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Lymphocytes Genetically Engineered to Express Anti-MAGE-A3 Antigens Provoke Anti-Propagating Immune Responses in Malignant Melanoma Patients

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Abstract

Current forms of treatment for melanoma such as chemotherapy, radiation, and the use of therapeutic agents such as IL-2 or vemurafenib are ineffective and highly toxic to melanoma patients. Increasing interest toward the field of immunotherapy has allowed for clinical trials and research to investigate the potential of immunotherapeutic treatment for melanoma. Increased knowledge of the tumor microenvironment as well as the biology of the human immune system and its pathways allows better, more improved therapeutic strategies. MAGE-A antigens, specific antigens present on melanoma tumor cells, indicate worse prognosis as well as lower rates of survival for melanoma patients. MAGE-A proteins promote aggressive cancer development, through affecting the p53 pathway, which is essential for cell growth regulation and apoptosis. Thus, MAGE-A presence on melanoma tumor cells is indicative of malignant phenotypes. Clinical studies indicate that intravenous administration of autologous genetically modified T-lymphocytes modified to express anti-MAGE-A3 antigen stimulate cellular and humoral immune responses, such as the production of antigen specific long term memory T cells, increased activity of effector T cells, disruption of interactions between the MAGE-A3 surface gene and the melanoma tumor cell, and increased frequency of anti-MAGE-A3 T lymphocytes in the blood. These immune responses all ultimately contribute to melanoma tumor regression. Clinical studies performed on a larger scale are needed to further verify these results. Lymphocytes genetically engineered to express anti-MAGE-A3 antigen provoke anti-propagating immune responses in malignant melanoma patients, demonstrating that they are the most effective form of immunotherapeutic treatment for malignant melanoma.

Keywords: Lymphocytes, Immune, Melanoma

1. Body of Paper

Human cutaneous malignant melanoma has undergone a noteworthy increase in recent years world-wide (Maio, 1996, p. 7). It is projected that over 4 million individuals will be afflicted with melanoma in the first 10 years of the 21st Century (Maio, 1996, p. 1). Even with protective measures, such as the use of sun lotions, melanoma occurrence increases. Scientific research and efforts have expanded in the search for a new, improved, and more efficient treatment for malignant melanoma. Melanoma can be described as a tumor most commonly presented as skin cancer. Melanoma arises as a result of changes occurring in melanin-producing cells, also termed as melanocytes (Clark, Clark, Sasse, Sasse, & Ulloa, 2006, p. 2). While the cause of melanoma is yet to be determined, certain risk factors significantly contribute to its development; some of these risk factors include exposure to high amounts of ultraviolet radiation, especially for individuals with pale skin, a prior family history of melanoma, and high nevi count [congenital growth or mark of some sort on the skin, such as a mole] (Clark et al., 2006, p. 2). Current

treatment for melanoma, including chemotherapy and radiation, has proven unsuccessful and results in detrimental effects to the body. The need for new, successful, and more efficient methods of melanoma treatment is unquestionable. Advancements in the understanding of the melanoma tumor cell as well as the biology of the human immune system allow great promise and hope for anti-cancer immunotherapeutic treatments. Activation of the immune system through the use of biomarkers is a newly discovered hidden treasure and key to the successful destruction of the melanoma tumor cell. Melanoma-associated antigens, also termed as MAGE, are a specific tumor-associated antigen of the Cancer/Testis antigen family. I hypothesize that the use of lymphocytes genetically engineered to express anti-MAGE-A3 antigen will provoke anti-propagating immune responses, ultimately resulting in a disruption of the MAGE-A bonds to the melanoma cell leading to melanoma tumor cell death.

Current forms of treatment for individuals diagnosed with melanoma, such as chemotherapy, biological therapy, or radiation therapy, are ineffective in the eradication of melanoma tumor cells and have largely produced detrimental and harsh side effects to the human body. Research suggests that chemotherapy and high doses of IL-2 as forms of treatment for melanoma produce high toxicity in patients as well as in the worst cases, severe side effects including organ dysfunction. Thus, the effective management of malignant melanoma currently remains an unresolved issue today.

Agarwala (2010) notes that treatment options for melanoma patients diagnosed with advanced disease are few and largely ineffective, resulting in these patients being enrolled in clinical trials for treatment, which has become "the preferred management option" (p. 1). He also states, "The only agent to be approved for advanced melanoma in the US in more than 30 years is high-dose bolus interleukin-2, but its use is associated with high toxicity and cost, and it has also failed to show a survival benefit" (p. 1). The adverse effects far outweigh the benefits of using IL-2 as a treatment for melanoma. Agarwala remarks that chemotherapy and current methods of cancer treatment have been unsuccessful in eradicating melanoma tumor cells, causing researchers to form combination treatments, in hopes of eliciting a wider response from the patient. However, Agarwala states that currently the approved chemotherapeutic treatments involve dacarbazine and fotemustine and "neither alone or in combination regimens has been shown to extend survival in clinical trials" (p. 1). The use of anti-MAGE-A3 antigens as treatment for melanoma does not present such adverse effects as IL-2 or chemotherapy. Anti-MAGE-A3 antigen is not costly or highly toxic to the body due to its derivation from autologous T cells in the cancer patient. This leads one to believe that better options for melanoma treatment, such as anti-MAGE-A3, are yet to be uncovered and thoroughly tested.

Immunotherapy involves harnessing the body's own defense mechanisms in order to fight off cancer tumor cells, rather than using drugs or medication. Immunotherapy is viewed by many researchers and physicians as an attractive form of cancer treatment, mainly due to its ability to use the patient's own immune response to eradicate tumor cells and the fact that it lasts a lifetime. Increasing interest toward the field of immunotherapy has allowed for clinical trials and research to investigate the potential of immunotherapeutic treatment for melanoma. The employment of specific immunotherapeutic agents in cancer treatment, such as autologous lymphocytes genetically modified to express anti-MAGE-A3, allows motivation of the antibody response in the immune system as well as "cytotoxic T cell response to specific tumor antigens" (Conforti et al., 1997, p. 56). The use of lymphocytes synthesized to express anti-MAGE-A3 proves to be the most effective form of immunotherapy available for melanoma patients.

In regard to cancer treatments not involving immunotherapeutic strategies, Farid Menaa (2013) investigates the benefits and negative effects of current FDA-approved metastatic melanoma therapies, including the $B-RAF^{V600E}$ inhibitor. B-RAF is part of a pathway that regulates processes involved in cell division. The V600E mutation acts to activate this pathway. Menaa states that the FDA approved $B-RAF^{V600E}$ inhibitor, Vemurafenib, seems to be an excellent candidate for anticancer therapy due to its causing tumor regression in "40% of patients who have B-RAF mutations of $V_{600}E$" however, the clinical benefits are offset by "high selectivity of the $B-RAF^{V600E}$ inhibitor, and related toxicities of the drug" (p. 2-3). Researchers can conclude that vemurafenib would not be an efficient therapy for melanoma due to $B-RAF^{V600E}$ presenting itself on only some melanoma tumor cells.

Conversely, anti-MAGE-A3 immunotherapeutic cancer treatment does not present this limited efficacy. Instead, anti-MAGE-A3 genetically engineered lymphocytes target MAGE-A3 antigens, present on the vast majority of melanoma tumor cells. A recent clinical study examining the presence of MAGE-A3 in various melanoma tumor samples from skin, lymph nodes, and internal organs of 316 patients reported that MAGE-A3 mRNA was identified in 72% of the metastases through use of a sensitive and precise gel analysis (Berchtold et al., 2005, p. 316). One can infer from these results that the high levels of MAGE-A3 presence on melanoma tumor antigens suggest that MAGE-A3 is frequent in melanomas. As well, the fact that MAGE-A3 mRNA was detected in varying sites of melanoma tumors indicates that MAGE-A3 presence is not affected or influenced by the melanoma tumor site. This evidence suggests that the use of anti-MAGE-A3 lymphocytes to target MAGE-A3 melanoma tumor cells would exhibit higher levels of success as compared to vemurafenib, a more selective inhibitor.

Agarwala (2010) similarly conveys low success rates of non-immunotherapeutic cancer treatment. Agarwala mentions that chemotherapeutic agents initiate extensive "tumor-cell lysis, which releases inflammatory components into [the surrounding] tissue and tumor-associated antigens" (p. 3). However, Agarwala found that use of chemotherapeutic drugs for cancer treatment exhibited "great toxicity and cost" and insignificant levels of survival benefit (p. 2). Agarwala suggests that finding an acceptable balance between the toxicity induced and effectiveness of the cancer therapy is yet to be solved.

Interestingly, research has revealed that anti-MAGE-A3 genetically engineered lymphocytes are capable of the very things Agarwala mentions to be the benefactors of the chemotherapeutic regimen. This is seen in a study conducted by Bregni et al. (2009) involving 10 patients diagnosed with stage IIIc/IV melanoma. Bregni et al. injected these patients intravenously with vaccinations containing autologous anti-MAGE-A3 T lymphocytes and analyzed the outcome. The results revealed that, "anti-MAGE-A3 T cells are able to migrate to the tumor site and to contribute to inflammatory response associated with recognition of the tumor antigen in peripheral tissues" (p. 1651). Bregni et al. explicate how anti-MAGE-A3 T lymphocytes travel to surrounding tissues, causing inflammation responses in the body, similar to Agarwala (2010) mentioning how chemotherapeutic drugs "release inflammatory components into [the surrounding] tissue" (p. 3). Bregni et al. therefore illustrate that anti-MAGE-A3 T lymphocytes are capable and indeed do perform the vital functions of chemotherapeutic agents. Thus, one can conclude from these studies that anti-MAGE-A3 T lymphocytes, with their ability to perform both the vital functions of chemotherapeutic agents and additional functions, demonstrate qualities of more effective melanoma treatment as compared to the use of chemotherapeutic agents.

Increased knowledge of the tumor microenvironment as well as the biology of the human immune system and its pathways allows better, more improved therapeutic strategies. The recent understandings of the biological functions of the MAGE-A3 antigen as well as the immune system have allowed the development of therapeutic strategies to prevent aggressive melanoma development and the successful eradication of the melanoma tumor cell.

The melanoma-associated antigen (MAGE) family can be divided into two categories, MAGE-I and MAGE-II. The MAGE-I family encompasses MAGE-A, -B, and –C (Marcar & Meek, 2012, p. 126). MAGE-I genes are more commonly associated with cancer than genes of the MAGE-II family due to their limited distribution in normal tissues. The MAGE-A family illustrates promising attributes to be a target for specific immunotherapy. One of MAGE-A family's unique characteristics is its limited presentation, with MAGE-A antigenic peptides exhibiting tumor-restricted expression. They are recognized by "both cytotoxic and helper T cells" which establishes MAGE-As significant potential as a target for immunotherapeutic regimens (p. 314). Moreover, the presence of MAGE-A proteins' on the surface of melanoma tumor cells indicate worse prognosis and lower rates of survival for melanoma patients. As well, MAGE-A proteins promote aggressive cancer development, through affecting the p53 pathway, which is essential for cell growth regulation and apoptosis (Ding et al., 2011, p. 88). P53 is a tumor protein associated with tumor suppression. Activation of the p-53 pathway results in cell cycle arrest, preventing the tumor cells from further dividing, thus preventing their duplication, and apoptosis. MAGE-A proteins serve as metastasis associated transcriptional regulators that impact the effective functioning of the p53 pathway by binding to 3 peptides on the DNA binding surface of the p53 tumor protein (Hupp, Maclaine, Marcar, & Meek, 2010, p. 10363). Therefore, MAGE-A proteins prevent the p53 from associating and binding to its proper sites in chromatin. This verifies that MAGE-A proteins inhibit proper functioning of this pathway, allowing growth and development of melanoma tumor cells. MAGE-A detection in tumors with "malignant phenotypes, such as invasiveness or metastasis" suggests that MAGE-A antigens are directly involved in the drive to tumor cell malignancy (Lian et al., 2011, p. 8498). Use of the MAGE-A3 antigen will allow scientists to target a unique melanoma associated antigen present only on melanoma tumor cells, thus effectively allowing the destruction of the melanoma cells.

Marcar and Meek (2012) advocate that MAGE-A expression on cancer cells may trigger malignancy, which is supported by several laboratory-based studies. They state, "MAGE-A3 expression stimulates cell-cycle progression, migration rate, and invasion of thyroid cells *in vitro*" which are all characteristics attributed to aggressive tumor cell behavior (p. 127). The authors further state that MAGE-A proteins have the capability of "conferring resistance to clinically relevant chemotherapeutic drugs by modulating the p53 pathway. Additionally, cell lines that express MAGE-A proteins show resistance to TNF-induced cytotoxicity" which is another form of cancer treatment (p. 127). Marcar and Meek further convey the harm of MAGE-A presence on tumor cells through articulating that MAGE-A3 expression in an orthotopic zenograft model for cancer "leads to increases in tumour size and in the number and size of metastatic foci in the lung" (p. 127). Marcar and Meek clearly indicate that MAGE-A presence on melanoma tumor cells stimulates malignancy and detrimental effects to the cancer patient, such as an increased migration rate of melanoma tumor cells. This indicates that the disruption of MAGE-A interactions between the melanoma tumor cell is pertinent and key in ending aggressive progression of melanoma tumor cells. Once the

MAGE-A interactions on the tumor cell are disrupted, a decrease in tumor cell size as well as an inhibition in the proliferation of metastatic foci will likely present itself in the patient.

Lian et al. substantiate Marcar and Meek's claim stating that MAGE-A proves a deciding role in human tumorigenesis. Lian et al. (2011) articulate that, "several MAGE-A proteins including MAGE-A1, -A2, -A3, and –A6 inhibit p53 transactivation function, in part by recruiting transcriptional HDAC to sites of p53 interaction at promoters, leading to resistance to anti-tumor drugs" (p. 8498). Furthermore, Lian et al. affirm that inhibition of MAGE-A binding to melanoma tumor cells that contain functional p53 would "lead to increased recruitment of p53 to p53-responsive promoters and increase in p-53 dependent transcription, cell-cycle arrest, and cell death" (p. 8498). Studies conducted indicated that alteration of MAGE-A3 led to apoptosis facilitated by p53 activation (p. 8498). Therefore, one can conclude that MAGE-A3 does indeed present oncogenic qualities, and thus the presence of MAGE-A3 antigen on melanoma tumor cells is detrimental to the cancer patient if unchecked. By targeting the MAGE-A3 antigen present on the melanoma tumor surface with anti-MAGE-A3 genetically modified lymphocytes, physicians will be able to allow the p53 pathway to function by disrupting the interactions between the MAGE-A3 surface antigen and the melanoma tumor cell. The functioning p53 pathway will allow regulation of tumor cell growth and apoptosis to occur, thus eliminating the melanoma tumor cell.

Ding et al. (2011) examine the activities of MAGE-A antigens as well as their involvement in tumor cell progression. Ding et al. convey agreement with Lian et al. concerning the involvement of MAGE-A antigens in management of the p53 pathway. Ding et al. (2011) state, "members of the MAGE-A family are able to repress p53 transactivation" which indicates that MAGE-A antigens may function as oncoproteins (p. 88). They also affirm that MAGE-A presence on tumor cells indicates worse prognosis through stating that increased frequencies of MAGE detection on melanoma tumor cells often correlated with poor outcome of the patient (p. 87). Moreover, research reveals that higher grade and metastatic tumors have increased levels of MAGE appearance (p. 87). It is thereby validated that MAGE-A occurrence on melanoma tumor cells insinuates worse outcome for the patients if they are not treated appropriately. Therefore, one must deduce from these studies that MAGE-A proteins exhibit an oncogenic factor through their inhibition of the p53 pathway as well as resulting in worse prognosis for the patient, further providing support and evidence that targeting the MAGE-A protein is the most effective treatment currently available for treating melanoma tumor cells.

Researchers can conclude from this agreement between various studies that MAGE-A manifestation on melanoma tumor cells does indeed denote worse prognosis of the patient as well as lower rates of survival. These negative effects can be attributed to MAGE-A's role in regulation of p53 function, increased malignancy, and resistance to chemotherapeutic medications. Thus, researchers must target the MAGE-A antigens in order to destroy the melanoma tumor cell and terminate its malignant functions. By targeting MAGE-A antigens, physicians will be able to successfully prevent the melanoma tumor cell from reproducing and ultimately cause its death. Since MAGE-A3, when present, is identifiable on all tumor cells, targeting and disrupting the functions of MAGE-A3 would provide successful therapy in both early and late stages of melanoma.

Genetically modified lymphocytes synthesized to express anti-MAGE-A3 antigen demonstrate unique qualities conveying the belief that their use is the most effective form of immunotherapeutic treatment available for melanoma patients. As previously mentioned, MAGE-A3 antigens were detected on 72% of melanoma metastases in a clinical study involving 316 melanoma tumor samples from various locations of the body, such as metastases from internal organs, lymph node, and the skin (Berchtold et al., 2005, p. 316). With MAGE-A3 antigens' high frequency on melanoma tumor sites, researchers can deduce that MAGE-A3 antigen is a viable candidate for immunotherapeutic targeting of melanoma tumor cells.

Employment of anti-MAGE-A3 T lymphocytes involves extraction of autologous lymphocytes from the melanoma cancer patient or lymphocytes genetically engineered in the laboratory. Currently, extraction of autologous lymphocytes from the melanoma patient is a long, strenuous process, which would have to be performed for each unique patient. One can conclude that the use of lymphocytes that are genetically engineered in the laboratory may be more successful and less costly. These lymphocytes, either autologous or genetically made, will be mutated in the laboratory to express anti-MAGE-A3 antigen. The lymphocyte is then injected with other substances, such as immunostimulants to increase efficacy, intravenously into the melanoma cancer patient (Becker et al., 2013, p. 2413).

Upon introduction into the body, anti-MAGE-A3 T lymphocytes stimulate several responses. The anti-MAGE-A3 T lymphocytes elicit immune response, "both humoral and cellular" as well as tumor cell response (Becker et al., 2013, p. 2414). The anti-MAGE-A3 T cells are thought to bind to MAGE-A3 antigens present on the surface of melanoma cells, thus disrupting the interactions between MAGE-A3 surface antigens and the melanoma tumor cells. This would in turn, result in the MAGE-A3 antigens to stop functioning, thus preventing further progression of the

melanoma tumor. As well, induction of anti-MAGE-A3 genetically modified lymphocytes into the body, allows their exposure to the immune system, stimulating the activity of effector T cells (Bregni et al., 2009, p. 1656).

In their study involving patients afflicted with malignant melanoma, Bregni et al. (2009) find that injection of anti-MAGE-A3 genetically modified lymphocytes intravenously allows an increase in MAGE-A3 specific T cells in the body, anti-MAGE-A3 T lymphocytes. Bregni et al. treated 10 malignant melanoma patients, who expressed the MAGE-A3 gene on the melanoma tumor surface, with autologous genetically modified lymphocytes synthesized to express anti-MAGE-A3 antigen (p. 1651). The study involved obtaining samples of patient peripheral blood mononuclear cells at specific intervals during treatment (p. 1652). Bregni et al. identified a significant response in 3 out of the 10 patients treated. In fact, one patient with significant measurable response experienced an increase in anti-MAGE-A3 T lymphocyte frequency in both their peripheral blood and tumor site at levels of "50-fold increase over the pre-vaccination frequency" (p. 1655). Additionally, another patient experienced specific T cell increase of "100-fold compared with the pretreatment blood sample" (p. 1656). Bregni et al. articulate that in addition to the specific T cell development, "both purified CD8+ and CD4+ recognized MAGE-A3-specific DTH reaction. The CD8+ effectors recognized the autologous tumor and were directed against epitopes of MAGE-A3 presented by different HLA-1 alleles" (p. 1656). Bregni et al. thus explicate that induction of anti-MAGE-A3 T lymphocytes into the body allows not only stimulation of effector T cells, such as CD8+, but also stimulation of these effector cells to target the MAGE-A3 genes on the melanoma tumor surface.

Becker et al. support the conclusions of Bregni et al in their clinical study involving 36 patients diagnosed with stage IIIc/IV melanoma. Becker et al. (2013) examined the effects of vaccination with anti-MAGE-A3 genetically modified T lymphocytes in combination with the use of two different immunostimulants, consisting of AS15 and $AS02_B$ (p. 2413). Becker et al. suggest that vaccinations containing anti-MAGE-A3 antigen as well as an immunostimulant will elicit robust immune responses. They observed that after 6 doses of vaccination, "76% of the patients in the AS15 arm presented CD4 T-cell activity greater than the cutoff, compared to only 21% of patients in the $AS02_B$ arm" (p. 2415). Becker et al. concluded that clinical efficacy was higher in patients treated with AS15 arm and anti-MAGE-A3 lymphocytes as compared to treatment with $AS02_B$ and anti-MAGE-A3 T lymphocytes. This illustrates that CD4 T cells, effector T cells, are indeed stimulated by induction of anti-MAGE-A3 T lymphocytes into the body.

Research and clinical studies performed have demonstrated that the intravenous route of administration of anti-MAGE-A3 genetically modified lymphocytes exhibits several advantages, such as the antigen delivery to whole lymphoid compartments allowing tumor regression, production of antigen specific long term memory T cells, as well as stimulation of immune response, which reduces tumor evasion mechanisms.

Paschen et al. (2000) studied the effectiveness and clinical responses elicited from both autologous tumor cells and genetically engineered cells in cancer vaccines. Paschen et al. determined that clinical trials involving the use of genetically engineered cells in vaccines were most feasible in "inducing anti-tumor immune responses even in far-advanced terminally-ill melanoma patients" (p. 70). Paschen et al. discovered that the clinical use of, "CD8+ T-lymphocytes derived from melanoma lesions or the peripheral blood and tumor tissue were shown to be capable to mediate impressive tumor regressions in vivo" (p. 68). Moreover, the studies performed revealed that the use of genetically modified cells brought an aspect of safety, which no other method of cancer treatment has brought. Paschen et al. remark that in comparing the use of autologous lymphocytes to current methods of cancer treatment, autologous lymphocytes display remarkably low levels of toxicity (p. 70). Researchers can apply this to the use of anti-MAGE-A3 autologous genetically modified lymphocytes and thus synthesize that anti-MAGE-A3 T lymphocytes induce tumor regression in melanoma cancer patients.

Bregni et al. (2009) support the findings of Paschen et al. and advocate that studies recently conducted on tumor murine models indicate that the use of genetically modified lymphocytes effectively triggers CD8+ and CD11+ dendritic cells in secondary lymphoid organs, which decreased the growth of the melanomas. In their study, Bregni et al. investigate the use of lymphocytes genetically modified to express the defined viral antigen anti-MAGE-A3 as an immunotherapeutic treatment for melanoma. Bregni et al. discovered that the anti-MAGE-A3 T lymphocytes migrated, "to the tumor site and to contribute to inflammatory response associated with recognition of the tumor antigen in peripheral tissues" (p. 1651). Bregni et al., thus uncovered the ability of anti-MAGE-A3 T lymphocytes to cause inflammatory responses in vivo at the tumor site, allowing the immune system to detect the melanoma tumor cell and reduce tumor evasion techniques.

Arienti et al. (1999) conducted a clinical study which investigated the clinical and immunological outcome of thirty-nine metastatic melanoma patients in response to 3 subcutaneous injections of MAGE-3.A1 peptide. They discovered significant tumor regressions occurred in 7 of the 25 patients who received a full treatment cycle. Five of the seven patients had regional metastases, while two had distant metastases (p. 223). Arienti et al. emphasize the successful results of each patient. Patient 1 exhibited "100 small cutaneous in-transit metastases on the left leg" and

received no forms of treatment prior to the clinical study other than surgery. The study involved the injection of MAGE-3 peptide into the thighs and arms of the patient and, "fifteen months after the first injection, the patient appeared to be completely free of disease, all the skin lesions had become impalpable" (p. 223). Moreover, Arienti et al. remarked that no substantial toxicity was observed in any of the patients treated (p. 220). They concluded that tumor regressions were directly tied to treatment with autologous lymphocytes modified to express anti-MAGE-A3 antigen.

Connerotte et al. (2008) support the findings of Arienti et al. in their clinical testing of melanoma patients with a vaccination containing a "MAGE-A3 peptide presented by the HLA-A1 allele" (p. 3931). Results indicated that anti-MAGE-A3 T-cells effectively stimulated tumor regression processes in patients. Connerotte et al. discovered that of the patients injected with a vaccination containing anti-MAGE-A3 cytotoxic T lymphocytes, "anti-MAGE-A3 CTL responses were found in 3 out of 4 patients who showed tumor regression and in 1 out of 11 who did not" (p. 3931). In addition, results suggested that intravenously injected anti-MAGE-A3 T lymphocytes triggered the restimulation of other antitumor cytotoxic T lymphocytes (p. 3939). Connerotte et al. (2008) concluded that antivaccine cytotoxic T lymphocyte responses in the immune system are directly connected to the occurrence of tumor regression (p. 3931). From these results, one can conclude that lymphocytes modified to express anti-MAGE-A3 antigens effectively lead to tumor regression as well as stimulation of additional cytotoxic T lymphocytes, which allows further aid in recognizing and destructing melanoma tumor cells.

2. Conclusion

Lastly, the induction of anti-MAGE-A3 genetically modified lymphocytes into the body has been determined to generate long term memory specific T cells. The production of long term memory specific T cells will prevent the melanoma tumor cell from reproducing as well as provide insurance that melanoma tumors will no longer thrive and survive in the host. Rather, the specific T cells will travel to any tumor site that has MAGE-A3 presence and eradicate its cells.

Bregni et al. (2009) discovered that the increase in frequency of anti-MAGE-A3 T lymphocytes in the blood, after vaccination with anti-MAGE-A3 genetically modified lymphocytes, was "paralleled with the development of DTH reactivity to MAGE-A3" (p. 1655). Bregni et al. revealed that strong DTH activity was detectable after as little as 5 injections and increased after further vaccinations. Bregni et al. state, "DTH reactivity against MAGE-A3 9 months after the last infusion" was detected in one patient tested (p. 1655). As well, they discovered in another patient that, "the induced anti-MAGE-A3 memory T cells were still detectable in vitro 1 year after the last injection." These results indicate that the immunotherapeutic approach involving use of anti-MAGE-A3 T lymphocytes as cancer treatment is able to establish long term memory T cells and that anti-MAGE-A3 T cell immune response is long lasting, preventing recurrence of melanoma tumor cells. With significant benefits of the use of autologous lymphocytes outweighing the few negative effects, the intravenous administration of anti-MAGE-A3 T cells is the most effective method to induce reaction from the body. The treatment can be increased in efficacy, as well, by adding immunostimulants in the intravenous injection.

The unchecked increase of melanoma occurrence is a prevalent issue in society today. Vaccination has established its worth in medical treatment and prevention of illness. Vaccinations, a technique for treatment that is both minimally invasive for the patient and coupled with few side effects, demonstrate great potential for cancer treatment (Cavallo et al., 2006, p. 204). Intravenously injected vaccinations are deemed to be exceptionally effective in targeting antigens of melanoma tumor cells. Research and clinical studies reveal that induction of antitumor antigens into the human body via vaccination has allowed the detection of melanoma tumor cells and ultimately, their termination. Genetically modified lymphocytes synthesized to express the anti-MAGE-A3 antigen prove to be the most effective form of immunotherapeutic regimen available for melanoma cancer treatment to date. The intravenous administration of these genetically modified lymphocytes into the body behaves as a typical vaccine would and elicits immune response against its target, the tumor antigen MAGE-A3. The injection of anti-MAGE-A3 antigens enhances immune response and exhibits several unique advantages not seen in other cancer treatments. Employing anti-MAGE-A3 antigens as immunotherapeutic treatment for melanoma allows production of long term memory specific T cells, substantially low levels of toxicity, increased frequency of effector T cells and anti-MAGE-A3 specific T cells, melanoma tumor detection, and tumor regression and ultimately tumor eradication. Moreover, these genetically modified lymphocytes will be derived from the patients' own body and thus result in minimal cost. While these studies have primarily addressed metastatic lesions in patients with advanced stage melanoma, the presence of MAGE-A3 on all melanoma tumor cells in a given patient would indicate effectiveness in treating the primary lesion as well. However, as primary lesions are typically treated by surgical excision, there is

no significant clinical need for adjuvant therapy in treating primary lesions of melanoma. The qualities fulfilled by use of anti-MAGE-A3 genetically modified lymphocytes suggest that anti-MAGE-A3 immunotherapeutic treatment is currently the best treatment for melanoma patients available. However, further testing and clinical studies should be performed on a larger scale in order to verify these results.

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