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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.

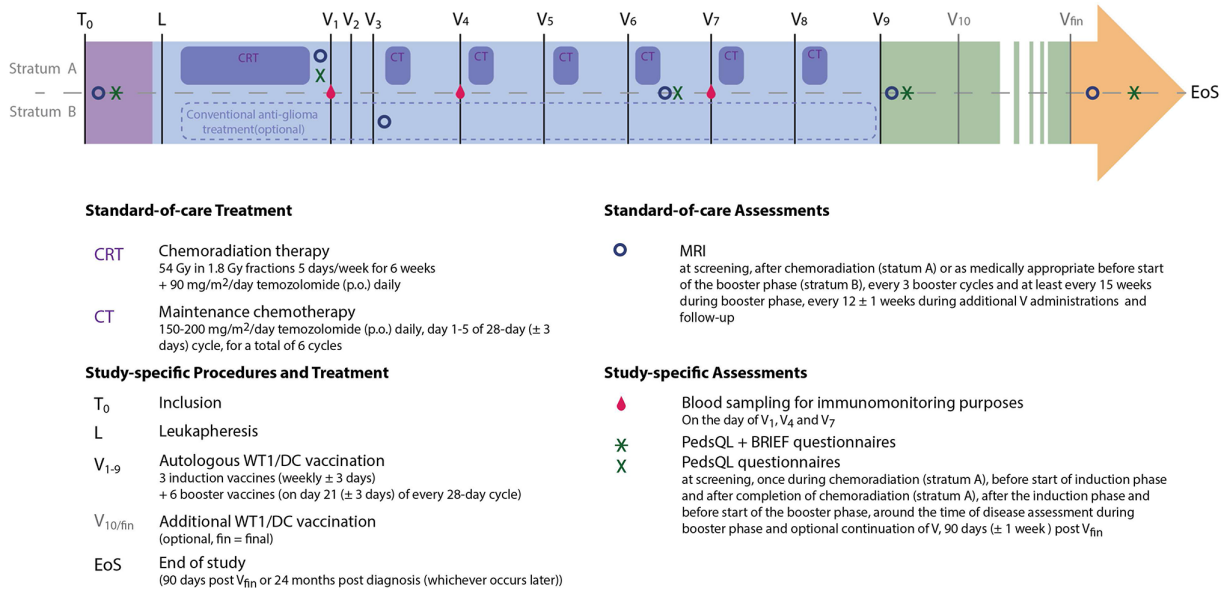

Figure 1 Schematic overview of the study treatment scheme and trial-related procedures.

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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Competing interests None declared.

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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REFERENCES

1 Hudson MM, Link MP, Simone JV. Milestones in the Curability of pediatric cancers. *J Clin Oncol* 2014;32:2391–7.
2 Burdach SEG, Westhoff M-A, Steinhauser MF, *et al*. Precision medicine in pediatric oncology. *Mol Cell Pediatr* 2018;5:6.
3 Esfahani K, Roudaia L, Buhlaiga N, *et al*. A review of cancer Immunotherapy: from the past, to the present, to the future. *Curr Oncol* 2020;27(Suppl 2):S87–97.
4 Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer Immunotherapy: from T cell basic science to clinical practice. *Nat Rev Immunol* 2020;20:651–68.
5 Malbari F. Pediatric neuro-oncology. *Neurol Clin* 2021;39:829–45.
6 Clymer J, Kieran MW. The integration of biology into the treatment of diffuse intrinsic pontine glioma: A review of the North American clinical trial perspective. *Front Oncol* 2018;8.
7 El-Ayadi M, Ansari M, Sturm D, *et al*. High-grade glioma in very young children: a rare and particular patient population. *Oncotarget* 2017;8:64564–78.
8 Frazier JL, Lee J, Thomale UW, *et al*. Treatment of diffuse intrinsic brainstem gliomas: failed approaches and future strategies. *J Neurosurg Pediatr* 2009;3:259–69.
9 Gielen GH, Baugh JN, van Vuurden DG, *et al*. Pediatric high-grade gliomas and the WHO CNS tumor classification—perspectives of pediatric neuro-Oncologists and Neuropathologists in light of recent updates. *Neurooncol Adv* 2022;4.
10 Anguille S, Smits EL, Bryant C, *et al*. Dendritic cells as pharmacological tools for cancer Immunotherapy. *Pharmacol Rev* 2015;67:731–53.
11 Lion E, Smits ELJM, Berneman ZN, *et al*. NK cells: key to success of DC-based cancer vaccines *Oncologist* 2012;17:1256–70.
12 Van Acker HH, Anguille S, Van Tendeloo VF, *et al*. Empowering gamma Delta T cells with antitumor immunity by Dendritic cell-based Immunotherapy. *Oncoimmunology* 2015;4:e1021538.
13 Palucka K, Banchereau J. Cancer Immunotherapy via Dendritic cells. *Nat Rev Cancer* 2012;12:265–77.
14 Sprooten J, Ceusters J, Coosemans A, *et al*. Trial watch: Dendritic cell vaccination for cancer Immunotherapy. *Oncoimmunology* 2019;8:e1638212.
15 Hsu FJ, Benike C, Fagnoni F, *et al*. Vaccination of patients with B-cell lymphoma using Autologous antigen-pulsed Dendritic cells. *Nat Med* 1996;2:52–8.
16 Caruso DA, Orme LM, Neale AM, *et al*. Results of a phase 1 study utilizing monocyte-derived Dendritic cells pulsed with tumor RNA in children and young adults with brain cancer. *Neuro Oncol* 2004;6:236–46.
17 Caruso DA, Orme LM, Amor GM, *et al*. Results of a phase I study utilizing monocyte-derived Dendritic cells pulsed with tumor RNA in children with stage 4 neuroblastoma. *Cancer* 2005;103:1280–91.
18 Lasky JL 3rd, Panosyan EH, Plant A, *et al*. Autologous tumor Lysate-pulsed Dendritic cell Immunotherapy for pediatric patients with newly diagnosed or recurrent high- grade gliomas. *Anticancer Res* 2013;33:2047–56.
19 de Bruijn S, Anguille S, Verlooy J, *et al*. Dendritic cell-based and other vaccination strategies for pediatric cancer. *Cancers (Basel)* 2019;11.
20 De Vleeschouwer S, Fieuws S, Rutkowski S, *et al*. Postoperative adjuvant Dendritic cell-based Immunotherapy in patients with Relapsed glioblastoma multiforme. *Clin Cancer Res* 2008;14:3098–104.

21 Hirabayashi K, Yanagisawa R, Saito S, *et al*. Feasibility and immune response of Wt1 peptide vaccination in combination with OK-432 for Paediatric solid tumors. *Anticancer Res* 2018;38:2227–34.
22 Berneman ZN, Anguille S, Willemen Y, *et al*. Vaccination of cancer patients with Dendritic cells Electroporated with mRNA Encoding the Wilms' tumor 1 protein (Wt1): correlation of clinical effect and overall survival with T-cell response. *Cytotherapy* 2019;21.
23 Cheever MA, Allison JP, Ferris AS, *et al*. The Prioritization of cancer antigens: a national cancer Institute pilot project for the acceleration of Translational research. *Clin Cancer Res* 2009;15:5323–37.
24 Yang L, Han Y, Suarez Saiz F, *et al*. A tumor Suppressor and Oncogene: the Wt1 story. *Leukemia* 2007;21:868–76.
25 Nakatsuka S, Oji Y, Horiuchi T, *et al*. Immunohistochemical detection of Wt1 protein in a variety of cancer cells. *Mod Pathol* 2006;19:804–14.
26 Nakahara Y, Okamoto H, Mineta T, *et al*. Expression of the Wilms' tumor gene product Wt1 in Glioblastomas and Medulloblastomas. *Brain Tumor Pathol* 2004;21:113–6.
27 Lee S, Kambhampati M, Yadavilli S, *et al*. Differential expression of Wilms' tumor protein in diffuse intrinsic pontine glioma. *Journal of Neuropathology & Experimental Neurology* 2019;78:380–8.
28 Oji Y, Suzuki T, Nakano Y, *et al*. Overexpression of the Wilms' tumor gene W T1 in primary Astrocytic tumors. *Cancer Sci* 2004;95:822–7.
29 Ohta H, Hashii Y, Yoneda A, *et al*. Wt1 (Wilms tumor 1) peptide Immunotherapy for childhood Rhabdomyosarcoma: a case report. *Pediatr Hematol Oncol* 2009;26:74–83.
30 Oka Y, Tsuboi A, Taguchi T, *et al*. Induction of Wt1 (Wilms' tumor Gene)-Specific cytotoxic T lymphocytes by Wt1 peptide vaccine and the resultant cancer regression. *Proc Natl Acad Sci U S A* 2004;101:13885–90.
31 Sawada A, Inoue M, Kondo O, *et al*. Feasibility of cancer Immunotherapy with Wt1 peptide vaccination for solid and hematological malignancies in children. *Pediatr Blood Cancer* 2016;63:234–41.
32 Hashii Y, Oka Y, Kagawa N, *et al*. Encouraging clinical evolution of a pediatric patient with Relapsed diffuse midline glioma who underwent Wt1-targeting Immunotherapy: A. *Front Oncol* 2020;10.
33 Anguille S, Van de Velde AL, Smits EL, *et al*. Dendritic cell vaccination as Postremission treatment to prevent or delay relapse in acute myeloid leukemia. *Blood* 2017;130:1713–21.
34 Van Tendeloo VF, Van de Velde A, Van Driessche A, *et al*. Induction of complete and molecular remissions in acute myeloid leukemia by Wilms' tumor 1 antigen-targeted Dendritic cell vaccination. *Proc Natl Acad Sci U S A* 2010;107:13824–9.
35 Garg AD, Vara Perez M, Schaaf M, *et al*. Trial watch: Dendritic cell-based anticancer Immunotherapy. *Oncoimmunology* 2017;6:e1328341.
36 Stupp R, Pavlidis N, Jelic S, *et al*. ESMO minimum clinical recommendations for diagnosis, treatment and follow-up of malignant glioma. *Ann Oncol* 2005;16 Suppl 1:i64–5.
37 Karachi A, Dastmalchi F, Mitchell DA, *et al*. Temozolomide for Immunomodulation in the treatment of glioblastoma. *Neuro Oncol* 2018;20:1566–72.
38 Emens LA, Middleton G. The interplay of Immunotherapy and chemotherapy: harnessing potential synergies. *Cancer Immunol Res* 2015;3:436–43.
39 Antonia SJ, Mirza N, Fricke I, *et al*. Combination of P53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer. *Clin Cancer Res* 2006;12(3 Pt 1):878–87.
40 Wheeler CJ, Black KL, Liu G, *et al*. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. *Cancer Res* 2008;68:5955–64.
41 Alhajala HS, Nguyen HS, Shabani S, *et al*. Irradiation of pediatric glioblastoma cells promotes Radioresistance and enhances glioma malignancy via genome-wide Transcriptome changes. *Oncotarget* 2018;9:34122–31.
42 Fadul CE, Fisher JL, Hampton TH, *et al*. Immune response in patients with newly diagnosed glioblastoma multiforme treated with Intranodal Autologous tumor Lysate-Dendritic cell vaccination after radiation chemotherapy. *J Immunother* 2011;34:382–9.
43 Westdorp H, Creemers JHA, van Oort IM, *et al*. Blood-derived Dendritic cell Vaccinations induce immune responses that correlate with clinical outcome in patients with Chemo-naive Castration-resistant prostate cancer. *J Immunother Cancer* 2019;7.
44 De Keersmaecker B, Claerhout S, Carrasco J, *et al*. Trimix and tumor antigen mRNA Electroporated Dendritic cell vaccination plus Ipilimumab: link between T-cell activation and clinical responses in advanced Melanoma. *J Immunother Cancer* 2020;8:e000329.
45 Chan A-W, Tetzlaff JM, Gøtzsche PC, *et al*. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ* 2013;346:e7586.
46 Chang SM, Wen PY, Vogelbaum MA, *et al*. Response assessment in neuro-oncology (RANO): more than imaging criteria for malignant glioma. *Neurooncol Pract* 2015;2:205–9.
47 Varni JW, Seid M, Rode CA. The Pedsql: measurement model for the pediatric quality of life inventory. *Med Care* 1999;37:126–39.
48 Varni JW, Burwinkle TM, Katz ER, *et al*. The Pedsql in pediatric cancer: Reliability and validity of the pediatric quality of life inventory generic core scales, multidimensional fatigue scale, and cancer Module. *Cancer* 2002;94:2090–106.
49 Baron IS. Behavior rating inventory of executive function. *Child Neuropsychol* 2000;6:235–8.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	1-2
Protocol version	#3	Date and version identifier	15
Funding	#4	Sources and types of financial, material, and other support	16
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	8
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	8,13
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	8
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	n/a
Objectives	#7	Specific objectives or hypotheses	7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4,7
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	8

		be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8,16
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-11
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	13
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	n/a
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	11
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	13-15
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	11-12
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	7,8
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	n/a
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	n/a
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	n/a
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	n/a
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	n/a
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known.	12-13

		Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	n/a
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-15
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13-15
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	n/a
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	13, 15
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	n/a
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events	14

		and other unintended effects of trial interventions or trial conduct	
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	13
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	15
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	n/a
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	15
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	15
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	16
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	15
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the	15

		public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	n/a
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	appendix 1
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	n/a

INFORMED CONSENT FORM

CCRG19-002 – PARTICIPANT (8-11 years)

A vaccine against brain tumors

What is this study about?

You have been told by your doctor that you are suffering from a malignant tumor which is growing in your brain or brain stem.
Tumor cells multiply quickly. In this way, the tumor can grow. This is an important property of tumor cells: tumor cells often divide and multiply more quickly than healthy cells do. We can use this property to fight the tumor cells.

The standard treatment for your disease consists of a combination of chemotherapy and radiotherapy.

- **Chemotherapy** is a type of medication that attacks and destroys cells which are rapidly dividing. In that way, chemotherapy will have a more profound effect on tumor cells than on healthy cells.

- **Radiotherapy** is also known as 'irradiation'. You cannot see, feel or smell irradiation. Irradiation are actually waves of energy. The doctor will target these waves onto the tumor cells using a special device. The irradiation will damage and destroy the tumor cells. Irradiation also has a stronger effect on rapidly dividing tumor cells than on healthy cells.

In the hospital, we are investigating a new manner to treat your brain tumor even better. This new treatment is called '**immunotherapy'**.
This immunotherapy treatment is made starting from **your own cells**, namely your white blood cells.
We will help your white blood cells to fight the tumor. To do so, your white blood cells will be transformed into **smart cells** in the laboratory.
These smart cells will be injected back into your body, like a **vaccine**.
In your body, these smart cells will help your other white blood cells to **find and destroy the tumor cells**.

You will receive this immunotherapy treatment together with or following other cancer treatments. The intention is that these different types of treatments will work together to have an even stronger effect. In this way, we hope to be able to treat your brain tumor even better.

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

What is going to happen?

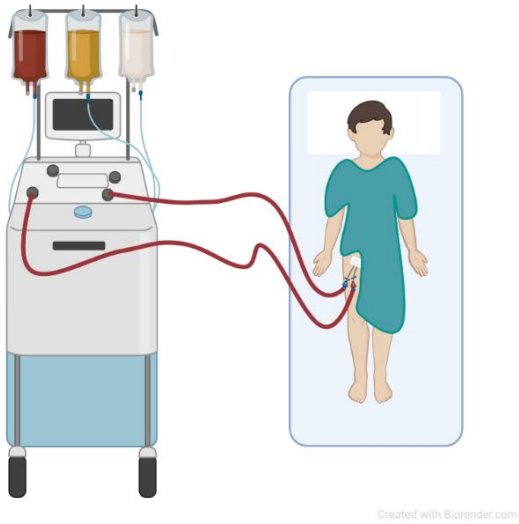
1. Production of the new treatment

This new treatment (immunotherapy) will be made in several different steps.

First, we will collect as many of your white blood cells as possible.
We have a special machine for that.
This machine will pump blood out of your body, will collect the white bloods cells in a small bag and will pump the rest of your blood back into your body.
This will be done using a special tube or catheter. Sometimes, this catheter will be placed in your groin. Sometimes, it may also be possible to use two separate catheters, one in each of both your arms. Placing the catheter in your groin will be done while you are sleeping, so that you won't feel anything.

The catheter will be removed when we have collected sufficient white blood cells.
Usually one collection procedure is enough, but it is possible that the procedure will need to be repeated the next day. In that case, you will have to stay in the hospital overnight.

It is important not to move too much while the machine is working.



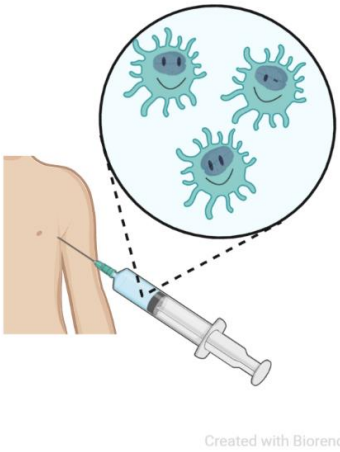
We will collect your white blood cells using this machine, while the rest of your blood is pumped back into your body. The procedure can be done via a catheter placed in your groin (see image) or through two separate catheters placed in both your arms (in case your blood vessels are strong and large enough).

After the procedure has ended, the bag with white blood cells will be transported to the laboratory.
In the laboratory, we will produce the new therapy starting from your white blood cells.
Your white blood cells will be transformed into smart cells in several steps.
Eventually, the smart cells will be transferred into a syringe.
We call this syringe a vaccine.
We try to produce as many vaccines as possible for you.

In a specialized laboratory, your white blood cells will be transformed into smart cells. Now they know how to help your body to fight the tumor. These smart cells are transferred into a syringe. This we call a vaccine.

2. Administration of the new therapy

The smart cells will be administered to you using a syringe.
Five injections will be given in your arm, near your armpit.
We will make sure this does not hurt too much.
For this, we could for example put some ointment onto your skin.



Every time you receive a vaccine, the doctor will give 5 injections into the skin, near the armpit cavity.

The smart cells will spread inside your body and will tell the other white blood cells in your body how they can find and destroy the tumor cells.
In this way, they can help to beat the tumor.
You will receive 9 vaccines in total.
Along with the vaccines, you will possibly receive other treatments, like chemotherapy or radiotherapy.
Your doctor will tell you more about this.
It is possible that we will be able to produce more than 9 vaccines.
In this case, it is possible for you to receive these additional vaccines afterwards, if you want this and if the doctor judges that the previous vaccines have been beneficial to you.

3. Follow-up in the hospital

It is important to come regularly to the hospital for a check-up.
During these visits we will investigate how your body and the tumor react to the new treatment with smart cells and to the other cancer treatments you are receiving (if any).
Your doctor will also take time to answer your questions.
Did you experience any pain during the past weeks, or was there something that bothered you? If yes, tell your doctor about it.

To monitor the tumor, we will make regular images (scans) of your brain.
We have a specialized machine for that too: an MRI machine.
You will have to lie inside of this machine, without moving, so that the machine can take a good picture of your brain and the tumor.
This machine will make a lot of noise, but it will not hurt. You will receive a separate brochure with additional information.

We will regularly collect blood from you. Blood drawing is also performed during standard treatment. For this study, we will collect some extra blood at specific time points. We will use this blood to investigate how your white blood cells react to the vaccine with smart cells. Drawing blood will be done using a needle. To make sure that the puncture does not hurt too much, the doctor can put some ointment onto your skin. Perhaps, the doctor has previously placed a port/tube (also called central catheter or port-a-cath). In that case, an additional puncture for blood drawing will not be required.

Also after you have received your last vaccine, you will have to come to the hospital regularly for check-ups.
The study will last approximately 2 years for you.

What are the advantages and disadvantages?

- For collecting your white blood cells using the specialized machine, we will have to place a catheter using a needle. Possibly, this catheter will be placed in your groin. This will be done while you are sleeping so that you won't feel anything. This is called 'under general anesthesia'. As with any anesthesia, there are risks involved. The 'sleep doctor' or anesthetist will discuss these risks with you.

- While the machine is collecting your white blood cells, you will not be able to move a lot, for several hours. This way, the machine can do its job.
  Your lips, arms or legs may start to tingle.
  You may also feel dizzy.
  Make sure to tell the nurse or doctor if this happens.
  They can give you something to make you feel better again.

- Your skin may become red or itchy after you have received a vaccine.
  You may also feel unwell and get fever.
  If this happens, make sure to tell your doctor about this immediately or during your next check-up.

- We are still investigating this new immunotherapy treatment.
  Therefore, it is possible that you experience other side effects that we are not yet aware of.
  Tell your doctor everything you feel or experience after you have received a vaccine.

- We are not sure whether or not this new immunotherapy treatment will prove to work for your disease.

Important to know!

- Is something unclear?
  Would you like to know more about this study?
  Your doctor will answer all questions you might have.
  You can find the contact details here:

  Name of your doctor:……

  Telephone number of your doctor: ……

- You do not have to participate in this study if you do not want to.

- You can at any time decide to stop participation in this study. You don't have to tell us why.

Do you want to participate?

Please sign this form.
Your doctor will also sign this form.

Name participant:……

Signature participant: Date:

Name doctor:……

Signature doctor: Date:

INFORMED CONSENT FORM

CCRG19-002 – PARTICIPANT (12-17 y)

Official title of the trial:

Adjuvant dendritic cell immunotherapy complementing conventional therapy for pediatric patients with high-grade glioma and diffuse intrinsic pontine glioma

Lay title of the trial:

Personalized dendritic cell therapy to improve the treatment of children and adolescents diagnosed with brain or brain stem tumors

EUDRACT-number:

2020-004125-23

Internal study number:

CCRG19-002

Sponsor of the trial:

Antwerp University Hospital
Drie Eikenstraat 655
2650 Edegem
Belgium

Study center name:

Center for Cell Therapy and Regenerative Medicine (CCRG)
Antwerp University Hospital (UZA)
Drie Eikenstraat 655
2650 Edegem
Belgium

Version number	Approval date	Revision description
V1.0	*Not applicable*	First version
V1.1	*To be determined*	Minor adjustments following advice obtained from the Ethics Committee and FAMHP

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

Who can I contact in case of questions?

Name	Function	In case of	Contact details
Berneman, Zwi	Principal Investigator	Information, problems or concerns about the investigational medicinal product.	0032 3 821 39 15 zwi.berneman@uza.be
Pediatrician	Investigator	Information, problems or concerns about your medical condition or treatment.	0032 3 821 38 10 0032 3 821 30 00
Caroline De Schepper	Contact person trial staff (Pediatric department)	Information, problems and concerns about the study and your rights as a participant in the trial.	0032 3 821 58 39
Kim De Rycke Maxime De Laere	Contact person trial staff (Study center CCRG)	Information, problems and concerns on practical aspect of the trial, for example planned visits.	0032 3 821 58 19 ccrg@uza.be
Nursing station Pediatrics	Emergency contact	Emergency	0032 3 275 73 79

Table of contents

THE TRIAL AT A GLANCE

Dear _____,

You have been diagnosed with a severe brain or brain stem tumor: a high-grade glioma or a diffuse intrinsic pontine glioma. The doctor has told you that the chance of the tumor reoccurring or progressing after treatment, is high. That is why you are invited to take part in a clinical trial (further referred to as "trial"). In this trial, we will evaluate a new kind of treatment (the “study treatment”) as a supporting treatment for your disease. “Supporting” means that the study treatment is given in addition to (together with or following) an available standard treatment (for example, chemotherapy and/or radiation).

Before you agree to participate in this trial, we want to make sure you are fully informed about the trial, and its possible risks and benefits. In this way, you can for yourself decide whether or not you want to participate. This we call “giving informed consent”.

After this introduction, you will already have an idea about what the trial is about. However, please make sure to read all pages of this informed consent form. It is important that you read and understand all of the information. Do not hesitate to ask any questions before you sign this document. By signing this document, you agree to participate in this trial.

About the study treatment

The study treatment which is under investigation in this trial, is a vaccine*[1] based on dendritic cells*. Dendritic cells are cells of our immune system, which is our body’s defense mechanism against different types of diseases. Vaccination with dendritic cells is a form of immunotherapy. This means that the patient’s own defense mechanisms (or immune system) are (is) stimulated to kill the cancer cells which are present in the body.

The dendritic cell vaccines will be produced from your own cells and will only be used as a therapy for yourself. To produce these vaccines, you will need to undergo a leukapheresis* procedure. Leukapheresis is a procedure to collect white blood cells from your blood. White blood cells are the cells of the immune system. The collected white blood cells will be processed in a specialized laboratory into dendritic cells. These dendritic cells will be administered to you, using a syringe, by injection into the skin. This we call “vaccination”.

This investigational medicinal product has not yet been approved by the Belgian authorities for your condition. It has not yet been proven that it can cure, improve or stabilize your disease. The main aim of this trial is to investigate safety, feasibility and efficacy of dendritic cell vaccination when it is combined with the available standard therapies. It is uncertain at this point whether you will benefit from it.

What will happen during the trial?

Over the course of the trial, you will receive 9 dendritic cell vaccinations, which will be administered to you over a course of several months. If you have not yet undergone any previous anti-glioma treatments, vaccination will be combined with the best available standard treatment, namely irradiation (radiotherapy) combined with chemotherapy, followed by chemotherapy alone. If you have already undergone an anti-glioma treatment, it is also still possible to participate in this trial. In this case, we look how we will optimally fit dendritic cell vaccination into your treatment schedule.

If after 9 vaccines, additional vaccines are still available, these can be administered to you as well, if you agree with this and if the investigators approve this. You will have to come to the Antwerp University Hospital for the administration of the dendritic cell vaccines.

If you agree to take part in the trial, you will first have to undergo a screening investigation to check whether you meet all the conditions to be accepted for this trial. During the study, you will regularly have to come the Antwerp University Hospital for clinical monitoring of your disease and your reactions to the study treatment. During these visits, blood samples will be collected for laboratory tests. In addition, you will undergo an imaging examination (MRI) of the tumor at specific time points. Finally, we will ask you and your parents to fill out questionnaires that assess your quality of life and your daily functioning at regular time points.

Overall, your participation in the trial will last about 2 years. After administration of the final dendritic cell vaccine, you will be followed-up for some more time.

Does taking part in the trial involve potential risks?

It is very important that you are aware that any medicinal product can cause side effects. Vaccination with dendritic cells has been performed in various medical centers around the world without signs of serious unwanted side effects. During and short after administration of a vaccine, you may experience a reaction at the injection site (pain, redness, swelling and itching). You may also temporarily feel unwell and/or have fever or chills.

If you experience any harm due to your participation in the trial, the trial sponsor is responsible for this. The sponsor has taken an appropriate insurance for this.

Important to know

You are not allowed to become pregnant or to get someone pregnant during the trial and for some time afterwards. We will discuss appropriate methods of birth control with you.

You are also not allowed to take part in another clinical trial at the same time, without informing the investigator or the trial staff. We may refuse participation to other trials for justified reasons. It is also very important that you cooperate and follow the instructions that the trial staff members give you with regard to the trial. You will receive an "emergency card", which says that you are taking part in a clinical trial. You must carry this card with you at all times; this is necessary to ensure your safety, in case you should have to undergo emergency treatment in a hospital where they don't know you.

Giving informed consent

If you agree to participate, you will have to sign the informed consent form. A trial staff member will also sign the form and thereby confirm that you have received the necessary information about the trial. You will receive a signed and dated copy of the form. Your parents will have to sign an informed consent form too.

The Belgian authorities and an ethics committee* have evaluated this trial. You should not under any circumstances feel obliged to participate, just because they approved this study. Even after you have started the trial, you can still decide to stop at any time. We will fully understand your decision and will continue to take care of you as before.

Are you considering to participate? In the following pages, you will find more information on the trial and about your rights as a trial participant. Please take your time to read the information carefully, so

[1] Words and expressions which are followed by '*' are explained in more detail in the glossary at the end of this document.

you are aware of the decisions that can be made by you. You can take your time to think about these decisions.

Your parents also receive information about this trial. You can discuss the trial with them so you can decide together. We need both your consent and that of your parents for you to participate in the trial.

The trial staff members are also available to help you if there is anything that is not clear. It is our job to make sure that you understand all of the information.

With our best regards,

The trial staff members

CHAPTER I – DESCRIPTION OF THE TRIAL AND YOUR RIGHTS WHEN PARTICIPATING

1. Why are we performing this trial?

Currently, for some types of brain tumors, including high-grade gliomas or diffuse intrinsic pontine gliomas, there is no treatment that always leads to a complete cure. Tumor resection during surgery (if possible) followed by radio- and chemotherapy is currently the best available treatment approach. However, the chance that the tumor reoccurs is very high. This is because often, some cancer cells persist after treatment. These may grow back into a tumor. In some cases, it is not possible at all to surgically remove the tumor. Therefore, there is an urgent need for new treatments that can prevent or postpone tumor progression or recurrence.

Boosting the immune system is a promising strategy to eliminate remaining tumor cells. The immune system disposes of natural defense mechanisms against tumor cells. Vaccination* with dendritic cells* is a manner to boost these natural defense mechanisms. Dendritic cells are immune cells which play an important role in organizing the body's defense against cancer. The goal of dendritic cell vaccination is to stimulate the body's own defense mechanisms against tumor cells. Therefore, this type of vaccination is called "active immunotherapy"*. Dendritic cell vaccination might help to delay or prevent disease progression or recurrence, and in this way, improve patients' survival.

Here, at the University of Antwerp (UAntwerp) and the Antwerp University Hospital (UZA), we are working very hard on the development and improvement of active cancer immunotherapy using dendritic cells. We developed a dendritic cell vaccine that has been tested in several clinical trials. More than 100 adult patients with different tumor types were treated with this dendritic cell vaccine at the Antwerp University Hospital, among which 30 adult patients with glioblastoma multiforme, a type of high-grade glioma.

The main aim of this clinical trial is to investigate whether it is possible and safe to combine dendritic cell vaccination with available standard therapies (such as radio- and chemotherapy) in children and adolescents diagnosed with high-grade glioma or diffuse intrinsic pontine glioma. In addition, we will also investigate the mechanism of action of dendritic cell vaccination as supporting therapy for these tumor types.

2. Why am I being asked to take part?

You have been diagnosed with a high-grade glioma or a diffuse intrinsic pontine glioma.

In this clinical trial, we will investigate an experimental treatment for these types of tumors. An experimental treatment is a treatment that is still under investigation to assess the safety, efficacy and/or mechanism of action.

It is unsure whether participating in this study will ameliorate your disease, improve your quality of life or extent your life expectancy.

The investigator or a trial staff member will discuss with you and your parents the conditions which need to be met for you to be accepted in the trial.

3. Do I have to take part in a trial?

Your participation in this trial is voluntary and cannot be coerced by anyone. This means that you have the right not to take part in the trial, even when your parents prefer you do.

If you want to participate, you will have to sign the informed consent form at the end of this document. Even if you agree to participate now, you can still withdraw from the trial at any time. You will not need to give a reason for doing so.

If other treatments are available for your disease, the investigator or his/her delegate will inform you on the treatments you might receive if you do not participate in this trial.

4. What will happen during the trial?

This trial is conducted at the Antwerp University Hospital. In total, 10 children and adolescents will participate.

Your participation in the trial will last about 2 years. Over the entire duration of the study, you will regularly need to come to the Antwerp University Hospital. An overview of all procedures and examinations that you will be undergoing in the context of this clinical trial is given below.

4.1. Screening visit

Before you can participate in this trial, we need to check whether you meet all conditions to be accepted into the trial. For this, we will do the following actions and examinations:

- We will go through this information and informed consent form. If you want to participate, you will need to sign the informed consent form. Your parents will need to consent too by signing an informed consent form.
- The doctor will perform a physical examination.
- An evaluation of the tumor will be performed. For this, we will perform a neurological examination, in which we assess the complaints you are experiencing because of the tumor. We will also perform an MRI scan, so we know the tumor's location and size.
- We will check whether the veins in both of your arms are fit to undergo a leukapheresis* procedure. For this, you need large and strong veins. If leukapheresis through the veins of the arms is not possible, a catheter will be placed in your groin.
- We will ask you to complete 2 questionnaires about your quality of life and one about your daily functioning. Your parents will complete these questionnaires as well.
- We will draw blood (3,8 mL[2], 6 tubes = about 1 teaspoon) for laboratory tests. Blood drawing will be done by means of a needle or using the central catheter (port-a-cath), if you have one. If these blood tests reveal any abnormalities, you and your parents will be infomed.

4.2. Leukapheresis

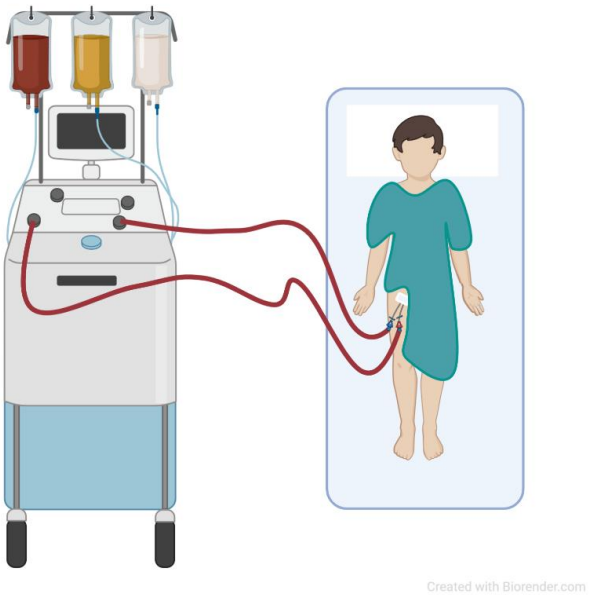
Leukapheresis* is a procedure in which white blood cells (these are the cells of your immune system) are selected and collected from your blood using a specialized machine, the leukapheresis device. For

[2] Indicated blood volumes are volumes minimally required to perform the test in the laboratory. If your body weight is sufficiently high, a higher volume may be taken to allow re-testing in the laboratory, if required.

this, a catheter is inserted into a vein in both your left and right arm using a needle. The arms should not be bent during the procedure to prevent the catheters from shifting.

If the veins in your arms are not strong enough to undergo the leukapheresis procedure, a catheter will be placed in your groin. Placement of the catheter in the groin will be done under local or general anesthesia. A specialized doctor will provide more information to you and your parents about this procedure.

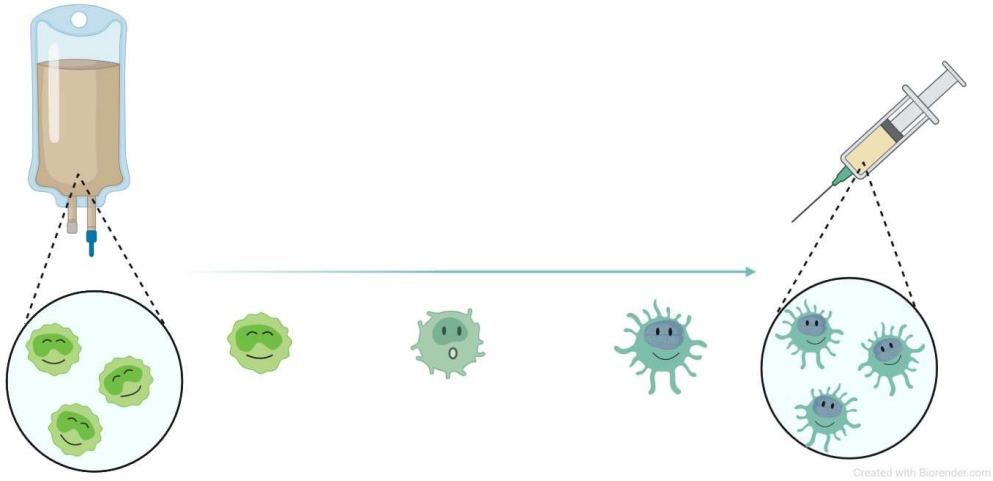
With the help of the machine, blood is taken via the catheter. In the machine, the white blood cells are separated from the other blood cells. The white blood cells are collected in a small amount of fluid and will later be used as starting material for the production of the vaccines. The rest of the blood goes back to your body, via the catheter in the other arm, or via the one in your groin. The leukapheresis procedure lasts four to six hours.



During the leukapheresis procedure, a specialized machine, the leukapheresis device, collects your blood. This can be done via a catheter in the groin area (as is the case here) or via 2 catheters, one in both your arms, if your veins are large and strong enough. The leukapheresis device collects the white blood cells, while the rest of your blood flows back into your body.

The collected white blood cells are processed in a specialized laboratory into dendritic cells for the production of the vaccines. We produce as many vaccines as possible from one leukapheresis procedure. If an insufficient number of cells are present in the leukapheresis product to produce 9 vaccines, a second leukapheresis procedure can be performed the next day. In that case, you will need to stay in the hospital overnight, until the second leukapheresis procedure is completed. After the (second) leukapheresis procedure , the catheter is removed (if applicable).

A maximum of 2 leukapheresis procedures is performed sequentially. An additional leukapheresis procedure at a later time point is possible, under certain conditions (discussed in more detail below).



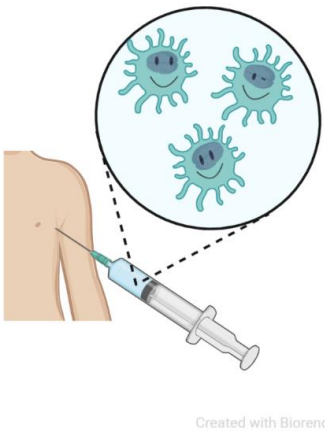
In a specialized laboratory, the white blood cells that were collected from your blood during the leukapheresis procedure, will be grown into dendritic cells. These dendritic cells are used to make the vaccines.

You will undergo following examinations and procedures on the day of leukapheresis.

- We will perform a physical examination. We will for example measure your blood pressure and heart rate.
- A blood sample will be taken before the start of the leukapheresis procedure (2,3 mL – 5 tubes = about half a tea spoon). Blood drawing will be done by means of a needle or using the central catheter (port-a-cath), if you have one.
- If applicable, a catheter will be placed under local or general anesthesia. A separate informed consent form will be provided for this procedure.
- After this, the leukapheresis procedure can start.
- Once the leukapheresis is finished, we will again take a blood sample (1 mL – 2 tubes = about 1/5th of a tea spoon) for laboratory tests.

4.3. Dendritic cell vaccination

You will receive 9 dendritic cell vaccines. The vaccines are administered into the skin at the inside of the arm, near the armpit cavity. Per vaccine, you will receive five injections. A numbing cream may be used to reduce pain associated with the injections.



Every time you receive a vaccine, the doctor will give 5 injections into the skin near the armpit cavity.

The dendritic cell vaccinations can be combined with standard cancer treatments. The treatment schedule depends on whether you have already undergone previous cancer treatments or not.

- **If you have not yet undergone previous anti-glioma treatments**, chemo- and radiotherapy will be started after the leukapheresis procedure for a period of 6 weeks. This is the first part of the standard treatment. After this, for a period of 3 weeks, you will receive one vaccine every week. Thereafter, you will again receive chemotherapy. This is called the “maintenance phase”. During this phase, you will receive chemotherapy for 5 days, every 4 weeks, until you have received 6 cycles in total. This is the second part of the standard therapy. During this maintenance phase, you will receive another 6 vaccines, each time 3 weeks after the chemotherapy.

- **If you have already undergone previous anti-glioma treatments**, your treating physician will decide in consultation with you and your parents whether or not dendritic cell vaccination will be combined with an available standard treatment. This could be continuation of a treatment that you already received or another type of treatment. In total, you will receive 9 vaccines. The first 3 vaccines will be administered weekly. Time intervals between the following 6 vaccines can be longer, up to 4 weeks. A personalized treatment schedule is designed for you by the trial staff to make sure combination of vaccinations with the standard treatment (if any) is optimally timed.

The doctor will provide you with more information about the standard treatments you will be receiving.

About what happens thereafter, you are able to decide yourself, together with your parents:

- If more than 9 vaccines are available, you can choose to receive additional vaccinations after the first 9 vaccines. The investigator needs to agree with additional vaccinations, based on your medical condition at that moment. You agree or disagree with continuation of dendritic cell vaccination after the planned study schedule by ticking the appropriate check-box in Chapter II, page 18
- In case your disease becomes worse during the trial, the study schedule is discontinued. Your doctor will assess your condition and determine the most appropriate treatment. He will discuss

this with you and your parents. If vaccines are still available, you can choose to continue dendritic cell vaccination in combination with another standard treatment. The investigator needs to approve this based on your medical condition at that moment. You agree or disagree with continuation of dendritic cell vaccination after disease progression and adjustment of your standard treatment by ticking the appropriate check-box in Chapter II, page 18.

- If, after you have received all vaccines, the investigator judges you have experienced any advantage of the vaccinations, additional vaccines may be produced. This is only possible if the principal investigator agrees and if your medical condition at that time permits you to undergo another leukapheresis procedure. This will be judged by the investigator. At the end of this informed consent form you agree or disagree to the option of an additional leukapheresis procedure by ticking the appropriate check-box in Chapter II, page 18.

After administration of the last dendritic cell vaccine, a follow-up period is initiated, during which you will have to undergo regular check-ups at the Antwerp University Hospital. This follow-up period will last until 90 days after the final dendritic cell vaccination or until 24 months after signing this informed consent form, whichever occurs last.

You have the right to stop receiving the study treatment or your participation in the study at any time. If you decide to stop receiving the study treatment, you will still regularly have to come to the Antwerp University Hospital for follow-up. If you decide to stop your participation in the study, no new data will be collected.

4.4. Clinical visits and follow-up

We will regularly check your health, the tumor, and your reaction to the standard treatment (if applicable) and the study treatment by means of:

- Physical examination
- Blood draws (1 mL, 2 tubes each time = about 1/5th of a tea spoon) for laboratory tests
- Tumor imaging (MRI of the brain)

For this, you will regularly need to come to the Antwerp University Hospital.

At three time points, additional blood samples will be collected to study how your immune system reacts to the dendritic cell vaccinations. How much blood will be taken, is dependent on your body weight. If you weigh 20 kg or less, this will be 10 mL (1 tube = about 2/3rd of a tablespoon), if you weigh more than 20 kg, this will be 25 mL (3 tubes = about 2 tablespoons) These additional blood draws will take place on the day of the 1st, 4th and 7th vaccination.

You and your parents will be regularly asked to complete questionnaires that assess your quality of life and daily functioning. These questionnaires take about 10-15 minutes to complete.

We also ask your parents' permission to collect and use your medical data relating to the cancer-related disease course (including further treatments and available imaging data) and survival after you have completed the trial.

If you have questions about the trial, do not hesitate to contact your doctor or a trial staff member (see page 2 for contact details).

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

5. Will I benefit from the trial?

We are not sure whether or not you will benefit from the trial. The vaccinations may have a beneficial impact on the tumor. They could prevent tumor growth or delay reoccurrence, and in this way prolong survival. This is however unsure. Even if it is beneficial to you, a potential return or worsening of symptoms, illness or disease is still possible.

Your participation will help the investigators to better understand whether the study treatment is safe and effective. In this way, we can develop improved therapies for future patients with your disease.

6. What are the possible risks and discomforts of taking part?

6.1. What are the possible side effects of dendritic cell vaccination?

Participating in a trial involves some risk. All medicinal products can have side effects. Some of these side effects are already known, and some are not known.

Dendritic cell vaccinations have so far been performed in various medical centers around the world without signs of serious unwanted side effects. At the Antwerp University Hospital, more than 100 adult patients with various cancer types were treated with dendritic cells in the context of clinical research, among which also 30 adult patients with glioblastoma, a specific type of malignant brain tumor.

You must be aware that you may experience the following (temporary) side effects during and shortly after administration of a vaccine:

- Reactions at the injection site (such as pain, redness, swelling and itching) *(often)*,
- Fever *(rarely),*
- general unwell feeling *(rarely),*
- shivering *(rarely).*

In theory, it is possible that the vaccination treatment could allow the immune system to react against normal healthy tissues of the body (= an autoimmune reaction) or weaken the immune system against the cancer cells instead of strengthening it. To date, based on previous trials, there are no concrete indications that these risks will occur.

Because this study treatment is still under investigation, other currently unknown risks and discomforts could occur. **Therefore, it is very important that you report <u>any</u> new or worsened health problems immediately to the investigator, regardless of whether or not you think it has to do with the trial, and even when it is already described in this document. If you need to use other medication, discuss this with the investigator before taking it.**

6.2. What are the possible risks or discomforts or the examinations during the trial?

The examinations and procedures during the trial may cause following discomforts and risks:

Leukapheresis:

- If required for performing the leukapheresis procedure, a catheter will be placed in your groin under local or general anesthesia. The risks related to this will be discussed in a separate informed consent form.
- If you weigh less than 25 kg and/or have a low amount of red blood cells, the leukapheresis device needs to be pre-loaded (‘primed’) with liquid. For this, either a solution containing proteins or blood can be used.

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

- If the protein solution is used for priming, it is possible that you will have to receive donor blood (a 'blood transfusion') after the leukapheresis procedure. This might be required to increase the amount of red blood cells in your blood. The most common side effects of a blood transfusion are chills, fever and symptoms of allergic nature, such as hives and itching. More serious, less frequent side effects may also occur. More information is available on the website of the Belgian Red Cross Flanders[3].
- If the device is primed with blood, blood from a donor is used as well. The risks associated with this are the same as with a blood transfusion, which are described above.

- During leukapheresis you may experience tingling in the lips, arms or legs. These symptoms are due to a decrease in calcium levels in your blood caused by the leukapheresis procedure. If required, you will be given a treatment with calcium for this.
- Blood pressure may drop, causing symptoms such as light-headedness, an increased heart rate and sweating. If necessary, the procedure will be temporarily interrupted to administer a physiological solution by infusion to restore blood pressure. The procedure can be restarted afterwards.
- The procedure is, except for placement of the catheter(s), painless and without loss of blood. As with a regular blood donation, a bruise can form at the puncture site during or after the leukapheresis. This can be unpleasant but is usually not serious. This may cause some discomfort for several days after the leukapheresis (e.g. sensitive or painful to touch or to move).

6.3. Can I take other medicines during the trial?

While participating in the study, you are not allowed to undergo any other cancer or investigational treatments than those prescribed or approved by the investigator in the context of this study.

We ask you to contact your doctor or someone from the trial staff before using any other medication or nutritional supplements.

6.4. Will my participation to the trial have an impact on my daily activities?

Except that your presence is required for the appointments at the Antwerp University Hospital, participation in the trial will not affect your daily activities.

Any effects of the standard therapies you will be receiving on your daily activities, will be discussed with you and your parents by the investigator or someone of the trial staff.

6.5. Can my partner or I get pregnant or can I breastfeed during the trial?

If applicable, based on your age and fertility, we ask you to follow the following instructions:

Female participant:

Because the effects of dendritic cell vaccination on an unborn child or infant are not known, you will not be allowed to take part in this trial if:

- you are pregnant,

[3] https://www.rodekruis.be/dienstvoorhetbloed/bloedproducten/info-bloedproducten/erytrocytenconcentraat/

- you wish to become pregnant in the near future or
- you are breastfeeding.

It is also not allowed to do egg donation during and after your participation in the trial for up to 100 days after the last vaccination.

Inform your partner of your participation in this trial and of the potential risk to an unborn child or infant. We ask you to use adequate birth control measures, such as hormonal contraceptives, from the moment of signing this informed consent form and up to 100 days after administration of the last dendritic cell vaccine. Please discuss this point with the investigator if this applies to you. If besides dendritic cell vaccines, you will be receiving another standard treatment, your doctor or a member of the trial staff will explain to you its effects on fertility as well as any additional birth control measures you need to take into account, if required.

During the screening visit for participation in the trial, a blood test will be performed to make sure you are not pregnant. Nevertheless, if you become pregnant during the trial, you should inform immediately your treating physician or a trial staff member.

Male participant:

Dendritic cell vaccination could have an effect on sperm quality and could lead to an unknown risk for an unborn child in case of pregnancy.

We ask you to use adequate birth control measures, such as condoms with spermicide, from the moment of signing this informed consent form and up to 100 days after administration of the last dendritic cell vaccine. It is also not allowed to be sperm donor during and after your participation in the trial for up to 100 days after the last vaccination. Please discuss this point with the investigator if this applies to you. If besides dendritic cell vaccines, you will be receiving another standard treatment, your doctor or a member of the trial staff will explain to you its effects on fertility as well as any additional birth control measures you need to take into account, if required.

Inform your partner of your participation in this trial and of the potential risk to an unborn child. Nevertheless, if your partner becomes pregnant during the trial, you should inform immediately the treating physician or a trial staff member.

7. What If something goes wrong within the trial?

If you experience any harm due to your participation in the trial, the trial sponsor is responsible for this. Even if there has not occurred any fault. The sponsor has taken an appropriate insurance for this.

More information is provided in the information and informed consent form provided to your parents. Additional information can also be obtained from the investigator or trial staff.

8. What if other treatment options or new information on the study treatment become available during the course of the trial?

During the course of the trial, important new information might become available, possibly affecting your decision to (further) participate. For example, other treatments for your disease or important new information on the study treatment may become available. It is the duty of the investigator to discuss this new information with you and to give you the opportunity to re-consider your participation in the trial.

If you decide to stop taking part in the trial or if you are no longer able to participate, your investigator will see to it that you continue to receive the best possible medical care.

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

9. Can my participation in the trial end prematurely?

Your trial participation may end prematurely when:

- you and/or your parents decide to withdraw your consent,
- the investigator decides to end your trial participation, or
- other entities interrupt or end the trial.

In any case, if your trial participation ends prematurely, the investigator will discuss your future medical care with you and your parents. Even after your trial participation has ended, you can still at any time contact the trial staff in case of questions or worries about potential side effects.

9.1. You decide to withdraw your consent

If you want to stop your participation in this trial, you should inform the investigator of your decision. This means you withdraw consent. Although it is not mandatory tell why you decide to stop, it may be useful for the investigator and for the sponsor to know the reason of your decision (for example if you are experiencing too many side effects, or if you feel you need to come to the hospital too frequently…).

9.2. The investigator decides to end your trial participation

The investigator may end your trial participation because

- you become pregnant during the trial,
- it is better for your health,
- he/she determines that you are not following the instructions given to participants, or
- he/she considers this is necessary for another reason that will be explained to you.

9.3. Other entities may interrupt or end the trial

The sponsor and the competent Belgian health authorities may interrupt or end the trial because

- the information gathered shows that the study treatment is not effective (does not deliver a sufficient level of improvement in the health of the trial participants),
- the study treatment causes more (serious) side effects than expected, or
- any other reason that will be explained by such party.

10. Which treatment will I get after my participation in the trial?

After you stopped the study treatment, the investigator will assess your health. If necessary, he/she will prescribe you the best standard treatment available or refer you to another treating physician of your choice.

Once the study has ended (this means, when you have received all vaccines, or when your participation/the trial has been ended prematurely, as described above), you are no longer have access to the investigational medicinal product.

11. Which data are collected about me during the trial and what will happen with them?

In the context of this trial, we will collect three types of information about you:

- Personal data (for example, your name, age, sex...)
- Data on your health (for example, your medical history, which medication you received and are currently taken, your medical condition, imaging of the tumor...)
- Results of the trial investigations (for example, results of blood tests, investigations on the tumor tissue, questionnaires...)

Your parents will provide consent for us to use these data. If you would like to know more about what will be happening with these data, you can discuss this with the investigator or someone of the trial staff, who will provide you with more information.

You have the right to access your data and to have them corrected in case they are incorrect.

When processing your data, your identity will be substituted by a code. In this way, no one will know these data are yours. Only the investigator and his personnel will know which code belongs to which participant, and they are bound by professional secrecy.

12. Which biological samples are collected from me during the trial and what will happen with them?

Biological samples are samples of human body material (for example, blood, tissue, urine, faecal stool...).

In this study, we will regularly perform blood draws. If tumor tissue is available (from a biopsy or resection), we may use this tissue for investigation. We will not ask you to undergo a biopsy or resection for this trial specifically.

Just like your personal data, your samples too will receive a code. If you would like to know more about what will be happening with your biological samples, you can discuss this with the investigator or someone of the trial staff, who will provide you with more information.

13. Who has reviewed and approved this trial?

This trial has been reviewed and approved by the Belgian authorities and an ethics committee*. It is their duty to protect individuals who participate in clinical trials. They also make sure that the trial is performed in accordance with the applicable laws. Just because they approved the trial, does not mean you should feel obliged to participate.

CHAPTER II – INFORMED CONSENT

Official study title:

Adjuvant dendritic cell immunotherapy complementing conventional therapy for pediatric patients with high-grade glioma and diffuse intrinsic pontine glioma

Lay title of the trial:

Personalized dendritic cell therapy to improve the treatment of children and adolescent diagnosed with brain or brain stem tumors

PARTICIPANT

Did you receive and understand all necessary information?

(Check the appropriate check-box: ☑)	**YES**	**NO**
I understand the information which was provided to me. I was able to ask my questions to the investigator or someone of the trial staff. My questions have been answered.	☐	☐
I have had enough time to think about taking part in this trial.	☐	☐
I understand that I don't have to participate.	☐	☐
I understand that I can stop my participation at any time.	☐	☐
I want to participate in this trial. For this, I agree to: - undergo investigations to determine whether I meet the conditions to be enrolled in the trial. - undergo a leukapheresis procedure. I am aware that this requires 2 catheters to be placed using a needle, one in each of my arms, or alternatively, a catheter in my groin. I am aware that general anesthesia might be required for the placement of a catheter in the groin. - receive dendritic cell vaccination. - cooperate to all requested investigations, including blood draws and completing questionnaires.	☐	☐

Do you consent with the following aspects of the trial, or do you prefer not to?

(Check the appropriate check-box: ☑)	**YES**	**NO**
Would you like to receive further vaccinations after the 9 vaccinations of the trial schedule, in case additional vaccines are available and the investigator agrees to this? (Still, you will be able to stop administration of the study treatment and/or study participation at any time.)	☐	☐

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

(Check the appropriate check-box: ☑)	**YES**	**NO**
Would you like that, in case your disease progresses, the investigator is able to decide to continue dendritic cell vaccination, in combination with an adjusted standard treatment, in case additional vaccines are still available? (Still, you will be able to stop administration of the investigational medicinal product and/or study participation at any time.)	☐	☐
Would you like to have the option to undergo an additional leukapheresis procedure to produce more vaccines, after all vaccines of the first leukapheresis procedure have been administered? This is only possible in case the investigator judges you experienced advantage of the first series of vaccinations, your medical condition allows an additional leukapheresis procedure and the principal investigator agrees. (Still, you will be able to stop administration of the investigational medicinal product and/or study participation at any time.)	☐	☐

Participant's surname and first name:

Date (DD/MMM/YYYY):

Participant's signature:

INVESTIGATOR

I, the undersigned investigator, confirm that

- the participant has been verbally provided with the necessary information about the trial, has been explained the content and has been given an original signed document.
- I have verified that the participant has understood the trial.
- I have given the participant sufficient time to agree to take part and to ask any questions.
- no pressure was applied to persuade the participant to agree to take part in the trial.
- I operate in accordance with the ethical principles set out in the latest version of the “Helsinki Declaration”, the “Good Clinical Practices” and the Belgian Law.

Investigator’s delegate, surname and first name:

Investigator’s delegate, qualification:

Date (DD/MM/YYYY):

Investigator’s delegate signature:

Investigator’s, Surname and first name:

Date (DD/MM/YYYY):

Investigator’s signature:

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

GLOSSARY

ACTIVE IMMUNOTHERAPY

A treatment to stimulate the patient's defense mechanisms (i.e. immune system) to destroy cancer cells.

DENDRITIC CELLS

Cells of the immune system that direct the body's defense mechanisms against diseases, including cancer. They can be regarded as the commandants of the immune system who direct the soldiers of the immune system to destroy cancer cells.

ETHICS COMMITTEE:

Provides advice on the ethical aspects of patient care and scientific research.

LEUKAPHERESIS:

Procedure to collect white blood cells from the blood.

VACCINE, VACCINATION:

The intention of a vaccine is to induce long term protection against a disease, by activating the immune system.

INFORMED CONSENT FORM

CCRG19-002 – PARENTS

Official title of the trial:

Adjuvant dendritic cell immunotherapy complementing conventional therapy for pediatric patients with high-grade glioma and diffuse intrinsic pontine glioma

Lay title of the trial:

Personalized dendritic cell therapy to improve the treatment of children and adolescents diagnosed with brain or brain stem tumors

EUDRACT-number:

2020-004125-23

Internal study number:

CCRG19-002

Sponsor of the trial:

Antwerp University Hospital (UZA)
Drie Eikenstraat 655
2650 Edegem
Belgium

Study center name:

Center for Cell Therapy and Regenerative Medicine (CCRG)
Antwerp University Hospital (UZA)
Drie Eikenstraat 655
2650 Edegem
Belgium

Version number	Approval date	Revision description
V1.0	*Not applicable*	First version
V1.1	*To be determined*	Minor adjustments following advice obtained from the Ethics Committee and FAMHP

Who can I contact in case of questions?

Name	Function	In case of	Co[redacted]
Berneman, Zwi	Principal Investigator	Information, problems or concerns about the investigational medicinal product.	0032 3 821 39 15 zwi[redacted]
Pediatrician	Investigator	Information, problems or concerns about the medical condition or treatment of your child.	0032 3 821 38 10 0032 3 821 30 00
Caroline De Schepper	Contact person trial staff (Pediatric department)	Information, problems and concerns about the study and your child's rights as a participant in the trial.	0032 3 821 58 39
Kim De Rycke Maxime De Laere	Contact person trial staff (Study center CCRG)	Information, problems and concerns on practical aspect of the trial, for example planned visits.	003[redacted] ccrg@uza.be
Nursing station Pediatrics	Emergency contact	Emergency	0032 3 275 73 79
Miranda Van Looveren	Patient rights ombudsperson	Concerns relating to your rights as a participant in a trial.	0032 3 821 31 60 ombudsdienst@uza.be
Amma Kunstlaan 39/1 1040 Brussel	Insurance Company of the sponsor	Disagreement or complaint relating to a damage claim.	Pol[redacted]
Filip Goyens	Data protection officer of the site	Questions relating to the confidentiality of your data.	0032 3 821 53 88 dpo@uza.be
Data Protection Authority*[1]	Belgian Data Protection Authority	Complaints relating to the confidentiality of your data.	contact@apd-gba.be

[1] Words and expressions which are followed by '*' are explained in more detail in the glossary at the end of this document.

TABLE OF CONTENTS

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

THE TRIAL AT A GLANCE

Dear parent,

Your child has been diagnosed with a severe brain or brain stem tumor: a high-grade glioma or a diffuse intrinsic pontine glioma. You have also been informed about the high risk of relapse (disease progression) after treatment with currently available (standard) therapies. For these reasons, you and your child are invited to take part in a clinical trial (further referred to as "trial") which aims to evaluate an investigational medicinal product as additional ("adjuvant") treatment for your child's disease. "Adjuvant" means that the investigational medicinal product is given in addition to (together with or following) an available standard treatment. The goal of the investigational treatment is to delay or prevent disease progression.

Before you agree for your child to participate in this trial, we want to make sure that you are fully informed about the trial, and its possible risks and benefits. In this way, you and your child can for yourselves decide whether or not you want to participate. This we call "giving informed consent".

After this introduction, you will already have an idea about what the trial is about. However, please make sure to read all pages of this informed consent form. It is important that you read and understand all of the information. Do not hesitate to ask any questions that come to your mind before signing this document. By signing this document, you consent for your child to participate in this trial.

About the investigational medicinal product

In this trial, a vaccine* based on a specific type of immune cells, namely dendritic cells*, is investigated. These dendritic cell vaccines (the 'investigational medicinal product') are investigated as an adjuvant treatment for children and adolescents suffering from a high-grade glioma or a diffuse intrinsic pontine glioma. Dendritic cell vaccination is a form of active immunotherapy*. This means that the patient's own defense mechanisms (or immune system) are (is) stimulated to kill the cancer cells which are present in the body.

The dendritic cell vaccines will be produced from your child's own cells and will only be used for your child as an investigational medicinal product. To produce the dendritic cell vaccines, he/she will be asked to undergo a leukapheresis procedure*. Leukapheresis is a procedure to collect white blood cells from the blood. The collected white blood cells will be processed into dendritic cells in a specialized laboratory. These dendritic cells will be administered to your child using a syringe, by injection into the skin. This we call 'vaccination'.

This investigational medicinal product has not yet been approved by the Belgian authorities for your child's condition. It has not yet been proven that it can cure, improve or stabilize your child's disease. The aim of this trial is to investigate safety, feasibility and efficacy of dendritic cell vaccination when it is combined with the available conventional therapies. It is uncertain at this point whether your child will benefit from it.

What does trial participation entail?

The study scheme consists of 9 dendritic cell vaccinations, which will be administered to your child over a course of several months, as explained in more detail below. If your child has not yet undergone any previous anti-glioma treatments, vaccination will be combined with the best available standard treatment, namely radiotherapy combined with chemotherapy, followed by chemotherapy alone. If your child has already undergone an anti-glioma treatment, it is also possible to participate in this trial. In this case, we will optimally fit dendritic cell vaccination into your child's treatment schedule.

Depending on your consent and in case vaccines are still available, additional vaccines may be administered after these 9 dendritic cell vaccinations until all available vaccines have been administered. This has to be approved by the investigators first. Your child will have to come to the Antwerp University Hospital for the administration of the dendritic cell vaccines.

If you agree to take part in the trial, your child will first have to undergo a screening investigation to check whether he/she meets all the conditions to be accepted into this trial. During the study, your child will regularly have to come to the Antwerp University Hospital for clinical monitoring of his/her disease and reactions to the investigational medicinal product. During these visits, blood samples will be collected for laboratory tests. In addition, your child will undergo an imaging examination (MRI scan) at specific time points to monitor the disease progress. Finally, we ask you and your child (from the age of 5 years) to fill out questionnaires that assess his/her quality of life and functioning on a regular basis.

Overall, your child's participation in the trial will last about 2 years. After administration of the final dendritic cell vaccine, your child will enter a follow-up period which lasts until 90 days after the final dendritic cell vaccination or 24 months after signing this informed consent form, whichever occurs later.

What will happen with my child's personal data and biological samples during and after the trial?

The data collected during this trial will be treated confidentially. After the end of the trial your pseudonymized* data will be retained for at least 30 years.

The blood samples that are collected during the trial will be stored for 15 years. Additional tests may be performed on these blood samples. The investigator or a trial staff member will provide you with information relating to this, and you will have to decide whether or not you consent with this. It is important to consider this carefully.

Does trial participation involve potential risks?

It is very important that you are aware that any medicinal product can cause side effects. Vaccination with dendritic cells has been performed in various medical centers around the world without signs of serious undesirable side effects. During and shortly after administration of a vaccine, your child may experience a reaction at the injection site (pain, redness, swelling and itching). He/she may also temporarily feel unwell and/or have fever or chills.

The sponsor is liable for any harm caused to your child whether directly or indirectly related to his/her participation in the trial. The sponsor of this trial, the Antwerp University Hospital, has taken out insurance for this.

Important to know

All treatments and examinations which your child will undergo and are specific to the trial will be free of charge for you. All other treatments and examinations, which he/she would have undergone too if he/she would not have taken part in the trial, must be paid for by your health insurance and by yourself.

It is not allowed for your child to become pregnant or to get someone pregnant during the course of the trial and for some time after. If this applies to your child (based on age and fertility), we will discuss appropriate methods of contraception with him/her.

Your child can not take part in another clinical trial simultaneously, without informing the investigator or the trial staff first. We may refuse participation to other trials for justified reasons. It is also very important that you and your child cooperate and follow the instructions that the trial staff members will give you with regard to the trial. Your child will receive an "emergency card", which says that he/she is taking part in a clinical trial. He/she must carry this card at all times. This is necessary to ensure

your child's safety, in case he/she should have to undergo emergency treatment in a hospital where they don't know him/her.

Giving informed consent

If you agree to participate, you will have to sign the informed consent form. A trial staff member will also sign the form and thereby confirm that you have received the necessary information about the trial. You will receive a signed and dated copy of the form.

The Belgian authorities and an ethics committee have evaluated this trial. You should not under any circumstances take their approval as an incentive to take part in the trial. Even after enrolment, you can still decide to stop at any time. We will fully understand your decision and will continue to take care of your child as before.

Now that you have an idea about what the trial is about, please take your time to read the other pages of this document. You do not have to do that all at once. It is important that you understand what you are reading. Feel free to discuss the trial with a trusted person (for example a friend, relative, your family doctor). The trial staff members are also available to help you if there is anything that is not clear. It is our job to make sure that you understand all of the information.

With our best regards,

The trial staff members

CHAPTER I – DESCRIPTION OF THE TRIAL AND YOUR RIGHTS WHEN PARTICIPATING

1. Why we are performing this trial?

Currently, for some types of brain tumors, including high-grade gliomas or diffuse intrinsic pontine gliomas, there is no standard treatment that uniformly leads to a complete cure. Tumor resection during surgery (if possible) followed by radio- and chemotherapy is currently the best available treatment approach. However, the risk for tumor recurrence is very high. This is due to cancer cells which persist after treatment, and which may give rise to re-emergence of the tumor. In some cases, tumor resection is not possible at all. Therefore, there is an urgent need for new, additional treatments that can prevent or postpone tumor progression or disease recurrence.

Boosting the patient's immune system is a promising strategy to eliminate remaining tumor cells. The immune system disposes of natural defense mechanisms against tumor cells. Vaccination* with dendritic cells* is a manner to boost these natural defense mechanisms. Dendritic cells are immune cells which play an important role in organizing the body's defense against cancer. The goal of dendritic cell vaccination is to stimulate the body's own defense mechanisms against tumor cells, without harming healthy tissues. Therefore, this type of vaccination is also called 'active immunotherapy'*. Dendritic cell vaccination might help to delay or prevent disease progression or recurrence, and in this way, improve patients' survival.

The Laboratory for Experimental Hematology (LEH) of the University of Antwerp (UAntwerp) and the Center for Cell Therapy and Regenerative Medicine (CCRG) of the Antwerp University Hospital (UZA) are working intensively on the development and improvement of active cancer immunotherapy using dendritic cells. The dendritic cell vaccine has been tested in several clinical trials. More than 100 adult patients with different tumor types were treated with this dendritic cell vaccine at the Antwerp University Hospital, among which 30 adult patients with glioblastoma multiforme, a type of high-grade glioma.

The main aim of this clinical trial is to investigate whether it is feasible and safe to combine dendritic cell vaccination with established therapies (such as radio- and chemotherapy) in children and adolescents diagnosed with high-grade glioma or diffuse intrinsic pontine glioma. In addition, we will also investigate the mechanism of action of dendritic cell vaccination as adjuvant therapy for these tumor types.

2. Why are we being asked to take part?

Your child has been diagnosed with a high-grade glioma or a diffuse intrinsic pontine glioma. He/she is invited to participate voluntarily in a clinical trial to evaluate an experimental treatment for these diseases. An experimental treatment is a treatment that is still under investigation to assess the safety, efficacy and/or mechanism of action.

Your child is invited to participate in this study because he/she:

- is aged between 1 year and 17 years,
- suffers from a high-grade glioma or a diffuse intrinsic pontine glioma,
- has not undergone any previous anti-glioma treatments or has sufficiently recovered from treatment-related toxicities in case he/she has already undergone and/or is receiving another anti-glioma treatment,
- is eligible to undergo a leukapheresis* procedure and immunotherapy,
- is not suffering from an active immunosuppressive disease (e.g. HIV) or autoimmune disease.

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

The investigator or a trial staff member will discuss with you the conditions which need to be met for your child to be accepted into this trial.

Before you agree that your child participates in this trial, it is important that you understand why this trial is being conducted. Please read this document carefully, so you can make your decision based on correct information. This is called "giving informed consent". If you require additional information, do not hesitate to contact your child's treating physician or a trial staff member.

3. Does my child have to take part in a trial?

Participation in a trial is voluntary and must remain free of any coercion. This means that you have the right not to take part in this trial or to withdraw at any time without giving a reason, even if you previously agreed to take part. Your decision will not affect your or your child's relationship with the investigator or treating physician nor will it affect the quality of your child's future medical care.

In case your child is 12 years of age or older, we also require his/her consent for him/her to participate in this trial. He/she may decide at any time to stop trial participation too. More information on your child's rights can be found in his/her own information and consent document ('Informed consent form – Participant (12-17 years)').

If other treatments are available for your child's disease, the investigator or his/her delegate will inform you on the treatments your child might receive if he/she does not participate in the study.

4. What will happen during the trial?

This trial is conducted at the Antwerp University Hospital and will include 10 children and adolescents.

If your child meets all the conditions required to be enrolled in the trial and you agree to take part in the trial, your child will undergo the treatments, examinations and procedures described below. Participation in the trial will last about 2 years. Over the entire duration of the study, your child will regularly need to come to the Antwerp University Hospital. An overview of all procedures and examinations in the context of this clinical trial is given below.

The treatments, examinations and procedures that are trial-specific (hereafter referred to as "SS") will be financed by the sponsor and will not be charged to you. If your child experiences any important side effects, the investigator may decide that it is necessary to perform additional investigations. These will also be considered to be specific to the trial.

4.1. Screening visit

Before your child can participate in this trial, eligibility will be evaluated during a screening visit (i.e. a complete medical examination). Following examinations and procedures will be performed during this screening visit:

- Discussing and signing of this informed consent form. If you wish to participate in the trial, you will need to sign this informed consent document. In case your child is 12 years or older and wishes to participate, he/she will also need to sign an informed consent document.
- Physical examination.
- Tumor evaluation and disease assessment, including clinical neurological examination and imaging (MRI scan),

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

- Clinical investigation of the blood vessels, in order to find a vessel suited for performing the leukapheresis procedure efficiently and safely. The leukapheresis procedure can be performed using a catheter in the groin area, or using 2 separate catheters, one in both arms.
- Completion of questionnaires by you and your child that assess his/her quality of life and executive functioning*,
- Blood sample collection for laboratory tests: 3,8 mL[2] (6 tubes = about 1 teaspoon) for general blood analysis, determination of hepatic and renal function, coagulation, blood group and assessment of markers for pregnancy (if applicable) and transmittable diseases such as HIV, Hepatitis B, Hepatitis C and syphilis. In case abnormalities are detected, you and your child will be informed on this by your treating physician.

4.2. Leukapheresis

If you and your child accept to participate in this trial and if your child meets all the conditions for participation, first, a leukapheresis* procedure[SS] will be performed.

For this, a catheter will be inserted into a vein in each arm using a needle. The arms should not be bent during the procedure to prevent the catheters from shifting. Alternatively, a catheter in the groin will be placed if the veins in the arms are not suitable to undergo a leukapheresis procedure. Placement of the catheter in the groin may be done under general anesthesia. If placement of a catheter under anesthesia is required, a separate informed consent form will be provided for this procedure.

With the help of a machine, blood is taken via one of the catheters in the arm or via the catheter in the groin. In the machine, the white blood cells are separated from the other blood cells. The white blood cells are collected in a small amount of liquid. The rest of the blood is pumped back into the circulatory system, via the catheter in the other arm, or the catheter in the groin. The leukapheresis procedure will last four to six hours.

The collected white blood cells will be processed into dendritic cells in a specialized laboratory for the production of the vaccines. We produce as many vaccines as possible from one leukapheresis procedure. If an insufficient number of cells are present in the leukapheresis product to produce 9 vaccines[3], a second leukapheresis procedure can be performed the next day. In that case, your child will be hospitalized until the second leukapheresis procedure is completed. After the (second) leukapheresis procedure, the catheter is removed (if applicable). A maximum of 2 leukapheresis procedures are performed consecutively. An additional leukapheresis procedure at a later time point is allowed under specific conditions (discussed below).

The following examinations and procedures are performed on the day of leukapheresis:

- physical examination,
- blood sample collection for laboratory testing before the start of the leukapheresis procedure (2,3 mL – 5 tubes = about half a tea spoon),

[2] Indicated blood volumes are volumes minimally required to perform the outlined tests. For patients with sufficiently high body weight/blood volume, increased blood volumes can be taken to allow retesting in case this should be required.

[3] "An insufficient number of cells" is a theoretical estimation based on previous leukapheresis procedures and vaccine production processes. Even when after leukapheresis, theoretically, a sufficient number of cells is available, in practice, there is no guarantee this will lead to the production of 9 vaccines.

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

- placement of the catheter under local or general anesthesia (if applicable, a separate informed consent form will be provided for this procedure),
- leukapheresis,
- blood sample collection for laboratory testing after the leukapheresis procedure has ended (1 mL – 2 tubes = about 1/5th of a tea spoon).

4.3. Dendritic cell vaccination

Nine dendritic cell vaccines^{SS} will be administered to your child, according to a pre-determined study schedule. The vaccines will be administered into the skin of the arm, near the armpit cavity. Each vaccine is administered through five injections.

The dendritic cell vaccinations will be combined with conventional anti-glioma treatments. The treatment schedule depends on whether your child has already undergone previous anti-glioma treatments or not.

If your child has not yet undergone previous anti-glioma treatments, chemo- and radiotherapy will be started after the leukapheresis procedure for a period of 6 weeks. This is the first part of the standard treatment. At the earliest one week after the end of the chemo- and radiotherapy, he/she will receive the first vaccinations^{SS}. One vaccine will be administered every week, for a total period of three weeks (induction phase). Thereafter, maintenance chemotherapy will be started. This is the second part of the standard treatment and consists of 6 cycles. This maintenance therapy will be combined with additional vaccinations^{SS} (maintenance phase). The vaccinations will be administered three weeks after the start of each chemotherapy cycle. Since one cycle takes about one month, the maintenance treatment with vaccinations will take about 6 months in total.

If your child has already undergone previous anti-glioma treatments, his/her treating physician will decide in consultation with you whether or not dendritic cell vaccination will be combined with an available standard treatment. This could be continuation of the treatment that your child already received or another type of treatment. The dendritic cell vaccinations will be administered in two phases: a first phase, consisting of three weekly vaccines^{SS} (induction phase), followed by a second phase, consisting of 6 vaccines^{SS} that will be administered at regular intervals of maximal 4 weeks (maintenance phase). Timing of the dendritic cell vaccinations and standard treatment will be optimally coordinated by the trial staff members.

Depending on your consent and the number of vaccines still available, additional vaccines^{SS} may be administered after the foreseen study schedule of 9 vaccines, provided that the treating physician considers that your child's medical condition allows additional vaccinations. You agree or disagree with continuation of dendritic cell vaccination after the foreseen study schedule by ticking the appropriate check-box in Chapter II, page 26. In case your child is 12 years or older, he/she will also need to provide consent for this.

In case of disease progression, the study schedule is discontinued, and the treating physician will assess your child's condition, determine the most appropriate treatment policy and discuss this with you and your child. Depending on your consent and the number of vaccines still available, the treating physician may decide to administer additional vaccines^{SS}, possibly in combination with another standard anticancer treatment. You agree or disagree with continuation of dendritic cell vaccination after disease progression and treatment adjustment by ticking the appropriate check-box in Chapter II, page 27. In case your child is 12 years or older, he/she will also need to provide consent for this.

If after depletion of the vaccines, continuation of dendritic cell vaccination is desired and warranted, additional leukapheresis procedures can be performed after agreement of the principal investigator and if following conditions are met:

- Your child has experienced clinical benefit from the previous vaccinations, in the opinion of the investigator.
- Your child's medical condition and blood values allow an additional leukapheresis procedure.

At the end of this informed consent form, you agree or disagree to the option of an additional leukapheresis procedure by ticking the appropriate check-box in Chapter II, page 27. In case your child is 12 years or older, he/she will also need to provide consent for this.

After administration of the last dendritic cell vaccine, a follow-up period is initiated, during which your child will have to undergo regular check-ups at the Antwerp University Hospital. This follow-up period will last until 90 days after the final dendritic cell vaccination or until 24 months after signing this informed consent form, whichever occurs last.

You have the right to discontinue dendritic cell vaccination for your child or his/her participation in the study at any time. If you decide to discontinue dendritic cell vaccination, your child will still regularly have to come to the Antwerp University Hospital for clinical monitoring. If you decide to terminate participation in the study, no new data will be collected (see Section 9.1, page 17).

4.4. Clinical visits and follow-up

Your child will have to come to the Antwerp University hospital for clinical monitoring at following time points:

- screening visits^{SS},
- each visit associated with the standard treatment (if applicable),
- each vaccination visit^{SS}, including additional vaccinations^{SS} after the anticipated study treatment schedule, and
- each follow-up visit at least every 15 weeks during the study schedule, and on average every 12 weeks during continuation of dendritic cell vaccination after the study schedule and in the follow-up phase.

During these visits, a blood sample of 1 mL (2 tubes = about 1/5th of a teaspoon) will be collected for routine laboratory tests. At three time points, an additional blood draw of 10 mL (1 tube = about 2/3rd of a tablespoon, if body weight ≤20 kg) or 25 mL (3 tubes = about 2 tablespoons, if body weight > 20 kg) will be collected to study the reactions of the immune system to dendritic cell vaccinations. These additional blood draws will take place at the following time points:

- on the day of the first vaccination,
- on the day of the fourth vaccination,
- and on the day of the seventh vaccination

Tumor imaging for clinical monitoring will be performed by means of a magnetic resonance imaging (MRI) scan at following time points:

- screening visit (if imaging was recently performed, the investigator may decide that new imaging is not necessary during the screening visit),
- after completion of the first part of the standard treatment regimen (chemoradiation) (if applicable according to medical standard practice, as judged by the investigator),

- at least every 15 weeks during the study schedule, and on average every 12 weeks during continuation of dendritic cell vaccination after the study schedule and in the follow-up phase. This frequency is in accordance with standard medical practices.

You and your child (if aged 5 years or older) will also be asked to complete questionnaires[SS] that assess your child's quality of life in general and with regard to his/her disease in particular. Completing these questionnaires takes about 10 minutes and will be done at the following time points:

- screening visit,
- once during standard treatment (if applicable), before the start of the dendritic cell vaccination
- once after standard treatment (if applicable) and before the start of the induction phase
- once after the induction phase and before the start of the maintenance vaccination phase,
- during the maintenance vaccination phase and continued vaccination (if applicable), at least every 15 weeks,
- once approximately 3 months after the last vaccination.

Finally, you and your child (if aged 11 years or older) will be requested to complete questionnaires about your child's executive functioning*. Completing this questionnaire takes about 15 minutes and will be done at the following time points:

- screening visit,
- after the planned study schedule (i.e. after the 9th vaccine), and/or after disease progression,
- and approximately 3 months after the last vaccine administered.

We also would like to ask your permission to collect and use your child's medical data relating to the cancer-related disease course (including further treatments and available imaging data) and survival after end of study, by consultation of his/her medical records. You agree or disagree with the collection of these data after end of study by ticking the appropriate box in Chapter II on page 27.

If you have questions about the study schedule or the nature of the interventions and examinations that take place in the context of this study, do not hesitate to contact your treating physician or a trial staff member (refer to page 2 for contact details).

5. Will my child benefit from the trial?

The information obtained during the trial may contribute to a better understanding of the use of the investigational medicinal product (referred to as "IMP") or to the development of a new medicinal product for the treatment of future patients.

Based on previous clinical trials, it can be expected that addition of the investigational medicinal product may contribute to an improved prognosis of the disease. Still, there is no guarantee that your child will benefit from the trial. The IMP may or may not be beneficial in treating your child's disease or relieving your child's symptoms. Even if it is beneficial to him/her, reoccurrence or worsening of symptoms, illness or disease is still possible.

6. What are the possible risks and discomforts of taking part?

6.1. What are the possible side effects of dendritic cell vaccination?

Participation in a trial involves a certain level of risk. All medicinal products can have side effects. Some of these side effects are already known, and some are not.

Dendritic cell vaccinations have so far been performed in various medical centers around the world without signs of serious undesirable side effects. More than 40 adult patients with blood cancer and more than 70 adult patients with other cancer types have been treated with dendritic cells in the context of clinical research at the Antwerp University Hospital. You must be aware that your child may experience the following (temporary) side effects during and shortly after administration of a vaccine:

- reactions at the injection site (such as pain, redness, swelling and itching) *(very common)*,
- general feeling of being unwell *(uncommon)*,
- fever *(uncommon)*,
- shivering *(uncommon)*.

Theoretically, it is possible that the vaccination treatment could allow the immune system to react against normal healthy tissues of the patient's own body (= autoimmune reaction) or weaken the immune system against the cancer cells instead of strengthening it. Based on previous trials, however, there are to date no concrete indications that these risks will occur.

Because this IMP is still under investigation, other risks and discomforts could occur which are currently unknown. **Therefore, it is very important that you report <u>any</u> new or worsened health problems immediately to the investigator, regardless of whether or not you think it has to do with the trial, and even when it has already been described in this document. If your child needs to use other medication, discuss this with the investigator before taking it. If, for any reason, you consult another treating physician during the trial you must inform him/her that your child is taking part in a trial and present the emergency card. This could be important in making a diagnosis and determining the correct treatment for your child's complaints if needed.**

The side effects typically related to the standard treatment that will be administered in combination with dendritic cell vaccination (if applicable), will be explained to you by your treating physician.

6.2. What are the possible risks or discomforts associated with the interventions and examinations during the trial?

The examinations and procedures during the trial may cause following discomforts and risks:

Leukapheresis:

- If required to perform the leukapheresis procedure, a catheter in the groin will be placed under local or general anesthesia. The risks related to these procedures will be discussed in a separate informed consent form.
- For children with a low body weight (< 25 kg) and/or low hematocrit* (< 30%), the leukapheresis device needs to be pre-loaded ('primed'). For this, either a solution containing proteins or blood can be used.
    - If the protein solution is used for priming, it is possible that your child has to undergo a blood transfusion after the leukapheresis procedure, to increase the amount of red blood cells in his/her blood. The most common side effects of a blood transfusion are chills, fever and symptoms of allergic nature, such as hives and itching. More serious,

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

less frequent side effects may also occur. More information is available on the website of the Belgian Red Cross Flanders[1].

- If the device is primed with blood, blood from a donor is used as well. The risks associated with this are the same as with a blood transfusion, as described above.

- During leukapheresis, your child may experience tingling in the lips, arms or legs. These symptoms are due to a decrease in calcium levels in the blood, which is caused by certain substances used during the leukapheresis procedure to prevent blood clotting in the leukapheresis device. A treatment with a calcic solution may be necessary.
- Blood pressure may drop, causing symptoms such as light-headedness, an increased heart rate and sweating. If necessary, the procedure will be temporarily interrupted to administer a saline solution by infusion to restore blood pressure. The procedure can be restarted afterwards.
- The procedure is, except for puncturing of the catheter, painless and without loss of blood. As with a regular blood donation, a hematoma ("bruise") can form at the puncture site during or after the leukapheresis.

Blood sample collection:

- Blood drawing may cause pain, bleeding, a hematoma (‘bruise”) or a local inflammation at the injection site. The trial staff will make every effort to minimize these inconveniences. For example, a local anesthetic ointment can be used.

6.3. Can my child take other medicines during the trial?

While participating in the study, your child is not allowed to undergo cancer treatments other than those prescribed or approved by the investigator. Experimental treatments other than the experimental treatment investigated in this study, namely vaccination with dendritic cells, are not allowed.

We ask you to contact the treating physician or someone from the trial staff before using any other medication or nutritional supplements.

6.4. Will my child’s participation to the trial have an impact on his/her daily activities?

Your child is required to attend the scheduled appointments at the Antwerp University Hospital. Other than that, participation in the trial will not affect his/her daily activities. An overview of all scheduled appointments will be provided after you confirm study participation.

The effects of the conventional anticancer treatments on the daily activities of your child, will be explained to you by the investigator or a trial staff member.

6.5. Can my child (or his partner) get pregnant or can she breastfeed during the trial?

If applicable, based on the age and fertility of your child, the following instructions are provided:

Female participant:

Because the effects of dendritic cell vaccination on an unborn child or infant are not known, your daughter will not be allowed to take part in this trial if:

- She is pregnant,
- She wishes to become pregnant in the near future or
- She is breastfeeding.

It is also not allowed to do egg/ovum donation during participation in this trial and for up to 100 days after the last vaccination.

Your daughter undertakes to inform her partner of her participation in this trial and of the potential risk to an unborn child or infant. We ask her to use adequate birth control measures, such as hormonal contraceptives, from the moment of signing this informed consent form and up to 100 days after administration of the last dendritic cell vaccine. Please discuss this point with the investigator if this applies to your daughter. If besides dendritic cell vaccines, your daughter will be receiving another standard treatment, the treating physician or a member of the trial staff will explain to you its effects on fertility as well as any additional birth control measures that need to be taken into account, if required.

During the screening visit for participation in the trial, a blood test will be performed to exclude pregnancy. Nevertheless, if your daughter becomes pregnant during the trial, her treating physician or a trial staff member should immediately be informed.

Male participant:

Dendritic cell vaccination could have an effect on sperm quality and could lead to an unknown risk for an unborn child in case of pregnancy. We ask your son to use adequate birth control measures, such as condoms with spermicide, from the moment of signing this informed consent form and up to 100 days after administration of the last dendritic cell vaccine. It is also not allowed to be sperm donor or to have sperm frozen for preservation during and after participation in the trial for up to 100 days after the last vaccination. Please discuss this point with the investigator if this applies to your son. If besides dendritic cell vaccines, your son will be receiving another standard treatment, the treating physician or a member of the trial staff will explain to you its effects on fertility as well as any additional birth control measures that need to be taken into account, if required.

Your son undertakes to inform his partner of his participation in this trial and of the potential risk to an unborn child. Nevertheless, if his partner becomes pregnant during the trial, the treating physician or a trial staff member should immediately be informed.

7. What If something goes wrong within the trial?

Even if there is no fault, the sponsor is liable for harm caused to your child whether directly or indirectly related to his/her participation in the trial. The sponsor has taken an appropriate insurance (a so called “No Fault insurance”*) for this liability (Ref. 2). A copy of the insurance certificate can be obtained from the investigator or trial staff.

If your child (or in the event of death, his/her rightful claimants) seeks compensation for a harm to his/her health as a direct or indirect result of participating in the trial, the investigator or trial staff should be informed promptly.

If the investigator believes that a link between the new or worsened health problem(s) and the trial is possible, he/she will inform the trial sponsor. The sponsor will then immediately initiate the declaration procedure to its insurance company. If the company considers it necessary, it will appoint an expert to assess whether there is a link between the reported health problem(s) and the trial. The insurance does not cover the natural progression of your child’s disease/condition or the known side effects of the treatment he/she would have received without taking part to the trial (*that is* the standard/conventional treatment).

Whenever you feel it is appropriate or if you, your child or your child’s rightful claimants disagree either with the investigator or with the expert appointed by the insurance company, you may contact the insurance company or proceedings may be brought against the insurance company. You will find the contact details on the front page of this form.

8. What if other treatment options or new information on the IMP become available during the course of the trial?

During the course of the trial, important new information might become available, possibly affecting your and/or your child's decision to (further) participate. For example, other treatments for his/her disease or important new information on the IMP may become available. It is the duty of the investigator to discuss this new information with you and to give you the opportunity to re-consider your child's participation in the trial.

If you and/or your child decide to stop taking part in the trial or if he/she is no longer able to participate, the investigator will see to it that your child continues to receive the best possible medical care.

9. Can my child's participation in the trial end prematurely?

As explained in detail below, your child's trial participation may end prematurely when:

- you and/or your child decide to withdraw consent,
- the investigator decides to end your child's trial participation, or
- other entities interrupt or end the trial.

In any case, if trial participation ends prematurely, the investigator will discuss your child's future medical care with you. The sponsor can retain and continue to use any data that have already been collected before the end of trial participation. This is to avoid skewing / biasing results of the trial (as described in I. § 12.5., page 20).

After ending trial participation, you can still contact the trial staff in case of questions or concerns about potential side effects.

9.1. You decide to withdraw your consent

You are entitled to withdraw your consent for trial participation for any reason, at any time, without having to justify your decision. However, for your child's safety, you should inform the investigator of your decision. Although it is not mandatory to explain why, it may be useful for the investigator and for the sponsor to know the reason of your decision (for example side effects, frequency of clinical visits…).

If you withdraw your consent, this means you decide to stop:

- the treatment with the IMP, and
- all trial-related visits and examinations.

Please discuss with the investigator the practical modalities of your withdrawal (in light of your child's situation), including follow-up of your child.

In any case, no new data will be sent to the sponsor, unless you agree that we collect and use your child's medical data relating to the cancer-related disease course (including further treatments and available imaging data) and survival after discontinuation of trial participation. You agree or disagree with the collection of these data after end of study by ticking the appropriate box in Chapter II on page 27.

If your child's blood samples have already been used or analyzed before the withdrawal of consent, the sponsor still has the right to use the results from those tests. Blood samples that have been collected (but not tested) before the withdrawal of consent and the data obtained from it, can also still be used by the sponsor. You may ask for a destruction of those samples. If this impacts the validity of the trial, the destruction may be postponed until the end of the trial.

9.2. The investigator decides to end your child's trial participation

The investigator may end your child's trial participation because

- she becomes pregnant during the trial,
- it is better for your child's health,
- he/she determines that you and/or your child are not following the instructions given to participants, or
- he/she considers this is necessary for another reason that will be explained to you.

9.3. Other entities may interrupt or end the trial

The sponsor and the competent Belgian health authorities may interrupt or end the trial because

- the information gathered shows that the IMP is not effective (does not deliver a sufficient level of improvement in the health of the trial participants),
- the IMP causes more (serious) side effects than anticipated, or
- any other reason that will be explained by such party.

10. Which treatment will my child get after participation in the trial?

After your child has discontinued the treatment with the IMP, the investigator will assess his/her health. If necessary, he/she will prescribe the best available standard treatment or refer you to another treating physician of your choice.

Once the study has ended (this means, when your child has received all vaccines for which you gave consent, or when your child's participation/the trial is ended prematurely for one of the reasons explained above in I.§9 page 17), your child is no longer eligible to receive the IMP.

11. Will participation in the trial involve extra costs for me?

11.1. Examinations and treatments paid by the sponsor

The sponsor has arranged to compensate the hospital for

- the time devoted to the trial by the investigator and the trial staff,
- the visits/consultations and all scheduled examinations specific to the trial,
- the investigational treatment (IMP and any other medication and material specifically used for the trial).

You will find in the table below the procedures and examinations which are specific for the trial and therefore will not be charged to you. The other procedures and examinations that belong to the standard of care for your child's disease, will be charged to you or the mutual insurance fund (Belgian social security). If you would like more details or in case you are not affiliated with a mutual insurance fund (Belgian social security), please contact the trial staff.

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

Following examinations/procedures are trial specific:

Screening visits
Discussing and signing of the informed consent form
Checking patient eligibility according to the study-specific criteria
Physical and neurological examinations
Blood tests
Leukapheresis
Checking of vascular accessibility
Pre-leukapheresis consult
Anesthesia, placement of the catheter and overnight stay in the hospital (as applicable)
Leukapheresis
Blood tests
Dendritic cell vaccination
Production dendritic cell vaccines
Dendritic cell vaccination
Visits and follow-up at predefined time points
Additional blood tests to investigate the reactions of the immune system on dendritic cell vaccination.

The visits and treatments which are a consequence of a side effect of the IMP are also considered to be trial specific.

11.2. Other expenses paid by the sponsor

No reimbursements will be provided for the participation of your child in the clinical trial.

12. Which data are collected about my child during the trial and what will happen with them?

12.1. Which data are collected and processed during the trial?

The collected and processed personal data concern information about your child's health and medical condition. These include medical history, background information (for example age, sex) and the results of examinations performed in the context of the trial.

12.2. How will the investigator treat my child's personal data?

The investigator is bound by professional secrecy about the data collected.

This means that he/she will never reveal your child's identity, including in a scientific publication or a lecture and that he/she will encode your child's data (*that is* he/she will replace your child's identity by an identification code).

Therefore, the investigator and the trial staff under the responsibility of the investigator, will be the only ones able to establish a link between your child's identity and the data transmitted during the trial, with the exceptions listed under section 12.6.

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

The data transmitted to the sponsor will not allow the sponsor to identify your child.

12.3. What will happen to information about my child collected during the trial?

Your child's participation in the trial means that his/her personal data

- are collected by the investigator, and
- are used in a pseudonymized form by the trial sponsor.

The investigator and the sponsor can only use the pseudonymized personal data for research purposes in connection with scientific publications within the context of the trial that your child participates in, or for a broader use of the pseudonymized data if described below.

In addition, the sponsor may provide access to the pseudonymized data to external researchers (that are not involved in this trial). In the event an external researcher wants to use the data in a project not yet described in this document, this project will have to be approved by an Ethics Committee*.

12.4. How will my child's data be handled?

Your child's trial data will be processed in accordance with the General Data Protection Regulation (GDPR, Ref. 3) and the Belgian law on data protection of 30th July 2018 (Ref. 4). The sponsor is responsible for this processing.

Processing your child's personal data in this trial is allowed because we are conducting scientific research and you have given your consent.

12.5. Do I have access to my child's data collected and processed during the trial and can I rectify them?

You are entitled to ask the investigator what data are being collected about your child and how those data will be used in connection with the trial.

You have the right:

- to access and inspect these data,
- to receive the personal data that are collected,
- to ask for correction if they are incorrect,
- to withdraw your consent for the processing of personal data. However personal data collected before withdrawal will be kept to avoid skewing of results in the trial.

It is not possible to:

- to have all personal data erased,
- to restrict the processing of your child's personal data,
- to object to the processing of your child's personal data

to avoid for example skewing of the results in the trial.

12.6. Who, other than the Investigator and his staff, has access to my child's personal data?

To verify the quality of the trial, it is possible that your child's personal unpseudonymized data or information in his/her medical records relevant for the trial, will be examined by people outside the trial staff but under the responsibility of the investigator. These persons must be subject to professional secrecy or a confidentiality agreement. This may consider:

- the personnel designated by the sponsor of the trial (monitors and auditors*), and people or organizations providing services for or collaborating with the sponsor. They will however never transfer your child's name and contact details to the sponsor.
- inspectors of competent health authorities worldwide
- an independent audit group
- people designated by the Ethics Committee

For the needs of the trial, the pseudonymized trial data may be sent to other European Union (EU) and non-EU countries and may be reviewed by

- personnel (other than the inspectors) of competent health authorities of Belgium (Federal agency for medicines and health products, FAMHP*) and of other EU and non-EU countries,
- the evaluating Belgian Ethics Committee(s)*,
- external researchers,
- the sponsor of the trial, personnel designated by the sponsor, and people or organizations providing services for or collaborating with the sponsor.

The European regulation and the Belgian legislation on data protection have requirements for transferring data to non-EU countries. The sponsor must ensure equivalent guarantees regarding personal data protection standards before transferring the pseudonymized data to non-EU countries. If for this purpose, there is a data protection agreement, a copy of this agreement may be obtained via the investigator.

You can always contact your investigator to obtain more information about any such transfers.

12.7. What will happen to the results of the trial?

After trial closure, a description and the results of this clinical trial will be published in specialized medical journals. A copy of the scientific publication can be obtained from the investigator or the trial staff.

A description of the trial will also be available on https://www.Clinicaltrials.gov. You can search this website at any time using the trial number given on the front page of the informed consent form. The website will include a summary of the results within 1 year after the end of the trial (Ref. 5).

This website or these publications will not include information that can identify your child.

12.8. Will my child's data be used for other purposes than for the trial in which he/she takes part?

The results of the trial will be used to answer the scientific questions of the trial. In addition, the sponsor would like to use your child's data obtained from this trial, in connection with other research and development activities (and the associated scientific publications) to gain more insights in the disease, the treatment for the disease and the response to that treatment.

Any additional research outside of the trial, must be approved by a Belgian recognized Ethics Committee*.

At the end of this form you agree or disagree to the use of your child's trial data for other purposes by ticking the appropriate check-box in Chapter II, page 26.

12.9. How long will my child's data be kept?

After the end of the trial your child's pseudonymized data will be retained for at least 30 years to ensure the validity of the research. This will also be the case if your child stopped trial participation prematurely.

12.10. Who can I contact in case of questions/complaints concerning the use of my child's personal data?

In case you have questions relating to your child's privacy, or the reasons for which or manner by which his/her personal data are collected and processed, in first instance, you can contact the data protection officer of the University Hospital Antwerp (for contact details, refer to page 2).

More information concerning the legal framework of personal data protection is available on the website of the Data Protection Authority (DPA).[6] The DPA is an independent body that ensures compliance with the fundamental principles of data protection. In addition, you can contact them to request mediation or to lodge a complaint in case you experience any problems relating to the protection of your child's personal data.

13. Which biological samples are collected from my child during the trial and what will happen with them?

13.1. Which biological samples are collected from my child during the trial?

Biological samples are samples of human body material (for example blood, tissue, urine, faecal stool….). In this trial, blood samples will be collected on a regular basis. If biopsy or resection tissue from the tumor is available, this material will also be investigated as part of the trial. However, tumor biopsy sampling or resection are not foreseen in the context of this trial specifically.

13.2. What will happen to the collected biological samples?

The collected biological samples will be managed and stored at the Center for Cell Therapy and Regenerative Medicine located at UZA for 15 years.

These biological samples will be analyzed for the objectives of the trial.

Genetic analyses will also be performed on your child's samples. The purpose of these analyses:

- to gain insight in the genetic profile of your child's disease,
- to identify parameters that can predict the course of the disease for future patients diagnosed with high-grade glioma or diffuse intrinsic pontine glioma,
- and to identify parameters that can predict whether or not future patients diagnosed with high-grade glioma or diffuse intrinsic pontine glioma will respond to dendritic cell vaccination.

These genetic analyses will deliver essential information for the trial. However, these genetic analyses are optional and not a prerequisite for participation to the trial. You agree or disagree to these genetic analyses by ticking the appropriate check-box in Chapter II, page 28.

It may happen by chance and in addition to the trial objectives, that the results of the analyses of your child's biological samples reveal information that may be important to your child's health or the health of his/her blood relatives. These data are called "incidental findings" and will be treated as described in Chapter I, § 16, page 24.

13.3. How will my child's biological samples be handled?

The procedure to encode biological samples is the same as that used for personal data (see I § 12.3, page 20, Ref. 7). Samples sent to the sponsor or to organizations working in collaboration with the sponsor, will only be labelled with your child's trial identification code.

As part of the trial, the sponsor might transfer (a part of) your child's samples to a laboratory that is working with them. This laboratory may only use your child's samples as specified in this document. Tracking of the samples will be ensured by the sponsor.

Your child's biological samples are deemed to be a "donation". You or your child will not receive any financial benefit associated with the development of new therapies derived from the use of his/her biological samples, and which may have commercial value.

13.4. What happens with any remainders of biological samples once the analyses described in this document have been carried out?

The sponsor shall use the biological samples within the context of the trial as described above.

Since scientific progress in this area is constant, the sponsor would like to, with your consent, retain the remainders of your child's biological samples for 15 years. The sponsor will use them for future research, outside the trial that your child will participate in, to better understand the disease, its treatment and the responses to this treatment. The retention of the remainders of samples goes together with the retention of the accompanying pseudonymized personal data.

The researchers undertake to destroy the samples at the end of the anticipated period of storage, unless you agree that the residual material will be included in the biobank* for other scientific research.

You agree or disagree to store your child's residual material for future research by ticking the appropriate check-box in Chapter II, page 28. If you agree, any future research additional to what is described above, may only be conducted according to the legislation on the use of human tissue material (Ref. 8) and with the approval of a Belgian recognized Ethics Committee*.

13.5. Will any additional biological samples be collected and used for additional research?

In this trial, no additional biological samples will be collected.

14. What will happen with residual dendritic cell vaccines after the end of the trial?

Residual dendritic cell vaccines can be used for validation* purposes. Validation is required to ensure the quality of the final product, for instance in case of minor adjustments to the production process. It concerns routine tests which are not considered to be scientific research.

The researchers undertake to destroy residual dendritic cell vaccines which have not been used for validation purposes, unless you agree that residual dendritic cell vaccines will be included in the biobank for future research. The sponsor will use them for future research, outside the context of the trial that your child will participate in, for instance to further optimize the IMP production process or to develop new types of vaccines.

You agree or disagree to store your child's residual dendritic cell vaccines for future research by ticking the appropriate check-box in Chapter II, page 28. If you agree, any future research additional to what is described above, may only be conducted according to the legislation on the use of human tissue material (Ref. 9) and with the approval of a Belgian recognized Ethics Committee*.

15. Who has reviewed and approved the trial documents?

The documents of the trial have been reviewed by

- The Belgian competent health authorities (FAMHP*)
- An independent Belgian Ethics Committee*

It is the task of the competent health authorities and the Ethics Committees to protect people who take part in a clinical trial. The health authorities will ensure that the trial is conducted in accordance with the applicable legislation.

You should not under any circumstances take their approval as an incentive to take part in the trial.

16. What happens in case of incidental findings?

If by chance and in addition to the trial objectives a result is discovered during the trial that may be important to your child's health or the health of your child's blood relatives (these are called "incidental findings"), the sponsor will inform the investigator. With your consent the investigator will notify you and your treating physician about your child's results and potential consequences thereof. If necessary, the investigator and/or the treating physician will advise you on the next steps. You agree or disagree to being informed of incidental findings by ticking the appropriate check-box in Chapter II, page 28.

CHAPTER II – INFORMED CONSENT

Official study title:

Adjuvant dendritic cell immunotherapy complementing conventional therapy for pediatric patients with high-grade glioma and diffuse intrinsic pontine glioma

Lay title of the trial:

Personalized dendritic cell therapy to improve the treatment of children and adolescents diagnosed with brain or brain stem tumors

PARENTS

PREREQUISITES FOR YOUR CHILD'S PARTICIPATION IN THE TRIAL

(Tick the appropriate check box: ☑)	**YES**	**NO**
I declare that I have been informed of and that I understand the purpose of the clinical trial, its duration, possible risks and discomforts, the precautions that my child has to take regarding pregnancy (if applicable) and what is expected of me and my child. My child's rights have been explained to me and I have understood those rights.	☐	☐
I have had enough time to think about taking part in this trial and to discuss it with a trusted person (for example friends, relatives, treating physician, …).	☐	☐
I have had the opportunity to ask any questions that came to mind and have obtained a satisfactory response to my questions.	☐	☐
I understand that my consent for my child's participation in this trial is voluntarily and free from any coercion and that I am free to stop at any time my child's trial participation.	☐	☐
I understand that data about my child will be collected and that they will be treated confidentially.	☐	☐
I agree to my child's personal data being processed as described in Chapter I, § 12, page 19. I understand that I have the right to access my personal data and to correct them if necessary.	☐	☐
I understand that the sponsor has taken out an insurance in case my child should suffer any damage in connection with his/her participation in this trial.	☐	☐
I understand that when participating in this trial, I will not have any costs except those related to the standard of care treatment of my child's disease.	☐	☐
I agree to my child's treating physician(s) being informed of his/her participation in this trial.	☐	☐
I agree for my child not to take part in any other trial at the same time without first informing the investigator or the trial staff, who might not permit this participation for a good reason.	☐	☐
I understand that my child and I need to cooperate and follow the investigator's and trial staff's instructions regarding the trial.	☐	☐

(Tick the appropriate check box: ☑)	**YES**	**NO**
I understand that participation to the trial might end for my child without my consent if he/she needs other treatment, does not follow the trial plan, has a trial-related injury, or for any other justified reason.	☐	☐
I certify that all the information I have given about my child's medical history is correct. I understand that my failure to inform the investigator or designee about any exclusion criteria may harm my child.	☐	☐
I agree that additional investigations will be conducted to determine if my child is eligible to participate in the study. This includes blood collection for laboratory analyses of markers for pregnancy, HIV, Hepatitis B and C, and syphilis. I am aware that any deviating results will be communicated to me.	☐	☐
I agree for my child to undergo a leukapheresis procedure in the context of this trial, for the production of dendritic cells intended for his/her own use. I understand that this requires catheters to be placed in both the arms, or alternatively one catheter in the groin area. I am aware that general anesthesia may be required for placement of the catheter in the groin area.	☐	☐
I agree for my child to receive dendritic cell vaccines in the context of this trial.	☐	☐
I give informed, conscious and free consent for my child to participate in this trial and for us to corporate with all requested investigations, including blood draws and completing of the mentioned questionnaires.	☐	☐

OPTIONAL CONSENTS WHICH ARE NO PREREQUISITE FOR YOUR CHILD'S PARTICIPATION IN THIS TRIAL.

1. As described in Chapter I, § 4.3 page 11, additional vaccines may be administered at regular time intervals after the final vaccine administration of the study scheme (i.e. vaccine 9), depending on the number of available vaccines and after agreement of the investigator.

   Do you agree that vaccination can be continued after vaccine 9?

   (Tick as appropriate. If you leave this question open, we assume the answer is 'yes, I want to be informed'.)

☐ **I agree**	☐ **I do not agree**

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

2. As described in Chapter I, § 4.3 page 11, an additional leukapheresis procedure to produce more vaccines can be performed, after agreement of the investigator and principal investigator.

   Do you agree that an additional leukapheresis procedure can be performed, provided all the conditions therefore are fulfilled?

   (Tick as appropriate. If you leave this question open, we assume the answer is 'yes, I want to be informed'.)

☐ **I agree**	☐ **I do not agree**

3. As described in Chapter I, § 4.3 page 11, additional vaccines may be administered at regular time intervals after my child's standard therapy has been adjusted in case of disease progression, depending on the number of available vaccines still available and after agreement of the investigator.

   Do you agree that vaccination can be continued after your child's standard therapy has changed in case of disease progression?

   (Tick as appropriate. If you leave this question open, we assume the answer is 'yes, I want to be informed'.)

☐ **I agree**	☐ **I do not agree**

4. As described in Chapter I, § 4.4 page 12 and § 9.1 page 17, the investigator would like to collect and use your child's medical data relating to the cancer-related disease course (including further treatment and available imaging data) and survival data after end of study or after termination of your child's trial participation.

   Do you agree that the investigator can collect and use these data after end of study or after termination of your child's trial participation?

   (Tick as appropriate. If you leave this question open, we assume the answer is 'yes, I want to be informed'.)

☐ **I agree**	☐ **I do not agree**

5. As specified in Chapter I, § 12.8, page 21, the sponsor would like to be able to use your child's pseudonymized data obtained from this trial in connection with other research and development activities (and the associated scientific publications) on the condition that such research purposes have been approved by a Belgian recognized Ethics Committee.

   Do you agree with the use of your child's data obtained in this trial for other research purposes?

   (Tick as appropriate. If you leave this question open, we assume the answer is 'I do not agree'.)

☐ **I agree**	☐ **I do not agree**

Van Genechten T, *et al. BMJ Open* 2024; 14:e077613. doi: 10.1136/bmjopen-2023-077613

6. As specified in Chapter I, § 13.2, page 22, the sponsor would like to conduct genetic analysis on your child's biological samples.

   Do you agree to the sponsor conducting genetic analysis on your child's biological samples?

   (Tick as appropriate. If you leave this question open, we assume the answer is 'I do not agree'.)

☐ **I agree**	☐ **I do not agree**

7. As specified in Chapter I, § 13.4, page 23, the sponsor would like to retain the remainders of your child's biological samples for 15 years for future research outside the trial that your child will participate in. The samples will be used to better understand the disease, its treatment and the responses to this treatment.

   Do you agree with the retention of the remainders of your child's biological samples and the accompanying personal data for future research outside the trial?

   (Tick as appropriate. If you leave this question open, we assume the answer is 'I do not agree'.)

☐ **I agree**	☐ **I do not agree**

8. As specified in Chapter I, § 13.4 page 23, the sponsor would like to include residual material from your child's biological samples in the biobank after the anticipated period of storage of 15 years for future research outside the trial that your child will participate in.

   Do you agree that after the initial period of 15 years, residual material from your child's biological samples and the accompanying personal data are stored in the biobank for future research outside the trial?

   (Tick as appropriate. If you leave this question open, we assume the answer is 'yes, I want to be informed'.)

☐ **I agree**	☐ **I do not agree**

9. As described in Chapter I, § 14, page 23, the sponsor would like to include residual dendritic cell vaccines in the biobank for future research, outside the trial that your child will participate in, for instance to further optimize the production process and to develop new types of vaccines.

   Do you agree that residual dendritic cell vaccines from your child and the accompanying personal data are stored for future research outside the trial?

   (Tick as appropriate. If you leave this question open, we assume the answer is 'yes, I want to be informed'.)

☐ **I agree**	☐ **I do not agree**

10. As described in Chapter I, § 13, page 22, and § 16, page 24, it may happen that incidental findings are discovered that may be important to your child's health or the health of his/her blood relatives.

    If this happens, do you want the investigator to inform you (directly or via your treating physician) of this result?

    (Tick as appropriate. If you leave this question open, we assume the answer is 'yes, I want to be informed'.)

☐ **No, I do not want to be informed**	☐ **Yes, I want to be informed**

I give informed, conscious and free consent to take part in the trial and I have received a signed and dated copy of all pages of this document.

Child's name:

Parent's / Legal guardian's surname, first name and signature (1):

Date (DD/MM/YYYY):

Parent's / Legal guardian's surname, first name and signature (2):

Date (DD/MM/YYYY):

INVESTIGATOR

I, the undersigned investigator, confirm that

- the participant's parents/guardians have been verbally provided with the necessary information about the trial, have been explained the content and have been given an original signed document.
- I have verified that the participant's parents/guardians have understood the trial.
- I have given the participant's parents/guardians sufficient time to agree to take part and to ask any questions.
- no pressure was applied to persuade the participant's parents/guardians to agree to take part in the trial.
- I operate in accordance with the ethical principles set out in the latest version of the "Helsinki Declaration", the "Good Clinical Practices" and the Belgian Law (Ref. 10).

Investigator's delegate, surname and first name:

Investigator's delegate, qualification:

Date (DD/MM/YYYY):

Investigator's delegate signature:

Investigator's, Surname and first name:

Date (DD/MM/YYYY):

Investigator's signature:

GLOSSARY

ACTIVE IMMUNOTHERAPY:

A treatment to stimulate the patient's defense mechanisms (i.e. immune system) to destroy cancer cells.

BIOBANK:

A biobank is a facility that stores human tissue material that is exclusively intended for scientific research and therefore will not be administered to humans.

DENDRITIC CELLS:

Cells of the immune system that direct the immune response. They can be regarded as the commandants of the immune system who direct the soldiers of the immune system to destroy cancer cells.

DATA PROTECTION AUTHORITY (DPA):

The Data Protection Authority ensures that personal data are handled with care and thoroughly protected, and that your future privacy also remains guaranteed.

ETHICS COMMITTEE:

Provides advice on the ethical aspects of patient care and scientific research.

EXECUTIVE FUNCTIONING:

Executive functions are the higher control functions of the brain, including planning & organizing, working memory, emotional control, …

FAMHP:

Federal agency for medicines and health products. This is the national competent health authority in Belgium.

HEMATOCRIT:

A measure for the amount of red blood cells in the blood.

IMP:

Investigational medicinal product.

LEUKAPHERESIS:

Procedure to collect white blood cells from the blood.

MONITOR and AUDITOR:

Both the monitor and auditor work for the sponsor. The monitor takes care of a continuous quality check during the course of a trial. The auditor performs a quality check after the trial. They verify if the trial is being/was conducted according to the protocol, if the reported data are liable and if the clinical trial was conducted according the applicable rules.

NO FAULT INSURANCE:

The sponsor is liable for any injury or any damage that the participant has suffered, and which is directly or indirectly related to the clinical trial. You do not have to prove any mistake in this respect.

PSEUDOMYNIZED PERSONAL DATA:

Pseudonymizing personal data ensures that the identity of the individual in question can no longer be directly deducted from the available data, for instance by replacing identifiable data with a code. Using a decoding key, it is still possible at any time to establish the link with the individual's identity. For this reason, the decoding key is only available to involved parties which have the right to access unencoded (unpseudomynized) personal data.

VACCINE, VACCINATION:

The intention of a vaccine is to induce long term protection against a disease, by activating the immune system

VALIDATION:

Validation of a certain method is the process of verifying whether the method meets all requirements and delivers the correct results.

REFERENCES

[1] https://www.rodekruis.be/dienstvoorhetbloed/bloedproducten/info-bloedproducten/erytrocytenconcentraat/

[2] This is in accordance with Article 29 of the Belgian Law of 7 May 2004 related to experiments on humans.

[3] General Data Protection Regulation No 2016/679 of the European Parliament and of the council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC.

[4] The Belgian Law of 30 July 2018 on the protection of natural persons with regard to the processing of personal data.

[5] In accordance with section 4.3. of the Commission Guideline: Guidance on posting and publication of result-related information on clinical trials in relation to the implementation of Article 57(2) of Regulation (EC) No 726/2004 and Article 41(2) of Regulation (EC) No 1901/2006 - (2012/C 302/03). [From the moment the Clinical trial regulation enters into force : In accordance with article 37 of the Clinical trial regulation No 536/2014 of the European Parliament and of the council of 16 April 2014 on clinical trials on medicinal products for human use, and repealing Directive 2001/20/EC; sponsor have to provide summary results of clinical trials in a format understandable to laypersons.]

[6] https://www.dataprotectionauthority.be/citizen

[7] Belgian Law of 19 December 2008 on the acquisition and use of human body material with a view to medical application to humans or scientific research, and the applicable royal decrees.

[8] This is in accordance with Article 21 of the Belgian Law of 19 December 2008 on the acquisition and use of human body material with a view to medical application to humans or scientific research, and the applicable royal decrees.

[9] This is in accordance with Article 21 of the Belgian Law of 19 December 2008 on the acquisition and use of human body material with a view to medical application to humans or scientific research, and the applicable royal decrees.

[10] Belgian Law of 7 May 2004 related to experiments on humans, and the applicable royal decrees.