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Ependymomas: Development of Immunotherapeutic Strategies

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Summary

Ependymomas are among the most challenging childhood brain tumors. Although 50–70% of ependymomas are cured with surgery and irradiation, a significant percentage of tumors recur. Ependymomas that are not amenable to complete resection at diagnosis have a particularly poor prognosis, and the vast majority of affected children experience tumor recurrence. Although transient responses have been observed in recurrent tumors treated with re-irradiation and several chemotherapy regimens, long-term disease control is rarely achieved. Children with recurrent disease commonly experience cumulative neurological morbidity from repeated surgical and adjuvant therapy interventions, and almost universally succumb to refractory tumor progression. Accordingly, conceptually new treatment approaches are needed, both to decrease the risk of tumor recurrence and to enhance disease control in those children who experience recurrent disease. This article reviews the current application of risk-based treatment stratification at diagnosis, the rationale for exploring the role of novel therapeutic strategies such as immunotherapy at recurrence, and the concept behind a vaccine-based trial for these tumors.

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Keywords

ependymoma; brain tumor; immunotherapy; pseudoprogression

Current Management and Challenges

Recent studies have demonstrated that the group of tumors histologically classified as ependymomas encompasses a number of subgroups of tumors that differ prognostically based on clinical, histological, and molecular features [1], and potentially based on their cell of origin [2]. Age has an important impact on tumor distribution and outcome. Adult ependymomas most commonly occur in the spinal cord are often treated with surgery alone, without adjuvant therapy, as are some intracranial lesions, if histologically low-grade and well circumscribed [3]. In the pediatric age group, tumors more commonly occur intracranially, tend to be less well circumscribed, and among young children often arise infratentorially {Godfraind, 2012 #239}, which complicates their management because of the potential morbidity of surgery around the brainstem and lower cranial nerves.

In both children and adults, the extent of tumor removal is the most important prognostic factor. Five-year survival rates generally exceed 60% after gross total resection, but are significantly lower after subtotal removal [1,3–6]. Improvements in imaging and surgical modalities have facilitated efforts to maximize resection extent. The use of image-guided conformal irradiation of the tumor bed has also been increasingly common and, when applied after extensive resection, has achieved five-year survival rates above 80% [7–9]. Tumor histology may also influence prognosis, with anaplastic (grade III) ependymomas having a significantly worse outcome than grade II lesions in several reports [1,4–5,8,10].

Recent studies for childhood ependymoma, such as the COG ACNS0121 trial, have stratified therapy based on resection extent and histology as well as tumor location, reflecting that supratentorial tumors may be more often more amenable to microscopically complete resection than infratentorial lesions. Patients undergoing gross total or near total resection have received conformal irradiation to the tumor bed plus a narrow (1 centimeter) margin, whereas the small subset of supratentorial lesions that have undergone microscopically complete resections have been observed without irradiation, based on favorable results in an institutional pilot study [11]. In contrast, patients who have had incomplete initial resections have received a short course of chemotherapy to determine whether this renders the residual disease amenable to complete removal at re-resection before irradiation. This study included children as young as one year of age in an effort to improve outcome in such patients, who have previously had suboptimal results using chemotherapy and delayed irradiation [8,12].

A currently open study for newly diagnosed ependymomas (ACNS0831) maintains the above stratification and is designed to address the issue of whether chemotherapy improves outcome when administered after irradiation. Although chemotherapy with agents such as the platinums have activity in that they can induce transient tumor regressions [13], and multi-agent regimens have shown some efficacy in delaying the need for irradiation in young children [14], a definitive role for chemotherapy in adjuvant management has heretofore been conjectural [15].

An ongoing effort of these and other studies is to identify patterns of genomic alterations and expression signatures that may supplement histological information to refine biological and prognostic classification of these tumors and identify new therapeutic targets [16–18]. Recent data suggest that there are likely to be biologically distinct subgroups of ependymomas that reflect different cellular pathways of tumorigenesis, in part as a function

of tumor location [2]. Insights regarding these pathways have been used to develop a mouse model of one of the supratentorial ependymoma subgroups, and identified molecularly targeted strategies that can be applied in their treatment [2]. These insights will likely refine the implementation of novel treatment approaches that are being explored by multi-institutional cooperative groups and consortia.

Unfortunately, tumor recurrence remains a major challenge for patients with ependymomas, and the management of recurrent disease in this setting is suboptimal. Although transient responses have been observed in recurrent tumors treated with re-irradiation and several chemotherapy regimens [13,19–20], long-term disease control is rarely achieved. Most tumors continue to progress despite additional surgery, irradiation, and conventional chemotherapy, and have proven refractory to a number of molecularly targeted agents [21–22]. Affected children commonly experience cumulative neurological morbidity from repeated surgical and adjuvant therapy interventions, and almost universally succumb to refractory tumor progression. Accordingly, conceptually new treatment approaches are needed. Based upon our recent evidence of promising immunological and clinical responses to immunotherapy-based approaches in pediatric astrocytomas [23–24], and our observation that ependymomas express a similar profile of tumor-associated antigens as astrocytic gliomas, but at even higher levels [25], we have launched a pilot study to evaluate the safety and efficacy of immunization in children with recurrent ependymomas.

Cancer Vaccines as a Novel Therapeutic Approach

Cancer vaccines are designed to induce a systemic immune response directed toward antigens expressed by tumor cells. In recent years, significant attention has been directed at the characterization of T-cell epitopes within tumor antigens that can be exploited in vaccine-based treatment approaches [26–29]. Most therapeutic studies to date have involved adult patients with advanced malignant tumors [26,28], although encouraging results have nonetheless been obtained [27,29]. Vaccine approaches may be even more effective if administered in clinical contexts where patients may have more robust immunity, such as the pediatric age group. Recent pilot studies of immunotherapy for children with malignant brain tumors have highlighted the potential for both clinical and immunological responses that may equal or exceed those noted in adults [23–24,30–32]. Although pediatric studies to date have largely focused on astrocytomas and medulloblastomas, other tumors have been examined, including ependymomas. In a study of vaccination with tumor lysate-pulsed dendritic cells, three of four children with recurrent ependymomas survived beyond 18 months [30]. In addition, recent data suggest that ependymomas exhibit a distinctive intrinsic potential for immune response that may correlate with prognosis [33]. These observations suggest that this tumor type may be particularly well suited for immune based therapies, provided appropriate antigen targets for immune-based therapy can be identified and an immune response to these antigens can be induced systemically to attack the tumor within the brain.

Immunological Constraints of the Brain

Although the brain has been referred to as an immunologically “privileged” site because of the absence of intrinsic lymphatic vessels and a lower rate of transplant rejection than systemic sites in allogeneic animal models, among other factors, it has long been recognized that immune responses can be induced in the brain under appropriate conditions, particularly following systemic stimulation with the targeted antigen [34–35]. This is exemplified by experimental allergic encephalomyelitis (EAE) in rodent models, which resembles in multiple sclerosis in humans [36–37]. Similarly, in paraneoplastic cerebellar degeneration [38], T cell reactivity directed toward an antigen expressed in systemic cancers leads to an

immune assault on cells within the normal cerebellum that express the same antigen. The response is induced by having the target antigen, which is normally "protected" within the brain, exposed to the systemic immune system by virtue of its expression in cancer tissues. These observations highlight the fact that the context of antigen presentation can impact the ability to induce an immune response against a target within the brain, and the possibility that systemic immunization strategies can be exploited against CNS tumors.

Based on these observations, attention has been focused on developing therapeutically applicable immunization approaches for patients with treatment-refractory brain tumors, particularly gliomas. Preliminary clinical studies by our group [39–43] and others [44–49] have shown that systemic vaccinations using autologous brain tumor-derived bulk antigens is safe and has some activity for adults with malignant gliomas. A challenge for the implementation of vaccines comprised of whole tumor cells, tumor lysates or tumor extracts is the potentially time consuming manipulation required to generate the vaccine, which can be problematic in patients at high risk for rapid tumor progression. Furthermore, the need for fresh tumor tissue for vaccine preparation can be a limiting factor in patients with unresectable disease, who may not be candidates for significant tumor debulking. An alternate approach that has been applied in our more recent trials has incorporated vaccines that are comprised of peptides derived from tumor-associated antigens (TAAs) [23–24,50].

Brain Tumor Antigen Targeted Vaccines

Because gliomas are the most common brain tumors in both children and adults, our initial efforts to identify TAAs focused on the discovery of cytotoxic T-lymphocyte (CTL) epitopes that recognized antigens overexpressed in gliomas. We first identified two human TAA-derived CTL epitopes in the interleukin-13 receptor (IL-13R)-α2 [51–52] and EphA2 [53], based on the hypothesis that these proteins might constitute promising vaccine antigens in gliomas. IL-13Rα2 is overexpressed by most malignant gliomas but not normal brain tissue or other normal organs except testis [54–56]. EphA2 is also frequently overexpressed in advanced cancers, such as malignant gliomas, promoting tumor growth [57–60]. Using algorithms for predicting binding affinity among Human Leukocyte Antigen-A2-positive (HLA-A2+) cells (the most common major histocompatibility class (MHC) 1 subgroup, present in 40–45% of the population) and proteosomal cleavage sites, we identified several IL-13Rα2 epitopes that could initiate a potent CTL response against IL-13Rα2-expressing HLA-A2+ glioma cells. We subsequently developed an analogue peptide ($IL\text{-}13R\alpha2_{345\text{-}353:1A9V}$) that could promote an even more potent effect than the wild-type peptide [51–52]. We next identified an EphA2 epitope that could elicit an HLA-A2-restricted CTL response against target-expressing glioma cells [53]. We also hypothesized that survivin, an apoptosis inhibitory protein that is overexpressed in many human cancers, including gliomas [61–65] would provide a promising immune target, particularly since an HLA-A2-restricted CTL epitope had already been identified in this protein and found to induce immune responses in patients with advanced cancers [66–68]. In addition to the above proteins, a variety of other targets with HLA-A2-restricted CTL epitopes (e.g., WT1, YKL-40, GP100) have been considered for brain tumor immunotherapy by our group and others [50,69], but based on our expression data, described below, our initial efforts in establishing a pediatric vaccination trial focused on these three targets.

TAA Expression in Ependymomas

As a primary step in evaluating the applicability of TAA vaccination for children with malignant brain tumors, we examined the frequency of antigen expression by immunohistochemistry in astrocytoma and ependymoma samples, with particular attention to EphA2, IL-13Rα2 and survivin, among others. Our initial studies in astrocytomas showed

that 13 of 15 pediatric brainstem gliomas and all 12 non-brainstem gliomas expressed at least one of these TAAs at high levels. EphA2 was expressed in 19 of 27 cases, survivin in 16 of 27, and IL-13Rα2 in 16 of 24 evaluable samples [70]. Building upon these observations and data from other groups that IL-13Rα2 was overexpressed in several non-astrocytic glial tumors [69], we examined the expression pattern of the above TAAs in a panel of 19 pediatric ependymomas, including grade I (myxopapillary, n=3), grade 2 (n=6), and grade 3 (anaplastic, n=10) lesions. These studies demonstrated that 16 tumors (84%) overexpressed IL-13Rα2, 18 overexpressed EphA2 (95%), and 18 overexpressed survivin (95%); several tumors showed patchy, intense overexpression of these antigens within discrete foci of the lesion [25]. The rate and magnitude of TAA overexpression exceeded our previous results in astrocytomas, providing a strong rationale for examining the efficacy of vaccine-based therapy in ependymomas, given the dismal results obtained with conventional chemotherapy and other molecularly targeted therapies. Of note, WT1, an antigen that has been previously targeted in our adult glioma vaccines, was expressed in only 37% of pediatric ependymomas, making this less suitable for inclusion as a vaccine component [25].

Because previous studies of TAA-specific T-lymphocyte responses in glioma patients demonstrated that T-cells in individual patients responded in a variable fashion to specific TAAs, we concluded that an optimal ependymoma vaccine should include multiple antigen-derived T-cell epitopes in order to enhance the likelihood of an immune response against an antigen overexpressed by the tumor [23–24,50,69,71–72], particularly given the diversity in epitope expression between tumor specimens. Based on our immunohistochemical analysis, EphA2, IL-13Rα2 and survivin appeared to be appropriate antigens for targeting in a peptide epitope-based vaccine.

Initial Experience with Peptide Vaccination in Adults with Recurrent Malignant Glioma

Prior to our embarking on a pediatric trial of peptide-based vaccination, safety and immunological efficacy were first examined in a cohort of adults with recurrent malignant gliomas at our institution. An initial trial incorporated dendritic cells (DCs) loaded with 4 peptides ($EphA2_{883\text{-}891}$, $IL\text{-}13R\alpha2_{345\text{-}353:1A9V}$, $YKL\text{-}40_{201\text{-}210}$ and $GP100_{209\text{-}217\ (T210M)}$) and a pan-DR epitope to stimulate helper T-cell responses and promote CTL responses [29,73–74], combined with i.m. poly-ICLC (20 μg/kg) [50], a Toll-like receptor (TLR) ligand [75–78], which has been shown to enhance immune response in preclinical models [78–79] and to be reasonably well tolerated in adults and children with malignant gliomas [80–82]. This study enrolled 22 patients (13 with glioblastoma (GBM, Grade IV), and 9 with anaplastic gliomas (AGs, Grade III), 19 of whom received at least four vaccinations. T-lymphocyte responses against TAA epitopes were assessed by enzyme-linked immunosorbent spot (ELISPOT) and HLA peptide tetramer assays.

The regimen was well tolerated [50] up to a total of 9 vaccinations without serious adverse events or evidence autoimmune responses in normal tissue. However, one patient demonstrated a CTCAE grade 4 anaphylactic reaction following the 18^{th} vaccine during the second booster phase (1×10^7 αDC1/dose), by which time the patient had demonstrated a complete response of recurrent anaplastic astrocytoma (WHO grade 3). This was considered dose-limiting toxicity, and the patient was withdrawn from the study.

The first 4 vaccines produced immunoreactivity against at least one of the vaccination-targeted antigens in 11 of 19 (58%) evaluable patients, five of whom exhibited MRI evidence of tumor regression. Two patients experienced complete responses, which were durable (29 and 37 months, and ongoing) and three had partial responses. In one of the latter

patients, biopsy of the residual intracranial lesion after vaccination revealed intense infiltration of T-cells and macrophages and no evidence of mitotically active tumor, suggesting that the residual area of enhancement reflected immune response within the tumor bed. Nine patients were progression-free for at least 12 months and 12-month overall survival was 55%, an encouraging result for a cohort of patients with recurrent malignant gliomas.

Pilot Application of Peptide Vaccination in a Pediatric Glioma Study

In view of the promising results in adults, plans were next made to transition peptide-based vaccination to the pediatric context, although modifications were made to the original study design. Whereas our initial vaccine trials in adults used a peptide cocktail administered with DCs, we questioned whether a non-cell-based delivery vehicle could provide comparable or perhaps superior immune priming, while avoiding the need for DC harvesting, which would enhance the feasibility and tolerability of the vaccine approach in children. An Incomplete Freund's Adjuvant (IFA)-type vaccine approach, which has been used extensively as a delivery strategy for peptide immunotherapy against systemic tumors in adults [27,29], provides a useful vaccination vehicle that is well suited to pediatric applications by avoiding the requirement for multiple DC harvests. Moreover, a preliminary study using this form of adjuvant was also launched for adults with recurrent low-grade gliomas using HLA-A2-restricted peptides and a pan HLA-DR peptide emulsified in a clinically approved IFA-like vehicle (Montanide ISA-51). This study demonstrated robust induction of TAA-specific $CD8^+$ T-cells and a high frequency of disease stabilization during vaccine therapy, highlighting the potential efficacy of using Montanide as a delivery vehicle [83].

Based upon the above data, we developed a vaccine trial for pediatric patients aged 1 to 21 years with poor prognosis astrocytomas that incorporated subcutaneous injections of TAA-derived HLA-A2-restricted peptides for IL-13Rα2, EphA2, and survivin and a pan-HLA-DR tetanus toxoid (TT) peptide (TetA830) emulsified in Montanide ISA-51 combined with poly-ICLC. The study has enrolled patients on five strata, determined by tumor histology, location, and prior therapy. Three strata enrolled newly diagnosed children in the following categories: 1) diffuse intrinsic pontine gliomas OR biopsy proven high-grade gliomas (HGG) involving the brainstem treated with irradiation alone; 2) biopsy proven non-brainstem HGG treated with radiation alone; and 3) HGG or brain stem glioma (BSG) treated with chemotherapy during radiation therapy. For the above groups, radiation was administered to a total dose of 5000–6000 cGy with standard fractionation and patients in these strata could not have received chemotherapy after completion of irradiation and must have met eligibility criteria and been enrolled within 4 to 12 weeks after completing radiotherapy. Two other strata enrolled children with recurrent gliomas, specifically: 1) unresectable low-grade gliomas that had progressed after two or more chemotherapy or biologic regimens; and 2) non-brainstem HGG that had recurred following treatment.

One key eligibility requirement was positivity for HLA-A2 on flow cytometry, because the peptide epitopes incorporated in the vaccine were HLA-A2-restricted. Another key requirement was that patients must be clinically stable off corticosteroids or on low-doses (i.e., no more than 0.1 mg/kg/day, max 4 mg/day Dexamethasone) for at least one week before beginning vaccination.

The vaccination regimen consisted of s.c. injections of TAA/TT-vaccines and i.m. poly-ICLC (30 μg/kg) provided on an outpatient basis every three weeks for 8 courses. Patients were monitored for adverse events (AEs), regimen-limiting toxicity (RLT), and response by clinic visits, laboratory testing, and MR imaging. Immune response was assessed by ELISPOT assay on peripheral blood mononuclear cells (PBMCs) 6, 15, and 21 weeks after

starting vaccination, the same time points used for the MRI scans. Patients demonstrating tumor regression or stable disease (SD) without RLT following the initial 8 courses of vaccination could continue to receive “booster” doses of vaccines at six-week intervals for up to 2 years of therapy. During the booster phase of the vaccine regimen, immunological and MRI evaluations were performed at 12-week intervals.

Although the trial is still ongoing (NCT01130077), initial observations in the first 32 patients (13 with newly diagnosed malignant BSG (all DIPGs) treated with irradiation alone, 6 with newly diagnosed malignant BSG (5 DIPGs) treated with irradiation and concurrent chemotherapy, 4 with newly diagnosed non-brainstem HGG treated with irradiation and concurrent chemotherapy, 4 with recurrent HGG, and 5 with recurrent low-grade astrocytomas) showed sufficient promise to warrant consideration of a subsequent study for children with ependymomas, and also provided guidance for development of this trial. Two children with BSGs had the sudden onset of neurologic deterioration associated with increased mass effect and enhancement of the tumor several months after starting vaccination that improved on corticosteroids. This course was consistent with immunological pseudoprogression, a phenomenon previously observed in our adult glioma trials [50,84]. In one of these cases, transient tumor enlargement and neurological decline were followed by a dramatic and prolonged MRI response. Three other children with BSG had probable pseudoprogression with transient tumor enlargement followed by prolonged survival, as did one child with a HGG, who had tumor regression after stopping vaccination, and another with a metastatic recurrent low-grade glioma who had initial asymptomatic tumor enlargement followed by >75% tumor shrinkage from baseline along with regression of extensive leptomeningeal and subependymal metastases.

Although the primary objective of this pilot study was to provide an analysis of safety, preliminary immunological and efficacy data have also been obtained. Only three children had disease progression during the initial two vaccine courses (6 weeks), and among the remainder, the best radiographic response has been stable disease in 23, partial response (PR, >50% tumor shrinkage) in 2, minor response (MR, 25 – 50% tumor shrinkage) in 3, and sustained disease-free status in 1 after a previous gross total resection. ELISPOT analysis showed TAA-specific immune responses in more than 50% of children.

Application of Peptide-Based Vaccines for Ependymoma Immunotherapy

The rationale for applying vaccine-based approaches to ependymomas was supported by three critical factors that characterize these tumors. First, recent studies indicate that ependymomas that express genes indicative of an antitumor immune response profile exhibit a significantly better prognosis than ependymomas lacking this feature [33], a unique association among primary childhood brain tumors, which suggests that efforts to boost immune surveillance and response to these tumors may lead to tangible improvements in outcome. Second, our observation that IL-13Rα2, EphA2, and survivin are not only commonly expressed in these tumors, but at a higher frequency and intensity than observed in our previous astrocytoma studies[25,70], highlights that these tumors may be particularly well suited to peptide-based immunotherapy targeted against CTL epitopes of these proteins. Third, given the poor response of recurrent ependymomas to conventional chemotherapy, the availability of an effective immunotherapeutic strategy would provide a major treatment advance for affected patients. However, because these tumors often arise adjacent to and impinging upon critical brainstem and cortical structures, there is clearly a potential for symptomatic “pseudoprogression” and neurological deterioration if a robust antitumor immune response is induced, which may differ in severity and time course from what we have observed in our previous trials for astrocytic gliomas in adults [50,84] and children [23–24]. The current pilot trial was therefore designed to take these issues into account.

The study cohort includes children > 1 and < 22 years of age with biopsy-proven ependymomas that have recurred or progressed after standard therapy. Because recent genomic data indicate that posterior fossa ependymomas exhibit molecular features distinct from those of ependymomas arising in other locations [2] and because tumor location may have a strong impact on the potential for symptomatic pseudoprogression in children who undergo immunotherapy, particularly if there is a robust intratumoral immune response, the study will incorporate separate strata for children with posterior fossa and non-posterior fossa ependymomas. This follows from our experience in astrocytic gliomas, in which pseudoprogression was observed in 5 of 19 brainstem gliomas versus only two of 13 non-brainstem astrocytomas [23–24].

Patients must be HLA-A2+ based on flow cytometry. Patients must have previously received standard initial therapy, which involves attempted gross total resection, where safely feasible, and in appropriate circumstances (e.g., those older than one year at initial diagnosis, with non-metastatic tumors and at least microscopic residual disease) involved field fractionated radiation therapy (RT) with total doses of 5000 to 6000 cGy. For patients who have received prior chemotherapy or biological therapy for recurrent disease, at least three weeks must have elapsed after prior myelosuppressive chemotherapy and one week after non-myelosuppressive chemotherapy. For those who have received radiotherapy, 3 months must have elapsed before beginning vaccination. All patients must have recovered from the acute toxic effects of prior therapy, and have no overt systemic disorders. Patients must be neurologically stable and off (or on no more than 0.1 mg/kg/day) dexamethasone for at least one week prior to registration.

As with our previous pediatric astrocytoma studies, patients receive s.c. injections of TAA/TT-vaccines in Montanide on an outpatient basis every three weeks for a total of 8 courses with the potential for receiving additional booster vaccines for up to two years. A topical TLR agonist with immunostimulatory properties, Imiquimod, which also has evidence of efficacy in CNS immunotherapy trials [85–86], and reasonable tolerability in the pediatric setting [87–88], is also administered. to an area involving and surrounding the peptide injection site. Participants are evaluated for adverse events (AEs), RLTs, and treatment response by clinic visits, laboratory testing, and MR imaging, similar to our astrocytoma trial.

As noted in our previous astrocytoma vaccine trials, the detection and appropriate management of pseudoprogression constitutes a particularly critical aspect of this study. This phenomenon reflects an immunologically mediated response in the tumor site that leads to transient enlargement and often, resultant symptoms, followed by tumor stabilization or regression. Accurately identifying which patients are experiencing pseudoprogression is essential in order to avoid premature termination of therapy in the setting of true treatment response [84]. However, if unchecked, this process can lead to irreversible neurological deterioration that can have lethal consequences, particularly in the setting of a robust immune response involving or adjacent to a critical brain region, such as the brainstem. Accordingly, the development of a management plan for this process presented a significant safety consideration in the design of our trial. We therefore incorporated close monitoring with serial MRI scans and neurologic evaluations to assess for treatment response as well as pseudoprogression, and developed detailed guidelines for pseudoprogression management, which are summarized below, as well as exploring the applicability of advanced MR techniques, such as MR spectroscopy and diffusion imaging, as noted by Vrabec et al. [89].

Once treatment is started, patients are closely monitored with serial MRI scans and neurologic evaluations to assess for treatment response as well as pseudoprogression. If pseudoprogression is suspected following the initiation of protocol treatment, (i.e., transient

increased edema and/or enhancement of the tumor), and the patient is neurologically worse, subsequent doses of vaccine and imiquimod are held. The patient may be placed on dexamethasone, and/or the dose increased, if clinically indicated. Re-imaging will be performed monthly thereafter, until it is determined whether this represents pseudoprogression or true progression. If the patient is clinically stable and on ≤ 0.1 mg/kg/d decadron for at least one week, and a repeat MRI indicates that tumor size has returned to less than 25% above pre-event baseline, the patient may restart vaccine and Imiquimod treatment.

In contrast, for patients in whom repeat imaging on increased steroids is unchanged or worse, and/or the patient's clinical status has not improved, a biopsy (or resection, if clinically indicated) may be considered to differentiate between pseudo- and true tumor progression. If inflammation or necrosis comprise the majority of the specimen, patients may remain on study and restart treatment once they are clinically stable and on ≤ 0.1 mg/kg/d decadron for at least one week. If the majority of the resected specimen consists of tumor, the patient is considered to have true progression and is taken off treatment. Patients who have progressively enlarging tumor size, or worsening symptoms despite increasing corticosteroids, or tumor enlargement that does not regress within 4 months, who are judged not to be candidates for biopsy/resection, are considered to have progressive disease and taken off treatment.

Although the primary goal of this study will be to determine the safety and tolerability of vaccination with TAA epitope peptides for children with recurrent ependymomas, we will also define the rate and magnitude of immune response in post-vaccine peripheral blood mononuclear cells against vaccine peptides, using ELISPOT. Preliminary data will also be obtained regarding clinical and imaging responses to therapy, associations between TAA expression and response, and mechanisms contributing to tumor response and resistance to immunotherapy. The results from this study will allow us to determine whether a subsequent larger phase II trial is warranted for these tumors.

CONCLUSIONS AND FUTURE DIRECTIONS

Based on preliminary studies that have shown activity of immunotherapy in adults and children with astrocytomas, and similarities between ependymomas and astrocytomas in terms of their tumor antigen expression profiles, there is significant interest in exploring the efficacy of tumor vaccination in patients with ependymoma. The trial described in this report builds upon lessons learned in previous studies, and is designed to make the vaccine as "user friendly" as possible in the pediatric setting. Because of the risk of pseudoprogression noted in our previous pediatric studies, detailed monitoring and management guidelines have been incorporated into the trial design.

In parallel with these efforts, a host of other new immune-based therapies are currently being examined in adult systemic tumors. Particularly exciting are antibodies directed against immune systemic checkpoints, such as the programmed death-1 (PD1) ligand and its receptor, and cytotoxic T lymphocyte associated antigen-4 (CTLA4) [90–92]. Although these approaches have yet to be systematically examined for brain tumors, much less childhood brain tumors, it is clear that the potential armamentarium for immune-based therapies is broadening rapidly.

EXPERT COMMENTARY

The management of childhood brain tumors, including ependymomas, is increasingly focusing on applying novel molecularly targeted treatment approaches in an effort to improve disease control for high-risk tumors, and reduce sequelae for tumors with favorable

outcomes. Immunotherapy is of particular interest in this regard, since it attempts to harness the body's immune system to attack antigen-expressing cells within the tumor. As a conceptually new therapeutic approach for patients with brain tumor types that have heretofore been refractory to conventional therapies, immunotherapy warrants examination as a novel approach that may offer the potential for achieving meaningful improvements in disease control. Time will tell which specific immunotherapy approach has sufficient safety and activity to justify further study in ependymomas and whether combination approaches may warrant consideration to optimize the magnitude and persistence of the therapeutic effects achieved.

FIVE-YEAR VIEW

Given that applications of molecularly targeted strategies are in their infancy, it is premature to infer which approach will manifest sufficient therapeutic activity to warrant inclusion in future therapy regimens. Small molecule-based treatment approaches are likely to gain further application in these tumors, as additional insights regarding the molecular underpinnings of the disease process become apparent. Insights regarding the molecular characteristics of these tumors may also inform the refinement of future immunotherapeutic approaches, particularly if common immunologically targetable mutations are identified. Defining the genomic landscape of these lesions and applying genomic analysis approaches in near real time may provide the opportunity to personalize immunotherapy to incorporate different epitopes in different patients, selecting preferred components of a vaccine cocktail from a menu of exploitable targets. The identification of additional antigenic targets in these tumors that directly influence tumor cell survival, such as EphA2 and survivin, may also enhance the chances that immunotherapy will have a favorable impact on the disease process, and delay the emergence of "antigen-negative" cell populations that circumvent immune targeting. In addition, the coupling of vaccine-based immunotherapy with approaches to positively influence the tumor immune milieu and counteract tumor-induced immunosuppression may also help to maximize the potential for achieving a beneficial therapeutic result. In this context, it is likely that vaccine-based immunotherapy will not be viewed as a stand-alone therapy, and instead, will probably form a component of a multi-modality management approach to treat these challenging tumors.

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KEY ISSUES

- The initial management of ependymomas has to take into account known prognostic factors, such as extent of tumor resection, histology, and location, as well as other clinical factors, such as patient age and metastatic stage.
- Advances in surgical techniques and the use of conformally delivered irradiation have improved the overall prognosis for children with ependymomas, but a substantial percentage of patients still experience disease progression, which is difficult to control with current therapies.
- Immunotherapy represents a novel treatment approach that has recently been explored in astrocytic tumors in both adults and children.
- Ependymomas express high levels of several tumor-associated antigens that are overexpressed in astrocytomas and have been incorporated in astrocytoma immunotherapy.
- Because the development of an immune response within brain tumors can lead to immunological pseudoprogression with resultant neurological deterioration, careful monitoring and management are required to minimize the risk of potentially dangerous adverse effects.