VRIJE UNIVERSITEIT BRUSSEL

Submitted to obtain the degree of Master in Medicine.

PHASE I CLINICAL TRIAL ON INTRA-TUMORAL IPILIMUMAB PLUS INTRAVENOUS NIVOLUMAB FOLLOWING THE RESECTION OF RECURRENT GLIOBLASTOMA

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ABSTRACT

Background: Glioblastoma represents the majority of malignant primary tumors of the central nervous system (1). Outcome remains poor with a median PFS of 6.9 months and median (OS) of 12.1 to 14.6 months (2-4). At the time of tumor recurrence, no treatment has demonstrated to improve the survival of GB patients in a randomized clinical trial. Median PFS at recurrence is 10 weeks and median OS from this moment is 30 weeks (5).

Methods: Twenty-one patients were treated. Patients in Cohort 1 (n=3) recieved 10 mg nivolumab IV 24h prior (day 0) to neurosurgical intervention. At day 1, a maximal safe resection of the GB was performed, followed by IT administration of 10 mg ipilimumab in the walls of the resection cavity. Every 2 weeks, they received 10 mg nivolumab IV (until total of 6 doses nivo IV). Patients in Cohort 2 (n=18) were administered 5 mg ipilimumab plus 10 mg nivolumab IT. The rest of the treatment phase remained the same as in Cohort 1.

Results: There were no patients who had to stop treatment due to toxicity. In both cohorts, most AEs were not-related to the study treatment. There were no high-grade immune-related AEs in Cohort 1, and in Cohort 2, only 12% of the immune-related AEs were high-grade (all grade 3). Median PFS in Cohort 1 and 2, was respectively 11,9 (95%CI 2,6 – 21,2) and 14,4 weeks (95%CI 10,5 – 18,3). Six-month PFS was 17,5% (95% CI 0 – 39,3). Median OS in Cohort 1 and 2, was respectively 116,9 (95%CI 0 – 242,5) and 33,3 weeks (95%CI 28,7 – 37,9). Six-month OS was 72,7% (95%CI 49,4 – 96,0).

Conclusion: Repeated IV administration of 10 mg nivolumab in combination with IT injection of 10 mg ipilimumab**,** following the gross total tumor resection in patients with recurrent GB, is tolerable and safe. A similar therapy, but with IT injection of 5 mg of ipilimumab plus 10 mg of nivolumab (as conducted in Cohort 2) is also tolerable and safe. This combined treatment may have therapeutically meaningful anti-tumor effects, but further investigations are indicated.

Key words: Recurrent glioblastoma – Nivolumab – Ipilimumab – Immune checkpoint inhibition

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2 Introduction

Glioblastoma represents the majority of malignant primary tumors of the central nervous system (1). Despite the development of innovative new diagnostic and therapeutic approaches, its prognosis remains very poor with a median overall survival of 12.1 to 14.6 months (2-4). The tumor's resistance to chemotherapy contributes to this unfortunate reality. To improve therapeutic options, it is important to understand the molecular mechanisms underlying the tumor's aggressive behavior and ability to escape the human immune system. Considering the key characteristics of glioblastoma, such as its invasive character and high angiogenic capacity, there is need for a tumor-specific targeted approach that inhibits cell migration, distribution and angiogenesis, rather than a sole maximal surgical resection (6).

This clinical trial aims to focus on a new therapeutic alternative based on a combined surgical resection and immune checkpoint inhibition.

2.1 Glioma

Gliomas are tumors that arise from glial cells (supporting cells of the central nervous system) or progenitor cells in the brain (7). Although multiple classifications can be made, based on different tumor characteristics, the most commonly used classification is the 'World Health Organization (WHO) Classification of Tumours of the Central Nervous System' (8). For example, they can be grouped by their growth rate: low-grade (slow growth), mid-grade (moderate growth), and high-grade (rapid growth) (9). The WHO classification has recently been updated, to take molecular markers into account, such as the IDH (isocitrate dehydrogenase) mutation status, in addition to histology to predict the tumors clinical behavior (7).

Classification
Grade I (pilocytic astrocytoma) and grade II (diffuse astrocytoma, oligodendroglioma and mixed oligo-astrocytoma) gliomas can be classified as 'low-grade', marked by a slow growth over many years. They are both considered benign, although surgical excision can only provide a complete cure for grade I. Grade II gliomas require additional follow-up every 6 to 12 months by MRI or CT scan (9) since they have the tendency to reoccur. Characteristic for grade III gliomas (anaplastic astrocytoma, anaplastic oligodendroglioma and anaplastic oligo-astrocytoma) is that they are malignant and mid-grade (moderate growth rate) tumors, known for their diffuse infiltration of the normal brain tissue. Grade IV gliomas are often called glioblastomas. They are not only the most aggressive, but also the most common malignant brain tumors. Glioblastoma grows rapidly and invasively, altering brain function (7, 9).

2.2 Glioblastoma

Epidemiology
Glioblastoma accounts for nearly half of all malignant primary tumors of the central nervous system (15.1% out of 32.8%) (1). It can be considered a rare tumor with a global incidence of 3 to 5 per 100.000 people per year (1, 10, 11). It affects men more frequently than women and twice as much Caucasian than black people. Also the incidence is higher in the western world than in developing countries, possibly because of underreporting, limited health care access and different diagnostic systems (11).

Subclassification
Subclassification of glioblastomas can be determined by assessing the IDH (isocitrate dehydrogenase) -status of the tumor. About 90% are IDH-wildtypes, correlating with clinical primary or de novo glioblastomas. The remaining 10% are IDH-mutants, which correlates with secondary glioblastomas in patients with a history of prior lower grade glioma (which is not always diagnosed). In case the IDH evaluation cannot be performed, the tumor is called a 'glioblastoma not otherwise specified (NOS)'. IDH-wildtypes and IDH-mutants have different key characteristics (see table 1) (6, 8, 9).

	IDH-wildtype GB	**IDH-mutant GB**
Synonym	Primary glioblastoma	Secondary glioblastoma
Precursor lesion	Not identifiable; develops de novo	Diffuse astrocytoma Anaplastic astrocytoma
Proportion of GB	90%	10%
Age at diagnosis (median age)	> 55 years (62 years)	Younger patients (44 years)
Male-to-female ratio	1.42:1	1.05:1
Mean length of clinical history	4 months	15 months
Median overall survival **Surgery + radiotherapy** **Surgery + radiochemotherapy**	 9.9 months 15 months	 24 months 31 months
Location	Supratentorial	Preferentially frontal

Table 1: Key characteristics of IDH-wildtype and IDH-mutant glioblastomas (8).

Mutational analysis of the tumor tissue adds value to prognostic predictions. The enzymatic activity of the proteins that were produced by IDH-mutants interferes with cellular mechanisms and epigenetic regulation, adding to oncogenesis (12). At the same time, aberrations in IDH1 and IDH2 have been associated with a more favorable clinical outcome (figure 1) (13).

Compared to IDH-wildtype, the mutants are more sensitized to alkylating agents such as temozolomide and lomustine due to an increased amount of DNA double-strand breaks after treatment (14, 15). As IDH-mutation is found in secondary GB, primary GB presents an EGFR-mutation in 35% of the cases, resulting in constitutive activity of the receptor, increased proliferation and tumor survival. EGFR-mutations are associated with shorter survival time (6, 16).

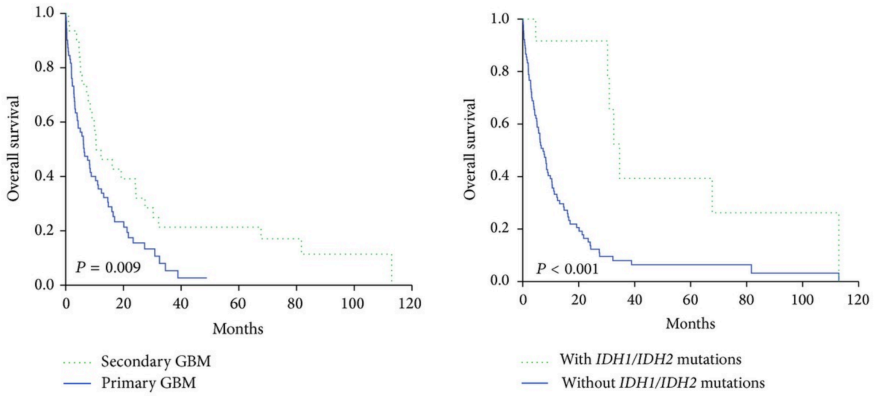

Figure 1. Kaplan-Meier plots of glioma patients showing the association of the following factors with overall survival: secondary vs. primary GB (left) and IDH1/IDH2 mutations (right) (13).

Both primary and secondary glioblastoma cannot be cured by sole radical tumor resection. The tumor contains a subpopulation of highly tumorigenic cells, 'glioblastoma stem cells', from which recurrent glioblastoma is thought to derive. This means recurrence was seeded by cancer stem cells derived from the initial tumor at an early stage of its evolution, resulting in the tumors capacity to evolve either linear or branched (6).

Macroscopic and histological features

In most cases glioblastoma develops in the cerebral hemispheres, preferentially in the white matter of the centrum semiovale. Outside the hemispheres, it preferentially distributes in the corpus callosum, although this localization is less frequent. The basal ganglia and grey matter are often preserved. Multifocal tumors are seen in 2-5%. Macroscopically, glioblastoma is a multilobulated tumor. This characteristic and the tumor's heterogenicity both correlate to its previous name 'glioblastoma multiforme' (GBM). The tumor represents as a large, irregular shaped lesion with relatively widespread vasogenic edema. It is not encapsulated and due to its invasive character, it often induces haemorrhages and, even more importantly, necrosis. Histologically, the tumor is pleomorphic and highly cellular, and contains undifferentiated multipolar astrocytes. Despite an important vascular endothelial proliferation, extracranial metastases are extremely rare whereas there are no lymphatics in the brain and it is difficult to invade the cranial blood vessels. Extracranial metastases have only been reported with a frequency of 0.44% (7).

Diagnosis
Peak incidence for the diagnosis of glioblastoma lies between 55 and 60 years (11), with an average age of 64 years (1), however the tumor can occur at any age. Diagnosis of a brain tumor can be suspected based on clinical presentation and medical imaging, yet histological confirmation on surgically obtained tissue is required. For deep tumors this can be realized by stereotactic or frameless neuronavigation-guided needle biopsy, whereas for superficial tumors open surgical excision with guidance by neuronavigation can be used (7).

Treatment and prognosis
Current standard of care for primary glioblastoma consists of a maximal safe resection, daily temozolomide chemotherapy (TMZ at a dose of 75mg/m^2/day) and concomitant fractionated radiotherapy (30x2Gy), followed by adjuvant TMZ (150-200 mg/m^2/day for 5 consecutive days every 28 days) (9, 10). Outcome remains poor with a median progression-free survival (PFS) of 6.9 months and median overall survival (OS) of 14.6 months (2-4). Furthermore, the recurrence rate is very high (about 90%) and less than 10% of patients are still alive 5 years after the diagnosis. Median PFS at recurrence is 10 weeks and median OS is 30 weeks (5). Although extracranial metastases are extremely rare, this worsens the prognosis even more and leaves chemotherapy as the only therapeutic option (4, 7, 10, 17). At the time of tumor recurrence, no treatment has demonstrated to improve the survival of glioblastoma patients in a randomized clinical trial.

Etiology
Despite research there have been no clear breakthroughs considering the genesis of the tumor. Environmental factors (smoking, diet, cell phones, …) are not conclusively associated with glioblastoma (18). In 5-10% of cases, genetic predisposition has been observed, corresponding with the fact that gliomas are observed in families, but the susceptibility gene remains unidentified (19). An increased incidence can be found in association with rare genetic disorders such as neurofibromatosis type 1 and 2 and tuberous sclerosis (19). Literature suggests that infection and allergic diseases may offer protection against the development of glioblastoma, which may be due to activation of the immune system. The chance of developing glioblastoma in patients with a history of allergy is found to be reduced with 40% (11, 20).

2.3 Cancer immunity cycle

Cancer is characterized by genetic and cellular alterations that trigger the immune system to provoke a response of lymphatic T-cells. The goal of these cells is to recognize and to eradicate the tumor cells. Elimination of cancer by T-cells requires a well-balanced equilibrum between recognition of non-self tumoral cells and prevention of auto-immunity. A series of steps, called the ‘cancer immunity cycle’, has to be run through in order to efficiently eradicate the tumor cells (21, 22).

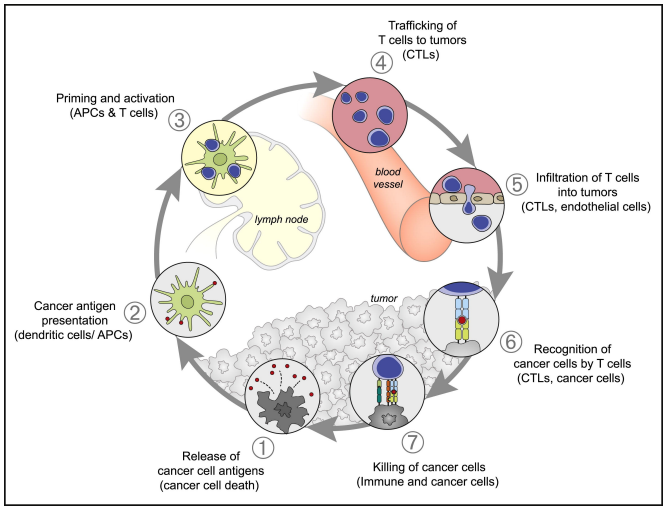

Figure 2. Steps of the cancer immunity cycle (21).

In the first step of the cancer immunity cycle, tumoral antigens are released, as a result of cancer cell death, and captured by antigen-presenting cells (APCs), for example dendritic cells (DCs). APCs present the antigens to T-cells on MHCI and MHCII molecules. This presentation will lead to priming and activation of effector T-cells when combined with a costimulatory interaction. The T-cells will attack non-self cancer-specific antigens and/or human antigens against which central tolerance has been incomplete. In this step, the balance between regulatory T-cells (Tregs) and effector T-cells (CTLs) is crucial. Following activation, the effector T-cells migrate to and infiltrate the tumor. Interaction between the T-cell receptor (TCR) and the corresponding tumoral antigen on MHCI of the APC results in a specific binding. Finally, the T-cell kills the bound cancer cell, resulting in a new tumoral antigen release, initiating a new amplified and expanded cancer immunity cycle (21, 22).

Both stimulatory and inhibitory factors can mediate the cycle by interaction between cell receptors and ligands. Stimulatory factors (pro-inflammatory cytokines, released tumoral antigens, immune checkpoints such as CD28, …) lead to amplification and anti-cancer activity, whereas inhibitory factors (anti-inflammatory cytokines, immune checkpoints such as CTLA-4, …) prevent damage to healthy tissue (auto-immunity) and reduce the provoked immune response (21-23).

2.4 Immune checkpoint inhibition

Inaccurate execution of one (or more) of the steps, or inhibition of the effector T-cells by the tumor micro-environment (‘immune rheostat’ or ‘immunostat’) results in a less than optimal functioning cancer immunity cycle. This is the case in cancer patients. Therefore, the aim of immunotherapy against cancer is to initiate or to resume the cycle so that it could increase in strength and extent without inducing auto-immunity (21, 22).

Amplification of the entire cycle could possibly induce an auto-immune response, so it is important to overcome the negative feedback mechanisms (inhibitory factors) by focusing on the speed-limiting step. Most frequently, this step seems to be the immunostat-function, where the tumor micro-environment (TME) induces immunosuppression (21). This environment induces specific conditions such as hypoxia and acidosis, that influence tumor progression and radiochemo-resistance. It consists of cancer stem cells (CSC) that recruit stromal, endothelial and immune cells to influence their phenotype and behavior, contributing to tumor recurrence (24).

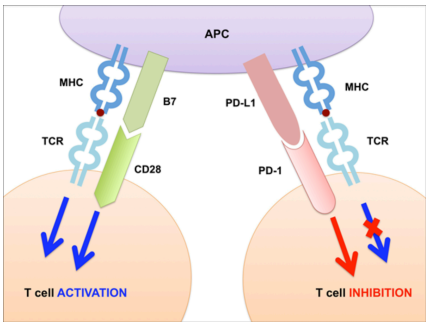

Figure 3. Illustration of an activating (CD28) and inhibiting (PD-1) immune checkpoint by secondary interaction (23).

Immune checkpoints are an example of costimulatory molecules. By secondary interaction with the effector T-cell they activate or suppress the T-cell. Primary interaction is established between the T-cell and the APC with its tumoral antigen/MHC complex. Because of this secondary interaction leading to suppression of the T-cell response, the immune checkpoints are a target for immunotherapy. A specific class of antibodies called the 'immune checkpoint inhibitors' have been developed to counteract the working mechanisms of the costimulatory molecules (23, 25).

2.4.1 Nivolumab

PD-1 (programmed cell death 1, CD279) is a cell receptor expressed on the membrane surface of T-cells. By interacting with its ligands PDL-1 and PDL-2, expressed by APC, it induces an apoptotic signal in the T-cell. Cancer cells may upregulate PDL-1 as a protective mechanism, resulting in immunosuppression in the tumor micro-environment ('immunostat'). This way PD-1 has an important role as an inhibiting immune checkpoint in the last step of the cancer immunity cycle.

Nivolumab (Opdivo®, Bristol-Myers Squibb) is a human monoclonal antibody (type IgG4) that binds the PD-1 receptor and blocks the interaction with its ligands (anti-PD-1 immune checkpoint inhibitor). This inhibits the downregulation of the PD-1 pathway, causing an amplification of the anti-tumoral T-cell response near the tumor (9, 21, 25).

Administration of anti-PD-1 was approved by the FDA (U.S. Food and Drug Administration) in September 2014 for the treatment of melanoma. Currently nivolumab is also being used in the treatment of non-small cell lung cancer, renal cell carcinoma, Hodgkins' lymphoma and squamous cell cancer of the head and neck (9, 23, 26). In 2016 the efficacy of the antibody was observed in children with recurrent glioblastoma suffering from a biallelic mismatch repair deficiency, which proved its potential regarding this tumor type (27).

When used as a monotherapy, the recommended dosing schedule for nivolumab is 3 mg/kg intravenous over 60 minutes, administered every two weeks. Treatment may continue as long as it is being tolerated by the patient and clinical improvement is being observed. Most frequent (≥ 10%) adverse events (AEs) are fatigue (24%), rash (18%), pruritus (13%), diarrhea (13%) and nausea (13%), however they are mostly mild to moderate in severeness (grade 1 or 2). A study regarding the safety profile of nivolumab (NCT00730639) demonstrated that only 14% of the patients showed grade 3 or 4 AEs, proving the antibody's low risk of high-grade toxicity (≥ grade 3) (28, 29).

2.4.2 Ipilimumab

Another major immune checkpoint and therefore target for immune therapy can be found in the third step of the cancer immunity cycle (priming and activation of the T-cells) (21). CTLA-4 (cytotoxic T-lymphocyte-associated molecule-4) is a cell receptor that is being upregulated on the membrane surface of activated T-cells (CD4+ and CD8+) two to three days after activation by antigen-presentation. Similar to its homologue CD28, but with an affinity that is 100 times as high, CTLA-4 binds its ligands, CD80 (B7.1) and CD86 (B7.2) on the membrane surface of APC. This interaction results in the downregulated development and proliferation of T-cells. The CTLA-4 receptor is also expressed by B-cells and regulatory T-cells (Tregs) where it contributes to the immunosuppression of these cells (21, 30-32).

Ipilimumab (Yervoy®, Bristol-Myers Squibb) is a fully human monoclonal antibody (type IgG1) that blocks the interaction between CTLA-4 and its ligands by binding the receptor itself. This way the expansion of T-cells after antigen-presentation is no longer downregulated and T-cell proliferation can increase both in the lymphoid organs as in the periphery (21, 31, 33).

FDA-approval for the use of immune checkpoint inhibitors was first obtained in March 2011 for the treatment of unresectable and metastatic melanoma in an advanced stadium in adults with CTLA-4 (23, 34, 35). The recommended dosing schedule is similar to that of nivolumab monotherapy (3 mg/kg), except for the fact that ipilimumab has to be administered over 90 minutes intravenous every 3 weeks with a total of four doses (35).

A phase III clinical trial showed that over 10% of all treated patients encountered adverse events such as diarrhea, pruritus, fatigue, nausea, vomiting, decreased appetite and abdominal pain. Even though most complaints were mild to moderate in severeness (grade 1 or 2), ipilimumab is more often associated with high-grade adverse events than nivolumab, due to amplified immune responses. The majority of these AEs disappear when treated appropriately (for example corticosteroids) or when ipilimumab administration is being interrupted/stopped. In 10% of patients the treatment had to be ended because of severe AEs. It mainly concerned cases of severe colitis, pneumonitis, hypophysitis, and other immune-related toxicities (34, 35).

Despite ipilimumab's potential high-grade toxicity, a phase III clinical trial in melanoma patients proved that it prolonged the mean overall survival (OS) significantly (10 months for ipilimumab + gp100 vs. 6.4 months for gp100 alone) (36). Therefore the CHMP (Committee for Medicinal Products for Human Use) concluded that the benefit is larger than the risks, which led to approval of the antibody (26, 35).

2.5 Combined anti-PD-1 and anti-CTLA-4 inhibition in glioblastoma

Considering the immunosuppressive capacity of the tumor micro-environment and the limited response rate (20-35%) for monoclonal antibody therapy (23) (e.g. 25-35% BORR for bevacizumab) (37-39), there is need for a combination of different immunomodulating agents in the treatment of recurrent glioblastoma. Previous research in melanoma-patients showed that the synergy of inhibitors acting on different immune checkpoints can increase efficacy in comparison to a monotherapy (34).

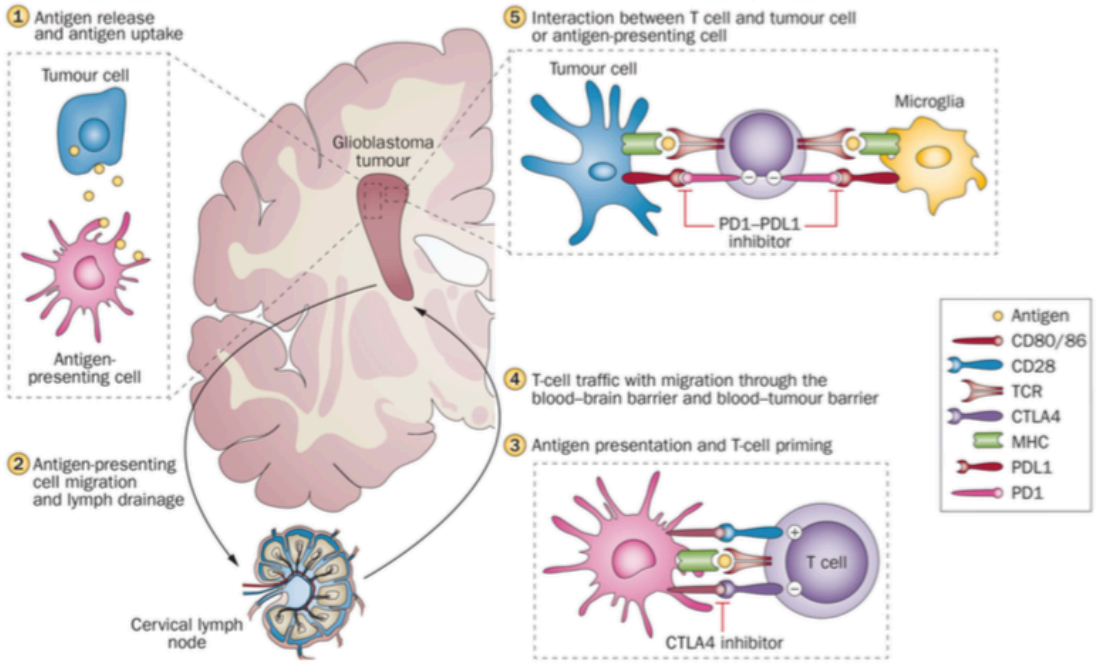

Figure 4. Overview of the immune response and intervention sites of anti-PD-1 and anti-CTLA-4 (immune checkpoint inhibitors) in the cancer immunity cycle (34).

A phase I clinical trial of the CHECKMATE-143 study (NCT02017717) indicated that a combined therapy of nivolumab and ipilimumab in patients with recurrent glioblastoma results in a high risk of high-grade toxicity, mainly due to the administration of ipilimumab. All nivolumab-related AEs were grade 1 or 2, however 15 out of 40 patients (=37,5%) treated with a combination therapy encountered grade 3 or 4 treatment-related AEs. Among the patients that received nivolumab monotherapy only one patient had to stop treatment as a result of AEs, while in the combined treatment group, 7 out of 40 patients (=17,5%) had to stop (40).

A partial response was documented in three patients (=7,5%) and eight patients (=20%) showed stable disease for ≥12 weeks, according to the Criteria for Immunotherapy Response Assessment in Neuro-Oncology (iRANO criteria) (41). The 6-month PFS was 10% in the nivolumab treatment group and 6,7% in the combined treatment group. In two patients immune-mediated effects resembling tumor progression on MRI occured ('pseudo-progression'). The 6-month OS for the monotherapy and combined therapy were respectively 70% and 47,5%. PDL-1 expression was detected in 68,5% of all patients (23, 40, 42).

This clinical trial was followed by a phase III clinical trial on the safety and efficacy of nivolumab monotherapy vs. bevacizumab monotherapy for the treatment of recurrent GB, which demonstrated no survival advantage for the nivolumab treatment. Median OS for nivolumab was 9.8 months, and 10.0 months for bevacizumab, and the 12-month OS was 42% in both arms. Although ORR with nivolumab was lower than with bevacizumab, the nivolumab responses showed longer duration (40).

3 Hypothesis

3.1 Rationale

Glioblastoma is characterized by a rapid and invasive growth that alters brain function. The tumor is highly capable of profound neo-angiogenesis and both linear and branched evolution, resulting in an extremely high recurrence rate (90%) (1, 6, 7). Outcome remains poor with a median progression-free survival (PFS) of 6.9 months and median overall survival (OS) of 12.1 to 14.6 months (2-4). The academic research group for Medical Oncology from the UZ Brussels and the VUB has investigated the utility of cetuximab (an EGFR-targeted mAb), bevacizumab (a VEGF-targeted mAb), and the small molecule VEGFR-inhibitors sunitinib (+/- lomustine) and axitinib (+/- lomustine) for the treatment of patients with recurrent glioblastoma (37, 43-47). Although they could observe objective tumor responses with all agents (up to 28% objective response rate (ORR) with axitinib), there was no significant improvement of the median OS of the patients in these studies. Thus, new therapeutic alternatives are needed.

The safety profile of nivolumab has been studied and showed low risk of high-grade toxicity (28, 29). The efficacy of this antibody has already been observed in children with recurrent glioblastoma suffering from biallelic mismatch repair deficiency (27). Ipilimumab prolonged the median OS in melanoma-patients in a phase III clinical trial, but indicated a higher risk of high-grade AEs (36). The CHECKMATE-143 study, combining both monoclonal antibodies in a phase I clinical trial, showed a numerous amount of patients with high-grade toxicity, mainly due to the administration of ipilimumab (42).

The blood brain barrier (BBB) makes it difficult for drugs to reach the tumor, contributing to chemo-resistance (24). Three preclinical animal studies provided evidence that equal anti-tumor activity can be obtained through intratumoral administration of ipilimumab in a lowered dose. This can be performed with less systemic toxicity as compared to systemic administration, while solving the delivery difficulties caused by the BBB (33, 48).

A phase I clinical trial combined intratumoral injection of ipilimumab with interleukin-2 (IL-2) in patients with unresectable stage III/IV melanoma. The study enrolled a total of 12 patients in a standard 3+3 design to assess the highest tolerable intratumoral dose of ipilimumab and IL-2. Dose escalation of ipilimumab (0.5mg – 1mg – 2mg) was performed by weekly injection in one lesion for 8 weeks in cohorts of 3 patients. IL-2 was injected in a fixed dose 3 times a week into the same lesion for 2 weeks, followed by 2 times a week for 6 weeks. The therapy did not cause any dose limiting toxicity and local responses were seen in 67% of the patients (95% CI, 40%-93%). Additionally, an abscopal response (regression of non-injected lesions) was observed in 89% (95% CI 68-100%). ORR for this study was 40% (95% CI 10-70%). The clinical benefit rate was evaluated based on immune-related response criteria (irRC) and corresponded with 50% (95% CI 19-81%). In most patients an intensified systemic immune response was observed, correlating with clinical appearances (49).

3.2 Aim

In this phase I clinical trial we aim at initiating or resuming the cancer immunity cycle by exploiting the synergetic combination of anti-PD1 and anti-CTLA-4 immune checkpoint inhibitors to treat patients at the time of resection of their recurrent glioblastoma. Risk for increased high-grade immune-related adverse events can be minimized by the use of intra-tumoral administration of the CTLA-4 blocking mAb ipilimumab instead of systemic administration. Considering the low risk of high-grade toxicity due to nivolumab, this antibody will be administered intravenously in Cohort 1. Once this therapy is considered safe and tolerable, an additional dose of nivolumab will be administered intratumorally in Cohort 2, besides administration of ipilimumab. Gross total surgical resection of the tumor cannot ensure complete extinction of all malignant cells, but it will establish a resection cavity that can contain potential (transient) swelling of the injected brain tissue or swelling as a result of post-injection inflammation. Therefore, the overall risk/benefit assessment can be considered acceptable.

3.2.1 Primary objectives and endpoints

The primary objective of this study is to document the feasibility and safety of intra-tumoral injection of ipilimumab in combination with repeated IV administration of nivolumab at a dose of 10 mg. In Cohort 1, ipilimumab will be administered at a dose of 10 mg, and in Cohort 2 at a dose of 5 mg, combined with an intra-tumoral injection of 10 mg nivolumab. The primary endpoint can be defined by reporting all AEs, and by determining feasibility at the protocol defined dose levels of both monoclonal antibodies.

3.2.2 Secondary objectives and endpoints

The secondary objective is to document a descriptive reporting of the tumor response on MRI and FET-PET of the brain. The secondary endpoint can be defined by documentation of progression-free survival (PFS) and overall survival (OS).

3.2.3 Exploratory objectives

In collaboration with the academic research group for Medical Oncology from the UZ Brussels and the VUB, the resected tumor tissue will be analysed for immunohisto-chemistry, molecular genetics, and gene expression.

HYPOTHESIS:
Repeated intravenous administration of the anti-PD-1 mAb *nivolumab* in combination with an intratumoral injection of the anti-CTLA-4 mAb *ipilimumab*, following the gross total tumor resection in patients diagnosed with recurrent glioblastoma, will be tolerable and may have therapeutically meaningful anti-tumor effects.

3.3 Methodology

3.3.1 Patient population

For recruitment to the study, all of the following criteria must be met.

3.3.1.1 Inclusion criteria

A. Diagnosis and tumor characteristics

. Histopathological diagnosis of glioblastoma (= WHO grade IV glioma of the central nervous system, de novo or secondary to a transformed lower-grade glioma WHO grade I, II or III);
. Recurrence and/or progression following prior treatment with surgery, radiation therapy and/or TMZ chemotherapy (significant growth on sequential MRI of the brain);
. Presence of a measurable tumor lesion characterized by gadolinium enhancement on T1-MRI of the brain (with a longest diameter of > 10 mm and a perpendicular diameter of >5mm) without evidence of clinically relevant spontaneous intra-tumor hemorrhage on baseline MRI-imaging or in the prior disease history;
. The tumor is (partially) resectable.

B. Age and reproductive status

. Male or female, 18 years or older;
. Study patients must be surgically sterile, postmenopausal or must agree to use effective contraception during the treatment period (continued for 12 weeks after the last dose of nivolumab). Woman must have a negative pregnancy test prior to enrollment and are not allowed to breastfeed during the treatment.

C. Informed consent and accepting general conditions

. Patients must have signed and dated an approved written informed consent form in accordance with regulatory and institutional guidelines before performing any protocol related procedures;
. Patients must be willing and able to comply with scheduled visits, treatment schedule, laboratory tests, tumor biopsies, and other requirements of the study.

D. Medical status

. Patients must have an ECOG performance status score of 0, 1 or 2;
. Resolution of all acute toxic effects of prior surgical procedures, radiotherapy and TMZ to NCI CTCAEv4.0 grade 0 or 1 except for alopecia;
. Patients must have an adequate hepatic, hematologic and renal function as defined by the following criteria: *total serum bilirubin < 1.5 x ULN (patients with Gilbert's disease exempt who should have bilirubin < 2x ULN), AST and ALT < 2.5 x upper limit of normal (ULN), serum creatinine ≤1.5 x ULN or calculated creatinine clearance ≥60 mL/min, absolute neutrophil count (ANC) > 1500/mm3 without growth factor support, platelets > 75000 cells/mm3, hemoglobin ≥9 g/dL (which may be obtained by transfusion or growth factor support), fT4 hormone levels within normal range.*

3.3.1.2 Exclusion criteria

E. Medical history and simultaneous conditions

- An interval of less than 4 months (: 16 weeks) after the end of postoperative radiation therapy for GB unless progression is confirmed on an MRI of the brain obtained > 4 week after the first observation of progression; and with an interval of at least 4 weeks after the last administration of TMZ;
- A non-resectable recurrence of the glioblastoma;
- Prior treatment on a nivolumab and/or ipilimumab trial; or with an anti-CTLA-4 or anti-PD-1: -L1 targeted therapy;
- Subjects with active, known, or suspected autoimmune disease;
- Subjects requiring systemic treatment with either corticosteroids (> 16 mg daily methylprednisolone equivalent) or other immunosuppressive medications within 14 days of study enrollment;
- Subjects with a known human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS)-related illness;
- Subjects with a severe or uncontrolled medical/psychiatric disorder, active infection or laboratory abnormality, that would impair their ability to receive the study treatment or that would add excessive risks to the study treatment in the judgment of the investigator.

F. Allergies, concomitant medications and adverse events

- Patients with a contra-indication for evaluation by gadolinium enhanced MRI or FET-PET of the brain;
- Patients in need of the following medications during the treatment and follow-up phase (before recurrence): any concurrent anti-neoplastic therapy or immunosuppressive agents, including systemic corticosteroids (: more than 8 mg of methylprednisolone on a daily basis), unless clinically indicated for controlling intracranial edema or treating a drug-related AE.
- Patients with a history of grade 3 toxicity due to administration of monoclonal antibodies.

G. Other

- Convicts or patients who are admitted or incarcerated against their will (no freedom of transportation);
- Pregnant women or women who are currently breastfeeding.

3.3.2 Study design

In this open label, single-centre phase I clinical trial all data were gathered prospectively. Approval for the study protocol by the medical ethical committee of the University Hospital of Brussels was obtained on 03-11-2016. An amendment for dose modification in the second cohort was submitted and approved on 24-02-2017. The clinical trial was registered on clinicaltrials.gov under the following identification number: NCT03233152. The study consisted of the following phases: study candidate identification, eligibility screening, recruitment, treatment, and follow-up.

Study candidate identification and eligibility screen: All patients who are diagnosed with recurrent glioblastoma following maximal surgical resection, radiation therapy and TMZ chemotherapy were considered as candidates for study participation. Once the subject reviewed the patient information brochure (PIB) and signed the informed consent (IC) form, he/she was screened for eligibility as described in the protocol. Subjects who met all eligibility criteria were enrolled in the clinical trial. Recruitment start date was the first of November 2016.

Treatment phase:
COHORT 1: 24 hours prior (day 0) to the neurosurgical intervention patients were administered the first IV injection of nivolumab 10 mg (: 1ml of Opdivo™, 40mg/4ml vial). At day 1, patients underwent a maximal safe resection of the recurrent glioblastoma, followed by injection of 10 mg ipilimumab (: 2ml of Yervoy™, 50mg/10ml vial) in the walls of the resection cavity. The day after the operation an MRI of the brain was performed. Every two weeks (day 15, 29, 43, 57, 71) an IV administration of nivolumab 10 mg was given until a total of six IV administrations was reached. During the treatment phase multiple assessments were conducted (monitoring of AEs, venous blood analyses and tumor response evaluation by MRI). Treatment phase was ended at day 127, after performance of an MRI six weeks after the last nivolumab administration.
COHORT 2: During the neurosurgical intervention these patients underwent a maximal safe resection of the recurrent glioblastoma, followed by injection of 5 mg ipilimumab (: 1ml of Yervoy™, 50mg/10ml vial) and 10 mg nivolumab (: 1ml of Opdivo™, 40mg/4ml vial) in the walls of the resection cavity. Rest of the treatment phase remained the same as in cohort 1.

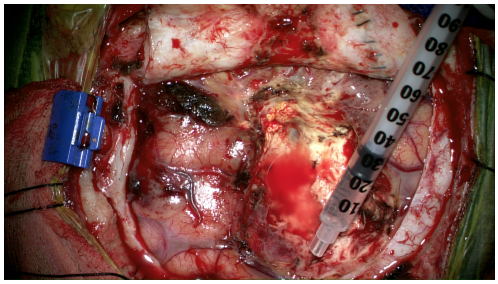

Figure 5. Picture of IT injection of ipilimumab in the walls of the resection cavity during the neurosurgical intervention.

Follow-up phase: In case of progression during the treatment phase and no ongoing treatment-related AEs (grade ≥ 2) patients were followed for survival for one year. In case of no progression on day 127, they were followed for duration of response and survival until progression, death or lost to follow-up, with performance of an MRI every six weeks during the first year.

This clinical trial used a classical phase I "3+3 patient" recruitment design. After the inclusion of 3 patients in Cohort 1 and 3 patients in Cohort 2, an amendment for expansion to treat a maximum of 27 patients in Cohort 2 was submitted and approved by the ethical committee. Four cohorts have been defined up until the moment of data cut-off on 05-06-2019.

3.3.2.1 Treatment plan

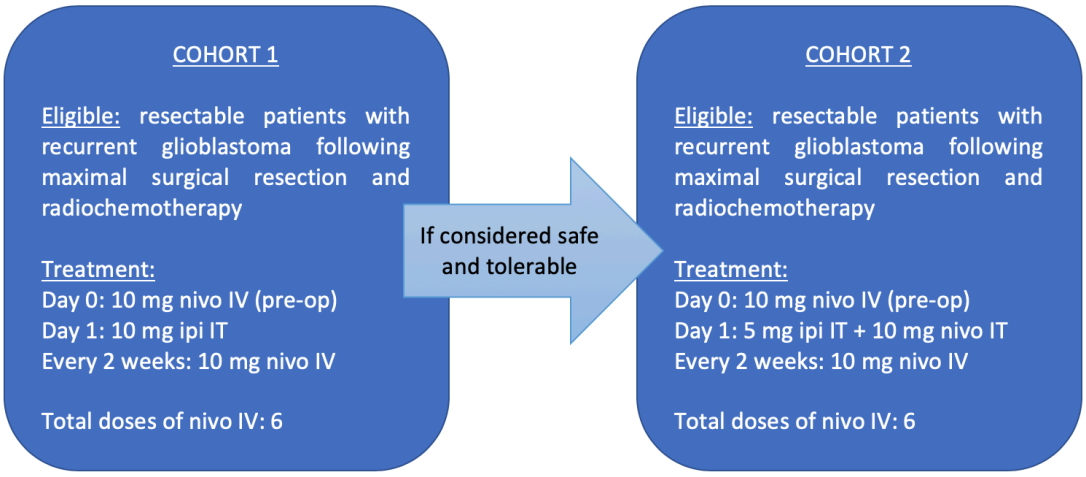

Figure 6. Simplified overview of the treatment plan for Cohort 1 and 2. Nivo = nivolumab; Ipi = Ipilimumab; IV = Intravenously; IT = Intratumorally.

3.3.3 Baseline characteristics, safety and progression

Baseline characteristics have been recorded for the study population. Safety was monitored by reporting the patients' clinical adverse events and most severe blood values, both treatment-related and not treatment-related. Blood events were monitored through venous blood analysis every two weeks, or more frequently if indicated. Treatment-related AEs were classified as either immune- or surgery-related events. Surgery-related AEs were defined as events occuring in the early treatment phase (first two weeks). Toxicity and blood events were graded by the Common Terminology Criteria for Adverse Events (CTCAE Version 4.03) from the National Cancer Institute (NCI). The moment of progression was determined based on radiological findings and clinical deterioration according to the professional judgement of the treating physician.

3.3.4 Statistical analysis

An interim analysis of the phase I clinical trial was performed on 05-06-2019. Only the data of patients who had a minimal treatment/follow-up phase of six weeks after their neurosurgical intervention, was included. Baseline characteristics are reported using descriptive statistics. A visual representation of survival was presented in a Swimmer plot. PFS and OS have been estimated by the Kaplan-Meier method using IBM SPSS Statistics 25.

4 Results

4.1 Consort flow diagram

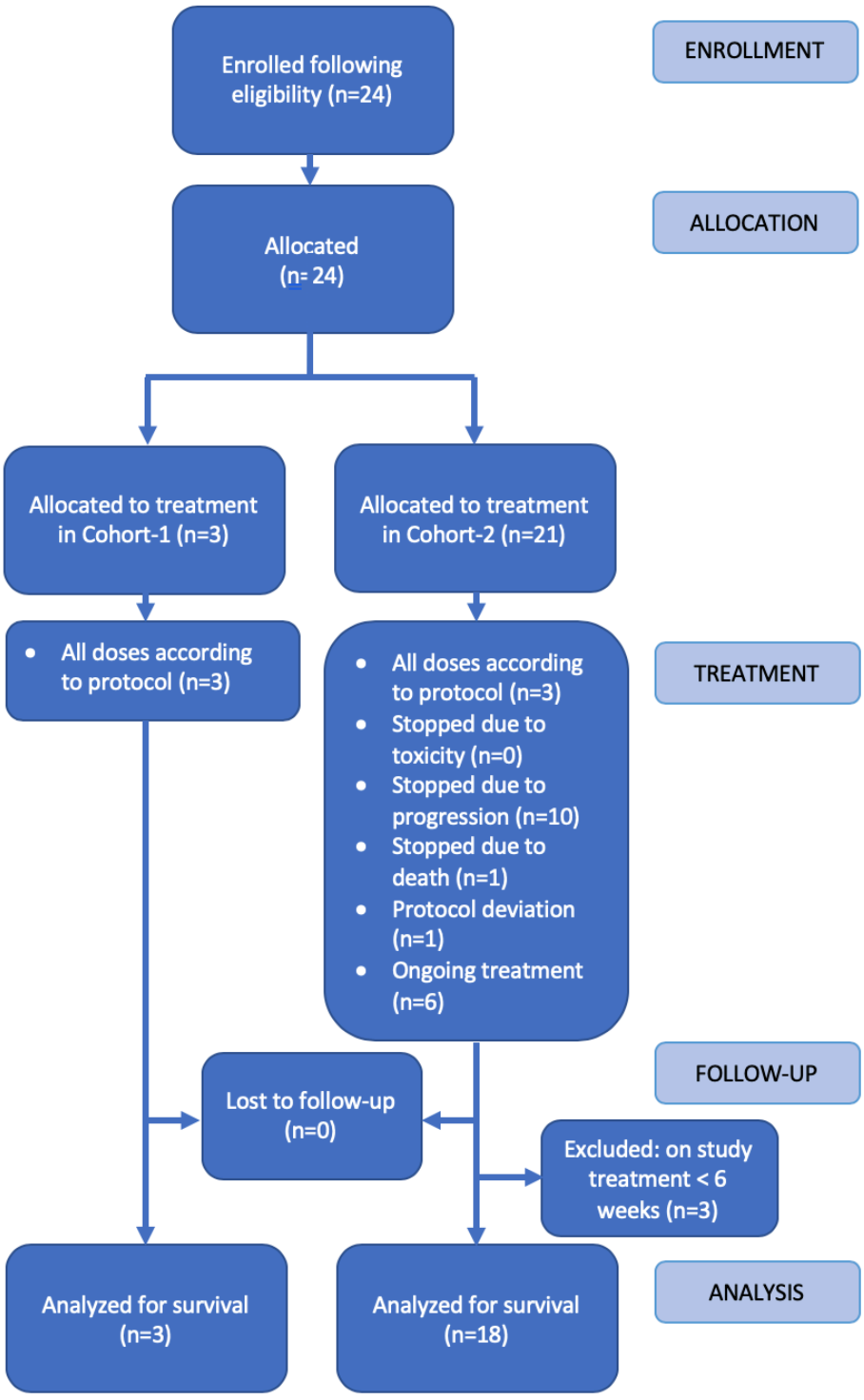

Figure 7. Flow chart of the phase I clinical trial on the combination therapy of nivolumab and ipilimumab for the treatment of patients with recurrent glioblastoma.

4.2 Baseline characteristics

Patients		Cohort 1 (3)	Cohort 2 (18)
Age (years)	Median (range)	67 (38-72)	55 (38-69)
Gender			
	Male	1 (33%)	12 (67%)
	Female	2 (67%)	6 (33%)
Ethnicity			
	Caucasian	3 (100%)	16 (89%)
	Asian	0 (0%)	1 (5,5%)
	North-African	0 (0%)	1 (5,5%)
WHO Performance Status			
	0	1 (33%)	7 (39%)
	1	2 (67%)	9 (50%)
	2	0 (0%)	2 (11%)
Histology			
	Primary glioblastoma	3 (100%)	14 (78%)
	Prior lower grade glioma	0 (0%)	4 (22%)
Predominant localisation of the glioma			
	Frontal	1 (33%)	7 (39%)
	Temporal	0 (0%)	6 (33%)
	Fronto-temporal	0 (0%)	1 (5,5%)
	Parietal	1 (33%)	3 (17%)
	Occipital	0 (0%)	1 (5,5%)
	Occipito-parietal	1 (33%)	0 (0%)
Cerebral hemisphere			
	Left	0 (0%)	8 (44%)
	Right	3 (100%)	10 (56%)
Prior therapy			
	Surgical resection	3 (100%)	18 (100%)
	Radiotherapy alone	0 (0%)	1 (5,5%)
	Chemotherapy alone	0 (0%)	1 (5,5%)
	RT/TMZ concom	0 (0%)	6 (33%)
	RT/TMZ concom + adj	3 (100%)	8 (44%)
	Radiotherapy + RT/TMZ concom + adj	0 (0%)	1 (5,5%)
	RT/TMZ concom + adj + Irinotecan	0 (0%)	1 (5,5%)
Baseline use of corticosteroids			
	Yes	1 (33%)	6 (33%)
	No	2 (67%)	12 (67%)

Table 2. Baseline characteristics of patients in Cohort 1 and 2 (n=21).

Between November 2016 and June 2019, a total of 21 patients (3 in Cohort 1 and 18 in Cohort 2) were analyzed in the phase I clinical trial on intratumoral ipilimumab plus intravenous nivolumab following the resection of recurrent glioblastoma.

Median age for the entire study population was 55 years (range 38-72). The median age in Cohort 1 was 67 years (range 38-72) and 55 years (range 38-69) in Cohort 2. Of all study patients, 81% (n=17) presented with a primary (de novo) glioblastoma. All patients in Cohort 1 (n=3) had a primary glioblastoma, compared to 78% (n=14) in Cohort 2.

Among all patients, 38% (n=8) were asymptomatic, 52% (n=11) showed minor symptoms of disease correlating with a Performance Status score of 1, and 10% (n=2) presented more prominent symptoms of disease correlating with a Performance Status score of 2. In Cohort 1, two out of three patients (67%) had a Performance Status score of 1. The third patient (33%) was asymptomatic. In Cohort 2, two patients (11%) had a Performance Status score of 2, nine patients (50%) had a score of 1 and seven patients (39%) were asymptomatic.

All 21 patients had undergone a surgical resection in the past, followed by different additional therapies. 33% of all patients (n=7) had a baseline use of corticosteroids. In Cohort 1, this was one patient out of three (33%), and in Cohort 2, six patients out of 18 (33%).

4.3 Treatment disposition

Patient Number	Ipilimumab (IT)	Nivolumab (IT)	Nivolumab (IV)	Treatment Status
1	1 x 10mg	0	6 x 10mg	All doses according to protocol
2	1 x 10mg	0	6 x 10mg	All doses according to protocol
3	1 x 10mg	0	6 x 10mg	All doses according to protocol
4	1 x 5mg	1 x 10mg	5 x 10mg	Stopped due to PD
5	1 x 5mg	1 x 10mg	3 x 10mg	Stopped due to PD
6	1 x 5mg	1 x 10mg	1 x 10mg	Stopped due to PD
7	1 x 5mg	1 x 10mg	5 x 10mg	All doses according to protocol
8	1 x 5mg	1 x 10mg	4 x 10mg	Stopped due to PD
9	1 x 5mg	1 x 10mg	3 x 10mg	Stopped due to PD
10	1 x 5mg	1 x 10mg	8 x 10mg	Protocol deviation
11	1 x 5mg	1 x 10mg	5 x 10mg	Stopped due to PD
12	1 x 5mg	1 x 10mg	2 x 10mg	Stopped due to PD
13	1 x 5mg	1 x 10mg	1 x 10mg	Stopped due to death
14	1 x 5mg	1 x 10mg	6 x 10mg	All doses according to protocol
15	1 x 5mg	1 x 10mg	3 x 10mg	Stopped due to PD
16	1 x 5mg	1 x 10mg	3 x 10mg	Stopped due to PD
17	1 x 5mg	1 x 10mg	5 x 10mg	Stopped due to PD
18	1 x 5mg	1 x 10mg	6 x 10mg	All doses according to protocol
19	1 x 5mg	1 x 10mg	5 x 10mg	Ongoing treatment
20	1 x 5mg	1 x 10mg	5 x 10mg	Ongoing treatment
21	1 x 5mg	1 x 10mg	5 x 10mg	Ongoing treatment

Table 3. Treatment disposition of Cohort 1 (n=3) and 2 (n=18). IT = Intratumoral; IV = Intravenous; PD = Progressive Disease.

All patients in Cohort 1 (n=3) received 10 mg ipilimumab administered by administering a volume of 2 ml by 20 injections in the wall of the resection cavity (further referred to as 'intratumoral administration') following maximally safe resection of the recurrent glioblastoma. All three patients were intravenously treated with nivolumab according to protocol. The patient received one intravenous dose of 10 mg 24 hours before the operation, and five doses post-operative, resulting in a total of six intravenous administrations of 10 mg nivolumab. In Cohort 1, none of the patients received an intratumoral administration of nivolumab.

In Cohort 2, an intratumoral administration of 5 mg ipilimumab (5mg/1ml) and 10 mg (10mg/1ml) nivolumab was administrated during the neurosurgical intervention, again by 20 injections of 0.1ml each, totaling 2ml administered volume. The pre-operative and post-operative intravenous administrations of nivolumab were identical to Cohort 1. At the moment of analysis three patients (17%) in this cohort were still on study treatment. Three out of 18 patients (17%) received all doses according to protocol. Ten patients (56%) stopped the study treatment earlier due to progressive disease. One patient (6%) deceased before progression or end of treatment. In one patient (6%) a protocol deviation occurred, resulting in eight intravenous administrations of nivolumab.

4.4 Adverse events

COHORT 1

Category	Adverse Event (AE)	All grades - Number (%)	Grade 1	Grade 2	Grade 3
Surgery-related					
General	Fever	2 (67%)	2	0	0
	Subfebrillitas	1 (33%)	1	0	0
Neurological	Ataxia	1 (33%)	0	1	0
	Globus	1 (33%)	0	1	0
	Headache	1 (33%)	1	0	0
	Hemianopsia	1 (33%)	0	0	1
	Hemiparesis	1 (33%)	0	1	0
	Proprioceptive dysfunction	1 (33%)	0	1	0
Immune-related					
Endocrine	Hyperthyroidism	1 (33%)	1	0	0
Skin	Pruritus	1 (33%)	1	0	0
Not related					
Gastrointestinal	Constipation	1 (33%)	1	0	0
	RBPA	1 (33%)	1	0	0
Neurological	Amnesia (short term memory)	1 (33%)	0	1	0
	Cognitive deterioration	1 (33%)	1	0	0
	Confusion	1 (33%)	0	1	0
	Dysphasia	1 (33%)	1	0	0
	Hemineglect	1 (33%)	0	1	0
	Hemiparesis	1 (33%)	0	0	1
	Insomnia	1 (33%)	0	1	0
	Paresthesia leg	1 (33%)	1	0	0
	Tremor hand	1 (33%)	1	0	0
Musculoskeletal	Tendinitis shoulder	1 (33%)	0	1	0
Cardiovascular	Hypertension	1 (33%)	0	1	0
Other	UTI	1 (33%)	0	1	0
	Positive HC (S. epidermidis)	1 (33%)	1	0	0

Table 4. Adverse Events in Cohort 1 (n=3). RBPA = Red Bloodloss Per Anum; UTI = Urinary Tract Infection; HC = Hemoculture.

In Cohort 1, a total of 26 AEs were reported, of which 2 AEs (8%) were high-grade. Nine out of 26 (35%) AEs were surgery-related, two (8%) were immune-related and 15 (58%) were not related to the study treatment.

Fever following the neurosurgical intervention was the most frequently reported AE (n=2; 67% of patients), in both cases not requiring specific treatment (grade 1). Of all AEs reported in Cohort 1, 13 (50%) were grade 1 and 11 (42%) were grade 2. Hemianopsia (surgery-related) and hemiparesis (not related to study treatment) were the only grade 3 AEs out of 26 reported AEs, together accounting for 8% of all reported AEs. There were no higher graded AEs.

The only immune-related AE in Cohort 1 were hyperthyroidism and pruritus (both n=1; 33% of patients) of grade 1.

COHORT 2

Category	Adverse Event (AE)	All grades – Number (%)	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Surgery-related							
General	Drowsiness	1 (6%)	0	0	1	0	0
	Fever	8 (44%)	6	2	0	0	0
	Subcutanous leakage of CSF	4 (22%)	3	1	0	0	0
Gastro-intestinal	Constipation	1 (6%)	1	0	0	0	0
	Nausea	4 (22%)	3	1	0	0	0
	Vomiting	2 (11%)	2	0	0	0	0
Neurological	Amnesia (short term memory)	2 (11%)	0	2	0	0	0
	Apathy	1 (6%)	1	0	0	0	0
	Aphasia	1 (6%)	0	0	1	0	0
	Cerebral hemorrhage	1 (6%)	0	0	1	0	0
	Cerebral edema	1 (6%)	0	0	0	1	0
	Confusion	3 (17%)	3	0	0	0	0
	Disturbed vision	1 (6%)	1	0	0	0	0
	Epileptic insult	2 (11%)	0	0	2	0	0
	Headache	8 (44%)	6	2	0	0	0
	Hemiparesis	4 (22%)	0	2	2	0	0
	Hypoesthesia	1 (6%)	0	1	0	0	0
	Meningitis	1 (6%)	0	0	1	0	0
	Photophobia	1 (6%)	1	0	0	0	0
	Urinary urge	1 (6%)	1	0	0	0	0
Respiratory	Respiratory insufficiency	1 (6%)	0	0	1	0	0
	Sarcoid-like pulmonary toxicity	1 (6%)	0	0	1	0	0
Musculo-skeletal	Neck pain	1 (6%)	1	0	0	0	0
Immune-related							
General	Dry eyes	1 (6%)	1	0	0	0	0
	Fatigue	11 (61%)	4	4	3	0	0
	Fever	2 (11%)	2	0	0	0	0
	Inflammatory reaction of unknown origin	1 (6%)	0	0	1	0	0
	Vasovagal malaise	1 (6%)	1	0	0	0	0
Endocrine	Hypothyroidism	1 (6%)	0	1	0	0	0
Skin	Dandruff	1 (6%)	1	0	0	0	0
	Pruritus	5 (28%)	4	1	0	0	0
	Swelling of scar	1 (6%)	1	0	0	0	0

	Xerosis cutis	1 (6%)	0	1	0	0	0
Gastro-intestinal	Xerostomia	1 (6%)	1	0	0	0	0
Neurological	Altered taste	1 (6%)	1	0	0	0	0
	Dysphasia	1 (6%)	1	0	0	0	0
	Headache	4 (22%)	2	2	0	0	0
	Tremor hand	1 (6%)	1	0	0	0	0
Not related							
Endocrine	Hyperthyroidism	1 (6%)	1	0	0	0	0
	Hypothyroidism	2 (11%)	1	1	0	0	0
Gastro-intestinal	Aphtosis tongue	1 (6%)	1	0	0	0	0
	Constipation	1 (6%)	1	0	0	0	0
	Digestive problems	1 (6%)	1	0	0	0	0
	Gastroenteritis	1 (6%)	1	0	0	0	0
	Nausea	3 (17%)	3	0	0	0	0
	RBPA	1 (6%)	1	0	0	0	0
	Vomiting	1 (6%)	1	0	0	0	0
Neurological	Amnesia (short term memory)	1 (6%)	0	1	0	0	0
	Ataxia	2 (11%)	0	2	0	0	0
	Cognitive deterioration	3 (17%)	0	1	2	0	0
	Disturbed vision	2 (11%)	0	2	0	0	0
	Dysphasia	2 (11%)	1	1	0	0	0
	Epileptic insult	3 (17%)	1	0	2	0	0
	Headache	1 (6%)	1	0	0	0	0
	Insomnia	1 (6%)	0	1	0	0	0
	Paresis distally	1 (6%)	1	0	0	0	0
	Paresthesia arm	1 (6%)	1	0	0	0	0
	Post herpetic neuralgia	1 (6%)	0	1	0	0	0
	Vertigo	2 (11%)	2	0	0	0	0
Respiratory	Bronchitis	1 (6%)	0	1	0	0	0
	Cardiorespiratoy arrest	1 (6%)	0	0	0	0	1
	Cough	1 (6%)	1	0	0	0	0
	Dyspnea	2 (11%)	2	0	0	0	0
	Pneumonia	1 (6%)	0	0	1	0	0
	Rhinitis	1 (6%)	1	0	0	0	0
	Sleep apnea	1 (6%)	0	1	0	0	0
	Upper airway infection	1 (6%)	1	0	0	0	0
Urogenital	AKI	1 (6%)	1	0	0	0	0
	Erectile dysfunction	1 (6%)	1	0	0	0	0
	Priapism	1 (6%)	0	1	0	0	0

	Urinary urge	1 (6%)	1	0	0	0	0
	UTI	1 (6%)	1	0	0	0	0
Skin	Cold sore lip	1 (6%)	1	0	0	0	0
	Rash	2 (11%)	2	0	0	0	0
Musculo-skeletal	Backache	1 (6%)	0	1	0	0	0
	Bone metastases	1 (6%)	0	0	1	0	0
	Fall	2 (11%)	2	0	0	0	0
	Pathological hip fracture	1 (6%)	0	0	1	0	0
	Shoulder pain	1 (6%)	1	0	0	0	0
Cardio-vascular	Edema leg	1 (6%)	1	0	0	0	0
	Hypertension	2 (11%)	1	1	0	0	0
Other	Otitis	1 (6%)	0	1	0	0	0

Table 5. Adverse Events in Cohort 2 (n=18). CSF = Cerebrospinal Fluid; RBPA = Red Bloodloss Per Anum; AKI = Acute Kidney Injury; UTI = Urinary Tract Infection.

In Cohort 2, 143 AEs were reported, of which 23 AEs (16%) were high-grade. 51 (36%) out of 143 AEs were surgery-related, 33 (23%) were immune-related and 59 (41%) were not related to the study treatment.

Amongst the surgery-related AEs, fever (n=8; 44% of the patients) and headache (n=8; 44% of the patients) were most frequently reported. For the immune-related AEs, this was fatigue (n=11; 61% of the patients) and pruritus (n=5; 28% of all patients). All other AEs related to the study treatment, both neurosurgical intervention and immunotherapy, occurred in less than one in four patients (<25%). Most frequently reported not related AEs were cognitive deterioration (n=3; 17% of all patients) and epileptic insults (n=3; 17% of all patients). To determine whether changes in thyroid hormone levels are immune-related or not, the patient's medical history, home medications and therapy compliance were taken into account.

Of all reported AEs in Cohort 2, 84 (59%) out of 143 were grade 1, 36 (25%) were grade 2, 21 (15%) were grade 3, 1 (1%) was grade 4 and 1 (1%) was grade 5. Cerebral edema, presenting within two weeks after the surgery, is the only reported grade 4 AE. The grade 5 AE was a patient that went into cardiorespiratory arrest, resulting in death.

There were no grade 4 or 5 AEs related to immunotherapy. Four patients experienced grade 3 AEs, being fatigue (n=3) and an inflammatory reaction of unknown origin (n=1). This high-grade toxicity (grade 3 or more) accounts for 12% of the reported immune-related AEs. All other immune-related AEs were grade 1 or 2.

4.5 Adverse blood events

COHORT 1

Category	Blood event	All grades - Number (%)	Grade 1	Grade 2	Grade 3
Hematology					
	Lymphopcytopenia	3 (100%)	0	1	2
	Leukocytosis	1 (33%)	0	1	0
	Neutropenia	1 (33%)	0	1	0
	Anemia	2 (67%)	1	1	0
	Thrombocytopenia	3 (100%)	3	0	0
Clinical Chemistry					
	CRP increased	3 (100%)	-	-	-
	Lipase increased	1 (33%)	0	1	0
	Hyperglycemia	3 (100%)	3	0	0
	Hyperuricemia	2 (67%)	2	0	0
	Hypoalbuminemia	2 (67%)	2	0	0
	Hypoglycemia	1 (33%)	1	0	0
	ALT increased	1 (33%)	1	0	0
	AST increased	1 (33%)	1	0	0
Electrolytes					
	Hypocalcemia	1 (33%)	0	1	0
	Hypokalemia	1 (33%)	0	1	0
	Hypophosphatemia	1 (33%)	0	1	0
	Hypermagnesemia	1 (33%)	1	0	0

Table 6. Most severe blood events in Cohort 1 (n=3), including the number of event occurrence and the percentage of patients the blood event was seen in. CRP = C-Reactive Protein; ALT = Alanine Transaminase; AST = Aspartate Aminotransferase.

A total of 28 blood events were reported in the first cohort. Ten (36%) out of 28 events were hematologic disorders, 14 (50%) were biochemical disorders and 4 (14%) were electrolyte disorders. Gradation of the increase in C-reactive Protein (CRP) cannot be performed, but was seen in all three patients of Cohort 1.

Lymphocytopenia was the highest graded blood event, and it occurred in all three patients (n=1 for grade 2; n=2 for grade 3). Other blood events that presented in all patients in Cohort 1 were thrombocytopenia (n=3 for grade 1), an increased CRP (not graded), and hyperglycemia (n=3 for grade 1).

COHORT 2

Category	Blood event	All grades – Number (%)	Grade 1	Grade 2	Grade 3	Grade 4
Hematology						
	Lymphocytopenia	17 (94%)	7	6	3	1
	Thrombocytopenia	10 (56%)	8	0	1	1
	Neutropenia	2 (11%)	1	0	0	1
	Leukocytosis	4 (22%)	0	1	3	0
	Leukocytopenia	5 (28%)	1	2	2	0
	Anemia	14 (78%)	11	3	0	0
	Lymphocytosis	1 (6%)	0	1	0	0
Clinical Chemistry						
	CRP increased	18 (100%)	-	-	-	-
	ALT increased	7 (39%)	5	1	1	0
	Bilirubin increased	4 (22%)	2	1	1	0
	Hypertriglyceridemia	2 (11%)	1	0	1	0
	Lipase increased	2 (11%)	1	0	1	0
	Hypoalbuminemia	13 (72%)	10	3	0	0
	Hyperglycemia	10 (56%)	7	3	0	0
	GGT increased	9 (50%)	6	3	0	0
	eGFR decreased	3 (17%)	0	3	0	0
	AST increased	6 (33%)	5	1	0	0
	AP increased	3 (17%)	2	1	0	0
	CPK increased	3 (17%)	2	1	0	0
	Hyperuricemia	8 (44%)	8	0	0	0
	Hypercholesterolemia	3 (17%)	3	0	0	0
	Creatinin increased	2 (11%)	2	0	0	0
	D-dimers increased	1 (6%)	1	0	0	0
Electrolytes						
	Hypocalcemia	8 (44%)	6	2	0	0
	Hypophosphatemia	1 (6%)	0	1	0	0
	Hyponatremia	7 (39%)	7	0	0	0
	Hypokalemia	6 (33%)	6	0	0	0
	Hypermagnesemia	4 (22%)	4	0	0	0
	Hyperkalemia	2 (11%)	2	0	0	0
	Hypercalcemia	1 (6%)	1	0	0	0

Table 7. Most severe blood events in Cohort 2 (n=18), including the number of event occurrence and the percentage of patients the blood event was seen in. CRP = C-Reactive Protein; ALT = Alanine Transaminase; GGT = Gamma-Glutamyl Transferase; eGFR = estimated Glomerular Filtration Rate; AST = Aspartate Aminotransferase; AP = Alkaline Phosphatase; CPK = Creatine Phosphokinase.

In the second cohort, a total of 176 blood events were reported. 53 (30%) out of 176 blood events were hematologic disorders, 94 (53%) were biochemical disorders, and 29 (16%) were electrolyte disorders. Comparable to Cohort 1, an increase in CRP (not graded) was seen in all 18 patients in Cohort 2.

Besides an increase in CRP, the most frequently reported blood events were lymphocytopenia (n=17; 94% of all patients), anemia (n=14; 78% of all patients), hypoalbuminemia (n=13; 72% of all patients), hyperglycemia (n=10; 56% of all patients), thrombocytopenia (n=10; 56% of all patients) and an increase in GGT (n=9; 50% of all patients). All other blood events occurred in less than 50% of the patients in Cohort 2.

Three (2%) out of 176 blood events were grade 4, all hematological (lymphocytopenia, thrombocytopenia and neutropenia). 13 (7%) blood events were grade 3, 33 (19%) were grade 2 and 109 (62%) were grade 1.

4.6 Progression-free and overall survival

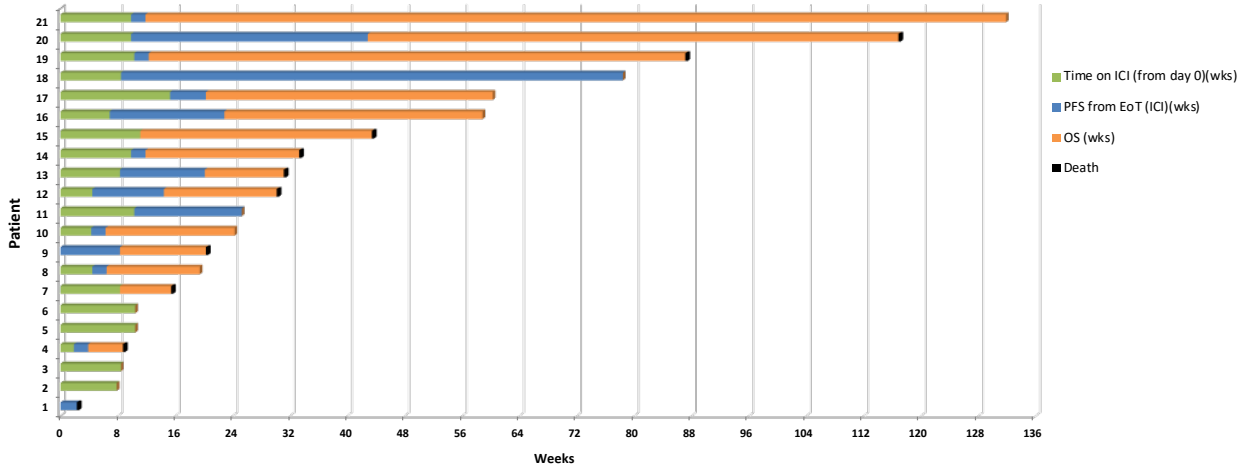

Figure 8. Swimmer plot of the patient population showing survival from start on study treatment (day 0: pre-operative intravenous administration of nivolumab). ICI = Immune Checkpoint Inhibitors; PFS = Progression-Free Survival; EoT = End of Treatment; OS = Overall Survival.

At the moment of analysis 21 patients were included in the phase I clinical trial, 3 in Cohort 1 and 18 in Cohort 2. During the treatment phase, ten (48%) out of 21 patients became progressive before end of study treatment. Six (29%) out of 21 patients received all doses according to protocol, and one (5%) patient received two additional administrations of nivolumab (protocol deviation). One patient (5%) deceased before progressive disease and before end of treatment. Three (14%) out of 21 patients were still on study treatment. The median treatment time was 8,4 weeks.

Ten (48%) out of 21 patients passed away so far (n=2 in Cohort 1; n=8 in Cohort 2). Six of the 11 patients that are still alive, were not progressive at the moment of analysis. This corresponds with a number of 15 (71%) out of 21 patients that are progressive and/or deceased. The median follow-up time was 25,3 weeks.

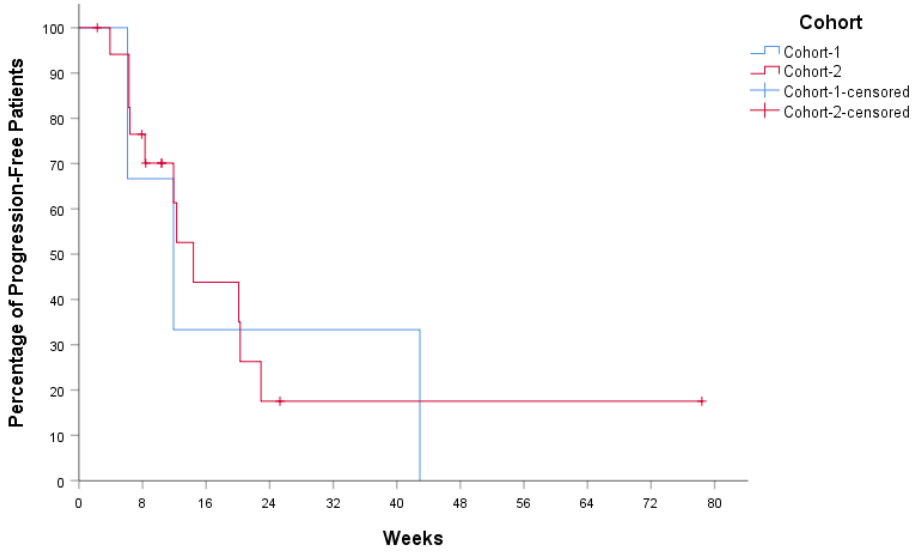

Figure 9. Progression-free survival of patients in Cohort 1 (n=3; blue line) and Cohort 2 (n=18; red line).

Median progression-free survival (PFS), as estimated by Kaplan-Meier, in Cohort 1 was 11,9 weeks (95%CI 2,6 – 21,2). In Cohort 2, median PFS was 14,4 weeks (95%CI 10,5 – 18,3). Six-month PFS for the entire study population was 17,5% (95% CI 0 – 39,3), meaning that 82,5% of all patients have progressed after six months on study treatment.

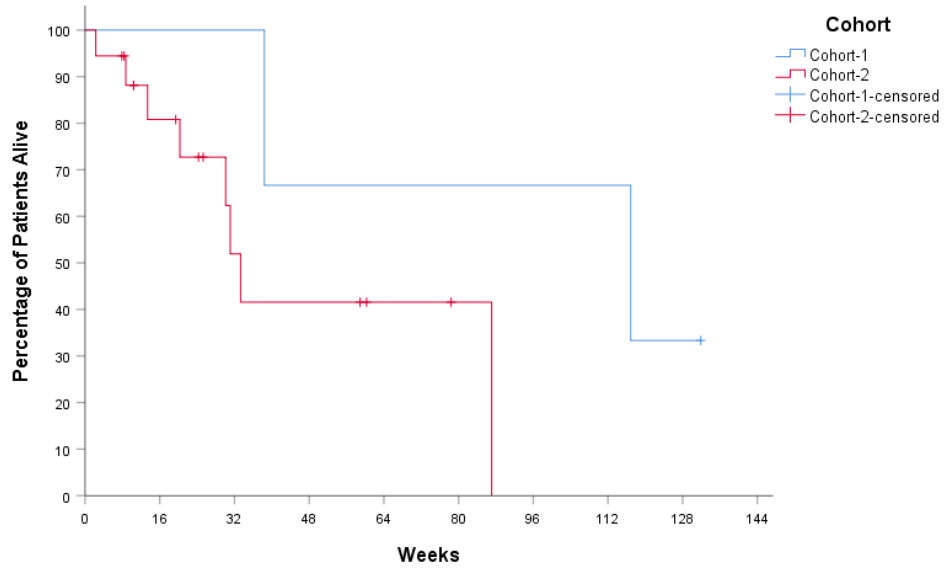

Figure 10. Overall survival of patiens in Cohort 1 (n=3; blue line) and Cohort 2 (n=18; red line).

Median overall survival (OS), as estimated by Kaplan-Meier, in Cohort 1 was 116,9 weeks (95%CI 0 – 242,5). In Cohort 2, median OS was 33,3 weeks (95%CI 28,7 – 37,9). However, this is only an estimation of the median OS to be expected, since 50% mortality of the cohort population has not been reached yet at the time of analysis. 6m-OS for the entire study population was 72,7% (95%CI 49,4 – 96,0), meaning that at least 7 out of 10 patients are still alive after six months on study treatment.

4.7 Case illustration

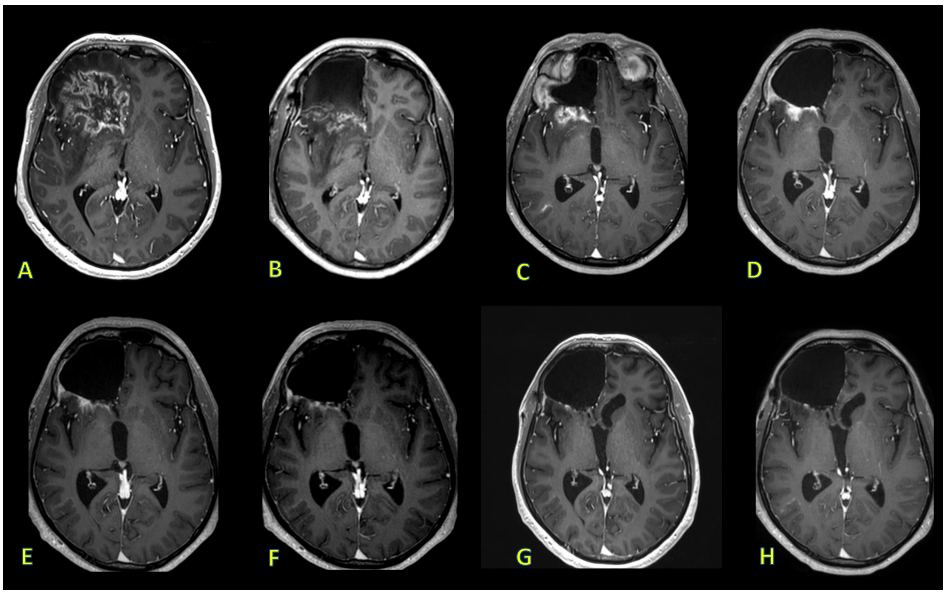

Figure 11. Case illustration of a patient in Cohort 2. Image A shows the baseline status on MRI before resection of the recurrent glioblastoma. Image B is one day post-op. The following images (C, D, E, F, G, H) were made with a six weeks interval.

The images above illustrate the case of a patient who was treated in Cohort 2 of the phase I clinical trial. It concerns a 41 year old Caucasian male that was diagnosed with a anaplastic astrocytoma (grade III) in January 2013. Treatment consisted of a surgical resection in 2013, followed by radiotherapy. In February 2016 a new lesion was found on MRI, stating tumor recurrence with possible progression to a grade IV glioma. Treatment continued under the form of radiotherapy with concomitant and adjuvant temozolomide.

Recurrence of the glioblastoma presented one year later. He signed the informed consent for the phase I clinical trial in September 2017, and got operated in the same month. He was treated in Cohort 2 with an intratumoral injection of ipilimumab (5mg) and nivolumab (10mg) plus six intravenous administrations of nivolumab (10mg, two-weekly). He is now off study treatment, alive and progression-free after 78 weeks from baseline. Post-operative captation of contrast at the margins of the resection cavity decreases through time. This suggests an inflammatory origin of the captation of contrast, rather than recurrent tumor tissue. Three study patients showed a transient gadolinium enhancing lesion on T1-MRI.

5 Discussion and conclusion

5.1 Interpretation of the results

This interim analysis of the phase I clinical trial on intratumoral ipilimumab plus intravenous nivolumab following the resection of recurrent glioblastoma offers the opportunity to interpret the preliminary results.

Safety and efficacy of the combined therapy of nivolumab and ipilimumab in patients with recurrent glioblastoma has been confirmed in a phase I clinical trial of the CHECKMATE-143 study (NCT02017717) (40, 42). In this study, both antibodies were administered intravenously. It showed that nivolumab monotherapy was better tolerated than the combined therapy, and that tolerability was influenced by ipilimumab doses. Ipilimumab was administered at a maximal dose of 3 mg/kg IV every 2 weeks.

A phase I clinical trial combined intratumoral injection of ipilimumab with interleukin-2 (IL-2) in patients with unresectable stage III/IV melanoma (49). Ipilimumab doses were escalated weekly (0.5mg – 1mg – 2mg) by injection in one lesion for 8 weeks in cohorts of 3 patients. There was no dose limiting toxicity and both local and abscopal responses were reported in respectively 67% and 89% of patients.

In this phase I clinical trial on intratumoral ipilimumab and intravenous nivolumab, ipilimumab was injected in the walls of the resection cavity at a dose of 10 mg in Cohort 1 and 5 mg in Cohort 2. By intratumoral injection we aim at limiting the systemic toxicity of the treatment while obtaining equal anti-tumor activity. Results show that there were no patients who had to stop treatment due to toxicity. In both cohorts, most AEs were not-related to the study treatment, and are often a precursor of progressive disease (e.g. amnesia, ataxia, hemiparesis, cognitive deterioration,…). There were no high-grade immune-related AEs in Cohort 1, and in Cohort 2, only 12% of the immune-related AEs were high-grade (all grade 3).

	Cohort 1		Cohort 2	
Surgery-related	35%		36%	
Immune-related	8%		23%	
	Low grade 100%	High grade 0%	Low grade 88%	High grade 12%
Not related	58%		41%	

Table 8. Frequency overview of the reported AEs in Cohort 1 and 2.

In comparison to the CHECKMATE-143 study, where ipilimumab was administered intravenously and 17,5% of the patients had to stop due to toxicity, these preliminary results suggest a safer and less toxic method of administration by intratumoral injection.

An increase in C-reactive protein was described in all treated patients. The cause remains unidentified, but it could possibly be a reaction to the neurosurgical intervention. Other disturbances in blood values were most often biochemical, however high-graded disturbances were mostly reported in hematologic values (lymphocytopenia, thrombocytopenia, neutropenia). A possible cause might be prior treatment with alkylating agents (chemotherapy).

Median PFS for primary glioblastoma is 6,9 months, and median OS is 12,1-14,6 months. Less than 10% of all patients are still alive 5 years after the initial diagnosis (2-4). At the time of tumor recurrence, no treatment has demonstrated to improve the survival of glioblastoma patients in a randomized clinical trial. Median PFS at recurrence is 10 weeks and median OS is 30 weeks (5). In the CHECKMATE-143 study, a 6-month PFS and 6-month OS of respectively 6,7% and 47,5% were reported in the combined treatment group (42). In this phase I clinical trial, a 6-month PFS of 17,5% and a 6-month OS of 72,7% was found. These preliminary results suggest that intra-tumoral injection of the monoclonal antibodies not only limits systemic toxicity, but also improves local delivery and activity, as suspected, based on preclinical animal studies.

In accordance with the findings of the CHECKMATE-143 study, immune-mediated effects resembling tumor progression on MRI occurred in three out of 21 study patients (‘pseudo-progression’). Considering the fact that post-operative captation of contrast at the margins of the resection cavity decreases through time, an inflammatory origin can be assumed, rather than recurrence of the glioblastoma. However, it remains difficult to determine, based on a sole MRI, whether we are dealing with a pseudo- or a true tumor progression. Therefor close follow-up by MRI seems to be pivotal.

This phase I clinical trial on intratumoral ipilimumab plus intravenous nivolumab may be subject to various forms of bias. For example, the trial excluded non-resectable patients in Cohort 1 and 2. This criterium could lead to a bias, considering the patients are often in a better general condition than non-resectable patients. Their baseline condition could contribute to a more favorable outcome in terms of PFS and OS. Therefore it is important to expand the study to non-resectable patients in order to draw more representative conclusions.

A classical 3+3 study design includes three patients per cohort. In this study, an amendment was approved to expand the second cohort up to 27 patients. Although this increases the original patients sample size, this study remains a phase I clinical trial in order to assess safety and feasibility, and it should be followed by a phase II trial to assess efficacy. No premature conclusions can be drawn regarding progression-free survival and overall survival.

The median PFS and OS that were estimated in this clinical trial were defined, respectively, as the time (in weeks) to progression and death from the start of study treatment (day 0). Comparing these survivals to the median for primary glioblastoma would result in a negatively biased outcome considering the study patients' recurrent disease at baseline. In comparison to other study treatments for recurrent glioblastoma, the combination of intratumoral ipilimumab and intravenous nivolumab seems to show promising results, especially regarding overall survival. Further investigations are indicated to assess the efficacy of the study treatment.

Target	Agent	Median PFS in weeks	6m-PFS	Median OS in weeks	6m-OS	Reference
Not applicable	Salvage agents	7-15	16%	26-40	55%	Lamborn et al. (50)
EGFR-targeted mAb	Cetuximab	8	7%	20	38%	Neyns et al. (45)
VEGF-targeted mAb	Bevacizumab	13	27%	26	52%	Duerinck et al. (37)
Small molecule VEGFR-inhibitor	Sunitinib (+/- lomustine)	7	15%	27	20%	Duerinck et al. (43)
Small molecule VEGFR-inhibitor	Axitinib	12	26%	29	54%	Duerinck et al. (44, 51)
Small molecule VEGFR-inhibitor + anti-PDL-1 mAb	Axitinib + avelumab (interim analysis)	15	18%	26	71%	Ben Salama et al. (52)
Anti-PD-1 mAb + anti-CTLA-4 mAb	Nivolumab + ipilimumab	6-8	6,7%	29-37	47,5%	Omuro et al. (42) CHECKMATE-143

Table 9. Overview of the results from clinical trials for the treatment of recurrent glioblastoma. mAb = monoclonal antibody; EGFR = Epidermal growth factor receptor; VEGF = Vascular endothelial growth factor; VEGFR = Vascular endothelial growth factor receptor; PDL = Programmed cell death ligand; CTLA-4 = Cytotoxic T-lymphocyte-associated molecule-4.

Distinguishing pseudoprogression from tumor progression is very complex and time-consuming. There is need for supporting software and future trials to identificate the cell population that shows increased captation of contrast on MRI-T1. Besides the complexicity of the radiological findings, best overall response rates (BORR) could not be determined in the resectable patient cohorts. In the non-resectable cohorts (Cohort 3 and 4) the criteria for Immunotherapy Response Assessment in Neuro-Oncology (iRANO) can be used to assess tumor responses to study treatment, but resection at day 1 makes it difficult to measure and compare to baseline (41). Radiological evolution can be used in addition to clincial findings to confirm suspicion of progressive disease.

5.2 Conclusion

Repeated intravenous administration of the anti-PD-1 monoclonal antibody nivolumab at a dose of 10 mg in combination with an intratumoral injection of the anti-CTLA-4 monoclonal antibody ipilimumab at a dose of 10 mg**,** following the gross total tumor resection in patients diagnosed with recurrent glioblastoma, is tolerable and safe. A similar therapy, but with an intratumoral injection of 5 mg of ipilimumab plus 10 mg of nivolumab (as conducted in Cohort 2) is also tolerable and safe. This combined treatment may have therapeutically meaningful anti-tumor effects, but further investigations are indicated.

5.3 Future perspectives

This interim analysis reports the preliminary results of Cohort 1 and 2 of the phase I clinical trial. Considering the tolerability and feasibility of the study treatment in resectable patients, inclusion criteria were expanded to non-resectable patients in Cohort 3 and 4. Patients in these cohorts will undergo a neurosurgical intervention, where a subcutanous Ommaya reservoir with a catheter will be placed in a previous resection cavity. This way, the monoclonal antibody ipilimumab can be administered through the reservoir on a regular basis, in combination with repeated intravenous administration of nivolumab.

6 Abbreviations

AE: Adverse event
AIDS: Acquired immunodeficiency syndrome
AKI: Acute kidney injury
ALT: Alanine transaminase
ANC: Absolute neutrophil count
AP: Alkaline phosphatase
APC: Antigen presenting cells
AST: Aspartate aminotransferase
BBB: Blood brain barrier
BORR: Best overall response rate
CD- : Cluster of differentiation-
CI: Confidence interval
CPK: Creatine phosphokinase
CRP: C-reactive protein
CSC: Cancer stem cells
CSF: Cerebrospinal fluid
CT: Computer tomography
CTCAE: Common terminology criteria for adverse events
CTLA-4: Cytotoxic T-lymphocyte-associated molecule-4
CTLs: Cytotoxic T-lymphocytes
DC: Dendritic cells
ECOG: Eastern cooperative oncology group
eGFR: Estimated glomerular filtration rate
EGFR: Epidermal growth factor receptor
EoT: End of treatment
FDA: U.S. Food and Drug Administration
FET-PET: 18F-fluoroethyl-tyrosine Positron emission tomography
fT4: Free thyroxine
GB: Glioblastoma
GBM: Glioblastoma multiforme
GGT: Gamma-glutamyl transferase
Gy: Gray
HC: Hemoculture
HIV: Human immunodeficiency virus
IC: Informed consent
ICI: Immune checkpoint inhibitors
IDH: Isocitrate dehydrogenase
IgG4: Immunoglobulin G4
IL-2: Interleukin-2
iRANO: Immunotherapy Response Assessment in Neuro-Oncology
irRC: Immune-related response criteria
IV: Intravenous
mAb: Monoclonal antibody
MHC: Major histocompatibility complex
MRI: Magnetic resonance imaging
NCI: National cancer institute

NOS: Not otherwise specified
ORR: Objective response rate
OS: Overall survival
PD: Progressive disease
PD-1: Programmed cell death
PDL-1: Programmed cell death ligand
PFS: Progression-free survival
PIB: Patient information brochure
RBPA: Red bloodloss per anum
TCR: T-cell receptor
TME: Tumor micro-environment
TMZ: Temozolomide
Tregs: Regulatory T-lymphocytes
ULN: Upper limit of normal
UTI: Urinary tract infection
UZB: Universitair Ziekenhuis Brussel
VEGF: Vascular endothelial growth factor
VEGFR: Vascular endothelial growth factor receptor
VUB: Vrije Universiteit Brussel
WHO: World Health Organization

7 Figures and tables

8 References

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APPENDIX

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Brussel, 09-11-2016

Ons Kenmerk: **2016/306**

ADVIES VAN DE COMMISSIE MEDISCHE ETHIEK

Betreft:
Phase I clinical trial on intra-tumoral ipilimumab plus intravenous nivolumab following the resection of recurrent glioblastoma - GLITIPNI
Protocol 2016-BN-002
EUDRACT 2016-003699-36

Op **03-11-2016** verleende de voorzitter van de Commissie Medische Ethiek een gunstig definitief advies aan bovenvermeld project.

Dit advies wordt vandaag bekrachtigd door de aanwezige leden (zie lijst als bijlage)

Deze goedkeuring blijft geldig voor de duur van het project. De Commissie wenst een jaarlijks overzicht van de stand van zake van het project te ontvangen. De studieresultaten dienen overgemaakt te worden aan de Commissie bij het beëindigen van de studie. Zij herinneren de verantwoordelijke van het experiment eraan dat dit experiment onder zijn persoonlijke verantwoordelijkheid zal worden uitgevoerd. Het gunstig advies van de Commissie betekent geenszins dat de Commissie de verantwoordelijkheid van het experiment op zich neemt. De Commissie Medische Ethiek werkt en is georganiseerd volgens de richtlijnen van ICH-GCP.

Met de meeste hoogachting,

J. van der Werff ten Bosch, ondervoorzitter

Cc: FAGG, Departement R&D, Eurostation blok 2, Victor Hortaplein 40 / 40, 1060 BRUSSEL

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Brussel, 24-02-2017

Ons Kenmerk: **2016/306**

ADVIES VAN DE COMMISSIE MEDISCHE ETHIEK

Betreft:
Phase I clinical trial on intra-tumoral ipilimumab plus intravenous nivolumab following the resection of recurrent glioblastoma - GLITIPNI
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De Commissie Medische Ethiek nam kennis van, en verleent een gunstig advies aan volgende documenten betreffende bovenvermelde studie:

- Aangepast "Request for authorisation of a clinical trial on a medicinal product for human use to the competent authorities and for opinion of the ethics committees in the community"-formulier, ondertekend door Prof. B. Neyns op 21-Feb-2017
- Protocol versie 2.0 dd 17-Feb-2017 + signature page in het Engels
- Patiënteninformatie- en toestemmingsformulier – Gltipni cohort 2 versie 2.0 dd 17-Feb-2017 in het Nederlands en het Frans (met en zonder track changes)

Ter uwer informatie :
Tijdens haar vergadering van 26/03/'98 besliste onze Ethische Commissie het volgende : *"De voorzitter (en in zijn afwezigheid de ondervoorzitter) mag, zonder de Commissie te raadplegen, beslissingen nemen i.v.m. aanvullende gegevens of ongewenste effecten die voor sommige dossiers later dan de oorspronkelijke aanvraag zouden ingestuurd worden . Mocht een punt van majeur belang blijken te zijn dan moet hij dit op de dagorde van de eerstvolgende commissievergadering plaatsen."*
Dit betekent dat de documenten enkel aan de voorzitter werden voorgelegd.

Hoogachtend,

A. Van Steirteghem, voorzitter

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