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Antidepressants And Melanoma: Is There A Link?

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Antidepressants and Melanoma: Is There a Link?

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ABSTRACT

ANTIDEPRESSANTS AND MELANOMA: IS THERE A LINK? Stephanie R. Douglas, Deepak Narayan. Section of Plastic and Reconstructive Surgery, Department of Surgery, Yale University School of Medicine, New Haven, CT.

Cutaneous melanoma is the sixth most common cancer in the United States, and its incidence continues to rise steadily at a rate of 2-3% per year. Meanwhile nearly 11% of the general population is treated with antidepressant medication. Laboratory studies suggest a number of theoretical links between antidepressants and melanoma. Serotonin is a growth factor for melanocytes, and antidepressants upregulate components of the Wnt/beta-catenin, mTOR, and MAP kinase signaling pathways, all of which may be involved in melanoma transformation and carcinogenesis. To investigate the potential link between antidepressants and melanoma *in vivo*, localized tumors were induced in a conditional mouse model of $Braf^{V600E}$-induced, *Pten*-deficient melanoma. Citalopram hydrobromide in 0.1% saccharin was administered at a dose of 20 mg/kg/day orally via drinking water beginning at the time of melanoma induction. Control animals received 0.1% saccharin. At weekly intervals, the overall tumor volume was assessed so that the tumor growth rate could be calculated for each mouse. At day 75 following tumor induction, necropsy was performed to assess for the presence of metastases. Antidepressant administration appeared to have no effect on tumor volume at any of the time points measured (day 29, p=0.997; day 36, p=0.761; day 44, p=0.612; day 50, p=0.682; day 57, p=0.797; day 66, p=0.691; day 75, p=0.736), or on metastasis. Secondly, the charts of 1271 patients treated for melanoma at Yale Cancer Center between 1997 and 2013 were reviewed, taking particular note of medication history. A health history questionnaire eliciting information about melanoma risk factors as well as medication histories was administered to patients seen at an outpatient surgery clinic at Yale-New Haven Hospital who had no history of melanoma. Age, sex, and group (cases or controls) were entered into a binary logistic regression model to calculate an adjusted odds ratio for antidepressant exposure as a function of group. Additionally, age, sex, and tumor stage were entered into a second model for the odds of antidepressant exposure as a function of tumor stage. Melanoma patients were less likely to have a history of antidepressant use relative to controls (*OR* 0.567, 95% CI 0.331-0.972, p=0.039). There was no association between antidepressants and melanoma stage at diagnosis. Further study is needed in order to clarify the nature of the association between antidepressants and melanoma risk.

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INTRODUCTION

Cutaneous melanoma is the sixth most common cancer in the United States and the most common fatal malignancy that afflicts young adults. The incidence of melanoma continues to rise steadily at a rate of 2-3% per year. (1) Melanoma risk is affected by both genetic and environmental factors, the most salient of which are skin color, sun exposure, immunocompromised status, and a personal or family history of melanoma. In addition to these known risk factors, however, certain hormones and medications (e.g. oral contraceptives, voriconazole) are postulated to play a role. (2, 3)

Antidepressants are one category of medications commonly used in the general population, including patients with melanoma or those at high risk for the disease. Nearly 10% of the general population is estimated to suffer from a mood disorder, and depression is the leading cause of disability in the United States for people between the ages of 15 and 44. (4, 5) Many of these patients are treated with antidepressant medication. Furthermore, use of antidepressant medication is not limited to the treatment of depression; these drugs are used to treat anxiety, chronic pain, and a variety of other conditions as well. According to National Health and Nutrition Examination surveys conducted in the years 2005-2008, antidepressants are used by nearly 11% of people over the age of 12. In some subsets of the population, the rate of antidepressant use may reach nearly 23%. (6)

Even with an extensive literature on antidepressants and overall cancer risk, the data are inconclusive. Adding to the uncertainty is the fact that few studies have directly investigated the effect of antidepressant exposure on melanoma incidence and prognosis.

Given the large number of people taking antidepressant medication and the potentially lethal nature of melanoma, any link between the two would be cause for grave concern.

Serotonin and melanocytes

Though there are many different classes of antidepressants, all are believed to affect serotonin pathways (**Table 1, Appendix I**). The most commonly prescribed antidepressants fall into the selective serotonin reuptake inhibitor (SSRI) or serotonin-norepinephrine reuptake inhibitor (SNRI) classes. These drugs increase the levels of neurotransmitters in the neural synapse by inhibiting the reuptake of serotonin (in the case of SSRIs) or both serotonin and norepinephrine (in the case of SNRIs) by the presynaptic neuron. Because of their favorable side-effect profile and relative safety in overdose, SSRIs are often first-line agents for the treatment of depression. Tricyclic antidepressants similarly inhibit reuptake of serotonin and norepinephrine, and monoamine oxidase inhibitors, or MAOIs, irreversibly inhibit the enzyme responsible for the breakdown of serotonin, norepinephrine, and dopamine. Even the atypical antidepressants are thought to have some effect on serotonin systems, though the importance of this in the efficacy of these drugs for treating depression is unknown.

Serotonin plays an important role in cutaneous physiology, and the skin is a site of both production and action of serotonin. Serotonin is synthesized from tryptophan in a reaction catalyzed by tryptophan hydroxylase, an enzyme that has been detected in biopsies of normal skin as well as normal melanocytes in culture and melanoma cell lines. Immunohistochemical studies have identified the presence of serotonin in epidermal and adnexal structures as well as within the dermis. (7, 8) The skin is also able to convert serotonin to melatonin, and functional receptors for serotonin and melatonin

are expressed in keratinocytes and fibroblasts, as well as melanocytes. (9) Melatonin activates pathways protective against oxidative stress, while serotonin has vasoactive and immunomodulating effects. (8)

Serotonin serves as a growth factor for many cell types, including hepatocytes, basal cells, fibroblasts, and smooth muscle cells. With regard to melanocytes, serotonin has been shown to either stimulate or inhibit cell proliferation depending on culture conditions. (7) Interestingly, the serum concentration of serotonin is elevated following exposure to ultraviolet light, which itself is the most important environmental risk factor for cutaneous malignancies. (10)

The precise mechanism of antidepressants' effects on mood has not been completely elucidated. However, antidepressants are believed to stimulate neurogenesis in regions of the brain such as the hippocampus, and this may play a role in the antidepressant effects of these compounds. (11) As derivatives of the neural crest, melanocytes may be similarly affected by antidepressant drugs.

Immunomodulatory effects of antidepressants

Immunosuppression is associated with a heightened risk for melanoma as well as a poorer prognosis. (12-14) Melanoma occurs at an increased rate in solid organ transplant recipients (RR 2.1-3.6), patients with lymphoma (RR 1.75-6.17) and patients with HIV (RR 2.6 [95% CI 1.9-3.6]). (15)

SSRIs have been shown to cause impairment of immune function. Herpes simplex virus reactivation has been observed in a number of patients taking fluoxetine, which is thought to be related to problems with cell-mediated immunity. (16) Furthermore, antidepressants can trigger a lupus-like illness in some patients. (17) Fluoxetine and

amitriptyline have been shown to inhibit normal lymphocyte proliferation *in vivo* in a rodent model. (18) Due to the important role of the immune system in clearance of melanoma cells, drugs that compromise immune function could allow melanoma tumors to grow unchecked.

Wnt/beta-catenin signaling

Antidepressants are known to interact with pathways that are thought to be involved in the pathogenesis of melanoma. The Wnt signaling pathway is involved in regulating cell proliferation and differentiation, and Wnt upregulation leads to carcinogenesis via activation of the β-catenin pathway. Increased Wnt2 is known to lead to carcinogenesis, and constitutive action of the Wnt/β-catenin pathway is commonly seen in melanoma. (19, 20)

At the same time, depressed subjects show decreased Wnt signaling in the prefrontal cortex, and this abnormal pattern is reversed with antidepressant treatment. (21) Antidepressants cause increased expression and function of components of the Wnt-Fz-GSK3 pathway, and hippocampal overexpression of Wnt2 or downregulation of GSK3 induces antidepressant-like effects. (21, 22) Given the ability of antidepressants to influence Wnt signaling in cortical and limbic regions of the brain, these drugs may also act on melanocytes, which are cells of neural crest origin.

mTOR activity

Mammalian target of rapamycin (mTOR) is a protein kinase responsible for regulating VEGF production and cell growth in response to signals indicating growth factor stimulation and nutrient availability. mTOR is activated in the majority of

melanomas and functions as a growth promoter. Immunostaining for phosphoribosomal protein S5, an indicator of mTOR activity, increases moving from benign nevus to melanoma in situ to invasive melanoma. (23) Inhibitors of mTOR have anti-mitotic and anti-angiogenic effects in melanoma and other cancers, and these qualities are being put to therapeutic use in a new class of drugs being developed for the treatment of melanoma. (24)

Meanwhile, depressed subjects show dysregulation of mTOR signaling, and there is some evidence to suggest that mTOR activation is required for antidepressant effects. (25) Ketamine, a fast-acting antidepressant, rapidly activates mTOR and increases synaptogenesis in the prefrontal cortex. (21, 26) Certain other antidepressants, including escitalopram, paroxetine, and tranylcypromine, activate mTOR as well. (27) Were this type of mTOR activation to occur within the cutaneous milieu, antidepressants could perhaps influence the incidence or clinical behavior of melanoma.

MAP kinase signaling

The BRAF gene encodes a protein involved in regulating MAP kinase and ERK signaling, which is involved in cell division and differentiation. BRAF activating mutations are present in as many as 80% of nevi and 50% of melanomas, leading to activation of the MAP kinase pathway and increased production of VEGF and matrix metalloproteinases. (28, 29) The MAP kinase pathway is thought to be an important pathway in melanoma transformation. (30) Likewise, the pathway seems to be involved in depression and the response to antidepressant treatment, with acute blockade of MAP kinase signaling leading to a depressive phenotype in behavioral models of depression.

(31) In addition, disruption of the MAP kinase signaling cascade leads to inhibition of antidepressant effects. (21, 31)

Antidepressants and cancer risk

Previous studies suggest a broad theoretical basis for an association between antidepressant medication and cancer risk. Many antidepressants are structurally similar to antihistamines and tamoxifen, which have known tumor-promoting ability. (32) SSRIs bind to growth-regulating intracellular histamine receptors associated with antiestrogen binding sites affecting the cytochrome P450 enzyme system, which is involved in the metabolism of carcinogens. (33, 34)

Preclinical studies have shown that serotonin may mediate cell proliferation in colonic tumor cells, and inhibition of serotonin uptake results in suppression of cell proliferation. (35) Some authors have described a biphasic response curve for fluoxetine and amitriptyline with low concentrations stimulating pancreatic cancer, colorectal cancer, and glioma growth and high concentrations inhibiting the growth of tumors. (36)

Clinical studies of antidepressants and cancer risk have yielded mixed results. (37) Most studies have shown no statistically significant difference in cancer risk following use of antidepressants from multiple drug classes. (38-42) However, a small number of studies have demonstrated an increased risk of epithelial ovarian cancer in association with tricyclic antidepressants. (43, 44) Still others have suggested that the effect of antidepressants with regard to breast, esophageal, and gastric cancer risk is dependent on the timing and duration of exposure. (45-47)

Antidepressants and melanoma: in vitro and animal literature

A small number of animal studies have directly studied the effect of antidepressants on melanoma growth and metastasis in animal models. In a rodent model of *in vivo* melanoma growth, tumors showed a two-to-three-fold greater rate of growth in mice receiving daily intraperitoneal injections of fluoxetine or amitriptyline at clinically relevant doses as compared to a control group of animals not injected with an antidepressant. (18)

Another study examined the effects of pretreatment with desipramine or fluoxetine on primary tumor growth, metastasis formation, and mortality rate in mice subsequently inoculated with B16F10 melanoma cells. (48) In young males, desipramine promoted development of metastases and increased the mortality rate in spite of inhibiting growth of the primary tumor. This prometastatic effect was associated with an increase in plasma levels of VEG-F and MMP-9. In aged animals, both antidepressants increased primary tumor growth and had a moderate stimulatory effect on metastasis formation.

In contrast, a similar study examined the effect of intraperitoneally injected fluoxetine, desipramine, and mirtazapine in the B16F10 melanoma model and found that fluoxetine had a dramatic inhibitory effect on melanoma tumor growth. (49) In this study, desipramine tended to decrease tumor growth as well, while mirtazapine had no effect. Previous studies by this group on the effect of fluoxetine on growth of S19 melanoma in female DBA/2 mice showed that fluoxetine had an inhibitory effect when low doses of cells were inoculated subcutaneously, but that effect disappeared when higher number of cells were introduced. (50)

While antidepressants upregulate activity in a number of pathways associated with the pathogenesis of melanoma, they may also influence other pathways in ways that serve to inhibit tumor growth. For example, one of the reasons melanoma is poorly responsive to chemotherapy and radiation is that tumor cells constitutively express Akt, protecting them against apoptosis. Sertraline is a potent inhibitor of the phosphorylation of Akt, and in vitro studies have demonstrated that sertraline can cause induction of endoplasmic reticulum and death of melanoma cells. (51) When sertraline was administered to mice with A375 melanoma xenografts, the drug appeared to inhibit tumor growth in vivo, perhaps due to downregulation of Akt, though the results did not achieve statistical significance.

Tricyclic antidepressants have likewise shown inhibitory effects on melanoma growth under certain conditions. Two cell lines and eight primary cell cultures from melanoma metastases were exposed to nortriptyline, clomipramine, and amitriptyline. All three drugs inhibited tumor cell growth, though nortriptyline was more active than the others. (52)

Interestingly, fluoxetine has been shown to act as an antioxidant in the B16F10 mouse model of melanoma, with fluoxetine preventing against melanoma-induced oxidative changes in the mouse spleen. (53) The implications of this in human disease are unknown.

Antidepressants and melanoma risk in humans

To date, few studies have investigated the relationship between antidepressants and melanoma in humans. Unpublished data from a cohort of patients with melanoma treated in the Yale Melanoma Unit demonstrated a higher incidence of multiple

melanomas in patients being treated with an SSRI or SNRI, with multiple primary lesions in 15% of such patients as compared to the overall rate of 6.4% identified in a prior study.

With conflicting data in the basic science literature and a paucity of data in human subjects, further study of the possible link between antidepressants and melanoma is clearly warranted. Antidepressants are widely prescribed to a large segment of the population for a variety of medical conditions. Thus, if antidepressants were to influence the incidence or progression of melanoma, the implications would be far-reaching, affecting treatment decisions not only for melanoma patients with coexisting depression but possibly also the larger body of patients with conditions for which antidepressants are often prescribed.

PURPOSE, HYPOTHESES, AND SPECIFIC AIMS

Despite the many theoretical links between serotonin and the development and progression of melanoma, the effect of antidepressants on the incidence and outcome of melanoma is unknown. By investigating the effect of citalopram on tumor latency, tumor number, and tumor growth trajectory in mice that are genetically programmed to develop melanoma, this study sheds light on an important question that remains poorly understood. The epidemiological component of the study serves as a first step to understanding the effect of antidepressants on melanoma risk in humans. Due to the growth-stimulatory effects of serotonin on melanocytes, the immunomodulatory effects of antidepressants, and the ability of antidepressants to interact with signaling networks (e.g. Wnt, mTOR, MAP kinase) in a way that may be favorable to melanoma tumorigenesis, we hypothesized that antidepressants may increase the risk for developing melanoma or may otherwise alter tumor growth characteristics.

Aim 1: To investigate the effect of antidepressants on tumor growth rate in vivo using a mouse model of melanoma.

Antidepressants are known to affect a number of pathways thought to be involved in melanoma pathogenesis, and serotonin is a growth factor for melanocytes. Citalopram is a highly serotonergic antidepressant of the SSRI class and is widely used to treat depression and anxiety due to its favorable side-effect profile. The Braf/Pten melanoma model described below features tumors with genetic lesions that are common in human melanoma and offers control over the timing and location of tumor initiation. This study is the first to examine the effect of antidepressant medication on the growth characteristics of melanoma tumors arising *in vivo*. We hypothesized that citalopram

would enhance melanoma tumor growth, leading to an increased tumor growth rate in a mouse model of melanoma.

Aim 2: To compare the prevalence of antidepressant use among patients treated for melanoma at Yale Cancer Center from 1997-2013 with that of control patients seen at an outpatient surgery clinic at Yale with no history of melanoma.

Preliminary review of data from a subset of patients treated at the Yale Melanoma Unit reveals a higher-than-average rate of antidepressant use as well as an increased number of patients with multiple primary melanomas among those taking antidepressants. We hypothesized that the melanoma patient population would exhibit a higher rate of antidepressant exposure (at or prior to the time of melanoma diagnosis) relative to an age- and sex-matched control population seen at an outpatient surgery clinic at Yale with no history of melanoma.

Aim 3: To compare the rate of antidepressant exposure among melanoma patients with early melanoma tumors and those with more advanced disease who were treated at Yale Cancer Center from 1997-2013.

Prior studies in animal models of melanoma have shown that antidepressants potentially affect not only the rate of growth of the primary tumor but also the propensity of the tumor to metastasize. We hypothesized that melanoma progression would be facilitated by the presence of antidepressants in patients taking these medications, such that melanoma patients presenting with late-stage disease would be more likely to have a history of antidepressant exposure.

METHODS

Aim 1: Mouse model

MELANOMA MODEL: The experiments in **Aim 1** utilized the conditional mouse model of *Braf*^{V600E}-induced, *Pten*-deficient melanoma described in Dankort et al. 2009 to investigate the effect of citalopram on melanoma growth parameters *in vivo*. (54) *BRAF* activating mutations are present in 80% of nevi and 50% of melanomas, forming the basis for this model. (54, 55) The combination of mutated *BRAF* and silencing of *PTEN* is found in 20% of melanomas. (55) The *Braf/Pten* melanoma model has been successfully used by investigators at Yale to study pharmacologic agents that influence signaling pathways implicated in the pathogenesis of melanoma. Because this model recapitulates the genetic lesions commonly found in human melanoma, the effect of pharmacologic agents on the development and progression of melanoma in these animals is thought to parallel the processes that occur in human patients with melanoma. *Braf*^{V600E} cell lines derived from Braf/Pten mice have been shown to respond to inhibition of mTorc1 and MEK1/2 using rapamycin and PD325901, respectively, suggesting that the biological behavior of tumors in these animals is similar to that of melanoma tumors in humans. (54)

The Braf/Pten melanoma model allows localized tumors to be induced by topical application of 4-hydroxytamoxifen (4-HT), with 100% penetrance within 10 weeks (**Figure 1**). (55) In these animals, the Cre-recombinase::estrogen receptor (CreER) fusion protein is constitutively expressed in melanocytes by Tyr transgenic promoter elements. Exposure to 4-HT causes CreER to be released from Hsp90 and translocate to the nucleus, where it results in the removal of DNA sequences between loxP sites and

consequent activation or inactivation of the gene of interest (here, *BRAF*). (55) In prior rodent studies investigating the possible association between antidepressants and melanoma, mice received subcutaneous or intravenous injections of B16F10 mouse melanoma cells that had been grown in culture, or melanoma xenografts. (18, 48-51, 53) The *Braf/Pten* model more closely replicates the biology of melanoma in humans, and the clinical behavior of such tumors likely has greater relevance to the human population.

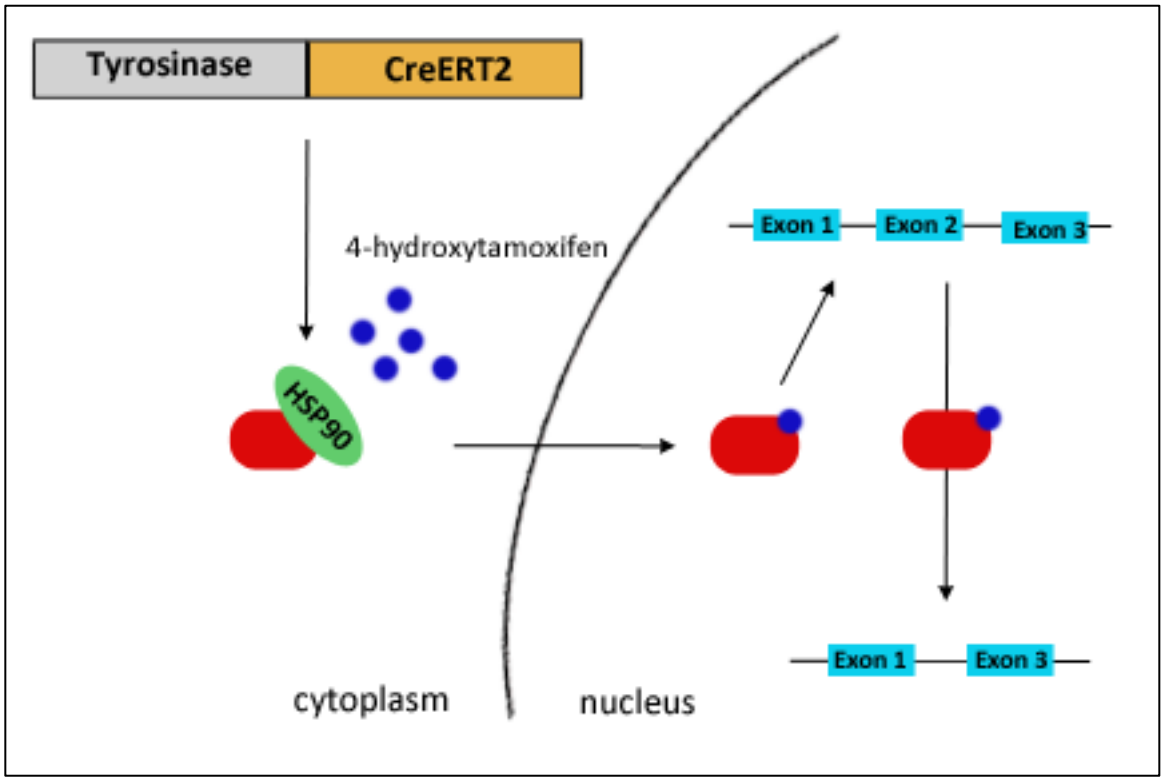

Figure 1: Braf/Pten mouse model of melanoma

Schematic showing induction of melanoma with 4-HT. The Cre-recombinase::estrogen receptor (CreER) fusion protein is constitutively expressed in melanocytes by Tyr transgenic promoter elements, Exposure to 4-HT causes CreER to be released from Hsp90 and translocate to the nucleus, where it results in removal of DNA sequences between loxP sites and subsequent activation or inactivation of the gene of interest.

Animals were housed in groups of 1-3 animals per cage in a temperature-controlled environment under a 12-hour light-dark cycle. Standard food and drinking water solutions (described below) were freely available. Animal experiments were performed in accordance with a protocol approved by the Institutional Animal Care and Use Committee at Yale University (IACUC protocol #2011-11211).

TUMOR INDUCTION: One major advantage of the Braf/Pten model of melanoma is that tumor induction with 4-HT offers control over the timing and location of melanoma initiation. Melanoma was induced on the back skin of the animals by treating adult mice with topical administration of 1-2 μl of 1.9 mg/ml (5 mM) 4-HT at 3 weeks of age.

DRUG ADMINISTRATION: Citalopram hydrobromide was chosen for these experiments because its high binding affinity for the serotonin transporter makes it highly selective for serotonin. In view of serotonin's role as a growth factor for melanocytes, a highly serotonergic antidepressant was felt to be desirable for these experiments. Citalopram has been used extensively in rodent studies and is commonly prescribed for the treatment of depression and anxiety due to its favorable side-effect profile.

Antidepressant administration began at the time of tumor induction with 4-HT and continued until the study endpoint (outlined below) was reached **(Figure 2)**. Citalopram hydrobromide (Sigma-Aldrich, St. Louis, MO) was administered orally via drinking water at a dose of 20 mg/kg/day to the animals in the experimental group. The drinking water solution contained 0.1% saccharin to mask the taste of the drug. Control animals received 0.1% saccharin. Animals were denied access to other sources of

drinking water. There were two animals in the control group receiving saccharin solution, while four animals received citalopram/saccharin solution.

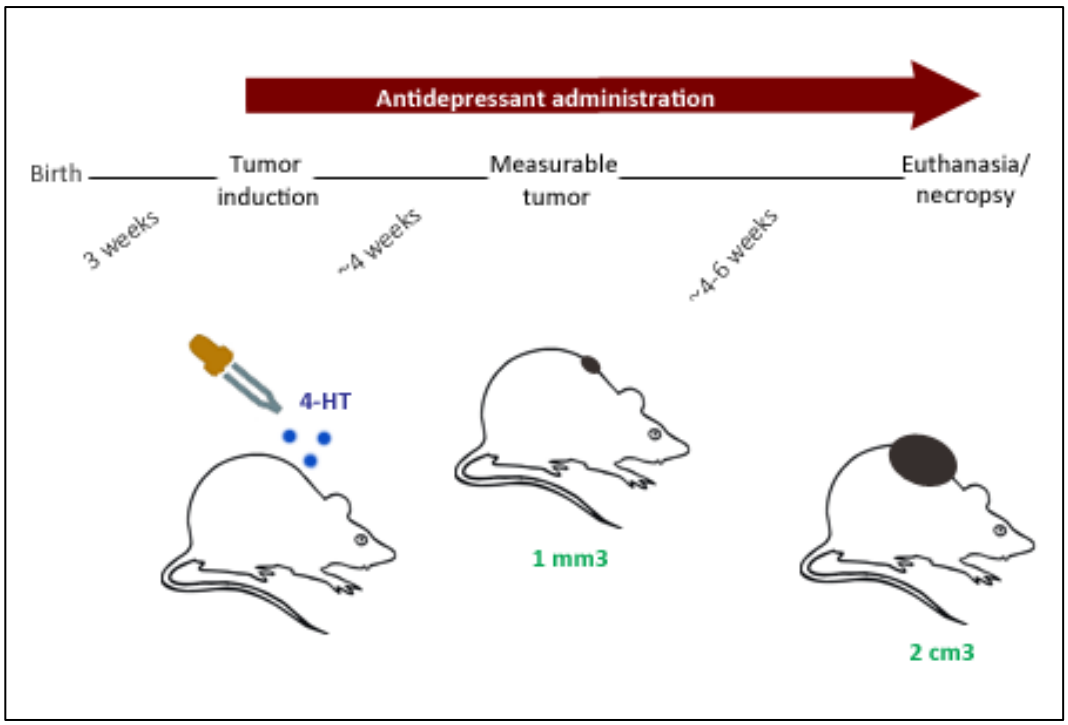

Figure 2: In vivo study design

Tumor is induced by topical application of 4-hydroxytamoxifen when the mouse is 3 weeks of age. Antidepressant/placebo administration begins at the time of tumor induction. Tumors reach 1 mm^3 in size by 4 weeks post-tumor induction. Study endpoint is reached when tumor measures 2 cm^3.

4-HT 4-hydroxytamoxifen

Saccharin solution (0.1%) was prepared by adding 200 mg saccharin (Sigma-Aldrich, St. Louis, MO) to 200 ml near-boiling water and stirring until dissolved. For the citalopram solution, 22 mg citalopram hydrobromide was added to the warm saccharin

solution to achieve a concentration of 0.11 mg/ml, and the solution was gently swirled until no visible solid remained. Solutions were allowed to cool to room temperature before being placed in the animals' cages. Fresh drinking water solutions were prepared every five days. The concentration of drug in solution was calculated to achieve a dose of 20 mg/kg/day assuming an average water intake of 6.7 ml/day per mouse and a weight of 30 g per mouse.

TUMOR ASSESSMENT: At weekly intervals, the overall tumor volume was assessed so that the *tumor growth rate* could be calculated for each mouse according to the following procedure: Each week, the maximal and minimal dimensions of each tumor were measured, along with tumor thickness. Tumor volumes (V) were calculated using the formula $V={}^{4}/_{3}\pi LWD$, where L was the longer dimension of the tumor, W the smaller dimension, and D the depth. Aggregate tumor volumes were calculated for each animal as the sum of the volume of the individual tumors, and this was plotted against time in days since the first melanoma lesion became visible.

STUDY ENDPOINT AND EVALUATION OF METASTASES: In accordance with the approved IACUC protocol, animals were euthanized by cervical dislocation when the largest melanoma tumor reached 2 cm^3. Necropsy was performed on control and experimental animals immediately following euthanasia. Lymph nodes (bilateral axillary and inguinal), lungs, liver, spleen, and brain were removed and assessed for visible metastases. For larger structures such as the lungs, liver, and brain, organs were serially sectioned to allow for more thorough evaluation. Ears, paws, and tail were examined for the presence of cutaneous metastases as well. Owing to the difficulty of assessing the precise size of

the metastatic deposits, metastases were categorized as either "present" or "absent."

STATISTICAL ANALYSES: Tumor latency, tumor number, and tumor growth rate were expressed as a mean value ± standard deviation (SD). *T*-tests were performed to evaluate differences between experimental and control groups, and *p*-values of <0.05 were considered to be statistically significant.

Aim 2: Case-control study

MELANOMA PATIENTS: Patients were selected from among those presenting to Yale Cancer Center between the years of 1997 and 2013 with a diagnosis of melanoma. Yale Cancer Center is the largest melanoma treatment center in Connecticut and has a catchment area covering much of New England. Patients were drawn from the case logs of the two surgeons responsible for the vast majority of surgical therapy for patients with cutaneous melanoma presenting to Yale Cancer Center. Patients who did not receive surgical treatment of melanoma at Yale-New Haven Hospital (e.g. those referred to the medical oncologists for participation in a clinical trial) were excluded from the study.

CONTROL SUBJECTS: Potential control subjects were identified from among patients and accompanying family members or friends presenting to two outpatient surgery clinics (orthopedic surgery and plastic surgery) at Yale University for reasons other than melanoma. Exclusion criteria included a personal history of prior melanoma and black race, due to the racial composition of the population of melanoma patients.

DATA COLLECTION: Charts of melanoma patients were reviewed for demographic variables; cancer risk factors, including family history; medical and surgical history, including medication history; and details of the primary tumor (location and number of

primary tumors, Breslow depth, Clark level, tumor stage, histologic subtype, presence of BRAF mutations), treatment history, and disease course. When available, details of a patient's antidepressant exposure were noted, including the particular medication used, the reason the antidepressant was prescribed, and the dose, timing, and duration of treatment. Patients whose charts did not contain a medication list were excluded from the study. Sun exposure data were not readily available in the patients' charts.

Data collection began with the charts of all patients treated by the primary thesis advisor (DN). Data collection then proceeded alphabetically through the remaining charts due to the large number of patients treated by the senior surgeon during the selected time frame. A subset of charts was examined by a second reviewer to confirm the accuracy and completeness of the data being collected.

The chart review was approved by the Human Investigations Committee at Yale University and conducted in accordance with protocol # 0609001869. Charts were reviewed in hospital-designated reading rooms. No identifying information was collected. Data were stored in a password-protected file on a computer protected by university-standard whole disk encryption accessible only by those conducting the chart review.

To obtain data from control subjects, an online health history questionnaire was administered in the waiting room of surgical subspecialty outpatient clinics (plastic surgery and orthopedic surgery) to patients and their accompanying family members or friends between August 2013 and January 2014. Questionnaires were administered using a laptop computer or an iPad belonging to a member of the research team. Participants were invited to take part in a research study "designed to learn more about the risk factors for melanoma." Participants were not given any indication that the study was specifically

aimed at investigating antidepressant exposure as a cancer risk factor.

The full content of the health history questionnaire can be found in **Appendix II**. Briefly, the questionnaire included demographic information (age, city/state of residence, reason for presenting to clinic); melanoma risk factors (hair and eye color, skin tone, sun reaction); social history (tobacco and alcohol use, occupation); past medical history, including malignancy history; past surgical history; family history of malignancy. The questionnaire also directly queried subjects about current or past use of NSAIDs, anticoagulants, and antidepressants.

STATISTICAL ANALYSES: Statistical analyses were performed using SPSS statistical software (IBM, Armonk, NY). Variables were first bivariately tested for differences between cases and controls using the chi-square test. Variables were then entered in a model of binary logistic regression, with antidepressants as the dependent variable and group as the first covariate. Age and sex were included in the second block of covariates to control for the effect of these variables. Results were expressed as an odds ratio with 95% confidence interval, with p values <0.05 considered statistically significant.

Aim 3: Melanoma cohort by stage

DATA COLLECTION: Data collected from the melanoma patients according to the methods described under Aim 2 above was used to determine and compare the rate of antidepressant exposure among patients with early and late disease. For the first set of analyses, patients were first grouped into stage 0, I, II, III, and IV. Because the detailed pathology reports revealing the presence or absence of ulceration and number of mitoses were not always present in the patients' charts, the A, B, and C subclassification was not specified. Data were then reanalyzed with patients divided into in situ, early-, and late-

stage melanomas. Early melanomas were defined as stage I, while late melanomas were defined as stage II, III, or IV.

STATISTICAL ANALYSES: Individual variables were initially tested using the chi-square test to determine any differences between groups. Binary logistic regression was then performed with antidepressant exposure as the dependent variable and age and sex as covariates. Tumor stage was included as a covariate in Block 2. Results were expressed as an odds ratio and 95% confidence interval, with p-values <0.05 considered statistically significant. SPSS software was used for these analyses.

RESULTS

Aim 1: Mouse model

Weekly tumor measurements began on post-induction day 29. Aggregate tumor volumes for control and experimental animals are shown in **Table 2**. The volumes listed represent the sum of the volumes of the individual tumors for each animal.

Table 2: Aggregate tumor volumes for control and experimental animals

Days Post-Induction[A]	**Aggregate Tumor Volume (mm^3)**[B,C]					
	Control		Experimental			
	C1	C2	E1	E2	E3	E4
29	129.35	82.65	24.35	2.07	99.11	297.22
36	252.90	163.48	160.56	5.98	105.86	428.96
44	352.86	217.07	118.79	21.24	105.96	579.00
50	444.82	246.33	129.19	22.41	174.67	727.38
57	550.41	265.75	328.44	42.16	389.79	1183.04
66	1102.32	480.49	361.11	28.73	433.29	1551.65
75	2200.49	477.64	545.13	41.96	706.01	2399.25

[A] Refers to the number of days after melanoma was induced with 4-HT.

[B] Tumor volumes (V) calculated using the formula $V=\frac{4}{3}\pi LWD$, where L was the longer dimension of the tumor, W the smaller dimension, and D the depth.

[C] Aggregate tumor volume calculated as the sum of the volume of individual tumors.

Aggregate tumor volume was plotted against days following tumor induction for each animal to yield the chart shown in **Figure 3** below. Animals C1 and E4 showed exponential tumor growth, whereas animals C2, E1, and E3 showed linear tumor growth

over the time period in which data were collected. Animal E2 demonstrated a very slow rate of tumor growth for the duration of the experiment.

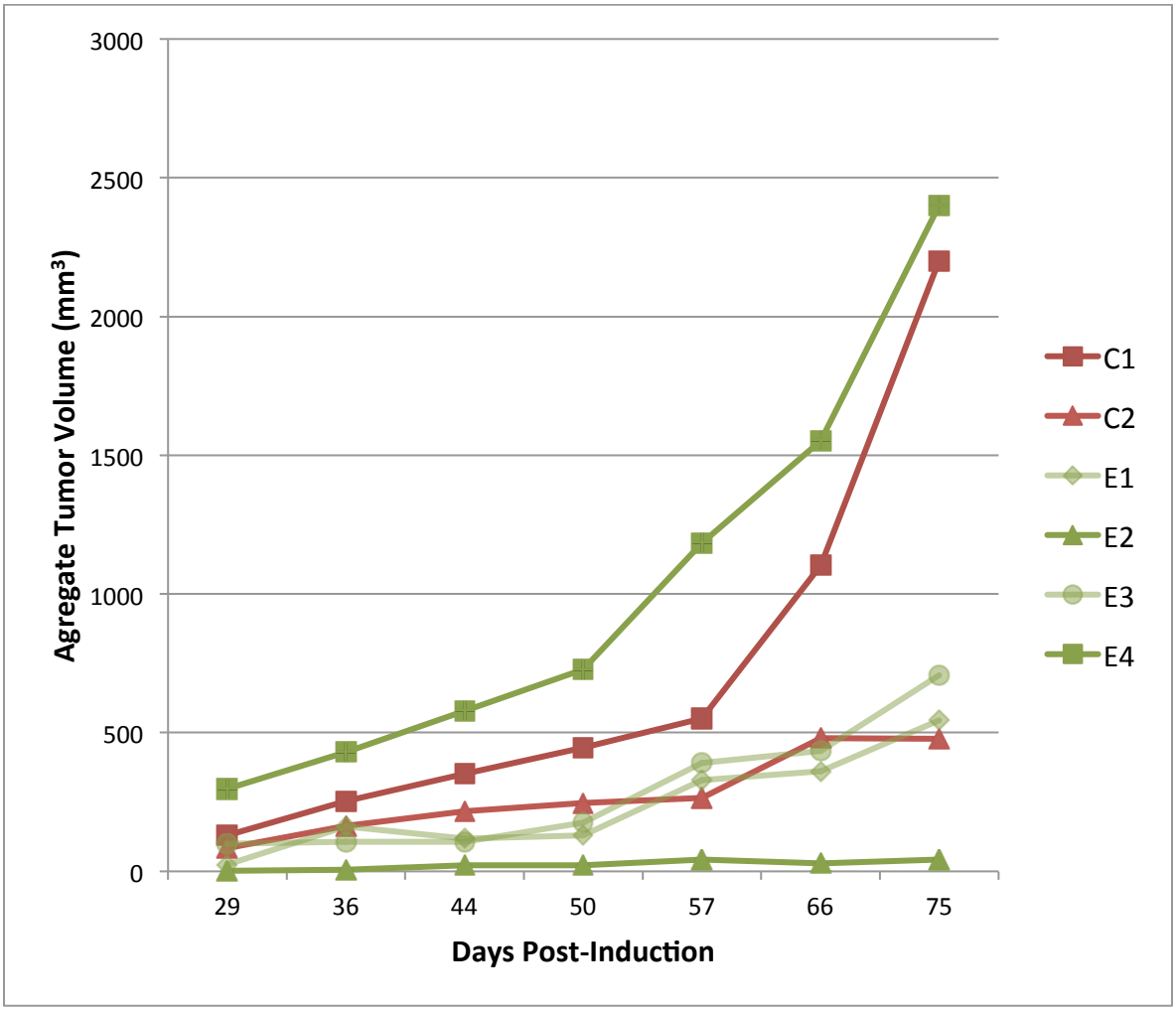

Figure 3: Melanoma growth trajectory in control and experimental animals

C1, *C2*: control animals

E1, *E2*, *E3*, *E4*: experimental animals

No difference was detected between control and antidepressant-exposed animals at any of the time points measured (p=0.612 to p=0.997; **Table 3**).

Table 3: Student's *t*-test for tumor growth

	Mean±SD		
	Control (n=2)	**Experimental (n=4)**	***p*-value**
Day 29	106.00±33.02	105.67±134.26	0.997
Day 36	208.19±63.23	175.34±180.79	0.761
Day 44	284.97±96.02	206.25±252.24	0.612
Day 50	345.58±140.35	263.41±315.82	0.682
Day 57	408.08±201.29	485.86±488.86	0.797
Day 66	791.40±439.71	593.70±662.49	0.691
Day 75	1324.07±1239.45	923.09±1023.95	0.736

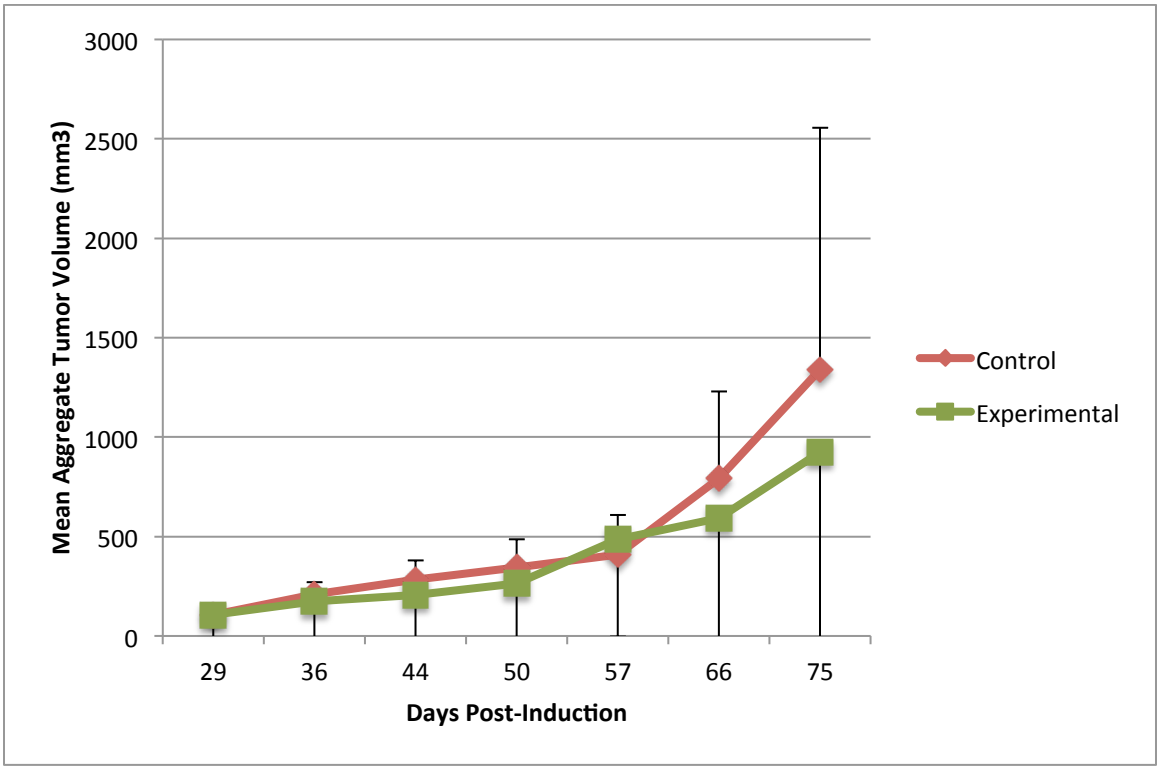

Figure 4: Mean tumor volumes for control and experimental animals

By day 75, one animal from each group had reached the study endpoint of a tumor volume of 2 cm^3, and all animals were euthanized. At the time of necropsy, four animals had evidence of cutaneous metastases at one or more location (ears—4, tail—3, paws—1). Four animals had axillary lymph nodes that were grossly positive for melanoma (ipsilateral—3; contralateral—1). Lung metastases were present in one animal, and another animal had a single focus of metastasis in the brain. No animals showed evidence of grossly visible liver metastases. Cutaneous and nodal metastases were present in control and experimental animals, whereas only animals treated with antidepressants displayed evidence of visceral metastases (**Table 4**).

Table 4: Patterns of metastases in control and experimental animals

Location	**Control**		**Experimental**				**Total (# animals)**
	C1	C2	E1	E2	E3	E4	
Ears	X			X	X	X	4
Tail				X	X	X	3
Paws						X	1
Contralateral LN	X						1
Ipsilateral LN		X	X		X		3
Lungs				X			1
Liver							0
Brain			X				1

LN, lymph node

Aim 2: Case-control study

The charts of 1412 melanoma patients were reviewed for this study. 141 patients were excluded because key information (medication list, date of melanoma diagnosis) could not be found in the patient's chart. Thus, data from 1271 melanoma patients were included in the analyses.

Ninety-eight control subjects agreed to participate in the study. Three subjects were excluded because they did not complete the health history questionnaire in its entirety or failed to provide data necessary for the analysis (e.g. age, gender, antidepressant history, melanoma history). Eight subjects were excluded due to black race so that the control population would more closely approximate the melanoma population. In total, 87 control subjects were included in the analysis.

Mean age was 59.4 ± 16.6 years for the melanoma patients and 48.8 ± 16.4 years for controls **(Table 5)**. Of the melanoma patients, 635/1271 (50.0%) were female. Among controls, 57/87 (65.5%) were female.

Table 5: Subject Characteristics

Variable	**Cases (n=1271)**	**Controls (n=87)**	***p*-value**
Age (mean ± SD)	59.4 ± 16.6	48.8 ± 16.4	<0.001
Sex (% female)	50.0	65.5	0.005

For both cases and controls, females were more likely to have been exposed to antidepressant medication (OR 2.148 [95% CI 1.564-2.950]; $p<0.001$). There was no significant effect of age on antidepressant exposure (OR 1.007 [95% CI 0.998-1.017];

p=0.117). Among melanoma patients, 182/1271 (14.3%) had a history of antidepressant use, compared to 20/87 (23.0%) controls (**Table 6**).

Table 6: Data for group and history of antidepressant use

Antidepressant Exposure	**Melanoma Patients**	**Controls**	**Total**
History of antidepressant use	182	20	202
No history of antidepressant use	1089	67	1156
Total	1271	87	1358

The logistical model with age and sex as covariates demonstrated that melanoma patients were less likely to have been exposed to antidepressant use prior to or at the time of melanoma diagnosis than were controls (OR 0.567 [95% CI 0.331-0.972]; *p*=0.039; **Table 7**).

Table 7: Logistic regression analysis of antidepressant exposure among melanoma patients and controls

Predictor	**β**	***SE* β**	**Wald's χ^2**	***df***	***p***	**e^{β} (odds ratio)**
Constant	-2.165	0.381	32.214	1	<0.001	0.115
Age	0.009	0.005	3.406	1	0.065	1.009
Gender (0=males, 1=females)	0.749	0.162	21.321	1	<0.001	2.116
Group (0=controls, 1=cases)	-0.567	0.275	4.265	1	0.039	0.567

ANALYSIS WITH SEPARATE MODELS FOR MALES AND FEMALES: When the data were reanalyzed using separate models for males and females, age remained insignificant (males: OR 1.009 [95% CI 0.993-1.025]; p=0.283; females: OR 1.007 [95% CI 0.995-1.018]; p=0.247). Among males, no association was seen between group (cases vs. controls) and antidepressant use (OR 0.658 [95% CI 0.219-1.982]; p=0.457). For females, an association between group and antidepressant use approached statistical significance, with melanoma patients less likely than controls to have a history of antidepressant exposure (OR 0.540 [95% CI 0.290-1.005]; p=0.052).

Aim 3: Melanoma cohort by stage

Of the 1271 melanoma patients included in the analysis for **Aim 2** (above), 1257 patients had staging information available. The characteristics of the cohort are shown in **Table 8** (below).

Mean age of the subjects was 60.9 ± 15.6 years for stage 0 disease, 58.0 ± 16.3 for stage I, 64.5 ± 17.7 for stage II, 56.4 ± 17.1 for stage III, and 55.3 ± 17.7 for stage IV. Of subjects who presented with stage 0 disease, 176/313 (56.2%) were female. 348/675 (51.6%) of stage I subjects were female, as were 61/177 (34.5%) of stage II subjects, 35/72 (48.6%) of stage III subjects, and 9/20 (45.0%) of stage IV subjects **(Table 9)**.

Table 8: Characteristics of the melanoma cohort (Aim 3).

Variable	Stage 0	Stage I	Stage II	Stage III	Stage IV	*p*-value
Age (mean ± SD)	60.9 ± 15.6	58.0 ± 16.3	64.5 ± 17.7	56.4 ± 17.1	55.3 ± 17.7	0.008
Sex (% female)	56.2	51.6	34.5	48.6	45.0	<0.001

Table 9: Data for tumor stage at diagnosis.

	Stage					
Sex	**0**	**I**	**II**	**III**	**IV**	**Total**
Male	137	327	116	37	11	628
Female	176	348	61	35	9	629
Total	313	675	177	72	20	1257

In the entire melanoma cohort, females were more likely to have been exposed to antidepressant medication (OR 2.067 [95% CI 1.485-2.877]; $p<0.001$). There was no significant effect of age on likelihood of antidepressant exposure (OR 1.008 [95% CI 0.998-1.017]; $p=0.127$).

The logistical model with age and sex as covariates demonstrated that there was no effect of tumor stage on the likelihood of antidepressant exposure at the time of melanoma diagnosis (OR 0.945 [95% CI 0.784-1.139]; $p=0.553$; **Table 10**).

Table 10: Logistic regression analysis of antidepressant exposure among melanoma patients by tumor stage

Predictor	β	*SE* β	Wald's χ^2	*df*	*p*	e^{β} (odds ratio)
Constant	-2.568	0.359	51.060	1	<0.001	0.077
Age	0.008	0.005	2.287	1	0.130	1.008
Gender (0=males, 1=females)	0.717	0.169	17.904	1	<0.001	2.048
Tumor stage (0=MIS, 1=stage I, 2=stage II, 3=stage III, 4=stage IV)	-0.57	0.095	0.353	1	0.553	0.945

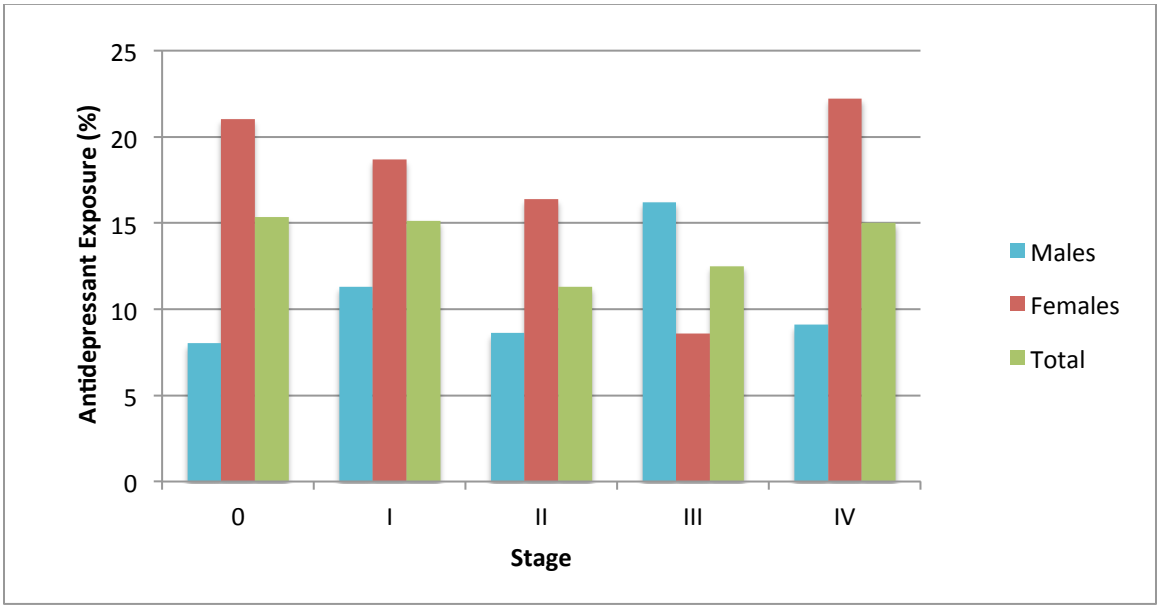

Figure 5: Percentage of melanoma patients with history of antidepressant use at time of melanoma diagnosis.

ANALYSIS OF EARLY VERSUS LATE AND IN SITU VERSUS INVASIVE LESIONS: When the melanoma cohort was divided into patients with melanoma in situ, early (stage I), or late (stage II-IV) disease, logistic regression demonstrated no effect of tumor stage on likelihood of antidepressant use before or at the time of melanoma diagnosis (OR 0.921 [95% CI 0.728-1.165]; p=0.492). Similarly, no differences in likelihood of antidepressant use were found when classifying patients as having in situ (stage 0) or invasive (stage I-stage IV) disease (OR 0.976 [95% CI 0.680-1.402]; p=0.895).

ANALYSIS USING SEPARATE MODELS FOR MALES AND FEMALES: When males and females were analyzed separately, there was no association between age and antidepressant use among males (OR 1.013 [95% CI 0.996-1.031]; p=0.130) or females (OR 1.005 [95% CI 0.993-1.017]; p=0.432). Males had a mean age of 62.07±15.87. Females had a mean age of 56.74±16.98. Melanoma stage at diagnosis was not a significant predictor of prior or concurrent antidepressant use for either males (OR 1.125 [95% CI 0.847-1.493]; p=0.417) or females (OR 0.835 [95% CI 0.650-1.073]; p=0.159).

DISCUSSION

To our knowledge, this is the first study to investigate the effect of an antidepressant on melanoma growth characteristics using an animal model that closely parallels human melanoma. Previously, other studies that attempted to address the issue of antidepressants and melanoma in vivo utilized melanoma models that do not replicate tumor biology, such as subcutaneous injection of melanoma cells grown in culture. Despite the small sample size, this project demonstrates the feasibility of administering antidepressants to melanoma-bearing mice via the drinking water and conducting weekly assessments of tumor volume.

In this experiment, oral citalopram at a dose of 20 mg/kg/day did not appear to affect the growth rate of melanoma tumors. Looking at **Figure 3**, there appear to be three distinct patterns of tumor growth: tumors in animals C1 and E4 showed exponential growth; C2, E1, and E3 showed linear growth over the time course of the experiment; and E2 had a relatively small tumor burden when the study endpoint was reached. Grossly, these patterns do not appear to correlate with antidepressant exposure. With marked variability in the patterns of growth between the two control animals that were tested, a larger sample size would be needed in order to draw firm conclusions about the effect of citalopram on tumor growth.

Citalopram was chosen for these experiments because it is a highly serotonergic antidepressant. However, other antidepressants may influence the growth characteristics of melanoma in different ways, or by different mechanisms. Thus, future studies could involve testing other SSRIs or different classes of antidepressants in the *Braf/Pten* mice. The 20 mg/kg/day dose of citalopram was in line with other rodent studies of

antidepressants in the psychiatry literature, but those studies were primarily interested in the CNS effects of antidepressants. Citalopram (and other antidepressants, as well) are metabolized differently by mice than by humans, and the action of antidepressants in the skin may vary across species as well; thus, different drug doses may be needed to achieve a bioequivalent effect in humans and in mice.

Furthermore, the patterns of antidepressant exposure in this experiment differ markedly from the ways in which such medications are typically used in humans. Often, patients with psychiatric diagnoses or chronic pain are maintained on antidepressant medication for many years prior to presenting with melanoma. The short exposure duration for the animals in this experiment may have been insufficient to generate an effect on tumor growth. Additionally, antidepressant exposure in the mice began simultaneously with tumor induction, whereas antidepressant use in humans frequently precedes melanoma tumorigenesis. In future studies, animals could be treated with antidepressants from birth, rather than beginning at the time of tumor induction.

Importantly, the study presented here did not assess tumor latency in control versus experimental animals, and this may in fact be an important parameter of interest. Tumor incidence and tumor growth rate may be quite different issues. Because the Braf/Pten mice develop melanoma with 100% penetrance, an effect of antidepressants on tumor incidence would be obscured. Tumor latency in the Braf/Pten mouse model might be a useful surrogate marker for tumor incidence in humans, as prolonging tumor latency in humans would give the immune system a better chance to mount a response against malignant cells.

Though the study was principally designed to investigate the effect of antidepressants on growth of the primary tumor, organs were visually assessed macroscopically for metastases. Histologic assessment could be employed in the future to more accurately determine the presence and/or size of metastatic deposits. With only two-to-three animals per experimental group involved in this experiment and the potential for a large number of possible combinations of visceral metastases, the results presented here must be viewed as inconclusive.

In the event that future studies demonstrate an effect of antidepressants on tumor growth characteristics, future work could be directed at clarifying the molecular pathways responsible for the association between antidepressants and melanoma. Two additional mouse models of melanoma are available at Yale, each with distinct genetic lesions resulting in tumors that show different clinical behavior. Investigating the differential effects of antidepressants on animals with different genetic backgrounds could shed light on the pathways through which antidepressants operate in malignant melanoma.

The results of the case-control analysis failed to support the hypothesis that melanoma patients would have a higher rate of antidepressant exposure than controls. Indeed, controls were 1.76 times more likely than melanoma patients to have taken antidepressant medication. This result is in line with some of the pre-clinical data and at odds with others. (7, 18, 48-53) While the study presented here represents a good first step toward characterizing the possible association between antidepressants and melanoma risk, the following methodological considerations may have impacted the study outcome.

One of the most important methodological challenges in designing a case-control

study such as the one presented here is deciding on an appropriate control group. Ideally, control subjects would be matched to melanoma patients according to race, age, and sex, at a minimum. Because the control group was younger and more female than the melanoma cases, age and sex were entered into the binary logistic regression model to correct for the differences statistically. However, this technique can obscure the effect of an exposure when it is strongly correlated with other variables in the model. Here, antidepressant use strongly correlated with female sex, so the analysis was repeated for males and females separately. No qualitative differences were observed in the repeat analysis.

Unfortunately, the patient's race was often not explicitly specified in the melanoma patients' charts, so the racial makeup of the melanoma cohort was not reported in this manuscript. However, melanoma is known to be largely a disease of whites, affecting only a miniscule number of non-white individuals. In order to maximize the number of controls available for the analysis, Hispanic subjects were included in the control group. The cases included a small proportion of subjects whose ethnicity was documented as Hispanic, but the proportion of Hispanic controls likely exceeded the proportion of Hispanic cases.

One risk factor that is of the utmost importance in melanoma is sun exposure. Sun exposure is incredibly difficult to quantify, particularly since the relevant exposure may occur rather remotely, such as in childhood. Studies attempting to quantify lifetime sun exposure have used a variety of methods, most relying on detailed surveys or questionnaires asking subjects to report the geographical locations in which they lived at various stages of life, as well as details of their occupation and leisure-time activities.

(56-59) Though a small subset of charts in this study contained information on the number of sunburns the patient had experienced, this information was insufficient to characterize overall sun exposure history. Thus, no attempt was made to match cases and controls for a history of sun exposure in this study.

In an effort to minimize the impact of physician practice patterns on the rate of antidepressant exposure in the two groups, a hospital-based control group was selected. Previous case-control studies investigating melanoma risk factors have used population-based controls recruited by means of random-digit dialing, population registries, and the like. (56, 57) Hospital-based controls were thought to be more reflective of the population presenting to Yale Cancer Center for treatment of melanoma.

Notably, the rates of antidepressant use in the control population presented here far exceeded the rates reported in the literature for the population as a whole, nationwide. (6) One thing that remains unanswered is whether this difference in rates of antidepressant use reflects a difference in prescribing habits of primary care physicians and psychiatrists in Connecticut as compared to the United States overall, or whether there was in fact something unrepresentative about patients presenting to the plastic surgery and orthopedic surgery outpatient clinic at Yale and/or those who elected to participate in the study. The subjects recruited from plastic surgery clinic included patients being seen in consultation or follow-up for both reconstructive and cosmetic surgical procedures. Cosmetic surgery patients may be more likely to carry psychiatric diagnoses and thus more likely to have been treated with antidepressants than the general population. (60-62) Similarly, reconstructive surgery practices often serve a population of medically complex, sicker patients, in comparison with the healthier, more active patients

who sustain orthopedic injuries or develop melanoma after years of sun exposure. Sicker patients may have higher rates of depression, or they may simply have a greater number or frequency of contacts with the healthcare system and thus a higher likelihood of being started on antidepressant medication.

Another methodological weakness of the study presented here is that antidepressant exposure was ascertained in different ways for cases and controls; for melanoma patients, information was collected via chart review, whereas control subjects were queried directly about their medical history and drug exposures. On one hand, people may be more willing to reveal sensitive information, such as a history of antidepressant use, to their doctors than to a member of the research staff with whom they have no preexisting relationship. Questionnaires were completed anonymously, but subjects may still have had reservations about volunteering information of a personal nature that seemed irrelevant to their care. On the other hand, the questionnaires completed by control subjects explicitly asked about antidepressant use, whereas the physician may have only asked about medications in more general terms. The methods utilized in this study were chosen for their feasibility in the setting of limited resources, but the method of data acquisition would have ideally been the same for cases and controls.

The data presented here failed to demonstrate an association between antidepressant use and melanoma stage at the time of diagnosis. This pattern held true whether patients were grouped into three or five categories of stages, as well as when they were divided into groups based on the presence of in situ or invasive disease. Neither males nor females showed an effect of tumor stage on likelihood of

antidepressant exposure. Based on the results of the case-control analysis, any interaction between antidepressant exposure and melanoma pathogenesis is presumed to be weak and confounded by a multitude of variables. Thus, this study may not have been adequately powered to detect an association when dividing the melanoma cohort into multiple groups.

With limited knowledge of the precise mechanism by which antidepressants interact with melanoma risk, the relevant details of antidepressant exposure are uncertain. Other studies in the cancer literature have found effects of antidepressants that were dependent on dose and duration of treatment, as well as the timing of exposure (i.e. temporal proximity to cancer diagnosis). Unfortunately, these details were often not available in the medical record, so for the present study, patients were categorized as "antidepressant exposed" if they had a record of current or past antidepressant treatment documented in the chart at the time of melanoma diagnosis. Likewise, the indication for treatment with an antidepressant would be important to consider when making recommendations about how to manage melanoma patients on antidepressants, should further study reveal a deleterious effect on prognosis, but this information was only infrequently recorded in the chart.

While it would be desirable to characterize the specific details of subjects' antidepressant use, it may not be practical to contact subjects individually when dealing with a large cohort of patients, and in some cases, it would not be possible due to death of a patient or incorrect contact information. Implementation of the electronic medical record for both inpatient and outpatient visits may yield a powerful tool for more accurately assessing a subject's past or present use of antidepressants or other

medications. Having a centralized medication list that is accessed by all providers may improve the chances that the charts of sick and non-sick patients alike will contain a complete medication list, and linking the chart with pharmacy records would provide a metric for determining whether patients are actually taking the medications that are prescribed to them.

An important consideration when it comes to evaluating the hypothesis that antidepressants might increase melanoma risk is whether there might be an underlying association between cancer risk and depression itself. Here, the data are conflicting. Depression is characterized by a proinflammatory state reflected by elevated plasma levels of circulating cytokines that could contribute to neoplastic growth. (63) Furthermore, a 1998 study of 4825 people over 71 years of age concluded that depression of at least six years' duration was associated with increased cancer mortality. (64) However, a majority of prospective studies point to a weak relationship or none at all between depression and overall cancer risk. (63, 65-70) Other studies claimed an increased cancer risk in depressed patients but failed to account for major confounders, including smoking. (71)

The relationship between depression and melanoma is poorly understood and undoubtedly complex. Sun exposure increases plasma levels of serotonin, and patients with mild depression may self-medicate by spending a greater amount of time in the sun. (10) Conversely, severely depressed patients may lack the energy or motivation to leave their homes and therefore have decreased sun exposure. For patients with a long history of depression, the effect of mood on sun exposure could perhaps be enough to impact overall melanoma risk. Interestingly, one group has shown that 46% of patients with

melanoma had a major life crisis in the five years preceding melanoma diagnosis, suggesting that stress may play a role. (72) However, the authors point out that these data must be interpreted with caution, as people may be more likely to consult a health practitioner following a stressful event, leading to an increase in melanoma diagnoses.

In the setting of unlimited resources, one way to address the issue of depression as a confounding factor in the interaction between antidepressants and melanoma would be to eliminate depression as a variable. This could be done by studying exclusively depressed subjects with and without a history of treatment with antidepressant medication, or by excluding patients with depression so that the antidepressant-exposed population would have been prescribed antidepressants for only non-psychiatric reasons. To achieve a sufficient number of subjects, such an effort would likely need to be multi-institutional.

Though the data presented here failed to uncover evidence in support of a causal link between citalopram and melanoma tumorigenesis, these results must be considered preliminary in nature. Further study is warranted, given the limitations of the project presented here. Chart review is ongoing, and we hope to include all patients at Yale treated surgically for melanoma in future studies on the effect of antidepressants on melanoma incidence and disease course.

A related question to investigate in the future is whether the proportion melanoma patients with *BRAF*-mutant tumors differs between those with and without a history of antidepressant exposure. *BRAF* is the most common genetic mutation found in melanoma, and *BRAF* mutations are also found at high frequency in benign nevi. Insofar as antidepressants may facilitate malignant transformation, the effect likely occurs only

atop a background of genetic mutations. Thus, one would expect to see a higher proportion of tumors from patients with antidepressant exposure carrying *BRAF* mutations, suggesting that they arose from a preexisting nevus.

In the event that further study demonstrates an effect of antidepressants on melanoma incidence, tumor growth kinetics and/or disease prognosis, the results could have ramifications for treatment of depression and the other conditions for which antidepressants are prescribed, particularly in patients diagnosed with melanoma. The possible implications of this line of research are made even more significant by the sheer number of patients using antidepressant medication. Evaluation of the hypothesis outlined in this paper potentially stands to inform the care of the many thousands of patients each year treated for depression, anxiety, chronic pain or various other medical conditions.

If, on the other hand, antidepressants prove to have an inhibitory effect on melanoma incidence, growth, and metastasis, perhaps the beneficial effects of these compounds could be harnessed and utilized in the treatment of melanoma or in the prevention of disease in high-risk groups.

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APPENDIX I

Table 1: Classes of antidepressant medication

Selective serotonin reuptake inhibitors (SSRIs)

citalopram (Celexa)

escitalopram (Lexapro)

fluoxetine (Prozac)

paroxetine (Paxil)

sertraline (Zoloft)

Serotonin-norepinephrine reuptake inhibitors (SNRIs)

desvenlafaxine (Khedezla, Pristiq)

duloxetine (Cymbalta)

milnacipran (Savella)

venlafaxine (Effexor)

Atypical antidepressants

agomelatine[A]

bupropion (Wellbutrin, Zyban)

mirtazapine (Remeron)

Tricyclics

amitriptyline (Tryptomer, Elavil, Endep)

clomipramine (Anafranil)

desipramine (Norpramin, Pertofrane)

doxepin (Adaptin, Sinequan)

imipramine (Tofranil, Janimine, Praminil)

nortriptyline (Pamelor, Aventyl, Norpress)

Monoamine oxidase inhibitors (MAOIs)

phenelzine (Nardil)

selegiline (Eldepryl, Emsam, Zelapar)

tranylcypromine (Parnate)

[A] not available in the United States

APPENDIX II

Table 11. Health History Questionnaire for Control Subjects

1. Sex:
   ○ Male
   ○ Female
2. Age:
3. Place of residence (City/Town, State):
4. Reason for coming into clinic:
5. Height (feet, inches):
6. Weight (lbs):
7. Occupation:
8. Race/ethnicity (select all that apply):
   ○ White
   ○ Black
   ○ Hispanic
   ○ Asian
   ○ American Indian or Alaskan Native
   ○ Native Hawaiian or Pacific Islander
9. Skin tone:
   ○ Pale white
   ○ Fair
   ○ Medium
   ○ Olive
   ○ Brown
   ○ Very dark brown
10. Hair color:
11. Eye color:
12. How does your skin react to the first summer sun exposure of the year?
    ○ Always burns, never tans
    ○ Usually burns, tans with difficulty
    ○ Sometimes burns, usually tans
    ○ Rarely burns, tans easily
    ○ Very rarely burns, tans very easily
    ○ Never burns
13. How many blistering sunburns have you experienced?
14. How many non-blistering sunburns have you experienced?
15. Do you sunbathe or use tanning oil or tanning beds?
    ○ Yes, currently
    ○ Yes, in my youth
    ○ Both
    ○ Neither
16. Smoking history:
    ○ I currently smoke
    ○ I used to smoke but do not currently

○ I have never smoked

17. If yes to 16: How many packs per day (on average)?

18. If yes to 16: For how many years?

19. How many alcoholic beverages do you drink in a typical week?

20. Have you ever been told you have any of the following medical conditions?

○ High blood pressure
○ High cholesterol
○ Diabetes
○ Heart disease
○ Hyperthyroidism (overactive thyroid)
○ Hypothyroidism (underactive thyroid)
○ Atrial fibrillation
○ DVT (blood clot in leg or arm)
○ Stroke
○ Pulmonary embolism
○ Depression
○ Anxiety
○ Asthma
○ COPD
○ Autoimmune disease (rheumatoid arthritis, lupus, scleroderma, etc.)
○ Eczema
○ Psoriasis
○ Kidney disease
○ Liver disease

21. Please list any medical conditions you have that are not listed above.

22. Please list the type of surgery and the year of surgery for any surgical procedures you have had, or write "none" if you have never had surgery.

23. Do you take any of the following anti-inflammatory medications?

	Use regularly	Use occasionally	Do not use
Aspirin	○	○	○
NSAIDs (e.g. ibuprofen, naproxen, Advil, Motrin, Aleve)	○	○	○

24. Have you ever taken steroid medications by mouth (e.g. prednisone) or other medications that suppress your immune system? (Do not include topical or inhaled medications, such as creams, ointments, or inhalers.)
○ No
○ Yes, short-term (less than 2 weeks)
○ Yes, long-term (greater than 2 weeks)

25. Have you ever taken blood thinners (e.g. warfarin, Coumadin, Lovenox)?
○ Currently taking
○ Have taken in the past
○ Have never taken

26. Please give the name of medication, dose, start date, and stop date (if applicable) for blood thinners you have taken, or write "N/A" if you have never taken blood thinners or "unsure" if you do not know the requested information.

27. Have you taken any of the following medications which are sometimes used to treat depression, anxiety, chronic pain, or migraine headaches?

	Currently taking	Have taken in the past	Have never taken
Celexa (citalopram)	○	○	○
Lexapro (escitalopram)	○	○	○
Zoloft (sertraline)	○	○	○
Prozac (fluoxetine)	○	○	○
Paxil (paroxetine)	○	○	○
Cymbalta (duloxetine)	○	○	○
Effexor (venlafaxine)	○	○	○
Amitriptyline	○	○	○
Nortriptyline	○	○	○
Desipramine	○	○	○
Other antidepressant not on list	○	○	○
Unsure of the name of the antidepressant	○	○	○

28. Please give the dose, start date, and stop date (if applicable) for any of the antidepressant medications listed above, along with the name of any antidepressants you have taken that are not listed. Write "N/A" if you have never taken antidepressant medication or "unsure" if you do not know the requested information.

29. Have you ever been prescribed thyroid medication (e.g. Synthroid, Levoxyl, levothyroxine, Cytomel, Thyrolar, Armour thyroid)?
○ Yes
○ No

30. Please list any other medications you currently take, along with the dose and frequency (if known). Include vitamins or herbal supplements you take, as well.

31. Have you ever had a suspicious mole removed?
○ Yes
○ No

32. Have you ever been diagnosed with melanoma?
○ Yes
○ No

33. Have you ever been diagnosed with a non-melanoma skin cancer (e.g. basal cell carcinoma, squamous cell carcinoma)?
○ Yes
○ No

34. Have you ever been diagnosed with another type of cancer?
○ Yes
○ No

35. If yes to 34: Please indicate the type of cancer and your age at diagnosis.

36. Has anyone in your family been diagnosed with melanoma?
    ○ Yes
    ○ No
37. If yes to 36: Please indicate which of your relatives was diagnosed with melanoma.
38. Has anyone in your family been diagnosed with another type of cancer besides melanoma?
    ○ Yes
    ○ No
39. If yes to 38: Please indicate the type of cancer and which of your relatives was affected.