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B7-H3: an attractive target for antibody-based immunotherapy

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Abstract

The recent impressive clinical responses to antibody-based immunotherapy have prompted the identification of clinically relevant tumor antigens that can serve as targets in solid tumors. Among them, B7-H3, a member of the B7 ligand family, represents an attractive target for antibody-based immunotherapy: it is overexpressed on differentiated malignant cells and cancer initiating cells, with limited heterogeneity, and high frequency (60% of 25,000 tumor samples) in many different cancer types, but has a limited expression at low level in normal tissues. In non-malignant tissues, B7-H3 has a predominantly inhibitory role in adaptive immunity, suppressing T-cell activation and proliferation. In malignant tissues, B7-H3 inhibits tumor antigen-specific immune responses, leading to a pro-tumorigenic effect. B7-H3 also has non-immunological pro-tumorigenic functions, such as promoting migration and invasion, angiogenesis, chemoresistance and endothelial-to-mesenchymal transition as well as affecting tumor cell metabolism. As a result, B7-H3 expression in tumors is associated with poor prognosis. Although experimental B7-H3 silencing reduces cancer cell malignant potential, there has been limited emphasis on the development of B7-H3 blocking antibodies, most likely because the B7-H3 receptor remains unknown. Instead, many antibody-based strategies utilizing distinct effector mechanisms to target B7-H3-expressing cancer cells have been developed. These strategies have demonstrated potent anti-tumor activity and acceptable safety profiles in preclinical models. Ongoing clinical trials are assessing their safety and efficacy in patients. Identification of the B7-H3 receptor will improve our understanding of its role in tumor immunity, and will suggest rational strategies to develop blocking antibodies which may enhance the therapeutic efficacy of tumor immunity.

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Author contributions

Conception and design: F.K., C.R.F., S.F.; Acquisition of data: F.K., T.M., T.K., A.S.; Analysis and interpretation of data: F.K., T.M., A.S., J.H.S., S.F.; Writing, review and/or revision of the manuscript: F.K., T.M., T.K., A.S., J.H.S., C.R.F., S.F.; Study supervision: S.F.

Conflicts of interest

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Keywords

B7-H3; CAR T-cells; immunotherapy; solid tumors; tumor antigen

INTRODUCTION

Monoclonal antibodies (mAbs), alone or as part of novel engineered structures, have had impressive clinical successes in cancer treatment (1). Furthermore, mAbs have facilitated the identification and molecular characterization of tumor antigens (TA) that can serve as antibody-based immunotherapy targets. Many TAs with a selective, although not specific expression on malignant cells have been identified. Among them, B7-H3, also known as CD276 or B7RP-2, a member of the B7 ligand family, appears to be the “right” TA for antibody-based immunotherapy (2), given that it is i) highly expressed with limited heterogeneity on differentiated tumor cells, ii) expressed on cancer initiating cells (CIC); the latter play a major role in metastasis and recurrence, and must be eradicated for a therapy to be effective (3) (Fig. 1), and iii) expressed on tumor-associated vasculature (TAV) and stroma; therefore, B7-H3 immunotargeting is expected to disrupt the tumor microenvironment (TME) and to inhibit neoangiogenesis. In contrast, B7-H3 has a restricted distribution in normal tissues. These characteristics have prompted significant interest in evaluating B7-H3 as a target of mAb-based immunotherapy.

STRUCTURE AND REGULATION

B7-H3 is a 316 amino-acid long type I transmembrane protein encoded by a gene in chromosome 9 in mice and 15q24 in humans. It exists in two isoforms determined by its extracellular domain. In mice, the extracellular domain comprises a single pair of immunoglobulin variable (IgV)-like and immunoglobulin constant (IgC)-like domains, whereas in humans it comprises one or two identical pairs due to exon duplication (4–6). Its intracellular domain is short without any known signaling motif (Fig. 2).

B7-H3 shares 20–27% amino-acid identity with other B7 family ligands (7). B7-H3 has maintained its amino-acid sequence through phylogenetic evolution, as it has an 88% amino-acid sequence homology with its murine counterpart (5). Despite this similarity, no human B7-H3-specific antibody cross-reacts with the endogenous murine B7-H3 expressed on murine cells. However, human B7-H3-specific mAbs cross-react with murine B7-H3 expressed by transfected human cells (8), likely because of different B7-H3 glycosylation in the two species and the role of carbohydrates in the expression of epitopes recognized by human B7-H3-specific mAbs (9). Thus, caution should be exercised in interpreting toxicity of strategies which test human B7-H3-specific mAbs in murine models, unless the mAb used has been shown to cross-react with the endogenous B7-H3 expressed by normal murine tissues.

The molecular weight of B7-H3 protein moiety is ~45–66kDa, while that of the glycosylated 4Ig-hB7-H3 isoform is ~100kDa (10). A B7-H3 “soluble” isoform has been detected in plasma, a questionable statement, since there is insufficient evidence about its solubility and B7-H3 is also expressed on exosomes (11). The correlation of B7-H3 level in cancer

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patients' serum with clinicopathologic variables (12) suggests its use as a non-invasive biomarker for diagnosis, prognosis, and/or treatment response.

B7-H3 mRNA is expressed in most normal tissues. In contrast, B7-H3 protein has a very limited expression in normal tissues (13,14) because of its post-transcriptional regulation by microRNAs (miRNA). Specifically, miRNA-29 suppresses B7-H3 expression in normal tissues by targeting the B7-H3 3'-untranslated mRNA region. Similarly, miRNA-29, miRNA-124, miRNA-199a and miRNA-1253 regulate B7-H3 expression in neuroblastoma (15), osteosarcoma (16), cervical cancer (17) and medulloblastoma (18), respectively. Furthermore, high miRNA-187, miRNA-1301-3p, miRNA-335-5p and miRNA-28-5p expression downregulates B7-H3 expression in CRC; this change leads to reduced aggressiveness, lower pathologic stage and decreased metastatic potential (19,20).

FUNCTIONS AND INTERACTIONS WITH MOLECULAR PATHWAYS

A. Immunological and non-immunological functions in non-malignant tissues

B7-H3 may inhibit NK-cell activation and have a pro-inflammatory role leading to cytokine release from monocytes and/or macrophages, in a Toll-like receptor (TLR)-2 and TLR-4-dependent manner (21). Nevertheless, B7-H3 involvement in innate immunity remains questionable.

In adaptive immunity, B7-H3 plays a role in T-cell regulation (22). Surprisingly, the molecular structure of its receptor remains unidentified, although soluble B7-H3 has been shown to bind to $CD4^+$ T, $CD8^+$ T, NK, and NKT-cells (23) and the extent of its binding is increased upon T-cell activation (2). In mice, B7-H3 binds to the $CD8^+$ T-cell trem-like transcript 2 (TLT-2) receptor leading to their activation (24), however, such interaction has not been found with human B7-H3 (25). At any rate, the B7-H3 receptor differs from CD28, CTLA-4 (cytotoxic T lymphocyte-associated protein 4), PD-1 (programmed cell death protein 1) and ICOS (inducible T cell costimulatory), receptors known to bind other B7 family ligands (2,5).

B7-H3 was initially considered necessary for T-cell co-stimulation (2). Their activation was associated with worse outcomes in auto-immune and infectious diseases (26). The co-stimulatory B7-H3 role is further supported by detection of B7-H3-positive mononuclear cells in acute renal rejection biopsies (27). Lastly, B7-H3 expression on mononuclear cells promoted cardiac allograft rejection in mice through $CD4^+$ and $CD8^+$ T-cell activation, while B7-H3 deletion in combination with immunosuppression decreased allograft rejection (27).

Nevertheless, B7-H3 likely has a predominantly inhibitory role in adaptive immunity, suppressing T-cell activation and proliferation. Indeed, B7-H3 expression on antigen-presenting cells (APCs) reduces $CD4^+$ and $CD8^+$ T-cell activation, as well as effector cytokine release (28). Furthermore, B7-H3 decreases T-cell activation and function by inhibiting the expression of major transcriptional factors such as Nuclear Factor-Kappa B (NF-κB). The B7-H3 inhibitory function is further supported by the capacity of APCs to stimulate T-cells in B7-H3-deficient mice (7). In addition, B7-H3 silencing accelerates auto-

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immune encephalomyelitis via T-helper (T_h)-type 1 immune response upregulation (7). Similarly, in graft-versus-host-disease, cardiac allograft transplantation and hypersensitivity reaction models, B7-H3 blockade with anti-B7-H3 mAb MIH35 or gene deletion resulted in uncontrolled T_h-type 1 responses and worse outcomes (26,29,30).

B7-H3 has also a non-immunological function in normal tissues: it is highly expressed on osteoblasts during embryogenesis, and is crucial for osteoblastic differentiation and bone mineralization (31).

B. Role in tumor microenvironment

B.1. Inhibitor of tumorigenesis—B7-H3 was first demonstrated to have anti-tumor activity in syngeneic murine lymphoma and mastocytoma where it induced $CD8^+$ T-cell and NK-cell activation-mediated tumor regression (32,33). Furthermore, B7-H3 transfection to CRC cells grafted to mice reduced their metastatic potential and prolonged the survival of tumor-bearing mice (34), indicating that B7-H3 upregulation on tumor cells can lead to immune cell recruitment and activation. Indeed, in mice, B7-H3 has been suggested to bind to the TLT-2 receptor on $CD8^+$ T-cells leading to their activation, and IFN-γ and IL-2 secretion (24).

Such evidence has been rarely found in humans, since an association between B7-H3 expression and favorable clinical course of the disease has been described in only 3 of the 61 analyzed studies (gastric (35) and pancreatic cancer (36), acute myeloid leukemia (37)). Whether this difference between mouse and man reflects the lack in humans of B7-H3 binding to T cell TLT-2 receptors (25) and/or the differential expression of the 4 and 2Ig B7-H3 isoforms in the two species, remains to be determined.

B.2. Promoter of tumorigenesis—B7-H3 appears to promote tumorigenesis mainly via immunological mechanisms. Preclinical and clinical evidence indicates that B7-H3 inhibits TA-specific immune responses, leading to a pro-tumorigenic effect. Indeed, B7-H3 expression in tumors correlates with aggressive biology, low tumor-infiltrating T-lymphocyte density and poor prognosis (38,39). Nevertheless, B7-H3-targeting strategies to date do not focus on blocking this inhibition, but rather on eliminating B7-H3-expressing cells. This tumorigenic effect may be mediated by immunoglobulin-like transcript (ILT)-4 overexpression on cancer cells. The latter increases B7-H3 expression via PI3K/AKT/mTOR pathway activation (40). B7-H3 is also correlated with activated regulatory T-cell (Treg) infiltration in non-small cell lung cancer (NSCLC) (41) and suppression of NK-cell-mediated glioma cell lysis (42).

Several mechanisms may explain the dual anti-/pro-tumorigenic B7-H3 role. Firstly, B7-H3, like other checkpoint inhibitor ligands, might interact with both stimulatory and inhibitory receptors (43). Secondly, the differential B7-H3 function may reflect its different binding affinity for the interacting receptors. Finally, tumor cells may express aberrant B7-H3 isoforms or splice variants with different immune functions.

B7-H3 promotes cancer progression and invasion also through non-immunological mechanisms. Indeed, siRNA-induced B7-H3 downregulation on melanoma cells, as well as

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on breast and prostate cancer cells decreases their *in vitro* migration and invasiveness (10,44). In contrast, high B7-H3 expression on CRC cells is associated with increased matrix metalloproteinase (MMP)-9 expression, thereby promoting tumor cell migration and invasion. Inhibition of the JAK2/STAT3 pathway, which regulates MMP-9 expression, decreases CRC cell *in vitro* aggressiveness (45). Furthermore, B7-H3 affects the metastatic potential of human melanoma cells in mice most likely by modulating the metastasis-associated proteins MMP-2 and STAT3 (46). Lastly, B7-H3 promotes angiogenesis in CRC, by upregulating vascular endothelial growth factor A expression through NF-κB pathway activation (47).

B7-H3 enhances chemoresistance, as shown by the decreased apoptosis of breast cancer and CRC cells treated with paclitaxel and oxaliplatin/5-FU, respectively, via JAK/STAT3/survivin pathway activation (48,49), or specifically in CRC by reducing the G2/M phase arrest (50). Similarly, B7-H3 silencing increases pancreatic ductal adenocarcinoma (PDAC) cell gemcitabine sensitivity (51). Therefore, B7-H3 is a potential chemoresistance target.

B7-H3 promotes epithelial-to-mesenchymal transition (EMT) in glioma and hepatoma cells through JAK2/STAT3/Slug pathway activation (52,53). Lastly, B7-H3 affects the metabolism of triple negative breast cancer cells: decreased B7-H3 expression reduces tumor cell glycolytic capacity and increases their AKT/mTOR inhibitor sensitivity (54). Consistent with these activities, B7-H3 protein expression correlated to significant positive enrichment in EMT, UV response, protein secretion, WNT/β-catenin signaling, and Notch signaling proteins in 378 cancer cell lines included in the Cancer Cell Line Encyclopedia (55) (Supplementary Tables 1–3).

EXPRESSION IN NORMAL AND MALIGNANT TISSUES, AND ASSOCIATION WITH MALIGNANT DISEASE PROGNOSIS

Immunohistochemical staining with mAb 8H9 of a large number of multiple normal tissues has detected only heterogeneous nonspecific cytoplasmic staining in stomach, liver, pancreas and adrenal cortex (56). Similarly, we have also reported only weak cytoplasmic immunohistochemical staining of salivary gland acinar cells, gastric epithelial cells, and adrenal gland cells (8).

In contrast, B7-H3 is highly expressed with limited heterogeneity in all cancer types tested. Representative results obtained by immunohistochemical and flow cytometric analysis of malignant and normal cells stained with the B7-H3-specific mAb 376.96 are shown in Fig. 3. In a comprehensive literature review comprising 94 studies on 21 cancer types including 26,703 patients, we found that the cumulative frequency of B7-H3 positivity among all tumors is 60% (Fig. 4). B7-H3 displays high expression on stromal fibroblasts and TAV even in tumors with low B7-H3 expression. Indeed, B7-H3 TAV expression frequency ranged between 86%–98% in hepatoma, CRC, renal cell carcinoma, and melanoma, with frequency in ovarian cancer outlying at 44% (unpublished).

Additionally, of the 61 studies that associated B7-H3 expression in tumors with prognosis, 67% demonstrated an association of positive/high expression of B7-H3 with poor prognosis,

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28% found no correlation, and only 3 (5%) (35–37) demonstrated an association of positive/high B7-H3 expression with better prognosis (unpublished).

ATTRACTIVE IMMUNOTHERAPY TARGET

The recent advances in molecular biology and antibody engineering have allowed targeting B7-H3 utilizing multiple effector mechanisms. Most of these strategies have been tested *in vitro* and in mice generating encouraging safety and/or anti-tumor activity data, paving the way for B7-H3-targeting clinical trials (Table 1).

A. Blocking mAbs

The significant changes induced in cancer cells by silencing B7-H3 (38,39) and the impressive clinical effect of mAbs blocking checkpoint molecules has provided a strong rationale to test B7-H3-specific inhibitory mAbs in solid tumors. B7-H3 blocking with mAbs has been shown to increase $CD8^+$ T and NK-cell tumor infiltration and reduce tumor growth, and/or prolong survival in mouse models of hematopoietic cancers, melanoma (57), CRC (58), and more recently ovarian cancer (23). However, translation to a clinical setting has been hampered by lack of human B7-H3-specific blocking mAbs.

B. Radioimmunotherapy

B7-H3-specific mAbs have been used as carriers to selectively target radioisotopes to tumors. In an early single-arm imaging study in patients with B7-H3-positive tumors, ^{131}I-murine mAb 8H9 (omburtamab, Y-mAbs) displayed moderate hepatic uptake (NCT00582608). Although no hepatotoxicity was reported, to avoid potential toxicity and to increase the therapeutic index, in subsequent studies systemic administration was replaced with compartmental administration. Specifically, in Phase I trials, intrathecal omburtamab in metastatic central nervous system neuroblastoma (NCT00089245) and intraperitoneal ^{131}I-mAb 8H9 in desmoplastic small round cell tumors (NCT01099644) were well-tolerated (59). Convection-enhanced brainstem delivery of ^{124}I-mAb 8H9 to diffuse pontine glioma resulted in negligible systemic exposure and no toxicity (NCT01502917). To facilitate rapid radioactivity clearance, mAb 8H9 was conjugated with the chelator diethylenetriamine pentaacetate and radiolabeled with lutetium-177 (60).

Furthermore, the B7-H3-specific mAb 376.96 has been conjugated with ^{212}Pb, a source of α-particles. Ovarian tumor-bearing mice treated with this conjugate, alone or with carboplatin, survived 2–3 times longer than controls (61). This conjugate also inhibited human PDAC cell growth *in vitro* and patient-derived xenograft growth in mice (62).

C. Antibody drug conjugates (ADCs)

The ADC approach has been tested utilizing MGC018 (humanized B7-H3 mAb with a cleavable linker-duocarmycin payload, MacroGenics) which delivers duocarmycin to tumors. A Phase I/II trial is assessing its safety alone or in combination with an anti-PD-1 mAb in B7-H3-expressing solid tumors (NCT03729596). DS-7300a (Daiichi Sankyo), a B7-H3-specific mAb conjugated to four topoisomerase I inhibitor particles is being tested in a Phase I/II trial (NCT04145622).

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D. mAbs mediating cellular cytotoxicity

The fully humanized mAb enoblituzumab (MGA271, MacroGenics) bearing an Fc domain engineered to enhance its anti-tumor function by increasing binding to the activating receptor CD16A and reducing that to the inhibitory receptor CD32B (63), was the first mAb tested against B7-H3-expressing tumors. Enoblituzumab was effective in multiple cancer types through antibody-dependent cellular cytotoxicity (ADCC) (63). Interim results from a Phase I trial (NCT01391143) including patients with B7-H3-expressing tumor or TAV cells, demonstrated that enoblituzumab was well-tolerated with no dose-limiting toxicity (64). A Phase II trial is assessing neoadjuvant enoblituzumab in prostate cancer (NCT02923180). Furthermore, a Phase I trial (NCT02475213) demonstrated that enoblituzumab with pembrolizumab in head and neck squamous cell cancer (HNSCC), NSCLC, urothelial cancer and melanoma was safe; the frequency of immune-related adverse events was comparable to pembrolizumab alone (65). Enoblituzumab with ipilimumab was also tested in a Phase I trial in NSCLC and melanoma (NCT02381314, results pending). More recently, a novel Fc enhanced bispecific anti-B7-H3 mAb/PD-1 fusion protein showed promising results in mice (66).

E. CD3-engaging bispecific antibodies (BsAbs)

BsAbs have been tested utilizing a B7-H3 mAb scFv linked to an anti-CD3 mAb scFv to recruit and activate T-cells against tumor cells (67). Only obrindatamab (MGD009, MacroGenics), a humanized CD3xB7-H3 dual affinity protein has been tested in humans in advanced B7-H3-expressing tumors (NCT02628535). In 2018 the FDA imposed a partial hold on this study due to hepatic adverse events likely due to cytokine release syndrome secondary to increased T-cell activation. These events were uncomplicated and short-lived, and the hold was lifted in 2019. However, the trial was terminated in November 2019 by MacroGenics due to administrative reasons (E. Bonvini, personal communication). Another B7-H3xCD3 BsAb created by coupling an anti-human B7-H3 mAb with an anti-CD3 mAb showed potent cytotoxicity toward hematological malignant cells *in vitro* (68).

F. Tri-specific Killer Engagers (TriKEs)

TriKEs form an antigen-specific immunological synapse between NK and tumor cells, thereby triggering NK-cell-mediated tumor cell lysis (69). TriKEs are composed of either 3 scFvs of antibodies with different specificity or 2 scFvs (CD16-specific and TA-specific) and a cytokine, most frequently IL-15. Vallera *et al.* have generated a B7-H3/IL-15 TriKE using the scFv of the B7-H3-specific mAb 376.96. This strategy demonstrated significant tumor burden decrease *in vitro* and in mice against HNSCC, PDAC and ovarian cancer (70,71). A second-generation TriKE has been bioengineered by the same group, with human IL-15 as a modified crosslinker between an anti-B7-H3 scFv and a humanized camelid anti-CD16 single domain antibody. The latter allows improved function of the IL15 moiety, improving NK-cell activation and proliferation, and augmenting killing of ovarian cancer cells *in vitro* and in mice (72).

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G. CAR T-cells and CAR NK-cells

CAR T-cells permit rapid generation of polyclonal T-cells with TA-specificity and potent cytotoxicity, and can recognize tumor cells independently of HLA class I antigen expression (73). B7-H3 CAR T-cells engineered with various B7-H3-specific scFvs have demonstrated potent *in vitro* anti-tumor activity against multiple cancer types (8,74–77). However, their efficacy remains limited in mice. This limitation which is common with CAR T-cells targeting different TA in mice and humans likely reflects the negative impact of TME escape mechanisms on tumor cell - CAR T-cell interactions (78). To counteract this limitation, combinatorial strategies with agents which can enhance the anti-tumor activity of CAR T-cells and/or tumor cell susceptibility to the effector mechanism are being tested. Preclinical studies suggest an acceptable safety profile. B7-H3 CAR T-cells are being evaluated in trials targeting glioblastoma (NCT04077866) and pediatric glioma (NCT04185038). More recently, NK-cells have been used to generate CAR NK-cells which have controlled growth of human NSCLC cells grafted in mice and prolonged their survival (79).

CONCLUSION

B7-H3 has a complex, still not entirely clear, role in the TME with several immunological and non-immunological functions. Endeavors to identify its receptor are of outmost importance; this information will elucidate its role in the TME, but, most importantly, will facilitate the development of B7-H3 blocking reagents. To date, this area remains unexploited, since most B7-H3-targeting strategies focus on eliminating B7-H3-expressing tumor cells. Blocking the B7-H3-mediated inhibition on T-cells is expected to greatly improve TA-specific immune responses and in turn, significantly ameliorate the disease course. As observed with other checkpoint inhibition strategies, B7-H3-blocknig mAbs may prove to be a significant addition to the anti-tumor strategy armamentarium.

B7-H3 is highly expressed, with limited heterogeneity, on cancer cells in primary tumors. Additional data on B7-H3 expression in metastases – crucial for designing B7-H3-targeting therapies – are needed. Other areas needing additional investigation include i) the assessment of the value of B7-H3 serum level as a diagnostic, prognostic and monitoring marker, and ii) the evaluation of B7-H3-targeting ability to eliminate CICs, given their postulated role in metastasis and recurrence.

The attractiveness of B7-H3 as a target for cancer antibody-based therapy has stimulated the development of B7-H3-targeting immunotherapeutic strategies. Preclinical results are encouraging. Additionally, data from clinical trials demonstrate a favorable safety profile with limited toxicity. Multiple ongoing clinical trials are evaluating the therapeutic efficacy of B7-H3-targeting strategies, alone or in combination with other checkpoint inhibitors. This information will assess its value as a target of antibody-based immunotherapy.

Supplementary Material

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Abbreviation list

ADC	antibody drug conjugate
ADCC	antibody-dependent cellular cytotoxicity
APC	antigen presenting cells
BsAb	bispecific antibody
CAR	chimeric antigen receptor
CIC	cancer initiating cell
CRC	colorectal cancer
CTLA-4	cytotoxic T lymphocyte-associated protein 4
EMT	epithelial-to-mesenchymal transition
HNSCC	head and neck squamous cell cancer
ICOS	inducible T-cell costimulatory
IgC	immunoglobulin constant domain
ILT	immunoglobulin-like transcript
IgV	immunoglobulin variable domain
mAb	monoclonal antibody
MHC	major histocompatibility complex
miRNA	microRNA
MMP	matrix metalloproteinase
NF-κB	nuclear factor kappa b
NSCLC	non-small cell lung cancer
PDAC	pancreatic ductal adenocarcinoma
PD-1	programmed cell death protein 1
TA	tumor antigen

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TAV	tumor-associated vasculature
T_h	T helper
TLR	toll-like receptor
TLT	trem-like transcript
TME	tumor microenvironment
Treg	regulatory T cell
TriKE	tri-specific killer engager

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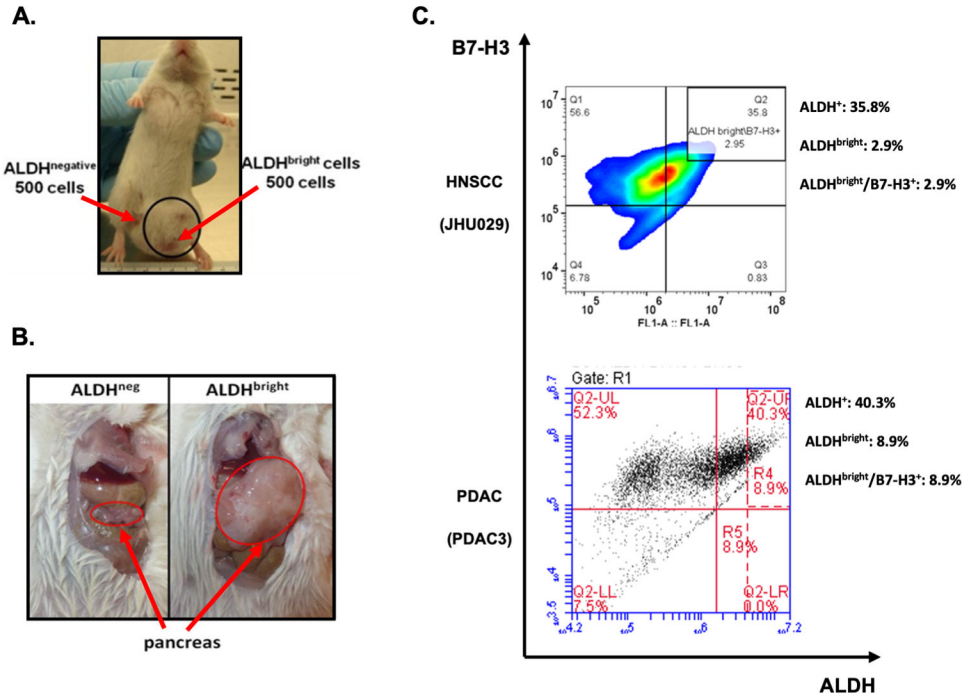

Figure 1. B7-H3 expression on tumorigenic cancer initiating cells isolated from human cancer cell lines.

High ALDH activity (ALDH^{bright} cells) is used as a marker to identify cancer initiating cells (CICs). Immunodeficient NOD/SCID mice were challenged with 500 ALDH^{bright} and ALDH^{neg} cells sorted from a (A) HNSCC or (B) PDAC cell line. In both models, tumors developed only in anatomic sites injected with ALDH^{bright} cells, but not ALDH^{neg} tumor cells. CICs from HNSCC (JHU029) and PDAC (PDAC3) human cell lines stained with the B7-H3-specific mAb 376.96 demonstrate high B7-H3 expression (C). ALDH: aldehyde dehydrogenase; HNSCC: head and neck squamous cell cancer; PDAC: pancreatic ductal adenocarcinoma. ALDH^{bright} cells are identified as those ALDH^{+} cells with twice the mean fluorescence intensity of the ALDH^{+} population.

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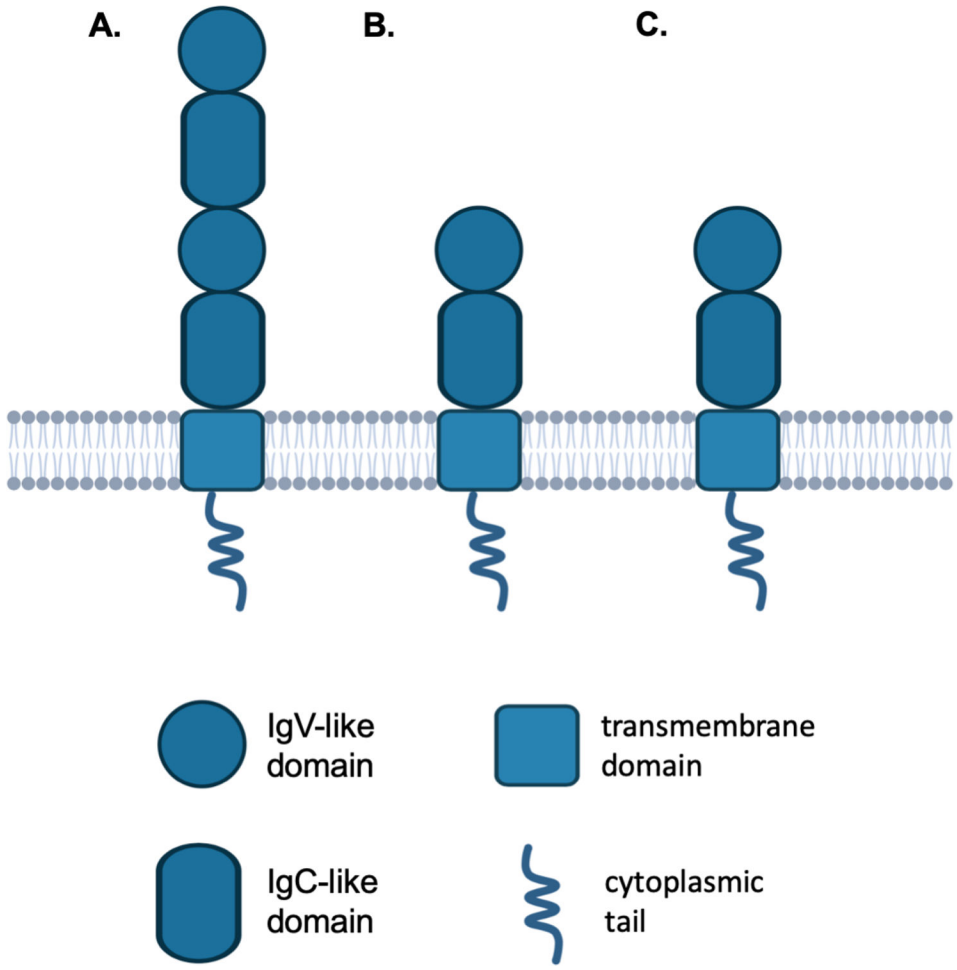

Figure 2. B7-H3 structure.

B7-H3 is a type I transmembrane protein composed of an extracellular, a transmembrane and a short intracellular domain. Human B7-H3 exists in two isoforms as determined by its extracellular domain: 4IgB7-H3 which comprises two identical pairs of IgV-like and IgC-like domains (A), and 2IgB7-H3 which comprises a single pair of IgV-like and IgC-like domains (B). 4IgB7-H3 is the predominant isoform in humans. B7-H3 in mouse is expressed only as the 2IgB7-H3 isoform (C). IgV: immunoglobulin variable; IgC: immunoglobulin constant.

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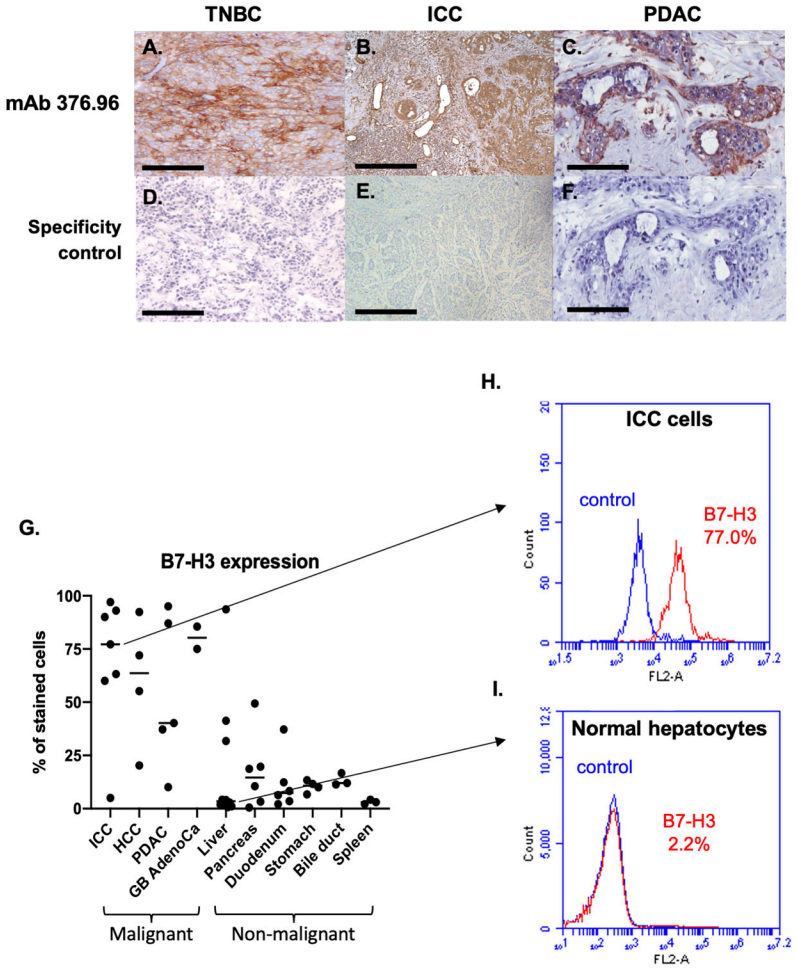

Figure 3. Higher B7-H3 expression on solid cancers than on normal tissues, as determined by immunohistochemical and flow cytometric staining with the B7-H3-specific mAb 376.96.

Representative micrographs of immunohistochemical staining with the B7-H3-specific mAb 376.96 of frozen tissue sections of TNBC (A), ICC (B), and PDAC (C) tumors are shown. Slides stained with the secondary antibody were used as a negative control (D, E, and F, respectively). (Magnification 200X). Surgically resected tumor specimens have a higher frequency of B7-H3-positive cells compared to normal tissues as determined by flow cytometric analysis (G). Surgically resected specimens were incubated with collagenase IV and single cell suspension was obtained. Cells were then stained with the B7-H3-specific mAb 376.96 (1ug/mL) and then analyzed by flow cytometry. Each dot on the plot represents a specimen from a different patient and the horizontal bars indicate the median value of each group. The frequency of B7-H3-positive cells was significantly higher in malignant tissues (Mann-Whitney U test, $p<0.001$). Of note, the liver specimen with the highest percentage of B7-H3-positive cells had underlying chronic hepatitis C, and the pancreas specimen with 50% B7-H3-positive cells had underlying chronic pancreatitis. Surgically removed ICC cells have a high frequency of B7-H3-positive cells (H), while normal liver cells adjacent to cancer tissue have a low frequency of B7-H3-positive cells (I), as determined by flow cytometric analyses. Cells were stained with the B7-H3-specific mAb 376.96 and rabbit anti-mouse IgG-PE antibodies. The isotype-matched mAb F3-C25 was used as a control for

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mAb 376.96. Stained cells were subjected to flow cytometry analysis. The percentage of cells stained with the B7-H3-specific mAb 376.96 are shown in each histogram. TNBC: triple negative breast cancer; ICC: intrahepatic cholangiocarcinoma; PDAC: pancreatic ductal adenocarcinoma; HCC: hepatocellular carcinoma; GB AdenoCa: gallbladder adenocarcinoma.

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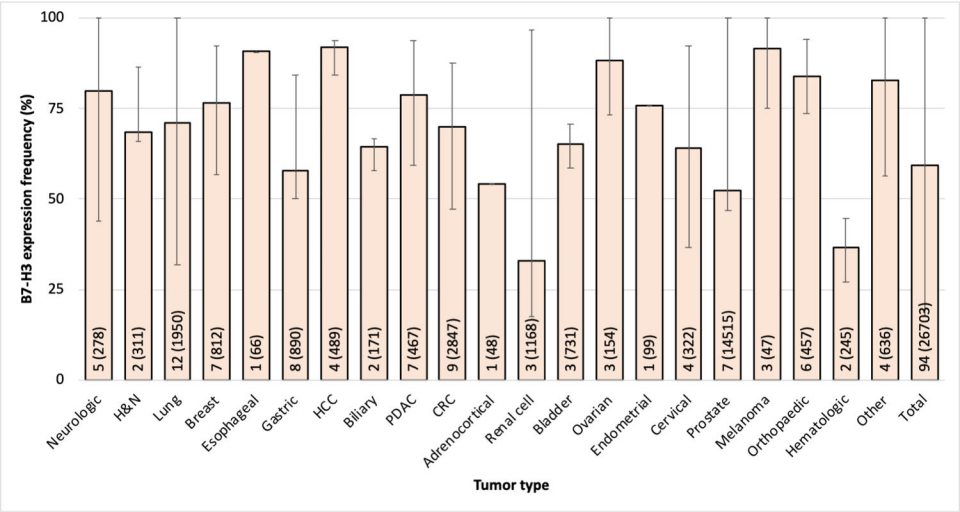

Figure 4. High frequency of B7-H3 expression in all the cancer types tested.

B7-H3 expression was assessed in surgically resected primary tumors by immunohistochemical staining with B7-H3-specific antibodies. Bar graphs represent the average of the frequency of B7-H3 expression in each cancer type yielded from a pooled data analysis. The number of included studies (number of tested samples) for each cancer type is presented within each bar. Error bars represent the range of B7-H3 expression frequency reported. B7-H3 expression was defined as positive by the criteria used by each individual study. H&N: head and neck; HCC: hepatocellular carcinoma; CRC: colorectal cancer. The tumors labeled as “other” include small round blue cell tumors of childhood, pediatric brain and solid tumors, Ewing’s family of tumors and renal angiomyolipoma.

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Table 1.

Clinical trials targeting B7-H3 on solid tumors with multiple effector mechanisms.

Trial number	Title	Status
NCT01391143	Safety Study of MGA271 in Refractory Cancer	Completed
NCT02381314	Safety Study of Enoblituzumab (MGA271) in Combination with Ipilimumab in Refractory Cancer	Completed
NCT02982941	Enoblituzumab (MGA271) in Children with B7-H3-expressing Solid Tumors	Completed
NCT02923180	Neoadjuvant Enoblituzumab (MGA271) in Men with Localized Intermediate and High-Risk Prostate Cancer	Active not recruiting
NCT04129320	Enoblituzumab Plus MGA012 in Squamous Cell Carcinoma of the Head and Neck	Not yet recruiting
NCT02475213	Safety Study of Enoblituzumab (MGA271) in Combination with Pembrolizumab or MGA012 in Refractory Cancer	Active not recruiting
NCT03729596	MGC018 With or Without MGA012 in Advanced Solid Tumors	Recruiting
NCT02628535	Safety Study of MGD009 in B7-H3-expressing Tumors	Terminated
NCT03406949	MGD009/MGA012 Combination in Relapsed/Refractory Cancer	Active not recruiting
NCT01502917	Convection-Enhanced Delivery of ^{124}I-8H9 for Patients with Non-Progressive Diffuse Pontine Gliomas Previously Treated with External Beam Radiation Therapy	Recruiting
NCT01099644	Intraperitoneal Radioimmunotherapy with ^{131}I-8H9 for Patients with Desmoplastic Small Round Cell Tumors and Other Solid Tumors Involving the Peritoneum	Active not Recruiting
NCT00089245	Radiolabeled Monoclonal Antibody Therapy in Treating Patients with Refractory, Recurrent, or Advanced CNS or Leptomeningeal Cancer	Active not recruiting
NCT04022213	A Study of the Drug I131-Omburtamab in People with Desmoplastic Small Round Cell Tumors and Other Solid Tumors in the Peritoneum	Recruiting
NCT04167618	177Lu-DTPA-Omburtamab Radioimmunotherapy for Recurrent or Refractory Medulloblastoma	Not yet recruiting
NCT04145622	Study of DS-7300a in Participants with Advanced Solid Malignant Tumors	Recruiting
NCT04185038	Study of B7-H3-Specific CAR T Cell Locoregional Immunotherapy for Diffuse Intrinsic Pontine Glioma/Diffuse Midline Glioma and Recurrent or Refractory Pediatric Central Nervous System Tumors	Recruiting
NCT04077866	B7-H3 CAR-T for Recurrent or Refractory Glioblastoma	Recruiting
NCT04432649	Targeting CD276 (B7-H3) Positive Solid Tumors by 4SCAR-276	Recruiting
NCT04385173	Pilot Study of B7-H3 CAR-T in Treating Patients with Recurrent and Refractory Glioblastoma	Recruiting
NCT04483778	B7H3 CAR T Cell Immunotherapy for Recurrent/Refractory Solid Tumors in Children and Young Adults	Recruiting
NCT04315246	177Lu-DTPA-Omburtamab Radioimmunotherapy for Leptomeningeal Metastasis from Solid Tumors	Not yet recruiting