

# Chapter 15

## Future Therapies for Malignant Brainstem Tumors



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### Abbreviations

AA	Anaplastic astrocytoma
ABC	ATP-binding cassette
ABCG	ATP-binding cassette, subfamily G
ACVR1	Activin A receptor, type I
Ad5	Adenovirus serotype 5
ADC	Antibody-drug conjugate
ADCC	Antibody-dependent cell-mediated cytotoxicity
ALA	5-aminolevulinic acid
AlPcS <sub>2a</sub>	Aluminum phthalocyanine disulfonate
AML	Acute myeloid leukemia
ATRT	Atypical teratoid rhabdoid tumor
ATRX	Alpha thalassemia/mental retardation syndrome X-linked
BBB	Blood-brain barrier
BMPs	Bone morphogenic proteins
CARs	Chimeric antigen receptors
CAR-T	Chimeric antigen receptor T-cell
CBTRUS	Central Brain Tumor Registry of the United States
CDC	Complement-dependent cytotoxicity
CED	Convection-enhanced delivery
CMV	Cytomegalovirus
CNS	Central nervous system
CPT-11	Camptothecin-11

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CSF	Cerebrospinal fluid
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CYP450s	Cytochromes P450
DIPG	Diffuse intrinsic pontine glioma
DNA	Deoxyribonucleic acid
DT	Diphtheria toxin
EGFR	Epidermal growth factor receptors
EGFRvIII	EGFR variant III
ETANTR	Embryonal tumor with abundant neuropil and true rosettes
ETX	Epsilon toxin, active form
ETXp	Epsilon prototoxin
EZH2	Enhancer of zeste homolog 2
FUS	Focused ultrasound
G34R	Glycine-to-arginine missense at position 34
GBM	Glioblastoma multiforme
Gd-DTPA	Gadolinium-diethylenetriamine penta-acetic acid
HDACi	Histone deacetylase inhibitor
HER2	Human epidermal growth factor receptor 2
HGG	High-grade glioma
HLA	Human leukocyte antigen
HSV	Herpes simplex virus
ICOS	Inducible costimulator
IDH	Isocitrate dehydrogenase
IDO1	Indoleamine 2,3-dioxygenase 1
IFN	Interferon
K27M	Lysine-to-methionine missense at position 27
LAG-3	Lymphocyte activation gene 3
LDLR	Low-density lipoproteins receptor
LRPs	LDLR-related proteins
MDR	Multidrug resistance
MHC	Major histocompatibility complex
MRI	Magnetic resonance imaging
MRP	Multidrug-resistance-associated protein
NK	Natural killer
PCI	Photochemical internalization
PD-1	Programmed death-1
PDGFR	Platelet-derived growth factor receptors
PDT	Photodynamic therapy
PE	Pseudomonas exotoxin
PET	Positron emission tomography
P-gp	P-glycoprotein
PNET	Primitive neuroectodermal tumor
PRC2	Polycomb repressive complex 2
PTEN	Phosphatase and tensin homolog
RNA	Ribonucleic acid

RTKs	Receptor tyrosine kinases
scFv	Single-chain variable fragment
SPECT	Single photon emission computed tomography
TCRs	T-cell receptors
Tf	Transferrin
TGF- $\beta$ 1	Transforming growth factor $\beta$ 1
TIL	Tumor-infiltrating lymphocyte
TIM-3	T-cell immunoglobulin and mucin domain-containing 3
TK	Thymidine kinase
Treg	Regulatory T cell
TTRNA-DC	Total tumor mRNA-pulsed autologous dendritic cell
VEGF	Vascular endothelial growth factors
WHO	World Health Organization
$\alpha$	Alpha
$\beta$	Beta

## 15.1 Introduction

Malignant brainstem tumors are a heterogeneous group of tumors occurring in the brainstem and cervicomedullary junction. According to the Central Brain Tumor Registry of the United States (CBTRUS), in all age groups, there were about 1200 primary brainstem tumors per year between 2010 and 2014 in the United States, among which 900 cases were malignant [1]. These numbers account for 1.6% of all primary central nervous system (CNS) tumors and 3.8% of all malignant primary CNS tumors. Brainstem tumors occur more often in children than in adults. Among 0–14 year-olds, about 450 cases of primary brainstem tumors occurred during the same period in the United States, accounting for 13.4% of all primary CNS tumors in this age group [1].

Approximately 90% of brainstem tumors are gliomas in origin [2]. In children, most of these are diffuse intrinsic pontine gliomas (DIPGs), accounting for at least 80% of brainstem gliomas [3, 4]. They have a dismal prognosis with a median survival of only 1 year [4]. Other malignant brainstem tumors in children include embryonal tumors such as atypical teratoid rhabdoid tumors (ATRTs), embryonal tumors with abundant neuropil and true rosettes (ETANTR) and primitive neuroectodermal tumors (PNETs) (the latter two disease entities were folded into embryonal tumors with multilayered rosettes [ETMR] in the WHO 2016 classification), and high-grade glial tumors that are not classified as DIPG [5]. These tumors are rarer than DIPG. Even rarer are high-grade mixed neuronal-glial tumors (anaplastic ganglioglioma) in the brainstem. Primary malignant tumors in the brainstem in adults are less common than in children and are mainly anaplastic astrocytoma (AA) and glioblastoma multiforme (GBM) [6]. Malignant tumors from nearby structures, such as choroid plexus carcinoma of the fourth ventricle, can also invade the brainstem.

The discussion in this chapter will focus on investigational therapeutic options for DIPG, sometimes with references to related diseases such as glioma in general.

## **15.2 Obstacles in the Treatment of Malignant Brainstem Tumors**

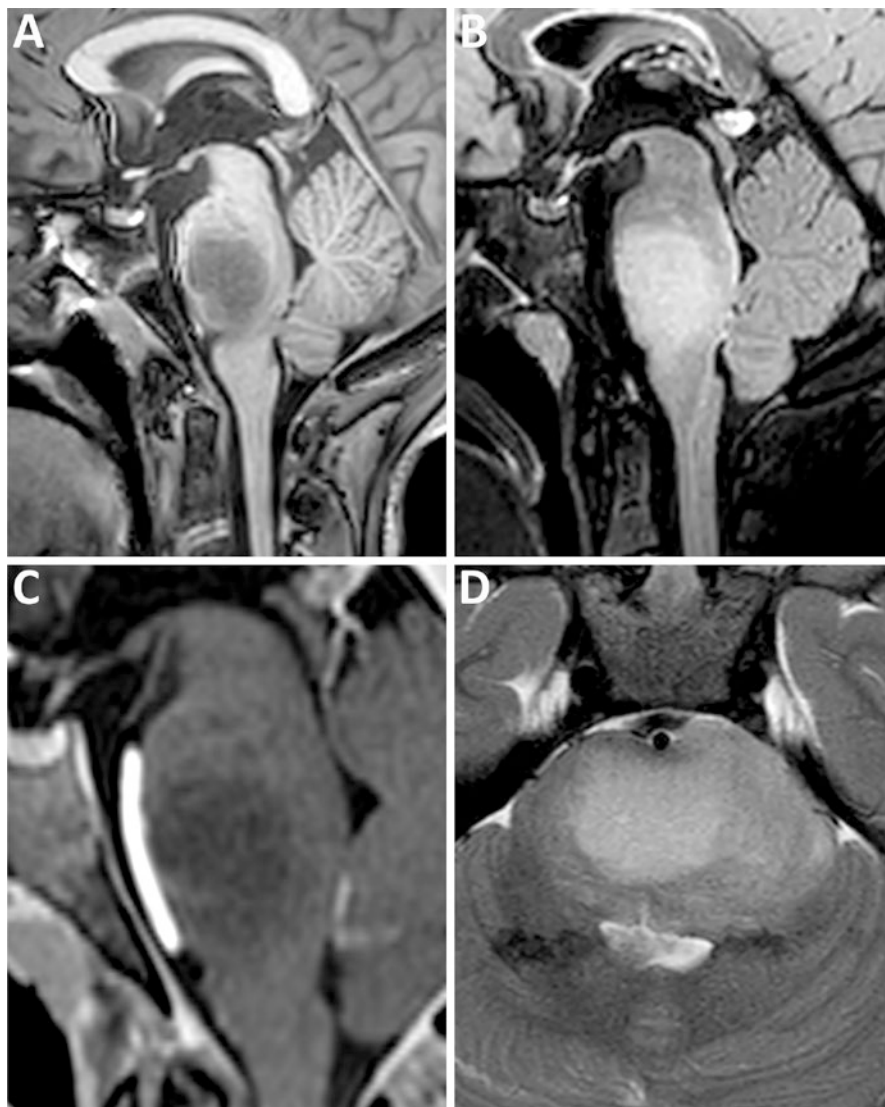
### ***15.2.1 Maximal Safe Cytoreduction Surgery***

The brainstem is a compact structure and plays pivotal roles in cardiovascular and respiratory control, alertness, awareness, and consciousness, as well as serving as the passageway for motor and sensory tracts and housing the cranial nerve nuclei. DIPG, the most common malignant primary brainstem tumor, infiltrates the brainstem extensively, precluding meaningful cytoreduction surgery. As a result, in the clinical management of DIPG, surgery is typically only used for relieving hydrocephalus or for biopsy. The diffuse growth pattern of DIPG is demonstrated in Fig. 15.1.

### ***15.2.2 Blood-Brain Barrier***

An important limitation of systemic chemotherapy in primary brain tumor treatment is the existence of the blood-brain barrier (BBB). The BBB is a barrier that isolates the circulating blood from the cerebrospinal fluid (CSF) and the interstitial fluid in the CNS. It occurs along the cerebral capillaries and consists of tight junctions (zona occludens) that do not exist in vasculatures in other organs. Endothelial cells restrict the diffusion of microscopic objects (e.g., bacteria) and large or hydrophilic molecules from the brain vasculature, while allowing the diffusion of small hydrophobic molecules (e.g., O<sub>2</sub>, CO<sub>2</sub>, and certain hormones). Typically, molecules larger than ~40 kD are unlikely to penetrate the intact barrier. For the brain's supply of nutrients and removal of metabolites, cells of the brain vasculature actively transport glucose and metabolic products across the barrier using transporters.

The BBB acts effectively to protect the brain from many common bacterial infections and some toxic substances. Yet, it presents a major challenge in delivering therapeutic agents to specific regions of the brain for the treatment of brain tumors and certain other disorders. Most cancer drugs are not able to permeate the BBB because they are polar in structure or too large in molecular weight. Even for drugs that are able to cross the cerebral capillary bed, it is difficult to achieve optimal concentrations in the brain due to the limitations posed by systemic toxicity.



**Fig. 15.1** Imaging presentation of a DIPG. These representative images were acquired from the same patient in one study. (a) The tumor is hypointense on T1-weighted images (sagittal view). (b) The tumor is hyperintense on FLAIR images (sagittal view). (c) The tumor lacks enhancement on post-contrast T1-weighted images (sagittal view). (d) The tumor is hyperintense on T2-weighted images (axial view). Note the diffuse growth pattern of the tumor and the ventral expansion of the pons caused by the tumor growth, partially engulfing the basilar artery

Another related challenge in the delivery of drugs for the treatment of primary brain tumors and certain other CNS diseases is how to direct drugs to the lesion while sparing healthy neural tissues from disturbance of normal neurological functions.

Clinically, gadolinium contrast enhancement on magnetic resonance imaging (MRI) serves as an indicator of the integrity of the BBB. DIPGs either do not show contrast enhancement or have only a small volume of contrast enhancement [7], suggesting that the BBB is largely intact in this tumor. Another piece of indirect evidence of the relatively intact BBB is that in a clinical study of tyrosine kinase inhibitors dasatinib and vandetanib in DIPG patients, the CSF to plasma exposure of the two drugs was only approximately 2% [8]. An animal study suggests that tumor location, instead of histone mutation status, may be the main reason for this relatively intact BBB in brainstem infiltrative gliomas [9]. While a more permeable BBB, as indicated by contrast enhancement, may allow chemotherapeutic drugs to reach the tumor more easily, it is also associated with shorter survival in DIPG patients [7].

### 15.2.3 *ATP-Binding Cassette Transporters*

ATP-binding cassette (ABC) transporters are a family of transporter proteins that contribute to drug resistance by functioning as ATP-dependent drug efflux pumps. At least four dozens of human ABC genes have been identified [10]. The best-known ABC transporter that is involved in multidrug resistance is P-glycoprotein (P-gp), an organic cation pump. P-gp is encoded by the MDR1 gene. The physiological function of P-gp is the excretion of toxins from cells, and it contributes to drug resistance in a pharmacological context. P-gp is overexpressed in chemotherapy-resistant tumors, conferring resistance to certain chemotherapeutic agents, and is upregulated after disease progression following chemotherapy in cancers. Other transporter proteins mediating drug resistance include those in the multidrug-resistance-associated protein (MRP) family and ABC, subfamily G (ABCG). Among members of the MRP family, only MRP1 has shown convincing evidence to be associated with clinical resistance. ABCG2, a half transporter in the ABCG subfamily, confers resistance to topotecan, camptothecin-11 (CPT-11), and mitoxantrone [11]. The expression and regulation of the MRP family and ABCG2 have not been extensively studied in cancers.

In the normal brain, ABC transporters are predominantly expressed on endothelial cells of micro blood vessels, but can also be found in astrocytes, microglia, and neurons [12, 13]. In brain capillary endothelial cells, P-gp is primarily found in the luminal (blood-facing) membrane [14, 15]. However, it is also expressed on the abluminal (brain-facing) membranes of capillary endothelial cells as well as adjacent pericytes and astrocytes [16].

A recent study demonstrated the expression of P-gp, MRP1, and ABCG2 in tumor vasculature, and the expression of MRP1 in glioma cells themselves in DIPG

as well as in pediatric supratentorial high-grade glioma (HGG) samples [17], suggesting that these drug efflux transporters may be a major factor in the failure of systemic chemotherapy in treating DIPG.

### ***15.2.4 Intratumoral Heterogeneity***

Intratumoral heterogeneity has been recognized as a common phenomenon in malignant solid tumors for several decades [18, 19]. Heterogeneity can be appreciated both histologically and molecularly. Intratumoral genetic heterogeneity has been documented in a large number of tumors recently [20–23]. Tumors are dynamically evolving genetically and epigenetically, both spatially within the local tumor and across metastatic sites, and temporally throughout the disease course. As a result of intratumoral heterogeneity, sampling different parts of the same tumor may produce different results for pathology studies and genetic and epigenetic profiling [24–29]. In glioblastoma, a single tumor consisted of a heterogeneous mixture of cells harboring diverse types of mutations, both by copy number analysis and transcription analysis [29, 30].

Intratumoral heterogeneity plays an important role in therapeutic resistance. Clonal variations in response to chemotherapeutic agents, hyperthermia or ionizing radiation have been well documented [31]. Most cancer therapies present selection pressure on the numerous and diverse clones of tumor cells [32, 33]. Clones that survive the therapy will dominate in the post-treatment or recurrent tumor. The impact of clonal variation on therapeutic resistance may be more pronounced for the contemporary signal transduction pathway-targeted therapies than the conventional cytotoxic chemotherapy. Targeting of receptor tyrosine kinases (RTKs), such as epidermal growth factor receptors (EGFR), platelet-derived growth factor receptors (PDGFR) and vascular endothelial growth factors (VEGF), has been a focus of some recent clinical trials for GBM. Therapy with single agents leads to clonal selection, enriching therapy-resistant clones that give rise to recurrent GBM [34]. Possibly a result of this phenomenon in part, clinical trials with signal transduction pathway-targeted therapies failed to show significant improvement in survival in GBM, as well as in DIPG patients [8, 35].

DIPGs show intratumoral T2-weighted signal heterogeneity [36, 37] as well as diffusivity heterogeneity [36, 38, 39]. Histologically, DIPGs show considerable intratumoral heterogeneity, with over 50% even showing focal areas resembling World Health Organization (WHO) grade I morphology [40]. Intratumoral molecular heterogeneity has also been demonstrated recently [40–42], with PDGFRA amplification and mutation as well as BCOR, ATRX, MYC and TP53 mutations [42], and H3-K27me3 mark [40] showing marked spatial heterogeneity. One of the studies also demonstrated that the histone 3 (H3) lysine-to-methionine missense at position 27 (K27M) mutation is spatially conserved [42], arguing that molecular intratumoral heterogeneity in DIPG is less significant than in adult GBM.

### ***15.2.5 Immune Privilege and Specialization of the Central Nervous System***

Immune privilege of the CNS refers to the experimental phenomenon where tissues grafted into the CNS survive for extended periods of time without rejection. Immune privilege of the CNS was thought as the result of CNS isolation from the immune system by the BBB, the lack of draining lymphatics, and the less immunocompetent microglia instead of regular macrophages. However, recent evidence shows that the CNS is neither isolated nor passive in its interactions with the immune system; rather, the CNS is immune-competent in that peripheral immune cells can cross the intact BBB. CNS neurons and glia actively interact with the peripheral immune system to regulate macrophage and lymphocyte responses. Microglia are immunocompetent but function differently from regular macrophage and dendritic cells. Thus, it may be more accurate to describe the CNS as a site of immune specialization.

Immune privilege or specialization of the CNS reflects the difference of initiating adaptive immune responses in the CNS compared to the process in the peripheral immune system, as a result of the composition of the immune system in the CNS, which is different from that of the peripheral system. The CNS can mount a robust immune response that can be used for immunotherapy. But the relative lack of understanding of the immune system in the CNS presents a bigger challenge in designing immunotherapy for brain tumors than for other tumors.

Parallel to evidence of immune competence of the CNS is the existence of immunosuppressive mechanisms in the CNS. One of the immunosuppressive pathways that are the focus of research is the programmed death-1 (PD-1) pathway. PD-1 is a member of the B7 family. Upon binding of programmed death-ligand 1 (PD-L1), the activated pathway leads to loss of the T-cell effector function. Both human GBM [43] and tumor-infiltrating macrophages [44] express high levels of PD-L1, and cytotoxic T-cells infiltrating GBM express high levels of PD-1 [45].

The expression and functions of PD-1 and other B7 family members have not been extensively studied in brainstem tumors. Recently, we found that in a small group of samples, all DIPGs expressed B7-H3 at various levels [46].

Another immunosuppressive pathway involved in the immune response to brain tumors is the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) pathway. CTLA-4 is upregulated during CD8(+) T-cell activation and is a negative regulator of this process [47]. CTLA-4 is also expressed on CD4(+) T-cells, including CD4(+) CD25(+) [48] and CD4(+)Foxp3(+) [49] regulatory T-cells (Tregs), and enhances Treg-mediated immunosuppression [48, 49]. CTLA-4 inhibits the activation and proliferation of effector T-cells in GBM [50]. The interaction of CTLA-4 with B7 on dendritic cells induces expression of indoleamine 2,3-dioxygenase 1 (IDO1) [51], another major immunosuppressive pathway involved in the immune response to brain tumors.



IDO1 is a tryptophan catabolic enzyme that converts tryptophan into kynurenines, a catabolite mediating the inhibition of effector T-cells and the induction of apoptosis in these cells [52]. It may also amplify immunosuppression by CD4(+)CD25(+)FoxP3(+) Tregs. IDO1 is expressed in over 90% of resected glioblastoma, with the upregulation correlating with a worse prognosis [53, 54]. In xenograft studies, malignant brain tumors deficient for IDO1 result in spontaneous rejection mediated by a T-cell-dependent mechanism [54], which implies that tumor-derived IDO1 is essential for Treg accumulation and immunosuppression.

A recent study characterized the immune microenvironment of DIPG [55] and found that these tumors do not have increased macrophage or T-cell infiltration compared to nontumor controls, nor do they overexpress immunosuppressive factors such as PD-L1 and/or transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1). H3.3-K27M DIPG cells do not repolarize macrophages but are ineffectively targeted by activated allogeneic T-cells. All DIPG cell cultures in the study could be lysed by natural killer (NK) cells. The results provide insights for the development of immunotherapy in the recruitment, activation and retention of tumor-specific effector cells.

### 15.3 Molecular Characteristics of Malignant Brainstem Tumors

DIPGs are genetically complex and distinct from both adult and childhood supratentorial HGGs. Recent evidence points to PDGF and its receptor PDGFR as among the major driving forces of tumorigenesis in the majority of cases [56–60]. Another growth factor receptor, EGFR, shows strong immunohistochemistry staining in about 27% of cases [57] and amplification of the gene at a rate of 7–9% [57, 59]. Approximately 50% of DIPGs have *TP53* mutations [61, 62], and three groups report loss of a region of 17p containing the *TP53* gene in 31%, 57% and 64% of cases, respectively [57, 63, 64]. In approximately 50% of DIPG patients, allelic loss of a region of 10q, where the phosphatase and tensin homolog (*PTEN*) gene is located, is observed [63, 65, 66].

Another commonly mutated gene in DIPG is the gene for activin A receptor, type I (ACVR1), which transduces signals of the bone morphogenic proteins (BMPs). The mutation occurs in approximately 20–32% of DIPGs [67–69]. ACVR1 mutations result in ligand-independent constitutive activation of the BMP signaling pathway [70–72]. Seven different ACVR1 mutations have been reported in DIPG, and they have been shown to increase the levels of phosphorylated SMAD1/5 [67–69, 73] as well as increased gene expression of the downstream BMP signaling targets, ID1 and ID2 [67].

Alpha thalassemia/mental retardation syndrome X-linked (ATR<sub>X</sub>) gene was found to be mutated in ~ 9% of DIPGs and predominantly in older children [74]. ATR<sub>X</sub> encodes a subunit of a chromatin remodeling complex required for histone H3.3 incorporation at telomeric regions.

Unlike the case in childhood supratentorial HGG, *CDKN2A* deletion is non-existent [59, 64] or only occurs at a low rate (3%) [58] in DIPG. Amplification of *CDK4* and *CDK6* in DIPG occurs at a rate of 7% and 11.6%, respectively [59].

Histone H3, which forms part of the nucleosome core, plays an essential role in the epigenetic regulation of deoxyribonucleic acid (DNA) replication and gene transcription. Recent studies of histone mutations indicate that DIPGs are also epigenetically distinct from pediatric supratentorial HGGs. Recurrent adenine-to-thymine transversions in the *H3F3A* gene, encoding a K27M mutation of histone H3.3, is seen in 60–75% of DIPGs [74, 75], significantly higher than that in pediatric supratentorial GBM (14–19%) [75, 76]. The *H3F3A* mutation is not present in the matching germline DNA samples [75], suggesting its somatic nature. The K27M mutation is found in 66–77% of pretreatment DIPG samples [74, 75], indicating that it is not the result of a selection or mutation process secondary to therapies.

In contrast to the H3.3-K27M mutation profiles, a guanine-to-adenine transition in *H3F3A*, resulting in a glycine-to-arginine missense at position 34 (G34R) of H3.3, is identified in 10–14% of pediatric supratentorial GBM [75, 76] but not in any of the 90 DIPG samples analyzed by two groups [74, 75].

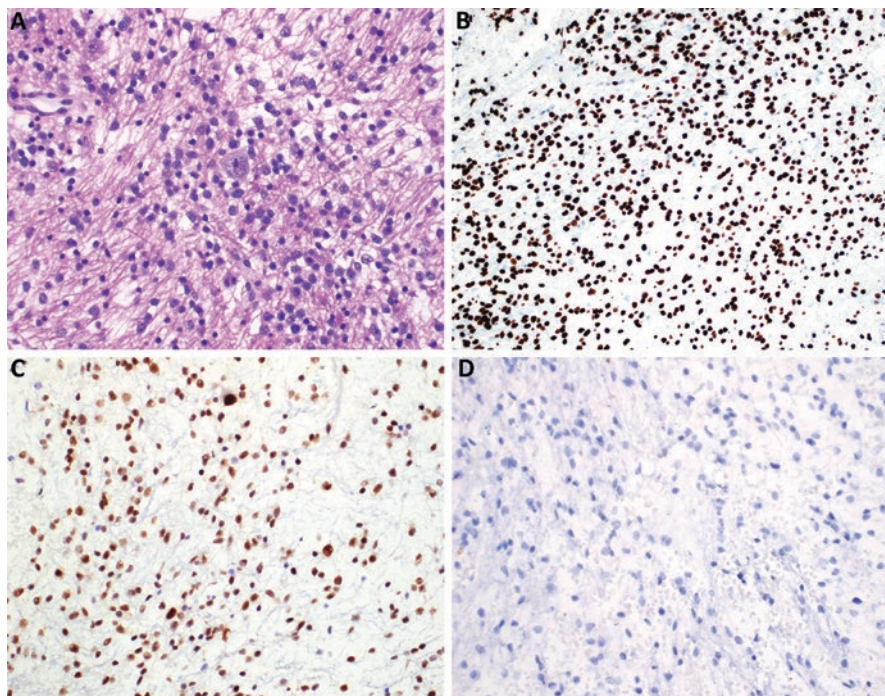
The presence of mutations in the *HIST1H3B* gene, which encodes histone H3.1, is less conclusive. One study found that the adenine-to-thymine transversion that encodes the K27M mutation was present in 18% (9/50) of DIPGs [75], whereas another group did not detect the mutation in any of their DIPG samples (0/27) [74].

The H3.3-K27M mutation is associated with poorer prognosis in DIPG patients [74, 77]. The significance of this mutation has led to the new category (diffuse midline glioma, H3 K27M-mutant) in the 2016 version of the WHO classification of CNS tumors [78].

Some of the described mutations of DIPG are demonstrated in Fig. 15.2.

## 15.4 Drug Delivery

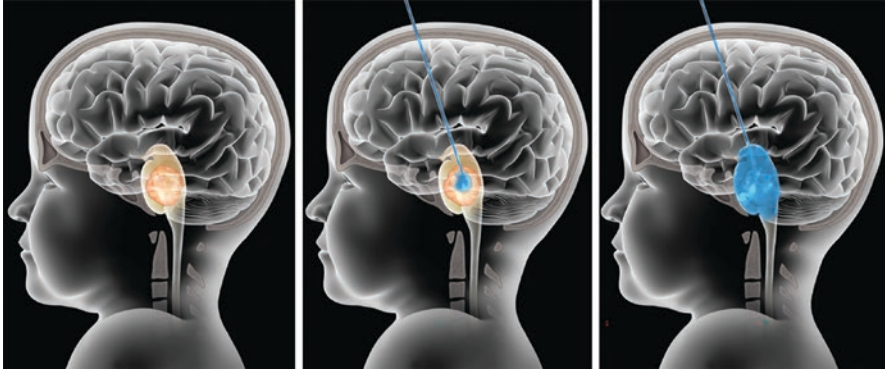
A number of strategies have been explored to address the BBB as a major obstacle in brainstem tumor treatment. In general, these strategies can be summarized into three categories: (1) bypassing the BBB via local delivery such as convection-enhanced delivery (CED); (2) opening the BBB using physical or chemical methods (paracellular approaches); and (3) delivery across the BBB (transcellular approaches).



**Fig. 15.2** Histology and immunohistochemistry staining of a DIPG sample. (a) H&E stain demonstrating a hypercellular, infiltrating astrocytoma with a non-neoplastic entrapped pontine neuron at center. (b) Immunohistochemical staining for H3 K27M showing positive labeling in neoplastic nuclei, confirming K27M mutation. (c) Immunohistochemical staining for ATRX demonstrating preserved expression, correlating with an absence of mutation. (d) Immunohistochemical staining for p53 demonstrating a complete absence of staining, correlating with a truncating mutation

### ***15.4.1 Convection-Enhanced Delivery***

CED is a drug delivery method first developed in the early 1990s [79]. In this method, a drug-containing solution is distributed into the interstitial space driven by a small, persistent hydraulic pressure (i.e., forced convection). In contrast to diffusion that depends on a concentration gradient to distribute the molecules, the use of hydraulic pressure in CED allows for a homogeneous distribution of small and large molecules over large distances by displacing the interstitial fluid with the infusate. In practice, the agent is delivered into the parenchyma or tumor driven by a pump through a microcatheter, or multiple microcatheters, inserted into the tissue. Infusion rates typically range from 0.1 to 10  $\mu\text{l}/\text{min}$  for application in the brainstem, and higher infusion rates are being explored (Fig. 15.3).



**Fig. 15.3** Convection-enhanced delivery into a diffuse pontine lesion

In CED, the distribution from a single point source results in an elliptical to spherical distribution, and spatial distribution is to some degree dependent on the tissue type (i.e., gray versus white matter). In a given tissue type, the distribution volume is roughly linear to the infusion volume.

CED into brain parenchyma, both white and gray matters, has shown reproducible large volumes of distribution with homogeneous drug concentration. Early work showed that the concentration fall-off at the border is steep [79], resulting in a potentially large benefit in cancer drug delivery whenever reducing toxicity to surrounding normal brain tissue is desired.

The volume of distribution can be affected by the retrograde movement of fluid along the outside of the catheter (backflow or reflux). Reflux is determined by catheter material, catheter diameter, infusion rate, and tissue density, among other factors. The larger the catheter diameter, the greater is the chance of backflow along its outer wall. If reflux reaches a low-pressure zone (necrosis or CSF space), the fluid will inadvertently be lost into these spaces; this leads to the accumulation of drug in these regions, which may cause toxicity. Increasing the infusion rate can increase the overall volume of distribution; however, this will also increase the chance of reflux, potentially shunting fluid away from the target region.

Ideally, agents delivered via CED should be contained within the target region of brain parenchyma or tumor mass. However, there are low-pressure regions in some tumors along which the infusate will flow, sometimes into the ventricles or subarachnoid space. This phenomenon is usually referred to as leakage and has often been observed in both humans and experimental animals. One study indicates that this can happen in 20% of CED procedures [80]. This obvious waste of therapeutic agent will consequently reduce the volume of distribution and drug concentration in the planned target region. It may also cause untoward effects on normal brain tissue. It is, therefore, critical to follow the flow of infused agents. When leakage happens, it might be helpful to adjust the catheter placement to move the opening away from the low-pressure region. It is unknown yet whether this leakage is reversible. If reversible, pausing the infusion for a period of time and subsequently restarting the infusion could eliminate the leakage.

Monitoring the distribution and concentration of an infused drug is critical for numerous reasons. In addition to its biological effectiveness, a drug would need to be distributed within the tumor in therapeutic concentrations to be effective. Exposure of normal tissue to the drug should be controlled to reduce the probability of toxicity. It is also highly desirable to monitor for possible reflux and leakage so that the cannula placement can be adjusted to correct any problems that may arise. In the brainstem, the transverse and longitudinal fiber bundles may direct the infusate flow, which also needs to be monitored. The importance of monitoring *in vivo* distribution and concentration is highlighted by the difficulty in achieving optimal therapeutic efficacy in recent clinical trials. In the recent TGF $\alpha$ -PE38 study and the phase III PRECISE trial for glioblastoma, poor drug distribution was cited as one of the reasons for the unsatisfactory efficacy results [81, 82].

Monitoring the distribution and concentration of the CED infusate in humans is difficult due to the fact that the majority of therapeutic agents cannot be seen on any of the clinical imaging methods. Nevertheless, the distribution can be visualized under certain circumstances. T2-weighted MR images are helpful in identifying the infusate distribution in regions of relatively normal intensity, but identifying the distribution is more difficult when infused into already hyperintense regions, such as in DIPG [83]. Another choice is to use surrogate tracers. Gadolinium-diethylenetriamine penta-acetic acid (Gd-DTPA) and  $^{123}\text{I}$ -albumin have been co-infused as surrogate tracers, viewable on T1-weighted and single photon emission computed tomography (SPECT) images, respectively, in clinical studies [81, 83–86]. The shortcomings of surrogate markers are that they are only able to accurately estimate the initial distribution. Differences in biological activities and clearance confound their ability to follow the distribution of the therapeutic agent over time. Moreover, neither T2-weighted signals nor surrogate tracers are able to provide information on the concentration of the infused therapeutic agent. The ideal scenario would be to directly image the therapeutic compound. With calibration, the concentration as well as the distribution of the drug can be determined.

The concept of using CED for DIPG treatment is appealing given that this particular tumor is relatively compact, has growth patterns simulating white matter tracts, seldom metastasizes before local relapse occurs, and no surgical resection is performed. Our group first established the feasibility of this delivery route in the brainstem in small animals for potential clinical application in 2002 [87]. Subsequently, the safety of inert agents, characteristics of distribution, and toxicity of potential therapeutic agents in the brainstem of small animals and non-human primates have been studied [88–93]. These studies showed that CED does not cause clinically relevant mechanical injury to the brainstem, and this approach has a promising therapeutic application in humans. In clinical practice, image-guided frameless stereotaxy can be utilized to target the brainstem in children for biopsy or cannula insertion with high accuracy and low risks of temporary or permanent morbidity [94–96], providing supportive evidence of the safety of applying CED in the brainstem.

A few groups in the United States and Europe are pursuing clinical studies of brainstem CED to treat DIPG [86, 97–99]. These small series reported reasonably good safety and tolerance. In a clinical trial to treat DIPG patients with IL13-PE38QQR CED in the brainstem (NCT00880061), all five patients tolerated the therapy well, and two patients showed temporary signs of anti-tumor effects on MRI [100]. The most comprehensive results of brainstem CED in DIPG patients have been reported by our group [101]. In this interim report of results of the phase I clinical trial (NCT01502917), 28 patients had been treated without any dose-limiting toxicities. Utilizing a theranostic agent ( $^{124}\text{I}$ -labelled 8H9 a.k.a. omburtamab), this study also reported that the radiation absorbed dose to the lesion was 1200-fold that of the systemic exposure. These results directly demonstrated the safety of CED in the human brainstem, as well as validated the principle that CED efficiently delivers therapeutics to the target with minimal systemic exposure.

Other ongoing CED clinical trials recruiting exclusively DIPG patients include NCT03086616 (CED of an irinotecan liposome formulation) and NCT03566199 (CED of a panobinostat nanoparticle formulation).

Future advances in CED for DIPG treatment will occur on a few fronts: (1) the selection or development of therapeutic agents for DIPG; this will depend on the better understanding of the disease biology and development of novel therapeutic strategies. (2) The improvement of the technique of CED; this includes a new design of the devices to facilitate easier and accurate deployment of the cannula, implementation of prolonged and repeated infusions lasting up to weeks for optimal time sequence of therapy, and better understanding of the infusate distribution and its influencing factors so that catheters can be placed to achieve optimal tumor coverage. It is also desirable to have devices that can be embedded to allow patients to remain ambulatory while undergoing continuous or multiple sessions of CED.

Perhaps more important is the need to evaluate the pharmacokinetics and regional therapeutic response in CED. Results from systemic pharmacokinetic and pharmacodynamic studies cannot be directly applied to CED applications of the same therapeutic agents. To evaluate pharmacokinetics and pharmacodynamics, imaging should accompany the therapy to ensure effective drug distribution and concentration as well as to determine the retention and clearance of therapeutic agents in the tumor and tumor-infiltrated brain tissue in individual patients whenever possible. This would require the improvement of current imaging techniques or the development of new imaging agents and methods, such as contrast agents that respond to effector molecules or end products of apoptosis. Such techniques as microdialysis may also have a place in the pharmacokinetic evaluations of CED. For evaluating the regional therapeutic response, an innovative noninvasive method is in desperate need, as the current methods of radiological evaluation are not sufficient. At this stage where CED is used as an investigational therapeutic platform, the pharmacokinetic and pharmacodynamic evaluation would allow for determination of the effectiveness of CED-based therapies. In the future, treatment strategies would be able to be dynamically adjusted based on the regional response, incorporating multiple modalities of therapeutic options.

### ***15.4.2 Intraarterial Delivery***

Intraarterial chemotherapy is now widely used for the treatment of retinoblastoma and advanced liver cancer, improving the quality of life and extending overall survival. In the case of retinoblastoma, super-selective intraarterial chemotherapy can produce high cure rates. Beyond these two well-established applications, intraarterial chemotherapy has been also used, with variable success, in the treatment of other cancers such as breast cancer, head and neck cancer, colorectal cancer, penile cancer, and pancreatic cancer.

Intraarterial therapy for malignant brain tumors, especially HGGs, has been administered since 1950s. Most of these attempts rely on the general belief that transiently generating high arterial blood concentrations would lead to the desired pharmacodynamic effects. However, in these early attempts, no objective comparisons with intravenous administration were performed. The advantages of intraarterial delivery were first demonstrated when intraarterial infusion led to a higher tissue concentration compared to non-targeted tissue [102]. In the 1970s, osmotic BBB disruption was studied to improve intraarterial delivery [103, 104]. With the miniaturization of catheters and other endovascular devices, selective and super-selective intraarterial delivery was developed and studied in the treatment of brain tumors since 1990s. They allow for accurate and super-selective targeting of the tumor's supplying vessels, compared to early intraarterial attempts of carotid or vertebral artery infusion.

A critical factor in intraarterial delivery is the BBB. Studies have shown that the BBB can be reversibly modified to be more permeable by hyperosmotic solutions such as mannitol [103]. Other methods of BBB disruption include vasoactive agents, such as bradykinin analogs, and focused ultrasound (FUS). Concurrent flow arrest appears to enhance the regional effectiveness of intraarterial delivery by achieving higher arterial concentrations, as well as more consistent concentrations in the arterial distribution, and increased transit time. Without flow arrest, for effective intraarterial delivery, drugs must be rapidly taken up during their first pass through the tissue circulation, lasting between 1 and 10 seconds in the brain. As another way of improving BBB penetration, intraarterial delivery can be combined with the transcellular approach of delivery using BBB-penetrating delivery vehicles such as cell-penetrating peptides. There is some evidence that intraarterial delivery of cell-penetrating peptides can penetrate the BBB, and preliminary results suggest that they can lead to tumor-specific drug uptake [105].

There are a number of clinical trials of intraarterial therapy for DIPG patients (NCT01688401) or for pediatric brain tumor patients, including DIPG (NCT01884740). Results have not been reported yet.

### 15.4.3 *Manipulating the Blood-Brain Barrier*

The BBB can be opened by hyperosmolar solutions (e.g., mannitol) or vasoactive drugs (e.g., bradykinin and adenosine) for drug delivery. The BBB, when opened this way, remains open for only a short period of time, and the procedure may need to be repeated in drug delivery sessions lasting longer than several minutes. Non-selective opening of the BBB exposes large volumes of normal brain to undesirable substances that may be toxic.

Site-specific disruption of the BBB represents an improvement to non-selective BBB disruption for drug delivery into the brain. This has been accomplished using either FUS [106] or laser-based approaches such as photodynamic therapy (PDT) [107] and photochemical internalization (PCI) [108]. These techniques have a number of advantages over non-selective BBB disruption. The site of BBB disruption is the only site receiving sufficient ultrasound or laser intensity. With imaging guidance, and through careful placement of the probes and adjustment of parameters, the site of BBB disruption can match the lesion with maximum coverage while the normal brain is minimally affected. In addition, these highly focused approaches do not cause permanent damage to the BBB, as long as the ultrasound or laser intensity remain below threshold levels. With these site-specific approaches, the BBB may remain open for relatively long periods of time, thus facilitating longer drug delivery.

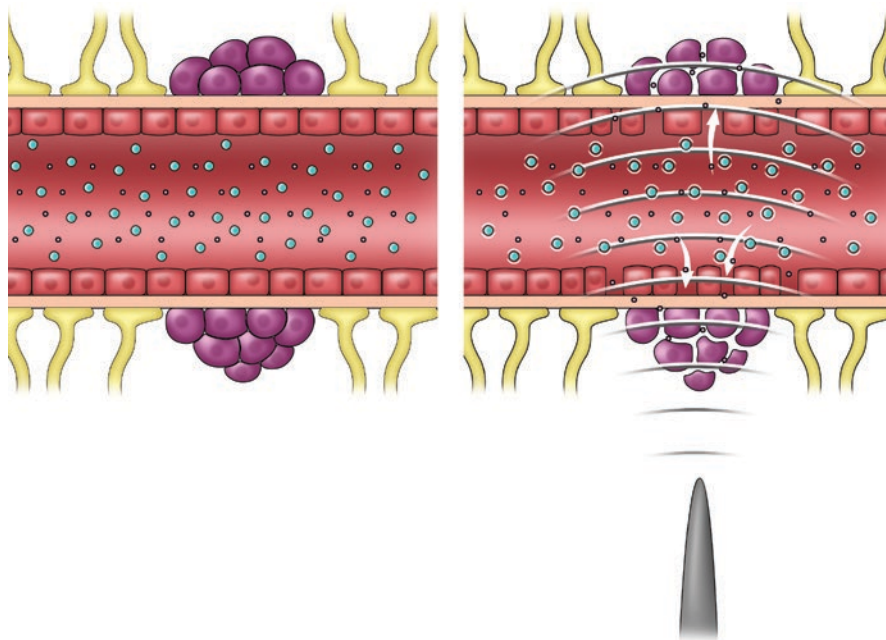
#### 15.4.3.1 **Focused Ultrasound**

FUS, which can be aimed at a spot of just a few mm in diameter, is capable of achieving selective disruption of the BBB. In this direct approach, it is difficult to identify parameters producing reliable opening of the BBB without damage to normal brain. Albumin-coated microbubbles were introduced to address this concern [109]. The microbubbles, when injected intravenously, confine the ultrasound effects to the walls of blood vessels resulting in BBB disruption with minimal damage to the surrounding brain tissue [110]. This has allowed for selective disruption of the BBB at much lower acoustic power levels than previously employed [111].

The exact mechanisms of BBB disruption by microbubble-enhanced FUS remain to be elucidated. The effect is likely due to a combination of cavitation and acoustic radiation forces [106]. Cavitation is the acoustically-induced activities of microscopic bubbles within the medium. The generation of microbubbles requires high acoustic power densities, which may result in tissue damage [112]. With the introduction of albumin-coated microbubbles, high powers are no longer required and, therefore, the risk of tissue damage has significantly decreased (Fig. 15.4).

The BBB opening by FUS is relatively short, ranging from 10 minutes to 5 hours following the sonication [106]. This may be sufficient for a single dose administration of drugs, but for prolonged drug administration, repeated sonication may be required, which can limit its feasibility.





**Fig. 15.4** Focused ultrasound for opening the BBB. (Left) Albumin-coated microbubbles are injected into the blood stream before focused ultrasound is applied. (Right) Upon application of focused ultrasound, the oscillation of the microbubbles helps open the BBB

Animal studies suggest that FUS-induced BBB disruption does not result in permanent damage to the brain as evidenced by the lack of ischemic or apoptotic changes [111]. The minor effects observed, such as small extravasations and mild inflammatory reactions, do not appear to affect the neurons up to four weeks following the sonication [111].

A major limitation of transcranial FUS is that the skull is highly absorbing of ultrasound. This causes strong attenuation and phase distortion [113]. At high powers, FUS also causes heating during the sonication.

The safety of FUS in opening the BBB is being investigated in a clinical trial (NCT02343991) in brain tumor patients.

#### 15.4.3.2 Photodynamic Therapy

PDT is the use of a tumor-localizing photosensitizer that is subsequently activated by an excitation light [114]. The photochemical and photobiological events would induce therapeutic effects.

PDT using either hematoporphyrin derivatives or 5-aminolevulinic acid (ALA) has been reported to induce brain edema surrounding the site of phototherapy [115–117], suggesting a local breakdown of the BBB.

ALA-PDT-mediated disruption of the BBB was found to be apparent as early as 2 hours following PDT, and the BBB was approximately 90% restored within 72 hours [107]. This is longer than the FUS-induced BBB opening.

The mechanisms of PDT-mediated BBB opening likely include rounding and contraction of endothelial cells mediated by PDT-induced microtubule depolarization [118]. The formation and enlargement of endothelial gaps have been observed in PDT [119]. Electron microscopy studies demonstrated that the treatment had minimal impact on the normal subcellular structures of endothelial cells [120], suggesting there were no permanent damages.

#### 15.4.3.3 Photochemical Internalization

PCI is the use of specially designed photosensitizers that localize preferentially in the membranes of endocytic vesicles. Upon light activation, the photosensitizer disrupts the vesicular membrane, releasing encapsulated macromolecules into the cell cytosol. This can be used to enhance the delivery of macromolecules in a site-specific manner [121].

PCI-delivered *Clostridium perfringens* epsilon prototoxin (ETXp) was used for localized BBB opening [108], because the active toxin (ETX) can cause widespread but reversible opening of the BBB [122–124]. Following administration, ETXp is converted to ETX by proteolytic cleavage. Disruption of the BBB was accomplished by combining sub-threshold doses of ETXp with sub-threshold light fluences. The membrane-localizing photosensitizer used was aluminum phthalocyanine disulfonate (AlPcS<sub>2a</sub>). The results show that ETXp-PCI is capable of causing localized BBB disruption at very low light fluences, and no significant damage was noted in rat brains at these conditions. In comparison, the BBB remained relatively intact when exposed to AlPcS<sub>2a</sub> without ETXp at these light levels. At higher fluences, the PDT effect was so pronounced that without ETXp, necrosis and inflammation were already evident, and the addition of ETXp had no apparent effect on BBB disruption.

#### 15.4.4 Inhibition of ATP-Binding Cassette Transporters

One mechanism that is responsible for multidrug resistance (MDR) is the active efflux of drugs by ABC transporters. Several agents have been developed to block ABC transporter-mediated drug efflux, and some have entered phase II/III clinical trials.

The first-generation inhibitors included drugs developed for other conditions such as verapamil, quinine, and cyclosporine A. Despite their efficacy in inhibiting P-gp1-dependent drug efflux *in vitro* [125], these inhibitors lack specificity and cause significant toxicity when used as ABC transporter inhibitors [126].

The second-generation P-gp1 inhibitors were designed to improve specificity. Valspodar is a derivative of cyclosporine A, with higher specificity and potency and no immunosuppressive effects [127]. However, it inhibits cytochromes P450 (CYP450s) [128] and causes pharmacokinetic effects. As a result, it failed to improve outcomes; it even produced worse outcomes in phase III clinical trials with the anticancer drugs vincristine and doxorubicin [129], or daunorubicin and etoposide [130] in patients with acute myeloid leukemia (AML).

The third-generation inhibitors were developed focusing on not to inhibit liver enzymes such as CYP450s. P-gp1 specificity was another focus. Tariquidar (an anthranilamide), elacridar (an acridone caroxamide), zosuquidar (quinolone derivative), CBT-1 (quinolone derivative) and laniquidar (a piperidine) are at various stages of clinical trials. Tariquidar and zosuquidar entered Phase II/III trials in combination with vinorelbine and doxorubicin, respectively, for a variety of advanced solid tumors or AML. Phase III trials of tariquidar in non-small cell lung cancer produced high rates of side effects without improving the patient response. Zosuquidar has also shown neurotoxicity [131] and drug-drug interactions with doxorubicin and vinorelbine [132].

These examples show that the strategies of inhibiting ABC transporters need further improvements. The way to these inhibitors' application to brainstem tumor therapies may take even longer than their use in non-CNS tumors. However, optimism is warranted with further improvement of such agents' specificity, strategies of more specific targeting of drugs beyond the drug's structure, and better selection of patients with improved diagnostic techniques.

### ***15.4.5 Carriers and Packaging Vehicles***

Several approaches for drug delivery across the BBB have been attempted, including encapsulation into liposomes and nanoparticles. By incorporation into liposomes or nanoparticles, the drugs are stabilized for more efficient direct delivery or paracellular delivery. The incorporation can also include targeting moieties, such as proteins (e.g., insulin, Apolipoprotein E, and transferrin) that are known to traverse the BBB by receptor-mediated endocytosis, making the formulation suitable for transcellular delivery. This molecular Trojan horse approach has been successful in delivering a number of therapeutic proteins. However, limitations still exist, including rapid removal from the circulation, low delivery yields, and the need for repeated injections.

#### **15.4.5.1 Liposomes**

Liposomes are small vesicles consisting of one or more lipid bilayers surrounding an aqueous compartment. They are nanometers-to-micrometers in diameter. They were discovered in early 1960s; the exploration of their potential use as a carrier

system for therapeutically active compounds began soon after that. In recent years, their application has been explored for the diagnosis and/or treatment of neurological diseases in particular. Due to the unique physicochemical characteristics of lipid bilayers, liposomes are able to incorporate hydrophilic, lipophilic, and hydrophobic therapeutic agents. Hydrophilic compounds may either be entrapped into the aqueous core of the liposomes or be located at the interface between the lipid bilayer and the external water phase. Lipophilic or hydrophobic drugs are generally entrapped almost completely in the hydrophobic core of the lipid bilayers. The use of cationic lipids further allows the adsorption of polyanions, such as DNA and ribonucleic acid (RNA). The surface of liposomes can be modified by the inclusion of other macromolecules, such as polysaccharides, peptides, antibodies, or aptamers, to improve its stability in blood circulation and brain-specific delivery. Several liposomal drugs are either approved for clinical use or in clinical trial studies [133, 134], but efficient brain-specific drug delivery by liposomes is not at the clinical stage yet.

Liposomes can be conjugated with specific antibodies or ligands to enhance their ability to cross the BBB through receptor-mediated endocytosis by the BBB cells. Several studies using transferrin (Tf), lactoferrin, insulin, glutathione, apolipoproteins and peptides reported successful delivery of liposomes to the brain parenchyma or tumor [135–137]. In one of the studies, it was also shown that Tf-conjugated liposomes were taken up by BBB cells more than unconjugated liposomes and were subjected to transcytosis [137].

Recently, magnetic liposomes have emerged as an interesting targeting moiety for delivery of therapeutic molecules across the BBB. In one study, one or more drug molecules could be reversibly bound to the surface of iron oxide nanoparticles and encapsulated within the core of liposomes [138]. When an external magnetic field was applied, the liposomes bypassed an *in vitro* model of the BBB. It has further been shown that magnetic liposomes can also be taken up into human monocytes, followed by the entry of nonmagnetic monocytes into the brain [138].

Various routes of administration have been tested for delivery using liposomes to the brain. Intravenous injection seems to be the preferred route in practice. Alternative routes of administration (oral, ocular, or mucosal) have also been explored. For example, intranasal administration is a noninvasive approach to deliver drugs to the brain. It was shown that a liposomal formulation of rivastigmine was able to prevent degradation of the drug in the nasal cavity and to carry it through the mucosal barriers [139]. The ability of cationic liposomes to deliver proteins to the brain via the intranasal route has also been demonstrated [140].

#### 15.4.5.2 Nanoparticles

Nanoparticles commonly refer to carriers with a size between 10 and 1000 nm. They can be made with a broad range of materials such as sugar derivatives, fatty acids, peptides and proteins.

Experimental evidence shows that nanoparticles enhance delivery across the BBB through mechanisms of passive (nonspecific endocytosis) or active targeting

(receptor-mediated endocytosis). In one study, the nanoparticles were mainly taken up via nonspecific endocytosis [141]. The size, chemical structure, and surface properties of nanoparticles are critical factors influencing their uptake by cells of the BBB. Nanoparticles smaller than 20 nm can pass through the BBB endothelial cells by transcytosis [142].

Surface features of nanoparticles, such as charge and coating, may be more important than their core structure in determining their ability to cross the BBB. Surface charge may determine the pathway through which nanoparticles are taken up. In one study [143], at 4 °C, when active endocytosis was stopped and only passive diffusion was present, cationic nanoparticles remained outside the BBB cells, neutral nanoparticles were associated with cell surface, and anionic nanoparticles were detected on cell surface and in paracellular space; at 37 °C, only neutral and anionic nanoparticles had undergone endocytosis and transcytosis. This study further showed that neutral and anionic nanoparticles followed the caveolae-mediated endocytotic pathway, whereas cationic nanoparticles did not.

The presence of noncovalently- or covalently-bound ligands on the surface of nanoparticles can further improve delivery across the BBB by receptor-mediated endocytosis [142, 144]. The most common receptors utilized for this purpose are the insulin receptor, the Tf receptor, the low-density lipoproteins receptor (LDLR), the LDLR-related proteins (LRPs), the folic acid receptor, and the diphtheria toxin receptor. The delivery of Tf-conjugated nanoparticles was more efficient than unconjugated ones within the CNS [145, 146] due to the abundance of Tf receptor in the BBB [142]. LDLR and LRPs trigger efficient receptor-driven endocytosis followed by transcytosis [147]. The major drawback of using Tf, folic acid or apolipoproteins as targeting ligands on nanoparticles is that their receptors are widespread; therefore, there is a risk of nanoparticle uptake by other tissues.

Current ongoing clinical trials of liposomes and nanoparticles recruiting exclusively DIPG patients include NCT03086616 (CED of an irinotecan liposome formulation) and NCT03566199 (CED of a panobinostat nanoparticle formulation).

## 15.5 Signal Transduction Pathway Targeted Therapy

As discussed in Sect. 15.3, a number of mutations have been discovered in DIPG. Some of the mutations observed in DIPG are targetable, such as the PDGFR pathway, while many others are not currently targetable yet. Targeting the RTK signal transduction pathways such as the PDGFR pathway has been a focus of some recent research. In GBM, therapy with single agents leads to clonal selection, enriching therapy-resistant clones that give rise to recurrent tumors [34]. Possibly and partially a result of this phenomenon, clinical trials with signal transduction pathway-targeted therapies failed to show significant improvement in survival in GBM, as well as in DIPG [8, 35]. In addition, RTKs downstream and parallel signal transduction pathways may be regulated in a compensatory fashion with redundancy that contributes to drug resistance, especially in single target therapies.

Therefore, it is not surprising that drug resistance has been inevitable in almost all single-drug targeted therapies. How to target multiple points in signal transduction pathways without causing much toxicity is the subject of active studies.

Ongoing clinical trials of signal transduction pathway targeted therapies recruiting DIPG patients include NCT03352427 (combination of dasatinib and everolimus), NCT01644773 (combination of crizotinib and dasatinib), NCT02420613 (combination of vorinostat and temsirolimus) and NCT03632317 (combination of panobinostat and everolimus).

As long as a molecule is differentially expressed between tumor cells and normal tissue, it does not need to be growth-promoting to be considered a therapeutic target. One example is IL-13R $\alpha$ 2, whose functions are not well understood in brain tumors. Like in adult malignant gliomas, IL-13R $\alpha$ 2 is highly expressed in DIPG [148, 149]; therefore, recombinant toxins using IL-13 as a targeting moiety are also potentially effective therapeutic agents for DIPG. CED of IL13-PE38QQR in the brainstem has shown a good safety profile in both a preclinical study [88] and a clinical trial in DIPG patients (NCT00880061) [100].

## 15.6 Modulating Gene Expression Status: Epigenetic Modulators

The importance of epigenetic changes in pediatric brainstem gliomas has recently been recognized. Most notable is the histone methylation status associated with the H3K27M mutation. It is thought that inhibition of the histone methyltransferase Polycomb repressive complex 2 (PRC2) and hypomethylation of H3K27 play an important role in the effects of H3.3K27M mutation on the tumorigenesis of DIPG [150].

One approach of investigational therapy used GSKJ4, an H3K27 demethylase inhibitor, to counter the effects of hypomethylation of H3K27 caused by H3.3K27M mutation. GSKJ4 increased cellular H3K27 methylation in K27M tumor cells, reduced tumor size of K27M xenografts, and prolonged survival of mice bearing these xenografts [151].

Another approach focuses on enhancer of zeste homolog 2 (EZH2). EZH2 is part of PRC2 and, hence, has been considered as a potential therapeutic target. However, preclinical studies of inhibiting EZH2 using tazemetostat showed different results from two research groups. In one study, tazemetostat did not show activity in pediatric glioma cells *in vitro* with or without H3.3 mutations [152]. In another, results showed that tazemetostat may affect growth of primary H3K27M-positive glioma cells in the presence of a functional p16INK4A [153]. Genetic studies showed that short-term EZH2 depletion in glioblastoma cells without H3 or isocitrate dehydrogenase (IDH) mutations has been associated with reduced proliferation [154], but prolonged EZH2 depletion caused a switch in cell fate, enhancing proliferation and DNA damage repair and resulting in tumor progression [155].

Other studies show that activation of PRC2 and hypermethylation of H3K27 may be driving the initiation of medulloblastoma [156], ependymoma [157], and lymphoma [158]. These pieces of evidence suggest that rebalancing the H3K27 methylation pathway for therapeutic purposes may not be an easy task.

Histone acetylation is another epigenetic target in cancer therapy. The rationale of using histone deacetylase inhibitors (HDACi) in cancer therapy is to reverse dysregulated gene expression by modulating histone acetylation. Acetylation of lysine residue on histones is a mark of active enhancers that control the expression of associated distal genes. HDAC inhibition causes hyperacetylation of histones and affects the expression of a large number of genes.

Vorinostat has been tested in clinical trials of recurrent glioblastoma. As a single agent, it showed good tolerability in glioblastoma patients and induced increased histone acetylation in the tumors [159]. However, when tested in combination with the protease inhibitor bortezomib, vorinostat did not show efficacy [160]. In newly diagnosed glioblastoma, the addition of vorinostat to the standard regimen of temozolomide and radiation therapy did not meet the primary endpoint of efficacy in a phase I/II trial [161]. An ongoing study is evaluating the safety of vorinostat in combination with temsirolimus in DIPG patients (NCT02420613).

Panobinostat, another HDAC inhibitor, reduced the viability of cultured DIPG cells, reduced tumor size of DIPG orthotopic xenografts, and prolonged the survival of mice bearing these xenografts [162]. Combination testing with GSKJ4 showed that they had synergistic effects [162]. Clinically, two patients with progressive DIPG tolerated concomitant panobinostat and reirradiation well [163]. An ongoing phase I clinical study is more systematically evaluating the safety and pharmacokinetics of panobinostat in both recurrent/progressive and non-progressed DIPG patients (NCT02717455). Other clinical trials of panobinostat with DIPG as an eligible disease include NCT03632317 (panobinostat in combination with everolimus). MTX110, a nanoparticle formulation of panobinostat, is being tested in treating DIPG patients via brainstem CED (NCT03566199).

## 15.7 Immunotherapy

### 15.7.1 *Therapeutic Antibodies, Radiolabeled Antibodies, and Immunotoxins*

To some degree, the main underlying assumption of using antibodies to treat malignant tumors is the specific recognition and elimination of malignant cells by antibodies. Several types of therapeutic antibodies have been developed to treat malignant tumors, including HGGs, where antibodies targeting growth factor receptors were among the most actively investigated recently.

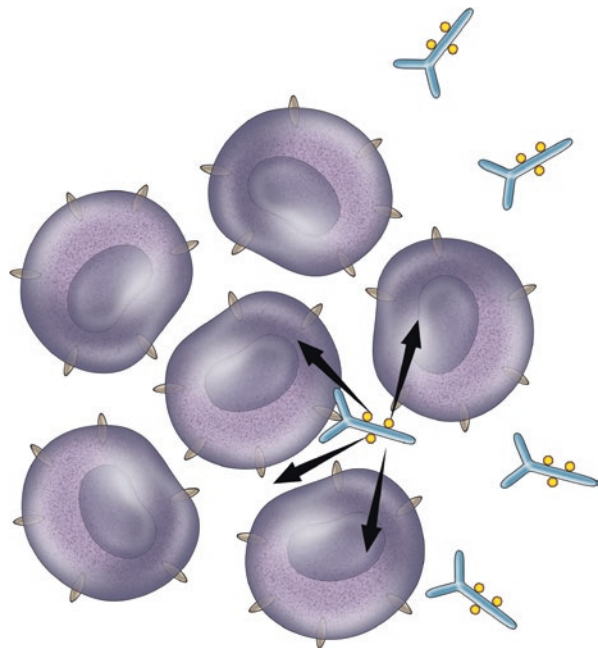
Growth factors have been shown to support tumor initiation and progression in malignant gliomas, including DIPG. Recent evidence showed that PDGF, along

with its receptor, PDGFR, is one of the most commonly involved oncogenic signal transduction pathways in the majority of DIPG cases [56–60]. Another growth factor receptor, EGFR, is also involved in a significant numbers of cases [57, 59].

Therapeutic antibodies against these growth factor receptors block activation of the receptor and have the potential to cause cell death. Additionally, binding of antibodies to cell surface antigens can induce antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), even though effector cells of ADCC and CDC are not abundant in the CNS. Antibodies against PDGFRs, such as olaratumab (IMC-3G3) and MEDI-575, have been proposed to treat DIPG. Currently, there are a few clinical trials of olaratumab (e.g., NCT02677116) on treating pediatric solid tumors via intravenous administration, with DIPG as an eligible disease. No results have been published from these trials yet. Due to the large molecular weight of the antibodies, we believe that CED would be an ideal delivery option of therapeutic antibodies in treating DIPG.

Therapeutic antibodies can be labeled with radionuclides to strengthen their therapeutic capability. In addition to the effects of the antibody itself, the therapeutic effects of radiolabeled antibodies are achieved by the energy deposited into the tissue by the radiation from the radionuclide that is tagged to the antibody (Fig. 15.5). The choice of a radionuclide for a specific application depends on the disease to be treated, physical characteristics of the nuclide, its commercial availability, and the labeling chemistry. Radionuclides used in oncologic therapy are typically Beta ( $\beta$ )- and alpha ( $\alpha$ )-emitters.  $\beta$  emissions from radionuclides such as  $^{131}\text{I}$ ,  $^{90}\text{Y}$ , and  $^{186}\text{Re}$  have a particle range of 2–12 mm in soft tissue; therefore, nearby tissue is impacted

**Fig. 15.5** Radiolabeled antibodies. In addition to the therapeutic effects of antibodies, emissions from radioisotopes on radiolabeled antibodies also have therapeutic effects. In the case of  $\alpha$  and  $\beta$  emissions, their ranges reach neighboring cells (“shoot the neighbor” effects)





along with the cells that the radiolabeled antibody binds to, which is suitable for the treatment of bulky tumors.  $\alpha$  emissions from radionuclides such as  $^{212}\text{Bi}$  and  $^{225}\text{Ac}$  have a particle range of tens of microns in soft tissue and can reach a few layers of cells surrounding the cells to which the radiolabeled antibodies are bound. The Auger and conversion electron emitters are also used for therapeutic purposes. Their particle range in soft tissue is approximately 1 micron, resulting in highly localized targeting without impacting adjacent cells, which is suitable for single cell situations as in certain micrometastatic diseases, minimal residual diseases, and blood malignancies.

The use of radiolabeled antibodies in the treatment of DIPG has been explored. Currently, there is a clinical trial (NCT01502917) of delivering  $^{124}\text{I}$ -labeled 8H9 (omburtamab) via CED to treat children with DIPG.  $^{124}\text{I}$  allows for the quantification of radiation dose by positron emission tomography (PET) imaging. The results showed that the drug as well as the delivery are safe. Uniquely, the quantification allowed for validating the principle that CED efficiently delivers therapeutics to the target with minimal systemic exposure by showing that the radiation absorbed dose to the lesion was 1200-fold that of the systemic exposure [101].

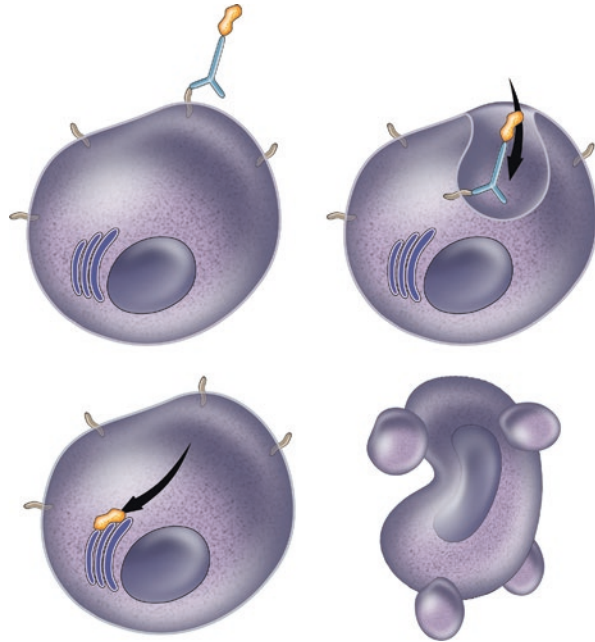
Immunotoxins are chimeric or recombinant molecules that contain a toxin linked to an antibody that binds specifically to its targets. Sometimes, growth factor or cytokine toxin fusions or conjugates are also considered immunotoxins, as they bind to target cells and contain a toxin that kills cells similar to classical immunotoxins [164].

In the early history of immunotoxin development, full-length antibodies were coupled with plant toxins like ricin or gelonin without the toxin's binding domain. Subsequently, bacterial protein toxins such as the *Pseudomonas* exotoxin (PE) and diphtheria toxin (DT) were used. The first-generation immunotoxins were made of full-length PE attached to whole monoclonal antibodies. These immunotoxins could bind to normal cells due to the existence of the toxin's binding domain. In second-generation immunotoxins, regions of the toxin that were not essential for cytotoxicity were removed to produce a truncated toxin that could not bind to normal cells, then the toxin was linked to an antibody. Second-generation immunotoxins such as PE38-based immunotoxins are more specific than first-generation immunotoxins.

Similarly, chemical drugs can be conjugated to antibodies forming antibody-drug conjugates (ADC). ADCs, like immunotoxins, take advantage of the antibody's specificity to improve targeting cells and reduce the drug's toxicity. While each specific immunotoxin or ADC has its specific mechanism of action, the general pathway is that upon binding to the antigens on the cell membrane, the immunotoxin or ADC molecules undergo internalization via receptor-mediated endocytosis and are then released into the cytosol to exert their toxicity on target organelles (Fig. 15.6). Some immunotoxins and ADCs may also undergo enzymatic conversion during the internalization and release process.

The potential clinical application of immunotoxins 8H9scFv-PE38 and IL13-PE38QQR in the treatment of DIPG has been investigated. In an animal study, 8H9scFv-PE38 was well tolerated and significantly shrank the tumor xenograft when delivered via CED [91]. Similarly, IL13-PE38QQR showed good safety and

**Fig. 15.6** Immunotoxins and antibody-drug conjugates (ADCs). Binding of immunotoxins and ADCs onto membrane receptors triggers receptor-mediated endocytosis. Internalized immunotoxins and ADCs reach end organelles (such as the rough endoplasmic reticulum) to exert their cytotoxic effects



efficacy in a xenograft DIPG model [88] and clinical safety in a phase I clinical trial (NCT00880061) [100], using CED to deliver the immunotoxin into the brainstem lesions in DIPG patients. IL-13 is an immune molecule normally present in the body. About 90% of malignant gliomas have high levels of IL-13 receptors while the normal brain tissue has only a low level of these receptors.

Current and future efforts in the development of antibody-based immunotherapies (including therapeutic antibodies, radiolabeled antibodies, immunotoxins, and ADCs) include the identification of therapeutic targets, development of antibodies against these targets, identification and/or development of radionuclides, drug conjugates and toxins with improved specificity and efficacy, and optimization of delivery methods.

### 15.7.2 Therapeutic Vaccines

Malignant tumors evade the host's immune surveillance. Therapeutic cancer vaccines induce immune responses against antigens specifically or highly selectively expressed by tumor cells or tumor-associated cells, such as stromal cells, while the host would not be able to mount such effective immune responses without assistance [165]. Such tumor antigens can be mutated peptides [166] or altered post-translational modifications [167]. Tumor antigen(s) are administered with immuno-stimulatory adjuvant(s) to enhance antigen presentation and subsequently

activate and expand tumor-reactive T-cells. The biggest challenge in designing such vaccines is the selection of optimal antigen(s) and adjuvant(s), because many tumor-associated antigens are not identified as foreign by the host's immune system [168]. Another reason for this challenge is that some antigens are not exclusively expressed by tumor cells, which can result in immunization against normal cells.

Results from clinical studies involving adult GBM patients suggest that immunization against a single tumor-associated peptide is not sufficient to control the progression of the tumor. In one study, where patients were immunized with dendritic cells loaded with glioma-associated peptides combined with adjuvant poly-ICLC, approximately 60% of patients demonstrated glioma-associated immune responses (vaccine immunogenicity), but only <10% of recurrent glioma patients demonstrated stable tumor regression [169].

Tumor neoantigens, generated by mutations occurring during tumor initiation and progression, are considered to have a higher potential for therapeutic vaccination. These neoantigens are often unique in individual patients [168, 170]. Some neoantigens, such as EGFR variant III (EGFRvIII), are present in a higher percentage of tumors and are rational targets for vaccination that would not be cost- and time-prohibitive. EGFRvIII is present in approximately 20–30% of newly diagnosed GBM [171] and is associated with worse prognosis for patients who survive more than 1 year [172]. EGFRvIII is capable of inducing both cellular and humoral immunity [173]. The clinical study results of an EGFRvIII peptide vaccine (rindopepimut) demonstrated vaccine immunogenicity and increased overall survival, with median survival of approximately 24 months [173–175], while the standard of care produces a median survival of approximately 15 months; the survival advantage also correlated with the induced tumor immunity. However, tumor relapse occurred with loss of EGFRvIII expression based on immunohistochemical detection [173–175]. The loss of antigen detection could be a result of clonal selection or the generation of antibodies by the hosts, which may have masked the antigens. A multicenter study confirmed that immune-mediated eradication of tumor cells bearing EGFRvIII contributed to prolonged progression-free survival and overall survival in patients having received the vaccine, as well as increased host-produced antibodies against EGFRvIII in some patients [174].

There are various recent efforts to address the immune evasion in vaccination by targeting a broad range of antigens simultaneously. One example is to utilize autologous dendritic cells that are pulsed with the tumor lysate. A vaccine using this approach (DCVax@-L) recently completed a phase III trial for patients with newly diagnosed GBM (NCT00045968). Results showed that the addition of DCVax-L to standard therapy is safe in glioblastoma patients and may extend survival [176].

There are several therapeutic vaccines in clinical trials for the treatment of DIPG. One peptide vaccine trial aims to vaccinate patients with HLA-A2-restricted glioma-associated antigen peptides with poly-ICLC in newly diagnosed DIPG and some other glioma patients (NCT01130077). Interim results showed that no dose-limiting non-CNS toxicity was encountered. In about 80% of the patients, immune responses were detected against glioma-associated antigens (IL-13R $\alpha$ 2, EphA2 and survivin). About 20% of patients had symptomatic pseudoprogression, which

responded to dexamethasone and was associated with prolonged survival [177]. Dexamethasone reduced the pseudoprogression, a presentation of inflammation, as well as immune responses to glioma-associated antigens. Another peptide vaccine trial aims to vaccinate patients with H3.3-K27M-specific peptides with poly-ICLC in patients with HLA-A2(+) H3.3-K27M(+) DIPG or other gliomas (NCT02960230).

One of the dendritic cell vaccines trials for DIPG patients is the NCT02840123, in which patients are vaccinated with autologous dendritic cells that are pulsed with allogenic tumor line lysate. Another dendritic cell vaccine, total tumor mRNA-pulsed autologous dendritic cell (TTRNA-DC) vaccine, is being tested as part of NCT03396575.

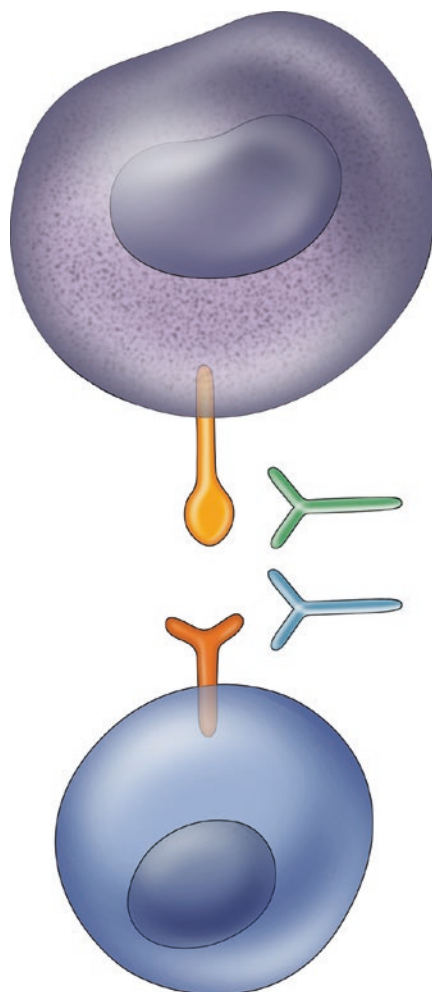
Recent advances in technology of genome-wide sequencing and peptide affinity algorithms can expedite the identification of mutations and related neoantigens, and the screening of peptides with high affinity to the major histocompatibility complex (MHC) for antigen presentation. The high efficiency of these new technologies may improve the feasibility of personalized vaccines.

### 15.7.3 Immune Checkpoint Inhibitors

Immune checkpoints are inhibitory receptors on T-cells that play an important role in suppressing T-cell-mediated antitumor responses [178]. Under physiological conditions, they prevent inappropriate activation and regulate the intensity and duration of activation. The most studied checkpoints for therapeutic purposes are CTLA-4 and PD-1. CTLA-4 plays a role of negative feedback during CD8(+) T-cell activation [47]. CTLA-4 expressed on CD4(+) T-cells enhances Treg-mediated immunosuppression [49]. In mice bearing intracranial gliomas, CTLA-4 monoclonal antibody (9H10) treatment induced robust antitumor immunity without affecting Treg function [50]. The humanized CTLA-4 antibody ipilimumab is the first Food and Drug Administration (FDA)-approved immune checkpoint inhibitor. Ipilimumab has only been used in a small number of GBM patients in the recurrent setting. On the other hand, it has been used in treating metastatic melanoma, with approximately 2% durable complete response rate [179]. Responses have been observed against both non-CNS and CNS-infiltrated melanoma metastases [180]. Based on the common neuroectodermal origin of gliomas and melanomas, the results from the melanoma studies may provide some insights into its application to glioma treatment.

Recent efforts at inhibiting the PD-1/PD-L1 pathway (Fig. 15.7) have produced robust clinical results. Tumor-infiltrating lymphocytes in GBM [45], among numerous cancers, express high levels of PD-1. The high expression levels of PD-1 is thought to be a result of chronic stimulation by the tumor antigen. When the T-cells with high PD-1 levels interact with PD-L1, their effector functions are inhibited [181]. A number of mechanisms, such as loss of PTEN, paracrine IL-10 signaling [44], and interferon (IFN)- $\gamma$  paracrine signaling [182], may contribute to the upregulation of PD-L1 in GBM. Clinical trials studying PD-1 and PD-L1 blockade in GBM patients (NCT02337491 and NCT02336165) recently completed patient recruitment, and their results may be reported soon.

**Fig. 15.7** PD-1/PD-L1 pathway and its blocking. PD-1/PD-L1 pathway can be blocked at either or both sides of the PD-1 – PD-L1 interaction. Blue: T-cell. Purple: tumor cell



Immune checkpoint inhibition has produced the most significant results with the combination of CTLA-4 and PD-1 blockade [183–185]. In a randomized trial of untreated advanced melanoma, which shares neuroectodermal origin with gliomas, dual CTLA-4 and PD-1 blockade produced an improved objective response rate (58%) compared to CTLA-4 only (19%) and PD-1 only (44%) [185]. For GBM, a preclinical study using an animal model showed high rates of survival with IDO, PD-L1 and CTLA-4 triple blockade, as compared to the respective monotherapies [186]. Few clinical trials of treating GBM patients with a combination of CTLA-4 and PD-1 inhibition have completed recruiting patients recently (NCT02311920 and NCT02017717), and the results may be reported soon. There are also clinical trials evaluating the combination of CTLA-4 and PD-1 inhibition in patients with brain melanoma metastasis (NCT02374242 and NCT02320058), which will conclude and report results soon.

A retrospective study showed that 12 patients with recurrent DIPG tolerated nivolumab (an anti-PD-1 monoclonal antibody) well [187]. However, recruitment of DIPG patients in clinical trials of nivolumab have all been discontinued in North America due to unexpected serious adverse events as of 2017. A phase I consortium clinical trial of pembrolizumab (another anti-PD-1 monoclonal antibody) treating brain tumors, including DIPG, is ongoing (NCT02359565).

Other immune pathways being investigated for therapeutic purposes include the inhibitory lymphocyte activation gene 3 (LAG-3) [188] and T-cell immunoglobulin and mucin domain-containing 3 (TIM-3) [189] pathways and the stimulatory inducible costimulator (ICOS) [190] and 4-1BB [191] pathways. LAG-3 and PD-1 dual blockade therapy has progressed to a clinical study in non-CNS solid tumors (NCT01968109).

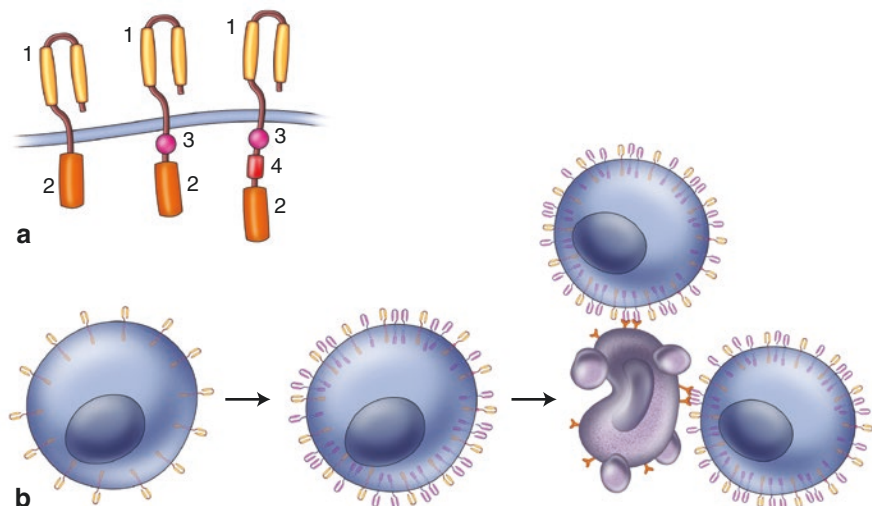
#### ***15.7.4 Adoptive Cell Therapies***

Adoptive cell therapies are the transfer of cells into a patient for therapeutic purposes. The two most investigated adoptive cell therapies for cancer treatment are tumor-infiltrating lymphocyte (TIL) therapy and chimeric antigen receptor T-cell (CAR-T) therapy.

In the TIL therapy, lymphocytes are isolated from resected tumor tissue and cultured with a high-dose cytokine (typically IL-2) in single cell suspensions. The cultures are expanded and tested for antigen specificity against the patient's tumor. Cultures with evidence of specific reactivity to the tumor are selected for rapid expansion before being infused into the patient.

Chimeric antigen receptors (CARs) in CAR-T therapy are receptors engineered to activate T-cells by binding to a specific antigen. They have both antigen-binding and T-cell activating functions by including an extracellular single-chain variable fragment (scFv) and a T-cell activating domain (typically the zeta chain of the CD3 complex) into a single receptor. Therefore, they redirect the specificity and functions of T-cells. The CARs with the minimal antigen-binding and T-cell activating domains are termed first-generation CARs. They recognize antigens without the human leukocyte antigen (HLA), but do not lead to sustained T-cell responses [192] because sustained activation of T-cells requires the engagement of both T-cell receptors (TCRs) and co-stimulatory molecules. Second-generation CARs also include a costimulatory domain (CD28 or 4-1BB), directing sustained T-cell responses upon activation [193] and generating persistent "living drugs," which showed great clinical success recently.

In CAR-T therapy, T-cells are harvested from the patient, then in a laboratory, the cells are purified, cultured and transfected with DNA for the chimeric antigen receptor. The cells with successful transfection (CAR-T cells) are expanded before being infused into the patient. The CAR-T cells continue to multiply after infusion and attack tumor cells expressing the targeted antigen (Fig. 15.8).



**Fig. 15.8** CAR-T therapy. **(a)** From left to right, first-generation CARs consist of a minimal design. Second-generation CARs contain a costimulatory domain to enable sustained T cell activation. Third-generation CARs contain at least two co-stimulatory domains. (1) scFv (2) T-cell activating domain (CD3 $\zeta$ ) (3) co-stimulatory domain (e.g., 4-1BB or CD28) (4) second co-stimulatory domain (e.g., CD28, ICOS or OX40). **(b)** General procedures of CAR-T therapy. T cells are collected, cultured and purified in a laboratory before being transfected with DNA constructs encoding the CAR. Successfully transfected T cells become CAR-T cells. These cells are cultured and purified before being infused into the blood stream of patients. CAR-T cells bind to tumor cells to exert their cytotoxic effects. In **(b)**, yellow color denotes native T-cell receptors and purple represents CARs

As a clinical success, both the TIL therapy and the CAR-T therapy demonstrated their capability in eradicating a large amount of tumor burden. The TIL therapy achieved a durable complete response in treating metastatic melanoma [194], and the CAR-T therapy was successful in treating CD19(+) B-cell leukemia [195].

TIL therapy targeting human cytomegalovirus (CMV) antigens expressed by tumor cells has been investigated for treating GBM. A clinical study treating recurrent GBM patients with autologous infusion of tumor-infiltrating T lymphocytes recognizing CMV antigens led to a median overall survival of over 57 weeks, with four patients remaining progression-free throughout the study period [196].

CAR-T therapies targeting human epidermal growth factor receptor 2 (HER2) and EGFRvIII produced impressive results in animal models of glioma [197, 198]. Clinical trials of CAR-T therapies targeting EGFRvIII (NCT02209376 and NCT01454596) and bispecific HER2/CMV (NCT01109095) in GBM patients have recently completed recruiting patients and may report detailed results soon. Preliminary results of the HER2/CMV bispecific CAR-T trial reported on the 30th Annual Meeting of the Society for Immunotherapy of Cancer (SITC, 2015, National Harbor, MD) showed that the therapy was well tolerated, and a durable clinical

benefit was observed in ~ 38 patients. A trial of CAR-T therapy targeting IL13Ra2 (NCT02208362) in malignant glioma patients is ongoing.

CAR-T therapy for DIPG is still at the preclinical stage. A recent preclinical CAR-T study targeting GD2, a disialoganglioside expressed on tumors of neuroectodermal origin, for DIPG was able to clear most of the tumor cells, but it produced dangerous levels of brain swelling in some animals [199]. Because of the essential anatomical and functional features of the brainstem, as well as its proximity to the thalamus, the safety issue of CAR-T therapy may be more difficult to solve in DIPG than in supratentorial gliomas.

## 15.8 Oncolytic Viruses

Oncolytic viruses are viruses that can infect and replicate in tumor cells in a tumor-selective conditional fashion, resulting in lytic destruction of tumor cells. The cell lysis results in the release of a large number of viral progenies, which go on to infect neighboring tumor cells.

A successful oncolytic virus requires a conditional, tumor-restricted viral replication with subsequent lysis of tumor cells. Certain tumor cell mutations would allow selective viral replication based on either inherent or engineered viral mechanisms. Such mechanisms can include any stage of the viral life cycle such as receptor-mediated viral attachment for infection initiation, DNA replication and protein synthesis, as well as the host's cytosolic antiviral mechanisms and innate and adaptive immune responses.

The most widely studied viruses for cancer treatment are herpes simplex virus (HSV) and adenovirus. The first experimental application of a genetically modified, replication-competent oncolytic virus for glioblastoma was reported in 1991 using a thymidine kinase (TK)-deficient HSV mutant, HSV-*dlsptk*, which was shown to be highly attenuated in non-dividing cells such as neurons but effective in infecting, replicating inside and lysing U87 cells *in vitro* and xenografts *in vivo* [200]. A number of oncolytic HSV mutants have been isolated or engineered since then, with R3616, HSV-1716, hrR3, G207, and G47 $\Delta$  being the most studied examples for targeting glioblastoma. After preclinical studies showed anti-glioma activity and good safety in rodents and non-human primates, G207 was tested in a clinical trial for glioblastoma that commenced in February 1998 [201]. HSV-1716 was tested in a clinical study in Europe at approximately the same time [202]. M032, an oncolytic HSV carrying human IL-12 gene, has shown enhanced glioma cell cytotoxicity in animal models [203], and a clinical trial of M032 in treating recurrent malignant gliomas is ongoing (NCT02062827).

Genetically engineered recombinants of adenovirus serotype 5 (Ad5) that show conditional replication are among the most studied oncolytic viruses. Early gene products of the adenovirus interact with Rb and p53. Adenoviruses with E1A or E1B deficiencies are replication-incompetent in normal cells and, therefore, tumor-selective. ONYX-015, a conditionally replicating adenovirus mutant with the



E1B-55 kD gene deletion was effective in killing p53-mutant U373 glioma cells but not p53-normal U87 cells [204]. However, a later study in glioma xenografts derived from primary human tumors reported increased oncolytic activity of ONYX-015 in p53 wild-type glioma xenografts compared to p53 mutants [205]. ONYX-015 was studied in a glioblastoma clinical trial [206]. DNX-2401, a newer adenovirus construct that combined Ad-delta-24 with Arg-Gly-Asp (RGD)-containing peptide modifications, has shown increased oncolytic activity against glioblastoma [207]. It was studied in a clinical trial showing dramatic responses with long-term survival in recurrent HGGs, which are probably due to direct oncolytic effects of the virus followed by elicitation of an immune-mediated anti-glioma response [208].

DNX-2401 is currently studied in DIPG patients in a phase I clinical trial (NCT03178032). Preliminary results reported at the 18th Biennial International Symposium for Pediatric Neuro-Oncology (ISPNO, 2018, Denver, Colorado, USA) showed that six DIPG patients were treated with DNX-2401 with no grade 3 or 4 adverse events, indicating that it may be a safe therapy for DIPG patients.

## 15.9 Conclusion

The most prominent example of malignant brainstem tumors is DIPG, which is considered one of the most difficult to treat in pediatric oncology. The standard of care for this tumor has not seen major changes in decades despite the disease's dismal outcome. A number of therapeutic options, many of which have shown dramatic therapeutic effects in other tumors, are being investigated for the treatment of DIPG. Despite the challenges presented by the critical anatomical and functional properties of the brainstem and its proximity to the thalamus and the unique biology of the tumor, it is hopeful that the outcome of this disease will improve with further refinement of these investigational therapies as well as development of new therapies.

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