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Anti-GD2 Antibody Therapy for GD2-expressing Tumors

Fariba Navid^{1,*}, Victor M. Santana¹, and Raymond C. Barfield²

¹Department of Oncology, St. Jude Children's Research Hospital, Memphis, Tennessee;
Department of Pediatrics, College of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee

²Division of Pediatric Hematology/Oncology, Department of Pediatrics, Duke University, Durham, North Carolina.

Abstract

In the development of novel immune therapies for high-risk cancers, one goal is to find tumor targets that are not widely shared by normal cells. One such target is the surface disialoganglioside GD2. This antigen is expressed on the surface of a variety of tumors for which no curative therapies exist for patients with advanced disease. In childhood, the most common GD2-expressing tumor is neuroblastoma. GD2 is also expressed on several other high-risk tumors, including those of neuroectodermal or epithelial origin, virtually all melanomas, and approximately 50% of tumor samples from osteosarcoma and soft-tissue sarcomas. Because of the tumor-selective expression of this molecule, it is an attractive target for tumor-specific therapies such as antibody therapy. Over the last 2 decades, several anti-GD2 antibodies have been developed. To reduce both the toxicity of the antibody and the development of human anti-mouse antibodies (HAMA), research efforts have primarily focused on exploring anti-GD2 antibodies that have progressively more human elements while at the same time reducing the mouse components. This review will examine antibodies currently undergoing clinical testing as well as the most recent advances to improve antibody therapy for patients with GD2-expressing tumors.

Keywords

monoclonal antibody; disialoganglioside; neuroblastoma; melanoma

INTRODUCTION

Anticancer monoclonal antibodies (mAbs) targeting specific antigens on the tumor surface are increasingly being applied in the treatment of solid and hematologic malignancies. Potential advantages include the long half-life, low toxicity, and high affinity and specificity. Several anticancer mAbs (e.g., cetuximab, bevacizumab, herceptin and rituximab) have been approved by the U.S. Food and Drug Administration, and many more are in development.

Mechanisms of action for the anti-tumor effect of monoclonal antibodies can be either dependent or independent of the immune system. Immune-mediated mechanisms include antibody-dependent, cell-mediated cytotoxicity (ADCC); complement-dependent cytotoxicity; and the ability of mAbs to alter the cytokine milieu or enhance development of an active anti-tumor immune response. Non-immune-mediated effects include blocking a survival signal for the cancer cell. Antibodies may also be used as targeting agents. When

*Correspondence to: Fariba Navid, MD St. Jude Children's Research Hospital 262 Danny Thomas Place Memphis, Tennessee 38105
Phone (901) 595-2573 Fax (901) 521-9005 fariba.navid@stjude.org..

linked to drugs, radioisotopes, and toxins they can kill tumors cells by delivering these agents at high concentrations directly to the tumor [1,2].

Disialoganglioside GD2 is a sialic acid-containing glycosphingolipid expressed primarily on the cell surface. The function of this carbohydrate antigen is not completely understood; however, it is thought to play an important role in the attachment of tumor cells to extracellular matrix proteins [3]. GD2 expression in normal fetal and adult tissues is primarily restricted to the central nervous system, peripheral nerves, and skin melanocytes [4-7], although GD2 expression has been described in the stromal component of some normal tissues and white pulp of the spleen [7,8]. In malignant cells, GD2 is uniformly expressed in neuroblastomas and most melanomas and to a variable degree in a variety of other tumors, including bone and soft-tissue sarcomas, small cell lung cancer, and brain tumors [4,9-13]. Because of the relatively tumor-selective expression combined with its presence on the cell surface, GD2 is an attractive target for tumor-specific antibody therapy. Several anti-GD2 antibodies have been developed for clinical use over the past 2 decades and are the subject of this review. Schematic representation of the structure of various genetically engineered antibodies is provided in Fig. (1) as a reference.

Anti-GD2 Antibodies Developed for Human Clinical Trials

Murine Anti-GD2 Antibodies

3F8: 3F8 is a murine IgG3 monoclonal anti-GD2 antibody developed by Cheung and colleagues [14] primarily to target neuroblastoma cells. This antibody has undergone extensive preclinical and clinical testing. In vitro studies suggest that the mechanism of tumor cell killing by 3F8 is mediated by human complement [14] and other human immune effector cells, including lymphocytes [15], neutrophils [16], and monocytes [17]. Published results of phase I and II clinical trials evaluating 3F8 as a single agent are summarized in Table I.

In phase I testing of 3F8, dosages of 5, 20, 50, and 100 mg/m² administered over 8 hours daily over 2-4 days have been evaluated. Hypertension was dose-limiting at 100 mg/m². Other toxicities observed with 3F8 administration included severe pain, fever, and urticaria. Pharmacokinetic analysis showed that mean serum concentrations of 3F8 increase with increasing dose administered. Two of 8 patients with neuroblastoma had a complete response (CR), and 2 of 9 patients with melanoma had a partial response (PR). All patients tested developed human anti-mouse antibodies (HAMA) to 3F8. Those patients with HAMA levels > 1000 U/ml had minimal side effects and no therapeutic benefit from 3F8 [18]. In a phase II trial of 3F8, 16 patients with refractory or recurrent neuroblastoma received 10 mg/m² doses of antibody daily for 5 days. Toxicities observed included pain, fever, urticaria, hypertension, hypotension, and anaphylactoid reactions. One of 13 patients evaluable for response had a CR. Three participants were alive without systemic therapy beyond 3F8 at 79 to 130+ months [19]. The limited antitumor activity seen in the initial phase I and phase II studies prompted further evaluation of 3F8 in a setting of minimal residual disease [20]. Thirty-four patients, 23 in CR, 8 in very good PR, and 1 in PR by conventional methods according to the International Neuroblastoma Staging System [21] were treated with 3F8 10 mg/m² doses over 90 minutes daily for 5 consecutive days for up to 4 courses based on disease status and HAMA titers. Thirteen patients remained disease-free 40 to 130 months after the first 3F8 treatment. Eleven of those patients had disease confirmed by either conventional methods (n=3) or non-conventional methods (n=8) at the start of antibody therapy. Non-conventional methods included immunoscintigraphy using 3F8 radiolabeled with iodine-131 (¹³¹I-3F8), bone marrow immunocytology, and molecular detection of the residual neuroblastoma marker GAGE by reverse transcriptase polymerase chain reaction (RT-PCR) in the bone marrow. In the entire cohort of 34 patients, evidence of response by immunocytology was noted in 6 of 9

patients, by GAGE RT-PCR in 7 of 12, and by ^{131}I -3F8 immunoscintigraphy in 6 of 6 patients. These findings suggest benefit from antibody therapy in the setting of minimal disease burden.

Strategies to augment the immune system to enhance the anti-tumor effects of 3F8 have included combining 3F8 with barley-derived β -glucans [22], interleukin 2 (IL-2) [15], and granulocyte-macrophage colony-stimulating factor (GM-CSF) [16]. GM-CSF enhances phagocyte-mediated antibody-dependent cellular cytotoxicity. 3F8 plus GM-CSF was evaluated in 45 patients with high-risk neuroblastoma. The side effects of this combination were manageable and appeared to benefit patients with bone marrow disease but was ineffective in patients with progressive disease and soft-tissue masses [23]. The investigators of this trial studied polymorphic alleles in the FCGR2A gene that encodes the Fc γ receptors (mediates ADCC and complement-dependent cytotoxicity) in patients who received 3F8 plus GM-CSF (N=136) [24]. Patients with the FCGR2A-R/R genotype had a better outcome than those with FCGR2A-R/H, FCGR2A-H/H, or FCGR3A genotype. A similar analysis in a smaller cohort of patients who received 3F8 alone did not show an improved outcome in patients with a specific polymorphism of FCGR. This finding suggests that the effects of FCGR2A polymorphism on outcome are probably due to the addition of GM-CSF in combination with the 3F8 antibody. A better understanding of the molecular basis for the activity of monoclonal antibodies in the presence or absence of cytokines may not only facilitate patient selection for these therapies but shed light on ways to improve cytotoxicity.

^{131}I -3F8 has been used for imaging of neuroblastoma. 3F8 does not cross the blood-brain barrier when administered systemically. Applications of this antibody for central nervous system disease is limited to direct administration of the antibody into the cerebrospinal fluid. The safety of intrathecal administration of ^{131}I -3F8 has been evaluated in patients with central nervous system and leptomeningeal malignancies. Fifteen patients were treated. The dose-limiting toxicity was increased intracranial pressure and chemical meningitis. Other transient side effects included headache, fever, and vomiting. Three of 13 evaluable patients had an objective radiographic and/or cytologic response [25].

14G2a: 14G2a is a murine monoclonal anti-GD2 antibody unrelated to 3F8. This antibody is an IgG2a-class switch variant of 14.18, an anti-GD2 antibody of an IgG3 isotype that can suppress the growth of human neuroblastoma and melanoma cells in athymic mice [4,26]. 14.18 never entered clinical testing because the switch variant, 14G2a, showed significantly better ADCC both in vitro and in vivo [26]. The 14G2a mAb has also undergone clinical evaluation. These studies are summarized in Table 1. The first phase I trial of 14G2a mAb was conducted in 18 patients with refractory melanoma, osteosarcoma or neuroblastoma. 14G2a was administered as a 120-hour continuous intravenous infusion. Dose-limiting toxicities were observed at 40 mg/m²/day for 5 days, including diarrhea, hypotension, and allergic reactions (fever, rash, dyspnea, and hypoxia). Pain was a major side effect in all patients treated. Two patients developed slowly reversible hyponatremia and postural hypotension. Evaluation of the hyponatremia could not distinguish between salt-losing nephropathy and the syndrome of inappropriate anti-diuretic hormone (SIADH) in either case. The hypotension was attributed to autonomic dysfunction in both cases. No other objective neurologic abnormalities were described in the study. The maximum tolerated dose (MTD) was 20 mg/m²/day for 5 days. Two PR (both with neuroblastoma) and 3 mixed responses (with 2 melanoma and 1 osteosarcoma) were observed [27].

An alternate dosing schedule using 14G2a was evaluated in 12 adult patients with melanoma. These patients received a 1-hour intravenous infusion of 14G2a mAb (total dose, 10-120 mg) on days 1, 3, 5, and 8 [28]. Patients (n=10) treated with 60 mg or 120 mg (total dose) experienced severe side effects including abdominal/pelvic pain requiring morphine sulfate infusions during antibody administration. Five patients had delayed neuropathic pain in the extremities.

One patient became confused, and hyponatremia consistent with SIADH developed in 2, 1 of whom had seizure as a complication of the low serum sodium. Within a week of finishing the 14G2a, both patients had complete resolution of the hyponatremia. Three patients receiving 60-120 mg of 14G2a developed sensorimotor neuropathy. Nerve conduction studies performed in these patients were consistent with demyelination. The neuropathy persisted for several weeks after antibody administration, but fully resolved in 2 patients, with persistent dysesthesia remaining in the third. The onset of neuropathy was typically within 48-72 hours after the antibody dose on day 8. Interestingly, 4 of 5 patients who developed neurologic side effects had a > 25% reduction in total hemolytic complement during therapy. All patients enrolled on this study developed high HAMA titers by day 22. There was no correlation between the degree of immune response and the dose of antibody. One patient had a mixed response, and 1 had a PR.

The pharmacokinetics of 14G2a were evaluated in the pediatric and adult clinical studies described above. The plasma clearance of 14G2a was determined to be biphasic and dose-dependent with a shorter β half-life in pediatric patients than in adults, 18.3 ± 11.8 hours versus 62 ± 20 hours, respectively [27,29]. In patients treated with a second course of antibody therapy, a significant decrease in the terminal half-life of the antibody was noted.

Based on preclinical studies demonstrating an increase in ADCC of tumor cells in response to antibody treatment when IL-2 or GM-CSF is administered to enhance effector cell function [15,17,30], a phase I study of 14G2a with either IL-2 alone or with both IL-2 and GM-CSF was undertaken in the Children's Oncology Group [31]. Accrual to the regimen containing both IL-2 and GM-CSF was discontinued when adult patients receiving this combination of cytokines developed neurologic toxicity coinciding with hyperleukocytosis. In combination with IL-2, the MTD in children of 14G2a was 15 mg/m^2 as a 2-hour infusion daily for 5 days. Dose-limiting toxicities included grade 4 thrombocytopenia, hyperbilirubinemia, diarrhea, neutropenia, bronchospasm, tachycardia, hypotension, angioedema, and generalized pain. One patient with neuroblastoma had a PR, and 1 patient with osteosarcoma had a CR. Nine of 21 patients developed a HAMA response.

Although administration of 14G2a proved to be feasible and antitumor effects were encouraging, one of the drawbacks of this and other murine mAbs for human use is the development of HAMA titers that limit further antibody therapy and potentially contribute to observed hypersensitivity reactions. These concerns and new advances in genetic engineering prompted the development of a human-mouse chimeric anti-GD2 antibody.

Human-Mouse Chimeric Anti-GD2 Antibodies

Ch14.18: Ch14.18 antibody consists of the Fab portion of the murine 14G2a antibody fused with the human Fc constant regions of an IgG1 immunoglobulin [32]. This antibody retains the anti-GD2 specificity and the ability to target GD2-positive tumors. Further, ch14.18 mediates tumor ADCC in vitro 50-100 times more efficiently than the murine 14G2a [33].

Clinical trials of single-agent ch14.18 are summarized in Table 2. Phase I testing of ch14.18 was first performed in 13 adult patients with metastatic melanoma [34]. Ch14.18 was administered as either a single dose of 5 mg to 45 mg (intravenous over 4 hours) or as 2 daily doses of 50 mg each (100 mg total). The 50 mg dose was chosen as the maximum daily dose because abdominal/pelvic pain during antibody infusion precluded the use of higher doses. This pain was severe in 3 of 4 patients receiving 100 mg but was also observed in patients receiving 15 mg or 45 mg of antibody. No other neurologic side effects occurred, and no other severe toxicity was noted. Eight of the 13 patients developed antibodies to ch14.18, but the observed titers were only about 10% as great as those detected in the trials of murine 14G2a. Anti-idiotypic and anti-isotype antibodies were both detected. Although no antitumor responses

occurred, antibody was detected on tumor cells through fluorescence-activated cell sorting analysis in some of the patients treated with ≥ 45 mg of ch14.18.

In a pediatric phase I trial, 9 children (ages 2-10 years) with neuroblastoma received up to 50 mg/m² of ch14.18 for 5 days [35]. Pain was the most common side effect during treatment and was most pronounced at 50 mg/m². Thus, the investigators considered 50 mg/m² the maximum tolerated dose. Other side effects included fever, urticaria, pruritus, and rash. One patient developed transient pupillotonia at the highest dose level. Optic nerve atrophy was observed in 2 patients, both of whom had received prior radiotherapy that was implicated in the adverse event. This toxic effect gradually resolved in the 6 months after therapy with ch14.18. HAMA was not detected in any of the participants in this study. Two of the participants had a CR, 2 a PR, and 1 a minor response.

An alternate dosing schedule of ch14.18 was tested in the phase I setting in 11 patients (10 with neuroblastoma and 1 with osteosarcoma). A total of 20 courses of ch14.18 were administered at doses of 10, 20, 50, 100, and 200 mg/m². The antibody was given as a 1-hour infusion for doses less than 50 mg/m², as a single infusion at 10-20 mg/m²/hour for a dose of 50 mg/m², and was fractionated to be given over 2-4 days for doses greater than 100 mg/m². The most common toxicities were pain, tachycardia, hypertension, fever and urticaria. An MTD was not established in this study, and the dosage was not further escalated because the supply of antibody had been exhausted. However, at the highest dose levels tested of 100 mg/m² and 200 mg/m² (total doses over 2-4 days), pain was graded as severe in 5 of 7 patients. Serum sickness was observed in 2 of 4 patients who received 200 mg/m². Repeated antibody administration (time interval of every 2-3 weeks) was not associated with increased toxicity. Anti-ch14.18 immune response was detectable in 7 of 10 patients. Of 10 patients evaluable for response, 1 had a PR, 3 mixed responses, and 1 stable disease [36].

Pharmacokinetic analysis of ch14.18 performed in the pediatric phase I study described above revealed that the area under the curve (AUC) for ch14.18 correlated strongly with dose. The higher the dose of antibody delivered, the higher the peak serum concentration. Further, similar to its murine counterpart, 14G2a, plasma clearance of the ch14.18 was biphasic and was significantly shorter in children than in adults. After the first course of treatment, the terminal half-life ($t_{1/2}$) alpha was 3.4 ± 3.1 hours and $t_{1/2}$ beta was 66.6 ± 27.4 hours. The $t_{1/2}$ beta decreased significantly from 72.9 ± 19.8 hours to 31.7 ± 18.4 hours in 5 patients who received a second course of antibody [37]. Three of those 5 patients also had a significant anti-chimeric-antibody response, suggesting that the rapid clearance may have been in part due to the formation of immune complexes cleared by the reticuloendothelial system.

Ch14.18 was administered in a subset of newly diagnosed patients with high-risk neuroblastoma during the maintenance phase of treatment in the Cooperative German Neuroblastoma Trials NB90 and NB97.[38]. These trials included 334 evaluable patients, of whom 166 received mAb ch14.18, 99 received a 12-month course of low-dose maintenance chemotherapy, and 65 had no further treatment after initial therapy. The mAb was administered over a period of 1 year at a schedule of 20 mg/m²/day for 5 days every 2 months (6 courses total). A total of 695 courses of mAb ch14.18 were administered, and the main toxicities were fever, abnormal C-reactive protein without infection, cough, rash, and pain. Three patients developed a capillary leak syndrome, and 7 required oxygen therapy during a mAb course. Ten of 85 patients treated with antibody on the NB97 developed mydriasis and accommodation deficits [39]. These symptoms were detected up to 2 months after therapy and lasted for 2-12 months. There was no statistically significant difference in event-free survival among patients treated with mAb ch14.18, maintenance chemotherapy, or no therapy after initial treatment. However, overall survival was better in the ch14.18 group (3-year overall survival, $68.5 \pm$

3.9%) than in groups receiving maintenance chemotherapy (3 year overall survival, $56.6 \pm 5\%$) or no further therapy (3-year overall survival, $46.8 \pm 6.2\%$).

Similar to other antibodies, ch14.18 has been evaluated in combination with GM-CSF and IL-2 in a number of clinical trials. In a phase I study of 24 adult patients with melanoma, the MTD of ch14.18 was $7.5 \text{ mg/m}^2/\text{day}$ daily for 5 consecutive days in combination with IL-2 continuous infusion $1.5 \text{ million units/m}^2/\text{day}$ for 4 days/week for 3 weeks. Dose-limiting toxicities were severe allergic reaction in 1 patient and weakness, pericardial effusion, and decreased performance status in another. One patient achieved a CR, 1 a PR, and 8 patients stable disease. Immunologic changes associated with this treatment were observed in all patients and included a treatment-related increase in lymphocyte count, induction of lymphokine-activated killer activity, and ADCC by peripheral blood mononuclear cells isolated from these patients [40]. Phase I evaluation in the Children's Oncology Group of ch14.18 administered in combination with GM-CSF was performed in 19 neuroblastoma patients after autologous stem cell transplantation. The MTD of ch14.18 in this combination was $40 \text{ mg/m}^2/\text{day}$ daily for 4 days with GM-CSF ($250 \text{ } \mu\text{g/m}^2/\text{day}$) started 3 days before and continued 3 days after [41]. Dose-limiting toxicities were recurrent urticaria, intractable neuropathic pain, and vomiting. Ten of the 19 patients experienced disease progression, with a median follow-up of 40 months (range, 25-50 months). In a phase II study (POG9347) ¹, ch14.18 at $50 \text{ mg/m}^2/\text{day}$ daily for 4 days and GM-CSF $10 \text{ } \mu\text{g/m}^2/\text{day}$ for 14 days was evaluated. Among 27 patients with refractory or recurrent neuroblastoma evaluable for response, one patient had a CR, 3 had a PR and 2 had stable disease. Patients with tumor response had an increase in neutrophil-mediated ADCC to > 20 lytic units; whereas, the majority of patients with progressive disease had ADCC activity of < 20 lytic units.

The manageable toxicities of ch14.18 combined with cytokines IL-2 and GM-CSF in the above studies prompted a pilot feasibility study [42] followed by a phase III randomized clinical trial in the Children's Oncology Group for patients with newly diagnosed high-risk neuroblastoma who achieved a CR or PR to induction chemotherapy. Patients in this latter study were randomized to receive standard maintenance therapy with cis-retinoic acid versus cis-retinoic acid plus ch14.18 in combination with IL-2 alternating with GM-CSF. Randomization was stopped early because interim monitoring revealed a significant 2-year overall survival ($86\% \pm 4\%$ versus $75\% \pm 5\%$, $p=0.0223$) and event-free survival ($66\% \pm 5\%$ versus $46\% \pm 5\%$, $p=0.0115$) advantage for patients receiving cis-retinoic acid plus ch14.18 and cytokine therapy versus those receiving cis-retinoic acid alone.² Based on these results, considerable effort is being put forth to provide ch14.18 to patients with high risk neuroblastoma in the United States.

Immunocytokines—Efforts to enhance the efficacy of antibody therapy and minimize the systemic toxicities associated with the addition of cytokine therapy have led to the development of “immunocytokines,” or fusion antibodies in which the cytokine is linked to the Fc end of the monoclonal antibody. In effect, the antibody binds to the target of interest on the tumor cell and delivers high concentrations of the cytokine directly to the tumor microenvironment to attract the immune effector cells required for tumor cell kill. Ch14.18 was initially linked to IL-2 (ch14.18–IL-2). This fusion protein was shown to retain the binding specificity of ch14.18 and stimulate proliferation of IL-2–responsive cells by the IL-2 component [43,44]. In a GD2-positive murine neuroblastoma and melanoma models, ch14.18–IL-2 eradicated metastatic

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neuroblastoma more efficiently than antibody administered with exogenously delivered IL-2 [45-47].

In an effort to reduce immunogenicity, a humanized hu14.18 (98% human derived) was genetically linked to IL-2 (hu14.18-IL-2, EMD 273063). One milligram of fusion protein contains approximately 0.8 mg of the hu14.18 antibody and approximately 3×10^6 U of IL-2. Clinical trials evaluating this molecule in children and adults are summarized in Table 3. The initial phase I study was performed in 33 adults with melanoma [48]. Dose levels tested ranged from 0.8-7.5 mg/m²/day given as a 4-hour infusion daily for 3 days every 28 days (1 course). Nineteen patients received 2 courses of therapy. Common toxicities included fever and chills (100%), pruritus (61%), hyperglycemia (55%), hypophosphatemia (39%), and transient neuropathic pain (39%). Dose-limiting toxicities included hypoxia and hypotension, which were believed to be IL-2 related. The MTD was determined to be 7.5 mg/m²/day. This is similar to the MTD of ch14.18 (7.5 mg/m²/dose) when given with IL-2 (equivalent to ~6.5 mg/m²/dose of hu14.18 in the fusion protein). Thus, the fusion protein did not ameliorate the systemic effects of IL-2 and limited the dose of antibody delivered to the patient. No objective responses were observed in this study. However, prolonged disease stabilization was noted in 4 patients with high-risk disease. Pharmacokinetic analysis showed that the overall mean half-life of hu14.18-IL-2 was 3.7 ± 0.9 hours. The peak serum levels and AUC showed significant dose-dependent increases. Immunologic changes included increases in lymphocyte count, NK cell count, NK lysis, and ADCC. Sixteen of 33 patients developed anti-idiotypic antibody. The clinical significance of this finding is unclear.

Pediatric phase I testing of hu14.18-IL-2 was conducted through the Children's Oncology Group [49]. Twenty-seven patients with neuroblastoma and 1 patient with melanoma were treated. Dosages tested ranged from 2-14.4 mg/m²/dose daily for 3 days as a 4-hour infusion every 28 days (1 course). Up to 4 courses of therapy were permitted. The MTD was 12 mg/m²/dose. The investigators of this study suggested that the higher MTD in pediatric patients may be related to more intensive prior immunosuppressive therapy received. Toxicities were similar to those reported in the adult phase I study. No objective responses were observed. The median half-life was 3.1 hours. There was a significant decrease in half-life, AUC, and peak concentration during the second course of therapy. More than 60% of the patients developed anti-idiotypic antibody to the hu14.18 portion of the antibody and ~50% to the Fc-IL-2 portion. The incidence of these anti-idiotypic responses increased with subsequent courses of therapy.

Phase II testing of hu14.18-IL-2 has been performed in adult patients with advanced melanoma and pediatric patients with neuroblastoma^{3, 4}. In the adult trial, 14 patients were treated with 6 mg/m²/day daily for 3 days every 28 days. Up to 4 cycles of treatment were permitted. One patient achieved a PR and 4 stable disease. In the pediatric study, 39 patients received 12 mg/m²/day for 3 consecutive days every 28 days and were evaluated on the basis of disease measurable by standard radiologic criteria (N=12) or by meta-iodo benzylguanine scanning or bone marrow histology (N=23). No responses were observed by standard radiologic criteria; however, in the other group, 5 patients had a CR, suggesting a benefit of this therapy in the setting of minimal residual disease. Most toxicities were expected and reversible (pain, rash, allergic reaction, fever, and hepatic transaminitis). Of note, 2 patients required dopamine for hypotension, and 1 required ventilatory support for capillary leak syndrome and hypoxia.

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⁴Albertini, M. R.; Hank, J. A.; Schalch, H.; Kostlevy, J.; Cassaday, R.; Gan, J.; Kim, K.; Clemets, B.; Gillies, S. D.; Sondel, P. M. Phase II Trial of hu14.18 – IL2 (EMD 273063) for Patients with Metastatic Melanoma. *Proc. Am. Soc. of Clin. Oncol.* **2008**, 26, Abstract 9039.

Future Directions—Anti-GD2 antibodies have shown promising anti-tumor activity in adult and pediatric patients with GD2-expressing tumors, primarily neuroblastoma and melanoma. However, despite advances in antibody technologies to humanize these antibodies and the addition of cytokines to boost immune effector cell responses, optimization of the therapeutic potential of these antibodies has yet to be achieved. Strategies to improve the efficacy of these antibodies have included GD2-targeted liposomes [50-53] and anti-GD2 antibodies in combination with novel chemokines [54] and cytokines [55], as well as further modifications in the structure of the antibody.

A major breakthrough in antibody engineering has been the derivation of single-chain molecules called scFv fragments and dimeric single-chain antibodies called minibodies, or small immunoproteins (SIPs). ScFv fragments consist of the variable heavy chain and the variable light chain joined by a flexible linker. These molecules maintain their antigen-specificity, yet, because of their size, can extravasate more efficiently than an IgG molecule and diffuse more readily within tumors but are also rapidly eliminated through the kidneys [56]. These molecules can be conjugated to toxins, radioisotopes, or effector molecules. Pretargeting of neuroblastoma xenografts in mice with an anti-GD2 scFv fragment ligated to streptavidin (5F11-scFv-SA) has been shown to improve the tumor-to-nontumor ratio of biotinylated radionucleotides and polypeptides [57]. SIPs that are constructed by connecting an scFv to the dimerizing domain of human immunoglobulin γ 1 chain penetrate tissues better than IgG molecules, yet have a slower clearance than scFv. Two anti-GD2 SIPs have been generated: one is a fully murine molecule containing the CH3 domain of mouse IgG1, and the second is a hybrid mouse-human molecule containing the CH4 domain of human IgE [58]. If the kinetics of these molecules prove to be favorable, these molecules alone or conjugated to other molecules may improve the therapeutic index of GD2-targeted therapies.

Alterations in antibody structure to reduce undesirable immune effects and enhance desirable antitumor effects have also been developed. At our institution, we are currently evaluating the safety in children and young adults with refractory/recurrent neuroblastoma or melanoma of a modified version of the hu14.18 antibody (hu14.18K322A), which has a single point mutation in the CH2 domain of the antibody. This region of the antibody has been shown to be critical for antibody-dependent complement activation [59]. Minimizing complement activation may ameliorate some of the side effects observed in patients receiving 14.18 antibodies, including pain, fever, rash, and capillary leak syndrome, which are probably due to increased production of inflammatory peptides such as C3a and C5a. These toxicities limit the dose of antibody that can be given. One potential drawback of decreasing complement activation is the potential to reduce the antitumor response of the antibody. However, Imai and colleagues [60] have shown in GD2-expressing–tumor-bearing wild-type, complement-deficient, complement-receptor–deficient, and Fc γ receptor I/III-deficient mice treated with anti-GD2 antibody 14G2a that the absence of complement does not appear to affect the anti-tumor effect of anti-GD2 antibody except at low concentrations of the antibody. By contrast, ADCC (absent in Fc γ receptor I/III-deficient mice) is required for eradication of tumors in the presence of antibody. In addition to the new design elements (i.e., point mutation K322A) incorporated into the hu14.18 molecule, the hu14.18K322A is expressed in a YB2/0 cell line. YB2/0 is a non-immunoglobulin-secreting rat myeloma that was derived from the hybrid rat myeloma YB2/3HL [61,62]. Due to the decreased fucosylation activity in these cells, anti-human antibodies produced by YB2/0 cell lines have higher levels of ADCC activity than antibodies produced by Chinese hamster ovary cell lines [63,64]; hence, the YB2/0 cells were chosen to produce hu14.18K322A. In vitro, hu14.18K322A demonstrates less complement activation than ch14.18 and has comparable to better dose-dependent ADCC activity than ch14.18 and hu14.18 antibodies (unpublished data), suggesting that this antibody has the potential to be less toxic, allowing for higher doses to be given, and more effective. The development of anti-GD2 antibodies has been challenging; however, the observed antitumor responses have been the impetus for further

research to improve this therapy. Beyond the strategies presented in this review, exploiting the optimal schedule of administration of these antibodies and the timing remain to be determined. As with other antibodies developed for therapeutic use, the promise of antibody therapy lies in treating minimal residual disease. These studies require large numbers of patients over an extended period of time to assess the benefit of treatment in this setting.

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ABBREVIATIONS

ADCC	Antibody-dependent cell-mediated cytotoxicity
AUC	Area under the curve
CNS	Central nervous system
CR	Complete response
GD2	Disialoganglioside
GM-CSF	Granulocyte-macrophage colony stimulating factor
HAMA	Human anti-mouse antibody
IL-2	Interleukin-2
mAb	Monoclonal antibody
MTD	Maximum tolerated dose
NK	Natural killer
PFS	Progression-free survival
PR	Partial response
RT-PCR	Reverse transcriptase polymerase chain reaction
SIADH	Syndrome of inappropriate anti-diuretic hormone
SIPs	Small immunoproteins
T _{1/2}	Terminal half-life

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Figure (1). Schematic structure of genetically engineered antibodies

A. Murine antibody is produced utilizing hybridoma technology following immunization of mice. All components, variable heavy (V_H) and light (V_L) chains and constant heavy (C_H) and light (C_L) chains are mouse derived. Examples of murine anti-GD2 antibodies are 3F8, 14G2a and 14.18. **B.** Chimeric antibody combines the murine antigen-binding variable region to the human constant regions. Example: Ch14.18. **C.** Humanized antibody is created by grafting the complementarity determining-regions of the murine antigen-binding variable regions into the human IgG molecule. Example: Hu14.18 **D.** Humanized fusion antibody has a cytokine (e.g. IL-2) linked to the Fc portion of the humanized antibody. Fusion antibodies have the advantage of attracting effector cells locally without the systemic toxicity of exogenous cytokine. Example: Hu14.18-IL2. **E.** Mutated humanized antibody has a single base mutation (e.g. lysine to alanine in the CH2 region critical for complement activation). Example: Hu14.18K322A. **F.** ScFv, single-chain variable fragment, is a small molecule composed of only a V_H and V_L from an IgG linked by a polypeptide chain. The reduced size allows for better tumor penetration and rapid plasma clearance, features important in developing radiopharmaceuticals. **G.** SIP, small immuno-protien, is composed of scFv fragments connected to the dimerizing CH3 domain of IgG. SIPs have higher tissue penetration than a complete antibody, but higher affinity and slower clearance than the single-chain scFv fragments.

Table 1

Clinical Trials of Single-Agent Murine Anti-GD2 Monoclonal Antibodies

mAb	Trial Design	Disease Type (No. Patients)	Dose	MTD	DLT	Response	Reference
3F8 (IgG3)	Phase I	NB (8) Melanoma (9)	5-100 mg/m ² over 2-4 days	50	HTN	2 CR NB 2 PR melanoma	Cheung et al. 1987 [18]
	Phase II	NB (16)	10 mg/m ² /dose over 90 minutes daily × 5 days	-	-	1 CR NB (of 13)	Cheung et al. 1998 [19]
14G2a (IgG2a)	Phase I	Melanoma (12)	10-120 mg total dose divided days 1, 3, 5, 8.	80	Neurologic toxicity	1 PR melanoma 1 mixed response	Saleh et al. 1992 [28]
	Phase I	NB (9)	100-400 mg/m ² total dose divided daily over 5-10 days	NR	NR	2 CR 2 PR (of 6)	Handgretinger 1992 [65]
	Phase I	OS (2) Melanoma (11) NB (5)	10, 20, 40 mg/m ² /day CI for 5 days	20	Hypotension	2PR NB 3 mixed responses (2 melanoma ; 1 OS)	Murray et al. 1994 [27]

Abbreviations: NB, neuroblastoma; OS, osteosarcoma; CI, continuous infusion; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; PR, partial response; CR, complete response; HTN, hypertension; NR, not reported.

Table 2

Clinical Trials of Single-Agent Anti-GD2 Ch14.18 Antibody

Trial Design	Disease Type (No. of Patients)	Dose and schedule	MTD	Toxicity	Response	Reference
Phase I	Melanoma (13)	5-100 mg 1-2 days	50 mg (daily × 2 days)	Pain	None	Saleh et al. [34]
Phase I	NB (9)	20-50 mg/m ² /dose over 8 hours daily × 5 days	50	Pain (DLT), pruritus, fever, urticaria, exanthem	2 CR NB 2 PR NB	Handgretinger et al. 1995 [35]
Phase I	NB (10) OS (1)	10-200 mg/m ² over 1-4 days every 2-3 weeks (MTD not reached)	Not established	Pain (most common), fever, hypertension, urticaria, serum sickness, and tachycardia	1 PR NB 4 mixed response NB	Yu et al. 1998 [36]
Retrospective review ^d	NB (166)	20 mg/m ² /dose over 8-12 hours daily × 5 days every 2 months × 6 courses	-	Fever, cough, rash, pain, capillary leak syndrome. Ocular symptoms 10 of 85 patients	3-year overall survival improved vs. cis- RA alone vs. no treatment. EFS no difference	Simon et al. 2004 [38]

Abbreviations: NB, neuroblastoma; OS, osteosarcoma; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; PR, partial response; CR, complete response; EFS, event-free survival; cis-RA, cis-retinoic acid.

^dRetrospective analysis of the ch14.18 cycles in the phase III Cooperative German Neuroblastoma Trials NB90 and NB97.

Table 3

Clinical Trials of Single-Agent Hu14.18-IL-2 Fusion Antibody

Trial Design	Disease Type (No. of Patients)	Dose (mg/m ² /day)	MTD (mg/m ² /day)	Toxicity	Response	Reference
Phase I	Melanoma (33)	0.8-7.5	7.5	DLT: hypoxia, hypotension	None	King et al. 2004 [48]
Phase I (COG)	NB (27) Melanoma (1)	2-14.4	12	DLT: allergic reaction, myelosuppression	None	Osenga et al. 2006 [49]
Phase II (COG)	NB (39)	12	-	Hypotension, capillary leak/hypoxia, fever, rash, pain, elevated liver transaminases	5 of 23 CR in BM or MIBG only disease; 0 of 12 in bulky disease	Shusterman et al. 2008 ¹
Phase II	Melanoma (14)	6	-	Oliguria, hypotension	1 PR	Albertini et al. 2008 ²

Abbreviations: COG, Children's Oncology Group; NB, neuroblastoma; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; CR, complete response; PR, partial response; BM, bone marrow histology; MIBG, meta-iodo benzylguanidine scanning.