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Hopes on immunotherapy targeting B7-H3 in neuroblastoma

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ABSTRACT

Neuroblastoma is one of the most aggressive cancer forms in children, with highly heterogenous clinical manifestations ranging from spontaneous regression to high metastatic capacity. High-risk neuroblastoma has the highest mortality rates of all pediatric cancers, highlighting the urgent need for effective novel therapeutic interventions. B7-H3 immune checkpoint protein is highly expressed in neuroblastoma, and it is involved in oncogenic signaling, tumor cell plasticity, and drug resistance. Immunotherapies based on immune checkpoint inhibition have improved patient survival in several human cancers, and recent reports provide preclinical evidence on the benefits of targeting B7-H3 in neuroblastoma, with emphasis on novel CAR T/NK-cell approaches. Here, we summarize the current status of neuroblastoma targeted therapies, with a focus on B7-H3 as a promising novel immunoregulatory therapeutic target for high-risk neuroblastoma.

Current neuroblastoma treatments

Neuroblastoma is the most frequently diagnosed extracranial solid pediatric tumor. Neuroblastoma develops from sympathetic-adrenal cells of the neural crest, and clinically manifests with primary tumors typically located in the abdomen, cervix, thorax and pelvis. Neuroblastoma tumors show a high clinical heterogeneity, from spontaneously regressing tumors to metastatic tumors refractory to multi-modal therapies. Approximately half of pediatric neuroblastomas are classified as high-risk neuroblastomas, with a predicted 5-year survival rate inferior to 50%. The transcription factor N-Myc (*MYCN*) and the receptor tyrosine kinase ALK are major oncogenic promoters in neuroblastoma. *MYCN* is amplified in most of high-risk neuroblastoma cases and constitutes a primary prognosis factor. However, the intrinsic molecular properties of MYCN make difficult its direct targeting, and mostly indirect approaches targeting MYCN upstream regulators have been explored in neuroblastoma. ALK small molecule inhibitors, as well as anti-ALK mAb, are being tested clinically or preclinically for therapeutic efficacy in neuroblastoma [1–3].

Clinical interventions of high-risk neuroblastoma include induction chemotherapy and surgery, followed by consolidation high-dose chemotherapy and autologous stem cell rescue, radiation therapy, isotretinoin (13-cis-retinoic acid), and targeted therapy in combination with cytokines. At present, the only targeted therapy currently approved for neuroblastoma consists on monoclonal antibodies (mAb) against the disialoganglioside GD2, an acidic glycolipid found on the outer cell membrane. GD2 is abundantly expressed on embryonal tumors of neuroectodermal origin, including human neuroblastoma. Currently approved anti-GD2 mAb therapies for treatment of high-risk neuroblastoma include the chimeric mAb dinutuximab and dinutuximab-beta (based on 14.18 mAb), and the humanized mAb naxitamab (based on 3F8 mAb). The mechanism of action of these mAb is mainly based on antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity, and although their use has improved the survival of high-risk neuroblastoma patients, almost 50% of anti-GD2 treated patients relapse [4]. Anti-GD2 tumor resistance is not fully elucidated, but some emerging mechanisms of resistance have been reported through downregulation of GD2 expression and by the action of neuroblastoma derived extracellular vesicles [5,6].

B7-H3 in neuroblastoma

B7-H3 (*CD276*) immune checkpoint protein, similar to GD2, is highly expressed in neuroblastoma [7–10], and increased B7-H3 mRNA expression is observed in advanced tumor stage (Fig. 1). Additionally, B7-H3 expression is a potential diagnostic/therapeutic target in patients negative for GD2, which could be advantageous in patients experiencing anti-GD2 resistance [11]. B7-H3 on cancer cells works in a dual fashion,

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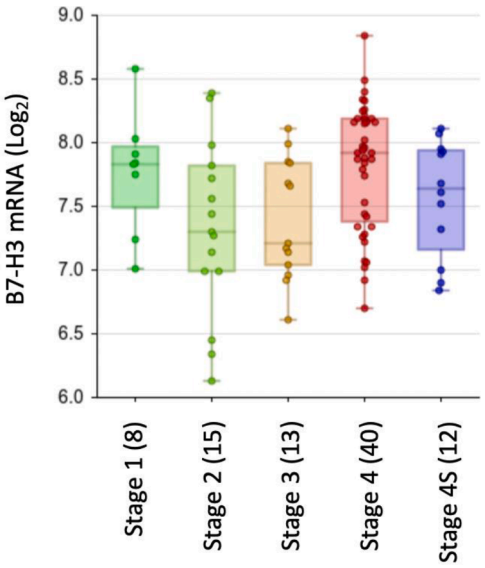

Fig. 1. Expression of B7-H3 mRNA in neuroblastoma stages. B7-H3 mRNA expression (Log_2) is shown in box plots grouped by stages according to International Neuroblastoma Staging System. Data is from study Tumor Neuroblastoma public, Versteeg 88, MAS5.0 u133p2 (number of patients 88). Plot is from R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl).

creating a molecular shield which favors both immune evasion and propagation of tumor-intrinsic promoting signals (Fig. 2) [12]. B7-H3 immunosuppressive function in neuroblastoma was revealed in early studies where blocking of B7-H3 with anti-B7-H3 mAb enhanced the NK-mediated killing of neuroblastoma cells [8]. More recently, high expression of B7-H3 mRNA in neuroblastoma tumors has been shown to associate with decreased patient overall survival, and B7-H3 shRNA knockdown induced cell cycle arrest and suppressed the proliferation of SH-SY5Y neuroblastoma cells [7]. In another report, overexpression of B7-H3 in SH-SY5Y cells was found to confer doxorubicin resistance in the presence of artemether, an anti-malaria drug that manifests cytotoxic effects in tumor cells [13].

Targeting B7-H3 in neuroblastoma

Targeting of B7-H3 is being assayed by multiple cancer therapeutic strategies, mostly based on anti-B7-H3 mAb or derivatives [14]. In summary, these include (I) use of blocking mAb, (II) mAb-dependent cellular cytotoxicity, (III) mAb-drug conjugates, (IV) mAb-targeted radiotherapy, (V) bi-specific mAb, and (VI) chimeric antigen receptor (CAR) T- and natural killer (NK)-cells (Fig. 2). Clinical trials targeting B7-H3 in neuroblastoma are summarized in Table 1. A phase I clinical trial using the anti-B7-H3 blocking mAb Enoblituzumab is finished, but results are not published. Although there are no described clinical trials using anti-B7-H3-drug conjugates in neuroblastoma patients, Kendersky et al. have recently reported the anti-tumor efficacy of an anti-B7-H3-pyrrolobenzodiazepine (PBD) conjugate in neuroblastoma patient- and cell line-derived xenograft mouse models [15]. Targeted radiotherapy trials using ^{131}I-Omburtamab, a radioconjugated anti-B7-H3 mouse mAb (8H9 mAb), are currently ongoing, and results are encouraging. Combination of anti-B7-H3 and anti-GD2 mAb constitutes a potential alternative approach to improve these therapies. In addition, trials using a variety of B7-H3 CAR methodologies, based on recent preclinical data (see below), are also in progress (Table 1).

B7-H3 CAR therapies in neuroblastoma

Grote et al. reported the specific and long-term cytotoxicity of B7-H3 CAR NK-92-cells towards neuroblastoma cells expressing B7-H3, in association with increased production of NK effector molecules and pro-inflammatory cytokines [16]. Further optimization of B7-H3 CAR NK-cells in neuroblastoma interventions will surely be pursued. Regarding CAR T-cell approaches, Birley et al. and Du et al. have reported the efficacy *in vitro* and in mouse models of the anti-tumor activity on neuroblastoma cells of B7-H3 CAR T-cells [17,18]. Additional methods are under preclinical exploration to augment the efficacy and to limit the toxicity of CAR T-cell therapies in neuroblastoma. Moghimi et al. have tested the synthetic Notch (SynNotch) gating strategy in metastatic neuroblastoma, using GD2 as the gate and B7-H3 as the inducible target, and they have found that SynNotch GD2-B7-H3 CAR T-cells display highly specificity and improved cytotoxicity against neuroblastoma cells *in vitro* and in xenograft mouse models [19]. More

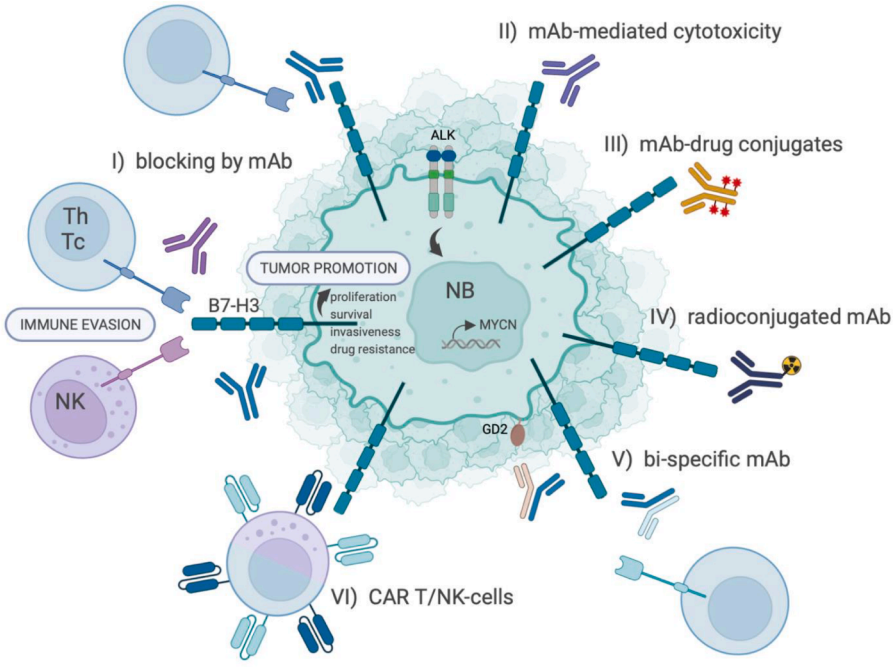

Fig. 2. Targeting B7-H3 by multiple therapeutic strategies in neuroblastoma. A neuroblastoma (NB) cell is represented, and schematic transmembrane B7-H3 proteins (containing four extracellular Ig-like domains and a short intracellular region) are depicted. The immune evasion mechanisms of B7-H3 are illustrated through binding of B7-H3 extracellular region to unidentified receptor(s) present on lymphoid cells (Th, T helper; Tc, T cytotoxic; NK, natural killer) in the tumor microenvironment. Tumor promoting mechanisms of B7-H3 link this protein to major pro-oncogenic and drug resistance signaling pathways. MYCN transcription factor and ALK tyrosine kinase receptor, two major pro-oncogenic proteins in neuroblastoma, are also represented. B7-H3 targeted therapies based on anti-B7-H3 mAb or byproducts are shown: (I) blocking by mAb of interactions mediating B7-H3 activity, (II) mAb-mediated cellular or complement cytotoxicity, (III) mAb conjugates with cytotoxic drugs, (IV) radioimmunotherapy by radioconjugated mAb, (V) bi-specific antibodies facilitating the proximity of cytotoxic effector cells and neuroblastoma cells, or recognizing in a bi-specific manner B7-H3 and other neuroblastoma tumor-associated antigen, such as GD2, and (VI) chimeric antigen receptor (CAR) T/NK-cells. Created with BioRender.com.

Table 1
Targeting B7-H3 in neuroblastoma.

Identifier # Phase / Study	Agent / Drug	Trial / Study	Status / estimated study completion / [Reference]
mAb-based CLINICAL TRIALS[1]			
NCT02982941 (Phase 1)	Enoblituzumab[2]	Enoblituzumab treatment in children with B7-H3-expressing solid tumors	Completed February 2022
NCT00582608 (NA)	^{131}I-Omburtamab[3]	Tumor detection in CNS cancer, NB, and sarcoma.	Completed May 2009
NCT05064306 (NA)	^{131}I-Omburtamab	Radioimmunotherapy for CNS/leptomeningeal neoplasms in children and young adults	Available NA
NCT00089245 (Phase 1)	^{131}I-Omburtamab	Radioimmunotherapy for refractory, recurrent, or advanced CNS or leptomeningeal cancer	Active, not recruiting July 2025
NCT03275402 (Phase 2)	^{131}I-Omburtamab	Radioimmunotherapy for NB, CNS/leptomeningeal metastases	Recruiting December 2026
CAR T-based CLINICAL TRIALS[1]			
NCT04691713 (NA)	B7-H3 CAR T-cells[4]	B7-H3-specific CAR T-cell therapy for treatment of patients with B7-H3-positive advanced solid tumors	Recruiting NA
NCT04637503 (Phase 1/2)	B7-H3 CAR T-cells[5]	CAR T-cell therapy targeting GD2, PSMA, and B7-H3 for treating NB	Recruiting December 2023
NCT04864821 (Early Phase 1)	B7-H3 CAR T-cells[4]	B7-H3-specific CAR T-cell therapy in patients with B7-H3-positive advanced solid tumors	Not yet recruiting May 2023
NCT04432649 (Phase 1/2)	B7-H3-iCasp9 CAR T-cells[6]	B7-H3-specific CAR T-cell therapy in patients with refractory and/or recurrent solid tumors	Recruiting May 2024
NCT04897321 (Phase 1)	B7-H3 CAR T-cells[4]	B7-H3-specific CAR T-cell therapy for pediatric patients with solid tumors	Recruiting March 2027
NCT04483778 (Phase 1)	4-1BBζ B7-H3-EGFRt-DHFR CAR T-cells[7]	B7-H3 CAR T-cell therapy for recurrent/refractory solid tumors in children and young adults	Recruiting December 2040
CAR T/NK PRECLINICAL STUDIES			
Grote et al.	B7-H3 CAR NK-cells	*In vitro* three-dimensional NB spheroid model of the anti-tumor activity of B7-H3 CAR NK-cells	[16]
Du et al.	B7-H3 CAR T-cells	*In vitro* and in mouse models of the anti-tumor activity on NB cells of B7-H3 CAR T-cells	[17]
Birley et al.	B7-H3 CAR T-cells	*In vitro* and in mouse models of the anti-tumor activity on NB cells of B7-H3 CAR T-cells	[18]
Moghimi et al.	GD2-B7-H3 CAR T-cells	*In vitro* and in xenograft mouse models of the anti-tumor activity on NB cells of GB2-B7-H3 CAR T-cells	[19]
Tian et al.	Bicistronic CAR T-cell	*In vitro* and in xenografts mouse models of the anti-tumor activity on NB cells of GPC2-B7-H3 CAR T-cells	[20]

[1]) Clinical trials including neuroblastoma patients are listed
[2]) Enoblituzumab, humanized mAb, also known as MGA271, based on murine BRCA84D mAb
[3]) ^{131}I-Omburtamab, radioactive iodine-labeled mAb, based on murine 8H9 mAb
[4]) Autologous B7-H3 CAR T-cells
[5]) Autologous B7-H3 CAR T-cells in combination with GD2 and/or PSMA CAR T-cells
[6]) Autologous B7-H3 CAR T-cells expressing B7-H3-iCasp9 (inducible apoptotic caspase 9 domain)
[7]) Autologous B7-H3 CAR CD4+ and CD8+ T-cells expressing 4-1BBζ (CD137) B7-H3-EGFRt-DHFR.

Abbreviations: CAR, Chimeric Antigen Receptor; CNS, Central Nervous System; mAb: monoclonal antibody; NA, not applicable; NB: neuroblastoma

recently, Tian et al. have performed a combinatorial approach to identify optimal combinations of tumor antigen targets into a bicistronic (BiCis) CAR T-cell against neuroblastoma cells. This has the advantage to potentially overcome the high heterogeneity found in the expression of tumor antigens in neuroblastoma, increasing the efficacy to eliminate heterogeneous populations of tumor cells. BiCis GPC2 (Glypican-2)/B7-H3 CAR T-cells were highly effective to eliminate neuroblastoma cells *in vitro* and in mice xenografts, and showed longer T-cell persistence and higher resistance to T-cell exhaustion, as compared with single antigen CAR T-cells [20]. It would be of interest to test the cytotoxic efficacy of additional BiCis CAR T-cell combinations of B7-H3 with other neuroblastoma tumor-associated antigens.

In summary, CAR T-cells targeting B7-H3 have demonstrated significant preclinical anti-tumor activity *in vivo* against pediatric solid tumors and brain tumors, including glioma and medulloblastoma [9, 21]. B7-H3 targeted CAR-based cell therapies shows tolerable safety and promising interim results [21], but specific target-organ damage and the potential of cytokine storm still needs to be analyzed in the upcoming clinical trials.

Concluding remarks

High-risk neuroblastoma patients respond poorly to classical immune checkpoint inhibitors, and a variety of alternative immunotherapy approaches are being explored for this pediatric cancer [22]. B7-H3 constitutes a suitable immunoregulatory therapeutic target in neuroblastoma, and several of the current mAb-based and CAR-based B7-H3-targeting approaches take advantage of the high selective expression of B7-H3 on the surface of neuroblastoma cells. The dual role of B7-H3 in tumor evasion and tumor promotion [23] provides additional mechanistic possibilities of therapeutic intervention, which need to be explored. The identification of receptor and intracellular binding partners of B7-H3, as well as the elucidation of its role in pro-oncogenic signal transduction, may provide information on therapeutically actionable vulnerabilities present in neuroblastoma. Establishing the fundaments of B7-H3 in neuroblastoma will help in the success of novel immunotherapies for the high-risk forms of this type of cancer.

CRediT authorship contribution statement

Rafael Pulido: Data curation, Funding acquisition, Conceptualization, Writing – review & editing. **Caroline E. Nunes-Xavier:** Conceptualization, Data curation, Funding acquisition, Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References

[1] A.K. Brenner, M.W. Gunnes, Therapeutic targeting of the anaplastic lymphoma kinase (ALK) in neuroblastoma-a comprehensive update, Pharmaceutics 13 (9) (2021).
[2] W.L. Furman, Monoclonal antibody therapies for high risk neuroblastoma, Biologics 15 (2021) 205–219.
[3] A. Zafar, et al., Molecular targeting therapies for neuroblastoma: progress and challenges, Med. Res. Rev. 41 (2) (2021) 961–1021.
[4] G.C. Chan, C.M. Chan, Anti-GD2 directed immunotherapy for high-risk and metastatic neuroblastoma, Biomolecules 12 (3) (2022).
[5] N.W. Mabe, et al., Transition to a mesenchymal state in neuroblastoma confers resistance to anti-GD2 antibody via reduced expression of ST8SIA1, Nat. Cancer 3 (8) (2022) 976–993.
[6] X. Liu, et al., Small extracellular vesicles induce resistance to anti-GD2 immunotherapy unveiling tipifarnib as an adjunct to neuroblastoma immunotherapy, J. Immunother. Cancer 10 (4) (2022).
[7] H. Zhang, et al., Survival association and cell cycle effects of B7H3 in neuroblastoma, J. Korean Neurosurg. Soc. 63 (6) (2020) 707–716.
[8] R. Castriconi, et al., Identification of 4Ig-B7-H3 as a neuroblastoma-associated molecule that exerts a protective role from an NK cell-mediated lysis, Proc. Natl. Acad. Sci. U. S. A. 101 (34) (2004) 12640–12645.
[9] R.G. Majzner, et al., CAR T cells targeting B7-H3, a pan-cancer antigen, demonstrate potent preclinical activity against pediatric solid tumors and brain tumors, Clin. Cancer Res. 25 (8) (2019) 2560–2574.
[10] U.B. Maachani, et al., B7-H3 as a prognostic biomarker and therapeutic target in pediatric central nervous system tumors, Transl. Oncol. 13 (2) (2020) 365–371.
[11] A. Dondero, et al., Multiparametric flow cytometry highlights B7-H3 as a novel diagnostic/therapeutic target in GD2neg/low neuroblastoma variants, J. Immunother. Cancer 9 (4) (2021).
[12] K. Flem-Karlsen, et al., B7-H3 immune checkpoint protein in human cancer, Curr. Med. Chem. 27 (24) (2020) 4062–4086.
[13] W.Q. Tan, et al., Artemether regulates chemosensitivity to doxorubicin via regulation of B7-H3 in human neuroblastoma cells, Med. Sci. Monit. 23 (2017) 4252–4259.
[14] F. Kontos, et al., B7-H3: an attractive target for antibody-based immunotherapy, Clin. Cancer Res. 27 (5) (2021) 1227–1235.
[15] N.M. Kendsersky, et al., The B7-H3-targeting antibody-drug conjugate m276-SL-PBD is potently effective against pediatric cancer preclinical solid tumor models, Clin. Cancer Res. 27 (10) (2021) 2938–2946.
[16] S. Grote, et al., CD276 as a novel CAR NK-92 therapeutic target for neuroblastoma, Adv. Cell Gene Therapy 4 (1) (2021), e105.
[17] H. Du, et al., Antitumor responses in the absence of toxicity in solid tumors by targeting B7-H3 via chimeric antigen receptor T cells, Cancer Cell 35 (2) (2019), e8.
[18] K. Birley, et al., A novel anti-B7-H3 chimeric antigen receptor from a single-chain antibody library for immunotherapy of solid cancers, Mol. Therapy Oncolyt. 26 (2022) 429–443.
[19] B. Moghimi, et al., Preclinical assessment of the efficacy and specificity of GD2-B7H3 SynNotch CAR-T in metastatic neuroblastoma, Nat. Commun. 12 (1) (2021), 511.
[20] M. Tian, et al., An optimized bicistronic chimeric antigen receptor against GPC2 or CD276 overcomes heterogeneous expression in neuroblastoma, J. Clin. Invest. 132 (16) (2022), e155621.
[21] G. Li, et al., B7-H3-targeted CAR-T cell therapy for solid tumors, Int. Rev. Immunol. (2022) 1–13.
[22] J. Anderson, et al., Immunotherapy of neuroblastoma: facts and hopes, Clin. Cancer Res. 28 (15) (2022) 3196–3206.
[23] K. Flem-Karlsen, et al., B7-H3 in cancer - beyond immune regulation, Trends Cancer 4 (6) (2018) 401–404.