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DCVax®-L—Developed by Northwest Biotherapeutics

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Abbreviations: BBB, blood brain barrier; CNS, central nervous system; CTL, cytotoxic T-lymphocyte; DC, dendritic cell; DTH, delayed tissue hypersensitivity; EORTC, European Organization for Research and Treatment of Cancer; FDA, Food and Drug Administration; GBM, glioblastoma multiforme; GM-CSF, granulocyte-macrophage colony-stimulating factor; HGG, high-grade glioma; IL-4, interleukin-4; IMP, investigational medicinal product; MHRA, Medicines and Healthcare products Regulatory Agency; MRI, magnetic resonance imaging; ND, newly diagnosed; NIHR, National Institute for Health Research; NWBT, Northwest Biotherapeutics Inc.; OS, overall survival; PEI, Paul-Ehrlich-Institute; PFS, progression-free survival; TAAs, tumor-associated antigens; UCLA, University of California, Los Angeles, U.S.A., United States of America.

Dendritic cell (DC) immunotherapy is emerging as a potential addition to the standard of care in the treatment of glioblastoma multiforme (GBM). In the last decade or so various research groups have conducted phase I and II trials of DC-immunotherapy on patients with newly diagnosed (ND) and recurrent GBM and other high-grade gliomas in an attempt to improve the poor prognosis. Results show an increase in overall survival (OS), while vaccination-related side effects are invariably mild. Northwest Biotherapeutics, Inc., Bethesda, Maryland, U.S.A. (NWBT) developed the DCVax®-L vaccine as an adjunct to the treatment of GBM. It is currently under evaluation in a phase III trial in patients with ND-GBM, which is the only ongoing trial of its kind. In this review current data and perspectives of this product are examined.

Nature of the Disease Being Prevented and the Basis in Human Biology / Pathology for the Vaccine or Immunotherapeutic.

Glioblastoma multiforme (GBM) is the most common primary malignant brain tumour and accounts for more than 50% of all intracranial gliomas.[1] Despite advances in standard of care and adjuvant therapy, GBM prognosis remains poor with a mean OS of 14.6 months for ND-GBM and a mean OS of 7.4 months for recurrent GBM.[2-4] The poor prognosis and the relatively young mean age of GBM patients at presentation (53 years), makes the disease not only devastating for the individual and the family, but also significant from a socioeconomic stand.

Current standard of care for ND-GBM is set by the landmark "Stupp" protocol.[2] This was introduced in 2005 and consists of a six-week regimen of concomitant radiotherapy and temozolomide chemotherapy followed by a six-month course of adjuvant temozolomide administered over six 28-day cycles, during which the patient is given temozolomide for five consecutive days per cycle. Even with the application of this optimized protocol combined with radical surgery mean OS is 14.6 months, two-year survival (2-YR S) is 26.5% and only ~3% of patients survive longer than five years.[5,6]

Despite advances in early diagnosis, especially the use of advanced MRI techniques, and multi-modal therapy, the continued poor prognosis of patients with GBM is likely to be due to a number of factors. The first factor to consider is GBM's unique interaction with the immune system both with respect to the immunosuppression that it causes in its environment and the fact that it resides in the immunologically "difficult to access" central nervous system (CNS). The former has been attributed to the ability of the tumor to secrete glioma-cell derived transforming mediators such as factor-β, interleukins and prostaglandin E2, which result in functional compromise of T-cell responsiveness.[7,8] The latter is mainly the result of the efficient isolation of nervous tissue from the peripheral immune system by the blood brain barrier (BBB). This results in a highly selective accessibility of the CNS to the immune system, predominantly to activated T lymphocytes, which are the only cells that can cross the BBB.

The second important factor is that GBM is characterized by a genetic profile that is highly diverse both in the paediatric and adult population. Tumors can express a wide variety of tumor-associated antigens (TAAs), which vary not only between different patients, but within the same individual as well. This results in tumors with different biological behaviors that respond differently to treatment. In contrast, current treatment options are uniform and thus unable to address this diversity. In the current genome era there is a clear need for novel GBM therapies designed to adapt to this genetic heterogeneity.[9,10]

In the last decade or so active DC-immunotherapy has emerged as one such novel treatment, addressing challenges posed by GBM through enhancing the immune-responses to overcome the tumor-derived immunosuppression, activating T-lymphocytes to cross the BBB and enter the tumor

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microenvironment, and utilising a tailored vaccine / immune-product that has been manufactured based on the specific genetics of each individual tumor.

Previous experience on DC-immunotherapy has shown favorable results in other types of malignancies such as metastatic melanoma, hepatocellular, ovarian, breast, lung and haematologic cancer.[11-16] The first ever DC-vaccine officially introduced in cancer therapy was sipuleucel-T (Provenge; Dendreon, Seattle, WA), which was approved by the FDA in 2010 for the use in advanced prostate cancer after clinical trials had shown increased survival.[17]

Along similar lines many research groups have conducted preclinical studies as well as phase I and II clinical trials to investigate the efficacy and safety of DC-vaccines in GBM and other high grade gliomas. The main type of immunotherapy evaluated in these studies is active autologous DC-based immunotherapy. DCs are immune cells with the most potent antigen presenting properties, which reside in tissues that come in contact with the external environment, such as the skin and the mucosa of the nose, stomach, intestines and lungs.

The fundamental step in production of DC-vaccine for GBM is the combination of autologous tumour antigens with patients' own dendritic cells. Tumor cells are obtained and procured during the surgical resection, while DCs are derived from ex vivo differentiation of the patient's peripheral blood monocytes, obtained via leukapheresis. DCs are then ex vivo pulsed with the tumor lysate or peptides, and subsequently "trained" to recognize the patient's tumor cells. The autologous fusion is then injected back to the patient enabling the DCs to present their surface tumor antigens to the CD4 and CD8 T-cells of the immune system, leading to the activation of both memory and naive T-cells. The activated T-cells then cross the BBB resulting in cytotoxic and cytolytic, antitumor immune responses of high specificity. DCVax®-L is the commercial vaccine produced by Northwest Biotherapeutics, Inc., Bethesda, MD, U.S.A. (NWBT) currently under evaluation in a phase III trial against ND-GBM. This forms the focus of this article.

Origin and Research Basis for Efficacy of the Product

Preclinical studies

The assumption that deficient or absent immune-responses to intracranial tumors play a decisive role in tumor genesis led to the conduction of experimental immunotherapeutic studies in rodent glioma models two to three decades ago.[18-20] In these studies active immunotherapy with the use of DC–based vaccines was tested for GBM treatment. It was found that this strategy provoked infiltration of the CNS by activated T-cells,[21,22] which was subsequently related to improved outcomes. Moreover, T-cell responses were found to be closely correlated to vaccine efficacy,[23-25] indicating that they can contribute in the prevention of tumor regrowth. Interestingly in one study there was evidence of vaccine related autoimmune encephalitis as a result of T-cells targeting normal brain tissue that partly shared antigens found in tumor cells. This has not however been reproduced in studies conducted in humans.[26]

Other research has focused on the interaction between other treatment modalities, such as radiotherapy, and DC-vaccinations in murine brain tumor models.[27] It was found that the combination of radiotherapy and DC-vaccinations can enhance the effect of the latter and improve outcomes. This was attributed to irradiation-induced upregulation of MHC molecules in tumor cells, which rendered them better immunological targets.

The potential of DC-vaccinations as revealed by the encouraging findings of preclinical studies directed clinical trials towards applying similar protocols on humans.

DCVax®-L phase I and II clinical trials

Data on these trials derive mainly from NWBT public reports. Prior to the ongoing phase III trial two phase I/II trials were carried out at the University of California, Los Angeles (UCLA) by Dr. Linda Liau and Dr. Robert Prins on this product. In these trials thirty-nine ($n = 39$) patients were enrolled in a dose-escalation scheme of 1, 5 or 10×10^6 DCs/injection. Enrollees received initially 3 biweekly courses of vaccinations, followed by up to 10 booster vaccinations at 3-moth intervals. Follow-up with brain MRI was every 2 months or when clinically indicated.[28] Twenty ($n = 20$) patients had ND-GBM and nineteen ($n = 19$) patients had recurrent GBM and other gliomas. For patients with ND-GBM, who received DCVax®-L in addition to standard of care treatment, progression-free survival (PFS, as evaluated with use of the McDonald's criteria) was around 24.0 months and OS was 36.0 months.[29]

The long-term data analysis (last updated in July 2011) showed that 33.0% of patients had reached or exceeded a median survival of 48.0 months and 27.0% had reached or exceeded a median survival of 72.0 months. By year 2013, two ($n = 2$) of the Phase I/II clinical trial patients were still alive reaching a survival of more than 10.0 years.

At the same institution (UCLA) historic controls sharing the same characteristics as the recruited patients in the trials [recursive partitioning analysis (R.P.A.) classes III and IV of the European Organization for Research and Treatment of Cancer (EORTC)] had a mean PFS of 8.9 months ($\pm$ 7.3 months), and a mean OS of 15.0 months ($\pm$ 13.9 months).[29]

The safety profile was favorable with only mild side effects (grade I and II). These included headache, nausea, loss of appetite, diarrhea, fatigue and low-grade fever. Other less common adverse events (AEs) included itching and redness at injection site, back or neck pain, lymph node swelling, arthromyalgia, depression, dehydration, dizziness, cough, somnolence and allergic rhinitis. Nil vaccination-related serious adverse events (SAEs) had been reported with the majority of AEs and all SAEs having been attributed to disease progression.[29]

Phase I and II clinical trials on other DC-based vaccines

Apart from the previously mentioned two phase I/II clinical trials on DCVax®-L, twenty-two ($n = 22$) phase I and II clinical trials and prospective studies have been conducted to evaluate the safety and efficacy of other DC-based vaccines on GBM and

other high-grade gliomas (HGG). Twenty (n = 20) of these were phase I and II trials, one was a pilot study towards a phase I/II trial and one was a prospective study. GBM patients were exclusively recruited in 12/22 studies, ND-GBM patients only were recruited in 7/22 studies, while 10/22 studies enrolled patients with any diagnosis of a HGG. In the vast majority of studies the vaccine was injected intradermally or subcutaneously and consisted of mature DCs pulsed with tumor lysate or peptides. Patients received an average of 17.62 (2-24) courses of vaccinations. Mean overall survival (OS) ranged between 16.0 and 38.4 months for ND-GBM and between 9.6 and 35.9 months for recurrent GBM.[28,30-50] Of interest, Ardon et al.[47] analyzed their results based on RPA classification and reported a mean OS of 39.7 months on patients with ND-GBM for RPA class III. Thus, it seems that specific subgroups of GBM patients may benefit from DC-vaccinations greater than others.

The vast majority of vaccine-related side effects were mild (grade I and II), with serious adverse events (grade II, IV and V) only reported rarely.

Timing of vaccination

In almost all trials autologous DCs were obtained via differentiation of autologous monocytes, which were collected by leukapheresis. DCs were then ex vivo matured and pulsed with tumor lysate or peptides and administered post-standard treatment. Timing of vaccination and its integration in the timeline of standard of care is a point of debate. Some research groups have commenced vaccinations immediately after surgery.[33,34,43] In most of the trials, and especially those conducted after the adoption of the "Stupp" protocol, vaccines were administered after completion of concomitant radiotherapy and chemotherapy with some doses coinciding with adjuvant temozolomide. This has been speculated to improve survival rates through possible interaction between vaccine and chemotherapy resulting in enhanced chemotherapy effect and higher tumor chemo-sensitivities, although the underlying mechanism has not yet been identified.[36,39,51,52]

Others have shown that DC-vaccination combines favorably with radiotherapy by increasing radio-sensitivity of tumor cells and up-regulating the expression of MHC antigens in animal models.[27] In contrast, Chang et al.[43] argue that the development of radiotherapy-induced mutant tumor cells, immunologically diverse from the ones obtained during surgery, may render vaccines inactive against residual or relapsed tumors.

DCVax®-L phase III clinical trial

The currently ongoing phase III clinical trial is a 312-patient randomized, placebo-controlled, double blinded, multi-center, international trial evaluating DCVax®-L on ND-GBM (clinical trial registration # NCT00045968). It is officially entitled "A Phase III Clinical Trial Evaluating DCVax®-L, Autologous Dendritic Cells Pulsed With Tumor Lysate Antigen For The Treatment Of Glioblastoma Multiforme (GBM)".[53] The trial is recruiting across two continents with currently fifty-one sites across the U.S.A. and one in Europe (present authors' institution) enrolling eligible patients. Additional sites are due for activation and are in varying stages of preparation in the U.K. and Germany.

The primary endpoint of the trial is PFS, i.e. time elapsed until disease progression, which could be either recurrence of the tumor or increase in size of residual tumor. Secondary endpoints of the trial are OS and parameters such as side effects, performance status and immune response. Of note in the trial PFS and OS times are estimated from time-point of randomization, which happens approximately three months after initial surgery, whereas in common clinical practice these are usually calculated from the time of surgery.

Patients recruited will be aged between eighteen and seventy years old and have newly diagnosed, unilateral GBM. Randomization occurs after total macroscopic or gross total surgical resection and completion of the six-week course of concomitant radiotherapy and chemotherapy. Patients without evidence of possible disease progression at baseline are randomized into two cohorts. Two thirds will be in the treatment cohort and one third in the placebo cohort. In the first cohort patients will receive the investigational medicinal product (IMP), while in the placebo cohort patients will be given only the autologous peripheral blood mononuclear cells obtained via leukapaheresis. During each session patients are administered two intradermal injections of either the vaccine or placebo in their upper arm. Entry into the trial is contingent on having sufficient tumor removed such that at least 5 vaccination sessions are possible. Vaccinations take place at 10 time-points, i.e. at days 0, 10 and 20 and at weeks 8, 16, 32, 48, 72, 96 and 120, depending on the patient's clinical condition and the number of vaccine doses that have been manufactured. In the case of <10 vaccination doses available, patients are injected for the remaining vaccinations with placebo while maintaining the blind.[53]

Subjects with possible disease progression or possible pseudoprogression (radio-necrosis) at baseline return after a set time period for a second baseline visit. Only if at this time it is confirmed that there is no disease progression, they will be enrolled. Additionally if patients in either cohort develop tumor recurrence at any point during the trial, they will then have the option of receiving DCVax®-L following a specific process that crosses them over to the IMP arm. From this point onwards subjects will be in the open label follow up arm, but without unblinding the previous trial data. Patients are monitored regularly by physical and neurological examination, blood tests and MRI imaging to evaluate effects and side effects. Immune responses are also tested for by blood withdrawals at baseline and follow up visits.

The trial is empowered such that achieving the set target for PFS (the primary endpoint) could result in a p value of 0.01 one-sided (0.02 two-sided), with a power of 82%. Statistical significance is deemed for a $p \leq 0.05$; therefore a p value of 0.02 provides a safety margin in case PFS is less than was initially anticipated during the protocol design. In case the primary and secondary endpoints are not achieved, it is planned for the data to be analyzed further based on sub-classification of the trial population. Finally, three interim analyses have been scheduled to take place for data evaluation while the trial is ongoing.[29]

Production

DCVax®-L is a tailored, immune-treatment based on DCs. The vaccine is essentially manufactured by fusing the patient's own GBM cells with the patient's own DCs. At surgery part of the excised tumor is sent for pathological studies and the remaining part is procured and added to a digestion buffer containing enzymes. Approximately two weeks after an uneventful operation the patient undergoes a session of leukapheresis, during which peripheral blood mononuclear cells are obtained. Then the cells get ex vivo differentiated to DCs with the addition of interleukin-4 (IL-4) and granulocyte-macrophage colony-stimulating factor (GM-CSF).

Next follows the process of combining the two ingredients, tumor tissue and DCs, to manufacture the vaccine. Whole tumor lysate is used in order to potentially pulse DCs with the entire spectrum of available tumor antigens. The incubation lasts 16 hours and after that the final product is harvested and stored under cryopreservation. If sufficient tumor lysate has been extracted, a significant amount of vaccines can be produced, which will be available for the patient as a course of treatment. Usually at least five ($n = 5$) doses of the vaccine are required.

Immune Monitoring of the Product

Accurate immuno monitoring assays/techniques to evaluate response to vaccinations are yet to be developed and uniformly used. In the past trials, increased peripheral immune markers, such as cytotoxic T-lymphocyte (CTL) activity and positive delayed tissue hypersensitivity (DTH) tests, have been reported, but their correlation with clinical outcomes was weak and therefore they lacked prognostic value.[28,36,38,44,47] Nevertheless Yamanaka et al.[37] and Wheeler et al.[39] reported some value in measuring such markers, while Fadul et al.[44] found that 50% of patients developed a measurable immune response which was associated with improved OS. Currently the most valid indicator of vaccination-induced immune responses to GBM is considered to be tumor infiltration by activated T-cells.

Regulatory Issues

The Food and Drug Administration (FDA) in the U.S.A., the Paul-Ehrlich-Institute (PEI) in Germany and the Medicines and Healthcare products Regulatory Agency (MHRA) in the U.K. have approved conduction of the phase III trial. The DCVax®-L technology and the Phase III trial have been evaluated by the National Institute for Health Research (NIHR) in the U.K. and "adopted" as a priority.

Presently in the U.K. the product may be offered to a limited quota of patients on a compassionate basis outside the trial. Though this is distinct from a formal approval, it does signify the potential that DCVax®-L technology carries and its favorable safety profile. Very recently DCVax®-L received a similar approval from the PEI in Germany. Moreover the German reimbursement authority (Institut Für Das Entgeltsystem Im Krankenhaus, or InEK) has ruled that DCVax®-L for gliomas is eligible for reimbursement from the Sickness Funds (health insurers) of the German healthcare system. Furthermore both in the U.S.A. and in Europe DCVax®-L has been granted orphan drug status for GBM and other gliomas. As a result DCVax®-L will have market exclusivity for 7 years in the U.S.A. and 10 years in Europe.[29]

Final approval of DCVax®-L will await the outcome of the phase III trial although if interim analysis results are favorable, then an early approval of the product may well be achieved.

Public-health Implications

Though GBM incidence is 2-3 per 100,000 people per year, it is the most commonly diagnosed primary brain tumor in adults and its effect is devastating for the patient, the family and the society.[54] Given the poor prognosis and in the absence of preventative measure, presently the majority of efforts are focused on broadening treatment options, as well as improving their efficacy and rendering them more tolerable. With this respect and if the phase III trial confirms the findings of phase I/II studies, an individualized novel treatment modality, such as active DC-immunotherapy with a favorable safety profile, could make a significant difference and have an important impact on the management of these patients.

Commercial Issues

DCVax®-L produced by NWBT is at present the only product of its kind in a phase III trial. If the results of the trial translate into approval of the product, then demand is likely to be a significant consideration. Therefore an international network of manufacturing sites will be required to enhance NWBT's infrastructure and ensure its capacity to address this demand. Another issue to consider is the manufacturing costs. With the increasing demand, it is likely that the costs will be reduced, but since DCVax®-L could potentially monopolize the market for a significant amount of time, a discussion with the international health systems and funders is required to ensure accessibility of the product to the patients.

Advantages and Disadvantages Relative to other Products for the Same Disease

DCVax®-L is an autologous vaccine that crystallizes the concept of personalized, targeted therapy. It generates an immune response that is underpinned by two distinct elements: a. it is triggered by autologous antigens and b. whole tumor lysate is used to obtain these antigens. The first spares the potential disadvantages caused by the use of artificial peptides, namely lower specificity and immunogenicity. The second allows exploitation of the whole spectrum of available tumor antigens accounting for genetic heterogeneity that is seen even within the same individual's tumor.

Table 1. Actively recruiting clinical trials evaluating DC-vaccines for ND-GBM, recurrent GBM or high-grade gliomas. Of these trials commercial company sponsors only #8, while the rest are conducted under Investigator Investigational New Drug (IND) or Institutional IND without a company sponsor.

	Sponsor	ClinicalTrials.gov Identifier	Trial Phase	Diagnosis	Study Design	Antigen Type	Comments
1	University of Miami Sylvester Comprehensive Cancer Center	NCT01808820	I	High Grade Glioma	Single-Center Open Label	Autologous lysate	DCs and antigens are separately injected.
2	Cedars-Sinai Medical Center	NCT02010606	I	ND- or recurrent GBM	Single-Center Non-Randomized Open Label	Allogeneic lysate	—
3	Jeremy Rudnick, M.D, Cedars-Sinai Medical Center	NCT02049489	NA	Recurrent GBM	Single-Center Open Label	Purified peptides from CD133 antigen	—
4	University of Miami Sylvester Comprehensive Cancer Center	NCT01902771	I	High Grade Glioma	Single-Center Open Label	Autologous lysate	DCs and antigens are separately injected.
5	Jonsson Comprehensive Cancer Center	NCT01204684	II	High Grade Glioma	Single-Center Open Label	Autologous lysate	+/- Administration of Toll-like Receptor Agonists
6	Huashan Hospital	NCT01567202	II	ND-GBM	Single-Center Randomized Double blind	Autogeneic glioma stem-like cells	—
7	John Sampson, Duke University Medical Center	NCT00890032	I	Recurrent GBM	Single-Center Open Label	Brain tumor stem cell messenger ribonucleic acid (mRNA)	—
8	NWBT	NCT00045968	III	ND-GBM	Multicenter Randomized Double blind	Autologous lysate	—

DCs, Dendritic cells; GBM, glioblastoma multiforme; NA, Not applicable; ND, Newly diagnosed; NWBT, Northwest Biotherapeutics

A potential disadvantage of the DCVax®-L technology could be that the use of whole tumor lysate, potentially containing healthy brain tissue, may result in immune responses against normal brain leading to autoimmune encephalitis. Presently however preliminary data from DCVax®-L and other active DC-immunotherapy trials have not revealed any SAEs related to autoimmunity. Additionally DC cancer vaccines in general may prove to be not as robust and durable as required to vanquish the intrinsic ability of cancers to suppress the immune system.[55] Long term data is required to shed light on this view point.

Presently according to the clinicaltrials.gov website there are 7 other ongoing clinical trials, evaluating DC-vaccines for either ND or recurrent GBM. These are exclusively phase I/ II single center trials primarily recruiting in the U.S.A. (**Table 1**).[56]

Conclusion

The lack of significant improvement in the prognosis of patients with GBM in the recent years warrants exploration of new therapeutic avenues. DCVax®-L as an autologous active DC-immunotherapy agent has achieved promising outcomes with little side effects in phase I/II trials. The ongoing phase III trial is aimed at verifying these preliminary results, which if achieved, will have a major impact in the management of patients with GBM.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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