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Therapeutic cancer vaccination for relapsed and refractory pediatric brain tumors

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Abstract

Relapsed and refractory paediatric brain tumors, including high-grade gliomas (HGG) such as diffuse hemispheric glioma, diffuse midline glioma (DMG), diffuse intrinsic pontine glioma (DIPG), and atypical teratoid/rhabdoid tumors (AT/RT), medulloblastoma (MB) and ependymoma (EPN), remain among the most treatment-resistant childhood malignancies. Despite multimodal therapy combining maximal safe resection, radiotherapy, and chemotherapy, long-term survival after relapse is exceedingly rare. The limitations of conventional cytotoxic regimens and the largely immune-silent nature of these tumors have driven interest in therapeutic vaccination as a strategy to induce durable, antigen-specific immune responses. Advances in molecular profiling and immunopeptidomics have enabled the identification of tumor-exclusive peptides, even within the low-mutational landscape typical of paediatric gliomas. Preclinical investigations demonstrate that dendritic-cell- (DC), peptide-, or mRNA-based vaccines can elicit tumor-specific cytotoxic T-cell responses and modify the tumor microenvironment. Early clinical experiences in relapsed paediatric HGG and DMG indicate safety and feasibility, with emerging evidence of immune activation and prolongation of survival. Features of paediatric immunity render children particularly responsive to cancer vaccines, and insights from paediatric trials may prove valuable for the design of immunotherapeutic strategies in aging-related cancers. This review summarizes current preclinical and clinical developments in paediatric brain-tumor vaccination, focusing on antigen discovery, immunologic mechanisms, vaccine platforms, and translational challenges that define this evolving frontier in paediatric neuro-oncology.

Keywords: Paediatric brain tumors, therapeutic vaccination, immunopeptidomics, dendritic cells, mRNA vaccine, high-grade glioma

1. Introduction

Paediatric malignant brain tumors constitute a biologically distinct and clinically devastating subset of childhood cancers. Together, high-grade gliomas including diffuse midline glioma/diffuse intrinsic pontine glioma (DMG/DIPG), atypical teratoid/rhabdoid tumors (AT/RT), medulloblastoma (MB) and anaplastic ependymoma (EPN) account for a major proportion of paediatric Central Nervous System (CNS) tumor mortality despite representing just 30% of all paediatric brain tumors[1-5]. Unlike their adult counterparts, these tumors often exhibit a low tumor-mutational burden and are rather driven by developmental epigenetic dysregulation than by accumulation of somatic mutations[6,7]. Consequently, the repertoire of actionable neoantigens is restricted, and immune-checkpoint inhibitors show limited efficacy despite acceptable safety profiles[8].

Therapeutic vaccination aims to overcome this limitation by *de novo* generating antigen-specific immunity. Unlike checkpoint blockade, which unleashes pre-existing T-cell responses, vaccination primes naïve T cells, establishes immunologic memory, and may convert immunologically "cold" tumors like gliomas into inflamed, T-cell-permissive microenvironments[9]. Proof-of-principle for successful neoantigen-directed vaccination has been demonstrated in isocitrate dehydrogenase 1 (IDH1)-mutant adult gliomas[10], supporting the rationale to explore similar strategies in paediatric high-grade gliomas (HGG) despite differing genomic drivers. Given the preserved thymic function, resulting in relative abundance of naïve T cells, preserved T-cell receptor diversity, and reduced age-related immune exhaustion in children, paediatric patients may be more capable of generating sustained and high-quality immune responses following therapeutic vaccination[11-13].

The post-relapse setting may provide a window of opportunity to evaluate therapeutic vaccination in a peculiar situation. Current data show the feasibility of vaccination in the post-relapse setting; however, the prerequisite of tumor burden reduction by surgical means is often not possible due to disseminated disease and patient tolerability. Instead, re-irradiation is a widely used *ad hoc* treatment modality in relapsed brain tumors[14]. Moreover, in a preclinical vaccine model irradiation of the tumor material improved

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the vaccine-mediated tumor rejection capacity and improved overall survival in glioma-challenged mice[15]. Whether recurrence is consistently associated with altered antigen-presentation capacity, and whether such changes influence vaccine efficacy, remains to be established.

Multi-omic profiling and immunopeptidomics support the notion that, despite the low genuine mutational load, paediatric CNS tumors share conserved sets of naturally presented tumor-exclusive peptides across different entities[16]. These insights provide the molecular foundation for vaccine development in paediatric brain tumors and highlight the necessity of age-adapted immunotherapy frameworks[9]. Moreover, early clinical trials with CAR T cells, another cellular immunotherapy, have shown first positive signals in children with relapsed/refractory CNS tumors. Infusions with chimeric antigen receptor (CAR) T directed against GD2, human erb-b2 receptor tyrosine kinase 2 (HER-2) and B7H3 have shown manageable toxicity and clinical signals in subgroups of patients[17-19]. Although beyond the scope of this review, these data underline the potential of T-cells to mount anti-tumor responses in the CNS.

This review highlights our current knowledge on cancer vaccines with a special emphasis on relapsed/refractory paediatric brain tumors. We summarize novel findings in immunopeptidomics-guided antigen discovery, present evidence for clinical efficacy of cancer vaccines in this patient cohort and discuss unsolved translational challenges such as appropriate patient selection, treatment timing, and integration into multimodal therapy regimens.

2. Immunological Rationale for Vaccination in Paediatric Brain Tumors

Historically, the CNS was regarded as immune-privileged based on the observation that skin homografts transplanted to the brain cannot elicit an immune state[20]. However, the discovery of meningeal lymphatic drainage and antigen-trafficking pathways established the brain as an immune-specialized rather than immune-isolated organ[21-23]. This paradigm shift legitimized active immunotherapies, including vaccination, for brain tumors.

While commonly described immune-evasion mechanisms in malignant gliomas include downregulation of major histocompatibility complex (MHC) class I, immunosuppressive cytokine signaling (e.g., transforming growth factor-beta (TGF-β) and IL-10), the accumulation of regulatory T cells (T_{regs}) and myeloid-derived suppressor cells (MDSCs)[24], the paediatric brain-tumor microenvironment also exhibits distinctive features related to microglial heterogeneity, developmental state, and functional plasticity[25].

These factors create a profoundly suppressive tumor microenvironment (TME) that might hamper cytotoxic responses. Although several of these mechanisms are detectable on a systemic level, glioma patients generally do not suffer from severe opportunistic infections and are still able to mount anti-tumor immune responses[26]. Collectively, current data suggest that vaccination strategies are capable of inducing systemic, antigen-specific $CD8^+$ and $CD4^+$ T cell responses in high-grade glioma patients; however, activated T cells will face challenges when infiltrating the tumor stroma.

The paediatric immune system with its preponderance of naïve elements is highly capable of mounting strong responses to novel epitopes. Indeed, early clinical data have shown that thymic function influences tumor antigen recognition and glioma-associated mortality[27]. Clinical trials have confirmed that younger age is a positive prognostic factor in immunotherapy trials[28]. Moreover, radiotherapy can synergize with vaccination by increasing antigen release and upregulating MHC expression[29,30]. Irradiation is frequently used as a standard therapy in children with brain tumors, both in the primary as well as in relapsed setting[31-33]. Insights from adult studies suggest that combining radiotherapy with vaccination can enhance antigen presentation and immune visibility of tumors, although paediatric-specific data are still limited[34].

In contrast, ageing is associated with thymic involution and a reduction in the naïve T-cell pool[35,36]. Changes in T-cell receptor (TCR) repertoire composition have also been described in older adults, although the relationship between thymic involution and TCR diversity in humans is complex and repertoire diversity may remain relatively well preserved despite age-related changes in clonal composition[35]. In addition, ageing is accompanied by the accumulation of highly differentiated and exhausted T-cell subsets[36]. Thus, immunological findings in pediatric populations cannot be directly extrapolated to older adults, and age-specific validation is required.

3. Antigen Discovery and Immunopeptidomic Insights

Antigen discovery pipelines in paediatric brain tumors have shifted from *in silico* prediction towards direct mapping of peptides naturally presented on human leukocyte antigen (HLA) molecules. Extrapolation of gene expression data to immune presentation by *in silico* prediction has yielded inconsistent results. Only for a minority of expressed genes a peptide fragment of the translated protein will make its way through the antigen processing and HLA loading machinery to the cell surface. In contrast, immunopeptidomics, large-scale mass-spectrometric (MS) identification of naturally expressed, HLA-bound ligands, provides an unbiased way of target identification. It is meanwhile considered the gold standard of antigen discovery and underpins rational vaccine design[9,37]. This novel technique has already been used to decipher the immunopeptidome of several paediatric brain tumors.

AT/RT, highly malignant brain tumors of infancy and early childhood, are characterised by a single deletion mutation in the

SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily B member 1 (*SMARCB1*) gene. Thus, not many neoantigens can be expected in such tumors. In melanoma (one of the cancers with the highest abundance of mutations) it has been demonstrated that single nucleotide variant (SNV)-derived peptides are exceedingly rare in the tumor ligandome[38]. Immunopeptidomics of AT/RTs in fact revealed that the majority of AT/RTs harbor a very low mutational load and no neoantigenic peptide could be detected by MS, particularly none of the *SMARCB1* locus[39]. However, abundant tumor-specific HLA class I and class II peptides from classically expressed proteins (coding sequence (CDS)-encoded) as well as from cryptic, i.e. non-canonical reading frames could be obtained. Notably, these peptides were not expressed on any other benign tissue including brain and the vast majority (> 85%) of peptides were recognized by naïve $CD8^+$ T cells. Interestingly, more than 50% of AT/RT-specific HLA class II peptides were also recognized by T cells from children with high-grade gliomas, suggesting a potential antigenic overlap in paediatric brain tumors[39].

MB is another paradigmatic paediatric brain tumor entity with embryonal features. Although few neoepitopes per tumor might exist[40], also in MB the vast majority of tumor-specific peptides is derived from classical proteins aberrantly expressed in the tumor[41]. After subtraction of peptides from benign tissues and careful manual curation 15 and 38 MB-specific source proteins could be delineated from which peptides in HLA class I and class II respectively are identifiable. Remarkably, most of the MB-associated peptides and proteins were subgroup-specific (WNT, SHH, Group 3, Group 4), with little overlap between the groups, yet a high level of immune reactivity. These data suggest that also in MB substantial numbers of actionable immune targets exist, which can be exploited for therapeutic approaches[41].

Next, Mühlenbruch *et al.* analyzed 22 EPN including samples from 5 young adults and identified over 6,000 unique HLA class I and II peptides, including 162 tumor-exclusive source HLA class I proteins[16]. Several epitopes elicited spontaneous T-cell responses in patient specimens. Again, 31% of class I and 13% of class II ligands overlapped with glioblastoma (GBM) ligandomes, revealing conserved antigenic networks across different entities of paediatric and adult brain tumors.

Finally, several published data sets exist on the immunopeptidome of HGG. These data were mainly obtained from adult specimen with just a few children included in these cohorts[42-44]. Like in AT/RT, MB, and EPN peptides with sequences derived from the oncogenic drivers of the tumors are usually not represented in the glioma HLA-ligandome. However, tumor-specific peptides from classic and cryptic source proteins offer a plethora of immunogenic targets that can be used for vaccination strategies. Cryptic peptides derive from fugacious short and aberrant proteins, encoded by non-canonical open reading frames, 5'- or 3'- untranslated regions, introns, or other non-coding strands[45]. Their frequency is not normally distributed between HLA haplotypes but they seem to be enriched in cancer[37]. Whether they can substitute for the general paucity of SNV-derived neoantigens in paediatric tumors is currently investigated by several groups.

In DMG, a paediatric subtype of high-grade gliomas, a special focus has been set on the oncogenic mutation in the H3 histone. The canonical H3.3 K27M mutation encodes the $CD8^+$ T-cell-restricted neoepitope $H3.3K27M_{26-35}$ with strong binding to HLA-A02:01 that provokes expansion of reactive $CD8^+$ T cells in vaccinated and responding patients[46]. However, this neoepitope could not be detected in unbiased immunopeptidomic analyses, even with highly sensitive mass-spectrometric methods[47]. Furthermore, T cells carrying a transgenic TCR with high avidity for the epitope were not able to recognize cells naturally expressing the H3.3K27M mutation, even when overexpressed as a transgene[48]. These data raise doubt about the suitability of this particular epitope for cancer immunotherapy and highlight the general need to validate *in silico* predicted epitopes for presence in the ligandome and suitability for cancer cell killing. Albeit not perfectly suited as an immune target itself, the H3.3K27M mutation seems to shape the immunopeptidome of DMGs resulting in unique peptide sequences that are undetectable in corresponding knock-out models[49]. The H3.3K27M mutation apparently promotes the turnover of other histones as peptides from the histone H2B1K were repetitively found in several DMG samples[50].

In past and present glioma peptide vaccination trials for HGG-related antigens such as IL-13R α2, EphA2, Survivin and WT1 have been used as immunization targets. E.g., a peptide vaccine targeting survivin (SurVaxM) has undergone evaluation in a PhaseIIa trial in adult glioblastoma[51]. However, it is important to understand that these frequently used peptides used for glioma treatment have not been consistently found in immunopeptidomic analyses of glioma tissue. This indicates that their prioritization in vaccine strategies has largely been driven by gene expression data rather than reproducible evidence of natural HLA presentation. Antigen prioritization based on unbiased immunopeptidomic data provides a much more robust biological rationale. We therefore compared the source-protein repertoires reported in the three available glioma immunopeptidomic studies by Dutoit *et al.* 2012, Neidert *et al.* 2018, and Hilf *et al.* 2019[42-44]. Despite differences in patient cohorts, HLA restriction, and analytical depth, these studies reveal a conserved set of recurrently presented glioma-associated proteins at the source-protein level. Notably, extracellular matrix and glial lineage–associated proteins, including brevican (BCAN), protein tyrosine phosphatase receptor type Z1 (PTPRZ1), tenascin-C (TNC), and, to a lesser extent, chondroitin sulfate proteoglycan 4 (CSPG4), emerge as shared antigens across datasets (Table 1). These proteins emerge from glial differentiation programs with limited expression in benign tissues, underscoring their suitability for vaccine strategies. The data also underline the importance of targeting the glioma extracellular matrix as the glioma microenvironment represents a pivotal tumor-promoting factor.

Table 1. Overview on glioma-associated source proteins, from which peptides repetitively have been described in glioma ligandome databases versus those used in clinical peptide vaccination trials.

Source protein	Dutoit 2012[42]	Neidert 2018[44]	Hilf 2019[43]	Comment
BCAN	✓	✓	✓	glial ECM-proteoglycan; strong glioma-association
PTPRZ1	✓	✓	✓	glial lineage marker, signal transduction; consistently found in glioma ligandomes
CSPG4 (NG2)	✓	✓	(✓)	frequently found in aggressive gliomas
TNC	✓	✓	✓	Glial ECM-protein, tumor associated isoforms
EGFR (wt)	✓	✓	(-)	presentation HLA-dependent
OLIG2	(–)	✓	✓	lineage-specific transcription factor
GFAP	✓	(–)	(–)	immunodominant peptides but not tumor-specific
SLC1A3 (EAAT1)	(–)	✓	✓	astroglial signature
ALDH1A1	✓	✓	(–)	metabolic enzyme, association with tumor stem cells
IDH1 (WT)	✓	(✓)	(–)	non-mutated isoform, little specificity
B7-H3	(–)	(–)	(–)	surface antigen, CAR T target, no evidence for presence in HLA-ligandome
Survivin	(–)	(–)	(–)	frequently expressed in glioma, but no sound evidence for presence in HLA-ligandome
WT1	(–)	(–)	(–)	expressed in glioma, but no sound evidence for presence in HLA-ligandome
EphA2	(–)	(–)	(–)	variably expressed in glioma, but no sound evidence for presence in HLA-ligandome

BCAN: brevican; WT1: Wilms' tumor protein; HLA: human leukocyte antigen; IDH1: isocitrate dehydrogenase 1; CAR: chimeric antigen receptor; TNC: tenascin-C; PTPRZ1: protein tyrosine phosphatase receptor type Z1; CSPG4: chondroitin sulfate proteoglycan 4; OLIG2: oligodendrocyte transcription factor 2; GFAP: glial fibrillary acidic protein; ALDH1A1: aldehyde dehydrogenase 1A1; SLC1A3: solute carrier family 1 member 3.

Collectively, these findings indicate that the naturally presented antigenic repertoire in paediatric brain tumors is enriched for lineage- and TME-associated epitopes rather than for mutational or overexpressed targets, reinforcing the value of immunopeptidomics-guided antigen selection. The conceptual distinction between antigens defined by surface expression and those validated by natural HLA presentation is highlighted in Box 1.

Box 1. Surface antigens *vs.* immunopeptidomic glioma-associated immunogenic antigens

Glioma-associated antigens comprise biologically distinct classes. Surface antigens (e.g., B7-H3) are defined by stable cell-surface expression, independent of antigen processing and HLA presentation, and are primarily relevant for antibody-based or CAR T-cell therapies[54]. In contrast, immunopeptidomic antigens are defined by naturally processed and HLA-presented peptides identified by mass spectrometry, which represents the gold standard for antigen validation in T-cell-based vaccine strategies[55,56]. These peptides have undergone natural processing and selection in the antigen processing and HLA loading machinery. Distinguishing between these classes is essential for rational antigen prioritization.

Most of the above-mentioned entities such as low or high-grade gliomas, AT/RTs or ependymomas may present with spinal manifestations in the primary or relapsed disease setting. Spinal tumor occurrences pose a particular challenge for immunotherapy as reviewed recently[52]. To overcome these hurdles, immunotherapeutic strategies have to be combined with additional disease modifiers to improve efficacy. Exemplarily, Pang *et al.* demonstrated that cytarabine not only induces growth inhibition and a senescence-associated secretory phenotype in spinal cord glioma cells but also upregulation of checkpoint molecules and immune cell infiltration. Cytarabine (Ara-C) plus programmed cell death protein 1 anti-PD-1 and Ig and ITIM domains (TIGIT) checkpoint blockade might emerge as a novel strategy to enhance immune response against spinal cord tumors. Three out of four patients treated with this combination in a pilot cohort showed a clinical benefit[53].

4. Preclinical Vaccine Platforms and Mechanistic Advances

Preclinical models have established multiple complementary vaccine approaches for paediatric brain tumors. The currently available therapeutic vaccine platforms, their mechanisms of action, and their respective advantages and limitations are summarized in Figure 1.

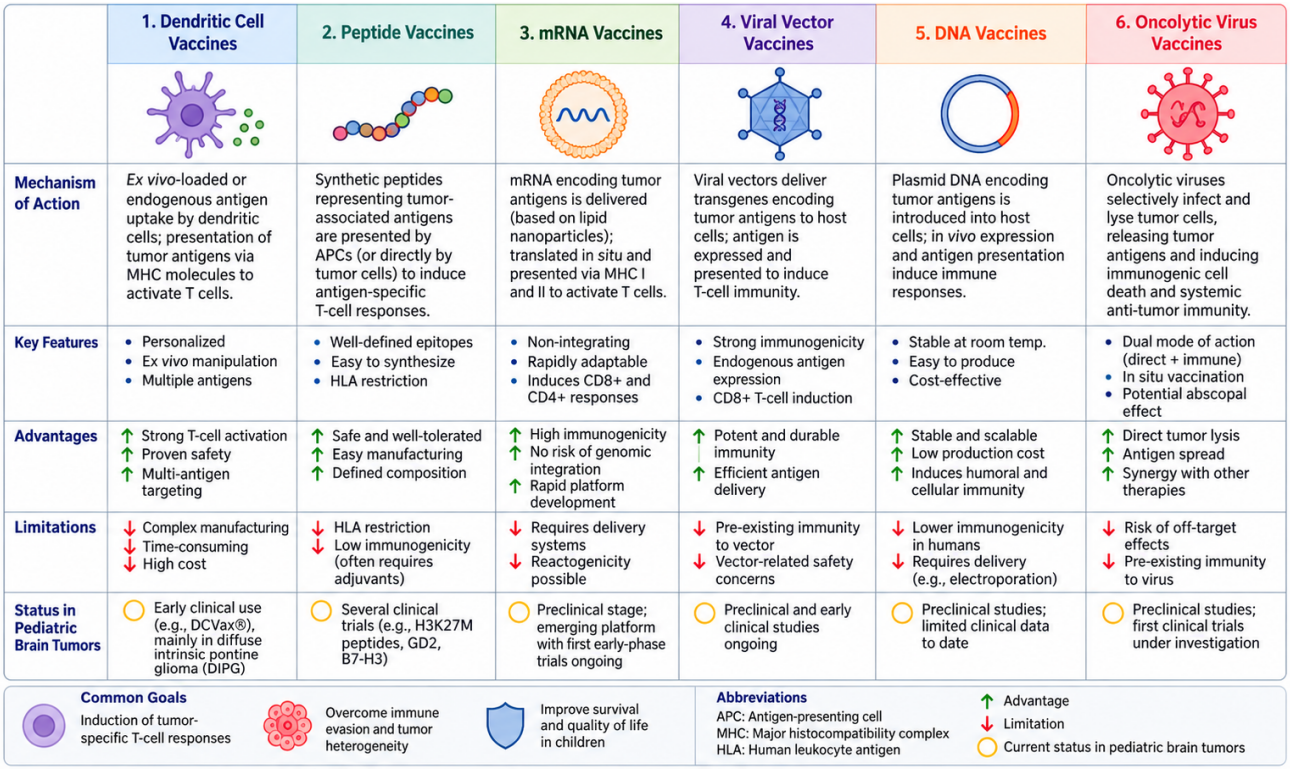


Figure 1. Overview of therapeutic cancer vaccine platforms for pediatric brain tumors: Mechanisms of action, advantages, and limitations of current vaccine approaches. The schematic provides a comparative overview of the main therapeutic cancer vaccine platforms investigated for pediatric brain tumors.

DC-based vaccines leverage professional antigen presentation to activate cytotoxic T lymphocyte (CTL) responses. In brain tumors this activation must take place in secondary lymphatic organs as DCs usually do not enter the CNS. The use of DCs and tumor lysate takes advantage of the fundamental property of DCs to process exogenous antigen and optimize peptide presentation. Furthermore, tumor lysate is relatively easy and inexpensive to produce and personalized by nature. In murine high-grade glioma models, DCs pulsed with tumor lysate or synthetic peptides elicit robust $CD8^+$ T-cell responses and delay tumor progression[57,58]. A lot of emphasis has been put on the DC maturation process. Adjuvants such as toll-like receptor 3 (TLR3) or TLR7/8 agonists can further enhance IL-12 production and Th1-type immune polarization in DCs[59,60], whereas prostaglandin E_2 (PGE_2) should rather be used with caution[61]. Transfection of DCs to secrete interferon (IFN)α or addition of inducible protein-10 (IP-10) can further enhance the stimulatory capacity of DCs[62-64]. Several published and validated good manufacturing practice (GMP)-compliant manufacturing protocols make DC generation for clinical use nowadays a straightforward process[65,66].

Replacing tumor lysate as the antigenic source by specific peptides offers the advantage of having the targeted epitope present in the vaccine in a pharmacologically relevant quantity. Additionally, immune monitoring after vaccination is greatly simplified and more precise. However, the use of the right target peptides remains an unsolved challenge. As mentioned above, peptides used in past glioma vaccination trials such as survivin, IL13Rα2 or Wilms' tumor protein (WT-1) were only partially confirmed in immunopeptidomic analyses. Building on these findings, novel vaccine strategies are being investigated to broaden the anti-tumor immune response, address the issue of clonal heterogeneity, and reduce the risk of antigen-loss variants at relapse[67,68]. These approaches combine multiple tumor-specific peptides or patient-specific neoepitopes, potentially enhancing the durability and breadth of immunogenicity in paediatric gliomas. The Glioma Actively Personalized Vaccine Consortium (GAPVAC) consortium has vaccinated adult HGG patients first with a set of glioma-specific warehouse peptides in a first round and then several weeks later with a second set of personalized peptide targets, mainly from SNV-derived neoantigens. Patients with newly diagnosed GBM in this trial showed a favourable overall survival (OS) of 29 months. However, epitope selection and peptide generation in this trial was extremely laborious and cost-intense since all targets had to be separately validated and manufactured under GMP conditions[43]. For a broader applicability of this approach, regulatory frameworks for early clinical trials have to be simplified to allow a more streamlined identification and manufacturing of personalized vaccine cocktails.

In parallel to multipeptide vaccines, multi-antigen mRNA platforms are rapidly being developed and explored in preclinical and early clinical studies[68,69]. A recently published mRNA-technique allows the targeting of multiple neoantigenic and aberrantly overexpressed tumor antigens in one personalized vaccine, which proved to be immunogenic and efficacious in a highly aggressive murine GBM model[68]. DC vaccines electroporated with WT-1 mRNA achieved measurable CTL expansion and were well tolerated in an adult GBM cohort[70].

In neuroblastoma, another paediatric neuronal tumor of embryonic origin, the tumor ligandome was found to be enriched for peptides derived from aberrantly expressed lineage-restricted genes, namely from noradrenergic neuron differentiation and sympathetic nervous system development[71]. The most significantly enriched group of parental genes were nucleic acid-binding proteins, particularly core regulatory circuit transcription factors, making peptides from these source proteins prime candidates for therapeutic targeting. Furthermore, the group succeeded to generate a peptide-centric chimeric antigen receptor, able to recognize peptides bound to structurally similar HLA molecules. This technique might potentially close the gap between immunopeptidomic research and CAR T technology[71].

Finally, preclinical studies provide a strong rationale for combining different immunotherapeutic principles in one treatment strategy. In murine GL261 glioma models, the combination of a therapeutic vaccine with aPD-1 checkpoint blockade significantly prolonged survival, increased tumor-homing properties of $CD8^+$ T-cells, promoted memory formation and finally prolonged survival in treated mice[72]. Similarly, oncolytic viruses such as IL-21-expressing Vaccinia or modified measles virus synergize with PD-1 blockade to strengthen antitumor immune responses and extend survival in GL261 or CT-2A models[73,74]. Radiotherapy combined with anti-PD-1 also demonstrates synergistic efficacy, with both single-dose and fractionated regimens outperforming monotherapy in preclinical settings[75,76].

Collectively, these findings support further exploration of combinatorial immunotherapy, integrating vaccination, checkpoint blockade, oncolytic viruses, and radiotherapy, to overcome immune suppression in paediatric CNS malignancies. Together with the advances in antigen discovery and vaccination design, these insights provide a translational bridge toward personalized and more effective immunotherapy in paediatric brain tumors.

One important factor that may affect vaccine efficacy is age. Age-related changes should therefore be considered when designing vaccination strategies. These considerations should include the optimal timing of vaccination, the choice of adjuvants, and the potential benefit of combination approaches to enhance antigen presentation or T-cell activation.

For instance, the structurally low tumor mutational burden (TMB) in HGG (only 3.5% of gliomas have a high tumor mutational load and a corresponding biomarker phenotype[77]) requires vaccines to rely on shared glioma-associated antigens (GAAs such as EphA2, interleukin-13 receptor alpha 2 (IL-13Rα2), and EGFRvIII) or specific clonal driver mutations, most notably the histone variant H3.3K27M.

Furthermore, functionality of paediatric immune cells exhibits a baseline propensity towards a Th2-skewed immune phenotype[78]. To overcome this barrier and enforce a robust, cytotoxic Th1-polarized anti-tumor response (characterized by IFN-γ secretion and $CD8^+$ T-cell activation), selection of optimal vaccine adjuvants is critical. In early-phase paediatric clinical trials, the combination of synthetic peptides with the TLR3 agonist polyinosinic-polycytidylic (poly-IC) stabilized with poly-lysine and carboxymethylcellulose (**poly-ICLC**) (Hiltonol) has been established to effectively prime dendritic cells and enhance the homing of antigen-specific cytotoxic T-lymphocytes into the central nervous system[79,80].

Optimizing timing between potentially lymphotoxic radiochemotherapy regimens, corticosteroid use (which can deplete naive T-cell populations), and vaccine delivery remains a major challenge[14]. A better preserved immunity and faster immune reconstitution after radiochemotherapy can be expected in children compared to older adults. To prevent premature T-cell exhaustion and turn immunologically “cold” paediatric tumors into “hot” therapeutic targets, multi-modal combination strategies are currently being evaluated. For example, multi-center trials conducted by the Pacific Pediatric Neuro-Oncology Consortium (PNOC), such as the PNOC007 trial, evaluate the synergistic triad of an H3.3K27M-specific peptide vaccine combined with poly-ICLC as an adjuvant and the PD-1 immune checkpoint inhibitor (ICI) nivolumab to prolong overall survival[46]. Whether such treatment designs are effective in the paediatric and adult setting alike remains to be evaluated in age-specific clinical studies.

5. Clinical Evidence in Relapsed and Refractory Paediatric Brain Tumors

DC–based vaccination has been the most extensively explored therapeutic vaccine platform in paediatric malignant brain tumors. Across multiple early-phase trials, autologous DCs loaded with tumor lysate, tumor RNA, or defined antigens have reproducibly demonstrated safety, feasible manufacturing, and consistent induction of tumor-reactive T-cell responses. A summary of paediatric trials in that indication is given in Table 2.

Interestingly, one of the first studies on therapeutic vaccines in paediatric brain tumors used DCs transfected with total tumor DNA instead of lysate. Three out of nine treated children, adolescents and young adults with recurrent brain tumors showed a clinical response (2 stable disease, 1 partial response)[81]. In the same year, a German-Belgian consortium reported in a mixed adult-paediatric

cohort that gross total resection (GTR) is a prognostic factor for patients with relapsed glioma treated with immunotherapy. 2/6 patients with GTR followed by DC-based vaccines remained in complete remission (CR) after 3 years, whereas all patients with partial resection relapsed[82]. A potential benefit of DC-based therapeutic vaccination in children with gliomas in whom a minimal residual disease situation can be achieved, was also confirmed in a smaller series of 7 patients[83]. De Vleeschouwer confirmed in a larger cohort of 56 adult and paediatric patients that GTR and younger age are pivotal prognostic factors[28].

Table 2. Overview on pediatric therapeutic vaccine trials in relapsed or refractory high-grade brain tumors.

Study/Year	Vaccine Platform	Target Antigen(s)	Study Population	Key Findings
Caruso *et al.*, 2004 Phase I	Total tumor RNA-loaded DC	total tumor proteome	9 children with recurrent brain tumors	feasibility, safety, in 2/7 patients T-cell activation, 2/7 SD, 1/7 PR
Rutkowski *et al.* 2004	Tumor lysate loaded DC	total tumor proteome	12 patients (11-78 years) with relapsed malignant glioma	2/6 with GTR in CR after 3 years after partial resection: 1/6 SD, 1/6 PR
De Vleeschouwer *et al.* 2008	tumor lysate loaded DC	total tumor proteome	56 patients with relapsed GBM (7-77 years)	median OS 9,6 mo, 2-year OS 14.8%, younger age and complete resection predictors for better outcome
Ardon *et al.*, 2010	tumor lysate loaded DC	total tumor proteome	45 children with relapsed malignant brain tumors	feasibility, safety; efficacy: HGG 7/33 alive after FU of 35.7 months, median OS 13.5 mo MB: median OS 5.7 mo EP: 1/4 patients alive after 22.3 mo AT/RT: 2/3 patients alive after 34.1 and 52.6 months, 1 died after 50.5 mo
Cho *et al*. 2012	lysate from primary cultures loaded on DCs	total tumor proteome	34 patients with GBM (14-70 years), randomisation: 18 treated, 16 controls	2- and 3-year OS 44.4 *vs.* 18.8% and 16.7 *vs.* 0% median OS 31.0 *vs.* 15 mo
Lasky *et al.*, 2013	tumor lysate loaded DC	total tumor proteome	children with recurrent HGG , 7 enrolled, 3 treated	2/3 alive after 51 and 40 mo 1/3 DOD after 9 mo
van Gool *et al.* 2016	tumor lysate loaded DC	total tumor proteome	7 children with AT/RT	3/7 alive after 143, 138, and 46 mo 2 of them harbor somatic mutation
Benitez-Riba *et al*. 2018	lysate from allogeneic DIPG cell lines loaded on DCs	total tumor proteome	9 children with DIPG	immune response in 8/9 patients no outcome data reported
Mueller *et al.*, 2020 Phase I/II	H3.3K27M$_{26-35}$ peptide, HLA-A02:01 restricted	H3.3 histone mutation	21 children and AYA with DMG/DIPG (3-21 years)	median OS 16.1 *vs.* 9.8 mo in immunologic responders *vs.* non-responders, below median MDSC prolong OS
Dunkel *et al.*, 2023 Phase I	-	anti-PD-1 ± aCTLA-4	166 children with high-grade CNS malignancies in DIPG, HGG, MB, EP and other	grade 3/4 adverse events in 14.1 *vs.* 27.2% anti-PD-1 monotherapy *vs.* combination therapy, no new safety signals, no detectable clinical benefit

			CNS tumors	to historical data in all five cohorts
Thompson *et al.*, 2025	CMV-pp65 synthetic long peptide (26 aa)	CMV pp65 protein	42 patients (4-35 years) with recurrent malignant brain tumors, 30 cases with HGG, 2 with MB	safety: 17 grade 1, 17 grade 2, 1 grade 3 (pyramidal tract syndrome), and 1 grade 4 (cerebral edema) AE efficacy: for HGG patients median OS was 6.5 mo, 12-month OS 28% for the two MB patients OS was 1.8 and 0.8 months increase of estimated 46 spots in IFN-γ pp65 ELISPOT assay
Trials ongoing:				
Neoepitope-Based Personalized DNA Vaccine Approach in Pediatric Patients With Recurrent Brain TumorsNCT03988283 Phase I/II *With Recurrent Brain Tumors NCT03988283* Phase I/II	Neoepitope-based personalized DNA vaccine delivered by Papivax Biotech TDS-IM v2.0	personalized neoantigen DNA vaccine	25 children and AYA (age 12-25 years) with recurrent CNS tumor	trial is recruiting primary endpoint: safety, feasibility and tolerability secondary endpoint: median PFS and OS
Adjuvant DC immunotherapy complementing conventional therapy for pediatric HGG and DIPG patients *Eudra-CT 2020-004125-23* Phase I/II	WT-1 mRNA electroporated DCs	WT-1	10 children with HGG and DIPG	recruitment completed, data analysis ongoing primary endpoint: feasibility of vaccine production, feasibility and safety of the vaccine in combination with SOC secondary endpoint: clinical activity, immunogenicity, quality of life
HIT-HGG Rez Immunovac *Eudra-CT 2013-000419-26* Phase I/II	tumor lysate loaded DCs + T_{reg} depletion + anti-PD-1/aCTLA-4	total tumor proteome	25 children and AYA with recurrent HGG	recruitment completed, data analysis ongoing primary endpoint: 6-months OS secondary endpoints: PFS, OS, safety, immune reactivity & immune parameters

AE: adverse event; AYA: adolescents and young adults; CNS: central nervous system; CR: complete remission; DC: dendritic cells; DMG: diffuse midline glioma; FU: follow-up; GBM: glioblastoma multiforme; GTR: gross total resection; HGG: high-grade glioma; MB: medulloblastoma; MDSC: myeloid-derived suppressor cell; mo: months; OS: overall survival; PR: partial response, SD: stable disease; SOC: standard of care; PD-1: programmed cell death protein 1; DIPG: diffuse intrinsic pontine glioma; WT-1: Wilms' tumor protein; CMV: cytomegalovirus; HLA: human leukocyte antigen; AT/RT: atypical teratoid/rhabdoid tumors; IFN: interferon; PFS: progression-free survival; TDS: TriGrid Delivery System; CTLA: cytotoxic T-lymphocyte-associated protein 4; ELISPOT: enzyme-linked immunospot assay; DOD: died of disease.

The only randomized trial in which children were included, published by Cho *et al.* in 2012, randomized 34 patients with newly diagnosed high-grade glioma (age range 14-70 years) into those receiving standard of care (SOC) *vs.* SOC plus DC vaccines. The vaccinated group had a significantly higher 2-year OS (44.4 *vs.* 18.8 in vaccinated *vs.* non-vaccinated patients, respectively) and a median overall survival of 31.9 *vs.* 15.0 months[84].

The largest purely paediatric cohort was published in 2010. 45 children suffering from different brain tumors were treated with tumor-lysate loaded DCs: 7 out of 22 glioma patients survived for a median of 13.5 months, 1 out of 4 EPN patients survived 22.3 months, 2 out of 3 AT/RT patients survived 34.1 and 52.6 months, whereas all MB patients died after a median of 5.7 months[85]. A positive effect of immunotherapy in children with AT/RTs was also reported in a survey of the HGG-immuno group, in which 3 out of 7 AT/RT patients treated with DC vaccines survived long-term[86].

More recently, therapeutic vaccines were also evaluated in DIPG. Benitez-Riba reported feasibility and safety of the approach in nine newly diagnosed DIPG patients. 8 of them showed vaccine-specific immune responses[87]. DIPG-vaccines in this trial were generated by loading DCs with lysate from five allogeneic DIPG cell lines. Another study used DCs electroporated with WT-1 mRNA for treatment of primary and progressive DIPG[88]. The trial has completed recruitment and results are awaited soon. As mentioned above, DMGs represent a paediatric subgroup of high-grade glioma in the deeper brain regions and are characterized by the oncogenic H3.3K27M histone mutation. An HLA-A02:01 specific epitope spanning the mutation was generated and used for vaccination in 10 DMG and 19 DIPG patients[46]. The OS at 12 months was 39% and 40% in the two cohorts, respectively. Patients with an expansion of vaccine-specific $CD8^+$ T cells had an improved OS compared to non-responders (median OS 16.1 *vs.* 9.8 months in responders *vs.* non-responders, respectively).

Finally, Thomson *et al.* reported in 2025 on the feasibility and safety of a cytomegalovirus (CMV)-pp65 peptide vaccine in paediatric brain tumor patients with either HGG or MB[89]. Background of this approach is the observation that CMV-DNA is frequently detectable in HGG and MB tumor DNA[90,91], raising the expectation that CMV-directed T-cell responses might also combat HGG or MB growth. Only patients with recurrent HGG were evaluable in that trial. In general, the vaccine was well tolerated and elicited pp65-specific immune responses; however, the median OS in the study population was only 6.5 months[89].

The profoundly immunosuppressive TME in paediatric high-grade CNS tumors has motivated investigation into combinatorial immunotherapy approaches[9]. In adults, anti-PD-1 treatment of HGG in the CheckMate 143 trial had no measurable survival benefit[92]. While safety profiles and exploratory outcome data from the initial phase I NRG-BN002 trial of combined ipilimumab and nivolumab in newly diagnosed glioblastoma have already been reported[93], the subsequent randomized phase II/III NRG-BN007 trial recently demonstrated no significant survival benefit over standard radiochemotherapy[94]. Alternative dual-targeting strategies are currently under evaluation in clinical trials. In children, double checkpoint blockade has been evaluated for safety and efficacy. In the CheckMate908 trial, anti-PD-1 was administered either as a monotherapy or in combination with aCTLA4 as a dual checkpoint blockade. Despite good tolerability, no survival benefit was noted for both regimens in all analyzed subcohorts (DIPG, HGG, MB, EPN, AT/RT and others)[8]. Our *HIT-HGG Rez Immunovac* trial (Eudra-CT 2013-000419-26), the first clinical study which combined T_{reg} depletion with therapeutic DC vaccines and double checkpoint blockade in children and adolescents and young adults (AYA) with recurrent HGG finished recruitment of the planned 25 patients and data are currently being analyzed.

Taken together, these studies establish DC-based vaccines as safe, immunogenic, and biologically active, even though their impact on progression-free survival (PFS) and OS remains exploratory in early-phase trials. The H3.3 K27M as well as the IDH1-peptide vaccine provide proof of concept for neoantigenic immunization in paediatric CNS tumors, eliciting systemic T-cell responses without observed toxicity. However, the apparent gap between immunogenicity, i.e. induction of measurable T-cell responses, and limited clinical efficacy points towards the need to combine vaccines with modulators of the TME to enhance homing, persistence and finally killing capabilities of induced T cells. While adjuvant checkpoint inhibition has shown no clinical benefit[8,92], neoadjuvant anti-PD-1 immunotherapy prolonged survival in relapsed HGG by promoting intratumoral immune responses[95]. For other agents such as antiangiogenic substances or sensitizing agents, preclinical and clinical evidence is emerging as reviewed recently[96]. Regarding optimal timing and placement of vaccines in multimodal treatment regimens, the available data suggest that therapeutic vaccination may be most effective in patients with minimal residual disease following maximal safe resection when tumor burden is low. Indeed, gross total resection and younger age were repeatedly identified as favorable prognostic factors in vaccination studies[28,82,97]. Furthermore, radiotherapy may synergize with vaccination[98] by promoting immunogenic cell death, enhancing antigen release, and increasing MHC expression, thereby facilitating T-cell priming and epitope spreading[15,29,30]. Patients with solid tumors might benefit from prolonged vaccination schedules[98]. Future clinical studies should therefore not only evaluate vaccine efficacy but also define the optimal sequencing of vaccination with surgery, radiotherapy, chemotherapy, and emerging immunomodulatory therapies.

6. Challenges and Ethical Considerations

The potential of therapeutic vaccines for children with relapsed or refractory brain tumors is not yet fully exploited. Several obstacles and challenges remain that ought to be addressed in preclinical research and clinical trials. Paradigmatic is the high clonal heterogeneity and development of antigen-loss variants under therapy which undermine the success of mono-antigen vaccines. Multipeptide or mRNA platforms may offset this issue. Loading synthetic long peptides (> 20 aa) on immature DCs instead of using 9mers suitable for external HLA peptide replacement will render the need for adaptation to HLA haplotypes unnecessary and lead to the generation of HLA class II peptides. For T cells, the TME of brain tumors is highly hostile and additional interventions are needed to alleviate T-cell function and survival in this setting.

For the design of clinical trials, it has to be considered that the study population is highly vulnerable and protected under the EU Regulation 1901/2006 and ICH E11A (2022) guideline. Care must be taken that the minor trial participants are not exposed to unjustifiable risks. On the other hand, also children have the right to partake in clinical trials and medical progress; therefore, a well-balanced risk-benefit assessment in all paediatric trials is warranted. Recruitment in clinical studies of childhood cancer frequently suffers from slow recruitment and low patient numbers. International collaboration is highly appreciated to allow for a sufficient number of cases to be recruited even in trials with rare diseases or subgroups.

7. Conclusions and Future Directions

Therapeutic cancer vaccination has progressed from concept to clinical feasibility in paediatric neuro-oncology. Preclinical data and early clinical trials demonstrate safety and robust immune activation in relapsed paediatric brain tumors. With robust and convincing data on safety and tolerability at hand, vaccine strategies should now be evaluated prospectively in newly diagnosed paediatric brain tumors as part of rational multimodal treatment protocols. Besides vaccines, other innovative treatment ideas such as CAR T cells, targeted therapies, tumor-treating fields or repurposed drugs are the new kids on the block and most of them have shown promising, yet not overwhelming data. The current challenge is to integrate these new options into a meaningful and validated concept given that the number of available patients for clinical trials is limited. Therapeutic vaccines most likely will find their place in the maintenance phase of treatment with the aim to establish immune surveillance and control of minimal residual disease. Precision antigen discovery by high-sensitivity immunopeptidomics, TME-mapping and -modulation, combination of different immunotherapeutic principles, and adaptive trial designs are key tools for overcoming some of the above-mentioned hurdles. Single-cell and spatial transcriptomics permit real-time response monitoring, enabling a more precise patient stratification. Global paediatric consortia like Brain Tumor Group of the European Society for Pediatric Oncology (SIOPE-BTG), PNOC, or Innovative Therapies for Children with Cancer (ITCC) will be key to conducting harmonized multi-center trials. Finally, paediatric vaccine research may provide valuable insights for the development of age-adapted cancer immunotherapy. Nevertheless, differences in immune function across human lifespan require that these approaches are independently evaluated and validated in cohorts of older cancer patients.

Declarations

Authors contribution

Knaup L, Eyrich M: Conceptualization, formal analysis, writing-original draft, writing-review & editing, validation.

Conflicts of interest

The authors declare no conflicts of interest.

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