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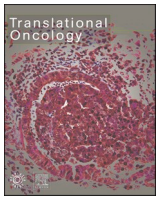

B7-H3/CD276 and small-cell lung cancer: What's new?

Federico Pio Fabrizio [a,*], Lucia Anna Muscarella [a], Antonio Rossi [b]

[a] Laboratory of Oncology, Fondazione IRCCS Ospedale Casa Sollievo della Sofferenza, San Giovanni Rotondo, Italy
[b] Oncology Centre of Excellence, Therapeutic Science & Strategy Unit, IQVIA, Milan 20019, Italy

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ABSTRACT

Immunotherapy revolutionized the treatment landscape of several cancers, including small-cell lung cancer (SCLC), with a huge number of practice-changing trials, and becoming a new frontier for their management. The addition of an anti-PD-L1, atezolizumab or durvalumab, to platinum/etoposide regimen became the standard of care for first-line therapy of extensive-stage (ES)-SCLC with the 12 months median survival exceeded for the first time.

Nevertheless, most patients show primary or acquired resistance to anti-PD-L1 therefore new promising therapeutic immune-targets are under clinical investigation in several solid tumors. Among these, B7-H3, also known as CD276, is a member of the B7 family overexpressed in tumor tissues, including SCLC, while showing limited expression in normal tissues becoming an attractive and promising target for cancer immunotherapy.

B7-H3 plays a dual role in the immune system during the T-cell activation, acting as a T-cell costimulatory/coinhibitory immunoregulatory protein, and promoting pro-tumorigenic functions such as tumor migration, invasion, metastases, resistance, and metabolism.

Immunohistochemistry, flow cytometry, and immunofluorescence were the most used methods to assess B7-H3 expression levels and validate a possible relationship between B7-H3 staining patterns and clinicopathological features in lung cancer patients.

To date, there are no clinically available therapeutics/drugs targeting B7-H3 in any solid tumors. The most promising preliminary clinical results have been reported by DS7300a and HS-20093, both are antibody-drug conjugates, that are under investigation in ongoing trials for the treatment of pretreated SCLC.

This review will provide an overview of B7-H3 and corresponding inhibitors and the clinical development in the management of SCLC.

Introduction

Immune checkpoint inhibitors directed against programmed death-1 (PD-1) receptor and its ligand (PD-L1), and against cytotoxic T-lymphocyte antigen-4 (CTLA-4) receptor, revolutionized the treatment landscape of several cancers with a huge number of practice-changing trials, and becoming a new frontier for their management [1]. Small-cell lung cancer (SCLC) is a very aggressive disease, characterized by a rapid doubling time, high growth fraction, paraneoplastic syndromes, and the early development of widespread metastases. SCLC is extremely sensitive to standard therapies, including conventional cytotoxic chemotherapies and radiotherapy, but the prognosis is very poor with short survival. Despite several treatment attempts and new strategic approaches investigated, in the last decades, for the management of SCLC, only immunotherapy led to an improvement in outcomes with the 12 months median survival exceeded for the first time for the extensive stage of disease (ES). The addition of an anti-PD-L1, atezolizumab or durvalumab, to platinum/etoposide regimen became the standard of care for first-line therapy of ES-SCLC [2,3]. However, after a variable period of activity of chemo-immunotherapy, most patients show primary or acquired resistance to these anti-PD-L1 [4]. T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), T-cell immunoglobulin and ITIM domain (TIGIT), lymphocyte activation gene-3 (LAG-3), B and T cell lymphocyte attenuator (BTLA), V-domain Ig suppressor of T cell activation (VISTA), and B7 homolog 3 protein (B7-H3) have onset as promising therapeutic targets and are under clinical investigation in several solid tumors [5].

B7-H3, also known as CD276, plays a dual role in the immune system during the T-cell activation, acting as a T-cell costimulatory/coinhibitory immunoregulatory protein, and represents an attractive target for

* Corresponding author.
E-mail address: fp.fabrizio@operapadrepio.it (F.P. Fabrizio).

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antibody-based immunotherapy [6]. B7-H3 has limited expression in normal tissue, while its overexpression was found in many cancer types, including SCLC, and it is often associated with worse survival [7].

This review will provide an overview of B7-H3 and corresponding inhibitors and the clinical development in the management of SCLC.

B7 family: a focus on B7-H3 isoforms and functions

In recent years, tumor immunotherapy has focused the attention on B7 family, considering that the abnormal expression of these predominant cell surface proteins in tumor microenvironment (TME) could lead to anti-tumor immune inhibition and immune escape mechanism [8,9]. The B7 family comprises ten protein members among which B7–1, B7–2, B7-H1, B7-DC, B7-H2, B7-H3, B7-H4, B7-H5, BTNL2, B7-H6, and B7-H7 [10]. It is characterized by co-inhibitory and co-stimulatory signals that modulate immune responses in several conditions. These effects are promoted by B7 ligands through the binding to CD28 family of receptors on lymphocytes [11,12]. The human *B7-H3* gene is located on chromosome 15q24.1 and counts 12 exons codifying for a total of 316 amino acids [9]. B7-H3 protein consists of several domains as well as an extracellular, a transmembrane and a short intracellular with no signaling activity, which allows its classification in type I transmembrane glycoprotein (as depicted in Fig. 1), [6,13]. In particular, human B7-H3 has two isoforms: 4IgB7-H3 that includes two pairs for both IgV (variable)-like and IgC (constant)-like domains (the main isoform in humans expressed on immunocytes) and 2IgB7-H3 with only single pair for IgV and IgC domains related to exon duplication and shared about 90% amino acid similarity with murine *B7-H3* gene [14–16]. Recently, it has been identified a soluble B7-H3 (sB7-H3) form in normal human serum as a result of alternative splicing that involves the fourth intron of *B7-H3*. The molecular characterization of this soluble form has been also performed on hepatocellular, gastric, bladder and ovarian cancers, where a negative association with long-term outcomes in these subsets of patients was reported [17–21].

In the context of the regulation of immune system, it is very important to consider that the signaling activity mediated by B7-H3 occurs in a distinct manner that depends on the involved immune cells and/or on the microenvironment, as well as non-immune pathways [22,23]. Firstly, it has been seen that the discovery of B7-H3 boils down to its co-stimulatory role through the activation and proliferation of CD4+ and CD8+ T-cells and the production of IFN-γ [24]. Additional experimental validations on orthotopic colon cancer and murine models suggested that the tumor growth inhibition and decreased metastasis spread led to an increased CD8+ T-cells and pro-inflammatory type 1 cytokine as well as interleukin-12 (IL-12) when subjected to adenoviral B7-H3 treatment [25,26]. On the opposite side, B7-H3 could exert a co-inhibitory function in the promotion of anti-cancer immune response. In fact, a growing number of evidences confirmed its importance in the

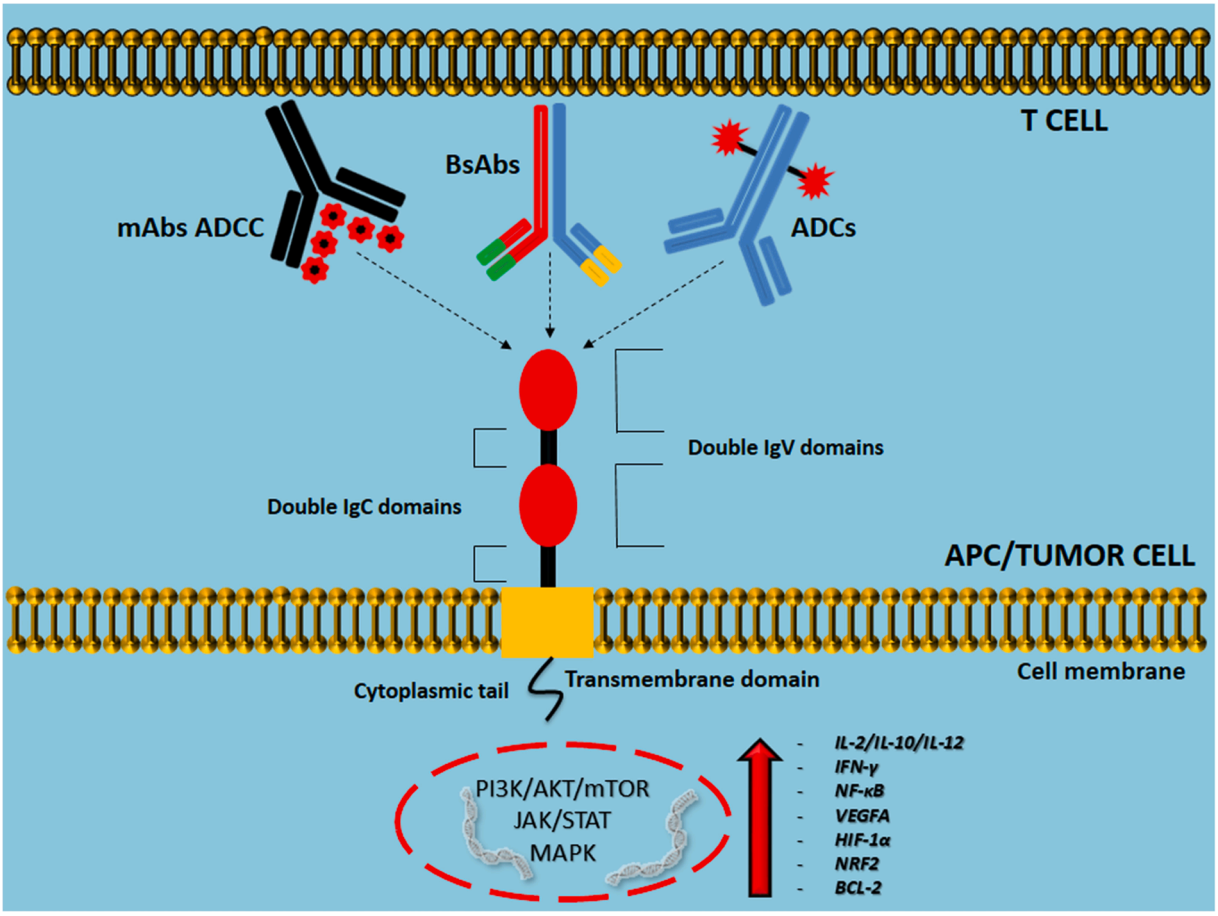

Fig. 1. The most frequent human isoform of B7-H3, 4IgB7-H3 composed of double IgV and IgC domains, a transmembrane domain and a cytoplasmic tail in the middle of the figure. In the upper, monoclonal antibodies-dependent cell cytotoxicity (mAbs), antibody–drug conjugates (ADCs), antibody-dependent cell-mediated cytotoxicity (ADCC) specifically targeting B7-H3 and contribute to an improvement in anticancer activity in recognizing and blocking tumor-associated, cell-surface antigens.In the bottom, intracellular signal transduction pathways, such as Janus kinase/signal transducer and activator of transcription (JAK/STAT), phosphatidylinositol 3-kinase/protein kinase B (also known as Akt)/mammalian target of rapamycin (PI3K/Akt/mTOR), mitogen-activated protein kinase (MAPK or MAP kinase) could be hyperactivated by B7-H3 deregulation and cause an increase of interleukin-2, interleukin-10, interleukin-12 (IL-2/IL-10/IL-12), interferon-γ (IFN), nuclear factor kappa B (NF-Kb), vascular endothelial growth factor-A (VEGFA), hypoxia-inducible factor 1-alpha (HIF-1α), nuclear factor erythroid 2–related factor 2 (NRF2), B-cell lymphoma-2 (BCL2).

suppression of CD4+ and CD8+ T-cells proliferation and NF-κB pathway due to the reduction of IL-2/IL-10/IL-13 and IFN-γ levels [27]. Moreover, it would be necessary to sum up a non-immunological pro-tumorigenic role of B7-H3 on the basis of some cellular processes as well as promotion of cancer invasion and metastasis, activation of anti-apoptosis pathway, intrinsic resistance to chemotherapy and radiotherapy that are implicated in several intracellular signaling (as simplified in Fig. 2), [9,28].

Recently, B7-H3 is getting more attention as an immune checkpoint regulator that concurs with the suppression of cytotoxic immune responses [29,30] but, unlike others, essentially affects adaptive and innate immune responses and, more important, modulates the aggressiveness of tumor cells strictly not dependent on immunological pathways [6]. In fact, B7-H3/TLT-2 was found to be involved in the increase of chemokine proinflammatory cytokine releases, by stimulating the phosphorylation of p38 downstream mitogen-activated protein kinase (MAPK), an important event related to the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activation [9, 31].

B7-H3 expression and detection methods in lung cancer with a spotlight on SCLC

B7-H3 protein is down-regulated in fibroblasts, immune cells and normal tissues, while it is overexpressed in tumor tissues, including different types of lung cancer, thus becoming an interesting and promising target in cancer immunotherapy so long as significantly contributes to the reduction of cancer cell proliferation and metastatization [32, 33]. From an immunity perspective, its expression is found to be associated with a reduction of T-cell and interferon-γ, and hence B7-H3 blockade can stimulate an early influx of CD8+ T-cells and, consequently, promotes an antitumorigenic activity [23,30]. Furthermore, it has to consider that these inhibitor effects on tumorigenesis are rare due to the fact that positive/high B7-H3 expression is a predictor of a favorable or better disease course, as described in 3 of 61 analyzed studies [34–36]. On the other hand, given that invasion, proliferation, and the capability of malignant neoplasms to metastasize through various mechanisms in lung cancer are strictly referred to B7-H3, especially in non-small cell lung cancer (NSCLC) [30,37], a huge interest in this field resulted from preclinical studies. By the way, in mouse models of NSCLC, it has been proven that the inhibition of B7-H3 consequently triggered both high levels of infiltrating CD8+ T-cells and recovery of effector function, thus demonstrating its potential role in cancer immunotherapy as a promising strategy for B7-H3–expressing NSCLCs in combination with anti-PD-1/PD-L1 treatment [37]. Indeed, B7-H3 expression in tumors correlates with aggressive behavior of cancer histology, number of infiltrating T-cells and worse prognosis [38, 39].

Moving on clinical point, emerging studies have highlighted the need to better clarify the assessment of B7-H3 surface expression pattern on immune, non-neoplastic and/or neoplastic cells in 84 NSCLC patients from a longitudinal study by flow cytometry (FC) and its clinicopathological correlation in a retrospective cohort of 484 formalin-fixed paraffin-embedded (FFPE) NSCLC patients by immunohistochemistry (IHC). At first glance, methodological results from FC confirmed the involvement of B7-H3 in NSCLC progression due to its expression being

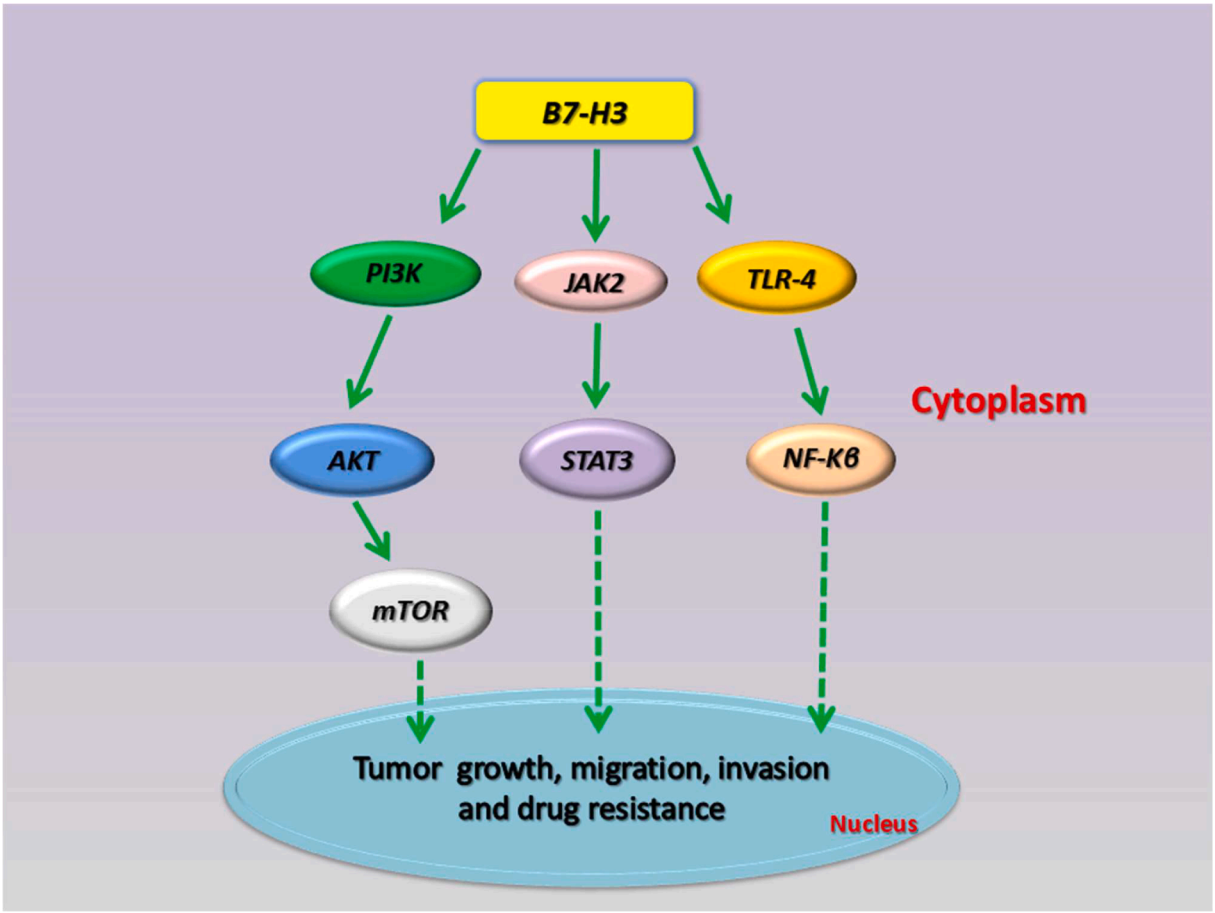

Fig. 2. B7-H3 and its role in oncogenic activation. The most known pathways mediated by B7-H3 are involved in promoting cell survival, proliferation, invasion and drug resistance through phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR), Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3), toll-like receptor 4/ Nuclear factor kappa B (TLR-4/NF-κB) signaling.

much higher in tumors than in normal tissues. Secondarily, a high tumor proportion score (TPS) equal to or greater than 25% on FFPE by IHC demonstrated its prognostic value as a predictor of clinical outcome in virtue of its association with smoking history, diagnostic molecular analysis of EGFR, tumor size and nodal status in these cohorts of NSCLCs [40]. The evaluation of B7-H3 expression was performed using quantitative immunofluorescence (QIF) by assessing both neoplastic epithelial and stromal compartments of TMA cores from 634 NSCLC patients. The relationship between high expression of B7-H3 and poor survival was found in the majority of NSCLC analyzed cases, in addition to that of smoking history [41]. In the same way, *Inamura et al.* confirmed a significant and negative correlation between worse prognosis in lung adenocarcinoma (LUAD) patients with medium or high cigarette consumption and the overexpression of B7-H3 as well as with tumor grading and staging [42]. *Yonesaka et al.* assessed B7-H3 expression levels by IHC in 82 NSCLC metastatic patients (with about 60% of those previously subjected to anti-PD-1 therapy) and observed that three-quarters had a staining pattern from 1+ to 3+ that it looks very interestingly in the association with refractory condition of the tumor [37]. *Yang et al.* assessed the predictive role of B7-H3 in a cohort of 56 treated EGFR-TKIs LUAD patients that harbored EGFR activating mutations as well as 19 Del and 21 L858R. Specifically, the histochemical score, which came from the multiplication of staining intensity with the percentage of positive tumor cells in the low group less than 6 and the high group ranging from 6 to 12, was useful to define the association of the latter group with poor progression-free survival (PFS) ($p = 0.001$, $p = 0.000$) and overall survival (OS) ($p = 0.03$, $p = 0.015$), [43].

Particularly, there are a few important papers (Table 1) that allow us to have some data about B7-H3 expression in SCLC cell lines and patients for a deeper understanding of the detection methods and the major variabilities that affect the evaluation of B7-H3.

Ohara et al. worked on SCLC-J1, a novel cell line obtained from a malignant pleural effusion in an advanced SCLC patient to characterize cytogenetic, genetic and cytologic aspects with immunocytochemical expression of selected antigens as well as ganglioside GD2, B7-H3, and delta-like protein 3 (DLL-3). The results put the focus on APC-conjugated anti-human B7-that is labeled for FC by which it was possible to confirm high levels of CD276 expression patterns on its cell surface of both floating and adherent SCLC-J1 sublines. The next step suggested by the authors will be providing significant insights for better therapeutic strategies for targeting tumor-specific antigens in SCLCs and decreasing intratumoral cellular heterogeneity [44].

Qiu et al. retrospectively evaluated the relationship between the expression of three negative co-stimulatory B7 family molecules such as PD-L1, B7-H3, and B7-H4 in 115 SCLC tissue specimens using IHC and the prognosis of these cancer patients [45]. The current evaluation of B7-H3 is based on the intensity score of the epithelial tumor cell-specific cytoplasmic/membranous staining as follows 0-negative; 1-very weak; 2-moderate; and 3–strong expression. Thus, according to this staining, scores of 0 and 1 were classified as negative, whereas 2 and 3 were defined as positive. As expected, B7-H3 (clone D9M2L) has lower expression in normal tissues but its positive expression in membranes or cytoplasms (or both) of tissue microarrays (TMAs) was found in different degrees of yellow-brown staining [45]. Among the SCLC patients ($n = 107$) that had a complete follow-up, sixty-nine (64.49%) expressed moderate to high B7-H3 levels, which demonstrated a positive correlation with tumor sizes ($p < 0.001$). Moreover, its positive expression significantly correlated with shortened overall survival (OS, $p = 0.006$). But even more impressive, the survival curve obtained from Kaplan Meier analysis shows that the total survival rate of the B7-H3 negative group is higher than that of the B7-H3 positive group ($p < 0.05$). Notably, a negative expression of B7-H3 was found be to an independent prognostic indicator that contributes to ameliorate OS ($p = 0.028$). This paper suggested that B7-H3 expression on cancer cells was found to be dispersed in the whole section locally or sporadically and correlated with larger tumor sizes and the tendency to metastasize as well as the authors claimed that its expression promotes the overactivation of inhibitory signals and biological activities in tumor cells, albeit it is limited to the relationship between expression and prognosis [45].

Carvajal-Hausdorf et al. performed a multiplexed QIF to purely investigate three markers' levels of B7 family ligands as well as PD-L1, B7-H3, B7-H4 and selected tumor infiltrating lymphocyte (TIL) subpopulations and correlate these expression data with clinicopathological features and survival of 90 SCLC samples (represented in TMA) retrospectively collected [46].

Focusing on B7-H3, the detection of B7-H3 (clone D9M2L) expression stands at 64.9%. The visual examination by QIF analysis showed that those cases with higher signals that had a broad range, appeared to be greater than the other considered markers. In addition to this, the authors have attempted to correlate the overexpression of B7-H3 with clinicopathological characteristics and CD3+, CD8+ TILs or CD20+ B-cell infiltrates and 5-year OS with no noteworthy results for all two-sided statistical tests. This may be explained by the fact that the major level of expression of B7-H3 could be associated with the ability to evade immunity in SCLC. At the same time, one of the main problems of analyzing SCLC cases is related to an underestimation or overestimation of the evaluation of proposed markers due to limited tumor tissue, without forgetting that, even though it comes from a single tumor location, could significantly restrict both representativeness and sensitivity of "satellite" lesions not considered in a pre-diagnostic setting. The lack of an independent validation did not provide clarification about marker stratification in SCLC cohort but confirmed only the limitation of this exploratory study [46].

The main focus of these papers was addressed on B7-H3, for which does not exist any standardized dyeing process and quality control for the IHC scoring until now. In this context, we should consider some

Table 1
B7-H3 expression, detection and clinical evaluation in SCLC cell lines and patients.

Tumor model	Expression	Detection methods	Clinical significance	Refs.
SCLC-J1, cell line from pleural-effusion fluid in a patient with clinically advanced SCLC	High levels of cell surface antigen expression patterns (floating 80.4%, adherent 85.9%)	FC analysis	Not evaluated	[44]
115 SCLC TMA specimens	Significantly high expression in TMA membranes or cytoplasms (or both) of SCLC samples showed different degrees of yellow-brown staining (from moderate to high)	IHC analysis	Positive correlation with tumor sizes ($P < 0.001$) and with shortened OS ($P = 0.006$). B7-H3 resulted as an independent prognostic indicator of OS ($P = 0.028$)	[45]
90 SCLC samples represented in TMA and retrospectively collected	The overexpression of B7-H3 was found in 64.9% of SCLC cases	QIF analysis	The correlation between expression, clinicopathological characteristics and CD3+, CD8+ TILs or CD20+ B-cell infiltrates and 5-year overall survival didn't show any significant results	[46]

Abbreviations: Refs, references; SCLC-J1, small cell lung cancer sublines; FC, Flow cytometry; SCLC, small cell lung cancer; TMA, Tissue microarrays; B7-H3, B7 homolog 3 protein; IHC, Immunohistochemistry; OS, Overall Survival; QIF, quantitative immunofluorescence; CD3, cluster of differentiation 3; CD8, cluster of differentiation 8; TIL, Tumor-infiltrating lymphocytes; CD20, cluster of differentiation 20.

discrepancies that came from the detection of negative co-stimulatory B7 family molecules and surely impact B7-H3 expression. Several limitations not yet overcome until now can be imputable also and above all from the pre-analytic phase related to the preparation of samples such as time and use of fixative, the selection of cancer species and histologies which depend on demographic and clinical features, to the analytic setting as well as the reliability and reproducibility of B7-H3 assessment (IHC and FC), the choice of clone for staining patterns and the possibility to analyze single or multiple tissue samples simultaneously, the sensitivity to reagents affecting research studies i.e. retrospective cohorts where, in most cases, B7-H3 was not related to surgery, radiotherapy and chemotherapy or others with no previous main treatment done [45, 46]. These findings highlight the importance of B7-H3 as a therapeutic target and open a new window about biological inhibitors under clinical investigation in SCLC patients.

B7-H3 inhibitors in SCLC

To date, there are no clinically available therapeutics/drugs targeting B7-H3 in any solid tumors. The clear difference in the B7-H3 expression levels, which is overexpressed in tumors but restricted in normal tissues, implies that targeting B7-H3 may selectively kill cancer cells and spare normal cells to minimize side effects. In this regard, several therapeutic approaches, such as antibody-drug conjugates (ADCs), monoclonal antibodies (mAbs) mediating cellular cytotoxicity (ADCC), bispecific antibodies (BsAbs), chimeric antigen receptor (CAR) T-cell therapy, and radioimmunotherapy (RIT) are being investigated to target B7-H3.

Here we report the main strategic approaches and related B7-H3 inhibitors under clinical investigation in solid tumors, focusing mainly on those being evaluated specifically in SCLC showing the preliminary results already available for the treatment of this disease (Tables 2 and 3), [47].

Antibody–drug conjugates (ADCs)

The ADCs are a novel approach for cancer therapy. They consist of the combination of a monoclonal antibody (mAb), targeting an overexpressed tumor cell surface protein, with a potent cytotoxic payload, that is delivered to a tumor, improving the therapeutic index.

MGC018, a humanized B7-H3 mAb with a cleavable linker duocarmycin payload, showed to be cytotoxic for B7-H3–positive human tumor cell lines sparing the B7-H3-negative tumor cells. MGC018 showed antitumor activity in both preclinical tumor models of melanoma, breast, ovarian, and lung cancer and patient-derived xenograft models of breast, prostate, and head and neck squamous cancer cell (HNSCC). The pharmacokinetic and safety profile was favorable in cynomolgus monkeys following repeat-dose administration. These data were considered enough interesting to support the further development of MGC018 for the treatment of solid cancers [49]. The preliminary results from a phase I/II trial, investigating MGC018 in solid tumors, no SCLC patients were included, showed a manageable safety profile with early evidence of clinical activity by prostate-specific antigen (PSA) and tumor responses in metastatic castration resistant prostate cancer (mCRPC), [50]. Another ongoing phase 1/1b trial is investigating the combination of MGC018 plus lorigerlimab, a bispecific PD-1/CTLA-4 inhibitor, in B7-H3-positive solid tumors [51].

Table 2
Main ongoing clinical trials investigating B7-H3 inhibitors in SCLC [47].

Protocol IDs	Title	SCLC eligibility	Primary endpoint	Status
NCT05280470	A phase 2, multicenter, randomized, open-label study of DS-7300a, a B7-H3 antibody drug conjugate, in subjects with pretreated extensive-stage small cell lung cancer	Only SCLC	ORR by BICR	Recruiting
NCT05405621	A phase 1, multi-center, open-label study to assess safety, tolerability, pharmacokinetics, and preliminary efficacy of BAT8009 in patients with advanced solid tumors	Solid tumors	DLT	Recruiting

Abbreviations: ES-SCLC: extensive-stage small-cell lung cancer; ORR: objective response rate; BICR: blinded independent central review; DLT: dose limiting toxicity.

DS-7300a (Ifinatamab deruxtecan, I-DXd), a B7-H3–specific mAb conjugated to an exatecan derivative payload with about four topoisomerase I inhibitor particles, specifically bound to B7-H3 inhibiting the growth of B7-H3-expressing cancer cells, but not that of B7-H3-negative cancer cells, *in vitro*. Moreover, DS-7300a demonstrated, *in vivo*, antitumor activities in high–B7-H3 tumor xenograft models with acceptable pharmacokinetic profiles in monkeys, and well tolerated in rats and monkeys [52]. DS7300a was administered at doses ranging from 0.8 to 16 mg/kg, intravenously, every 3 weeks, in a phase 1–2 trial, in patients with pretreated solid tumors, including SCLC. The preliminary results showed no dose limiting toxicities (DLT) among the 147 patients with solid tumors. Treatment-emergent adverse events (TEAEs) were reported in 98% of patients, with the most common being all-grade nausea in 63%, anemia (33%), infusion-related reactions (IRRs) in 32%, decreased appetite in 31% and vomiting in 30% of cases. Grade $\geq$ 3 TEAEs occurred in 45% of patients, with 1 TEAEs leading to death (1%-interstitial lung disease in the 16.0 mg/kg cohort), while a dose discontinuation was reported in 8% of cases. A total of 118 patients with solid tumors were evaluable for a confirmed partial response of 28%. Nineteen pretreated SCLC patients were enrolled reporting 10 (53%) confirmed partial responses. Median time to response was 1.2 months with a median duration of response of 5.5 months [47]. The 16.0 mg/kg cohort was closed for safety reasons reporting a higher rate of serious and grade $\geq$ 3 TEAEs occurring within a shorter median treatment duration than the 8.0 and 12.0 mg/kg cohorts. These data support further clinical development of DS-7300a, including a phase 2 dose-optimization study in SCLC with doses of 8.0 mg/kg and 12.0 mg/kg, every 3 weeks. The aim of the study is to define the recommended phase 2 dose of DS7300a based on the efficacy (primary endpoint is the ORR based on the Blinded Independent Central Review [BICR] assessment), safety, and pharmacokinetics (PK) results in ES-SCLC patients who received 1–3 prior lines of therapy, including a platinum-based chemotherapy (NCT05280470).

A first-in-human, multicenter, open-label phase 1 study investigated HS-20093, a B7-H3-targeted ADC, in advanced pretreated solid tumors. The dose escalation part assessed safety and tolerability of intravenous HS-20093 with doses ranging from 1.0 to 16.0 mg/kg, every 3 weeks. In the dose escalation study, 53 patients were enrolled including 11 with SCLC. The maximum tolerated dose was determined to be 12.0 mg/kg with the most common TEAEs being leukopenia, neutropenia, anemia, pyrexia, nausea, thrombocytopenia, hypoalbuminemia, vomiting, lymphopenia, infusion-related reaction and fatigue. No interstitial lung disease was reported. ORR was reported in 35% out of the 40 response-evaluable patients, regardless of baseline B7-H3 expression level, with a disease control rate of 85.0%. In the subset of 11 evaluable SCLC patients, the ORR was observed in 63.6% of cases, all occurring at the first disease assessment with a median time to first response of 6 weeks. The disease control rate was 81.8% with a median PFS of 4.7 months and a 3-month PFS rate of 72.7% [48].

BAT8009, another B7-H3 ADC, linked to the camptothecin analog exatecan, with potential antineoplastic activity, is being investigated in phase 1 trial in solid tumors, including ES-SCLC (NCT05405621).

Table 3
Available clinical results of B7-H3 inhibitors in SCLC.

Study	Phase of Study	Treatment	Starting Doses	No. pts	ORR (%)	Confirmed ORR (%)	Median time to response (95 % CI)	Median duration of response (months [95 % CI])	Median follow-up (months [95 % CI])
[47]	1/2	DS7300a (Ifinatamab deruxtecan, I-DXd)	6.4 mg/kg 8.0 mg/kg 12.0 mg/kg 16.0 mg/kg	19	58	53	1.2 months (NA-1.4)	5.5 (2.8-NR)	4.9 (3.3–8.8)
[48]	1	HS-20093	1.0 mg/kg 2.0 mg/kg 4.0 mg/kg 6.0 mg/kg 8.0 mg/kg 12.0 mg/kg 16.0 mg/kg	11	63.6	NRep	6 weeks (NRep)	NRep	NRep

Abbreviations: SCLC: small-cell lung cancer; No.pts: number of patients; ORR, objective response rate; CI: confidence intervals; NA: not applicable; NR: not reached; NRep: not reported.

mAbs ADCC

ADCCs work as a bridge that links the effector, via their fragment crystallizable region (Fc) portions, to a target through their antigen-binding fragment (Fab) portions [53].

Enoblituzumab (MGA271) is a fully humanized IgG1 B7-H3 targeting mAb containing a five amino acid change at its humanized Fc site for increased activation affinity. Enoblituzumab, the first mAb tested against B7-H3-expressing tumors, showed potent antitumor activity in renal cell carcinoma and bladder cancer xenograft models [54]. Several trials investigated enoblituzumab alone or combined with other immune checkpoint inhibitors in solid tumors, including melanoma, prostate cancer, bladder cancer, breast cancer, clear cell renal carcinoma, NSCLC, and HNSCC, reporting interesting results [55,56]. On the other hand, a phase II trial, investigating enoblituzumab plus retifanlimab or tebotelimab as first-line treatment for patients with recurrent or metastatic HNSCC, was closed early due to safety concerns with 7 fatalities potentially linked to hemorrhagic events [56]. Currently, no data are reported with enoblituzumab in SCLC and no clear evidence of ongoing trials in this setting are available.

Other mAbs targeting B7-H3 via ADCC, such as DS-5573a and omburtamab (8H9), are in early stages of clinical research in solid tumors with no evidence of investigation in SCLC. In particular, omburtamab has been the most widely used carrier for radioimmunoconjugates in fact, radioactive iodine labeled omburtamab has been developed and evaluated in rhabdomyosarcoma, recurrent metastatic neuroblastoma peritoneal tumors with interesting results [9].

Bispecific antibodies (BsAbs)

BsAbs are artificially generated by combining antibody fragments recognizing two distinct antigens. At least four types of BsAbs targeting B7-H3, such as CD3/B7-H3, CD16/B7-H3, PD-1/B7-H3, and 4–1BB/B7-H3, have been engineered and showed antitumor activities against tumor cell lines *in vitro*, and, with the exception of PD-1/B7-H3, suppressed tumors in murine xenograft models, too [9].

Among the CD3xB7-H3 BsAbs, obrindatamab (MGD009), a humanized dual affinity protein, was investigated in previously treated solid B7-H3-expressing tumors. The Food and Drug Administration (FDA) initially suspended this study due to hepatic adverse events, and subsequently reactivated, because these events were uncomplicated and short-lived. However, the trial was permanently terminated due to a business decision (NCT02628535).

Currently, MGD009 is the only BsAb targeting B7-H3 clinically evaluated in solid tumors with no activity results released yet.

Conclusion

Significant challenges remain in expanding the research targeting B7-H3 and, even more important, the clinical relevance of B7-H3 expression and its detection in SCLC, the most aggressive form of lung cancer characterized by high proliferative rate, early metastatization and poor prognosis [57]. B7-H3 overexpression has been reported in a variety of cancer types, including SCLC, and its correlation with poor prognosis is also widely established. Therefore, targeting B7-H3, via various immunotherapeutic approaches, might provide a novel and promising option for cancer therapy.

Although B7-H3 protein expression in lung tumors has been largely explored by different quantitative immunostaining methods, it remains very difficult to reduce some discrepancies related to the lack of standardization that could allow us to define a real association between B7-H3 expression and clinicopathological characteristics.

Although SCLC is a strongly chemo-sensitive disease, a rapid resistance to standard chemo-immunotherapy frequently occurs in the ES. Thus, further strategic approaches are welcome. The most promising preliminary clinical results have been reported by DS7300a and HS-20093, both are ADCs, that, to date, are under investigation in ongoing trials for the treatment of pretreated SCLC.

The expression of B7-H3 tends to be mutually exclusive to PD-L1 and CTLA4 expressions. This led to hypothesize that combining B7-H3 and other immune checkpoint inhibitors might be a strategic approach to pursue, paying particular attention to the safety profile.

Overall, B7-H3 might be a promising target for future immunotherapy, contributing to an improvement in cancer immunotherapy becoming a novel and promising option for cancer therapy.

CRediT authorship contribution statement

Federico Pio Fabrizio: Writing – review & editing, Data curation, Formal analysis, Writing – original draft. **Lucia Anna Muscarella:** Writing – review & editing, Data curation, Formal analysis, Writing – original draft. **Antonio Rossi:** Writing – review & editing, Data curation, Formal analysis, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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