High-Grade-Glioma-IMMUNO-2003 protocol, D cohort

A phase I/II experimental therapy for the treatment of relapse of high-grade glioma with dendritic cell vaccination loaded with tumor homogenate

Explanation and declaration to participate

For a treatment according to High-Grade Glioma-IMMUNO-2003, cohort D protocol

Madam, Sir,

Your medical doctor proposes you an experimental immune therapy for the treatment of a malignant glioma.

Aim of the treatment

The aim of the immune therapy in this protocol is a prolongation of the survival period due to vaccination in patients with high grade glioma. It is aimed to try to block tumor growth. Maybe we can even induce a reduction of existing minimal tumoral mass. The tolerability of this new type of treatment will also be assessed.

Patients who can enroll in the experimental immune therapy stategy

The patients who can enroll in this protocol have a relapse of a malignant glioma. It is assumed that the patients received classical oncological treatment at time of the primary (first time diagnosed) tumor, which exists of surgery, radiotherapy with or without chemotherapy. If the tumor can be surgically removed completely or at least subtotally in case of relapse, tumor tissue becomes available to prepare the tumor vaccine, and the patient is ready to enroll into the trial.

Background

High grade glioma's, also called high grade astrocytoma's, are brain tumors that grow quickly, that infiltrate in the normal brain parenchyma and that have capacity to metastasize. According to their aggressivity, a distinction is made into a grade III glioma or anaplastic astrocytoma and a grade IV glioma or glioblastoma multiforme. The treatment of high grade gliomas remains extremely difficult, in spite of maximal surgery, radiotherapy and chemotherapy. Moreover, there is no therapy available that attacks specifically the tumor cells. For other tumors, the medical researchers have been able to develop immune therapy that targets tumor cells, with good clinical results. We know that specific immune cells, called T cells, can be directed against tumor cells. Of course, the type of tumor plays a role, and this is based on the expression of tumor-specific molecules on the surface of the cell.

In specific immune therapy, tumor cells are selectively attacked by T cells. For this, tumor antigens play an important role. These are proteins that are expressed on the membrane surface of the tumor cells, but that are wrongly build up due to the tumoral character of the cells, and hence are foreign to the body. We know that in these types of tumors, T cells enter into the tumor tissue (tumor-infiltrating lymphocytes or TIL). In high grade gliomas, TIL have been detected on pathological examination. But we know from the literature that tumor cells produce cytokines (hormones with a very localized effect on the surrounding cells) that suppress T cell function. Therefore, although T cells might be able to kill the tumor cells, their effects are suppressed.

There is another problem. For adequate T cell activation, T cells have to recognize tumor antigens as foreign, but costimulatory signals are required as well. There seem to be several keys and signals necessary to start up the process of T cell activation. The cell that have all these necessary keys to deliver all necessary signals is the dendritic cell (DC). These DC are the most potent cells to start up immune activation. Although these cells also can infiltrate into some tumoral tissues to pick up tumor antigens, we do not find them in malignant gliomas, likely because of the blood-brain barrier.

Based on the combination of different evolutions in the basic sciences, we are able to perform as follows: The tumor tissue can be transformed into a homogenate, in which tumor antigens are present. Out of the blood of the patient, we can isolate white blood cells and let them differentiate into DC. In the laboratory, we can load these DC with the tumor proteins from the homogenate, enabling the DC to handle these tumor proteins in an appropriate way towards the membrane, to present the tumor antigens to the T cells. The loaded DC will be injected in the skin, so that they can move, as they usually do, to the lymph nodes to activate T cells against the antigens, in this case tumor antigens. These activated T cells against tumor antigens will circulate in the blood, and will go to the tumor cells to destroy them. Because high tumor load would suppress the antitumoral activity of the activated T cells, the technique is performed in a situation of minimal residual tumor load.

The principle of antitumoral therapy by tumor antigen-loaded DC has been demonstrated for patients with melanoma, renal cell carcinoma and prostate cancer. Als in lymphoma and breast tumor, similar techniques have been developed. For the brain tumors, there is the particular problem of the blood-brain barrier. But in the tumoral tissue, the blood-brain barrier is locally disturbed. Moreover, animal experiments demonstrate that immune therapy with DC can target tumors in the brain.

In patients, only a few research groups are active to develop such strategy. The report from the group in Los Angeles shows that such therapy is tolerable, and that there are no major adverse events. No conclusions could be drawn from this report on the efficacy. In our own

experience with more than 200 patients being vaccinated thusfar, no serious adverse events have been demonstrated.

Elements in the treatment

- **Tumor antigens**. The tumor is transformed in a sterile way to a homogenate. The exisiting tumor proteins or tumor antigens are used to load DC.

- **DC**. The DC are differentiated out of monocytes, that are a specific sort of white blood cells circulating into the blood. All the white blood cells required are taken from the blood circulation through a special apparatus. This procedure is called leukapheresis. During leukapheresis, blood from the patient is taken out via a intravenous catheter. The blood is centrifuged in the apparatus, and in this way, the white blood cells are isolated. The platelets, the red blood cells and the plasma go back to the patient via a second intravenous catheter. Everything is done in a sterile way. The total procedure takes some 4 hous. The technique is widespread and is used in a lot of patients for a lot of indications and has proven to be safe. There are no side effects with this technique. The with blood cells will be frozen in the laboratory. For making a vaccine, part of the white blood cells will be thawed, to take out the monocytes that will be then cultured further for 1 week in the presence of cytokines to differentiate them to immature DC. These immature DC will then be loaded with tumor antigens out of the tumor homogenate, and will be stimulated further to end up with mature loaded DC.

- **Vaccination**. The loaded mature DC are injected into the skin (intradermal) in the upper arm close to the shoulder of both arms. The amount of injections depends on the amount of cells derived after the week of culture. Each intradermal injection is similar to the well-known tuberculin test. All the injections of cells at one moment are called 1 vaccine. The first 4 vaccines are administered weekly. They consist of loaded DC and are called ‘induction vaccines’. The next 3 vaccins are given monthly. Afterwards, one vaccine is given each three months. From the 5th vaccine on, the vaccines consist of tumor proteins; they are called ‘booster vaccines’.

- **Aldara® Cream (Imiquimod).**

  Aldara cream consists of a local immune enhancing substance. It originately has been developed for gynecological use but it is used in the context of our tumor vaccines to prepare the skin which will be injected with the vaccine. The evening before the vaccine, one little sack of cream is used to apply to the skin of both shoulders (upper arms). The exact place where the cream is applied should be marked on the skin (e.g. with a pen or skin marker). This area will be injected by the physician administering the vaccine. This procedure is repeated exactly the same, the evening the vaccination has been given and the day after the vaccine. It is adviced to apply the cream in the evening, as it should be on the skin for at least 10 hours after each application. It can be washed away however in the morning without any problem. Before and after applying the cream the hands should be washed with soap. The cream is provided to you by our team. Apart from rare local and transitory dermal reactions, very rarely headaches or a short-term viral-like syndrome have been described. No specific actions however are needed if these rare side effects might appear.

- **Skin tests**. (not mandatory and only performed upon specific indication). By injection of a little bit of tumor homogenate in the skin (a technique that is also used to screen for allergy and that is similar to the tuberculin test to screen for TBC), we can evaluate if the patient has an immune reaction against the tumor. For read-out of the skin test, redness and induration will be looked for. A negative control skin test will be performed with the solution buffer of the tumor homogenate. Normally, this injection will not induce redness nor induration. A positive skin test will be performed with Tetanos-Toxoid, to which an immune reaction is expected as almost everybody is vaccinated against Tetanos.

- **Evaluation of the treatment**.
  - Besides regular clinical and neurological examination, three-monthly controls with MRI will be performed. Tumor volume and peritumoral oedema will be assessed. A PET scan will also be performed if there might be uncertainty in interpreting the MRI findings, to be able to maximally interprete the local processes in the tumoral region.
  - Immunization will be followed-up via skin tests, and via specialized laboratory investigations. These examinations however are not mandatory and only performed rather rarely upon specific indications.

Risks and side effects

- By repetitive injections, redness and induration at the sites of injections of the vaccin can occur. Lymph nodes can also enlarge and cause some pain due to immune activation. These side effects were till now very minimal and did not require any treatment.
- There are no side effects due to the skin tests.
- Allergic reactions are not expected because the blood cells and tumor proteins are derived from the patient. There is no use of substances derived from other people or animals.
- The induction of an autoimmune reaction is a *theoretical* risk. Indeed, the immune system is stimulated against brain molecules. We can not exclude the risk that proteins from normal brain cells, that were not targeted by immune cells due to the blood-brain barrier, now become an immune target. Theoreticall, all brain functions, even vital brain functions can be affected as a consequence of that. In such situation, we can treat immediately with anti-inflammatory drugs, mainly high dose corticosteroids. We have to mention that this side effect has not yet been observed in any (unmanipulated) animal model, nor in the patients reported by other research groups or in the more than 200 patients treated in Leuven thusfar.

Duration of the treatment

- The first 4 vaccines are administered weekly. They consist of loaded DC and are called ‘induction vaccines’. The next 3 vaccins are given monthly. Afterwards, one vaccine is given each three months. From the 5th vaccine on, the vaccines consist of tumor proteins; they are called ‘booster vaccines’. The vaccines continue to be administered as long as the patients support the treatment well and as long as tumor material is present.

Although there are scientific arguments to expect a positive effect from the immune therapy, it is possible that the tumor growth is not affected. Taking into account the minimal effect of

radiotherapy and chemotherapy in high grade glioma, also a slowering of tumoral growth is considered as a positive result.

Follow-up of the treatment

After treatment, a classical oncological follow-up will be done. For the first year after vaccination, a clinical examination is scheduled at each vaccination moment. Each " months, a MRI scan is scheduled. A PET scan is performed upon specific indications. The controls will be less frequently in the following years.

Investigators group

The **pre-clinical data** to prove that human T cells can be activated against glioblastoma tumor cells after stimulation with loaded DC, and that these T cells can affect growth of glioblastoma tumor cells, have been generated in the Laboratory of Experimental Immunology, conducted by Professor Dr. Jan Ceuppens, within the group of Professor Dr. Stefaan Van Gool, who is a pediatric hemato/neuro-oncologist and Prof Dr. Steven De Vleeschouwer, who is neurosurgeon.
The early **pilot phases of the clinical protocol** has been set-up by Dr. Stefan Rutkowski under the supervision of Professor Dr. Joachim Kühl, in the Universitäts-Kinderklinik in Würzburg (Germany). Within this university, Professor Dr. Ecki Kämpgen from the Klinik und Poliklinik für Haut- und Geschlechtskrankheiten der Universität Würzburg was co-investigator.
The **clinical protocol HGG-IMMUNO-2003** has been developed by Professor Dr. Stefaan Van Gool, pediatric hemato/neuro-oncologist at the university hospital Gasthuisberg in Leuven, in cooperation with Professor Dr Steven De Vleeschouwer from the department of neurosurgery in Leuven, and in cooperation with the universities of Würzburg, Köln and Regensburg (Germany).
Professor Dr. Johannes Wolff, pediatric oncologist at MD Anderson Cancer Centre, Houston, Texas, is also involved in the clinical protocol. Professor Dr. Johannes Wolff was responsible for the HIT-GBM studies (the treatment protocols for high grade glioma in childhood) from the Gesellschaft für Pädiatrische Onkologie und Hämatologie in Germany until 2005.

Responsible doctors

The responsibility of the treatment is taken by 1/ the staff members of neurosurgery (Prof Dr Steven De Vleeschouwer and 2/ the radiologists (Professor Dr. Guido Wilms andy Professor Dr. Philippe Demaerel) The final responsibility of the immune therapy is taken by Professor Dr. Stefaan Van Gool (Pediatric hemato/neuro-oncologie and Laboratory of Experimental Immunology).
The responsible doctors will use the clinical data for scientific communications and discussions, except those data that would allow identification of the patient.

Costs

All direct costs from the vaccination production and administration will be taken by the group of investigators: the honoraria for clinical follow-up (apart from the vaccination moments) and intake consultations, MRI and PET during and after the vaccination period are facturated at normal rates according to the Belgian Health Care Insurance System, as they constitute the

normal follow-up for patients under brain tumor treatment. The leucapheresis procedure will be charged at 500 Euros by the University Hospital Leuven.

***Declaration of* Agreement**

- I am completely informed about immune therapy with dendritic cells, that are loaded in the laboratory with tumor antigens
- I am completely informed about the experimental character of the treatment, and I understand the theoretical background behind the development of this therapy
- I am completely informed about all procedures due to the therapy
- I am completely informed about potential side effects
- I have had the occasion to ask all my questions and that I understand completely the responses
- I understand the advantage of immune therapy within the scope of the potential risks
- I have the right to finish the therapy, even without being obliged to explain why, while keeping the absolute garanty that the treating medical doctors will look for adapted treatment modalities if available.

Leuven, _____/_____/_____

Patient

Independent witness

Professor Dr. Stefaan Van Gool

Professor Dr. Neurosurgeon