Ombretta Melaiu, Valeria Lucarini, Roberto Giovannoni, Doriana Fruci, Federica Gemignani, News on immune checkpoint inhibitors as immunotherapy strategies in adult and pediatric solid tumors, Seminars in Cancer Biology, 2020, ISSN 1044-579X,
https://doi.org/10.1016/j.semcancer.2020.07.001

News on immune checkpoint inhibitors as immunotherapy strategies in adult and pediatric solid tumors

Ombretta Melaiu[2], Valeria Lucarini[1], Roberto Giovannoni[2], Doriana Fruci[1,*] and Federica Gemignani[2,*]

[1]Paediatric Haematology/Oncology Department, Ospedale Pediatrico Bambino Gesù, Rome, Italy
[2]Department of Biology, University of Pisa, Pisa, Italy

[*] equally contributed

Corresponding authors:
Doriana Fruci, Immuno-Oncology Laboratory, Department of Paediatric Haematology/Oncology and of Cell and Gene Therapy, Ospedale Pediatrico Bambino Gesù, IRCCS, 00146 Rome, Italy. Phone: +39-06-68592657, email: doriana.fruci@opbg.net
Federica Gemignani, Department of Biology, University of Pisa, 56126 Pisa, Italy. Phone: Phone: +39-050-2211529, email: federica.gemignani@unipi.it

Running Title: Immune checkpoint inhibitors and cancer

Abstract

Immune checkpoint inhibitors (ICIs) have shown unprecedented benefits in various adult cancers, and this success has prompted the exploration of ICI therapy even in childhood malignances. Although the use of ICIs as individual agents has achieved disappointing response rates, combinational therapies are likely to promise better results. However, only a subset of patients experienced prolonged clinical effects, thus suggesting the need to identify robust bio-markers that predict individual clinical response or resistance to ICI therapy as the main challenge. In this review, we focus on how the use of ICIs in adult cancers can be translated into pediatric malignances. We discuss the physiological mechanism of action of each IC, including the most widely studied (PD-1, PD-L1 and CTLA-4), and the new emerging ones (LAG-3, TIM-3, TIGIT, B7-H3, BTLA and IDO-1), and evaluate their prognostic value in both adult and childhood tumors. Furthermore, we offer an overview of preclinical models and clinical trials currently under investigation to improve the effectiveness of cancer immunotherapies in these patients. Finally, we outline the main predictive factors that influence the efficacy of ICIs, in order to lay the basis for the development of a pan-cancer immunogenomic model, able to direct young patients towards more specific immunotherapy.

Keywords: adult cancers, pediatric cancers, immune checkpoint inhibitors, clinical trials, immunotherapy

1. Introduction

Pediatric cancer represent the leading cause of death by disease among children. Overall, the most common types of cancer are leukemia, lymphoma and brain and central nervous system (CNS) tumors, followed by soft tissue sarcomas (STS), neuroblastoma (NB) and kidney tumors in children (age 0 to 14 years), and by gonadal germ cell tumors (meGCTs), thyroid cancer and melanoma (MM) in adolescents (ages 15 to 19) [1]. Solid tumors account for almost 60% of all pediatric cancers [2]. Although survival rates for most childhood malignances have significantly improved in the last decades, thanks to the combined action of chemotherapy, surgery and radiation therapy, the clinical outcome of some cancer patients still remains extremely low, with a high risk of recurrence or consequent onset of malignant neoplasms, thus determining the need for long-term clinical monitoring of childhood cancer survivors [3]. Furthermore, the side-effects and long-term sequelae of anti-cancer chemotherapy remain a major source of concern for both patients and clinicians, with the consequential cost of cure for the majority of children diagnosed with cancer [4, 5]. For all these reasons, new approaches to improve tolerance and reduce adverse consequences of cancer chemotherapy are needed [6]. Cancer immunotherapy represents a new alternative option to conventional therapies. The ability to target the host immune system rather than the cancer itself has revolutionized the treatment of multiple malignancies over the past decades [7]. Cancer immunotherapy can be classified into i) synthetic immunotherapies, designed to initiate new immune responses directed toward tumor-expressed targets, such as monoclonal antibodies (mAbs) and chimeric antigen receptors (CARs), and ii) agents designed to amplify natural immune responses, such as immune checkpoint inhibitors (ICIs) [7]. In general, mAbs have been found to be effective when administrated as bispecific molecule, able to simultaneously bind a tumor antigen, and activate T cells (by binding CD3) to direct kill the tumor cells brought into close proximity by the antibody. Blinatumomamab, is an anti-CD3/anti-CD19 bispecific Ab that target CD19-expressing B cell acute lymphoblastoid leukemia both in adult and pediatric patients [8, 9]. Another effective bispecific mAb is the anti-CD3/anti-GD2 able to redirect activated T cells to target GD2-expressing NB and osteosarcoma (OSS) [10]. Adoptive transfer of T cells expressing CARs targeting tumor antigens has also produced encouraging results in pediatric cancers. For a comprehensive overview of the effects of synthetic immunotherapies against childhood cancers, please refer to Majzner 2017 Cancer Cell, etc [7].

The recent development of immune checkpoint inhibitors (ICIs), object of the present perspective, represents a crucial milestone in the immuno-oncology field for their ability to make immune cells able to control tumor growth [11-13]. The impressive results achieved in several adult cancer patients

[14-17], has prompted the exploration of the ICIs therapy also in childhood malignances, as a promising alternative therapy, aimed to reinvigorate anti-tumor responses also in pediatric cancers. Here, we review the current state of the art of IC molecules in solid adult neoplasms, and how this knowledge can be translated into pediatric solid tumor therapies. To this aim, we summarize i) the physiological mechanism of action of ICs and their prognostic value in both adult and childhood malignances, ii) the preclinical models employed for ICIs studies in adult and pediatric solid tumors, iii) the most recent ICI clinical trials aimed to strengthen the immunotherapy strategies and overcome the existing obstacles on the effectiveness of checkpoint inhibition in cancers, and iv) the approved and emerging predictive biomarkers of ICI therapy response.

2. Immune-checkpoints: physiological function and mechanisms of regulation

T-cell function represents a perfect balance between positive and negative signals by which transformed cells are eliminated. The recognition and destruction of damaged cells typically occurs through binding of the T-cell receptor (TCR), expressed by T-cells, to peptide-major histocompatibility complexes (MHC) on antigen-presenting cells (APC) and tumor cells. IC molecules are one of the main players in maintaining the individual's immune homeostasis, by regulating the level and duration of physiological immune responses. The different IC molecules are important for limiting tissue damage and promoting self-tolerance by suppressing the inflammatory activity of T-cells.

At this regard**, PD-1** (Programmed cell Death 1, CD279) is a crucial regulator of normal host physiology and programmed cell death of lymphocytes [18]. PD-1 was discovered in 1992 at the University of Kyoto [19] with a project aimed at selecting all the genes involved in apoptosis. The protein encoded by the *PDCD1* gene located on chromosome 2q37.3 [20] is a type I membrane protein belonging to the CD28 superfamily [19]. The critical role of PD-1 in maintaining peripheral tolerance was evident even before the discovery of its ligands. Indeed, *PD-1*-deficient mice spontaneously developed autoimmune diseases (AD), such as lupus-like proliferative arthritis and glomerulonephritis [21]. PD-1 binds two members of the B7 family, PD-L1 (CD274) and PD-L2 (CD273) [22, 23]. PD-L1 is expressed on a wide range of tissues, including primary and secondary lymphoid organs and healthy non-hematopoietic tissues. In contrast, the expression of PD-L2 is restricted to APCs in lymphoid tissues [24, 25]. Structurally, PD-1 contains a cytoplasmic domain with two tyrosine-based signaling motifs, a transmembrane domain, and an Ig-V like extracellular domain [19] (**Figure 1**). PD-L1 includes a short cytoplasmic tail, a transmembrane domain, and the Ig-V- and Ig-C-like extracellular domains [26-28] (**Figure 1**). The interactions between the extracellular domains of PD-L1 and its receptor determine the recruitment of type 11 and 6 non-

receptor tyrosine-protein phosphatases (also known as SHP-2 and SHP-1 proteins, respectively), which attenuate the T-cell activating signals [19, 29]. T-cell activity is affected by PD-1/PD-L1 interaction in several ways, including inhibition of T-cell proliferation, survival and cytokine production [22, 26, 28, 30-33]. PD-1 is expressed during the initial antigen activation on different subsets of T cells, B cells, natural killer (NK) cells, some myeloid cells and cancer cells [34, 35].

CTLA-4 (Cytotoxic T-lymphocyte antigen 4, CD152) is an inhibitory receptor able of arresting potential autoreactive T-cells in the initial stage of their activation [36]. It is a member of the immunoglobulin (Ig) superfamily and is structurally homologous to the CD28 molecule, the counterpart with a costimulatory function [37]. The human *CTLA4* gene is mapped on chromosome 2q33 [38] near the *CD28* gene, from which it is 25 to 150 kb away [39]. The discovery of CTLA-4 function as a negative T-cell regulator dates back to 1995 [40]. CTLA-4 competes with CD28 to bind to the same ligands, CD80 and CD86 inhibiting immune responses [37]. CTLA-4 is able to bind to CD80 and CD86 with greater affinity and avidity than CD28 [41], thus overcoming the risk of excessive T-cell activation and blocking immune responses against self. CTLA-4 can exists in different isoforms due to alternative mRNA splicing [42]. The main isoform is a transmembrane protein, containing an extracellular domain, a transmembrane domain and a cytoplasmic tail (**Figure 1**). CTLA-4 exerts its inhibitory action directly through the de-phosphorylation of TCR via SHP-2 and PP2A [43]. More recently, CTLA-4 was shown to capture and remove CD80 and CD86 from the membranes of APCs, making them unavailable for CD28 binding [44]. The soluble CTLA-4 isoform has been shown to inhibit early T-cell activation, and its serum levels have increased in several ADs [45]. CTLA-4 is upregulated in conventional T-cells after their activation and constitutively expressed by regulatory T (Treg) cells [46]. *CTLA-4*-deficient mice have been frequently reported to develop a lethal AD with strong lympho-proliferation [47, 48]. Moreover, several polymorphisms of the *CTLA-4* gene detected through genome-wide association studies (GWAS) are associated with various AD, including systemic lupus erythematosus, rheumatoid arthritis (RA), Grave's disease (GD), autoimmune hypothyroidism and type 1 diabetes [47].

LAG-3 (lymphocyte activation gene 3) is a lymphocyte checkpoint protein whose mechanism of action is still unknown [49]. LAG-3 was discovered by Triebel *et al*. in 1990 [50], and is encoded by the homonym homologous gene mapped on chromosome 12p13 [50]. Given the structural homology with CD4, *LAG-3* and *CTLA-4* was supposed to derive from a common evolutionary ancestor gene [50]. One of the first functions attributed to LAG-3 was the ability to induce dendritic cell (DC) maturation [51]. Structurally, LAG-3 is a transmembrane protein belonging to the Ig superfamily consisting of four extracellular domains referred as D1-D4 [50] (**Figure 1**). The canonical ligand of LAG-3 is MHC class II molecule (MHC-II) [52], however direct evidence on their physical

interaction is still missing [53]. MHC-II interacts with the CD4 receptor stimulating activation of helper T cells [54]. Moreover, the fact that LAG-3 also regulates the activity of $CD8^+$ T cells and NK cells, which do not interact with MHC-II [55], led researchers to identify additional unknown LAG-3 ligands [53]. Recently, LAG-3 has been shown to bind Galectin3 and FGL1 [53, 56]. The latter is now considered an important functional ligand of LAG-3. LAG-3 is expressed on activated T-cells, both $CD4^+$ and $CD8^+$ T-cells, and NK cells, in which negatively regulates effector function, homeostasis and proliferation [54, 57-61]. In addition, LAG-3 is also expressed on Treg cells [62-64], B cells [65] and plasmacytoid DCs [66]. *LAG3*-deficient mice in nonobese diabetic background exhibited accelerated autoimmune diabetes with 100% penetrance [67].

TIM-3 (T-cell Ig and mucin domain 3) is a type I transmembrane protein identified for the first time on Type 1 CD4 helper (Th1) and cytotoxic $CD8^+$ T cells in 2002 [68]. TIM-3 is encoded by the *HAVCR2* gene located on chromosome 5q33.2 [69]. It belongs to the Ig super family. Structurally, it contains an N-terminal IgV domain, a mucin-like domain with glycosylation sites, and a C-terminal cytoplasmic domain with two out of five tyrosine residues, which have been shown to be phosphorylated and critical for TIM-3-mediated signaling [70, 71] (**Figure 1**). TIM-3 is defined as an IC molecule, and several studies have reported an association between *HAVCR2* polymorphisms and the risk of developing AD, such as GD, Hashimoto's disease, idiopathic thrombocytopenic purpura, multiple sclerosis , and RA [68, 72-75]. However, more recently, TIM-3 has been shown to play a co-stimulatory enhancing function, thus suggesting its dual role with both stimulating and inhibitory functions [70, 76, 77]. TIM-3 can bind four ligands: Gal-9 (galectin-9), PtdSer (phospatidyl serine), HMGB1 (high-mobility group protein B1) and Ceacam-1 (Carcinoembryonic Antigen Related Cell Adhesion Molecule 1) [78]. The interaction of TIM-3 with Gal-9 causes apoptosis of Th1 cells [79]. PtdSer is over-expressed in apoptotic cells and causes the clearing of apoptotic bodies and the reduction of antigens cross-presentation by DCs [80, 81]. The binding of HMGB1 dampens the activation of innate immune responses thus interfering with the stimulation of nucleic acids [82, 83]. The mutual interaction between TIM-3 and Ceacam-1 is required to promote T-cell exhaustion, thus suggesting that the inhibitory function of TIM-3 depends on Ceacam-1 co-expression [84]. This could explain the apparently contradictory reports of TIM-3 function in T cells. $TIM\text{-}3^+PD\text{-}1^+CD8^+$ T-cells have been shown to represent a “deeply” exhausted T-cell population compared to $TIM\text{-}3^-$ $PD\text{-}1^+$ $CD8^+$ T-cells [83]. Indeed, TIM-3 is generally co-expressed with other checkpoint receptors in T-cell exhaustion contexts [85]. TIM-3 is also expressed by innate immune cells, including DCs [82, 86] and NK cells [87]. During chronic HIV infection, TIM-3 is able to inhibit NK and DC function by interfering with TLR signaling via the recruitment of IRF7 and p85 to lysosomes [88].

TIGIT (T-cell Ig and ITIM domain) is a cell surface protein encoded by the homonymous gene, mapped on chromosome 3q13.3. It was discovered in 2009 following a bioinformatics analysis aimed at identifying all the genes expressed in activated T-cells [89]. Structurally, TIGIT has a single Ig domain, followed by a type I transmembrane domain and a single intracellular immune-receptor tyrosine-based inhibitory motif (ITIM) [89] (**Figure 1**). It belongs to the PVR family [89] and, together with CD96 and CD226 (DNAM-1), forms a pathway similar to that of CTLA-4/CD28 [90]. Indeed, TIGIT and CD96 exert an inhibitory function, whereas CD226 is a co-stimulating receptor. They compete to bind to the same ligands, i.e., CD155 (PVR, necl-5) and CD112 (PVRL2, nectin-2) [89]. TIGIT preferably binds to PVR rather than nectin-2, and the interaction between TIGIT and PVR is greater than that between DNAM-1 and PVR [89]. This allows to destroy the co-stimulation driven by DNAM-1, thus providing a cell-inhibitory signal [90]. Homodimerization between TIGIT and PVR is required to recruit SHP-2 molecules [91], which inhibits ERK activation in DC [89], dampening T-cell responses [89]. Another mechanism by which TIGIT regulates the responses of effector T-cells is the activation of Treg cells [92]. Flow cytometry analysis showed that TIGIT is expressed on the surface of αβ T-cells upon activation [93, 94], memory T-cells, and Treg cells [89, 95]. Interestingly, TIGIT is also expressed on NK cells in which it inhibits NK cell-mediated killing [96].

There are other immune checkpoints whose role has not yet been defined, including B7-H3, BTLA and IDO-1.

B7-H3 (CD276 molecule, cytogenetic locations: 15q24.1) is a quite intriguing molecule (Figure 1). It was initially defined as a stimulatory molecule [97], whereas now is believed to be part of inhibitory receptors [98]. Controversial evidences have been described for B7-H3 in humans (where it inhibits the activation of naive T-cells and the production of cytotoxic cytokines [98]) and in mice (where it stimulates T cell proliferation [99, 100]). However, since neither its receptor nor stimulating factors have been identified, a clear functional picture cannot be defined [101].

Another molecule similar to PD-1 and CTLA-4 is **BTLA** (B- and T-lymphocyte attenuator, CD272) [102], whose protein in humans is encoded by the homonymous gene mapping on chromosome 3q13.2. It belongs to the CD28 family and is structurally an Ig with two immunoreceptor tyrosine-based inhibitory motifs (**Figure 1**). The interaction with its herpes virus entry mediator (HVEM) (herpes virus entry mediator) ligand, a member of the TNF receptor [103, 104], led to phosphorylation of the immune-receptor tyrosine-based inhibitory motifs and recruitment of the SHP2 protein, which result in decrease of IL-2 production and T-cell proliferation [105, 106]. BTLA is constitutively expressed on Th1 cells, but not on naive T-cells, and its depletion causes an increase in T-cell

proliferation [102]. *BTLA*-deficient mice have an increased severity of allergic airway inflammation [107], autoimmune hepatitis–like disease [108] and autoimmune encephalomyelitis [102].

Finally, **IDO-1** (indoleamine 2,3-dioxygenase) is a tryptophan catabolic enzyme encoded by the homonym gene on chromosome 8p11.21, which could be classified as an immune checkpoint due to its immune-inhibitory properties. Indeed, evidence highlighted that IDO-1 promotes cancer progression trough different mechanisms. Among these, the role of IDO-1 in modulating the immune system is becoming increasingly important. IDO-1 has been shown to suppress both T cells [109, 110] and NK cells [111, 112], and activate Treg cells [113] and myeloid-derived suppressor cells (MDSC) [114].

Given the emerging evidence indicating how the level of ICs on different immune cells can influence normal homeostasis, it is critical to understand how the levels of IC expression at the cell surface are regulated, and how this regulation may vary between immune cells and tumors cells.

3. Prognostic value of ICs in adult and childhood cancers

ICs molecules are frequently overexpressed during cancer development as mechanism of immune subversion in response of oncogenic signaling and inflammatory cytokines, able to enhance tumor tolerance and evade cancer eradication by the immune system. Therefore, the precise quantification and characterization of ICs within tumor microenvironment (TME) is receiving increasing attention in order to understand whether IC protein expression can be used as a potential biomarker for patient survival prediction. To date, the expression and prognostic significance of IC molecules in TME have been widely explored in various adult cancers, and to a lesser extent in children with solid tumors.

3.1 ICs expression in adult cancers

A recent bibliometric analysis of global research on **PD-1** and **PD-L1** in cancer revealed a total of 7359 articles published after 2014 [115]. In this section, we will focus on the most recent findings related to the prognostic role of PD-1 and PD-L1 in adult malignancies. The analysis of the PD-L1/PD-1 axis was mainly performed through transcriptomic and immunohistochemistry (IHC) techniques, important in distinguishing PD-L1 expressed on tumor-infiltrating lymphocytes (TILs) and tumor cells. A meta-analysis conducted on different types of cancer revealed that the expression of PD-L1 by TILs (immune PD-L1) correlates with a good prognosis [116]. This evidence was confirmed on breast cancer (BC) [117], colon cancer (CC) [118] and ovarian cancer (OC). In OC, stromal TILs expressing PD-L1 may serve as a favorable prognostic factor, particularly in the serous carcinoma subtype [119]. In contrast, various studies have reported that the high density of tumor cells expressing PD-L1 (tumor PD-L1) has a negative impact on disease progression of different

tumors [120-123]. A systemic analysis conducted on 11,383 patients, revealed that PD-L1 is associated with several clinic-pathological features, and acts as a poor prognostic biomarker for patients with lung cancer (LC) [124]. Similarly, another study on a total of 14,367 BC patients showed that the density of PD-L1$^+$ tumor cells is associated with large tumor size, histological grade tumors, high Ki-67 expression, and a shorter disease-free survival (DFS) [117]. The negative prognostic role of PD-L1 expression on tumor cells has also been confirmed in CC [125]. However, other studies have found no consistent effects of PD-L1 expression on survival rates, thus suggesting the need for further investigation [126-128]. To date, growing evidence has highlighted the discordance between the expression of PD-L1 on the primary tumor and metastases. Frequency of PD-1$^+$ and PD-L1$^+$ cells was detected in primary BCs and their corresponding distant metastases by IHC [129]. Analysis showed that PD-1 was differently expressed between primary tumor and metastases in half of the patients. In primary tumors, there was a correlation between PD-1 positivity and a higher tumor grade, as well as between immune PD-L1 and estrogen receptor negativity. Moreover, in survival analyses, an acquired expression of PD-L1 in metastases seems to indicate better survival [129]. PD-L1 expression is temporally and spatially discordant even between lesions of primary and metastatic urothelial carcinoma (UC) [130]. Indeed, PD-L1 levels in primary tumors were not correlated with metastatic lesions [130]. Similarly, the differential expression of PD-1 and PD-L1 was also detected between primary and metastatic sites in renal cell carcinoma (RCC), with a higher detection rate in metastases rather than the primary site [131]. Finally, since tumor cells exert their immunosuppressive role by up-regulating PD-L1 and engaging the PD-1 receptor expressed on TILs, several authors have also focused on the prognostic role of PD-1 and its expansion in the TME of various malignancies [132, 133].

Increasing findings have shown that a high expression of **CTLA-4** in TME is linked to an unfavorable prognosis in several adult cancers. High tumor CTLA-4 expression was associated with poor prognosis in patients with nasopharyngeal carcinoma. No difference in terms of clinical outcome was observed between groups with high and low expression of immune CTLA-4 [134]. A study on 158 patients with esophageal cancer (EC) showed a membrane and cytoplasmic expression of CTLA-4 on both tumor cells and TILs, and in both cases, high expression was associated with shorter overall survival (OS) [135]. Furthermore, overexpression of CTLA-4 represents a negative prognostic factor also for patients with thymoma [136] and Her2-negative-BC [137]. A study on 536 patients with stage I-IIIA of non-small-cell lung cancer (NSCLC) showed discordant CTLA-4 expression levels between primary and metastatic tumor lesions. Indeed, in primary tumors the expression of CTLA-4 was not significantly associated with DFS neither in tumor epithelial cells (T-CTLA-4) nor in stromal cells. In contrast, there was an independent negative prognostic impact of T-CTLA-4 expression in

metastatic lymph nodes [138]. Recently, the combination of PD-1/CTLA-4 expression in TILs had a prognostic role in high-risk RCC patients [139]. Some recent reports have shown a significant accumulation of exhausted T-cells expressing multiple ICs, including PD-1 and CTLA-4 in TME. In BC patients, an increase in CTLA-4^+ PD-1^+ Treg cells within TME has been shown [140]. Similar results are reported in CC patients [141].

The first evidence on the prognostic role of **LAG-3** occurred in 2006 in a cohort of 246 sera analyzed for the soluble form (sLAG-3) in BC patients. The authors found that both DFS and OS rates were higher in patients with estrogen or progesterone receptor positive tumor cells with detectable levels of sLAG-3 at diagnosis compared to patients with non-sLAG-3 detectable levels [142]. Similar results were obtained on gastric cancer (GC), in which sLAG-3 positively correlates with the frequency of $CD8^+$ T-cells, production of IL-12 and IFN-γ, and better prognosis [143]. Conversely, LAG-3^+ TILs were significantly increased in tumor tissues compared to peritumoral regions, and associated with the progression of colorectal cancer (CRC) [144]. Another study revealed that patients with CC had an enrichment of circulating Treg cells, typically associated with poor prognosis, and that most of them exhibited a LAG-3^+ TIM-3^+ phenotype [145]. LAG-3 expression was detected on TILs in STS and significantly associated with a poor clinical outcome [146]. In contrast, another study revealed that expression of LAG-3, PD-L1 and IDO-1 on TILs showed a better prognosis for patients with high microsatellite instability (MSI-H) CC [147]. In NSCLC, LAG-3^+ TILs were analyzed by IHC staining in both intraepithelial and stromal compartments of primary tumors, as well as in the intraepithelial and extraepithelial compartments of metastatic lymph nodes. A good association was detected between their infiltration and improved DFS, thus representing an independent positive prognostic factor [148]. Recent studies have reported that elevated LAG-3 expression correlates with better OS and DFS in the EC patients [149]. LAG-3 and PD-1 are frequently co-expressed and upregulated within TME and lead to immune exhaustion and tumor growth [150]. Expression of LAG-3 and PD-1 on stromal or intra-epithelial TILs and tumor PD-L1 were evaluated by IHC on 4322 primary BC specimens. The presence of LAG-3^+ TILs in BC had significantly improved survival. Moreover, since a high percentage of PD-1/PD-$L1^+$ tumors were co-infiltrated with LAG-3^+ TILs, the authors supported potential IC block combination strategies as a treatment option for BC patients [151]. A further study confirmed that PD-1 and LAG-3 were expressed simultaneously in approximately 15% of patients with triple-negative-BCs (TNBC). The expression of both checkpoint receptors was positively correlated with the presence of TILs [152].

TIM-3 has been shown to be correlated with the clinic-pathological features of several cancers, suggesting its potential role in tumor immunity [153, 154]. The presence of intratumoral TIM-3^+ PD-1^+ $CD8^+$ T-cells has been established as a critical mediator of an aggressive phenotype in RCC [155].

Similar results were obtained by analyzing the TIM-3 expression on RCC [156]. A cohort of 3992 BC specimens was investigated for the expression of TIM-3 in both intraepithelial and stromal TILs. BC patients with TIM-3^+ intraepithelial TILs represent a minority of cases (11%), whereas TIM-3^+ stromal TILs occurred in 20% of cases. In prognostic analyses, patients with early BC with TIM-3^+ intraepithelial TILs significantly improved survival, whereas TIM-3^+ stromal TILs did not reach statistical significance [157]. Conversely, the expression of tumor TIM-3 was correlated with poor clinical outcome in BC [158]. TIM-3 overexpression was associated with poor prognosis even in patients with OSS [159], hepatocellular carcinoma (HCC) [160] and CRC tissues [161]. Moreover, patients with MSI-H CC showed an association with high expression of TIM-3, thus representing a potential tool for the development of therapeutic targets [162]. Recently, the single-cell CyTOF analysis allowed to determine the distribution, functional associations and clinical significance of TIM-3, PD-1 and LAG-3 in NSCLC. The authors demonstrated that expression of TIM-3 was higher in macrophages and NK cells, whereas PD-1 and LAG-3 were predominantly localized on T-cell subsets and NKT-cells. The co-expression of PD-1, LAG-3 and TIM-3 has been associated with prominent T-cell activation (CD69/CD137), effector function (Granzyme-B), and proliferation (Ki-67), as well as high levels of pro-apoptotic markers (FAS/BIM) [163]. The combination of Gal-9 and TIM-3 expression was proposed as an independent prognostic predictor for patients with GC, where high Gal-9 expression and low TIM-3 expression were significantly associated with long OS [164]. The prognostic effect of Gal-9 has recently been reviewed through a meta-analysis and, in general, its expression indicates a beneficial outcome in patients with solid tumors [165].

More than 1700 tumor samples from 86 different tumor entities were examined for expression of **TIGIT** and/or PD-1. The expression of TIGIT overlapped that of PD-1, highlighting significant opportunities for co-targeting with the corresponding checkpoint inhibitors. Interestingly, the highest density of immune TIGIT was found in immune-inflamed tumors, such as Warthin's tumors, medullary-BC, seminoma, GC and squamous cell cancers of various origins [166]. Similarly, MM patients were characterized by $CD8^+$ TILs expressing both TIGIT and PD-1 at high levels. $CD8^+$ TILs isolated from MM patients exhibited a downregulation of DNAM-1 [167]. Expression of TIGIT and PVR was detected on tumor and T-cells of CC tissues at a higher rate [168]. Expression levels of TIGIT and PVR were increased in HCC compared to para-cancerous tissues. TIGIT and PVR expression was inversely correlated based on the different degree of liver tumor cell differentiation [169]. Variable frequency of $TIGIT^+$ TILs was detected in seminomas (ranging from 2.3 to 69.4%) [170]. PVR/TIGIT and PD-L1/PD-1 were found expressed on small cell lung cancer (SCLC). High expression of TIGIT exhibited no significant association with survival, whereas high expression of

PVR alone, or in combination with PD-L1, was significantly associated with shorter survival and considered as an independent prognostic factor in patients with SCLC [171].

B7-H3 is widely overexpressed on solid tumors [172-177], making it an attractive target for cancer immunotherapy. In addition to its immune-regulatory role, with which it contributes to the tumor immune evasion [178], B7-H3 has intrinsic pro-tumorigenic activities related to enhanced cell proliferation, migration, invasion, angiogenesis, metastatic capacity and anti-cancer drug resistance [179]. Soluble form of B7-H3 has been detected at high levels in the serum of cancer patients [180, 181]. Recent evidence has supported the pro-tumoral role of B7-H3 also for Squamous Cell Carcinoma of the Head and Neck (SCCHN) [182], cranio-pharyngiomas [183] and glioma patients [184]. High expression of B7-H3 is also detected in tumor-associated endothelial cells, and associated with advanced tumor grade [181]. At this regard, B7-H3 has also been observed in tumor cells and vascularization of the RCC, and found positively associated with a high number of $FOXP3^+$ Treg cells [185]. Through multiplexed quantitative immunofluorescence, Carvajal-Hausdorf D *et al.*, measured the levels of B7-H3 in 90 SCLC samples and found its presence in 65% of SCLC patients [186]. Aung *et al.*, proposed to evaluate the co-localized expression of B7-H3/CD31 as a poor prognostic indicator and suggested strategies targeting B7-H3 as an effective approach to increase immune-activating therapies for Merkel cell carcinoma (MCC). Indeed, the increase co-localized expression of B7-H3 with CD31 significantly associated with an increased tumor size, invasion depth, presence of lymphovascular invasion and invasion beyond the skin in the primary MCC [187].

Few studies are available on **BTLA** expression, and its prognostic role in adult cancer patients. Jiayu Liu *et al.*, have shown high expression of BTLA and HVEM on circulating $CD4^+$ T cells of HCC patients [188]. Similar results were obtained by Zhao Q *et al.*, thus providing evidence that BTLA signals can participate in suppressing function of $CD4^+$ T cells in those patients [189]. Conversely, patients with metastatic MM, subject to adoptive cell therapy (ACT), showed high levels of $CD8^+BTLA^+$TILs, response to IL-2 and less-differentiated effector-memory cells that persisted longer *in vivo* after infusion, thus suggesting BTLA as a useful marker of T-cell differentiation in ACT [190]. In pancreatic cancer (PC), plasma levels of soluble PD-1, PD-L1 and BTLA were strongly correlated to each other and associated with very short OS [191]. A multivariate analysis established that high HVEM expression was an independent prognostic factor of GC associated with the worst OS along with depth of invasion, lymph node metastasis and histological grade. Similarly, BTLA was significantly associated with lymph node metastases and short OS [192].

Finally, the expression of **IDO-1** is associated with tumor-induced tolerance and resistance to T-cell-targeting therapies due to the recruitment and activation of MDSCs [193, 194]. The prognostic role of IDO-1 has been poorly investigated on TILs until a few years ago. IDO-1 activity has been

associated with poor prognosis and low survival rates in some adult cancers. The expression level, associations, and biological role of IDO-1 and PD-L1 have recently been determined in 552 NSCLC patients. Even though they showed limited co-expression, PD-L1 and IDO-1 were associated with an increase in TILs and IFNγ stimulation [195]. The prognostic value of PD-1, PD-L1, and IDO-1 was investigated in the sinonasal mucosal MM. The authors showed that the low expression of IDO-1 was correlated with worse DFS, and together with high PD-1 expression, was associated with poorer survival. These data led the authors to propose IDO-1 and PD-1 as further markers, together with clinical/pathologic staging, brain metastases, age and pigmentation, to evaluate the clinical outcome [196]. On GC, IDO-1 is associated with immuno-tolerance through attenuation of Treg cell activation; its expression constitutes an independent prognostic factor for OS and DFS, being associated with poor prognosis in patients with stage III [197]. The role of IDO-1, able to interact with immunocompetent cells and humoral immune factors affecting tumor progression and survival of GC patients, has also recently been confirmed by Li F *et al.* [198]. In surgically treated thymic carcinoma, significant survival benefit has been noted in patients with complete resection, high expression of $FOXP3^+$ Treg cells and low levels of IDO-1 [199]. Conversely, high expression of PD-L1 and IDO-1 was associated with high-grade thyme tumor histology [199].

3.2 ICs expression in pediatric cancers

Based on the role of **PD-1** and **PD-L1** as predictive biomarkers in adult cancers, numerous studies have also evaluated the PD-1/PD-L1 axis in pediatric tumors, mainly by IHC analysis. The results are generally discordant, with some studies reporting either high or low expression levels of PD-1 and PD-L1 in a wide range of pediatric malignancies. Chowdhury F *et al.*, evaluated 115 pediatric tumors and found that over 50% were $PD\text{-}L1^+$ (86% of alveolar rhabdomyosarcoma, 72% of high-risk NB, 57% of Ewing's sarcoma (EWS), 50% of embryonal rhabdomyosarcoma and 47% of OSS). The authors demonstrated that, when grouped by cancer type, samples with the highest percentage of PD-L1 showed poorer survival, and an increase of $CD8^+$TILs expressing PD-1 [200]. Majzner RG *et al.*, showed PD-L1 expression in 39 out of 451 tested tumors (9%), with the highest frequency of PD-L1 in glioblastoma multiforme (GBM) (36%; 5 of 14 tumors) and NB (14%; 17 of 118 tumors), that was associated with low survival [201]. Conversely, a further study comprising 124 different malignances, reported low expression of PD-1, PD-L1, and PD-L2 in many pediatric solid tumors and a poor correlation between the IHC score and mRNA expression [202]. Similar results had also been previously found by Aoki T *et al.*, who reported that only 1 out of 53 cases showed PD-L1 membrane staining, thus suggesting an inactivity of PD-1/PD-L1 pathway in pediatric cancers [203]. Several other studies have focused on the expression of PD-L1 and/or PD-1 on individual tumor

types. Dondero *et al.*, have shown that PD-L1 can be acquired from NB cell lines and metastatic neuroblasts isolated from bone marrow (BM) aspirates of high-risk NB patients with different MYCN amplification status. The authors also documented the presence of lymphocytes expressing PD-1 [204]. Melaiu *et al.*, studied the relationship between frequency of PD-L1$^+$ and HLA class I$^+$ (HLA-I) tumor cells, PD-1$^+$ and LAG-3$^+$ TILs, and clinical outcome in 77 NB patients [205]. The authors defined two groups of patients based on PD-L1 and HLA-I tumor cell densities: a group in which PD-L1 is absent and the clinical outcome is driven by HLA-I, and a second group where the presence of PD-L1 directly affects the prognosis [205]. Multivariate Cox regression analysis revealed that the combined PD-L1 and HLA-I tumor cell density predicts the clinical outcome in NB patients [205]. In addition, they found that both MYC and MYCN control PD-L1 expression in NB, indicating that their pharmacological inhibition may represent a novel treatment strategy for targeting PD-L1 expression in high-risk NB patients [205]. Liao *et al*., demonstrated a correlation between PD-L1 and the clinical features of NB patients [206]. In order to identify new markers predicting clinical outcome of pediatric meGCTs, Boldrini *et al.*, recently described three different cancer phenotypes, including i) tumors not infiltrated by T-cells, ii) tumors highly infiltrated by PD-1$^+$ CD8$^+$ T-cells, and iii) tumors highly infiltrated by CD8$^+$ T-cells within an immunosuppressive TME characterized by CD4$^+$FOXP3$^+$ Treg cells and PD-L1$^+$ tumor cells. These findings supported the role of immune TME in the development of meGCTs [207]. Several studies have examined the expression of PD-L1 in pediatric sarcoma [208]. Chan Kim *et al.*, investigated PD-L1 expression in 82 STSs including rhabdomyosarcoma (RMS), synovial sarcoma, EWS, epithelioid sarcoma and mesenchymal chondrosarcoma. PD-L1 was expressed in 43% of cases and significantly associated with shorter OS [208]. In OSS patients PD-L1 expression was significantly associated with increased infiltration of T cells, DC, and NK cells [209]. The PD-1/PD-L1 axis did not appear to be a predominant feature of EWS. Indeed, none of the 60 cases analyzed expressed PD-L1 on tumor cells, and only 5 showed PD-L1$^+$ TILs [210]. In RMS, the PD-L1 expression was found variable and strictly dependent on the antibody used [211]. Bertolini *et al.*, reported a peculiar pattern of PD-L1 expression with tumor cells scantly positive for PD-L1, and recurrent PD-L1 expression in surrounding immune cells or infiltrating tumor burden [212]. Recently, immune evasion strategies have also been investigated in a series of tumors of the CNS. PD-L1 was found correlated with malignancy of astrocytoma and mainly expressed on Ki67-negative tumor cells [213]. In pediatric medulloblastoma (MB), with the exception of the sonic hedgehog subtype [214], a limited number of PD-1$^+$ T cells and complete absence or low levels of PD-L1 were found [215]. Among pediatric ependymomas (PE), PD-L1 was expressed only by tumor and myeloid cells of supratentorial RELA fusion (RELA) tumors, which also expressed PD-1 on both CD4$^+$ and CD8$^+$ T cells. By contrast, the other subtypes had little PD-

L1 with no prognostic significance [216, 217]. Finally, similarly to adult cancers, the expression of PD-L1 is dynamic also in pediatric tumors and it can be modulated by disease progression. In OSS, PD-L1 was detected in metastatic, but not primary tumors, thus suggesting that this pathway may limit cytotoxic control of metastatic OSS [218].

Contardi E *et al.*, showed that **CTLA-4** was expressed by immune and tumor cells of NB, RMS, and OSS [219]. Two studies on NB have focused on LAG-3 expression. Morandi and colleagues found altered Treg cell trafficking in NB, with $CD4^+CD45R0^+CD49b^+LAG\text{-}3^+$ type 1 regulatory cell subtype present at a lower level both in the BM and peripheral blood from patients with NB, compared to their healthy controls [220]. A study of 77 cases of NB showed that the density of $LAG\text{-}3^+$ lymphocytes, together with that of PD-1, was proportional to tumor-infiltrating immune cells and distributed mainly in tertiary lymphoid aggregates, structures that resemble lymph nodes. However, survival analysis performed by stratifying patients according to the median cut-off values of $LAG\text{-}3^+$ lymphocyte densities did not significantly correlate with patient outcome [205].

A comprehensive analysis of various checkpoint molecules (Gal-9, MHC-II and HVEM on tumor cells, and **TIM-3**, **LAG-3** and **BTLA** on TILs) has recently been performed in common solid pediatric tumors, including 16 NB, 11 RMS, 12 OSS, 10 hepatoblastomas (HB), 10 Wilms tumors (WT), and 6 EWSs. While the expression of Gal-9 and MHC-II on tumor cells was limited, that of HVEM has been found at high levels in many types of tumor. NB, RMS, OSS and HB expressed moderate levels of TIM-3 on TILs. LAG-3 was expressed at moderate to high levels in TILs of NB, RMS, OSS, HB and WT. BTLA was detected at varying levels in each type of tumor included in the study [221]. The expression of the soluble form of TIM-3 was investigated in an independent set of pediatric OSS patients, and found associated with larger tumor size, late stages, distant metastases and lower survival [222, 223].

Similar to other checkpoint molecules, few studies examined **B7-H3**. It is expressed at high levels in NB and diffuse intrinsic pontine glioma (DIPG) [224]. B7-H3 expression was also evaluated by IHC on 68 primary OSS tissues and 37 osteochondroma. The intensity of B7-H3 in OSS was significantly increased compared to adjacent normal tissues, and inversely correlated with the number of TILs. Moreover, patients with high tumor B7-H3 levels had significantly shorter OS and recurrence [225]. Recently Majzner RG *et al.*, conducted the largest screen to date of pediatric tumor tissues for B7-H3 expression, and found that it is highly and homogeneously expressed on numerous pediatric solid tumors, including EWS, RMS, WT, NB and MB [226].

Finally, **IDO-1** expression was assessed by IHC in tumor specimens from 15 patients with primary CNS tumors. A diffuse IDO-1 expression was detected in low-grade CNS tumor, whereas in high-grade CNS tumors IDO-1 was absent or largely confined to endothelial cells [227]. Thirty pediatric

patients with OSS were immunohistochemically scored as five grades for IDO-1 expression. No significant correlation was detected between the intensity of IDO-1 expression and various variables, including gender, age, anatomical site, chemotherapy regimen, post-chemotherapy necrosis and surgical stage. However, patients with high IDO-1 expression had lower OS, suggesting that IDO-1-mediated immune tolerance could play an important role in the metastatic potential of OSS affecting the clinical outcome [228].

Overall, the expression of PD-L1, B7-H3, and IDO-1 on tumor cells, and that of the other IC molecules on TILs is associated with poor prognosis. However, the presence of discordant results, among patients with the same tumor, could be due to different factors that influence the OS, such as ethnicity and therapeutic treatment, as well as to different technical aspects, such as the use of different monoclonal and polyclonal antibodies and the cut-off values. This makes it difficult to draw solid conclusions on the usefulness of IC molecules as universal prognostic biomarkers. **Table 1** and **2** show a schematic overview of the prognostic role of each checkpoint, of which FDA approved drugs are available, in different adult and pediatric cancers, respectively.

4. Preclinical studies of Immune Checkpoint Inhibitors in cancer models.

The discovery of antibodies able to block IC proteins, has paved the way for the development of an immune-mediated therapy, as traditional approach for many types of cancer. The rationale behind this immunotherapeutic strategy is based on the fact that, blocking these ICs could allow counteracting the immune suppressive TME, improving antigen recognition by TCRs, increasing anti-tumor cytotoxicity and preventing immune cell exhaustion. The preclinical cancer models to evaluate immunotherapies are designed with a peculiar set of features such as, among others, a functionally intact and tumour adapted immune system [229]. Several preclinical studies reliably showed that drugs interrupting ICs are able to mediate durable cancer regressions. This evidence supported the clinical development of ICIs, highlighting that many of the therapeutic effects detected in humans, had already been predicted in animal models. For this reason, the development of predictive and solid preclinical models to reduce translational failures in immuno-oncology has intensively increased in the last years. To date, particular attention has been paid on the preclinical evaluation of combined therapies, using ICIs, such as anti-CTLA-4, anti-PD-1, anti-PD-L1, and others in early development, as a promising strategy to compensate conventional therapies for different types of tumours.

4.1 Immune Checkpoint Inhibitors in adult cancer models

Single anti-**PD-1** or anti-**PD-L1** therapy generated strong antitumor activity against CC and MM mouse models [230] and efficiently inhibited pre-established tumors in a murine PC model [231]. In

a mouse model of OC, blockade of PD-1/PD-L1 induced an increase in the function of tumor-specific $CD8^+$ T cells [232]. To overcome resistance to single treatment and improve patient survival, combined-based therapies were developed with preclinical success. The importance of combined treatment was evaluated *in vivo* in several solid tumors. Administration of anti-PD-1 or anti-PD-L1 together with a BRAF inhibitor in a MM mouse model resulted in an enhanced response, significantly prolonging survival and increased number and activity of TILs [233]. Another *in vivo* study revealed the strong therapeutic efficacy of combined inhibition of TGFβ signaling and anti-PD-L1 therapy in the CRC model resulting in improved long-term survival associated with increased recruitment of $CD8^+$ T cells within TME [234]. Furthermore, the co-administration of anti-TGFβ and anti-PD-L1 antibodies reduced TGFβ signaling in stromal cells facilitating recruitment of T cells within TME which cause strong anti-tumor immunity and tumor regression [235].

Many *in vitro* studies have revealed that the treatment of **CTLA-4**$^+$ human OSS cells lines with recombinant forms of CD80 and CD86 induced a caspase-dependent tumor cell apoptosis [219]. Several preclinical studies demonstrated that CTLA-4 inhibition cause tumour eradication and durable antitumor immunity [236, 237]. Moreover, *CTLA-4* deficient mice developed a progressive accumulation of activated T cells and died of lympho-proliferative disease 3 to 4 weeks after birth [238]. Preclinical studies have shown that anti-CTLA-4 monoclonal antibody is able to deplete $FOXP3^+$ Treg cells especially in tumor tissues [239, 240]. Different authors demonstrated that treatment with anti-CTLA-4 antibody selectively depleted $CTLA\text{-}4^+FOXP3^+$ Treg cells and expanded tumor specific $CD8^+$T-cells, resulting in a potent antitumor response *in vitro* and *in vivo* against MM [241] and colon adenocarcinoma (COAD) [240]. The combination of direct enhancement of function of effector T cells and inhibition of Treg cells is essential for mediating the full therapeutic effects of anti-CTLA-4 antibodies. Differences in timing, location, and non-redundant effects of their actions have suggested that anti-CTLA-4 and anti-PD-1 therapies have the potential for additive or possibly synergistic effects in the treatment of advanced malignances [242]. Therapeutic inhibition of CTLA-4 in a glioma mouse model resulted in a reduced recruitment of tumor-infiltrating Treg cells and significantly increased in T-cell-mediated long-term survival [243]. Moreover, blockade of CTLA-4, PD-1, or PD-L1 can affect GBM growing, and this antitumor activity can be increased by combination therapy targeting CTLA-4 and PD-1 [244]. Indeed, despite the effect of anti-CTLA-4 antibodies or other ICI alone, combination therapy could improve the anti-tumor effect in several solid tumors [245, 246].

Blocking **LAG-3** with a specific antibody in a mouse GBM model has been effective against cancer cells. Moreover, combination therapy with other ICIs has led to complete eradication of GBM tumors [247]. Furthermore, other studies have confirmed that the dual anti-LAG-3/anti-PD-1 antibody

treatment is able to eradicate most of the fibrosarcoma and adeno colocarcinoma that were resistant to single treatment [248]. Finally, the dual blockade of LAG-3 and PD-1 efficiently promoted the proliferation of tumor-specific $CD8^+$ T-cells, and increased the cytokine production [249].

Regarding **TIM-3** inhibition, Ngiow *et al.*, reported an extensive characterization of the therapeutic activity and mechanism of action of an anti-TIM-3 antibody against experimental and carcinogen-induced tumors, such as sarcoma, MM and COAD [250]. Moreover, in the mouse model of SCCHN, blockade of TIM-3 by the anti-TIM-3 monoclonal antibody induced a reduction of $CD4^+CD25^+Foxp3^+$ Treg cells, MDSC and an increase in $CD8^+$ T cells [251, 252]. A recent study revealed that the dual therapy with anti-TIM-3 and anti-PD-1 antibodies supplemented with radiosurgery, reduced tumor growth of a murine glioma model [253]. This dual treatment restored the exhausted phenotype of T cells, and efficiently controls tumor growth in CRC [254].

Another promising IC molecule to be targeted is **TIGIT**. Anti-TIGIT antibody treatment significantly decreased the immunosuppressive ability of MDSC and Treg cells *in vitro* and delayed tumor growth *in vivo* in transgenic HNSCC mouse models, activating $CD8^+$ T-cell effector function and reducing the Treg cell population [255]. The antitumor efficacy of anti-TIGIT antibody was improved with anti-PD-1 and anti-TIGIT combination therapy. Hung *et al.*, showed that the use of anti-PD-1 and anti-TIGIT antibodies significantly improved survival of a mouse GBM model [256]. The relevance of TIGIT to the NK cell dysfunction has also emerged. The blockade of TIGIT prevented NK cell exhaustion and promoted NK cell-dependent tumor immunity in several tumor mouse models. The use of anti-TIGIT antibody in combination with the PD-1 and PD-L1 antibodies improved antitumor therapy representing a promising anti-cancer therapeutic strategy [257, 258].

Recently, the importance of the **B7-H3** inhibition has emerged as a further anti-tumoral strategy. In particular, the blocking of B7-H3 antibody promoted T-cell proliferation [259], reduced growth and glycolytic capacity of tumor cells, and increased sensitivity to chemotherapy and various targeted therapies [260, 261]. I*n vivo* treatment with anti-B7-H3, alone or combined with chemotherapeutic drugs, was able to increase survival of various tumor mice models [261, 262].

A recent study showed that inhibition of **BTLA** significantly limited tumor growth and lung metastases in a mouse model of BC and was correlated with increase activation of lymphocytes [263].

In vivo treatment with an **IDO-1** inhibitor reversed tumor-associated immunosuppression by decreasing the number of tumor-infiltrating MDSCs and Treg cells and abolishing their suppressive function [193]. The IC blockade with anti-IDO-1 antibody increased the efficacy of the HCC treatment, overcame the resistance to the anti-CTLA-4 and anti-PD-1 therapies [264]. Combination therapy based on the use of anti-CTLA-4, anti-PD-L1, and IDO-1 inhibitors in a MM mouse model

showed marked improvement in tumor control, with many mice achieving complete tumor rejection, and a potent ability to recruit and activate intratumoral $CD8^+$ T cells [265].

Currently, alternative approaches are under investigation. Among them, great hope lies in the development of bispecific antibodies able of simultaneously blocking two inhibitory pathways with a single therapeutic agent. FS118, for example, is a bispecific antibody that targets LAG-3 and PD-L1 by providing a dual pathway blockade. *In vitro* studies have revealed that FS118 improves the activation of $CD8^+$ T cells stimulated with antigenic peptides better than anti-PD-L1 alone [266]. In addition, bispecific antibodies to TIM-3 have been developed and showed promising results in preclinical and clinical experiments [266]. Orlotamab is a humanized bispecific dual-affinity targeting both CD3 and B7-H3 designed to redirect cytotoxic T cells towards B7-H3-overexpressing tumor cells [266]. Another strategy is based on the development of neoantigen vaccines in combination with IC blockade. Specifically, the authors have shown that early therapeutic vaccination with adenoviruses derived from non-human Great Apes (GAd) efficiently controls tumor growth in mice, and that in combination with ICIs is able to eradicate large tumors [267].

4.2 *Immune Checkpoint Inhibitors in pediatric cancer models*

Although numerous studies have been developed to treat adult cancers with ICIs, the pediatric experience is very limited. Indeed, even if many IC molecules have been analyzed for their prognostic value, their role and inhibition *in vivo* and *in vitro* have not been extensively analyzed yet in pediatric tumors. Despite this, several preclinical studies on pediatric tumors have revealed the antitumoral role of PD-1/PD-L1 inhibitors. Blockade of PD-1/PD-L1 interactions in metastatic OSS tumors significantly improved the function of reactive $CD8^+$ T cells *in vitro* and *in vivo* resulting in a significant reduction in tumor burden and increased survival of mice [218]. In addition, PD-1 blockade conferred antitumor efficacy in animals with MB resulted in a marked increase of TILs [268]. In a murine NB model, the combination of anti-PD-1/PD-L1 antibodies with molecules selectively blocking the induction of MDSCs worked better than single anti-PD-1/PD-L1 therapy, resulting in a significant control of tumor growth [269, 270]. Similarly, the combination of anti-PD-1 and anti-GD2, which is a standard treatment for this cancer type, showed a strong reduction of tumor growth, prolonged survival and the highest cytotoxicity against NB cells [271]. Rigo *et al.*, also demonstrated that mono-therapy with anti-PD-1/PD-L1 mAbs had no effect on the progression of systemic NB *in vivo* [272]. Conversely, the use of anti-PD-1 with an anti-CD4 antibodies mediated a potent synergistic effect CD8-dependent, leading to significant increase of tumor-free survival of mice, complete tumor regression and durable antitumor immunity [272].

While preclinical studies in adult malignancies have shown significant responses to anti-CTLA-4 antibodies, few pediatric studies have been performed. The combined strategy based on the use of ICI (anti-CTLA-4 and anti-PD-L1) with high intensity focused ultrasound (HIFU) significantly enhanced anti-tumor responses by improving the survival of a NB mouse model [273]. Despite the promising results of preclinical studies on the use of LAG-3, TIM-3, TIGIT, B7-H3, BTLA and IDO inhibitors in adult solid tumors, information on the inhibition of these molecules in pediatric tumors is still unknown.

5. Clinical targeting immune checkpoint molecules

With the discovery of ICs as one of the main tumor mechanisms to evade immune control Tasuku Honjo and James Allison won the Nobel Prize in 2018 [274]. The encouraging results obtained from the preclinical experimentation have led to a rapid use of ICI also in the clinic both for the treatment of adult and pediatric tumors (**Figures 2 and 3**).

5.1 Immune checkpoint inhibitors in clinic of adult solid tumors

Ipilimumab acting against CTLA-4 was the first IC blocking antibody approved by the US Food and Drug Administration (FDA) in 2011. The first MM patients treated with Ipilimumab showed clinical benefits with prolonged OS and durable responses [275, 276]. Subsequent studies reported immune-related side effects, including dermatitis, hepatitis, enterocolitis, hypophysitis, and uveitis [277]. Later, early-phase trials investigating humanized monoclonal IgG4 antibodies targeting PD-1 and PD-L1 in advanced adult solid tumors paved the way for the development of PD-1 and PD-L1 inhibitors. The first anti-PD-1 antibodies approved by the FDA were nivolumab and pembrolizumab in 2014. Since then, the number of new FDA-approved inhibitors have grown enormously, with promising results for several types of cancers [278]. In NSCLC, MM and RCC for example, antibody-mediated blockade of PD-L1 resulted in durable tumor regression and prolonged stabilization of disease (NCT00729664) [279]. Recently, the clinical trials that led to FDA-approved indications of anti-PD-1 and anti-PD-L1 therapies have been extensively revised, focusing on MM, NSCLC, UC, RCC, SCCHN, GC, CR, HCC and MCC [278]. Other studies highlighted the importance of atezolizumab, a humanized anti-PD-L1 monoclonal antibody, in restoring anti-cancer immunity. Rittmeyer and colleagues assessed its efficacy and safety compared to standard therapy and demonstrated a clinically relevant improvement in overall survival in patients with NSCLC (NCT02008227) [280]. In addition, atezolizumab has shown encouraging response rates, survival and tolerability in metastatic UC (NCT02108652) [281]. Avelumab is another anti-PD-L1 blocking antibody that has shown lasting antitumor activity and disease control with an acceptable safety

profile in a heavily pretreated cohort of patients with mesothelioma (MPM) (NCT01772004) [282] and progressive NSCLC (NCT01772004) [283]. Finally, Cemiplimab (another anti-PD-1 monoclonal antibody) showed an anti-tumor activity and increase of survival in patients with cutaneous squamous cell carcinoma (SCC) compared to conventional systemic therapy (NCT02760498) [284].

Despite the initial success of anti-CTLA-4 and anti-PD-1/PD-L1 monotherapies, only a fraction of patients (10-30%) benefits from ICIs for long-term with durable responses. Many patients develop adaptive resistance and relapse, while others have no response since the start of treatment [285-287]. In CRC, for example, IC therapy received regulatory approval in 2017 for the treatment of strongly mutated tumors that are mismatch-repair-deficient or exhibit MSI-H. Conversely, ICIs are ineffective in tumors that are mismatch-repair-proficient and are microsatellite-stable or have low levels of MSI [288].

To increase the therapeutic efficacy of ICIs, a number of clinical studies is examining whether the combination of ICIs with conventional treatments, such as radiation therapies and chemotherapies or other immunotherapeutic agents, could improve patient outcomes [289]. Radiation therapy is known to augment the generation of neoantigens thereby increasing immune activation and response to ICIs by other means [290, 291]. For this reason, immunotherapy combined with radiotherapy may represent a successful strategy for maximizing the anti-tumor immune response and inducing durable disease control. Optimism regarding the potential synergy between radiotherapy and immunotherapy has led to a large increase in the number of clinical trials evaluating this combination in adult cancers; as of November 2019, 364 clinical trials have been registered on ClinicalTrials.gov. However, controversial results have been obtained so far. Indeed, some prospective phase I and II trials have reported encouraging results on the potential synergy between this combined treatment [292-294], whereas other have not shown a substantial improvement in patient survival [295]. A detailed review of this topic has recently been published [296].

The use of ICIs combined with chemotherapy is also emerging as an effective first-line treatment for many cancers. To date, November 2019, 506 studies are ongoing with this combined treatment for adult cancer treatment (clinicaltrials.gov). Zhou *et al.*, analyzed the results derived from 6 trials conducted on 3144 patients with LC treated with chemotherapy alone or chemotherapy plus PD-1/PD-L1 inhibitors and outlined a better prognosis in terms of DFS and OS for those receiving the combined therapy [297]. In SCCHN, the randomized phase III trial KEYNOTE-048 showed that pembrolizumab with chemotherapy was superior than chemotherapy regimen alone in all patients, and that pembrolizumab monotherapy was superior in patients whose tumors express PD-L1 (combined positive score ≥1). Pembrolizumab is now approved as monotherapy in PD-L1 expressing diseases, or combined with chemotherapy for all other patients, thus highlighting the use of PD-L1

expression as an important biomarker in predicting clinical response [298]. The addition of atezolizumab and chemotherapy in the first-line treatment resulted in significantly improved overall survival and progression-free survival compared to chemotherapy alone in patients with SCLC (NCT02763579) [299]. Furthermore, the combination of atezolizumab and bevacizumab showed encouraging anti-tumor activity in a phase 1b trial involving HCC patients led to better overall and progression-free survival (NCT03434379) [300]. Durvalumab (anti-PD-L1) in combination with chemotherapy significantly improved overall survival in patients with SCLC compared to a clinically relevant control group. (NCT03043872) [301]. Similar results were obtained in patients with locally advanced/metastatic UC (NCT01693562) [302]. Moreover, cemiplimab in combination with radiotherapy and/or chemotherapy has shown encouraging anti-tumor activity with a better durable response [303].

Another approach evaluated the use of multiple combination of ICIs to trigger non-redundant pathways to promote effective anti-tumor responses [304, 305]. A significant improvement in patient prognosis was achieved, for example, with the combined treatment of anti-CTLA-4 and anti-PD-1 antibodies. Among patients with advanced MM, sustained long-term OS at 5 years was observed in a higher percentage of patients who received nivolumab plus ipilimumab than in those receiving only ipilimumab (NCT01844505) [306]. A phase II trials recruiting 125 patients with malignant pleural MPM showed promising activity in relapsed patients treated with nivolumab and ipilimumab, as well as with nivolumab monotherapy, without unexpected toxicity (NCT02716272) [307]. A recent meta-analysis, which involved eight different clinical trials, has shown that the combined treatment of anti-PD-1 and anti-CTLA-4 is a feasible strategy with improved efficacy compared to single agents and acceptable adverse events. Moreover, in the context of patients with low PD-L1 expression (determined as $< 1\%$ in most studies), those receiving combination therapy tended to have higher response rate than anti-PD-1 monotherapy [308].

To date, various clinical trials examine the combination of classical ICIs with new inhibitor receptors and ligands, such as LAG-3, TIM-3, TIGIT and IDO-1 [266]. However, as all clinical trials are ongoing, preliminary results on the efficacy and safety of most of these new drugs are not always available. LAG-3 is the third IC to be clinically investigated as a target for cancer immunotherapy. The first agent tested in various human cancers was IMP321 (Immutep), a recombinant sLAG-3-Ig fusion protein designed to activate monocytes and DC via stimulation with MHC-II. IMP321 has completed three clinical trials of RCC, metastatic-BC and MM, with only modest clinical responses [309]. Most patients (90%) with metastatic-BC receiving as first-line therapy IMP321 in combination with paclitaxel, showed a clinical benefit, with only 3 progressors at 6 months and no toxicity (phase I/II, NCT00349934) [310]. Relatlimab (BMS-986016) initially entered the clinic as a fully human

LAG-3-specific antibody that acts by destroying the interaction with MHC-II. Simultaneous blockade of LAG-3 and PD-1 can synergistically restore T-cell activation and improve antitumor immunity. In a phase I/II study (NCT01968109), BMS-986016 (anti–LAG-3) plus nivolumab (anti–PD-1) showed promising antitumor activity for patients with advanced solid malignancies who were not previously exposed to immunotherapy, as MM patients who progressed during prior anti-PD-1/PD-L1 therapy [311].

Based on results from preclinical studies, TIM-3 blocking agents are currently being investigated in patients with adult solid tumors as monotherapy or more frequently in combination with other ICIs. LY3321367 mAb targeting TIM-3 on immune cells and LY3300054 mAb targeting PD-L1 on tumor cells and TILs for example, are evaluated in a phase Ia/Ib study on patients with advanced cancers. Clinical effects seem to be promising in terms of interim safety, efficacy, and pharmacokinetic (NCT03099109). A phase 1 study instead included administrating the drug TSR-022 as an anti-TIM-3 antibody, as monotherapy and in combination with an anti-PD-1 antibody, in patients with advanced solid tumors (NCT02817633). Another clinical trial based on anti-TIM-3 (MBG453) combined with anti-PD-1 (PDR001) is ongoing (NCT02608268).

Seven TIGIT-targeting therapeutics are currently in early phase clinical trials: MK-7684, AB154, tiragolumab, BMS-986207, etigilimab, ASP8374 and BGB-A1217 [266]. A very recent clinical study aims to evaluate the efficacy of anti-LAG-3 and anti-TIGIT as single agents and in combination with pomalidomide and dexamethasone on relapsed refractory MM patients who relapsed after prior treatments (NCT04150965). Another recent study is recruiting patients with advanced solid tumors to test the safety and anti-tumor effect of BGB-A1217, a humanized IgG1 monoclonal antibody against TIGIT, in combination with tislelizumab, a humanized IgG4-variant monoclonal antibody against PD-1 (NCT04047862). However, as all ongoing clinical trials are very recent, preliminary results on the efficacy and safety of anti-TIGIT are not currently available.

Currently, a number of clinical trials targeting B7-H3 are in progress. Enoblituzumab is a monoclonal antibody targeting B7-H3 whose effectiveness has been evaluated in a phase I clinical trial in combination with an anti-PD-1 monoclonal antibody in patients with MM, SCCHN, NSCLC and UC. The first results indicate antitumor properties and an increase of TILs, without dose-limiting toxicity and serious immune-related side effects [181]. A randomized, open-label phase 2/3 study is currently ongoing to evaluate the safety and efficacy of enoblituzumab in combination with MGA012, with and without chemotherapy, in the first-line treatment of patients with recurrent or metastatic SCCHN (NCT04129320).

Interestingly, a very novel approved clinical trial (NCT04137900) is recruiting patients with advanced solid malignances to treat with TAB004 which is a recombinant humanized IgG4κ monoclonal

antibody specific to BTLA. The objectives of this study concern several aspects: i) pharmacodynamic effects of TAB004 on the target receptor BTLA and immune system; ii) biomarkers that can be correlated to TAB004 activity; and iii) the utility of HVEM and further exploratory biomarkers helping in the selection of patients for TAB004 therapy.

The results of multiple phase I/II trials have encouraged the idea that IDO-1 inhibitors may improve patient responses to anti-PD-1 IC therapy [312]. Recent results of ECHO-301, the first large phase III trial to evaluate epacadostat (an IDO-1-selective enzyme inhibitor) in combination with pembrolizumab in advanced MM, showed no indication that epacadostat provided an increased benefit (NCT02752074). However, phase II clinical data continue to encourage further testing of IDO-1 inhibitors in combination with anti-PD-1 antibodies in other settings [313]. Indoximod (1-methyl-tryptophan), an orally available IDO-1 inhibitor, for example, is currently being studied in several phase I and II trials for adults alone, in combination with conventional chemotherapy and/or radiation, and with ipilimumab (NCT02073123), pembrolizumab (NCT03301636, NCT02073123), or nivolumab (NCT02073123, NCT03301636).

In summary, hundreds of phase I-IV clinical trials have been carried out around the world to find the optimal therapeutic strategy for each type of adult cancer patients based on ICIs as monotherapy or in combination. Given the different chronology on the discovery and therapeutic use of the various ICIs, the CTLA-4 and PD-1 blockade is under phases III/IV investigation in various clinical studies, whereas agents targeting LAG-3, TIM-3, TIGIT, B7-H3, BTLA and IDO-1 are mainly in early phase trials. Details on CTLA-4 and PD-1/PD-L1 blockade in phase III/IV trials (recruiting or completed in the last year, 01/01/2019-31/12/2019), and those of early phase clinical studies ongoing on LAG-3, TIM-3, TIGIT, BTLA and IDO-1 inhibitors (recruiting in the last three years, 01/01/2017-31/12/2019) for adult solid tumors are shown in **Figure 2A** and **Table 3**.

5.2 Immune checkpoint inhibitors in clinic of pediatric solid tumors

Following the approval and development of a large number of clinical trials on adult cancers, therapy with a number of ICIs (e.g., ipilimumab, nivolumab and pembrolizumab) is currently being explored also in pediatric tumors. Similar to adult patients, the strategies adopted for the treatment of pediatric cancers include both monotherapy and combined approaches with ICIs. Although few studies have focused exclusively on pediatric cancer patients, numerous trials evaluating ICIs in all age groups of cancer patients are ongoing (https://clinicaltrials.gov). Since these studies are still in their early stages, results in terms of effect and improved survival are not available for all types of drugs tested.

The first published phase I protocol concerned the administration of ipilimumab in 33 patients under the age of 21 with MM (n=12), sarcoma (n=17), or other solid refractory tumors (n=4). Patients experienced immune-related adverse events similar to those described in adults. No objective tumor regressions were observed, but subjects with immune-related toxicities had an increase in OS compared to those without evidence of breaking tolerance [314]. Similar results were obtained with ipilimumab in MM patients [315], nivolumab in a GBM patient [316], and pembrolizumab in patients with progressive primary CNS [317].

Despite the lack of encouraging results, a limited number of clinical trials testing PD-L1 and PD-1 inhibitors as monotherapy are still ongoing. The iMATRIX-Atezolizumab study (NCT02541604) assessed the safety, pharmacokinetics and activity of atezolizumab in pediatric patients with solid tumors, showing a preliminary antitumor activity of this drug. Phase II clinical trials test the safety and efficacy of pembrolizumab in pediatric patients with solid tumors (NCT02332668 or KEYNOTE-051) and in HB, together with the exploration of different biological factors of the tumor and immune system that could help predict the response to treatment (NCT04134559). A second study (SARC028) evaluated the efficacy of pembrolizumab in the context of adult and adolescent bone and STS. In this trial, 18% of patients with STS and 5% with bone sarcoma were determined to have an objective response. This led to enrollment of enlarged cohorts to confirm and characterize the activity of pembrolizumab (NCT02301039 [318]). Another phase I trial in progress is evaluating the side effects and the best dose of pembrolizumab for treating younger patients with high-grade gliomas, DIPGs, CNS tumors with a high number of genetic mutations, PE and MB who have progressed or have not responded to previous treatment (NCT02359565).

The reason for the overall poor success achieved so far with ICIs administered as single agents could be attributed to the poor immunogenicity of most pediatric cancers. The exception that proves the rule is represented by cancers arising in children born with biallelic mismatch repair deficiency (bMMRD), following germline-inactivating mutations in DNA-mismatch repair genes. In these type of cancers (generally affecting brain, gastrointestinal tract and lymphoid system), impressive antitumor effects have been obtained with PD-1/PD-L1 inhibitor treatment, probably due to the high number of non-synonymous somatic mutations by which they are characterized [319]. The authors provided the proof of principle that GBMs with DNA-repair defects treated with ICIs can result in the immune activation of the CNS, leading to clinically and immunologically significant responses [320]. Clinical trials testing the efficacy of nivolumab (NCT02992964) or pembrolizumab (NCT02359565) in pediatric patients with recurrent or refractory hypermutant malignancies are currently ongoing.

As previously shown for adult malignances, therapeutic strategies aimed to exploit the functional principle of IC blockade and improve its effectiveness in pediatric cancers include the combination of multiple IC antibodies together with chemotherapy or radiotherapy. The safety and efficacy of nivolumab in combination with ipilimumab is currently under investigation in high-grade primary CNS pediatric malignancies (NCT03130959), recurrent or refractory solid tumors (NCT02304458), and MM (NCT03068455). Another study was designed to evaluate the safety and tolerability of durvalumab in combination with increasing doses of tremelimumab in pediatric patients with advanced solid and hematological malignancies. This study will also explore potential biological activity and immunogenicity by assessing pharmacodynamics, anti-drug antibody levels, and anti-tumor activity (NCT03837899).

Given the local and systemic effects of radiotherapy on the immune system [290, 291], this is a promising approach not only for adult cancer patients, but also for children. In NB, anti-GD2 antibody is combined with 131-l Metaiodobenzylguanidine (mlBG) and nivolumab, in order to generate sustained anti-NB immunity. In particular, this trial aimed to determine the safety and tolerability of the new combination, as well as document the evidence of efficacy in pediatric patients with relapsed and refractory high-risk NB (NCT02914405). The combination of cemiplimab with radiotherapy is also studied in pediatric patients with newly diagnosed or recurrent glioma (NCT03690869).

Different clinical trials are evaluating the effectiveness of ICIs administered together with chemotherapeutics drugs. This strategy derived from preclinical evidence showing that tumors lacking T-cell infiltration and refractory to current treatment options could be successfully sensitized to accommodate antitumor T-cell immunity, when appropriately selected immunogenic drugs were used [321]. Ten different drugs were tested in combination with nivolumab in a clinical trial started on 2016 estimating to enroll up to 397 pediatric participants with relapsed or refractory tumors (NCT02813135). A study started in 2018 is evaluating the efficacy of vinblastine, cyclophosphamide and capecitabine in combination with nivolumab in children and adolescents with refractory/relapsing solid tumors or lymphoma (NCT03585465). Further trials are underway to test different combinations in various cancer types in all age groups, including children (NCT01738139, NCT02775292, NCT02621021, NCT03056001, NCT02693535).

Few trials are currently ongoing to examine the effectiveness of the new ICIs. In particular, Relatlimab is under investigation in three clinical trials combined with nivolumab for patients over 12 years of age with cancer per site (NCT01968109), MM (NCT03470922) and chordoma (NCT03623854). A single study focused on the use of Enoblituzumab. Children and young adults with NB, RMS, OSS, EWS/primitive neuroectodermal tumor, WT, desmoplastic small round cell tumor (DSRCT) or malignant solid tumors of any other histology expressing B7-H3 were subject to

an increase of the dose in a phase 1 study (NCT02982941), designed to characterize the safety, tolerability, pharmacokinetics, pharmacodynamics, and preliminary antitumor activity of enoblituzumab. Three clinical trials are evaluating the inhibition of IDO-1 in patients with primary malignant CNS tumors, aged 3 to 21 years (NCT02502708, NCT04049669) or more than 12 years (NCT02052648), on the assumption that indoximod will improve antitumor immune responses and consequently reduce tumor growth. Indeed, the central clinical hypothesis of these studies is that inhibiting the pivotal IDO-1 pathway by adding indoximod during chemotherapy and/or radiation, is a powerful approach for breaking immune tolerance for pediatric tumors and improve clinical outcomes, compared to standard therapies alone.

Finally, unlike what has been seen in adult cancers, no studies on the efficacy of TIM-3, TIGIT and BTLA inhibitors in childhood neoplasms have been performed so far. Details on clinical trials with ICI therapy for pediatric solid tumors (recruiting in the last three years, 01/01/2017-31/12/2019) are shown in **Figure 2B** and **Table 4**.

6. Approved and emerging biomarkers of response to ICI therapy

The current understanding of the clinical efficacy to ICI therapy indicates the need to identify robust biomarkers to select patients who are likely to benefit from this type of immunotherapy.

PD-L1 expression has been investigated not only for its prognostic value, but also as predictive biomarker, starting from the initial trials on PD-1 blockade in adult cancers. IHC analysis performed on pretreatment tumor specimens of patients with MM, NSLC, PrC, RCC and CRC enrolled in the first pivotal phase 1 nivolumab study, showed that only patients with PD-L1-positive tumors (36%) had an objective response. In line with this first evidence, PD-L1 expression has been reported to correlate with improved objective response rates in many other adult cancers [322]. As extensively reviewed by Arora et al. [323], the FDA approved several agents concurrently with specific tests for PD-L1 expression. One of the major issue concerns the fact that each company adopted different scoring schemes for the readout and developed distinct IHC protocols for assessing PD-L1 expression, either on tumor cells alone or in combination with infiltrating immune cells. This resulted in a considerable confusion in the interpretation of various tests and their utility [323], in both adult and pediatric cancers. Furthermore, assessing PD-L1 expression as a single predictive biomarker for ICI therapy efficacy has other limitations including the heterogeneous expression of PD-L1 in tumors, the different expression between primary and metastatic tumors [324], and the fact that PD-L1 negative patients also responded to PD-1 blockade alone or in combinatorial treatment [325, 326]. For this reason, many attempts have been made over the past years to evaluate promising novel biomarkers able to estimate response or duration of response in patients. In this regard, the in-depth

study of each immune TME component, including both lymphoid and myeloid compartments, should be considered in clinical practice as a first step.

To date, the density, spatial distribution and localization of TILs within TME are parameters already considered important predictor of good prognosis [327, 328] and response of ICI therapy for tumors in adults [329-331]. Clinical studies, performed by examining histological sections of tumors biopsies collected from patients before receiving any therapy, have made possible to distinguish three basic immune profiles related to a person's response to the administration of ICIs [332] (**Figure 3**). The "immune-inflamed profile" is characterized by the presence of $CD8^+$ and $CD4^+$ T cells in the tumor parenchyma, spatially positioned in proximity to the cancer cells, and usually expressing IC molecules, suggesting pre-existing antitumor immune responses [333, 334]. These "hot tumors" have typically been associated with a better response to ICI therapy [335]. The "immune-excluded" phenotype is characterized by the presence of different types of immune cells trapped in the stroma surrounding cancer [332]. Strategies that allow to recruit immune cells into the tumor bed can convert TME into a more inflamed state, responsive to ICI therapy [336]. The immune-desert phenotype is characterized by the absence of abundant T cells in the parenchyma or stroma of the tumor and defines the so-called "cold tumors" that likely do not fully respond to IC blocking [332].

The specific composition of the immune TME of solid pediatric cancers is under investigation and further efforts should be warranted to shed further light. In general, the number of TILs appears to be significantly lower in pediatric tumors compared to adult malignancies [337]. However, in some tumors, such as NB, TILs have a higher prognostic value than the criteria currently used to stage this neoplasm [338], whereas several pediatric brain tumors exhibit distinct immunophenotypes, suggesting that specific immunotherapeutic approaches may be more effective for each type of tumor [339].

It is important to underline that, in order to obtain a more comprehensive picture of the TME and a more reliable prediction of the efficacy of the ICI, the measure of myeloid cells infiltration is also strongly recommended [336]. In the RCC for example, a high IC expression in the absence of mature DC was associated with an increased risk of disease progression, while low IC expression with localization of mature DC was associated with a good prognosis [340]. In patients with MM, tumor-associated macrophages and MDSC were found to correlate with poor anti-PD-1 [341] and anti-CTLA-4 [342] responses, respectively.

Elucidating the contribution of the myeloid component in prediction of ICI therapy efficacy would be of considerable help, especially for those cases where the evaluation of TILs alone does not lead to exhaustive response. The optimal characterization of the immune TME could be achieved through both the implementation of multiplex-IHC protocols, as well as a pan-cancer immune-genomic

analysis, able to decipher the effects of immune cells on ICI response rates, starting from adequate biological material for both adult and pediatric malignances.

Beyond the immune compartment, malignant cells develop and evolve in a complex and strongly interconnected TME, also comprising a vast variety of non-immune stromal cells, such as endothelial cells and fibroblasts [343], which contribute to treatment resistance [344].

The involvement of blood and lymphatic vessels spraying a neoplasm has been analyzed also in response to ICI therapy. Indeed, abnormal angiogenesis reduces the infiltration of lymphocytes through a series of mechanisms, including i) the down-regulation of adhesion molecules, known to impair the extravasation of T cells [345], ii) the concurrent hypoxia which directly undermines the functions of TILs through the upregulation of some inhibitory signals such as PD-L1, IDO, IL-6, and IL-10 [346, 347], and iii) the expression of Fas ligand which eliminates effector $CD8^+$ T cells rather than Treg [348]. In addition, circulating VEGF impedes the maturation and function of DC to help tumor escape immune surveillance [349, 350]. Several preclinical and clinical studies have demonstrated that normalizing the vasculature facilitated the entry of immune cells into the tumor, thus suggesting the neo-angiogenic process as a marker to be monitored, and its targeting as a strategy to enhance responsiveness to ICB [351-354].

Cancer-associated fibroblasts (CAFs) constitute the main cellular component of the tumor-associated stroma able of driving the invasion of cancer cells and, similarly to endothelial cells, compromising the migration and activation of TILs [355]. The fibroblast activation protein-alpha (FAP) is known to inhibit T cell activity, compromising the success rate of ICI therapy [356, 357].

Recent studies have highlighted the role of TGFb in stroma-mediated resistance to PD-L1 blockade [235, 357, 358]. In retrospective analyses of patient with tumors, the role of TGFb pathway activation emerged as a critical mediator of primary resistance to ICI therapy. Transcriptional profiling of pretreatment MM biopsies from patients who do not respond to anti-PD-1 treatment revealed an enrichment of the TGFb-associated pathways [359]. Similar analyses in metastatic UC have also discovered a correlation between the transcriptional signatures associated with TGFb and the lack of response to the PD-L1 blockade, particularly in tumors in which $CD8^+$ T cells appear to be excluded from tumor entry [235]. Interestingly, Hikmet et al., demonstrated an important role of TGFb in tumor metastastis of childhood solid tumors, including NB, OSS, EWS, and RMS, suggesting its evaluation in relation with both prognosis and response to treatment [360].

Together, these recent data unveil the central role of stroma-derived TGFb in inducing immune evasion and ICB resistance, thus suggesting its evaluation as a predictive biomarker [361].

It has been hypothesized that a high tumor mutation load may affect the probability of generating immunogenic neoantigens, representing the mutations effectively targeted by activated TILs [362].

In light of this, a large number of neoantigens can influence the ICI response in patients, and should be considered as an additional marker to be explored (**Figure 3**). Much effort has been made in recent years to identify immunogenic neoantigens from genomic data analyses, as well as to enable new strategies to further increase the recognition of neoantigens by T cells [363]. In general, adult cancers are characterized by a high tumor mutational load which in an ideal scenario, causes the activation of distinct T cell clones able of recognizing and attacking tumor cells [364]. Conversely, pediatric cancers are characterized by a paucity of neoantigens [365, 366]. Indeed, unlike adult patients, which are chronically exposed to environmental insults causing DNA damage and cancer development, mainly of epithelial origin [367], the lower mutational load of childhood cancers may be due to a smaller contribution of environmental carcinogens in combination with the embryonal origin of many of these cancers [368]. The low immunogenicity of pediatric cancers may be a weak point in the effectiveness of the ICI response in these patients. However, some pediatric tumors have shown spontaneous remission and inducing patients' immune responses to their tumor cells is one of the suggested mechanisms contributing to this phenomenon [369].

Interestingly, in addition to the deeply rooted ICI therapy predictors, other factors have recently been investigated, including systemic markers analysis, gene signatures and microbiota influence, mainly in adult cancers. Thus, the study of these new aspects also in pediatric malignancies could be of further help to direct young patients towards the right therapy.

In this regard, a great interest is paid to the development of standardized methods that allow the identification of analytical markers in peripheral blood with which to monitor the ICI response in a non-invasive way [370]. Research on this topic is being carried out with two different approaches: i) direct count of total circulating leukocytes (lymphocytes, monocytes, Treg cells, eosinophils, MDSCs), secreted cytokines (i.e, IL-6, IL-8 and IL-10) or other factors (i.e. lactate dehydrogenase, VEGFC) [371, 372] and ii) analysis of the so-called "liquid biopsies" to determine the exosomes expressing ICs or isolate the circulating tumor cells and perform sequencing mutation analyses for the identification of the patient-specific neoantigens [373].

Recent technological innovations have allowed us to delineate gene signatures that can be correlated with clinical outcome [374, 375], and predict both toxicity and response to immunotherapy in various malignancies [372, 376]. For example, in a phase II study evaluating ipilimumab in patients with MM, gene expression profiling was performed on tumor biopsies before and after treatment. Patients with high expression levels of immune-related genes responded better to ipilimumab than those with lower basal expression [377]. In the RCC patients, “ERV signatures” had a prognostic role with increased transcriptional expression of specific ERVs genes being associated with response to ICI treatment [378].

The pivotal role of the commensal microbiota in influencing the individual's health and response to ICI therapy is starting to be determined [379]. Various studies have demonstrated strains of bacteria associated with response or resistance in MM [380-382], NSCLC, RCC and UC [383]; moreover, patients with high microbiota diversity, showed a better ICI response, regardless of the identity of the bacterial species [382, 383]. It is interesting to note that patients treated with antibiotics, during ICI therapy had decreased anti-tumor response [383], thus suggesting that the gut microbiota is a key influencing factor.

In summary, the search for new biomarkers able of predicting the ICI response with greater efficiency and precision, is constantly growing. However, the intrinsic complexity of these assays and the development of independent protocols between laboratories lead to high data variability and poor reproducibility. Overcoming this limitation, through harmonization based on integration of specific laboratory protocols with standard operating procedures, will allow the objective interpretation and comparison of data across clinical trial sites and will facilitate the identification of relevant immune biomarkers, guiding the development of new therapies [323].

7. Conclusion and future perspectives

Although most of the current studies focus on adult tumors, considerable efforts are being made to use ICI also in the pediatric field, where the treatment of patients with advanced, relapsing or refractory tumors still remains a great challenge. Currently, immunotherapy, with ICIs as a top-player, represents the most promising strategy. However, preclinical and clinical data highlight the inefficacy of ICI as monotherapy in most pediatric tumors, known to be characterized by a low mutation load and relative lack of neoantigens. For this reason, ICIs are increasingly making their way as components of other combined therapies (with other ICIs, or with standard chemotherapies and radiotherapy), thanks to their general safety and the importance of T cell activation in the establishment of antitumor immunity [384]. However, in light of these general considerations, the specific biological features of pediatric tumors should be considered in order to design specific protocols combining ICIs with other agents that have the potential to favorably modulate TME and further amplify the antitumor immune response. Agents targeting VEGF/VEGFR, for example, have immunomodulatory and antiangiogenic effects, with beneficial consequences on TAM, MDSC, Treg, and cytotoxic T cells [385]. The abolition CAFs was found to synergize with anti-CTLA-4 therapy in an adjuvant setting by releasing checkpoint blocking targets during immune suppression in the PC [386]. Interestingly, targeting CAF mediated production of PGE2 through the inhibition of a key enzyme (microsomal prostaglandin E synthase-1) in its synthesis, suppressed the infiltration of CAF leading to a reduction in the angiogenesis and tumor growth [387].

In summary, the number of possible combinations with ICIs is enormous and an ideal scenario, which involves the development of a predictive model that takes into account the different components affecting tumor-host interactions, as well as the use of standardized biomarkers, can be extremely useful for setting up new clinical trials, carefully guided by the knowledge of pediatric TME, in order to increase the survival of children and their overall quality of life.

Abbreviations:

Adoptive cell therapy: ACT; Advanced Solid Tumors: AST; Alveolar Soft Part Sarcoma: ASPS; Androgen deprivation therapy: ADT; Antigen-presenting cells: APC; Autoimmune diseases: AD; B- and T-lymphocyte attenuator: BTLA; Biallelic mismatch repair deficiency: bMMRD; Biliary Tract carcinoma: BTC; Bladder carcinoma: BlC; Bone marrow: BM; Breast cancer: BC; Carcinoembryonic Antigen Related Cell Adhesion Molecule 1: Ceacam-1; Central nervous system: CNS; Cervical Cancer: CeC; Childhood Solid Tumors: CST; Colon adenocarcinoma: COAD; Colon cancer: CC; Colorectal cancer: CRC; Cytotoxic T-lymphocyte antigen 4: CTLA-4; Dendritic cell: DC; Desmoplastic Small Round Cell Tumor: DSRCT; Diffuse intrinsic pontine glioma: DIPG; Disease free survival: DFS; Endometrial Cancer: EnC; Ependymoma: PE; Eptide-major histocompatibility complexes: MHC; Esophageal cancer: EC; Ewing's sarcoma: EWS; Galectin-9: Gal-9; Gastric cancer: GC; Glioblastoma: GBM; Grave's disease: GD; Hepatoblastoma: HB; Hepatocellular carcinoma: HCC; Herpes virus entry mediator: HVEM; High-mobility group protein B1: HMGB1; IC: immune checkpoint; Immune checkpoint inhibitors: ICIs; Immunoglobulin: Ig; Immunohistochemistry: IHC; Immunoreceptor tyrosine-based inhibitory motif: ITIM; Indoleamine 2,3-dioxygenase: IDO-1; Lung cancer: LC; Lymphocyte activation gene 3: LAG-3; Malignant Melanoma: MM; Medulloblastoma: MB; Merkel cell carcinoma: MCC; Malignant pleural mesothelioma: MPM; Metaiodobenzylguanidine: mlBG; Microsatellite instability: MSI; Myeloid-derived suppressor cells: MDSC; Nasopharyngeal Carcinoma: NC; Natural killer: NK; Neuroblastoma: NB; Non–small-cell lung cancer: NSCLC; Oropharyngeal Carcinoma: OrC; Osteosarcoma: OSS; Ovarian cancer: OC; Overall survival: OS; Pancreatic cancer: PC; Phospatidyl serine: PtdSer; Programmed cell Death 1: PD-1; Prostate cancer: PrC; Regulatory T cells: Treg cells; Renal cell carcinoma: RCC; Rhabdomyosarcoma: RMS; Rheumatoid arthritis: RA; Small cell lung cancer: SCLC; Soft tissue sarcoma: STS; Squamous Cell Carcinoma: SCC; Squamous cell carcinoma of the head and neck: SCCHN; T-cell Ig and ITIM domain: TIGIT; T-cell Ig and mucin domain 3: TIM-3; T-cell receptor: TCR; Triple negative breast cancer: TNBC; Tumor infiltrating lymphocytes: TILs; Tumor microenvironment: TME; Type 1 CD4 helper: Th1; Urothelial carcinoma: UC; Wilm's tumor: WT

Acknowledgments

This work was supported by grants from the Associazione Italiana Ricerca Cancro (AIRC) #IG18495 and the Italian Ministry of Health #PE-2011-02351866 to DF and from Istituto Toscano Tumori (ITT) #I57G12000330002 and PRIN-MIUR #2008AMJSER to FG. The Fondazione Umberto Veronesi supported this work with a fellowship to VL.

Bibliography

[1] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2018, CA: a cancer journal for clinicians 68(1) (2018) 7-30.
[2] E.K. Ring, J.M. Markert, G.Y. Gillespie, G.K. Friedman, Checkpoint Proteins in Pediatric Brain and Extracranial Solid Tumors: Opportunities for Immunotherapy, Clinical cancer research : an official journal of the American Association for Cancer Research 23(2) (2017) 342-350.
[3] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2019, CA: a cancer journal for clinicians 69(1) (2019) 7-34.
[4] C. Frobisher, P.M. Gurung, A. Leiper, R.C. Reulen, D.L. Winter, A.J. Taylor, E.R. Lancashire, C.R. Woodhouse, M.M. Hawkins, Risk of bladder tumours after childhood cancer: the British Childhood Cancer Survivor Study, BJU Int 106(7) (2010) 1060-9.
[5] E.R. Lancashire, C. Frobisher, R.C. Reulen, D.L. Winter, A. Glaser, M.M. Hawkins, Educational attainment among adult survivors of childhood cancer in Great Britain: a population-based cohort study, J Natl Cancer Inst 102(4) (2010) 254-70.
[6] K. Nurgali, R.T. Jagoe, R. Abalo, Editorial: Adverse Effects of Cancer Chemotherapy: Anything New to Improve Tolerance and Reduce Sequelae?, Frontiers in pharmacology 9 (2018) 245.
[7] R.G. Majzner, S. Heitzeneder, C.L. Mackall, Harnessing the Immunotherapy Revolution for the Treatment of Childhood Cancers, Cancer cell 31(4) (2017) 476-485.
[8] M.S. Topp, N. Gokbuget, A.S. Stein, G. Zugmaier, S. O'Brien, R.C. Bargou, H. Dombret, A.K. Fielding, L. Heffner, R.A. Larson, S. Neumann, R. Foa, M. Litzow, J.M. Ribera, A. Rambaldi, G. Schiller, M. Bruggemann, H.A. Horst, C. Holland, C. Jia, T. Maniar, B. Huber, D. Nagorsen, S.J. Forman, H.M. Kantarjian, Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study, The Lancet. Oncology 16(1) (2015) 57-66.
[9] P. Schlegel, P. Lang, G. Zugmaier, M. Ebinger, H. Kreyenberg, K.E. Witte, J. Feucht, M. Pfeiffer, H.M. Teltschik, C. Kyzirakos, T. Feuchtinger, R. Handgretinger, Pediatric posttransplant relapsed/refractory B-precursor acute lymphoblastic leukemia shows durable remission by therapy with the T-cell engaging bispecific antibody blinatumomab, Haematologica 99(7) (2014) 1212-9.
[10] M. Yankelevich, S.V. Kondadasula, A. Thakur, S. Buck, N.K. Cheung, L.G. Lum, Anti-CD3 x anti-GD2 bispecific antibody redirects T-cell cytolytic activity to neuroblastoma targets, Pediatric blood & cancer 59(7) (2012) 1198-205.
[11] M.A. Postow, J. Chesney, A.C. Pavlick, C. Robert, K. Grossmann, D. McDermott, G.P. Linette, N. Meyer, J.K. Giguere, S.S. Agarwala, M. Shaheen, M.S. Ernstoff, D. Minor, A.K. Salama, M. Taylor, P.A. Ott, L.M. Rollin, C. Horak, P. Gagnier, J.D. Wolchok, F.S. Hodi, Nivolumab and ipilimumab versus ipilimumab in untreated melanoma, The New England journal of medicine 372(21) (2015) 2006-17.
[12] T. Powles, J.P. Eder, G.D. Fine, F.S. Braiteh, Y. Loriot, C. Cruz, J. Bellmunt, H.A. Burris, D.P. Petrylak, S.L. Teng, X. Shen, Z. Boyd, P.S. Hegde, D.S. Chen, N.J. Vogelzang, MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer, Nature 515(7528) (2014) 558-62.
[13] S.L. Topalian, C.G. Drake, D.M. Pardoll, Immune checkpoint blockade: a common denominator approach to cancer therapy, Cancer cell 27(4) (2015) 450-61.
[14] J. Brahmer, K.L. Reckamp, P. Baas, L. Crino, W.E. Eberhardt, E. Poddubskaya, S. Antonia, A. Pluzanski, E.E. Vokes, E. Holgado, D. Waterhouse, N. Ready, J. Gainor, O. Aren Frontera, L. Havel, M. Steins, M.C. Garassino, J.G. Aerts, M. Domine, L. Paz-Ares, M. Reck, C. Baudelet, C.T. Harbison, B. Lestini, D.R. Spigel, Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer, The New England journal of medicine 373(2) (2015) 123-35.

[15] J.E. Rosenberg, J. Hoffman-Censits, T. Powles, M.S. van der Heijden, A.V. Balar, A. Necchi, N. Dawson, P.H. O'Donnell, A. Balmanoukian, Y. Loriot, S. Srinivas, M.M. Retz, P. Grivas, R.W. Joseph, M.D. Galsky, M.T. Fleming, D.P. Petrylak, J.L. Perez-Gracia, H.A. Burris, D. Castellano, C. Canil, J. Bellmunt, D. Bajorin, D. Nickles, R. Bourgon, G.M. Frampton, N. Cui, S. Mariathasan, O. Abidoye, G.D. Fine, R. Dreicer, Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial, Lancet 387(10031) (2016) 1909-20.
[16] R.J. Motzer, B. Escudier, D.F. McDermott, S. George, H.J. Hammers, S. Srinivas, S.S. Tykodi, J.A. Sosman, G. Procopio, E.R. Plimack, D. Castellano, T.K. Choueiri, H. Gurney, F. Donskov, P. Bono, J. Wagstaff, T.C. Gauler, T. Ueda, Y. Tomita, F.A. Schutz, C. Kollmannsberger, J. Larkin, A. Ravaud, J.S. Simon, L.A. Xu, I.M. Waxman, P. Sharma, I. CheckMate, Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma, The New England journal of medicine 373(19) (2015) 1803-13.
[17] J. Larkin, V. Chiarion-Sileni, R. Gonzalez, J.J. Grob, C.L. Cowey, C.D. Lao, D. Schadendorf, R. Dummer, M. Smylie, P. Rutkowski, P.F. Ferrucci, A. Hill, J. Wagstaff, M.S. Carlino, J.B. Haanen, M. Maio, I. Marquez-Rodas, G.A. McArthur, P.A. Ascierto, G.V. Long, M.K. Callahan, M.A. Postow, K. Grossmann, M. Sznol, B. Dreno, L. Bastholt, A. Yang, L.M. Rollin, C. Horak, F.S. Hodi, J.D. Wolchok, Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma, The New England journal of medicine 373(1) (2015) 23-34.
[18] B.T. Fife, K.E. Pauken, The role of the PD-1 pathway in autoimmunity and peripheral tolerance, Annals of the New York Academy of Sciences 1217 (2011) 45-59.
[19] Y. Ishida, Y. Agata, K. Shibahara, T. Honjo, Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death, The EMBO journal 11(11) (1992) 3887-95.
[20] L.R. Finger, J. Pu, R. Wasserman, R. Vibhakar, E. Louie, R.R. Hardy, P.D. Burrows, L.G. Billips, The human PD-1 gene: complete cDNA, genomic organization, and developmentally regulated expression in B cell progenitors, Gene 197(1-2) (1997) 177-87.
[21] H. Nishimura, M. Nose, H. Hiai, N. Minato, T. Honjo, Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor, Immunity 11(2) (1999) 141-51.
[22] G.J. Freeman, A.J. Long, Y. Iwai, K. Bourque, T. Chernova, H. Nishimura, L.J. Fitz, N. Malenkovich, T. Okazaki, M.C. Byrne, H.F. Horton, L. Fouser, L. Carter, V. Ling, M.R. Bowman, B.M. Carreno, M. Collins, C.R. Wood, T. Honjo, Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation, The Journal of experimental medicine 192(7) (2000) 1027-34.
[23] Y. Latchman, C.R. Wood, T. Chernova, D. Chaudhary, M. Borde, I. Chernova, Y. Iwai, A.J. Long, J.A. Brown, R. Nunes, E.A. Greenfield, K. Bourque, V.A. Boussiotis, L.L. Carter, B.M. Carreno, N. Malenkovich, H. Nishimura, T. Okazaki, T. Honjo, A.H. Sharpe, G.J. Freeman, PD-L2 is a second ligand for PD-1 and inhibits T cell activation, Nature immunology 2(3) (2001) 261-8.
[24] J. Hori, M. Wang, M. Miyashita, K. Tanemoto, H. Takahashi, T. Takemori, K. Okumura, H. Yagita, M. Azuma, B7-H1-induced apoptosis as a mechanism of immune privilege of corneal allografts, Journal of immunology 177(9) (2006) 5928-35.
[25] M.G. Petroff, L. Chen, T.A. Phillips, J.S. Hunt, B7 family molecules: novel immunomodulators at the maternal-fetal interface, Placenta 23 Suppl A (2002) S95-101.
[26] H. Dong, G. Zhu, K. Tamada, L. Chen, B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion, Nature medicine 5(12) (1999) 1365-9.
[27] D.Y. Lin, Y. Tanaka, M. Iwasaki, A.G. Gittis, H.P. Su, B. Mikami, T. Okazaki, T. Honjo, N. Minato, D.N. Garboczi, The PD-1/PD-L1 complex resembles the antigen-binding Fv domains of antibodies and T cell receptors, Proceedings of the National Academy of Sciences of the United States of America 105(8) (2008) 3011-6.
[28] M.E. Keir, S.C. Liang, I. Guleria, Y.E. Latchman, A. Qipo, L.A. Albacker, M. Koulmanda, G.J. Freeman, M.H. Sayegh, A.H. Sharpe, Tissue expression of PD-L1 mediates peripheral T cell tolerance, The Journal of experimental medicine 203(4) (2006) 883-95.
[29] C. Blank, A. Mackensen, Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion, Cancer immunology, immunotherapy : CII 56(5) (2007) 739-45.

[30] M.J. Butte, M.E. Keir, T.B. Phamduy, A.H. Sharpe, G.J. Freeman, Programmed death-1 ligand 1 interacts specifically with the B7-1 costimulatory molecule to inhibit T cell responses, Immunity 27(1) (2007) 111-22.
[31] T.T. Chang, C. Jabs, R.A. Sobel, V.K. Kuchroo, A.H. Sharpe, Studies in B7-deficient mice reveal a critical role for B7 costimulation in both induction and effector phases of experimental autoimmune encephalomyelitis, The Journal of experimental medicine 190(5) (1999) 733-40.
[32] T.J. Curiel, S. Wei, H. Dong, X. Alvarez, P. Cheng, P. Mottram, R. Krzysiek, K.L. Knutson, B. Daniel, M.C. Zimmermann, O. David, M. Burow, A. Gordon, N. Dhurandhar, L. Myers, R. Berggren, A. Hemminki, R.D. Alvarez, D. Emilie, D.T. Curiel, L. Chen, W. Zou, Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity, Nature medicine 9(5) (2003) 562-7.
[33] Y.E. Latchman, S.C. Liang, Y. Wu, T. Chernova, R.A. Sobel, M. Klemm, V.K. Kuchroo, G.J. Freeman, A.H. Sharpe, PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells, Proceedings of the National Academy of Sciences of the United States of America 101(29) (2004) 10691-6.
[34] A.H. Sharpe, K.E. Pauken, The diverse functions of the PD1 inhibitory pathway, Nature reviews. Immunology 18(3) (2018) 153-167.
[35] Y. Agata, A. Kawasaki, H. Nishimura, Y. Ishida, T. Tsubata, H. Yagita, T. Honjo, Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes, International immunology 8(5) (1996) 765-72.
[36] C. Huang, H.X. Zhu, Y. Yao, Z.H. Bian, Y.J. Zheng, L. Li, H.M. Moutsopoulos, M.E. Gershwin, Z.X. Lian, Immune checkpoint molecules. Possible future therapeutic implications in autoimmune diseases, Journal of autoimmunity 104 (2019) 102333.
[37] M.F. Krummel, J.P. Allison, CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation, The Journal of experimental medicine 182(2) (1995) 459-65.
[38] P. Dariavach, M.G. Mattei, P. Golstein, M.P. Lefranc, Human Ig superfamily CTLA-4 gene: chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains, European journal of immunology 18(12) (1988) 1901-5.
[39] N. Buonavista, C. Balzano, P. Pontarotti, D. Le Paslier, P. Golstein, Molecular linkage of the human CTLA4 and CD28 Ig-superfamily genes in yeast artificial chromosomes, Genomics 13(3) (1992) 856-61.
[40] P. Waterhouse, J.M. Penninger, E. Timms, A. Wakeham, A. Shahinian, K.P. Lee, C.B. Thompson, H. Griesser, T.W. Mak, Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4, Science 270(5238) (1995) 985-8.
[41] P.S. Linsley, S.G. Nadler, J. Bajorath, R. Peach, H.T. Leung, J. Rogers, J. Bradshaw, M. Stebbins, G. Leytze, W. Brady, et al., Binding stoichiometry of the cytotoxic T lymphocyte-associated molecule-4 (CTLA-4). A disulfide-linked homodimer binds two CD86 molecules, The Journal of biological chemistry 270(25) (1995) 15417-24.
[42] M.K. Oaks, K.M. Hallett, R.T. Penwell, E.C. Stauber, S.J. Warren, A.J. Tector, A native soluble form of CTLA-4, Cellular immunology 201(2) (2000) 144-53.
[43] K.M. Lee, E. Chuang, M. Griffin, R. Khattri, D.K. Hong, W. Zhang, D. Straus, L.E. Samelson, C.B. Thompson, J.A. Bluestone, Molecular basis of T cell inactivation by CTLA-4, Science 282(5397) (1998) 2263-6.
[44] O.S. Qureshi, Y. Zheng, K. Nakamura, K. Attridge, C. Manzotti, E.M. Schmidt, J. Baker, L.E. Jeffery, S. Kaur, Z. Briggs, T.Z. Hou, C.E. Futter, G. Anderson, L.S. Walker, D.M. Sansom, Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4, Science 332(6029) (2011) 600-3.
[45] D. Saverino, R. Simone, M. Bagnasco, G. Pesce, The soluble CTLA-4 receptor and its role in autoimmune diseases: an update, Auto- immunity highlights 1(2) (2010) 73-81.
[46] N.L. Syn, M.W.L. Teng, T.S.K. Mok, R.A. Soo, De-novo and acquired resistance to immune checkpoint targeting, The Lancet. Oncology 18(12) (2017) e731-e741.
[47] C.A. Chambers, T.J. Sullivan, J.P. Allison, Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells, Immunity 7(6) (1997) 885-95.
[48] K. Klocke, S. Sakaguchi, R. Holmdahl, K. Wing, Induction of autoimmune disease by deletion of CTLA-4 in mice in adulthood, Proceedings of the National Academy of Sciences of the United States of America 113(17) (2016) E2383-92.

[49] Y. Lui, S.J. Davis, LAG-3: a very singular immune checkpoint, Nature immunology 19(12) (2018) 1278-1279.
[50] F. Triebel, S. Jitsukawa, E. Baixeras, S. Roman-Roman, C. Genevee, E. Viegas-Pequignot, T. Hercend, LAG-3, a novel lymphocyte activation gene closely related to CD4, The Journal of experimental medicine 171(5) (1990) 1393-405.
[51] S. Andreae, F. Piras, N. Burdin, F. Triebel, Maturation and activation of dendritic cells induced by lymphocyte activation gene-3 (CD223), Journal of immunology 168(8) (2002) 3874-80.
[52] E. Baixeras, B. Huard, C. Miossec, S. Jitsukawa, M. Martin, T. Hercend, C. Auffray, F. Triebel, D. Piatier-Tonneau, Characterization of the lymphocyte activation gene 3-encoded protein. A new ligand for human leukocyte antigen class II antigens, The Journal of experimental medicine 176(2) (1992) 327-37.
[53] J. Wang, M.F. Sanmamed, I. Datar, T.T. Su, L. Ji, J. Sun, L. Chen, Y. Chen, G. Zhu, W. Yin, L. Zheng, T. Zhou, T. Badri, S. Yao, S. Zhu, A. Boto, M. Sznol, I. Melero, D.A.A. Vignali, K. Schalper, L. Chen, Fibrinogen-like Protein 1 Is a Major Immune Inhibitory Ligand of LAG-3, Cell 176(1-2) (2019) 334-347 e12.
[54] C.J. Workman, D.A. Vignali, The CD4-related molecule, LAG-3 (CD223), regulates the expansion of activated T cells, European journal of immunology 33(4) (2003) 970-9.
[55] A.C. Anderson, N. Joller, V.K. Kuchroo, Lag-3, Tim-3, and TIGIT: Co-inhibitory Receptors with Specialized Functions in Immune Regulation, Immunity 44(5) (2016) 989-1004.
[56] T. Kouo, L. Huang, A.B. Pucsek, M. Cao, S. Solt, T. Armstrong, E. Jaffee, Galectin-3 Shapes Antitumor Immune Responses by Suppressing CD8+ T Cells via LAG-3 and Inhibiting Expansion of Plasmacytoid Dendritic Cells, Cancer Immunol Res 3(4) (2015) 412-23.
[57] B. Huard, M. Tournier, T. Hercend, F. Triebel, F. Faure, Lymphocyte-activation gene 3/major histocompatibility complex class II interaction modulates the antigenic response of CD4+ T lymphocytes, European journal of immunology 24(12) (1994) 3216-21.
[58] C.J. Workman, K.J. Dugger, D.A. Vignali, Cutting edge: molecular analysis of the negative regulatory function of lymphocyte activation gene-3, Journal of immunology 169(10) (2002) 5392-5.
[59] C.J. Workman, D.S. Rice, K.J. Dugger, C. Kurschner, D.A. Vignali, Phenotypic analysis of the murine CD4-related glycoprotein, CD223 (LAG-3), European journal of immunology 32(8) (2002) 2255-63.
[60] C.J. Workman, L.S. Cauley, I.J. Kim, M.A. Blackman, D.L. Woodland, D.A. Vignali, Lymphocyte activation gene-3 (CD223) regulates the size of the expanding T cell population following antigen activation in vivo, Journal of immunology 172(9) (2004) 5450-5.
[61] C.J. Workman, D.A. Vignali, Negative regulation of T cell homeostasis by lymphocyte activation gene-3 (CD223), Journal of immunology 174(2) (2005) 688-95.
[62] C. Camisaschi, C. Casati, F. Rini, M. Perego, A. De Filippo, F. Triebel, G. Parmiani, F. Belli, L. Rivoltini, C. Castelli, LAG-3 expression defines a subset of CD4(+)CD25(high)Foxp3(+) regulatory T cells that are expanded at tumor sites, Journal of immunology 184(11) (2010) 6545-51.
[63] N. Gagliani, C.F. Magnani, S. Huber, M.E. Gianolini, M. Pala, P. Licona-Limon, B. Guo, D.R. Herbert, A. Bulfone, F. Trentini, C. Di Serio, R. Bacchetta, M. Andreani, L. Brockmann, S. Gregori, R.A. Flavell, M.G. Roncarolo, Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells, Nature medicine 19(6) (2013) 739-46.
[64] C.T. Huang, C.J. Workman, D. Flies, X. Pan, A.L. Marson, G. Zhou, E.L. Hipkiss, S. Ravi, J. Kowalski, H.I. Levitsky, J.D. Powell, D.M. Pardoll, C.G. Drake, D.A. Vignali, Role of LAG-3 in regulatory T cells, Immunity 21(4) (2004) 503-13.
[65] M. Kisielow, J. Kisielow, G. Capoferri-Sollami, K. Karjalainen, Expression of lymphocyte activation gene 3 (LAG-3) on B cells is induced by T cells, European journal of immunology 35(7) (2005) 2081-8.
[66] C.J. Workman, Y. Wang, K.C. El Kasmi, D.M. Pardoll, P.J. Murray, C.G. Drake, D.A. Vignali, LAG-3 regulates plasmacytoid dendritic cell homeostasis, Journal of immunology 182(4) (2009) 1885-91.
[67] M. Bettini, A.L. Szymczak-Workman, K. Forbes, A.H. Castellaw, M. Selby, X. Pan, C.G. Drake, A.J. Korman, D.A. Vignali, Cutting edge: accelerated autoimmune diabetes in the absence of LAG-3, J Immunol 187(7) (2011) 3493-8.
[68] L. Monney, C.A. Sabatos, J.L. Gaglia, A. Ryu, H. Waldner, T. Chernova, S. Manning, E.A. Greenfield, A.J. Coyle, R.A. Sobel, G.J. Freeman, V.K. Kuchroo, Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease, Nature 415(6871) (2002) 536-41.

[69] J.J. McIntire, S.E. Umetsu, O. Akbari, M. Potter, V.K. Kuchroo, G.S. Barsh, G.J. Freeman, D.T. Umetsu, R.H. DeKruyff, Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family, Nature immunology 2(12) (2001) 1109-16.
[70] R.L. Ferris, B. Lu, L.P. Kane, Too much of a good thing? Tim-3 and TCR signaling in T cell exhaustion, Journal of immunology 193(4) (2014) 1525-30.
[71] A.C. Anderson, D.E. Anderson, L. Bregoli, W.D. Hastings, N. Kassam, C. Lei, R. Chandwaskar, J. Karman, E.W. Su, M. Hirashima, J.N. Bruce, L.P. Kane, V.K. Kuchroo, D.A. Hafler, Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells, Science 318(5853) (2007) 1141-3.
[72] R. Liu, X. Wang, X. Chen, S. Wang, H. Zhang, TIM-3 rs1036199 polymorphism increases susceptibility to autoimmune diseases: evidence based on 4200 subjects, Bioscience reports 38(6) (2018).
[73] S.C. Chae, Y.R. Park, S.C. Shim, K.S. Yoon, H.T. Chung, The polymorphisms of Th1 cell surface gene Tim-3 are associated in a Korean population with rheumatoid arthritis, Immunology letters 95(1) (2004) 91-5.
[74] S.C. Chae, Y.R. Park, Y.C. Lee, J.H. Lee, H.T. Chung, The association of TIM-3 gene polymorphism with atopic disease in Korean population, Human immunology 65(12) (2004) 1427-31.
[75] P.E. Graves, V. Siroux, S. Guerra, W.T. Klimecki, F.D. Martinez, Association of atopy and eczema with polymorphisms in T-cell immunoglobulin domain and mucin domain-IL-2-inducible T-cell kinase gene cluster in chromosome 5 q 33, The Journal of allergy and clinical immunology 116(3) (2005) 650-6.
[76] J.V. Gorman, G. Starbeck-Miller, N.L. Pham, G.L. Traver, P.B. Rothman, J.T. Harty, J.D. Colgan, Tim-3 directly enhances CD8 T cell responses to acute Listeria monocytogenes infection, Journal of immunology 192(7) (2014) 3133-42.
[77] B.L. Phong, L. Avery, T.L. Sumpter, J.V. Gorman, S.C. Watkins, J.D. Colgan, L.P. Kane, Tim-3 enhances FcepsilonRI-proximal signaling to modulate mast cell activation, The Journal of experimental medicine 212(13) (2015) 2289-304.
[78] H. Banerjee, L.P. Kane, Immune regulation by Tim-3, F1000Research 7 (2018) 316.
[79] C. Zhu, A.C. Anderson, A. Schubart, H. Xiong, J. Imitola, S.J. Khoury, X.X. Zheng, T.B. Strom, V.K. Kuchroo, The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity, Nature immunology 6(12) (2005) 1245-52.
[80] M. Nakayama, H. Akiba, K. Takeda, Y. Kojima, M. Hashiguchi, M. Azuma, H. Yagita, K. Okumura, Tim-3 mediates phagocytosis of apoptotic cells and cross-presentation, Blood 113(16) (2009) 3821-30.
[81] R.H. DeKruyff, X. Bu, A. Ballesteros, C. Santiago, Y.L. Chim, H.H. Lee, P. Karisola, M. Pichavant, G.G. Kaplan, D.T. Umetsu, G.J. Freeman, J.M. Casasnovas, T cell/transmembrane, Ig, and mucin-3 allelic variants differentially recognize phosphatidylserine and mediate phagocytosis of apoptotic cells, Journal of immunology 184(4) (2010) 1918-30.
[82] S. Chiba, M. Baghdadi, H. Akiba, H. Yoshiyama, I. Kinoshita, H. Dosaka-Akita, Y. Fujioka, Y. Ohba, J.V. Gorman, J.D. Colgan, M. Hirashima, T. Uede, A. Takaoka, H. Yagita, M. Jinushi, Tumor-infiltrating DCs suppress nucleic acid-mediated innate immune responses through interactions between the receptor TIM-3 and the alarmin HMGB1, Nature immunology 13(9) (2012) 832-42.
[83] M. Das, C. Zhu, V.K. Kuchroo, Tim-3 and its role in regulating anti-tumor immunity, Immunological reviews 276(1) (2017) 97-111.
[84] Y.H. Huang, C. Zhu, Y. Kondo, A.C. Anderson, A. Gandhi, A. Russell, S.K. Dougan, B.S. Petersen, E. Melum, T. Pertel, K.L. Clayton, M. Raab, Q. Chen, N. Beauchemin, P.J. Yazaki, M. Pyzik, M.A. Ostrowski, J.N. Glickman, C.E. Rudd, H.L. Ploegh, A. Franke, G.A. Petsko, V.K. Kuchroo, R.S. Blumberg, CEACAM1 regulates TIM-3-mediated tolerance and exhaustion, Nature 517(7534) (2015) 386-90.
[85] D. Zehn, E.J. Wherry, Immune Memory and Exhaustion: Clinically Relevant Lessons from the LCMV Model, Advances in experimental medicine and biology 850 (2015) 137-52.
[86] N. Maurya, R. Gujar, M. Gupta, V. Yadav, S. Verma, P. Sen, Immunoregulation of dendritic cells by the receptor T cell Ig and mucin protein-3 via Bruton's tyrosine kinase and c-Src, Journal of immunology 193(7) (2014) 3417-25.
[87] N. Nielsen, N. Odum, B. Urso, L.L. Lanier, P. Spee, Cytotoxicity of CD56(bright) NK cells towards autologous activated CD4+ T cells is mediated through NKG2D, LFA-1 and TRAIL and dampened via CD94/NKG2A, PloS one 7(2) (2012) e31959.

[88] J.A. Schwartz, K.L. Clayton, S. Mujib, H. Zhang, A.K. Rahman, J. Liu, F.Y. Yue, E. Benko, C. Kovacs, M.A. Ostrowski, Tim-3 is a Marker of Plasmacytoid Dendritic Cell Dysfunction during HIV Infection and Is Associated with the Recruitment of IRF7 and p85 into Lysosomes and with the Submembrane Displacement of TLR9, Journal of immunology 198(8) (2017) 3181-3194.
[89] X. Yu, K. Harden, L.C. Gonzalez, M. Francesco, E. Chiang, B. Irving, I. Tom, S. Ivelja, C.J. Refino, H. Clark, D. Eaton, J.L. Grogan, The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells, Nature immunology 10(1) (2009) 48-57.
[90] W.C. Dougall, S. Kurtulus, M.J. Smyth, A.C. Anderson, TIGIT and CD96: new checkpoint receptor targets for cancer immunotherapy, Immunological reviews 276(1) (2017) 112-120.
[91] C.B. Coyne, K.S. Kim, J.M. Bergelson, Poliovirus entry into human brain microvascular cells requires receptor-induced activation of SHP-2, The EMBO journal 26(17) (2007) 4016-28.
[92] N. Joller, E. Lozano, P.R. Burkett, B. Patel, S. Xiao, C. Zhu, J. Xia, T.G. Tan, E. Sefik, V. Yajnik, A.H. Sharpe, F.J. Quintana, D. Mathis, C. Benoist, D.A. Hafler, V.K. Kuchroo, Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses, Immunity 40(4) (2014) 569-81.
[93] S.D. Levin, D.W. Taft, C.S. Brandt, C. Bucher, E.D. Howard, E.M. Chadwick, J. Johnston, A. Hammond, K. Bontadelli, D. Ardourel, L. Hebb, A. Wolf, T.R. Bukowski, M.W. Rixon, J.L. Kuijper, C.D. Ostrander, J.W. West, J. Bilsborough, B. Fox, Z. Gao, W. Xu, F. Ramsdell, B.R. Blazar, K.E. Lewis, Vstm3 is a member of the CD28 family and an important modulator of T-cell function, European journal of immunology 41(4) (2011) 902-15.
[94] N. Joller, J.P. Hafler, B. Brynedal, N. Kassam, S. Spoerl, S.D. Levin, A.H. Sharpe, V.K. Kuchroo, Cutting edge: TIGIT has T cell-intrinsic inhibitory functions, Journal of immunology 186(3) (2011) 1338-42.
[95] M. Amiezer, T.G. Phan, Disentangling Tfr cells from Treg cells and Tfh cells: How to untie the Gordian knot, European journal of immunology 46(5) (2016) 1101-4.
[96] N. Stanietsky, H. Simic, J. Arapovic, A. Toporik, O. Levy, A. Novik, Z. Levine, M. Beiman, L. Dassa, H. Achdout, N. Stern-Ginossar, P. Tsukerman, S. Jonjic, O. Mandelboim, The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity, Proceedings of the National Academy of Sciences of the United States of America 106(42) (2009) 17858-63.
[97] A.I. Chapoval, J. Ni, J.S. Lau, R.A. Wilcox, D.B. Flies, D. Liu, H. Dong, G.L. Sica, G. Zhu, K. Tamada, L. Chen, B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production, Nature immunology 2(3) (2001) 269-74.
[98] J. Leitner, C. Klauser, W.F. Pickl, J. Stockl, O. Majdic, A.F. Bardet, D.P. Kreil, C. Dong, T. Yamazaki, G. Zlabinger, K. Pfistershammer, P. Steinberger, B7-H3 is a potent inhibitor of human T-cell activation: No evidence for B7-H3 and TREML2 interaction, European journal of immunology 39(7) (2009) 1754-64.
[99] L. Luo, A.I. Chapoval, D.B. Flies, G. Zhu, F. Hirano, S. Wang, J.S. Lau, H. Dong, K. Tamada, A.S. Flies, Y. Liu, L. Chen, B7-H3 enhances tumor immunity in vivo by costimulating rapid clonal expansion of antigen-specific CD8+ cytolytic T cells, Journal of immunology 173(9) (2004) 5445-50.
[100] C.N. Tran, S.G. Thacker, D.M. Louie, J. Oliver, P.T. White, J.L. Endres, A.G. Urquhart, K.C. Chung, D.A. Fox, Interactions of T cells with fibroblast-like synoviocytes: role of the B7 family costimulatory ligand B7-H3, Journal of immunology 180(5) (2008) 2989-98.
[101] Y. Mao, W. Li, K. Chen, Y. Xie, Q. Liu, M. Yao, W. Duan, X. Zhou, R. Liang, M. Tao, B7-H1 and B7-H3 are independent predictors of poor prognosis in patients with non-small cell lung cancer, Oncotarget 6(5) (2015) 3452-61.
[102] N. Watanabe, M. Gavrieli, J.R. Sedy, J. Yang, F. Fallarino, S.K. Loftin, M.A. Hurchla, N. Zimmerman, J. Sim, X. Zang, T.L. Murphy, J.H. Russell, J.P. Allison, K.M. Murphy, BTLA is a lymphocyte inhibitory receptor with similarities to CTLA-4 and PD-1, Nature immunology 4(7) (2003) 670-9.
[103] K.M. Murphy, C.A. Nelson, J.R. Sedy, Balancing co-stimulation and inhibition with BTLA and HVEM, Nature reviews. Immunology 6(9) (2006) 671-81.
[104] J.R. Sedy, M. Gavrieli, K.G. Potter, M.A. Hurchla, R.C. Lindsley, K. Hildner, S. Scheu, K. Pfeffer, C.F. Ware, T.L. Murphy, K.M. Murphy, B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator, Nature immunology 6(1) (2005) 90-8.
[105] J.M. Chemnitz, A.R. Lanfranco, I. Braunstein, J.L. Riley, B and T lymphocyte attenuator-mediated signal transduction provides a potent inhibitory signal to primary human CD4 T cells that can be initiated by multiple phosphotyrosine motifs, Journal of immunology 176(11) (2006) 6603-14.

[106] M. Gavrieli, K.M. Murphy, Association of Grb-2 and PI3K p85 with phosphotyrosile peptides derived from BTLA, Biochemical and biophysical research communications 345(4) (2006) 1440-5.
[107] C. Deppong, T.I. Juehne, M. Hurchla, L.D. Friend, D.D. Shah, C.M. Rose, T.L. Bricker, L.P. Shornick, E.C. Crouch, T.L. Murphy, M.J. Holtzman, K.M. Murphy, J.M. Green, Cutting edge: B and T lymphocyte attenuator and programmed death receptor-1 inhibitory receptors are required for termination of acute allergic airway inflammation, Journal of immunology 176(7) (2006) 3909-13.
[108] Y. Oya, N. Watanabe, T. Owada, M. Oki, K. Hirose, A. Suto, S. Kagami, H. Nakajima, T. Kishimoto, I. Iwamoto, T.L. Murphy, K.M. Murphy, Y. Saito, Development of autoimmune hepatitis-like disease and production of autoantibodies to nuclear antigens in mice lacking B and T lymphocyte attenuator, Arthritis and rheumatism 58(8) (2008) 2498-510.
[109] F. Fallarino, U. Grohmann, C. Vacca, R. Bianchi, C. Orabona, A. Spreca, M.C. Fioretti, P. Puccetti, T cell apoptosis by tryptophan catabolism, Cell death and differentiation 9(10) (2002) 1069-77.
[110] P. Terness, T.M. Bauer, L. Rose, C. Dufter, A. Watzlik, H. Simon, G. Opelz, Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites, The Journal of experimental medicine 196(4) (2002) 447-57.
[111] G. Frumento, R. Rotondo, M. Tonetti, G. Damonte, U. Benatti, G.B. Ferrara, Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase, The Journal of experimental medicine 196(4) (2002) 459-68.
[112] M. Della Chiesa, S. Carlomagno, G. Frumento, M. Balsamo, C. Cantoni, R. Conte, L. Moretta, A. Moretta, M. Vitale, The tryptophan catabolite L-kynurenine inhibits the surface expression of NKp46- and NKG2D-activating receptors and regulates NK-cell function, Blood 108(13) (2006) 4118-25.
[113] F. Fallarino, U. Grohmann, K.W. Hwang, C. Orabona, C. Vacca, R. Bianchi, M.L. Belladonna, M.C. Fioretti, M.L. Alegre, P. Puccetti, Modulation of tryptophan catabolism by regulatory T cells, Nature immunology 4(12) (2003) 1206-12.
[114] C. Smith, M.Y. Chang, K.H. Parker, D.W. Beury, J.B. DuHadaway, H.E. Flick, J. Boulden, E. Sutanto-Ward, A.P. Soler, L.D. Laury-Kleintop, L. Mandik-Nayak, R. Metz, S. Ostrand-Rosenberg, G.C. Prendergast, A.J. Muller, IDO is a nodal pathogenic driver of lung cancer and metastasis development, Cancer discovery 2(8) (2012) 722-35.
[115] Y. Gao, S. Shi, W. Ma, J. Chen, Y. Cai, L. Ge, L. Li, J. Wu, J. Tian, Bibliometric analysis of global research on PD-1 and PD-L1 in the field of cancer, International immunopharmacology 72 (2019) 374-384.
[116] T. Zhao, C. Li, Y. Wu, B. Li, B. Zhang, Prognostic value of PD-L1 expression in tumor infiltrating immune cells in cancers: A meta-analysis, PloS one 12(4) (2017) e0176822.
[117] W. Huang, R. Ran, B. Shao, H. Li, Prognostic and clinicopathological value of PD-L1 expression in primary breast cancer: a meta-analysis, Breast cancer research and treatment 178(1) (2019) 17-33.
[118] P. Kong, J. Wang, Z. Song, S. Liu, W. He, C. Jiang, Q. Xie, L. Yang, X. Xia, L. Xia, Circulating Lymphocytes, PD-L1 Expression on Tumor-infiltrating Lymphocytes, and Survival of Colorectal Cancer Patients with Different Mismatch Repair Gene Status, Journal of Cancer 10(7) (2019) 1745-1754.
[119] K.H. Kim, K.U. Choi, A. Kim, S.J. Lee, J.H. Lee, D.S. Suh, B.S. Kwon, C. Hwang, PD-L1 expression on stromal tumor-infiltrating lymphocytes is a favorable prognostic factor in ovarian serous carcinoma, Journal of ovarian research 12(1) (2019) 56.
[120] S. Brosseau, C. Danel, A. Scherpereel, J. Mazieres, S. Lantuejoul, J. Margery, L. Greillier, C. Audigier-Valette, V. Gounant, M. Antoine, D. Moro-Sibilot, I. Rouquette, O. Molinier, R. Corre, I. Monnet, A. Langlais, F. Morin, E. Bergot, G. Zalcman, G. Levallet, Shorter Survival in Malignant Pleural Mesothelioma Patients With High PD-L1 Expression Associated With Sarcomatoid or Biphasic Histology Subtype: A Series of 214 Cases From the Bio-MAPS Cohort, Clinical lung cancer 20(5) (2019) e564-e575.
[121] X. Gu, M. Dong, Z. Liu, Y. Mi, J. Yang, Z. Zhang, K. Liu, L. Jiang, Y. Zhang, S. Dong, Y. Shi, Elevated PD-L1 expression predicts poor survival outcomes in patients with cervical cancer, Cancer cell international 19 (2019) 146.
[122] J. Carlsson, P. Sundqvist, V. Kosuta, A. Falt, F. Giunchi, M. Fiorentino, S. Davidsson, PD-L1 Expression is Associated With Poor Prognosis in Renal Cell Carcinoma, Applied immunohistochemistry & molecular morphology : AIMM (2019).

[123] M. Aghajani, S. Graham, C. McCafferty, C.A. Shaheed, T. Roberts, P. DeSouza, T. Yang, N. Niles, Clinicopathologic and Prognostic Significance of Programmed Cell Death Ligand 1 Expression in Patients with Non-Medullary Thyroid Cancer: A Systematic Review and Meta-Analysis, Thyroid : official journal of the American Thyroid Association 28(3) (2018) 349-361.
[124] H. Li, Y. Xu, B. Wan, Y. Song, P. Zhan, Y. Hu, Q. Zhang, F. Zhang, H. Liu, T. Li, H. Sugimura, F. Cappuzzo, D. Lin, T. Lv, A.M.E.L.C.C.G. written on behalf of, The clinicopathological and prognostic significance of PD-L1 expression assessed by immunohistochemistry in lung cancer: a meta-analysis of 50 studies with 11,383 patients, Translational lung cancer research 8(4) (2019) 429-449.
[125] Y. Li, M. He, Y. Zhou, C. Yang, S. Wei, X. Bian, O. Christopher, L. Xie, The Prognostic and Clinicopathological Roles of PD-L1 Expression in Colorectal Cancer: A Systematic Review and Meta-Analysis, Frontiers in pharmacology 10 (2019) 139.
[126] C. Bence, V. Hofman, E. Chamorey, E. Long-Mira, S. Lassalle, A.F. Albertini, I. Liolios, K. Zahaf, A. Picard, H. Montaudie, J.P. Lacour, T. Passeron, A.A. Andea, M. Ilie, P. Hofman, Association of combined PD-L1 expression and tumour-infiltrating lymphocyte features with survival and treatment outcomes in patients with metastatic melanoma, Journal of the European Academy of Dermatology and Venereology : JEADV (2019).
[127] X. Huang, W. Zhang, Z. Zhang, D. Shi, F. Wu, B. Zhong, Z. Shao, Prognostic Value of Programmed Cell Death 1 Ligand-1 (PD-L1) or PD-1 Expression in Patients with Osteosarcoma: A Meta-Analysis, Journal of Cancer 9(14) (2018) 2525-2531.
[128] G. Xu, L. Sun, Y. Li, F. Xie, X. Zhou, H. Yang, S. Du, H. Xu, Y. Mao, The Clinicopathological and Prognostic Value of PD-L1 Expression in Cholangiocarcinoma: A Meta-Analysis, Frontiers in oncology 9 (2019) 897.
[129] Q.F. Manson, W. Schrijver, N.D. Ter Hoeve, C.B. Moelans, P.J. van Diest, Frequent discordance in PD-1 and PD-L1 expression between primary breast tumors and their matched distant metastases, Clinical & experimental metastasis 36(1) (2019) 29-37.
[130] E.F. Burgess, C. Livasy, A. Hartman, M.M. Robinson, J. Symanowski, C. Naso, S. Doherty, R. Guerrieri, S. Riggs, C.M. Grigg, P.E. Clark, D. Raghavan, Discordance of high PD-L1 expression in primary and metastatic urothelial carcinoma lesions, Urologic oncology 37(5) (2019) 299 e19-299 e25.
[131] X. Zhang, X. Yin, H. Zhang, G. Sun, Y. Yang, J. Chen, X. Zhu, P. Zhao, J. Zhao, J. Liu, N. Chen, J. Wang, P. Shen, H. Zeng, Differential expressions of PD-1, PD-L1 and PD-L2 between primary and metastatic sites in renal cell carcinoma, BMC cancer 19(1) (2019) 360.
[132] H.D. Kim, G.W. Song, S. Park, M.K. Jung, M.H. Kim, H.J. Kang, C. Yoo, K. Yi, K.H. Kim, S. Eo, D.B. Moon, S.M. Hong, Y.S. Ju, E.C. Shin, S. Hwang, S.H. Park, Association Between Expression Level of PD1 by Tumor-Infiltrating CD8(+) T Cells and Features of Hepatocellular Carcinoma, Gastroenterology 155(6) (2018) 1936-1950 e17.
[133] A. Kitano, M. Ono, M. Yoshida, E. Noguchi, A. Shimomura, T. Shimoi, M. Kodaira, M. Yunokawa, K. Yonemori, C. Shimizu, T. Kinoshita, Y. Fujiwara, H. Tsuda, K. Tamura, Tumour-infiltrating lymphocytes are correlated with higher expression levels of PD-1 and PD-L1 in early breast cancer, ESMO open 2(2) (2017) e000150.
[134] P.Y. Huang, S.S. Guo, Y. Zhang, J.B. Lu, Q.Y. Chen, L.Q. Tang, L. Zhang, L.T. Liu, L. Zhang, H.Q. Mai, Tumor CTLA-4 overexpression predicts poor survival in patients with nasopharyngeal carcinoma, Oncotarget 7(11) (2016) 13060-8.
[135] X.F. Zhang, K. Pan, D.S. Weng, C.L. Chen, Q.J. Wang, J.J. Zhao, Q.Z. Pan, Q. Liu, S.S. Jiang, Y.Q. Li, H.X. Zhang, J.C. Xia, Cytotoxic T lymphocyte antigen-4 expression in esophageal carcinoma: implications for prognosis, Oncotarget 7(18) (2016) 26670-9.
[136] G. Santoni, C. Amantini, M.B. Morelli, D. Tomassoni, M. Santoni, O. Marinelli, M. Nabissi, C. Cardinali, V. Paolucci, M. Torniai, S. Rinaldi, F. Morgese, G. Bernardini, R. Berardi, High CTLA-4 expression correlates with poor prognosis in thymoma patients, Oncotarget 9(24) (2018) 16665-16677.
[137] G. Lan, J. Li, Q. Wen, L. Lin, L. Chen, L. Chen, X. Chen, Cytotoxic T lymphocyte associated antigen 4 expression predicts poor prognosis in luminal B HER2-negative breast cancer, Oncology letters 15(4) (2018) 5093-5097.
[138] E.E. Paulsen, T.K. Kilvaer, M. Rakaee, E. Richardsen, S.M. Hald, S. Andersen, L.T. Busund, R.M. Bremnes, T. Donnem, CTLA-4 expression in the non-small cell lung cancer patient tumor microenvironment: diverging

prognostic impact in primary tumors and lymph node metastases, Cancer immunology, immunotherapy : CII 66(11) (2017) 1449-1461.
[139] A. Kahlmeyer, C.G. Stohr, A. Hartmann, P.J. Goebell, B. Wullich, S. Wach, H. Taubert, F. Erlmeier, Expression of PD-1 and CTLA-4 Are Negative Prognostic Markers in Renal Cell Carcinoma, Journal of clinical medicine 8(5) (2019).
[140] A.S. Syed Khaja, S.M. Toor, H. El Salhat, I. Faour, N. Ul Haq, B.R. Ali, E. Elkord, Preferential accumulation of regulatory T cells with highly immunosuppressive characteristics in breast tumor microenvironment, Oncotarget 8(20) (2017) 33159-33171.
[141] A.S. Syed Khaja, S.M. Toor, H. El Salhat, B.R. Ali, E. Elkord, Intratumoral FoxP3(+)Helios(+) Regulatory T Cells Upregulating Immunosuppressive Molecules Are Expanded in Human Colorectal Cancer, Frontiers in immunology 8 (2017) 619.
[142] F. Triebel, K. Hacene, M.F. Pichon, A soluble lymphocyte activation gene-3 (sLAG-3) protein as a prognostic factor in human breast cancer expressing estrogen or progesterone receptors, Cancer letters 235(1) (2006) 147-53.
[143] N. Li, B. Jilisihan, W. Wang, Y. Tang, S. Keyoumu, Soluble LAG3 acts as a potential prognostic marker of gastric cancer and its positive correlation with CD8+T cell frequency and secretion of IL-12 and INF-gamma in peripheral blood, Cancer biomarkers : section A of Disease markers 23(3) (2018) 341-351.
[144] J. Chen, Z. Chen, The effect of immune microenvironment on the progression and prognosis of colorectal cancer, Medical oncology 31(8) (2014) 82.
[145] Q. Ma, J. Liu, G. Wu, M. Teng, S. Wang, M. Cui, Y. Li, Co-expression of LAG3 and TIM3 identifies a potent Treg population that suppresses macrophage functions in colorectal cancer patients, Clinical and experimental pharmacology & physiology 45(10) (2018) 1002-1009.
[146] Y. Que, Z. Fang, Y. Guan, W. Xiao, B. Xu, J. Zhao, H. Chen, X. Zhang, M. Zeng, Y. Liang, X. Zhang, LAG-3 expression on tumor-infiltrating T cells in soft tissue sarcoma correlates with poor survival, Cancer biology & medicine 16(2) (2019) 331-340.
[147] S.J. Lee, S.Y. Jun, I.H. Lee, B.W. Kang, S.Y. Park, H.J. Kim, J.S. Park, G.S. Choi, G. Yoon, J.G. Kim, CD274, LAG3, and IDO1 expressions in tumor-infiltrating immune cells as prognostic biomarker for patients with MSI-high colon cancer, Journal of cancer research and clinical oncology 144(6) (2018) 1005-1014.
[148] S.M. Hald, M. Rakaee, I. Martinez, E. Richardsen, S. Al-Saad, E.E. Paulsen, E.S. Blix, T. Kilvaer, S. Andersen, L.T. Busund, R.M. Bremnes, T. Donnem, LAG-3 in Non-Small-cell Lung Cancer: Expression in Primary Tumors and Metastatic Lymph Nodes Is Associated With Improved Survival, Clinical lung cancer 19(3) (2018) 249-259 e2.
[149] Y. Zhang, Y.D. Liu, Y.L. Luo, B.L. Liu, Q.T. Huang, F. Wang, Q. Zhong, Prognostic Value of Lymphocyte Activation Gene-3 (LAG-3) Expression in Esophageal Squamous Cell Carcinoma, Journal of Cancer 9(22) (2018) 4287-4293.
[150] F.S. Lichtenegger, M. Rothe, F.M. Schnorfeil, K. Deiser, C. Krupka, C. Augsberger, M. Schluter, J. Neitz, M. Subklewe, Targeting LAG-3 and PD-1 to Enhance T Cell Activation by Antigen-Presenting Cells, Frontiers in immunology 9 (2018) 385.
[151] S. Burugu, D. Gao, S. Leung, S.K. Chia, T.O. Nielsen, LAG-3+ tumor infiltrating lymphocytes in breast cancer: clinical correlates and association with PD-1/PD-L1+ tumors, Annals of oncology : official journal of the European Society for Medical Oncology 28(12) (2017) 2977-2984.
[152] G. Bottai, C. Raschioni, A. Losurdo, L. Di Tommaso, C. Tinterri, R. Torrisi, J.S. Reis-Filho, M. Roncalli, C. Sotiriou, A. Santoro, A. Mantovani, S. Loi, L. Santarpia, An immune stratification reveals a subset of PD-1/LAG-3 double-positive triple-negative breast cancers, Breast cancer research : BCR 18(1) (2016) 121.
[153] H. Su, H. Xie, C. Dai, Y. Ren, Y. She, L. Xu, D. Chen, D. Xie, L. Zhang, G. Jiang, C. Chen, Characterization of TIM-3 expression and its prognostic value in patients with surgically resected lung adenocarcinoma, Lung cancer 121 (2018) 18-24.
[154] P.J. Peng, Y. Li, S. Sun, On the significance of Tim-3 expression in pancreatic cancer, Saudi journal of biological sciences 24(8) (2017) 1754-1757.
[155] C. Granier, C. Dariane, P. Combe, V. Verkarre, S. Urien, C. Badoual, H. Roussel, M. Mandavit, P. Ravel, M. Sibony, L. Biard, C. Radulescu, E. Vinatier, N. Benhamouda, M. Peyromaure, S. Oudard, A. Mejean, M.O.

Timsit, A. Gey, E. Tartour, Tim-3 Expression on Tumor-Infiltrating PD-1(+)CD8(+) T Cells Correlates with Poor Clinical Outcome in Renal Cell Carcinoma, Cancer research 77(5) (2017) 1075-1082.
[156] J. Yuan, B. Jiang, H. Zhao, Q. Huang, Prognostic implication of TIM-3 in clear cell renal cell carcinoma, Neoplasma 61(1) (2014) 35-40.
[157] S. Burugu, D. Gao, S. Leung, S.K. Chia, T.O. Nielsen, TIM-3 expression in breast cancer, Oncoimmunology 7(11) (2018) e1502128.
[158] S. Cheng, F.H. , Y.X. , T.Q. , Y.J. , Expression of Tim-3 in breast cancer tissue promotes

tumor progression Int J Clin Exp Pathol 11(3) (2018).
[159] F. Pu, F. Chen, Z. Zhang, X. Qing, H. Lin, L. Zhao, P. Xia, Z. Shao, TIM-3 expression and its association with overall survival in primary osteosarcoma, Oncology letters 18(5) (2019) 5294-5300.
[160] F. Liu, Y. Liu, Z. Chen, Tim-3 expression and its role in hepatocellular carcinoma, Journal of hematology & oncology 11(1) (2018) 126.
[161] M. Yu, B. Lu, Y. Liu, Y. Me, L. Wang, P. Zhang, Tim-3 is upregulated in human colorectal carcinoma and associated with tumor progression, Molecular medicine reports 15(2) (2017) 689-695.
[162] S. Narayanan, T. Kawaguchi, X. Peng, Q. Qi, S. Liu, L. Yan, K. Takabe, Tumor Infiltrating Lymphocytes and Macrophages Improve Survival in Microsatellite Unstable Colorectal Cancer, Scientific reports 9(1) (2019) 13455.
[163] I. Datar, M.F. Sanmamed, J. Wang, B.S. Henick, J. Choi, T. Badri, W. Dong, N. Mani, M. Toki, L.D. Mejias, M.D. Lozano, J.L. Perez-Gracia, V. Velcheti, M.D. Hellmann, J.F. Gainor, K. McEachern, D. Jenkins, K. Syrigos, K. Politi, S. Gettinger, D.L. Rimm, R.S. Herbst, I. Melero, L. Chen, K.A. Schalper, Expression Analysis and Significance of PD-1, LAG-3, and TIM-3 in Human Non-Small Cell Lung Cancer Using Spatially Resolved and Multiparametric Single-Cell Analysis, Clinical cancer research : an official journal of the American Association for Cancer Research 25(15) (2019) 4663-4673.
[164] J. Jiang, M.S. Jin, F. Kong, D. Cao, H.X. Ma, Z. Jia, Y.P. Wang, J. Suo, X. Cao, Decreased galectin-9 and increased Tim-3 expression are related to poor prognosis in gastric cancer, PloS one 8(12) (2013) e81799.
[165] X. Zhou, L. Sun, D. Jing, G. Xu, J. Zhang, L. Lin, J. Zhao, Z. Yao, H. Lin, Galectin-9 Expression Predicts Favorable Clinical Outcome in Solid Tumors: A Systematic Review and Meta-Analysis, Frontiers in physiology 9 (2018) 452.
[166] N.C. Blessin, R. Simon, M. Kluth, K. Fischer, C. Hube-Magg, W. Li, G. Makrypidi-Fraune, B. Wellge, T. Mandelkow, N.F. Debatin, D. Hoflmayer, M. Lennartz, G. Sauter, J.R. Izbicki, S. Minner, F. Buscheck, R. Uhlig, D. Dum, T. Krech, A.M. Luebke, C. Wittmer, F. Jacobsen, E.C. Burandt, S. Steurer, W. Wilczak, A. Hinsch, Patterns of TIGIT Expression in Lymphatic Tissue, Inflammation, and Cancer, Disease markers 2019 (2019) 5160565.
[167] J.M. Chauvin, O. Pagliano, J. Fourcade, Z. Sun, H. Wang, C. Sander, J.M. Kirkwood, T.H. Chen, M. Maurer, A.J. Korman, H.M. Zarour, TIGIT and PD-1 impair tumor antigen-specific CD8(+) T cells in melanoma patients, The Journal of clinical investigation 125(5) (2015) 2046-58.
[168] B. Ma, X. Duan, Q. Zhou, J. Liu, X. Yang, D. Zhang, S. Yang, Y. Du, H. Li, C. Su, Use of aspirin in the prevention of colorectal cancer through TIGIT-CD155 pathway, Journal of cellular and molecular medicine 23(7) (2019) 4514-4522.
[169] X. Duan, J. Liu, J. Cui, B. Ma, Q. Zhou, X. Yang, Z. Lu, Y. Du, C. Su, Expression of TIGIT/CD155 and correlations with clinical pathological features in human hepatocellular carcinoma, Molecular medicine reports 20(4) (2019) 3773-3781.
[170] A. Hinsch, N.C. Blessin, R. Simon, M. Kluth, K. Fischer, C. Hube-Magg, W. Li, G. Makrypidi-Fraune, B. Wellge, T. Mandelkow, N.F. Debatin, D. Hoflmayer, M. Lennartz, G. Sauter, J.R. Izbicki, S. Minner, F. Buscheck, R. Uhlig, D. Dum, T. Krech, A.M. Luebke, C. Wittmer, F. Jacobsen, E. Burandt, S. Steurer, W. Wilczak, Expression of the immune checkpoint receptor TIGIT in seminoma, Oncology letters 18(2) (2019) 1497-1502.
[171] Y. Xu, G. Cui, Z. Jiang, N. Li, X. Zhang, Survival analysis with regard to PD-L1 and CD155 expression in human small cell lung cancer and a comparison with associated receptors, Oncology letters 17(3) (2019) 2960-2968.
[172] J. Wang, K.K. Chong, Y. Nakamura, L. Nguyen, S.K. Huang, C. Kuo, W. Zhang, H. Yu, D.L. Morton, D.S. Hoon, B7-H3 associated with tumor progression and epigenetic regulatory activity in cutaneous melanoma, The Journal of investigative dermatology 133(8) (2013) 2050-8.

[173] X. Zang, R.H. Thompson, H.A. Al-Ahmadie, A.M. Serio, V.E. Reuter, J.A. Eastham, P.T. Scardino, P. Sharma, J.P. Allison, B7-H3 and B7x are highly expressed in human prostate cancer and associated with disease spread and poor outcome, Proceedings of the National Academy of Sciences of the United States of America 104(49) (2007) 19458-63.
[174] V.A. Ingebrigtsen, K. Boye, J.M. Nesland, A. Nesbakken, K. Flatmark, O. Fodstad, B7-H3 expression in colorectal cancer: associations with clinicopathological parameters and patient outcome, BMC cancer 14 (2014) 602.
[175] V.A. Ingebrigtsen, K. Boye, C. Tekle, J.M. Nesland, K. Flatmark, O. Fodstad, B7-H3 expression in colorectal cancer: nuclear localization strongly predicts poor outcome in colon cancer, International journal of cancer 131(11) (2012) 2528-36.
[176] T.W. Sun, Q. Gao, S.J. Qiu, J. Zhou, X.Y. Wang, Y. Yi, J.Y. Shi, Y.F. Xu, Y.H. Shi, K. Song, Y.S. Xiao, J. Fan, B7-H3 is expressed in human hepatocellular carcinoma and is associated with tumor aggressiveness and postoperative recurrence, Cancer immunology, immunotherapy : CII 61(11) (2012) 2171-82.
[177] S. Han, Y. Wang, X. Shi, L. Zong, L. Liu, J. Zhang, Q. Qian, J. Jin, Y. Ma, B. Cui, X. Yang, B. Kong, Y. Zhang, Negative roles of B7-H3 and B7-H4 in the microenvironment of cervical cancer, Experimental cell research 371(1) (2018) 222-230.
[178] L. He, Z. Li, B7-H3 and its role in bone cancers, Pathology, research and practice 215(6) (2019) 152420.
[179] K. Flem-Karlsen, Y. Fodstad, C.E. Nunes-Xavier, B7-H3 immune checkpoint protein in human cancer, Current medicinal chemistry (2019).
[180] C. Xie, D. Liu, Q. Chen, C. Yang, B. Wang, H. Wu, Soluble B7-H3 promotes the invasion and metastasis of pancreatic carcinoma cells through the TLR4/NF-kappaB pathway, Scientific reports 6 (2016) 27528.
[181] K. Flem-Karlsen, O. Fodstad, M. Tan, C.E. Nunes-Xavier, B7-H3 in Cancer - Beyond Immune Regulation, Trends in cancer 4(6) (2018) 401-404.
[182] J. Hu, C. Jiang, M. Zheng, Y. Guo, X. Tang, J. Ren, D. Lu, L. Yu, W. Gan, S. Liu, A. Tong, H. Yang, Overexpression of B7-H3 as an opportunity for targeted therapy in head and neck cancers, American journal of translational research 11(8) (2019) 5183-5196.
[183] C. Chen, Y. Wang, K. Zhong, C. Jiang, L. Wang, Z. Yuan, C. Nie, J. Xu, G. Guo, L. Zhou, M. Yang, A. Tong, Frequent B7-H3 overexpression in craniopharyngioma, Biochemical and biophysical research communications 514(2) (2019) 379-385.
[184] C. Zhang, Z. Zhang, F. Li, Z. Shen, Y. Qiao, L. Li, S. Liu, M. Song, X. Zhao, F. Ren, Q. He, B. Yang, R. Fan, Y. Zhang, Large-scale analysis reveals the specific clinical and immune features of B7-H3 in glioma, Oncoimmunology 7(11) (2018) e1461304.
[185] K. Inamura, G. Amori, T. Yuasa, S. Yamamoto, J. Yonese, Y. Ishikawa, Relationship of B7-H3 expression in tumor cells and tumor vasculature with FOXP3+ regulatory T cells in renal cell carcinoma, Cancer management and research 11 (2019) 7021-7030.
[186] D. Carvajal-Hausdorf, M. Altan, V. Velcheti, S.N. Gettinger, R.S. Herbst, D.L. Rimm, K.A. Schalper, Expression and clinical significance of PD-L1, B7-H3, B7-H4 and TILs in human small cell lung Cancer (SCLC), Journal for immunotherapy of cancer 7(1) (2019) 65.
[187] P.P. Aung, E.R. Parra, S. Barua, D. Sui, J. Ning, B. Mino, D.A. Ledesma, J.L. Curry, P. Nagarajan, C.A. Torres-Cabala, E. Efstathiou, A.G. Hoang, M.K. Wong, J.A. Wargo, A.J. Lazar, A. Rao, V.G. Prieto, I. Wistuba, M.T. Tetzlaff, B7-H3 Expression in Merkel Cell Carcinoma-Associated Endothelial Cells Correlates with Locally Aggressive Primary Tumor Features and Increased Vascular Density, Clinical cancer research : an official journal of the American Association for Cancer Research 25(11) (2019) 3455-3467.
[188] J. Liu, J. Li, M. He, G.L. Zhang, Q. Zhao, Distinct Changes of BTLA and HVEM Expressions in Circulating CD4(+) and CD8(+) T Cells in Hepatocellular Carcinoma Patients, Journal of immunology research 2018 (2018) 4561571.
[189] Q. Zhao, Z.L. Huang, M. He, Z. Gao, D.M. Kuang, BTLA identifies dysfunctional PD-1-expressing CD4(+) T cells in human hepatocellular carcinoma, Oncoimmunology 5(12) (2016) e1254855.
[190] L.G. Radvanyi, C. Bernatchez, M. Zhang, P.S. Fox, P. Miller, J. Chacon, R. Wu, G. Lizee, S. Mahoney, G. Alvarado, M. Glass, V.E. Johnson, J.D. McMannis, E. Shpall, V. Prieto, N. Papadopoulos, K. Kim, J. Homsi, A. Bedikian, W.J. Hwu, S. Patel, M.I. Ross, J.E. Lee, J.E. Gershenwald, A. Lucci, R. Royal, J.N. Cormier, M.A. Davies, R. Mansaray, O.J. Fulbright, C. Toth, R. Ramachandran, S. Wardell, A. Gonzalez, P. Hwu, Specific lymphocyte

subsets predict response to adoptive cell therapy using expanded autologous tumor-infiltrating lymphocytes in metastatic melanoma patients, Clinical cancer research : an official journal of the American Association for Cancer Research 18(24) (2012) 6758-70.
[191] B. Bian, D. Fanale, N. Dusetti, J. Roque, S. Pastor, A.S. Chretien, L. Incorvaia, A. Russo, D. Olive, J. Iovanna, Prognostic significance of circulating PD-1, PD-L1, pan-BTN3As, BTN3A1 and BTLA in patients with pancreatic adenocarcinoma, Oncoimmunology 8(4) (2019) e1561120.
[192] X. Lan, S. Li, H. Gao, A. Nanding, L. Quan, C. Yang, S. Ding, Y. Xue, Increased BTLA and HVEM in gastric cancer are associated with progression and poor prognosis, OncoTargets and therapy 10 (2017) 919-926.
[193] R.B. Holmgaard, D. Zamarin, Y. Li, B. Gasmi, D.H. Munn, J.P. Allison, T. Merghoub, J.D. Wolchok, Tumor-Expressed IDO Recruits and Activates MDSCs in a Treg-Dependent Manner, Cell reports 13(2) (2015) 412-24.
[194] C. Uyttenhove, L. Pilotte, I. Theate, V. Stroobant, D. Colau, N. Parmentier, T. Boon, B.J. Van den Eynde, Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase, Nature medicine 9(10) (2003) 1269-74.
[195] K.A. Schalper, D. Carvajal-Hausdorf, J. McLaughlin, M. Altan, V. Velcheti, P. Gaule, M.F. Sanmamed, L. Chen, R.S. Herbst, D.L. Rimm, Differential Expression and Significance of PD-L1, IDO-1, and B7-H4 in Human Lung Cancer, Clinical cancer research : an official journal of the American Association for Cancer Research 23(2) (2017) 370-378.
[196] H.Q. Liu, B.Q. Zou, S.Y. Wang, [Expression and prognostic values of PD-1, PD-L1 and IDO-1 in sinonasal malignant mucosal melanoma], Zhonghua bing li xue za zhi = Chinese journal of pathology 46(11) (2017) 782-788.
[197] M. Nishi, K. Yoshikawa, J. Higashijima, T. Tokunaga, H. Kashihara, C. Takasu, D. Ishikawa, Y. Wada, M. Shimada, The Impact of Indoleamine 2,3-dioxygenase (IDO) Expression on Stage III Gastric Cancer, Anticancer research 38(6) (2018) 3387-3392.
[198] F. Li, Y. Sun, J. Huang, W. Xu, J. Liu, Z. Yuan, CD4/CD8 + T cells, DC subsets, Foxp3, and IDO expression are predictive indictors of gastric cancer prognosis, Cancer medicine 8(17) (2019) 7330-7344.
[199] Y.F. Wei, C.Y. Chu, C.C. Chang, S.H. Lin, W.C. Su, Y.L. Tseng, C.C. Lin, Y.T. Yen, Different pattern of PD-L1, IDO, and FOXP3 Tregs expression with survival in thymoma and thymic carcinoma, Lung cancer 125 (2018) 35-42.
[200] S.D. Ferdousi Chowdhury, Simon Mitchell,Toby Mellows,Margaret Ashton-Key &Juliet C Gray, PD-L1 and CD8+PD1+ lymphocytes exist as targets in the pediatric tumor microenvironment for immunomodulatory therapy, Oncoimmunology 4(10) (2015).
[201] R.G. Majzner, J.S. Simon, J.F. Grosso, D. Martinez, B.R. Pawel, M. Santi, M.S. Merchant, B. Geoerger, I. Hezam, V. Marty, P. Vielh, M. Daugaard, P.H. Sorensen, C.L. Mackall, J.M. Maris, Assessment of programmed death-ligand 1 expression and tumor-associated immune cells in pediatric cancer tissues, Cancer 123(19) (2017) 3807-3815.
[202] N. Pinto, J.R. Park, E. Murphy, J. Yearley, T. McClanahan, L. Annamalai, D.S. Hawkins, E.R. Rudzinski, Patterns of PD-1, PD-L1, and PD-L2 expression in pediatric solid tumors, Pediatric blood & cancer 64(11) (2017).
[203] T. Aoki, M. Hino, K. Koh, M. Kyushiki, H. Kishimoto, Y. Arakawa, R. Hanada, H. Kawashima, J. Kurihara, N. Shimojo, S. Motohashi, Low Frequency of Programmed Death Ligand 1 Expression in Pediatric Cancers, Pediatric blood & cancer 63(8) (2016) 1461-4.
[204] A. Dondero, F. Pastorino, M. Della Chiesa, M.V. Corrias, F. Morandi, V. Pistoia, D. Olive, F. Bellora, F. Locatelli, A. Castellano, L. Moretta, A. Moretta, C. Bottino, R. Castriconi, PD-L1 expression in metastatic neuroblastoma as an additional mechanism for limiting immune surveillance, Oncoimmunology 5(1) (2016) e1064578.
[205] O. Melaiu, M. Mina, M. Chierici, R. Boldrini, G. Jurman, P. Romania, V. D'Alicandro, M.C. Benedetti, A. Castellano, T. Liu, C. Furlanello, F. Locatelli, D. Fruci, PD-L1 Is a Therapeutic Target of the Bromodomain Inhibitor JQ1 and, Combined with HLA Class I, a Promising Prognostic Biomarker in Neuroblastoma, Clinical cancer research : an official journal of the American Association for Cancer Research 23(15) (2017) 4462-4472.
[206] R. Liao, X.F. Sun, Z.Z. Zhen, D.S. Huang, [Expression and significance of programmed cell death ligand-1 in neuroblastoma tissues], Zhonghua er ke za zhi = Chinese journal of pediatrics 56(10) (2018) 735-740.

[207] R. Boldrini, M.D. De Pasquale, O. Melaiu, M. Chierici, G. Jurman, M.C. Benedetti, N.C. Salfi, A. Castellano, P. Collini, C. Furlanello, V. Pistoia, L. Cifaldi, M. Terenziani, D. Fruci, Tumor-infiltrating T cells and PD-L1 expression in childhood malignant extracranial germ-cell tumors, Oncoimmunology 8(2) (2019) e1542245.
[208] C. Kim, E.K. Kim, H. Jung, H.J. Chon, J.W. Han, K.H. Shin, H. Hu, K.S. Kim, Y.D. Choi, S. Kim, Y.H. Lee, J.S. Suh, J.B. Ahn, H.C. Chung, S.H. Noh, S.Y. Rha, S.H. Kim, H.S. Kim, Prognostic implications of PD-L1 expression in patients with soft tissue sarcoma, BMC cancer 16 (2016) 434.
[209] P. Koirala, M.E. Roth, J. Gill, S. Piperdi, J.M. Chinai, D.S. Geller, B.H. Hoang, A. Park, M.A. Fremed, X. Zang, R. Gorlick, Immune infiltration and PD-L1 expression in the tumor microenvironment are prognostic in osteosarcoma, Scientific reports 6 (2016) 30093.
[210] C. Spurny, S. Kailayangiri, S. Jamitzky, B. Altvater, E. Wardelmann, U. Dirksen, J. Hardes, W. Hartmann, C. Rossig, Programmed cell death ligand 1 (PD-L1) expression is not a predominant feature in Ewing sarcomas, Pediatric blood & cancer 65(1) (2018).
[211] P.R. Gabrycha A, Kuncb M, Krawczyka M, Swieszewskac EI, Biernatb W, Bieńa E, The PD-L1/PD-1 axis expression on tumor-infiltrating immune cells and

tumor cells in pediatric rhabdomyosarcoma, Pathology - Research and Practice

215

(12

) (2019) 6.
[212] G. Bertolini, L. Bergamaschi, A. Ferrari, S.L. Renne, P. Collini, C. Gardelli, M. Barisella, G. Centonze, S. Chiaravalli, C. Paolino, M. Milione, M. Massimino, M. Casanova, P. Gasparini, PD-L1 assessment in pediatric rhabdomyosarcoma: a pilot study, BMC cancer 18(1) (2018) 652.
[213] Y. Yao, R. Tao, X. Wang, Y. Wang, Y. Mao, L.F. Zhou, B7-H1 is correlated with malignancy-grade gliomas but is not expressed exclusively on tumor stem-like cells, Neuro-oncology 11(6) (2009) 757-66.
[214] A.M. Martin, C.J. Nirschl, M.J. Polanczyk, W.R. Bell, T.R. Nirschl, S. Harris-Bookman, J. Phallen, J. Hicks, D. Martinez, A. Ogurtsova, H. Xu, L.M. Sullivan, A.K. Meeker, E.H. Raabe, K.J. Cohen, C.G. Eberhart, P.C. Burger, M. Santi, J.M. Taube, D.M. Pardoll, C.G. Drake, M. Lim, PD-L1 expression in medulloblastoma: an evaluation by subgroup, Oncotarget 9(27) (2018) 19177-19191.
[215] J.F. Vermeulen, W. Van Hecke, E.J.M. Adriaansen, M.K. Jansen, R.G. Bouma, J. Villacorta Hidalgo, P. Fisch, R. Broekhuizen, W.G.M. Spliet, M. Kool, N. Bovenschen, Prognostic relevance of tumor-infiltrating lymphocytes and immune checkpoints in pediatric medulloblastoma, Oncoimmunology 7(3) (2018) e1398877.
[216] S.J. Nam, Y.H. Kim, J.E. Park, Y.S. Ra, S.K. Khang, Y.H. Cho, J.H. Kim, C.O. Sung, Tumor-infiltrating immune cell subpopulations and programmed death ligand 1 (PD-L1) expression associated with clinicopathological and prognostic parameters in ependymoma, Cancer immunology, immunotherapy : CII 68(2) (2019) 305-318.
[217] D.A. Witt, A.M. Donson, V. Amani, D.C. Moreira, B. Sanford, L.M. Hoffman, M.H. Handler, J.M.M. Levy, K.L. Jones, A. Nellan, N.K. Foreman, A.M. Griesinger, Specific expression of PD-L1 in RELA-fusion supratentorial ependymoma: Implications for PD-1-targeted therapy, Pediatric blood & cancer 65(5) (2018) e26960.
[218] D.M. Lussier, L. O'Neill, L.M. Nieves, M.S. McAfee, S.A. Holechek, A.W. Collins, P. Dickman, J. Jacobsen, P. Hingorani, J.N. Blattman, Enhanced T-cell immunity to osteosarcoma through antibody blockade of PD-1/PD-L1 interactions, Journal of immunotherapy 38(3) (2015) 96-106.
[219] E. Contardi, G.L. Palmisano, P.L. Tazzari, A.M. Martelli, F. Fala, M. Fabbi, T. Kato, E. Lucarelli, D. Donati, L. Polito, A. Bolognesi, F. Ricci, S. Salvi, V. Gargaglione, S. Mantero, M. Alberghini, G.B. Ferrara, M.P. Pistillo, CTLA-4 is constitutively expressed on tumor cells and can trigger apoptosis upon ligand interaction, International journal of cancer 117(4) (2005) 538-50.
[220] F. Morandi, S. Pozzi, S. Barco, G. Cangemi, L. Amoroso, B. Carlini, V. Pistoia, M.V. Corrias, CD4(+)CD25(hi)CD127(-) Treg and CD4(+)CD45R0(+)CD49b(+)LAG3(+) Tr1 cells in bone marrow and peripheral blood samples from children with neuroblastoma, Oncoimmunology 5(12) (2016) e1249553.
[221] K. Mochizuki, S. Kawana, S. Yamada, M. Muramatsu, H. Sano, S. Kobayashi, Y. Ohara, N. Takahashi, M. Hakozaki, H. Yamada, Y. Hashimoto, A. Kikuta, Various checkpoint molecules, and tumor-infiltrating

lymphocytes in common pediatric solid tumors: Possibilities for novel immunotherapy, Pediatric hematology and oncology 36(1) (2019) 17-27.
[222] W. Ge, J. Li, W. Fan, D. Xu, S. Sun, Tim-3 as a diagnostic and prognostic biomarker of osteosarcoma, Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 39(7) (2017) 1010428317715643.
[223] H. Liu, L. Zhi, N. Duan, P. Su, Abnormal expression of Tim-3 antigen on peripheral blood T cells is associated with progressive disease in osteosarcoma patients, FEBS open bio 6(8) (2016) 807-15.
[224] Z. Zhou, N. Luther, G.M. Ibrahim, C. Hawkins, R. Vibhakar, M.H. Handler, M.M. Souweidane, B7-H3, a potential therapeutic target, is expressed in diffuse intrinsic pontine glioma, Journal of neuro-oncology 111(3) (2013) 257-64.
[225] L. Wang, Q. Zhang, W. Chen, B. Shan, Y. Ding, G. Zhang, N. Cao, L. Liu, Y. Zhang, B7-H3 is overexpressed in patients suffering osteosarcoma and associated with tumor aggressiveness and metastasis, PloS one 8(8) (2013) e70689.
[226] R.G. Majzner, J.L. Theruvath, A. Nellan, S. Heitzeneder, Y. Cui, C.W. Mount, S.P. Rietberg, M.H. Linde, P. Xu, C. Rota, E. Sotillo, L. Labanieh, D.W. Lee, R.J. Orentas, D.S. Dimitrov, Z. Zhu, B.S. Croix, A. Delaidelli, A. Sekunova, E. Bonvini, S.S. Mitra, M.M. Quezado, R. Majeti, M. Monje, P.H.B. Sorensen, J.M. Maris, C.L. Mackall, CAR T Cells Targeting B7-H3, a Pan-Cancer Antigen, Demonstrate Potent Preclinical Activity Against Pediatric Solid Tumors and Brain Tumors, Clinical cancer research : an official journal of the American Association for Cancer Research 25(8) (2019) 2560-2574.
[227] C.E. Batista, C. Juhasz, O. Muzik, W.J. Kupsky, G. Barger, H.T. Chugani, S. Mittal, S. Sood, P.K. Chakraborty, D.C. Chugani, Imaging correlates of differential expression of indoleamine 2,3-dioxygenase in human brain tumors, Molecular imaging and biology 11(6) (2009) 460-6.
[228] H. Urakawa, Y. Nishida, H. Nakashima, Y. Shimoyama, S. Nakamura, N. Ishiguro, Prognostic value of indoleamine 2,3-dioxygenase expression in high grade osteosarcoma, Clinical & experimental metastasis 26(8) (2009) 1005-12.
[229] B. Olson, Y. Li, Y. Lin, E.T. Liu, A. Patnaik, Mouse Models for Cancer Immunotherapy Research, Cancer Discov 8(11) (2018) 1358-1365.
[230] B. Homet Moreno, J.M. Zaretsky, A. Garcia-Diaz, J. Tsoi, G. Parisi, L. Robert, K. Meeth, A. Ndoye, M. Bosenberg, A.T. Weeraratna, T.G. Graeber, B. Comin-Anduix, S. Hu-Lieskovan, A. Ribas, Response to Programmed Cell Death-1 Blockade in a Murine Melanoma Syngeneic Model Requires Costimulation, CD4, and CD8 T Cells, Cancer immunology research 4(10) (2016) 845-857.
[231] M. Feng, G. Xiong, Z. Cao, G. Yang, S. Zheng, X. Song, L. You, L. Zheng, T. Zhang, Y. Zhao, PD-1/PD-L1 and immunotherapy for pancreatic cancer, Cancer letters 407 (2017) 57-65.
[232] J. Duraiswamy, G.J. Freeman, G. Coukos, Therapeutic PD-1 pathway blockade augments with other modalities of immunotherapy T-cell function to prevent immune decline in ovarian cancer, Cancer research 73(23) (2013) 6900-12.
[233] Z.A. Cooper, V.R. Juneja, P.T. Sage, D.T. Frederick, A. Piris, D. Mitra, J.A. Lo, F.S. Hodi, G.J. Freeman, M.W. Bosenberg, M. McMahon, K.T. Flaherty, D.E. Fisher, A.H. Sharpe, J.A. Wargo, Response to BRAF inhibition in melanoma is enhanced when combined with immune checkpoint blockade, Cancer immunology research 2(7) (2014) 643-54.
[234] H.S. Sow, J. Ren, M. Camps, F. Ossendorp, P. Ten Dijke, Combined Inhibition of TGF-beta Signaling and the PD-L1 Immune Checkpoint Is Differentially Effective in Tumor Models, Cells 8(4) (2019).
[235] S. Mariathasan, S.J. Turley, D. Nickles, A. Castiglioni, K. Yuen, Y. Wang, E.E. Kadel, III, H. Koeppen, J.L. Astarita, R. Cubas, S. Jhunjhunwala, R. Banchereau, Y. Yang, Y. Guan, C. Chalouni, J. Ziai, Y. Senbabaoglu, S. Santoro, D. Sheinson, J. Hung, J.M. Giltnane, A.A. Pierce, K. Mesh, S. Lianoglou, J. Riegler, R.A.D. Carano, P. Eriksson, M. Hoglund, L. Somarriba, D.L. Halligan, M.S. van der Heijden, Y. Loriot, J.E. Rosenberg, L. Fong, I. Mellman, D.S. Chen, M. Green, C. Derleth, G.D. Fine, P.S. Hegde, R. Bourgon, T. Powles, TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells, Nature 554(7693) (2018) 544-548.
[236] D.R. Leach, M.F. Krummel, J.P. Allison, Enhancement of antitumor immunity by CTLA-4 blockade, Science 271(5256) (1996) 1734-6.
[237] J.F. Grosso, M.N. Jure-Kunkel, CTLA-4 blockade in tumor models: an overview of preclinical and translational research, Cancer immunity 13 (2013) 5.

[238] E.J. Lipson, C.G. Drake, Ipilimumab: an anti-CTLA-4 antibody for metastatic melanoma, Clinical cancer research : an official journal of the American Association for Cancer Research 17(22) (2011) 6958-62.
[239] Y. Bulliard, R. Jolicoeur, M. Windman, S.M. Rue, S. Ettenberg, D.A. Knee, N.S. Wilson, G. Dranoff, J.L. Brogdon, Activating Fc gamma receptors contribute to the antitumor activities of immunoregulatory receptor-targeting antibodies, The Journal of experimental medicine 210(9) (2013) 1685-93.
[240] M.J. Selby, J.J. Engelhardt, M. Quigley, K.A. Henning, T. Chen, M. Srinivasan, A.J. Korman, Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells, Cancer immunology research 1(1) (2013) 32-42.
[241] D. Ha, A. Tanaka, T. Kibayashi, A. Tanemura, D. Sugiyama, J.B. Wing, E.L. Lim, K.W.W. Teng, D. Adeegbe, E.W. Newell, I. Katayama, H. Nishikawa, S. Sakaguchi, Differential control of human Treg and effector T cells in tumor immunity by Fc-engineered anti-CTLA-4 antibody, Proceedings of the National Academy of Sciences of the United States of America 116(2) (2019) 609-618.
[242] J.A. Seidel, A. Otsuka, K. Kabashima, Anti-PD-1 and Anti-CTLA-4 Therapies in Cancer: Mechanisms of Action, Efficacy, and Limitations, Frontiers in oncology 8 (2018) 86.
[243] D.A. Wainwright, A.L. Chang, M. Dey, I.V. Balyasnikova, C.K. Kim, A. Tobias, Y. Cheng, J.W. Kim, J. Qiao, L. Zhang, Y. Han, M.S. Lesniak, Durable therapeutic efficacy utilizing combinatorial blockade against IDO, CTLA-4, and PD-L1 in mice with brain tumors, Clinical cancer research : an official journal of the American Association for Cancer Research 20(20) (2014) 5290-301.
[244] D.A. Reardon, P.C. Gokhale, S.R. Klein, K.L. Ligon, S.J. Rodig, S.H. Ramkissoon, K.L. Jones, A.S. Conway, X. Liao, J. Zhou, P.Y. Wen, A.D. Van Den Abbeele, F.S. Hodi, L. Qin, N.E. Kohl, A.H. Sharpe, G. Dranoff, G.J. Freeman, Glioblastoma Eradication Following Immune Checkpoint Blockade in an Orthotopic, Immunocompetent Model, Cancer immunology research 4(2) (2016) 124-35.
[245] A. Rotte, Combination of CTLA-4 and PD-1 blockers for treatment of cancer, Journal of experimental & clinical cancer research : CR 38(1) (2019) 255.
[246] A. Rotte, J.Y. Jin, V. Lemaire, Mechanistic overview of immune checkpoints to support the rational design of their combinations in cancer immunotherapy, Annals of oncology : official journal of the European Society for Medical Oncology 29(1) (2018) 71-83.
[247] S. Harris-Bookman, D. Mathios, A.M. Martin, Y. Xia, E. Kim, H. Xu, Z. Belcaid, M. Polanczyk, T. Barberi, D. Theodros, J. Kim, J.M. Taube, P.C. Burger, M. Selby, C. Taitt, A. Korman, X. Ye, C.G. Drake, H. Brem, D.M. Pardoll, M. Lim, Expression of LAG-3 and efficacy of combination treatment with anti-LAG-3 and anti-PD-1 monoclonal antibodies in glioblastoma, International journal of cancer 143(12) (2018) 3201-3208.
[248] S.R. Woo, M.E. Turnis, M.V. Goldberg, J. Bankoti, M. Selby, C.J. Nirschl, M.L. Bettini, D.M. Gravano, P. Vogel, C.L. Liu, S. Tangsombatvisit, J.F. Grosso, G. Netto, M.P. Smeltzer, A. Chaux, P.J. Utz, C.J. Workman, D.M. Pardoll, A.J. Korman, C.G. Drake, D.A. Vignali, Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape, Cancer research 72(4) (2012) 917-27.
[249] J. Matsuzaki, S. Gnjatic, P. Mhawech-Fauceglia, A. Beck, A. Miller, T. Tsuji, C. Eppolito, F. Qian, S. Lele, P. Shrikant, L.J. Old, K. Odunsi, Tumor-infiltrating NY-ESO-1-specific CD8+ T cells are negatively regulated by LAG-3 and PD-1 in human ovarian cancer, Proceedings of the National Academy of Sciences of the United States of America 107(17) (2010) 7875-80.
[250] S.F. Ngiow, B. von Scheidt, H. Akiba, H. Yagita, M.W. Teng, M.J. Smyth, Anti-TIM3 antibody promotes T cell IFN-gamma-mediated antitumor immunity and suppresses established tumors, Cancer research 71(10) (2011) 3540-51.
[251] J.F. Liu, L. Wu, L.L. Yang, W.W. Deng, L. Mao, H. Wu, W.F. Zhang, Z.J. Sun, Blockade of TIM3 relieves immunosuppression through reducing regulatory T cells in head and neck cancer, Journal of experimental & clinical cancer research : CR 37(1) (2018) 44.
[252] J.F. Liu, S.R. Ma, L. Mao, L.L. Bu, G.T. Yu, Y.C. Li, C.F. Huang, W.W. Deng, A.B. Kulkarni, W.F. Zhang, Z.J. Sun, T-cell immunoglobulin mucin 3 blockade drives an antitumor immune response in head and neck cancer, Molecular oncology 11(2) (2017) 235-247.
[253] J.E. Kim, M.A. Patel, A. Mangraviti, E.S. Kim, D. Theodros, E. Velarde, A. Liu, E.W. Sankey, A. Tam, H. Xu, D. Mathios, C.M. Jackson, S. Harris-Bookman, T. Garzon-Muvdi, M. Sheu, A.M. Martin, B.M. Tyler, P.T. Tran, X. Ye, A. Olivi, J.M. Taube, P.C. Burger, C.G. Drake, H. Brem, D.M. Pardoll, M. Lim, Combination Therapy with

Anti-PD-1, Anti-TIM-3, and Focal Radiation Results in Regression of Murine Gliomas, Clinical cancer research : an official journal of the American Association for Cancer Research 23(1) (2017) 124-136.
[254] K. Sakuishi, L. Apetoh, J.M. Sullivan, B.R. Blazar, V.K. Kuchroo, A.C. Anderson, Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity, The Journal of experimental medicine 207(10) (2010) 2187-94.
[255] L. Wu, L. Mao, J.F. Liu, L. Chen, G.T. Yu, L.L. Yang, H. Wu, L.L. Bu, A.B. Kulkarni, W.F. Zhang, Z.J. Sun, Blockade of TIGIT/CD155 Signaling Reverses T-cell Exhaustion and Enhances Antitumor Capability in Head and Neck Squamous Cell Carcinoma, Cancer immunology research 7(10) (2019) 1700-1713.
[256] A.L. Hung, R. Maxwell, D. Theodros, Z. Belcaid, D. Mathios, A.S. Luksik, E. Kim, A. Wu, Y. Xia, T. Garzon-Muvdi, C. Jackson, X. Ye, B. Tyler, M. Selby, A. Korman, B. Barnhart, S.M. Park, J.I. Youn, T. Chowdhury, C.K. Park, H. Brem, D.M. Pardoll, M. Lim, TIGIT and PD-1 dual checkpoint blockade enhances antitumor immunity and survival in GBM, Oncoimmunology 7(8) (2018) e1466769.
[257] Q. Zhang, J. Bi, X. Zheng, Y. Chen, H. Wang, W. Wu, Z. Wang, Q. Wu, H. Peng, H. Wei, R. Sun, Z. Tian, Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity, Nature immunology 19(7) (2018) 723-732.
[258] O. Melaiu, V. Lucarini, L. Cifaldi, D. Fruci, Influence of the tumor microenvironment on NK cell function in solid tumors, Front. Immunol. (2020).
[259] J. Shi, D.L. Zhang, Z.C. Cui, H.M. Wang, Preparation and application of a novel monoclonal antibody specific for human B7-H3, Molecular medicine reports 14(1) (2016) 943-8.
[260] K. Flem-Karlsen, C. Tekle, Y. Andersson, K. Flatmark, O. Fodstad, C.E. Nunes-Xavier, Immunoregulatory protein B7-H3 promotes growth and decreases sensitivity to therapy in metastatic melanoma cells, Pigment cell & melanoma research 30(5) (2017) 467-476.
[261] H. Liu, C. Tekle, Y.W. Chen, A. Kristian, Y. Zhao, M. Zhou, Z. Liu, Y. Ding, B. Wang, G.M. Maelandsmo, J.M. Nesland, O. Fodstad, M. Tan, B7-H3 silencing increases paclitaxel sensitivity by abrogating Jak2/Stat3 phosphorylation, Molecular cancer therapeutics 10(6) (2011) 960-71.
[262] B.B. Kasten, R.C. Arend, A.A. Katre, H. Kim, J. Fan, S. Ferrone, K.R. Zinn, D.J. Buchsbaum, B7-H3-targeted (212)Pb radioimmunotherapy of ovarian cancer in preclinical models, Nuclear medicine and biology 47 (2017) 23-30.
[263] D. Sekar, L. Govene, M.L. Del Rio, E. Sirait-Fischer, A.F. Fink, B. Brune, J.I. Rodriguez-Barbosa, A. Weigert, Downregulation of BTLA on NKT Cells Promotes Tumor Immune Control in a Mouse Model of Mammary Carcinoma, International journal of molecular sciences 19(3) (2018).
[264] Z.J. Brown, S.J. Yu, B. Heinrich, C. Ma, Q. Fu, M. Sandhu, D. Agdashian, Q. Zhang, F. Korangy, T.F. Greten, Indoleamine 2,3-dioxygenase provides adaptive resistance to immune checkpoint inhibitors in hepatocellular carcinoma, Cancer immunology, immunotherapy : CII 67(8) (2018) 1305-1315.
[265] S. Spranger, H.K. Koblish, B. Horton, P.A. Scherle, R. Newton, T.F. Gajewski, Mechanism of tumor rejection with doublets of CTLA-4, PD-1/PD-L1, or IDO blockade involves restored IL-2 production and proliferation of CD8(+) T cells directly within the tumor microenvironment, Journal for immunotherapy of cancer 2 (2014) 3.
[266] L.P. Andrews, H. Yano, D.A.A. Vignali, Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups, Nature immunology 20(11) (2019) 1425-1434.
[267] A.M. D'Alise, G. Leoni, G. Cotugno, F. Troise, F. Langone, I. Fichera, M. De Lucia, L. Avalle, R. Vitale, A. Leuzzi, V. Bignone, E. Di Matteo, F.G. Tucci, V. Poli, A. Lahm, M.T. Catanese, A. Folgori, S. Colloca, A. Nicosia, E. Scarselli, Adenoviral vaccine targeting multiple neoantigens as strategy to eradicate large tumors combined with checkpoint blockade, Nature communications 10(1) (2019) 2688.
[268] C.D. Pham, C. Flores, C. Yang, E.M. Pinheiro, J.H. Yearley, E.J. Sayour, Y. Pei, C. Moore, R.E. McLendon, J. Huang, J.H. Sampson, R. Wechsler-Reya, D.A. Mitchell, Differential Immune Microenvironments and Response to Immune Checkpoint Blockade among Molecular Subtypes of Murine Medulloblastoma, Clinical cancer research : an official journal of the American Association for Cancer Research 22(3) (2016) 582-95.
[269] Y. Mao, N. Eissler, K.L. Blanc, J.I. Johnsen, P. Kogner, R. Kiessling, Targeting Suppressive Myeloid Cells Potentiates Checkpoint Inhibitors to Control Spontaneous Neuroblastoma, Clinical cancer research : an official journal of the American Association for Cancer Research 22(15) (2016) 3849-59.

[270] N. Eissler, Y. Mao, D. Brodin, P. Reutersward, H. Andersson Svahn, J.I. Johnsen, R. Kiessling, P. Kogner, Regulation of myeloid cells by activated T cells determines the efficacy of PD-1 blockade, Oncoimmunology 5(12) (2016) e1232222.
[271] N. Siebert, M. Zumpe, M. Juttner, S. Troschke-Meurer, H.N. Lode, PD-1 blockade augments anti-neuroblastoma immune response induced by anti-GD2 antibody ch14.18/CHO, Oncoimmunology 6(10) (2017) e1343775.
[272] V. Rigo, L. Emionite, A. Daga, S. Astigiano, M.V. Corrias, C. Quintarelli, F. Locatelli, S. Ferrini, M. Croce, Combined immunotherapy with anti-PDL-1/PD-1 and anti-CD4 antibodies cures syngeneic disseminated neuroblastoma, Scientific reports 7(1) (2017) 14049.
[273] A. Eranki, P. Srinivasan, M. Ries, A. Kim, C.A. Lazarski, C.T. Rossi, T.D. Khokhlova, E. Wilson, S.M. Knoblach, K.V. Sharma, B.J. Wood, C. Moonen, A.D. Sandler, P.C.W. Kim, High-Intensity Focused Ultrasound (HIFU) Triggers Immune Sensitization of Refractory Murine Neuroblastoma to Checkpoint Inhibitor Therapy, Clinical cancer research : an official journal of the American Association for Cancer Research (2019).
[274] H. Ledford, H. Else, M. Warren, Cancer immunologists scoop medicine Nobel prize, Nature 562(7725) (2018) 20-21.
[275] F.S. Hodi, S.J. O'Day, D.F. McDermott, R.W. Weber, J.A. Sosman, J.B. Haanen, R. Gonzalez, C. Robert, D. Schadendorf, J.C. Hassel, W. Akerley, A.J. van den Eertwegh, J. Lutzky, P. Lorigan, J.M. Vaubel, G.P. Linette, D. Hogg, C.H. Ottensmeier, C. Lebbe, C. Peschel, I. Quirt, J.I. Clark, J.D. Wolchok, J.S. Weber, J. Tian, M.J. Yellin, G.M. Nichol, A. Hoos, W.J. Urba, Improved survival with ipilimumab in patients with metastatic melanoma, The New England journal of medicine 363(8) (2010) 711-23.
[276] D. Schadendorf, F.S. Hodi, C. Robert, J.S. Weber, K. Margolin, O. Hamid, D. Patt, T.T. Chen, D.M. Berman, J.D. Wolchok, Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma, Journal of clinical oncology : official journal of the American Society of Clinical Oncology 33(17) (2015) 1889-94.
[277] G. Della Vittoria Scarpati, C. Fusciello, F. Perri, F. Sabbatino, S. Ferrone, C. Carlomagno, S. Pepe, Ipilimumab in the treatment of metastatic melanoma: management of adverse events, OncoTargets and therapy 7 (2014) 203-9.
[278] J. Gong, A. Chehrazi-Raffle, S. Reddi, R. Salgia, Development of PD-1 and PD-L1 inhibitors as a form of cancer immunotherapy: a comprehensive review of registration trials and future considerations, Journal for immunotherapy of cancer 6(1) (2018) 8.
[279] J.R. Brahmer, S.S. Tykodi, L.Q. Chow, W.J. Hwu, S.L. Topalian, P. Hwu, C.G. Drake, L.H. Camacho, J. Kauh, K. Odunsi, H.C. Pitot, O. Hamid, S. Bhatia, R. Martins, K. Eaton, S. Chen, T.M. Salay, S. Alaparthy, J.F. Grosso, A.J. Korman, S.M. Parker, S. Agrawal, S.M. Goldberg, D.M. Pardoll, A. Gupta, J.M. Wigginton, Safety and activity of anti-PD-L1 antibody in patients with advanced cancer, The New England journal of medicine 366(26) (2012) 2455-65.
[280] A. Rittmeyer, F. Barlesi, D. Waterkamp, K. Park, F. Ciardiello, J. von Pawel, S.M. Gadgeel, T. Hida, D.M. Kowalski, M.C. Dols, D.L. Cortinovis, J. Leach, J. Polikoff, C. Barrios, F. Kabbinavar, O.A. Frontera, F. De Marinis, H. Turna, J.S. Lee, M. Ballinger, M. Kowanetz, P. He, D.S. Chen, A. Sandler, D.R. Gandara, O.A.K.S. Group, Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial, Lancet 389(10066) (2017) 255-265.
[281] A.V. Balar, M.D. Galsky, J.E. Rosenberg, T. Powles, D.P. Petrylak, J. Bellmunt, Y. Loriot, A. Necchi, J. Hoffman-Censits, J.L. Perez-Gracia, N.A. Dawson, M.S. van der Heijden, R. Dreicer, S. Srinivas, M.M. Retz, R.W. Joseph, A. Drakaki, U.N. Vaishampayan, S.S. Sridhar, D.I. Quinn, I. Duran, D.R. Shaffer, B.J. Eigl, P.D. Grivas, E.Y. Yu, S. Li, E.E. Kadel, 3rd, Z. Boyd, R. Bourgon, P.S. Hegde, S. Mariathasan, A. Thastrom, O.O. Abidoye, G.D. Fine, D.F. Bajorin, I.M.S. Group, Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial, Lancet 389(10064) (2017) 67-76.
[282] R. Hassan, A. Thomas, J.J. Nemunaitis, M.R. Patel, J. Bennouna, F.L. Chen, J.P. Delord, A. Dowlati, S.T. Kochuparambil, M.H. Taylor, J.D. Powderly, U.N. Vaishampayan, C. Verschraegen, H.J. Grote, A. von Heydebreck, K. Chin, J.L. Gulley, Efficacy and Safety of Avelumab Treatment in Patients With Advanced Unresectable Mesothelioma: Phase 1b Results From the JAVELIN Solid Tumor Trial, JAMA Oncol 5(3) (2019) 351-357.

[283] J.L. Gulley, A. Rajan, D.R. Spigel, N. Iannotti, J. Chandler, D.J.L. Wong, J. Leach, W.J. Edenfield, D. Wang, H.J. Grote, A.V. Heydebreck, K. Chin, J.M. Cuillerot, K. Kelly, Avelumab for patients with previously treated metastatic or recurrent non-small-cell lung cancer (JAVELIN Solid Tumor): dose-expansion cohort of a multicentre, open-label, phase 1b trial, The Lancet. Oncology 18(5) (2017) 599-610.
[284] M.R. Migden, N.I. Khushalani, A.L.S. Chang, K.D. Lewis, C.D. Schmults, L. Hernandez-Aya, F. Meier, D. Schadendorf, A. Guminski, A. Hauschild, D.J. Wong, G.A. Daniels, C. Berking, V. Jankovic, E. Stankevich, J. Booth, S. Li, D.M. Weinreich, G.D. Yancopoulos, I. Lowy, M.G. Fury, D. Rischin, Cemiplimab in locally advanced cutaneous squamous cell carcinoma: results from an open-label, phase 2, single-arm trial, The Lancet. Oncology 21(2) (2020) 294-305.
[285] C. Robert, J. Schachter, G.V. Long, A. Arance, J.J. Grob, L. Mortier, A. Daud, M.S. Carlino, C. McNeil, M. Lotem, J. Larkin, P. Lorigan, B. Neyns, C.U. Blank, O. Hamid, C. Mateus, R. Shapira-Frommer, M. Kosh, H. Zhou, N. Ibrahim, S. Ebbinghaus, A. Ribas, K.-. investigators, Pembrolizumab versus Ipilimumab in Advanced Melanoma, The New England journal of medicine 372(26) (2015) 2521-32.
[286] N.P. Restifo, M.J. Smyth, A. Snyder, Acquired resistance to immunotherapy and future challenges, Nature reviews. Cancer 16(2) (2016) 121-6.
[287] P. Sharma, S. Hu-Lieskovan, J.A. Wargo, A. Ribas, Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy, Cell 168(4) (2017) 707-723.
[288] K. Ganesh, Z.K. Stadler, A. Cercek, R.B. Mendelsohn, J. Shia, N.H. Segal, L.A. Diaz, Jr., Immunotherapy in colorectal cancer: rationale, challenges and potential, Nature reviews. Gastroenterology & hepatology 16(6) (2019) 361-375.
[289] Y.J. Park, D.S. Kuen, Y. Chung, Future prospects of immune checkpoint blockade in cancer: from response prediction to overcoming resistance, Experimental & molecular medicine 50(8) (2018) 109.
[290] E.A. Reits, J.W. Hodge, C.A. Herberts, T.A. Groothuis, M. Chakraborty, E.K. Wansley, K. Camphausen, R.M. Luiten, A.H. de Ru, J. Neijssen, A. Griekspoor, E. Mesman, F.A. Verreck, H. Spits, J. Schlom, P. van Veelen, J.J. Neefjes, Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy, The Journal of experimental medicine 203(5) (2006) 1259-71.
[291] K.W. Mouw, J.M. Cleary, B. Reardon, J. Pike, L.Z. Braunstein, J. Kim, A. Amin-Mansour, D. Miao, A. Damish, J. Chin, P.A. Ott, C.S. Fuchs, N.E. Martin, G. Getz, S. Carter, H.J. Mamon, J.L. Hornick, E.M. Van Allen, A.D. D'Andrea, Genomic Evolution after Chemoradiotherapy in Anal Squamous Cell Carcinoma, Clinical cancer research : an official journal of the American Association for Cancer Research 23(12) (2017) 3214-3222.
[292] L.R.G. Pike, A. Bang, B.A. Mahal, A. Taylor, M. Krishnan, A. Spektor, D.N. Cagney, A.A. Aizer, B.M. Alexander, O. Rahma, T. Balboni, P.A. Ott, F.S. Hodi, J.D. Schoenfeld, The Impact of Radiation Therapy on Lymphocyte Count and Survival in Metastatic Cancer Patients Receiving PD-1 Immune Checkpoint Inhibitors, International journal of radiation oncology, biology, physics 103(1) (2019) 142-151.
[293] C. Tang, J.W. Welsh, P. de Groot, E. Massarelli, J.Y. Chang, K.R. Hess, S. Basu, M.A. Curran, M.E. Cabanillas, V. Subbiah, S. Fu, A.M. Tsimberidou, D. Karp, D.R. Gomez, A. Diab, R. Komaki, J.V. Heymach, P. Sharma, A. Naing, D.S. Hong, Ipilimumab with Stereotactic Ablative Radiation Therapy: Phase I Results and Immunologic Correlates from Peripheral T Cells, Clinical cancer research : an official journal of the American Association for Cancer Research 23(6) (2017) 1388-1396.
[294] N. Shaverdian, A.E. Lisberg, K. Bornazyan, D. Veruttipong, J.W. Goldman, S.C. Formenti, E.B. Garon, P. Lee, Previous radiotherapy and the clinical activity and toxicity of pembrolizumab in the treatment of non-small-cell lung cancer: a secondary analysis of the KEYNOTE-001 phase 1 trial, The Lancet. Oncology 18(7) (2017) 895-903.
[295] C. Twyman-Saint Victor, A.J. Rech, A. Maity, R. Rengan, K.E. Pauken, E. Stelekati, J.L. Benci, B. Xu, H. Dada, P.M. Odorizzi, R.S. Herati, K.D. Mansfield, D. Patsch, R.K. Amaravadi, L.M. Schuchter, H. Ishwaran, R. Mick, D.A. Pryma, X. Xu, M.D. Feldman, T.C. Gangadhar, S.M. Hahn, E.J. Wherry, R.H. Vonderheide, A.J. Minn, Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer, Nature 520(7547) (2015) 373-7.
[296] C. Grassberger, S.G. Ellsworth, M.Q. Wilks, F.K. Keane, J.S. Loeffler, Assessing the interactions between radiotherapy and antitumour immunity, Nature reviews. Clinical oncology 16(12) (2019) 729-745.

[297] Y. Zhou, C. Chen, X. Zhang, S. Fu, C. Xue, Y. Ma, W. Fang, Y. Yang, X. Hou, Y. Huang, H. Zhao, S. Hong, L. Zhang, Immune-checkpoint inhibitor plus chemotherapy versus conventional chemotherapy for first-line treatment in advanced non-small cell lung carcinoma: a systematic review and meta-analysis, Journal for immunotherapy of cancer 6(1) (2018) 155.
[298] J.D. Cramer, B. Burtness, R.L. Ferris, Immunotherapy for head and neck cancer: Recent advances and future directions, Oral oncology 99 (2019) 104460.
[299] L. Horn, A.S. Mansfield, A. Szczesna, L. Havel, M. Krzakowski, M.J. Hochmair, F. Huemer, G. Losonczy, M.L. Johnson, M. Nishio, M. Reck, T. Mok, S. Lam, D.S. Shames, J. Liu, B. Ding, A. Lopez-Chavez, F. Kabbinavar, W. Lin, A. Sandler, S.V. Liu, I.M.S. Group, First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer, The New England journal of medicine 379(23) (2018) 2220-2229.
[300] R.S. Finn, S. Qin, M. Ikeda, P.R. Galle, M. Ducreux, T.Y. Kim, M. Kudo, V. Breder, P. Merle, A.O. Kaseb, D. Li, W. Verret, D.Z. Xu, S. Hernandez, J. Liu, C. Huang, S. Mulla, Y. Wang, H.Y. Lim, A.X. Zhu, A.L. Cheng, I.M. Investigators, Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma, The New England journal of medicine 382(20) (2020) 1894-1905.
[301] L. Paz-Ares, M. Dvorkin, Y. Chen, N. Reinmuth, K. Hotta, D. Trukhin, G. Statsenko, M.J. Hochmair, M. Ozguroglu, J.H. Ji, O. Voitko, A. Poltoratskiy, S. Ponce, F. Verderame, L. Havel, I. Bondarenko, A. Kazarnowicz, G. Losonczy, N.V. Conev, J. Armstrong, N. Byrne, N. Shire, H. Jiang, J.W. Goldman, C. investigators, Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial, Lancet 394(10212) (2019) 1929-1939.
[302] T. Powles, P.H. O'Donnell, C. Massard, H.T. Arkenau, T.W. Friedlander, C.J. Hoimes, J.L. Lee, M. Ong, S.S. Sridhar, N.J. Vogelzang, M.N. Fishman, J. Zhang, S. Srinivas, J. Parikh, J. Antal, X. Jin, A.K. Gupta, Y. Ben, N.M. Hahn, Efficacy and Safety of Durvalumab in Locally Advanced or Metastatic Urothelial Carcinoma: Updated Results From a Phase 1/2 Open-label Study, JAMA Oncol 3(9) (2017) e172411.
[303] K.P. Papadopoulos, M.L. Johnson, A.C. Lockhart, K. Moore, G.S. Falchook, S.C. Formenti, A. Naing, R.D. Carvajal, L.S. Rosen, G.J. Weiss, R.S. Leidner, J. Li, A. Paccaly, M. Feng, E. Stankevich, I. Lowy, M.G. Fury, M.R. Crittenden, First-In-Human Study of Cemiplimab Alone or In Combination with Radiotherapy and/or Low-dose Cyclophosphamide in Patients with Advanced Malignancies, Clinical cancer research : an official journal of the American Association for Cancer Research 26(5) (2020) 1025-1033.
[304] M.A. Curran, W. Montalvo, H. Yagita, J.P. Allison, PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors, Proceedings of the National Academy of Sciences of the United States of America 107(9) (2010) 4275-80.
[305] D.M. Lussier, J.L. Johnson, P. Hingorani, J.N. Blattman, Combination immunotherapy with alpha-CTLA-4 and alpha-PD-L1 antibody blockade prevents immune escape and leads to complete control of metastatic osteosarcoma, Journal for immunotherapy of cancer 3 (2015) 21.
[306] J. Larkin, V. Chiarion-Sileni, R. Gonzalez, J.J. Grob, P. Rutkowski, C.D. Lao, C.L. Cowey, D. Schadendorf, J. Wagstaff, R. Dummer, P.F. Ferrucci, M. Smylie, D. Hogg, A. Hill, I. Marquez-Rodas, J. Haanen, M. Guidoboni, M. Maio, P. Schoffski, M.S. Carlino, C. Lebbe, G. McArthur, P.A. Ascierto, G.A. Daniels, G.V. Long, L. Bastholt, J.I. Rizzo, A. Balogh, A. Moshyk, F.S. Hodi, J.D. Wolchok, Five-Year Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma, The New England journal of medicine 381(16) (2019) 1535-1546.
[307] A. Scherpereel, J. Mazieres, L. Greillier, S. Lantuejoul, P. Do, O. Bylicki, I. Monnet, R. Corre, C. Audigier-Valette, M. Locatelli-Sanchez, O. Molinier, F. Guisier, T. Urban, C. Ligeza-Poisson, D. Planchard, E. Amour, F. Morin, D. Moro-Sibilot, G. Zalcman, I. French Cooperative Thoracic, Nivolumab or nivolumab plus ipilimumab in patients with relapsed malignant pleural mesothelioma (IFCT-1501 MAPS2): a multicentre, open-label, randomised, non-comparative, phase 2 trial, The Lancet. Oncology 20(2) (2019) 239-253.
[308] K. Wu, M. Yi, S. Qin, Q. Chu, X. Zheng, K. Wu, The efficacy and safety of combination of PD-1 and CTLA-4 inhibitors: a meta-analysis, Experimental hematology & oncology 8 (2019) 26.
[309] L.P. Andrews, A.E. Marciscano, C.G. Drake, D.A. Vignali, LAG3 (CD223) as a cancer immunotherapy target, Immunological reviews 276(1) (2017) 80-96.
[310] C. Brignone, M. Gutierrez, F. Mefti, E. Brain, R. Jarcau, F. Cvitkovic, N. Bousetta, J. Medioni, J. Gligorov, C. Grygar, M. Marcu, F. Triebel, First-line chemoimmunotherapy in metastatic breast carcinoma: combination

of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity, Journal of translational medicine 8 (2010) 71.
[311] A. P, B.P. , B.S. , M.I. , N.M. , S.I. , L.J. , G.-R.C. , S.D. , D.R. , A. Marabelle A, C. Hoeller C,, C.T.H.C. M. Maurer M, P. Mitra P, S. Suryawanshi S, K. Thudium K, E. Munoz ~, C. E, Efficacy of BMS-986016, a monoclonal antibody that targets lymphocyte activation gene-3 (LAG-3), in combination with nivolumab in pts with melanoma who progressed during prior anti–PD-1/PD-L1 therapy (mel prior IO) in all-comer and biomarker-enriched populations, ANNALS of ONCOLOGY 28(5) (2017).
[312] A.J. Muller, M.G. Manfredi, Y. Zakharia, G.C. Prendergast, Inhibiting IDO pathways to treat cancer: lessons from the ECHO-301 trial and beyond, Seminars in immunopathology 41(1) (2019) 41-48.
[313] K.G. Lillian L. Siu, Quincy Chu, Russell Pachynski, Olatunji Alese, Paul Basciano, Justine Walker, Priyam Mitra, Li Zhu, Penny Phillips, John Hunt and Jayesh Desai, Abstract CT116: BMS-986205, an optimized indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor, is well tolerated with potent pharmacodynamic (PD) activity, alone and in combination with nivolumab (nivo) in advanced cancers in a phase 1/2a trial, (2017).
[314] M.S. Merchant, M. Wright, K. Baird, L.H. Wexler, C. Rodriguez-Galindo, D. Bernstein, C. Delbrook, M. Lodish, R. Bishop, J.D. Wolchok, H. Streicher, C.L. Mackall, Phase I Clinical Trial of Ipilimumab in Pediatric Patients with Advanced Solid Tumors, Clinical cancer research : an official journal of the American Association for Cancer Research 22(6) (2016) 1364-70.
[315] B. Geoerger, C. Bergeron, L. Gore, L. Sender, I.J. Dunkel, C. Herzog, L. Brochez, O. Cruz, K. Nysom, E. Berghorn, B. Simsek, J. Shen, A. Pappo, Phase II study of ipilimumab in adolescents with unresectable stage III or IV malignant melanoma, European journal of cancer 86 (2017) 358-363.
[316] X. Zhu, M.M. McDowell, W.C. Newman, G.E. Mason, S. Greene, M.S. Tamber, Severe cerebral edema following nivolumab treatment for pediatric glioblastoma: case report, Journal of neurosurgery. Pediatrics 19(2) (2017) 249-253.
[317] D.T. Blumenthal, M. Yalon, G.W. Vainer, A. Lossos, S. Yust, L. Tzach, E. Cagnano, D. Limon, F. Bokstein, Pembrolizumab: first experience with recurrent primary central nervous system (CNS) tumors, Journal of neuro-oncology 129(3) (2016) 453-460.
[318] H.A. Tawbi, M. Burgess, V. Bolejack, B.A. Van Tine, S.M. Schuetze, J. Hu, S. D'Angelo, S. Attia, R.F. Riedel, D.A. Priebat, S. Movva, L.E. Davis, S.H. Okuno, D.R. Reed, J. Crowley, L.H. Butterfield, R. Salazar, J. Rodriguez-Canales, A.J. Lazar, Wistuba, II, L.H. Baker, R.G. Maki, D. Reinke, S. Patel, Pembrolizumab in advanced soft-tissue sarcoma and bone sarcoma (SARC028): a multicentre, two-cohort, single-arm, open-label, phase 2 trial, The Lancet. Oncology 18(11) (2017) 1493-1501.
[319] E. Bouffet, V. Larouche, B.B. Campbell, D. Merico, R. de Borja, M. Aronson, C. Durno, J. Krueger, V. Cabric, V. Ramaswamy, N. Zhukova, G. Mason, R. Farah, S. Afzal, M. Yalon, G. Rechavi, V. Magimairajan, M.F. Walsh, S. Constantini, R. Dvir, R. Elhasid, A. Reddy, M. Osborn, M. Sullivan, J. Hansford, A. Dodgshun, N. Klauber-Demore, L. Peterson, S. Patel, S. Lindhorst, J. Atkinson, Z. Cohen, R. Laframboise, P. Dirks, M. Taylor, D. Malkin, S. Albrecht, R.W. Dudley, N. Jabado, C.E. Hawkins, A. Shlien, U. Tabori, Immune Checkpoint Inhibition for Hypermutant Glioblastoma Multiforme Resulting From Germline Biallelic Mismatch Repair Deficiency, Journal of clinical oncology : official journal of the American Society of Clinical Oncology 34(19) (2016) 2206-11.
[320] T.M. Johanns, C.A. Miller, I.G. Dorward, C. Tsien, E. Chang, A. Perry, R. Uppaluri, C. Ferguson, R.E. Schmidt, S. Dahiya, G. Ansstas, E.R. Mardis, G.P. Dunn, Immunogenomics of Hypermutated Glioblastoma: A Patient with Germline POLE Deficiency Treated with Checkpoint Blockade Immunotherapy, Cancer discovery 6(11) (2016) 1230-1236.
[321] C. Pfirschke, C. Engblom, S. Rickelt, V. Cortez-Retamozo, C. Garris, F. Pucci, T. Yamazaki, V. Poirier-Colame, A. Newton, Y. Redouane, Y.J. Lin, G. Wojtkiewicz, Y. Iwamoto, M. Mino-Kenudson, T.G. Huynh, R.O. Hynes, G.J. Freeman, G. Kroemer, L. Zitvogel, R. Weissleder, M.J. Pittet, Immunogenic Chemotherapy Sensitizes Tumors to Checkpoint Blockade Therapy, Immunity 44(2) (2016) 343-54.
[322] S.P. Patel, R. Kurzrock, PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy, Molecular cancer therapeutics 14(4) (2015) 847-56.
[323] S. Arora, R. Velichinskii, R.W. Lesh, U. Ali, M. Kubiak, P. Bansal, H. Borghaei, M.J. Edelman, Y. Boumber, Existing and Emerging Biomarkers for Immune Checkpoint Immunotherapy in Solid Tumors, Adv Ther 36(10) (2019) 2638-2678.

[324] Erratum to: Pyo KH, Lim SM, Kim HR, Sung YH, Yun MR, Kim S-M, et al. Establishment of a Conditional Transgenic Mouse Model Recapitulating EML4-ALK-Postive Human Non-Small Cell Lung Cancer. J Thorac Oncol. 2017;12(3):491-500, J Thorac Oncol 12(12) (2017) 1859.
[325] S.J. Antonia, J.A. Lopez-Martin, J. Bendell, P.A. Ott, M. Taylor, J.P. Eder, D. Jager, M.C. Pietanza, D.T. Le, F. de Braud, M.A. Morse, P.A. Ascierto, L. Horn, A. Amin, R.N. Pillai, J. Evans, I. Chau, P. Bono, A. Atmaca, P. Sharma, C.T. Harbison, C.S. Lin, O. Christensen, E. Calvo, Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial, The Lancet. Oncology 17(7) (2016) 883-895.
[326] J. Larkin, F.S. Hodi, J.D. Wolchok, Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma, The New England journal of medicine 373(13) (2015) 1270-1.
[327] J. Galon, A. Costes, F. Sanchez-Cabo, A. Kirilovsky, B. Mlecnik, C. Lagorce-Pages, M. Tosolini, M. Camus, A. Berger, P. Wind, F. Zinzindohoue, P. Bruneval, P.H. Cugnenc, Z. Trajanoski, W.H. Fridman, F. Pages, Type, density, and location of immune cells within human colorectal tumors predict clinical outcome, Science 313(5795) (2006) 1960-4.
[328] F. Pages, J. Galon, M.C. Dieu-Nosjean, E. Tartour, C. Sautes-Fridman, W.H. Fridman, Immune infiltration in human tumors: a prognostic factor that should not be ignored, Oncogene 29(8) (2010) 1093-102.
[329] P.C. Tumeh, C.L. Harview, J.H. Yearley, I.P. Shintaku, E.J. Taylor, L. Robert, B. Chmielowski, M. Spasic, G. Henry, V. Ciobanu, A.N. West, M. Carmona, C. Kivork, E. Seja, G. Cherry, A.J. Gutierrez, T.R. Grogan, C. Mateus, G. Tomasic, J.A. Glaspy, R.O. Emerson, H. Robins, R.H. Pierce, D.A. Elashoff, C. Robert, A. Ribas, PD-1 blockade induces responses by inhibiting adaptive immune resistance, Nature 515(7528) (2014) 568-71.
[330] D.S. Thommen, V.H. Koelzer, P. Herzig, A. Roller, M. Trefny, S. Dimeloe, A. Kiialainen, J. Hanhart, C. Schill, C. Hess, S. Savic Prince, M. Wiese, D. Lardinois, P.C. Ho, C. Klein, V. Karanikas, K.D. Mertz, T.N. Schumacher, A. Zippelius, A transcriptionally and functionally distinct PD-1(+) CD8(+) T cell pool with predictive potential in non-small-cell lung cancer treated with PD-1 blockade, Nature medicine 24(7) (2018) 994-1004.
[331] R. Zappasodi, S. Budhu, M.D. Hellmann, M.A. Postow, Y. Senbabaoglu, S. Manne, B. Gasmi, C. Liu, H. Zhong, Y. Li, A.C. Huang, D. Hirschhorn-Cymerman, K.S. Panageas, E.J. Wherry, T. Merghoub, J.D. Wolchok, Non-conventional Inhibitory CD4(+)Foxp3(-)PD-1(hi) T Cells as a Biomarker of Immune Checkpoint Blockade Activity, Cancer cell 33(6) (2018) 1017-1032 e7.
[332] D.S. Chen, I. Mellman, Elements of cancer immunity and the cancer-immune set point, Nature 541(7637) (2017) 321-330.
[333] J.M. Taube, A. Klein, J.R. Brahmer, H. Xu, X. Pan, J.H. Kim, L. Chen, D.M. Pardoll, S.L. Topalian, R.A. Anders, Association of PD-1, PD-1 ligands, and other features of the tumor immune microenvironment with response to anti-PD-1 therapy, Clinical cancer research : an official journal of the American Association for Cancer Research 20(19) (2014) 5064-74.
[334] N.J. Llosa, M. Cruise, A. Tam, E.C. Wicks, E.M. Hechenbleikner, J.M. Taube, R.L. Blosser, H. Fan, H. Wang, B.S. Luber, M. Zhang, N. Papadopoulos, K.W. Kinzler, B. Vogelstein, C.L. Sears, R.A. Anders, D.M. Pardoll, F. Housseau, The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints, Cancer discovery 5(1) (2015) 43-51.
[335] R.S. Herbst, J.C. Soria, M. Kowanetz, G.D. Fine, O. Hamid, M.S. Gordon, J.A. Sosman, D.F. McDermott, J.D. Powderly, S.N. Gettinger, H.E. Kohrt, L. Horn, D.P. Lawrence, S. Rost, M. Leabman, Y. Xiao, A. Mokatrin, H. Koeppen, P.S. Hegde, I. Mellman, D.S. Chen, F.S. Hodi, Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients, Nature 515(7528) (2014) 563-7.
[336] J.J. Havel, D. Chowell, T.A. Chan, The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy, Nature reviews. Cancer 19(3) (2019) 133-150.
[337] J. Vakkila, R. Jaffe, M. Michelow, M.T. Lotze, Pediatric cancers are infiltrated predominantly by macrophages and contain a paucity of dendritic cells: a major nosologic difference with adult tumors, Clinical cancer research : an official journal of the American Association for Cancer Research 12(7 Pt 1) (2006) 2049-54.
[338] M. Mina, R. Boldrini, A. Citti, P. Romania, V. D'Alicandro, M. De Ioris, A. Castellano, C. Furlanello, F. Locatelli, D. Fruci, Tumor-infiltrating T lymphocytes improve clinical outcome of therapy-resistant neuroblastoma, Oncoimmunology 4(9) (2015) e1019981.

[339] A.M. Griesinger, D.K. Birks, A.M. Donson, V. Amani, L.M. Hoffman, A. Waziri, M. Wang, M.H. Handler, N.K. Foreman, Characterization of distinct immunophenotypes across pediatric brain tumor types, Journal of immunology 191(9) (2013) 4880-8.
[340] N.A. Giraldo, E. Becht, F. Pages, G. Skliris, V. Verkarre, Y. Vano, A. Mejean, N. Saint-Aubert, L. Lacroix, I. Natario, A. Lupo, M. Alifano, D. Damotte, A. Cazes, F. Triebel, G.J. Freeman, M.C. Dieu-Nosjean, S. Oudard, W.H. Fridman, C. Sautes-Fridman, Orchestration and Prognostic Significance of Immune Checkpoints in the Microenvironment of Primary and Metastatic Renal Cell Cancer, Clinical cancer research : an official journal of the American Association for Cancer Research 21(13) (2015) 3031-40.
[341] N.J. Neubert, M. Schmittnaegel, N. Bordry, S. Nassiri, N. Wald, C. Martignier, L. Tille, K. Homicsko, W. Damsky, H. Maby-El Hajjami, I. Klaman, E. Danenberg, K. Ioannidou, L. Kandalaft, G. Coukos, S. Hoves, C.H. Ries, S.A. Fuertes Marraco, P.G. Foukas, M. De Palma, D.E. Speiser, T cell-induced CSF1 promotes melanoma resistance to PD1 blockade, Science translational medicine 10(436) (2018).
[342] M. Sade-Feldman, J. Kanterman, Y. Klieger, E. Ish-Shalom, M. Olga, A. Saragovi, H. Shtainberg, M. Lotem, M. Baniyash, Clinical Significance of Circulating CD33+CD11b+HLA-DR- Myeloid Cells in Patients with Stage IV Melanoma Treated with Ipilimumab, Clinical cancer research : an official journal of the American Association for Cancer Research 22(23) (2016) 5661-5672.
[343] W.H. Fridman, F. Pages, C. Sautes-Fridman, J. Galon, The immune contexture in human tumours: impact on clinical outcome, Nature reviews. Cancer 12(4) (2012) 298-306.
[344] M. Georganaki, L. van Hooren, A. Dimberg, Vascular Targeting to Increase the Efficiency of Immune Checkpoint Blockade in Cancer, Frontiers in immunology 9 (2018) 3081.
[345] M.W. Teng, S.F. Ngiow, A. Ribas, M.J. Smyth, Classifying Cancers Based on T-cell Infiltration and PD-L1, Cancer research 75(11) (2015) 2139-45.
[346] R.R. Ramjiawan, A.W. Griffioen, D.G. Duda, Anti-angiogenesis for cancer revisited: Is there a role for combinations with immunotherapy?, Angiogenesis 20(2) (2017) 185-204.
[347] M. Liu, X. Wang, L. Wang, X. Ma, Z. Gong, S. Zhang, Y. Li, Targeting the IDO1 pathway in cancer: from bench to bedside, Journal of hematology & oncology 11(1) (2018) 100.
[348] G.T. Motz, S.P. Santoro, L.P. Wang, T. Garrabrant, R.R. Lastra, I.S. Hagemann, P. Lal, M.D. Feldman, F. Benencia, G. Coukos, Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors, Nature medicine 20(6) (2014) 607-15.
[349] D.I. Gabrilovich, H.L. Chen, K.R. Girgis, H.T. Cunningham, G.M. Meny, S. Nadaf, D. Kavanaugh, D.P. Carbone, Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells, Nature medicine 2(10) (1996) 1096-103.
[350] Y. Huang, X. Chen, M.M. Dikov, S.V. Novitskiy, C.A. Mosse, L. Yang, D.P. Carbone, Distinct roles of VEGFR-1 and VEGFR-2 in the aberrant hematopoiesis associated with elevated levels of VEGF, Blood 110(2) (2007) 624-31.
[351] M. Yi, D. Jiao, S. Qin, Q. Chu, K. Wu, A. Li, Synergistic effect of immune checkpoint blockade and anti-angiogenesis in cancer treatment, Mol Cancer 18(1) (2019) 60.
[352] A.R. Elia, M. Grioni, V. Basso, F. Curnis, M. Freschi, A. Corti, A. Mondino, M. Bellone, Targeting Tumor Vasculature with TNF Leads Effector T Cells to the Tumor and Enhances Therapeutic Efficacy of Immune Checkpoint Blockers in Combination with Adoptive Cell Therapy, Clinical cancer research : an official journal of the American Association for Cancer Research 24(9) (2018) 2171-2181.
[353] X. Zheng, Z. Fang, X. Liu, S. Deng, P. Zhou, X. Wang, C. Zhang, R. Yin, H. Hu, X. Chen, Y. Han, Y. Zhao, S.H. Lin, S. Qin, X. Wang, B.Y. Kim, P. Zhou, W. Jiang, Q. Wu, Y. Huang, Increased vessel perfusion predicts the efficacy of immune checkpoint blockade, The Journal of clinical investigation 128(5) (2018) 2104-2115.
[354] A. Calcinotto, M. Grioni, E. Jachetti, F. Curnis, A. Mondino, G. Parmiani, A. Corti, M. Bellone, Targeting TNF-alpha to neoangiogenic vessels enhances lymphocyte infiltration in tumors and increases the therapeutic potential of immunotherapy, Journal of immunology 188(6) (2012) 2687-94.
[355] T.J. Harryvan, E.M.E. Verdegaal, J.C.H. Hardwick, L. Hawinkels, S.H. van der Burg, Targeting of the Cancer-Associated Fibroblast-T-Cell Axis in Solid Malignancies, Journal of clinical medicine 8(11) (2019).
[356] L. Chen, X. Qiu, X. Wang, J. He, FAP positive fibroblasts induce immune checkpoint blockade resistance in colorectal cancer via promoting immunosuppression, Biochemical and biophysical research communications 487(1) (2017) 8-14.

[357] C.J. Martin, A. Datta, C. Littlefield, A. Kalra, C. Chapron, S. Wawersik, K.B. Dagbay, C.T. Brueckner, A. Nikiforov, F.T. Danehy, Jr., F.C. Streich, Jr., C. Boston, A. Simpson, J.W. Jackson, S. Lin, N. Danek, R.R. Faucette, P. Raman, A.D. Capili, A. Buckler, G.J. Carven, T. Schurpf, Selective inhibition of TGFbeta1 activation overcomes primary resistance to checkpoint blockade therapy by altering tumor immune landscape, Science translational medicine 12(536) (2020).
[358] D.V.F. Tauriello, S. Palomo-Ponce, D. Stork, A. Berenguer-Llergo, J. Badia-Ramentol, M. Iglesias, M. Sevillano, S. Ibiza, A. Canellas, X. Hernando-Momblona, D. Byrom, J.A. Matarin, A. Calon, E.I. Rivas, A.R. Nebreda, A. Riera, C.S. Attolini, E. Batlle, TGFbeta drives immune evasion in genetically reconstituted colon cancer metastasis, Nature 554(7693) (2018) 538-543.
[359] W. Hugo, J.M. Zaretsky, L. Sun, C. Song, B.H. Moreno, S. Hu-Lieskovan, B. Berent-Maoz, J. Pang, B. Chmielowski, G. Cherry, E. Seja, S. Lomeli, X. Kong, M.C. Kelley, J.A. Sosman, D.B. Johnson, A. Ribas, R.S. Lo, Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma, Cell 168(3) (2017) 542.
[360] H.G. Tanyildiz, G. Kaygusuz, E. Unal, N. Tacyildiz, H. Dincaslan, G. Yavuz, The prognostic importance of TGF-beta, TGF-beta receptor, and fascin in childhood solid tumors, Pediatric hematology and oncology 34(4) (2017) 238-253.
[361] F. Petitprez, M. Meylan, A. de Reynies, C. Sautes-Fridman, W.H. Fridman, The Tumor Microenvironment in the Response to Immune Checkpoint Blockade Therapies, Frontiers in immunology 11 (2020) 784.
[362] T.N. Schumacher, R.D. Schreiber, Neoantigens in cancer immunotherapy, Science 348(6230) (2015) 69-74.
[363] S.K. Saini, N. Rekers, S.R. Hadrup, Novel tools to assist neoepitope targeting in personalized cancer immunotherapy, Annals of oncology : official journal of the European Society for Medical Oncology 28(suppl_12) (2017) xii3-xii10.
[364] R.M. Samstein, C.H. Lee, A.N. Shoushtari, M.D. Hellmann, R. Shen, Y.Y. Janjigian, D.A. Barron, A. Zehir, E.J. Jordan, A. Omuro, T.J. Kaley, S.M. Kendall, R.J. Motzer, A.A. Hakimi, M.H. Voss, P. Russo, J. Rosenberg, G. Iyer, B.H. Bochner, D.F. Bajorin, H.A. Al-Ahmadie, J.E. Chaft, C.M. Rudin, G.J. Riely, S. Baxi, A.L. Ho, R.J. Wong, D.G. Pfister, J.D. Wolchok, C.A. Barker, P.H. Gutin, C.W. Brennan, V. Tabar, I.K. Mellinghoff, L.M. DeAngelis, C.E. Ariyan, N. Lee, W.D. Tap, M.M. Gounder, S.P. D'Angelo, L. Saltz, Z.K. Stadler, H.I. Scher, J. Baselga, P. Razavi, C.A. Klebanoff, R. Yaeger, N.H. Segal, G.Y. Ku, R.P. DeMatteo, M. Ladanyi, N.A. Rizvi, M.F. Berger, N. Riaz, D.B. Solit, T.A. Chan, L.G.T. Morris, Tumor mutational load predicts survival after immunotherapy across multiple cancer types, Nature genetics 51(2) (2019) 202-206.
[365] M.S. Lawrence, P. Stojanov, P. Polak, G.V. Kryukov, K. Cibulskis, A. Sivachenko, S.L. Carter, C. Stewart, C.H. Mermel, S.A. Roberts, A. Kiezun, P.S. Hammerman, A. McKenna, Y. Drier, L. Zou, A.H. Ramos, T.J. Pugh, N. Stransky, E. Helman, J. Kim, C. Sougnez, L. Ambrogio, E. Nickerson, E. Shefler, M.L. Cortes, D. Auclair, G. Saksena, D. Voet, M. Noble, D. DiCara, P. Lin, L. Lichtenstein, D.I. Heiman, T. Fennell, M. Imielinski, B. Hernandez, E. Hodis, S. Baca, A.M. Dulak, J. Lohr, D.A. Landau, C.J. Wu, J. Melendez-Zajgla, A. Hidalgo-Miranda, A. Koren, S.A. McCarroll, J. Mora, B. Crompton, R. Onofrio, M. Parkin, W. Winckler, K. Ardlie, S.B. Gabriel, C.W.M. Roberts, J.A. Biegel, K. Stegmaier, A.J. Bass, L.A. Garraway, M. Meyerson, T.R. Golub, D.A. Gordenin, S. Sunyaev, E.S. Lander, G. Getz, Mutational heterogeneity in cancer and the search for new cancer-associated genes, Nature 499(7457) (2013) 214-218.
[366] B. Vogelstein, N. Papadopoulos, V.E. Velculescu, S. Zhou, L.A. Diaz, Jr., K.W. Kinzler, Cancer genome landscapes, Science 339(6127) (2013) 1546-58.
[367] R.A. DePinho, The age of cancer, Nature 408(6809) (2000) 248-54.
[368] E.A. Sweet-Cordero, J.A. Biegel, The genomic landscape of pediatric cancers: Implications for diagnosis and treatment, Science 363(6432) (2019) 1170-1175.
[369] K. Yamamoto, R. Hanada, A. Kikuchi, M. Ichikawa, T. Aihara, E. Oguma, T. Moritani, Y. Shimanuki, M. Tanimura, Y. Hayashi, Spontaneous regression of localized neuroblastoma detected by mass screening, Journal of clinical oncology : official journal of the American Society of Clinical Oncology 16(4) (1998) 1265-9.
[370] K. Buder-Bakhaya, J.C. Hassel, Biomarkers for Clinical Benefit of Immune Checkpoint Inhibitor Treatment-A Review From the Melanoma Perspective and Beyond, Frontiers in immunology 9 (2018) 1474.

[371] M. Fankhauser, M.A.S. Broggi, L. Potin, N. Bordry, L. Jeanbart, A.W. Lund, E. Da Costa, S. Hauert, M. Rincon-Restrepo, C. Tremblay, E. Cabello, K. Homicsko, O. Michielin, D. Hanahan, D.E. Speiser, M.A. Swartz, Tumor lymphangiogenesis promotes T cell infiltration and potentiates immunotherapy in melanoma, Science translational medicine 9(407) (2017).
[372] K.R. Voong, J. Feliciano, D. Becker, B. Levy, Beyond PD-L1 testing-emerging biomarkers for immunotherapy in non-small cell lung cancer, Annals of translational medicine 5(18) (2017) 376.
[373] J.R. Thompson, S.P. Menon, Liquid Biopsies and Cancer Immunotherapy, Cancer journal 24(2) (2018) 78-83.
[374] L. Liotta, E. Petricoin, Molecular profiling of human cancer, Nature reviews. Genetics 1(1) (2000) 48-56.
[375] J. Chifman, A. Pullikuth, J.W. Chou, D. Bedognetti, L.D. Miller, Conservation of immune gene signatures in solid tumors and prognostic implications, BMC cancer 16(1) (2016) 911.
[376] V. Shahabi, D. Berman, S.D. Chasalow, L. Wang, Z. Tsuchihashi, B. Hu, L. Panting, M. Jure-Kunkel, R.R. Ji, Gene expression profiling of whole blood in ipilimumab-treated patients for identification of potential biomarkers of immune-related gastrointestinal adverse events, Journal of translational medicine 11 (2013) 75.
[377] R.R. Ji, S.D. Chasalow, L. Wang, O. Hamid, H. Schmidt, J. Cogswell, S. Alaparthy, D. Berman, M. Jure-Kunkel, N.O. Siemers, J.R. Jackson, V. Shahabi, An immune-active tumor microenvironment favors clinical response to ipilimumab, Cancer immunology, immunotherapy : CII 61(7) (2012) 1019-31.
[378] C.C. Smith, K.E. Beckermann, D.S. Bortone, A.A. De Cubas, L.M. Bixby, S.J. Lee, A. Panda, S. Ganesan, G. Bhanot, E.M. Wallen, M.I. Milowsky, W.Y. Kim, W.K. Rathmell, R. Swanstrom, J.S. Parker, J.S. Serody, S.R. Selitsky, B.G. Vincent, Endogenous retroviral signatures predict immunotherapy response in clear cell renal cell carcinoma, The Journal of clinical investigation 128(11) (2018) 4804-4820.
[379] S.C. Ganal-Vonarburg, C.U. Duerr, The interaction of intestinal microbiota and innate lymphoid cells in health and disease throughout life, Immunology 159(1) (2020) 39-51.
[380] N. Chaput, P. Lepage, C. Coutzac, E. Soularue, K. Le Roux, C. Monot, L. Boselli, E. Routier, L. Cassard, M. Collins, T. Vaysse, L. Marthey, A. Eggermont, V. Asvatourian, E. Lanoy, C. Mateus, C. Robert, F. Carbonnel, Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab, Annals of oncology : official journal of the European Society for Medical Oncology 30(12) (2019) 2012.
[381] V. Matson, J. Fessler, R. Bao, T. Chongsuwat, Y. Zha, M.L. Alegre, J.J. Luke, T.F. Gajewski, The commensal microbiome is associated with anti-PD-1 efficacy in metastatic melanoma patients, Science 359(6371) (2018) 104-108.
[382] V. Gopalakrishnan, C.N. Spencer, L. Nezi, A. Reuben, M.C. Andrews, T.V. Karpinets, P.A. Prieto, D. Vicente, K. Hoffman, S.C. Wei, A.P. Cogdill, L. Zhao, C.W. Hudgens, D.S. Hutchinson, T. Manzo, M. Petaccia de Macedo, T. Cotechini, T. Kumar, W.S. Chen, S.M. Reddy, R. Szczepaniak Sloane, J. Galloway-Pena, H. Jiang, P.L. Chen, E.J. Shpall, K. Rezvani, A.M. Alousi, R.F. Chemaly, S. Shelburne, L.M. Vence, P.C. Okhuysen, V.B. Jensen, A.G. Swennes, F. McAllister, E. Marcelo Riquelme Sanchez, Y. Zhang, E. Le Chatelier, L. Zitvogel, N. Pons, J.L. Austin-Breneman, L.E. Haydu, E.M. Burton, J.M. Gardner, E. Sirmans, J. Hu, A.J. Lazar, T. Tsujikawa, A. Diab, H. Tawbi, I.C. Glitza, W.J. Hwu, S.P. Patel, S.E. Woodman, R.N. Amaria, M.A. Davies, J.E. Gershenwald, P. Hwu, J.E. Lee, J. Zhang, L.M. Coussens, Z.A. Cooper, P.A. Futreal, C.R. Daniel, N.J. Ajami, J.F. Petrosino, M.T. Tetzlaff, P. Sharma, J.P. Allison, R.R. Jenq, J.A. Wargo, Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients, Science 359(6371) (2018) 97-103.
[383] B. Routy, E. Le Chatelier, L. Derosa, C.P.M. Duong, M.T. Alou, R. Daillere, A. Fluckiger, M. Messaoudene, C. Rauber, M.P. Roberti, M. Fidelle, C. Flament, V. Poirier-Colame, P. Opolon, C. Klein, K. Iribarren, L. Mondragon, N. Jacquelot, B. Qu, G. Ferrere, C. Clemenson, L. Mezquita, J.R. Masip, C. Naltet, S. Brosseau, C. Kaderbhai, C. Richard, H. Rizvi, F. Levenez, N. Galleron, B. Quinquis, N. Pons, B. Ryffel, V. Minard-Colin, P. Gonin, J.C. Soria, E. Deutsch, Y. Loriot, F. Ghiringhelli, G. Zalcman, F. Goldwasser, B. Escudier, M.D. Hellmann, A. Eggermont, D. Raoult, L. Albiges, G. Kroemer, L. Zitvogel, Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors, Science 359(6371) (2018) 91-97.
[384] B. Hutzen, S.N. Paudel, M. Naeimi Kararoudi, K.A. Cassady, D.A. Lee, T.P. Cripe, Immunotherapies for pediatric cancer: current landscape and future perspectives, Cancer Metastasis Rev 38(4) (2019) 573-594.

[385] J. Yang, J. Yan, B. Liu, Targeting VEGF/VEGFR to Modulate Antitumor Immunity, Frontiers in immunology 9 (2018) 978.
[386] B.C. Ozdemir, T. Pentcheva-Hoang, J.L. Carstens, X. Zheng, C.C. Wu, T.R. Simpson, H. Laklai, H. Sugimoto, C. Kahlert, S.V. Novitskiy, A. De Jesus-Acosta, P. Sharma, P. Heidari, U. Mahmood, L. Chin, H.L. Moses, V.M. Weaver, A. Maitra, J.P. Allison, V.S. LeBleu, R. Kalluri, Depletion of Carcinoma-Associated Fibroblasts and Fibrosis Induces Immunosuppression and Accelerates Pancreas Cancer with Reduced Survival, Cancer cell 28(6) (2015) 831-833.
[387] A. Kock, K. Larsson, F. Bergqvist, N. Eissler, L.H.M. Elfman, J. Raouf, M. Korotkova, J.I. Johnsen, P.J. Jakobsson, P. Kogner, Inhibition of Microsomal Prostaglandin E Synthase-1 in Cancer-Associated Fibroblasts Suppresses Neuroblastoma Tumor Growth, EBioMedicine 32 (2018) 84-92.

Figure legends

Figure 1: Structure of Immune Checkpoint receptors

PD-1, CTLA-4, LAG-3, TIM-3, TIGIT, B7-H3 and BTLA showed a similar structure, which include N-terminal IgV domain, a transmembrane domain, a cytoplasmic tail and distinct signaling motifs. ITIM: immunoreceptor tyrosine-based inhibitory motif. The figure was performed with https://biorender.com/.

Figure 2: Number of clinical trials in adult and pediatric cancers

Number of clinical trials with ICI therapy performed in adult (A) and pediatric (B) cancers.
In A, clinical trials recruiting or completed in the last year (2019) for CTLA-4 and PD-1/PD-L1 blockades, and in the last three years (2017-2019) for LAG-3, TIM-3, TIGIT, BTLA and IDO-1 inhibitors are included. In B, clinical trials recruiting in the last three years (2017-2019) are included.

Figure 3: Immune checkpoint blockade approaches in adult and pediatric solid malignances

A. Anticancer immunity impairment through interaction of IC receptors and the respective ligands. **B**. Overview of the most common ICIs employed in solid tumors. **C**. Summary of TME compositions, highlighting T cell infiltration, IC expression and neoantigens formation as predictive biomarkers of ICI therapy. **D**. Therapeutic strategies employed for the treatment of adult and pediatric solid tumors. IC, immune checkpoint; ICIs, immune checkpoint inhibitors. The figure was performed with https://biorender.com/.

Table 1. Expression and prognostic values of IC molecules in different solid adult cancers.

IC molecule	Cancer Type	Prognosis	Technique	Ab (clone used)	Cut-off of positivity	References
PD-L1	CC	Good (if high on TILs)	IHC	27A2	⩾ 5 % of positive cells	[118]
PD-L1	OC	Good (if high on TILs)	IHC	SP263	Intensity scale (0-3^{+})	[119]
PD-L1	MPM	Poor (if high on Tum)	IHC	E1L3N	⩾ 5 % of positive cells	[120]
PD-L1	MM	Discordant (primary *vs* metastasis)	IHC	28-8	⩾ 5 % of positive cells	[126]
PD-L1	BC	Discordant (primary *vs* metastasis)	IHC	SP263	⩾ 5 % of positive cells	[129]
PD-L1	UC	Discordant (primary *vs* metastasis)	IHC	SP142	⩾ 5 % of positive cells	[130]
PD-L1	RCC	Discordant (primary *vs* metastasis)	IHC	ZM-0170	Intensity scale (0-3^{+})	[131]
PD-1	HCC	Poor (if high on Tum)	RNA seq	–	–	[132]
PD-1	BC	No differences	IHC	NAT105	–	[133]
CTLA-4	NPC	Poor (if high on Tum)	IHC	251548	Intensity scale	[134]
CTLA-4	EC	Poor (if high on Tum)	IHC	EPR1476	Intensity scale	[135]
CTLA-4	Thymoma	Poor (if high on Tum)	IHC	BNI3	⩾ 5 % of positive cells	[136]
CTLA-4	BC	Poor (if high on Tum)	IHC	Polyclonal	⩾ 1 % of positive cells	[137]
CTLA-4	NSCLC	Discordant (primary *vs* metastasis)	IHC	14D3	Intensity scale (0-3^{+})	[138]
PD-1/CTLA-4	RCC	Poor (if high on TILs)	IHC	BSB-88/ NAT105	Intensity scale (0-3^{+})	[139]
CTLA-4	BC	Poor (if high on TILs)	Flow cytometry	46-1529-42	–	[140]
CTLA-4	CC	Poor (if high on TILs)	Flow cytometry	14D3	–	[141]
sLAG-3	BC	Good if high on serum	ELISA	–	–	[142]
sLAG-3	GC	Good if high on serum	ELISA	–	–	[143]
LAG-3	CRC	Poor (if high on TILs)	IHC	–	–	[144]
LAG-3	CC	Poor (if high on TILs)	Flow cytometry	11C3C65	–	[145]
LAG-3	STS	Poor (if high on TILs)	IHC	17B4	–	[146]
LAG-3	CC	Good (if high on TILs)	IHC	–	–	[147]
LAG-3	NSCLC	Good (if high on TILs)	IHC	D2G4O	Counting scale (0–3)	[148]
LAG-3	EC	Good (if high on EC)	IHC	17B4	Median value of count	[149]
LAG-3	BC	Good (if high on TILs)	IHC	17B4	⩾ 1 positive cells	[151]
LAG-3	TNBC	Good (if high on TILs)	IHC	17B4	⩾ 5 % of positive cells	[152]
TIM-3	LC	Poor (if high)	IHC	Polyclonal	⩾ 11 or 24 % of positive cells	[153]
TIM-3	PrC	Poor (if high)	IHC	–	Counting scale (0–3)	[154]
TIM-3	RCC	Poor (if high on TILs)	Flow cytometry/IF	F38-2E2	–	[155]
TIM-3	BC	Good (if high on TILs)	IHC	D5D5R	⩾ 1 % of positive cells	[157]
TIM-3	BC	Poor (if high on Tum)	IHC	Polyclonal	Intensity scale (0-3^{+})	[158]
TIM-3	OSS	Poor (if high on Tum)	IHC	MAB2365R	⩾ 5 % of positive cells	[159]
TIM-3	CRC	Poor (if high on Tum)	IHC	Polyclonal	Staining intensity (high/low)	[161]
TIM-3	NSCLC	No differences	CyTOF	–	–	[163]
TIM-3	GC	Poor (if high on Tum)	IHC	Polyclonal	Intensity scale (0-3^{+})	[164]
TIGIT	HCC	Poor (if high on TILs)	IHC	–	–	[169]
TIGIT	Seminoma	Not specified	IHC	TG1	⩾ 2 % of positive cells	[170]
CD155	SCLC	Poor (if high on Tum)	IHC	B-6	Staining intensity	[171]
B7-H3	MM	Poor (if high on Tum)	IHC	Polyclonal	Intensity scale (0-3^{+})	[172]
B7-H3	PrC	Poor (if high on Tum)	IHC	Polyclonal	Staining intensity	[173]
B7-H3	CC	No differences	IHC	MIH42	⩾ 10 % of positive cells	[174]
B7-H3	CC	Poor (if high on Tum)	IHC	Polyclonal	⩾ 10 % of positive cells	[175]
B7-H3	HCC	Poor (if high on Tum)	IHC	Polyclonal	Intensity scale (0-3^{+})	[176]
B7-H3	CeC	Poor (if high on Tum)	IHC	6A1	Intensity scale (0-3^{+})	[177]
B7-H3	SCCHN	Poor (if high on Tum)	IHC	14058	Score > 3	[182]
B7-H3	NC	Poor (if high on Tum)	IHC	14058	Intensity scale (0-3^{+})	[183]
B7-H3	DIPG	Poor (if high on Tum)	IHC/RNAseq	–	–	[184]
B7-H3	RCC	Poor (if high on Tum)	IHC	BD/5A11	Staining intensity (high/low)	[185]
B7-H3	SCLC	Poor (if high on Tum)	QIF	D9M2L	top 25-percentile	[186]
BTLA	PrC	Poor if high on serum	ELISA	–	–	[191]

BTLA	GC	Poor (if high on Tum)	IHC	Polyclonal	⩾ 5 % of positive cells	[192]
IDO-1	MM	Poor (if low on TILs)	IHC	–	–	[196]
IDO-1	GC	Poor (if high on Tum)	IHC	998743	Intensity scale (0-3^{+})	[197]
IDO-1	GC	Poor (if high on Tum)	IHC	Polyclonal	⩾ 5 % of positive cells	[198]
IDO-1	Thymoma	Poor (if high on Tum)	IHC	10,1	Staining intensity (0–3)	[199]

Table 2. Expression and prognostic values of IC molecules in different solid pediatric cancers.

IC molecule	Cancer Type	Prognosis	Technique	Ab (clone used)	Cut-off of positivity	References
PD-L1	RMS, OSS, NB, EWS	Poor (if high on Tum)	IHC	28-8	⩾ 5 % of positive cells	[200]
PD-L1	Burkitt lymphoma, GBM, NB	Poor (if high on Tum)	IHC	28-8	1 % of positive cells	[201]
PD-L1	EWS, NB, OSS, RMS, WT	Poor (if high on Tum)	IHC	22C3	0–5 (0 = negative to 5 = very high)	[202]
PD-L1	NB, HB, MB, RMS	–	IHC	E1L3N	⩾ 5 % of positive cells	[203]
PD-L1	NB	–	Flow cytometry	PD-L1.3.1	–	[204]
PD-L1	NB	No differences	IHC	RBT-PDL1	1 tumor cell per mm^2	[205]
PD-L1	meGCTs	No differences	IHC	RBT-PDL1	1 tumor cell per mm^2	[207]
PD-L1	OSS	Poor (if high on Tum)	IHC	130021	Intensity scale	[208]
PD-L1	OSS	Poor (if high on Tum)	IHC	SP142	% of positive cells (0=negative to 4 = > 75 %)	[209]
PD-L1	EWS	Poor (if high on Tum)	IHC	E1L3N	Intensity scale	[210]
PD-L1	RMS	–	IHC	SP142/ 22C3	–	[211]
PD-L1	RMS	–	IHC/Flow cytometry	SP142/ 22C3	% of positive cells (0= < 1 % to 3= ≥50 %)	[212]
PD-L1	glioma	Poor (if high on Tum)	IHC	MIH1	–	[213]
PD-L1	MB	–	IHC	5H1/SP142	Intensity Scale (0–3)	[214]
PD-L1	MB	–	IHC	28-8	⩾ 5 % of positive cells	[215]
PD-L1	PE	No differences	IHC	SP263	⩾ 10 % of positive cells	[216]
PD-L1	PE	–	IHC	SP263	Intensity Scale (0–3)	[217]
CTLA-4	Solid Tumors	–	Flow cytometry	50.18.21	–	[219]
LAG-3	NB	No differences	IHC	EPR4392(2)	1 lymphocytes per mm^2	[220]
TIM-3	OSS	Poor (if high on TILs)	Flow cytometry/ELISA	–	–	[222]
TIM-3	OSS	Poor (if high on TILs)	Flow cytometry	–	–	[223]
B7-H3	Glioma	–	IHC	–	Intensity scale	[224]
B7-H3	OSS	Poor (if high on Tum)	IHC	–	Intensity scale (0–3)	[225]
B7-H3	GBM, MB, EWS, RMS	Poor (if high on Tum)	IHC	–	Intensity scale (0–3)	[226]
IDO-1	GBM	–	IHC	10,1	Intensity scale	[227]
IDO-1	OSS	Poor (if high on Tum)	IHC	AB9252	% of positive cells (0= negative to 4 = 75–100 %)	[228]

Table 3. The most recent clinical trials with immune checkpoint inhibitors as monotherapy or in combination in different solid adult cancers.

Trials	Cancer Types	ICI* Therapy	In combination with	Other procedures	Phases
NCT04008030	CRC#	Ipilimumab	Nivolumab, Oxaliplatin, Leucovorin,Fluorouracil, Irinotecan,	–	III
NCT03793166	AST#	Ipilimumab	Bevacizumab, Cetuximab Nivolumab, Cabozantinib	–	III
NCT04026412	NSCLC	Ipilimumab	Nivolumab, Durvalumab	–	III
NCT03879122	Hormone-sensitive PrC#	Ipilimumab	Nivolumab, Docetaxel, ADT	–	II-III
NCT03873402	RCC	Ipilimumab	Nivolumab	–	III
NCT03604991	Adenocarcinoma	Ipilimumab	Nivolumab,Carboplatin, Paclitaxel,	Radiation Therapy	II-III
NCT03937219	RCC	Ipilimumab	Nivolumab, Cabozantinib	–	III
NCT04039607	HCC	Ipilimumab	Nivolumab, Sorafenib, lenvatinib	–	III
NCT04109066	BC	Nivolumab	paclitaxel, anthracycline, Cyclophosphamide	Endocrine Therapy, Surgery	III
NCT03906071	Non-Squamous NSCLC	Nivolumab	Sitravatinib, Docetaxel	–	III
NCT03774732	NSCLC	Nivolumab	–	Radiation Therapy	III
NCT03952585	Basaloid SCC, OrC	Nivolumab	Cisplatin	Image Guided Radiation Therapy	II-III
NCT04025879	NSCLC	Nivolumab	Carboplatin, Cisplatin, Paclitaxel, Pemetrexed	–	III
NCT03811015	OrC	Nivolumab	Cisplatin	Intensity-Modulated Radiation Therapy	II-III
NCT03873402	RCC	Nivolumab	Ipilimumab	–	III
NCT03834493	PrC	Pembrolizumab	Enzalutamide	–	III
NCT03834506	PrC	Pembrolizumab	Docetaxel, Prednisone, Dexamethasone	–	III
NCT03834519	PrC	Pembrolizumab	Olaparib, Abiraterone acetate, Prednisone, Enzalutamide	–	III
NCT03924895	Urinary BlC	Pembrolizumab	–	Surgery	III
NCT03924856	Urinary BlC	Pembrolizumab	Gemcitabine, Cisplatin	Surgery	III
NCT03829332	NSCLC	Pembrolizumab	Lenvatinib	–	III
NCT03924869	NSCLC	Pembrolizumab	–	Stereotactic Body Radiation Therapy	III
NCT03829319	Non squamous NSCLC	Pembrolizumab	Carboplatin, Cisplatin, Pemetrexed, Lenvatinib	–	III
NCT03976375	NSCLC#	Pembrolizumab	Lenvatinib, Docetaxel	–	III
NCT03881111	EC	Pembrolizumab	Cisplatin	–	III
NCT03793179	NSCLC	Pembrolizumab	Carboplatin, Pemetrexed	–	III
NCT03867175	LC#	Pembrolizumab	–	Stereotactic Body Radiation Therapy	III
NCT03884101	EnC	Pembrolizumab	Lenvatinib, Paclitaxel, Carboplatin	–	III
NCT03976323	NSCLC	Pembrolizumab	Pemetrexed, Carboplatin, Cisplatin, Olaparib	–	III
NCT03914612	EnC	Pembrolizumab	Carboplatin, Paclitaxel	–	III
NCT03898180	UC	Pembrolizumab	Lenvatinib	–	III
NCT03715205	NSCLC, MM	Pembrolizumab	–	–	IV
NCT03976362	SCC, NSCLC	Pembrolizumab	Carboplatin, Paclitaxel, Nab-paclitaxel, Olaparib	–	III
NCT04003636	BTC	Pembrolizumab	Gemcitabine, Cisplatin	–	III
NCT03975036	AST	Pembrolizumab	Anlotinib	–	II-III
NCT03833167	SCC	Pembrolizumab	–	–	III
NCT03867084	HCC	Pembrolizumab	–	–	III
NCT03820986	MM	Pembrolizumab	Lenvatinib	–	III
NCT03969004	Cutaneous SCC	Cemiplimab	–	–	III
NCT03815643	AST	Avelumab	–	–	III
NCT04177108	TNBC	Atezolizumab	Ipatasertib, Paclitaxel	–	III
NCT03726879	BC	Atezolizumab	Doxorubicin, Cyclophosphamide, Paclitaxel, Trastuzumab, Pertuzumab	–	III
NCT03799835	BlC	Atezolizumab	BCG	–	III
NCT03922997	NSCLC	Atezolizumab	–	–	III
NCT03775265	BlC	Atezolizumab	Cisplatin, Fluorouracil, Gemcitabine, Mitomycin	Radiation Therapy	III
NCT03199885	BC	Atezolizumab	Paclitaxel, Biological, Trastuzumab, Pertuzumab	–	III
NCT03811002	LC, SCLC	Atezolizumab	Carboplatin, Cisplatin, Etoposide	Radiation Therapy	II-III
NCT04028050	SCLC	Atezolizumab	Carboplatin, Etoposide	–	III
NCT03762018	MPM	Atezolizumab	Carboplatin, Pemetrexed, Bevacizumab	–	III

NCT04102098	HCC	Atezolizumab	Bevacizumab	–	III
NCT03875235	BTC	Durvalumab	–	–	III
NCT03830866	Locally Advanced CeC	Durvalumab	Cisplatin, Carboplatin	External beam radiation therapy, brachytherapy	III
NCT03737643	Advanced OC	Durvalumab	Bevacizumab, Olaparib, Carboplatin, Paclitaxel	–	III
NCT03833154	NSCLC	Durvalumab	–	–	III
NCT04078152	AST	Durvalumab	–	–	IV
NCT03847428	HCC	Durvalumab	Bevacizumab	–	III
NCT03784014	STS	Durvalumab	Nilotinib, Ceritinib, Capmatinib, Lapatinib, Trametinib, Dabrafenib, Olaparib, Palbociclib, Glasdegib	Next Generation sequencing exome	III
NCT03607890	AST	Relatlimab	Nivolumab		II
NCT03044613	GC, EC, GEC	Relatlimab	Nivolumab, Carboplatin, Paclitaxel	Radiation Therapy	I
NCT03642067	COAD	Relatlimab	Nivolumab	–	II
NCT04080804	SCCHN	Relatlimab	Nivolumab, Ipilimumab		II
NCT03459222	AST	Relatlimab	Nivolumab, BMS-986205, Ipilimumab	–	I-II
NCT03610711	GEC	Relatlimab	Nivolumab	–	I-II
NCT04112498	AST	Relatlimab	nivolumab, rHuPH20	–	I
NCT04062656	GC	Relatlimab	Nivolumab, Ipilimumab, Oxaliplatin, Docetaxel, 5-Fluorouracil	Folic acid	II
NCT02996110	AST	Relatlimab	Nivolumab, Ipilimumab, BMS-986205, BMS-813160	–	II
NCT03335540	AST	Relatlimab	Nivolumab, Cabiralizumab, Ipilimumab, IDO Inhibitor	Radiation Therapy	I
NCT03743766	MM	Relatlimab	Nivolumab, Relatlimab	–	II
NCT03724968	MM[#]	Relatlimab	Nivolumab, Ipilimumab	–	II
NCT03978611	MM	Relatlimab	Ipilimumab	–	I
NCT03099109	AST	LY3321367	LY3300054		I
NCT03680508	HCC	TSR-022	TSR-042	–	II
NCT03307785	AST, NSCLC	TSR-022	Niraparib, Carboplatin-Paclitaxel, Bevacizumab, Carboplatin Pemetrexed, Carboplatin-Nab-Paclitaxel	–	I
NCT03945253	AST	ASP8374	–	–	I
NCT03260322	AST	ASP8374	Pembrolizumab	–	I
NCT04047862	AST[#]	BGB-A1217	Tislelizumab	–	I
NCT03628677	AST	AB154	AB122	–	I
NCT03563716	NSCLC	MTIG7192A	Atezolizumab	–	II
NCT03563716	AST	TAB004	–	–	I
NCT03301636	MM	Indoximod	Pembrolizumab, Nivolumab	–	II

Table 4. The most recent clinical trials with immune checkpoint inhibitors as monotherapy or in combination in different pediatric solid cancers.

Trials	Cancer Types	ICI* Therapy	In combination with	Other procedures	Age	Phases
NCT01445379	Sarcoma, WT, Lymphoma, NB	Ipilimumab	/	/	3 yrs to 21 yrs	I
NCT02304458	MM#, EWS#, HL#, NB#, OSS#, RMS#, AST#	Ipilimumab	Nivolumab	Biomarker Analysis, Pharmacological Study	12 mths to 30 yrs	I, II
NCT01738139	AST; Unresectable MM; GC#	Ipilimumab	Imatinib Mesylate	/	> 15 yrs	I
NCT00057889	RCC	Ipilimumab	/	/	> 16 yrs	II
NCT00623766	MM	Ipilimumab	Corticosteroids	/	> 16 yrs	II
NCT01274338	MM	Ipilimumab	Recombinant Interferon Alfa-2b	/	> 12 yrs	III
NCT01827111	MM	Ipilimumab	ABI-007	/	12 yrs to 70 yrs	II
NCT00289640	MM	Ipilimumab	MDX-010, BMS-734016	/	> 16 yrs	II
NCT00058279	Intraocular and skin MM	Ipilimumab	aldesleukin	/	> 16 yrs	I, II
NCT00077532	Skin MM	Ipilimumab	gp100 antigen, incomplete Freund's adjuvant	/	> 16 yrs	II
NCT00032045	Intraocular and skin MM	Ipilimumab	gp100 antigen, incomplete Freund's adjuvant	/	> 16 yrs	II
NCT03837899	CST, Hematological Malignancies	Tremelimumab	Durvalumab	/	up to 18 yrs	I, II
NCT02879162	AST	Tremelimumab	Durvalumab	/	> 16 yrs	II
NCT03838042	CNS Tumor; CST	Nivolumab	Entinostat	/	6 yrs to 21	I, II
NCT03130959	AST	Nivolumab	Ipilimumab	/	6 mths to 21	II
NCT03668119	CST	Nivolumab	Ipilimumab	/	> 12 yrs	II
NCT02992964	Refractory or Recurrent Hypermutated Malignancies	Nivolumab	/	/	12 mths to 18	I, II
NCT03585465	CST; Childhood Lymphoma	Nivolumab	Vinblastine; Cyclophosphamide; Capecitabine;	/	4 yrs to 18	I, II
NCT02775292	CST; Metastatic Tumors	Nivolumab	Aldesleukin; Cyclophosphamide; Fludarabine Phosphate	NY-ESO-1(157–165) Peptide-pulsed Autologous Dendritic Cell Vaccine	> 16 yrs	I
NCT03465592	Sarcoma; CST	Nivolumab	/	/	12 mths to 40	I, II
NCT02419417	AST	Nivolumab	BMS-986158	/	> 12 yrs	I, II
NCT03329846	Skin MM	Nivolumab	BMS-986205	Placebo	> 12 yrs	III
NCT02813135	AST	Nivolumab	Various chemotherapics	/	up to 18	I, II
NCT03595124	RCC	Nivolumab	Axitinib	/	> 12 mths	II
NCT03282344	Sarcoma	Nivolumab	NKTR-214		> 12 yrs	II
NCT02989636	Chordoma	Nivolumab	Radiation: Stereotactic Radiosurgery	Biomarker Analysis	> 15 yrs	I
NCT03628209	OSS	Nivolumab	Azacitidine	Post Treatment Surgery	up to 39 yrs	I, II
NCT02914405	NB	Nivolumab	Ch14.18/CHO	/	1 year to 18	I
NCT02550249	GBM	Nivolumab	/	/	> 1 year	II
NCT03635983	MM	Nivolumab	NKTR-214	/	> 12 yrs	III
NCT02970981	MM	Nivolumab	Ipilimumab	/	> 16 yrs	II
NCT04099251	MM	Nivolumab	/	/	> 12 yrs	III
NCT03068455	MM	Nivolumab	ipilimumab	/	> 12 yrs	III
NCT02388906	MM	Nivolumab	Ipilimumab	Placebo matching Ipilimumab	> 15 yrs	III
NCT01176474	Skin MM	Nivolumab	Ipilimumab; NY-ESO-1 157–165 (165 V); gp100:280-288 (288 V); Montanide ISA 51 vegetable grade	Apheresis	> 16 yrs	I
NCT02332668	MM; Lymphoma; CST	Pembrolizumab	/	/	6 mths to 17 yrs	I, II
NCT03145961	TNBC	Pembrolizumab	/	/	> 16 yrs	II
NCT03532737	SCCHN	Pembrolizumab	/	/	> 16 yrs	II
NCT03445858	CST; Lymphoma	Pembrolizumab	Decitabine	Hypofractionated Index Site Radiation	12 mths to 40 yrs	Early I
NCT02359565	Glioma; PE; DIPG; MB	Pembrolizumab	/	Imaging	1 year to 29 yrs	I
NCT03012620	Sarcoma; OC; CNS tumors; Germ Cell and Embryonal; NK/T-cell Lymphoma	Pembrolizumab	/	/	> 15 yrs	II
NCT04134559	HCC	Pembrolizumab	/	/	up to 30 yrs	II
NCT02621021	MM	Pembrolizumab	Cyclophosphamide; Fludarabine; Aldeslaukin	/	> 16 yrs	II
NCT03769467	NC	Pembrolizumab	tabelecleucel	/	> 12 yrs	I, II
NCT02748564	MM#	Pembrolizumab	Aldesleukin	Biomarker Analysis	> 15 yrs	II
NCT03092323	STS	Pembrolizumab	/	/	> 12 yrs	II
NCT03056001	STS	Pembrolizumab	Doxorubicin	/	> 12 yrs	II
NCT02636725	ASPS; STSs	Pembrolizumab	Axitinib	Tumor Specimen Collection	> 16 yrs	II
NCT03063632	Myxoid Liposarcoma#	Pembrolizumab	Interferon Gamma-1b	Biomarker Analysis	> 12 yrs	II

NCT02693535	Lymphoma; MM; AST	Pembrolizumab	Nivolumab; Ipilimumab; plus Chemotherapy	/	> 12 yrs	II
NCT02301039	STS; Bone Sarcoma	Pembrolizumab	/	/	> 12 yrs	II
NCT03783078	MCC	Pembrolizumab	/	/	> 12 yrs	III
NCT03553836	MM	Pembrolizumab	/	Placebo	> 12 yrs	III
NCT03690869	CST; CNS Tumor; DIPG; High Grade Glioma	Cemipilmab	Radiation: Conventional or hypofractionated	Re-irradiation	up to 25 yrs	I, II
NCT03141684	ASPS#	Atezolizumab	/	Biomarker Analysis	> 2 yrs	II
NCT02793466	CST; Lymphoma; CNS Tumors	Durvalumab	MEDI4736	/	1 yrs to 17	I
NCT03451825	CST; Lymphoma	Avelumab	/	/	up to 18 yrs	I, II
NCT03006848	OSS	Avelumab	/	Questionnaires	12 yrs to 49	II
NCT01968109	Neoplasms by Site	Relatlimab	Nivolumab; BMS-986213	/	> 12 yrs	I, II
NCT03470922	MM	Relatlimab	Nivolumab	/	> 12 yrs	II, III
NCT03623854	Chordoma	Relatlimab	Relatlimab	/	> 12 yrs	II, III
NCT02982941	NB; RMS; OSS; EWS; WT; DSRCT	Enoblituzumab	/	/	1 yrs to 35	I
NCT02502708	GBM; Glioma; Gliosarcoma; PE; MB; DIPG; CNS	Indoximod	Temozolomide; Cyclophosphamide; Etoposide	Conformal Radiation	3 yrs to 21	I
NCT04049669	GBM; MB; PE; DIPG	Indoximod	Temozolomide;Cyclophosphamide; Etoposide;Lomustine	Full-dose Radiation	3 yrs to 21	II
NCT02052648	GBM; Glioma; Gliosarcoma; CNS Tumor	Indoximod	Temozolomide; Bevacizumab	Stereotactic Radiation	> 16 yrs	I, II

Figure 1

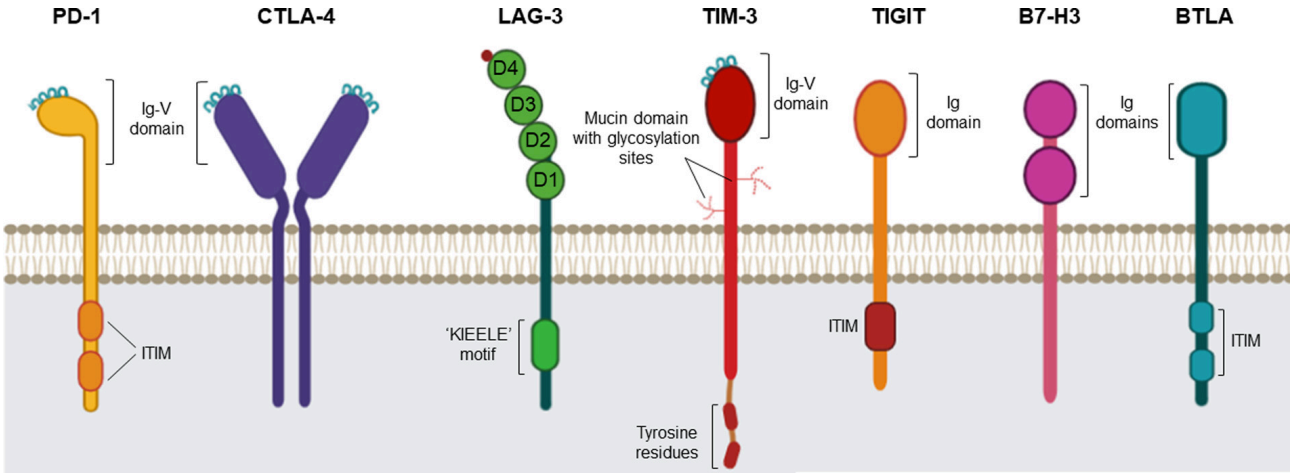

PD-1
CTLA-4
LAG-3
TIM-3
TIGIT
B7-H3
BTLA
Ig-V domain
ITIM
D4
D3
D2
D1
'KIEELE' motif
Mucin domain with glycosylation sites
Ig-V domain
Tyrosine residues
Ig domain
ITIM
Ig domains
ITIM

Figure 2

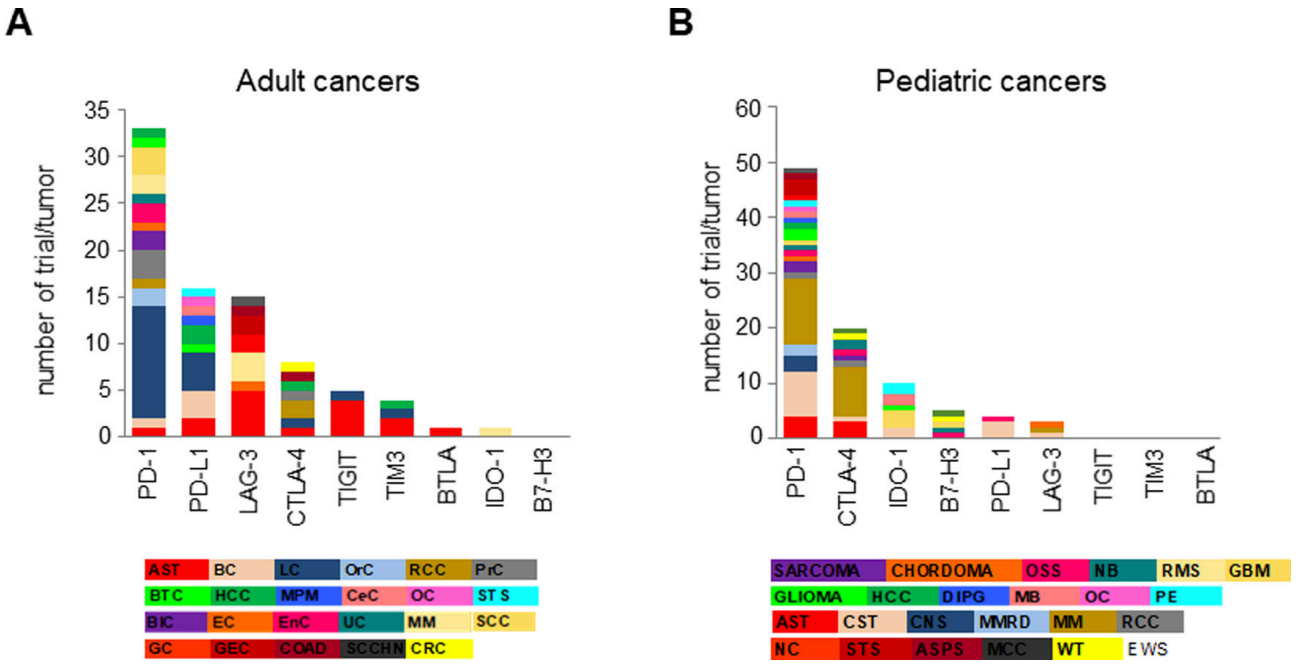
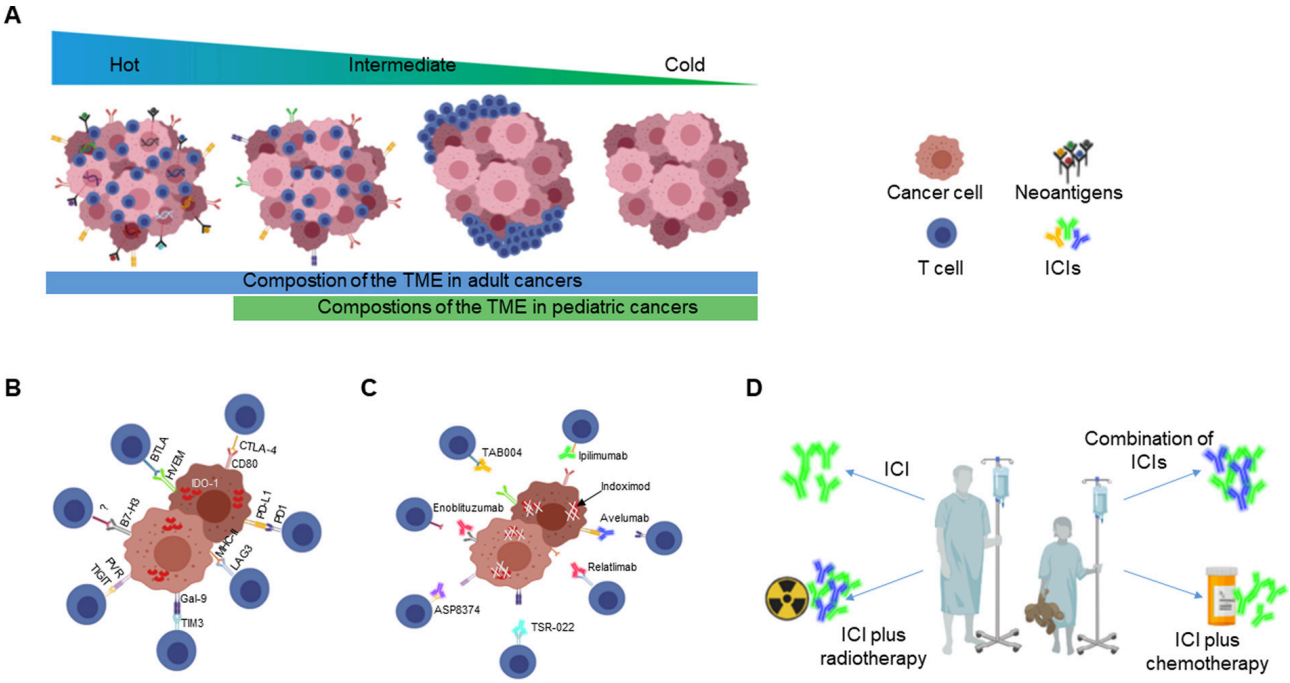

A
Adult cancers
number of trial/tumor
0
5
10
15
20
25
30
35
PD-1
PD-L1
LAG-3
CTLA-4
TIGIT
TIM3
BTLA
IDO-1
B7-H3
AST
BC
LC
OrC
RCC
PrC
BTC
HCC
MPM
CeC
OC
STS
BlC
EC
EnC
UC
MM
SCC
GC
GEC
COAD
SCCHN
CRC
B
Pediatric cancers
number of trial/tumor
0
10
20
30
40
50
60
PD-1
CTLA-4
IDO-1
B7-H3
PD-L1
LAG-3
TIGIT
TIM3
BTLA
SARCOMA
CHORDOMA
OSS
NB
RMS
GBM
GLIOMA
HCC
DIPG
MB
OC
PE
AST
CST
CNS
MMRD
MM
RCC
NC
STS
ASPS
MCC
WT
EWS

Figure 3


A
Hot
Intermediate
Cold
Compostion of the TME in adult cancers
Compostions of the TME in pediatric cancers
Cancer cell
Neoantigens
T cell
ICIs
B
BTLA
CTLA-4
CD80
IDO-1
PD-L1
PD1
LAG3
Gal-9
TIM3
TIGIT
PVR
C
TAB004
Ipilimumab
Indoximod
Enoblituzumab
Avelumab
Relatlimab
ASP8374
TSR-022
D
ICI
Combination of ICIs
ICI plus radiotherapy
ICI plus chemotherapy