

A Systemic Review of Clinical Trials on Dendritic-Cells Based Vaccine Against Malignant Glioma

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Abstract

The prognosis of patients with high grade gliomas (HGG) is quite poor, in spite of the standard combination of surgery, radiotherapy and chemotherapy. Recently, many clinical researchers have been focusing their efforts on the safety, feasibility and efficiency of dendritic cells (DCs) based vaccine against HGG. According to the literature search, 23 phase I/II clinic studies were picked and systemically reviewed to assess the application, the effect, and the future of DC vaccine for HGG. DC vaccine appears to have potential to increase overall survival with minimal complications. However, there still remains to be many challenges during vaccination, such as targeting specific/ associated antigens, adjuvants, clinical status, and the evaluation of the response.

Keywords: Glioma; Dendritic cell; Immunotherapy; Gliomas; Brain tumor

Introduction

Glioma is one of the most common primary brain tumors. High grade gliomas (HGG), such as anaplastic astrocytoma (AA), glioblastoma multiforme (GBM) have a poor prognosis with tendency to recur, even after combined treatment. Immunotherapy has been considered as the sword of Damocles hanging over cancer. The antigen-specific immunotherapy has been developed with the identification of cancer-specific antigens. Adoptive transfer of autologous antigen-specific T cells might be effective but last not that long. Antigen peptide vaccination may be specific, but the tumors could develop selective immune escape. Central to the success of a vaccine is its ability to harness the potent antigen-presenting capabilities of dendritic cells (DCs), which is the most professional and powerful antigen-presenting cells. Immunotherapeutic strategies have attempted to exploit the intrinsic capacity of dendritic cells to stimulate antitumor immune effector cells, such as tumor antigen-specific cytotoxic T lymphocytes, Th1 cells, etc. (Figure 1). Since the publication of the first major clinical trial in melanoma patients in 1998 [1], therapeutic DC based vaccines have been extensively studied in numerous phase I and II trials. While advances have been encountered (especially with prostate cancer and melanoma), there are still considerable challenges that need to be addressed in future clinical trials. In this review, we describe the current methodology and highlight trials which have contributed to the development of DC vaccines. We review strategies to optimize DC vaccines in order to improve antitumor responses in patients with gliomas.

We searched on PubMed with the keywords of “glioma”, “dendritic cell”, “clinical trial” and “immunotherapy” to get clinical trials of DCs based immunotherapy against HGG. Finally, 23 manuscripts [2-24] on DC vaccines against HGG were found, including 2 case reports, 21 phase I/II clinic studies. All the studies were performed in a single medical center, and inclusion criteria, design, and evaluation criterion are not consistent amongst different studies. The most important issue is that the antigens for priming DCs also varied in different trials considerably, so informative meta-analysis is not possible. Here we systematically reviewed all previous clinical trials to get better understanding of DCs based immunotherapy for HGG. The safety and feasibility of DC vaccine against HGG were well illustrated, and a tendency that HGG patients could benefit from DC vaccine was observed.

Application of DC Vaccine against Malignant Glioma

Tumor antigens

The key of immunotherapy is tumor-specific antigen (TSA) which could ignite strong anti-tumor immune response. EGFRvIII (epidermal growth factor receptor variant III) [25] is frequently expressed on GBM (27%) [26] and not on normal brain tissues, so it could serve as an ideal target for anti-tumor active-specific immunotherapy. This approach has already been tested by several clinical trials [27]. Many other HGG proteins such as EphA2 (Ephrin type-A receptor 2) [28], IL-13 receptor α 2 chain [29], surviving [30], tenascin [31], and SOX2 [32] might serve as markers for gliomas. These studies need further exploration due to the limited sample size and heterogeneous expression level of glioma-specific proteins. Traditional SEREX (serological analysis of recombinant expressed cDNA clone) approach may pick up new antigens like EFTUD1 (Elongation factor Tu GTP binding domain containing protein 1) [33]. CT (cancer testis) antigen family members are also potential candidates [34], but the expression of CT antigens on glioma varies considerably. To date, it has been a big challenge to search TSA, and the lack of TSA arrests the progress of active-specific immunotherapy (Figure 2).

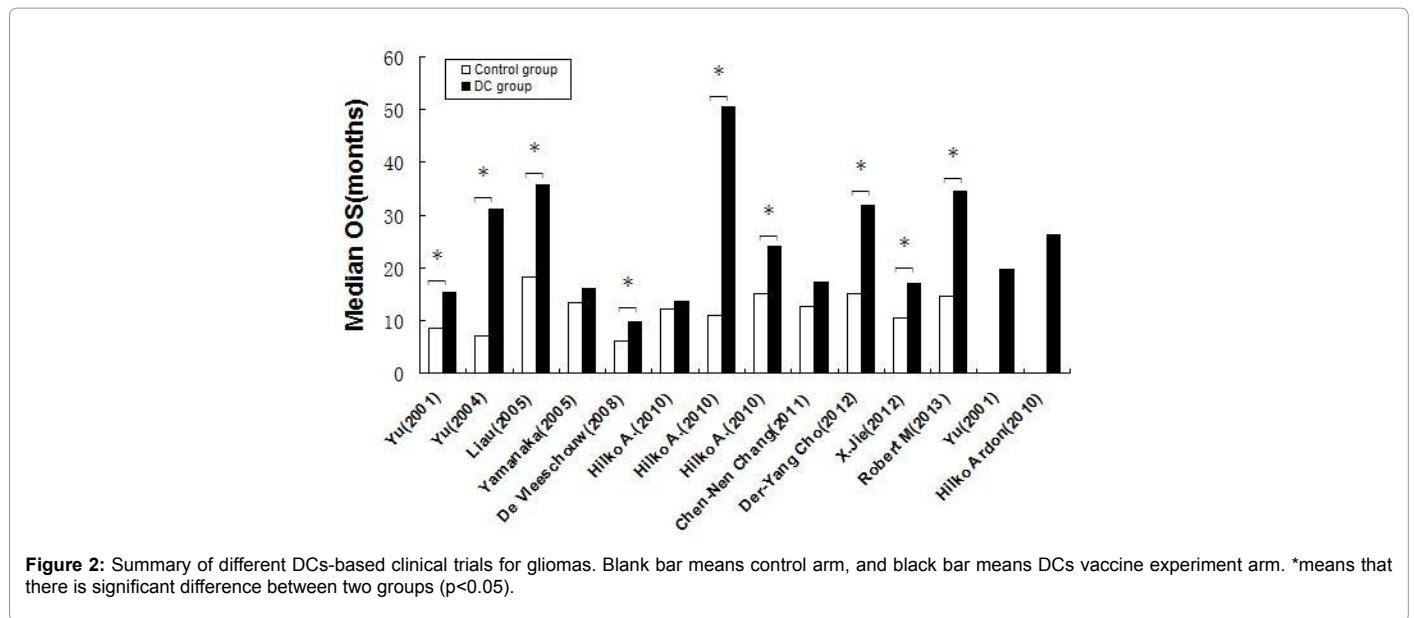
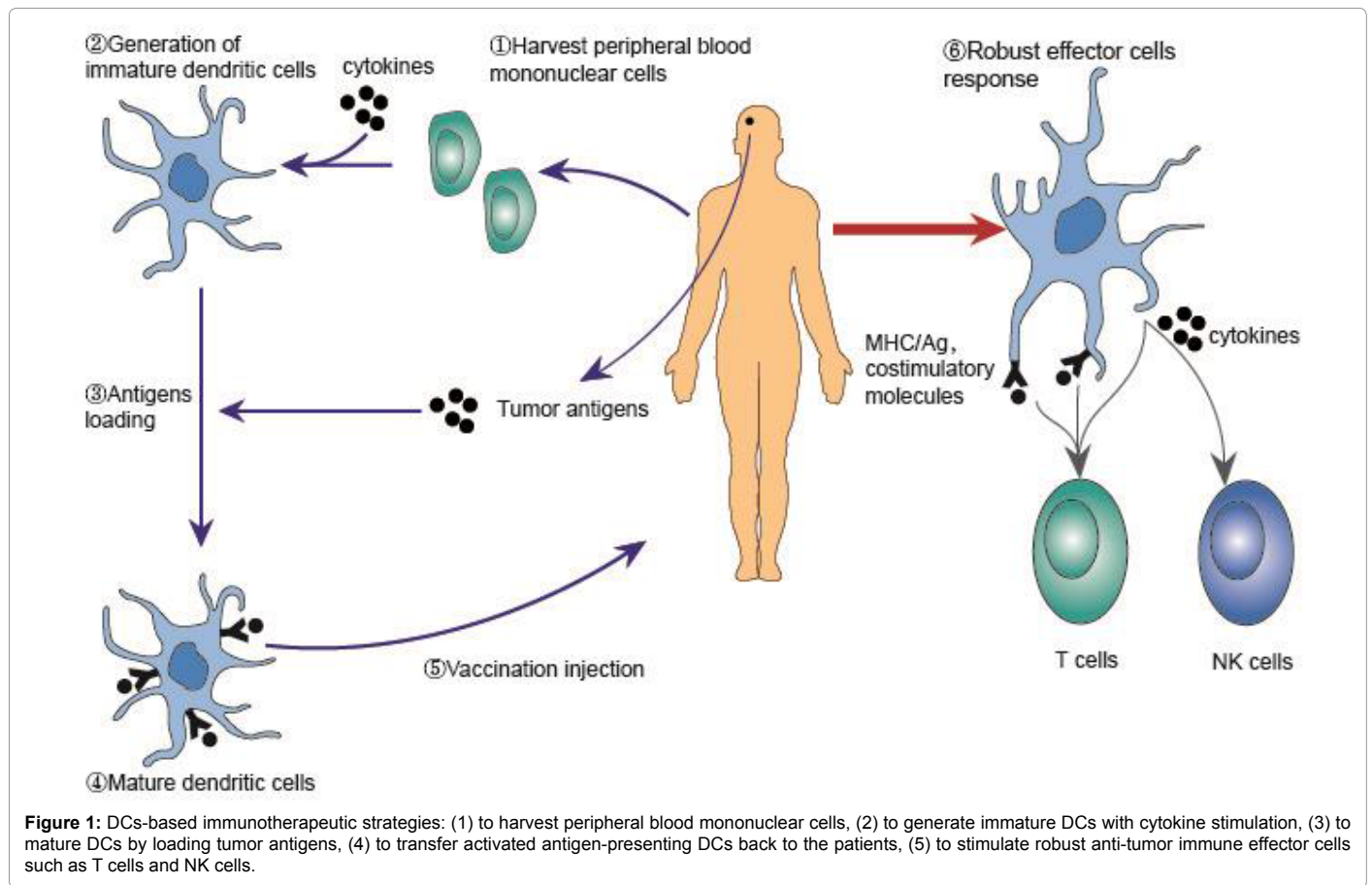
Genomic and proteomics profiling might offer us better information about TSA and tumor-associated antigen (TAA), and many efforts have been put on them. Kalinina J et al. reviewed the proteomics screening of glioma TAA from U87 and clinical GBM samples [35]. Unfortunately, they found that not a single protein identified as a specific marker could be verified in other independent studies. The hybridoma technology, gene or antibody screening

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technology, and predication of immunogenicity of proteins might bring us a breakthrough in TAA screening. Since single TSA/TAA proteins may have limited expression, the combination with multiple antigens could provide better target strategy in immunization. Freitas M et al. [36] found that CT antigen family such as ACTL8 (actin-like 8, 57%), OIP5 (Opa interacting protein 5, 54%), XAGE3 (X antigen

family, member 3, 44%) and CTCFL (CCCTC-binding factor [zinc finger protein]-like, 15%) were heterogeneously expressed in 48 GBM samples. Interestingly, 85% of the tumors expressed at least one of the CT antigens, and mRNA positivity for 3-4 CT antigens could serve as an independent predictor of better overall survival (OS) [36]. Okada et al mixed four TAA peptides including EphA2 (EPH Receptor A2), IL-

13Ra2 (Interleukin 13 receptor, alpha 2), YKL-40, and gp100 to mature DCs to treat recurrent HGG patients [37]. Terasaki et al. [38] screened 14 common HLA-A24-restricted antigens including EGFR, EZH2 (enhancer of zeste homolog 2), MRP3 (multidrug resistance-associated protein 3), Lck (lymphocyte-specific protein tyrosine kinase), SART (squamous cell carcinoma antigen recognized by T cells), etc. And then top four antigenic peptides were picked and produced in GMP laboratory serving as personalized mixed peptide vaccine and induced dose-dependent immune boosting.

The efforts have been switched to mixed total tumor antigens (TTA) [1], which could compensate the low expression of single TAA or certain antigen mutation. It would be theoretically more effective. Many clinical trials have extensively used TTA as antigen source to prime DCs [2-24]. Liao et al. used an allogenic MHC-I peptides in a case report [2], but the efficacy of this approach need large controlled study to confirm. The autologous TTA appeared to be most commonly used [3-24], many different methods were applied to isolate TTA autologous from tumor tissue lysates or cells, such as repeated freezing and thaw lysates [5,8,11,12,16,18], X-ray irradiation [3,10,14,24] acid elution [13]. Autologous tumor specific peptides [4] or RNA [7] was also used. These studies all reported minimal risk of toxicity or side effects and improved survival. The main concern about TTA is still its low immunogenicity, whereby various techniques have been explored to improve it. The fusion of DC and tumor cells with PEG (Poly Ethylene Glycol) was explored [39]. Prahlad P et al. compared DCs primed with different tumor lysates, RNA, and apoptotic cells, and DC primed with apoptotic cells seemed to have the optimal immune response [40]. The progress in stem cell research encouraged researchers [41,42] to employ tumor stem cells (TSCs) for antigens preparation. The mixed TTA antigens from TSCs show better effect *in vitro* and *in vivo* studies against GBM. We have further sorted CD133 positive TSCs to produce TTA, and have achieved even better responses *in vitro* [42]. Phase I clinical trials with DCs primed with TTA from TSCs against GBM are ongoing [20]. Antigens derived from tumor exosome [43] or autophagosome [44] have been explored also. These potential strategies may be useful in specific active immunotherapy.

It is quite important to choose the most efficient antigens. However, all of the results to date have shown that each antigen has its own shortcomings. For example, EGFRvIII vaccine only could be applied to the patients with EGFRvIII variant. For TAA, it is unclear which of the antigens will benefit the patients, so, further refinement of TAA is necessary. Another important clinical issue is the isolation of sufficient TAA or TTA, since it usually requires a significant amount of tissue or cells to process.

Dendritic cells preparation

DCs could be derived from peripheral blood or bone marrow. Peripheral blood mononuclear cells (PBMCs) are the main source to generate DCs with stimulation of GM-CSF and IL-4 [45]. TNF- α and IL-1 β were added by some investigators before DC maturation [46], and IL-1 β , TNF- α , IFN- α and IFN- β were applied to generate aDC1 which could initiate more effective anti-tumor immune response by Okada et al. [14,47]. For patients without sufficient tumor tissues for tumor antigens, transfection with mRNA or DNA into DCs to express more cytokines and co-stimulatory molecules might be feasible [48].

After maturation, DC vaccine could be delivered subcutaneously or intramuscularly. However, the post-injective lymphatic return rate (LRR) is always limited. So, ultrasound-guided intranodal injection [37] and injection through Ommaya reservoir connected to tumor

area or ventricles was also tried [12,49,50]. No evidence demonstrated any significant effect or more severe complications like edema after the administration of DCs. Although there is no criterion for the course of vaccine, it was deemed that both low quantity and limited dosages were likely to limit DCs' effect [51]. As shown in the Table 1, DCs were delivered every 1-2 weeks for more than 3 times in most studies [2-24].

Combination with adjuvants or other therapies

Not only the tumor antigen, but also the specific adjuvant, and combination with other therapies as well, should be considered during the design and evaluation of tumor vaccine. Bacillus Calmette Guerin (BCG) and Freund's adjuvant were used as adjuvants traditionally. More new effective and targeted adjuvants, such as cytokines (IL-12), MF59, cholera toxin B subunit (CTB), AS04 and Toll-like receptor agonist (CpG oligonucleotides, Imiquimod and poly I:C), have demonstrated some efficiency and safety [52]. Development of adjuvants has been a major issue in tumor vaccine strategy to improve immunogenicity. The main concern is that there are no effective universal adjuvants for tumor immunization protocols. Adjuvants can enhance specific type of immune responses albeit cellular or humoral of poorly immunogenic antigens. Emerging evidence from clinical trials indicates that DCs could elicit cellular immune response [53], induce tumor-specific cytotoxic T cells and also enhance natural-killer cell immunity [54]. Therefore adjuvants that can boost specific type of cell-mediated vaccine responses would be more helpful. This may also require temporal events that reflect tumor status and vaccine effectiveness during the course of immunotherapy.

Chemotherapy and radiotherapy are the main adjuvant treatment modalities for HGG after surgical resection. Prins et al. [55] reported that immunotherapy has good synergism with chemotherapy and radiotherapy, and have speculated that apoptotic tumor cells after adjuvant therapies might provide abundant tumor antigens to DCs. Another study by Hilko Ardon [18] found the CD8+T cells could increase significantly in GBM after treatment with the combo of DC vaccine and temozomide. As for the time frame to administrate DC vaccines, Walker [24] and Chang [19] recommended vaccine first, since ionizing radiation will not only kill the tumor cells, but also may impair the immune responses.

Evaluation of curative effect

No standard has been proposed to assess the effect of DC vaccine. OS and progression free survival (PFS) are often adopted to evaluate the effect. Interestingly, OS seems to have more chance to be prolonged by vaccine than PFS. Age, tumor invasiveness status and the extent of tumor resection have been proven to be independent prognostic predictors [15]. The bias of selection of patients with potential good prognosis will significantly sway the survival time. Maximal tumor resection still dominates other considerations, and obviously DC vaccines are inept to big tumor burdens. So the strategy is to remove all clinically evident tumor, and then to vaccinate to inhibit subclinical micro-metastatic disease. Some immune index might provide us some reference to select patients or to predict the response as previously discussed.

In the reviewed 23 phase I/II clinical trials (Table 1), 13 studies with OS data available were further analyzed, and DCs vaccine seems effective on the improvement of OS (Figure 1). Yu et al. [4,11] reported two trials consecutively, and the median OS of GBM patients received DCs vaccine primed with acid-eluted tumor peptides was much longer than the control patients. In the clinical trials by Liao et al. [13] and Hilko et al. [17,18], the improvement of OS in pediatric patients with

Citation	Study type	Case	DC-vaccine treated	Antigen source	Culture of DC	Dosage	Immunological reaction	Clinical response
[2]	Case report	1	R-GBM	allogeneic MHC-I GBM peptides	GM-CSF and IL-4;	i.d. ½ wks (× 3)	Increased TILs	PD, OS:21m
[4]	Phase I	9	N-GBM(n=7), AA(n=2)	autologous tumor specific, MHC-I	GM-CSF and IL-4;	s.c. ½ wks (× 3)	SCR, TILs (CTL and Tm)	Med OS; 455d
[3]	Phase I	8	GBM(n=5), AA(n=3)	ATC	GM-CSF, IL-4 and TNF-α	i.d. 1/3 wks (× 9)	Increased NK cells, IFN-γ	PR(n=2); SD(n=4); PD(n=2);
[6]	Phase I/II	10	GBM(n=7), AA(n=3)	ATL	GM-CSF and IL-4	i.d. and/or i.t. (ommayaya) 1/3 wks (× 10)	Increased NK cells; DTH+; infiltration of T cells	--
[5]	Phase I/II	17	N-GBM, R-GBM	ATL	GM-CSF and IL-4; 3	s.c., 1/2 wks (× 3)+1 at 6 th wks	Increased IFN-γ	--
[11]	Phase I/II	14	N-GBM(n=1) and AA (n=1); R-GBM(n=9) and AA(n=3)	ATL	CM-CSF and IL-4	s.c. 1/2 wks (× 3)	Increased IFN-γ mRNA	Med OS; 133 wks
[7]	Phase I	7	EM (n=3); GBM(N=2); AA(n=1); PA(n=1); MB(n=1); PXAs (n=1)	Tumor tissue RNA	GM-CSF and IL-4	i.v. and i.d. ½ wks × 2+1/1 mth (× 5)	-	-
[9]	Case report	1	AA	ATL	GM-CSF and IL-4; TNF-α, IL-1β, and PGE2	i.d. ½ wks (× 2)+1/1 mth (× 6)	DTH(+)	PFS(60m)
[8]	Phase I	12	HGG (n=8), AA(n=4)	ATL	GM-CSF and IL-4; TNF-α, IL-1β, and PGE2	i.d. 1 at 1 st w+2 at 2 nd +1/1 mth	DTH(+)	CCR(1), NC(3), PD(3); Med OS: 42.0 wks
[10]	Phase I/II	15	GBM(n=6); AA(n=9)	ATC	FCs and rhIL-12 GM-CSF, IL-4, and TNF-α;	i.d. , s.c. ½ wks (× 6)	increased IFN-γ,	PR (1), MR(1), NC (1), PD(3)
[13]	Phase I	12	N-GBM(n=7); R-GBM(n=5)	Acid-eluted ATCP	GM-CSF and IL-4	i.d. 1/2-4 wks (× 3)	Tumor specific CTL;	Med PFS: 15.5 m Med OS: 23.4 m
[12]	Phase/ II	24	R-AA(n=6); GBM(n=18)	ATL	GM-CSF and IL-4; OK-432	i.d. or i.d. combined i.t. (ommayaya) 1/3 wks	DTH(+); increased tumor specific CTLs	Med OS 480d; PR(1), MR(3), NC(6), PD(8)
[14]	Phase I	7	R-GBM ; AA	irradiated ATC and TFG-IL4-Neo- TK-transfected fibroblast	UPCI95-033 UPCI 99-111	i.d. 1 st in D1+2 nd in D7 +1/2 wks	CD4+, CD8+ IFN-γ against EphA2883–89; HLA-A2	UPCI95033: Stable disease 4m (n=2) UPCI99-111: Med PFS 6m
[16]	Phase II	34	N-GBM(n=11); R-GBM(n=23)	ATL	GM-CSF and IL-4	s.c. ½ wks (× 3)+1 st wk#6	increased IFN-γ	N-GBM OS: 642 ± 61d; PFS: 308 ± 55d; R-GBM OS: 599 ± 75d; PFS: 401 ± 53d
[24]	Phase I	13	GBM(n=9) and AA(n=4)	ATC	GM-CSF and IL-4	i.d. ½ wks (× 6)+1/6 wks	Increased T cell infiltration	Med OS: 11m
[15]	Phase/ II	56	R-GBM	ATL	GM-CSF and IL-4; TNF-α, IL-1β, and PGE2	i.d. G1: 1 st , 1w, 2 nd , 3 w, ¼ wks; G2: ½ wks (× 5), ¼ w; G3: 1/1 w (× 4)	DTH(+)	Med PFS 3m; OS, 9.6m.
[17]	Phase/ II	45	HGG(n=33), MB/ PNET(n=5), EM(n=4) and ATRT(n=3)	ATL	GM-CSF and IL-4	i.d. G1: 1 st , 1 w, 2 nd , 3 w, ¼ w; G2: ½ w (× 5), ¼ w; G3: 1/1 w (× 4), ATL; G4: 1/1 w (× 4)	-	HGG: Med OS 13.5m
[18]	Phase/ II	8	N-GBM	ATL	GM-CSF and IL-4; TNF-α, IL-1β, and PGE2	i.d. 1/1 w (× 4)+1/2 wks (vacc+ATL)	increased CD8+CD25+cell, T-cell IFN-γ	Med OS: 24m; PFS: 18m
[19]	Phase I/II	17	N-GBM, AA and R-GBM, AA	ATL	INF-Y	S.C 1/1wks (× 4)+1/2 wks (× 2)+1/2 mth (× 4)	Increased TIL	GBM:Med:OS 520d
[42]	Phase I	5	R-HGG	Stem-like associate antigen	GM-CSF and IL-4	½ w (× 3)+poly- ICICI M1/2 wks	-	-
[21]	RCT Phase II	34	N- GBM and R- GBM	ATL	GM-CSF and IL-4	s.c. 1/1 w (× 4)+½ wks (× 2) +1/4 wks (× 4)	-	Med OS: 31.9m; Med PFS: 8.5m
[22]	Phase II	25	N-and R- GBM	ATC antigen by heat-shock	GM-CSF and IL-4	i.d. 1 st in D7+2 nd in D14+3 rd in D28+4 th in D42	-	Med OS: 17 m
[23]	Phase I	34	GBM(27) and AA(7)	ATL, GAA	GM-CSF and IL-4	½ wks+1/3 mth	Lymphocyte subset change	ATL/GAA; Med OS 34.4m/15.5m

Abbreviations: R-GBM: Recurrent GBM; GBM: Glioblastoma Multiforme; I.D.: Intradermally; Wks: Weeks; PD: Progressive Disease; OS: Overall Survival; N-GBM: Newly Diagnosed GBM; AA: Anaplastic Astrocytoma; S.C.: Subcutaneously; Med: Media; ATC: Autologous Tumor Cell; PR: Partial Response; SD: Stable Disease; PD: Progressive Disease; ATL: Autologous Tumor Lysate; EM: Ependymoma; PA: Pilocytic Astrocytoma; MB: Medulloblastoma; PXA: Pleomorphic Xanthoastrocytoma; I.V.: Intravenously; Mth: Months; PFS: Progression Free Survival; HGG: High Grade Gliomas; CCR: Continued Complete Remission; NC: No Change; MR: Mixed Response; ATCP: Autologous Tumor Cell Peptide; PNET: Primitive Neuroectodermal Tumors; GAA: Glioma Associated Antigen.

Table 1: Characteristics of clinical trials against gliomas using DCs based vaccine immunotherapy.

HGG treated with DCs vaccine was not observed, while an effective responses with an significant increase of OS was observed in adult patients. The longest follow-up was reported by Chang [19], and the survival rate after five years were 18.8% for newly diagnosed HGG. The only RCT (Randomized Clinical Trial) was performed by Der-Yang Cho et al. [21], and the results showed that the vaccinated patients got an impressive improvement of OS. However, in this RCT study, most patients in the experiment arm received salvage therapy. As a group, the numbers of enrolled patients and RCT studies were so limited, phase II/III RCT and long-term evaluation are urgently needed. Overall, DCs based vaccine appears to benefit certain HGG patients (Figure 1).

As for the immunological index, tumor infiltrating lymphocytes (TILs) is one objective indicator, which has been verified in several clinical trials [2,4,14,19,24]. Delayed type hypersensitivity (DTH) were observed in a number of clinical trials [6,8,9,12,15]. Wheeler et al found that the PFS and OS of the patients with the elevation of IFN- γ (more than 1.5 times in serum) were longer than those without IFN- γ elevation after vaccination [16]. Other factors such as the dosage of vaccine, regulatory T cells, the ratio of Th1/Th2 showed no correlations with the prognosis [24,55]. Yu et al. used the MAGE-1 (melanoma antigen family A-1) specific CTLs as a marker to address the antigen-specific immune response of the patients [11].

Evaluation of side effect

DC vaccine for HGG seems to be well tolerated across all variations in all clinical trials. The adverse events associated with immunotherapy were evaluated by the RECSIT (new Response Evaluation Criteria in Solid Tumors). Skin itch, erythema on the injection location, headache, fever, and lymphopenia were observed in some cases. Notably, seizure were found in two studies with a frequency of 16.6% [19] and 8.3% [13]. Since there is no comparison with other treatment modalities, no statistical analysis or definite conclusion could be drawn. By and large, no evidence of toxicity or severe side effect was observed.

Challenge of HGG immune escape

GBM themselves could develop various immunosuppressive methods to circumvent surveillance which lead to the failure of immunotherapy. Like other malignant tumors, GBM cells could express less MHC or tumor antigens to reduce the first signal. In the meantime, GBM might cut down co-stimulatory molecules like CD80, CD86, CD40, LFA-1 (lymphocyte function associated antigen-1), and ICAM-1 (intercellular cell adhesion molecule-1) [56] or express negative regulators such as PDL-1 (programmed cell death ligand 1), TGF- β , IL-10, FASL (Fas ligand) [57], and B7 family members such as B7H4 [58]. All these factors can hamper the activation of T cells and NK cells, thus impede the antitumor effect. The subpopulation of TSCs in glioma are the only cells able to generate tumors in immune compromised hosts, and TSCs can better escape from the immune attack than non-stem cells do [57]. TSCs in glioma have been shown to be immune privileged, making immunotherapy more challenging. Accordingly, the translation of DC-induced antitumor immunity into clinical activity needs to overcome the GBM-associated immunosuppression, blockade of negative co-stimulatory molecules, and immunotherapy targeted TSCs might improve the antitumor effect.

Besides the tumor ability of immune escape, microenvironment or niche in the GBM tumor mass make matters even more complicated. Pellegatta S et al. used GL261 cells with good immunogenicity to generate brain tumors in C57BL6 mice. Interestingly intratumoral

and subcutaneous injection with DCs could ignite stronger immune response than subcutaneous injection only, and then prolong the survival of GL261 beard mice [50]. This indicated that brain tumors may have their unique niche which is different from other solid tumors. Many immune suppressive lymphocytes such as Treg [59], MDSC (myeloid-derived suppressor cells) [60], M2 microglia [61] have been reported to be recruited to GBM to suppress the local immune system, and even favor the tumor growth. Other FAP+ stromal cells might also contribute to the immunosuppressive microenvironment [62]. Some factors including IL-6 [63], CSF-1 (colony stimulating factor 1) [64], and TGF- β [65] are secreted into tumor niche, and they also played an important role in the "cross-talk" between tumor cells and host immune system.

As tumor vaccination studies continue, many new approaches of tumor immunization strategies have come about such as use of PD-1 (programmed death receptor-1). HGG was considered to have better immunogenicity, and then have better response to immunotherapy. Prin et al. showed that patients with mesenchymal GBM were more sensitive to immunotherapy according to the gene analysis [24]. However, Okada et al reported that low grade gliomas also have a good response to DC vaccine [66].

Conclusion

DCs based immunotherapy might improve the overall survival of HGG, and could serve as a novel treatment modality. However, the design of the tumor antigens, administration of DCs, and the prediction of the response need more progress. Although, many of the phase II studies have been promising, there are still considerable challenges that need to be addressed. Larger phase II and III immunotherapy trials are warranted to confirm the effectiveness. The other important factor to be determined is which patients will best benefit vaccine therapy and disease outcome expected.

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