Hopes on immunotherapy targeting B7-H3 in neuroblastoma

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ARTICLE INFO

Keywords:
Neuroblastoma
B7-H3
Immunotherapy
Immune checkpoint protein
CAR therapy

ABSTRACT

Neuroblastoma is one of the most aggressive cancer forms in children, with highly heterogeneous 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 of 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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https://doi.org/10.1016/j.tranon.2022.101580
Received 20 August 2022; Received in revised form 29 September 2022; Accepted 25 October 2022
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SH-SY5Y neuroblastoma cells [7]. In another report, overexpression of B7-H3 associates with decreased patient overall survival, and B7-H3 shRNA associated NK-mediated killing of neuroblastoma cells [8]. More recently, high expression of B7-H3 mRNA in neuroblastoma tumors has been shown to confer doxorubicin resistance in knockdown induced cell cycle arrest and suppressed the proliferation of SH-SY5Y cells was found to confer doxorubicin resistance 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 mAbs 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).

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 mAbs 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...
Table 1
Targeting B7-H3 in neuroblastoma.

| Identifier | Phase / Study | Agent / Drug | Trial / Study | Status / estimated study completion / [Reference] |
|------------|---------------|--------------|---------------|----------------------------------------------|
| NCT02982941 | (Phase 1) | Enoblituzumab | 1 | Completed February 2022 |
| NCT00582608 | (NA) | Omburtamab | 2 | Completed May 2009 |
| NCT05064306 | (NA) | Omburtamab | 3 | Available NA |
| NCT00089245 | (Phase 1) | Omburtamab | 4 | Active, not recruiting July 2025 |
| NCT03275402 | (Phase 2) | Omburtamab | 5 | Recruiting December 2026 |

CAR T-based CLINICAL TRIALS

| Identifier | Phase / Study | Agent / Drug | Trial / Study | Status / estimated study completion / [Reference] |
|------------|---------------|--------------|---------------|----------------------------------------------|
| NCT04691713 | (NA) | B7-H3 CAR T-cells | 6 | Recruiting NA |
| NCT04637503 | (Phase 1/2) | B7-H3 CAR T-cells | 7 | Recruiting December 2023 |
| NCT04864821 | (Early Phase 1) | B7-H3 CAR T-cells | 8 | Not yet recruiting May 2023 |
| NCT04432649 | (Phase 1/2) | B7-H3-iCasp9 CAR T-cells | 9 | Recruiting May 2024 |
| NCT04897321 | (Phase 1) | B7-H3 CAR T-cells | 10 | Recruiting March 2027 |
| NCT04483778 | (Phase 1) | B7-H3 EGFRI-DHFR CAR T-cells | 11 | Recruiting December 2040 |

CAR T/NK PRECLINICAL STUDIES

| Identifier | Phase / Study | Agent / Drug | Trial / Study | Status / estimated study completion / [Reference] |
|------------|---------------|--------------|---------------|----------------------------------------------|
| Grote et al. | | B7-H3 CAR NK-cells | 12 | Recruiting March 2023 |
| Du et al. | | B7-H3 CAR T-cells | 13 | Recruiting December 2023 |
| Birley et al. | | B7-H3 CAR T-cells | 14 | Recruiting December 2023 |
| Moghimi et al. | | GD2-B7-H3 CAR T-cells | 15 | Recruiting December 2023 |
| Tian et al. | | Bicistronic CAR T-cell | 16 | Recruiting December 2023 |

1) Clinical trials including neuroblastoma patients are listed
2) Enoblituzumab, humanized mAb, also known as MGA271, based on murine BRCA84D mAb
3) Omburtamab, radioactive iodine-labeled mAb, based on murine BH9 mAb
4) Autoologous B7-H3 CAR T-cells

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 fundamentals 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.

Acknowledgments

We apologize for not citing, due to limitations in space, many of the papers covering important aspects summarized in this short communication. This work was partially supported by grants BIO20/01/004 from BIOEF (EITB Maratoia, Basque Country, Spain), and Asociación NEN (Spain) (to RP and CENX). RP is funded by Ikerbasque, Basque Foundation for Science (Basque Country, Spain), and CENX is funded by...
Miguel Servet program, Instituto de Salud Carlos III (Spain and the European Social Fund+; "Investing in your future"); Grant number CP20/00088. We thank all collaborators in our neuroblastoma projects for their continuous support.

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