

# Investigating The Potential for Immunotherapies in Diffuse Intrinsic Pontine Glioma (DIPG)

Anika Verma<sup>1</sup> and Alexander Wesolowski<sup>#</sup>

<sup>1</sup>Saratoga High School, Saratoga, FL, USA

<sup>#</sup>Advisor

## ABSTRACT

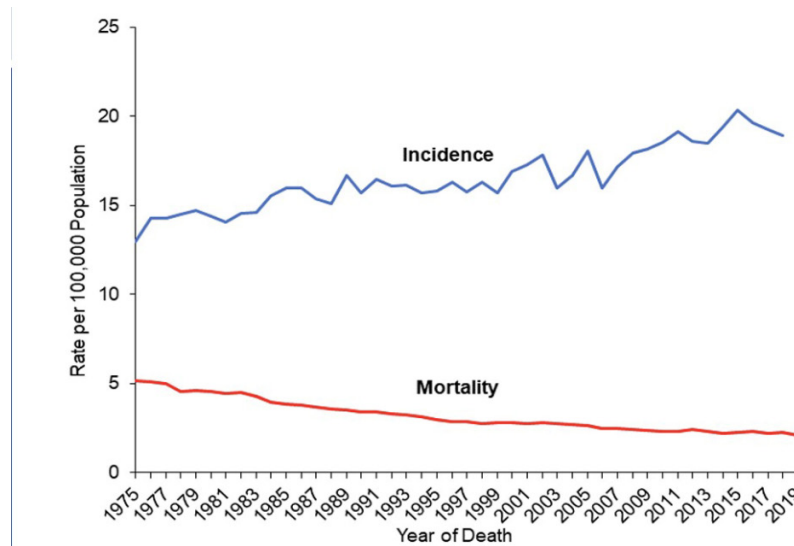
Cancer is the second leading cause of death worldwide, and has significant economic, social, and emotional effects. Diffuse Intrinsic Pontine Glioma (DIPG), a rare type of cancer, is a devastating pediatric brain tumor that currently has no effective therapy. This disease affects around 150 to 300 children in the United States every year and poses a greater than 98 percent mortality rate within five years of diagnosis. DIPG has been suggested as a potential candidate for immunotherapies and this report's main focus is on exploring immunotherapies as a treatment for DIPG. It first dives into the impact of cancer on society, introduces DIPG, and explores its biology and prevalence. It then explains the current treatment options, roadblocks, and advantages of immunotherapies over other diseases. This paper concludes by suggesting three most promising immunotherapies for the treatment of DIPG based on their effectiveness in similar brain cancers: immune checkpoint inhibitors, cancer vaccines, and adoptive T-cell therapy.

## **Introduction**

### Impact of Diffuse Intrinsic Pontine Glioma

Cancer is the leading cause of premature death globally, accounting for nearly 10 million deaths in 2020 and approximately one in six deaths.<sup>1</sup> It is a disease in which abnormal body cells divide uncontrollably and spread, destroying tissues.<sup>2</sup> The most common cancers are breast, lung, colon, rectum, and prostate cancer.<sup>1</sup>

Cancer incidence rates are continuously increasing with over 1.9 million cancer cases expected to be diagnosed in the US in 2022, and out of these approximately 609,360 deaths.<sup>3</sup> This would mean about 1,670 deaths per day.<sup>3</sup> The graph below demonstrates the trends in cancer incidence and death rates over the years.



**Figure 1.** Trends in Cancer Incidence and Death Rates in Children and Adolescents from 1975 to 2019.<sup>4</sup>

Although cancer rates have increased slightly by about 0.8% per year, mortality rates have declined by about 61-71% for children and adolescents from 1975 to 2019 (Figure 1). This large decrease in mortality rates can be attributed to the overall advances in early detection and treatment for certain cancers. Though, progress still lags for children and adolescents with certain cancers whose tumor distribution and biology make it difficult to effectively treat and diagnose them.

These more challenging cancers are having an increased impact on populations and are entering the forefront of clinical research. An example is Diffuse Intrinsic Pontine Glioma (DIPG), a rare and fatal pediatric brain cancer that is difficult to treat because of its location in the pons of the brain and other properties.<sup>5</sup> Given this cancer and many others that are so deadly and have such a large economic, social, and emotional impact on society, a treatment that will cure the cancer and not delay the inevitable is needed. Immunotherapies are a type of treatment that have displayed curative abilities and are therefore setting the stage for DIPG treatment.<sup>5</sup>

### Diffuse Intrinsic Pontine Glioma

Diffuse Intrinsic Pontine Glioma(DIPG) is a rare and fatal pediatric brain cancer that mostly affects children from ages 7-9.<sup>6</sup> It is extremely difficult to treat because of its specific location in the pons of the brain, where many of the body’s most vital functions such as breathing, blood pressure, and heart rate are controlled.<sup>6</sup> The DIPG tumor is a type of glioma, brain tumors that arise from the glial cells that protect and support the neurons in the brain.<sup>5</sup> Due to the tumor’s location and how rapidly it progresses, DIPG is considered a “high grade” malignant brain tumor.<sup>7</sup> High grade tumors are those that are fast-growing and spread quickly throughout tissue, making them extremely difficult to treat.<sup>8</sup> There are currently no identifiable causes for this disease but this cancer most probably forms when issues with cellular reproduction during the brain’s development arise. Hence, the prominence of this cancer in children is when their brains are developing.

Brain tumors are currently the most common type of cancer and cause of cancer-related death in children less than 15 years old.<sup>9</sup> Out of these brain tumors, DIPG tumors are the most common and deadly brain stem tumors in children, representing around 75-80% of all pediatric brainstem tumors.<sup>9</sup> Approximately 10,000 children are affected every year worldwide, and fewer than 10% of the children diagnosed are able to survive beyond 2 years and fewer than 2% for beyond 5 years.<sup>10</sup> Nearly half of the patients diagnosed with this cancer die within 9 months of being diagnosed.<sup>10</sup> This devastating disease affects mostly children and is seldom in adults. Doctors believe that this is

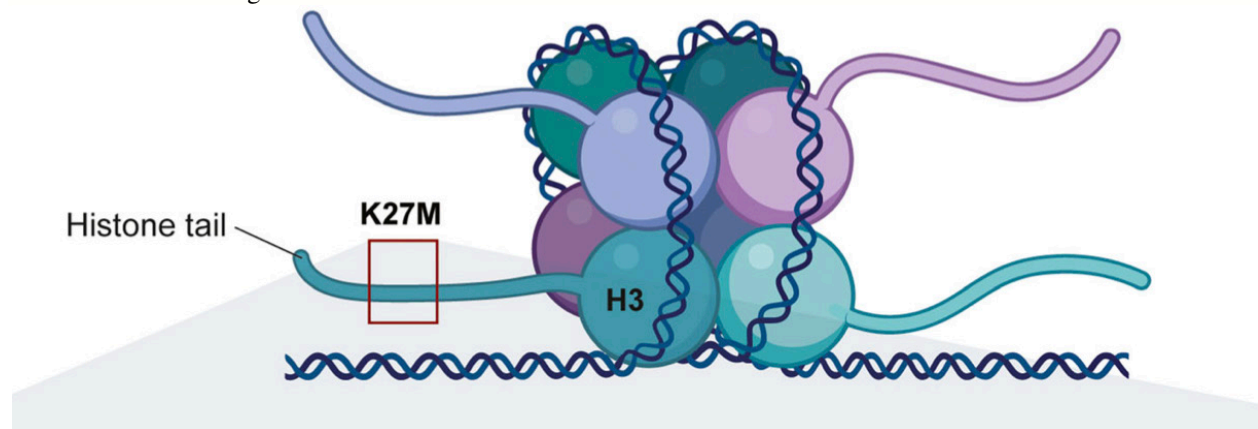
because DIPG may be linked to how a child's brain grows.<sup>11</sup> Tumors tend to appear at an age when the brain is changing fast like during the first couple years of life. During this time, there is also a high amount of a type of brain cell that can drive DIPG tumor growth.<sup>11</sup>

## Molecular Hallmarks of DIPG

DIPG can fall into three different molecular defined groups: H3K27M, MYCN, and silent mutated.<sup>5</sup> These subgroups differ in their number and type of methylation, expression, copy number alteration (CNA), and genetic mutations.<sup>5</sup> Currently, the biology of DIPG is not fully understood, but more research is being done to improve our understanding.

### *H3K27M*

DIPG tumors within the H3K27M subgroup are characterized by histone mutations, whose identification has redefined the focus of research on this disease.<sup>5</sup> Nearly 80% percent of the histone mutations in DIPG are H3K27M mutations.<sup>5</sup> This mutation causes the isoforms H3.1 and H3.3, which are encoded by the genes HIST1H3B and H3F3A respectively, to undergo a substitution of lysine with methionine.<sup>5</sup> This substitution leads to the loss of histone trimethylation, ultimately producing epigenetic silencing at key genomic loci.<sup>5</sup> These epigenetic alterations can then lead to altered gene expression and the expression of pro-tumorigenic onco-genes that ultimately drive the cell to become tumorous.<sup>5</sup> Figure 2 illustrates this mechanism.



**Figure 2.** The K27M mutation on the histone tail.<sup>5</sup>

A modification in the histone tails can change the degree to which DNA wraps around the histones, allowing for genes within traditionally silent loci of the genome to be actively transcribed and expressed. If a mutation causes the DNA to be wrapped around the histone loosely, specific genes can be transcribed. In terms of DIPG, the K27M mutation in the histone tail causes epigenetic remodeling and facilitates the opening of the DNA in specific regions, resulting in an overexpression of the PDGFRA gene. PDGFRA is a growth factor signaling receptor that is one of the most amplified genes in this disease and is found in around 10% of DIPGs.<sup>5</sup> When this gene is overexpressed, it can lead to uncontrolled growth factor signaling and cell division, satisfying one of the hallmarks of cancer.<sup>5</sup>

### *MYCN*

DIPGs in the MYCN subgroup are characterized by MYC and MYCN genetic aberrations that act as transcriptional regulators to enhance gene expression throughout the genome.<sup>5</sup> These genetic aberrations don't contain any histone mutations, but are instead characterized by high-level amplifications of MYCN and ID2 genes caused by chromothripsis on chromosome 2p.<sup>12</sup> Chromothripsis is a mutational process in which up to thousands of clustered chromosomal

rearrangements occur at once in localized genomic regions.<sup>13</sup> Furthermore, these MYCN amplified DIPG don't contain PVT-1/MYC, PDGFRA, or RB1 CNAs (copy number alterations) which were previously described to be common in these brainstem neoplasms.<sup>12</sup>

### *Silent*

In the silent subgroup, there are not many DIPGs with CNAs or mutations.<sup>5</sup> This subgroup is actually characterized by stable genomes in comparison to both MYCN and H3-K27M subgroups.<sup>5</sup> Though some DIPGs in this subgroup do contain histone H3, TP53, and ACVR1 mutations, they are found at much lower frequencies.<sup>5</sup> Instead, the silent subgroup usually shows over-expression of WNT pathway genes like *MDM2*, *MSMP*, and *ADAM33* when compared with the other two subgroups.<sup>14</sup>

### *Other Mutations*

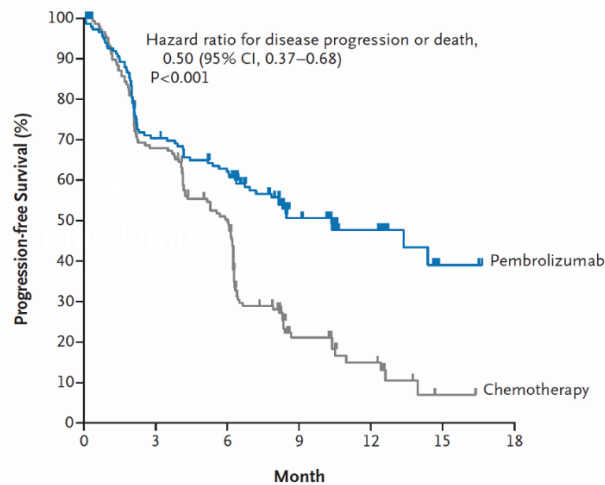
The ACVR1 mutation co-segregates with H3 mutations and has been identified in approximately 30% of DIPG tumors.<sup>5</sup> This mutation has been shown to ultimately upregulate the bone morphogenetic protein developmental signaling pathway that facilitates early tumor progression.<sup>5</sup> Additionally, TP53 mutations have been identified in approximately 77% of DIPGs and are often coupled with H3K27M and PPM1D mutations to allow tumor cells to evade cell death and senescence by influencing epi-genetic regulation.<sup>5</sup>

## Coming of Age of Immunotherapies

Due to the eloquent location of the DIPG tumor, surgical resection to remove the tumor is rarely an option for patients.<sup>5</sup> Only in certain circumstances can incisional biopsies under direct observation in safe-entry zones be performed.<sup>5</sup> This makes it difficult to obtain a comprehensive tumor tissue that accurately reflects the heterogeneity of this disease. The location also creates an obstacle as there is an intact blood-brain barrier that causes a lack of effective drug delivery to the tumor site.<sup>5</sup> For this reason, the current standard of care for DIPG involves only a 54-59 Gy dose of standard fractionated radiation.<sup>5</sup> Monotreatments and combination chemotherapies have also had no substantial benefit.<sup>5</sup> The current treatments are extremely limited but there is a scope for more modern and advanced targeted cancer therapeutics to have greater success in DIPG management.

The field of cancer immunology is considered a relatively young field.<sup>15</sup> The immune system has been shown to be a potent regulator of tumor growth and is often dysregulated or functionally suppressed in advanced cancers. Immunotherapies are a type of treatment that uses a person's own immune system to fight the cancer by boosting or changing the immune response.<sup>16</sup> Immunotherapies aimed at restoring the endogenous function of the immune system have had profound effects in the clinic, providing substantially improved progression-free survival in several cancer types.<sup>17</sup> Immunotherapies can be administered by doctors with or after another treatment like surgery, radiation, or chemotherapy. They can also be administered by themselves as the first treatment or as a part of a clinical trial if the other treatments haven't worked and the cancer has spread.<sup>17</sup>

There are many benefits of immunotherapies compared to traditional cancer treatments like chemotherapy. An advantage of immunotherapies is that they are more robust than other treatments. This is because resistance by cancer cells emerges less frequently than other therapies due to the immune system's ability to co-evolve with the disease. Immunotherapies can also synergize with other cancer treatments like chemotherapy and can cause fewer side effects since they only target the immune system and not all the cells in the body.<sup>18</sup> Additionally, immunotherapies can provide long-term protection against cancer due to the immune system's ability to recognize and remember what cancer cells look like, making longer-lasting remissions more possible.<sup>19</sup>



**Figure 3.** Comparing the percent of patient survival with lung cancer when treated with traditional chemotherapy versus Pembrolizumab, a humanized antibody used in cancer immunotherapy.<sup>20</sup>

According to the trial demonstrated in the figure, patients with lung cancer had a higher progression-free survival rate over 18 months when treated with Pembrolizumab as compared to chemotherapy (Figure 3).

Despite all the pros, immunotherapies can also have some risks. These risks include patients developing bad reactions like pain, itching, swelling, or soreness in the area that the medicine is administered. In some cases, immunotherapies can also rev up the immune system, leading to flu-like symptoms like fever and chills. Swelling, weight gain from extra fluids, heart palpitations, a stuffy head, and diarrhea can also result, but these symptoms usually ease up after the first treatment.<sup>21</sup>

## Discussion

### Immunotherapeutic approaches to DIPG

There are several types of immunotherapies that are currently being used in clinical trials. Most of them can be broadly categorized into drugs that target tumor immune evasion through the blockade of negative regulatory signals like immune checkpoint inhibitors and cancer vaccines, and agents that enhance immune function like agonists of costimulatory receptors and adoptive T-cell transfers.<sup>22</sup>

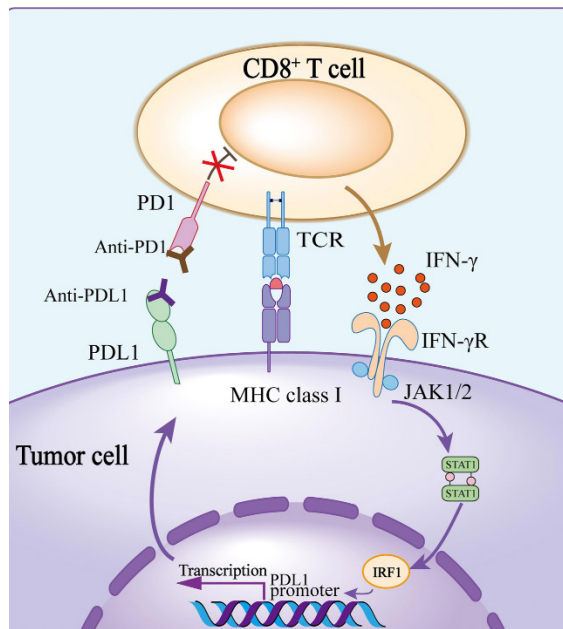
### *Immune Checkpoint Inhibition*

The immune system has a set of checkpoints that stop it from killing the healthy cells of the body and calm down the immune response post infection. However, cancer cells can take advantage of these checkpoints and hide from or suppress the defense system.<sup>23</sup> Therefore, there is a need for immune checkpoint inhibitors that can help the immune system see the cancer cells as a problem, prevent cancer induced immunosuppression, and fight them. Monoclonal antibodies are types of checkpoint inhibitors that are made in a laboratory and attach to cancer cells, making them more visible for the immune system to attack them.<sup>23</sup> There are also molecular inhibitors but monoclonal antibodies are more specific and don't have a problem crossing the plasma membrane.<sup>23</sup> Some checkpoint pathways that these

monoclonal antibodies target are CTLA-4, PD-1, TIM-3, VISTA, LAG-3, and TIGIT.<sup>17</sup> Out of these, the PD-1 blockade has had the greatest clinical success so far.<sup>24</sup>

### PD-1

Programmed death-1 (PD-1) is a cell surface receptor that functions as a T-cell checkpoint and plays a central role in regulating T-cell exhaustion.<sup>25</sup> Binding of PD-1 to its ligand, programmed death-ligand 1 (PD-L1), activates downstream signaling pathways and inhibits T-cell activation.<sup>25</sup> Moreover, abnormally high PD-L1 expression on tumor cells and antigen-presenting cells in the tumor microenvironment can mediate tumor immune escape.<sup>25</sup> In order to counteract this, there has been the development of monoclonal anti-PD-1/PD-L1 antibodies such as Nivolumab and Pembrolizumab.<sup>24</sup> Figure 4 illustrates the mechanisms of these anti-PD/PD-L1 antibodies.



**Figure 4.** Mechanisms of PD1/PDL1 blockade.<sup>26</sup> The CD8+ T-cell activates when it recognizes the tumor antigen presented on the MHC class I molecule on the tumor cell. INF $\gamma$  is then released to promote inflammation and ultimate immune clearance of the tumor cell.<sup>26</sup> To avoid immune destruction, tumor cells can overexpress PDL1 on their surface that interacts with PD1 on the T-cell surface, triggering inhibitory T-cell signaling.

### Immune Checkpoint Potential in DIPG

Immune checkpoint inhibitors like PD1 and PDL1 antibodies have shown positive and promising results in melanoma, lung, and other cancers.<sup>27</sup> However, they have not demonstrated very positive results in other brain cancers similar to DIPG like glioblastoma(GMB).<sup>24</sup> Even though preclinical studies have shown some potential for treatment in GMB, most trials for anti-PD-1/PD-L1 monotherapy have revealed only a low tumor response and did not prolong patient survival.<sup>24</sup> This is surprising as there seems to be a lot of expression of PD-1 and PDL1 found in brain cancers.<sup>24</sup> A reason for this could be that the antibodies may be unable to cross the blood brain barrier.

Though, an exciting development is the use of combined therapies in which two or more inhibitors are administered together.<sup>27</sup> The concept of a combined immune checkpoint blockade like anti-PD1 and anti-CTLA-4 inhibitors does seem to have promising results on brain tumors. An example of this are the findings that anti-PD-1 and anti-

CTLA-4 therapy induces a novel subset of effector T-cells and supports administration of induction chemotherapy in children with high-risk neuroblastoma, a pediatric tumor that results from nerve cells.<sup>27</sup> In one study, GBM tumor-bearing mice were treated with a combination of anti-PD-1 and anti-CTLA-4 antibodies, and significant improvement in survival was noted in comparison to the controls.<sup>27</sup> Another study showed that when mice with GBM were treated with just anti-TIGIT therapy, there was no significant effect on the survival rate, but when combined with anti-PD-1, there was a significant effect.<sup>27</sup>

Since combination immune checkpoint inhibitors, which are being used more frequently, have shown promising results in brain cancers like DIPG, they may also be a good treatment option for DIPG.

### *Cancer Vaccines*

Cancer vaccines work similar to pre-existing vaccines. In the context of cancer, they work to enhance and generate the adaptive antitumor response by increasing tumor antigen presentation and generating tumor specific T-cells.<sup>28</sup> They are useful when tumors downregulate the presentation of cancer-specific antigens or suppress T-cell priming at the tumor site.<sup>29</sup> These vaccines are often combined with other substances or cells called *adjuvants* that help boost the immune response even further.<sup>29</sup> In some cases, a patient's own immune cells are removed and exposed to these substances in a lab to create the vaccine.<sup>29</sup> There are mostly two types: preventative vaccines, which are given before the disease arises, and treatment vaccines, which are given after the disease arises. Both the prevention and treatment vaccines can have whole-cell effects or contain specific prepared peptide antigens. They are both ways of training the immune system to target a specific pathogen/cancer, and they are both currently being investigated in clinical trials.

### Cancer Vaccine Potential in DIPG

Cancer vaccines have the potential to elicit a widespread and durable response. They are beneficial to DIPG patients for several reasons. Since vaccine therapy is just like getting a shot, it is a quick treatment that causes little discomfort.<sup>18</sup> Most people taking the vaccine also experience minimal side effects including an occasional itch or mild sting at the injection site if none at all. Studies also show that patients receiving the vaccines are surviving significantly longer than patients who don't receive the vaccines.<sup>18</sup> Additionally, taking vaccines allows the patient to receive other treatments. For example, patients who receive brain tumor vaccines can receive additional treatments like new therapies available through other clinical trials, increasing their chances for better outcomes.<sup>18</sup> For patients with glioblastoma, brain tumor vaccines have been found to increase progression-free survival by approximately 50%.<sup>30</sup> Types of these vaccines are AV-GBM-1 and EGFRvIII.

AV-GBM-1 is AIVITA Biomedical Inc's personalized cancer vaccine consisting of autologous dendritic cells loaded with autologous tumor neoantigens that are derived from self-renewing tumor-initiating cells.<sup>30</sup> AV-GBM-1 is mixed with granulocyte-macrophage colony stimulating factor, an immune modulator, as an adjuvant prior to administration by subcutaneous injection.<sup>30</sup> This treatment is good because it is "pan-antigenic," meaning that it targets many antigens from tumor-initiating cells that are responsible for tumor growth.<sup>30</sup> In a trial focusing on 57 glioblastoma patients who received eight doses of AV-GBM-1 over the course of approximately six months, all survived and were followed between 10.1 and 27.6 months from enrollment.<sup>30</sup> In EGFRvIII vaccine trials, patients who underwent re-resection no longer expressed EGFRvIII, demonstrating that the vaccination strategy is effective.<sup>31</sup>

Cancer vaccines have also been very successful in another brain cancer similar to DIPG called ependymoma.<sup>32</sup> In a study of vaccination with tumor lysate-pulsed dendritic cells, three of four children with recurrent ependymomas survived beyond 18 months.<sup>32</sup> In another trial also including DIPG patients, nine patients were progression-free for at least 12 months and overall survival was 55%, an encouraging result for a cohort of patients with recurrent malignant gliomas.<sup>32</sup> One concern that people could have is that an immune reaction against a tumor in the brain could lead to an inflammatory or autoimmune reaction in the CNS, but thankfully this has not been an issue in any trials.<sup>32</sup>

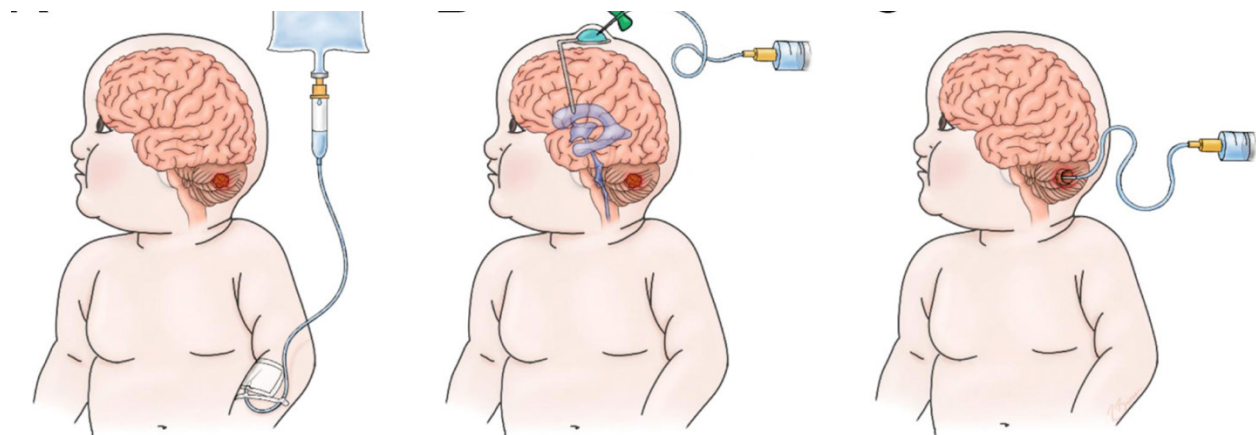
Additionally, even though the brain is thought of to be an immune privileged organ, there have not only been specific immune responses detected in the peripheral blood, but also evidence of immune cells reaching the tumor.<sup>32</sup>

All in all, two of the most successful vaccines used in gliomas include epidermal growth factor receptor variant III (EGFRvIII) junctional epitope combined with GM-CSF, and AV-GBM-1, whole tumor cell antigen pools presented by autologous dendritic cells.<sup>30</sup> Since these cancer vaccines are safe and successful in GBM and ependyoma, there is also a probability of them working in DIPG.

### *Adoptive and CAR T-cell Immunotherapy*

Adoptive T-cell immunotherapies, in which autologous T-cells are infused into patients with cancer, are designed to boost key immune cells.<sup>23</sup> Researchers essentially remove T-cells from the patient's blood or tumor and figure out which ones are the most efficient at tumor targeting. Once they identify the T-cells, they genetically engineer the genes in those cells to be even more effective, then stimulate the cells to grow and expand in an in vitro culture system, and then return them back into the body through administering an I.V..<sup>23</sup> This approach currently shows a lot of promise in the treatment of many cancers including brain cancers.<sup>23</sup>

A type of adoptive T-cell therapy (ACT) is called CAR T-cell therapy, which has been around for 25 years.<sup>33</sup> This treatment is sometimes used to treat acute lymphoblastic leukemia in children as well as certain types of B-cell lymphoma in adults that are unable to get better with other treatments.<sup>34</sup> In the manufacturing of CAR T-cells, a protein complex called chimeric antigen receptor (CAR) is added to the T-cell's surface to help the T-cells focus their attention towards finding antigens.<sup>34</sup> These CAR receptors are specific to a particular cancer expressed antigen. The CAR protein is made up of three other proteins: one protein that recognizes antigens on the cancer cell, and two others that signal the T-cell to activate when the first protein attaches to an antigen.<sup>34</sup> The CAR T-cells work by circulating around the body and looking for cells that carry the antigen programmed into the CAR protein.<sup>34</sup> When a CAR T-cell comes in contact with the antigen on a cancer cell, it activates, multiplies, and sends signals to other parts of the immune system to come to the site of the cancer cell.<sup>34</sup> These signaling proteins called cytokines and activated T-cells then cause significant inflammation, which ultimately causes the cancer cell to die.<sup>34</sup> Recognition of a target antigen usually requires presentation by MHC, but the ingenuity of CAR T-cells allows for this recognition without presentation by MHC.<sup>35</sup> Additionally, a key factor of CAR T-cells is that they can also be engineered to be resistant to immunosuppressive mechanisms employed in the tumor site. Figure 5 demonstrates the three different ways in which CAR T-cells can be injected into the body.



**Figure 5.** The three different ways that CAR T-cells can be administered: through a vein in the arm, a spot near the top of the brain, and directly into the spot of the tumor.<sup>33</sup>



Directly injecting the CAR T-cells into the tumor site can also get around the obstacle of the BBB, which usually stops other treatments like immune checkpoint inhibitors from working.<sup>33</sup> Though, a negative of this is that it can be very problematic if the extra immune cells start damaging neurons.

## Adoptive T-cell Therapy Potential in DIPG

There are usually very few immune cells in the brain to fight the tumor, but ACT and CAR T-cell therapy can overcome this problem as they flood the tumor environment with immune cells. CAR T-cells are also being developed that can overcome some of the suppressive features of the tumor environment.<sup>33</sup> Additionally, T-cells often lack the required costimulatory signaling to target tumor cells, but CAR T-cells are being developed that don't require this costimulation.<sup>17</sup>

Adoptive T-cell therapy has been extremely successful in the past with complete regressions in patients with melanoma and lymphoma by using naturally tumor-reactive T-cells and genetically engineered T-cells expressing the chimeric anti-CD19 receptor.<sup>36</sup> Regressions of brain metastases have also been observed in patients, suggesting that adoptive T-cells and CAR T-cells can pass the blood-brain barrier, infiltrate into brain tumors, and even exist in peripheral blood 1 year after infusion.<sup>36</sup>

The administration of ACT and CAR T-cells have also shown therapeutic potentials for GBM treatment.<sup>37</sup> Clinical trials have reported exciting results including complete regression of bulky, multifocal cancer in the brain and spinal canal following intraventricular infusion.<sup>33</sup> Additionally, due to the heterogeneity of cell surface antigen expression in brain tumors like GBM, multivalent CAR T-cells have been designed that are capable of targeting multiple antigens simultaneously.<sup>33</sup> Examples of these targets are EPHA2, HER2, and IL13Ra2, and efficacy in preclinical models of recurrent medulloblastoma and GBM has been demonstrated when they were targeted by CAR T-cell.<sup>33</sup>

Although not in human models, CAR T-cells have also shown efficacy in vitro models. Disialoganglioside 2 (GD2) is highly expressed in DIPG and GD2-targeted CAR T-cells have shown killing of DIPG cells *in vitro* and complete regression of DIPG in a mouse model.<sup>33</sup> Additionally, an anti-GD2 CAR T-cell study also produced positive results in DIPG in vivo models.<sup>5</sup> Due to the success and benefits of ACT and CAR T-cell therapy in similar brain cancers and more, ACT and CAR T-cell therapy are strong candidates for the treatment of DIPG.

## Conclusion

DIPG is increasingly affecting young children around the world and cutting their lives short as more than 90% of patients with DIPG die within 2 years of diagnosis.<sup>38</sup> Due to the location of the tumor in the pons of the brain behind the blood brain barrier, it is difficult to get a proper image of the tumor on an MRI and also difficult to deliver a treatment like immune checkpoint inhibitors to the site of the tumor. Due to this, a T2-weighted MRI works the best and a standard radiation therapy and chemotherapy are the only current treatments available. Our understanding of DIPG biology compared to other cancer types is very limited as it is very difficult to get a tumor sample for analyses given their inaccessibility.

Immunotherapies are a new type of treatment that have shown positive results in other brain cancers like glioblastoma and medulloblastoma, and since DIPG has similar physiology and biology, immunotherapies also have the potential to be successful in DIPG. They also may be more effective in DIPG because they target the immune system in younger patients where the immune system is generally more effective. According to Girish Dhall MD, director in the division of Pediatric Hematology & Oncology at Children's Hospital of Alabama, there is "lots of promise for immunotherapies in brain tumor treatment as compared to regular drugs because regular drugs are unable to penetrate the blood brain barrier whereas the beauty of immune cells is that they can cross this barrier. We just need to figure out how to exactly get these immune cells to the tumor". Also, in an interview with Brooke Vittimberga, a former research assistant at the Stanford Cancer Institute, she stated that "childhood cancers are the best targets for

immunotherapies because they are more specific in their mutations. This is because children don't acquire as many mutations over time as adults do".

This report has gone on to evaluate the three major areas of immunotherapies: immune checkpoint inhibitors, cancer vaccines, and adoptive T-cell therapy. Immune checkpoint inhibitors, which are usually monoclonal antibodies, attach to cancer cells and make them more visible for the immune system to attack them. They block the binding of T-cell checkpoint molecules to their surface receptors, effectively allowing T-cells to be activated and attack the tumor. Studies have shown that they work best in brain cancers when administered in a combination of different antibodies. Cancer vaccines inject tumor antigens into the tumor site to increase tumor antigen presentation and generate tumor specific T-cells, in order to enhance and generate the adaptive antitumor response. Two of the most successful cancer vaccines in gliomas have been EGFRvIII combined with GM-CSF, and AV-GBM-1. Adoptive T-cell therapy identifies, modifies, and enhances the number of effective T-cells, and a special type of adoptive T-cell therapy that has shown very promising results in brain cancers is CAR T-cell therapy.

There are many more types of immunotherapies as well, but out of these three main ones, CAR T-cells seem to be the best option. This is because there have shown more positive results in many similar brain cancers, they are able to cross the blood brain barrier, and reduce if not eliminate the tumor suppressive environment by bringing in stronger and more effective T-cells to the tumor site and being specific and precise in their target mechanisms. Dr. Girish Dhall also supports this as in an interview he stated that "we still have to work on making immunotherapies a possibility but so far CAR T-cells are what are showing the most promise, especially CAR T-cells for anti-GD2". However, the cost of CAR T-cells is one of the biggest challenges as its price ranges from \$373,000 - \$475,000 depending on the specific drug and indication.<sup>39</sup> Given this, it is still important to understand that this treatment is fairly new and not as commonly used. Due to this, it is extremely expensive right now, but if it is more commonly used, it can become cheaper in the future. Additionally, it takes around 10 weeks for the CAR T-cell treatment process to start as it takes time to first collect the T-cells, engineer them, multiply them, and infuse them back. Though, this treatment is curative compared to other treatments and with a disease like DIPG, it is so important that the cancer be treated immediately and fully cured instead of delaying the inevitable.

To make ACT, CAR T-cells, and other immunotherapies more of a reality, more money for research and treatment is desperately needed as currently childhood cancer research only gets 4% of NCI funding and out of this only 0.1% funding for DIPG research.<sup>40</sup>

## Limitations

Due to how rare of a condition DIPG is, there is limited primary focus and clinical trials on DIPG patients. Additionally, due to the location of the tumor in the pons of the brain, it is extremely difficult to obtain samples of the tumor for inspection and analysis. Given these limitations, there is currently an incomplete understanding of the biology of DIPG.

In terms of immunotherapy as a treatment, some limitations include the inability to predict treatment efficiency and patient response, the need for additional biomarkers, the development of resistance to cancer immunotherapies, the lack of clinical designs to be optimized to determine efficiency, and high treatment costs.<sup>41</sup>

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