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MONITORING AND TARGETING OF MYELOID-DERIVED SUPPRESSOR CELLS (MDSC) IN DIFFERENT MOUSE CANCER MODELS

By

EVANGELIA MARIA ASSIMACOPOULOS

A Thesis Submitted to The Honors College
In Partial Fulfillment of the Bachelors degree
With Honors in
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THE UNIVERSITY OF ARIZONA
MAY 2014

Approved by:

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University of Arizona, Department of Pediatrics
Monitoring and Targeting of Myeloid-Derived Suppressor Cells (MDSC) in Different Mouse Cancer Models

Evangelia M. Assimacopoulos, under the direction of Dr. Emmanuel Katsanis and Dr. Nicolas Larmonier; University of Arizona, Department of Pediatrics

Abstract

Myeloid-derived suppressor cells (MDSC) undermine immunotherapeutic efforts to treat cancer because of their ability to suppress both innate and adaptive immunity of the host and contribute to tumor progression. Therefore, MDSC hold great potential as a therapeutic target in cancer treatment. Multiple strategies to eliminate MDSC have been described but most lack selectivity. A number of selected anticancer drugs have been described for their ability to eliminate MDSC or to mitigate the immunosuppressive function of these cells, thus improving responses to immunotherapeutic interventions. The potential of Vitamin E derivatives and the chemotherapeutic drug Imatinib mesylate to selectively reduce the number and function of MDSC has not been previously investigated. The purpose of this study was to monitor MDSC induction and target this population in different murine cancer models. γ-Tocotrienol, a derivative of Vitamin E, and Imatinib mesylate, a tyrosine kinase inhibitor, were tested for their ability to reduce MDSC and increase the ratio of effector to suppressor cells in mice bearing either 4T1 breast cancer or MCA205 sarcoma, respectively.
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Abstract
Myeloid-derived suppressor cells (MDSC) undermine immunotherapeutic efforts to treat cancer because of their ability to suppress both innate and adaptive immunity of the host and contribute to tumor progression. Therefore, MDSC hold great potential as a therapeutic target in cancer treatment. Multiple strategies to eliminate MDSC have been described but most lack selectivity. A number of selected anticancer drugs have been described for their ability to eliminate MDSC or to mitigate the immunosuppressive function of these cells, thus improving responses to immunotherapeutic interventions. The potential of Vitamin E derivatives and the chemotherapeutic drug Imatinib mesylate to selectively reduce the number and function of MDSC has not been previously investigated. The purpose of this study was to monitor MDSC induction and target this population in different murine cancer models. γ-Tocotrienol, a derivative of Vitamin E, and Imatinib mesylate, a tyrosine kinase inhibitor, were tested for their ability to reduce MDSC and increase the ratio of effector to suppressor cells in mice bearing either 4T1 breast cancer or MCA205 sarcoma, respectively.

Introduction
Despite extensive research on the subject, cancer remains a continuously evasive culprit to conquer. Characteristic of cancer cells is a generous arsenal of survival tactics including a lack of dependence on the host for growth and evolvement, as well as the ability to resist and evade the host’s efforts to inhibit replication and promote apoptosis (1). A significant problem in cancer treatment is immune suppression by chemotherapeutic agents, radiation therapy, and the tumor itself (2, 3). Immunotherapeutic treatment of cancer would greatly benefit from a novel approach in which anticancer drugs could be used to inhibit tumor-induced immune suppression. Myeloid-Derived Suppressor Cells (MDSC) have recently been identified as a new therapeutic target in cancer treatment due to their significant role in the suppression of host immune response (3).

MDSC are a heterogeneous population of cells that originate in bone marrow as immature myeloid cells (IMCs). In healthy individuals, IMCs differentiate into mature myeloid lineages such as granulocytes, macrophages, or dendritic cells (DCs). However, in cases of cancer, inflammation, and infection, IMCs become unable to terminally differentiate into mature myeloid cells, causing the population of IMCs to expand (3, 4, 5). In cancer, this stimulation of myelopoiesis and block on differentiation is caused by multiple factors mainly produced by tumor cells. Most of these factors play a role in signaling cascades that trigger various JAK-STAT pathways. Janus kinase (JAK), a tyrosine kinase, and Signal Transducer and Activator of Transcription 3 (STAT3), a transcription factor, are involved in the regulation of the growth, survival, differentiation and death of cells. In pathological conditions, the continual, abnormal activation of JAK-STAT pathways in IMCs is associated with their increased survival and proliferation, thereby preventing their differentiation and promoting MDSC expansion (3, 4, 5).

In order for MDSC to acquire their suppressive function, however, they must also be activated by the expression of certain factors, produced mainly by activated T cells and tumor stromal cells (4). Once activated, MDSC are capable of inhibiting innate and adaptive immunity, as well as contributing to angiogenesis, tumor invasion and metastasis (3, 4, 5). MDSC-mediated suppression of T cell function is caused by MDSC expression of high levels of immune suppressive factors, including arginase 1 and inducible nitric oxide synthase (iNOS), as well as an increased release of reactive oxygen species (ROS) and nitric oxide (NO). MDSC alter innate immune responses by modulating the cytokine production of macrophages to secrete immune-suppressive cytokines (3, 4, 5).

Phenotypically, MDSC in mice co-express the antigens Gr-1 and CD11b. The marker Gr-1 consists of two epitopes (Ly6G and Ly6C), creating two distinct
populations of MDSC based on their relative expression of Ly6G and/or Ly6C. The two morphologies of MDSC are granulocytic MDSC (CD11b+Ly6G+Ly6C+low) and monocytic MDSC (CD11b+Ly6G+Ly6C+high). Both subpopulations of MDSC are found to expand in cancer, granulocytic MDSC more than monocytic MDSC, and may have different mechanisms of suppressing host immune function (3, 4, 5, 6).

In order for cancer immunotherapy to be successful, immune suppressants must be eliminated as much as possible in the host, as they are largely responsible for tumor progression and the frequent failure of cancer vaccines. MDSC are one of the most important populations of cells contributing to host immunosuppression in cancer and other pathological states. There are four main strategies that have been described for therapeutic targeting of MDSC; 1) Deactivate MDSC by factors that inhibit NO, arginase 1, ROS and others; 2) Promote MDSC differentiation to mature myeloid cell lineages using agents such as all-trans retinoic acid (ATRA) and Vitamins D3 or A; 3) Block development of myeloid cells before they become MDSC mainly by cell signaling modulators, such as JAK2/STAT3 inhibitors; 4) Deplete MDSC using cytotoxic agents, HSP90 inhibitors and others (3, 4).

For example, Doxorubicin, a chemotherapeutic drug, has recently been shown to be an effective immunomodulatory agent aside from its direct cytotoxic activity against tumor cells. In a study that showed the drug’s selective elimination of MDSC from the spleen, blood, and tumor beds of mice bearing 4T1 breast cancer, it was suggested that triggering of apoptosis was dependent on the induction of ROS (5). Gemcitabine and 5-Fluorouracil, both inhibitors of nucleoside metabolism, are two more chemotherapeutic drugs mentioned often in the literature shown to selectively eliminate MDSC (7, 8, 9). We hypothesize that a Vitamin E derivative and the drug Imatinib mesylate may target MDSC through the JAK/STAT signaling pathway, and potentially other mechanisms as well (1, 2, 10, 11, 13-22).

Studies have demonstrated the potential use of Vitamin E, a lipid-soluble micronutrient, in a novel approach to immunotherapy. Vitamin E, consisting of two groups of compounds; tocopherols and tocotrienols, are found in nature and are already consumed regularly; therefore their use as antineoplastic agents provides low toxicity and no harmful secondary effects (2, 10). Most edible oils from coconut, barley, oat, wheat, rice bran, and palm contain Vitamin E. Vitamin E is also found in hazelnut, walnut, rye, flaxseed, grape seeds, pumpkin seeds, and other natural sources (1). To cite a few examples of these promising properties, one review stated that the Vitamin E analogue α-Tocopheryl succinate, or α-TOS, demonstrated high apoptotic activity in as many as 50 different carcinoma models tested (2). Results of another study testing the oral administration of α-tocopherol and a mixture of tocotrienols, suggest that these compounds affect spleen and mesenteric lymph node (MLN) lymphocyte production and productivity (11).

It is important to note that the different analogues of Vitamin E have different structures which may allow them differing biological activity (2, 12). This activity can also be enhanced, for instance by modifying different functional groups (10). Vitamin E refers to a total of eight different compounds; tocopherols and tocotrienols, each consisting of α, β, γ, and δ derivatives, although succinyl derivates of each isoform can also be synthesized (2, 12). Their antioxidant properties are provided by a redox-active hydroxyl group, which serves as a scavenger of free-radicals, neutralizing potential damage (2).

Originally, tocotrienols were somewhat ignored compared to tocopherols, however there is now evidence to suggest that the former may have greater potential in physiologic functioning (1). One factor in the greater biological effects of tocotrienols is their unsaturated phytol side chain, which allows them to more easily traverse the membrane bilayer and therefore have better uptake and accumulation within cells, as compared to tocopherols with their saturated side chain (13). A few characteristics unique to tocotrienols are their ability to inhibit the inflammatory transcription factors Nuclear Factor (NF)-kB and STAT3, the enzyme HMG-CoA reductase, DNA polymerases in mammals, and select tyrosine kinases (1). These findings as well as results from other studies provide evidence that multiple cell signaling pathways are targeted by tocotrienols in fighting cancer (1, 14, 15, 16, 17, 18).

Further evidence exists that the γ- isoform of tocotrienol might provide the most health benefit compared to the other forms of tocotrienols (19, 20). In an in vivo study investigating the antitumor effect for hormone-refractory prostate cancer (Pca), intraperitoneal injection of γ-tocotrienol sensitized the tumor cells to apoptosis induced by the chemotherapeutic agent docetaxel (DTX) (20). Another study found that γ-tocotrienol, but not γ-tocopherol, inhibits activation of the STAT3 cell signaling pathway and the effects were dose- and time- dependent (19). As discussed before, targeting of STAT3 plays an important role in cancer treatment, as STAT3 activation promotes proliferation, angiogenesis, and tumor immune evasion (19, 21).

The targeted therapeutic agent Imatinib mesylate, an effective tyrosine kinase inhibitor, revolutionized
‘targeted’ anticancer therapy with its approval in 2001 for treating chronic myelogenous leukemia (CML), increasing 5-year survival rates by 60-65% (22). In the case of CML, Imatinib is able to efficiently inhibit the constitutively active tyrosine kinase encoded by the breakpoint cluster region (BCR)-ABL oncogene; the most common cause of CML (22, 23).

Of significant interest is the fact that of those CML patients who are in remission and undergoing treatment with Imatinib, most display strong immunological responses against the leukemia. These immunogenic effects are often brought about by stimulation of antileukemic tumor necrosis factor (TNF)-secreting CD4+ T cell development. In some patients, Imatinib may also stimulate the production of carbohydrate-specific antibodies that are capable of an antitumor effect (22).

Imatinib has also proven effective in the treatment of gastrointestinal stromal tumors (GISTs). Studies suggest that the molecular mechanism behind the drug’s immunogenic effects in cases of GISTs occurs by inhibiting the expression of indoleamine 2,3-dioxygenase, an immunosuppressive enzyme, by tumor cells (22, 23).

In a study previously performed in our laboratory, the modulation of regulatory T lymphocyte (Treg) activity by Imatinib was investigated. Regulatory T lymphocytes are an important population of immunosuppressive cells that are expanded in many cancers. This subpopulation of T cells aids the tumor in host immune evasion, much like MDSC (6, 23). The rationale for this experiment was that, since Treg proliferation, survival and suppressive function rely on tyrosine kinases, and Imatinib is a tyrosine kinase inhibitor, perhaps Imatinib may be capable of impacting Treg. Results showed that Imatinib inhibits phosphorylation of transcription factors STAT3 and STAT5 in Treg, as well as reduces the frequency of Treg cells and impairs immunosuppressive capabilities of Treg in vivo at concentrations relevant to a clinical setting (23).

The goal of our current study was to monitor MDSC induction and evaluate whether the Vitamin E derivative, γ-tocotrienol, and Imatinib mesylate may selectively reduce the number of these cells. The cancer cell lines utilized in the murine models were 4T1 breast cancer (to study γ-tocotrienol) and MCA205 (to study Imatinib mesylate).

A pilot experiment had been previously performed to determine which Vitamin E compound had the greatest effect on the ratio of effector to suppressor cells in mice bearing 4T1 breast cancer. The γ- forms of tocopherol, tocotrienol, tocopherol succinate, and tocotrienol succinate had been chosen for the experiment based on our findings from the literature that the γ- forms had the most potential (19,20). From this pilot study, γ-tocotrienol was determined to be the most promising at increasing the number of effector CD4+ and CD8+ T cells to suppressive MDSC.

Three dose-titration experiments were carried out with γ-tocotrienol. The observed trend in all three cases was a decrease in MDSC and increase in ratio of effector to suppressor cell populations with increasing doses of γ-tocotrienol. However, small sample sizes and a number of outliers prevented the data from being conclusive.

Next, we addressed therapeutic targeting of MDSC by the drug Imatinib mesylate via oral gavage. Results confirmed the hypothesis that Imatinib significantly reduces the population of MDSC in an MCA205 murine model.

Materials and Methods

Animals.

Six- to eight-week-old female BALB/c mice, (Vitamin E studies), and C57Bl/6 mice, (Imatinib studies), were obtained from the National Cancer Institute (NCI). All mice were housed and cared for following the University of Arizona Institutional Animal and Care Guidelines and Use Committee (IACUC).

Tumor cell lines.

Experiments testing the effects of γ-tocotrienol utilized the murine mammary carcinoma cell line 4T1; cultured in RPMI 1640 medium supplemented with 10% Fetal Bovine Serum (FBS), 0.5X Minimal Essential Medium (MEM) Non-Essential Amino Acids, and 1mM sodium pyruvate. The same culture medium was used to culture the mouse sarcoma MCA205 cells, utilized in the Imatinib experiments. Both tumor cell lines were obtained from the American Type Culture Collection.

Therapeutic agents.

The Vitamin E compound, a pure form of γ-tocotrienol, was a generous gift provided by Dr. Klearchos Papas (University of Arizona, Department of Surgery). The olive oil vehicle and the γ-tocotrienol were sterile filtered before experimentation. The drug Imatinib mesylate was obtained from the pharmacy at the University of Arizona Medical Center.

Experimental design: γ-Tocotrienol.

Three dose-titration experiments were performed with the same general outline. On day 0, 4T1 tumor cells were washed and resuspended in PBS to 1 × 10⁶ cells/100μL. 100μL (1 × 10⁴ cells) 4T1 were injected orthotopically into the mammary fat pad of the mice.
On day 1, the average weight of the mice was determined and the mice were randomly divided into treatment groups. In the first dose-titration experiment there were four groups (n=4 mice per group). The groups received 50μL intraperitoneal (i.p.) injections of either the olive oil vehicle (50μL), or 50-, 100-, or 200-mg/kg of γ-tocotrienol. In the second dose-titration experiment, the groups were as follows: 50μL olive oil control (n=4) and 100-, 200-, or 400-mg/kg γ-tocotrienol (n=6 each). The injections were 50μL i.p. as the first dose-titration. For the third dose-titration experiment, the i.p. injections were increased to 100μL for all groups and the groups consisted of the olive oil vehicle (n=4), and 200-, or 400-mg/kg γ-tocotrienol (n=8 each). Mice were treated once daily for 8 days.

On day 9, mice were euthanized and their spleens harvested. Spleens were dissociated, mixed with supplemented RPMI 1640 medium and centrifuged. The supernatant was discarded and the pellets were resuspended in 3mL 1X BD Pharmlyse lysis buffer. 10mL supplemented media was added after 30 seconds to inactivate the lysis buffer. Splenocytes were spun again, the supernatant discarded, and the pellets resuspended in 30mL media. Cells were counted, centrifuged, and 1 x 10⁶ splenocytes (per tube needed) from each spleen were washed in 10mL flow cytometry staining buffer (PBS + 2% FBS), spun, and the supernatant discarded. Cells were resuspended in 100μL/1 x 10⁶ cells of flow cytometry staining buffer and incubated with 2μL/1 x 10⁶ cells of Fc block in flow tubes (1 x 10⁶ cells per tube) for 15 minutes at 4°C. For extracellular staining, fluorochrome-labeled antibodies for Gr-1, CD11b, CD4, and CD8, and their isotype controls were added and the cells incubated in the dark for 30-40 minutes at 4°C. Cells were washed in 1mL PBS, centrifuged, the supernatant discarded, then washed and centrifuged again before suspension in 300μL PBS.

Flow cytometry (Fortessa, BD) was used to analyze the cells. A minimum of 10,000 events were collected. The results were analyzed using the Flowjo software. The ratio of effector (CD4⁺, CD8⁺) to suppressor MDSC (Gr-1⁺CD11b⁺) populations were calculated for each individual mouse.

**Experimental design: Imatinib mesylate.**

Before using Imatinib, pilot experiments were performed to determine at what day and what tumor size MDSC induction occurred in an MCA205 murine model. The experiments followed the same general outline. On day 0, C57Bl/6 mice were given a subcutaneous injection of 1 x 10⁶ viable (less than 5% dead) MCA205 sarcoma cells in 100μL PBS in the lower right quadrant of the abdomen. MCA205 cells were washed twice in PBS prior to injections.

The experimental design was modified slightly for the next and last experiment testing the chemotherapeutic drug Imatinib mesylate. The mice were subcutaneously injected with 5 x 10⁶ MCA205 cells on day 0. Tumors were allowed to grow until 6 mice had tumors of sufficient size. At this point, day 24 after tumor cell injection, Imatinib was administered by oral gavage once daily for 10 days. One day rest was given before the mice were euthanized on day 35. Spleens were harvested and flow cytometry performed in the same way as detailed above for the Vitamin E studies.

**Results**

**Impact of γ-tocotrienol on MDSC.**

Dose-titration experiments were done with the Vitamin E derivative γ-tocotrienol to determine if there was a dose effect of the compound on the percentage of spleen MDSC and the ratio of effector (CD4⁺, CD8⁺) to suppressor (MDSC) cells. These experiments yielded somewhat hopeful results as to the potential use of γ-tocotrienol as a therapeutic agent, however the data was not significant enough to draw any clear conclusions. Statistical analysis was not appropriate in any of the three cases due to outliers and the small sample size.

There were a number of obstacles in getting clear-cut data. First, the tumor injections, although improved over the course of the three experiments, continued to yield tumors of varying sizes (Fig. 2A). A number of factors come into question, such as the health and viability of the 4T1 tumor cells at the time of injection, the quality and consistency of injections, and possible natural variance in tumor growth due to host differences.

Another issue that came up during experimentation was the measuring, administration, and absorption of the Vitamin E compound. γ-Tocotrienol is extremely viscous, which renders measurement of the correct dosage and administration of the compound inconsistent. Intraperitoneal injections were difficult due to the viscosity of the compound. In order to lessen the extent of dosing inconsistencies, the injection volume was increased from 50μL to 100μL in the final experiment. However, the extra olive oil vehicle may have caused poor i.p. absorption, as there was a lot of oil left in the abdomen when the spleens were harvested. This may explain why the differences between the 200 mg/kg and 400 mg/kg group in the third dose-titration experiment are so minimal compared to the differences between doses in the previous two experiments.

What can be observed are general trends that occurred in all experiments to varying degrees. First, it seems that increased doses of γ-tocotrienol are correlated with decreased myeloid-derived suppressor cells.
cell populations (Fig. 1A, Fig. 2B). The data would also suggest a possible positive correlation between the dose of γ-tocotrienol and the ratio of effector cell (CD4⁺, CD8⁺) populations to suppressor cell (Gr-1⁺CD11b⁺) populations (Fig. 2D).

However, these trends are not very apparent by simply looking at averages of the data. For example, averages in the dot plots in Figure 2B-D of the third dose-titration experiment are not very indicative of a dose-titration effect because they are skewed toward the outliers; for instance, there was one sick mouse in the 200 mg/kg group and one in the 400 mg/kg group. The lungs were checked for metastases but none were observed. However both had splenomegaly and looked somewhat cachetic. These mice demonstrated very low T cell counts, exaggerating the proportion of MDSC in the spleen and understating the ratio of effector to suppressor cell populations. The trends discussed above are more apparent when observing the average of the clusters within groups, not the averages including the outliers.

**Impact of Imatinib mesylate on MDSC.**

First, time-point experiments were carried out to characterize the modulation of immunosuppression, specifically induction of MDSC, associated with an MCA205 murine model. Results from these pilot studies yielded inconclusive results. The intended plan for the first experiment was for the tumor to be monitored until tumor size reached approximately 2,500-3,000 mm³. At this time, expected 2-3 weeks after tumor injection, mice were to be euthanized and their spleens harvested for flow cytometry analysis. However, due to poor injections and perhaps too little tumor injected, two of the mice became very sick, one dying on day 25 and the other euthanized on the same day. The experiment was discontinued on day 43, at which time the spleens from the remaining two mice were harvested and analyzed by flow cytometry when tumor size had only reached 782 mm³ and 1,759 mm³. The lungs were checked for metastases but none were observed. However both had splenomegaly and looked somewhat cachetic. These mice demonstrated very low T cell counts, exaggerating the proportion of MDSC in the spleen and understating the ratio of effector to suppressor cell populations. The trends discussed above are more apparent when observing the average of the clusters within groups, not the averages including the outliers.

One of the most critical challenges in cancer treatment is the absence of a functional immune system capable of efficiently controlling tumor development. This is caused by a number of factors, significantly tumor-induced immune suppression. Most chemotherapeutic agents, particularly when used at high doses, and radiation therapy, also suppress the immune system (3). A novel approach to cancer treatment focuses on eliminating factors that weaken immune system functioning, thereby increasing the efficacy of immune-based intervention. Along these lines, a selective panel of anticancer agents have been recently reported for their ability to selectively target immunosuppressive cells such as Treg and MDSC, thereby enhancing the efficacy of immunotherapy (3-6).

Based on our findings from the literature, we hypothesize that the Vitamin E derivative, γ-tocotrienol, and the drug Imatinib mesylate, a conventional chemotherapeutic agent, may also have the potential to selectively reduce the number and function of MDSC. The main rationale behind choosing these agents is that both γ-tocotrienol and Imatinib mesylate are capable of interfering with the JAK-STAT signaling pathway (6, 19, 22, 23). This pathway has been described as an important mechanism in targeting MDSC, as MDSC arise from IMCs when JAK-STAT pathways become constitutively active, thereby preventing IMC differentiation and promoting MDSC expansion (3-5).

A potential dose-titration effect of the Vitamin E derivative γ-tocotrienol on MDSC was demonstrated. However, multiple challenges in the measurement, administration, and absorption of the compound, as well as variability in tumor injections, will need to be addressed before future experiments in order to obtain more consistent data for analysis. Statistical analysis of the data obtained in these experiments is not appropriate due to the small sample size and a number of outliers caused by factors stated previously. For the same reason, average percentages of MDSC, CD4⁺, and CD8⁺ populations do not give an accurate description of the trend observed for most mice in each group, as the
few outliers with such small sample sizes have skewed the averages in directions that underestimated the difference in effect between groups. What is roughly observed is that increasing dose of γ-tocotrienol is associated with a decrease in percent MDSC and an increase in the ratio of effector to suppressor cell populations. In the least, it is obvious that γ-tocotrienol causes some difference in MDSC compared to the control population, although stronger studies are needed to prove a more definitive impact of γ-tocotrienol on MDSC.

A few significant questions need to be addressed concerning how to administer the Vitamin E compound with accuracy, as far as measurement and absorption. Could γ-tocotrienol be modified to a state that was not as viscous? If not, how can more accurate results be obtained using the current viscous form of the compound? Also, is there another method of administration that would allow for greater absorption? Once these inconsistencies have been managed, the focus of future experiments would be to determine the dose of Vitamin E that has the greatest effect on MDSC, as well as the degree of that effect and the mechanism of action behind the outcome. Also, is there a critical window of time in tumor progression at which point the effects of Vitamin E are greatest, and at what point, if any, do the effects diminish? Is treatment with Vitamin E effective at all stages of tumor growth and progression? If Vitamin E is proven capable of selectively inhibiting MDSC, could it be used in combination therapy with current immunotherapeutic strategies such as adoptive T cell transfer and dendritic cell (DC) vaccination?

Promising results were obtained with Imatinib mesylate, which was shown to reduce MDSC in mice bearing MCA205 sarcoma. Average percent MDSC was significantly reduced from the Control to the group treated with Imatinib. The percent MDSC of the Imatinib group resembles that of the mice in the Non-treated group without tumor.

Many of the same questions proposed for Vitamin E need to be addressed here as well. Is there a dose titration effect of Imatinib? What is the most effective mode of administration? Does a critical window of time exist for the effects? What is the mechanism of action in MDSC reduction? In the case of Imatinib, how can immunologic effects be maximized while minimizing negative side effects of a chemotherapy drug? What other molecular mechanisms and immunomodulatory agents could be used to inhibit the suppressive function of residual MDSC which were not eliminated by Imatinib? Recent discovery that Imatinib mesylate elicits antitumor immune responses, aside from its direct cytotoxic activity against cancer cells, points to a potentially more potent future use for the drug in combination with immunotherapy as opposed to its current use as a conventional chemotherapeutic agent (6).

The eventual goal of this research is determining how treatment with γ-tocotrienol and Imatinib mesylate can be carried over to clinically relevant settings. Currently, it seems the future of cancer treatment rests in combination therapy where substances such as γ-tocotrienol or Imatinib mesylate will be used to augment immunotherapy (2, 5, 6, 14, 22, 23).

Our laboratory has already proposed the use of a conventional chemotherapeutic agent, Doxorubicin, to be used for its immunogenic capabilities rather than its direct tumoricidal activity in the treatment of cancer. As stated earlier, Doxorubicin selectively eliminates tumor-induced MDSC. Our laboratory tested a potential chemo-immunotherapeutic treatment in mice that consisted of Doxorubicin treatment followed by Th1 or Th17 cell therapy. Results showed that pairing with Doxorubicin led to increased antitumoral efficacy of the T helper lymphocytes (5).

Our laboratory also found that Imatinib is effective in a similar way as Doxorubicin; as an immunomodulator in combination therapy. This study however, looked at Imatinib’s effect on Treg, rather than MDSC populations. Here they found that Imatinib paired with DC-based immunization was more effective in reducing immunosuppressive Treg than either Imatinib or DC therapy alone (23).

Another study looked at the use of tocotrienol-rich fraction (TRF) in enhancing dendritic cell (DC) based cancer vaccines, which have only been proven minimally effective without the use of an adjuvant like TRF. They found that TRF does, in fact, improve the outcome of using DC based immunotherapy (14).

The results of this study in combination with the current literature provide motivation to pursue agents like γ-tocotrienol and Imatinib mesylate for their ability to target immunosuppressive MDSC, ultimately improving immunotherapeutic interventions in cancer treatment.

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Figure 1. γ-Tocotrienol has an effect on immune cell subsets. Figures are representative flow cytometry analyses from the third and last Vitamin E dose-titration experiment. A, percentage of MDSC (Gr-1+CD11b+) in the spleens of mice bearing 4T1 tumor from either the olive oil control group, or the 200- or 400-mg/kg γ-tocotrienol treatment groups. Population of MDSC decreases with increasing dose of γ-tocotrienol. B, proportion of CD4+ T cells from the same representative sample as shown in A. Population of CD4+ T cells increases with increasing dose of γ-tocotrienol. C, proportion of CD8+ T cells from the same representative samples as in A and B. CD8+ T lymphocytes increase with increasing dose of γ-tocotrienol.
Figure 2. γ-Tocotrienol has an effect on the percentage of MDSC, CD4\(^+\) and CD8\(^+\) immune cell populations. Data is from dose-titration experiment 3. A, tumor volume (mm\(^3\)) variability within each group. B, dot plot of the percentage of MDSC in each mouse analyzed. Olive oil control (n=4), 200mg/kg (n=8), and 400mg/kg (n=8). C, dot plot of the percentage of CD4\(^+\) and CD8\(^+\) T cells in each mouse analyzed. D, ratio of CD4\(^+\) or CD8\(^+\) T cells to MDSC. Percentage of MDSC and ratios appear to increase with γ-tocotrienol, although outliers render data inconclusive.
Figure 3. Imatinib mesylate reduces MDSC in tumor-bearing mice. A, representative flow cytometry analyses. Proportion of MDSC (Gr-1+CD11b+) is decreased in mice that received Imatinib treatment. B, increase in tumor volume over the duration of treatment. C, average percentage of MDSC in each group with standard error bar.

References


