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Anti-GD2 immunotherapy for neuroblastomas

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Abstract

Introduction: Current therapeutic approaches for high-risk neuroblastoma (HR-NB) include high-dose chemotherapy, surgery and radiotherapy; interventions that are associated with long and short-term toxicities. Effective immunotherapy holds particular promise for improving survival and quality of life by reducing exposure to cytotoxic agents. GD2, a surface glycolipid is the most common target for immunotherapy.

Areas covered: We review the status of anti-GD2 immunotherapies currently in clinical use for neuroblastomas and novel GD2-targeted strategies in preclinical development.

Expert commentary: Anti-GD2 monoclonal antibodies are associated with improved survival in patients in their first remission and are increasingly being used for chemorefractory and relapsed neuroblastoma. As protein engineering technology has become more accessible, newer antibody constructs are being tested. GD2 is also being targeted by natural killer cells and T-cells. Active immunity can be elicited by anti-GD2 vaccines. The rational combination of currently available and soon-to-emerge immunotherapeutic approaches, and their integration into conventional multimodality therapies will require further investigation to optimize their use for HR-NB.

Keywords

neuroblastoma; immunotherapy; monoclonal antibodies; anti-cancer vaccines; adoptive immunotherapy; GD2

1. Introduction

Neuroblastoma is the most common extracranial solid tumor of childhood accounting for 8% of all childhood cancers. More than 650 cases are diagnosed each year in North America where the incidence is 10.5 cases/million/year in children younger than 15 years. 90% of patients are younger than 5 years at diagnosis. Prognostic features include age at diagnosis, tumor stage, presence of absence of *MYCN* amplification, tumor histology and DNA index. [1] Approximately 55% of patients have metastatic disease at diagnosis. Patients with stage

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Declaration of interests

S Modak is a paid consultant for Y-mabs Therapeutics Inc.; The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

4 disease who are older than 18 months of age at diagnosis and those with >stage 1 *MYCN*-amplified neuroblastoma are considered to have high-risk disease.[2] Therapy for patients with high-risk neuroblastoma has evolved through the results of randomized clinical trials performed by national and international cooperative groups and currently includes multi-agent doseintensive induction chemotherapy and surgical resection to effect remission. Strategies to consolidate remission include high-dose chemotherapy with autologous hematopoietic stem cell transplant (ASCT), external beam radiotherapy, the differentiating agent isotretinoin and immunotherapy with anti- GD2 monoclonal antibodies (MoAb) plus cytokines. The addition of anti-GD2 MoAbs significantly improved event-free survival (EFS) and overall survival (OS) in patients with high-risk neuroblastoma.[3,4] However, current therapeutic approaches remain suboptimal with long-term EFS of 40–50% due to failure to achieve remission (i.e. persistent chemorefractory disease) and relapse after achieving remission resulting from failure to eradicate minimal residual disease (MRD).[5,6] Significant immediate and late toxicities limit further dose intensification of chemotherapy and radiotherapy. Immunotherapy has not been associated with significant cumulative or long term toxicities and therefore remains an attractive treatment option to be further explored and enhanced.[7] Unlike malignancies arising in adults, neoantigens are rare in neuroblastoma,[8] resulting in few target for immunotherapy. GD2 remains the most frequently targeted antigen for neuroblastoma immunotherapy.[9]

1.1 Biology of GD2

Gangliosides, such as GD2 are complex, acidic glycolipids found on the outer cell membrane. Antenatally, GD2 is expressed on neural and mesenchymal stem cells,[10] while postnatal expression on normal tissues is restricted to peripheral neurons, central nervous system, and skin melanocytes.[11] GD2 is biosynthesized from precursor gangliosides GD3/GM3 by β -1,4-N-acetyl galactosaminyltransferase (GD2 synthase).[12] Neuroblastoma cells have high levels of the GM2/GD2 synthase transcript, enzyme activity and GD2 expression with an estimated 5–10 million molecules/cell.[13] The role of GD2 in normal development is not well understood, though it is thought to play a role in neural differentiation and repair.[14] Gangliosides including GD2 may function as receptors for microbial toxins,[15] as well as mediators of cell adhesion.[16] In addition to its expression on breast cancer stem cells,[17] evidence for a tumorigenic role for GD2 includes its phosphorylation of tyrosine kinases,[18] enhancement of invasion and motility,[19] and immunosuppressive effect on effector cells.[20]

1.2 GD2 as an immunotherapeutic antigen on neuroblastoma

GD2, a disialoganglioside, is a part of the immunological identity of mammalian cells and generally nonimmunogenic.[21] Besides neuroblastoma, GD2 is expressed on a variety of embryonal cancers including brain tumors, retinoblastoma, Ewing's sarcoma, rhabdomyosarcoma, osteosarcoma; and on neural crest derived cancers in adults.[22] Based on multiple criteria, the National Cancer Institute program for prioritization of cancer antigens ranked GD2 12th among a list of top cancer antigens.[23] GD2 expression on neuroblastoma cell membrane is ubiquitous, being expressed on all primary tumors regardless of stage, and abundant, with an estimated 5–10⁶ molecules/cell.[13] Furthermore, GD2 is not immunomodulated off cell membrane and persists on neuroblastoma cell

membrane post-therapy.[24] Circulating GD2 levels both in the blood and cerebrospinal fluid (CSF) are not high enough to interfere with binding to anti- GD2 specific MoAbs.[25] These properties make it an attractive target for immunotherapy. Targeting GD2 antigen with immune effectors, including MoAbs has the potential to achieve substantial clinical benefits in NB therapy. Dinutuximab, a chimeric anti-GD2 IgG1 was the first antibody to be approved by the FDA and EMA for neuroblastoma and indeed for any pediatric solid tumor. [26] Here we review established and experimental anti-GD2 immunotherapeutic approaches for neuroblastoma (Tables 1 and 2).

2.0 Anti-GD2 monoclonal antibodies

Anti-GD2 MoAbs bind to the end-terminal penta-oligosaccharide of GD2[9] and take advantage of antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-mediated cytotoxicity (CMC) for their anti-neuroblastoma effect. Granulocytes and NK cells have Fc-receptors (FcγRIIA/CD32 and FcγRIIIA/CD16A respectively) on their cell surface which are engaged by tumorbound MoAb and subsequently release cytotoxic granules and cytokines causing tumor cell destruction. Binding of C1q to the Fc of tumor-bound MoAb activates the complement cascade with formation of membrane attack complexes which create pores in NB cell membrane, and lyse tumor cells leading to CMC.[27] In addition, non-immune-mediated effects, including survival signal blockade and anoikis, an induced apoptotic process in which adherent cells detach from the extracellular matrix, might also be enhanced by MoAbs. Tumor cells escape anoikis by constitutive activation of focal adhesion kinase (FAK). Anti-GD2 MoAbs cause dephosphorylation of FAK and inhibit activation of PI3K/Akt pathways, thereby inducing apoptosis and decreasing the invasiveness of cancer cells, respectively.[28] Although NB patients undergoing high dose chemotherapy have significant attenuated lymphocyte responses, neutrophils and macrophages are only transiently suppressed. Hence, CMC and ADCC, which are dependent upon the latter effector cells, can be utilized for tumor cell destruction. Furthermore, the relative lack of complement-inhibitory proteins on NB cell surface renders them more susceptible to CMC and ADCC.[29] Cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin- 2 (IL-2) can enhance ADCC. GM-CSF directly activates clonal expansion and maturation of partially committed progenitor cells in the granulocyte-macrophage pathways to form monocytes, macrophages and dendritic cells. In addition, it indirectly activates T cells via secretion of tumor necrosis factor, interferon, and IL-1.[30] IL-2 stimulates a broad range of immune cells, including T and B cells, monocytes, macrophages, lymphokine-activated killer and NK cells.[31]

2.1 Murine antibodies

The anti-GD2 MoAbs m3F8 and 14G2a, murine IgG3 and IgG2a respectively, were produced in the 1980s. In preclinical testing, both m3F8[32] and 14G2a[33] demonstrated in vivo and in vitro activity against neuroblastoma thus providing a strong rationale for testing in clinical trials.

2.1.1 m3F8—m3F8 mediates dose-response-related destruction of NB by human complement and by human lymphocytes[29], monocytes and granulocytes.[34] Both FcγRII

and Fc γ RIII Fc receptors are utilized for neutrophil ADCC.[27] m3F8 deposits iC3b on the surface of tumor cells and enhances neutrophil and NK cell mediated cytotoxicity through the CR3 receptor.[35] CMC can be further enhanced by the naturally-occurring complex polysaccharide β -glucan (BG) which increases the adhesion of complement receptors on myeloid cells to their natural ligands including iC3b.[36] In the initial Phase I study carried out at Memorial Sloan Kettering Cancer Center (MSKCC), the toxicity profile of m3F8 was established; major adverse events included pain, fever, hypertension and urticarial reactions, which were sometimes severe, but controllable. Anti-NB activity was observed in 4/8 patients. As expected with a xenogeneic protein, m3F8 was immunogenic: human anti-mouse antibody (HAMA) responses were detected and patients with elevated HAMA levels had lack of pain suggesting high levels of serum autoantibodies with m3F8 activity.[37] The recommended phase II dose was established at 20mg/m²/day $\times$ 5 days. Specific binding of m3F8 to tumor was proven using gamma camera imaging after administration of ¹³¹I-m3F8.[38] When m3F8 was heat-modified, multifold dose-escalation was possible without significant enhancement of toxicity or blunting of anti-NB activity.[39] Clinical benefit was demonstrated in a phase II trial in which m3F8 alone was used as consolidation therapy in patients with high-risk neuroblastoma. Although 31/34 patients were in complete remission (CR) or very good partial remission (VGPR) per International NB Response Criteria (INRC), 75% had evidence of minimal residual disease (MRD) by immunocytology, ¹³¹I-m3F8 scans or RTPCR. There was evidence of response by immunocytology, RT-PCR and follow-up ¹³¹I-m3F8 scans. Long-term progression-free survival (PFS) without further systemic therapy was 38%. This study demonstrated that m3F8 could maintain long-term remission and established its efficacy in the setting of MRD.[40]

Based on preclinical data showing an increase in activation markers and enhancement of ADCC when GM-CSF was combined with m3F8,[41] two phase II studies combining m3F8 with IV[42] or subcutaneous (SC)[43] GM-CSF were performed. GM-CSF did not enhance known toxicities of m3F8 and the therapy could be administered out-patient. In patients with primary, chemorefractory osteomedullary neuroblastoma (i.e. in patients not achieving CR after induction chemotherapy), highly impressive responses were observed in both studies (CR rates of 68% for SC and 65% or IV GM-CSF), however, patients receiving SC GM-CSF +m3F8 appeared to have better outcomes with five-year PFS of 24% $\pm$ 6% for SC versus 11 $\pm$ 7% for IV GM-CSF (p=0.002).[44] In multivariate analyses, besides route of GM-CSF administration, early neuroblastoma MRD response as assessed by qRT-PCR, missing ligand for inhibitory immunoglobulin like receptor (KIR), HAMA response and FCGR2A polymorphism were significantly prognostic.[43] Responses in patients with relapsed or PD were less impressive.[42] In patients in first remission, therapy with m3F8+GMCSF significantly improved long-term PFS and OS compared to historical outcomes in the pre-immunotherapy era, when long-term PFS rates were <30%.[45] In an analysis of 169 children with high-risk NB treated with 3F8+GM-CSF and cis-retinoic acid, 5-year PFS improved from 44% for m3F8 alone to 56% for m3F8+ IV GMCSF to 62% for m3F8+ SC GMCSF. (Figure 1) Five-year OS for the SC group was 81%. Again, MRD[46] after two cycles, HAMA and route of GMCSF administration were prognostic.[4] GM-CSF induced activation of peripheral-blood granulocytes was associated with improved outcome,[43] but autologous stem cell transplant did not impact survival in patients treated with m3F8+GM-

CSF.[47] Patients receiving m3F8+GMCSF in second remission also benefited from immunotherapy albeit with less impressive results compared to those in first CR. Five-year PFS and OS in 101 high-risk NB patients treated in second remission were 33% and 48% respectively. Early MRD response was prognostic. Of the 33 long-term progression-free survivors, 19 (58%) had previously received m3F8+GMCSF suggesting its effectiveness in eradicating MRD even when used for re-treatment.[48]

In addition to GM-CSF, BG has also been demonstrated to enhance m3F8-mediated NB cytotoxicity.[36] A phase I study of oral barley-derived β -glucan plus m3F8 noted improvement in 13 of 22 patients by MIBG scintigraphy.[49] A subsequent phase I study of oral yeast-derived β -glucan (NCT00492167) plus m3F8 noted responses in resistant neuroblastoma.[50] Both studies demonstrated an acceptable safety profile with neither reaching MTD for BG. However, in another clinical trial, the combination of m3F8+ IL-2 was associated with unfavorable m3F8 pharmacokinetics and capillary leak syndrome in some patients, and the study was terminated after 12 patients were treated (unpublished data).

Since m3F8 is a murine antibody, it induces HAMA of which anti-idiotypic (Ab2) human anti-mouse antibodies are a component.[51] HAMA can neutralize m3F8 and Ab2 can interfere with the binding of m3F8 to GD2, thereby reducing cytotoxic efficacy. HAMA titers can be abrogated by treating patients with a combination of rituximab and cyclophosphamide allowing continuation of m3F8 therapy.[52] Paradoxically, patients developing low-level HAMA consistently had higher long term survival rates than those who did not.[4,53] This observation supports the hypothesis of an anti-idiotypic network in patients treated with m3F8 with Ab2 induction of anti-anti-idiotypic antibodies (Ab3) that bind with GD2. HAMA thus serves as a surrogate measure of the host anti-idiotypic network.

2.1.2 14G2a—14.G2a, a murine monoclonal anti-GD2 antibody unrelated to 3F8 demonstrated in vivo and in vitro anti-NB activity[54] and was clinically tested in several studies. In a radioimmunoassay trial, radioiodinated 14.G2a was shown to target NB in patients.[55] In a phase I therapeutic trial, the maximum tolerated dose was 15–20mg/m²/day $\times$ 5 days with pain and allergic reactions being the main toxicities. 14.G2a alone or in combination with IL2 and GM-CSF had modest anti-tumor activity but adults developed neurotoxicity coinciding with hyperleukocytosis and HAMA developed in a majority of patients.[56]

Although murine anti-GD2 MoAbs are tolerable and have proven antineuroblastoma activity, associated hypersensitivity reactions and the development of HAMA often limits repeated administration. These concerns and new advances in genetic engineering prompted the development of newer generation chimeric and humanized anti-GD2 MoAbs.

2.2 Chimeric monoclonal antibodies

2.2.1 Dinutuximab—The human-murine chimeric Ab ch14.18 subsequently renamed dinutuximab was produced in an SP2/0 cell line by combining the variable regions of the original murine IgG3 anti-GD2 MoAb 14.18 and the constant regions of human IgG1.[57] Compared to 14G2a, it maintained anti-GD2 specificity, had equal CMC and 50–100 fold

higher ADCC.[58] Toxicities, studied in phase I studies with different schedules were similar to 14G2a: pain, hypertension, fever and allergic reactions. A unique toxicity of reversible internal ophthalmoplegia manifesting as mydriasis and loss of accommodation was also observed.[59,60] Recently, rare cases of motor peripheral neuropathy and transverse myelitis have been reported. The addition of GM-CSF did not significantly enhance dinutuximab toxicities,[61] however IL-2 resulted in additional toxicities, primarily capillary leak syndrome and hepatic transaminitis.[62] A dinutuximab dose of 20mg/m²/day × 5 days was established for further testing in combination with GMCSF and IL-2.[62] Dinutuximab did not undergo formal phase II testing to determine response in patients with evaluable disease, however, its efficacy in neuroblastoma patients achieving remission was tested in two large studies. In the randomized cooperative German Neuroblastoma Trials, NB90 and NB97, dinutuximab (administered without cytokines) after myeloablative transplant was compared to maintenance chemotherapy. Dinutuximab-treated patients did not have improved EFS, but OS was superior: respective 3-year OS rates being 68%, 57% and 47% for patients receiving dinutuximab, maintenance chemotherapy or no further therapy post-transplant.[63] The seminal dinutuximab trial was performed by the Children's Oncology Group (COG).[3] 226 patients with high-risk neuroblastoma in remission after induction chemotherapy and ASCT were randomized to receive either five cycles of dinutuximab-based immunotherapy+ cis-retinoic acid (CRA) or CRA alone. Patients treated with immunotherapy received 5 cycles of dinutuximab: 3 in combination with GM-CSF and 2 with IL-2. At a median followup of 2 years, dinutuximab recipients had significantly higher EFS (66% vs. 46%; p = 0.01) and OS rates (86 vs. 75%; p=0.02) compared with CRA (Figure 2). This study provided definitive evidence for the effectiveness of anti-GD2 immunotherapy and established it as the new standard of care for maintenance therapy in patients with HR NB post ASCT. At follow-up 4-year EFS rate was no longer significant though 4-year OS rate was superior (p=0.02) for the immunotherapy group.[64]

In 2015, the US FDA and the European Medicines Agency (EMA) approved dinutuximab in combination with GM-CSF, IL-2, and CRA after ASCT for the treatment of pediatric patients with HR-NB who achieved at least PR with prior first-line multi agent, multimodality therapy. The recommended dosage of dinutuximab was 17.5 mg/m²/day × 4 days/cycle for 5 cycles which was bioequivalent to 25 mg/m²/day previously tested in earlier trials.

Attempts to develop novel approaches to enhance antibody efficacy include the addition of lenalidomide which increases cytokine levels, activates natural killer cells and has immunomodulatory/anti-tumor effects in neuroblastoma when combined with dinutuximab. [65] A phase I trial of the combination of dinutuximab, lenalidomide and CRA is currently underway (NCT01711554).

2.2.2 Ch14.18/CHO (Dinutuximab-beta)—Ch14.18 was later produced in Chinese hamster ovary (CHO) cells resulting in a MoAb named dinutuximab-beta which had a more favorable glycosylation pattern than dinutuximab and avoided the murine virus contamination that can be associated with SP2/0 cells. Dinutuximab-beta had identical GD2 binding, equal CMC and superior ADCC compared to dinutuximab; toxicity and pharmacokinetics were similar.[66,67] A continuous dinutuximab-beta infusion over 10 days

appeared to be associated with reduced pain while immune function and immunogenicity were unchanged.[68] In a large randomized trial conducted by SIOPEN (NCT01704716), HR-NB patients were randomized to receiving dinutuximab-beta (over 8 hours) with or without SC IL-2 after induction chemotherapy and ASCT. Toxicities were higher in patients receiving IL-2 resulting in a much higher termination rate than those receiving dinutuximab-beta alone (44% vs 18%). Intent-to-treat analysis EFS and OS rates were similar for both groups, suggesting that SC IL-2 was not beneficial. However, for patients completing all assigned therapy, there appeared to be a favorable trend for IL-2 treated patients.[69] In March 2017 the EMA approved dinutuximab-beta for treatment of HR-NB.

2.3 Humanized monoclonal antibodies

Chimeric Abs are less immunogenic than murine MoAbs, however, the residual mouse component in the antigen binding region can still be immunogenic leading to the development of human anti-chimeric antibody (HACA). Significant HACA responses were observed in a majority of patients in a phase I study resulting in reduced elimination half-life.[70] Creation of a humanized antibody can be accomplished by inserting the coding segments for complementarity-determining regions responsible for the desired binding properties into a human antibody “scaffold”. This is achieved through recombinant DNA methods using an appropriate vector and expression in mammalian cells.[71]

2.3.1 Hu3F8—Murine 3F8 was humanized to CHO cells and a candidate IgG1 with nM affinity for GD2 with a Koff ~10-fold slower than other anti-GD2 MoAbs selected for clinical use. Longer retention on NB cell surface and improved Fc-related functionality translated to 200-fold enhanced ADCC; CMC was reduced to 60% of m3F8.[72] Hu3F8 is currently being studied in 6 ongoing clinical trials: 5 in patients with NB and 1 in osteosarcoma. Preliminary data indicate that adverse events in phase I monotherapy[73] (NCT01419834) and the recently completed (NCT01757626)[74] combination trial with GM-CSF were transient and similar to those in patients treated with m3F8: pain and hypertension. MTD was not reached and hu3F8 dose could be escalated to several-fold higher than the equivalent m3F8 dose. The recommended phase II dose established was 3 mg/kg/dose administered every other day $\times$ 3 doses, about 3-fold higher than m3F8. Each infusion is administered over 30 minutes in an outpatient setting. As expected, allergic reactions and immunogenicity as assessed by human antihuman antibody (HAHA) titers were far less pronounced and occurred chiefly in patients who had received MoAb therapy (including m3F8 and dinutuximab) prior to hu3F8. When hu3F8 was combined with SC IL-2 (NCT01662804) the expected additional toxicities related to SC IL-2 were observed, however, there was a significant shortening in hu3F8 half-life ($T_{1/2}$), suggesting a negative effect of IL-2. Preliminary pharmacokinetic analysis indicated a dose-independent hu3F8 $T_{1/2}$ of 3.26 ± 1.09 days. Both maximal concentration (C_{max}) and area under the curve (AUC) were strongly correlated with dose level. Pharmacokinetic parameters remained statistically unchanged during subsequent treatment cycles suggesting lack of neutralizing HAHA. Interestingly, there was a strong positive linear correlation between patient’s weight and AUC, C_{max} and C_{min} ($R=0.88+/-0.14$).[75] Robust and durable clinical responses have been observed both for hu3F8 monotherapy and when combined with GM-CSF. Two phase

II studies are underway for NB in first and second remission (NCT03033303 and NCT01757626) and for osteosarcoma after pulmonary metastasectomy (NCT02502786).

2.3.2 Hu14.18K322A—Hu14.18K322A was biochemically modified from 14G2a to improve its clinical attributes. Firstly, by substituting a single amino acid in the Fc region, complement activation could be eliminated thereby reducing neuropathic toxicity. Secondly, production was carried out in YB2/0 cell lines to decrease fucosylation in the Fc region, augment interaction with Fc receptors and increase ADCC. Preclinical studies in mice demonstrated robust anti-tumor effects through interactions with myeloid and NK cells. In a phase I study of hu14.18K322A, dose-limiting toxicities included sensory neuropathy, serum sickness, and PRES. Grade 3 or 4 pain was observed in most patients, though dose-escalation was possible to higher doses than dinutuximab with a recommended phase II dose of 60 mg/m²/day × 4 days.[76,77] Pilot studies utilizing combination treatments including chemotherapy, IL-2, GM-CSF and infusion of NK cells are currently underway. (NCT 01857934, NCT 02130869)

2.4 Immunocytokines

Immunocytokines are MoAbs with cytokine linked to Fc ends. Upon binding to target antigen, cytokines are released directly into the tumor microenvironment, achieving high local concentrations and minimizing systemic side effects. When compared to dinutuximab plus co-administered IL-2 the immunocytokine hu14.18-IL-2 activated human effector cells more effectively.[78,79] However, in early phase studies the toxicity profile in NB patients was similar: pain and vascular leak.[80] Responses were observed, but were restricted to patients with low-burden osteomedullary disease.[81]

2.5 Chemotherapies

The role of dinutuximab in the maintenance phase of therapy in high risk neuroblastoma is well-established but few data are available on responses in evaluable disease.[82] Preliminary results from a randomized phase II study conducted by the COG (NCT01767194) indicate a significantly higher response rate to a combination of dinutuximab, irinotecan, temozolomide and GMCSF compared to the combination of dinutuximab and temsirolimus (52.9% versus 5.5%), though the former group also had a higher complication rate including respiratory and renal toxicities.[83] A more definitive study of the chemoimmunotherapy arm is under way to further evaluate response and toxicity. A further chemoimmunotherapy strategy using hu3F8, though with different timing of therapeutic components is also being tested at MSKCC.

2.6 Radioimmunotherapy

MoAbs may be used as carriers to selectively deliver radioactivity to NB tumor sites, either as tracers or therapeutics, thereby avoiding the side effects associated with external beam radiation. Radioimaging with radioimmunoconjugates can potentially enhance sensitivity and specificity of disease detection and provide key pharmacokinetic and dosimetry information, parameters not easily available with conventional imaging modalities. Radioimmunoconjugates targeting GD2: ¹³¹I-3F8, ¹³¹I-14G2a and ^{99m}Tc-ch14.18 can target NB with a high degree of specificity and sensitivity even when compared to MIBG

scanning which is considered the “gold standard” for imaging NB.[38] However, their potential immunogenicity precludes wider use. An ongoing study using ^{124}I -hu3F8 for radioimmuno-PET might overcome this obstacle (NCT02307630). ^{131}I -3F8 is the only radioimmunoconjugate to be used for radioimmunotherapy thus far. At doses of 6–28 mCi/kg, the main toxicity (in addition to known 3F8-related toxicities) was myelosuppression requiring bone marrow rescue and hypothyroidism. Responses were observed both for soft tissue and osteomedullary disease. Maximum estimated total body dose was estimated at <700 cGy, substantially less than total body irradiation dose used for myeloablative regimens for NB.[84] Based on the lack of major extramedullary toxicities, ^{131}I -3F8 (20 mCi/kg) was added to a multimodality program for all high-risk NB patients in an attempt to target minimal residual disease (MSKCC N7 protocol). Thirty-five patients received ^{131}I -3F8, all engrafting after bone marrow rescue. Long-term EFS was 40%, [85] similar to that observed with 3F8+GM-CSF therapy for patients in first CR.[4] A phase I trial of ^{131}I -3F8-mediated radioimmunotherapy and bevacizumab-mediated anti-angiogenic therapy has also been completed (NCT01114555). Although toxicities were acceptable and anti-NB responses noted, myelosuppression (38% patients required stem cell rescue) and immunogenicity were observed. Bevacizumab did not appear to impair ^{131}I -3F8 targeting to sites of NB or BM recovery after ASCR.[86]

With improved systemic control of NB metastases, the central nervous system and leptomeninges appear to be an increasingly common metastatic site. The CSF compartment can be accessed by intra-ventricular radioimmunotherapy. In a phase I study, patients with GD2-positive tumors (including NB) metastatic to the CNS received intra-ventricular ^{131}I -3F8. Toxicities included self-limited headache, fever and vomiting. DLT was reached at 20mCi, when transient elevations in intracranial pressure and chemical meningitis were seen. Highly favorable dosimetry was observed with radiation doses of 14.9–56 cGy/mCi to the cerebrospinal fluid and <2 cGy/mCi to blood and non-CNS organs.[87] The addition of compartmental therapy with ^{131}I -3F8 or ^{131}I -8H9 to a multi-modality treatment regimen has succeeded in radically changing the natural history of patients with CNS relapse of NB, achieving long-term survival rates of 65%.[88]

3.0 Adoptive cell immunotherapy

3.1 Natural killer cells

Natural Killer cells (NK cells) are part of the innate immune system and are capable of rapid killing via degranulation with subsequent release of toxic granules. NK cells are maintained in a delicate equilibrium between self-tolerance and the ability to respond to malignant transformation primarily by the interaction between polymorphic killer immunoglobulin receptors (KIR) and HLA class I ligands. Fc γ RIII-mediated signaling via MoAb therapy can overcome the effect of inhibitory KIRs leading to NK-mediated ADCC.[89] KIR-HLA genotypes have been shown to influence the anti-NB activity of 3F8 as well as hu14.18-IL2 with an advantage for patients missing HLA ligands for specific KIR.[90,91] Based on this observation, a pilot study combining 3F8 with haploidentical NK cells selected to optimize KIR-HLA “mismatch” was conducted (NCT00877110). The combination was safely administered and preliminary data suggest an anti-NB effect for NK cells.[92] Other studies

are investigating combinations of dinutuximab, hu3F8 or hu14.18 with haploidentical NK cells or expanded autologous NK cells ex vivo in combination with dinutuximab (NCT02650648, NCT02130869 and NOT 02573896)

3.2 T cells

Activated T-cells have improved tumor trafficking compared to other effectors and long-term memory against cancer cells. However, the general lack of HLA expression on NB makes it a suboptimal tumor for adoptive T-cell therapy, even with immune checkpoint inhibition strategies. T-cells can be directed to tumor cells by strategies including chimeric antigen receptors and bispecific antibodies.

3.3 Chimeric antigen receptor T-cells

Chimeric antigen receptor T-cells (CAR T-cells) are produced by genetically transducing a chimeric antigen receptor into autologous T-cells enabling recognition and targeting of GD2 on neuroblastoma irrespective of HLA expression. First generation anti-GD2 CAR T-cells were safely administered to patients with neuroblastoma without neuropathic pain,[93] showed promising anti- neuroblastoma activity and persisted for up to 192 weeks post-injection.[94] However, newer-generation CAR T-cells against neuroblastoma have not been able to reproduce the success of their counterparts targeting hematological malignancies. Other anti-GD2 CAR-T cell approaches currently undergoing clinical investigation include third-generation CAR T-cells incorporating caspase 9 (NCT01822652) and CD20 (NCT02761915) safety switches to reduce the risk of serious toxicities including cytokine release syndrome that have been 5d with CAR T-cells targeted at CD19.

3.4 Armed T-cells

Instead of genetically engineering T-cells, they can be “armed” using a bispecific antibody molecule that coats T-cells via a CD3 portion and redirects them to kill NB via an anti-GD2 portion. Armed T-cells (ATC) therapy is usually not associated with cytokine release syndrome (CRS) described with CAR T-cells against hematological malignancies. It has been speculated that as ATC divide, they dilute out the amount of bispecific antibody coating them, allowing the T-cells to quench, thus limiting non-specific T-cell activation and CRS. ATC with anti-CD3 × anti-Her2 bispecific antibody have been used to treat patients with breast cancer[95] with multiple infusions being administered almost entirely on an outpatient basis. A similar approach using ATC armed with anti-CD3 × anti-GD2 (hu3F8) is currently being tested in a phase I trial in children with NB and other GD2-expressing tumors (NCT02173093).

4.0 Vaccines

Passive immunotherapy typically results in short-lived immune responses. Eliciting active immunotherapy against neuroblastoma has been difficult since the glycolipid GD2 is poorly immunogenic and does not engage T cells. To overcome these obstacles, two clinical approaches have been developed: anti-idiotypic vaccines and the combination of multiple adjuvants with gangliosides.

4.1 Anti-idiotypic vaccines

HAMA elicited by 3F8 has consistently been associated with favorable survival outcomes. This paradoxical observation strongly suggests the presence of a host anti- idiotypic network that results in low levels of Ab3 which might have anti-NB activity.⁵⁹ This observation formed the basis for testing anti-idiotypic antibodies as surrogate antigens to induce anti GD2 immune responses. Ab2 vaccines have several advantages over native gangliosides: being proteins, they induce stronger T-cell responses and are easier to synthesize. The antiidiotypic GD2 mimic MoAbs, anti-ch14.18 mouse IgG1 1A7[96] and anti-3F8 rat IgG1 antibody A1G4,[97] were tested in phase I trials.ere noted and anti-GD2 responses observed.

4.2 Bivalent ganglioside vaccines

Initial preclinical studies involved conjugation of gangliosides to the highly immunogenic, foreign keyhole limpet hemocyanin (KLH) in combination with the immune adjuvant QS-21. Preclinical studies validated the effectiveness of this approach in treating GD2+ micro-metastatic tumors. However, antibody response was variable, driving the development of a more potent GD2 lactone (GD2L)-KLH vaccine.[98] In a cohort of patients with HR-NB in second remission, the bivalent vaccine GD2L-KLH+GD3L-KLH combined with OPT-821 (QS 21 equivalent) and oral BG induced anti-GD2 and anti-GD3 IgG antibody responses, with long-term EFS of 75%.[99] Twelve of 15 patients had antibody responses against GD2 and/or GD3 and disappearance of MRD documented in 6/10 assessable patients. Based on these encouraging results, phase II studies in further cohorts of patients in first and second remission have been initiated. (NCT00911560)

5.0 Preclinical approaches

Preclinical investigations to improve GD2-targeted immunotherapy are being carried out in several research areas.

5.1 Modifying anti-GD2 antibodies

The activity of GD2-specific MoAbs can be further enhanced by increasing specificity and improving effector function. Cross-reactivity with other gangliosides e.g. GD1b expressed on sensory neurons might result in worsening the toxicity of pain. Newer technologies such as crystal structure analysis and *in silico* design can result in algorithms that enhance specificity to GD2.[100] Affinity maturation by de-fucosylation results in the production of MoAbs with highly improved ADCC.[101]

5.2 Enhancing payloads delivered by monoclonal antibodies

MoAbs have the potential to selectively deliver cytotoxic payloads to NB cells while sparing normal tissues the toxicities associated with these agents. Radioimmunotherapy has been the only such strategy tested clinically thus far. The long serum half-life of MoAbs results in a relatively low therapeutic index for radiotherapy resulting in systemic radiation exposure particularly to bone marrow. A multi-step approach separates the MoAb from the payload. The anti-GD2 scFv 5F11 recombinantly fused to streptavidin formed a homo-tetramer and showed high GD2 binding. Excess 5F11 was cleared from the circulation by a clearing agent followed by injection of a small radioactive ligand (111In-DOTA) conjugated to biotin. In

NB xenograft experiments this multi-step approach significantly improved therapeutic index. However, the high immunogenicity of streptavidin prevented repeat administration.[102] When a bispecific antibody with affinity for GD2 (scFv hu3F8) and DOTA (scFv C825) was used, the multistep approach resulted in complete responses in NB xenografts without any toxicity being observed and has a much reduced risk of immunogenicity.[103]

Other payloads include drugs delivered via conjugates (ADC). Anti-GD2 ADCs have not been tested clinically in NB, though in preclinical experiments, ch14.18/14G2a anti-GD2 Fab' fragments were demonstrated to deliver liposomal doxorubicin or fenretinide to NB xenografts.[104] Liposomes or other nanoparticles have also been shown to deliver anti-sense oligonucleotides,[105] inhibitory micro RNAs,[106] ALK-siRNA,[107] and thermoablative molecules[108] to NB via GD2 targeting with resultant anti-NB activity. Immunotoxins produced by fusing anti-GD2 MoAbs with bacterial or plant-derived toxins have also shown anti-NB activity in xenograft models. Diphtheria toxins fused to scFv 5F11 and pseudomonas toxin fused to scFv14.18 demonstrated specific killing of GD2+ tumor cells independent of host immune function.[109] However, immunogenicity of foreign toxin proteins has remained a major concern and no GD2-directed immunotoxins are currently in human trials. Finally, the immunocytokine ch14.18-IL15[110] demonstrated superior tumor suppression to ch14.18 alone in preclinical testing and has the potential to be safer and more effective than hu14.18-IL2.

5.3 Harnessing T-Cells

5.3.1 Improving CAR T-cell function—Early T-cell exhaustion and tonic signaling are considered to be primary factors limiting the antitumor efficacy of CAR-T cells against solid tumors including neuroblastoma. Other obstacles to optimal CAR-T cell function are related to tumor microenvironment and include lack of chemotactic homing signals, lack of costimulatory molecules for T-cells, abnormal tumor vasculature and immunosuppressive cells.[111] Approaches to overcome obstacles to anti-GD2 CAR T-cell activity against neuroblastoma include incorporation of costimulatory molecules such as 4-1BB[112] and combination with PD-1 blockade.[113] The expansion of T-cell subpopulations such as CD4+ cells or central memory cells transfected with CAR is also being investigated.[114]

5.3.2 Bispecific antibodies (BiAb)—NB cells evade T-cell mediated anti-tumor immunity by down-regulating HLA expression and releasing proteins to inhibit or kill T-cells.[115] Moreover, carbohydrate antigens like GD2 are poorly immunogenic and must be attached to a peptide to enable binding to MHC.[115] BiAbs circumvent these escape mechanisms because they are impervious to the immunosuppressive NB microenvironment and do not require tumor HLA or costimulatory molecules. BiAb produced by fusing anti-CD3 scFv with anti-GD2 IgG MoAb (5F11) or hetero-conjugating full anti-CD3 and 3F8 can effectively recruit CTLs to kill NB cells. Recent advances in protein engineering and recombinant DNA technologies permit packaging of the VH and VL of an IgG into a smaller scFv. These constructs increase efficacy by monovalently binding T-cells with lower affinity and prevent non-specific T-cell activation until presented in a multivalent manner by a target cell. A scFv-based bispecific antibody (scBA) against GD2 based on the anti-GD2 murine 5F11-scFv and the anti-CD3 huOKT3-scFv (5F11- scBA) was constructed to explore

the critical structural properties that affect biologic function. The effect of VH-VL orientation, linker length and antigen affinity on stability, T-cell cytotoxicity, and in vivo antitumor effect was investigated. More recent studies have substituted the 5F11-scFv with the higher affinity hu3F8-scFv to form hu3F8-scBA. This scBA redirected T cells to kill GD2+ cancer cell lines with up to 5,000-fold higher potency, induced stronger T-cell activation in vitro, and more effectively suppressed NB xenograft growth in vivo compared to 5F11-scBA.[116] It will be studied in patients with GD2-positive tumors including neuroblastoma.

6.0 Conclusions

Immunotherapy promises to achieve the dual goals of improving long term survival and reducing long and short-term toxicities in children with high-risk neuroblastoma. Achieving these goals depends on developing immunotherapeutics and demonstrating their improved efficacy over standard treatments, allowing for reduced exposure to broadly cytotoxic agents. GD2 is an antigen against which a large majority of the current therapies have been designed due to its specificity and expression in neuroblastoma. A number of antibody-based and vaccine approaches are currently in clinical use or being developed in research studies. Both engineered antibodies and T-cell based therapies targeting GD2 are in preclinical and clinical development and aim to harness the effects of cytotoxic immune cells against tumor and residual disease. Given the relative success in treatment of HR-NB, and their integration into conventional multimodality therapies, we expect the development of both currently available and soon-to-emerge immunotherapeutic approaches to continue.

7.0 Expert commentary

Children with high-risk malignancies and NB in particular require multimodality therapies to improve outcomes. Immunotherapy, by virtue of its specificity for identified tumor targets and mechanisms of action that are not cross-reactive with other anti-tumor modalities (chemotherapy and radiotherapy), is an attractive therapeutic option especially in the pediatric age group where the latter therapies can lead to major long-term morbidities. Among all pediatric solid tumors, NB has been the most successfully targeted by immunotherapy and GD2 has been the most well-studied target. Therapeutic efficacy and an acceptable safety profile have firmly established anti-GD2 MoAb therapy as the standard of care for maintenance therapy in patients with HR-NB who achieved first CR following induction chemotherapy.[3,4] Dinutuximab was the first immunotherapy to be FDA and EMA approved for children with any cancer. Major responses have also been reported in patients with refractory and relapsed osteomedullary NB treated with 3F8 leading to improved OS compared to historical data.[43] Compartmental RIT into the ventricular space with ¹³¹I-3F8 has contributed to major survival improvements in patients with relapsed CNS NB.[88] Active immunity elicited by bivalent anti-GD2 (and anti-GD3) vaccine appears to improve survival rates for patients with NB in second CR.[99] However, major challenges remain in optimizing anti-GD2 immunotherapy for NB. Severe pain is experienced by almost all children receiving anti-GD2 MoAbs and efforts to reduce pain by reducing or abrogating complement activation, thought to be a major mechanism for pain, have been only partially successful. A chimeric MoAb c.8B6 binds to O-acetyl-GD2, which is

expressed on NB cell surface but not on pain fibers. In preclinical studies, in vitro and in vivo ADCC against NB xenografts was observed but without allodynia in rats.[117] Currently available anti-GD2 MoAbs are ineffective for the treatment of bulky disease, and patients receiving dinutuximab experience late relapses as evidenced by the fact that EFS advantage appeared to decline with longer follow-up of patients on the seminal study.[64] Humanized MoAbs have the potential to be more effective and have less immunogenicity than their murine or chimeric counterparts. Chemoimmunotherapy and radioimmunotherapy with anti-GD2 MoAbs has the potential to reduce soft tissue or high-burden disease resistant to chemotherapy. With a better understanding of HLA/KIR interactions and development of ex vivo improvements in patients with relapsed CNS NB.[88] Active immunity elicited by expansion methods, NK cells can potentially be mobilized to target NB. Effective T-cell immunotherapy targeted against GD2 remains an elusive goal, though efforts to reverse CAR T-cell exhaustion and the introduction of BiAb might succeed in harnessing T-cells against NB. Finally, the optimal combination of anti-GD2 immunotherapies against NB is unclear. At MSKCC, NK cell and T-cell mediated investigational strategies are being tested in patients with high-burden osteomedullary and soft tissue diseases, while bivalent anti-NB vaccines are administered after hu3F8+GM-CSF to maintain first and second remissions in HR-NB.

8.0 Five year view

NB is a rare pediatric cancer; <750 patients are diagnosed every year in North America, half being considered high-risk and candidates for immunotherapy. A recent update of the seminal randomized CCG study of ASCT for HR-NB indicated no long-term OS advantage for ASCT.[45] Conversely, MoAb-based anti-GD2 immunotherapy both with dinutuximab, and 3F8 continues to demonstrate significantly improved OS both with and without transplant[3,4] suggesting that anti-GD2 therapy is an integral part of multimodality therapy for HR-NB. The chimeric MoAbs dinutuximab and dinutuximab-beta have been approved for HR-NB therapy, though they are not optimal due to concerns about toxicity, immunogenicity and lack of activity against bulk disease. The humanized MoAbs hu3F8 and hu14.18K322A demonstrate encouraging early results in phase I trials suggesting reduced pain and immunogenicity; phase II studies will determine if they have superior efficacy. Dinutuximab is indicated for use with IV IL-2 and SC GM-CSF, however, IL-2 adds significantly to toxicity, particularly serious toxicities such as capillary leaks. The role of each of the cytokines particularly the use of IL-2 requires further investigation. Indeed 3F8, hu3F8 and dinutuximab-based chemoimmunotherapy regimens use GM-CSF alone without IL-2

There are substantial biochemical differences among anti-GD2 MoAbs (Table 3). 3F8 and hu3F8 have a much slower koff (410-fold) than 14G2a or ch14.18[72] which could translate into longer retention time on the target. Murine and chimeric MoAbs are more immunogenic than their humanized counterparts stimulating the production of neutralizing auto-antibodies, though the consistent association of improved survival with HAMA responses observed in 3F8-treated patients suggests a potential benefit of enhanced immunogenicity. Proteins produced in CHO cell lines have theoretical advantages over those produced in SP2/0 eliciting lower levels of interfering anti-Gal91 and anti-NeuGc autoantibodies.

[118] Whether these biochemical differences translate to clinical benefit remains to be seen and currently ongoing clinical trials seek to identify the optimal anti-GD2 MoAb for immunotherapy of HR-NB. The design of newer anti-GD2 MoAbs with further enhancement of antigen-affinity and effector function can be achieved with investigation of structure-function relationships and implementation of newly-accessible technologies and algorithms.[44,119]

Early reports from a recent COG study on a small number of patients with chemoresistant NB indicate that combining irinotecan plus temozolomide, with dinutuximab plus GM-CSF yields significantly enhanced anti-NB responses compared to those reported with either component alone.[83] Although these findings need to be confirmed in a larger population of patients, and the mechanism of synergy elucidated, the possible role of chemoimmunotherapy as part of first-line chemotherapy for HR-NB is being considered for investigation. MoAb-based radioimmunotherapy has thus far been hampered by collateral BM damage due to off-target effects. Preclinical multistep approaches that separate payloads from MoAb targeting to tumor can precisely deliver radioisotopes, nanoparticles and drugs, vastly improve therapeutic index, and are being developed for clinical use.[103]

The relative lack of expression of HLA Class I and PD1 molecules on NB discourages expectations that immune checkpoint inhibitors, which have revolutionized immunotherapy of several solid tumors in adults, will be effective against NB. CAR T-cells and bispecific antibodies have the potential to target T-cells to NB via HLA-independent mechanisms. Bispecific antibodies are 'off-the-shelf reagents and may be discontinued in the event of toxicity. CAR T cells need to be produced individually for each patient, but they expand in response to tumor and can result in long-term antitumor immune responses. Both approaches can result in the rapid activation of large numbers of T cells interacting with large numbers of cells expressing the target antigen. One major concern is their potential toxicity. Both BiAb and CAR T-cells can be associated with major toxicities including cytokine release syndrome (CRS) which is characterized by fever and constitutional symptoms that can progress to cardiac, renal, hepatic or neurological dysfunction requiring critical care. The recent establishment of treatment algorithms for the management of CRS including in pediatric populations should result in a safer administration of these therapies.[120] Another major concern remains efficacy. Although first-generation CAR T-cells were noted to have anti-GD2 activity,[93] subsequent CAR T-cell studies for NB, similar to those in solid tumors of adults, showed T-cell exhaustion impeding anti-NB effect.[112] Understanding and overcoming the causes of T-cell exhaustion is necessary for their incorporation into immunotherapy for NB. If efficacy for this rare, typically metastatic, pediatric malignancy is demonstrated, other limiting issues, such as cost and accessibility in the community, are likely to be overcome.

A bivalent anti-GD2/GD3 vaccine therapy has been shown to be safe and tolerable in children and to elicit active anti-humoral immunity against NB.[99] Early data from an ongoing phase II study indicates promising results and it is being tested in expanded groups of NB patients in first and second remission. However, due to the biological and clinical heterogeneity of this population without measurable and evaluable disease, the efficacy of

vaccine can only be demonstrated in randomized studies with survival as a statistical endpoint.

Finally the rational combination of currently available and soon-to-emerge immunotherapeutic approaches, and their integration into conventional multimodality therapies will require investigation over the next five years to optimize their use for HR-NB.

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Abbreviations

NB	Neuroblastoma
HR	High risk
MoAb	Monoclonal antibody
ADCC	Antibody dependant cell mediated cytotoxicity
CDC	Complement mediated cytotoxicity
MRD	Minimal residual disease
BM	Bone marrow
3F8	Monoclonal antibody of murine origin
Hu3F8	Humanized 3F8
GM-CSF	Granulocyte macrophage colony stimulating factor
IL-2	Interleukin-2
CR	Complete response
PR	Partial response
SD	Stable disease
PD	Progressive disease
OS	Overall survival
EFS	Event free survival
PFS	Progression free survival
CHO	Chinese hamster ovary

FAK	Focal adhesion kinase
PI3K/AKT	Phosphatidyl inositol 3 kinase/serine threonine kinase
ScFv	Single chain variable fragments
BiAb	Bispecific antibody
scBA	Single chain variable fragment-based bispecific antibody

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Reference annotations

* Of interest

** Of considerable interest

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Active immunity elicited by anti-GD2 (and anti-GD3) vaccine when combined with the immune adjuvant QS21 and oral glucan appears to improve survival rates for NB patients in second CR.

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Key issues

- NB is the first pediatric solid tumor for which immunotherapy has been proven to be effective. Anti-GD2 MoAb therapy is now an essential component of multimodality therapy for HR-NB.
- GD2 is an oncofetal glycolipid antigen which is ubiquitously expressed in NB with restricted expression in normal tissues, making GD2 an attractive target for immunotherapy
- Anti-GD2 MoAbs avail of ADCC and CDC as effector mechanisms
- M3F8, dinutuximab and dinutuximab-beta are the most well-studied anti-GD2 immunotherapies when used in combination with cytokines. The latter two have been approved by regulatory agencies to treat patients with HR-NB. Pain and allergic reactions are experienced by most patients, though long-term toxicities are rare. All have shown benefit for patients in first CR. The combinations of 3F8 plus GM-CSF; and dinutuximab
- irinotecan, temozolomide plus GM-CSF have also demonstrated antitumor responses in patients with chemoresistant NB.
- The humanized anti-GD2 MoAbs hu3F8 and hu14.18K322A can be safely administered at higher doses than their murine or chimeric counterparts, but also have the toxicity of pain. Hu3F8 is being studied in phase II studies in combination with GM-CSF and chemotherapy.
- Early phase clinical and pre-clinical investigations in enhancing MoAb-based anti-tumor strategies include radioimmunoconjugates, immunocytokines, and immunotoxins.
- Adoptive immune cell anti-GD2 approaches tested clinically include haploidentical NK cells, autologous T-cells armed with bispecific MoAbs or transduced to form CAR T-cells. Cell therapies for NB are yet to achieve clinical success. BiAb for CD3 and GD2 demonstrates robust responses in mouse models and will soon be investigated in clinical trials.
- Active anti-GD2 immunity can be elicited by bivalent (anti-GD2 and GD3) and by anti-idiotypic vaccines.

Fig 1.

(A) Progression-free survival (PFS) for 169 patients with stage 4 neuroblastoma in first remission after consecutive immunotherapy regimens: 3F8 alone (regimen A-high risk [HR]; n 43), 3F8 plus intravenous granulocyte-macrophage colony-stimulating factor (GM-CSF) 13-cis-retinoic acid (CRA; regimen B-HR; n 41), and 3F8 plus subcutaneous GM-CSF/CRA (regimen C-HR; n57 and regimen C-ultra HR [UHR]; n28); P.018 (derived from log-rank test to compare PFS among these four groups).

(B) Overall survival (OS) among same cohort of patients; P .003 (derived from log-rank test to compare OS among these four groups). Reprinted from [4] with permission from the American Society of Clinical Oncology. All rights reserved.

Figure 2. Kaplan-Meier Estimates of Survival among the 226 study patients who had been randomly assigned, according to treatment group.

Data are shown for event-free survival (Panel A) and overall survival (Panel B) for all 226 patients and for event-free survival (Panel C) and overall survival (Panel D) for the 179 patients 1 year of age or older at enrollment. The estimated survival ($\pm$ SE) at 2 years is indicated in each plot. Reprinted from [3] with permission from Massachusetts Medical Society.

Table 1:

Results of salient completed trials of Anti-GD2 Immunotherapy for Neuroblastoma

Agent	Combination	Phase	Summary of Results	Refs
Murine Antibodies				
m3F8	-	I	<ul style="list-style-type: none"> • Toxicity profile established: common toxicities included pain and allergic reactions • Anti-NB activity observed • HAMA response noted 	[37]
m3F8	-	II	<ul style="list-style-type: none"> • Activity against MRD after induction chemotherapy demonstrated • Improved survival compared to pre-immunotherapy historical data 	[40]
m3F8	IV GMCSF	II	<ul style="list-style-type: none"> • No additional toxicities of GM-CSF • Activity against chemorefractory osteomedullary NB 	[42]
m3F8	SC GMCSF	II	<ul style="list-style-type: none"> • Activity against chemorefractory NB • Improved PFS and OS compared to historical data for patients in first and subsequent remissions • Improved outcomes when GMCSF given SC rather than IV • Biomarkers influencing outcome identified 	[43,44, 46]
Heat-modified m3F8	SC GMCSF	I	<ul style="list-style-type: none"> • Heat modification permitted further dose-escalation of m3F8 • Anti-NB activity observed 	[39]
m3F8	barley- or yeast-derived- β -D- glucan	I	<ul style="list-style-type: none"> • No additional toxicities of BG • Activity against chemorefractory NB 	[49,50]
14.G2a		I	<ul style="list-style-type: none"> • Toxicity profile established: common toxicities included pain and allergic reactions 	[121]
14.G2a	IL-2/IL-2+GMCSF	I	<ul style="list-style-type: none"> • Additional toxicities noted with IL-2 chiefly capillary leak • Neurological toxicities observed in adults 	[56]
Chimeric Antibodies				
Dinutuximab	-	I	<ul style="list-style-type: none"> • Toxicity profile established 	[59,60]
Dinutuximab	IL-2 + GM-CSF	I	<ul style="list-style-type: none"> • Additional toxicities noted with IL-2 chiefly capillary leak • No additional toxicities with GM-CSF 	[62]
Dinutuximab	-	Randomized II/III	<ul style="list-style-type: none"> • 3-year EFS superior for immunotherapy group compared to maintenance chemotherapy or no therapy • 3-year OS superior for immunotherapy 	[63]
Dinutuximab	IL-2 + GM-CSF; CRA	Randomized III	<ul style="list-style-type: none"> • 2-year EFS and OS superior for immunotherapy+CRA versus CRA 	[3]
Dinutuximab	I/T/GMCSF; temsirolimus	Randomized II	<ul style="list-style-type: none"> • Superior response rate for dinutuximab+I/T/GMCSF 	[83]
Dinutuximab-beta		I	<ul style="list-style-type: none"> • Toxicity profile and pharmacokinetics similar to dinutuximab 	[67]
Dinutuximab-beta		I	<ul style="list-style-type: none"> • Pain toxicity appeared to be reduced when infused over a prolonged 	[68]
Dinutuximab-beta	SC IL-2	Randomized III	<ul style="list-style-type: none"> • Higher toxicities in IL-2 treated group • EFS and OS similar for intent-to-treat analysis but favorable trend for combination in patients completing planned therapy 	[69]
Humanized Antibodies				

Agent	Combination	Phase	Summary of Results	Refs
hu14.18K322A		I	<ul style="list-style-type: none"> • Toxicity profile similar to dinutuximab though higher doses tolerable • Anti-NB activity observed 	80
Hu3F8	GM-CSF	I	<ul style="list-style-type: none"> • Toxicity profile similar to hu3F8 though allergic reactions not encountered; higher doses tolerable compared to m3F8 • Anti-NB activity observed • Lower immunogenicity compared to m3F8 	[74]
Immunocytokines				
Hu14.18-IL-2		I	<ul style="list-style-type: none"> • Toxicity profile similar to dinutuximab+IL-2 • Anti-NB activity observed 	[80]
Hu14.18-IL-2		II	<ul style="list-style-type: none"> • Responses observed only for osteomedullary NB 	[81]
Radioimmunocytokines				
¹³¹ I- m3F8		I	<ul style="list-style-type: none"> • Myelosuppression requiring bone marrow rescue required in most patients • Anti-NB activity noted • Dosimetry data obtained; radiation exposure was well below tolerable doses for all organs 	[84]
¹³¹ I-m3F8		II	<ul style="list-style-type: none"> • Patients received myeloablative doses after induction chemotherapy • Myelosuppression was reversible with autologous bone marrow rescue • Long-term EFS for HR-NB was 40% 	[85]
¹³¹ I-m3F8	Bevacizumab	I	<ul style="list-style-type: none"> • Myelosuppression requiring stem cell rescue required in most patients • Bevacizumab did not interfere with 3F8 targeting to tumor sites • Anti-NB activity noted 	[86]
Cell-Mediated Immunotherapy				
Haploidentical NK cells +m3F8	CTV chemotherapy	I	<ul style="list-style-type: none"> • No unexpected toxicities (including GvHD) beyond those expected with chemotherapy and m3F8 • NK-cell dose-dependent anti-NB activity observed 	[92]
CAR T cells		I	<ul style="list-style-type: none"> • Well-tolerated without neuropathic pain • Anti-NB activity noted • Persistence of CAR T cells for several years after infusion 	[93]
Vaccines				
Anti-m3F8 anti-idiotypic	BCG	I	<ul style="list-style-type: none"> • Minimal toxicities 	[96]
Anti-ch14.18 anti-idiotypic		I	<ul style="list-style-type: none"> • Minimal toxicities 	[96]
Bivalent anti-GD2/GD3	OPT821 + yeast-derived- β -D- glucan	I	<ul style="list-style-type: none"> • Minimal toxicities • Prolonged EFS and OS for patients in second remission 	[98]

Table 2:

Currently recruiting trials of anti-GD2 immunotherapy for NB

Immunotherapy	Agents combined	Phase	Clinicaltrials.gov identifier
Dinutuximab	Lenalidomide+isotretinoin	I	NCT01711554
Dinutuximab	Irinotecan, temozolomide, GMCSF	I	NCT01767194
Dinutuximab	NK cells, lenalidomide	I	NCT02573896
Dinutuximab-beta	None	II	NCT02743429
Hu3F8	None	I	NCT01419834
Hu3F8	GMCSF	II	NCT01757626
Hu3F8	GM-CSF+Isotretinoin	II	NCT03033303
Hu3F8	Haploidentical NK cells	I	NCT02650648
Hu14.18-K322A	Haploidentical NK cells	Pilot	NCT02130869
Hu14.18-K322A	Combination chemotherapy	II	NCT01857934
Activated T-Cells armed with h3F8xCD3 bispecific antibody	GMCSF+ IL-2	I/II	NCT02173093
Bivalent GD2 and GD3 lactone vaccine	OPT-821	I/II	NCT00911560

Table 3:

Anti-GD2 monoclonal antibodies

MoAb	m3F8	Dinutuximab	Dinutuximab-beta	Hu3F8	Hu14.18K322A
Cell line	SP2/0	SP2/0	CHO	CHO	YB2/0
Origin	Murine	Chimeric (VH and VL domains Mab14.18 grafted onto human IgG Fc)	Chimeric (VH and VL domains 14.G2a grafted onto human IgG Fc)	Humanized (CDR loops and structurally significant residues of m3F8 grafted onto a fully human MoAb framework)	Humanized (CDR loops and structurally significant residues of 14.G2a grafted onto a fully human MoAb framework)
GD2 binding (Koff)	5 nM	Kds 11.2 nM	-	13 nM (slower wash-off in vitro)	-
ADCC	Significant	Significant (50-100 fold higher than 14.G2a)	Significant (superior to Dinutuximab)	Significant (200 fold increase over m3F8)	Significant
CMC	Significant	Significant	Significant	Decreased	Decreased
Immunogenicity	Highly immunogenic; HAMA develops	Moderately immunogenic; HACA develops	Moderately immunogenic; HACA develops	Less immunogenic; HAHA rare	Less immunogenic; HAHA rare
Neuropathic pain	Significant	Significant	Significant	Decreased	Decreased
Other toxicities (	- Fever, hypertension, allergic reactions	Fever, hypertension, allergic reactions Transverse myelitis, internal ophthalmoplegia,	Fever, hypertension, allergic reactions	Fever, hypertension,	Fever, hypertension
Efficacy	Noted in patients in remission; and against evaluable disease	Noted in patients in remission, limited efficacy in patients with evaluable disease but maybe enhanced when combined with cytokines (IL-2 and GM-CSF)	Noted in patients in remission, limited efficacy in patients with evaluable disease, combination with IL-2 increases toxicity	Promising results in the context of Phase I studies phase II studies underway	Phase II studies underway