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 (ICls), 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 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%. In addition, compartmental radioimmunotherapy (RIT) with iodine-131-8H9 has contributed to major survival improvements in patients with CNS relapsed NB. 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. 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, oncolytic...
viral therapy, dendritic cell vaccines, and chimeric antigen receptor (CAR) T cells 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. 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 (NB). The TAM promotes T-cell apoptosis via Fas-FasL interactions, while activating myeloid-derived suppressor cells (MDSCs) and regulatory T cells, suppressing active immune response. 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, and neurofilament light chain in CSF is markedly increased, consistent with neuronal damage. 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.

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. 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. Second, by downregulating HLA class I antigens and NKGD2 ligands, activating immunoreceptor expressed by natural killer (NK) cells, NBs make themselves nearly invisible to classic T cells or NK cells. Third, NB cells express high levels of gangliosides and sialic acid–containing sugars and proteins, which are immunosuppressive when they shed into TME. Fourth, lymphocytes in the NB-infiltrated bone marrow (stage 4 metastatic NB) express programmed cell death 1 (PD-1) receptor, whereas HLAs are expressed highly. CD20+ B cells 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.

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 homogeneous 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. 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. 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. 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). Anti-GD2 immunoglobulin G (IgG) mAbs and anti-GD2 radioimmunoconjugates have shown successes in preclinical and clinical studies. T-cell–based approaches targeting GD2 are also actively pursued using both bispecific antibodies (BsAbs) and CAR T-cells.

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. This protein is homogeneously expressed in both primary and metastatic NBs 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. 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 (ClinicalTrials.gov identifier: NCT005282608).
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) multiple mechanisms, including the following: (1) immunosuppressive tumor microenvironment mediated by myeloid-derived suppressor cells (MDSCs); (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.

ALK

Aberrant anaplastic lymphoma kinase (ALK) expression is found in anaplastic large-cell lymphoma (ALCL), NSCLC, rhabdomyosarcoma, and NB. ALK is ranked 33rd among the NCI cancer antigens, 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. Mutations in ALK have been implicated in 9% of NBs, and it is adversely prognostic, especially in the presence of MYCN amplification. 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. Prediction of T-Cell Epitopes for Cancer Therapy (ProTECT) analyses 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. Intrathecal (through an Ommaya reservoir) 131I- or 124I-conjugated omburtamab has increased the cure rate for patients with CNS involvement. A phase I clinical trial of intraperitoneal 131I-8H9 for patients with desmoplastic small round cell tumors and other solid tumors involving the peritoneum is ongoing (ClinicalTrials.gov identifier: NCT01099644). Another B7-H3–targeting antibody, enoblituzumab, notable for its nonreactivity with liver, 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.

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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. 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. 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). 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 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, Fcγ receptor (FcγR)-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) by NK cells, neutrophils, and macrophages; and complement-mediated cytotoxicity (CMC) through complement breakdown of macrophages and complement-mediated cytotoxicity (ADCC) 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). 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. 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, whereas hypertension and posterior reversible encephalopathy syndrome were more noticeable for 3F8. 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. Postconsolidation treatment with 3F8 plus granulocyte-macrophage colony-stimulating factor (GM-CSF) improved overall survival to > 65% among patients with high-risk metastatic NB. 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. A subsequent randomized study using dinutuximab-β showed no benefit of IL-2 over mAb alone, 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.

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 reducethese ADAs while enhancing ADCC through the human IgG1-Fc, as well as retaining CMC potency through its high affinity for GD2. 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. 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 Fc-γRIIA/B binding, while alanine substitution at K322 significantly decreases complement activation.

**Arming IgG Antibodies With Conjugates**

Another strategy to enhance IgG functions is to arm them with therapeutic agents such as drugs, radionuclides, or cytokines. 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 G2/M phase cell cycle arrest. DNA-damaging agents such as anthracyclines, calicheamicin, duocarmycin, and pyrroloindazoleazines (PBDS) function by binding the minor groove of DNA and cause DNA strand scission, alklylation, or cross-linking. Antibody-drug conjugates targeting neural cell adhesion molecule (NCAM; CD56), HuN901-D1M, maytansinoid (DM1)-conjugated anti-NCAM mAb (lorvotuzumab, hN901), showed antitumor activity against NB, and lorvotuzumab mertansine (IMGN901) is in a phase II
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 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. Early studies showing clinical benefit in non-Hodgkin lymphoma have resulted in FDA approval of both 131I-tositumomab (Bexxar; GlaxoSmithKline, London, United Kingdom) and 90Y-ibritumomab tiuxetan (Zevalin; Acrotext 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. 131I-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. To increase the TI and to avoid liver uptake of intravenous 8H9, compartmental RIT was adopted among patients with CNS metastasis. 4,79,80 131I-3F8 and 131I-ombrutamab 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 131I-3F8 for GD2-positive CNS disease achieved high TI with major antitumor responses. 79 Intra-Ommaya 131I-ombrutamab administered as part of a salvage regimen produced long-term survival after CNS relapse. 7 In addition, convection-enhanced delivery of 131I-ombrutamab directly into diffuse intrinsic pontine glioma showed favorable dosimetry with a potential for escalation to curative doses. 80 Alpha-Particle–emitting actinium-225 [225Ac] has also been conjugated to 3F8 [225Ac-1,4,7,10-tetra-azacyclododecane (DOTA)-3F8; 225Ac-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 effector arms of the immune response. The expectation is to deliver cytokines into the tumor, avoiding systemic toxicities. 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. 85 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-15Ra 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. 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). 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. 91 Yet, because the Fc domain is associated with undesirable cytokine release syndrome and interferes with T-cell infiltration into tumor, 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 Fab5 fused to the N or C termini of IgGs, resulting in tetravalent molecules with bivalent binding specificities. 92,93

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. 90 BsAbs containing anti-GD2 murine 5F11-scFv and anti-CD3 huOKT3-scFv (BiTE; Amgen, Thousand Oaks, CA) used in blinatumomab, the first BsAb to receive FDA approval. 88 Other IgG-like BsAb formats include additional single-chain or disulfide stabilized Fvs or Fab5 fused to the N or C termini of IgGs, resulting in tetravalent molecules with bivalent binding specificities. 92,93

Exploiting the IgG-like platform, a chemically conjugated anti-GD2 BsAb was developed,95 and a phase I/II clinical trial using BsAbs against 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 Fe region to prevent FcγRs binding to reduce complement activation and cytokine storm.34,31 Its high tumor killing potency (femtomolar half-maximal effective concentration [EC50]), wide margin of safety (104-fold EC50 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 (177Lu and 90Y), α-emitters (225Ac, 212Pb), or diagnostic emitters (64Ga, 89Zr) 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.57,58,99 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,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 KIR3DL1 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 reaction124,125 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 sequencing126 and circulating microRNA127 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 Multimomics 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-O13 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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Targets and Antibody Formats for Immunotherapy of Neuroblastoma

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### 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 |
|                     |          |                  | 123I-3F8 in treating CNS or leptomeningeal NB | I     | NCT00492167, NCT00037022 |
|                     |          |                  | 3F8/GM-CSF immunotherapy for high-risk NB | I     | NCT00450307 |
|                     |          |                  | 3F8/GM-CSF immunotherapy for high-risk NB | I     | NCT00003022 |
|                     |          |                  | 3F8 and allogeneic NK cells for high-risk NB | I     | NCT00025660 |
|                     |          |                  | 3F8 and allogeneic NK cells for high-risk NB | I     | NCT00024585 |
|                     |          |                  | 123I-3F8 and bevacizumab for relapsed or refractory NB | I     | NCT00450827 |
|                     |          |                  | Ch14.18/GM-CSF/IL-2 with isotretinoin for high-risk NB | I     | NCT00004110 |
|                     |          |                  | Ch14.18/GM-CSF/IL-2 after ACT for high-risk NB | II    | NCT00026312 |
|                     |          |                  | Ch14.18/GM-CSF/IL-2 after ACT for high-risk NB | II    | NCT0005576 |
|                     |          |                  | Ch14.18 pharmacokinetic study in high-risk NB | I     | NCT01592054 |
|                     |          |                  | 123I-MIBG with ch14.18 for relapsed/refractory NB | I     | NCT03330667 |
|                     |          |                  | Ch14.18 and lenalidomide ± isotretinoin for relapsed/refractory NB | I     | NCT01711594 |
|                     |          |                  | 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 with isotretinoin after ACT for high-risk NB | II    | NCT01041638 |
|                     |          |                  | Ch14.18 with 123I-MIBG or citotinib 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) | Chimeric IgG1 | Chimeric IgG1 | Ch14.18/CHO for refractory or relapsed NB | II    | NCT01704872 |
|                     |          |                  | Ch14.18/CHO for refractory or relapsed NB | I, II | NCT01704872 |
|                     |          |                  | 123I-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/refractory 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)
| 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 | 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 | |   |
|                     |          | PET imaging of solid tumors using 124I-hu3F8 | II | NCT02307630 |
|                     |          | Hu3F8 and alligeneic NK cells for high-risk NB | I | NCT02650648 |
|                     |          | Hu3F8, irinotecan, temozolomide, and GM-CSF for high-risk NB | |   |
|                     |          | Hu3F8 and GM-CSF in patients with high-risk NB with osteosarcoma, and other solid tumor cancers | III | NCT03363373 |
| OKT3 x 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               | MH9 (omburtamab) | Murine IgG1 | Intrathecal 131I-MH9 for CNS/leptomeningeal disease | I | NCT0089245 |
|                     | 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 (hnN901-DM1, lorvotuzumab mertansine) | Humanized IgG1 N902-maytansinoid 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 |
|                     |          | Bevacizumab, cyclophosphamide, and zoledronic acid for relapsed/refractory NB | II | NCT02308527 |
|                     |          | 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; 131I, iodine-131; IgG, immunoglobulin G; IL, interleukin; MIBG, metadiobenzylguanidine; 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 (lgG) (hu3F8) | Hu3F8 showed enhanced antitumor activities in vitro and in vivo | Cheung et al. |
|                     | Aglycosylated hu3F8 mAb produced in GnT1-deficient CHO cells (hu3F8-IgG1n) | Hu3F8-IgG1n elicited improved antitumor effect in vivo | Xu et al. |
|                     | HuGD2 mAb, hu14.18 K322A | Hu14.18 K322 reduced complement fixation in vitro and decreased antibody-induced autodidynia in vivo | Sorkin LS, et al: Pain 149:135-142, 2010 |
|                     | 225Ac-1,4,7,10-tetra-azacylododecane-3F8 radioimmunoconjugate | 225Ac-3F8 showed specific targeting of NB and acceptable toxicities in vivo; 1T 225Ac-3F8 improved survival in mouse xenograft models | Miederer et al. |
|                     | 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-RL1 immunocytokine showed strong antitumor activities in vivo | Anti-GD2-RL1 immunocytokine showed strong antitumor activities in vivo | Vincent et al. |
|                     | Bispecific Fab × Fab anti-GD2 and anti-FcyRI (CD64) Ab (MDX-260) | MDX-260 localized GD2-positive NB in vivo and showed effective cytotoxicity in vitro | Michon et al. |
|                     | Anti-GD2 murine 5F11-scFv and anti-CD3 huOKT3-scFv (5F11-BiTE) | 5F11-BiTE induced strong TPCC in vitro and could efficiently inhibit NB xenograft growth | Cheng et al. |
|                     | Anti-GD2 h3F8-scFv and anti-CD3 huOKT3-scFv (hu3F8-BiTE) | Hu3F8-BiTE hu3F8-scBA induced Strong T-cell activation and suppressed tumor growth and prolonged mice survival more effectively than 5F11-scBA | Cheng et al. |
|                     | 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. |
|                     | Anti-GD2 mAb, ch14.18 | Dinutuximab, temozolomide, and Y4 T-cell immunotherapy reduced tumor burden and prolonged survival in vivo | Zaine 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. |
|                     | 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 | Horwack 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 | Gholidzadeh et al. |
| GD2 CAR T cells     | Anti-GD2 CAR T cells induced strong cytotoxicity in vitro and abrogated NB growth in vivo | 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 | 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 | 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 | 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 | GD2–CAR–IL–15 T cells enhanced antitumor activity and survival in vivo | Chen Y, et al: Clin Cancer Res 25:3915-3924, 2019 |
| GD2 CAR NK cells    | NK-92-scFvCh14.18: T cells are effective against drug-resistant NB | NK-92-scFvCh14.18: T cells are effective against drug-resistant NB | Seidel D, et al: Cancer Immunol Immunother 64:1261-134, 2015 |
| GD2 CAR NKT cells  | GD2 CAR NKT cells effectively localized to the tumor site had potent antitumor activity, and | GD2 CAR NKT cells effectively localized to the tumor site had potent antitumor activity, and | Heczey A, et al: Blood 124:2824-2833, 2014 |

(continued on following page)
### TABLE A2. Preclinical Developments of Immunotherapeutic Targets for Neuroblastoma (continued)

| Cell Surface Targets | Immunotherapy | Preclinical Study Results | Study |
|----------------------|---------------|---------------------------|-------|
| 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 al86 |
| 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 (m9066)-PBD conjugate | Treatment with m9066PBD conjugate resulted in potent cytotoxicity in CD56+ NB cell lines | Feng et al76 |
| | 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 vivo | | 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) | L1CAM mAb 131I-chCE7 showed superior growth inhibition compared with 131I -MIBCG treatment in NB xenograft model | | Hoefnagel CA, et al: Eur J Nucl Med 28: 359-368, 2001 |
| | 131I-chCE7 and 67/64Cu-chCE7 immunoconjugates 131I- and 67/64Cu-chCE7 was successful for L1CAM-positive tumor imaging | | Grünewald 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 al51 |
| | ALK-targeting antibody-drug conjugate (CDX-0125-TEI) CDX-0125-TEI had antitumor effect both in ALK-wild and -mutant PDXs | | Sano et al52 |
| GPC2 | GPC2-directed antibody-drug conjugate GPC2-directed antibody-drug conjugate that is potently cytotoxic to GPC2-expressing NB cells | | Bosse et al132 |
| | 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 al133 |

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; NK-T, natural killer T; PD-1, programmed cell death 1; PDX, patient-derived xenograft; SCLC, small-cell lung cancer; TDCC, T-cell–dependent cellular cytotoxicity.