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REVIEW ARTICLE

Active dendritic cell immunotherapy for glioblastoma: Current status and challenges

Stavros Polyzoidis[1], Juel Tuazon[2], Lucy Brazil[3], Ronald Beaney[3], Safa Taha Al-Sarraj[4], Lawrence Doey[4], Jamie Logan[5], Victoria Hurwitz[5], Jozef Jarosz[6], Ranjeev Bhangoo[1], Richard Gullan[1], Aleksandar Mijovic[7], Mark Richardson[8], Farzin Farzaneh[9] & Keyoumars Ashkan[1]

[1]*Department of Neurosurgery, King's College Hospital, London, UK,* [2]*Neurosciences Clinical Trials Unit, King's College Hospital, London, UK,* [3]*Department of Neuro-oncology, Guy's and St Thomas' Hospital, London, UK,* [4]*Department of Neuropathology, King's College Hospital, London, UK,* [5]*Department of Neuro-oncology, King's College Hospital, London, UK,* [6]*Department of Neuroradiology, King's College Hospital, London, UK,* [7]*Department of Haematology, King's College Hospital, London, UK,* [8]*Institute of Epileptology, Institute of Psychiatry, King's College London, London, UK,* [9]*Department of Haematological Medicine, The Rayne Institute, King's College London, London, UK*

Abstract

Dendritic cell (DC) immunotherapy is developing as a promising treatment modality for patients with glioblastoma multiforme (GBM). The aim of this article is to review the data from clinical trials and prospective studies evaluating the safety and efficacy of DC vaccines for newly diagnosed (ND)- and recurrent (Rec)-GBM and for other high-grade gliomas (HGGs). By searching all major databases we identified and reviewed twenty-two (n = 22) such studies, twenty (n = 20) of which 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, while 10/22 studies enrolled patients with any diagnosis of a HGG. In 7/22 studies GBM was newly diagnosed. In the vast majority of studies the vaccine was injected subcutaneously or intradermally and consisted of mature DCs pulsed with tumour lysate or peptides. Median overall survival ranged between 16.0 and 38.4 months for ND-GBM and between 9.6 and 35.9 months for Rec-GBM. Vaccine-related side effects were in general mild (grade I and II), with serious adverse events (grade III, IV and V) reported only rarely. DC immunotherapy therefore appears to have the potential to increase the overall survival in patients with HGG, with an acceptable side effect profile. The findings will require confirmation by the ongoing and future phase III trials.

Keywords: dendritic cells; glioblastoma multiforme; high-grade glioma , immunotherapy, review

Introduction

Comprising more than 50% of all gliomas, glioblastoma multiforme (GBM) represents the commonest high-grade primary neoplastic lesion of the brain. In the vast majority of cases GBM is primary, while in the rest it is the result of transformation of a pre-existing glioma of a lower grade. It is mainly seen in adulthood with a slight male predominance over females (3:2), but it can also rarely present in childhood. Patients' mean age at presentation is approximately 53 years, which implies that GBM usually affects patients in the peak of their productive life.[1] Existing multimodality treatment protocols for GBM result in an unfavourable prognosis even for patients who are able to receive radical treatment; median overall survival (OS) is 14.6 months, 2-year survival is 26.5%, while at 5 years only 9.8% of patients are still alive.[2] GBM is therefore difficult to treat and novel targets and therapeutic interventions are needed. In the recent years, novel agents such as anti-angiogenic factors (mainly bevacizumab), selective inhibitors of receptor tyrosine kinases and notch inhibitors, amongst others, have provided potential therapeutic avenues to explore.[3] Gene therapy (DNA damage and oncolytic virotherapy) showed initial promising results in preclinical animal models with acceptable safety profiles in phase I clinical trials. The results however could not be replicated in phase III clinical trials, leaving this area in need of further evaluation.[4]

Active immunotherapy represents another area of research in glioma management. The concept formally entered into the mainstream cancer therapy in 2010 after the FDA's (Food and Drug Administration-USA) approval of sipuleucel-T (Provenge®; Dendreon, Seattle, WA), which moderately increased survival in advanced prostate cancer.[5] Since it has been tested in a range of malignancies including haematological, hepatocellular, ovarian and melanoma.[6]

Theoretical background of immunotherapy for GBM

The central nervous system (CNS) represents an immunologically discrete environment, separated from the peripheral

Correspondence: Professor Keyoumars Ashkan, Department of Neurosurgery, King's College Hospital, Denmark Hill, SE5 9RS, London, UK. Tel: 0044(0)2032993285. Fax: 0044(0)2032993280. E-mail: k.ashkan@nhs.net

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immune system by the function of the blood–brain barrier (BBB). Of relevance in this environment: malignant gliomas express major histocompatibility complex (MHC) class I (which can be upregulated), but not class II molecules; only activated T lymphocytes cross the BBB, the brain seems to have a very unique lymphatic drainage[7] and the resident microglia seems to act in a distinct and localised manner representing a category of immune cells that is not met anywhere else in the body. Thus the CNS may provide a 'protected' niche for the tumours to grow. In fact the observation that primary brain tumours rarely metastasise outside the CNS has led to the speculation of an endogenous need for these tumours to remain within the immunological environment of CNS, in which they can flourish.[8]

Moreover and specifically for GBMs, it is generally accepted that such tumours tend to provoke tumour-induced immune suppression; more specifically these tumours render T-cell immune responses less potent by secreting glioma-cell-derived mediators, including transforming growth factor-β, interleukins and prostaglandin E2, among others.[9,10] Thus, if the immune response could be altered, boosted or even just enhanced, anti-tumour benefits might arise.

Another equally important property of immunotherapy is its specificity. Gliomas are known to be genetically diverse.[11] As such therefore, 'generalised' treatments such as surgery and radiotherapy are unlikely to benefit all uniformly. Immunotherapy, on the other hand, by stimulating one's immune response against one's own tumour, individualises the treatment, addressing the genetic diversity.

The emerging approach of active immunotherapy for GBM consists mainly of autologous dendritic cell (DC)-based immunotherapy, while synthetic tumour-associated peptide-pulsed DC therapy has been tested as well. DCs are cells of the immune system that live in tissues exposed to ambient pathogens such as the skin and mucosa. They have the property of processing and presenting antigens to the rest of the immune system to stimulate a response. For manufacturing vaccine, the DCs are ex vivo differentiated from the peripheral blood monocytes that are usually harvested during a leucapheresis session that patients undergo.[6] Following this cells are pulsed ex vivo with glioma lysate or peptides to produce the vaccine. When administered to the patient, the DCs perform in vivo the glioma antigen presentation to the CD4 and CD8 T-cells, with which they interact, and activate both the memory and naive T-cells. Subsequently the activated T-cells cross the BBB and infiltrate the brain and the tumour leading to a highly specific cytotoxic and cytolytic, anti-tumour immune activity (Fig. 1).[12] Therefore, DCs are not only active against the initially diagnosed tumour, but can potentially perform a protective immune surveillance role against tumour relapses by activating the memory T-cells.

In preclinical studies on intracranial gliomas, active DC immunotherapy was found to be associated with increased infiltration of the CNS by T-cells, which resulted in more favourable OS.[12] This led to the development of phase I and II clinical trials in humans with promising results and culminated in a phase III study, which is currently recruiting patients in the USA and Europe.

Aims

The purpose of the study is to systematically review the literature on phase I and II clinical trials and prospective studies evaluating the safety and efficacy of DC vaccines for newly diagnosed (ND)- and recurrent (Rec)-GBM and other high-grade gliomas (HGGs).

Materials and methods

Review process: study selection

A systematic review of the literature was conducted by a thorough online search in PubMed, EMBASE, Google Scholar and Turning Research into Practice (TRIP) to retrieve all phase I/II clinical trials and prospective studies evaluating the safety and efficacy of DC-based vaccines on ND-, Rec-GBM

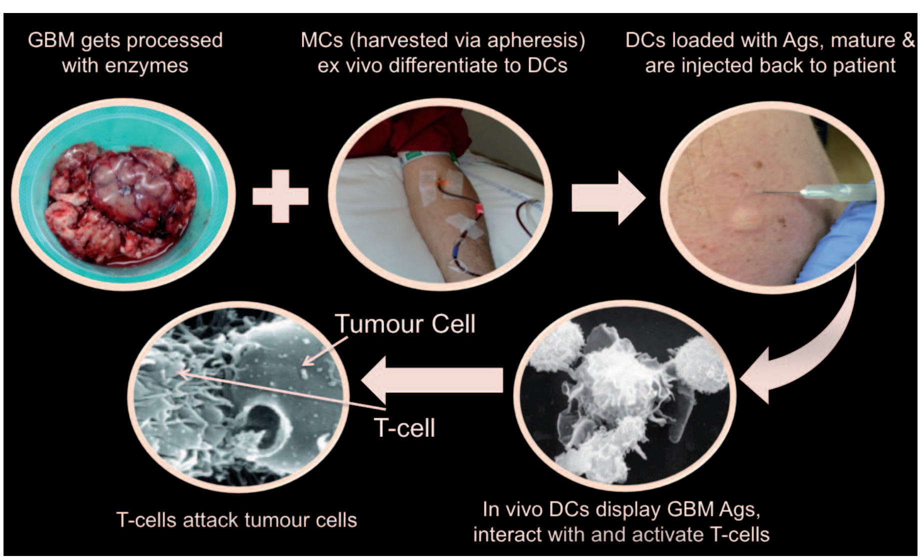

Fig. 1. Schematic illustration of the biology underlying DC-based vaccination against GBM. Key: Ags, Antigens; DCs, dendritic cells; GBM, glioblastoma multiforme; MCs, monocytes. Bottom row photograph on the right: Reprinted with permission from Professor SC Knight (http://www1.imperial.ac.uk/resources/A5C7BF81-1EAA-426D-A143-2F6B54E6AB5A/dendriticcellcropped.jpg). Bottom row photograph on the left: Reprinted with permission from Prostate Cancer Research Institute (Mrs J Manarite, http://prostate-cancer.org/cancer-cryo-immunotherapy-a-battle-between-the-immune-system-and-cancer/).

and HGG. The last date of search was 31.12.2013. Furthermore, to eliminate the chance of missing any published results of trials that might have not been revealed by the initial search we thoroughly searched http://clinicaltrials.gov/.

Study parameters

The data were collected and classified under three basic categories:

1. General study data

Authors
Country
Study type/Trial phase
Number of Participants and Diagnosis
Mean age
Type of Injection (vaccinations)

2. Immunotherapeutic protocols

Pre-vaccine Karnofsky Performance Score (KPS)
Pre-vaccine treatment
Time from surgery to commencement of vaccinations
Antigen-Presenting Cells (APC and Number of cells/vial)
Antigen (Type of antigen and quantity)
Site of Injection
Duration of therapy

3. Outcomes

Post-Vaccination KPS
Completion of therapy (percentage of patients)
Objective Responses (magnetic resonance imaging [MRI]-based)
Immune Responses
Progression-Free Survival (PFS)
OS
2-year survival
Safety – Vaccination-related side effects

Results

Studies

The initial search returned 49 research studies. By restricting our focus on papers in humans, on DC immunotherapy, clinical trials and prospective studies, written in English language the results were limited to 29. Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses or PRISMA guidelines[13] full-text articles were assessed for eligibility and 7 were excluded resulting in twenty-two (n = 22) studies to be reviewed. None of the publications were found to be duplicates, while no case reports were included. Study data and outcomes of these studies are reported in Table I.

1. General study data

Country:

The country in which the study was conducted was U.S.A. for 9 (40.9%), Japan for 5 (22.7%), Belgium for 4 (18.2%), China (Taiwan) for 2, Australia for 1 and Norway for 1.

Trial Phase/Study type:

The majority (n = 20) of publications reviewed were clinical trials, one (n = 1) was a pilot study towards a phase I/II trial and one (n = 1) was a prospective study. Of the clinical trials ten (n = 10) were phase I, two were phase II (n = 2) and eight (n = 8) were phase I/II.

No of participants-Diagnosis:

The size of study populations ranged between 7[35] and 77.[32] Although the vast majority of the trials focussed on GBM, either ND or Rec, some research groups did not exclusively enrol patients with that diagnosis. The breakdown of diagnoses per study was as follows: 12/22 (54.5%) studies recruited GBM patients only [ND: 7/22 (31.8%), Rec: 1/22 (4.5%) and both: 4/22 (18.2%)], while 10/22 (45.5%) studies enrolled patients with any diagnosis of a HGG [ND: 1/22 (4.5%), Rec: 7/22 (31.8%) and both: 2/22 (9.0%)].

Mean age:

The mean age of study populations ranged from 38.4 years[15] to 60.0 years.[27] One study also enrolled children.[22]

Vaccine ingredients:

In all 22 studies the injected product was DCs loaded with antigens. In almost all trials autologous DCs were obtained through differentiation of autologous monocytes, which were collected by leucapheresis in 68.0% (15/22) of studies[16,17,20,21,23,25–30,32–35], via centrifugation of withdrawn peripheral blood in 23.0% (5/22) of studies[14,15,19,24,31] or by a combination of both techniques in 9.0% (2/22) of studies.[18,22]

In 95.5% (21/22) of studies the DCs injected were mature (after contact with tumour antigens). In one study[21] the protocol was divided into a phase I cohort where immature (before contact with tumour antigens) DCs plus tumour lysate were administered intradermally (ID) or ID + intratumourally (IT) and a phase II cohort where mature DCs plus tumour lysate were administered ID and immature DCs were administered IT.

To manufacture the vaccine, the DCs were pulsed with an antigen ex vivo. The exact nature of the antigen varied and is further analysed below in 'Antigen' section of 'Immunotherapeutic protocols'. In addition to the DC–antigen fusion, in one study[29] the researchers injected intramuscular (IM) polyinosinic–polycytidylic acid stabilised by lysine and carboxymethylcellulose (poly-ICLC); in another study[18] they injected subcutaneous (SC) recombinant human interleukin-12, in an attempt to enhance the anti-tumour effect of DC vaccinations.

Boost injections followed the initial vaccination. These invariably consisted of DC–tumour antigen fusions apart from three trials,[22,25,32] in which boosts comprised only tumour antigen without any DCs. In another trial[30] during the booster phase, DC vaccines were injected along with either IM injection of poly-ICLC or pre-treatment of the skin with imiquimod cream (an immunostimulant that improves the outcomes of anti-glioma DC vaccinations).

2. Immunotherapeutic protocols

Pre- and Post-Vaccination Performance Score:

Pre-vaccination KPS was used in 82.0% (18/22) of studies[14–17,19,20–23,25,26,28–32,34] as a measure of the patients' general

Table I. Study data – outcomes.

	Authors	Trial phase/Study type	No of Participants & Diagnosis	Mean age	Vaccine ingredients/Route of injection	OS (months)	2-YR S	Side effects (Vaccination-related)
1.	Yu et al. (2001)[13]	I	9 ND-HGG (7 GBM + 2 AA)	48.8	DC + TP/SC	15.6	0/9	Minor side effects
2.	Kikuchi et al. (2001)[14]	I	8 Rec-HGG (5 GBM + 2 AA + 1AO)	38.4	DC–glioma cells/ID	NDis	NDis	Minor side effects
3.	Yamanaka et al. (2003)[15]	I/II	10 Rec-HGG (7 GBM + 1 AOA + 1 Glioma + 1 A mixed glioma)	46.1	DC + TL/ID & ID + IT	Overall 40.2 ID cohort: 39.5 ID + IT cohort: 40.8	40%	Nil side effects. 1/10 Gr 1
4.	Yu et al. (2004)[16]	I	14 HGG (1 ND-GBM + 9 Rec-GBM + 1 ND-AA + 3 Rec-AA)	44.7	DC + TL/SC	17.6	42.9%	Minor side effects
5.	Rutkowski et al. (2004)[17]	I	12 Rec-HGG (10 GBM, 1 PXA, 1 nil pathology)	39.7	DC + TL/ID	10.5	16.7%	Nil SAEs 1 pt repetitively developed vac-related peritumoural oedema with Gr 4 neurotoxicity, responding to steroid.
6.	Kikuchi et al. (2004)[18]	I	15 Rec HGG (6 GBM + 7 AA + 2 AO)	46.3	DC-glioma cells + rIL-12/ ID	9.8	0.0%	Nil SAEs Minor side effects
7.	Liau et al. (2005)[19]	I	12 GBM (7 ND + 5 Rec)	40.4	DC + TP/ID	23.4	50%	Gr 1 & Gr 2 only
8.	Yamanaka et al. (2005)[20]	I/II	24 Rec-HGG (18 GBM + 1 AO + 2AA + 1 AOA + 1 Glioma + 1 A mixed glioma)	48.9	DC + TL/ID & ID + IT	GBM: 16 ID: 13.6 ID + IT: 25.0 Longer OS for GBM pts with DC OK-432 maturation than that without it.	23.5%	Minor side effects
9.	De Vleeschouwer et al. (2008)[21]	I (Prospective study)	56 Rec GBM (children included)	45.0	DC + TL/ID	9.6 (since re-operation)	14.8%	Only mild AEs. 1 SAE: Gr 4 neurotoxicity (stupor) due to vac-related peritumoural oedema (gross residual tumor)
10.	Wheeler et al. (2008)[22]	II	32 GBM (11 ND + 21 Rec)	51.9	DC + TL/ID	R: 21.4 NR: 14.3	<u>All GBM</u> R: 41.0% NR: 7.0% <u>Rec GBM</u> R: 56.0% NR: 8.0%	No Gr 3 or 4 AEs. 1 pt with cutaneous GBM at site of DTH testing
11.	Walker et al. (2008)[23]	I	13 HGG (7 ND-GBM, 2 Rec-GBM, 1 ND-AA, 3 Rec-AA)	51.0	DC + Glioma cells/ID	14.0	11%	Nil AEs

(*Continued*)

Table I. (Continued)

	Authors	Trial phase/Study type	No of Participants & Diagnosis	Mean age	Vaccine ingredients/Route of injection	OS (months)	2-YR S	Side effects (Vaccination-related)
12.	Ardon et al. (2010)[24]	Pilot study towards I/II	8 ND-GBM	50.0	DC + TL/ID	24.0	50%	1 SAE Ischaemic stroke 8m post-op. Others Gr 1& 2
13.	Chang et al (2011)[25]	I/II	17 HGG (8 ND-GBM + 6 Rec-GBM + 2 MO + 1 AA)	44.7	DC + Glioma cells /SC	17.3	41.2%	47.1% (8/17) Gr 1 & 2 AST/ALT elevation 52.9% (9/17) Gr 1–4 lymphopenia 5.9% (1/17) anaemia
14.	Fadul et al. (2011)[26]	I/II	10 ND-GBM	60.0	DC + TL /IN	28.0	60%	Gr 1 unilateral neck pain after 1 cervical lymph node vac in 1 pt that persisted for several wk
15.	Okada et al. (2011)[27]	I/II	22 Rec-HGG (13 GBM, 5 AA, 3AO, 1 AOA)	48.0	αDC1 + Synthetic TP/IN + IM poly-ICLC	GBM 12.0 AG Undefined (5/9 pts still alive)	GBM ~36% AG ~63% (based on supplemental online data)	Only Gr 1 & 2 symptoms
16.	Prins et al. (2011)[28]	I	23 GBM (15 ND + 8 Rec)	51.0	DC + TL/ID	31.4 ND-GBM 35.9 Rec-GBM 17.9	55% ND-GBM 77%	Only Gr 1 & 2 symptoms
17.	Cho et al. (2012)[29]	II	34 ND-GBM DCg 18 Cg 16	DCg 53.8 Cg 55.8	DC + GBM cell/SC	DCg 31.9 Cg 15.0	DCg 44.4% Cg 18.8%	Few Gr 1 only
18.	Jie et al. (2012)[30]	I/II	25 ND-GBM (13 DCg 12 Cgl)	DCg 40.2 Cg 43.1	DC + post heat-shock apoptosis GBM cells/SC	DCg 16.0 Cg 12.3	DCg 7.7% Cg 0.0%	Nil
19.	Ardon et al. (2012)[31]	I/II	77 ND-GBM	57.0	DC + TL/ID	Overall (from leuc) 18.3 RPA III 39.7 RPA IV 18.3 RPA V 10.7	NDis	38 SAEs (NCI CTC Gr 3, 4, and 5) in 30 pts (39%), including 19 haematological AEs in 18 pts (23%)
20.	Akiyama et al. (2012)[32]	I	9 Rec-HGG (7 GBM + 1 AA + 1AO)	48.6	DC + synthetic TP (WT-1, HER2, MAGE-A3, and MAGE-A1 or gp100)/ID	NDis	1/9	All vacs were well tolerated except for transient liver dysfunction with Gr 2
21.	Phuphanich et al. (2013)[33]	I	16 ND-GBM 3 Rec GBM	51.8	DC + TP (HER2, TRP-2, gp100, MAGE-1, IL13Ra2, and AIM-2)/ID	38.4 (ND-GBM) OS exhibited a trend towards better survival in R versus NR, but was SNS	80.3% (3-YR 55.6%)	Gr 1 & 2
22.	Vik-Mo et al. (2013)[34]	I/II	7 ND-GBM	56.2	DC + GSC-mRNA/ID	25.3 (759 d) SNS	5/7	19/21 Gr 1 Mainly fatigue, pain, anorexia

Key - AA: Anaplastic Astrocytoma, AEs: Adverse Events, AO: Anaplastic Oligodendroglioma, AOA: Anaplastic Oligoastrocytoma, Cg: Control group, DC: Dendritic Cells, DCg: Dendritic Cell group, ND: Not Disclosed, GBM: Glioblastoma Multiforme, Gr: Grade, HGG: High-grade Glioma, IC: Intracranial, ID: Intradermal, IM: Intramuscular, IN: Intranodal, MO: Malignant Oligodendroglioma, ND: Newly Diagnosed, NDis: Non-Disclosed, NR: Non-Responders, OR: Objective regressions, i.e. approximate tumour decrease of 25% or greater on MRI, PXA: Malignant Pleomorphic Xanthoastrocytoma, OS: overall survival, 2-YR S: 2-Year Survival, pt: patient, R: Responders, Rec: Recurrent, SAEs: Serious Adverse Events, SD: Stable Disease, SNS: Statistically Non-Significant compared with control, SS: Statistically Significant compared with control, TL: Tumour Lysate, TP: Tumour peptide, vac: vaccination.

physical condition and quality of life before the treatment. Use of the Eastern Cooperative Oncology Group (ECOG) score was reported in 9.0% (2/22) of studies,[24,35] while no pre- or post-vaccination performance score was recorded in one trial.[18] In another publication the same performance score must have been used, though not clearly stated.[33]

In those studies using KPS, the minimum score for enrolment was 30 in 17.0% (3/18), 50 in 6.0% (1/18), 60 in 50.0% (9/18) and 70 in 27.0% (5/18). The ECOG performance score was ≤ 2 in the studies that used this scale.

Pre-vaccination treatment:

In 36.0% (8/22) of studies, all patients within the study group had the same pre-vaccination treatment.[15,25,27,30–32,34,35] This included surgery + radiotherapy + chemotherapy, with the latter varying from temozolomide alone to Gliadel® wafers, bevacizumab, procarbazine and other chemotherapeutics in addition to temozolomide. In two studies (9.0%), which recruited either ND- or Rec-HGG patients, pre-vaccination treatment consisted of surgery + radiotherapy,[14,16] whilst the group of Okada et al. in 2011,[28] recruiting Rec-HGG, proceeded to vaccinations immediately after surgery only (4.5% - 1/22).

In 8/22 (36.4%) studies pre-vaccination treatment within the same trial population was variable,[17,23,24,33,18,19,21,22] while in 3/22 (13.6%) trials patients with ND-GBM and Rec-GBM received differing pre-vaccination treatment.[20,26,29]

Time from surgery to commencement of vaccinations:

This parameter varied a great deal between the reviewed studies. In 32.0% (7/22) of studies a specific time frame from surgery to commencement of vaccinations was not reported. In the remaining studies time from surgery to first vaccine varied widely from immediately after surgery and/or radiotherapy[14,18,19,26,31] to a mean of 7.32 months after completion of chemo/radiotherapy[34] while in two trials from the same authors[16,21] vaccinations commenced upon disease recurrence.

Antigen-presenting cells:

As mentioned in the 'Vaccine ingredients' section, all studies used DCs. The exact amount of DCs per vial administered with every injection was reported in all studies. In 45.5% of studies (10/22) the quantity of cells was $\leq 10^7$,[14–16,18–20,22,24,25,29,31,32] while in 41.0% of studies (9/22) it was $\geq 10^7$.[17,21,23,26–28 30,33–35]

Antigen:

The antigens used in the trials varied. These were autologous tumour cell lysates in 10/22 (45,5%) studies,[16–18,21–23,25,27,29,32] autologous tumour cells in 6/22 (27.0%) studies,[15,19,24,26,30,31] autologous tumour cell peptides in 3/22 (14.0%) studies,[14,20] synthetic peptides in 2/22 (9.0%) studies[27,33] and transfected DCs with gamma-glutamylcysteine synthetase (GCS) mRNA in 1 study.[35]

Route and Site of Injection:

The route of vaccination was SC in 5/22 studies (22.7%), ID in 15/22 studies (68.2%) or intranodal (IN) in 2/22 studies (9.1%).

In the SC group, the site of injection varied between the deltoid,[14,17] the subaxillary area,[30] the region over the lymph nodes of either axillae[27] and the region over the inguinal lymph nodes.[31]

In the ID group, the site of injection included the upper third of both arms,[18,22,25,29,32] close to cervical lymph nodes,[15,19] below the axillae,[20] the deltoid region,[23] the abdomen,[24] the posterior neck[33] and the axilla.[34] Of note, there were two studies from the same authors,[16,21] who reported ID administration close to the cervical lymph nodes to a subgroup of the study population and/or IT administration via an Ommaya reservoir to another. No specific site of ID injection was indicated in one study.[35]

In the IN group, vaccines were injected with ultrasound guidance either into 2 bilateral cervical nodes[28] or into inguinal/axillary nodes.[28]

Duration of therapy:

The number of vaccinations delivered per patient varied greatly contingent on the immunotherapeutic protocol followed and the clinical condition of each patient. In total it ranged between 2 and 24 with an average of 17.6 vaccinations per patient.[14–35]

3. Outcomes

Post-Vaccination Performance Score:

Post-vaccination performance score was not reported in the majority (77.0%) of trials (17/22). In 4 trials (18.0%) post-vaccination performance score was published,[26,30,31,35] while in one study[25] authors refer to post-vaccination performance, but without using a specific scale.

Chang et al. in 2011,[26] not surprisingly, reported a decrease in KPS in 7 out of 17 patients after completion of vaccinations, but of note an increase was noted in 3 out of 17 patients. Jie et al. in 2012[31] reported that 9 months after vaccination ($n = 13$), the KPS had deteriorated below 80 in 1. In the series by Cho et al.,[30] at 33-month follow-up, the survivors maintained a KPS of 50. Ardon et al. in 2010 reported that 'patients could take care for themselves and perform daily life activities during treatment', but no specific scores were provided.[25]

Completion of therapy:

In 12/22 (54.6%) studies 100% of recruited patients completed the full course of the therapy.[14,16–19,21–23,27,29–31] In the remaining 10 studies a minimum of 50.7% [32] to a maximum of 86.4% of patients completed the protocol.[28]

Objective Responses (based on neuroimaging):

In 10/22 studies (45.5%) a response to treatment based on neuroimaging was not documented.[14,17,20,22,26,27,29,30,32,34] The remaining studies can be grouped based on the criteria used to classify radiological (predominantly MRI) response to treatment.

In 3/22 studies (13.6%)[16,21,28] responses to treatment were classified using the McDonald criteria consisting of 5 classes[21] as follows: 27 progressive disease (PD), 23 no change (NC), 5 minor response (MR) and 2 complete or partial response (CR or PR).

In 5/22 studies (22.7%)[15,19,31,33,35] responses to treatment were classified using the Response Evaluation Criteria In Solid Tumours (RECIST or WHO Response Evaluation in Tumours) consisting of 4 classes[31] as follows: 32 PD, 9 NC, 2 MR and 24 CR or PR.

In 4/22 studies (18.2%) radiological response to treatment was published, but authors did not report the criteria or type of classification used and therefore cannot be grouped.[18,23,24,25]

Immune Responses:

In all studies but one[26] positive immune responses, either systemic or intracranial, were reported. The latter was based on investigating tumour specimens obtained by re-operation when tumours recurred.

Post-vaccination increase in anti-tumour T-cells was reported in 13/22 (59.0%) studies,[15,16,19,20,25,27,28,29,31–35] in interferon-gamma was reported in 4/22 studies (18.0%)[15,23,25,29] and in tumour/intracranial T-cells was reported in 7/22 (32.0%) studies.[14,16,17,20,24,26,29]

Skin delayed-type hypersensitivity (DTH) tests were performed in 5/22 (22.7%) studies.[18,21–23,33]

Progression-Free Survival:

PFS was not reported in 8/22 (36.4%) studies.[14–17,19,21,24,26] In the rest of the studies, grouped according to tumour pathology, PFS was as follows:

1. For ND-GBM: 23.1 months[35]; 9.5 months[27]; 6.0 months (for 75% of patients)[25] and 6.0 months (for 70% of patients)[32]; Cho et al.[30] reported a PFS of 8.5 months for the dendritic cell group versus 8.0 months for the control group, whilst Jie et al.[31] found a PFS of 11.92 months for the dendritic cell group versus 7.75 months for the control group.

2. For Rec-GBM: 3.0 months[22] and 16.9 months.[34] In the latter study, PFS exhibited a non-significant trend towards better survival in vaccine responders versus non-responders.

3. For ND- and Rec-GBM combined: 15.5 months (n = 7 ND + 5 Rec)[20] and 15.9 months (n = 15 ND + 8 Rec).[29] Wheeler et al.[23] reported PFS of 10.3 months for vaccine responders versus 5.6 months for non-responders: (n = 11 ND + 22 Rec).

4. For Rec-HGG: 3.0 months[18]; 4.0 months for GBM and 13.0 months for anaplastic glioma.[28] In the study by Akiyama et al.[33] only 1/9 had not progressed at 2 years.

Overall Survival:

OS was not reported in 2/22 studies.[15,33] In the rest of the studies, grouped according to tumour pathology, OS was as follows:

1. For ND-GBM: 24.0 months[25]; 28.0 months[27]; 18.3 months [39.7, 18.3 and 10.7 months for recursive partitioning analysis (RPA) classes III, IV and V, respectively][32]; 25.3 months[35]; and 38.4 months, with a non-significant trend towards better survival in responders compared with that in non-responders.[34] Cho et al.[30] reported an OS of 31.9 months for the dendritic cell group versus 15.0 months for the control group, whilst Jie et al.[31] found an OS of 16.0 months for the dendritic cell group versus 12.3 months for the control group.

2. For Rec-GBM: 9.6 months after re-operation.[22]

3. For ND- and Rec-GBM combined: 23.4 months (n = 7 ND + 5 Rec)[20]; 31.4 months for ND-GBM and 35.9 for Rec-GBM[29]; 21.4 months for vaccine responders versus 14.3 months for non-responders (n = 11 ND + 22 Rec).[23]

4. For Rec-HGG: 40.2 months[16]; 10.5 months[18]; 9.8 months[19]; 16.0 months[21]; and 13.1 months for GBM and 17.2 months for anaplastic gliomas [5/9 patients were still alive at time of publication].[28]

5. Others: 17.3 months for ND- and Rec-GBM and anaplastic astrocytoma (AA)[26]; 14.0 months for ND- and Rec-HGG[24]; 17.6 months for ND- and Rec-HGG[17]; and 15.6 months for ND-HGG.[14]

2-Year Survival:

In 2/22 studies[15,32] 2-year survival was not indicated. Based on categorisation of the studies according to tumour pathology, 2-year survival was as follows:

1. For ND-GBM: 50.0%[25]; 60.0%[27]; and 71%.[35] Cho et al.[30] reported a 2-year survival of 44.4% for the dendritic cell group versus 18.8% for the control group, whilst Jie et al.[31] indicated a 2-year survival of 7.7% for the dendritic cell group versus 0.0% for the control group.

2. For Rec-GBM: 80.3%[34]; and 14.8% after reoperation.[22]

3. For ND- and Rec-GBM combined: 50.0%[20] and 55.0%.[29] Wheeler et al.[23] (n = 11 ND + 22 Rec) reported a 2-year survival of 41.0% for vaccine responders versus 7.0% for non-responders.

4. For Rec-HGG: 40.0%[16]; 16.7%[18]; 0.0%[19]; 23.5%[21]; 11.0%[33]; 36.0% for GBM and 63.0% for anaplastic gliomas.[28]

5. Others: 41.2% for ND- and Rec-GBM and AA[26]; 11.0% for ND- and Rec-HGG[24]; 42.9% for ND- and Rec-HGG[17]; and 0.0% for ND-HGG.[14]

Safety – Vaccination-related side effects:

All 22 studies reported mild vaccination-related side effects with their grade ranging mostly between I and II according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) for Adverse Events. These consisted mainly of mild toxicity reactions such as erythema, swelling and pain around the injection site, lymph node swelling, fatigue, anorexia, nausea, lymphopenia, leucopenia and transient liver dysfunction. No autoimmune encephalitis or other autoimmune disease was reported.

In the study of Ardon et al. in 2012[32] 39% of patients experienced NCI CTC grade III, IV and V serious adverse events mainly consisting of chemotherapy-related lymphopenia and leucopenia. Rare adverse events reported consisted of vaccination-related peritumoural oedema, with grade IV neurotoxicity responding to steroids, in 1 patient,[18] grade IV neurotoxicity (stupor) due to vaccination-related peritumoural oedema in the presence of gross residual tumour in 1 patient[22] and cutaneous GBM at site of DTH testing in 1 patient.[23]

Discussion

From the above results, it is clear that there is a great deal of diversity in terms of patient population, timing and protocol for vaccination in the phase I/II trials investigating DC vaccine therapy. Within these limitations and potential biases, however, these trials have revealed effective stimulation of anti-glioma immunoresponse with low toxicity. This has translated into improved mean overall survival of around 3 years in GBM patients in multiple phase I and II trials.[29,34]

The study by Ardon et al. in 2012[32] is of particular interest, because the authors assessed the efficacy of DC vaccinations based on the RPA classification of the European Organisation for Research and Treatment of Cancer (EORTC). This resulted in a mean OS, which is favourably comparable to the EORTC 26981/22981-NCIC CE3 Trial in all RPA classes.[36] In particular, the RPA class III demonstrated a significantly higher OS of 39.7 months. Therefore, it seems that at least a subgroup of GBM patients could potentially benefit greatly from this treatment.

Of note and of particular relevance to this patient group, serious adverse events were uncommon, consisting mainly of minor side effects at the site of injection, rendering the treatment safe. By and large, the vaccine was delivered by the SC or ID route, making the procedure minimally invasive and fast to administer.

In almost all trials, autologous DCs were pulsed ex vivo with tumour lysate or peptides for vaccine production. This is crucial for bringing specificity to the vaccine. Gliomas are characterised by antigen heterogeneity amongst patients and even within the same individual. Whole tumour lysate could provide multiple and comprehensive range of antigens for recognition by specific T-cells. Moreover, the targeting of multiple antigens could preclude immune selection and enhance the immune system to interact with the maximum number of tumour variants.

The administration of poly-ICLC[28,29] and imiquimod[29] represents an attempt to further enhance the effect of DC vaccination. Poly-ICLC is a toll-like receptor (TLR)-3 agonist and imiquimod is a TLR-7 agonist, both stimulating the immune system. Their actual effect on the efficacy of DC vaccinations, however, is hard to evaluate from the current studies and requires further work.

The extent of surgical resection was one variable not easily extractable from the published data. This was only reported in a handful of the studies, and even then not necessarily comprehensively, making comparative evaluation difficult. This is important since total or gross total surgical resection of the tumour is increasingly thought to confer better outcomes, both directly and by increasing the efficacy of the adjuvant therapy.[22,37,38] Furthermore from the immunological prospective, a lower volume of residual glioma cells may translate to less glioma-induced immunosuppression.[39] However, on the other hand, there are authors who believe that minimal residual tumour is a prerequisite for effective immunotherapy.[10,18,20]

The optimal timing for the commencement of vaccination varied widely in the trials and remains uncertain. Chang et al.[26] showed that paradoxically, the survival of ND-GBM was lower than that of Rec-GBM. They attributed this to the early initiation of immunotherapy soon after surgery in the Rec-GBM group versus delayed commencement, after radiotherapy, in the ND-GBM patients. Radiotherapy was thought to result in the development of mutant tumour cells, which were immunologically diverse from those obtained at surgery, rendering the vaccine less effective. On the contrary Kjaergaard et al.,[40] in an experimental murine brain tumour model, showed that the best outcomes were derived from the combination of radiotherapy and DC vaccination. According to the authors, this was due to radiation-induced increased expression of MHC molecules in the glioma cells, which resulted in the increased immunogenicity of the tumours. Moreover, there is now some evidence supporting improved survival rates and higher chemosensitivity of tumours when adjuvant temozolomide is combined with immunotherapy[20,23,41] although comprehensive data are still pending. In practice, given that 'the Stupp protocol' is widely accepted as the milestone in the standard of care for GBM, it is ethically difficult to consider any vaccination schedule that disrupts this protocol. Indeed almost all the more recent DC vaccine phase I/II trials adopt 'the Stupp protocol', with the vaccine being delivered after the concomitant phase. This approach has also been incorporated into the ongoing phase III trial of the vaccine.

Given that both systemic and intracranial immunoresponses were reported following DC vaccination, the question of biomarkers of tumour response prediction is raised. Thus, Fadul et al.[27] found that 50% of recruited patients developed a measurable immune response that was associated with improved OS. Moreover, Liau et al. in 2005[20] reported that in 6 patients without pre-existing peripheral cytotoxic T lymphocyte activity, who did develop cytotoxic activity post-vaccination, overall survival was statistically significantly longer. On the other hand, Prins et al.[29] and Ardon et al. in 2012[32] found no evidence that the immunological changes contained any predictive value for either PFS or OS. Presently, therefore the most valid indicator of vaccination-enhanced immune response to GBM is tumour infiltration by cytotoxic and memory T-cells as shown in tumours resected after surgical relapse.[14,16,17,19,20,24,26] More sensitive blood, neuroimaging, including PET, and CSF biomarkers are therefore required to predict immunotherapy response.

Conclusion

Active DC immunotherapy has emerged as a promising means of stimulating anti-tumour immune responses. This has resulted in significant improvement in survival for patients with HGGs in a number of phase I and II clinical trials, although such trials recruited small numbers and were heterogeneous in design. There is therefore the need for further studies to fully evaluate this therapy. The ongoing phase III randomised blinded trials, initially opened in the USA and now recruiting in Europe, are designed to address this need and will define the role of DC immunotherapy in the management of patients with this most devastating disease.

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Jarosz, Dr. Aleksandar Mijovic and Prof. Farzin Farzaneh are sub-investigators in the same trial. Dr. Ronald Beaney, Jamie Logan, Victoria Hurwitz, Mr. Ranjeev Bhangoo, Mr. Richard Gullan and Prof. Mark Richardson have no conflict of interest to declare.

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