REVIEW

Coming in from the cold: overcoming the hostile immune microenvironment of medulloblastoma

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Medulloblastoma is an aggressive brain tumor that occurs predominantly in children. Despite intensive therapy, many patients die of the disease, and novel therapies are desperately needed. Although immunotherapy has shown promise in many cancers, the low mutational burden, limited infiltration of immune effector cells, and immune-suppressive microenvironment of medulloblastoma have led to the assumption that it is unlikely to respond to immunotherapy. However, emerging evidence is challenging this view. Here we review recent preclinical and clinical studies that have identified mechanisms of immune evasion in medulloblastoma, and highlight possible therapeutic interventions that may give new hope to medulloblastoma patients and their families.

Medulloblastoma is among the most common malignant brain tumors in children, with an estimated 450 new cases per year in the United States (Ostrom et al. 2018). Progress in diagnosis and treatment has led to an increased 10-yr survival rate of 65% in 2015 (Ostrom et al. 2018) compared with 44% in 1984 (Hughes et al. 1988). Molecular profiling of medulloblastoma has identified four major subgroups of the disease: the Wingless (WNT) and Sonic hedgehog (SHH) subgroups, which are driven by deregulation of the eponymous signaling pathways; group 3, characterized by overexpression of MYC; and group 4, whose genetic signature is more heterogeneous, without a common genetic driver (Taylor et al. 2012; Juraschka and Taylor 2019). The stratification of medulloblastoma into subclasses is of great clinical relevance, as the identification of oncogenic drivers may enable the use of subgroup-specific targeted therapies (Robinson et al. 2012) and de-escalation strategies for treatment of

tumors with a better prognosis (NCT01878617, NCT02724579, and EORTC-1634-BTG). Despite this progress, up to 40% of patients remain affected with an incurable recurrent and metastatic form of this disease, which is the primary cause of death. Moreover, patients who do survive suffer from severe long-term side effects, including cerebrovascular disease; visual, auditory, and other neurosensory impairments; neurocognitive deficits; and increased incidence of secondary malignancies (for review, see Chevignard et al. 2017). Thus, the development of more effective therapies with less toxicity is essential. Here we review what is known about interactions between medulloblastoma and the immune system, as well as efforts to develop immunotherapies against this disease.

The immune system provides a potent defense against pathogens and can also identify and eradicate malignant cells. This concept was first postulated by the Nobel prize-winning scientist Paul Ehrlich in the early 20th century (Ehrlich 1909; for review, see Galon and Bruni 2020), 50 yr after Rudolf Virchow had discovered the first immune infiltrates in cancer specimens (Virchow 1863; Galon and Bruni 2020). At about the same time, without understanding the underlying mechanisms, the surgeon William Coley successfully treated cancer patients with injections of heat-inactivated bacteria, laying the foundation for modern immunotherapy (Coley 1893; Galon and Bruni 2020). However, it was several decades before experimental evidence supported the hypothesis of immune surveillance (Burnet 1957). One key observation was that inbred mice could be immunized against syngeneic transplants of tumors induced by chemical carcinogens or viruses, establishing the existence of tumor antigens that mark tumor cells for immune-mediated elimination (Old and Boyse 1966). This was further corroborated by observations of immune cell infiltrates correlating with improved prognosis of cancer patients (Dunn et al. 2004).

Since then, numerous studies have identified immune cells that are involved in antitumor immunity. Accumulating evidence suggests that the adaptive and innate immune systems cooperate to recognize and kill tumor cells. However, in medulloblastoma and other brain tumors, a variety of factors impede the antitumor activity of these cells and thwart efforts to use the immune system as a mode of therapy. These factors are discussed below.

Barriers to effective immunotherapy of brain tumors

Currently, the FDA has approved at least 55 viral and adoptive cell therapies, immune modulators, vaccines, and targeted antibody-based immunotherapies for >20 cancer types (The Cancer Research Institute, https://www.cancerresearch.org/en-us/immunotherapy/treatment-types). Unfortunately, the development of immunotherapies for brain tumor patients has remained challenging, and their clinical implementation is clearly lagging behind other cancer types. For pediatric patients suffering from brain tumors, there is no FDA-approved immunotherapeutic option yet. Tumor immunology research has identified various barriers to an effective immune response that remain challenges for the development of immunotherapies for medulloblastoma.

Lack of infiltrating immune cells

Slight variations in the composition of immune infiltrates between the different subgroups of medulloblastoma have been reported (Bockmayr et al. 2018; Grabovska et al. 2020; Riemondy et al. 2022). However, all medulloblastoma tumors are characterized by low numbers of infiltrating immune cells. Some studies have indicated that medulloblastomas harbor even fewer immune cells than glioblastoma (Griesinger et al. 2013; Bockmayr et al. 2018), which has been described as a hostile "immunologic desert with rare to nonexistent infiltrating immune effector cells" (Reardon et al. 2017). The number of T cells present in medulloblastoma tumors was found to be not statistically higher than in pediatric control tissue (Griesinger et al. 2013). In particular, the low percentage of effector cells such as granzyme B-expressing $CD8^+$ T cells and natural killer (NK) cells suggests a very low level of active antitumor immune responses in medulloblastoma (Bockmayr et al. 2018; Vermeulen et al. 2018).

The blood–brain barrier tightly controls immune cell infiltration

The limited immune infiltration in medulloblastoma might have several causes. The blood–brain barrier (BBB), which is made up of endothelial cells, pericytes, and astrocytic endfeet, serves to protect the brain from pathogens, toxins, and other harmful factors in the blood. It also represents a physical barrier for immune cells. The classical concept of the brain as an immune-privileged organ that is protected from immune surveillance has been called into question over the past decade (Carson et al. 2006). Increasing evidence has supported the existence of CNS immune surveillance; however, it remains a highly controlled process granting only limited access to immune cells (for review, see Carson et al. 2006; Engelhardt et al. 2017).

Despite restricted immune cell trafficking across the BBB under physiological conditions, there may be an increase in trafficking under pathological conditions due to induced leakiness or disruption of the barrier (Weiss et al. 2009). However, with the exception of WNT tumors, most medulloblastomas show large regions of intact BBB (Phoenix et al. 2016), suggesting sustained limited immune cell infiltration. Moreover, reactive astrocytes have been reported to form perivascular scar-like barriers that serve to restrict the infiltration of leukocytes in an experimental autoimmune encephalomyelitis model (Voskuhl et al. 2009). It remains to be determined whether reactive astrocytes can also function as a physical barrier to immune cell infiltration in medulloblastoma.

Therapy-induced immunosuppression

Chemotherapy and radiotherapy are part of the standard of care for medulloblastoma and are intended to target rapidly dividing cancer cells. However, this form of treatment does not exclusively kill cancer cells, but can also cause collateral damage to immune cells. A recent study investigated how radiotherapy and chemotherapy affect circulating T cells in medulloblastoma patients and found that treated patients exhibited $CD4^+$ T-cell lymphopenia (Gururangan et al. 2017). Moreover, medulloblastoma treatment commonly includes application of corticosteroids to relieve symptoms such as headache and nausea caused by edema, hydrocephalus, increased intracranial pressure, and compression of the fourth ventricle (for review, see De Braganca and Packer 2013). However, a common side effect of steroids is immunosuppression. As a result, steroid-treated patients with melanoma or non-small cell lung cancer brain metastasis have been shown to respond less efficiently to immunotherapy (Margolin 2012; Kotecha et al. 2019). Thus, it can be presumed that in addition to radiotherapy and chemotherapy, corticosteroid treatment induces systemic immunosuppression and reduced antitumor immunity in medulloblastoma patients.

Low mutational load

A high mutational load correlates with higher numbers of neoantigens that can elicit tumor-directed T-cell responses (Rizvi et al. 2015) and, accordingly, tumors with a high mutation burden have been found to respond more efficiently to immune checkpoint blockade compared with tumors with a low or intermediate number of mutations (Rizvi et al. 2015; Goodman et al. 2017). Pediatric tumors, and in particular brain tumors like medulloblastoma, have a relatively low mutation rate (Parsons et al. 2011; Chalmers et al. 2017). Thus, it has been concluded that medulloblastoma is minimally immunogenic and unlikely to respond to therapeutic

approaches that promote the endogenous T-cell immune response, such as immune checkpoint inhibition (Landi et al. 2018).

Dysfunctional antigen presentation

Beyond the expression of immunogenic antigens, a key determinant of tumor immunogenicity is presentation of these antigens by professional antigen-presenting cells (APCs) and tumor cells.

APCs, such as dendritic cells, macrophages, microglia, and B cells, have been reported to infiltrate medulloblastoma tumors. However, medulloblastoma and atypical teratoid/rhabdoid tumors (ATRT) showed the lowest infiltration of APCs among all brain tumors tested (Bockmayr et al. 2018). It has not been explored whether medulloblastoma-associated APCs are functional and which APC type is the predominant one in inducing antimedulloblastoma T-cell responses. Microglia, as brain-resident macrophages, may represent the most abundant APCs in brain tumors. There is little evidence that microglia migrate out of the CNS to present antigens to naïve T cells in lymph nodes, suggesting that T cells are initially primed by a different type of APC. Once activated T cells enter the brain, microglia can act as local APCs to modulate and enhance T-cell activity. There are no data available on whether microglia can promote antimedulloblastoma immune responses; however, studies on other neuropathologies, such as experimental rodent autoimmune encephalomyelitis, have reported inefficient antigen presentation (Ford et al. 1996; Mundt et al. 2019) and showed that microglia are dispensable for T-cell entry into the brain and for local reactivation of T cells (Mundt et al. 2019). In mouse models of glioma, microglia exhibit a reduced capacity to process or present antigens, up-regulate MHC class II, and stimulate T cells (Badie et al. 2002; Schartner et al. 2005; Qian et al. 2018). Interestingly, single-cell sequencing of myeloid cells isolated from a glioblastoma model revealed that the extent of MHC class II up-regulation in microglia is sex-dependent (Ochocka et al. 2021).

In summary, little is known about the function of microglia as APCs in medulloblastoma, but it appears that efficient antigen presentation and T-cell activation in the brain tumor setting may rely on other APCs. Of note, these APCs can be suppressed in their maturation and antigen presentation capacity as well (Herber et al. 2010; Cubillos-Ruiz et al. 2015).

In addition to the presentation of tumor antigens by APCs to activate T cells, antigens must be presented by MHC class I on tumor cells for these cells to be recognized and eliminated by CD8^{+} T cells. In a process called immunoediting (Dunn et al. 2004), CD8^{+} T cells may progressively clear immunogenic MHC class I-presenting tumor cells, resulting in the selection of tumor cells that have a deficiency in the antigen presentation machinery. The loss of MHC class I presentation on the tumor cell surface is a common mechanism of immune escape and has been reported for medulloblastoma (Raffaghello et al. 2007; Vermeulen et al. 2018).

Tumor-induced immunosuppression

Medulloblastoma cells can escape NK cell attack   Although MHC class I is necessary to activate CD8^{+} T cells, it acts as a negative regulator of natural killer cells. Thus, tumors lacking MHC class I could show increased susceptibility to NK cell-mediated elimination. However, recent studies of medulloblastoma have demonstrated minimal infiltration of NK cells (Haberthur et al. 2016; Vermeulen et al. 2018). Moreover, NK cell activation depends on positive signals from target cells, and tumor cells often downregulate these signals to evade NK cell attack. For example, low expression of NKG2D ligands on tumor cells, as well as low expression of the cognate receptor NKG2D on NK cells, hinders NK cell activation in medulloblastoma (Haberthur et al. 2016). Mechanistically, medulloblastomas may inhibit NKG2D expression and NK cell cytotoxicity by release of the immunosuppressive cytokine transforming growth factor β (TGFβ) (Gate et al. 2014; Bockmayr et al. 2018; Powell et al. 2019), as shown for other cancer entities including glioma (Friese et al. 2004; Crane et al. 2010). In a PDX model of glioblastoma, TGFβ release by glioblastoma stem cells was triggered by cell–cell interactions with adoptively transferred human NK cells, resulting in NK cell dysfunction and enhanced tumor growth (Shaim et al. 2021). TGFβ also suppresses T-cell proliferation, T-cell cytotoxicity, and antigen presentation and recruits regulatory T cells (Tregs) (for review, see Flavell et al. 2010), creating a hostile, immunosuppressive microenvironment.

Regulatory T cells suppress antitumor immune responses   Tregs control the activity of effector immune cells by secreting anti-inflammatory cytokines such as TGFβ and interleukin-10 (IL-10), removing costimulatory signals from APCs by CTLA4-mediated trogocytosis (Gu et al. 2012) or *trans*-endocytosis (processes in which plasma membrane fragments are transferred between cells) (Qureshi et al. 2011), or consumption of interleukin-2, limiting the availability of this stimulatory cytokine for effector T cells (Pandiyan et al. 2007). Tumors may benefit from Treg-mediated immunosuppression, as in some cancer entities Tregs have been correlated with tumor grade (El Andaloussi and Lesniak 2007), decreased survival (Curiel et al. 2004), and resistance to immune checkpoint inhibition (Amoozgar et al. 2021). Treg infiltration has been described for medulloblastoma (Gate et al. 2014; Bockmayr et al. 2018; Vermeulen et al. 2018; Grabovska et al. 2020) and can be facilitated by tumor-derived TGFβ (Gate et al. 2014). TGFβ drives the conversion of CD4^{+} T cells to Tregs, which in turn secrete high levels of TGFβ, generating a feed-forward loop that may ensure the continued presence of immunosuppressive Tregs in the tumor. Elevated Treg infiltration in medulloblastoma tumors may also be therapy-induced, as Treg expansion has been observed in the blood of treated medulloblastoma patients (Gururangan et al. 2017) as well as in an experimental in vitro setting using mTOR inhibitor-treated medulloblastoma cell lines (Folgiero et al. 2016).

Reactive astrocytes and their tumor-promoting potential A recent study of melanoma brain metastasis found that STAT3-positive reactive astrocytes can act as a physical barrier, shielding the lesion from lymphocytes and repressing the activity of CD8^{+} T cells, presumably by expression of PD-L1 and VEGF-A, which drive T-cell exhaustion (Priego et al. 2018). In medulloblastoma, there is substantial cross-talk between astrocytes and microglia and other myeloid cells. A recent study has elegantly shown that SHH medulloblastoma cells can transdifferentiate into interleukin-4-secreting astrocytes, which stimulate microglia to release insulin-like growth factor 1 (IGF1) and IL-10 (Yao et al. 2020). The study focused on IGF1, demonstrating an essential role for this growth factor in tumor development and progression. The effect of IL-10 in medulloblastoma is yet to be determined, but it is commonly reported to function as an immunosuppressive cytokine that substantially represses antitumor immunity by inhibiting APCs (for review, see Widodo et al. 2021). Furthermore, medulloblastoma-associated astrocytes, especially in group 3 medulloblastoma, have recently been shown to produce high levels of the tumor-promoting chemokine CCL2 (Liu et al. 2020). CCL2 signaling in medulloblastoma cells has been shown to promote leptomeningeal metastasis (Garzia et al. 2018) and to maintain stem-like properties and proliferation of disseminated tumor cells (Liu et al. 2020). CCL2 is also a very potent chemoattractant for numerous immune cells, including myeloid cells (for review, see Gschwandtner et al. 2019), and has been shown to facilitate infiltration of bone marrow-derived macrophages into SHH medulloblastoma lesions (Maximov et al. 2019).

Medulloblastoma-associated myeloid cells can adopt an immunosuppressive phenotype The role of myeloid cells in medulloblastoma is controversial, with some studies identifying an antitumoral function and others reporting a tumor-promoting function for these cells. The tumoricidal function of medulloblastoma-associated macrophages was postulated based on the observation that their depletion in a model of SHH medulloblastoma (the *NeuroD2-SmoA1* mouse) resulted in enhanced tumor growth (Maximov et al. 2019). Moreover, blockade of the surface molecule CD47 on tumor cells led to macrophage-mediated phagocytosis in a patient-derived xenograft (PDX) model (Gholamin et al. 2017). These studies highlight the capacity of tumor-associated myeloid cells to impair the growth and survival of medulloblastoma cells.

However, there is also strong evidence for a tumor-supportive function of medulloblastoma-associated myeloid cells. Gene expression profiling of human medulloblastoma samples showed that SHH tumors, which are characterized by high myeloid infiltration, are enriched for an M2-like gene expression profile, which is associated with immunosuppressive functions of myeloid cells (Margol et al. 2015). Consistently, Margol et al. (2015) suggested an inverse correlation of CD163 expression (an M2-like marker) and survival of SHH medulloblastoma patients, but highlighted the need for validation with a larger sample size. In group 4 medulloblastoma patients, a high infiltration of mono-

cytes has also been associated with poor prognosis (Grabovska et al. 2020). This notion of a tumor-promoting macrophage function was further affirmed by two recent studies. In a mouse model of SHH medulloblastoma (*Atoh1-SmoM2*), Tan et al. (2021) showed delayed tumor growth and prolonged mouse survival in response to pharmacological inhibition of colony-stimulating factor 1 receptor (CSF1R or CD115), which depletes microglia and macrophages. Likewise, Dang et al. (2021) reported increased infiltration of immunosuppressive monocyte-derived macrophage cells in response to radiotherapy in another mouse model of SHH medulloblastoma (*Ptch1^{+/−}; Tp53^{−/−}*). Coculture of these tumor-infiltrating monocyte-derived Ly6C^{hi}/Ccr2^{hi} myeloid cells with ex vivo stimulated T cells resulted in significantly decreased T-cell proliferation. In vivo, knockout of *Ccr2* (which encodes the receptor for CCL2) decreased monocyte-derived macrophage infiltration in irradiated tumors and resulted in increased CD8^{+} T-cell levels. Despite increased intratumoral CD8^{+} T-cell numbers, no survival benefit was reported, most likely due to drastically increased numbers of tumor-associated neutrophils. These neutrophils are characterized by elevated IL-10 signaling and resemble granulocytic myeloid-derived suppressor cells (MDSCs), indicating an immunoregulating phenotype that could compensate for the loss of immunosuppressive monocyte-derived macrophages in this model (Dang et al. 2021). The presence of MDSCs has also been reported for another SHH-associated medulloblastoma model, where the investigators show that MDSCs increase Treg infiltration and reduce numbers of effector T cells in the tumor (Abad et al. 2014).

In line with the putative immunosuppressive phenotype of medulloblastoma-associated macrophages, infiltrating myeloid cells have been identified as the predominant source of PD-L1 expression in mouse models of SHH and group 3 medulloblastoma (Pham et al. 2016). Binding of PD-L1 to PD-1 on effector T cells promotes T-cell exhaustion and leads to immune escape of tumor cells (Freeman et al. 2000; Dong et al. 2002).

In summary, medulloblastoma tumors are sparsely infiltrated by immune cells and seem to have a broad repertoire of mechanisms for evading the few immune cells that do find their way into the tumor. However, the field of medulloblastoma immunology is relatively unexplored, and emerging evidence indicates that medulloblastoma cells can be susceptible to immune-mediated attack. Below, we discuss the approaches that have been used to enhance the antimedulloblastoma immune response and present findings from other cancer types that might be relevant for the future development of immunotherapies for medulloblastoma.

Approaches to overcoming immune response barriers

Corticosteroid substitution and metronomic chemotherapy can minimize therapy-induced immunosuppression

In medulloblastoma patients, corticosteroids are administered in order to relieve edema-related symptoms. Steroid

treatment is often accompanied by immunosuppression, which could contribute to the lack of therapy response and relapse. However, multiple studies have demonstrated that corticosteroids can be replaced with bevacizumab, an anti-VEGF monoclonal antibody that seems highly effective in the control of peritumoral edema in brain tumor patients (Liu et al. 2009; Nagpal et al. 2011; Banks et al. 2019). Combination therapy using bevacizumab and anti-CTLA-4 in metastatic melanoma was associated with enhanced effector immune cell infiltration, increased numbers of circulating memory T cells, and a more favorable outcome compared with anti-CTLA-4 treatment alone (Hodi et al. 2014). The high cost of anti-VEGF therapy represents a financial burden for the health care system, limiting its clinical use. However, combining costly immunotherapies with corticosteroids that likely reduce the efficiency of these very same therapies may be equally challenging from an ethical and financial standpoint. At least in clinical studies where the efficacy of immunotherapies is evaluated, the use of immunosuppressive steroids should be avoided.

Conventional chemotherapy and radiotherapy have been associated with systemic immunosuppression as well. Metronomic treatment, which involves the application of chemotherapeutic drugs in lower but more frequent doses, has been proposed to have milder side effects and, moreover, to enhance antitumor immune responses (Wu and Waxman 2018). On the one hand, this might be due to the lower overall toxicity that also spares effector immune cells; on the other hand, metronomic therapy has also been shown to reduce the numbers of immunosuppressive immune cells such as Tregs and MDSCs in glioma (Banissi et al. 2009; Peereboom et al. 2019). Moreover, the metronomic administration of certain chemotherapeutic drugs induces an immunogenic tumor cell death, which is characterized by the release of inflammatory factors such as HMGB1 and ATP and exposure of phagocytosis-stimulating calreticulin, greatly increasing the efficacy of antitumor responses (Wang et al. 2018). A recent report describing a metronomic antiangiogenic combination treatment of patients with recurrent medulloblastoma has shown promising preliminary results (Slavc et al. 2018). The combination of metronomic therapy with immune checkpoint blockade has not been tested yet in medulloblastoma, but it is possible that these approaches may have synergistic effects.

Enhancing trafficking of immune cells into tumors

Although the BBB represents a physical barrier to immune cells, the CNS is constantly surveilled by immune cells. T cells can patrol perivascular and leptomeningeal compartments until local recognition of their specific antigen initiates their infiltration into the brain parenchyma, which is normally sealed off by the glia limitans (for review, see Engelhardt and Ransohoff 2012). In addition, peripherally activated T cells have been shown to traverse the BBB. Peripheral presentation of brain tumor antigens and T-cell activation has been reported for brain-draining lymph nodes (Calzascia et al. 2005). Despite the lack of conventional lymphatic vessels in the brain parenchyma, evidence has emerged that the glymphatic system, a draining system that clears metabolic by-products from the brain, as well as recently discovered meningeal lymphatic vessels, allows drainage of soluble factors and immune cells from the brain to peripheral lymph nodes (Louveau et al. 2015; Hu et al. 2020).

Increased leukocyte trafficking into the brain has been reported in certain pathological conditions. Activated T cells have been shown to alter the BBB characteristics, allowing for recruitment and entry of immune cells across the glia limitans into the brain parenchyma (for review, see Engelhardt et al. 2017). Disruption of the BBB can also be caused by radiotherapy, factors secreted by tumor cells (Phoenix et al. 2016), or, as observed in glioblastoma, mechanical dislocation of astrocytic endfeet from the vasculature by tumor cells (Watkins et al. 2014). A recent study investigated the BBB phenotype in medulloblastoma subgroups and demonstrated that WNT-driven tumors have aberrant fenestrated vasculature, which facilitates the influx of chemotherapeutics and immune cells (Phoenix et al. 2016). Paradoxically, WNT tumors secrete large amounts of Wnt inhibitors, including Wnt inhibitory factor 1 (WIF1) and Dickkopf 1 (DKK1), which act in a paracrine fashion on adjacent endothelial cells to impair BBB integrity (Phoenix et al. 2016). In contrast, SHH tumors do not secrete Wnt inhibitors and overall show a more intact BBB (Phoenix et al. 2016). Although it might be tempting to induce BBB permeability by administering Wnt inhibitors, this therapeutic strategy requires careful investigation, as recent studies have shown that Wnt signaling is beneficial in non-WNT medulloblastoma tumors. For example, activation of Wnt signaling in SHH, group 3, and group 4 tumors decreased proliferation and prolonged survival in medulloblastoma mouse models (Pöschl et al. 2014; Manoranjan et al. 2020). Thus, administration of Wnt inhibitors could exacerbate tumor growth.

Another approach to therapeutically disrupting the BBB is the application of low-intensity pulsed ultrasound (LIPU). The ultrasound-induced oscillation of intravenously administered microbubbles results in a temporary opening of endothelial tight junctions and increased transcytosis (Sabbagh et al. 2021). Whereas LIPU alone showed no significant effect in a glioblastoma mouse model, the combination of LIPU with anti-PD-1 immune checkpoint blockade or chimeric antigen receptor T (CAR-T) cell therapy led to enhanced antibody penetration into the brain, a significantly higher CAR-T cell infiltration, and prolonged survival of treated mice (Sabbagh et al. 2021). Thus, combining this technique with other immunotherapies could significantly enhance treatment efficacy (see Fig. 1).

Another determinant of immune infiltration is recruitment or homing of immune cells to the tumor site. Generally, activated lymphocytes migrate from the lymph nodes along a gradient of chemoattractants to their target site, and this requires expression of the appropriate chemokine receptors on lymphocytes. The subsequent extravasation of these cells into tumor tissue is facilitated by expression of selectins and integrins by immune and endothelial cells, which facilitate tethering, rolling, arrest, and diapedesis.
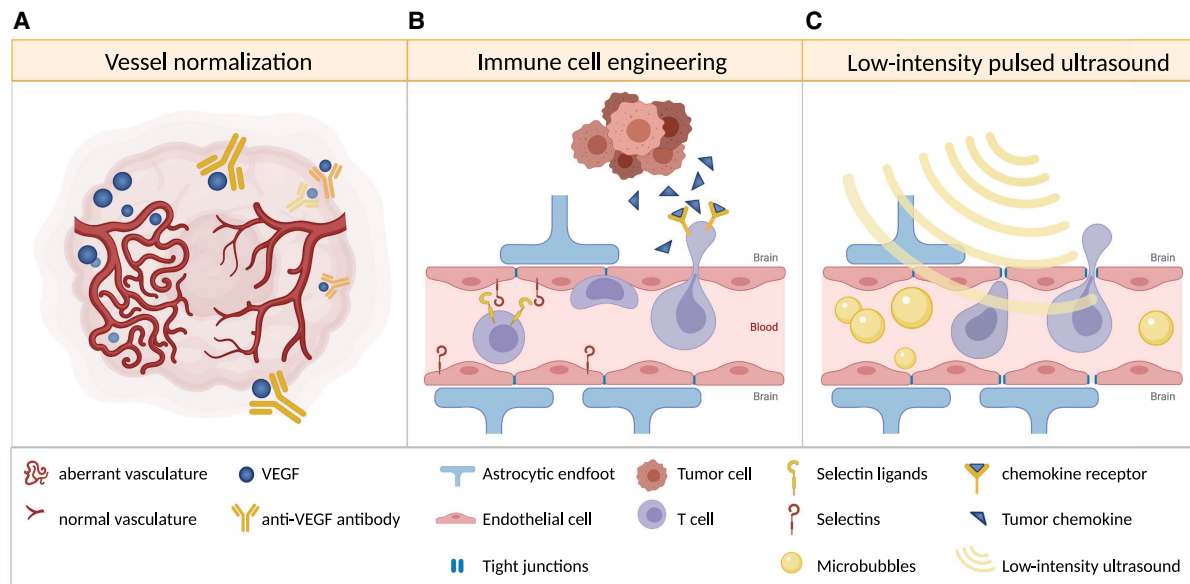
Figure 1. Strategies to enhance immune cell trafficking into brain tumors. (*A*) Vessel normalization induced by anti-VEGF antibody therapy can improve immune cell infiltration. (*B*) Immune cells engineered to express selectin ligands and chemokine receptors that match tumor-derived chemokines may show increased homing to and extravasation at the tumor site. (*C*) Low-intensity pulsed ultrasound temporarily opens the blood–brain barrier and may allow increased immune cell infiltration.

Thus, mismatching of chemokine receptors expressed by lymphocytes and chemokines released by the tumor, down-regulation of adhesion molecules, and aberrant vasculature may all contribute to poor homing of effector immune cells (for review, see Slaney et al. 2014). With this in mind, efforts are being made to engineer immune cells to overcome these challenges and to adoptively transfer them back into cancer patients. For example, cell surface glycoengineering is being used to enforce expression and modification of E-selectin ligands in T cells to enhance their extravasation at the target site (see Fig. 1; Mondal et al. 2019). Several studies have shown that ectopic expression of chemokine receptors matching tumor-derived chemokines enhances tumor infiltration of CAR-T cells (Di Stasi et al. 2009; Craddock et al. 2010; Moon et al. 2011). Medulloblastoma cells as well as medulloblastoma-associated reactive astrocytes secrete the chemoattractant CCL2 (Garzia et al. 2018; Maximov et al. 2019; Liu et al. 2020), but its corresponding receptor CCR2 is poorly expressed on activated T cells; thus, ectopic CCR2 expression in adoptively transferred CAR-T cells may substantially improve their homing to medulloblastoma tumors (see Fig. 1), as has been reported for neuroblastoma (Craddock et al. 2010) and mesothelioma (Moon et al. 2011) models.

In addition to the aforementioned ability of bevacizumab to relieve severe edema in brain tumor patients, anti-VEGF therapy may also enhance immune cell infiltration by promoting tumor vessel normalization (see Fig. 1). The administration of low, vascular-normalizing doses of bevacizumab has been shown to result in restoration of functional tumor vessels and increased infiltration of T cells in a breast cancer model (Huang et al. 2012). In a

mouse model of glioblastoma, vessel normalization has been shown to prolong survival in combination with immune checkpoint inhibition, which has limited therapeutic efficacy as a monotherapy in this disease (Di Tacchio et al. 2019). Importantly, in addition to blood vessel normalization, lymphangiogenesis has also been shown to positively impact immune infiltration. Two recent studies demonstrated that ectopic VEGF-C expression by glioblastoma cells (or other cells in the brain and meninges) resulted in increased lymphangiogenesis in the meninges and enabled enhanced T-cell infiltration in mouse models of glioblastoma. This approach, combined with immune checkpoint blockade, led to tumor regression and significantly prolonged survival of the mice (Hu et al. 2020; Song et al. 2020).

Medulloblastomas still express neoantigens despite their low mutational load

A high tumor mutation burden has been suggested to contribute to immunogenicity and to predict response to immunotherapy. Concordantly, a recent study has found a sustained response to anti-PD-1 therapy in pediatric cancer patients with an exceptionally high load of mutations and microsatellite insertion–deletion events due to germline DNA mismatch repair deficiency or polymerase proofreading deficiency (Das et al. 2022). However, this correlation found in ultrahypermutated tumors does not necessarily extrapolate to other cancers, as recently shown by a large-scale study by Touat et al. (2020). The investigators showed that chemotherapy-induced hypermutation in glioblastomas does not promote an immune response following anti-PD-1 immunotherapy (Touat

et al. 2020). Another recent study identified low mutational burden as a common feature of immunotherapy-responsive recurrent glioblastoma tumors (Gromeier et al. 2021). Although the investigators note that this unexpected correlation may not be causal, it still calls into question the assumption that a high mutational burden is required for an efficient antitumor immune response. Rather, it supports the idea that it is not the quantity but the quality of neoantigens and the ability to present them that determine the antitumor immune response.

Medulloblastomas are rarely ultrahypermutated and are usually characterized by a rather low mutation burden (Parsons et al. 2011). Nevertheless, neoantigen expression has been identified in medulloblastoma tumors. These neoantigens were able to elicit a T-cell response in vitro (Blaeschke et al. 2019) and a humoral response in patients (Behrends et al. 2003). Neoantigens can be derived from errors at the genetic level, such as point or frameshift mutations, deletions, and fusions, and at the transcriptional or post-transcriptional level, such as frameshifts generated by errors in transcription of microsatellites and missplicing of exons (Shen et al. 2019). The latter study by Shen et al. (2019) has intriguingly demonstrated a high load of RNA-based neoantigens in glioblastoma, a low mutation rate cancer, which elicited overall immune responses similar to neoantigens from other high mutation cancers.

Recent studies have revealed aberrations in the splicing and translational machinery of medulloblastomas: SHH subgroup-specific mutations in U1 spliceosomal small nuclear RNA have been associated with enhanced alternative splicing, which not only activates oncogenes and inactivates tumor suppressor genes like *PTCH1* but has also been suggested to result in generation of neoepitopes (Suzuki et al. 2019). SHH tumors have also been shown to be enriched for mutations in the elongator complex protein 1, which results in loss of elongator-dependent U34 tRNA modifications (Waszak et al. 2020). The absence of these modifications results in translational inefficiency of AA-ending codons and protein misfolding and aggregation. Consequently, the translation of proteins rich in AA-ending codons is down-regulated, whereas proteins that encode the same amino acids by AG-ending codons are translationally up-regulated. In addition to aberrantly translated proteins, phosphorylated proteins can serve as potent tumor-associated antigens. Leukemia cells show increased MHC class I presentation of phospho-peptides derived from signaling molecules with well-established roles as oncogenic drivers such as MAP kinases, Myc, and Gfi1. Many of these phospho-peptides have been shown to induce a T-cell response (Cobbold et al. 2013). A recent study described a profound increase in global tyrosine phosphorylation in group 4 medulloblastomas (Forget et al. 2018). Thus, similar to leukemia, phospho-peptides could function as tumor antigens in medulloblastoma.

The identification of medulloblastoma neoantigens could enable the promotion of tumor targeting T-cell responses, either by vaccination with tumor-specific epitopes or by engineering CAR-T cells expressing tumor antigen-specific TCRs.

Therapeutic approaches to increase antigen presentation

It has been suggested that there is minimal infiltration of APCs in medulloblastoma. Nonetheless, a positive correlation of dendritic cell expression markers and survival of group 3 and 4 patients suggests a beneficial role of this cell type in medulloblastoma (Bockmayr et al. 2018). Successful approaches to increasing the number of APCs in medulloblastoma have been reported. In mouse models of SHH medulloblastoma and glioblastoma, Flores et al. (2018) have demonstrated that adoptively transferred CCR2+ hematopoietic stem cells (HSCs) migrate to the tumor site and differentiate into dendritic cells. These dendritic cells then home to draining lymph nodes where they prime T cells by presentation of tumor antigens. The transfer of CCR2+ HSCs together with anti-PD-1 treatment or adoptive T-cell transfer resulted in significantly prolonged survival of the animals (Flores et al. 2018).

Adoptive transfer or vaccination with dendritic cells that were loaded ex vivo with tumor RNA or peptides has shown promising effects in some cancer types (Lesterhuis et al. 2006; Kantoff et al. 2010; Mitchell et al. 2015). One study investigated the effect of tumor lysate-loaded dendritic cells in 45 children with recurrent malignant brain tumors. Patients with high-grade glioma and atypical teratoid–rhabdoid tumors responded favorably to the vaccine treatment, whereas no clear benefit was achieved for the five medulloblastoma patients who were included in the study (Ardon et al. 2010). In contrast, preclinical studies in group 3 and SHH medulloblastoma models have indicated that dendritic cell vaccination is beneficial when combined with the autologous transfer of ex vivo propagated T cells (Flores et al. 2018; Flores et al. 2019). This approach is currently being evaluated in a clinical trial for patients with recurrent medulloblastoma (NCT01326104, Re-MATCH) (see Table 1 for an overview of clinical trials of medulloblastoma).

In addition to APC infiltration, antigen uptake, processing, and presentation are essential for a potent antitumor response. However, tumor antigen presentation might be inefficient and fail to elicit antitumor T-cell responses. One immunotherapeutic approach whose success presumably relies on the release of large quantities of tumor antigens is oncolytic virus therapy. Oncolytic viruses can selectively replicate in tumor cells until they burst and release more infectious particles, which spread within the tumor. While the virus-mediated lysis of tumor cells was initially considered the primary mode of action, stimulation of the host antitumor immune response is now considered an essential aspect of oncolytic virus therapy (for review, see Davola and Mossman 2019). Virus-induced lysis of tumor cells releases cytosolic damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), endogenous danger signals that attract and activate the innate immune system. Importantly, cell lysis also releases large quantities of tumor and viral antigens that are phagocytosed and presented by recruited APCs, eliciting an adaptive antitumor immune response (see Fig. 2). New generations of oncolytic

Table 1. *Ongoing and recently completed medulloblastoma immunotherapy trials*[a]

Trial identifier	Study title	Study goal	Eligibility
NCT01326104	Vaccine Immunotherapy for Recurrent Medulloblastoma and Primitive Neuroectodermal Tumor (Re-MATCH)	To determine the efficacy of tumor-specific immune cells and dendritic cell vaccines	Patients of up to 30 yr of age with recurrent medulloblastoma
NCT03911388	HSV G207 in Children with Recurrent or Refractory Cerebellar Brain Tumors	To determine the safety and effectiveness of the oncolytic herpes simplex virus-1 G207 treatment of brain tumors, with or without a single low dose of radiation	Patients between 36 mo and 19 yr of age with recurrent or refractory cerebellar brain tumor
NCT02962167	Modified Measles Virus (MV-NIS) for Children and Young Adults with Recurrent Medulloblastoma or Recurrent ATRT	To determine the safety of the modified oncolytic measles virus	Patients between 12 mo and 39 yr of age with recurrent medulloblastoma or ATRT
NCT03043391	Phase 1b Study PVSRIPO for Recurrent Malignant Glioma in Children	To determine the safety and efficacy of the oncolytic modified poliovirus PVSRIPO	Patients between 12 yr and 21 yr of age with recurrent grade III or grade IV malignant glioma, medulloblastoma, or ATRT
NCT02444546	Wild-Type Reovirus in Combination with Sargramostim in Treating Younger Patients with High-Grade Relapsed or Refractory Brain Tumors	To assess the safety and efficacy of the combined treatment with wild-type reovirus (Reolysin) and sargramostim (GM-CSF)	Patients between 10 yr and 21 yr of age with recurrent grade III or grade IV malignant glioma, medulloblastoma, ATRT, or PNET
NCT03389802	Phase I Study of APX005M in Pediatric CNS Tumors	To determine the safety and efficacy of the monoclonal humanized anti-CD40 antibody in childhood brain tumors	Patients between 1 and 21 yr of age with recurrent or refractory primary malignant central nervous system tumor or newly diagnosed diffuse intrinsic pontine glioma
NCT03173950	Immune Checkpoint Inhibitor Nivolumab in People with Recurrent Select Rare CNS Cancers	To evaluate the efficacy of anti-PD1 therapy in patients with rare CNS tumors	Patients older than 18 diagnosed with medulloblastoma, ependymoma, parenchymal pineal region tumors, choroid plexus tumors, atypical/malignant meningioma, histone mutated gliomas, gliomatosis cerebri, ATRT, gliosarcoma, primary CNS sarcoma, (anaplastic) pleomorphic xanthoastrocytoma (PXA), PNETs, CNS neuroblastoma, or CNS Ewing sarcomas
NCT02359565	Pembrolizumab in Treating Younger Patients with Recurrent, Progressive, or Refractory High-Grade Gliomas, Diffuse Intrinsic Pontine Gliomas, Hypermutated Brain Tumors, Ependymoma, or Medulloblastoma	To determine the safety and efficacy of anti-PD-1 therapy	Patients between 1 and 29 yr of age with recurrent, progressive, or refractory high-grade glioma, ependymoma, DIPG, or medulloblastoma
NCT03130959	A Study to Evaluate the Safety and Efficacy of Nivolumab Monotherapy and Nivolumab in Combination with Ipilimumab in Pediatric Participants with High-Grade Primary Central Nervous System (CNS) Malignancies (CheckMate 908)	To determine the safety and effectiveness of anti-PD-1 therapy alone or in combination with anti-CTLA4 treatment	Patients between 6 mo and 29 yr of age with newly diagnosed DIPG; recurrent or progressive high-grade glioma, both previously treated with radiotherapy but no chemotherapy; relapsed or resistant medulloblastoma or ependymoma; or other high-grade progressive or recurrent CNS malignancy
NCT02502708	Study of the IDO Pathway Inhibitor, Indoximod, and Temozolomide for Pediatric Patients with Progressive Primary Malignant Brain Tumors	To test the efficacy of IDO inhibition combined with radiation and temozolomide	Patients between 3 and 21 yr of age with progressive high-grade glioma, ependymoma, medulloblastoma, other primary CNS tumors, or newly diagnosed DIPG

Continued

Table 1. *Continued*

Trial identifier	Study title	Study goal	Eligibility
NCT02748135	A Study of TB-403 in Pediatric Subjects with Relapsed or Refractory Medulloblastoma	To assess the safety and tolerability profile of a humanized monoclonal anti-PlGF antibody in pediatric subjects with relapsed or refractory medulloblastoma	Patients between 6 mo and 19 yr of age with relapsed or refractory medulloblastoma, neuroblastoma, Ewing sarcoma, or alveolar rhabdomyosarcoma
NCT03638167	EGFR806-Specific CAR-T Cell Locoregional Immunotherapy for EGFR-Positive Recurrent or Refractory Pediatric CNS Tumors	To evaluate the safety of CNS-delivered anti-EGFR CAR-T cell infusions	Patients between 1 and 26 yr of age with EGFR-positive recurrent or refractory glioma, ependymoma, medulloblastoma, germ cell tumor, ATRT, PNET, choroid plexus carcinoma, or pineoblastoma
NCT04185038	Study of B7-H3-Specific CAR-T Cell Locoregional Immunotherapy for Diffuse Intrinsic Pontine Glioma/ Diffuse Midline Glioma and Recurrent or Refractory Pediatric Central Nervous System Tumors	To assess the safety of B7-H3-specific CAR-T cell infusions delivered into the tumor resection cavity or ventricular system	Patients between 1 and 26 yr of age with recurrent or refractory glioma, ependymoma, medulloblastoma, germ cell tumor, ATRT, PNET, choroid plexus carcinoma, pineoblastoma, or DIPG
NCT03500991	HER2-Specific CAR-T Cell Locoregional Immunotherapy for HER2-Positive Recurrent/ Refractory Pediatric CNS Tumors	To analyze the safety of Her2-specific CAR-T cell infusions delivered into the tumor resection cavity or ventricular system	Patients between 1 and 26 yr of age with recurrent or refractory glioma, ependymoma, medulloblastoma, germ cell tumor, ATRT, PNET, choroid plexus carcinoma, or pineoblastoma

[a]Retrieved from ClinicalTrials.gov on May 5, 2022.

viruses are equipped with genes that encode immune checkpoint inhibitors such as soluble PD-1-IgG Fc (Wang et al. 2020) or immune cell-recruiting or -stimulating factors such as IL-12, GM-CSF, OX40L, or Flt3L (Liu et al. 2003; Barnard et al. 2012; Jiang et al. 2017; Saha et al. 2017). In medulloblastoma, several preclinical studies have been performed analyzing the efficacy of oncolytic viruses (Lal et al. 2018; Thompson et al. 2018). A recent study of G207, a genetically engineered herpes simplex virus (HSV-1), demonstrated efficient elimination of murine group 3 medulloblastoma tumors without induction of systemic toxicity in an immunocompetent, HSV-1-sensitive mouse strain (Bernstock et al. 2020). G207 (NCT03911388) and other oncolytic viruses such as modified measles virus (NCT02962167), poliovirus (NCT03043391), and wild-type reovirus (NCT02444546) are currently being investigated in clinical trials for medulloblastoma (see Table 1).

Another approach to increase the efficacy of antigen presentation is targeted stimulation of CD40 using agonistic monoclonal antibodies (see Fig. 2). Activation of CD40 on APCs induces up-regulation of costimulatory molecules, production of cytokines, increased expression of MHC, and prosurvival genes (Suek et al. 2019). Although direct cytotoxic effects on tumor cells have also been reported, experiments in immunodeficient and immunocompetent mice suggest that the prolonged survival of anti-CD40-treated glioblastoma-bearing mice is due to its immune stimulatory effect (Shoji et al. 2016). Currently, an agonistic CD40 targeting antibody is being evaluated in a clinical trial in patients with medulloblastoma and other pediatric brain tumors (NCT03389802).

APCs are crucial for the initiation of T-cell-mediated tumor rejection, yet the final cytotoxic T-cell-induced tumor cell killing relies on MHC presentation by tumor cells. The genetic loss or transcriptional, translational, or post-translational suppression of MHC class I in tumor cells (for review, see Dhatchinamoorthy et al. 2021) is a frequent event in immune escape and has been reported for medulloblastoma (Raffaghello et al. 2007). Studies in cancer cell lines have shown that the suppression of MHC class I expression is often reversible and can be restored by histone deacetylase inhibitors (Khan et al. 2008), DNA methyltransferase inhibitors (Serrano et al. 2001), γ interferon (IFNγ) (Zhang et al. 2019), or SMAC mimetics (see Fig. 2; Gu et al. 2021). More preclinical studies will be required to determine whether these measures can also sensitize MHC class I-low or -negative brain tumor cells to CD8^{+} T-cell-mediated tumor cell rejection.

Inhibition of tumor-induced immunosuppression

Immune checkpoint blockade   Immune checkpoint proteins, including the intensively studied surface proteins PD-1 and CTLA-4, decrease the proliferation and activity of T cells by inducing cell cycle arrest and down-regulation of T-cell receptor signaling, by promoting death of activated T cells, and by suppressing prosurvival signals and cytokine secretion (Krummel and Allison 1996; Freeman et al. 2000; Dong et al. 2002). Their blockade has emerged as a promising immunotherapy in a variety of cancer types (see Fig. 3). Several approaches have been taken to achieve immune checkpoint inhibition in brain tumors. Dorand
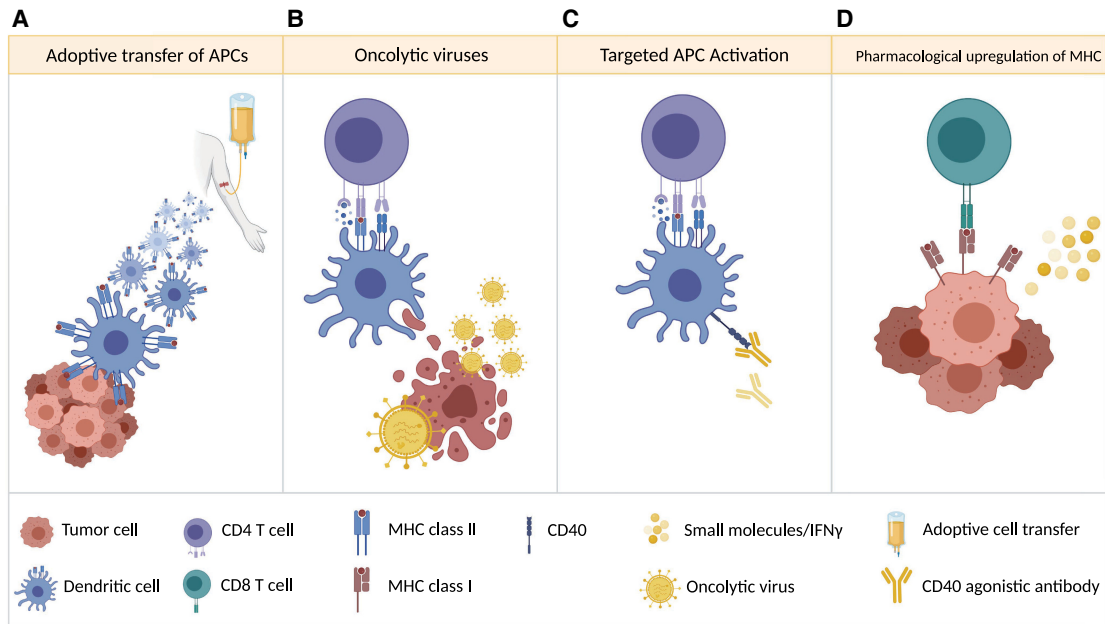
Figure 2. Strategies to increase antigen presentation. (*A*) Autologous dendritic cells, pulsed with total tumor RNA or lysate, are infused into the patient. (*B*) Oncolytic viruses replicate in tumor cells, which ultimately leads to tumor cell lysis and release of tumor-associated antigens, damage-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs) that can enhance antigen presentation. (*C*) Targeted activation of APCs (for example, by agonistic anti-CD40 antibody treatment) enhances APC activity and antigen presentation. (*D*) Antigen presentation by tumor cells may be enhanced by treating with molecules (e.g., IFNγ) that up-regulate MHC class I expression and presentation.

et al. (2016) have shown that Cdk5 deletion in mouse SHH medulloblastoma tumors abrogates the IFNγ-induced up-regulation of the PD-1 ligand PD-L1 in tumor cells, and this results in CD4$^+$ T-cell-mediated tumor rejection, suggesting Cdk5 as a therapeutic target. Although medulloblastomas are generally characterized by the presence of very few PD-L1-positive tumor cells, a preclinical study has demonstrated a modest response of murine group 3 medulloblastoma tumors to anti-PD-1 monoclonal antibody treatment (Pham et al. 2016). Similarly, nonsmall cell lung cancer patients scored as tumor PD-L1-null have been found to respond to anti-PD-L1 therapy (Bocanegra et al. 2019). The study has identified circulating immune cells, predominantly of myeloid origin, that express PD-L1 and whose number significantly correlates with clinical responses to immune checkpoint inhibition (Bocanegra et al. 2019). Finally, two recent studies used various combinations of PD-L1 knockout and wild-type cancer cells and mouse recipients to demonstrate that *Cd274* (which encodes PD-L1) expression by host APCs, and not by tumor cells, determines the response to anti-PD-L1 and anti-PD-1 therapy (Lin et al. 2018; Tang et al. 2018).

It is important to note that PD-1 activation can also be initiated by PD-L2. However, its expression in medulloblastoma has not been examined, with the exception of a study that found low PD-L2 expression in two medulloblastoma cell lines (Martin et al. 2018). This underscores the complexity of immune checkpoints and suggests that PD-L1 expression in tumor specimens cannot necessarily predict responses to anti-PD-1 treatment.

Comparatively little is known about CTLA-4 expression on medulloblastoma-infiltrating T cells. Bockmayr et al. (2018) have described the microenvironment of human group 3 and group 4 tumors as having an increased immunosuppressive gene signature characterized by PD-L1 and CTLA-4 expression. No CTLA-4 expression was detected on infiltrating CD3$^+$ T cells in mouse models of SHH and group 3 medulloblastoma (Pham et al. 2016). Accordingly, anti-CTLA-4 monoclonal antibody treatment showed no effect on survival (Pham et al. 2016).

Recent studies have identified several other inhibitory checkpoint molecules, including LAG3, TIM-3, B7-H3, TIGIT, and VISTA, most of which are being investigated as therapeutic targets (Kelly et al. 2020). Likewise, the activation of T-cell stimulatory molecules like ICOS, GITR, and OX40 have been suggested to enhance antitumor responses. However, preclinical and clinical data on these second-generation immune checkpoint inhibitors or activators in medulloblastoma tumors are lacking. A detailed review of immune checkpoint modulation in brain tumors is provided in Kelly et al. (2020).

Despite limited knowledge about immune checkpoint expression and function in medulloblastoma, several clinical trials have been initiated to investigate the safety and efficacy of anti-PD-1, PD-L1, and CTLA-4 monoclonal antibody therapy in medulloblastoma patients (NCT03173950, NCT02359565, and NCT03130959) (see Table 1).
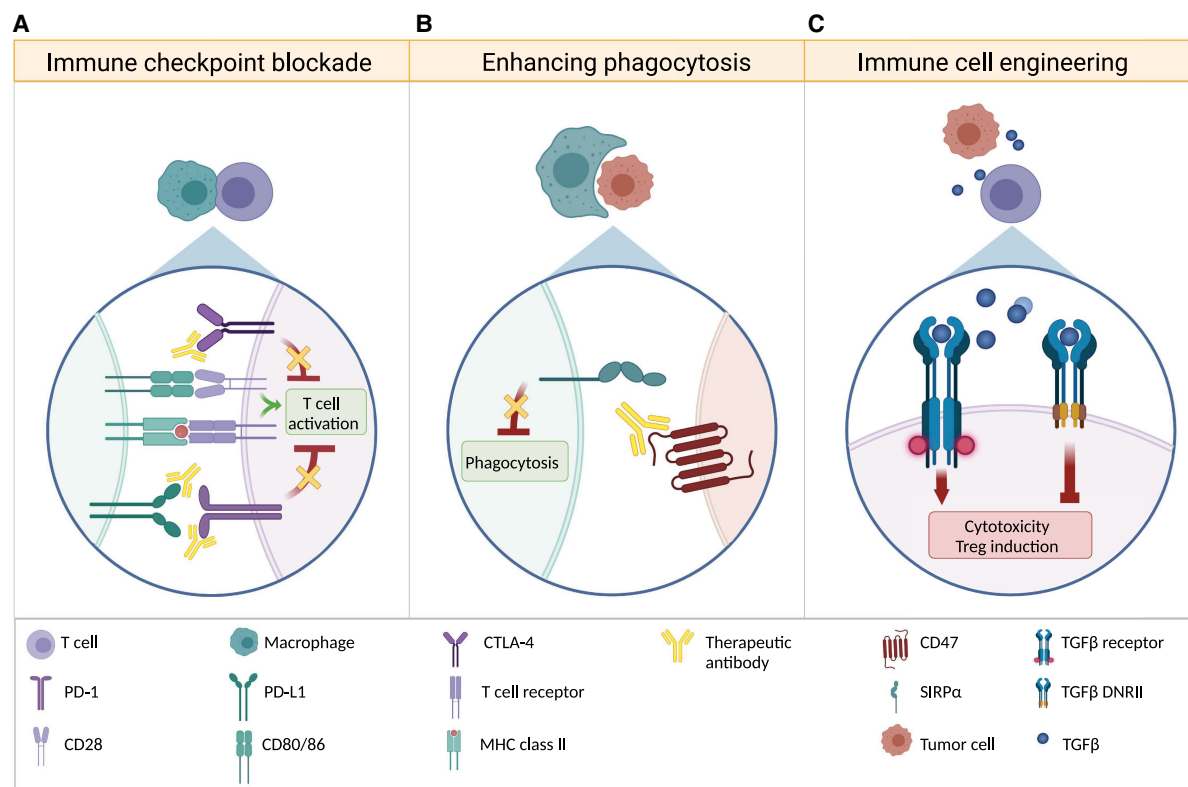
Figure 3. Strategies to overcome immune suppression. (*A*) Monoclonal antibodies block immune checkpoint proteins that serve as intrinsic negative regulators of immune responses, allowing for continued immune cell activity. Antibodies directed against CTLA-4, PD-1, or its ligand PD-L1 inhibit the suppression of activated T cells. (*B*) CD47 is expressed on tumor cells and, upon binding to SIRPα on macrophages, prevents phagocytosis. Anti-CD47 antibodies block this interaction, allowing tumor cell phagocytosis by macrophages. (*C*) Genetic engineering can render immune cells resistant to tumor cell-induced suppression. Expression of a dominant-negative TGFβ receptor on immune cells prevents TGFβ-induced cytotoxicity as well as differentiation of Tregs and sequesters soluble TGFβ, preventing immunosuppressive TGFβ signaling in endogenous immune cells.

Overcoming the immunosuppressive milieu Given that tumor cells generate an immune-hostile environment by releasing immunosuppressive molecules, one therapeutic approach is to equip immune cells with resistance to the anti-inflammatory milieu before their adoptive transfer into the patient. Along these lines, first attempts have been made to render NK and T cells insensitive to immune-suppressive TGFβ secreted by medulloblastoma cells. The exogenous expression of a dominant-negative TGFβ receptor type II (TGFβRII DNR) in NK cells restored their cytotoxic activity in in vitro coculture experiments with TGFβ-secreting medulloblastoma cells (Powell et al. 2019). As the engineered NK cells sequestered TGFβ, the investigators suggested that the use of TGFβRII DNR-expressing NK cells might not only have therapeutic benefits due to restoration of tumor-directed cytotoxicity but could also help reverting the immunosuppressive environment in medulloblastomas, improving the efficacy of other immune cells.

Abrogation of TGFβ signaling can also promote antitumor activity of other immune cell types, particularly T cells (for review, see Thomas and Massagué 2005). A mouse model of medulloblastoma that expresses the TGFβRII DNR under the control of a CD4 promoter has

shown enhanced antitumor immune responses and a clear survival benefit compared with TGFβ wild-type receptor littermates (Gate et al. 2014). TGFβ signaling blockade in T cells not only reduced the number of medulloblastoma-infiltrating Tregs but also promoted CD8^{+} T-cell differentiation and formation of T-cell memory (Gate et al. 2014).

Thus, engineering immune cells for TGFβ signaling resistance and their subsequent adoptive transfer may be one option to enhance antitumor responses (see Fig. 3). Alternatively, small molecule inhibitors or neutralizing antibodies specific for TGFβ could be applied to abrogate tumor-induced immunosuppression. The safety and efficacy of TGFβ inhibitors as well as of TGFβ-resistant CAR-T cells are currently being investigated in clinical trials for other solid cancers (for review, see Patel et al. 2019; Huang et al. 2021), including studies that combine TGFβ inhibition with immune checkpoint blockade (NCT02423343 and NCT02734160). Furthermore, as TGFβ signaling is a known driver of SHH and group 3 medulloblastoma progression and metastasis (Aref et al. 2013; Ferrucci et al. 2018; Morabito et al. 2019; Sarić et al. 2020), its therapeutic targeting might have additional benefits in inhibiting tumor growth.

Other ongoing clinical trials that target the immune-suppressive medulloblastoma milieu include assessment of indoximod, an indoleamine 2,3-dioxygenase (IDO) inhibitor (NCT02502708), and a humanized monoclonal antibody against placental growth factor (PlGF; NCT02748135). IDO1 inhibition results in reduced catalytic depletion of tryptophan, an amino acid that is critical for T-cell activity and survival. In glioblastoma mouse models, IDO1 inhibition has been shown to restore antitumor immune responses in a multimodal treatment approach with immune checkpoint blockade (Wainwright et al. 2014) or checkpoint blockade and radiotherapy (Ladomersky et al. 2018). PlGF is a member of the VEGF family and may be a good therapeutic target, as it has been reported to suppress the immune system by inhibition of dendritic cell maturation (Lin et al. 2007) and, moreover, to promote medulloblastoma growth and metastasis (Snuderl et al. 2013).

The extent to which macrophages are suitable as therapeutic targets is controversial. As discussed above, several studies have indicated an immunosuppressive function of medulloblastoma-associated myeloid cells, which makes them an attractive target in cancer therapy. However, there is also increasing evidence indicating no or even adverse effects of myeloid cell depletion from medulloblastoma tumors. The pharmacological CSF1R inhibition in a syngeneic mouse model of SHH medulloblastoma ($Ptch1^{+/-}$; $Trp53^{-/-}$) and in a group 3 PDX model (grown in immune-compromised mice) did reduce the number of tumor-associated macrophages; however, no effect on survival or tumor progression was observed (Crotty et al. 2021). Interestingly, as opposed to the above-mentioned studies, the CSF1R-inhibited SHH mouse model showed reduced numbers of T-cell infiltrates. Moreover, other studies have shown that macrophages and other myeloid cells can have a beneficial effect, and their depletion may even enhance tumor growth. Gholamin et al. (2017) described a high phagocytic potential of medulloblastoma-associated macrophages that was unleashed by blocking the surface molecule CD47 on tumor cells (see Fig. 3), and this resulted in elimination of primary and metastatic tumor cells in a medulloblastoma PDX model. Consistent with this, another study has demonstrated a beneficial effect of medulloblastoma-associated macrophages, as their depletion from SHH (*NeuroD2-SmoA1*) tumors by *Ccr2* knockout, CSF1R inhibition, or clodrosome administration promoted tumor progression (Maximov et al. 2019). More studies are needed to investigate the conditions that determine tumor-promoting or tumor-opposing functions of medulloblastoma-associated macrophages. Until then, re-education of medulloblastoma-associated myeloid cells to become tumoricidal, rather than nonspecific depletion of these cells, may be a more effective therapeutic strategy.

Re-education, reprogramming, or repolarization of tumor-associated myeloid cells involves loss of tumor-promoting and immunosuppressive signatures and acquisition of proinflammatory signatures. In a mouse model of proneural glioblastoma, CSF1R inhibition was reported to reprogram tumor-associated macrophages from M2-like tumor-promoting to more tumor phagocytic cells, resulting in significant inhibition of tumor growth and prolonged survival (Pyonteck et al. 2013). However, clinical trials in glioblastoma patients have not confirmed the beneficial effects of CSF1R inhibition observed in preclinical mouse models (ClinicalTrials.gov identifier NCT01790503, https://clinicaltrials.gov/ct2/show/results/NCT01790503; Butowski et al. 2016). Another promising strategy is the inhibition of the γ isoform of phosphoinositide-3-kinase (PI3Kγ), which is predominantly expressed by myeloid cells. Pharmacological inhibition of PI3Kγ in mouse models of glioblastoma changed the cytokine profile of tumor-associated myeloid cells, which sensitized tumors to antiangiogenic (Rivera et al. 2015) or temozolomide (Li et al. 2021) therapy. In particular, Li et al. (2021) found that glioblastoma-associated microglia induced MYC expression in tumor cells by secretion of IL-11, which was abrogated by inhibition of PI3Kγ. The effect of PI3Kγ inhibition in medulloblastoma, and in particular in MYC-driven medulloblastoma, remains to be determined.

CAR-T cells have gained interest as an alternative to pharmacological intervention in the reprogramming of the tumor microenvironment. A recent study used CAR-T cells for the selective targeting of folate receptor β (FRβ)-positive, tumor-promoting myeloid cells in mouse models of ovarian cancer, colon cancer, and melanoma. Specific depletion of the FRβ⁺ macrophage subpopulation led to increased numbers of classical/inflammatory monocytes and endogenous CD8⁺ T cells that displayed an activated phenotype, resulting in delayed tumor growth and prolonged survival (Rodriguez-Garcia et al. 2021). More studies will be required to identify markers of tumor-promoting myeloid populations in medulloblastoma in order to apply the CAR-T cell technology for the reprogramming of the medulloblastoma microenvironment.

Engineering CAR-T cells to overcome limitations of endogenous T cells   Endogenous T cells engineered to express chimeric antigen receptors against common tumor antigens represent an important pillar of immunotherapy. A major advantage of the chimeric antigen receptor is that it does not require tumor antigen presentation by MHC class I and interaction with costimulatory ligands, but instead can be designed against any surface protein expressed by tumor cells. In medulloblastoma, several promising CAR-T cell targets have been identified. Preclinical studies have shown high efficacy of HER2-directed (Ahmed et al. 2007; Nellan et al. 2018; Donovan et al. 2020), EPHA2-directed (Donovan et al. 2020), and B7-H3-directed (Majzner et al. 2019) CAR-T cells. To overcome CAR-T cell resistance by intratumoral antigen heterogeneity, trivalent CAR-T cells have been developed that are able to target tumor cells that express any one of three target genes. Although multivalent HER-2/IL13Rα2/EPHA2 CAR-T have proved more efficacious than their monovalent CAR-T cell counterpart in glioblastoma xenografts (Bielamowicz et al. 2018), this superiority has not been shown in PDX models of group 3 medulloblastoma (Donovan et al. 2020).

Additional modifications such as overexpression of surface proteins and immune stimulatory cytokines that improve T-cell homing, expansion, and persistence or equipping cells with resistance against tumor-induced immunosuppression may augment antitumor responses. As encouraging as engineered immune cells such as CAR-T cells may seem, their safety must be carefully evaluated. Cell engineering may harbor unknown risks; for example, expression of the TGFβRII DNR in T cells has been shown to cause lymphoproliferative disorders in mice (Lucas et al. 2000; Ishigame et al. 2013). Identified risks can be overcome by further modifications, such as an inducible TGFβRII DNR expression regulated by TCR activation, coupling TGF-β resistance to tumor responsiveness (Hartley and Abken 2019).

Currently, multiple clinical trials are dedicated to assessing the safety and efficacy of CAR-T cells directed against HER2 (NCT03500991), the tumor-specific variant EGFR806 (NCT03638167), and B7-H3 (NCT04185038) in medulloblastoma and other pediatric brain tumors.

Future perspectives

Multiple studies have shown that medulloblastoma tumors largely lack effector immune cells. Like many other brain tumors, medulloblastoma might be protected from immune infiltration by the fact that the immune surveillance of the central nervous system is tightly controlled by the BBB and is restricted to specific subsets of immune cells. Although our knowledge of the BBB and its common disruption by tumor growth has remained very limited, immune cell infiltration seems to be continuously impaired in the tumor setting. The limited numbers of tumor-infiltrating immune cells will eventually encounter a hostile environment that by various means either suppresses, kills, or polarizes them toward a tumor-promoting phenotype. Although these facts suggest that medulloblastoma may be refractory to endogenous antitumor immune responses, there are intriguing data indicating great potential of intervening strategies. Slight but effective changes in chemotherapy and radiotherapy regimens and use of anti-VEGF instead of corticosteroids have been shown to spare immune cells, enabling them to participate in antitumor immune responses. Engineering of immune cells, particularly T cells, may enable their enhanced and persistent tumor infiltration by overexpression of tumor-matching chemokine receptors or cell adhesion molecules. Several strategies are being established to increase tumor antigen release (for example, by oncolytic viruses) and enhance antigen presentation by APCs and tumor cells themselves. Furthermore, the immunosuppressive environment created by the tumor has been shown to be reversible; for example, by targeting anti-inflammatory molecules such as TGFβ or by immune checkpoint inhibition. Moreover, T-cell engineering enables the equipment of T cells with resistance against tumor-initiated immunosuppression and with an MHC class I-independent T-cell receptor.

Despite great successes of individual preclinical studies, clinical trials of various brain and other solid tumors exhibiting immunotherapy resistance have highlighted the need for more detailed mechanistic studies on interactions between immune cells and tumor cells in order to develop novel therapies and to predict and prevent immunotherapy resistance. One critical approach to bypassing resistance may be combining several of the above-described strategies to initiate, enhance, and maintain antitumor responses. With more preclinical and clinical studies analyzing potent and safe combinations of immunotherapeutic agents, immunotherapy might offer a durable cure for medulloblastoma patients.

Competing interest statement

Acknowledgments

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Coming in from the cold: overcoming the hostile immune microenvironment of medulloblastoma

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