



Review Article

Updates on the Importance of CD200:CD200R Checkpoint Blockade in Solid Tumors and B cell Malignancies

Reginald M Gorczyński

University of Toronto and Institute of Medical Sciences, University of Toronto, Toronto, ON, Canada

***Corresponding author:** Reginald M Gorczyński, University of Toronto and Institute of Medical Sciences, University of Toronto, Toronto, ON, Canada**Citation:** Gorczyński RM (2023) Updates on the Importance of CD200:CD200R Checkpoint Blockade in Solid Tumors and B cell Malignancies. J Oncol Res Ther 8: 10185. DOI: 10.29011/2574-710X.10185**Received Date:** 24 September, 2023; **Accepted Date:** 29 September, 2023; **Published Date:** 03 October, 2023**Abstract**

The last several years have seen the introduction into clinical medicine of a family of reagents directed towards so-called “checkpoint inhibitors”, which act at gateways in a developing immune response to regulate unwanted and/or harmful self-directed activation responses. The molecules involved at such gateways generally belong to an extended immunoglobulin supergene family, and contribute inhibitory signals to dampen over-exuberant responses. They include, but are not limited to, molecules of the CD28/cytotoxic T-lymphocyte antigen-4 (CTLA-4):B7.1/B7.2 receptor/ligand family; PD-1 and PDL-1; CD200 and CD200R; TIGIT and VISTA and their respective ligands (VSIG-3/IGSF11, Nectin), all of which are presumed to play a physiological role in maintaining natural self-tolerance. In the field of cancer immunotherapy, where the ultimate clinical goal is to improve immuno-targeting of cancer cells, triggering these checkpoint inhibitory signaling pathways, has the potential to thwart effective tumor immunity. This in turn has led to the characterization and application of multiple reagents, including antibodies and other designed inhibitory molecules, which can act as checkpoint blockade agents. Such reagents have had a dramatic effect on human cancer treatment, with marked success for anti-CTLA-4 and PD-1 in particular in clinical trials. This review elaborates on the promise on other more under-appreciated target molecules for checkpoint blockade in human B cell malignancies and solid tumors, particularly CD200:CD200R, and describes both the background, and newer studies, which highlight the potential importance of targeting the CD200:CD200R dyad in cancer immunobiology/therapy.

Keywords: Checkpoint blockade; Immunotherapy; Oncology; Inhibitory pathways; Stimulatory pathways; Activated T cells**Introduction**

T cell activated immune responses to both nominal antigen and tumor-expressed antigens follows T cell receptor engagement of antigen expressed on suitable antigen-presenting-cells and delivery of suitable co-stimulatory signals (generally via the CD28: CD80/CD86 axis), along with an activation signal to antigen-presenting cells [1]. Early studies directed at improving cancer immunotherapy were directed towards enhancing antigen presentation, and/or co-stimulatory signals for cancer therapy [2,3]. However, it has become clear over the past 10-20 years that immunity is also regulated by the expression and engagement of

inhibitory molecules and their receptors in the immune system [4-7], and activation of such inhibitory ligand: receptors leads to so-called checkpoint blockade [8-10]. In turn, reversal of checkpoint blockade can release activation of anti-tumor responses [11-13].

The discussion that follows highlights some of the more recent data exploring checkpoint blockade in cancer care with the most common molecules currently explored in both model systems and in clinical situations [12,13] (a summary of checkpoint blockade studies is included in Table 1, and a schematic for the action of many reagents targeting key checkpoints in tumor therapy in Figure 1). A major emphasis of this review however, as will be apparent later, is to focus on the growing body of evidence to support the notion that targeting a novel ligand:receptor dyad which my laboratory has explored at length, CD200:CD200R, is

an important, and to date overlooked, area with great therapeutic potential in both solid tumors and B cell malignancies [14].

Targeted interaction ^a (ref) ^c	Checkpoint blockade reagent ^b	Experimental(E)/Clinical(C)+
CTLA4:CD28	Anti-CTLA4	E: (18,19, 29-31)
		C: melanoma (17, 18)
		C:solid tumor (19, 21,22)
PD-1:PDL-1	Anti-PD-1	E: (25,29)
		C: melanoma (35)
		C: solid tumor (26,30)
	Anti-PDL-1	E: (35, 36)
		C: solid tumor (39)
TIGIT	Anti-TIGIT	E: (38)
Tim3	Tim3 KO mice	E: (42)
VISTA: VSIG-3/IGSF11	Anti-VISTA	E: (39)
CD47	Anti-CD47	E: (43)
CD200:CD200R		E: hematopoietic tumor (36,46)
	Anti-CD200 and anti-CD200R	C: leukemias (48-57; 59)
	+KO mice	E: solid tumor (47,71-75;89-99; 105-106)
		C: Solid tumors (73,76-84;89,96,104)
Combined therapies	Anti-CTLA4/Anti-PD-1	C: melanoma (20)
	Anti-CD47/Anti-PD-1	E: (44,45)
	Anti-TIGIT/Anti-PD-1	C: solid tumor (24)
	VISTA/Anti-PD-1	E: (40)
		C: solid tumor (41)
	Anti-PD-1/vaccination	E: solid tumor (27, 28,32)
	TEM ^d +Anti-PD-1/vaccination	E: (33,34,77,87)
	TEM ^d + anti-CD200/CD200R	E: (110,111)
	CAR-T ^d cells/PD-1	E: (31)

Footnotes to Table:

a. Co-stimulatory/inhibitor pathway targeted for manipulation

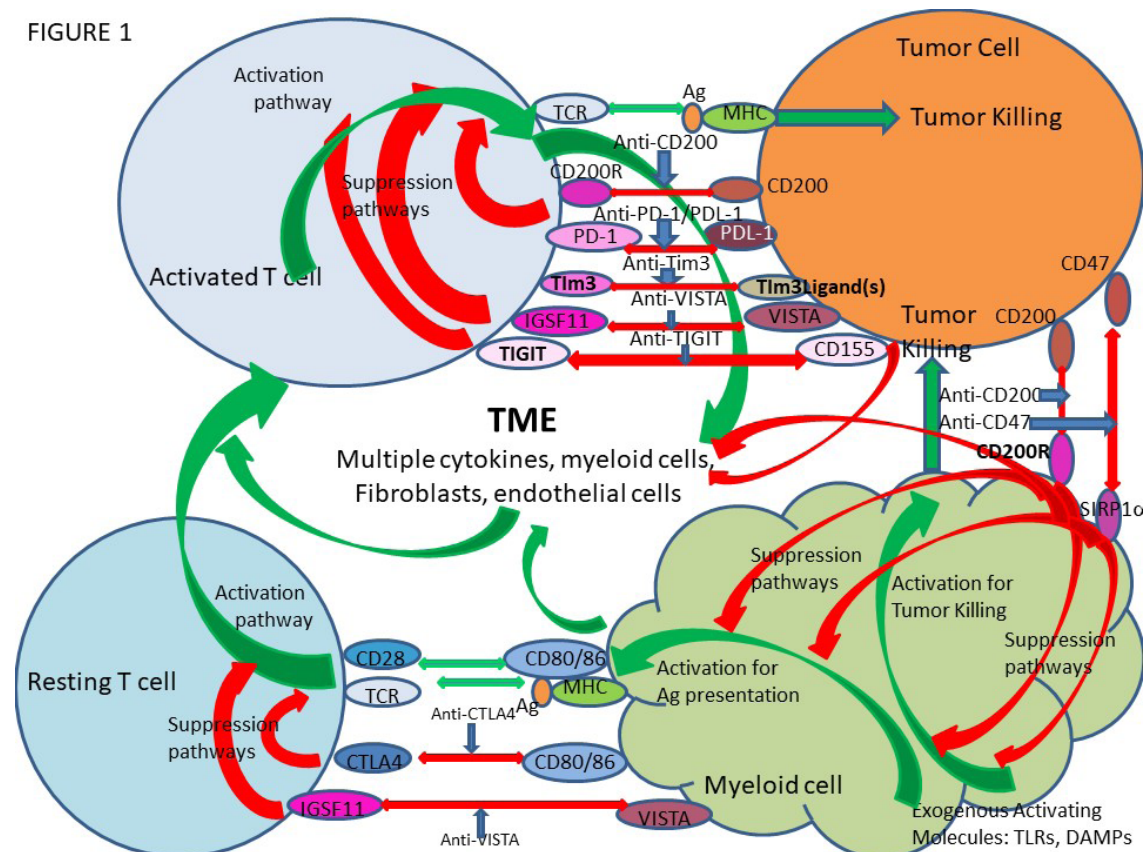
b. Reagents used to target interaction under investigation

c. Situation in which reagents are used; E=experimental model system; C=clinical scenario

d. TEM=tumor microenvironment; CAR-T=genetically engineered chimeric antigen-receptor T cells

Table 1: Summary of checkpoint blockade, alone or in combination, in tumor Immunotherapy.

FIGURE 1



Modified from: Gorczynski RM, Zhu F. *Cancer Manag Res.* 2017 Nov 13;9:601-609.

CTLA-4, PD-1:PDL-1, TIGIT, VISTA, Tim-3 and other inhibitory receptors in cancer therapy:

Given that CTLA4 is an alternate ligand for CD80/86, and unlike the activating ligand CD28, induces regulation of T cell responses rather than T cell activation, it was no surprise that neutralization of CTLA4 showed a beneficial effect on anti-tumor immunity [15,16], with such neutralizing antibodies being the first approved inhibitors of checkpoint blockade to be used clinically in melanoma [17,18] and subsequently in solid tumors [19]. More recent studies have focused on the possible use of combinations of reagents targeting different checkpoints to improve tumor immunotherapy eg. CTLA4 and PD-1 (below) blockade [20], as well as the use of checkpoint blockade along with vaccination (see below). This latter strategy is well documented in studies showing that patients with advanced prostate cancer responding to treatment with anti-CTLA4 therapy developed enhanced responses to a number of other tumor-related antigens, both patient specific and disease specific, compared with non-responders [21].

As note above, a current evolving trend has been to use anti-CTLA4 therapy in association with other therapies, either

immunotherapy (vaccination) or other checkpoint blockade reagents. Huang et al, in a murine model of ovarian cancer [22] found that multiple immune checkpoint molecules were expressed in tumor-associated or tumor-infiltrating cells in ovarian tumor-bearing mice, but simple blockade of any one of PD-1, LAG-3, or CTLA-4 alone using blocking antibodies or knockout mice merely led to an up-regulation of the other checkpoint pathways with failure to attenuate tumor outgrowth. However, dual antibody blockade against PD-1/CTLA-4 or triple blockade against PD-1/LAG-3/CTLA-4 led to tumor-free survival in ~20% of treated mice [22]. Interestingly, dual blockade of LAG-3 and CTLA-4 pathways in PD-1 knockout mice resulted in tumor-free survival in ~40% of treated mice, implying the existence of a hierarchy in the importance of immune checkpoints in some tumors, though this remains to be explored in depth. Similar results with combination checkpoint inhibitor blockade has been reported by others [23,24].

Focus on the importance of the PD-1:PDL-1 dyad [25] in cancer growth was stimulated by evidence that up-regulation of PDL-1 was often seen on cancer cells [26] and the ligand PD-1 was in turn expressed on T cells [27]. At least one potential important

effect of blockade of PDL-1 signaling may involve augmentation of DC maturation, proliferation, and IL-12 secretion, with resultant improved immunity induced by DCs [28]. Results from such model systems are borne out by positive clinical trial results with antibodies targeting the PD-1:PDL-1 pathway in metastatic melanoma, prostate cancer, non-small-cell lung cancer (NSCLC), renal cell carcinoma (RCC) and colorectal cancer (CRC) [29,30]. Such therapy is often used in combination with additional treatments, including CAR-T cell therapy [31], tumor vaccine administration [32], and manipulation of exogenous/endogenous cytokines [33,34]. It is interesting in this context too that there are reports that high levels of soluble PD-L1 are potentially a useful biomarker of poorer prognosis in renal cell carcinoma/multiple myeloma/melanoma patients [35], analogous to the observation we had reported earlier in regards to soluble serum CD200 [36,37].

The T cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT), which binds two ligands, CD155 (PVR) and CD112 (PVRL2, nectin-2) expressed by tumor and other cells in the tumor microenvironment, has also been acknowledged as another potential target for cancer immunotherapy. Expression of TIGIT is increased on activated T cells, natural killer cells, and regulatory T cells, and there is evidence that combined PD-1/TIGIT blockade increased tumor antigen-specific CD8⁺ T cell expansion/function and promoted tumor rejection in mouse models [38]. Similarly, targeting V-domain immunoglobulin suppressor of T-cell activation (VISTA), a molecule expressed on hematopoietic cells, and on myeloid cells infiltrating tumors [39] has been reported to prove effective in attenuating tumor growth in combination with PD-1 blockade [40], with preliminary data lending credence to a potential value in clinical therapy in gastric cancer patients [41]. More recently it has been suggested that targeting Tim-3, a molecule expressed on Foxp3⁺Tregs (along with other cells of the innate immune system), has a role in leading to augmentation of anti-tumor immunity [42].

Another interesting target molecule is CD47, a (relatively) ubiquitously expressed glycoprotein of the immunoglobulin superfamily, over-represented on many solid and hematologic cancer cells, with high expression correlated with poor clinical prognosis. CD47 is implicated in the regulation of self-recognition [43] through interaction with its receptor, SIRP α , expressed on myeloid cells. This interaction in turn activates cytoplasmic immunoreceptor tyrosine-based inhibition motifs, recruitment of Src homology 2 domain-containing tyrosine phosphatases, and delivery of the anti-phagocytic-"don't eat me"-signal. Thus CD47-SIRP α acts as a negative checkpoint bridging innate immunity and adaptive immunity, and CD47 blocking antibodies diminish tumor growth in several animal models [43]. While to date no clinical studies targeting this pathway are available, there are a number of animal model studies which suggest there may be clinical utility

in targeting such a molecule [44,45]. As is discussed next, there is independent precedence for such an approach in a plethora of data investigating a role for targeting a similar molecular dyad, the CD200:CD200R axis, in cancer therapy.

The importance of CD200:CD200 interactions in controlling tumor growth

CD200 is a molecule known to be relatively ubiquitously expressed, while expression of the receptors (CD200R1-5 in mouse; CD200R1,R2 in man) is seen mainly on cells of the myeloid and macrophage lineage. There are a number of studies which suggest that blockade of CD200:CD200R interactions attenuates inflammatory (innate) immune reactions while augmenting development of acquired immunity [46], which in turn promoted investigations into the potential role of regulation of CD200:CD200R interactions in tumors [47].

Regulation of hematopoietic tumor growth by CD200:CD200R

Abundant data indicates expression of CD200 both by activated normal B cells, by tumor cells in patients with B lymphoid malignancies, and even by precursor stem cells for B cell malignancies. Expression of CD200 is generally a poor prognostic indicator in B cell malignancies [48], and therapies aimed at neutralizing CD200 expression favour modulation of tumor growth [49-52], including in both pediatric AML and ALL [53,54]. There is now too a growing body of evidence implicating a role for CD200 expression levels in regulation of growth of CLL [55-57]. Interestingly, often the effects seen in different patients, at least in CLL, were not reflective of relative expression levels of CD200 by tumor cells, suggesting that the mechanism of action was not simply one of neutralization of expressed CD200 [58].

Wong et al reported [36] that soluble CD200 found in the serum of CLL patients was essential for promotion of CLL growth in a NOD.SCID humanized mouse model. There are now similar reports of the prognostic significance of sCD200 expression in patients with CLL [59]. Newer evidence suggests that CD200 expression controls Treg expansion and disease progression in both acute myeloid leukemia and CLL [60]. In a study with 14 CLL patients, CD200 blockade using an anti-CD200 antibody we described earlier [36] led to disruption of T cell suppression as measured in autologous MLCs using CD40 ligand (CD40L)-stimulated CLL cells as antigen-presenting cells (APCs) [60], a mechanism inferred by Wong et al in studies in NOD.SCID mice with CLL cells [36]. A similar mechanism has also been suggested as contributing to the mechanism behind increases in Foxp3⁺T cell numbers in PTLN patients following overexpression of CD200 [61].

An alternate mechanism of action of CD200:CD200 blockade was suggested by Poh et al. [62], who reported that

checkpoint blockade augmented cytotoxicity of cytokine-induced killer cells against human myeloid leukemia blasts. Interestingly, CD200 blockade has also been reported to improve regulation of AML growth by Tr1 cells engineered to overexpress IL-10 [63]. This is in turn reminiscent of the report that Ibrutinib, an inhibitor of Bruton's tyrosine kinase (BTK) and IL-2-inducible T cell kinase (ITK), which is used in treatment of CLL, may impact CLL induced immunosuppression by modulating CD200 expression [64]. Zhu et al (personal communication) more recently combined a vaccination approach to CLL (using PMA and ionomycin stimulated CLLs as vaccine) in concert with blockade using anti-human CD200 antibody, to attenuate both local disease and secondary organ spread of CLL cells in a NOD.SCID mouse model.

Before concluding this section on discussion of the importance of CD200:CD200R in control of myeloid tumors, it is worthy of note that even in studies where there was little direct evidence for a role of attenuating CD200 expression in tumor growth control, there has been reported value in monitoring expression in both the diagnosis of B cell malignancies [65,66], and in monitoring dynamic changes during the response to treatment [67]. This is the case also in high risk myelodysplastic patients [68]. A final comment, which will be elaborated on below in a discussion of the effect of CD200:CD200R interactions in solid tumors, concerns the potential importance of this dyad, beyond any direct effect on tumor cells themselves, in controlling tumor growth through regulation of the tumor microenvironment (TME) [69]. Tumor associated myeloid cells (TAMCs) are important in cancer immunotherapy, and CD200-CD200 receptor (CD200R) interactions are reported to be implicated in regulating the TME by affecting TAMCs [70]. Studies in an in vitro/in vivo model exploring control of growth of CLL, showed that bone marrow mesenchymal stem cells (BMMSCs) and the cytokines they produce, including IL-6, are important components of the TME in CLL, and that BMMSCs support the survival of CLL cells in vitro through an IL-6 dependent mechanism [70]. IL-17, which can induce IL-6 generation in many cells, increased production of IL-6 both in CLL cells and BMMSCs in vitro. It must be acknowledged that the effect of CD200-target therapy in this particular system was not investigated.

Regulation of solid tumor growth by CD200:CD200R

The role of CD200:CD200R as a crucial checkpoint for immunity in solid tumors was implied from an earlier study by Moreaux et al [71], who described overexpression of CD200 in renal carcinoma, head and neck carcinoma, testicular cancer, malignant mesothelioma, colon carcinoma, myeloma, and CLL, all compared to their normal cells or their tissue counterparts. These data also showed that CD200 expression was associated with tumor progression in many cancers. Similarly, Siva et al

[72] reported expression of CD200 on cell lines derived from ovarian cancer, melanoma, neuroblastoma and renal carcinoma cell patients. Addition of CD200⁺ but not CD200⁻ tumor cells to MLCs led to a decline in Th1 cytokine production, and this effect was attenuated by anti-CD200 antibody [72]. Increased staining of CD200 and CD200R protein expression was also found on liver tissue specimens in subjects with hepatocellular carcinoma compared with healthy controls [73], which was correlated with tumor size, alpha-fetoprotein levels, and pathological tumor grade compared with healthy controls. Overall and recurrence-free survival rates were lower in patients with high CD200R [73] expression compared to those with low CD200R expression. These data are consistent with those from Matsuo et al, who investigated immune responsiveness in patients with colorectal liver metastasis [74]. CD4⁺, CD8⁺, and CD45RO⁺ tumor-infiltrating lymphocytes were significantly higher in the low-CD200 group than in the high-CD200 group, and overall survival was significantly worse in patients in the high-CD200 group who had received preoperative chemotherapy. Similarly, in a rat model of hepatocellular carcinoma where treatment involved thermal ablation of tumor, Huang et al concluded that the CD200/CD200R pathway participated in tumor growth and the expression of CD4⁺T cell subsets in cancer tissue, and that thermal ablation treatment inhibited cancer recurrence through a mechanism potentially involving this same CD200:CD200R axis [75].

Multiple studies have investigated the importance of checkpoint inhibition in lung cancer besides the antagonism of the PD1:PDL1 and CTLA4 axes discussed above (see earlier). Vathiotis et al found that CD200:CD200R was frequently expressed in non small cell lung cancer patients (NSCLCs), and that expression was correlated with PD-L1 expression, with implications for targeting this pathway alone, or in combination with PD1:PDL1 in NSCLCs [76]. In an animal model system Wang et al [77] also concluded that the CD200:CD200R and PD-1:PDL-1 axes might interact favourably to regulate tumor growth, while Yoshimura et al [78] independently argued for the potential importance of assessing CD200:CD200R expression in NSCLCs. CD200 expression was reported by Zhang et al as one of six signature marker genes whose expression was a prognostic predictor of survival in NSCLCs [79]. The favourable interaction between CD200:CD200R and PD-1:PDL-1 in regulating tumor growth was also highlighted in studies of pancreatic adenocarcinomas by Choueiry et al [80], who concluded that one of the primary mechanisms of action involved modulating MDSCs, known to express CD200R, and possibly their expansion in response to local cytokines in the TME. The role of CD200 expression in modifying the local TME, in turn resulting in regulation of tumor growth, was also noted by Zhang et al in studies of cervical cancer [81], and by Feng et al in prostate cancer [82]. Interestingly, in a study of metastatic pancreatic cancer, liver metastases in both animal models and humans were reported to

contain highly anergic T cells and MHCII^{lo}IL10⁺ macrophages which could not present tumor-antigen, along with CD24⁺CD44⁺CD40⁺ B cells [83]. These B cells were apparently recruited to the metastatic milieu by Muc1hiIL18hi metastatic tumor cells, and were implicated in driving macrophage-mediated immune-tolerance through CD200 and BTLA. These authors reported that depletion of these B cells and targeting CD200/BTLA led to enhanced macrophage and T-cell immunogenicity with subsequent effective immunotherapeutic of liver metastases [83]. A similar result showing therapeutic efficacy of CD200 blockade in reducing Merkel cell carcinoma tumor growth was seen in tumors which were infiltrated by suppressive CD200⁺ macrophages and Tregs [84]. Along similar lines, in animal models of lung cancer, within the TME there was preferential differentiation of DCs away from CD103⁺ DC1s, which have the ability to induce tumor immunity, towards CD103^{lo}CD11b⁺ DCs, expressing the DC2 markers IRF4 and Sirpα, along with high levels of T-cell inhibitory molecules PD-L1/2 and CD200 [85]. The effect of CD200 blockade on DC differentiation within the tumor was not investigated in these studies-see also [86]. A model which might help explain much of these data is suggested by recent studies of adrenocortical carcinoma patients [87], which were subdivided into low/high hypoxia risk scores (HRS) by gene expression data. The low hypoxia risk score group showed a “fit” to an inflammatory immunophenotype, while the high HRS group represented a non-inflammatory immunophenotype. Importantly, high HRS correlated with expression of immune checkpoint molecules including PD-L1, CD200, CTLA-4, and TIGIT, consistent with data that patients with low HRSs responded better to immunotherapy.

The potential importance of the CD200:CD200R axis in regulation of tumor growth independent of tumor expression of CD200 (or CD200R) was also described by Rygiel et al in animal studies exploring regulation of skin malignancies induced in an animal model of chemical carcinogenesis [58]. CD200KO mice were resistant to tumor induction, an effect which was correlated with an alteration in the Th17:Treg ratio, and which occurred independent of any CD200 expression by tumor cells themselves. In stark contrast to these data are those of Talebain et al, investigating the effect of CD200 blockade in a CD200⁺ Yumml.7 mouse melanoma model [88]. It is known that Yumml.7 cells bear Braf/Pten mutations resembling human melanoma, yet they observed that anti-CD200 therapy failed to show efficacy either alone or in combination with checkpoint inhibitors such as anti-PD-1 or anti-CTLA4 in inhibiting Yumml.7 tumor growth, and that both CD200R-deficiency and anti-CD200 treatment resulted in reduced T cell responses in the TME-they highlighted these studies as indicating some caution in using blockade of CD200 as an immunotherapy for melanoma [88]. Somewhat analogous to studies above indicating the potential importance of CD200:CD200-R interactions in regulating the TME are reports by Belkin et al, who

explored the ability of this same dyad to modulate squamous cell carcinoma tumor diapedesis at the endothelium of blood vessels [89]. CD200 expression on local blood vessels was thought to promote tumor progression by suppressing CD200R myeloid cells during diapedesis leading to immune evasion by SCC, and again suggesting a novel therapeutic target for control of metastasis of SCC.

There has been intense interest in the past decade on the role of CD200 blockade in cancers of the brain, which had generally been poorly responsive to tumor-derived vaccines and chemotherapy, yet are known to express CD200 [90,91]. These poor results may reflect the effect of expression of immune-checkpoint ligands including PD-L1 and CD200 by glioblastoma (GBM) cells which interact with their respective receptors on tumor-infiltrating effector T cells/myeloid cells thus curtailing the activation of anti-GBM immunity. Studies of expression of genes involved in autophagy, apoptosis and necrosis in 518 GBM patients from The Cancer Genome Atlas (TCGA) database revealed a cohort of 16-genes grouped into a cell death index (CDI), with patients subsequently clustered into either a high risk or low risk group according to the CDI score, with overall survival subsequently correlated with higher infiltration of neutrophils, macrophages, Treg, T helper cells, and lower expression levels of immune checkpoint molecules PDCD1 and CD200 [92]. Subsequent studies by Olin’s group confirmed that overexpression of CD200 attenuated the efficacy of a tumor derived vaccine in glioblastoma (GBM) [93], which could in turn be blocked using a CD200 checkpoint inhibitor peptide [94]. The mechanism of action of this peptide inhibitor, CD200AR-L was hypothesized to reflect the targeting of a CD200 activation receptors (CD200AR) to overcome tumor-induced immunosuppression [95]. The same group subsequently used a proteomics approach to produce a humanized CD200AR-L, which was shown to induce a unique chemokine response, stimulating immature dendritic cell differentiation and significantly enhancing an antigen-specific response, which also led to downregulation of the expression of CD200 inhibitory and PD-1 receptors [95]. More recent studies showed that CD200AR-L/CD200AR binding induced early activation of both the DAP10&12 pathways, followed by a decrease in activity within 30 min, followed by reactivation via a positive feedback loop [96]. In vivo studies with DAP10&12KO mice showed the importance of DAP10, not DAP12, for tumor control. This immunotherapy aimed at modulating immune checkpoints in GMB is currently being analyzed in a phase I adult trial [96]. An interesting variant of a similar approach was described by Kobayashi et al [97], who described expression of both full-length CD200 (CD200L) and a truncated form, CD200S, in human carcinoma tissues. In a murine transplant model, we previously reported that this truncated variant could itself antagonize immunosuppression induced by full-length CD200, likely through engaging alternate receptors

and/or by acting as a competitive inhibitor to full-length CD200 at an inhibitor receptor [98]. Kobayashi used rat C6 glioma cell lines that expressed either CD200L or CD200S and showed that CD200S activated TAMs to become DC-like antigen presenting cells, resulting in activation of CD8⁽⁺⁾ cytotoxic T lymphocytes and subsequent apoptotic elimination of tumor cells. They suggest this may in itself highlight a novel therapeutic modality for the treatment of carcinomas [97].

A focus of my own group, besides the CLL studies discussed earlier, has been to investigate the role of CD200:CD200R as an immune checkpoint in breast cancer. Two quite different BALB/c mouse models have been characterized. The one, 4T1 is a highly aggressive, metastasizing, poorly immunogenic tumor, while EMT6 in contrast is an example of a moderately immunogenic, poorly metastasizing, and slower growing tumor [99]. In the case of the former, an inflammatory cascade is growth promoting, while for EMT6 tumors, suppression of inflammation results in development of T cell immunity and delayed tumor growth-similar heterogeneous findings have been made with human breast cancer [100-104]. Consistent with this dichotomy, over-expression of CD200 led to improved control of growth of 4T1 tumors, while augmenting growth of EMT6 cells [99]. In contrast, in the absence of endogenous expression of CD200, or more particularly CD200R, tumor bearing mice showed markedly reduced local and metastatic EMT6 growth and could be immunized for sterile immunity with resistance to further tumor challenge, again with the opposite effects seen for 4T1 tumor [105]. It was clear that an active immune process developed in EMT6 tumor-injected mice in association with CD200 (or CD200R) blockade since we showed that conventional chemotherapy acted synergistically with CD200 blockade to cure wild-type mice and produce immune mice resistant to re-challenge and/or metastasis even at 1 year post treatment-such an effect was not seen with chemotherapy alone [106].

It has also become clearer over the past few years that checkpoint inhibitors modulate not simply the innate or adaptive immune responses to the tumor, but can, as already mentioned, have a equally important effect on the TME [69,107,108]. This dual effect likely helps explain some anomalies observed in the effect of either CD200 or CD200R blockade on tumor growth [109], where, for example, Pilch et al in a mouse melanoma observed that blockade of endogenous CD200 expression prevented the tumorigenic effect of CD200R-expressing myeloid cells in the tumor microenvironment, although agonistic anti-CD200R had no effect on tumor development. A further effect of manipulation of CD200:CD200R signaling to influence tumor growth indirectly through the TME was reported from studies of papilloma tumors in mice [110]. Toll-like receptor 7 (TLR7) stimulation is known to augment immunity through activation of myeloid cells,

decreasing papilloma growth, an effect antagonized by CD200R stimulation which suppresses TLR7 signaling. However, the immediate antitumor effects of TLR7 signaling alone were independent of lymphocytes, with similar results seen in wild-type and Rag1^{tm1Mom} mice [110]. Moreover, TLR7 stimulation, particularly in combination with anti-CD200R, changed the phenotype of intratumoral myeloid cells, decreasing expression of the macrophage markers F4/80, CD206, CD86, CD115, and the ability to produce IL1 β , suggesting a shift in the composition of intratumor myeloid cells. Indeed, CD11b⁺ myeloid cells, isolated from the tumors of mice receiving combined treatment inhibited tumor outgrowth on adoptive transfer suggesting that administration of agonistic anti-CD200R improved the antitumor effects of TLR7 signaling and altered the local TME to become one less supportive of tumor progression [110]. More recently an intriguing effect of a CD200 mimetic aptamer given in conjunction with pegylated doxorubicin was reported in regulation of growth of breast cancer cells in a mouse model [111]. Peg-M49 and Peg-Dox co-treatment induced complete tumor regression and loss of macroscopic lung metastasis in four out of seven BALB/c mice [111]. Synergism was associated with Peg-M49-induced inhibition of Gr1⁺CD11b⁺ cells and Peg-Dox-induced increases in tumor-infiltrating CD8⁺ and CD8⁺CD4⁺ cells. Importantly, similar changes were observed in CD200R1^{-/-} mice suggesting that the primary effects of Peg-M49 were mediated by non-inhibitory CD200R receptors. Moreover, tumor growth, metastasis, and tumor infiltrating GR1⁺ CD11b⁺ cells were markedly increased in CD200R1^{-/-} mice, implying that there existed an anti-inflammatory and protective role for CD200. The authors concluded that in fact CD200 mimetics might be safe and effective therapeutics, in conjunction with classical chemotherapy, in treatment of aggressive metastatic breast carcinoma. In this regard it might be noted that a recent study of T cells engineered following single-cell mRNA sequencing (scRNA-seq) with T-cell receptors (TCRs) from naturally occurring tumor antigen-specific T cells (TAS cells) in different patients, presumably targeting personal TSAs in that individual's tumor, were observed to express CXCL13 as a unique marker for both CD4⁺ and CD8⁺ TAS cells [112]. TCR-T cells expressing TCRs from TAS cells attenuated growth of autologous patient-derived xenograft tumors, with intratumoral TAS cell levels (CXCL13 expression) predicting the response to immune checkpoint blockade. Importantly, CD200 was a surface marker for CD4⁺ which enabled the isolation of TAS cells from tumor by FACS sorting, implying a non-suppressive (overall) effect in this model.

Summary

There can be little doubt that immune checkpoint blockade has transformed cancer immunotherapy producing some quite remarkable and long-lasting effects, particularly in melanoma

and some hematopoietic tumors and now expanding to multiple solid tumor studies. As has been highlighted, the use of genomic expression analysis has both guided characterization of the molecules involved, and helped gain insight into their mechanism(s) of action [65,68,73,74,79,87,92,101-103]. As but one further example, analysis of sorted CD4⁺CD26⁻ and CD4⁺CD26⁺ T cells from classical Hodgkins Lymphoma lymph node cell suspensions (HLN) by RNA sequencing and T cell receptor variable gene segment usage analysis showed that while CD4⁺CD26⁻ T cells were antigen experienced, they were not clonally expanded [113]. In part at least this is explained by the over-expression of exhaustion associated transcription factors TOX and TOX2, immune checkpoints PDCD1 and CD200, and the chemokine CXCL13, which were all significantly enriched genes compared with the CD4⁺CD26⁺ T cells. This overwhelming dominance of a CD4⁺CD26⁻ T cell population in HLN which is antigen experienced, polyclonal, and exhausted likely is itself predictive of, and contributes to, the very high response rates to immune checkpoint inhibitors in such patients [113].

With experience in use of single agents for checkpoint blockade it is also becoming increasingly apparent that the overall response rate for the currently approved reagents used alone is limited. This has encouraged studies using multiple checkpoint blockade reagents in combination (see Figure 1 and Table 1 suggesting the potential for use of checkpoint blockade of T cell expressed and myeloid expressed markers), and/or with additional immunotherapy, as well as further biomarker analysis (gene expression data) which might predict both the success of these therapies; can monitor their efficacy [114]; and may even highlight new molecules for consideration [115]-see also studies on VISTA/TIGIT/CD47 highlighted above, and more recent reports on use of combined checkpoint blockade for NK-mediated tumor killing [116]. In this regard, the very fact that some 40% of humans and Tasmanian devils (*Sarcophilus harrisii*) develop cancer in their lifetime, compared to less than 10% for most species, and that CD200:CD200R is one of the immune checkpoint interactions which has been conserved across 160,000,000 years of evolution, suggests that CD200, which is highly expressed on transmissible tumor cells, may be an important molecule to understand [117].

The final, though by no means the least important, observation to be made concerns the demographics of the at-risk population, and those reflected in clinical trials. Studies of cancer diagnosis and death by age groups shows that elderly patients (≥ 65 years) are over-represented with even further increases expected over the next decade, for example for non-small cell lung cancer [118]. Nevertheless, for a variety of reasons this population is quite under-represented in clinical trials, despite the fact that it has been acknowledged for many years that ageing per se is associated with both qualitative and quantitative changes in

immunity [119,120]. It would not be surprising to find that many of the reagents used as checkpoint inhibitors in younger subjects might be more/less efficacious in elderly ones and may produce quite unexpected responses in the two different cohorts. Moreover, there may be novel molecules which take on important roles in immunoregulation, or TME control, in the elderly which have yet to be identified. It seems self-evident that that over the next decade, with more attention paid to this discrepancy, an improved understanding of checkpoint blockade in aging; more specific clinical trials for elderly cancer patients; and increased use of combination immunotherapies will likely further improve cancer survival across multiple disease types.

References

1. Harris NL and Ronchese F (1999) The role of B7 costimulation in T-cell immunity *Immunol and Cell Biol* 77: 304-311.
2. Spaepen R, Blomen V, van Veelen P, Jongsma M, Janssen L, et al. (2014) Identification of potential immunotherapeutic targets in antigen presentation and costimulation networks *Immunother Cancer*: 2.
3. Maj T, Wei S, Welling T, Zou W (2013) T cells and costimulation in cancer. *Cancer J* 19: 473-482.
4. Ansari MJ, Salama AD, Chitnis T, Smith RN, Yagita H, et al. (2003) The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice. *J Exp Med* 198: 63-69.
5. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, et al. (1995) Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 3: 541-547.
6. Chambers CA, Sullivan TJ, Allison JP (1997) Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4⁺ T cells. *Immunity* 7: 885-895.
7. Zhang J, Zhang M, Jiang W, Wang L, Fu Z, et al. (2009) B7- H4 gene polymorphisms are associated with sporadic breast cancer in a Chinese Han population. *BMC Cancer* 9: 394.
8. Lines JL, Pantazi E, Mak J, Sempere LF, Wang L, et al. (2014) VISTA is an immune checkpoint molecule for human T cells *Cancer Res* 74: 1924-1932.
9. Monney L, Sabatos CA, Gaglia JL, Ryu A, Waldner H, et al. (2002) Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease *Nature* 415: 536-541.
10. Okazaki T, Okazaki IM, Wang J, Sugira D, Nakaki F, et al. (2011) PD-1 and LAG-3 inhibitory co-receptors act synergistically to prevent autoimmunity in mice *J Exp Med* 208: 395-407.
11. Korman AJ, Peggs KS, Allison JP (2006) Checkpoint blockade in cancer immunotherapy. *Adv Immunol* 90: 297-339.
12. Pardoll DM (2012) The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 12: 252-64.
13. Ni L, Dong C (2017) New checkpoints in cancer immunotherapy. *Immunol Rev* 276: 52-65.
14. Gorczynski RM, Zhu F (2017) Checkpoint blockade in solid tumors and B-cell malignancies, with special consideration of the role of CD200.

- Cancer Manag Res 13; 9:601-609.
15. Chambers CA, Kuhns MS, Egen JG, Allison JP (2001) CTLA-4-mediated inhibition in regulation of T cell responses: mechanisms and manipulation in tumor immunotherapy. *Annu Rev Immunol* 19: 565-94.
 16. Egen JG, Kuhns MS, Allison JP (2002) CTLA-4: new insights into its biological function and use in tumor immunotherapy. *Nat Immunol* 3: 611-8.
 17. Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, et al. (2003) Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc Natl Acad Sci U S A* 100: 8372-7.
 18. Hodi FS, O'Day SJ, McDermott DF et al. (2010) Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med* 363: 711-723
 19. May KF Jr, Gulley JL, Drake CG, Dranoff G, Kantoff PW (2011) Prostate cancer immunotherapy. *Clin Cancer Res* 17: 5233-8.
 20. Willmsore ZN, Coumbe BGT, Crescioli S, Reci S, Gupta A, et al. (2021) Combined anti-PD-1 and anti-CTLA-4 checkpoint blockade: Treatment of melanoma and immune mechanisms of action. *Eur J Immunol* 51: 544-556.
 21. Kwek SS, Dao V, Roy R, Hou Y, Alajajian D, et al. (2012) Diversity of antigen-specific responses induced in vivo with CTLA-4 blockade in prostate cancer patients. *J Immunol.* 189: 3759-66.
 22. Huang RY, Francois A, McGray AR, Miliotto A, Odunsi K (2016) Compensatory upregulation of PD-1, LAG-3, and CTLA-4 limits the efficacy of single-agent checkpoint blockade in metastatic ovarian cancer. *Oncoimmunology* 6: e1249561.
 23. Yi M, Zheng X, Niu M, Zhu S, Ge H, et al. (2022) Combination strategies with PD-1/PD-L1 blockade: current advances and future directions. *Mol Cancer* 21:28.
 24. Niu J, Nagrial A, Voskoboinik M, Chung H, Lee D, Ahn M, et al. (2020) 1410P Safety and Efficacy of Vibostolimab, an Anti-TIGIT Antibody, Plus Pembrolizumab in Patients With Anti-PD-1/PD-L1-Naive NSCLC. *Ann Oncol* 31: S891-S92.
 25. Balar AV, Weber JS (2017) PD-1 and PD-L1 antibodies in cancer: current status and future directions. *Cancer Immunol Immunother* 66: 551-564.
 26. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, et al. (2010) Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol* 28: 3167-75.
 27. Fu J, Malm IJ, Kadayakkara DK, Levitsky H, Pardoll D, et al. (2014) Preclinical evidence that PD1 blockade cooperates with cancer vaccine TEGVAX to elicit regression of established tumors. *Cancer Res* 74: 4042-52.
 28. Ge Y, Xi H, Ju S, Zhang X (2013) Blockade of PD-1/PD-L1 immune checkpoint during DC vaccination induces potent protective immunity against breastcancer in hu-SCID mice. *Cancer Lett.* 336: 253-9.29.
 29. Topalian SL, Drake CG, Pardoll DM (2012) Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. *Curr Opin Immunol* 24: 207-12
 30. Herbst RS, Baas P, Kim DW, et al. (2016) Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 387: 1540-50.
 31. Hegde UP, Mukherji B (2017) Current status of chimeric antigen receptor engineered T cell-based and immune checkpoint blockade-based cancerimmunotherapies. *Cancer Immunol Immunother.*
 32. Santegoets SJ, Stam AG, Loughheed SM, Gall H, Scholten PE, et al. (2013) T cell profiling reveals high CD4+CTLA-4 + T cell frequency as dominant predictor for survival after prostate GVAX/ipilimumab treatment. *Cancer Immunol Immunother* 62: 245-56
 33. Yu P, Steel JC, Zhang M, Morris JC, Waldmann TA (2010) Simultaneous blockade of multiple immune system inhibitory checkpoints enhances antitumor activity mediated by interleukin-15 in a murine metastatic colon carcinoma model. *Clin Cancer Res* 16: 6019-28.
 34. Terabe M, Robertson FC, Clark K, De Ravin E, Bloom A, et al. (2017) Blockade of only TGF- β 1 and 2 is sufficient to enhance the efficacy of vaccine and PD-1 checkpoint blockadeimmunotherapy. *Oncoimmunology* 6: e1308616.
 35. Zhou J, Mahoney KM, Giobbie-Hurder A, Zhao F, et al. (2017) Soluble PD-L1 as a Biomarker in Malignant Melanoma Treated with Checkpoint Blockade. *Cancer Immunol Res* 5: 480-492.
 36. Wong KK, Bannerman B, Chesny E, Spaner DD and Gorczynski RM (2012) Soluble CD200 Is Critical to Engraft Chronic Lymphocytic Leukemia Cells in Immunocompromised Mice. *Cancer Research* 72: 4931.
 37. Gorczynski RM, Erin N and Khatri I (2011) Role of CD200 expression in regulation of metastasis of EMT6 tumor cells in mice. *Breast Cancer Research and Treatment* 130: 49-60.
 38. Chauvin JM, Zarour HM (2020) TIGIT in cancer immunotherapy. *J Immunother Cancer* 8: e000957.
 39. Lines JL, Sempere LF, Broughton T, Wang L, Noelle R (2014) VISTA is a novel broad-spectrum negative checkpoint regulator for cancer immunotherapy. *Cancer Immunol Res* 2: 510-7.
 40. Liu J, Yuan Y, Chen W, Putra J, Suriawinata AA, et al. (2015). Immune-checkpoint proteins VISTA and PD-1 non-redundantly regulate murine T-cell responses. *Proc Natl Acad Sci U S A.* 112: 6682-7.
 41. Böger C, Behrens HM, Krüger S, Röcken C (2017) The novel negative checkpoint regulator VISTA is expressed in gastric carcinoma and associated with PD-L1/PD-1: A future perspective for a combined gastric cancer therapy? *Oncoimmunology* 6: e1293215.
 42. Das M, Zhu C, Kuchroo VK (2017) Tim-3 and its role in regulating anti-tumor immunity. *Immunol Rev* 276: 97-111.
 43. Liu X, Kwon H, Li Z, Fu YX (2017) Is CD47 an innate immune checkpoint for tumor evasion? *J Hematol Oncol.* 10: 12.
 44. Chen SH, Dominik PK, Stanfield J, Ding S, Yang W, et al. (2021) Dual checkpoint blockade of CD47 and PD-L1 using an affinity-tuned bispecific antibody maximizes antitumor immunity. *J Immunother Cancer* 9: e003464.
 45. Zhang X, Wang Y, Fan J, Chen W, Luan J, et al. (2019) Blocking CD47 efficiently potentiated therapeutic effects of anti-angiogenic therapy in non-small cell lung cancer. *J Immunother Cancer.* 7: 346.
 46. Gorczynski RM (2005) CD200 and its receptors as targets for

- immunoregulation. *Current Opin Investig Drugs. Reviews* 6: 483-8.
47. Kretz-Rommel A, Qin F, Dakappagari N, Ravey EP, McWhirter J, et al. (2007) CD200 expression on tumor cells suppresses antitumor immunity: new approaches to cancer immunotherapy. *J Immunol* 178: 5595-605.
48. Aref S, Abousamra N, El-Helaly E, Mabed M (2020) Clinical Significance of CD200 and CD56 Expression in Patients with Acute Myeloid Leukemia. *Asian Pac J Cancer Prev* 21: 743-748.
49. Herbrich S, Baran N, Cai T, Weng C, Aitken MJL, et al. (2021) Overexpression of CD200 is a Stem Cell-Specific Mechanism of Immune Evasion in AML. *J Immunother Cancer*. 9: e002968.
50. Conticello C, Giuffrida R, Parrinello N, Buccheri S, Adamo L, et al. (2013) CD200 expression in patients with Multiple Myeloma: another piece of the puzzle. *Leuk Res* 37: 1616-21.
51. Tiribelli M, Geromin A, Cavallin M, Di Giusto S, Simeone E, et al. (2017) ABCG2 and CD200 define patients at high risk of relapse in ELN favorable subgroup of AML. *Eur J Haematol*. 99: 269-274.
52. Rastogi N, Baker S, Man S, Uger RA, Wong M, et al. (2021) Use of an anti-CD200-blocking antibody improves immune responses to AML in vitro and in vivo. *Br J Haematol*. 193: 155-159.
53. Kandeel EZ, Madney Y, Eldin DN, Shafik NF (2021) Overexpression of CD200 and CD123 is a major influential factor in the clinical course of pediatric acute myeloid leukemia. *Exp Mol Pathol*. 118: 104597.
54. Diamanti P, Cox CV, Ede BC, Uger RA, Moppett JP, et al. (2021) Targeting pediatric leukemia-propagating cells with anti-CD200 antibody therapy. *Blood Adv*. 5: 3694-3708.
55. D'Arena G, De Feo V, Pietrantonio G, Seneca E, Mansueto G, et al. (2020) CD200 and Chronic Lymphocytic Leukemia: Biological and Clinical Relevance. *Front Oncol*. 10: 584427.
56. Mahadevan D, Lanasa MC, Farber C, Pandey M, Whelden M, et al. (2019) Phase I study of samalizumab in chronic lymphocytic leukemia and multiple myeloma: blockade of the immune checkpoint CD200. *J Immunother Cancer*. 7: 227.
57. Wong KK, Khatri I, Shaha S, Spaner DE, Gorczynski RM (2010) The role of CD200 in immunity to B cell lymphoma. *J Leukoc Biol* 88: 361-72.
58. Rygiel TP, Karnam G, Goverse G, van der Marel AP, Greuter MJ, et al. (2012) CD200-CD200R signaling suppresses anti-tumor responses independently of CD200 expression on the tumor. *Oncogene* 31: 2979-88.
59. D'Arena G, Vitale C, Coscia M, Lamorte D, Pietrantonio G, et al. (2021) CD200 Baseline Serum Levels Predict Prognosis of Chronic Lymphocytic Leukemia. *Cancers (Basel)* 13: 4239.
60. Pallasch CP, Ulbrich S, Brinker R, Hallek M, Uger RA, et al. (2009) Disruption of T cell suppression in chronic lymphocytic leukemia by CD200 blockade. *Leuk Res* 33: 460-4.
61. Vaughan JW, Shi M, Horna P, Olteanu H (2020) Increased CD200 expression in post-transplant lymphoproliferative disorders correlates with an increased frequency of FoxP3(+) regulatory T cells. *Ann Diagn Pathol* 48: 151585.
62. Poh SL, Linn YC (2016) Immune checkpoint inhibitors enhance cytotoxicity of cytokine-induced killer cells against human myeloid leukaemic blasts. *Cancer Immunol Immunother* 65: 525-36.
63. Cieniewicz B, Uyeda MJ, Chen PP, Sayitoglu EC, Liu JM, et al. (2021) Engineered type 1 regulatory T cells designed for clinical use kill primary pediatric acute myeloid leukemia cells. *Haematologica* 106: 2588-2597.
64. Long M, Beckwith K, Do P, Mundy BL, Gordon A, et al. (2017) Ibrutinib treatment improves T cell number and function in CLL patients. *J Clin Invest* 127: 3052-3064.
65. El Din Fouad NB, Ibrahim NY, Aziz RSA, Ibrahim SK (2018) CD200 Expression in Diagnostic and Prognostic Assessment of Mature B Cell Lymphoproliferative Neoplasms *Asian Pac J Cancer Prev* 19: 3383-3392.
66. Hu Z, Sun Y, Schlette EJ, Tang G, Li S, et al. (2018) CD200 expression in mantle cell lymphoma identifies a unique subgroup of patients with frequent IGHV mutations, absence of SOX11 expression, and an indolent clinical course. *Mod Pathol* 31: 327-336.
67. Shi Q, Wu C, Han W, Zhao S, Wu Y, et al. (2021) Clinical significance of CD200 expression in newly diagnosed multiple myeloma patients and dynamic changing during treatment. *Leuk Lymphoma* 62: 709-715.
68. Aref S, El Agdar M, El Sebaie A, Abouzeid T, Sabry M, Ibrahim L (2020) Prognostic Value of CD200 Expression and Soluble CTLA-4 Concentrations in Intermediate and High-Risk Myelodysplastic Syndrome Patients. *Asian Pac J Cancer Prev* 21: 2225-2230.
69. Liu JQ, Hu A, Zhu J, Yu J, Talebian F, Bai XF. (2020) CD200-CD200R Pathway in the Regulation of Tumor Immune Microenvironment and Immunotherapy. *Adv Exp Med Biol* 1223: 155-165.
70. Zhu F, McCaw L, Spaner DE, Gorczynski RM (2018) Targeting the IL-17/IL-6 axis can alter growth of Chronic Lymphocytic Leukemia,72 in vivo/in vitro. *Leukemia Res* 66: 28-38
71. Moreaux J, Veyrune JL, Reme T, De Vos J, Klein B (2008) CD200: a putative therapeutic target in cancer. *Biochem Biophys Res Commun* 366: 117-22.
72. Siva A, Xin H, Qin F, Oltean D, Bowdish KS, Kretz-Rommel A (2008) Immune modulation by melanoma and ovarian tumor cells through expression of the immunosuppressive molecule CD200. *Cancer Immunol Immunother* 57: 987-96.
73. Sun H, Xu J, Huang M, Huang Q, Sun R, et al. (2016) CD200R, a co-inhibitory receptor on immune cells, predicts the prognosis of human hepatocellular carcinoma. *Immunol Lett* 178: 105-13.
74. Matsuo Y, Sho M, Nomi T, Hokuto D, Yoshikawa T (2021) Kamitani N, Nakamura K, Iwasa Y. Clinical Importance of CD200 Expression in Colorectal Liver Metastasis. *Ann Surg Oncol* 28: 5362-5372.
75. Huang S, Pan Y, Zhang Q, Sun W (2019) Role of CD200/CD200R Signaling Pathway in Regulation of CD4+T Cell Subsets During Thermal Ablation of Hepatocellular Carcinoma. *Med Sci Monit* 25: 1718-1728.
76. Vathiotis IA, MacNeil T, Zugazagoitia J, Syrigos KN, Aung TN, et al. (2021) Quantitative Assessment of CD200 and CD200R E110expression in Lung Cancer. *Cancers (Basel)* 13: 1024.
77. Wang X, Zha H, Wu W, Yuan T, Xie S, et al. (2023) CD200⁺ cytotoxic T lymphocytes in the tumor microenvironment are crucial for efficacious anti-PD-1/PD-L1 therapy. *Sci Transl Med* 15:eabn5029.
78. Yoshimura K, Suzuki Y, Inoue Y, Tsuchiya K, Karayama M, et al. (2020) CD200 and CD200R1 are differentially expressed and have differential

- prognostic roles in non-small cell lung cancer. *Oncoimmunology* 9: 1746554.
79. Zhang X, Shi X, Zhao H, Jia X, Yang Y (2021) Identification and Validation of a Tumor Microenvironment-Related Gene Signature for Prognostic Prediction in Advanced-Stage Non-Small-Cell Lung Cancer. *Biomed Res Int*: 8864436.
 80. Choueiry F, Torok M, Shakya R, Agrawal K, Deems A, et al. (2020) CD200 promotes immunosuppression in the pancreatic tumor microenvironment. *J Immunother Cancer*. 8: e000189.
 81. Zhang Y, Yu M, Jing Y, Cheng J, Zhang C, et al. (2021) Baseline immunity and impact of chemotherapy on immune microenvironment in cervical cancer. *Br J Cancer*. 124: 414-424.
 82. Feng D, Xiong Q, Wei Q, Yang L (2023) Cellular landscape of tumour microenvironment in prostate cancer. *Immunology* 168: 199-202.
 83. Diskin B, Adam S, Soto GS, Liria M, Aykut B, et al. (2022) BTLA⁺CD200⁺ B cells dictate the divergent immune landscape and immunotherapeutic resistance in metastatic vs. primary pancreatic cancer. *Oncogene* 41: 4349-4360.
 84. Gaiser MR, Weis CA, Gaiser T, Jiang H, Buder-Bakhaya K, et al. (2018) Merkel cell carcinoma expresses the immunoregulatory ligand CD200 and induces immunosuppressive macrophages and regulatory T cells. *Oncoimmunology* 7: e1426517.
 85. Brassard J, Gill ME, Bernatchez E, Desjardins V, Roy J, et al. (2021) Countering the adverse effects of lung cancer on the anticancer potential of dendritic cell populations reinstates sensitivity to anti-PD-1 therapy. *PLoS One* 16: e0260636.
 86. Maier B, Leader AM, Chen ST, Tung N, Chang C, et al. (2020) Author Correction: A conserved dendritic-cell regulatory program limits antitumour immunity. *Nature*. 582: E17.
 87. Deng Y, Li H, Fu J, Pu Y, Zhang Y, et al. (2022) A hypoxia risk score for prognosis prediction and tumor microenvironment in adrenocortical carcinoma. *Front Genet* 13: 796681.
 88. Talebian F, Yu J, Lynch K, Liu JQ, Carson WE, et al. (2021) CD200 Blockade Modulates Tumor Immune Microenvironment but Fails to Show Efficacy in Inhibiting Tumor Growth in a Murine Model of Melanoma. *Front Cell Dev Biol* 9: 739816.
 89. Belkin DA, Mitsui H, Wang CQ, Gonzalez J, Zhang S, Shah KR, et al. (2013) CD200 upregulation in vascular endothelium surrounding cutaneous squamous cell carcinoma. *JAMA Dermatol* 149: 178-86.
 90. Moertel CL, Xia J, LaRue R, Waldron NN, Andersen BM, et al. (2014) CD200 in CNS tumor-induced immunosuppression: the role for CD200 pathway blockade in targeted immunotherapy. *J Immunother Cancer* 2: 46.
 91. Ring EK, Markert JM, Gillespie GY, Friedman GK (2017) Checkpoint Proteins in Pediatric Brain and Extracranial Solid Tumors: Opportunities for Immunotherapy. *Clin Cancer Res* 23: 342-350
 92. Bi Y, Wu ZH, Cao F (2022) Prognostic value and immune relevancy of a combined autophagy-, apoptosis- and necrosis-related gene signature in glioblastoma. *BMC Cancer*. 22: 233.
 93. Xiong Z, Ampudia-Mesias E, Shaver R, Horbinski CM, Moertel CL, et al. (2016) Tumor-derived vaccines containing CD200 inhibit immune activation: implications for immunotherapy. *Immunotherapy* 8: 1059-71.
 94. Olin MR, Ampudia-Mesias E, Pennell CA, Sarver A, Chen CC, et al. (2019) Treatment Combining CD200 Immune Checkpoint Inhibitor and Tumor-Lysate Vaccination after Surgery for Pet Dogs with High-Grade Glioma. *Cancers (Basel)* 11: 137.
 95. Xiong Z, Ampudia Mesias E, Pluhar GE, Rathe SK, Largaespada DA, et al. (2020) CD200 Checkpoint Reversal: A Novel Approach to Immunotherapy. *Clin Cancer Res* 26: 232-241.
 96. Ampudia-Mesias E, Puerta-Martinez F, Bridges M, Zellmer D, Janeiro A, et al. (2021) CD200 Immune-Checkpoint Peptide Elicits an Anti-glioma Response Through the DAP10 Signaling Pathway. *Neurotherapeutics* 18: 1980-1994.
 97. Kobayashi K, Yano H, Umakoshi A, Matsumoto S, Mise A, et al. (2016) A Truncated form of CD200 (CD200S) Expressed on Glioma Cells Prolonged Survival in a Rat Glioma Model by Induction of a Dendritic Cell-Like Phenotype in Tumor-Associated Macrophages. *Neoplasia* 18: 229-41.
 98. Chen Z, Chen DX, Kai Y, Khatri I, Lamptey B, Gorczynski RM (2008) Identification of an expressed truncated form of CD200, CD200tr, which is a physiologic antagonist of CD200-induced suppression. *Transplantation* 86: 1116-24.
 99. Erin N, Podnos A, Tanriver G, Duymuş O, Cote E, et al. (2015) Bidirectional effect of CD200 on breast cancer development and metastasis, with ultimate outcome determined by tumor aggressiveness and a cancer-induced inflammatory response. *Oncogene* 34: 3860-70.
 100. Rygiel TP, Meyaard L (2012) CD200R signaling in tumor tolerance and inflammation: A tricky balance. *Curr Opin Immunol* 24: 233-8.
 101. Shi H, Yang Y (2021) Identification of inhibitory immune checkpoints and relevant regulatory pathways in breast cancer stem cells. *Cancer Med*. 10: 3794-3807.
 102. Fang J, Chen F, Liu D, Gu F, Chen Z, Wang Y (2020) Prognostic value of immune checkpoint molecules in breast cancer. *Biosci Rep* 40: BSR20201054.
 103. Donnenberg VS, Zhang JJ, Moravcikova E, Meyer EM, Lu H, et al. (2018) Antibody-based cell-surface proteome profiling of metastatic breast cancer primary explants and cell lines. *Cytometry A*. 93: 448-457.
 104. Gorczynski RM, Podnos A, Kos O, Oyedele A, Zhu F, Khatri I (2016) Significance of Soluble CD200 in plasma and serum of human breast cancer patients. *Int. Med Rev*: 2.
 105. Gorczynski RM, Chen Z, Khatri I, Podnos A, Yu K (2013) Cure of metastatic growth of EMT6 tumor cells in mice following manipulation of CD200:CD200R signaling. *Breast Cancer Res Treat*. 142: 271-82.
 106. Gorczynski RM, Chen Z, Erin N, Khatri I, Podnos A (2014) Comparison of immunity in mice cured of primary/metastatic growth of EMT6 or 4THM breast cancer by chemotherapy or immunotherapy. *PLoS One* 9: e113597.
 107. Shao A, Owens DM (2023) The immunoregulatory protein CD200 as a potentially lucrative yet elusive target for cancer therapy. *Oncotarget* 14: 96-103.
 108. Gorczynski RM (2022) Interaction of Immune Cells and Soluble Factors Which Contribute to Local and Metastatic Tumor Control within the Tumor Microenvironment. *J Cancer Sci Ther* 14: 524.
 109. Pilch Z, Tonecka K, Skorzynski M, Sas Z, Braniewska A, et al. (2019) The pro-tumor effect of CD200 expression is not mimicked by agonistic CD200R antibodies. *PLoS One* 14: e0210796.

110. Pilch Z, Tonecka K, Braniewska A, Sas Z, Skorzynski M, et al. (2018) Antitumor Activity of TLR7 Is Potentiated by CD200R Antibody Leading to Changes in the Tumor Microenvironment. *Cancer Immunol Res.* 6: 930-940.
111. Erin N, Dilmaç S, Curry A, Duymuş Ö, Tanriover G, Prodeus A, et al. (2020) CD200 mimetic aptamer PEG-M49 markedly increases the therapeutic effects of pegylated liposomal doxorubicin in a mouse model of metastatic breast carcinoma: an effect independent of CD200 receptor 1. *Cancer Immunol Immunother.* 69: 103-114.
112. He J, Xiong X, Yang H, Li D, Liu X, Li S, et al. (2022) Defined tumor antigen-specific T cells potentiate personalized TCR-T cell therapy and prediction of immunotherapy response. *Cell Res.* 32: 530-542.
113. Veldman J, Rodrigues Praça J, Chong L, Terpstra MM, Mastik M, et al. (2022) CD4+ T cells in classical Hodgkin lymphoma express exhaustion associated transcription factors TOX and TOX2: Characterizing CD4+ T cells in Hodgkin lymphoma. *Oncoimmunology.* 11: 2033433.
114. Wang W, Liu J, He Y, McLeod HL (2017) Prospect for immune checkpoint blockade: dynamic and comprehensive monitorings pave the way. *Pharmacogenomics.*
115. Tronik-Le Roux D, Sautreuil M, Bentrion M, Véline J, Palma MB, et al. (2020) Comprehensive landscape of immune-checkpoints uncovered in clear cell renal cell carcinoma reveals new and emerging therapeutic targets. *Cancer Immunol Immunother.* 69: 1237-1252.
116. Khan M, Arooj S, Wang H (2020) NK Cell-Based Immune Checkpoint Inhibition. *Front Immunol* 11: 167.
117. Flies AS, Darby JM, Lennard PR, Murphy PR, Ong CEB, et al. (2020) A novel system to map protein interactions reveals evolutionarily conserved immune evasion pathways on transmissible cancers. *Sci Adv.* 6: eaba5031.
118. Elias R, Morales J, Presley C (2017) Checkpoint Inhibitors for non-small cell lung cancer among older adults. *Curr Oncol Rep.* 19: 62.
119. Daste A, Domblides C, Gross-Goupil M, Chakiba C, et al. (2017) Immune checkpoint inhibitors and elderly people: a review. *Eur J Cancer* 68: 155-166.
120. Marrone KA, Forde PM (2017) Cancer Immunotherapy in older patients. *Cancer J* 23: 219-222.