Targets and Antibody Formats for Immunotherapy of Neuroblastoma

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ABSTRACT

Neuroblastoma (NB) is a malignant embryonal tumor of the sympathetic nervous system that is most commonly diagnosed in the abdomen, often presenting with signs and symptoms of metastatic spread. Three decades ago, high-risk NB metastatic to bone and bone marrow in children was not curable. Today, with multimodality treatment, 50% of these patients will survive, but most suffer from debilitating treatment-related complications. Novel targeted therapies to improve cure rates while minimizing toxicities are urgently needed. Recent molecular discoveries in oncology have spawned the development of an impressive array of targeted therapies for adult cancers, yet the paucity of recurrent somatic mutations or activated oncogenes in pediatric cancers poses a major challenge to the evolving paradigm of personalized medicine. Although low tumor mutational burden is a major hurdle for immune checkpoint inhibitors, an immature or impaired immune system and inhibitory tumor microenvironment can further complicate the prospects for successful immunotherapy. In this regard, despite the poor immunogenic properties of NB, the success of antibody-based immunotherapy and radioimmunotherapy directed at single targets (eg, GD2 and B7-H3) is both encouraging and surprising, given that most solid tumor antibodies that use Fc-dependent mechanisms or radioimmunotargeting have largely failed. Here, we summarize the current information on the immunologic properties of this tumor, its potential immunotherapeutic targets, and novel antibody-based strategies on the horizon.

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INTRODUCTION

Most metastatic solid tumors are not curable with chemotherapies alone. Immunotherapy, a modality that achieves durable and sometimes complete tumor regression in metastatic melanoma, renal cell cancer, or chemotherapy-resistant non–small-cell lung cancers (NSCLCs), is emerging as a viable alternative or adjuvant to current standards of care. However, major hurdles persist. Intensive chemotherapy and its sequelae severely compromise both innate and adaptive immunities in patients. With low tumor mutation burdens (TMBs) and the downregulation or absence of surface HLA expression in some cancers (eg, neuroblastoma [NB]), classic T-cell immunity, which relies on tumor-derived peptides presented on the HLA molecule, is no longer functional. Although low TMB is a major hurdle for immune checkpoint inhibitors (ICIs), additional roadblocks such as an immature or impaired immune system (eg, from chemotherapy), the paucity of tumor-infiltrating lymphocytes, and immune suppression by tumor microenvironment (TME) combine to derail the antitumor immune response. As of 2019, there are 33 US Food and Drug Administration (FDA)–approved antibodies or conjugates for human cancer, 2 vaccines (sipuleucel-T [Provenge; Dendreon, Seal Beach, CA] and talimogene laherparepvec), and 2 cell therapies (axicabtagene ciloleucel [Yescarta; Kite Pharma, Santa Monica, CA] and tisagenlecleucel [Kymriah; Novartis, Basel, Switzerland]). This review will provide a focused update on antibody-based immunotherapy for high-risk metastatic NB, which has achieved the most success among pediatric solid tumors, with an emphasis on the immunologic properties of this tumor and its potential immunotherapeutic targets for novel antibody formats[1] and their clinical applications.

Treatment of high-risk NB currently includes induction chemotherapy, surgical resection, radiotherapy, high-dose chemotherapy with autologous hematopoietic stem-cell transplantation, the differentiating agent isotretinoin, and immunotherapy with anti-GD2 monoclonal antibodies (mAbs; dinutuximab [ch14.18] or 3F8) plus cytokines, achieving long-term overall survival of $> 50\%$.[2,3] In addition, compartmental radioimmunotherapy (RIT) with iodine-131 [^{131}I]-8H9 has contributed to major survival improvements in patients with CNS relapsed NB.[4] Active immunity elicited by a bivalent anti-GD2 and anti-GD3 vaccine trial also improved survival rates for patients with NB with a history of prior relapse.[5] However, major challenges remain in optimizing anti-GD2 immunotherapy and expanding therapeutic targets for NB immunotherapy. A better understanding of the limitations and opportunities of antibody-based immunotherapy is critical in shaping the new treatment perspective. Classic T-cell cytotherapy,[6] oncolytic

ASSOCIATED CONTENT

Appendix

Author affiliations and support information (if applicable) appear at the end of this article.

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viral therapy,[7] dendritic cell vaccines,[8] and chimeric antigen receptor (CAR) T cells[9] will not be discussed; readers are referred to reviews that address these topics in depth.

IMMUNOLOGIC PROPERTIES OF NB

Clinically, a subset of NB undergoes spontaneous regression or maturation, whereas others will rapidly progress despite intensive multimodal treatment. Although low-risk NBs show whole chromosome gains without segmental aberrations or gene amplifications, high-risk metastatic NBs frequently show segmental aberrations and *MYCN* amplification.[10] Within these clinical and genetic heterogeneities, 2 distinct immunologic profiles emerge. Among low-risk subtypes, NB has the characteristics of hot tumor, where spontaneous regression or maturation is not uncommon (eg, among locoregional disease and stage 4S NB). Most stage 4S tumors express normal levels of HLA class I antigen and have strong $CD3^+$ T-cell infiltration,[11] suggesting recognition of NB cells by T cells.[12,13] In addition, patients with low-risk NB can manifest the opsoclonus-myoclonus-ataxia syndrome associated with the presence of antineuronal antibodies. Cerebellar gray matter volume and visual and motor cortex thickness can be significantly reduced,[14] and neurofilament light chain in CSF is markedly increased, consistent with neuronal damage.[15] These ganglioneuroblastomas or differentiating NBs are characterized by the presence of diffuse immune cell infiltrates and tumor-associated lymphoid follicles (containing $CD20^+$ B cells), suggesting an active immune reaction against NB.[16]

In contrast, high-risk metastatic NBs have the characteristics of cold tumors, armed with immune evasion mechanisms (Fig 1). First, these tumors are embedded in an immunosuppressive TME, typically infiltrated by $CD163^+$ tumor-associated macrophages (TAMs) that paralyze T-cell responses.[17,18] The TAM promotes T-cell apoptosis via Fas-Fas ligand (FasL) interactions, while activating myeloid-derived suppressor cells (MDSCs) and regulatory T cells, suppressing active immune response.[19-21] Second, by downregulating HLA class I antigens and NKG2D ligands, activating immunoreceptor expressed by natural killer (NK) cells, NBs make themselves nearly invisible to classic T cells or NK cells.[11,22] Third, NB cells express high levels of gangliosides and sialic acid–containing sugars and proteins, which are immunosuppressive when they shed into TME.[23,24] Fourth, lymphocytes in the NB-infiltrated bone marrow (stage 4 metastatic NB) express programmed cell death 1 (PD-1) receptor, whereas HLA class I–positive NB cell lines constitutively express programmed death ligand 1 (PD-L1); interferon-γ (IFN-γ) could also induce PD-L1 expression in NB tumors. This PD-1/PD-L1 pathway is thought to mediate immune resistance mechanisms in metastatic NB.[25,26]

IMMUNOTHERAPEUTIC TARGETS FOR NB

Disialoganglioside GD2

Among the immune surface targets for NB (Appendix Tables A1 and A2, online only), disialoganglioside GD2 is one of the most often studied clinically. It belongs to a unique class of carbohydrate antigens expressed at high density on all primary or metastatic tumors regardless of stage, with proximity to the cell membrane and homogenous distribution within and across NBs, as well as rare antigen loss, which are all properties highly desirable for cancer immunotherapy; they ranked 12th among National Cancer Institute (NCI) cancer antigens.[27,28] As an oncofetal antigen, GD2 is expressed during fetal development, and after birth, its expression is restricted to the CNS, predominantly on neurons, as well as peripheral nerves and skin melanocytes.[29] Although monosialogangliosides, such as GM1 or GM3, function as negative regulators of receptor tyrosine kinases (RTK) signaling, disialoganglioside GD2 activates RTK-mediated signal transduction, leading to the activation of c-Met, engaging the MEK/ERK and PI3K/Akt pathways, and resulting in increased cancer cell proliferation and migration.[30-32] Changes in ganglioside and glycan profiles occur in pathologic conditions and are observed in a variety of embryonal cancers (eg, NB, brain tumor, retinoblastoma, Ewing sarcoma, rhabdomyosarcoma), bone tumors (eg, osteosarcoma), soft tissue sarcomas (eg, leiomyosarcoma, liposarcoma, fibrosarcoma), and neural crest–derived tumors (eg, small-cell lung cancer, melanoma).[27] Anti-GD2 immunoglobulin G (IgG) mAbs and anti-GD2 radioimmunoconjugates have shown successes in preclinical and clinical studies.[27,33] T-cell–based approaches targeting GD2 are also actively pursued using both bispecific antibodies (BsAbs)[34] and CAR T-cells.[9,35]

B7-H3

B7-H3 (CD276), a type I transmembrane glycoprotein molecule, is ubiquitously transcribed in normal human tissues, but its protein expression is restricted by a tight post-transcriptional control. In some tumors, B7-H3 is highly overexpressed by microRNA-29, IFN-γ stimulation, and immunoglobulin-like transcript 4 (ILT-4) signaling, enabling immunotherapies targeting B7-H3 to circumvent on-target off-tumor toxicity.[36-39] This protein is homogeneously expressed in both primary and metastatic NBs[40] and many pediatric and adult solid cancers, including primary and metastatic brain cancers. It is correlated with worse prognosis and increased potential for metastasis, and this protein ranked 66th among NCI cancer antigens.[28] The mAb 8H9 (omburtamab) is specific for 4Ig-B7-H3, the long and principal form of B7-H3. Although most normal tissues were negative for 8H9 staining, liver tissue showed positive, and moderate uptake of 8H9 in the liver was observed in patient imaging studies using IgG1 ^{131}I-8H9 (ClinicalTrials.gov identifier: NCT00582608). To increase

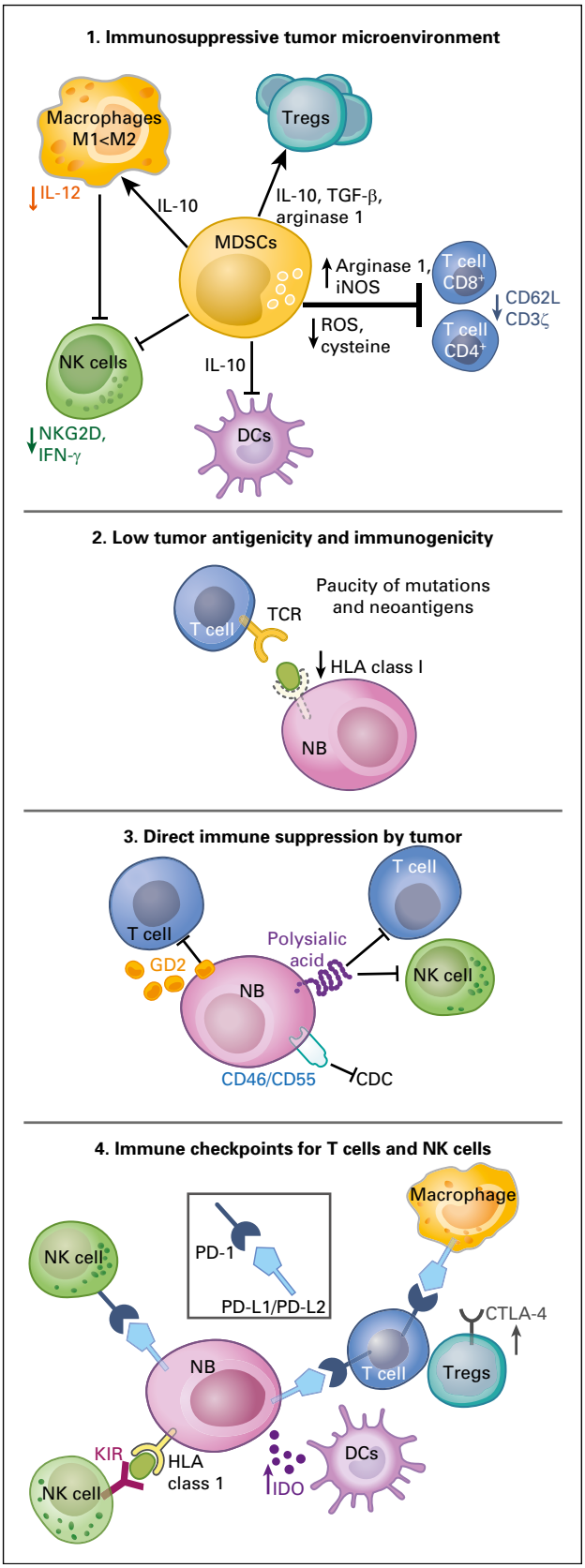


FIG 1. Mechanisms of immune evasion of neuroblastoma (NB). NBs may evade the immune destruction mediated by cytotoxic T cells (CTLs) and natural killer (NK) cells through (continued on next column)

the therapeutic index (TI) and to avoid liver uptake of intravenous 8H9 and subsequent liver toxicity, compartmental radioimmunotherapy (RIT) was given among patients with CNS metastasis, and radioimmunoconjugates using omburtamab have shown the most success so far.[4] Intrathecal (through an Ommaya reservoir) ^{131}I- or ^{124}I-conjugated omburtamab has increased the cure rate for patients with CNS involvement.[4] A phase I clinical trial of intraperitoneal ^{131}I-8H9 for patients with desmoplastic small round cell tumors and other solid tumors involving the peritoneum is ongoing (ClinicalTrials.gov identifier: NCT01099644).[41] Another B7-H3–targeting antibody, enoblituzumab, notable for its nonreactivity with liver,[42] is currently in phase I trials for diverse solid tumors including refractory tumors and pediatric cancers. Furthermore, a clinical trial of a T-cell–engaging BsAb built on the dual-affinity retargeting (DART) platform (MGD009) is underway in patients with B7-H3–positive advanced solid tumors (ClinicalTrials.gov identifier: NCT02628535). The prevalence of B7-H3 overexpression across NB, lung, breast, brain, kidney, and prostate cancers, and dendritic cells makes B7-H3 a particularly intriguing tumor target or a checkpoint ligand.[43]

ALK

Aberrant anaplastic lymphoma kinase (ALK) expression is found in anaplastic large-cell lymphoma (ALCL), NSCLC, rhabdomyosarcoma,[44] and NB.[45] ALK is ranked 33rd among the NCI cancer antigens,[28] and the majority of NBs (22 of 24 NBs) and half of 29 cell lines of neural origin were found to express *ALK* transcripts and ALK protein.[45] Mutations in *ALK* have been implicated in 9% of NBs, and it is adversely prognostic, especially in the presence of *MYCN* amplification.[46,47] *ALK* mutations hyperactivate the RAS-MAPK signaling pathways in NB, promoting cancer formation. Immunodominant peptide epitopes of ALK for both class I and II major histocompatibility complex (MHC) and circulating ALK-specific T cells have been identified in patients with ALCL, providing the basis for peptide vaccine immunotherapy for ALK-driven tumors.[48,49] Prediction of T-Cell Epitopes for Cancer Therapy (ProTECT) analyses

(Continued). multiple mechanisms, including the following: (1) immunosuppressive tumor microenvironment mediated by myeloid-derived suppressor cells (MDSCs)[147]; (2) rarity of somatic mutations or neoantigens recognizable by classic T-cell receptors (TCRs) and downregulation of HLA class I molecules and antigen processing and presenting pathways; (3) expression of immunosuppressive tumor antigens such as gangliosides and sialic acids and membrane complement inhibitors; and (4) upregulation of multiple immune checkpoint inhibitors on immune effector cells and NB tumor cells. DCs, dendritic cells; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; ROS, reactive oxygen species; TGF-β, transforming growth factor-β; Treg, regulatory T cells.

have identified 2 neoepitopes created by the R1275Q mutation in the ALK protein that could complex with HLA-B*15:01 to drive cytotoxic T-cell response.[50] IgGs targeting the ALK ectodomain have also shown activity against NB tumors in preclinical models irrespective of *ALK* mutation, and the combination of crizotinib with anti-ALK mAb induced cell surface accumulation of ALK, resulting in enhanced apoptosis of NB cells.[51] In addition, an antibody-drug conjugate directly targeting ALK receptor, CDX-0125-TEI, exhibited efficient ALK antigen binding and internalization, showing cytotoxicity against both *ALK*-wild and *ALK*-mutant patient-derived xenografts (PDXs).[52] ALK could be a viable immunotherapeutic target, with relevance for NB and other ALK-positive cancers, irrespective of *ALK* mutation.

ANTIBODY-BASED IMMUNOTHERAPY FOR NB

IgG mAbs

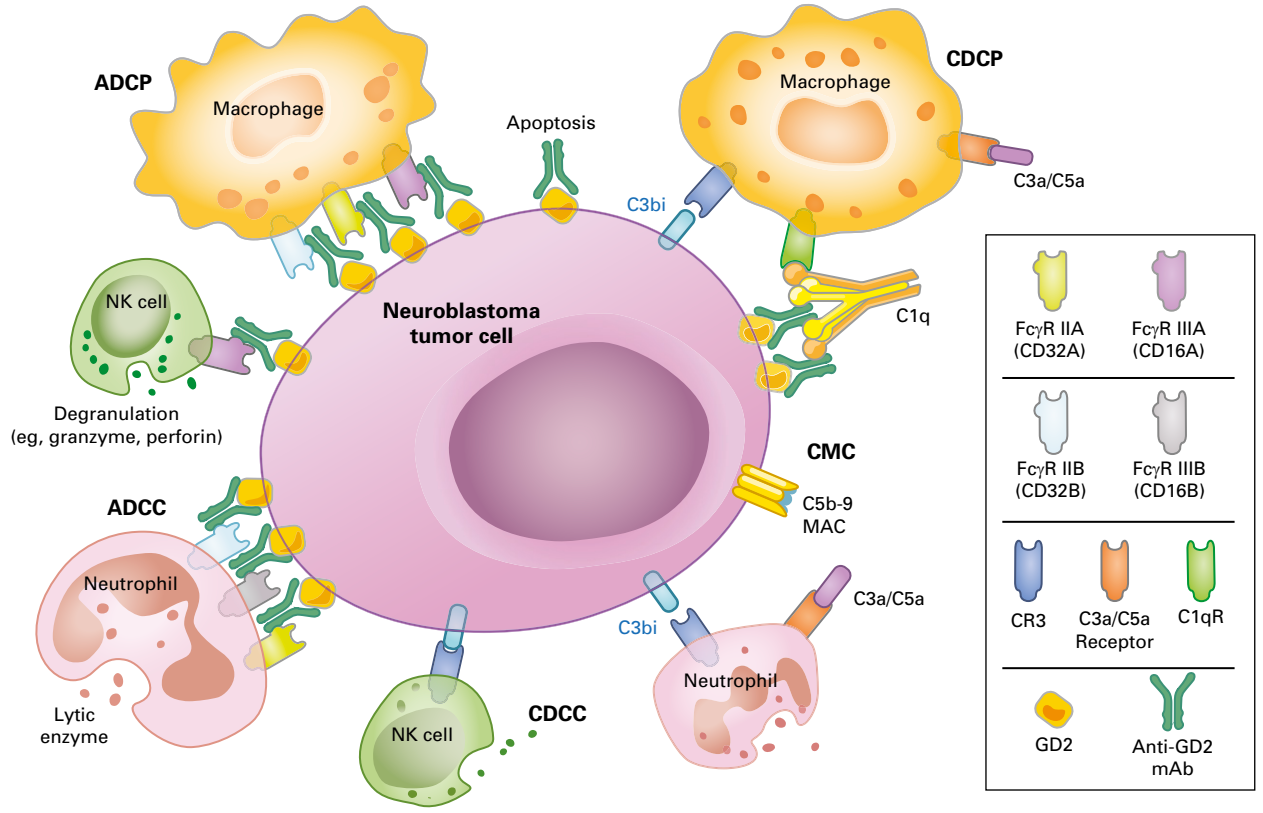
Hybridoma technology first introduced by Köhler and Milstein[53] has generated numerous mAbs targeting human malignancies and immune cells, leading to major breakthroughs in cancer therapy in the past 3 decades. Anti-GD2 mAbs can induce direct cell death[54]; Fcγ receptor (FcγR)–mediated antibody-dependent cell-mediated cytotoxicity (ADCC) by NK cells,[55,56] neutrophils,[57] and macrophages[58]; and complement-mediated cytotoxicity (CMC)[59,60] (Fig 2). Through complement breakdown products (eg, C3bi) deposited on NB, complement-dependent cell-mediated cytotoxicity (CDCC) or phagocytosis (CDCP) could potentially become relevant.

Two anti-GD2 mouse IgG3 antibody families have been the most studied (ie, 3F8 and 14.18). Early on, 14.18 was class switched to IgG2a and chimerized with human IgG1-Fc (ch14.18, dinutuximab) and manufactured in SP2/0 mouse myeloma cells. Ch14.18 was later produced in Chinese hamster ovary (CHO) cells and renamed ch14.18/CHO (dinutuximab-β).[61] Although dinutuximab families have efficient ADCC activity, mouse 3F8 has strong CMC activity as a result of the difference between human IgG1 and mouse IgG3.[59,62] Regarding toxicities, both antibodies induce neuropathic pain in nearly all patients; fever and allergic reactions are also common. Motor neuropathy, ophthalmoplegia, and transverse myelitis seemed to be more prevalent with dinutuximab,[3,63] whereas hypertension and posterior reversible encephalopathy syndrome were more noticeable for 3F8.[64] The difference in toxicity profile is partly explained by the difference in plasma half-life of 3F8 versus dinutuximab (2 *v* 8-10 days, respectively). Despite these differences, the clinical impact on survival appeared similar.[2,3,65] Postconsolidation treatment with 3F8 plus granulocyte-macrophage colony-stimulating factor (GM-CSF) improved overall survival to > 65% among patients with high-risk metastatic NB.[2] Dinutuximab (Unituxin; United Therapeutics, Silver Spring, MD) plus interleukin (IL)-2, GM-CSF, and 13-*cis*-retinoic acids also significantly improved survival when compared with standard of care.[3] A subsequent randomized study using dinutuximab-β showed no benefit of IL-2 over mAb alone,[61] suggesting that NK-ADCC may not be the dominant contributor to clinical benefit of anti-GD2 mAbs. The unexpected impact on survival after mouse 3F8, which has stronger CMC but substantially inferior ADCC compared with dinutuximab and naxitamab (humanized 3F8 [hu3F8]), suggests that complement activation pathways could be important in the immunotherapy of NB. This high sensitivity of NB to CMC is partly attributed to low expression of complement decay-accelerating factor (DAF or CD55) on NB cells.[59,60]

Although active against minimal residual disease (MRD), anti-GD2 mAbs have been less successful against bulky soft tissue tumors, and neuropathic pain and on-target off-tumor adverse effects (because of the presence of GD2 on peripheral pain fibers) have been major management challenges. Furthermore, antidrug antibodies (ADAs), including human antimouse antibodies or human anti-chimeric antibodies, are causing treatment delays or even terminations and, most importantly, abrogating the anti-tumor effect. Naxitamab was created to reduce these ADAs while enhancing ADCC through the human IgG1-Fc, as well as retaining CMC potency through its high affinity for GD2.[66] Phase I and II trials of hu3F8 (ClinicalTrials.gov identifiers: NCT01419834, NCT01757626, and NCT03033303) have confirmed its low immunogenicity, favorable pharmacokinetics (4 days instead of 8-10 days), and improved toxicity profile.[66-68] Another humanized anti-GD2 mAb with *K322A* point mutation, hu14.18K322A, was developed to increase ADCC by lowering fucosylation and to remove CMC to reduce the adverse effect of pain. Reduced fucosylation of the carbohydrate attached to the Asn297 glycosylation site of the Fc region can greatly enhance ADCC by increasing FcγRIIIA/B binding,[69] while alanine substitution at K322 significantly decreases complement activation.[70]

Arming IgG Antibodies With Conjugates

Another strategy to enhance IgG functions is to arm them with therapeutic agents such as drugs,[71] radionuclides,[72] or cytokines.[73] Inactive prodrugs selectively delivered by antibodies can be activated in the tumor stroma or after internalization. The most common conjugates are microtubule inhibitors and DNA-damaging agents. Microtubule inhibitors, including auristatins and maytansines, bind tubulin, destabilize microtubules, and cause G_2/M phase cell cycle arrest. DNA-damaging agents such as anthracyclines, calicheamicin, duocarmycin, and pyrrolobenzodiazepines (PBDs) function by binding the minor groove of DNA and cause DNA strand scission, alkylation, or cross-linking. Antibody-drug conjugates targeting neural cell adhesion molecule (NCAM; CD56), HuN901-DM1, maytansinoid (DM1)-conjugated anti-NCAM mAb (lorvotuzumab, hN901), showed antitumor activity against NB,[74] and lorvotuzumab mertansine (IMGN901) is in a phase II



	Killing Mechanism					Affinity	Pharmacokinetics	
	NK-ADCC	PMN-ADCC	ADCP	CMC	CDCC/CDCP	(fold higher)	C_{max}	Half-Life
Mouse 3F8	+	+	+	+++	+++	10	High	Short
hu3F8 (naxitamab)	++	++	++	++	++	10	High	Short
ch14.18 (dinutuximab)	++	++	++	+	+	1	Low	Long
hu14.18-K322A	+++	+++	++	-	-	1	Low	Long

FIG 2. Mechanisms of action of anti-GD2 monoclonal antibodies. Anti-GD2 monoclonal antibodies (mAbs) mediate active immune response against disialoganglioside (GD2)–positive tumor cells. Anti-GD2 mAbs bind to cell surface GD2 and induce immune reactions including direct tumor cell apoptosis. Recruitment and signaling of type I receptors (FcγR I-III and their isoforms) through antigen-antibody complexes trigger antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). Alternatively, activation of complement pathway leads to tumor cell killing by the following 2 distinct processes: first, direct tumor cell lysis through complement-mediated cytotoxicity (CMC) by assembly of membrane attack complex (MAC; C5b-C9); and second, complement receptors (CRs) on effector cells recognize opsonins, such as C3b, and trigger complement-dependent cellular cytotoxicity (CDCC) and complement-dependent cellular phagocytosis (CDCP). These various immune responses by anti-GD2 mAbs can be modified further through Fc engineering by mutation and/or glycomodification to reduce immunogenicity or toxicity and increase the antitumor effect of engaging immune effector cells. Cmax, maximum concentration; NK, natural killer; PMN, polymorphonuclear leukocyte.

clinical trial for relapsed or refractory solid tumors including NB (ClinicalTrials.gov identifier: NCT02452554). In addition, m906, another human anti-NCAM mAb, was conjugated to the cytotoxic drug PBD and showed antitumor effect against $CD56^+$ NB in vitro.[75] For anti-GD2 antibodies, pegylated anti-GD2 immunoliposomes for targeted delivery of the survivin inhibitor sepantronium bromide (YM155) were successfully formulated to improve serum half-life and tumor accumulation of YM155.[76] Other pegylated anti-GD2 etoposide-loaded immunoliposomes have also shown antitumor potential in preclinical studies.[77]

Built on centuries of knowledge in radiation biology, radionuclides are powerful payloads with major therapeutic and diagnostic potential. Using antibodies as delivery vehicles, RIT exploits radionuclides that emit α- or β-particles or Auger electrons, with the potential to rival the precision

and intensity of external-beam radiation.[72,78] Early studies showing clinical benefit in non-Hodgkin lymphoma have resulted in FDA approval of both ^{131}I-tositumomab (Bexxar; GlaxoSmithKline, London, United Kingdom) and ^{90}Y-ibritumomab tiuxetan (Zevalin; Acrotech Biopharma, East Windsor, NJ). However, clinical development in solid tumors has lagged behind, mostly because of the unfavorable pharmacokinetics of large molecules, such as IgG, with slow clearance or of small molecules, such as single-chain Fv, with rapid renal clearance leading to insufficient tumor uptake.[72] ^{131}I-labeled GD2 or B7-H3 mAbs have been tested for NB, but systemic administration has encountered 2 major drawbacks, namely myelotoxicity and insufficient tumor dose, which is a limitation of IgG pharmacokinetics where the TI (payload area under curve for tumor *v* that for blood or normal tissues) is at best 5:1.[72] To increase the TI and to avoid liver uptake of intravenous 8H9, compartmental RIT was adopted among patients with CNS metastasis.[4,79,80] ^{131}I-3F8 and ^{131}I-omburtamab have been administered intrathecally to overcome the blood-brain barrier and to achieve a high TI for the treatment of recurrent leptomeningeal disease. In a phase I trial, intra-Ommaya ^{131}I-3F8 for GD2-positive CNS disease achieved high TI with major antitumor responses.[79] Intra-Ommaya ^{131}I-omburtamab administered as part of a salvage regimen produced long-term survival after CNS relapse.[4] In addition, convection-enhanced delivery of ^{124}I-omburtamab directly into diffuse intrinsic pontine glioma showed favorable dosimetry with a potential for escalation to curative doses.[80] α-Particle–emitting actinium-225 [^{225}Ac] has also been conjugated to 3F8 (^{225}Ac-1,4,7,10-tetra-azacyclododecane [DOTA]-3F8; ^{225}Ac-3F8) and administered intrathecally without toxicities, which improved survival in a xenograft model of meningeal carcinomatosis.[81]

Another class of ligands targetable by mAbs are cytokines that can enhance both the afferent and the efferent arms of the immune response. The expectation is to deliver cytokines into the tumor, avoiding systemic toxicities.[73] Different cytokines have been tested, including IL-2, IL-12, IL-13, IL-15, and GM-CSF, each fused to the amino and/or carboxy terminus of the IgG, and each showing antitumor benefits in preclinical studies.[82] Hu14.18-IL2 (EMD273063) immunocytokine is a genetic fusion protein where IL-2 is attached to the carboxy terminus of each of the IgG heavy chain on hu14.18. A phase II study of hu14.18-IL2 in relapsed or refractory NB has shown antitumor effect in patients with MRD in the bone marrow, but the response was difficult to separate from hu14.18 alone.[83] Intratumoral injection of hu14.18-IL2 in preclinical models achieved better immunocytokine retention and induced more potent antitumor responses than systemic injection by activating intratumoral NK cells and T cells.[84,85] Moreover, IL-15/IL-15Rα fusion protein (RLI) linked to the carboxy terminus of the heavy chain of anti-GD2 IgG showed superior antitumor effect compared with RLI or antibody alone.[86]

BsAbs

Unlike classic mAbs, BsAbs possess 2 binding specificities, built chemically or genetically based on a wide selection of structural platforms.[1,87] NK cell–engaging BsAbs have 2 specificities, one toward a tumor target and the other toward an NK-activating receptor such as CD16. T-cell BsAbs have the second specificity at the activating receptor CD3 and recruit polyclonal T cells without the restriction of HLA to overcome the low clonal frequency of classic cytotoxic T cells in tumor. BsAbs can be structurally grouped into the following 2 general classes: those built on the IgG framework (IgG-like BsAbs) and those built using antibody fragments such as a single-chain fragment (non–IgG-like BsAbs).[1] The most common non–IgG-like format is the tandem single-chain variable fragment (scFv; bispecific T-cell engager [BiTE; Amgen, Thousand Oaks, CA]) used in blinatumomab, the first BsAb to receive FDA approval.[88] Non–IgG-like BsAbs usually have short serum half-lives as a result of their small size (< 65 kDa) and absent interaction with neonatal Fc receptor (FcRn). Although their small size facilitates fast tissue penetration, their fast clearance requires repeated daily injections. Besides BiTE, various formats such as diabody, tandem diabody, DART, tandem triple scFv, and, dock-and-rock, Fab3 have been developed; however, most have encountered short half-lives as potential limitations.[89] IgG-like BsAbs are larger molecules (> 150 kDa) with longer serum half-lives because of their size above the renal clearance threshold and recycling through the FcRn-IgG complex.[90] The presence of Fc in IgG-like BsAb has other advantages over non-IgG BsAbs, such as structural symmetry, ease of manufacturing, drug stability during formulation, and distribution in vivo.[1,87] Yet, because the Fc domain is associated with undesirable cytokine release syndrome and interferes with T-cell infiltration into tumor,[91] silencing the Fc function is now routinely adopted in building IgG-like BsAbs. Other IgG-like BsAb formats include additional single-chain or disulfide stabilized Fvs or Fabs fused to the N or C termini of IgGs, resulting in tetravalent molecules with bivalent binding specificities.[87,89]

A number of BsAbs targeting GD2 have been built. At first, a bispecific Fab × Fab anti-GD2/anti-FcγRI (CD64) antibody was developed to engage antigen-presenting cells, monocytes, and macrophages against NB.[92] BsAbs containing anti-GD2 murine 5F11-scFv and anti-CD3 huOKT3-scFv (BiTE) recruited T cells and demonstrated antitumor effect against NB.[93] Substituting 5F11-scFv with the higher affinity hu3F8-scFv significantly improved T-cell activation and tumor cell killing in vitro.[94] Exploiting the IgG-like platform, a chemically conjugated anti-GD2 BsAb was developed,[95] and a phase I/II clinical trial using BsAb-armed T cells is ongoing (ClinicalTrials.gov identifier: NCT02173093). Using genetic engineering, a more recent IgG-like anti-GD2 BsAb, hu3F8-BsAb, where the anti-CD3 huOKT3-scFv is linked to the carboxyl end of the light

chain of hu3F8 IgG1 [IgG(L)-scFv], has been developed. Hu3F8-BsAb had N297A aglycosylation and K322A mutation of the Fc region to prevent FcγRs binding to reduce complement activation and cytokine storm.[34,91] Its high tumor killing potency (femtomolar half-maximal effective concentration [EC_{50}]), wide margin of safety (10^5-fold EC_{50} selectivity of tumor *v* normal tissue), ability to drive circulating T cells into solid tumors, and absence of neurotoxicity in preclinical models warranted the initiation of its clinical trial (ClinicalTrials.gov identifier: NCT03860207).[34] In parallel, pretargeted RIT (PRIT) using radiolabeled hu3F8-C825 BsAb, where anti-CD3 scFv is replaced by an anti-DOTA(metal) scFv (C825), achieved high TI (> 100:1) and cured NB tumors without toxicities in preclinical models.[96,97] This PRIT can adapt therapeutic β-emitters (^{177}Lu and ^{90}Y), α-emitters (^{225}Ac, ^{212}Pb), or diagnostic emitters (^{66}Ga, ^{89}Zr) and expand its clinical application.

ANTIBODY-BASED THERAPY OF NB AT THE CROSSROADS: A NEW PERSPECTIVE

Limitations of GD2 Immunotherapy

Two anti-GD2 mAb families, 3F8/hu3F8 (naxitamab) and ch14.18 (dinutuximab)/dinutuximab-β/hu14.18-K322A, have produced long-term cures among patients with high-risk metastatic NB. Antibody engineering through humanization and Fc modification to optimize their structure and function can reduce immunogenicity, improve effectiveness, and decrease on-target off-tumor adverse effects.[67,98,99] Engaging T cells using T-BsAbs also improved the potency of GD2 immunotherapy, and furthermore, the combination of BiTE-expressing oncolytic virus with CAR T-cell therapy has demonstrated successful outcomes for patients with advanced solid tumors.[100] Attaching payloads to IgGs enabled the delivery of therapeutic agents to the tumor even more efficiently. Of note, PRIT based on BsAb structure has produced cures in preclinical models without physical, chemical, or histologic toxicities and may provide an alternative to dose-intensive chemotherapy, which is deemed necessary for rapidly progressing metastatic NB.

Damaged Immune System

Partly because of intensive chemotherapy, immune effector cells in patients with NB are insufficient or incapacitated. Supplemented cytokines such as GM-CSF and IL-2 have been instrumental in enhancing myeloid cell–associated ADCC in NB.[3,101,102] Although IL-2 seemed to have failed in augmenting NK cell function,[61] IL-15 is a viable alternative given its pleiotropic effects on NK cells and T cells.[103] Immunocytokines have shown early promise, but competing affinities for cytokine receptor versus tumor target can derail the intended driver function of IgGs, such that cytokines fail to accumulate in the tumor.[104] Intratumoral injection of immunocytokine may be an alternative with the potential for inducing adaptive immunity.[105]

Suppressive TME

Among the key elements of the TME, TAMs, MDSCs, and immune checkpoints provide viable options to counter immune evasion.[106,107] Anti-CD105 antibody to deplete tumor-infiltrating myeloid cells has shown synergy with dinutuximab to overcome immunosuppressive TME.[108] The histone deacetylase inhibitor vorinostat decreases MDSCs and increases macrophage effector cells, which express high levels of FcγRs, thereby enhancing anti-GD2 mAb potency.[109] NK cell or myeloid cell inhibitory receptors, as members of immune checkpoints, provide biologic reasons for treatment failures as well as predictive biomarkers for clinical response. The sensitivity of NB to NK-ADCC and myeloid-ADCC derives partly from the downregulation or absence of HLA, hence missing-self recognition by inhibitory killer cell immunoglobulin-like receptors (KIRs) or inhibitory leukocyte immunoglobulin-like receptor subfamily B receptors (LILRBs).[110,111] For NK cells, checkpoint receptors and molecules include KIRs, CD94/NKG2A,TIGIT, CD96, TIM-3, CTLA-4, LAG-3, and PD-1; for macrophages, CD47 is the most studied.[112] Inhibition of NK checkpoints has the potential to reverse NK cell dysfunction and to boost antitumor activity, both in preclinical (anti-TIGIT and anti-CD96) and clinical studies (anti-NKG2A and anti-KIR).[113-115] The PD-1/PD-L1 axis also acts as a checkpoint in regulating NK-ADCC in NB,[26,116] and its modulation by nivolumab is being tested in combination with dinutuximab-β both in preclinical and clinical studies (ClinicalTrials.gov identifier: NCT02914405).[116] More recently, the gut microbiome might offer another tool to reboot or recruit antitumor responses through direct or indirect effects on antigen presentation, effector cell function, and vaccine efficacy.[117-119] In the phase I GD2 vaccine study, the effect of microbiome on anti-GD2 antibody titer is actively being investigated (ClinicalTrials.gov identifier: NCT00911560).

Biomarkers to Guide Treatment

The missing KIR ligand for NK-ADCC is associated with improved survival in patients treated with anti-GD2 IgGs, and KIR polymorphism KIR3LD1 and HLA-B allele combinations have been implicated as strong prognostic factors.[120,121] Moreover, FcγR2A polymorphisms,[122] the proportion of GD2-positive tumor cells in tumor,[123] and quantitation of bone marrow MRD by quantitative reverse transcription polymerase chain reaction[124,125] can be highly prognostic for survival after anti-GD2 immunotherapy. The utility of MRD measured early on after 2 cycles of immunotherapy was particularly relevant to provide rationale for stopping futile toxic therapies.[124] MRD panels including patient-specific DNA markers using whole-genome sequencing[126] and circulating microRNA[127] may provide additional insights into prognosis and treatment responses.

With the clinical introduction of BsAbs with or without checkpoint inhibitors, other biomarkers for both response and toxicities could be highly relevant.[128]

Chemoimmunotherapy

Induction and stem-cell transplantation followed by anti-GD2 antibody therapy has produced long-term cures.[3] Under the hypothesis that chemotherapy-induced microvascular or TME modification could enhance IgG-mediated antitumor response, moving anti-GD2 antibody hu14.18K322A or 3F8 up front to be administered concurrently with induction chemotherapy is feasible.[129,130] Hu14.18K322A incorporated into induction chemotherapy significantly improved early responses, reduced tumor volumes, and improved 2-year event-free survival (ClinicalTrials.gov identifier: NCT01857934).[131] For relapsed or refractory diseases, dinutuximab plus GM-CSF, when combined with irinotecan and temozolomide, and hu14.18K322A plus GM-CSF combined with chemotherapy and haploidentical NK cells have produced favorable response rates and survival.[129,130]

Alternative Targets

GD2 has provided a proof of principle for antibody-based targeting of NB. If it represents the tip of the iceberg, uncovering novel high-payoff targets should continue. So far NB antigens targeted by antibodies have included surface receptors or ligands shared with the neural crest (eg, GD2, CD56, L1CAM, ALK, and polysialic acid), immune checkpoint (eg, B7-H3), and signaling receptors (eg, glypicans).[132-135] Internal antigens, classically recognized only by T cells when presented as peptides buried in the HLA pocket, have just recently become druggable with T-cell receptor mimic antibodies.[136,137] These antigens include oncoproteins unique to NB (eg, MYCN),[138] cancer testis antigens (eg, PRAME),[139-142] transcription factors (eg, WT1),[143] or telomerase.[144] Multiomics approaches continue to uncover both cell surface and internal proteins as potential therapeutic targets.[132,145,146] However, the low density of these peptide-MHC complexes, their HLA allele restriction, potential tissue cross-reactivity, and tumor downregulation of HLA class I could limit their utility in clinical applications that rely on CMC and ADCC. Because normal tissue expression of antibody targets can influence the pharmacokinetics of mAbs, monitoring of their biodistribution in preclinical models and in patients should help prioritize their clinical development. Unexpected liver or lung uptakes have blunted enthusiasm for some antibodies in pediatrics; for example, a phase I trial of anti-CD99 MAB-013 for Ewing sarcoma was terminated because of liver and lung uptake associated with hypotension and chills (Memorial Sloan Kettering Institutional Review Board No. 90140), whereas liver uptake after intravenous anti–B7-H3 antibody forced its clinical development toward compartmental approaches (ClinicalTrials.gov identifier: NCT00582608). In vitro cytotoxicity directed at GD2, whether through CMC, ADCC, or antibody-dependent T-cell–mediated cytotoxicity, tends to be substantially stronger than that observed against other surface antigens, most likely attributable to its unique properties for immunotherapy. Despite the cross-reactivity to neural tissues, irreversible or chronic neurologic damage has rarely been reported through decades of clinical development, allowing GD2 to stand out among NCI priority antigens for immunotherapy.[28]

Integration of immunotherapy Into the Standard of Care

Finally, integrating antibody-based immunotherapy into the overall standard of care is still challenging. Many variables can affect the clinical outcome, such as passive versus active immunotherapy, up front versus sequential combinations, the type of chemotherapy, and the timing and the dose of radiation. These combinations are best optimized in appropriate animal models.[101] Yet, because most biologics are designed for human use, they are highly immunogenic in immunocompetent animals, hence the limitation of transgenic mouse or dog models. Immune-deficient mice engrafted with human cells can be constrained by graft-versus-host reactions that can confound both efficacy and toxicity measurements. In addition, NB xenografts and PDXs typically become admixed with substantial murine stroma content, thereby confounding conclusions on the TME. Despite these limitations, for diseases as rare as NB, skipping animal models and adopting a trial-and-error clinical approach is highly inefficient and should be discouraged. Here, a scientific consensus is sorely needed.

CONCLUSION

Cancer immunotherapy will improve long-term patient survival while reducing acute or chronic toxicities from genotoxic therapies. High-risk NB is one of the few cancers transformed by immunotherapy, changing its natural history from a uniformly lethal disease to a potentially curable one in more than half of patients. Yet, our understanding of immunobiology of NB and anti-GD2 therapy needs to be improved, with implications for future antibody-based therapies in NB and cancer immunotherapy in general. With the advances in protein engineering, novel antibody formats have the potential to deliver high-dose radiation to achieve responses without long-term toxicities, offering powerful alternatives to dose-intensive chemotherapy deemed necessary to treat rapidly growing NB. The combination of Fc-dependent and T-cell–mediated antibody approaches plus high-TI antibody-targeting strategies should change the outlook for children devastated by metastatic NB.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Targets and Antibody Formats for Immunotherapy of Neuroblastoma

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APPENDIX

TABLE A1. Targets and Their Antibody-Based Clinical Trials for Neuroblastoma

Cell Surface Target	Antibody	Molecular Format	NCI Clinical Trial	Phase	ClinicalTrials.gov Identifier
GD2	3F8	Murine IgG3	3F8/GM-CSF with isotretinoin for high-risk NB	II	NCT01183897, NCT01183884, NCT01183429, NCT00072358, NCT01183416
			3F8 and allogeneic NK cells for high-risk NB	I	NCT00877110
			β-Glucan and 3F8 in treating metastatic NB	I	NCT00037011
				I	NCT00492167
			^{131}I-3F8 in treating CNS or leptomeningeal NB	II	NCT00445965
				I	NCT00003022
			3F8/GM-CSF immunotherapy for high-risk NB	I	NCT00450307
				I	NCT00089258
				II	NCT00002560
			Adjuvant therapy with 3F8 for metastatic NB in second remission	II	NCT00002458
			^{131}I-3F8 and bevacizumab for relapsed or refractory NB	I	NCT00450827
			3F8 and oral etoposide for high-risk NB	I	NCT00004110
	Ch14.18 (dinutuximab)	Chimeric IgG1	Ch14.18/GM-CSF/IL-2 after ACT for high-risk NB	III	NCT00026312
				I	NCT00005576
			Ch14.18 pharmacokinetic study in high-risk NB	I	NCT01592045
			^{131}I-MIBG with ch14.18 for relapsed/refractory NB	I	NCT03332667
			Ch14.18 and lenalidomide ± isotretinoin for relapsed/refractory NB	I	NCT01711554
			Ch14.18 with NK cells and lenalidomide for relapsed/refractory NB	I	NCT02573896
			Ch14.18/GM-CSF/IL-2 with isotretinoin for high-risk NB	II	NCT02743429
			Irinotecan/temozolomide with temsirolimus or ch14.18 for relapsed/refractory NB	II	NCT01767194
			Ch14.18/GM-CSF/IL-2 and isotretinoin after ACT for high-risk NB	III	NCT01041638
			Ch14.18 with ^{131}I-MIBG or crizotinib for newly diagnosed high-risk NB	III	NCT03126916
			Ch14.18 plus irinotecan and temozolomide ± eflornithine (DFMO)	II	NCT03794349
			Ch14.18/GM-CSF with chemotherapy for patients with newly diagnosed high-risk NB undergoing stem-cell transplantation	II	NCT03786783
	Ch14.18/CHO (dinutuximab beta)	Chimeric IgG1	Ch14.18/CHO for refractory or relapsed NB	II	NCT02743429
				I	NCT01704872
			^{131}I-MIBG, nivolumab, and ch14.18/CHO for relapsed/refractory NB	I	NCT02914405
			Isotretinoin and Ch14.18/CHO with or without IL-2 for high-risk NB	III	NCT01704716
			Ch14.18/CHO plus IL-2 for refractory/relapsed NB	I, II	NCT01701479
			Ch14.18/CHO and IL-2 after haploidentical stem-cell transplantation for relapsed NB	II	NCT02258815
			Ch14.18/CHO plus NK cells for relapsed NB	I, II	NCT03242603

(continued on following page)

TABLE A1. Targets and Their Antibody-Based Clinical Trials for Neuroblastoma (continued)

Cell Surface Target	Antibody	Molecular Format	NCI Clinical Trial	Phase	ClinicalTrials.gov Identifier
	Hu14.18	Humanized IgG1	Ex vivo expanded haploidentical NK cells and hu14.18-IL-2 for relapsed/refractory NB	I	NCT03209869
			Hu14.18-IL-2 fusion protein for recurrent/refractory NB	II	NCT00082758
			Hu14.18-IL-2 fusion protein with GM-CSF and isotretinoin for relapsed/refractory NB	II	NCT01334515
			Hu14.18K322A with induction chemotherapy (cyclophosphamide and topotecan) for high-risk NB	II	NCT01857934
			Hu14.18-IL-2 fusion protein for refractory NB	I	NCT00003750
			Hu14.18K322A with NK cells for recurrent/refractory NB	I	NCT01576692
			Hu14.18K322A for recurrent/refractory NB	I	NCT00743496
				I	NCT02159443
			Hu14.18K322A with haploidentical NK cells after $CD33^+$ selected autologous stem-cell transplantation for high-risk NB	I	NCT02130869
	Hu3F8 (naxitamab)	Humanized IgG3	Hu3F8 combined with IL-2 for high-risk NB	II	NCT01662804
			Hu3F8/GM-CSF for relapsed/refractory NB	I, II	NCT01757626
			Hu3F8 for high-risk NB	I	NCT01419834
			Hu3F8/GM-CSF plus isotretinoin in first remission of high-risk NB	II	NCT03033303
			Hu3F8/GM-CSF plus isotretinoin for primary refractory NB in BM	II	NCT01183897
			PET imaging of solid tumors using ^{124}I-hu3F8	I	NCT02307630
			Hu3F8 and allogeneic NK cells for high-risk NB	I	NCT02650648
			Hu3F8, irinotecan, temozolomide, and GM-CSF for high-risk NB	I	NCT03189706
			Hu3F8 and GM-CSF in patients with high-risk NB with osteomedullary disease	III	NCT03363373
	OKT3 × hu3F8 BsAb (GD2Bi-ATC)	Chemical conjugate of IgG	Activated T cells armed with GD2Bi for high-risk NB	I, II	NCT02173093
	Hu3F8-BsAb	IgG(L)-scFv	Hu3F8-BsAb in patients with relapsed/refractory NB, osteosarcoma, and other solid tumor cancers	I, II	NCT03860207
B7-H3	8H9 (omburtamab)	Murine IgG1	Intrathecal ^{131}I-8H9 for CNS/leptomeningeal disease	I	NCT00089245
				II, III	NCT03275402
	MGA271 (enoblituzumab)	Humanized IgG1	MGA271 for B7-H3–expressing solid tumors	I	NCT02982941
	B7-H3 xCD3 BsAb (MGD009)	DART	MGD009 plus anti–PD-1 antibody in B7-H3–expressing relapsed/refractory cancers	I	NCT03406949
NCAM (CD56)	IMGN901 (hN901-DM1, lorvotuzumab mertansine)	Humanized IgG1 N902-maytasinoid DM1 drug conjugate	IMGN901 in children with relapsed/refractory tumors	II	NCT02452554
VEGF	Bevacizumab	Humanized IgG1	Bevacizumab, irinotecan, and temozolomide for relapsed/refractory NB	II	NCT01114555
				II	NCT02308527
			Bevacizumab, cyclophosphamide, and zoledronic acid for relapsed/refractory NB	I	NCT00885326
			Cyclophosphamide, topotecan, and bevacizumab for relapsed/refractory NB	II	NCT01492673

Abbreviations: ACT, adoptive cell therapy; BM, bone marrow; BsAb, bispecific antibody; CHO, Chinese hamster ovary; DART, dual-affinity retargeting; DFMO, difluoromethylornithine; GM-CSF, granulocyte-macrophage colony-stimulating factor; ^{131}I, iodine-131; IgG, immunoglobulin G; IL, interleukin; MIBG, metaiodobenzylguanidine; NB, neuroblastoma; NCAM, neural cell adhesion molecule; NK, natural killer; PD-1, programmed cell death 1; PET, positron emission tomography; VEGF, vascular endothelial growth factor.

TABLE A2. Preclinical Developments of Immunotherapeutic Targets for Neuroblastoma

Cell Surface Targets	Immunotherapy	Preclinical Study Results	Study
GD2	Humanized anti-GD2 mAb (IgG) (hu3F8)	Hu3F8 showed enhanced antitumor activities in vitro and in vivo	Cheung et al[2]
	Aglycosylated hu3F8 mAb produced in GnT1-deficient CHO cells (hu3F8-IgG1n)	Hu3F8-IgG1n elicited improved antitumor effect in vivo	Xu et al[99]
	HuGD2 mAb, hu14.18 K322A	Hu14.18 K322 reduced complement fixation in vitro and decreased antibody-induced allodynia in vivo	Sorkin LS, et al: Pain 149:135-142, 2010
	^{225}Ac-1,4,7,10-tetra-azacylododecane-3F8 radioimmunoconjugate	^{225}Ac-3F8 showed specific targeting of NB and acceptable toxicities in vivo; IT ^{225}Ac-3F8 improved survival in mouse xenograft models	Miederer et al[81]
	Hu14.18-IL2 (EMD273063) immunocytokine	Intratumoral hu14.18-IL2 enhanced inhibition of tumor growth and improved survival in vivo	Yang RK, et al: Cancer Immunol Immunother 62:1303-1313, 2013
	Anti-GD2-IL-15/IL-15Rα fusion protein (RLI)	Anti–GD2-RLI immunocytokine showed strong antitumor activities in vivo	Vincent et al[86]
	Bispecific Fab × Fab anti-GD2 and anti-FcγRI (CD64) Ab (MDX-260)	MDX-260 localized GD2-positive NB in vivo and showed effective cytotoxicity in vitro	Michon et al[92]
	Anti-GD2 murine 5F11-scFv and anti-CD3 huOKT3-scFv (5F11-BiTE)	5F11-BiTE induced strong TDCC in vitro and could efficiently inhibit NB xenograft growth	Cheng et al[93]
	Anti-GD2 h3F8-scFv and anti-CD3 huOKT3-scFv (hu3F8-BITE)	Hu3F8-BiTE hu3F8-scBA induced stronger T-cell activation and suppressed tumor growth and prolonged mice survival more effectively than 5F11-scBA	Cheng et al[93]
	Anti-GD2 anti-idiotype antibody (ganglidiximab)	Chimeric GD2-mimicking anti-idiotype antibody ganglidiximab for NB	Eger C, et al: PLoS One 11:e0150479, 2016
	Bispecific IgG-LC-scFv immunofusion (hu3F8-BsAb)	Hu3F8-BsAb activated and recruited T cells for tumor ablation, significantly prolonging survival in NB xenograft models	Xu et al[34]
	Anti-GD2 mAb, ch14.18	Dinutuximab, temozolomide, and γδ T-cell immunotherapy reduced tumor burden and prolonged survival in vivo	Zoine JT, et al: Oncoimmunology 8:1593804, 2019
	Anti-GD2 mAb, ch14.18	Anti-CD105 eliminated tumor microenvironment cells and enhanced the antitumor effect of anti-GD2 antibody and NK cell immunotherapy	Wu et al[108]
	Anti-GD2 mAb, ch14.18	Activated NK cells and dinutuximab improve survival after surgical resection of primary NB	Barry WE, et al: Clin Cancer Res 25:325-333, 2019
	Anti-GD2 14G2a mAb	Anti-GD2 14G2a plus MK-5108–specific aurora A kinase inhibitor potentiated cytotoxicity against NB cells in vitro	Horwacik I, et al: Cancer Lett 341:248-264, 2013
	Anti-GD2 immunoliposome	PEGylated sepantronium bromide (YM155)–loaded anti-GD2 immunoliposome increased half-lives and NB tumor accumulation of YM155	Gholizadeh et al[76]
	GD2 CAR T cells	Anti-GD2 CAR T cells induced strong cytotoxicity in vitro and abrogated NB growth in vivo	Prapa M, et al: Oncotarget 6:24884-24894, 2015
		High-affinity GD2 (GD2-E101K) CAR T cells induce fatal encephalitis	Richman SA, et al: Cancer Immunol Res 6: 36-46, 2018
		GD2-targeting retroviral cassette for NB	Thomas S, et al: PLoS One 11:e0152196, 2016
		GD2 CAR T cells undergo potent activation and deletion after antigen encounter but can be protected from AICD by PD-1 blockade	Gargett T, et al: Mol Ther 24:1135-1149, 2016
		GD2–CAR–IL-15 T cells enhanced antitumor activity and survival in vivo	Chen Y, et al: Clin Cancer Res 25:2915-2924, 2019
	GD2 CAR NK cells	NK-92-scFv(ch14.18)-ζ cells are effective against drug-resistant NB	Seidel D, et al: Cancer Immunol Immunother 64:621-634, 2015
	GD2 CAR NKT cells	GD2 CAR NKT cells effectively localized to the tumor site had potent antitumor activity, and	Heczey A, et al: Blood 124:2824-2833, 2014

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TABLE A2. Preclinical Developments of Immunotherapeutic Targets for Neuroblastoma (continued)

Cell Surface Targets	Immunotherapy	Preclinical Study Results	Study
		repeat injections significantly improved the long-term survival of mice with metastatic NB	
		GD2–CAR–IL-15 NKT cells increased in vivo persistence and antitumor activity against NB	Xu X, et al: Clin Cancer Res 25:7126-7138, 2019
B7H3	B7-H3 mAb conjugated with *Pseudomonas* endotoxin [8H9(dsFv)-PE38]	Recombinant IT 8H9(scFv)-PE38 showed cytotoxic and antitumor activities in vitro and in vivo	Onda M, et al: Cancer Res 64:1419-1424, 2004
	B7-H3–specific mAb (8H9)	8H9 has potent antitumor activity against NB cell lines in vitro	Ahmed M, et al: J Biol Chem 290: 30018-30029, 2015
	B7-H3–CAR T cells	B7-H3–CAR T cells (41BB costimulated) decreased PD-1 expression and significantly controlled NB tumor growth in vivo without toxicity	Du et al[38]
NCAM (CD56)	huN901-DM1, maytansinoid (DM1)–conjugated anti-NCAM mAb (hN901) (IMGN901)	IMGN901 has antitumor activity against some CD56-expressing pediatric cancer xenograft models, including NB	Wood AC, et al: Pediatr Blood Cancer 60: 1860-1867, 2013
	huNCAM mAb (m906)–PBD conjugate	Treatment with m906PBD conjugate resulted in potent cytotoxicity in $CD56^+$ NB cell lines	Feng et al[75]
	Anti-CD3 and NCAM targeting bispecific antibodies (OKT3/ERIC1)	OKT3/ERIC1 induced T-cell activation, expansion, and effector function and exerted antitumor effect on NB in vitro	Jensen M, et al: Clin Exp Immunol 134: 253-263, 2003
	Anti-CD56 CAR T cells	CD56 CAR T cells were effective against $CD56^+$ NB, glioma, and SCLC cells in vitro and suppressed tumor growth in vivo	Crossland DL, et al: Oncogene 37: 3686-3697, 2018
	NCAM-targeting peptide–polyglutamic acid–paclitaxel conjugates (PGX-PTX-NTX)	NCAM-targeted conjugates of polyglutamic acid with paclitaxel increased maximum-tolerated dose of paclitaxel and achieved better antitumor activity without increasing toxicity	Markovsky E, et al: J Control Release 249: 162-172, 2017
L1CAM (CD171)	^{131}I-L1CAM mAb	^{131}I-chCE7 showed superior growth inhibition compared with ^{131}I -MIBCG treatment in NB xenograft model	Hoefnagel CA, et al: Eur J Nucl Med 28: 359-368, 2001
	^{177}Lu- and $^{67/64}$Cu-chCE7 immunoconjugates	^{177}Lu- and $^{67/64}$Cu-chCE7 was successful for L1CAM-positive tumor imaging	Grünberg J, et al: Clin Cancer Res 11: 5112-5120, 2005
	CE7-specific CAR T cells	CE7 CAR T cells demonstrated in vitro and in vivo antitumor activity	Künkele A, et al: Clin Cancer Res 23: 466-477, 2017; Hong H, et al: J Immunother 37:93-104, 2014
ALK (CD246)	ALK-directed CAR T-cells	ALK CAR T cells can eradicate ALK-positive NB in mouse model	Walker AJ, et al: Mol Ther 25:2189-2201, 2017
	Mouse mAb IgG1	Anti-ALK mAb (mAb30 plus mAb49) induced significant dose-dependent growth inhibition and significant cytotoxicity in NB	Carpenter et al[51]
	ALK-targeting antibody-drug conjugate (CDX-0125-TEI)	CDX-0125-TEI had antitumor effect both in ALK-wild and -mutant PDXs	Sano et al[52]
GPC2	GPC2-directed antibody-drug conjugate	GPC2-directed antibody-drug conjugate that is potently cytotoxic to GPC2-expressing NB cells	Bosse et al[132]
	Anti-GPC2 immunotoxins and CAR T cells	Immunotoxin treatment was demonstrated to inhibit NB growth in vivo, and CAR T cells targeting GPC2 eliminated tumors in a disseminated NB mouse model	Li et al[133]

Abbreviations: AICD, activation-induced cell death; BsAb, bispecific antibody; CAR, chimeric antigen receptor; CHO, Chinese hamster ovary; IgG, immunoglobulin G; IL, interleukin; IT, intrathecal; mAb, monoclonal antibody; NB, neuroblastoma; NCAM, neural cell adhesion molecule; NK, natural killer; NKT, natural killer T; PD-1, programmed cell death 1; PDX, patient-derived xenograft; SCLC, small-cell lung cancer; TDCC, T-cell–dependent cellular cytotoxicity.