

Steven de Vleeschouwer, M.D., Ph.D.

Department of Neurosurgery,
University Hospital Gasthuisberg,
Leuven, Belgium; and
Laboratory of Experimental
Immunology,
Catholic University Leuven,
Leuven, Belgium

Marion Rapp, M.D.

Department of Neurosurgery,
University Hospitals Heinrich
Heine University,
Duesseldorf, Germany

Rüdiger V. Sorg, Ph.D.

Institute for Transplantation
Diagnostics and Cell Therapeutics,
University Hospitals Heinrich
Heine University,
Duesseldorf, Germany

Hans-Jakob Steiger, M.D.

Department of Neurosurgery,
University Hospitals Heinrich
Heine University,
Duesseldorf, Germany

Walter Stummer, M.D.

Department of Neurosurgery,
University Hospitals Heinrich
Heine University,
Duesseldorf, Germany

Stefaan van Gool, M.D., Ph.D.

Department of Paediatric Oncology,
University Hospital Gasthuisberg,
Leuven, Belgium; and
Laboratory of Experimental
Immunology,
Catholic University Leuven,
Leuven, Belgium

Michael Sabel, M.D.

Department of Neurosurgery,
University Hospitals Heinrich
Heine University,
Duesseldorf, Germany

Reprint requests:

Steven de Vleeschouwer, M.D., Ph.D.,
Department of Neurosurgery,
University Hospital Leuven,
Herestraat 49,
B-3000 Leuven, Belgium.
Email: Steven.devleeschouwer@
uz.kuleuven.ac.be

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DENDRITIC CELL VACCINATION IN PATIENTS WITH MALIGNANT GLIOMAS: CURRENT STATUS AND FUTURE DIRECTIONS

OBJECTIVE: Despite recent advances in neurosurgical resection techniques, radiation therapy, and chemotherapy, malignant gliomas continue to have a dismal prognosis because relapses are unavoidable.

METHODS: Dendritic cell vaccination has recently emerged as a promising type of active immunotherapy that aims to induce rather than transfer specific antitumor immune responses in patients. Active immunotherapy is the only type of immunotherapy able to induce immunological memory.

RESULTS: Although an increasing number of small clinical trials show safety, feasibility, and immunological and clinical responses, this technology requires further clarification of some critical basic and clinical issues before its presumed place in the treatment of malignant gliomas can be specified. This article addresses the basic and clinical pitfalls that, more than with conventional therapies, may interfere with the potential benefits of this approach.

CONCLUSION: Considering the particular mechanisms involved in the immune modulation of tumor biology using dendritic cell-based vaccinations, the authors summarize the arguments in favor of a further, appropriate assessment of this technology.

KEY WORDS: Dendritic cell, Glioblastoma, Immunotherapy, Review, Tumor vaccination

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THEORETICAL CONCEPT OF DENDRITIC CELL VACCINATION

Dendritic cells (DCs) are a subset of white blood cells that are critical to most aspects of adaptive immunity because of their central role as specialized antigen-presenting cells in the initiation phase of T cell responses (*Table 1*). Typically, DCs reside as immature cells in almost every organ and tissue at the interface of potential pathogen entry sites. In this state, they continuously sample antigens. This sampling, however, results in effective antigen presentation only when DCs are also triggered by danger signals derived from pathogens, tissue damage, or signs of inflammation. Danger-triggered DCs start to mature and they up-regulate chemokine receptors, which guide them to draining lymph nodes. There, the mature DCs are capable of inducing primary T cell responses because of their high levels of

major histocompatibility complex (MHC), adhesion, and costimulatory molecule expression. Only mature DCs show the typical dendritic morphological features with multiple cytoplasmic extensions (*Fig. 1A*) (7). As opposed to the other antigen-presenting cells, such as macrophages, monocytes, and B cells, DCs are able to present and crosspresent the antigenic peptides in the context of both MHC Class II and I molecules, respectively (75, 76). In this way, they can prime not only CD4⁺ T helper cells, but also CD8⁺ cytotoxic T-cells (18). Both effector cell types are thought to be necessary to induce an effective cell-mediated immune response (27, 52, 90). Direct recognition of tumor cells by unprimed T cells, in contrast, induces T-cell anergy rather than immunity because the costimulatory molecules are missing on the tumor cells (96, 97).

DCs are not only sentinels in the adaptive immune response, but have also been shown to be strong activators of natural killer (NK)

TABLE 1. Definitions of general immunological terminology

Adaptive immunity	Includes all immunological responses that have been “learned” by the organism on encountering the pathogen. Typically involves an antigen-presenting cell such as the dendritic cell as well as B and T lymphocytes for the humoral (antibody production) and cellular (cytotoxic cells) immune response, respectively.
Innate immunity	Includes all immunological responses that have been acquired by the organism before being exposed to the pathogen. Typically involves phagocytic cells (e.g., macrophages, dendritic cells), neutrophils, eosinophils, natural killer cells.
Class I major histocompatibility complex	Self-antigens expressed on the membrane of human cells consisting of proteins, encoded for in the human leucocyte antigen A, B, and C loci. Antigenic epitopes presented to CD8+ T lymphocytes have to be presented in the context of a Class I major histocompatibility complex molecule.
Class II major histocompatibility complex	Self-antigens expressed on the membrane of certain human cells (especially antigen-presenting cells), consisting of proteins, encoded in the human leucocyte antigen DR, DP, and DQ loci. Antigenic epitopes presented to CD4+ T lymphocytes have to be presented in the context of a Class II major histocompatibility complex molecule.
Syngeneic	In syngeneic animal models, the transplanted tumor cell lines are inoculated in inbred animals of the same genetic background as the animals from which the transplanted tumor cell lines originally were derived spontaneously or after carcinogen induction.
Orthotopic	In orthotopic tumor inoculation, transplanted tumor cell lines are inoculated in the same organ as the organ from which they derive. In case of brain tumor cells, the tumor cell lines are inoculated in the animal's brain.
Allogeneic	Allogeneic material has been harvested from another donor individual of the same species and as a consequence expresses a different set of self-antigens as compared with the receptor individual, which, after transplantation, spontaneously leads to a rejection of the transplanted graft or cells.

cells and NK T (NKT) cells (25), thus linking the innate and adaptive immune responses (*Table 1*). In this way, both tumor cells with and without expression of MHC Class I molecules can theoretically be killed (9). All of these particular characteristics make DC a perfect adjuvant in active specific immunotherapeutic strategies in which one aims to induce a specific immune response *in vivo* (71).

JUSTIFICATION OF THE USE OF DC TECHNOLOGY IN GLIOMA THERAPY

Gliomas have been shown to express several tumor-associated antigens (TAAs), such as epidermal growth factor receptor isoform III (62), glycoprotein 240 (49), tenascin (98), survivin (105), squamous cell carcinoma-associated reactive antigen for cytotoxic T cells 1 (39), the α -2 chain of interleukin-13 receptor (70), and melanoma-associated antigens, such as tyrosinase, tyrosinase-related protein 1 and 2, glycoprotein 100, melanoma antigen-1, and melanoma antigen-3 (17). Until now, however, identification of a universally expressed glioma TAA with a critical downstream cell survival-related function has not been identified. Therefore, just targeting the known TAA using individual peptides would inherently lead to immune escape because of the positive clonal selection of antigen-loss variants (33); those tumor cell clones that do not express the

particular, targeted TAA (anymore) escape from the immune rejection and, thus, have an important proliferation advantage compared with the cell clones that do express the targeted TAA. That heterogeneity in TAA expression in gliomas represents the main reason why we favor the use of whole-tumor cell lysates as a source of TAA to load the DCs. In case the TAAs are not expressed exclusively on the tumor cells, but also on normal, healthy cells, both tolerance and induction of autoimmunity are possible, theoretical hurdles to a beneficial immune response. In the former case, a rejective immune response cannot be induced because the TAA is considered a self-antigen, and, in the latter case, a pathological immune response against normal tissues is mounted.

Tumor vaccination strategies are not new (10). Several vaccination strategies have been used in the past, especially for the spontaneously more immunogenic tumors, such as malignant melanoma (66), renal cell carcinoma (37), and prostate carcinoma (65). Only recently, the clinical implementation of tumor vaccinations, either with or without DCs, have led to real enthusiasm in larger prospective, randomized trials for the treatment of prostate carcinoma (13) and renal cell carcinoma (42), respectively.

Malignant gliomas, however, reside within the relatively immune-privileged central nervous system (100). Moreover, several reports have described various immune-suppressive properties of these tumors (11, 72, 78). Nevertheless, proof of the prin-

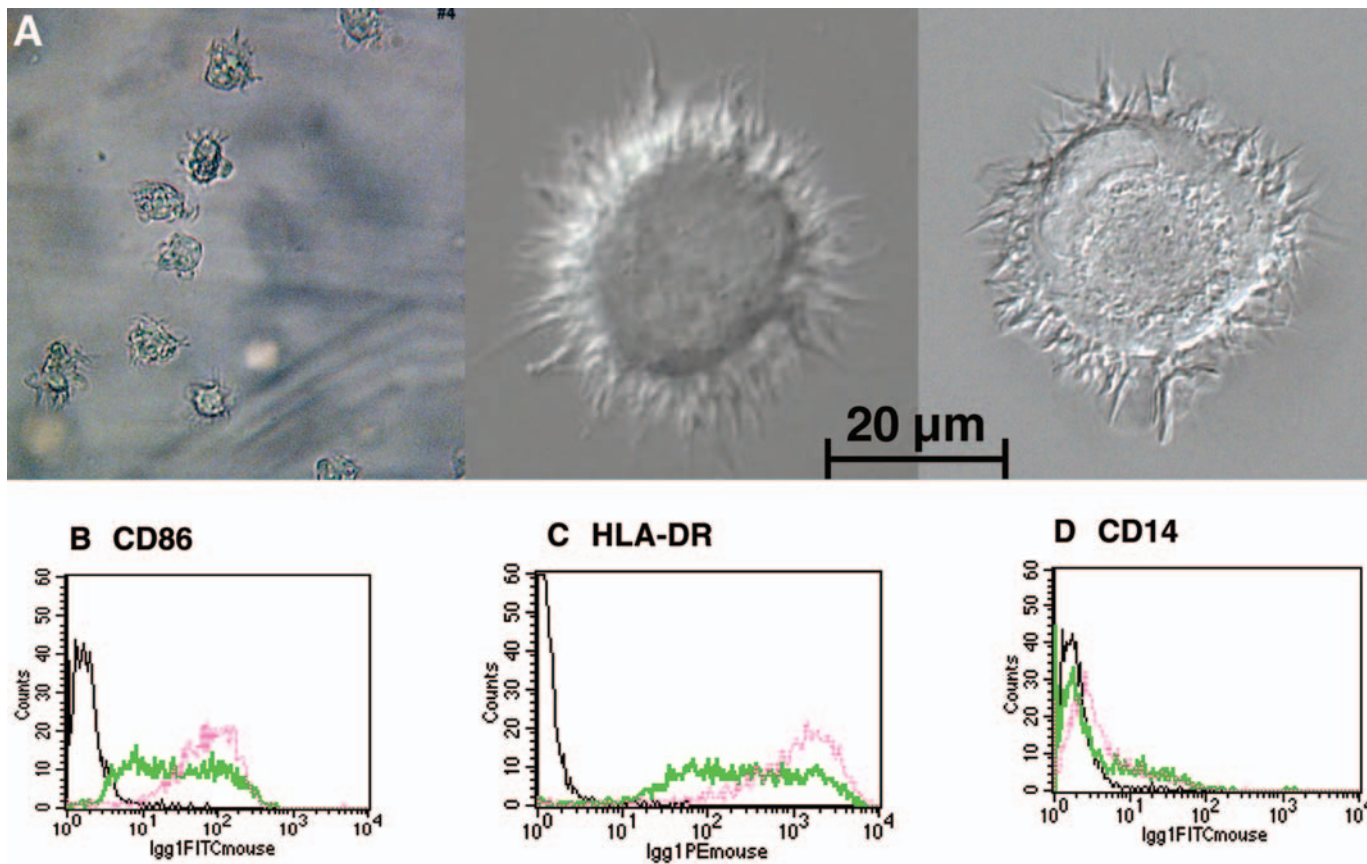


FIGURE 1. Minimal phenotypical criteria for monocyte-derived dendritic cells (Mo-DCs). A, (left panel) light microscopy image and (middle and right panels) phase-contrast microscopy images of mature DCs in culture displaying the typical cytoplasmic veils. B–D, histograms depicting the typical phenotype assessed by fluorescence-activated cell sorter (FACS) analysis of (green line) immature DC and (pink line) mature DC in comparison with an isotype control (black line; mouse antihuman immunoglobulin G1). CD86 (B) and HLA-DR (C) is expressed in immature DC and is upregulated further in mature DC. D, CD14, a typical monocyte marker, is downregulated in both immature and mature DC.

ciple has been demonstrated in in vitro experiments (21) and in several rodent models (69, 83). Our group showed that DCs are capable of taking up antigens from glioblastoma tumor cell lysate and of presenting tumor antigen-derived peptides to T cells in an MHC Class I and II-restricted manner (21), leading to tumor cell-specific cytotoxicity. Yoshida et al. (109) also showed that T cells can be primed in vitro against glioblastoma cells by autologous DCs loaded with glioblastoma tumor cell lysate. Recently, in addition to the induction of a specific immune response, the potential use of DC to enhance NK cell- and NKT cell-mediated antitumor immunity has been demonstrated clearly (25). In several rodent models (reviewed in Reference 23), induction of protective immunity and immunological memory against syngeneic orthotopic gliomas have been shown after vaccination with DCs loaded with TAA of different antigen sources. Although both prophylactic vaccination (35) and therapeutic vaccination (54) seem to be possible, the relative time frame of tumor inoculation and DC vaccination have been shown to be crucial, further documenting that an established brain tumor can modulate and escape the immune response.

In the clinical field, several early-phase clinical trials have shown feasibility and safety as well as immunological and clinical responses after DC-based vaccination therapies (Table 2). Recently, large-scale production of clinical grade DCs became possible (92), including the development of several closed culture systems to obtain large amounts of DCs for clinical use (63, 86, 94). Regardless of the source and nature of TAA used, neither a single clinical trial nor any animal model of DC vaccinations for malignant gliomas without further manipulation showed evidence for the induction of autoimmune encephalomyelitis.

REPORTS ON CLINICAL TRIALS OF DC VACCINATION FOR PATIENTS WITH MALIGNANT GLIOMA

Eleven Phase I/II trials or case reports have been published to date, in which 123 patients with malignant glioma have been treated using slightly different variants of DC vaccina-

TABLE 2. Clinical trials and reports of dendritic cell-based vaccination in patients with malignant gliomas*

Series (ref. no.)	No. of patients (type of trial)	Toxicity data	Immunological response	Clinical response
Liau et al., 2000 (53)	1 (case report)	None	In vitro T-cell proliferation assay: measurable cellular immune response	None
Yu et al., 2001 (111)	9 (Phase I)	Mild fever, nausea and vomiting (n = 1), general lymph node swelling (n = 1)	JAM assay: systemic T-cell cytotoxicity against tumor (n = 4)	Not reported
Kikuchi et al., 2001 (45)	8 (Phase I)	Erythema at the injection site (n = 1)	FACS assay: increase of percentage of NK cells in PBMC (n = 4); ELISA in supernatant: increase of IFN- γ production (n = 6)	Mixed response (n = 1) (steroid administration during vaccination [n = 5])
Yamanaka et al., 2003 (106)	10 (Phase I and II)	Mild headache (n = 1)	FACS assay: increase of percentage of NK cells in PBMC (n = 5); positive DTH reaction to tumor lysate (n = 3); ELISPOT: increased T-cell mediated antitumor activity (n = 2)	Minor response (n = 2)
Wheeler et al., 2003 (102)	1717 (Phase I) 1717 (Phase II)	Not reported (trial designated to study the influence of CD8 + RTE on survival)	Not reported	Not reported
Yu et al., 2004 (110)	14 (Phase I and II)	Transient headache (n = 3), fatigue (n = 2), erythema at the injection site (n = 1), generalized convulsions (n = 2)	qPCR assay: IFN- γ release in PBMC (n = 6); tetramer staining: expansion of CD8 + antigen-specific T-cell clones (n = 4); JAM assay: systemic T-cell cytotoxicity against tumor (n = 1)	Significantly increased median survival
de Vleeschouwer et al., 2004 (22)	1 (case report)	Transient increase in morning stiffness in the left leg	Positive DTH reaction to tumor lysate (n = 1); transient contrast enhancement after fifth vaccine in MRI scan; transient increase in metabolic uptake ratio around resection cavity in Meth-PET	Ongoing tumor-free survival (60 mo after vaccination)
Rutkowski et al., 2004 (79)	12 (Phase I)	Reversible Grade IV neurotoxicity (n = 1), transient Grade II hematotoxicity (n = 2), meningismus after third vaccine (n = 1), nocturnal transpiration after fourth vaccine (n = 1)	Positive DTH reaction to tumor lysate (n = 6)	Partial response (n = 1); tumor-free survival (n = 2; 5 yr after vaccination)
Kikuchi et al., 2004 (44)	15 (Phase I and II)	Grade I fever (n = 4), generalized convulsions (n = 1), transient liver dysfunction (n = 6), transient Grade II leukocytopenia (n = 7)	Positive DTH reaction to tumor lysate (n = 15); Cr51+-release assay: increase in cytotoxic activity (n = 2); intracellular (CD8 + T-cells) ELISA staining: increased IFN- γ (n = 1)	Partial response (n = 4); mixed response (n = 1)
Yamanaka et al., 2005 (107)	24 (Phase I and II)	Mild erythema at the injection site (n = 7), headaches (n = 1)	Positive DTH reaction (n = 8); ELISPOT assay: positive (n = 7); positive immune monitoring predicts good clinical outcome	Partial response (n = 1); minor response (n = 3); significantly increased median survival
Liau et al., 2005 (55)	12 (Phase I, dose escalation)	Nausea and vomiting (n = 3), headaches (n = 2), seizures (n = 1), erythema and swelling (n = 3), fatigue (n = 5), fever (n = 2)	Alamar blue CTL assay: systemic antitumor CTL activity (n = 6)	Partial response (n = 1); significantly increased median survival

* RTE, recent thymic emigrants; FACS, fluorescence-activated cell sorter; NK, natural killer; PBMC, peripheral blood mononuclear cells; ELISA, enzyme-linked immunosorbent assay; IFN, interferon; DTH, delayed-type hypersensitivity; qPCR, quantitative polymerase chain reaction; MRI, magnetic resonance imaging; Meth-PET, methionine-positron emission tomography; Cr51, chromium 51; CD3, general T-cell marker; CD4, helper T-cell marker; CD8, cytotoxic T-cell marker; CD45RO, memory T-cell marker; GBM, glioblastoma multiforme; DC, dendritic cell; rh-IL12, recombinant human interleukin 12. Toxicity data are scored according to the National Cancer Institute Common Toxicity criteria. Clinical responses are defined as follows: partial response is an incomplete regression of the tumor of 50% or more; mixed response is a partial response in one lesion together with a growing second lesion; minor response is a documented regression of the tumor of between 25 and 50%. Stable diseases were not included in this table.

tion. Safety, feasibility, and clinical and immunological bioactivity have been demonstrated in these publications, and proof of the principle has been confirmed in humans. A short overview and interpretation is given below.

Liau et al. (53) reported on a patient with a recurrent brainstem glioblastoma multiforme (GBM) in whom they demonstrated a measurable cellular immune response, but no clinical response, after three intradermal injections of autologous DCs pulsed with allogeneic acid-eluted GBM peptides, which could theoretically differ from the TAA in the vaccinated patient.

Yu et al. (111) treated nine patients with malignant glioma with three intradermal injections of DCs loaded with autologous glioma cell peptides and found a robust intratumoral infiltration of cytotoxic and memory T cells in two of four reoperated patients. The vaccinated group had a prolonged median survival as compared with a larger matched control group treated at the same institution, thus providing preliminary evidence of a beneficial effect of vaccination.

Kikuchi et al. (45) fused glioma cells and DCs to vaccinate eight patients with malignant glioma and reported two minor responses and an increase in NK cells in the peripheral blood, thereby pointing toward a possible aspecific activation of the innate immune response after DC vaccination.

Yamanaka et al. (106) treated 10 patients with DCs loaded with GBM tumor cell lysate, intradermally in five patients and intradermally plus intratumorally in another five patients. They reported unspecific and specific T cell-mediated immunity, minor clinical responses, and T-cell infiltration in the tumor.

Wheeler et al. (102) described a striking correlation between thymic function (CD8⁺ recent thymic emigrants), age, and outcome in 17 patients with GBM who had been vaccinated with DC loaded with autologous tumor cell lysate. This complex finding may point toward an important prognostic value of age in immunotherapeutic strategies, probably even more than for conventional therapies.

Yu et al. (110) reported a significant increase in median survival of eight patients with recurrent GBM (133 wk) treated with DCs loaded with autologous tumor cell lysate, as compared with a matched control group of 26 patients treated in the same institution (30 wk). The total group of vaccinated patients consisted of 14 patients with malignant glioma. No adverse events were noted. Immunological responses were measured using quantitative reverse-transcriptase polymerase chain reaction analysis of interferon (IFN)- γ production and human leukocyte antigen (HLA)-restricted tetramer staining of CD8⁺ T cells.

We found immunological and clinical responses in a pilot group of 12 patients with recurrent malignant gliomas who were treated with intradermal injections of DCs loaded with autologous tumor cell lysate after reoperation. We stressed the importance of a minimal residual disease status after surgery before starting the adjuvant DC vaccinations for safety and efficacy reasons (79). Two of six patients with a macroscopically complete resection before vaccination remain in complete

remission 5 years later. This underscores the importance of appropriate assessment techniques of clinical efficacy if DC vaccination is used as a postoperative adjuvant treatment when no measurable disease is present.

In a pilot patient with a radiation-induced recurrent malignant glioma, we demonstrated a transient immune response after DC vaccination after surgery using the combination of magnetic resonance imaging and methionine positron emission tomography (22). This report illustrates that imaging findings after active immunotherapy can be hard to interpret because inflammatory reactions can occur.

Kikuchi et al. (44) vaccinated 15 patients with high-grade glioma using fusions of autologous tumor cells and DCs together with recombinant human IL-12 and found partial regressions in four out of 15 patients.

Yamanaka et al. (107) reported on the vaccination of 24 patients with recurrent malignant gliomas (18 GBM and six Grade III tumors) using DCs loaded with autologous lysate plus intratumoral that were injected either intradermally or intratumorally. In general, the vaccinations were well tolerated. The study was conceived to be a Phase I trial (17 patients) in which immature DCs were used and a Phase II trial (seven patients) in which immature DCs were injected intratumorally and mature DCs were injected intradermally. They described one partial response, three minor responses, stable disease in 10 patients, and progressive disease in 10 patients. They reported several interesting findings, such as a significantly better overall survival and a much higher rate of 2-year survival of the vaccinated patients (23%) compared with a matched control group (3%), a longer survival for the patients who received both intradermal and intratumoral injections, and a clear prediction of the clinical outcome by positive findings in the immune monitoring assays.

Recently, Liau et al. (55) reported a Phase I dose-escalation study in 12 newly diagnosed GBM patients whom they vaccinated with DCs loaded with acid-eluted peptides from established cultures of autologous tumor cells. They did not find any indication of dose-limiting toxicity. Furthermore, they documented systemic antitumor cytotoxic lymphocyte activity and intratumoral T-cell infiltration in 50% of the tested patients. The vaccinated patients exhibited a significantly increased median progression-free survival (8.2 mo) and overall survival (OS) (18.3 mo). They also postulated that a minimal residual disease status may be an important prerequisite for a clinical benefit of DC vaccination based on the finding that tumor-derived transforming growth factor β (TGF β) modulates the local immune response; TGF β production was inversely correlated with intratumoral T-cell infiltration and overall patient survival.

The main objection raised against cancer vaccine approaches is the perceived low rate of reported objective clinical responses (77) despite the clearly documented immunological efficacy, which is still considered to be a surrogate marker of clinical efficacy (19). We feel strongly that the conventional oncological response criteria (58, 91), essentially developed to monitor

effects of radiotherapy and/or chemotherapy, may not be fully appropriate to measure the beneficial effect of an active immunotherapeutic approach. Moreover, if DC vaccinations in patients with malignant gliomas are applied as an adjuvant therapy after gross total tumor removal by the neurosurgeon, no measurable disease is left to assess the clinical efficacy in terms of volume reductions. Although several objective magnetic resonance imaging-measurable and positron emission tomography-measurable responses have been documented in patients with malignant gliomas treated with DC-based vaccinations (22, 79), we favor assessment of the net OS benefit. Significantly better OS of vaccinated patients in large prospective, randomized trials for renal cell carcinoma (42) and prostate carcinoma (13) shows the importance of this crucial outcome parameter. In the latter trial, the fact that OS, but not progression-free survival, was prolonged in the vaccination arm of the trial clearly demonstrates that cancer vaccines probably act in a slower, more indirect way to modulate tumor biology compared with the conventional cancer therapies. Neglecting the particular nature of the immune mechanisms that modulate tumor evolution after vaccination can be a major pitfall for correct assessment of its value in an oncological treatment.

QUESTIONS THAT REMAIN TO BE ELUCIDATED

Table 3 outlines the remaining issues discussed below.

Vaccine Preparation

Different subsets and lineages of DCs have been described, and recent classifications distinguish between myeloid

(CD11c⁺) and lymphoid (CD11c⁻ DC precursor cells), both deriving from CD34⁺ hematopoietic stem cells (6, 57). The former give rise to Langerhans DCs (CD11c⁺, CD1a⁺) in the epidermis and to interstitial DCs (CD11c⁺, CD1a⁻) in the other tissues. Lymphoid DC precursor cells give rise to plasmacytoid DCs, which preferentially produce IFN- α , whereas the interstitial DCs preferentially produce IL-12 on activation. Most clinical studies to date have been carried out with ex vivo-generated monocyte-derived DCs, which resemble myeloid, interstitial DCs. Clinical grade DCs can also be generated ex vivo from CD34⁺ blood stem cells (59) or they can be isolated directly from fresh human blood, in which they represent only approximately 0.3% of leukocytes (15). Using FMS-like tyrosine kinase-3 ligand, however, they can be mobilized in vivo before their direct isolation (31). To date, none of these DC subtypes have been proven to be superior in comparative clinical trials, so any preference for a certain subtype is based mainly on perceived preclinical differences deemed to be important and pragmatic considerations regarding availability and harvesting procedures.

The choice of the optimal DCs may be even more troubling if one considers the heterogeneity in the literature about the phenotypic characteristics. The minimal, common phenotypic criteria are the stellate morphological features (cytoplasmic veils; Fig. 1A), the upregulation of CD86 and HLA-DR, and the downregulation of CD14 (Fig. 1, B–D) (80). Further phenotypic markers are more inconsistent and include a variable expression of CD1a, CD80, CD83, CD11b, CD11c, CD25, CD54, CD58, and CD40 (5, 30, 32, 38, 63, 68, 86, 92, 94, 106, 111). However, these different phenotypic characteristics may play an important role. In particular, the maturation status of the DC seems to be crucial. In physiological conditions, the maturation of DCs takes place during the migration of the immature DC toward the draining lymph nodes, after they capture the pathogen and are triggered by a danger signal (7). The upregulation of HLA-DR, CD80, CD86, and CD83 is considered to be a typical sign of maturation and is deemed to be important for the capacity of DCs to activate T cells properly (29, 50). Immature DCs take up antigen well, but can induce tolerance rather than immunity (87, 99) and are thought to have less migratory capacities to draining lymph nodes compared with mature DC, partially based on their lack of CCR7 expression, which is a chemokine receptor involved in lymph node homing of DCs (24). However, this is not a consistent finding, and Barratt-Boyes et al. (8) did not find any difference in migratory capacities of immature and mature DCs in their monkey model. The few available comparative clinical data also support the use of mature rather than immature DCs for tumor vaccination (24, 43, 61).

Several different methods to mature DCs have been described and include the use of a classical proinflammatory cytokine mixture of tumor necrosis factor α , IL-1 β , prostaglandin E2, and IL-6 (92); CpG motifs (36); bacterial lipopolysaccharides (4); viral double-stranded ribonucleic acid (16); poly I:C (3); and CD40 ligand (82). No direct comparative data of these methods exist regarding the DC's ability to induce a clinically relevant immunization in oncological

TABLE 3. Basic and clinical issues in dendritic cell vaccination to be solved^a

Vaccine preparation	Optimal subset and source of DCs Optimal phenotype and purity of DCs Maturation status of DCs Source, nature, and dose of tumor antigens Optimal number of DCs per vaccination Antigen loading and DC maturation protocol
Clinical implementation	Optimal (sub-)group of patients Timing of PBMC harvesting Timing and frequency of DC vaccination Site of DC injection Immune monitoring

^a DC, dendritic cell; PBMC, peripheral blood mononuclear cell.

patients. Some reports, however, mention the presumed better cytokine production profile of immature DCs (93), and some groups continue to work with immature DCs in tumor vaccination trials (110).

DCs can capture, process, and present tumor antigens from different sources, including purified defined peptides (60), undefined acid-eluted peptides from autologous or allogeneic tumor cells (112), tumor cell lysate (28), viral vectors containing genes (85), apoptotic bodies (2), necrotic tumor cells (68), tumor homogenate (35), and messenger ribonucleic acid (12). Although none of these sources has been proven to be superior, the use of total tumor-derived material as a source of tumor antigens may be preferable for glioma vaccination strategies; the positive clonal selection of antigen-loss variants if the immunization is targeted to only one TAA, which may not be expressed by a subpopulation of tumor cells and which may not be essential for tumor cell survival, leads to a classical tumor-escape phenomenon (33). Secondly, loading the DC with unfractionated tumor material may provide peptides for both MHC Class I and II presentation (75, 76). The main disadvantage of using a mixture of undefined tumor antigens, however, is the difficulty in immune monitoring of the vaccinated patient because the molecular target of the response is unknown, in addition to the potential transfer of factors used by the tumor cells to evade immune responses onto the DC. Recently, Akasaki et al. (1) showed the potential harmful influence of apoptotic glioma tumor cells on the DC, especially if the tumor contains many cyclooxygenase 2 overexpressing cells. In contrast, the risk of autoimmunity, which theoretically may be increased when using unfractionated tumor cell lysates (101), seems to be low because no autoimmune encephalomyelitis or other autoimmune phenomenon have been reported after DC vaccination for malignant gliomas to date. If DC-based tumor vaccination for malignant gliomas is used as a postoperative, adjuvant treatment against remaining infiltrating cells as suggested (79), it should still be pointed out that proliferating and infiltrating tumor cells display a comparable set of TAA (ongoing research).

Finally, no hard data are available about the optimal number of DCs that should be injected per vaccination. Because the immune system seems to function as an on-or-off system, rather than following classical dose responses, a typical dose-escalation study is probably not useful. Commonly, the arbitrary amount of 5×10^6 DC per vaccination is often used as a minimum (80). If, however, a lower number of DC may suffice to induce clinically relevant responses, then the overall costs of the treatment may be reduced considerably. An enhanced immunogenicity of DC-based vaccination can be expected if certain additional adjuvants, for instance, human IL-12 (44) or granulocyte-macrophage colony-stimulating factor (84) are injected together with the DCs. Recent data also suggest a potential immune-enhancing role of small interference ribonucleic acid technology to modify DC biology and to reduce immunosuppressive activities of the tumor cells (46).

TABLE 4. Potential combination therapies together with dendritic cell vaccination^a

Systemic therapies	Nonimmunosuppressive chemotherapy Inhibitors of angiogenesis Immune adjuvants (e.g., rh-IL12, GM-CSF)
Local therapies	Enhanced resection techniques Local delivery of immune modulating agents, e.g., TGF β antisense

^a rh-IL 12, recombinant human interleukin 12; GM-CSF, granulocyte-macrophage colony-stimulating factor; TGF β , transforming growth factor β .

Clinical Implementation of DC-based Vaccination in Glioma Patients

Which Patients Should Be Vaccinated?

DC-based vaccination in malignant glioma patients should essentially be considered as an adjuvant therapy after gross total resection of the bulky tumor volume. Based on the well-documented local and systemic immune suppressive effects of malignant glioma (26, 78), a macroscopically total resection creates a more immune-favorable local environment and is expected to result in a more immune-competent status of the patient systemically (ongoing research). Moreover, published safety and efficacy data clearly suggest that a minimal residual disease status may be the ideal condition for a relevant clinical effect of DC-based vaccination in glioma patients (79). Several reported (22, 79, 107) and unpublished data showing objective clinical responses in vaccinated glioma patients with a residual tumor after surgery, however, also support the further assessment of DC-based vaccination in this patient group, probably in combination with a second adjuvant treatment (Table 4).

What Should Be the Timing of This Adjuvant Treatment Strategy?

As for any adjuvant strategy, we think that the potential benefit of this vaccination will be at its maximum if the vaccines are administered as soon as possible after the conventional therapy of maximal, safe surgery and radiotherapy or chemotherapy, or both. If this treatment option is considered in recurrent malignant gliomas, which was the case in the vast majority of the reported patients so far, the aggressive and rapid (re)growth of the tumor requires a vaccination quickly after reoperation. Several aspects that can cause a possible delay, however, should be considered. Almost all patients operated on for malignant gliomas receive steroids in the perioperative period. A fast weaning from steroids, generally accepted to be easier after gross total resection than in cases of partial resection, provides better chances for a faster immune normalization, thus allowing more rapid generation and application of the vaccines. Because of the reported systemic immune suppression in patients harboring a malignant glioma (26, 78), performing the leukapheresis procedure to harvest autologous monocytes before surgery cannot be rec-

ommended. Moreover, ongoing research suggests a dramatic and fast restoration of the systemic immune status of patients with a malignant glioma in the early postoperative period. This would be consistent with the findings that CD8⁺ T-cell function is correlated with the tumor load, as shown in patients with advanced ovarian carcinoma (20). Because no hard data about the number and frequency of vaccinations are available, any schedule remains essentially empirical. In most vaccination trials, however, three vaccination sessions seems to be the minimum.

How and Where Should We Vaccinate?

Theoretically, injected DCs should reach the adjacent, draining lymph nodes where they prime the (naïve) T cells (7). Although it has been shown that DCs migrate to the adjacent lymph nodes fewer than 30 minutes after subcutaneous injection, the maximum accumulation of DCs in the draining lymph nodes occurs between 48 and 72 hours after injection (48, 74). Activation of the T cells in the lymph nodes is thought to take at least another 1 to 2 days (51). Several reports have shown that intradermal or subcutaneous injection of DC is better than intravenous injection (30). Moreover, the different routes of application seem to be related to the immune protection of different sites in the body, with a more complete protection in the case of subcutaneous or intradermal administration compared with intravenous injection (64). As far as the induction of a protective immunity in the brain is concerned, recent animal experiments indicate that, although the cervical lymph node is the draining lymph node for spontaneous immune responses to brain tumors, it is not the identity of this lymph node that determines efficient brain tropism (14). Thus, for DC vaccination, a vaccination site in the proximity of inguinal lymph nodes would be expected to be as efficient as a site near the cervical lymph nodes, because it is the properties of the DC that are predicted (from this study) to be paramount in determining tissue tropism. The functional importance of this was demonstrated by homing studies of T cells primed *in vivo* by endogenous DCs having captured antigen from tumor implanted in different sites. A 2.5-fold preferential entry to the brain was demonstrated for T cells primed in cervical lymph nodes with the tumor implanted in the animal's brain, compared with T cells primed in inguinal lymph nodes with the tumor implanted subcutaneously in the flank of the animals (14).

Exploring Synergistic Activity with Other Therapies

Because malignant gliomas inevitably relapse after any combination of conventional therapies and because growing evidence suggests that tumor vaccination may modulate tumor biology in a slower, more indirect way than the conventional therapies (13), it seems to be justified to look for possible synergistic effects between distinct therapies (Table 4).

Enhanced Surgical Resection Techniques

As already discussed above, a macroscopically complete resection induces the optimal condition to apply adjuvant

DC-based vaccination therapy in patients with malignant gliomas. This implies that advanced surgical resection techniques, for instance, fluorescence-guided resection of malignant gliomas, which substantially increases the extent and the percentage of gross total resections (88), may not only lead to a net survival benefit of its own (89), but also creates the most ideal situation in a greater number of operated patients to start DC vaccination after surgery.

Radiotherapy and Chemotherapy

Secondly, several theoretical considerations and preliminary reports (56, 103) support a combination of vaccination therapy and (nonimmunosuppressive) chemotherapy. Sublethal triggering of apoptosis pathways by vaccination, followed by further tumor cell killing by effective chemotherapeutics, such as temozolomide, is one of the suggested mechanisms possibly leading to a higher response rate after chemotherapy in vaccinated patients (103). Recently, a molecular basis for this perceived synergism has been described (56). In addition, the perceived relative depletion of regulatory T cells, which compromise the induction of an immune response after chemotherapy (73) and the higher yield of specifically primed effector T cells because of the principle of T-cell homeostasis if vaccination is performed during the phase of immune reconstitution (41), could be important arguments furthering the assessment of vaccination after nonmyeloablative, lymphodepletive chemotherapy. It has already been suggested that local radiotherapy may remove suppressor T cells, thus permitting a more effective T-cell stimulation *in loco* (67). Very recently, Kjaergaard et al. (47) showed that active immunotherapy with a single administration of a DC-tumor fusion vaccine in combination with local cranial radiotherapy and adjuvant anti-CD134 monoclonal antibodies induced a potent and tumor-specific immune response and cured more than 80% of mice with advanced brain tumors. Considering all these data, there is preliminary evidence to support the application of a DC-based vaccination in close combination with radiotherapy and chemotherapy.

Tumor Biology Modulation

Thirdly, the combination of DC vaccination with inhibitors of angiogenesis may theoretically increase the time window in which active immunization can be performed. Preliminary findings show that DC-based vaccinations can be combined with vinblastine in children with malignant brain tumors without compromising the immune status of the patient (unpublished data).

Finally, it may prove useful to combine the systemic effect of DC-based vaccination together with a modulation of the local tumor environment. The simultaneous use of DC vaccination and convection-enhanced delivery of substances neutralizing, for example, TGFβ (40), could be an attractive strategy, especially in patients with subtotal (or partial) tumor resection. TGFβ is the most prominent glioblastoma-associated immunosuppressant. In addition, it stimulates tumor migration,

invasion, proliferation, and angiogenesis (104). Several anti-TGF β strategies, such as TGF β receptor I kinase inhibitor (95), or a TGF β 2 antisense oligodeoxynucleotide (81), which is currently being evaluated in a randomized clinical trial for recurrent malignant gliomas, could be promising, especially in conjunction with systemic DC vaccination. Based on their capacity to upregulate MHC Class I molecules in malignant glioma cells, IFN- α and IFN- γ may be good candidates for local delivery because they can increase tumor cell susceptibility to T cell-mediated killing (108).

FINAL AIMS AND FUTURE PROSPECTS

Several in vitro assays can give an idea about the ability of DCs to stimulate T-cell immunity, mainly comprising T-cell proliferation, cytokine production, HLA-restricted tetramer staining, and cytotoxicity assays. All of these parameters are important to assess proof of the principle, but inherently remain surrogate markers of efficacy for which no hard data exist regarding their respective clinical relevance as an oncological treatment strategy. As illustrated in *Table 2*, there is no clear correlation between an (in vitro) immune response and classical, objectively measured clinical responses, although this is suggested for other types of tumors (34). Although no hard data are available, there may be a correlation between immune responses and survival time, as recently suggested by Yamanaka et al. (107). At least some data revealed the striking correlation between age, CD8⁺ recent thymic emigrants, and prognosis in vaccinated patients with malignant glioma (102), indicating the crucial involvement of age and thymic function in this type of therapy. The final assessment of the true value of all the strategy variants outlined in the previous sections requires the clinical evaluation of consecutive cohorts of patients, with postoperative minimal residual disease status, each treated according to a slightly modified protocol of DC vaccination in a cohort-comparison model. Parameters such as progression-free survival and especially OS should be guiding end points in the analysis of these cohorts more than exclusively the classical objective response measurements on magnetic resonance imaging, positron emission tomography, and immunomonitoring.

Ideally, this approach may lead us to the optimal strategy with an enhanced immunogenicity and preserved selectivity. Theoretically, the use of a battery of TAAs, which, combined, cover the phenotypes of all the remaining tumor cells, can be a potent and safe alternative for the whole tumor cell approach as a source of TAA. Finally, this optimized DC vaccination strategy for malignant gliomas should then be validated in a prospective, multicenter, randomized Phase III trial, probably as a treatment following surgery, radiotherapy, and chemotherapy before vaccination and consolidating non-immunosuppressive chemotherapy after vaccination. Therefore, several centers with clinical neuro-oncological expertise and the appropriate Good Manufacturing Practice facilities for DC therapy should cooperate.

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de Vleeschouwer et al. have written an interesting and fairly comprehensive review on the topic of dendritic cell (DC) immunotherapy for malignant gliomas. This field is moving forward rapidly, with several independent Phase I clinical trials completed throughout the world (e.g., United States, Japan, Europe). Although many immunological and technical questions remain, this form of active immunotherapy is gradually evolving as a potential adjunct to the treatment of patients with malignant glial neoplasms.

The organization of this review into sections composed of theoretical concepts, justification for the use of DC technology, reports on clinical trials, and a discussion of the questions that remain to be elucidated, is outstanding. In this comprehensive and timely review, de Vleeschouwer et al. adequately describe some of the fundamental aspects of DC-based immunotherapy, as well as the current difficulties associated with its clinical application.

Of great interest and relevance is the authors' contention that conventional imaging response criteria (e.g., magnetic resonance imaging or positron emission tomographic scanning), essentially developed to monitor the effects of radio- and/or chemotherapy, might not be fully appropriate to measure the beneficial effects of active immunotherapeutic approaches. I strongly agree with this statement, and would also favor the assessment of net overall survival (with good quality of life) over any imaging or other surrogate measures of "response." As we further develop cellular and other immunotherapies for malignant gliomas, our traditional approaches to measuring response to such novel therapeutics must also develop accordingly.

Another important point brought up by the authors is the potential application of DC-based vaccination in close combination with radiochemotherapy and/or other novel agents (e.g., antiangiogenesis inhibitors, immune modulators, etc.). My opinion is that immunotherapy, like any other form of treatment for malignant gliomas, cannot operate in a vacuum in lieu of other therapies. It is unlikely that any single treatment modality will serve as a magic bullet for brain tumor therapy. The potential role of tumor vaccination approaches, if any, will only come in combination with other conventional and novel therapeutic strategies. Therefore, with further advances in the field of DC-based tumor vaccines and tumor immunology, the prospects of effective clinical trials for the treatment of glioma are optimistic, as long as we carefully apply the lessons that we have already learned. Overall, this is a well-written review that will be helpful for readers interested in staying abreast of recent advances in DC-based immunotherapy and tying together these advances in a logical framework.

Linda M. Liau
Los Angeles, California

The authors have prepared an informative review of an emerging field of research along with the challenges to further experimentation and clinical activity. A clear presentation regarding overall approach at conceptual, experimental, and practical levels is provided. As noted, although the clinical value of DC vaccinations has yet to be seen, preliminary Phase I and II data is noted in the discussion and the extensive bibliography.

Jack P. Rock
Detroit, Michigan

This is a reasonably well written review on dendritic cell vaccination in malignant gliomas. It is an important and relevant subject for

neurosurgeons and investigators. The authors are leaders in the field. There are other recent related reviews on the topic that are available to the international readership, including:

1. Sikorski CW, Lesniak MS: Immunotherapy for malignant glioma: Current approaches and future directions. *Neurol Res* 27:703–716, 2005. (England)
2. Pellegatta S, Finocchiaro G: Cell therapies in neuro-oncology. *Neurol Sci* 26 [Suppl 1]:S43–S45, 2005. (Italy)
3. Wheeler CJ, Black KL: Dendritic cell vaccines and obstacles to beneficial immunity in glioma patients. *Curr Opin Mol Ther* 7:35–47, 2005. (England)
4. Okada H, Pollack IF: Cytokine gene therapy for malignant glioma. *Expert Opin Biol Ther* 4:1609–1620, 2004. (England)
5. Parajuli P, Sloan AE: Dendritic cell-based immunotherapy of malignant gliomas. *Cancer Invest* 22:405–416, 2004. (United States)

The authors are writing a trial of 60 patients and a large Phase II trial has recently started at their institutions. Furthermore, a Phase III randomized, double-blinded trial is being conceived for Spring 2007. These results will be eagerly anticipated by readers of this review.

Nelson M. Oyesiku
Atlanta, Georgia

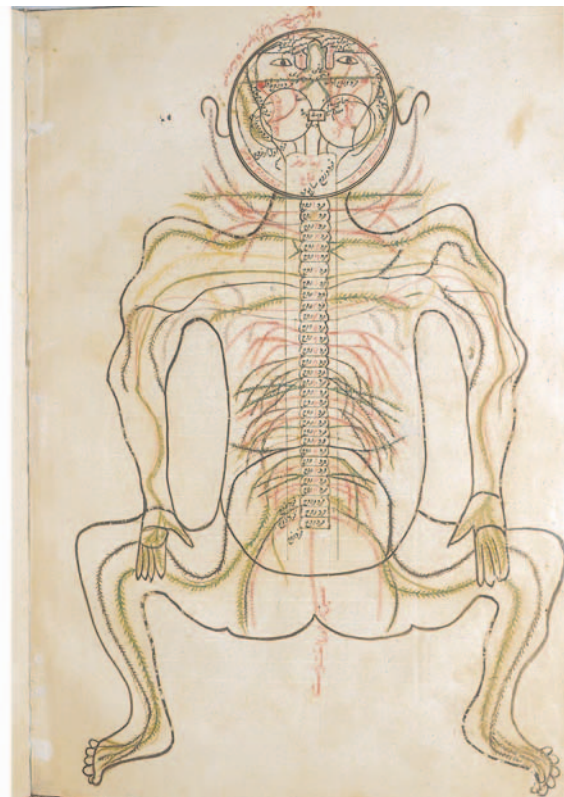
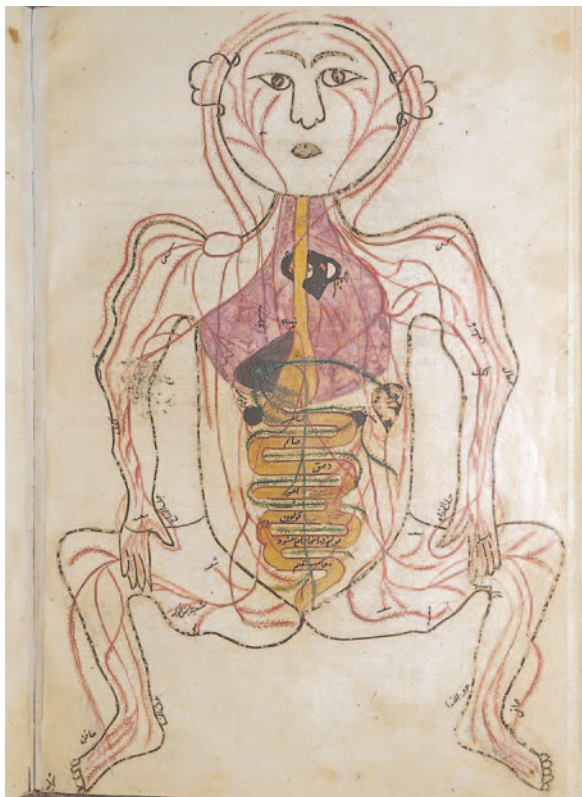
This review by de Vleeschouwer et al. deals with the current state of malignant glioma therapy based on DC vaccination. It is clearly a challenge to cover this subject for a nonspecialized readership because interpreting the clinical data also requires an appreciation of many immunological complexities. The authors' attempt to explain these

issues is commendable, and most of the critical issues in developing this sort of therapy are discussed.

Those in the field may feel that other considerations should be added to the "future prospects" section of this review. To validate the approach of DC vaccination, the necessity of randomized Phase III trials goes without saying. But, in view of the complexity in standardizing DC vaccines for such a trial, the way in which an optimal strategy is chosen is critical. In terms of assessing clinical trial outcomes, the authors rightly mention the difficulties in applying appropriate oncological response criteria and discuss possible improvements.

I would suggest that the other issue that requires further discussion is the apparent lack of correlation between immunological efficacy and clinical efficacy. Unfortunately, immune monitoring in many clinical trials consists of what has been possible to measure within the feasibility, resources, and capabilities of the centers involved. The reported immunological responses are rarely unequivocal tumor-specific responses with functionality at the tumor site. Taking into account such limitations of the data, it is thus difficult to assess the relevant immunological efficacy of a vaccine or to correlate this with clinical efficacy. Because the only proposed mechanism for DC-based vaccination is the induction of tumor immunity, it would seem that we cannot escape the necessity of improving the monitoring of immunological responses in parallel with oncological responses if we are to rationally propose optimal protocols for Phase III testing.

Paul R. Walker
Tumor Immunologist
Nicolas de Tribolet
Geneva, Switzerland



Mansur ibn Ilyas, *Tashrih-i badan-i insan [Anatomy of the Human Body]*. Iran, ca. 1390. (Courtesy of the U.S. National Library of Medicine, National Institutes of Health, Bethesda, Maryland).