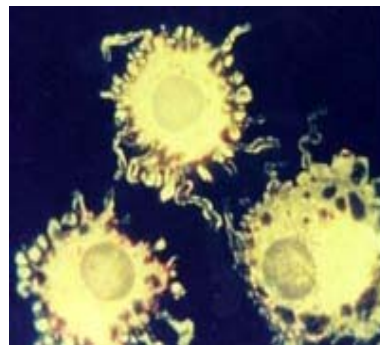


Dendritic Cells

1.1. Introduction

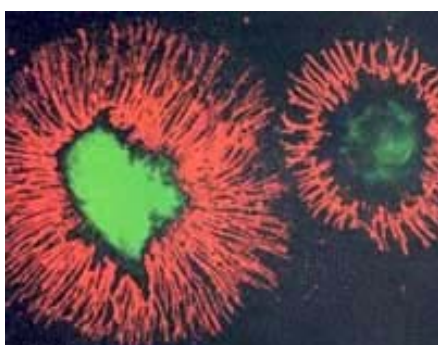
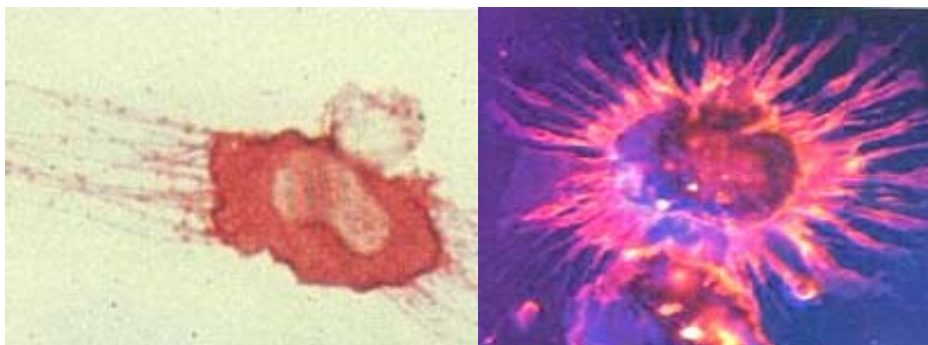
Dendritic cells (DC) belong to the group of antigen-presenting cells (APC). One of the first functions discovered of DC was the very important role they play in the activation of specific immune responses against pathogens. Because of this function, they are often called nature's adjuvant. In recent years, through increasing understanding of the role of DC in the immune system, new areas of clinical application were discovered and conducted. One example may be the improved immune response in vaccination of non-responders. Very recently, the possible role of DC in the biological immune response against malignancies (solid tumors) was discovered and clinically investigated.



DC form a complex network of APC within the organism.[1] In early stages of differentiation, in skin and mucosa, DC have been described as so-called Cells of Langerhans (CL). CL function as perceivers and incorporators of, for instance, viral and bacterial antigens, and toxins. After perception of an antigen, CL are activated by local inflammatory signals (through the production of certain cytokines, including IL-2 and IL-6), and start wandering mainly through the lymphatic system, and to a certain degree through the blood flow, to the secondary lymph nodes. Here, as fully differentiated, so-called inter-digitating dendritic cells (IDC), they settle in the T-cell areas and begin to activate T-cells antigen-specifically. Thus, the function of DC is the transportation of antigen from the location of infection to the secondary lymph nodes, where the specific immune response against the antigens and toxins is orchestrated. Through this transportation of antigens by DC to the lymph nodes, it is made easier for T-cells to come into contact with the antigens, as non-activated T-cells circulate through the peripheral lymph system several times during a 24-hour period. After being activated by DC, T-cells change their wandering pattern, and become effector cells at the location of infection. As cytotoxic T-cells, they destroy infected cells in the body, and, interestingly, also tumor cells. As T-helper cells of the TH-1 type, they stimulate macrophages in their phagocytic and bactericidal activities, by producing inflammatory mediators, to eradicate intra-cellular pathogens, like tuberculosis bacteria. As TH-2 cells, they support the humoral immune response by activating the anti-body producing B-cells.

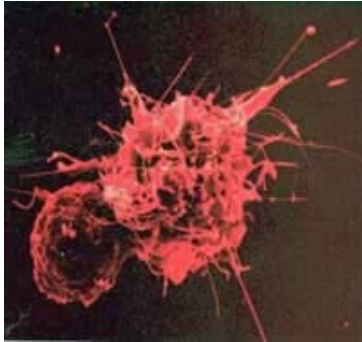
APC play an important role in recruiting antigen-specific T-cells to adapt properly the immune response. Usually, T-cells by themselves do not recognize antigens. APC have to prepare this recognition process. This means that within the APC, the (specific) antigen is dissected into much smaller peptides, and bound to the major histocompatibility complex class I (MHC-I). Afterwards, this MHC-I is presented at the surface of the cell membrane. Only then, T-cells are able to perceive the complex between the MHC-I molecule and the antigen peptide. This leads to the activation of the resting T-cells. In the periphery of the body CL are specialized in antigen recognition and antigen processing, but still have little potential for activation of resting T-cells. After their wandering to the secondary lymph nodes, and maturation into IDC, the process is reversed. Now, the IDC have lost the ability to recognize and process the antigen almost completely. Instead, IDC, after having incorporated and presented the antigen on the cell surface in its CL stage, are now able to activate resting T-cells. Thus, within the lymph node, IDC guarantee that a most accurate picture is transmitted to the T-cells about the nature and the characteristics of the infection in the periphery. The specific capability of DC to activate naïve T-cells can be used in immune therapies, like in cancer. The purpose of our clinical and research activities will be, to sensitize DC for tumor-specific antigens. Thus, returned to the cancer patient through an infusion (as a cellular vaccine), in this way specifically activated T-cells of the patient will be able to eliminate tumor cells and pathogens, including chronic viral infections, like hepatitis C, human papilloma virus (HPV) in persistent cervix dysplasia, and HIV. Recently, it was found that pre-loaded DC with human immunodeficiency virus type 1 (HIV-1) provoked strong responses from CD8+ T-lymphocytes of late-stage HIV-1 infected individuals. These responses were enhanced under application of gamma-interferon and interleukin-2, strongly suggesting that DC of HIV-1 positives can be engineered to evoke a stronger anti-HIV-1 CD8+ T-lymphocyte reactivity as a strategy to augment anti-retroviral therapy.[2]

Spiky arms are common to mature dendritic cells from humans...



...but also by the mice and rats.

The rat dendritic cell is interacting with what is probably a helper T cell. Through such interactions, dendritic cells teach the immune system what it should attack. Cells matured in the laboratory, are being used in cancer vaccines.



Dendritic Cells

1.2. Production and Differentiation of Human Dendritic Cells in vitro

DC are spread out throughout the body and are very difficult to isolate. In addition, DC lose their capability for mitosis in their CL and IDC stage. Therefore, even after isolation of DC, no further cell replication is possible. Through years of intensive research by several groups of researchers, like ours, ways have been developed to gain DC out of omnipotent peripheral blood stem cells (CD 34+ cells) or peripheral blood progenitor cells (PBPC). Alternatively, monocytes from peripheral blood can be used initially to produce DC in very large quantities by exposing them to GM-CSF, IL-4, TNF-alpha and other cytokines.[3,4,5,6,7,8,9] Since monocytes are available in large quantities, the strategy of the Cologne Model is to harvest monocytes from small amounts of peripheral blood taken from the patient and develop them into active DC in this way.

DC are pivotal regulators of immune reactivity and immune tolerance. The observation that DC can recruit naïve T-cells has invigorated cancer immunology and stimulated clinical trials of DC in immunotherapy. However, variables inherent in preparation and use of dendritic cell grafts remain to be tested. Clinical trials with dendritic cell vaccines in myelogenous leukaemia are ongoing.[x]

1.3. Sensibilisation of Newly Produced DC with Tumor-Specific Peptides

Antigen can be introduced into the DC as DNA, which is transcribed and translated by the DC themselves. This foreign protein is then broken down and eliminated by the regular cell metabolism. The peptides, produced in this way, are bound to MHC molecules and presented on the cell membrane surface. In this way, CD8+ cytotoxic T-cells are activated. They play an important role in the immune response against tumor cells.

Lysates of pathogens and tumor-specific antigen can be used to sensitize DC. But most antigen presented in this way to DC are not metabolized in the cytoplasm but in the endosomes. Therefore, these peptides are mainly bound to the major histocompatibility complex class II (MHC-II) molecules, which initiates mainly the

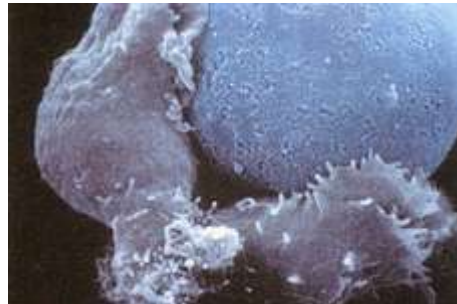
activation of CD4+ T-cells. This effect is advantageous in immune responses to infectious antigen and in immunization of non-responder. However, in the treatment of tumor patients, the TH-1 response with its cytotoxic activation is most wanted. Therefore, incubating DC with proteins and lysates as a form of antigen presentation is not optimal.

Recently, it was shown that extra-cellular proteins and heat-shock proteins have additional value to enhance the TH-1 type cytotoxic CD8+ T-cell responses. This is an important observation for the development of new treatments of oncological patients. In the Cologne Model, therefore, in addition, hyperthermia is performed in the care of cancer patients.

DCs are used which have been exposed to tumor-specific antigen, which are directly bound to the MHC-I molecule. In this way, one can circumvent the usual route of antigen incorporation and processing and make sure that the TH-1 response is dominant.

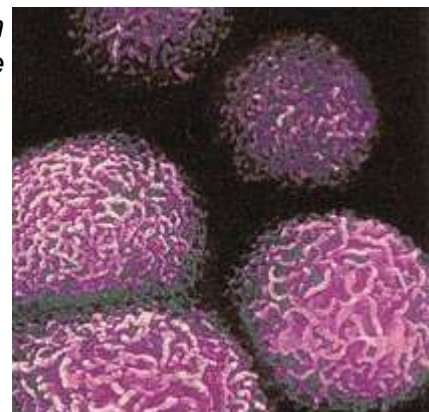
In this way, DC that have been differentiated in vitro and sensitized with tumor-specific antigen were tested very successfully in animal settings. Tumor regression was seen in most animals.

In the Cologne Model, the patient comes to the clinic for a blood draw (50 ml) at day 1. Our own laboratory processes the blood, monocytes are harvested, and, in a six-day period, changed into DC. Then, if tumor tissue is available, the DC are exposed to the specific cancer antigen of the patient. Finally, these sensitized and programmed DC are re-infused into the patient on day 7. It is recommended that the patient undergoes an infusion (vaccination) with DC at least six times. Usually, there is a four-week interval between two DC infusions.

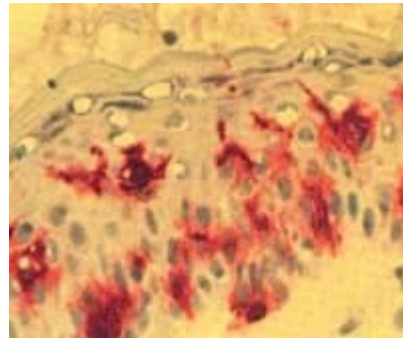
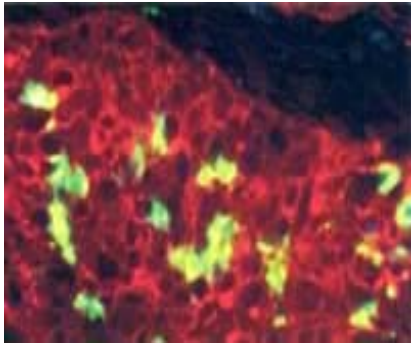


Nachcolorierte Raster-EM-Aufnahmen einer Tumorzelle, die von mehreren natürlichen Killerzellen attackiert wird.

Tumorzellen sind gegen höhere Temperaturen deutlich empfindlicher als gesundes Körpergewebe



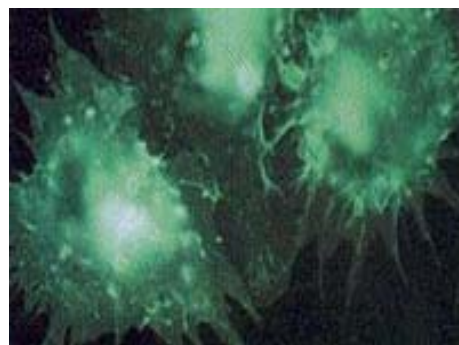
Immature dendritic cells can be stained to show up green in breast cancer tissue (left) or red in normal skin (right).



As the cells mature, they make proteins that allow them to stick to one another (left).



They also produce forklike receptors (green dots, right) which they use to show bits of invaders to other immune cells.



Dendritic Cells

1.4. Summary Dendritic Cell Therapy

The suggestion that the immune system might target tumors is admittedly not new. Attempts to derive tumorcidal cancer vaccines date back to at least 1909 with Coley's efforts to treat inoperable sarcoma with bacterial toxins.[32]

It was not until many years later that pioneering work by Zinkernagel, Van Pel, and Boon established that the immune system, via CD8+ cytotoxic T lymphocytes (CTL),

can kill neoplastic cells following recognition of tumor antigens bound to tumor cell major histocompatibility complex (MHC) class I molecules. [32]

The dendritic cell (DC) was championed by Steinman and Chon In 1973, as a novel stellate cell, DC were identified in the spleen of mice. Because of their shape under the microscope with all their tentacles, they were called dendritic cells (Greek: dendros = tree, branches). [32, 15]

Not until the mid-80s of the last century, it was noticed that DC belong to the same family of cells, which had been discovered more than one hundred years ago by Langerhans.¹⁶ Later on, DC were also documented in other lymphatic organs and in non-lymphatic tissues.

In the past decade, much has been learned regarding the use of DC and their role in the development of cancer vaccines. Contrary to what was originally thought, dendritic cells are not capable of inducing an immune response unless they undergo activation. Current vaccine approaches may incorporate foreign carrier proteins, adjuvants, cytokines and genetically engineered viruses in an attempt to increase immunogenicity. [24]

DC express high levels of major histocompatibility complex class I and II antigens. In 1990, Freudenthal and Steinman showed, that DC also express high levels of the immunomodulatory proteins B7.1 (CD80), B7.2 (CD86), CD 40, and two adhesins, the intracellular adhesion molecule ICAM-1 (CD54) and the lymphocyte function-associate protein LFA.3 (CD58). DC can also produce γ -producing cells.[25]IL-12, a potent cytokine,that activates interferon-

The initial group of trials involving DC for the treatment of cancer used cells obtained from peripheral blood but they had difficulties because DC constitute less than 5% but in 1990 Markowics et al²⁶ found that GM-CSF not only promotes dendritic cell survival, but also induces dendritic cell differentiation to mobile, reversibly-adherent cells with long-branched projections, changing the survival for up to 6 weeks.[26]

Lately, in 1994, Mehta-Damani et al²⁷ were able to use DC to generate antigen-specific CD8+ cytotoxic T lymphocytes (CTLs) from naïve precursors in vitro and in 1995 Mehta-Damani et al²⁸ described an in vitro system for generating antigen-specific CD4+ T cells.

In 1996, Hsu et al²⁹ reported on the first clinical trial of antigen-pulsed dendritic cells. This trial was designed to evaluate the efficacy of tumor-specific idiotype (Id) protein-pulsed autologous dendritic cells in the treatment of B-cell lymphoma.

Because of the success of the study by Hsu et al,[29] Timmerman et al [30] used the same approach in a larger number of patients with non-Hodgkin's B-cell lymphoma. Three monthly infusions of antigen-pulsed dendritic cells were administered intravenously to each patient, followed by a fourth vaccination 2 to 6 months later. The study concluded that Id-pulsed dendritic cell vaccination can induce T-cell and humoral anti-Id responses and (complete) tumor regression

1.4.a. Characteristics of dendritic cells

There are a number of challenges associated with the development and optimization of dendritic cell-based cancer vaccines. The current approach of isolating and pulsing dendritic cells is difficult and expensive. Ultimately, targeting and activating dendritic cells should be performed in vivo instead of the current in vitro approach. To attract dendritic cells into an accessible location into which an antigen and a dendritic cell activator can be injected or introduced, the numbers of DC may need to be expanded, using an approach. [24]

Another challenge is the determination of which clinical endpoints should be measured. Most of the focus in cancer immunology has been on CD8+ CTL responses. However, recent evidence has indicated that CD4+ T cells may be an equally critical component of the antitumor immune response.[38]

In practically all tissues in the body, DC form a close network of watch-dog cells, which identify and incorporate extracellular antigens through phagocytosis and endocytosis and thus, analyze their surroundings. Incorporated proteins (antigens) are then split into smaller peptides, linked to MHC-molecules and then transported to the surface (cell membrane) of the DC.

In this way, antigen peptides are made visible for T-Lymphocytes and cytotoxic cells. DC migrate actively from peripheral tissue to regional lymph nodes, where they communicate with resting T-Lymphocytes, and transmit their information.

1.4.b. Activation and maturation of dendritic cells

A well-functioning surveillance system recognizes damaging processes fast and with great detail, and to augment a quick and effective response. For this purpose, DC carry on their surface receptors for millions of possible danger signals, which can be activated by micro-organisms, damaged- or cancerous cells, and interleukins and cytokines, which are produced by the body's own tissues and T-Lymphocytes.

1.4.c. Induction of an immune response

In lymph nodes, DC interact with various lymphocyte sub-populations. Especially naïve T-lymphocytes communicate with DC by active searching over the surface of DC. Naïve T-lymphocytes are activated when they recognize an antigen, presented by the DC. Both CD4+ lymphocytes and CD8+ lymphocytes are activated in this way by DC and this process is called priming.

DC develop their full capacity for activating CD4+ and CD8+ lymphocytes after they have gone through their own maturation process, on their way from location of their activation by, for instance a cancerous cell, to the regional lymph nodes.

Today, it is still not certain how many activated and matured DC are necessary to induce a significant immune response against, for instance, a malignancy. In animal models and in clinical studies, 100,000 to (rarely) 100,000,000 DC per vaccination have been used. In the Cologne Model, 5,000,000 to 15,000,000 DC per vaccination

are commonly used. Numbers of DC might vary secondary to the number and the fitness of monocytes, harvested from 100ml of peripheral blood of a patient. In the Cologne Model, DC are manufactured from the patients own monocytes ("autologous, monocyte-derived dendritic cells"), and applied intracutaneously, intranodally, intra-tumorally, intra-theically or intravenously, depending on location, kind of tumor, and other factors. In addition, through leucopharese, many more monocytes can be harvested so that more dendritic cells for a vaccination can be manufactured.

2.1. Dendritic cells in various tumors

Tanaka et al. investigated the in vivo antitumor effects of intratumoral administration of dendritic cells after low-dose chemotherapy using cisplatin + 5-FU. Combination of intratumoral injection of dendritic cells and systemic chemotherapy induced complete rejection of the treated tumor, MC38 murine adenocarcinoma. Furthermore, the antitumor effects were also observed on a distant tumor inoculated in the contralateral flank of the animal. When 10x the number of tumor cells were inoculated, the antitumor effect of the combination of dendritic cells after chemotherapy was also confirmed and in comparison to that of dendritic cells or chemotherapy alone, thereafter contributed to a greater prolongation of survival. This study by Tanaka et al. suggests that the strategy of intratumoral injection of dendritic cells after low-dose chemotherapy could be a powerful weapon to treat patients with cancer in the clinical settings. [12]

Stift et al. validated in their study the toxicity and immunological response induced by autologous dendritic cells (DCs) pulsed with allogeneic tumor lysate in a pancreatic cancer patient. The lack of available tumor peptides in pancreatic cancer strongly supports the idea to use allogeneic tumor cells as a source of antigens. The patient suffering from a stage IV pancreatic cancer received $1-2 \times 10^7$ autologous monocyte-derived DC's in three-week intervals injected into a groin lymph node. Monocytes from peripheral blood were isolated by magnetic bead selection. For the first ten vaccinations DC's were loaded with autologous tumor cell lysate obtained during surgical exploration. After consumption of the autologous lysate, equal numbers of DC's were pulsed with lysate of the tumor cell line AsPc-1 and BxPc-3 for a further five vaccinations. Peripheral mononuclear cells (PMNC's) were harvested after the seventh and compared with PMNC's obtained after the fourteenth vaccination for immunological response. Delayed type hypersensitivity reactivity to DC's pulsed with autologous and allogeneic tumor lysate was also assessed. The patient received a total of fifteen vaccinations. There was no toxicity or evidence of autoimmunity observed. Delayed type hypersensitivity was found to be positive for the autologous as well as the allogeneic tumor lysate pulsed DC's. in vitro cytotoxicity assays demonstrated a dramatic increase of the PMNC killing capacity against the pancreatic cancer cell lines AsPc-1 and BxPc-3 after the fourteenth compared to the seventh vaccination. CT scans revealed a stable disease for six months. The administration of autologous DC's pulsed with allogeneic tumor lysate is non-toxic and suitable for inducing an immunological anti-tumor response. Even though this study was confined to a single patient, the data might open a door for novel immunotherapeutic strategies. [13]

2.1.a Liver Cancers

Sangro et al. found when dendritic cells are injected directly into a colorectal tumor, or into liver metastasis of colorectal cancer, there is an intense anti-tumor effect.[11]

Qiu et al. found that dendritic cells, which are primed ex vivo with adenovirus vector highly expressing HBsAg, induce strong and specific anti-tumor effects, stronger than that induced by recombinant HBsAg vaccine.[12]

Liu et al. found that dendritic cells can induce significant in vitro cytotoxicity against human hepatocellular carcinoma.[13]

Liu et al. found that sensitized Dendritic cells can induce obvious antitumor activities in the murine liver cancer models and strongly suggest new strategies for constructing a new type of dendritic cell therapy for liver cancer.[14]

2.1.b Chronic (B-cell) lymphatic Leukaemia

Chronic lymphatic leukaemia (CLL) B-cells has defects in apoptosis pathways and therefore accumulate in vivo. However, when removed from the patient and cultured in vitro, these malignant cells rapidly undergo apoptosis. Recent studies suggest that leukaemia cell survival is influenced by interactions with non-leukaemia cells in the micro-environment of lymph nodes, bone marrow, and other tissues. Pedersen et al. documented that under certain circumstances, dendritic cells can induce apoptosis of CLL B-cells.[15]

2.1.c Plasmacytoma (multiple myeloma or Kahler's Disease)

Failures of chemotherapy to cure a significant proportion of multiple myeloma patients and increasing knowledge of tumor immunology have generated considerable interest in immunotherapy for this lethal disease. Immunotherapy for multiple myeloma can be divided into three categories: passive antibody-mediated immunotherapy, active specific immunization (vaccination), and adoptive T-cell immunotherapy. Dendritic cell vaccination is a very good example of a promising new immunotherapy in myeloma patients.[16]

Gong et al. demonstrated that the induction of antitumor humoral and cytotoxic T-lymphocytes (CTL) responses of dendritic cells can be enhanced by simultaneous application of IL-12, and thus, potentiates antitumor immunity and the treatment of multiple myeloma.[17]

Liu et al. found that dendritic cells have a powerful antitumor activity against plasmacytoma (myeloma) cells. [18]

Zeis et al. could demonstrate that antitumor immunity of dendritic cells in multiple myeloma can be enhanced by syngeneic peripheral blood stem cell transplantation (PBSCT) given in conjunction with dendritic cells.[19]

Cull et al. found that dendritic cells have a powerful potential as adjuvant immunotherapy of hematological malignancies, including multiple myeloma. Recently, Human Herpes Virus-8 (HHV-8) has been associated with multiple myeloma. Interestingly, in patients with myeloma, in their dendritic cells no HHV-8 has been identified. Therefore, autologous dendritic cells remain a suitable vehicle for immunotherapy of myeloma patients and for further investigation in the immunotherapy of myeloma.[20]

Another hope-provoking study is by Valone et al. His group demonstrated the effective use of dendritic cells in several malignancies, including hematological malignancies, like CLL and multiple myeloma.[21]

Liso A: et al³¹ studied 26 patients with Multiple Myeloma who were in minimal disease state after receiving chemotherapy and peripheral blood progenitor cell transplantation. All patients were given a series of monthly immunization of two intravenous infusions of dendritics. The treatments were well tolerated. The study showed that responses could be induced in patients who are in complete remission, but there was no clear evidence of therapeutic effect in patients with active disease. [31]

2.1.d Breast Cancer

Breast Cancer is a major health problem implying strong emotional impact. Its incidence in Europe from 43 to 73 per 100,000; peaking in developed countries exceeding the cumulative number of lung cancer, leukaemias, and colorectal cancer in women. The mortality rate ranges from 10 to 30 in 100,000, and the 5 years survival is 43-75%[33, 34]

The recent reports of Wojas K, et al demonstrate that the number and function of dendritic cells changes dramatically in cancer patients. In the present study, they evaluated the percentage of myeloid and lymphoid DCs in patients with breast cancer, non-small cell lung cancer (NSCLC) and in healthy donors. The percentage of both DC populations was significantly lower in patients with NSCLC than in the control group. In patients with breast cancer, the number of lymphoid DCs was significantly higher than in NSCLC patients. The obtained results suggest influence of pathological states on host immune system. The decrease in the number of DCs in the peripheral blood from cancer patients may be closely correlated with the type of tumor.[35]

There is evidence that DCs are of diverse origin, with at least two types of myeloid and lymphoid precursors implicated in their generation. Recent reports demonstrate that the number and function of dendritic cells changes dramatically in cancer patients.[35]

Pockaj, BA. et al³⁶ found that in several neoplastic diseases, including breast cancer, immuno-suppression correlates with disease stage, progression, and outcome. Thus, thorough analysis of immune parameters in breast cancer patients (and very likely, in ALL cancer patients) may be beneficial in designing effective anticancer immune-based therapies. Data revealed increased synthesis of PGE₂, an immune suppressor, along with increased expression of COX-2, a key regulator of

PGE2 synthesis. COX-2-induced PGE2 may contribute to immuno-suppression and may directly block antitumor immunity while promoting tumor growth, providing the rationale for using COX-2 inhibition combined with immunotherapy, like dendritic cells.[36]

Manna PP et al. researched Mammaglobin-A, which is exclusively expressed by breast cancer cells. Thus, mammaglobin-A-specific T cell immune responses may be useful for the design of new breast cancer-specific immuno-therapies. They showed in that CD8+ T cells generated against recombinant mammaglobin-A-pulsed dendritic cells display a marked cytotoxic activity against mammaglobin-A-positive breast cancer cell lines. Indicating the immunotherapeutic potential of this novel antigen for the treatment of breast cancer, and might enhance efficacy of vaccinations with autologous dendritic cells.[37]

2.1.e Prostate Cancer

Bigotti G, et al, investigated Langerhans cell (LC) distribution in 38 prostatic carcinomas, of various degrees of differentiation, furthermore evaluating the expression of HLA class II-DR by neoplastic cells. They showed that the number of LC is inversely correlated to the histopathological grade and directly to the expression of HLA class II-DR molecules by tumor cells. In this context, macrophages might play a primary role in controlling tumor progression. They also suggest that the presence of LCs and HLA class II molecules, either singly or in combination, in carcinoma of the prostate represents a good prognostic indicator, being constantly associated with the clinically less aggressive low-grade tumors. [39]

Tjoa B, et al, determined that dendritic cells (DC) are "professional" antigen-presenting cells, capable of stimulating T-cell proliferation and cytotoxicity when loaded with and presenting specific antigens, including tumor antigens. They found that the stimulation of an autologous cytotoxic T-cell response elicited by DC loaded with autologous tumor cell lysate derived from primary prostate tumor. And the ability to use DC for presentation of either tumor or peptide antigen in an HLA-restricted fashion in order to stimulate T-cell proliferation and cytotoxicity demonstrates the potential of this technology for development of a potent prostate cancer vaccine. [40]

Murphy G, et al., found that conventional treatment for metastatic prostate cancer have failed to demonstrate curative potential in all patients. Development of T-cell immunotherapy may give a new approach to the treatment of advanced metastatic prostate cancer.

In these results, they noticed cellular response and decrease in PSA level in some patients who received DC pulsed with PSM-P2, which indicates that this method has great potential in prostate cancer. [41]

Tjoa BA, et al, did a clinical monitoring for hematological studies and prostate markers up to 370 days from the start of the phase I study with autologous dendritic cells, pulsed, or primed, with PSMA peptides.

They concluded that the responses observed in this phase I clinical trial are significant and of long duration. Most of the responders were in treatment groups

infused with DC pulsed with PSM-P1 or -P2, suggesting the requirement of both components for effective immunotherapy. [42]

Slovin SF, et al, studied conventional therapies for patients with early-stage relapsed prostate cancer, which often, unfortunately, do not provide an acceptable quality of life. These patients often have increasing PSA values as the sole manifestation of their disease recurrence and represent a unique subgroup of patients for whom alternative treatment strategies are urgently needed. These patients are asymptomatic and may be an appropriate population for targeted immunological approaches. Vaccine therapies, based on synthetically constructed, naturally occurring prostate-associated antigens or genetically modified immune cells, offer exciting new approaches toward treating this disease with resulting antitumor effects and minimal toxicities. The results of clinical trials using these technologies reinforces the use of immunological approaches for the treatment of prostate cancer. [43]

In this paper, Salgaller ML, et al, describe their program for immune monitoring of phase II participants given dendritic cell (DC)/prostate-specific membrane antigen (PSMA)-based immunotherapy. All study subjects received six administrations of autologous dendritic cells. Observations indicate that, in this way, cellular immunity in prostate cancer patients is enhanced. Several methods are underway to comprehensively monitor both cell-mediated and humoral immune responsiveness, including: determining anti-PSMA serum antibody titers, testing immunogen-restricted responder-cell proliferation and cytotoxicity, assessing aberrations in signal transduction, antigen processing, and presentation, and measuring soluble factors that may promote tumor outgrowth. [44]

Salgaller ML, et al, found that immunotherapy of cancer, based on eliciting or enhancing the body's own capacity to mount an effective antitumor response, has produced encouraging early results in the areas of melanoma and renal-cell carcinoma. Such treatments utilizing dendritic cells (DC), immune cells that are excellent antigen presenters, are especially promising. They performed a phase I clinical trial assessing the administration of autologous DC pulsed with HLA-A0201-specific prostate-specific membrane antigen (PSMA) for the treatment of 51 men with hormone-refractory prostate cancer. No significant toxicity was observed. The excellent tolerance of this treatment approach, as well as the enhanced cellular responses, decreased PSA levels, and partial clinical responses in some patients suggests that it holds great potential in prostate cancer therapy. [45]

Tjoa BA, et al, included thirty-three patients in a phase-I study, and subsequently in a phase-II trial, which involved six infusions of DC pulsed with PSM-P1 and -P2 peptides. Clinical monitoring was conducted up to 770 days from the start of the phase-I study. They found 9 partial responders in the phase-II study based on National Prostate Cancer Project (NPCP) criteria, plus 50% reduction of prostate-specific antigen. Four of the partial responders were also responders in the phase-I study, with an average response duration of 225 days. Five other responders were nonresponders in the phase I study. Their average partial response period was 196 days. They concluded that the responses observed in the phase I and II clinical trials were significant and of long duration. The partial-responder group included patients who continued to respond from phase I, as well as those who started to respond during the phase-II trial. [46]

Peshwa MV, et al, determined that most strategies in cancer immunotherapy are aimed at the induction of a strong cellular immune response against the tumor. Particularly, CD8+ T lymphocytes. They used dendritic cells (DC), selected peptides from prostatic acid phosphatase (PAP), and a prostate tissue-specific antigen.

The obtained PAP-peptide-specific CTL lysed peptide-coated target cells, vaccinia-infected target cells, and HLA-A2-positive prostate-tumor cells in vitro in an antigen-specific manner. In conclusion, the results indicate that CTL precursors to the PAP gene product exist and could be potentially recruited to elicit an antitumor response. Thus, PAP is a suitable antigen for inclusion in prostate cancer vaccines. [47]

Simmons et al., found that when recombinant human granulocyte-macrophage colony-stimulating factor was administered to a subgroup of 44 patients in a phase-II clinical trial for prostate cancer using DC pulsed with HLA-A2-specific prostate-specific membrane antigen (PSMA) peptides, there was a significant anti tumor activity against prostate cancer. Their purpose was to determine if GM-CSF caused any enhancement of patients' immune responses, including enhancement of clinical response to the DC-peptide treatment. The results suggest GM-CSF as employed in this trial did not detectably enhance clinical response to DC-peptide infusions, or significantly enhance the measured immune response. Thus, DC vaccinations can be conducted without simultaneous application of GMCSF. [48]

Tjoa et al., recently completed a phase-II trial in prostate cancer patients, involving infusions of autologous dendritic cells (DC) and two human histocompatibility antigen (HLA-A2)-specific prostate-specific membrane antigen (PSMA) peptides. Subjects were observed for an average of 291 days, with a metastatic group and a local recurrence group, which included treatment and follow-up periods. The average duration of response was 149 days a majority of responders (11/19; 58%) and were still responsive at the end of the current follow-up. They concluded that the responses observed may be significant and relatively durable, which suggests that DC-based cancer vaccines in the future may provide an additional therapy for advanced prostate cancer. [49]

Murphy et al., compared the importance of over 22 measurements used in evaluating the clinical responses of patients with metastatic or locally recurrent prostate cancer, treated by dendritic cell (DC) infusions with prostate-specific membrane antigen (PSMA) peptides. Patients with metastases showed a sharp rate of response secondary to the level of DC infusion, in contrast to those patients with local recurrence, in which it was more gradual. They concluded that the importance of level of DC infusion, immune parameters, cytokines, and markers such as PSMA in determining the response to PSMA peptide immunotherapy are important parameters in predicting clinical outcome and efficacy of DC vaccinations in prostate cancer. [50]

McNeel DG, et al, discusses the rationale in animal models for particular immunization strategies and describes vaccines, currently being used in patients with prostate cancer. The ongoing identification of tumor antigens and proteins involved in prostate cancer progression and the development of better immunologic animal models suggest a hopeful future for the design of effective prostate cancer vaccines, especially vaccines with dendritic cells. [51]

Prostate-specific Ag (PSA) is a self Ag expressed by both normal and malignant prostatic epithelium, and therefore offers a unique opportunity to examine the ability of self Ags to serve as specific CTL targets. In this study, Heiser A, et al, investigated the efficacy of autologous dendritic cells (DC) transfected with mRNA encoding PSA to stimulate CTL against PSA Ags in vitro. Ag in form of RNA carries the advantage to encode multiple epitopes for many HLA alleles, thus permitting induction of CTL responses among many cancer patients independent of their HLA repertoire. In this study, we show that PSA mRNA-transfected DC were capable of stimulating primary CTL responses against PSA Ags in vitro. The PSA-specific CTL did not cross-react with kallikrein Ags, a protein, which shares significant homology with PSA, suggesting that harmful autoimmune toxicity may not represent a significant problem with this approach. PSA RNA-transfected DC generated from male or female healthy volunteers or from cancer patients were equally effective in stimulating PSA-specific CTL in vitro, implying that neither natural tolerance to PSA Ags nor tumor-mediated T cell anergy may represent major barriers for CTL generation against the self Ag PSA. This study provides a preclinical rationale for using PSA RNA-transfected DC in active or adoptive immunization protocols. [52]

Meidenbauer et al., studied JBT 1001, a vaccine used for therapy of prostate cancer (CA), which consists of recombinant prostate-specific antigen (PSA) with lipid A formulated in liposomes. The hypothesis tested was that PSA-based vaccines induce T cell responses to human PSA. Vaccination with PSA formulated into liposomes induced T-cell responses in 8/10 patients with prostate carcinoma. In their conclusion Meidenbauer N, et al, found that the frequency of PSA-reactive precursor T cells was relatively low in the blood of these patients, and IVS, leading to amplification of the precursor cells prior to ELISPOT, was necessary for quantification of the PSA-responding T cells. Cellular responses to PSA were predominantly mediated by CD4 (+) T lymphocytes. [53]

Pirtskhalaishvili G, et al, have shown that prostate cancer (PCa) causes apoptosis of dendritic cells (DC), which might block the development of specific antitumor immune responses. They demonstrated that the cytokine-mediated increase in DC survival was accompanied by an elevated expression of the anti-apoptotic protein Bcl-xL. Next, they evaluated the resistance to tumor-induced apoptosis and the antitumor efficiency of genetically engineered DC over-expressing Bcl-xL. DC were transduced with an adenoviral vector encoding the murine Bcl-xL gene and injected intratumorally. Data analysis revealed that treatment of PCa-bearing mice with Bcl-xL-transduced DC resulted in significant inhibition of tumor growth compared with the administration of non-transduced DC. In conclusion, they strongly suggest that the protection of DC from PCa-induced apoptosis might significantly increase the efficacy of DC-based therapies in cancer; even in the absence of available tumor-specific Ags. [54]

In this review, Tjoa BA, et al discuss the progress in active specific immunotherapeutic approaches as potential alternative methods in the treatment of metastatic prostate cancer. Current efforts are now directed towards developments of novel strategies for the treatment of metastatic prostate cancer. Cancer immunotherapeutic strategies utilize patient immune system components to kill cancer cells. One of the newest advances in cancer immunotherapy is the use of dendritic cells as the vehicle to deliver cancer antigens for an effective in vivo T cell activation. The development of dendritic cell-based prostate cancer vaccine, as well

as results of several clinical trials in prostate cancer involving the administration of peptide-pulsed autologous dendritic cell pulsed are discussed. [55]

Nouri-Shirazi M, et al, could show that immature human monocyte-derived DC capture various killed tumor cells, including Jurkat T cell lymphoma, malignant melanoma, and prostate carcinoma. CTL elicited by DC loaded with the PC3 prostate carcinoma cell bodies kill another prostate carcinoma cell line, DU145, suggesting recognition of shared Ags. Finally, CTL elicited by DC loaded with killed LNCap prostate carcinoma cells, which express prostate specific Ag (PSA), are able to kill PSA peptide-pulsed T2 cells. This demonstrates that induced CTL activity is not only due to allo-antigens, and that alloantigens do not prevent the activation of T cells specific for tumor-associated Ags. This approach opens the possibility of using allogeneic tumor cells as a source of tumor Ag for antitumor therapies, like with autologous monocyte-derived dendritic cells, like used in the Cologne Modell. [56]

Provenge (Dendreon Corp, Seattle, WA) is a company in the USA which manufactures an immunotherapy product consisting of autologous dendritic cells loaded ex vivo with a recombinant fusion protein consisting of prostatic acid phosphatase (PAP) linked to granulocyte-macrophage colony-stimulating factor. Sequential phase-I and phase-II trials were performed to determine the safety and efficacy of Provenge and to assess its capacity to break immune tolerance to the normal tissue antigen PAP. All patients developed immune responses to the recombinant fusion protein used to prepare Provenge, and 38% developed immune responses to PAP. Three patients had a more than 50% decline in prostate-specific antigen (PSA) level, and another three patients had 25% to 49% decreases in PSA. They concluded that Provenge is a novel immunotherapy agent that is safe and breaks tolerance to the tissue antigen PAP.[57]

Pirtskhalaishvili G, et al, studied progression of prostate cancer which is accompanied by a marked suppression of the immune system, including the apoptotic death of dendritic cells (DC) responsible for the induction of antitumor immunity. Evaluated was whether prostate cancer might inhibit DC generation and maturation in vitro. Prostate cancer significantly inhibited the conversion of monocytes into DC, which was assessed by the expression of DC markers CD1a and CD83. These cells were weak stimulators of T-cell proliferation, suggesting that DC generated in the prostate cancer microenvironment are functionally inhibited. In conclusion, prostate cancer not only kills mature DC, but also inhibits their generation and maturation, resulting in decreased production of antigen-presenting cells and inhibition of their functional activity.[58]

Recent studies have suggested that the best strategy for achieving an intense immune response may be priming with naked DNA followed by boosting with a viral vector. Mincheff M, et al have successfully completed a phase-I and phase-II clinical trials on immunotherapy of prostate cancer using naked DNA and adenoviral immunizations against the prostate-specific membrane antigen (PSMA) and phase-I clinical trial on colorectal cancer using naked DNA immunization against the carcinoembryonic antigen (CEA). The vaccination was tolerated well and no side effects have been observed so far. The therapy has proven to be effective in a number of patients treated solely by immunizations. The success of the treatment clearly depends on the stage of the disease proving to be most efficient in patients with minimal disease or no metastases. A panel of changes in the phenotype of

peripheral blood lymphocytes and the expression of intra-T-cell lymphokines, as routinely measured in the Cologne Modell, seems to correlate with clinical improvement. [59]

Many tumor-associated Ags represent tissue differentiation Ags that are poorly immunogenic. Their weak immunogenicity may be due to immune tolerance to self-Ags. Prostatic acid phosphatase (PAP) is just such an Ag that is expressed by both normal and malignant prostate tissue. Fong L, et al, has previously demonstrated that PAP can be immunogenic in a rodent model. To explore the potential role of xenoantigen immunization in cancer patients, they performed a phase-I clinical trial using dendritic cells pulsed with recombinant mouse PAP as a tumor vaccine. Finally, 6 of 21 patients included in the study had clinical stabilization of their previously progressing prostate cancer. All six of these patients developed T cell immunity to human PAP following vaccination. These results demonstrate that xenoantigen immunization can break tolerance to a self-Ag in humans, resulting in a clinically significant antitumor effect. [60]

In this report, Kiessling A, et al define immunogenic peptides of PSCA which are recognized by circulating CD8(+) T cells from prostate cancer patients and able to activate CTLs in vitro. Screening the amino acid sequence of PSCA for peptides containing a binding motif for HLA-A*0201 resulted in 8 candidate peptides. CTLs from prostate cancer patients were raised against these 2 peptides in vitro when presented by autologous DCs. They specifically recognized peptide-pulsed T2 target cells and prostate cancer cells that were HLA-A*0201- and PSCA-positive, indicating that these peptides were naturally generated by tumor cells. These data strongly suggest that PSCA is a promising target for immunotherapy of prostate cancer. [61]

The development of effective cancer vaccines depends heavily on the ability to deliver target antigens to generate an immune response. Dendritic cells express high levels of major histocompatibility complex class I and II antigens, which are crucial to cancer immunotherapy, as well as a variety of important immunomodulatory proteins, adhesins, and a potent cytokine. EG Engelman has listed and discussed numerous clinical trials of antigen-pulsed dendritic cells, which have been conducted in various types of cancer, including non-Hodgkin lymphoma, multiple myeloma, prostate cancer, malignant melanoma, colorectal cancer, and non-small cell lung cancer. These studies show that antigen-loaded dendritic cell vaccinations are safe and promising in the treatment of cancer. This review discusses the use of dendritic cells in immunotherapy and some of the clinical trials that have successfully been conducted. [62]

Ciavarra et al., have previously reported that Fms-like tyrosine kinase-3 ligand (flt3-L) induced tumor stabilization and regression of palpable ectopic prostate tumors (TRAMP-C1. Taken together, these data reveal several important immunosuppressive characteristics of the prostate tumor microenvironment (TME) that immunotherapeutic interventions must first overcome to achieve longterm cures. These data also highlight the importance of utilizing treatment versus vaccination models in the evaluation of immunotherapeutic modalities. [63]

The impact of different routes of administration of (autologous) dendritic cells on their anti-tumor effects is uncertain. They examined the effect of injection of cloned dendritic cells, which were stably transfected with IL-12 and exposed to an extract of

murine RM-9 prostate carcinoma cell antigens on tumor growth in vivo. Compared with the wild type dendritic cells, the IL-12-transfected dendritic cells delayed tumor engraftment by 7 days, and reduced tumor growth by up to 80%. In contrast to wild type dendritic cells, IL-12-transfected dendritic cells induced infiltration of mononuclear cells into the tumors, and induced apoptosis and necrosis of tumor cells. In conclusion they conclude that antigen-exposed, IL-12-transfected dendritic cells have potential as an immunotherapy for prostate carcinoma. Routes of administration of dendritic cells are critical for maximal anti-tumor effect.[64]

Parathyroid hormone-related protein (PTH-rP), a protein produced by prostate carcinoma and other epithelial cancers, is a key agent in the development of bone metastases. Kiessling A, et al, investigated whether the protein follows the self-tolerance paradigm or can be used as a target Ag for anticancer immunotherapy by investigating the immunogenicity of two HLA-A(*)02.01-binding PTH-rP-derived peptides (PTR-2 and -4) with different affinity qualities. The two peptides were also able to elicit a strong antitumor PTH-rP-specific CTL response in HLA-A(*)02.01 (HHD) transgenic mice. In this study we describe two immunogenic and toxic-free PTH-rP peptides as valid candidates for the design of successful peptide-based vaccination strategies against prostate cancer. [65]

Here, Chakraborty NG, et al., identified for the first time an immunogenic peptide derived from the prostate-specific protein transient receptor potential-p8 (trp-p8) that is recognized by cytotoxic T lymphocytes (CTLs) from PCa patients. Trp-p8 mRNA was found to be expressed in all prostate tumors and in the corresponding normal prostate tissue. Of five selected trp-p8-derived peptides, only peptide GLMKYIGEV was shown to activate specific CTLs, which effectively lysed PCa cells confirming the endogenous generation and presentation of this peptide by tumor cells. In conclusion they suggest this antigen as a suitable target for the T-cell-based immunotherapy of prostate cancer. [66]

Immunotherapy is currently being investigated as a treatment for patients with asymptomatic, recurrent prostate cancer manifested only by a rising prostate-specific antigen (PSA) level. Immunization with DNA overcomes many of the obstacles noted in previous studies. Use of the xenogeneic DNA (ie, human PSMA DNA injected into mouse) has been shown to be an absolute requirement to overcome immunologic tolerance. Wolchok JD, et al are currently conducting a phase I trial of human and mouse PSMA DNA vaccines in patients with recurrent prostate cancer, based on preclinical experiments described below. [67]

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2.1.f Multiple myeloma and dendritic cells

Dendritic cells (DCs) are professional antigen-presenting cells and are frequently used in current immunotherapy protocols. The administration of DCs loaded with tumor-associated proteins or peptides results in the induction of immune responses against different types of malignant cells. Methods for large-scale generation of DCs in a sufficient quality and quantity have permitted their use in clinical experiments.

DC-based vaccines have already shown promise in follicular non-Hodgkin's lymphoma, and to some extent, in other hematological malignancies. Several strategies have been developed to boost their potency as a new and relatively non-toxic treatment modality. Buchler *et al.* reviewed clinical trials using DCs in the treatment of hematologic malignancies, like **Multiple Myeloma**, and recent studies of the immunophenotype, development, and maturation of DCs may have an important impact on designing DC-based antitumor vaccines.⁽¹⁾

The poor response to immunotherapy in patients with **multiple myeloma (MM)** indicates that a better understanding of any defects in the immune response in these patients is required before effective therapeutic strategies can be developed. Recently, Brown *et al.* reported that high potency (CMRF44(+)) dendritic cells (DC) in the peripheral blood of patients with MM failed to significantly up-regulate the expression of the B7 co-stimulatory molecules, CD80 and CD86, in response to an appropriate signal from soluble trimeric human CD40 ligand. This defect was caused by transforming growth factor beta(1) (TGFbeta(1)) and interleukin (IL)-10, produced by malignant plasma cells, and the defect was neutralized *in vitro* with anti-TGFbeta(1). As this defect could impact on immunotherapeutic strategies and may be a major cause of the failure of recent trials, it was important to identify a more clinically useful agent that could correct the defect *in vivo*.⁽²⁾

Effective adoptive cancer immunotherapy depends on an ability to generate tumor-antigen-presenting cells and tumor-reactive effector lymphocytes and to deliver these effector cells to the tumor. The current experimental and clinical statuses of adoptive transfer of tumor antigen-pulsed DCs and vaccine-primed activated T cells are summarized herein. Clinical trials of antigen-pulsed DCs have been conducted in patients with various types of cancer, including non-Hodgkin lymphoma, **multiple myeloma**, prostate cancer, renal cell carcinoma, malignant melanoma, colorectal cancer, and non-small cell lung cancer. These studies have shown that antigen-loaded DC vaccination is safe and promising for the treatment of cancer. In addition, tumor vaccine-primed T cells have been shown to induce antitumor activity *in vivo*. Several clinical studies are being conducted on the use of vaccine-primed T cells such as tumor-drainage lymph node. It is reasonable to consider using both tumor antigen-pulsed DCs and vaccine-primed lymphocytes as adjuvants. Morisaki *T et al.* are now investigating the use of autologous whole tumor antigen-pulsed DCs and the DC vaccine-primed activated lymphocytes in patients with multiple metastases of solid tumors.⁽³⁾

Multiple myeloma (MM) cells produce monoclonal immunoglobulin (Ig) which serves as a truly tumor-specific antigen. The tumor-specific antigenic determinants are localized in the variable (V)-regions of the monoclonal Ig and are called idiotopes (Id). Corthay *et al.* reviewed the evidence obtained in a T-cell receptor (TCR) transgenic mouse model that Id-specific, MHC class II-restricted CD4(+) T cells play a pivotal role in immunosurveillance and eradication of MHC class II-negative MM cells. In brief, monoclonal Ig secreted by MM cells is endocytosed and processed by antigen-presenting cells (APCs) in the tumor. Such tumor-resident dendritic cell APCs in turn present Id peptide on their class II molecules to Id-specific CD4(+) T cells which become activated and indirectly kill the MHC class II-negative myeloma cells. However, if the Id-specific CD4(+) cells fail to eliminate the MM cells during their initial encounter, the increasing number of tumor cells secretes so much monoclonal

Ig that T-cell tolerance to Id is induced. Extending these findings to MM patients, Id-specific immunotherapy should be applied at a time of minimal residual disease and when new Id-specific T cells have been educated in the thymus, like after high-dose chemotherapy and autologous stem cell transplantation.⁽⁴⁾

Multiple myeloma (MM) cells express certain tumour-associated antigens (TAAs) that could serve as targets for active-specific immunotherapy. The aim of the present study was to test the MM/dendritic cell (DC) fusion as a vaccination strategy. *Raje N, et al;* fused MM cells with DC to generate fusion cells (FCs) and tested their antigen presenting cell (APC) function in mixed lymphocyte reactions and cytotoxicity assays. The FCs demonstrated a biphenotypic profile, confirmed both by flow-cytometry and dual immunofluorescence microscopy. These FCs induced MM-specific cytotoxicity. FCs, but not MM cells or DCs alone, were potent stimulators of autologous patient T cells. More importantly, FC-primed autologous peripheral blood mononuclear cells demonstrated major histocompatibility complex-restricted MM-specific cytotoxicity. These studies therefore demonstrated that MM/DC FC can trigger an autologous immune response to MM cells and formed the framework for a clinical trial with autologous monocyte-derived DCs currently underway.⁽⁵⁾

Multiple myeloma (MM) is a lymphoproliferative disorder that is characterized by a proliferation of clonal B cells in various stages of maturation that then infiltrate the bone marrow. MM has been reported to accompany various T cell abnormalities including quantitative and functional defects of CD4+ and CD8+ T cells. Recently, immunotherapy such as dendritic cell therapy, vaccination therapy, and anti-tumor antibody therapy, has been attempted in patients with MM. To develop more effective immunotherapy for patients with MM, further studies are required to identify the immunological abnormalities, especially in T cells, associated with MM. The T helper 1 (Th1) and T helper 2 (Th2) cells are characterized by distinct cytokine production patterns. The Th1 cells produce interferon gamma and interleukin-2 (IL-2), and are involved in cell-mediated immunity. The Th2 cells produce IL-4 and promote humoral immunity by stimulating antibody production, particularly IgE responses. Furthermore, Th1 and Th2 cells have been found to cross-regulate each other's development. The Th1/Th2 combination has an important role in immune response to many disorders including infection, autoimmune diseases, and malignancies. In this review, *Murakami H, et al;* report a Th1/Th2 imbalance in cases of MM, and discuss the relationship between T cell abnormalities and the pathology of MM.⁽⁶⁾

Most patients with **multiple myeloma (MM)** cannot be cured with currently available therapies. Although complete remission could be achieved in about 30-50% of newly diagnosed patients with high-dose chemotherapy and tandem transplantation, relapses of the underlying disease occur frequently. To realize long-term disease-free survival, it will be necessary to develop complementary therapies that are non-cross-resistant with chemotherapy. To this end, immunotherapy aimed at inducing or enhancing tumor-specific immunity that may control or eradicate remaining tumor cells may be an appealing method. Dendritic cells (DCs) are professional antigen-presenting cells and considered the best natural adjuvants for immunotherapy in malignancies. Vaccination with tumor antigen-pulsed DCs has been shown to be protective and therapeutic in animal tumor models, and induced a strong tumor-specific immunity and durable tumor regression in human solid tumors and B-cell lymphoma. As a result, clinical trials in various human malignancies have been initiated. This review will focus on DC-based immunotherapy in MM. *Yi Q, et al;*

discussed myeloma antigens and antigen-specific immune responses, the capacity of DCs to present myeloma antigens and induce cytotoxic T-cell responses, and clinical experience of DC vaccination in myeloma patients.⁽⁷⁾

Dendritic cells (DC) from distinct DC subsets are essential contributors to normal human immune responses. Despite this, reliable assays that enable DC to be counted precisely have been slow to evolve. *Vuckovic S et al.* have now developed a new single-platform flow cytometric assay based on TruCOUNT beads and the whole blood "Lyse/No-Wash" protocol that allows precise counting of the CD14(-) blood DC subsets: CD11c(+)CD16(-) DC, CD11c(+)CD16(+) DC, CD123(hi) DC, CD1c(+) DC and BDCA-3(+) DC. The data is highly reproducible with intra-assay and inter-assay coefficients of variation less than 3% and 11%, respectively. This assay does not produce the DC-T lymphocyte conjugates that result in DC counting abnormalities in conventional gradient-density separation procedures. Using the TruCOUNT assay, we established that absolute blood DC counts reduce with age in healthy individuals. In preliminary studies, we found a significantly lower absolute blood CD11c(+)CD16(+) DC count in stage III/IV versus stage I/II breast carcinoma patients and a lower absolute blood CD123(hi) DC count in multiple myeloma patients, compared to age-matched controls. These data indicate that scientific progress in DC counting technology will lead to the global standardization of DC counting and allow clinically meaningful data to be obtained for DC vaccination in this patient population.⁽⁸⁾

Dendritic cells (DCs) are potent antigen-presenting cells that have the ability to stimulate primary T cell antitumor immune responses in animals and humans. Since the first published clinical trial of dendritic cell vaccination in 1995, 98 studies describing more than 1000 vaccinees. Trials have been performed in 15 countries. Trials included patients with more than two dozen tumor types; most trials studied patients with malignant melanoma, prostate cancer, colorectal carcinoma, or multiple myeloma, using autologous DCs pulsed with synthetic antigens or idiotype antibodies. *The DC vaccines were also prepared by pulsing DCs with tumor lysates or RNA, by transfection with tumor DNA, or by creating tumor cell/DC fusions.* Various approaches to vaccine cell numbers, length of vaccine program, site of vaccination, frozen preservation of vaccine, and use of a maturation step for DCs were used. Adverse effects associated with DC vaccination were uncommon; most were mild and self-limited and none were serious. Clinical responses were observed in approximately half the trials. That is the reason why Ridgway *D*, support the DC vaccination may provide a safe approach to cancer immunotherapy that can overcome the limited reach and immunogenicity of peptide vaccines.⁽⁹⁾

Tumor-specific genes delivered to dendritic cells (DCs) have been used for the generation of cytotoxic T cells (CTLs), but their application has been limited on the one hand by low viral titers resulting in low transduction efficiency and poor protein production, and on the other hand by immunogenicity of the selectable marker and poor viability of the DCs. Batchu RB, et al; addressed these limitations by creating a multipurpose master vector (pMV) and cloning the tumor gene NY-ESO-1, which is highly expressed in more than 50% of advanced myeloma patients. pMV was constructed from a Moloney murine leukemia virus (Mo-MuLV)-based retroviral backbone with the following features: (1) an extended packaging signal to achieve high viral titers, (2) a splice acceptor region to facilitate protein production, (3) a nonimmunogenic selectable marker, dihydrofolate reductase-L22Y (DHFR(L22Y)), to

exclude the generation of CTLs against the selectable marker, (4) an internal ribosomal entry site between the tumor-specific gene (NY-ESO-1) and the selectable marker DHFR(L22Y) for coexpression of two heterologous gene products from a single bicistronic mRNA, minimizing the possibility of differential expression of these two genes, and (5) human granulocyte-macrophage colony-stimulating factor (hGM-CSF) cDNA driven by the human T-lymphotropic virus promoter to enhance DC function and viability. Recombinant virus of pMV-NY-ESO-1 was generated with vesicular stomatitis virus G envelope protein (VSV-G) in the GP2-293 cell line for efficient transduction. We present evidence that the DC phenotype is unaltered after transduction and that more than 85% of DCs express NY-ESO-1, which secrete approximately 40 ng of GM-CSF per 10⁶ DCs.⁽¹⁰⁾

Despite advancements in therapeutic regimens, the prognosis remains under search. Specificity has been an elusive goal for current modalities, but immunotherapy has emerged as a potential means of designing more tumor-specific treatments. Dendritic cells (DC) are the specialized antigen presenting cells of the immune system and have served now as a platform for therapeutic immunizations against such cancers as lymphoma, **multiple myeloma**, melanoma, prostate cancer, renal cell carcinoma, non-small cell lung carcinoma, colon cancer, and even malignant gliomas. DC-based immunizations offer a number of advantages over traditional immunotherapeutic approaches to brain tumors, approaches that have proved promising despite concerns over central nervous system immune privilege and glioma-mediated immunosuppression. Fecci PE, et al; say that the future success of clinical trials will depend on the optimization and standardizing of procedures for DC generation, loading, and administration.⁽¹¹⁾

In vitro priming of T cells with dendritic cells (DC) pulsed with clinically relevant, but weak antigens such as tumor idiotype (Id), is an attractive strategy to generate tumor-specific T lymphocytes. In order to enhance the specific antitumor effect of allogeneic stem cell grafts, *Kim SB, et al;* investigated whether induction of tumor specific T cells using autologous DC pulsed with patient's myeloma Id could be maintained and potentiated by in vitro priming. Similarly, in vitro priming of T cells to Id-pulsed DC resulted in marked increases in cytokine production for both myeloma Id proteins tested. These data suggest that multiple in vitro immunization using DC could be beneficial in generating tumor specific T cells from normal donor PBMC, which may be used for adoptive immunotherapy (e.g. "tumor-specific" donor lymphocyte infusion) of B cell malignancies. In vitro immunization may also offer an alternative to immunization of healthy stem cell transplant donors with tumor antigen.⁽¹²⁾

Multiple myeloma is still a fatal disease. Despite advances in high-dose chemotherapy supported by autologous transplantations, relapse of the underlying disease remains the primary cause of treatment failure. Strategies for post-transplantation immunomodulation would be desirable for eradication of remaining tumor cells. Toward this end, immunotherapy aimed at inducing myeloma-specific immunity in patients has been exploited. Idiotype protein, secreted by myeloma cells, has been the main target for immunotherapy as it is the best-defined, tumor-specific antigen. *Yi Q, et al;* focused this review article is the use of idiotype as a form of protein antigen to immunize patients, to load dendritic cells, or as part of DNA vaccines.⁽¹³⁾

Multiple myeloma (MM) affects 15,000 new patients annually in the US, with 50,000 total patients, and remains incurable. *Hideshima T, et al;* in preliminary in vitro and animal studies suggest a role for MM-host interactions in regulating MM cell growth, drug resistance, and migration in the bone marrow. ⁽¹⁴⁾

Recent studies demonstrate that recombinant adeno-associated virus (rAAV)-based antigen loading of dendritic cells (DCs) generates significant and rapid (one stimulation per week) cytotoxic T-lymphocyte (CTL) responses in vitro against viral antigens. As a more extensive analysis of the rAAV system, *Chiriva-Internati M, et al;* have used a self-antigen, HM1.24, expressed in multiple myeloma (MM). Again, with one stimulation, significant major histocompatibility complex (MHC) class 1-restricted, anti-HM1.24-specific CTL killing was demonstrated against MM cells. Furthermore, higher expression of interferon-gamma (IFN-gamma) in T cells and higher expression levels of, in order of significance, CD80 (2.6- to 3.8-fold increase), CD86, and CD40 on DCs were also observed. We also show that HM1.24 may be an effective antigen for targeting MM. ⁽¹⁵⁾

This review of *Engelman EG, et al;* show that the development of effective cancer vaccines depends heavily on the ability to deliver target antigens to generate an immune response. Dendritic cells must undergo activation to induce an immune response, and this can be achieved through the use of certain carrier proteins, adjuvants, cytokines, or genetically engineered viruses. Dendritic cells are scattered throughout many tissues of the body, as well as bone marrow and peripheral blood. The cytokine, granulocyte-macrophage colony-stimulating factor, has been found to induce the maturation and enhance the viability of dendritic cells isolated from peripheral blood. Numerous clinical trials of antigen-pulsed dendritic cells have been conducted in various types of cancer, including non-Hodgkin lymphoma, multiple myeloma, prostate cancer, malignant melanoma, colorectal cancer, and non-small cell lung cancer. ⁽¹⁶⁾

Dhodapkar MV, et al; studied the function of antitumor T and natural killer T (NKT) cells from the blood and tumor bed in 23 patients with premalignant gammopathy, nonprogressive myeloma, or progressive multiple myeloma. They show that antitumor killer T cells can be detected in patients with both progressive or nonprogressive myeloma. However, freshly isolated NKT cells from both the blood and tumor bed of patients with progressive disease, but not nonprogressive myeloma or premalignant gammopathy, have a marked deficiency of ligand-dependent interferon-gamma production. This functional defect can be overcome in vitro using dendritic cells pulsed with the NKT ligand, alpha-galactosylceramide (alpha-GalCer). Fresh myeloma cells express CD1d, and can be efficiently killed by autologous NKT cells. We hypothesize that presentation of tumor derived glycolipids by myeloma cells leads to NKT dysfunction in vivo. These data demonstrate that clinical progression in patients with monoclonal gammopathies is associated with an acquired but potentially reversible defect in NKT cell function and support the possibility that these innate lymphocytes play a role in controlling the malignant growth of this incurable B cell tumor in patients. ⁽¹⁷⁾

In order to improve the treatment and cure rate of multiple myeloma (MM), immunotherapy is a novel therapeutic approach. Since neoplastic plasma cells do not undergo further hypermutation, the variable region of the immunoglobulin light chain obtained from MM patients (V(L)IgMM) could serve as a tumor-specific antigen. In

addition, dendritic cells (DC) have been identified as potent stimulators of an antigen-specific immune response. Here, Ramadan G, et al; analyze in vitro autologous T-cell proliferation against the V(L)IgMM on presentation by retrovirally transduced dendritic cells. Obtained PI-PLC releases cell surface FLAG-antigen from transduced CD34(+) cells indicating that the vector directs the fusion protein to the cell surface via GPI-anchor. V(L)IgMM transgene expression in DC using our retroviral vector elicited an autologous T-cell proliferation restricted to MHC class I molecules. The proliferative response is more prominent in PMA-derived DC compared to cytokine-derived DC indicating that PMA-derived DC are more potent in activating autologous T-cell proliferation. In conclusion V(L)IgMM is an immunogenic peptide, which under certain conditions could provide a basis for a V(L)Ig-based immunotherapy in MM.⁽¹⁸⁾

In this study, Hayashi T, et al; identified factors in patients' bone marrow (BM) sera inhibiting autologous anti-MM immunity and developed an ex vivo strategy for inducing MM-specific cytotoxic T lymphocytes (CTLs). They found that sera from BM of MM patients inhibited induction of dendritic cells (DCs), evidenced by both phenotype and only weak stimulation of T-cell proliferation. These CTLs from MM patients demonstrated specific cytotoxicity (24.7% at the effector-target [E/T] ratio of 40:1) against autologous primary MM cells. These studies therefore show that CTLs from MM patients can recognize and lyse autologous tumor cells and provide the framework for novel immunotherapy to improve patient outcome in MM.⁽¹⁹⁾

Molta MR, et al; developed dendritic cells (DCs) from circulating monocytes of multiple myeloma. Cells were then grown, according to good manufacturing practice guidelines, in fetal-calf-serum-free medium in cell culture bags and differentiated to dendritic cells (DC) with granulocyte-macrophage colony stimulating factor plus interleukin 4 (IL-4), followed by either tumour necrosis factor-alpha (TNF-alpha) or a cocktail of IL-1beta, IL-6, TNF-alpha and prostaglandin-E2. The CD14+ cell yield was increased from 17.6 +/- 6.5% to 93.8 +/- 6.3% (recovery 64.4 +/- 15.4%, viability > 97%). After cell culture, phenotypic analysis showed that 86.7 +/- 6.8% of the cells were DC: 2.27 +/- 0.9 x 10⁸ DC/leukapheresis were obtained, which represented 20.7 +/- 4.6% of the initial number of CD14+ cells. Notably, the cytokine cocktail induced a significantly higher percentage and yield (28.6 +/- 3% of initial CD14+ cells) of DC than TNF-alpha alone, with secretion of larger amounts of IL-12, potent stimulatory activity on allogeneic T cells and efficient presentation of tumour idotype to autologous T cells. Storage in liquid nitrogen did not modify the phenotype or functional characteristics of preloaded DC. The recovery of thawed, viable DC was 78 +/- 10%. Finally, interferon-alpha-2b was at least as efficient as IL-4 in inducing the differentiation of mature, functional DC from monocytes.⁽²⁰⁾

Two common features in human immunodeficiency virus infection and acquired immunodeficiency syndrome, rheumatoid arthritis, and hematologic malignancies including multiple myeloma are elevated serum levels of beta(2)-microglobulin (beta(2)M) and activation or inhibition of the immune system. We hypothesized that beta(2)M at high concentrations may have a negative impact on the immune system. In this study, Xie J, et al; examined the effects of beta(2)M on monocyte-derived dendritic cells (MoDCs). Thus, their study demonstrates that beta(2)M at high concentrations retards the generation of MoDCs, which may involve down-regulation of major histocompatibility complex class I molecules, inactivation of Raf/MEK/ERK cascade and NF-kappaB, and activation of STAT3, and it merits further study to elucidate the underlying mechanisms.⁽²¹⁾

Dendritic cells (DCs) are antigen-presenting cells that play a key role in the induction of cytotoxic T-lymphocytes. Adjuvant immunotherapy with antigen-loaded DCs represents an attractive anticancer strategy for multiple myeloma (MM). Autologous DCs loaded with idiotypic protein or other myeloma-associated antigen have been used in several clinical trials. Preclinical and first clinical experience have provided valuable insights in the mechanisms of cellular immunity, but few, if any, patients with MM benefited from such vaccination. Taken together, the data suggest that antitumor T-cell responses fail in MM because of a deregulated cytokine network, downregulation of costimulatory surface receptor expression, and changes in T-cell repertoire, enabling tumor cells to escape immune effectors by preventing the antitumor immune response. *Buchler T, et al;* discuss current clinical protocols for DC-based immunotherapy in MM and review some strategies that may increase the efficacy of DC vaccines.⁽²²⁾

Myeloma cells secrete monoclonal immunoglobulin (Ig), called myeloma protein. The variable (V) regions of myeloma proteins are unique to each plasma cell tumor, and therefore contain highly tumor-specific antigenic determinants called idiotopes (Id). In ongoing clinical trials, myeloma patients are vaccinated against the Id of their own myeloma protein. T cells with specificity for Id are thought to be of importance in eradication of multiple myeloma. *Bogen B, et al;* have developed a mouse model to study the molecular and cellular mechanisms for how Id-specific T cell protect against myeloma. The Id-peptide represents an 11-mer of the variable region L chain of a particular mouse myeloma, MOPC315. Id-specific CD4⁺ cells protect in the absence of anti-Id antibodies. Dendritic cells in s.c. tumors take up myeloma protein and present Id-peptide on class II molecules to CD4⁺ cells which become activated and then kill bystander myeloma cells by an unknown mechanism. In conclusion, in an experimental model, Id-specific CD4⁺ T cells protect against myeloma. However, once a tumor is established, Id-specific T cells become incapacitated. Based on these results, it is suggested that Id-vaccination in humans should be reserved for eradication of minimal residual disease, eg after high dose chemotherapy and stem cell transplantation.⁽²³⁾

Sperm protein 17 (Sp17) is a protein recently identified as a novel cancer-testis (CT) antigen in multiple myeloma (MM). Because this tumor antigen demonstrates a very restricted normal tissue expression, Sp17 may be an excellent target for tumor vaccine of MM. In this study, *Chiriva-Inetrnati M, et al;* determined the ability to generate Sp17-specific HLA class I-restricted cytotoxic T lymphocytes (CTLs) from the peripheral blood of 4 patients with MM, 3 consecutive Sp17(+) patients, and 1 Sp17(-) patient. Tumor cell lysis in all cases appeared to be mainly mediated by perforin and could be blocked by concanamycin A. They conclude that Sp17 is a suitable target for immunotherapy of MM. Their findings provide the basis for a clinical study aimed at inducing a cellular immune response directed at Sp17(+) MM.⁽²⁴⁾

To determine how widely applicable this approach is, *Chiriva-Inetrnati M, et al;* have determined the ability to generate Sp17-specific CTLs from four consecutive healthy donors with other HLA class I phenotypes. They found that Sp17-specific HLA class I-restricted CTLs could be easily generated from all four donors. Sp17-specific CTLs were primarily CD8 in phenotype and produced interferon-gamma and very little interleukin-4. These T cells killed target cells primarily via the perforin-mediated route. These results therefore suggest that myeloma-specific donor T-cell infusion

that targets Sp17 to selectively enhance GVM could be applicable to patients with Sp17+ MM.⁽²⁵⁾

Ratta M, et al; studied concentration, phenotype, and function of peripheral blood (PB) dendritic cells (DCs) from patients with multiple myeloma (MM). The absolute number of circulating precursors of myeloid and plasmacytoid DCs was significantly lower in MM patients than in healthy subjects. After maturation, PBDCs from MM patients showed significantly lower expression of HLA-DR, CD40, and CD80 antigens and impaired induction of allogeneic T-cell proliferation compared with controls. Remarkably, they were not capable of presenting the patient-specific tumor idiotype to autologous T cells. Conversely, DCs generated in vitro from CD14(+) monocytes from the same patients, and PBDCs freshly isolated from healthy donors efficiently stimulated allogeneic and autologous T cells. To clarify the mechanism of PBDC deficiency in MM, we investigated the effects of the main plasma cell growth factor, interleukin-6 (IL-6), on the development of DCs from CD34(+) cells. IL-6 inhibited the colony growth of CD34(+) DC progenitors and switched the commitment of CD34(+) cells from DCs to CD14(+) CD1a(-) CD86(-)CD80(-) CD40(+/-)HLA-DR +/- monocytic cells exerting potent phagocytic activity but no antigen-presentation capacity. This effect was reversed by anti-IL-6 antibodies. Growing CD34(+) cells in the presence of autologous serum (without IL-6) also suppressed the development of functional DCs. This study demonstrates that PBDCs from MM patients are functionally defective, partially because of IL-6-mediated inhibition of development. This brings into question the advisability of using PBDCs as antigen carriers for immunotherapy trials in MM. The results also suggest a novel mechanism whereby myeloma cells escape immune recognition.⁽²⁶⁾

Vaccination with idiotype protein-pulsed dendritic cells (DCs) has been explored in multiple myeloma and the results have been disappointing. These studies used immature DCs, which are less potent at activating T cells and could differentiate to macrophages once the cytokines were withdrawn. After intravenous administration, DCs accumulate in the lungs and liver for up to 48 h, thus reducing their potential to migrate to lymphoid organs and interact with T cells. To improve the efficacy of DC vaccination in myeloma, *Yi Q, et al*; investigated the use of idiotype-pulsed mature DCs administered subcutaneously. Five patients (three IgG and two IgA myeloma) with stable partial remission following high-dose chemotherapy were enrolled. DC vaccines were administered three times at 2-week intervals at least 4 months post transplantation. No major side-effects were noted. Thus, subcutaneous administration of idiotype-pulsed mature DCs induced idiotype-specific T- and B-cell responses. Current efforts are geared towards optimizing the conditions of DC generation and administration, and the development of in vitro assays to monitor the cytotoxicity of the T cells.⁽²⁷⁾

The idiotype protein, secreted by myeloma plasma cells, is a tumor-specific but weak antigen. Idiotype-based immunotherapy has been explored in myeloma patients with disappointing results. It is conceivable that myeloma cells contain a multitude of tumor antigens that can more effectively stimulate antitumor T cells. To explore the possibility of using whole myeloma cells as a source of tumor antigens for immunotherapy, *Wen JY, et al*; in this current study was undertaken to generate and examine the function of myeloma-specific cytotoxic T lymphocytes (CTLs) by using dendritic cells (DCs) pulsed with myeloma cell lysates as stimulating cells. Them

results show that these T cells not only recognized and lysed autologous myeloma protein-pulsed DCs, they also killed autologous primary myeloma cells.⁽²⁸⁾

The mechanism of antitumor effect of monoclonal antibodies (mAbs) is not fully understood. Here *Dhodapkar KM, et al;* show that coating myeloma cells with anti-syndecan-1 antibody promotes cross-presentation of cellular antigens by dendritic cells (DCs) to autologous T cells from healthy donors. The tumor cells treated with anti-syndecan-1 or isotype-matched control antibody were fed to HLA-mismatched monocyte-derived immature DCs. Tumor cell-loaded mature DCs induced a strong CD8(+) T cell response that was specific for the cancer-testis (C-T) antigens expressed in the tumor. The CD8(+) T cells killed peptide-pulsed targets, as well as myeloma tumor cells. Importantly, mAbs-coated tumor-loaded DCs were consistently superior to DCs loaded with peptides or dying cells for eliciting tumor-specific killer T cells. This enhanced cross-presentation was not due to enhanced tumor cell uptake or to DC maturation. When mixtures of NY-Eso-1-positive and -negative myeloma cells were captured by DCs, the anti-syndecan-1 antibody had to be on the NY-Eso-1-positive cells to elicit NY-Eso-1-specific response. Cross-presentation was inhibited by pretreatment of DCs with Fc gamma receptor blocking antibodies. Targeting of mAb-coated tumors to DCs may contribute to the efficacy of tumor-reactive mAb and offers a new strategy for immunotherapy.⁽²⁹⁾

Limited response to idotype vaccination in patients with myeloma suggests that there is a need to develop better immunotherapy strategies. *Brown RD, et al;* found that thus patients with myeloma have normal numbers of DCs, but CD80 expression may fail to be up-regulated in the presence of huCD40LT because of tumor-derived TGF-beta1 or IL-10. Autologous high-potency DCs may have to be tested for CD80 up-regulation and biologically modified ex vivo before idotype priming for immunotherapy.⁽³⁰⁾

We recently found that sperm protein 17 (Sp17), a spermatozoa-restricted protein, is aberrantly expressed on the tumor cells in patients with multiple myeloma (MM). It may therefore be possible to generate donor-derived Sp17-specific CTL for administration following allogeneic stem cell transplant to augment graft-versus-myeloma (GVM) effect without inducing a global GVHD. *Chiriva-Internati M, et al;* in their findings suggest the potential for the generation and administration of donor-derived Sp17-specific CTL to augment GVM without inducing GVHD following allogeneic stem cell transplant for MM.⁽³¹⁾

Myeloma protein is a unique tumor antigen that can be used to devise tumor-specific vaccination strategies. As dendritic cells (DCs) are extremely potent at inducing T-cell responses, clinical protocols have been designed using myeloma protein-pulsed DCs to elicit anti-tumor cell responses in vivo. To optimize antigen pulsing of DCs, *Butch AW, et al;* investigated mechanisms of antigen uptake and evaluated various laboratory parameters including class of myeloma protein, antigen exposure time, and DC maturational stage. Pulse-chase experiments revealed that the majority of internalized myeloma protein disappeared within 4 hours but was retained in the presence of chloroquine, indicating antigen processing had occurred. Cultured DCs from myeloma patients are functional and can efficiently endocytose different classes of myeloma protein by the mechanism of macropinocytosis. This demonstrates the feasibility of using all classes of myeloma protein for producing DC vaccines, and

defines culture conditions for optimizing antigen loading of DCs for induction of anti-myeloma responses.⁽³²⁾

The idiotype (Id) determinants on the multiple myeloma immunoglobulin can serve as tumor-specific antigens. An anti-Id immune response may stem the growth of the malignant clone. Liso A, et al; report on 26 patients treated at with chemotherapy and peripheral blood progenitor cell transplantation (PBPC) and vaccinated with the Id protein. The DC infusions and the administration of Id-KLH boosts were well tolerated, with patients experiencing only minor and transient side effects. Of the patients, 24 of 26 generated a KLH-specific cellular proliferative immune response. Only 4 patients developed an Id-specific proliferative immune response. Three of these immune responders were in complete remission at the time of vaccination. A total of 17 patients are alive at a median follow-up of 30 months after transplantation. Id vaccination with autologous DCs is feasible for myeloma patients after transplantation. Id-specific cellular responses can be induced in patients who are in complete remission. Further studies are needed to increase the rate of anti-Id immune responses in patients who do not achieve complete remission.⁽³³⁾

Although DCs are normally present in extremely small numbers in the circulation, recent advances in DC biology have made it possible to generate DCs in culture. DCs can be generated in vitro from various cellular sources including bone marrow, cord blood and peripheral blood. The ability to obtain DCs in numbers suitable for manipulating immune responses has pushed DC-based immunotherapies into the spotlight for treatment of various malignancies, including **multiple myeloma**, a B cell malignancy that is presently incurable. Several studies have demonstrated that myeloma protein, also called idiotype (Id), is sufficiently immunogenic and can be used to generate in vivo T cell responses in myeloma patients. *Hajek R, et al;* determined that clinical trials using Id-pulsed DCs as a vaccine to treat minimal residual disease or relapsed myeloma are currently underway, action necessary for the development of new treatments against multiple myeloma.⁽³⁴⁾

Immunization with ex vivo-generated, tumor antigen-loaded dendritic cells (DC) has been proposed as a strategy for reducing relapses following high-dose chemotherapy, but the ideal time and method for obtaining DC progenitors are unknown. *Morse MA, et al;* determined the percentage yield, phenotype, and function of DC generated over 7 days in GM-CSF and IL-4-supplemented, serum-free medium from PBMC obtained from breast cancer and lymphoma patients at the time of their initial presentation for transplant, cytokine or chemotherapy plus cytokine-mobilized leukapheresis, and following granulocyte recovery from high-dose chemotherapy. The phenotype of the generated cells was similar for the various mobilization procedures, and there were no differences in allostimulatory function of the DC from any of the groups. They conclude that functional DC may be generated equally well from mobilized PBPC and PBPC obtained after high-dose chemotherapy.⁽³⁵⁾

The confirmation that most cancers express one or more molecular changes, which may act as tumour-associated antigens (TAA), combined with the knowledge that T lymphocytes recognize even single amino acid differences in MHC presented peptides has stimulated renewed clinical interest in immunotherapeutic strategies. Dendritic cells (DC) are now recognized as specialist antigen-presenting cells, which initiate, direct and regulate immune responses. Recent data suggest that DC are not

recruited into, or activated by, cancers and that other abnormalities in DC function are associated with malignancy, including multiple myeloma. This provides a rationale for designing immunotherapeutic strategies, which exploit DC as nature's adjuvant either in vivo or in vitro. Low-grade lymphoma and multiple myeloma are slowly progressive malignancies, which generally express a unique immunoglobulin idiotype as a potential TAA. *Hart DN, et al;* suggest from animal models and clinical studies that DC-based immunotherapy strategies, applied when the patient has minimal residual disease, may improve the long-term prognosis in these diseases. ⁽³⁶⁾

Anderson K; did a review where he explained all the recent advances in the biology of multiple myeloma cell growth and survival have suggested new avenues for treatment and potential cure of this disease. Adhesion molecules on the myeloma cell surface mediate their localization in the bone marrow via binding to extracellular matrix proteins and stromal cells. New immune therapies offer the opportunity to treat minimal residual disease after stem cell transplantation, thereby improving outcome. For the first time, a variety of novel treatment strategies derived from advances in understanding the disease pathogenesis offer the potential to achieve long-term disease-free survival in patients with multiple myeloma. ⁽³⁷⁾

Berenson JR. did a review of the tumor cells and the microenvironment of dendritic stromal which it looks appear to be involved in the etiology of multiple myeloma. Switch translocations in myeloma tumor cells often involve oncogenes. These translocations have a clearly established role in the etiology of lymphoma and may prove to have a role in the transformation process of myeloma. Dendritic stromal cells infected with human herpesvirus-8 may provide a growth and antiapoptosis advantage for myeloma bone marrow stromal cells via viral interferon regulatory factor expression. In addition, increased vascular endothelial growth factor expression secondary to viral interleukin-8 receptor gene expression stimulates angiogenesis and inhibits development of uninfected dendritic cells, providing an advantage to infected dendritic cells. These recent advances in the understanding of the etiology of multiple myeloma provide potential new genetic, viral, and cytokine targets for therapy of this fatal malignancy. ⁽³⁸⁾

Melanomas are promising targets for immunotherapy, as they express a number of tissue-specific antigens against which immune responses can be elicited. We have previously described transgenic mice in which malignant cutaneous melanomas are produced. The 1042 melanoma cell line, derived from a primary melanoma in one of these mice, was used here to generate tumours by subcutaneous inoculation in syngeneic animals. All mice injected with 1×10^6 cells of the 1042 cell line developed a tumour. CD4 T cells, CD8 T cells and macrophages infiltrated the tumours. Treatment with dendritic cells pulsed with peptides from melanogenic proteins slowed tumour growth and resulted in increased numbers of infiltrating lymphocytes and macrophages, expansion of CD4 T cells specific for 1042 cell antigens, and increased levels of 1042-specific immunoglobulin G1 (IgG1) and IgM in serum. The frequency of cytotoxic T lymphocytes (CTLs) specific for the MART-1 melanocytic antigen did not increase after dendritic cell treatment. Indeed, the presence of CD8 T cells was apparently not required for the anti-tumour effects: slowing of tumour growth was not abrogated in animals depleted of CD8 T cells using antibodies, or in syngeneic CD8 animals. In contrast, treatment with dendritic cells+peptides was ineffective after depletion of CD4 T cells and in syngeneic CD4 mice. This experimental system therefore provides an opportunity to investigate CD4-dependent

anti-tumour effector mechanisms, and for studies designed to activate the quiescent CTLs which infiltrate melanomas. (39)

CD1 molecules are expressed by antigen-presenting cells such as dendritic cells and mediate primary immune responses to lipids and glycolipids which have been shown to be expressed by various tumors. Glycolipids are expressed by melanoma cells but, despite their immunogenicity, no efficient spontaneous immune responses are elicited. As IL-10 has previously been shown to down-regulate CD1a on dendritic cells and is known to be expressed by various melanoma cell lines, we investigated if melanoma-derived IL-10 could down-regulate CD1 molecule expression on dendritic cells as a possible way to circumvent immune recognition. We found that CD1a, CD1b, CD1c, and CD1d were significantly down-regulated on dendritic cells in metastatic (n = 10) but not in primary melanoma lesions (n = 10). We further detected significantly higher IL-10 protein levels in metastatic than in primary melanomas. Moreover, supernatants from metastatic melanomas were significantly more effective in down-regulating CD1 molecules on dendritic cells than supernatants from primary melanoma cultures. This effect was blocked using a neutralizing IL-10 antibody in a dose dependent manner. Our findings suggest that metastatic but not primary melanomas can down-regulate CD1 molecules on infiltrating dendritic cells by secreting IL-10 which may represent a novel way to escape the immune response directed against the tumor. (40)

An alternative strategy for cancer treatment is the manipulation of the immune system, denominated cancer immunotherapy. The immunotherapeutical use of cells of the immune system, like dendritic cells (DC), is being explored in different clinical protocols. Recently, we finalized a clinical phase I protocol, for the treatment of malignant melanoma, using DCs loaded with tumor lysates. Our results indicate that the subcutaneous application of DCs do not produce adverse effects. We also observed an increase of tumor specific T lymphocytes precursors in the blood, associated to hypersensitivity reactions (DTH) in 60% of the treated patients. In most cases, an stability in the disease was observed, although without a significant association between vaccination and survival. Additionally, therapies based on Interleukin-2 (IL-2) have been used with relative success in the treatment of some kind of tumors since 1985. However, problems associated to the toxicity of IL-2 still restrict its massive use. Our direct experience with the use of IL-2, indicates that low doses and its subcutaneous application, maintains the beneficial effects for patients, eliminating the adverse effects. Based on the accumulated evidence during last the five years, we decided to implement an optimized clinical protocol, which alternatively combines dendritic cells vaccines with the use of low doses of IL-2 for the reinforcement of the immunological system. (41)

Cellular immunotherapy (CI), as we now know it, began in the early 1980s with the use of lymphokine-activated killer cells (LAK) and progressed to the use of the immunologically specific, tumor-infiltrating lymphocytes (TIL). TIL were shown to be particularly effective against melanoma and it was in these trials that we learned the importance of immunologic specificity for tumor. With the identification and characterization of tumor antigens recognized by TIL, we now see the use of these antigens in various forms constituting vaccines. Investigators are using tumor antigens alone or in combination with dendritic cells (DCs), the body's most efficient and powerful antigen-presenting cell. Therapies are being delivered to many patients with different types of cancer in order to combat bulky disease, eliminate micro-

metastatic disease, and provide a memory mechanism to fight tumor recurrence. This review will detail the past 18 years and present the developments that have been made in this therapy. Many believe that with continued development, immunotherapy will provide a fourth modality of cancer therapy. (42)

Dendritic cells (DCs) are antigen-presenting cells that play an important role in the body's immune defence against cancer. Strategies using antigen-primed DCs as tumour vaccines show promise in patients, but the approach is cumbersome to use clinically. Soluble tumour antigens can be targeted to DCs in vivo, but this often induces antigenic tolerance rather than immunity. Liposomes are vesicular lipid structures with adjuvant-like properties. Importantly, liposomes can encapsulate antigen and immunomodulatory factors, thus serving as potent delivery vehicles. Different strategies are being explored to target liposomal antigens to DCs in vivo. One approach has employed single-chain antibody fragments to the DC surface molecules CD11c and DEC-205, attached to the vesicle surface by metal-chelating linkage, to target liposomal membranes containing antigen and either interferon-gamma or lipopolysaccharide to DCs. Such membranes induce dramatic antitumour responses and immunotherapeutic effects when used as a vaccine in the murine tumour model B16-OVA melanoma. Liposomal targeting of antigen and maturation signals directly to DCs in vivo, therefore, represents a much simpler strategy for cancer immunotherapy than antigen loading DCs ex vivo. (43)

The cancer-germline gene MAGE-3 codes for tumor-specific antigens recognized on many tumors by T lymphocytes. A MAGE-3 antigen presented by HLA-A1 has been used in several vaccination trials on metastatic melanoma patients. Only a small minority of patients have shown evidence of tumor regression. Attempts to correlate the tumor rejections with the cytotoxic T lymphocyte (CTL) response against the vaccine have been hampered by the low level of these responses. In noncancerous individuals, the frequency of the T cell precursors against antigen MAGE-3.A1 is approximately 4×10^{-7} CD8 T cells. The diversity of the T cell receptor repertoire of these anti-MAGE-3.A1 precursors was analyzed in one individual. The results indicate that it is very likely that the repertoire comprises >100 clonotypes. On this basis, it is possible to use not only the frequency of CTL precursors in the blood but also the presence of dominant clonotypes to ascertain in patients the existence of anti-MAGE-3.A1 responses as low as 10^{-6} of CD8. With this approach, we observed a correlation between tumor regression and anti-MAGE-3.A1 CTL responses in patients vaccinated with a recombinant virus encoding the antigen and also in patients vaccinated with peptide-pulsed dendritic cells. In contrast, for patients showing tumor regression after vaccination with peptide alone, CTL responses were almost never observed. It is possible that even those CTL responses that are below our present detection level can trigger a sequence of events that leads to tumor regression. (44)

Dendritic cells (DCs) have been well characterized for their ability to initiate cell-mediated immune responses by stimulating naive T cells. However, the use of DCs to stimulate antigen-activated T cells in vivo has not been investigated. In this study, we determined whether DC vaccination could improve the efficacy of activated, adoptively transferred T cells to induce an enhanced antitumor immune response. Mice bearing B16 melanoma tumors expressing the gp100 tumor antigen were treated with cultured, activated T cells transgenic for a T-cell receptor specifically recognizing gp100, with or without concurrent peptide-pulsed DC vaccination. In this

model, antigen-specific DC vaccination induced cytokine production, enhanced proliferation, and increased tumor infiltration of adoptively transferred T cells. Furthermore, the combination of DC vaccination and adoptive T-cell transfer led to a more robust antitumor response than the use of each treatment individually. Collectively, these findings illuminate a new potential application for DCs in the in vivo stimulation of adoptively transferred T cells and may be a useful approach for the immunotherapy of cancer. (45)

Slingluff et al¹ compared in a prospective randomized phase II trial two vaccine approaches for the treatment of 26 patients with advanced melanoma. The primary end point of this trial was clinical response rate according to the Response Evaluation Criteria in Solid Tumors criteria. In addition, immune monitoring assays were performed to assess peptide-specific immune responses in blood and draining sentinel immunized node (SIN). Patients received several melanoma-associated, major histocompatibility I-restricted peptides with a tetanus helper peptide either in an emulsion of granulocyte macrophage colony-stimulating factor (GM-CSF) and Montanide adjuvant (arm 2) or pulsed onto dendritic cells (arm 1). Dendritic cells (DCs) were generated from autologous blood monocytes by in vitro culture with interleukin (IL) -4 and GM-CSF and displayed an immature phenotype. The investigators found a significantly higher presence of cytotoxic T lymphocytes in the SIN of the GM-CSF arm. There were no statistically significant differences in T-cell responses in the peripheral blood or in clinical benefit between the two groups, though potentially confounding factors were present (previous treatments, concurrent low-dose IL-2, additional booster vaccinations, nonblinded arms). The authors conclude that multipeptide vaccines with GM-CSF and adjuvant deserve further investigation. (46)

In our view, the use of immature DCs in this trial does not establish the inferiority of DC-based vaccination. It has been definitively demonstrated that the efficacy of DC vaccines is critically dependent on the maturation state of the cells. Maturation is a terminal differentiation process that transforms DCs from poorly immunostimulatory cells specialized for antigen capture into cells specialized for T-cell stimulation. DC maturation is accompanied by reduced phagocytic uptake, migration to lymphoid tissues, and enhanced T-cell activation potential. Maturation is induced by stimuli that alert the resting DC to the presence of pathogens, inflammation, or tissue injury. This can be exploited in cancer vaccines either ex vivo by the addition of such stimuli (IL-1, IL-6, tumor necrosis factor [TNF] alpha, and prostaglandin E-2 are commonly used) to DC cultures or in situ maturation through the injection of immature DCs into adjuvant-treated skin.

A direct comparison of peptide-loaded immature and mature DCs in patients with metastatic melanoma has shown that only mature DCs induce antigen-specific cytolytic effector responses.² An analysis of several published melanoma vaccine trials indicated that TNF alpha-induced maturation of DCs correlated with favorable clinical outcomes.³ In addition, immature DCs might not only fail to immunize, but can induce tolerance to presented antigens.⁴ In situ matured DCs might have superior migratory and immunostimulatory capacities when compared with DCs matured ex vivo.⁵

In summary, Slingluff et al¹ present an elegant study comparing two vaccine adjuvants. However, since immature DCs are now known to be less potent than

matured DCs, no conclusion in regard to the better adjuvant can be drawn. Although optimal DC preparations are not yet defined for use in cancer immunotherapy, we believe that the incorporation of matured DCs into protocols is important in the search for the most effective vaccine adjuvant.

Previous experiments have shown that tumour-associated antigens can be exploited for a successful anti-tumour immunisation. Previous reports demonstrated that oncoprotein MDM2 (HDM2) contains two highly conserved MHC class I binding motifs, MDM2100 and MDM2441, and that dendritic cells (DC) presenting MDM2100 stimulate an effective CTL reaction against melanoma cells. **MATERIALS AND METHODS:** In this study, we investigated the CTL-inducing capacity of autologous human dendritic cells pulsed with fragment HDM2441. **RESULTS:** In vitro HDM2441-primed T lymphocytes revealed a strong proliferation activity, released Th-1-associated cytokines, and possessed an effective anti-tumour activity causing apoptosis in HDM2441-overexpressing melanoma cells. Cytotoxic assay demonstrated that in parallel to melanoma cells, up to 65% of primed T cells also underwent apoptosis. **CONCLUSION:** These data suggest that HDM2441 may be exploited for broad-spectrum DC-based trials against metastatic melanomas overexpressing HDM2, and point out that the efficacy of such immunotherapeutical approaches may be limited via T cell apoptosis. (47)

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2.1.g Brain Tumor and dendritic cells

The central nervous system is an immunologically privileged site hidden behind the blood brain barrier. Nevertheless, immune effector cells, induced peripherally, can be recruited into the central nervous system. Active immunotherapy of intracranial malignancies is thus potentially feasible. In this study Insug O, *et al*, describe a vaccine regimen, based on bone marrow-derived dendritic cells pulsed with the RNA derived from GL261 glioma cells that induces a specific T cell response and protection against intracerebrally implanted GL261 tumors. Immunohistochemical analysis of brain tumors from vaccinated mice was characterized by pronounced intratumoral infiltrates predominantly of CD4+ as well as CD8+ T cells. The efficacy of the vaccine was improved further by administration of recombinant interleukin-12 into the vaccine regimen.¹

It has been thought that a systemic immune response does not play a role in regression of central nervous system (CNS) tumors, because the CNS is an immunologically privileged site. Here, Aoki H, *et al*, investigated whether DCs pulsed with tumor extract could induce an antitumor effect against malignant glioma. Furthermore, they also investigated whether the antitumor effect become higher by pulsation with tumor extract-liposome complex, compared to pulsation with tumor extract alone. The CTLs showed a specific antitumor activity for GL261 mouse glioma cells. These findings indicated that DCs pulsed with tumor extract and

liposomes might play an important role in the activation of an immune response in malignant glioma.²

The aim of Yamanaka R, *et al*, in this study was to investigate further immunogene treatment of malignant brain tumor to improve its therapeutic efficacy. Thru application of Intratumoral dendritic cells pulsed with Semliki Forest virus (SFV), IL-18 and/or systemic IL-12 were injected into mice bearing the B16 brain tumor. They concluded that Immunogene therapy combines the local administration of dendritic cells pulsed with IL-18 bound by SFV and the systemic administration of IL-12 may be an excellent candidate for the development of a new treatment protocol. A self-replicating SFV system may therefore open a novel approach for the treatment of malignant brain tumor.³

In this Phase I/II trial, the patient's peripheral blood dendritic cells were pulsed with an autologous tumour lysate of the glioma. Yamanaka R, *et al* studied seven patients with glioblastoma and three patients with anaplastic glioma, ranging in age from 20 to 69 years, participated in this study. The mean numbers of vaccinations of tumour lysate-pulsed dendritic cells were 3.7 times intradermally and 3.2 times intratumorally. This study demonstrated the safety and antitumour effects of autologous tumour lysate-pulsed dendritic cell therapy for patients with malignant glioma.⁴

Fecci PE, *et al*, have documented advancements in therapeutic regimens, the prognosis remains poor for patients with malignant gliomas. Specificity has been an elusive goal for current modalities, but immunotherapy has emerged as a potential means of designing more tumor-specific treatments. Dendritic cells have served now as a platform for therapeutic immunizations against such cancers as lymphoma, multiple myeloma, melanoma, prostate cancer, renal cell carcinoma, non-small cell lung carcinoma, colon cancer, and even malignant gliomas. They concluded that the future success of clinical trials will depend on the optimization and standardizing of procedures for DC generation, loading, and administration.⁵

Herrlinger U, *et al* found that subcutaneous vaccination using granulocyte-macrophage colony-stimulating factor (GM-CSF)-transduced glioma cells substantially prolongs survival in the mouse glioma model. To potentiate the efficacy of GM-CSF-based vaccination, syngeneic mice bearing pre-implanted intracerebral gliomas were vaccinated twice subcutaneously with various combinations of glioma cells retrovirally engineered to release GM-CSF, interleukin (IL)-4 or macrophage inflammatory protein (MIP)-1alpha. More than 80% of the animals vaccinated with GM-CSF-secreting or GM-CSF- and IL-4-secreting cells were long-term survivors. Their survival was significantly prolonged compared with animals vaccinated with wild-type cells, which died after a median survival time.⁶

Jean WC, *et al* found that the cytokines play a major role in the regulation of the immune system. In the present study they postulated that peripheral infusion of GM-CSF along with either IL-2 or IL-12 and irradiated tumor cells can lead to increased survival from 9L brain tumors. The addition of IL-2 or IL-12 to the GM-CSF/tumor cell therapy further increased the survival rate up to 90%. The anti-tumor response was associated with vigorous DTH against 9L cells and increased infiltration of CD4+ and

CD8+ lymphocytes into the tumor. These results suggest that the combined infusion of GM-CSF and other cytokines may be effective adjuvants in treating brain tumors.⁷

In this review, Yamanaka R, *et al*, discuss the implications of findings for glioma therapy. A literature review of dendritic cell-based glioma immunotherapy was used to overview the dendritic cell in immunobiology, in the central nervous system and in tumor immunology, glioma-associated antigens, dendritic cell therapy in animal glioma model, dendritic cell therapy in clinical trials and future directions in dendritic cell therapy. They concluded that the dendritic cell-based immunotherapy strategies appear promising as an approach to successfully induce an antitumor immune response and increase survival in patients with glioma. Dendritic cell therapy of glioma seems to be safe and without major side effects. Its efficacy should be further determined in randomized, controlled clinical trials. The development of methods for manipulating dendritic cells for the purpose of vaccination will enhance the clinical usefulness of these cells for biotherapy for malignant glioma.⁸

Intracranial occurrence of follicular dendritic cell (FDC) sarcoma, a rare tumor derived from dendritic cells of the lymphoid follicle, has not yet been described. Therefore, the case of a 53-year-old man presenting with an intracranial mass invading the clivus is reported. The diagnosis of FDC sarcoma was confirmed by immunohistochemical staining for dendritic cell markers, that is, CD21, CD23, and CD35. Due to some similarities with meningioma, intracranial FDC sarcoma might be an underdiagnosed disease.⁹

One strategy utilizing DC-tumor fusion hybrids as cancer vaccine is particularly attractive because of polyclonal presentation of a diverse array of unaltered tumor antigens. Kjaergaard J, *et al*, have recently developed a large-scale electrofusion technique for generating DC-tumor heterokaryons and demonstrated their superb immunogenicity. Here, employing the weakly immunogenic sarcoma, a single vaccination with electrofusion hybrids eradicated tumors established in the lung, skin, and brain. Immunotherapy required intra-lymphoid vaccine delivery and co-administration of adjuvants such as OX-40R antibody. Tumor eradication was immunologically specific and involved the participation of both CD4 and CD8 T cells. Consistent with DC's functionality of MHC-restriction, the use of syngeneic DCs for fusion was an obligatory requirement. Fusion with allogeneic DCs completely lacked therapeutic effects. These findings provide a strong impetus for treating cancer patients with similarly generated DC-tumor hybrids.¹⁰

Yamanaka R, *et al*, determines in their review that despite advances in radiation and chemotherapy along with surgical resectioning, the prognosis of patients with malignant glioma is poor. Among the new treatments currently being investigated for malignant glioma, immunotherapy is theoretically very attractive, since it offers the potential for high tumor-specific cytotoxicity. Therefore, dendritic cell-based immunotherapy could be a new treatment modality for patients with glioma. Dendritic cell-based immunotherapy strategies appear promising as an approach to successfully induce an antitumor immune response and increase survival in patients with glioma. The development of methods for manipulating dendritic cells for the purpose of vaccination will enhance the clinical usefulness of these cells for biotherapy for malignant glioma.¹¹

Broder H, *et al* studied about a recombinant adenovirus vector encoding the melanoma-associated antigen, MART-1, was used to transduce murine DCs, which were then tested for their ability to activate cytotoxic T lymphocytes (CTLs) and induce protective immunity against B16 melanoma tumor cells implanted intracranially. Immunization DCs transduced with an adenoviral vector encoding the MART-1 antigen elicited the development of antigen-specific CTL responses. As evidenced by a prolonged survival curve when compared to control-immunized mice with intracranial B16 tumors, AdMART-1-DC vaccination was able to elicit partial protection against central nervous system tumor challenge *in vivo*.¹²

In this study, Kobayashi T, *et al*, demonstrate that tumor mRNA-loaded dendritic cells can elicit a specific CD8(+) cytotoxic T-lymphocyte (CTL) response against autologous tumor cells in patients with malignant glioma. Cytotoxicity against autologous glioma cells could be significantly inhibited by anti-HLA class I antibody. These data demonstrate that tumor mRNA-loaded DC can be an effective tool in inducing glioma-specific CD8(+) CTLs able to kill autologous glioma cells *in vitro*. They concluded that, DCs transfected with total tumor RNA may represent a method for inducing immune responses against the entire repertoire of glioma antigens.¹³

Fecci PE, *et al*, determine as an immunization platform for brain tumors, dendritic cells supply an impressive host of advantages. On the simplest level, they provide the safety and tumor-specificity so wanted by current therapeutic options. Directions to take now include the identification of new tumor-specific and tumor-associated antigens; the determination of the optimal dendritic cell subtype, generation, loading method, maturation state, dose, and route of delivery for immunizations; further characterization of dendritic cells and their activities; and, potentially, the discovery of ways to pulse dendritic cells efficiently *in vivo*.¹⁴

Ehtesham M, *et al* with the aim of generating antitumor immunity, they enhance *in vivo* tumor antigen presentation by using an intratumoral DC vaccination strategy in the setting of partially irradiated intracranial brain tumors. Fisher rats, implanted with 9L gliomas, were treated with freshly cultured DC inoculated directly into the tumor bed. Intracranially inoculated DCs were found to drain to ipsilateral deep cervical lymph nodes. DC therapy resulted in prolonged survival and immunity to subsequent intracranial tumor re-challenge. These results demonstrate the viability of intratumoral DC vaccination as an effective therapeutic strategy for intracranial glioma.¹⁵

Yamanaka R, *et al*, evaluated dendritic cell (DC)-based immunotherapy for malignant brain tumor to improve its therapeutic efficacy. Dendritic cells were pulsed with phosphate-buffered saline and Semliki Forest virus to treat mice bearing brain tumors of the B16 cell line. The results indicated that therapeutic immunization with DCs pulsed with SFV-IL-12 prolonged the survival of mice with established tumors. Semliki Forest virus induced apoptosis in DCs, which in turn facilitated the uptake of apoptotic cells by other DCs, thus providing a potential mechanism for enhanced immunogenicity.¹⁶

Knutson KL, studied about DCVax, a dendritic cell-based immunotherapy, is an active immunization platform being developed by Northwest Biotherapeutics for the potential treatment of multiple malignancies. The DCVax is tailored to a specific cancer type with either purified tumor-specific antigen or tumor cell extracts derived

from patients at the time of resection. Phase I/II clinical trials of DCVax-Prostate have been completed, and phase III clinical trials have recently been initiated. DCVax-Brain is currently undergoing phase II clinical trials, and DCVax-Lung recently received approval from the US FDA for phase I clinical trials.¹⁷

Witham TF, *et al*, explored the efficacy of vaccination with glioma apoptotic body-pulsed dendritic cells (DCs) for inhibiting tumor growth in the syngeneic 9L glioma/Fischer rat model. These studies suggest that induction of apoptosis in glioma cells may promote the uptake of tumor antigens by DCs. This finding is important because apoptotic body-stimulated DCs may hold promise in promoting a host response against an established intracranial glioma, particularly if the parameters for apoptotic induction, duration of co-culture, and vaccination can be optimized.¹⁸

Heimberger AB, *et al*, showed that immune responses generated by DCs have also been demonstrated to produce clinically significant autoimmunity. Targeting the epidermal growth factor receptor variant III (EGFRvIII), which is a mutation specific to tumor tissue, could eliminate this risk. The purpose of this study was to demonstrate that DC-based immunizations directed solely against this tumor-specific antigen, which is commonly found on tumors that originate within or metastasize to the brain, could be efficacious. They concluded in a murine melanoma model, immunization with DCs mixed with tumor-specific peptide results in an antigen-specific immunological response that recognizes the EGFRvIII mutation, has potent antitumor efficacy against intracerebral tumors that express EGFRvIII, and results in long-lasting antitumor immunity.¹⁹

Soling A and Rainov NG, made a review about current treatment modalities for malignant gliomas, such as surgery, radiation and chemotherapy, have been improved markedly in the past two decades, the prognosis of these neoplasms remains poor, the two year survival rate being approximately 5%. Now one of the most promising immunotherapeutic approaches for the treatment of cancer is the vaccination of cancer patients with dendritic cells (DC) pulsed with tumor antigens. Immunotherapy with DC seems to be able to overcome, at least partially, the immunosuppressive state associated with primary malignant gliomas routes of administration either were subcutaneous, intradermal or intraperitoneal, with multiple injections of DC to enhance antitumor immunity. DC therapy as an adjuvant treatment for patients with malignant glioma seems to be biologically safe. Further clinical studies are warranted.²⁰

Fujita N, *et al*, demonstrate that antitumor reactivity induced in regional lymph nodes (LNs) by s.c. injection of CD40 ligand (CD40L)-transduced tumor (MCA205 CD40L) showed far superior therapeutic efficacy against established brain tumors of a weakly immunogenic fibrosarcoma, MCA205, when adoptively transferred. This is the first report that fully potent antitumor CD4(+) T cell priming was promoted by s.c. injection of CD40L-transduced tumor in the presence of apoptotic tumor cells.²¹

Osada T, *et al*, report a 22-year-old male patient with a history of intracranial malignant germ cell tumor (GCT) who had undergone tumor resection twice, followed by radiation and chemotherapy. The previous histological diagnosis of germinoma and elevated serum beta-hCG levels suggested recurrence of malignant GCT. The

patient declined chemotherapy but accepted dendritic cell (DC)-based immunotherapy. DC inoculation five times resulted in rapid tumor shrinkage and a significant decrease in the serum level of beta-hCG.²²

Yoshida S, *et al*, analysed the in vitro-responses against brain tumor cells using DCs from the peripheral blood of patients with brain tumors. They found that the matured DCs displayed the typical surface phenotype of CD3+, CD45+, CD80+ and CD86+. After the pulsation treatment with tumor lysate, DCs were found to have strong cytotoxic T lymphocyte activity, showing 42.5+12.7% killing of autologous tumor cells. They also found an enhancement of allogeneic T cell proliferation after pulsing the DC with tumor lysate. These data support the efficacy of DC-based immunotherapy for patients with malignant brain tumors.²³

The purpose of Ni HT *et al.* study is to investigate the efficacy of dendritic cell-mediated immunotherapy against intracranial gliomas. Tumor extract-pulsed DC2.4 dendritic cells were then used for the treatment of C57BL/6 mice with syngeneic GL261 gliomas. Animals with intracranial GL261 gliomas and vaccinated i.p. with pulsed DC2.4 dendritic cells exhibited significantly enhanced survival, relative to animals treated with saline or non-pulsed DC2.4 cells alone. In summary these results indicate that dendritic cells pulsed with tumor extract can enhance immune responses to tumor antigen and therefore represent a potential immunotherapeutic approach for treating patients with intracranial gliomas.²⁴

Akasaki Y, *et al*, studied antitumor immunity conferred by fusions of dendritic and glioma cells in a mouse brain tumor model. Previous immunization with fusion cells (FCs) prevented tumor formation on challenge with glioma cells in the flank or in the brain. Efficacy was decreased when studies were performed in mice depleted of CD8+ cells. In a treatment model, FCs were injected subcutaneously after tumor development in the brain. The administration of FCs alone had limited effects on survival of mice bearing brain tumors. Importantly, however, administration of FCs and recombinant interleukin-12 (rIL-12) remarkably prolonged the survival of mice with brain tumors. Cytotoxic T lymphocyte activity against glioma cells from immunized mice was also stimulated by coadministration of FCs and rIL-12 compared with that obtained with FCs or rIL-12 alone. These data support the therapeutic efficacy of combining FC-based vaccine therapy and rIL-12.²⁶

The aim of this study of Yamanaka R, *et al*, was to further investigate dendritic cell (DC)-based immunotherapy for malignant glioma to improve its therapeutic efficacy. The results indicated that pre-immunization with DCs pulsed with the same type of cDNA as in the tumor by a self-replicating RNA vector (that is, SFV) protected mice from tumor challenge, and that therapeutic immunization prolonged the survival of mice with established tumors. The SFV induced apoptosis in DCs and their death facilitated the uptake of apoptotic cells by other DCs, thus providing a potential mechanism for enhanced immunogenicity. They concluded that the therapy with DCs that have been pulsed with SFV-mediated tumor cDNA may be an excellent procedure for the development of new cancer vaccines.²⁷

Yu JS, *et al*, in this Phase I trial, they took patients' peripheral blood dendritic cells were pulsed with peptides eluted from the surface of autologous glioma cells. Three biweekly intradermal vaccinations of peptide-pulsed dendritic cells were administered to seven patients with glioblastoma multiforme and two patients with anaplastic

astrocytoma. This Phase I study demonstrated the feasibility, safety, and bioactivity of an autologous peptide-pulsed dendritic cell vaccine for patients with malignant glioma.²⁸

Tjoa BA, *et al*, summarized in this review brain tumours as one of the most lethal causes in the beginning of this new millennium as they were 30 years ago. Among the promising treatment modalities being tested are various immunotherapeutic approaches. Development of cancer vaccines, also known as active-specific immunotherapy, for malignant brain. Preclinical work on the use of dendritic cell-based vaccine for malignant brain tumours are encouraging. The move from these preliminary studies to the clinic is anticipated with high hope.²⁹

Heimberger AB, *et al*, evaluate the efficacy and toxicity of dendritic cell (DC) based therapy for intracerebral gliomas, they utilized a cell line derived from an astrocytoma that arose spontaneously in a VM/Dk mouse. There was no evidence that autoimmune encephalomyelitis was induced by DC vaccination. Therefore, vaccination with DCs pulsed with glioma tumor homogenate is a safe and effective therapy against a syngeneic glioma located in the immunologically privileged central nervous system (CNS).³⁰

Liau LM, *et al*, investigated the ability of "professional" antigen-presenting cells (dendritic cells) to enhance host antitumor immune responses when injected as a vaccine into tumor-bearing animals. These dendritic cells were then pulsed (cocultured) *ex vivo* with acid-eluted tumor antigens from 9L glioma cells. The results indicate that tumor peptide-pulsed dendritic cell therapy led to prolonged survival in rats with established intracranial 9L tumors implanted. In addition, the results of *in vitro* cytotoxicity assays suggest that vaccination with these peptide-pulsed dendritic cells can induce specific cytotoxic T lymphocytes against 9L tumor cells. They concluded that dendritic antigen-presenting cells pulsed with acid-eluted peptides derived from autologous tumors represent a promising approach to the immunotherapy of established intracranial gliomas.³¹

Glioblastoma multiforme (GBM) is a malignant tumor of the central nervous system that directly suppresses immunological defenses *in vitro* and *in vivo*. Wallenfriedman MA, *et al*, used the peripheral delivery of continuously infused granulocyte-macrophage colony-stimulating factor (GM-CSF) in the presence of irradiated tumor antigens as a tumor-specific stimulant to dendritic cells to initiate an immune response to GBM in rats. The results suggest that the continuous localized delivery of subcutaneous GM-CSF in conjunction with inactivated tumor antigens can initiate a systemic response that leads to the regression of distant peripheral and intracerebral tumors. The success of this treatment illustrates the feasibility of tumor-specific peripheral immunological stimulation after tumor resection to prevent the **recurrence** of malignant brain tumors.³²

Okada H, *et al*, to explore a role for DC-based immunization strategies for the treatment of CNS tumors, they developed a brain tumor model using the C3 sarcoma cell line which expresses the tumor-specific, major histocompatibility complex (MHC) class I. Syngeneic mice receiving intravenous (i.v.) injections of bone marrow-derived DCs pulsed with E7 peptide were effectively protected against a subsequent intracerebral challenge with C3 tumor cells. *In vivo* depletion of CD8+ cells, but not CD4+ or asialo-GM1+ cells, abrogated the efficacy of E7 peptide-pulsed DC therapy

of established tumors, indicating a pivotal role of specific CD8+ T-cell responses in mediating the anti-tumor effect. These findings support the hypothesis that effective CNS anti-tumor immunoreactivity can be generated with DC-based tumor vaccines.³³

Siesjo P *et al*, in this study show that prolonged survival and cures of rats with established gliomas in their brains can be achieved by therapeutic immunizations with tumor cell mutants, combined with in vitro and in vivo interferon (IFN)-gamma (adjuvant) treatment, or tumor cells admixed with semipurified syngeneic dendritic cells. This demonstrates that effective immunizations against a weakly immunogenic brain tumor can be achieved by different adjuvant concepts. The overall results show that therapeutic immunizations can indeed be effective against an established and growing intracerebral tumor.³⁴

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Hyperthermia-induced proteasome inhibition and loss of androgen receptor expression in human prostate cancer cells.

[Pajonk F](#), [van Ophoven A](#), [McBride WH](#).

Department of Radiation Oncology, Experimental Division, David Geffen School of Medicine at UCLA, Los Angeles, California 90095-1714, USA. fpajonk@mac.com

Prostate cancer is the second leading cause of death in men in western countries and is usually treated by surgery and/or radiotherapy. More recently, hyperthermia has been introduced into clinical trials investigating a possible effect in the first-line treatment of prostate cancer. However, the molecular mechanisms of hyperthermia are not completely understood. In this study, we investigated the effects of hyperthermia on proteasome function and its significance for signal transduction, cell death and androgen receptor (AR) expression in PC-3, LnCaP, and DU-145 human and TRAMP-C2 murine prostate cancer cells. Hyperthermia caused apoptosis and radiosensitization and decreased 26S proteasome activity in all three human cell lines to about 40% of untreated control cells. 20S proteasome activity was not affected by heat. Heat treatment inhibited constitutive and radiation-induced activation of nuclear factor kappaB caused by stabilization of IkappaB. Although stabilization of AR by proteasome inhibitors has been reported previously, AR protein levels in LnCaP cells decreased dramatically after heat. Our data suggest that inhibition of proteasome function and dependent signal transduction pathways might be a major molecular mechanisms of heat-induced apoptosis and radiosensitization. Hyperthermia abrogates AR expression in androgen-dependent cells and might thus promote malignant progression of prostate cancer.

PMID: 15930304 [PubMed - indexed for MEDLINE]

Increased expression of the major heat shock protein Hsp72 in human prostate carcinoma cells is dispensable for their viability but confers resistance to a variety of anticancer agents.

[Gabai VL](#), [Budagova KR](#), [Sherman MY](#).

Department of Biochemistry, Boston University School of Medicine, 715 Albany Street, Boston, MA 02118, USA.

The major heat shock protein Hsp72 is expressed at high levels in various types of cancer. Here we attempt to clarify the role of Hsp72 in prostate cancer cells by studying the effects of specific downregulation of this protein using siRNA and antisense RNA approaches. Contrary to previous reports, specific depletion of Hsp72 did not reduce viability of the prostate carcinoma cell lines PC-3 and DU-145. However, even short-term downregulation of Hsp72 in these cells made them more sensitive to hyperthermia, inhibitors of proteasome and Hsp90, and tumor necrosis factor. Interestingly, prolonged downregulation of Hsp72 in PC-3 cells over 3 weeks aggravated these effects, as well as enhanced the sensitivity of cells to oxidative stress, radiation, cis-platinum, vinblastin and taxol. The increased sensitivity to the anticancer agents was due to increased apoptosis, as well as other types of cell death, which resulted in the loss of clonogenic survival. Prolonged downregulation of Hsp72 led to severe suppression of the major survival pathways, ERK and NF-kappaB, which may be responsible for enhanced sensitivity of prostate carcinoma cells to a variety of anticancer treatments, as well as reduction of the cell's capability of forming colonies in soft agar.

PMID: 15735699 [PubMed - indexed for MEDLINE]