immunotherapy; neuroblastoma

Introduction

Cancer immunotherapy was named “Breakthrough of the Year” in 2013 by the journal *Science*.¹ Research in the field continues to expand exponentially, harnessing the ability of immunotherapy to: (1) enable tumor-selective killing; (2) minimize patient harm by avoiding immune recognition of normal tissues; and (3) achieve complete and long-lasting cures by using the immune system to eradicate identifiable cancer as well as microscopic sites of cancer spread, thereby preventing recurrence. Because of these attributes, including the ability to destroy cancers that are resistant to chemotherapy, immunotherapy has the potential to achieve lasting cures in heavily pre-treated patients with poor prognosis.

Neuroblastoma (NBL) is the most common extracranial solid tumor in pediatrics, yet overall survival is poor for high-risk cases. Following high-dose chemotherapy, surgery, tandem autologous stem cell transplant and radiation therapy, immunotherapy using a tumor-specific anti-disialoganglioside (anti-GD2) monoclonal antibody (mAb) together with immunostimulatory agents [interleukin-2 (IL2) and granulocyte-macrophage colony-

stimulating factor (GM-CSF)] and isotretinoin has already been successfully incorporated into standard of care treatment per a Children's Oncology Group (COG) protocol for patients with high-risk NBL, which has significantly improved survival rates for these patients.²

GD2 is a surface antigen that is expressed on tumors of neuroectodermal origin, such as neuroblastoma and melanoma, with little heterogeneity between cells and, usually, without loss of expression after treatment with mAb.^{3,4} In normal tissues, GD2 expression is limited to neurons, melanocytes and peripheral pain fibers, making it a suitable target for anti-tumor therapy.⁵ Here we describe a brief history of the development of anti-GD2 immunotherapy for patients with high-risk NBL, explore important advances in anti-GD2 immunotherapy to augment its effect, and describe ongoing investigations to determine predictors of outcome and toxicity in response to treatment with anti-GD2 immunotherapy, as summarized in Table 1. Most of these concepts in anti-GD2 directed immunotherapy of NBL were featured at the recent Advances in Neuroblastoma Research meeting (May 9–12, 2018; San Francisco CA).

Overview of Anti-GD2 mAb Immunotherapy for High-Risk Neuroblastoma

Tumor-reactive mAbs affect their anti-tumor function via antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and direct cytotoxicity.⁶ Several anti-GD2 mAb have been tested for clinical use, including murine 3F8, humanized 3F8 (hu3F8), murine 14.G2a, chimeric 14.18 (ch14.18), humanized 14.18 (hu14.18K322A), and humanized 14.18 fused with interleukin-2 (hu14.18-IL2). The first anti-GD2 mAb, 3F8, has been shown to be effective against chemotherapy-resistant marrow minimal residual disease (MRD), but has had limited responses in patients with bulky disease.^{7,8,9} Hu3F8 in combination with GM-CSF is currently being tested for patients with relapsed or refractory disease.¹⁰

Ch14.18 is a chimeric mAb that joins the variable chains of mouse 14.18 anti-GD2 mAb with the human constant regions for heavy IgG1 chain and light κ chain to reduce unwanted immunogenicity of foreign antigens on mouse proteins. Immunotherapy with ch14.18 was shown in a COG phase III randomized clinical trial to have superior event-free survival (EFS, $66 \pm 5\%$ vs $46 \pm 5\%$) and overall survival (OS, $86 \pm 4\%$ vs $75 \pm 5\%$) at 2 years when compared to standard therapy with isotretinoin alone.² Several studies have since recapitulated these results.^{19,20} In 2015, the FDA approved the use of dinutuximab, or ch14.18 produced in SP2/0 cells,¹¹ which is now incorporated into standard of care therapy for patients with high-risk NBL. Dinutuximab beta, or ch14.18 manufactured in Chinese hamster ovary cells (CHO), was approved for marketing in Europe in 2017.

Other anti-GD2-based immunotherapies are now in development and clinical trial testing. By reducing complement-mediated cytotoxicity, hu14.18K322A is associated with decreased pain toxicity and hypersensitivity reactions and still demonstrated an 18% objective response rate in a phase I trial.^{12,13} In a phase II study, monotherapy with the hu14.18-IL2 immunocytokine achieved complete responses in 22% of patients with MRD.¹⁴

Important treatment-related side effects of ch14.18 include pain, hypotension, capillary leak syndrome, and hypersensitivity reactions. In a single-center study including patients with

relapsed or refractory disease, long-term constant infusion (LTI) of ch14.18/CHO over a 10-day period given with subcutaneous IL2 and retinoic acid was associated with decreased morphine usage and lower pain scores, while still having a best response rate of 40.5% (15/37; 5 CR, 10 PR), 4-year progression free survival (PFS) of 33.1% (observation 0.1–4.9 years, mean: 2.2 years), and a 4-year OS of 47.7% (observation 0.27–5.20 years, mean: 3.6 years).¹⁵ Survival of the entire cohort (53/53) and the relapsed patients (29/53) was significantly improved compared to historical controls. Early observations in an ongoing phase II trial is revealing similar results.^{16,17} A phase I trial is ongoing to assess the benefit of drug delivery by LTI in combination with ¹³¹I-metaiodobenzylguanidine (MIBG) and anti-PD1 immune checkpoint inhibition.¹⁸ Future studies could compare LTI of anti-GD2 antibody to standard delivery in a randomized control trial for upfront treatment of newly diagnosed patients and/or those with relapsed or refractory disease.

Augmenting the Immunotherapeutic Effect of anti-GD2 mAb

Studies are now ongoing to investigate other regimens combining chemotherapy with ch14.18. In a preclinical study of NOD/SCID mice, anti-GD2 immunotherapy was improved by administration with lenalidomide, an immunostimulatory molecule which enhances natural killer (NK) cell activation and inhibits their suppression by IL-6 and TGFβ1.²¹ A phase I trial combining lenalidomide with ch14.18 and retinoic acid showed the regimen was tolerable and associated with statistically significant increases in circulating NK cells, CD4+ memory T cells, and ADCC.^{22,23} In a COG phase II trial which included patients with relapsed or refractory NBL, irinotecan and temozolomide combined with dinutuximab and GM-CSF showed notable anti-tumor activity with significantly higher objective response rate (53%) compared to the same chemotherapy regimen combined with temsirolimus (6%).²⁴ In this trial, responses were seen in patients that had previously progressed with anti-GD2 mAb treatment. A similar approach has been taken by the St. Jude team in a study adding hu14.18K322A mAb treatment to chemotherapy for patients with relapsed or refractory NBL, with response rates higher than expected for chemotherapy alone.²⁵ This concept has been moved into a protocol incorporating hu14.18K322A mAb into the induction chemotherapy regimen for newly diagnosed NBL patients at St. Jude; the response rate thus far is greater than that seen for a COG historical control using the same induction chemotherapy but without anti-GD2 mAb.²⁶ A preclinical trial investigating a combination therapy with anti-GD2 and vorinostat, a histone deacetylase inhibitor, showed a synergistic effect to reduce tumor growth, upregulate GD2 expression on tumor cells, and create a permissive tumor microenvironment (TME) by increasing high FcR expressing macrophage effector cells and reducing the number and function of myeloid-derived suppressor cells.²⁷

Other immunotherapeutic strategies have been found to augment the effect of anti-GD2 mAb by increasing tumor killing by cytotoxic cells and establishing a permissive TME. Activated NK cells and haploidentical NK cells are being studied in the preclinical and clinical settings to augment ADCC by anti-GD2 mAb,^{28–30} while a preclinical study found that activated plasmacytoid dendritic cells can further stimulate NK cell-mediated killing.³¹ Patient expanded γδ T cells augment ch14.18 and temozolomide responses in a murine neuroblastoma model and allows for reductions in exposure to cytotoxic chemotherapy.³² A preclinical study found that PD-L1/PD1 immune checkpoints were upregulated by ADCC

with ch14.18/CHO—an effect that was further augmented with the addition of IL2 but abrogated with the addition of an anti-idiotypic mAb, ganglidiomab—suggesting a role for adding anti-PD1 checkpoint inhibition with anti-GD2 immunotherapy in clinical trials.³³

Cellular Therapies Targeting GD2

Now approved by the FDA for use in pediatric patients with relapsed or refractory B-cell acute lymphoblastic leukemia, CD19 chimeric antigen receptor (CAR) T cells have made groundbreaking strides in gene therapy with overall remission rates of 82%.³⁴ Autologous T cells are engineered to express CARs that specifically target tumor antigens—in this case CD19—via an extracellular domain, thereby introducing tumor specificity into adoptive cell therapy through a major histocompatibility complex (MHC)-independent manner.³⁵ Because of these impressive successes in hematologic malignancies, there is an active search for effective CAR T cell therapy for NBL and other solid tumors.³⁶ GD2 has been at the forefront of this investigation as a NBL target for CAR T cells because of its tumor specificity and its clinical successes as an immunotherapeutic target.

For CAR T cell therapy to be successful, there must be a high target antigen density on the tumor³⁷ and sufficient T cell expansion and persistence. GD2 is an exemplary tumor-antigen target as it is ubiquitously expressed on NBL cell membrane while restricted in normal tissues to peripheral nerves, the central nervous system, and skin melanocytes.^{38,39} This differential expression ensures sufficient cytolytic T cell activation and anti-tumor cytokine production, while expanding the therapeutic window by allowing for a low background level of GD2 expression on normal tissues.

Optimal co-stimulation improves the survival and function of CAR T cells, leading to greater tumor killing and sustained remissions. Whereas first generation CARs only produce an activation signal via their cytoplasmic domain, second and third generation CARs have one or more co-stimulatory domains that potentiate the activation signaling.⁴⁰ However, molecules such as CD28, 4-1BB, and OX40 have shown variable successes in pre-clinical models.^{41–44} CAR T cells engineered from Epstein-Barr virus-specific cytotoxic T cells (EBV-CTLs) may have greater proliferation and persistence in patients compared to those derived from activated T cells (ATCs) likely due to co-stimulation via native anti-virus receptors by latent EBV antigen on endogenous antigen-presenting cells.⁴⁵ A trial infusing anti-GD2 CAR expressed in EBV-CTLs and ATCs achieved complete remission in three of 11 patients with active disease, with long-term persistence of EBV-CTLs and ATCs associated with improved outcomes but impressively without side effects of neuropathic pain as seen with treatment with anti-GD2 mAb.^{46,47} An alternative approach is to incorporate constitutively active cytokine receptors to enhance anti-tumor activity and favorably alter the tumor microenvironment, with translation of preclinical investigations into co-stimulation with 4-1BB and IL7 underway.⁴⁸ One trial is currently open for patients with relapsed/refractory GD2-positive osteosarcoma or neuroblastoma to find the largest safe dose of an anti-GD2 CAR that has CD28 and OX40 co-stimulatory domains and expressed in varicella zoster virus (VZV) activated T cells.⁴⁹

Other Targeted Therapies Utilizing GD2

Because of its relative specificity for neuroblastoma, GD2 has been utilized as a vehicle to deliver drug or toxin directly to the tumor, where it is endocytosed and selectively released into the tumor cell for killing. Preclinical studies have shown increased tumor killing by immunotoxins linking GD2 with agents such as ricin-A chain toxin, gelonin, diphtheria toxin and pseudomonas exotoxin, but none of these have been studied clinically with primary concerns relating to immunogenicity of a foreign toxin protein.⁵⁰ Similarly, antibody-drug conjugates, such as calicheamicin-14G2a,⁵¹ have been tested preclinically but have not yet been expanded to the clinical setting.

Radiolabeled GD2 has both diagnostic and therapeutic potential. In a murine neuroblastoma model, ⁶⁴Cu-labeled ch14.18 was detected by Cerenkov luminescence imaging (CLI), which has a shorter acquisition time than, but tumor detection as reliable as, Position Emission Tomography (PET); however, CLI has the potential advantage for visualization of mAb labeled with therapeutic isotopes like electron emitters.⁵² ¹³¹I-3F8 scintigraphy has been reported to have improved sensitivity of tumor detection compared to ¹³¹I-MIBG in patients, particularly for those with extensive disease.⁵³ Therapeutic testing of ¹³¹I-3F8 is ongoing, following phase I trials of systemic and intrathecal delivery of drug.^{54,55} Another radioimmunotherapeutic approach has been studied preclinically with the bispecific antibody hu3F8 linked to C825, a murine antibody with high affinity for the chelator 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). Via a three-step regimen consisting of hu3F8-C825 (where C825 is a murine single-chain variable fragment antibody with high affinity for DOTA) followed by a dextran-based clearing agent and a radioactive payload of DOTA complexed with lutetium-177, radiation therapy can safely be delivered at high doses to neuroblastoma without harming normal tissues.⁵⁶

Ongoing preclinical development has shown that the combination of low-dose, immunomodulatory radiotherapy [12 Gray (Gy)] together with intratumoral injection of the hu14.18-IL2 immunocytokine (a fusion protein linking humanized anti-GD2 mAb to IL2) can induce striking eradication of relatively large GD2-positive tumors. This response involves both innate and adaptive immunity, and enables the tumor receiving the local radiotherapy and the intratumoral injection to serve as an *in situ* vaccine, generating protective, tumor-specific T-cell memory. Most of this work has been done in the GD2-positive B78 melanoma model,⁵⁷ and even greater activity is seen when checkpoint blockade or T regulatory cell depletion is added.⁵⁸ Clinical testing of this concept is being initiated in refractory melanoma, and initial data are demonstrating this *in situ* vaccine approach can be extended to treatment of neuroblastoma in immunocompetent mice.⁵⁹

Finally, GD2 vaccines are being tested to induce an active humoral (and potentially cellular) immune response in the MRD and disease-free settings to produce lasting remissions. Initial results from a phase II trial using a ganglioside vaccine, GD2-keyhole limpet hemocyanin (KLH)/GD3-KLH conjugate, showed 51% 2-year PFS and 90% 2-year OS, which correlated with increased anti-GD2 antibody titer.^{60,61} Anti-idiotypic antibodies (anti-Id Ab) are also being tested as vaccines. Anti-Id Abs recognize idiotype epitopes in the variable region of mAbs, including the antigen binding site, of the therapeutic mAb. In this way, the anti-Id is acting as a surrogate of the original GD2 antigen. By presenting an immunogenic GD2-

surrogate as a protein epitope to T and B cells, anti-Id vaccines may be more immunogenic than active immunization with GD2 itself. MAb1A7, an anti-Id Ab directed against murine 14G2a (that also recognizes the idiotype of ch14.18), and ganglidiomab, a human/mouse chimeric anti-Id Ab directed against ch14.18 anti-GD2 mAb, have shown similar potential for active immunotherapy against NBL.^{62,63}

Predicting Response to Immunotherapy with Anti-GD2 mAb

Development of human anti-chimeric antibody (HACA), human anti-mouse antibody (HAMA), and human anti-human antibody (HABA) can be a mechanism of resistance to tumor immunotherapy with anti-GD2 mAb. Patients with strong HACA/HAMA responses following treatment with anti-GD2 mAb show significantly decreased detectable circulating anti-GD2 mAb following subsequent mAb infusions; this was associated with decreased, but not complete abrogation, of ADCC and CDC effector function.^{64,65} Similarly, HAMA responses can occur against murine mAbs such as 3F8 and reduce the drug's efficacy by neutralization. Interestingly, a transient HAMA response can induce a network of anti-anti-idiotypic antibodies that has been associated with long-term tumor control and increased survival.⁶⁶ Detecting levels of anti-therapeutic antibodies may be useful in guiding treatment-related decisions; however, a Clinical Laboratory Improvement Amendments (CLIA)-approved standardized clinical assay is not yet available for serially evaluating this important parameter in patients receiving anti-GD2 mAb-based therapy with ch14.18 mAb or its genetically engineered derivatives.

GD2 expression on tumor cells is important for tumor-specific anti-GD2 mAb therapy. Therefore, quantitation of GD2 synthase mRNA may be a useful surrogate marker for measuring response to immunotherapy.⁶⁷ Moreover, neuroblastoma cells may lack GD2 expression at diagnosis or at relapse.^{68,69} While this is a rare occurrence, this has diagnostic and therapeutic implications, therefore it may also be important to evaluate in future studies whether a defined level of GD2 expression is needed for treatment response.

Cytokine release in response to treatment with anti-GD2 mAb is thought to play an important role in anti-tumor killing and development of an immune memory response. Cytokines are also implicated in treatment-related toxicities such as allergic reactions, anaphylaxis, capillary leak syndrome, and pain. Therefore, several investigations are ongoing to query the utility of measuring serum cytokine levels to predict toxicities and outcome. For example, patients with higher levels of CXCL9, a cytokine involved in pro-tumor activities, at the start of treatment may be associated with poorer EFS, while IL1 receptor antagonist (IL1-RA) and interferon gamma (IFN γ) may be associated with allergic reaction.²⁰ Preliminary studies have also implicated pre-existing serum antibodies against Neu5Gc and alpha-gal, which are non-human glycan residues present on ch14.18, to be associated with treatment-related toxicities and outcome for patients.⁷⁰ Studies are ongoing to further investigate these associations and further elucidate the origin of these pre-existing antibodies in patients.

Killer immunoglobulin-like receptors (KIRs), a family of highly polymorphic receptors that regulate NK cell function, may also have an association with clinical outcome. Several studies have shown improved outcomes among patients with unlicensed NK cells (those

with at least one inhibitory KIR ligand missing) compared to patients who have all inhibitory KIR ligands present.^{71,72} This finding was not recapitulated in a recently published analysis of newly diagnosed patients participating in the COG phase III trial testing the role of dinutuximab combined with IL2 and GM-CSF. In this trial, it was possible to evaluate whether the addition of the immunotherapy regimen provided significant improvement in outcome for NBL patients with distinct KIR/KIR-ligand genotypes. A subset of these patients (30%) were found to have a specific genotype with all KIR ligands present that was associated with improved outcome if the patient had received maintenance treatment after autologous stem cell transplant with immunotherapy compared to isotretinoin alone.⁷³ In addition to specific KIR haplotypes, high affinity Fcγ-receptor polymorphisms may be correlated with increased ADCC and improved clinical outcome among patients receiving long-term infusion of ch14.18/CHO.⁷⁴ While further investigations are ongoing, these data suggest that KIR/KIR-ligand and FcR genotyping can be used as a biomarker for potentially predicting response to immunotherapy.

Conclusion

Anti-GD2 mAbs have been studied for several decades now, with known clinical activity against NBL, but have only recently been incorporated into standard of care treatment for patients with high-risk NBL. As we begin to learn more about genotypic and phenotypic variability between patients and tumors, GD2 will remain at the forefront of research in cancer immunotherapy for NBL and potentially other GD2-positive tumors (including rhabdomyosarcoma, osteosarcoma, leiomyosarcoma, liposarcoma, fibrosarcoma, small cell lung cancer and melanoma) because of its high-density expression on these tumors, its relative resistance to down-regulation, and its relative paucity on most normal tissues.⁷⁵

Acknowledgement

The authors thank Dr. Katherine Matthay of University of California San Francisco, the meeting director for the Advances in Neuroblastoma Research (ANR) meeting, held May 9–12, 2018, in San Francisco, California. The topics highlighted in this review represent many of the important and developing approaches, strategies, and concepts in anti-GD2-based neuroblastoma therapy that were featured at this year's ANR meeting. The authors also thank Dr. Holger Lode for his helpful review of the details pertaining to the long-term infusion studies of anti-GD2 antibody.

Grant Support:

This work was supported NIH Grants CA032685, CA87025, CA166105, CA197078, GM067386, The University of Wisconsin ICTR Grant 1TL1RR025013–01, The University of Wisconsin Carbone Cancer Center Core Grant, P30CA014520, The University of Wisconsin UW2020 grant, The Midwest Athletes for Childhood Cancer Fund, The Hyundai Hope on Wheels Foundation and The St. Baldrick's-Stand Up To Cancer Pediatric Dream Team Translational Research Grant (SU2C-AACR-DT1113). Stand Up To Cancer is a program of the Entertainment Industry Foundation administered by the American Association for Cancer Research.

References

1. Couzin-Frankel J Breakthrough of the year 2013: Cancer immunotherapy. *Science*. 2013 12 20;342(6165):1432–3. [PubMed: 24357284]
2. 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;363:1324–34. [PubMed: 20879881]

3. Schulz G, Cheresch DA, Varki NM, et al. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. *Cancer Res.* 1984 12;44(12 Pt 1):5914–5920. [PubMed: 6498849]
4. Kramer K, Gerald WL, Kushner BH, et al. Disialoganglioside G(D2) loss following monoclonal antibody therapy is rare in neuroblastoma. *Clin Cancer Res.* 1998 9;4(9):2135–2139. [PubMed: 9748131]
5. Yang RK, Sondel PM. Anti-GD2 Strategy in the Treatment of Neuroblastoma. *Drugs of the future.* 2010;35(8):665-. [PubMed: 21037966]
6. Perez Horta Z, Goldberg JL, Sondel PM. Anti-GD2 mAbs and next-generation mAb-based agents for cancer therapy. *Immunotherapy.* 2016;8(9):1097–1117. [PubMed: 27485082]
7. Cheung NK, Kushner BH, Yeh SD, et al. 3F8 monoclonal antibody treatment of patients with stage 4 neuroblastoma: a phase II study. *Int J Oncol.* 1998 6;12(6):1299–1306. [PubMed: 9592190]
8. Kushner BH, Kramer K, Cheung NK. Phase II trial of the anti-G(D2) monoclonal antibody 3F8 and granulocyte-macrophage colony-stimulating factor for neuroblastoma. *J Clin Oncol.* 2001 11 15;19(22):4189–4194. [PubMed: 11709561]
9. Cheung N-KV, Cheung IY, Kushner BH, et al. 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(26):3264–3270. [PubMed: 22869886]
10. [ClinicalTrials.gov](https://clinicaltrials.gov). Bethesda (MD): National Library of Medicine (US) 2012 12 31 Identifier: NCT01757626, Combination Therapy of Antibody Hu3F8 With Granulocyte- Macrophage Colony Stimulating Factor (GM-CSF) in Patients With Relapsed/Refractory High-Risk Neuroblastoma; 2018 Apr 18. Available at: <https://clinicaltrials.gov/ct2/show/NCT01757626>.
11. FDA Approves First Therapy for High-Risk Neuroblastoma. NIH National Cancer Institute <https://www.cancer.gov/news-events/cancer-currents-blog/2015/dinutuximab-neuroblastoma>. Published 3 27, 2015 Accessed July 19, 2018.
12. Anghelescu DL, Goldberg JL, Faughnan LG, et al. Comparison of Pain Outcomes between Two Anti-GD2 Antibodies in Patients with Neuroblastoma. *Pediatr Blood Cancer.* 2015;62(2):224–228. [PubMed: 25382742]
13. Navid F, Sondel PM, Barfield R, et al. Phase I Trial of a Novel Anti-GD2 Monoclonal Antibody, Hu14.18K322A, Designed to Decrease Toxicity in Children With Refractory or Recurrent Neuroblastoma. *J Clin Oncol.* 2014;32(14):1445–1452. [PubMed: 24711551]
14. Shusterman S, London WB, Gillies SD, et al. Antitumor Activity of Hu14.18-IL2 in Patients With Relapsed/Refractory Neuroblastoma: A Children's Oncology Group (COG) Phase II Study. *J Clin Oncol.* 2010;28(33):4969–4975. [PubMed: 20921469]
15. Mueller I, Ehler K, Endres S, et al. Tolerability, response and outcome of high-risk neuroblastoma patients treated with long-term infusion of anti-GD2 antibody ch14.18/CHO. *mAbs.* 2018;10(1):55–61. [PubMed: 29120699]
16. Lode HN, Valteau-Couanet D, Garaventa A, et al. Long-term infusion of anti-GD2 antibody ch14.18/CHO in combination with interleukin-2 (IL2) activity and efficacy in high-risk relapsed/refractory neuroblastoma patients. *J Clin Oncol.* 2015;33(15_suppl):TPS10080.
17. [ClinicalTrials.gov](https://clinicaltrials.gov). Bethesda (MD): National Library of Medicine (US) 2012 10 5 Identifier: NCT01701479, Long Term Continuous Infusion ch14.18/CHO Plus s.c. Aldesleukin (IL-2) (LTI); 2012 October 8. Available at: <https://clinicaltrials.gov/ct2/show/NCT01701479>.
18. [ClinicalTrials.gov](https://clinicaltrials.gov). Bethesda (MD): National Library of Medicine (US) 2018 6 25 Identifier: NCT02914405, Phase I Study of Investigational Medicinal Products in Children with Relapsed/Refractory Neuroblastoma; 2018 Jun 25. Available at: <https://clinicaltrials.gov/ct2/show/NCT02914405>.
19. Ladenstein R, Poetschger U, Couanet D, et al. Immunotherapy with anti-GD2 antibody ch14.18/CHO±IL2 within the HR-NBL1/SIOPEN trial to improve outcome of high-risk neuroblastoma patients compared to historical controls. *J Clin Oncol.* 2018;36(15_suppl):10539.
20. Ozkaynak MF, Gilman AL, London WB, et al. A Comprehensive Safety Trial of Chimeric Antibody 14.18 with GM-CSF, IL-2 and Isotretinoin in High-Risk Neuroblastoma Patients Following Myeloablative Therapy: Children's Oncology Group Study ANBL0931. *Front Immunol.* 2018 6 18;9:1355. [PubMed: 29967609]

21. Xu Y, Sun J, Sheard MA, et al. Lenalidomide overcomes suppression of human natural killer cell anti-tumor functions by neuroblastoma microenvironment-associated IL-6 and TGF β 1. *Cancer Immunol Immunother*. 2013 10;62(10):1637–48. [PubMed: 23982484]
22. Marachelian A, Villablanca JG, Duvalyan A, et al. A Phase I Study of Lenalidomide in Combination with ch14.18 and Isotretinoin (RA) in Patients with Refractory/Recurrent Neuroblastoma (RR-NB). In: *Proceedings from the Advances in Neuroblastoma Research*; May 9–12, 2018; San Francisco, CA Abstract 400.
23. [ClinicalTrials.gov](https://clinicaltrials.gov). Bethesda (MD): National Library of Medicine (US) 2012 10 22 Identifier: NCT01711554, Lenalidomide and Dinutuximab With or Without Isotretinoin in Treating Younger Patients With Refractory or Recurrent Neuroblastoma; 2018 Jul 17. Available at: <https://clinicaltrials.gov/ct2/show/NCT01711554?term=lenalidomide&cond=Neuroblastoma&cntry=US&rank=1>.
24. Mody R, Naranjo A, Van Ryn C, et al. Irinotecan-temozolomide with temsirolimus or dinutuximab in children with refractory or relapsed neuroblastoma (COG ANBL1221): an open-label, randomised, phase 2 trial. *Lancet Oncol*. 2017 7;18(7):946–957. [PubMed: 28549783]
25. Federico SM, McCarville B, Shulkin B, et al. A pilot trial of humanized anti-GD2 monoclonal antibody (hu14.18K322A) in combination with chemotherapy with and without natural killer cells in children with recurrent/refractory neuroblastoma. *Clin Cancer Res*. 2017 11 1;23(21):6441–6449. [PubMed: 28939747]
26. Furman WL, Federico SM, McCarville NB, et al. Improved clinical responses with the concomitant use of an anti-GD2 monoclonal antibody and chemotherapy in newly diagnosed children with high-risk (HR) neuroblastoma (NB): Preliminary results of a phase II study. *J Clin Onc*. 2016;34(15_suppl):10501.
27. Kroesen M, Büll C, Gielen PR, et al. Anti-GD2 mAb and Vorinostat synergize in the treatment of neuroblastoma. *Oncoimmunology*. 2016 3 28;5(6):e1164919. [PubMed: 27471639]
28. [ClinicalTrials.gov](https://clinicaltrials.gov). Bethesda (MD): National Library of Medicine (US) 2017 7 6 Identifier: NCT03209869, Treatment of Relapsed or Refractory Neuroblastoma With Expanded Haploidentical NK Cells and Hu14.18-IL2; 2018 Jun 1. Available at: <https://clinicaltrials.gov/ct2/show/NCT03209869>.
29. [ClinicalTrials.gov](https://clinicaltrials.gov). Bethesda (MD): National Library of Medicine (US) 2015 10 12 Identifier: NCT02573896, Immunotherapy of Relapsed Refractory Neuroblastoma With Expanded NK Cells; 2018 Apr 11. Available at: <https://clinicaltrials.gov/ct2/show/NCT02573896>.
30. Tran HC, Wan Z, Sheard MA, et al. TGF β R1 Blockade with Galunisertib (LY2157299) Enhances Anti-Neuroblastoma Activity of Anti-GD2 Antibody Dinutuximab (ch14.18) with Natural Killer Cells. *Clin Cancer Res*. 2017 2 1;23(3):804–813. [PubMed: 27756784]
31. Cordeau M, Belounis A, Lelaidier M, et al. Efficient Killing of High Risk Neuroblastoma Using Natural Killer Cells Activated by Plasmacytoid Dendritic Cells. *PLoS ONE*. 2016 10 7;11(10):e0164401. [PubMed: 27716850]
32. Zoine J, Sutton K, Knight K, et al. Gamma Delta ($\gamma\delta$) T Cell-Based Immunotherapy Augments Dinutuximab And Low Dose Chemotherapy to Regress Neuroblastoma Tumors In Vivo. In: *Proceedings from the Advances in Neuroblastoma Research*; May 9–12, 2018; San Francisco, CA Abstract 246.
33. Siebert N, Zumpfe M, Marx M, et al. Upregulation of the PD1/PDL1 Immune Checkpoint Pathway in Neuroblastoma by ch14.18/CHO and hu14.18-IL2 and effects of PD-1 blockade in vivo. In: *Proceedings from the Advances in Neuroblastoma Research*; May 9–12, 2018; San Francisco, CA Abstract 46.
34. FDA approves tisagenlecleucel for B-cell ALL and tocilizumab for cytokine release syndrome US Food and Drug Administration <https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm574154.htm>. Last updated September 7, 2017 Accessed July 19, 2018.
35. Maude SL, Teachey DT, Porter DL, et al. CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia. *Blood*. 2015;125(26):4017–4023. [PubMed: 25999455]
36. Lee DW, Barrett DM, Mackall C, et al. The Future Is Now: Chimeric Antigen Receptors as New Targeted Therapies for Childhood Cancer. *Clin Cancer Res*. 2012;18(10):2780–2790. [PubMed: 22589486]

37. Walker AJ, Majzner RG, Zhang L, et al. Tumor Antigen and Receptor Densities Regulate Efficacy of a Chimeric Antigen Receptor Targeting Anaplastic Lymphoma Kinase. *Mol Ther.* 2017 9 6;25(9):2189–2201. [PubMed: 28676342]
38. Mujoo K, Cheresh DA, Yang HM, et al. Disialoganglioside GD2 human neuroblastoma cells: target antigen for monoclonal antibody-mediated cytotoxicity and suppression of tumor growth. *Cancer Res.* 1987;47:1098–1104. [PubMed: 3100030]
39. Modak S, Cheung NK. Disialoganglioside Directed Immunotherapy of Neuroblastoma. *Cancer Invest.* 2007 2; 25(1):67–77. [PubMed: 17364560]
40. Sadelain M, Brentjens R, Riviere I. The basic principles of chimeric antigen receptor (CAR) design. *Cancer discovery.* 2013;3(4):388–398. [PubMed: 23550147]
41. Thomas S, Straathof K, Himoudi N, et al. An Optimized GD2-Targeting Retroviral Cassette for More Potent and Safer Cellular Therapy of Neuroblastoma and Other Cancers. *PLoS ONE.* 2016;11(3):e0152196. [PubMed: 27030986]
42. Li W, Guo L, Rath P, et al. Redirecting T Cells to Glypican-3 with 4–1BB Zeta Chimeric Antigen Receptors Results in Th1 Polarization and Potent Antitumor Activity. *Human Gene Therapy.* 2017;28(5):437–448. [PubMed: 27530312]
43. Carpenito C, Milone MC, Hassan R, et al. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proc Natl Acad Sci USA.* 2009;106:3360–3365. [PubMed: 19211796]
44. Zhong XS, Matsushita M, Plotkin J, et al. Chimeric antigen receptors combining 4–1BB and CD28 signaling domains augment PI3kinase/AKT/Bcl-XL activation and CD8+ T cell-mediated tumor eradication. *Mol Ther.* 2010;18:413–420. [PubMed: 19773745]
45. Pule MA, Savoldo B, Myers GD, et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. *Nature medicine.* 2008;14(11):1264–1270.
46. 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. [PubMed: 21984804]
47. [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/NCT00085930). Bethesda (MD): National Library of Medicine (US) 2004 6 21 Identifier: NCT00085930, Blood T-Cells and EBV Specific CTLs Expressing GD-2 Specific Chimeric T Cell Receptors to Neuroblastoma Patients (NESTLES); 2018 May 7. Available at: <https://clinicaltrials.gov/ct2/show/NCT00085930>.
48. Omer B, Shum T, Parikh K, et al. Optimizing GD2 Directed T Cells for The Treatment of Neuroblastoma. In: *Proceedings from the Advances in Neuroblastoma Research*; May 9–12, 2018; San Francisco, CA Abstract 47.
49. [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/NCT01953900). Bethesda (MD): National Library of Medicine (US) 2013 10 1 Identifier: NCT01953900, iC9-GD2-CAR-VZV-CTLs/Refractory or Metastatic GD2-positive Sarcoma and Neuroblastoma (VEGAS); 2018 Sept 25. Available at: <https://clinicaltrials.gov/ct2/show/NCT01953900>.
50. Ahmed M, Cheung NK. Engineering anti-GD2 monoclonal antibodies for cancer immunotherapy. *FEBS Lett.* 2014 1 21;588(2):288–97. [PubMed: 24295643]
51. Lode HN, Reisfeld RA, Handgretinger R, et al. Targeted Therapy with a Novel Eneidiyene Antibiotic Calicheamicin Theta(I)1 Effectively Suppresses Growth and Dissemination of Liver Metastases in a Syngeneic Model of Murine Neuroblastoma. *Cancer Res.* 1998;58(14):2925–2928. [PubMed: 9679947]
52. Maier FC, Schmitt J, Maurer A, et al. Correlation between positron emission tomography and Cerenkov luminescence imaging in vivo and ex vivo using ⁶⁴Cu-labeled antibodies in a neuroblastoma mouse model. *Oncotarget.* 2016;7(41):67403–67411. [PubMed: 27602580]
53. Yeh SDJ, Larson SM, Burch L, et al. Radioimmunodetection of neuroblastoma with iodine-131–3F8: Correlation with biopsy, iodine-131-metaiodobenzylguanidine and standard diagnostic modalities. *J Nuclear Med.* 1991;32(5):769–776.
54. Dauer LT, St. Germain J, Williamson MJ, et al. Whole-body clearance kinetics and external dosimetry of 131I-3F8 monoclonal antibody for radioimmunotherapy of neuroblastoma. *Health Phys.* 2007 1;92(1):33–9. [PubMed: 17164597]

55. Kramer K, Humm JL, Souweidane MM, et al. Phase I Study of Targeted Radioimmunotherapy for Leptomeningeal Cancers Using Intra-Ommaya 131-I-3F8. *J Clin Onc.* 2007;25(34):5465–5470.
56. Cheal SM, Xu H, Guo HF, et al. Preclinical evaluation of multistep targeting of diasialoganglioside GD2 using an IgG-scFv bispecific antibody with high affinity for GD2 and DOTA metal complex. *Mol Cancer Ther.* 2014 7;13(7):1803–12. [PubMed: 24944121]
57. Morris ZS, Guy EI, Francis DM, et al. In situ Tumor Vaccination by Combining Local Radiation and Tumor-specific Antibody or Immunocytokine Treatments. *Cancer Research.* 2016;76:3929–3941. [PubMed: 27197149]
58. Morris ZS, Guy EI, Werner LR, et al. Tumor-specific inhibition of in situ vaccination by distant untreated tumor sites. *Cancer Immunology Research.* 2018; 6:825–834. [PubMed: 29748391]
59. Voeller J, Erbe AK, Rasmussen K, et al. Local radiation with intratumoral anti-diasialoganglioside (anti-GD2) and interleukin-2 (IL2) induces significant tumor responses with immunologic memory in a syngeneic murine NXS2 neuroblastoma model. Accepted for 33rd Annual Society for Immunotherapy of Cancer, 2018 Nov 7–11.
60. Kushner BH, Cheung IY, Modak S, et al. Phase I trial of a bivalent gangliosides vaccine in combination with β -glucan for high-risk neuroblastoma in second or later remission. *Clin Cancer Res.* 2014 3 1;20(5):1375–82. [PubMed: 24520094]
61. Cheung I, Kushner B, Modak S, et al. Phase II Trial of GD2-KLH/GD3-KLH Vaccine for Stage 4 Neuroblastoma In 2nd Remission: Induced Anti-GD2 Titer Strongly Correlates with Survival. In: Proceedings from the Advances in Neuroblastoma Research; May 9–12, 2018; San Francisco, CA Abstract 116.
62. Yu AL, Eskenazi A, Strother D, et al. A pilot study of anti-idiotypic monoclonal antibody as tumor vaccine in patients with high risk neuroblastoma. In: Proceedings from the 37th Annual American Society of Clinical Oncology Meeting; May 12–15, 2001; San Francisco, CA Abstract 1470.
63. Eger C, Siebert N, Seidel D, et al. Generation and Characterization of a Human/Mouse Chimeric GD2-Mimicking Anti-Idiotypic Antibody Ganglidximab for Active Immunotherapy against Neuroblastoma. *PLOS ONE.* 2016 3;11(3):e0150479. [PubMed: 26967324]
64. Siebert N, Eger C, Seidel D, et al. Pharmacokinetics and pharmacodynamics of ch14.18/CHO in relapsed/refractory high-risk neuroblastoma patients treated by long-term infusion in combination with IL-2. *mAbs.* 2016;8(3):604–616. [PubMed: 26785755]
65. Hank JA, Gan J, Ryu H, et al. Immunogenicity of the Hu14.18-IL2 Immunocytokine Molecule in Adults with Melanoma and Children with Neuroblastoma. *Clin Cancer Res.* 2009 9 15;15(18): 5923–30. [PubMed: 19737959]
66. Cheung NK, Guo HF, Heller G, et al. Induction of Ab3 and Ab3' antibody was associated with long-term survival after anti-G(D2) antibody therapy of stage 4 neuroblastoma. *Clin Cancer Res.* 2000 7;6(7):2653–60. [PubMed: 10914706]
67. Cheung IY, Lo Piccolo MS, Kushner BH, et al. Quantitation of GD2 synthase mRNA by real-time reverse transcriptase polymerase chain reaction: clinical utility in evaluating adjuvant therapy in neuroblastoma. *J Clin Oncol.* 2003 3 15;21(6):1087–93. [PubMed: 12637475]
68. Schumacher-Kuckelkorn R, Hero B, Ernestus K, et al. Lacking immunocytological GD2 expression in neuroblastoma: report of 3 cases. *Pediatr Blood Cancer.* 2005 8;45(2):195–201. [PubMed: 15800908]
69. Schumacher-Kuckelkorn R, Volland R, Gradehandt A, et al. Lack of immunocytological GD2 expression on neuroblastoma cells in bone marrow at diagnosis, during treatment, and at recurrence. *Pediatr Blood Cancer.* 2017;64(1):46–56. [PubMed: 27654028]
70. Dicciani M, Gilman A, Ozkaynak F, et al. Impact of Immune Cell Profiles on High-Risk Neuroblastoma Treated with A COG Phase III Randomized Immunotherapy Study. In: Proceedings from the 37th Annual American Society of Clinical Oncology Meeting; May 12–15; San Francisco, CA Abstract 376.
71. Venstrom JM, Zheng J, Noor N, et al. KIR and HLA genotypes are associated with disease progression and survival following autologous hematopoietic stem cell transplantation for high-risk neuroblastoma. *Clin Cancer Res.* 2009 12 1;15(23):7330–4. [PubMed: 19934297]
72. Tarek N, Le Luduec JB, Gallagher MM, et al. Unlicensed NK cells target neuroblastoma following anti-GD2 antibody treatment. *J Clin Invest.* 2012 9;122(9):3260–70. [PubMed: 22863621]

73. Erbe AK, Wang W, Carmichael L, et al. Neuroblastoma Patients' KIR and KIR-Ligand Genotypes Influence Clinical Outcome for Dinutuximab-based Immunotherapy: A Report from the Children's Oncology Group. *Clin Cancer Res.* 2018 1 1;24(1):189–196. [PubMed: 28972044]
74. Lode HN, Troschke-Meurer S, Valteau-Couanet D, et al. Correlation of killer-cell Ig-like receptor (KIR) haplotypes and Fc γ -receptor polymorphisms with survival of high-risk relapsed/refractory neuroblastoma patients treated by long-term infusion of anti-GD2 antibody ch14.18/CHO. *J Clin Onc.* 2016;34:15_suppl,10548–10548.
75. Suzuki M and Cheung NK. Disialoganglioside GD2 as a therapeutic target for human diseases. *Expert Opin Ther Targets.* 2015;19(3):349–62. [PubMed: 25604432]

TABLE 1

Current Immunotherapy Strategies for NBL Using Anti-GD2 mAb

Augmenting the Immunotherapeutic Effect of GD2		
Ch14.18 + IL2 + GM-CSF	COG ⁺	Phase III ²
Ch14.18 + lenalidomide + retinoic acid	NANT ⁺⁺	Phase I ²³
Ch14.18 + irinotecan + temozolomide + GM-CSF	COG	Phase II ²⁴
Hu14.18K322A + induction chemotherapy	St Jude	Phase II ²⁶
I4.G2a + vorinostat		Preclinical
Hu14.18-IL2 + expanded haploidentical NK cells	UW Madison [¥]	Phase I ²⁸
Ch14.18 + lenalidomide + expanded autologous NK cells	NANT	Phase I ²⁹
Ch14.18 + galunisertib + human ex vivo activated NK cells		Preclinical ³⁰
Ch14.18 + activated NK cells by plasmacytoid dendritic cells		Preclinical ³¹
Ch14.18 + temozolomide + expanded $\gamma\delta$ T cells		Preclinical ³²
Ch14.18/CHO + PD-L1/PD1		Preclinical ³³
Ch14.18/CHO + nivolumab (anti-PD1) + ¹³¹ I-MIBG	Multicenter	Phase I ¹⁸
3F8 + GM-CSF +/- cis-retinoic acid	MSKCC ^{¥¥}	Phase II ^{8,9}
Hu3F8 + GM-CSF	MSKCC	Phase II ¹⁰
Hu14.18-IL2 (intratumoral) + local radiotherapy		Preclinical
Targeted Therapies Against GD2		
Anti-GD2 CARs Co-stimulation with CD28, 4-1BB, OX40, IL7 Expressed in EBV-CTLs and ATCs	BCM [¥]	Preclinical Phase I ^{46,47}
Anti-GD2 vaccines GD2-KLH/GD3-KLH mAb1A7 Ganglidiomab	MSKCC	Preclinical ^{50,51} Phase I and II ^{60,61}
Radiolabeled GD2 With Diagnostic and Therapeutic Applications		
⁶⁴ Cu-labeled ch14.18		Preclinical
¹³¹ I-3F8 scintigraphy	MSKCC	Preclinical, clinical ^{54,55}
Hu3F8-C825 + DOTA		Preclinical
Diagnostic Tools to Predict Response to anti-GD2 Immunotherapy		
Anti-therapeutic antibodies (HACA, HAMA, HABA)		
GD2 synthase mRNA		
Cytokine levels (CXCL9, IL1-RA, INF γ)		
Pre-existing anti-glycan antibodies		
KIR/KIR-ligand genotyping		
FcR genotyping		

⁺ Children's Oncology Group⁺⁺ New Approaches to Neuroblastoma Therapy[¥] University of Wisconsin, Madison

[¶]Memorial Sloan Kettering Cancer Center

^YBaylor College of Medicine.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript