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Adjuvant Wilms' tumour 1-specific dendritic cell immunotherapy complementing conventional therapy for paediatric patients with high-grade glioma and diffuse intrinsic pontine glioma: protocol of a monocentric phase I/II clinical trial in Belgium

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ABSTRACT

Introduction Diffuse intrinsic pontine glioma (DIPG) and paediatric high-grade glioma (pHGG) are aggressive glial tumours, for which conventional treatment modalities fall short. Dendritic cell (DC)-based immunotherapy is being investigated as a promising and safe adjuvant therapy. The Wilms' tumour protein (WT1) is a potent target for this type of antigen-specific immunotherapy and is overexpressed in DIPG and pHGG. Based on this, we designed a non-randomised phase I/II trial, assessing the feasibility and safety of *WT1* mRNA-loaded DC (WT1/DC) immunotherapy in combination with conventional treatment in pHGG and DIPG.

Methods and analysis 10 paediatric patients with newly diagnosed or pretreated HGG or DIPG were treated according to the trial protocol. The trial protocol consists of leukapheresis of mononuclear cells, the manufacturing of autologous WT1/DC vaccines and the combination of WT1/DC-vaccine immunotherapy with conventional antiglioma treatment. In newly diagnosed patients, this comprises chemoradiation (oral temozolomide 90 mg/m^2 daily+radiotherapy 54 Gy in 1.8 Gy fractions) followed by three induction WT1/DC vaccines (8–10×10^6 cells/vaccine) given on a weekly basis and a chemoimmunotherapy booster phase consisting of six 28-day cycles of oral temozolomide (150–200 mg/m^2 on days 1–5) and a WT1/DC vaccine on day 21. In pretreated patients, the induction and booster phase are combined with best possible antiglioma treatment at hand. Primary objectives are to assess the feasibility of the production of mRNA-electroporated WT1/DC vaccines in this patient population and to assess the safety and feasibility of combining conventional antiglioma treatment with the proposed immunotherapy. Secondary objectives are to investigate in vivo immunogenicity of WT1/DC vaccination and to assess disease-specific and general quality of life.

Ethics and dissemination The ethics committee of the Antwerp University Hospital and the University of Antwerp granted ethics approval. Results of the clinical trial will be shared through publication in a peer-reviewed journal and presentations at conferences.

Trial registration number NCT04911621

STRENGTHS AND LIMITATIONS

⇒ Offering immunotherapy complementing standard of care treatment in difficult-to-treat and rare paediatric neuro-oncological care.
⇒ In-depth analysis of immunological response to Wilms' tumour 1 directed dendritic cell (DC) vaccination.
⇒ Assessing the quality of life when adding DC immunotherapy to an already intensive therapy plan in patients with limited life expectancy.
⇒ Small sample size of 10 patients, this in light of the trial purpose being a phase I feasibility trial.

INTRODUCTION

For different types of paediatric malignancies, the implementation and use of international standard treatment protocols have yielded significant improvements in overall survival (OS) and event-free survival (EFS) over the last decades.[1] The combination of conventional chemotherapy, radical surgery and radiotherapy resulted in a first important wave of

improvement of prognosis. Since recently, the addition of targeted therapy and immunotherapy has shown promise for further progress.[1–4] This is especially true for haematological and to some extent for solid paediatric tumours. However, in paediatric neuro-oncology, this progress lags behind, making brain tumours the leading cause of death in paediatric oncology.[5] Two distinct cancer entities are associated with exceptionally poor OS and EFS: paediatric high-grade gliomas (pHGGs) and diffuse intrinsic pontine gliomas (DIPGs), which make up 10%–12% of all paediatric central nervous system tumours. Despite a growing molecular understanding of both entities, the prognosis remains extremely grim, with a 5-year OS of 5%–30% and <5% for pHGG and DIPG, respectively.[6–9]

Clinical research is essential in the quest for improved treatment options for difficult-to-treat tumours like DIPG and pHGG. Unfortunately, so far, the majority of investigated agents have failed to demonstrate a significant improvement of EFS and OS.[5] Based on preclinical research and significant successes obtained in other tumour types, there is an expectation that real breakthroughs can be obtained with next-generation therapies, including immunotherapy, cell-based therapy or precision medicine. However, patient access to these promising treatments remains limited. Currently, on clinicaltrials.gov (date of consultation: 10 January 2024), there are 35 recruiting interventional clinical trials registered worldwide for paediatric patients with DIPG (search terms, 'DIPG Brain Tumor'; status, 'recruiting'; age, 'child (birth-17)'; study type, 'Interventional (clinical trial)'). For HGG (search terms, 'High-grade glioma'; status, 'recruiting'; age, 'child (birth-17)'; study type, 'interventional'), the number of recruiting trials is 30, with a significant overlap (n=13) with the trials currently open for DIPG. For newly diagnosed HGG (search terms, HGG, newly diagnosed; status, 'recruiting'; age, 'child (birth-17)'; study type, 'interventional'), there are only seven recruiting trials. A significant proportion of the recruiting trials are basket trials and not specific for DIPG or pHGG. While there is a clear rationale for such basket trials, making new treatments available for all kinds of difficult-to-treat (paediatric) malignancies, clinical trials specifically designed for DIPG and pHGG will better tailor to the need of these patients. In addition, most of the early phase trials are not conducted in Europe (in the case of DIPG, only 7/35 are accessible in Europe), making them practically inaccessible for European patients. In this way, we fall short in providing maximal experimental options for patients with pHGG and DIPG and their families, tempting them to seek refuge in usually expensive alternative medicinal approaches or clinical trials far from home, jeopardising patients' or their family's psychosocial well-being.

In light of this unmet need and the ever-evolving knowledge of the role of the immune system in tumour control, the Antwerp University Hospital designed a phase I/II trial to investigate the safety and feasibility of adding autologous dendritic cell (DC)-based immunotherapy to the currently available standard-of-care treatments for pHGG and DIPG.

The goal of active immunotherapy is to stimulate and arm the body's own immune system to establish a more vigorous antitumour immune activation. DCs, being the most proficient antigen-presenting cells of the immune system, play a critical role in this process. By activating T cells in an antigen-specific manner, they are key to induce an immune response immediately directed against malignant cells expressing the antigen in question. Besides their important role in the adaptive immune response, DCs are also important modulators of natural killer cells, effectively linking innate and adaptive immunity.[10–12] Owing to these particular properties, DCs have claimed central stage in the development of cell-based cancer immunotherapy over recent decades.[13 14] Since the publication of the first clinical trial in 1996,[15] DC vaccination was repeatedly shown to be safe and well tolerated, with side effects generally being limited to local injection site reactions.[16–21]

The selection of a powerful tumour-associated target antigen was driven by promising results obtained in the phase I trial investigating Wilms' tumour 1 (WT1)-targeted DC (WT1/DC) vaccination in adult patients with solid tumours (NCT01291420)[22] and later the WT1/DC vaccination trial in adult glioblastoma (NCT02649582), both conducted at the Antwerp University Hospital (Belgium). The WT1 antigen was ranked as the most interesting tumour antigen to be targeted by immunotherapeutic approaches in a variety of tumour types according to a pilot project of the National Cancer Institute (NCI).[23] Knowledge of WT1's function has evolved from being a tumour suppressor gene, where biallelic loss can cause nephroblastoma in, for instance, the WAGR-syndrome (Wilms tumor, Aniridia, Genitourinary anomalies, and a Range of developmental delays syndrome), to equally being an oncogene, where overexpression of wild-type WT1 seems to be one of the main drivers of oncogenesis in different tumour types.[24 25] In pHGG and DIPG, overexpression of WT1 has also been documented,[26 27] while this is not the case in healthy surrounding tissue.[27 28] Different case reports and early phase clinical trials, in different paediatric tumour types including pHGG, have already proven immunological and clinical responses in specific WT1-targeted activation of the patients' immune system by means of peptide vaccination.[29–32] This particular form of WT1-targeted immunotherapy requires human leucocyte antigen (HLA)-matched epitopes of the protein to be available, limiting its use to a selection of patients. By loading DC ex vivo with full-length WT1 mRNA, the encoded protein is processed to express the complete WT1 epitope repertoire, overcoming the limits of HLA restrictions.[10 33 34]

As WT1 is a self-antigen also expressed in healthy tissues (eg, gonads, kidney and haematological progenitor cells), theoretically autoimmunity after vaccination with WT1 antigens might be a concern. However, based on the toxicity data from 21 phase I and II clinical trials with

WT1-targeted immunotherapy in patients with cancer (n=158), the risk of WT1-mediated autoimmunity appears to be low.[33] Our own clinical experience with autologous *WT1* mRNA-loaded DC vaccination in patients with different haematological and solid malignancies (n=155) confirms the safety of WT1-targeted therapy.[22 33 34] Moreover, both we and others have demonstrated that WT1/DC vaccination is capable of inducing immunological and clinical responses in patients with various haematological and solid malignancies.[14 22 33–35]

Autologous WT1/DC vaccination in 47 adult patients with limited spread metastatic solid tumours, including 13 patients with glioblastoma multiforme (GBM), was evaluated as adjuvant therapy on top of standard-of-care treatment in an open-label, single-arm clinical trial at the university hospital between May 2010 and April 2016 (NCT01291420). None of the vaccinated patients developed any vaccine-related grade III or IV toxicity, and there was a suggestion of increased median OS.[22] For the cohort of patients with GBM (n=13) specifically, comparing WT1/DC-treated patients' OS with equivalent data from literature—taking into account small sample size and heterogeneity of the study population—median OS was 43.7 months from the time of diagnosis[22] versus a median OS of 14.7 months in the literature.[36] These results suggest that adjuvant WT1/DC-based immunotherapy provides a clinical benefit for these patients and have led to the initiation of a subsequent clinical study to investigate the potential benefit of adding WT1/DC vaccination to standard-of-care treatment with chemoradiation following surgery in adult patients newly diagnosed with glioblastoma (ADDIT-GLIO trial, NCT02649582).

It can be rationalised that combining DC vaccination with conventional chemotherapy and radiation therapy results in therapeutic synergism. Tumour-cell damage induced by chemotherapy or radiation leads to increased release of antigens, stimulatory cytokines and damage-associated patterns, facilitating the induction of antitumour immune responses and creating a state of overall enhanced immune responsiveness.[37] In addition, the transient state of lymphopenia induced by chemotherapy allows for the selective DC-induced expansion of tumour antigen-specific T cells, thereby skewing the T cell repertoire in the desired antigenic specificity.[38] Conversely, increased chemosensitivity after DC vaccination has also been reported in different types of cancer,[39] including for GBM and the subsequent use of temozolomide,[40] but the mechanisms behind this phenomenon remain elusive. Of interest for this particular trial is the observed in vitro upregulated expression of WT1 in a (paediatric) glioblastoma cell line model following irradiation, suggesting that prior radiotherapy could sensitise tumour cells to WT1-targeted immunotherapy.[41] Based on these arguments, the ADDIT-GLIO trial was designed to combine chemoradiation, DC vaccination and maintenance chemotherapy in the first-line treatment of adult GBM. The first 15 evaluable study patients did not report any serious adverse events (SAEs) possibly, probably or definitely related to the vaccine during this trial, anticipating that WT1/DC vaccination in combination with conventional chemoradiation is well tolerated and confirming its overall beneficial safety profile. Based on these interim data, a parallel study was designed for paediatric patients with HGG and DIPG (ADDICT-pedGLIO trial, NCT04911621).

As for the majority of advances in immunotherapy, most experience with autologous DC vaccination is with adult cancer patients. A limited number of phase I/II trials evaluating DC vaccination have been conducted in the paediatric oncological setting, and a significant proportion of them included children with pHGG and DIPG.[19] A particular challenge in paediatric patient populations is the collection of starting material for the manufacturing of the cell therapy product. For the generation of autologous monocyte-derived DC vaccine doses, patients need to undergo a leukapheresis procedure to obtain large amounts of mononuclear cells for subsequent purification of monocytes, the precursors of DC. In adults, these mononuclear cells are collected by means of a peripheral access leukapheresis procedure. For young children with low body weight/blood volume and smaller vessel size, such a leukapheresis procedure is more invasive considering the need for a femoral catheter and for general anaesthesia to safely obtain this venous access. In smaller children (eg <20 kg), more pronounced intravascular volume fluctuations and/or changes in hematocrit and electrolytes should be anticipated. Therefore, a specific paediatric leukapheresis protocol and supportive care procedures should be at hand. Referring to published paediatric trial results investigating DC vaccination, manufacturing of and treatment with DC vaccines was deemed feasible and safe.[19] In line with what has been observed in adults, injection site reactions were the most commonly reported AEs, while systemic toxicities, if any, were generally mild. Grade IV toxicities were rare and manageable in all cases.[18 20]

Taken together, DC immunotherapy has proven to be safe and feasible, including for (paediatric) patients with brain tumours, and clinical successes have been demonstrated for WT1-targeted therapy. Scientific evidence of bidirectional beneficial effects between conventional chemoradiation and this type of personalised cellular immunotherapy is growing. This clinical trial was designed to evaluate for the first time the feasibility and safety of treatment in children with pHGG and DIPG with autologous WT1/DC vaccination in combination with conventional antitumour treatments. Despite a small sample size of ten patients, this study will allow us to collect relevant data on safety and feasibility. While statements concerning results on progression-free survival (PFS) or OS will be descriptive rather than statistically relevant, we will be able to detect any immunological response induced by WT1/DC vaccination, which is known to correlate with clinical responses.[33 42–44]

METHODS AND ANALYSIS

Trial design and organisation

The ADDICT-pedGLIO trial is an investigator-driven, academic, non-randomised, single-centre phase I/II trial designed to investigate the safety and feasibility of adding autologous WT1/DC vaccination to currently available therapies in pHGG and DIPG (registered at www.clinicaltrials.gov as NCT04911621 and in the EudraCT database with reference number 2020-004125-23). The trial sponsor is the Antwerp University Hospital (UZA, Edegem, Belgium). Recruitment is coordinated by the Division of Pediatric Oncology and Hematology of the Antwerp University Hospital (UZA, Edegem, Belgium), on a national level in collaboration with the Belgian Society for Pediatric Hematology and Oncology and internationally based on individual referrals. The collection of starting material via leukapheresis is organised by the Divisions of Nephrology, Pediatric Oncology and Hematology of UZA. Manufacturing of autologous WT1/DC vaccines is performed at the registered Good Manufacturing Practices (GMP) production facility Anicells (Niel, Belgium). DC vaccination and patient follow-up are performed at the Division of Pediatric Oncology and Hematology (UZA). Standard oncological care and radiological assessment can be conducted in the referring centre; tumour imaging is being centrally reviewed by the neuroradiologist associated with the trial.

The Standard Protocol Items Recommendations for Interventional Trials reporting guidelines were used to ensure all valuable information was included in the publication of the trial protocol (online supplemental appendix 1).[45]

Patient population and inclusion and exclusion criteria

This single-arm, phase I/II study is designed to include a total of 10 evaluable paediatric patients with HGG or DIPG. Children from the age of 1 until <18 years, presenting with a biopsy-proven HGG (WHO grade III or IV) or a histologically or radiologically confirmed DIPG, are considered for inclusion. Both newly diagnosed and pretreated patients are eligible for participation. Newly diagnosed patients are allocated to stratum A. In case of any previous treatment, patients are allocated to stratum B. Patients in stratum B should have recovered from earlier antiglioma treatment-related toxicities before enrolment in the study treatment protocol. The exhaustive list of inclusion and exclusion criteria is provided in table 1.

Objectives

The primary objective of this phase I/II clinical study is to evaluate the feasibility of *WT1* mRNA-loaded autologous monocyte-derived DC vaccine production and to demonstrate that intradermal administration of WT1/DC vaccines, either combined with first-line chemoradiation treatment or administered as adjuvant therapy following previous therapies, is feasible and safe. Secondary objectives are to study vaccine-induced in vivo immune responses, to assess efficacy-related indicators of clinical activity and to collect patient-reported outcome of disease-related quality of life for comparison with current patients' outcome, allowing indication of the added value. Exploratory objectives are to characterise changes in patient and proxy-reported general and executive

Table 1 Inclusion and exclusion criteria ADDICT-pedGLIO clinical trial

Inclusion criteria	Exclusion criteria
- Diagnosis of: HGG (WHO grade III or IV), histologically verified DIPG (radiological diagnosis will suffice) - Aged ≥12 months and <18 years - Body weight ≥10 kg - Lansky/Karnofsky score (as applicable based on age) of ≥50% - Life expectancy ≥8 weeks - Stratum B: recovery from treatment-related (haematological) toxicities (>grade I) following previous antiglioma treatments - Written informed consent of parents/legal guardian and of patients aged ≥12 years - Willing and able to comply with the study protocol - Negative serum or urine pregnancy test for female patients of childbearing potential - Woman of childbearing potential and men should agree to use effective contraception before, during and for at least a hundred days after the last study treatment administration - Women breastfeeding should discontinue nursing prior to the first dose of study treatment and until at least a hundred days after the last study treatment administration	- Use of any investigational agents ≤4 weeks before leukapheresis - Concomitant malignancy or history of another malignancy - Known concomitant presence of any active immunosuppressive disease (eg, HIV) or active autoimmune condition - Pre-existing contraindication for contrast-enhanced MRI - Pregnant or breastfeeding - Any other condition, either physical or psychological, or reasonable suspicion thereof on clinical or special investigation, which contraindicates the use of the vaccine, or may negatively affect patient compliance, or may place the patient at higher risk of potential treatment complications

DIPG, diffuse intrinsic pontine glioma; HGG, high-grade glioma.

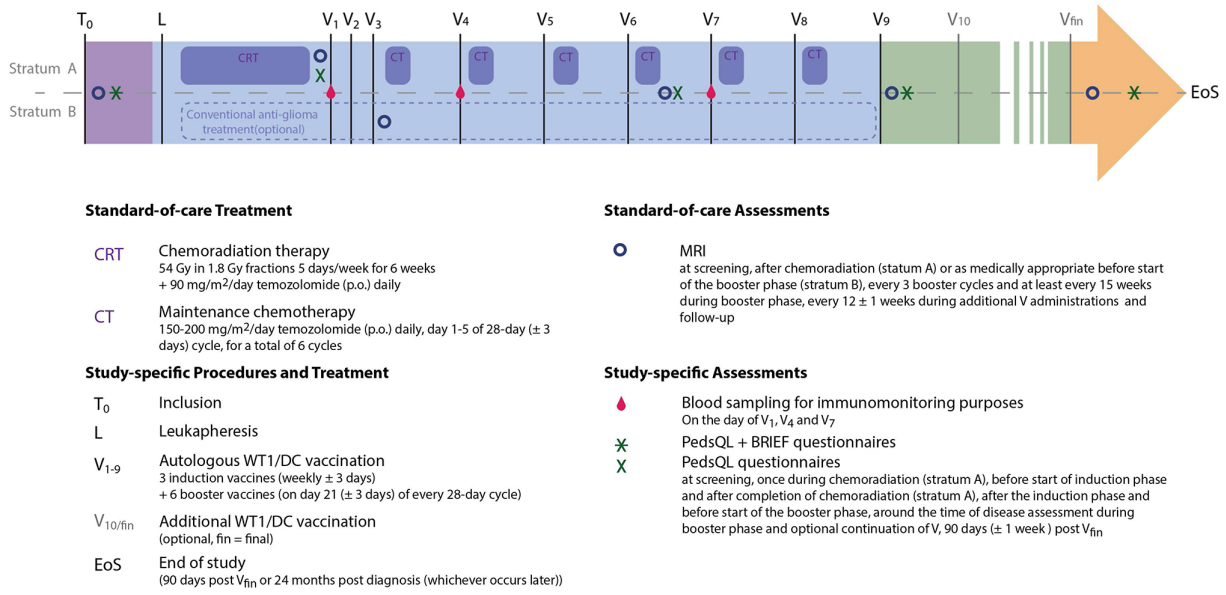

Figure 1 Schematic overview of the study treatment scheme and trial-related procedures.

function and to identify prognostic, predictive and therapeutic biomarkers.

Patient and public involvement

There was no direct patient or public involvement in the conduct, reporting or dissemination plans of our research. However, different funders (Olivia Hendrickx Research fund, Stichting Semmy) have parents of paediatric oncological patients on their board and gave critical feedback on the trial protocol, which we included in the final protocol. They actively disseminate information about the trial, increasing awareness among the general public.

Description of processes and interventions

An overview of the study treatment scheme and all trial-related procedures is provided in figure 1.

Screening

At screening visit, the patient's demographics, medical history and active concomitant medication are collected as well as any prior anticancer treatment in non-treatment-naïve patients. Clinical disease assessment at this time point includes a full neurological and standard paediatric physical examination including evaluation of performance status (Lansky/Karnofsky, as appropriate for age), registration of vital signs and measurement of height and weight. A radiological assessment by MRI is performed, unless an assessment performed within 4 weeks prior to T_0 (and after the last surgical intervention, if applicable) is already available. Peripheral blood analysis comprises determination of complete and differential blood counts, evaluation of kidney and liver function by relevant biochemical analyses, coagulation analysis, determination of serology (herpes simplex virus, varicella zoster virus, HIV, hepatitis B, hepatitis C and syphilis) and blood type and Rhesus blood groups. When applicable, a pregnancy test is conducted. The eligibility of the patient to undergo leukapheresis and the optimal route of vascular access (peripheral or via central venous catheter) is determined by a delegated nephrologist. Finally, a patient and parents are asked to the general (PedsQL) and disease-specific (PedsQL Cancer Module) quality-of-life questionnaires, as well as the 'Behavior Rating Inventory of Executive Function' (BRIEF) questionnaire.

Apheresis

Within 24 hours before the scheduled leukapheresis procedure (figure 1, L), the patient's differential blood count and hemostasis are evaluated for adequacy to undergo the leukapheresis procedure. In addition, the patient's ABO and Rhesus blood groups are verified. It is recommended to discontinue corticosteroid treatment three days prior to apheresis. If not feasible, patients can be maintained on corticosteroid therapy at the lowest possible dose.

Apheresis is performed using a Spectra Optia device (Terumo, Leuven, Belgium) using settings appropriate for monocyte collection. Depending on the patient's age, body weight and vascular accessibility, vascular access is obtained either via a central venous double-lumen femoral catheter or via cannulation of peripheral veins in the arm. Priming of the apheresis device for patients with a body weight of <25 kg and/or a hematocrit of <30% is performed with matched packed red blood cells.

During apheresis, clinical condition, cardiorespiratory parameters and serum electrolytes (eg, ionised calcium) are closely monitored. Fluid or electrolyte imbalances are corrected following institutional guidelines. A maximum of four times the patient's total blood volume or 12 L, whichever is smaller, is processed per session. Determination of complete blood count is repeated after apheresis, to check the need for transfusion.

After the release of the apheresis product by the UZA Cell and Tissue Bank, the number of CD14-positive

mononuclear cells is determined. The intent is to harvest at least 1×10^9 CD14-positive mononuclear cells, in view of producing at least nine WT1/DC vaccine doses of $8–10\times10^6$ viable DCs/dose. When the number of monocytes in the apheresis product is $<1\times10^9$, a second apheresis procedure is scheduled to be performed the following day.

WT1/DC vaccine manufacturing

Patient-derived WT1/DC vaccine manufacturing and quality control testing are performed in a period of 4 weeks, while patients receive first-line chemoradiation therapy (CRT) or conventional next-line antiglioma treatment for patients in stratum A or stratum B, respectively (figure 1, upper and lower parts of the arrow). In brief, CD14-positive monocytes are isolated from the peripheral blood mononuclear cell fraction of the apheresis product by means of magnetic bead-labelled anti-CD14 monoclonal antibodies using the CliniMACS Cell Separation System (Miltenyi Biotech, Germany). Subsequently, these CD14+ monocytes are differentiated ex vivo into immature DCs in 5 days, in the presence of 80 ng/mL granulocyte-macrophage colony-stimulating factor and 250 IU/mL interleukin-4. DC cultures are maintained in CellGenix GMP-grade DC medium supplemented with 1% pretested human AB serum. On day 6, immature DCs are matured for 48 hours through an addition of 20 ng/mL tumour necrosis factor-α, 2.5 µg/mL prostaglandin E2 and 10 µg/mL pyrogen-free keyhole limpet hemocyanin as a CD4+ T cell helper antigen. On day 8, mature DCs are harvested and washed for subsequent antigen loading through electroporation with mRNA.

Mature DCs are resuspended in sterile phenol red-free Opti-MEM electroporation medium and electroporated with *WT1-DC-LAMP* mRNA using a Gene Pulser Xcell electroporation device (Bio-Rad, Ghent, Belgium). Immediately after electroporation, cells are allowed to recover for 2 hours in the DC culture medium.

Electroporated DCs are then harvested and cryopreserved in aliquots of $15\pm1.6\times10^6$ cells in pretested human AB serum supplemented with 10% dimethyl sulfoxide and 2% (w/v) glucose, at temperatures below −130°C. Frozen aliquots remain under embargo until the quality control test results are available and all release criteria are met. Quality control testing performed on the cryopreserved WT1/DC aliquots consists of determination of cell count and viability, sterility, endotoxin contents, flow cytometric analysis of DC morphology and phenotype (CD86, HLA-DR, CCR7, CD80, CD83 and CD14) and contamination by T lymphocytes (CD3), immunohistochemical analysis for WT1 protein expression and analysis of functional migratory capacity.

WT1/DC vaccine reconstitution and administration

On the day of vaccination (figure 1, V), one dose of prealiquoted cryopreserved *WT1*/DC is thawed for reconstitution. The cell product is washed three times, counted and resuspended in a saline solution containing 5% human albumin at a concentration of $8–10\times10^6$ viable cells/500 µL and transferred to a 1 mL syringe for intradermal injection at five sites (100 µL/site) in the ventral region of the upper arm (2–5 cm from the axillary lymph node region). Per WT1/DC vaccine dose (figure 1, V), the injection site is alternated between the left and right arm to maximise the exposure of different lymph node regions.

Treatment schedule

Stratum A

Patients eligible for stratum A undergo apheresis before the start of chemoradiation, providing time to produce, test and release the WT1/DC vaccines. Temozolomide-based chemoradiation can be initiated as soon as the patient's haematological blood values are adequate after apheresis and must start ≤6 weeks after surgery in case of resectable disease and ≤6 weeks after histological and/or radiographically confirmed diagnosis in case of non-resectable disease (ie, date of tumour biopsy or imaging). Chemoradiation consists of involved field radiation 5 days per week for 6 weeks (54 Gy in 1.8 Gy fractions) and 90 mg/m² of oral temozolomide daily from the first until the last day of radiotherapy, that is, 42 consecutive days.

After completion, the induction immunotherapy phase is initiated (figure 1, V_1–V_3). Patients are vaccinated three times on a weekly (−1 day, +2 days) basis with $8–10\times10^6$ autologous monocyte-derived *WT1* mRNA-electroporated DCs per vaccine. The first vaccine (figure 1, V_1) must be administered after baseline imaging and ≥1 week after completing chemoradiation.

Following the induction phase, patients enter the booster phase consisting of oral temozolomide treatment (figure 1, CT) in combination with WT1/DC vaccination (figure 1, V_4–V_9), for a total of six 28-day (±3 days) cycles. The first cycle of maintenance treatment with oral temozolomide should start ≥4 weeks and ≤8 weeks after the last day of chemoradiation and ≥3 days after the end of the induction phase. Patients start maintenance treatment with 150 mg/m² of oral temozolomide once daily on days 1–5 of the first cycle. From the second cycle onwards, the temozolomide dose must be escalated to 200 mg/m²/day, if toxicity allows. During maintenance temozolomide treatment, one WT1/DC vaccine is administered on day 21 (± 3 days) of each cycle. The rationale is to administer the immunotherapy coinciding with the expected haematological recovery phase and surge in immunologically active cells. Maintenance treatment continues for a total of six cycles or until intolerance or disease progression. Continuation of DC vaccination beyond the study treatment schedule is possible as described below (continuation of DC vaccination beyond the study treatment schedule).

Stratum B

For patients recruited in stratum B, the decision to continue or reinitiate conventional antiglioma treatment and, if applicable, its dose and scheme are at the

investigator's discretion and will depend on the patient's previous treatment scheme and condition.

The backbone WT1/DC immunotherapy scheme for the induction and booster phase as described for stratum A is followed with minor modifications. Timing of the start of the induction phase and the booster phases and the intervals between booster vaccinations are based on the administration of concomitant treatment(s), taking into account the degree and kinetics of its leukodepleting effects. WT1/DC vaccine administration should be scheduled to coincide with the haematological recovery phase. In this way, a personalised vaccination scheme is established per patient.

Induction vaccination ($V_{1–3}$), consisting of 3 weekly (−1 day, + 2 days) vaccines, can be initiated ≥4 weeks after apheresis and should at that point be initiated as soon as possible, taking into account compatibility with ongoing conventional treatments.

The booster phase can be initiated ≥3 weeks after the last induction vaccine and should at that point be initiated as soon as possible, again taking into account compatibility with ongoing conventional treatments. A total of six booster vaccinations ($V_{4–9}$) are administered at regular intervals. It is advised that the interval between subsequent booster vaccinations is no longer than 4 weeks. Timing and intervals of the personalised vaccination scheme are determined by the investigator to optimise the timing between the administration of immunotherapy and other antiglioma treatments, if any.

Continuation of DC vaccination beyond the study treatment schedule

Continuation of WT1/DC vaccination after nine doses is optional (figure 1, $V_{10–fin}$), on the condition that the investigator judges that the participant's clinical situation justifies additional vaccinations, consent for the continuation of vaccination of the parents/guardian and the participant (if aged 12 years or older) has been obtained and residual vaccine aliquots are available. In case of disease progression, concomitant glioma treatment and WT1/DC vaccination are re-evaluated, but continuation of DC vaccination under an investigator's discretion is allowed. In case of insufficient vaccines to complete the study treatment protocol ($V_{1–9}$) or in case of suspected or documented benefit of treatment protocol and exhaustion of vaccine doses manufactured from first leukapheresis, a second leukapheresis and vaccine manufacturing procedure is allowed.

Patient evaluation, safety evaluation, follow-up and data collection

During every study-related visit, the assessment of disease-specific features (eg, neurological examination), safety-related features (haematological evaluation, organ function and inflammatory signs or symptoms) and evaluation of patients' well-being are conducted. All AEs occurring during the study are recorded, and newly started concomitant medication is documented. Patients are evaluated at trial entry, during chemoradiation (if applicable) and at least at every WT1/DC vaccination visit during the study treatment scheme and continued WT1/DC vaccination. After the final DC vaccine dose, patients enter a follow-up period, during which they are investigated clinically at regular intervals coinciding with the radiological disease assessment by MRI, at least every 12 (±1) weeks. Follow-up continues up until two years after diagnosis or until 90 days after the last DC vaccination, whatever comes last.

The severity of AEs is assessed according to the latest version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) scale (at the time of trial opening: CTCAE V.5.0). The relationship of an AE to the investigational treatment should be assessed by the investigator as either related (definitely, probably, possibly or unlikely) or non-related, based on their clinical judgement. Disease evolution is assessed radiologically according to the Response Assessment in Neuro-Oncology (RANO) criteria.[46] Apart from imaging findings, also clinical status and corticosteroid use are closely monitored as part of patient follow-up. Radiological evaluation is performed at the time of screening, after chemoradiation (for stratum A) or as medically appropriate before the start of the booster phase (for stratum B) and subsequently every three booster cycles and at least every 15 weeks. After completion of the treatment protocol, in case of early cessation or in case of continuation of immunotherapy after completion of the initial protocol, radiological assessment is being conducted every 12 (±1) weeks, until the end of follow-up.

Immunological responses to the vaccine are evaluated ex vivo. Blood samples are collected from patients on the day of the first, fourth and seventh WT1/DC vaccine dose. Blood samples are processed and cryopreserved for later bulk in-depth T cell analysis by means of flow cytometry and/or RNA sequencing. Tumour resection or biopsy specimens, if available, are assessed for WT1 expression and other relevant tumour characteristics by means of immunohistochemistry. If possible, biomarkers will be identified based on associations with clinical and immunological responses following DC vaccination (if homogeneity of population allows).

To assess changes in general and disease-specific quality of life during the study, parents/legal guardians and participants aged 5 years and older are asked to complete general and disease-specific quality-of-life questionnaires (standard PedsQL Generic Core Scales and PedsQL Cancer Module, respectively).[47 48] Evaluation takes place at the time of screening, once during chemoradiation (stratum A), before the start of induction phase and after completion of chemoradiation (stratum A), after the induction phase and before the start of the booster phase, around the time of disease assessment during the booster phase and possible continuation of therapy thereafter, and a last time 90 days (±1 week) after the last WT1/DC vaccine (figure 1, asterisks). Executive function is assessed using the BRIEF questionnaire[49] at trial entry, at

the end of study treatment scheme and during follow-up, 90 days (± 1 week) after the last DC vaccine.

Data and safety monitoring

Compliance with Good Clinical Practice (GCP) guidelines is monitored by independent monitors of the Clinical Trial Center of UZA. An independent international data safety monitoring board (DSMB) is instated to protect the interests of the patients. The DSMB receives a monthly summary of trial progress and meets at least every 6 months to review the latest study results as well as data that have become available from other related studies. Based on their review, the DSMB provides recommendations to the sponsor on study continuation, amendment or discontinuation. In case of the occurrence of severe toxicities, the DSMB will immediately review the available data and formulate recommendations to the sponsor.

Analysis of endpoints

For the purpose of data analysis, the following study populations are defined. The intention-to-treat (ITT) population includes all patients enrolled in the study. The efficacy evaluable population includes all eligible patients enrolled in the study who have started the investigational treatment (administration of at least one DC vaccine) and did not have a major protocol violation. The safety population includes all patients who were administered at least one DC vaccine. The immunogenicity population includes all patients of whom sufficient blood sample material from at least before and after the DC vaccine induction phase is available for analysis.

Evaluation of feasibility (primary endpoint) is done by assessing the proportion of patients in the ITT population that had successful leukapheresis and successful vaccine production (ie, production of nine or more vaccine doses meeting quality control requirements) as well as the proportion of patients who completed the study treatment schedule (ie, from leukapheresis until the administration of the ninth vaccine). The proportion of efficacy evaluable patients in the ITT population is another measure of feasibility. Results will be presented as percentage with 95% CI. Safety (primary endpoint) is evaluated by assessing the occurrence of AEs and SAEs during the DC vaccine administration and follow-up period, taking into account their relationship with DC vaccination. (S)AEs and their grade are reported per patient in the safety population and, if homogeneity of the population allows, reported as frequencies.

Secondary endpoints for clinical activity are determined in the efficacy evaluable population and include:

1. Best overall response (BOR), which is determined per patient as the best response designation over the study, based on radiological RANO criteria.[46] The response categories are complete response, partial response, stable disease and progressive disease.
2. PFS, defined as the time (in months) between diagnosis/study entry and the date of progression (recurrence in the case of total resection) or death due to any cause, whichever occurs first.
3. OS, defined as the time (in months) between diagnosis/study entry and death due to any cause.

In-depth T cell reactivity is assessed to evaluate immunogenicity (secondary endpoint) for all patients of the immunogenicity population. They include, but are not limited to, the following measures of (antitumour) immune responses:

1. Occurrence of WT1-specific CD8+ T cells.
2. Functional WT1-specific T cell responses.

Patient-reported outcome measures are secondary endpoints, assessed by means of general and disease-specific quality-of-life questionnaires, completed at different time points throughout the course of the study. We evaluate:

1. How patients experience different phases of the study treatment schedule
2. How patient-reported and proxy-reported disease-related symptoms evolve over time during the study
3. How patient-reported and proxy-reported general quality of life evolves over time during the study

Secondary endpoints for clinical activity (BOR, PFS and OS), immunogenicity and quality-of-life evaluation are reported per patient. If homogeneity of population allows, summary measures will be calculated. In addition, for quality-of-life evaluation, associations with endpoints for clinical activity are studied graphically, and if homogeneity of population allows, association measures will be calculated.

By means of associative analyses, prognostic, predictive and/or therapeutic biomarkers (exploratory endpoint) are identified (if homogeneity of population allows). By means of questionnaires, completed before and after the study treatment scheme, we assess how the patient's executive function (exploratory endpoint) changes from baseline. For biomarker identification, associations are studied graphically, and if homogeneity of population allows, association measures will be calculated. Exploratory endpoints relating to patient-reported and proxy-reported executive function are reported per patient. If homogeneity of population allows, summary measures will be calculated.

Ethics and dissemination

The trial is conducted according to the principles of the Declaration of Helsinki and has been approved by the Ethics Committee of the Antwerp University Hospital and the University of Antwerp (Edegem, Belgium) and by the Belgian Federal Agency for Medicines and Health Products. Trial insurance is foreseen by the trial sponsor, the Antwerp University Hospital. An independent international DSMB has been installed and is in place to protect the interests of the patients.

After an informed discussion with the investigator, informed consent documents (online supplemental appendix 2) are signed by the patient (required if aged ≥12 years, optional if younger) and parents. Patient

samples and data are stored in a pseudonymised manner for a duration of 30 years and can potentially be used for ancillary studies, informed consent of patient/parents and additional ethics committee approval was obtained.

Results of the clinical trial will be shared at international conferences and in peer-reviewed scientific journals and on the Clinical Trials Information System and clinicaltrials.gov.

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Contributors TVG, MDL, M-MC, JV, KN, SA, ZB and EL conceived and designed the trial protocol. TVG, MDL, JVdB, BS, KDR, RP, KVDW, MH, KC, GN and SV participated in logistical planning and execution of the clinical trial. TVG and MDL wrote the initial draft of the manuscript. M-MC, SM, SVB, SD, MH, NC, JV, KN, SA, ZB and EL reviewed the manuscript. CD and KH collected data as representatives of the pediatric oncology clinical trial unit. ER provided the statistical support for the sample size estimates and the design of the statistical analysis. ZB is the principal investigator, and TVG, JV and KN are subinvestigators of the clinical trial. SVB and SD are reference radiologists of the clinical trial. NC is responsible for manufacturing of investigational medicinal product. TVG is responsible for patient recruitment and follow-up. TVG, MDL, JVdB, BS, KDR, CD, KH, RP, M-MC, KVDW, ER, SM, SVB, SD, MH, KC, GN, NC, JV, KN, SV, SA, ZB and EL made significant contributions to the development and conceptualisation of the protocol, reviewed the draft versions of this paper and have read and approved the final manuscript.

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Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the Ethics Committee of UZA, Edge Number 683. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data will not be freely accessible, but will be saved and made available for clinical reseachers upon request.

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