Antidepressant Use and Risk of Central Nervous System Metastasis

by

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Biographical Sketch

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The following publications were a result of work conducted during doctoral study:

- Holkova, B, M Kmiecik, P Bose, VY Yazbeck, P Barr, MB Tombes, E Shrader, EM Cebula, E Pierce, **M Herr**, H Sankala, KT Hogan, C Feng, D Petersen, RI Fisher, S Grant, and JW Friedberg. Phase 1 Trial of Carfilzomib (PR-171) in Combination with Vorinostat (SAHA) in Patients with Relapsed or Refractory B-Cell Lymphomas. *Leukemia and Lymphoma*. DOI: 10.3109/10428194.2015.1075019.
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Abstract

Central nervous system (CNS) metastasis is the spread of a primary cancer to the CNS and occurs in up to 25% of cancer patients. Antidepressant therapy, used in 15-30% of cancer patients, affects the blood-brain barrier, potentially allowing CNS metastasis. One study found selective serotonin reuptake inhibitors (SSRIs), a class of antidepressants, increased CNS metastasis in mice. Our study determined whether antidepressants, and specifically SSRIs, increased the relative odds of CNS metastasis.

Using a case-control study design, subjects were identified from the three types of cancers with the highest cumulative incidence of CNS metastasis (excluding lung cancer) at our institution: breast cancer, melanoma, and lymphoma. Our case ascertainment rate was 27.6% resulting in 189 cases (patients with CNS metastasis) and 945 controls (patients without CNS metastasis). Using logistic regression models (Aim 1), we separately estimated the relative odds of CNS metastasis associated with ‘any antidepressant use’ (any antidepressant irrespective of class), ‘any SSRI use’ (SSRI use, nonexclusively), and ‘exclusive SSRI use’ (only SSRI use and no other class of antidepressant), all compared to ‘no antidepressant use’. Using linear regression and only breast cancer controls, we separately estimated the change in white blood cell (WBC) count associated with ‘any antidepressant use’ and ‘any SSRI use’ (Aim 2).

Although not statistically significant, the odds of CNS metastasis in breast cancer associated with ‘any SSRI use’ (Aim 1) was greater than 1.0 (OR=1.73, 95% CI = 0.75, 4.04). However, the smaller than expected sample size may, in part, explain the lack of statistical significance. In Aim 2, WBC counts of those breast cancer controls who took SSRIs were $0.75 \times 10^9/\text{L}$ higher than those breast cancer controls who did not take antidepressants (95% CI = 0.01, 1.45).

This was the first study to examine the association between antidepressant use and CNS metastasis. Our results found no clear statistically significant associations but suggested an increase in CNS metastasis and WBC count associated with antidepressant use.

However, these estimates were imprecise with wide confidence intervals. Future studies, with larger sample sizes, are needed to examine these associations, and perhaps focus specifically on breast cancer patients.

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Abbreviations

AJ	adherens junctions
AJCC	American Joint Committee on Cancer
ALM	acral lentiginous melanoma
ALS	amyotrophic lateral sclerosis
AQP4	aquaporin-4
BBB	blood brain barrier
CNS	central nervous system
CT	computed tomography
CYP2D6	Cytochrome P-450 2D6
Da	Daltons
DLBCL	diffuse large B-cell lymphoma
DM	desmoplastic melanoma
EBV	Epstein-Barr virus
ECOG	Easter Cooperative Oncology Group
EGFR	epidermal growth factor receptor
ER	estrogen receptor
FDA	Food and Drug Administration
FNA	fine needle aspirate
FLIPI	follicular lymphoma international prognostic index
HER2	human epidermal growth factor type 2 receptor
HIV	human immunodeficiency virus

ICAM	intercellular adhesion molecule
ICD	International Classification of Diseases
IL-2	interleukin-2
IPI	international prognostic index
IRB	institutional review board
JAM	junction adhesion molecule
KPS	Karnofsky performance score
LMD	leptomeningeal disease
LMM	lentigo maligna melanoma
LPS	lipopolysaccharide
MALT	mucosa-associated lymphoid tissue
MAOI	monoamine oxidase inhibitor
MHC-II	major histocompatibility complex class II
MRI	magnetic resonance imaging
MS	multiple sclerosis
NHL	non-Hodgkin lymphoma
NK	natural killer
NM	nodular melanoma
NO	nitric oxide
NSAID	non-steroidal anti-inflammatory drug
OCD	obsessive-compulsive disorder
P-gp	P-glycoprotein

PC	pericytes
PCR	polymerase chain reaction
PMDD	premenstrual dysphoric disorder
PNN	perineuronal nets
PR	progesterone receptor
PTSD	posttraumatic stress disorder
SEER	Surveillance, Epidemiology, and End Results
SNRI	norepinephrine reuptake inhibitor
SSM	superficial spreading melanoma
SSRI	selective serotonin reuptake inhibitor
TJ	tight junction
TNF	tumor necrosis factor
TNM	tumor, node, metastasis
URMC	University of Rochester Medical Center
UV	ultraviolet
WBC	white blood cell
WHO	World Health Organization
ZO	zonula occludens

Chapter 1: Background

Brain metastasis is the spread of a primary cancer to the brain and occurs in up to 25% of cancer patients [1]. From 1978 to 2003, brain metastasis increased 120% among breast cancer patients [2], and, from 1988 to 2000, surgical resections for brain metastasis of all cancers increased 79% [3]. This cumulative incidence appears to be increasing [1], and is attributed in part to an aging population, better cancer treatment allowing longer survival with cancer, and thus more chance of brain metastases [4-6]. Brain metastasis diagnoses are particularly devastating to the patient, since the average survival time from diagnoses of brain metastasis to death is approximately 4 to 6 months [7].

Although this cumulative incidence is increasing and a diagnosis of brain metastasis is frequently terminal, knowledge about modifiable risk factors of brain metastasis is limited and focuses mainly on smoking [8, 9]. Antidepressants have been used more frequently in cancer patients than healthy individuals [10], since the cancer population has a higher incidence rate of depression and utilizes antidepressants for hot flashes, sleep disturbances, pain, and multidrug resistance [11-13].

To date, no study has analyzed the risk of brain metastasis, or more widely central nervous system (CNS) metastasis which also includes metastasis to the leptomeninges, among cancer patients from antidepressant use. Antidepressants affect the blood-brain barrier via inflammation, which may allow cancer to spread to the CNS [14]. For this study we focused on breast cancer, non-Hodgkin lymphoma (NHL), and melanoma, since

these three cancer types comprise the majority of cases at our institution (with the exception of lung cancer) where CNS metastasis occur.

1.1 Cancer

1.1.1 Overview of de novo neoplasms

Cancer, the uncontrollable growth of abnormal cells, affected over 750,000 people in the United States in 2009, the last year of complete data [15]. For women, lymphoma, melanoma, and breast cancer are included in the top 10 highest incidence rates (Figure 1). The incidence rates of NHL and melanoma are increasing, although the mortality rates are decreasing. The estimated number of new NHL cancer cases and deaths in the United States are 70,130 and 18,940 in 2012, respectively [16]. Together with Hodgkin lymphoma, they represent 5% of all cancers [17, 18]. In the same year, 76,250 incident melanoma cases and 9,180 deaths were estimated to occur, and, for women, 226,870 incident breast cancer cases and 39,510 deaths were estimated [16]. These numbers were estimated because cancer statistic reporting lags 3 to 4 years and cancer reporting is incomplete in some states. Estimates were derived from incident data from 1995 through 2008 [16].

Figure 1: Ten Leading Cancer Types for the Estimated New Cancer Cases and Deaths by Sex, United States, 2012 [16]

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In the United States, 1 out of every 4 deaths is attributed to cancer. NHL contributes 3% of the estimated deaths, while breast cancer adds another 14% of deaths in females (Figure 1) [16]. These deaths can occur at a young age leading to significant years of potential life lost. Melanoma also has substantial years of potential life lost since this cancer can occur at any age, and the cumulative incidence has been rising in recent decades [16, 19].

Racial and ethnic differences in cancer incidence and mortality rates exist. The incidence rate among black men is 15% higher than white men, but 6% lower among women. The mortality rate among black men is 33% higher in men and 16% higher in women in comparison to those of white race [16]. In addition to the differences in incidence and mortality rates, black cancer patients, in comparison to white cancer patients, have worse survival once a cancer diagnosis is made [16, 19]. For example, among all stages of female breast cancer, the 5-year survival rate for those of black race is 77%, while those of white race have a 90% 5-year survival rate. The 5-year survival rate for black and white races with melanoma was 72% and 91%, respectively, and 68% and 60%, respectively, for of non-Hodgkin lymphoma [16]. However, cancer mortality rates declined among all races/ethnicities, with the exception of American Indians/Alaskan Natives [16, 19]. Black individuals had a 2.4% reduction in overall mortality rate for males and 1.5% for females, the largest decline among all races [16].

Treatment generally differs by type of cancer but can include chemotherapy, radiation, targeted therapies, transplantation, surgery, immunotherapy, or usually a combination of these treatments. Treatment options are determined by numerous patient characteristics, such stage, age, and prognosis. Chemotherapy can be used for curative purposes, cancer control, or palliative care. Radiation and chemotherapy both aim to kill or stop the cancer cells from dividing. Surgery can be used to either remove just the tumor or, if needed, the surrounding tissue, but it is only useful in solid tumors, not tumors of the blood, such as lymphomas. Unlike radiation and chemotherapy which also affect the healthy cells, targeted therapies only destroy cancer cells.

1.1.2 Lymphomas

Hodgkin lymphoma and NHL are cancers that begin in the cells of the immune system. This thesis excluded Hodgkin lymphoma, since only 0.08% to 0.5% of Hodgkin lymphoma patients develop CNS disease [20]. The National Cancer Institute estimated that 19,020 deaths in 2013 were due to non-Hodgkin lymphoma [17] and the incidence rate was increasing until leveling off in 2004. The mortality rate began decreasing in 1997 with the introduction of Rituxan[®] (Rituximab), a treatment for NHL [17, 18].

According to the WHO classification, non-Hodgkin lymphoma is classified into B-cell lymphoma, T-cell lymphoma, and natural killer (NK)-cell lymphoma. These subtypes are separated according to the stage of differentiation [21]. B-cells originate in the bone marrow and T-cell lymphomas originate in the thymus [22]. B-cell lymphomas include Burkitt lymphoma, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT-lymphoma), mantle cell lymphoma (MCL), follicular lymphoma (FL) and diffuse large B cell lymphoma (DLBCL), while T-cell lymphomas include adult T-cell lymphoma and unspecified peripheral T-cell lymphoma. Although these cancers lie within lymphoma classification, they are different clinically and histologically and may have different etiologies [22].

Another way of describing and defining NHL is indolent or aggressive. Indolent lymphomas have few symptoms and grow and spread slowly, while aggressive lymphomas grow and spread quickly and often have symptoms such as drenching night sweats, weight loss and unexplained fevers. Indolent lymphomas include FL and MCL,

and types of aggressive lymphomas include DLBCL, Burkitt lymphoma, and human immunodeficiency virus (HIV)-associated lymphomas [17]. Additionally, certain subtypes of indolent cancers behave more aggressively, such as the blastoid variant of MCL. It is important to note that follicular lymphoma and other indolent lymphomas can transform into DLBCL, and then behave more aggressively.

Another subtype of NHL, primary DLBCL of the central nervous system (CNS), transpires in the CNS without any other systemic lymphoma. It comprises 90% to 95% of primary CNS lymphomas, another subtype of lymphoma [23]. Primary CNS lymphomas can occur in the brain, leptomeninges, cerebral spinal fluid, and eyes [23, 24]. The age-adjusted two-year incidence rate of this disease increased in the 1970's to the 1980's from 2.7 to 7.5 per 10 million people ($p < 0.001$) [23, 25]. This increase has since plateaued [26] and primary CNS lymphoma occurrence seems to be decreasing, potentially as a result of better HIV control [23]. Treatment options generally include whole brain irradiation, chemotherapy, or a combination of the two; however, severe neurotoxicity can be a complication of treatment [23].

The Ann Arbor staging scheme is used in most NHLs and ranges from Stage I through IV [17, 22]. Stage I is defined by cells that are not yet in the lymph nodes or only in one lymph node group [17]. If the lymphoma cells are in at least two lymph node groups on the same side of the diaphragm, it is defined as Stage II. The cells may be in other organs and lymph nodes near that organ but remain on the same side of the diaphragm. Stage III is defined as the spread of lymphoma to both above and below the diaphragm or found in tissues or organs near these lymph node groups. Stage IV is

defined as when the lymphoma has entered the liver, blood, bone marrow, or several parts of one or more organs or tissues, in addition to the lymph nodes [17]. These tissues include lymphoid tissues of respiratory tract and gastrointestinal tract [22]. These stages can be further broken down into A and B. A patient is defined as having B symptoms if they are experiencing weight loss, drenching night sweats, or fever, while classification A is without these symptoms [17]. Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) uses the Rai system for staging and defines stage 0 to IV with 0 as low risk, I and II as intermediate risk, and III and IV as high risk [27]. Interesting, all primary CNS lymphomas are technically stage IE, but this disease is much more aggressive than systemic stage IEs [23].

In addition to stage, prognosis can also be affected by other factors. The international prognostic index (IPI) was created to determine which patients had a higher probability for relapse. Each factor was given a point for a score ranging from 0 to 5, one point for each: age (<60 years vs. >60 years), serum LDH (normal vs. elevated), performance status (0 or 1 vs. 2 to 4), stage (stage I or II vs. stage III or IV), extranodal site involvement (0 or 1 vs. 2 to 4) [28]. This tool is mainly for DLBCL while the follicular lymphoma international prognostic index (FLIPI) is used for follicular lymphoma. The main difference between the FLIPI and IPI is the FLIPI includes serum hemoglobin (less than 12 g/dL) instead of performance status [29]. These prognostic indices divide patients into low (0 to 1 risk factors), intermediate (2 risk factors) or high risk (3 to 5 risk factors) [18].

Standard treatment options are dependent upon the type of lymphoma, whether it is indolent or aggressive, new or recurrent, stage I, II, III, or IV. For example, a treatment for an asymptomatic, stage I or II, follicular lymphoma could be watchful waiting. Other potential treatments for NHL include radiation therapy, lenalidomide, radiolabeled anti-CD20 monoclonal antibodies, rituximab with or without chemotherapy, or other novel clinical trial agents. As previously mentioned, the era of rituximab changed survival and has become the new standard of therapy. Diffuse large b-cell lymphoma (DLBCL), the most common type of NHL, is treated with R-CHOP (Rituximab with cyclophosphamide, hydroxydaunorubicin (doxorubicin), Oncovin (vincristine), and prednisone (prednisolone)).

1.1.3 Melanoma

Normal melanocytes protect against ultraviolet (UV) damage by synthesizing melanin. Cutaneous melanoma develops in melanocytes, which are found in the basal layer of the epidermis or benign common nevi. Uveal and mucosal melanoma also exist but are infrequent [30].

Distinct etiology, clinical features, and anatomic and ethnic distribution exist for five clinical growth patterns of melanoma: superficial spreading melanoma (SSM), nodular melanoma (NM), lentigo maligna melanoma (LMM), acral lentiginous melanoma (ALM), and desmoplastic melanoma (DM), in order of frequency. SSM is the most common growth pattern of melanoma among the white population accounting for the majority of the increase in cumulative incidence over the past few decades, with an

average age of 51 years. SSM may take a few months to a few years to develop from pre-existing nevus. NM patients are similar to SSM patients in age and anatomic distribution, but the tumors are usually thicker, and, consequently, patients are more advanced in stage at diagnosis. Occurring mainly in a population over 60 years of age, LMM usually develops on frequently sun-exposed skin, such as the head and neck. In contrast to SSM, NM, and LMM, which usually occur in fair-skinned individuals, ALM arises in any ethnic group and is relatively uncommon. ALM develops on palms, soles of the feet, and nail beds. They are more locally advanced in comparison to other melanoma types when they are identified, and, even after controlling for tumor thickness at presentation, the tumors are more aggressive. The most uncommon type of melanoma is DM, which is mainly located on the head and neck. It is often identified as a nondescript papule, plaque, or nodule and may be nonpigmented. DM behaves as a soft tissue sarcoma would, infiltrating across fascia and peripheral nerves, causing neurotropism. This subtype has the highest rate of local recurrence [30].

Prognosis differs by stage of melanoma. Localized disease is mainly influenced by tumor thickness and ulceration, although other statistically significant prognostic factors include age, site of primary melanoma, level of invasion, and gender [31]. Stage of melanoma can be defined by microstaging methods such as Breslow thickness [32] or Clark's level of invasion [33]. Breslow method of microstaging is based upon tumor thickness; an inverse relationship exists between tumor thickness and survival [32, 34]. Inferior to Breslow thickness in survival prediction and reproducibility, Clark's level of invasion measures the degree of tumor invasion [33, 34]. This measurement is primarily

used in T1 tumors, since Clark's level of invasion is only a good predictor of survival in patients with a tumor thickness less than 1.0mm [35]. A multifactorial analysis conducted by Balch *et al.* [34] compared the different microstaging models and found them to be accurate in low stage disease.

Metastases and overall survival are also influenced by mitotic rate. Prognosis for patients with regional metastases is dependent upon the number of metastatic lymph nodes, the tumor burden, ulceration, and metastases [36, 37]. Patients with advanced disease have poor prognoses, which are influenced by the site of metastasis, the number of metastatic sites, and elevated lipid dehydrogenase (LDH) levels [30, 38, 39]. Melanoma patients with metastasis to the lung in comparison to other visceral sites have better prognoses [31]. Overall, the median survival for patients with metastatic disease is 6 to 9 months [30].

Melanoma uses the American Joint Committee on Cancer (AJCC) staging criteria (Table 1). This criteria was updated in 2009 with the seventh edition [40]. The majority of the staging criteria remained the same. The change in new criteria uses mitotic rate and immunochemical detection of nodal metastases. The 0.2mm threshold of defined N+ that was implied in the sixth edition was removed so any isolated tumor cells or deposits less than 0.01mm are scored as N+, as long as they meet also meet the criteria for histologic or immunochemical detection of melanoma [41]. Pathologic staging provides more accuracy in defining distinctive subgroups in comparison to the traditional clinical staging if regional lymph node pathology is available [40, 41].

Different stages of melanoma require different treatment options. The treatment options include surgery, chemotherapy, radiation, targeted therapy, and immunotherapy. Melanoma surgery options include wide local excisions, which remove the tumor and some surrounding tissue, lymphadenectomy, the removal of the lymph tissue, and sentinel lymph node biopsy, where the closest lymph node is biopsied. Chemotherapy can either be given systemically or regionally, such as hyperthermic isolated limb perfusion where drugs are injected into a particular limb that has been clamped with a tourniquet. Both interferon and interleukin-2 (IL-2) can be used as types of immunotherapies and tumor necrosis factor (TNF) therapy can be used in conjunction with other treatments. Interestingly, IL-2 showed durable complete responses in a minority of patients previously treated, but no overall survival benefits were demonstrated. Targeted therapies include monoclonal antibody therapy, signal transduction inhibitors, oncolytic virus therapy, and angiogenesis inhibitors [42].

Table 1: Melanoma Staging [40, 41]

Clinical Staging	Pathologic Staging	Prognostic Group	Prognostic Group Definitions
Stage 0	Stage 0	Tis N0 M0	PRIMARY TUMOR (T) Tis: Melanoma in situ. (The tumor remains in the epidermis, the outermost layer of skin.) T1: 0.1–1.0mm thick T1a: without ulceration and level II/III T1b: with ulceration or level IV/V T2: 1.01–2.0mm thick T2a: without ulceration T2b: with ulceration T3: 2.01–4.0mm thick T3a: without ulceration T3b: with ulceration T4: >4.0mm thick T4a: without ulceration T4b: with ulceration
Stage IA	Stage IA	T1a N0 M0	
Stage IB	Stage IB	T1b N0 M0	
		T2a N0 M0	
Stage IIA	Stage IIA	T2b N0 M0	
		T3aN0M0	
Stage IIB	Stage IIB	T3bN0M0	
		T4aN0M0	
Stage IIC	Stage IIC	T4bN0M0	
Stage III	Stage IIIA	T1-4a N1aM0	REGIONAL LYMPH NODES (N) Nx: Cannot be assessed N0: No regional lymph node metastasis N1: metastasis in 1 node N1a: micrometastasis (clinically occult) N1b: macrometastasis (clinically apparent) N2: metastasis in 2 to 3 nodes or in-transit metastases without nodal metastases N2a: micrometastases N2b: macrometastases N2c: in-transit met(s)/satellite(s) without metastasis in nodes N3: metastasis in 4 or more regional nodes, matted nodes, or in transit metastasis or satellite(s) with metastasis in node(s)
		T1-4a N2a M0	
	Stage IIIB	T1-4b N1aM0	
		T1-4b N2a M0	
		T1-4a N1bM0	
		T1-4a N2b M0	
		T1-4a N2c M0	
	Stage IIIC	T1-4b N1b M0	
		T1-4b N2b M0	
		T1-4b N2c M0	
		Any T N3 M0	
Stage IV	Stage IV	Any T Any N M1	DISTANT METASTASES (M) Mx: cannot be assessed M0: no distant metastasis M1: Distant metastasis M1a: skin, subcutaneous, distant lymph node metastases M1b: lung metastases M1c: all other visceral metastases, or distant metastases at any site with elevated serum LDH

1.1.4 Breast Cancer

Within this document, “breast cancer” refers solely to primary epithelial breast cancer. Lymphomas, sarcomas, and melanomas can also involve the breast. Breast cancer generally occurs within the ducts or lobules of the breast tissue. Ductal carcinoma occurs in 75% to 80% of breast cancer cases, while lobule carcinoma occurs in 5% to 10% of cases. The remaining 10% to 20% of breast cancer types have a more favorable prognosis, including medullary, adenoid cystic, mucinous, and tubular breast cancers [43]. The disease develops in both genders, but male breast cancer is extremely rare, occurring in less than 1% of incident breast cancer cases [16]. This thesis focuses only on female breast cancer.

Breast cancer can be defined by stage, grade, and molecular subtype. TNM staging is used also for breast cancer. As with melanoma, TNM staging criteria changed with the seventh edition in January 2010 to reflect updated technology and recent data (Table 2). A large difference with the seventh edition is the division of Stage I into Stage IA and IB. Breast cancer is also defined by histologic grade: Grade I (well differentiated), Grade II (moderately differentiated), and Grade III (poorly differentiated) [43]. Furthermore, five molecularly distinct tumor subtypes exist: basal-like/triple negative (HER2-, ER-, PR-), HER2, luminal A, and luminal B [44-46].

Table 2: TNM Staging of Breast Cancer [40]

6 th ed. Stage	7 th ed. Stage	Prognostic Group	Prognostic Group Definitions	
Stage 0	Stage 0	TisN0M0	Primary Tumor (T)	
Stage I	Stage IA	T1N0M0	Tis	Carcinoma <i>in situ</i> (including DCIS and LCIS)
			T1	Tumor ≤ 20mm in greatest dimension
	Stage IB	T0N1miM0	T1mi	Tumor ≤ 1mm in greatest dimension
		T1N1miM0	T1a	Tumor > 1mm but ≤ 5mm in greatest dimension
Stage IIA	Stage IIA		T1b	Tumor > 5mm but ≤ 10mm in greatest dimension
			T1c	Tumor > 10mm but ≤ 20mm in greatest dimension
		T0N1M0	T2	Tumor > 20mm but ≤ 50mm in greatest dimension
		T1N1M0	T3	Tumor > 50mm in greatest dimension
		T2N0M0	T4	Tumor of any size with direct extension to the chest wall and/or to the skin (ulceration or skin nodules) Note: invasion of the dermis alone does not qualify as T4
			T4a	Extension to the chest wall, not including only pectoralis muscle adherence/invasion
Stage IIB	Stage IIB	T2N1 M0	T4b	Ulceration and/or ipsilateral satellite nodules and/or edema (including peau d'orange) of the skin, which do not meet the criteria for inflammatory carcinoma
		T3N0 M0	T4c	Both T4a and T4b
			T4d	Inflammatory carcinoma
Stage IIIA	Stage IIIA	T0N2 M0	Regional Lymph Nodes (N)	
		T1N2 M0	N0	No regional lymph node metastases
		T2N2 M0	N1	Metastases to movable ipsilateral level I, II axillary lymph node(s)
		T3N1 M0	N2	Metastases in ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted; or in clinically detected ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastases
		T3N2 M0	N2a	Metastases in ipsilateral level I, II axillary lymph nodes fixed to one another (matted) or to other structures
			N2b	Metastases only in clinically detected* ipsilateral internal mammary nodes and in the absence of clinically evident level I, II axillary lymph node metastases
Stage IIIB	Stage IIIB	T4N0 M0	N3	Metastases in ipsilateral infraclavicular (level III axillary) lymph node(s) with or without level I, II axillary lymph node involvement; or in clinically detected* ipsilateral internal mammary lymph node(s) with clinically evident level I, II axillary lymph node metastases; or metastases in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
		T4N1 M0	N3a	Metastases in ipsilateral infraclavicular lymph node(s)
			N3b	Metastases in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
		T4N2 M0	N3c	Metastases in ipsilateral supraclavicular lymph node(s)
Stage IIIC	Stage IIIC		Distant Metastases (M)	
		Any T N3 M0	M0	No clinical or radiographic evidence of distant metastases
			cM0(i+)	No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor

Stage IV	Stage IV	Any T Any N M1		M1	cells in circulating blood, bone marrow, or other nonregional nodal tissue that are no < 0.2mm in a patient without symptoms or signs of metastases Distant detectable metastases as determined by classic clinical and radiographic means and/or histologically proven>0.2mm
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Tumor subtype and histologic grade influence prognosis as well as tumor size, age, menopausal status [43, 47-49], number of nodes involved [43, 50], estrogen (ER) and progesterone (PR) receptor status [43, 51, 52], human epidermal growth factor type 2 receptor (HER2/*neu*) overexpression [43, 53], and possibly Ki67 status, a proliferative marker [43, 54, 55]. HER2/*neu* overexpression, also referred to as HER2/*neu* and c-*erbB-2*, occurs in 25% to 30% of all breast cancer patients [53, 56, 57] and, in one study, was the strongest predictor of relapse [58]. With the development of trastuzumab (Herceptin; Genentech, San Francisco, CA), a humanized anti-HER2 monoclonal antibody, patients overexpressing HER2/*neu* experienced improved systemic control and overall survival [43, 59, 60]. Although breast cancer incidence rates increase with age, patients diagnosed at a younger age have worse survival because of the aggressiveness of the disease [43].

This type of treatment, the monoclonal antibody trastuzumab, may be used in combination with chemotherapy. Other breast cancer treatments include surgery, radiation therapy, hormone therapy, targeted therapy. These therapies may be prescribed singly or, more commonly, concurrently with other therapies. The type of treatment or treatment combination is dependent upon the patient's risk of reoccurrence, including prognostic factors such as stage of disease, age, menopausal status, and type of receptor status (ER, PR, or HER2) [42].

Chemotherapy can be used for curative purposes, control purposes, or palliative care. Both radiation and chemotherapy aim to kill or stop the cancer cells from dividing. Unlike radiation and chemotherapy which also affect healthy cells, targeted therapies

only destroy cancer cells. Surgery can be used to either remove just the tumor or, if needed, the surrounding tissue [42]. Surgical options include a lumpectomy, a partial mastectomy, or a total mastectomy. The lumpectomy only removes the tumor and a small amount of surrounding normal tissue, while the total mastectomy removes the whole breast that has cancer and possibly some lymph nodes. A partial mastectomy removes part of the breast and normal surrounding tissue that has the tumor, while a segmental mastectomy additionally removes the lining over the chest muscles. A modified radical mastectomy removes the complete breast with the tumor, the lymph nodes under the respective arm, the lining over the chest muscles, and, sometimes, the chest wall muscles. In combination with or without surgery, chemotherapy options include neoadjuvant therapy, in attempt to shrink the tumor and limit the amount of surgical tissue removal. Other chemotherapeutic options include chemotherapy followed by a stem cell transplant.

Another treatment, tamoxifen, a hormone therapy, is usually prescribed for patients diagnosed with early stage disease or metastatic breast cancer. The beneficial effect of tamoxifen is limited to ER-positive patients [61, 62] and is seen for the first 10 years, but appears to diminish after this time period. In ER-positive disease, a 47% reduction in breast cancer recurrence occurred in years 0 to 4 and a 32% reduction for years 5 through 9, although this benefit is not apparent in ER-negative disease [63]. The general recommendation for tamoxifen use is 5 years after diagnosis, although, a recent study conducted by Davies *et al.* [64] found continuing adjuvant tamoxifen for a total of 10 years, in comparison to stopping at 5 years, further reduced recurrence and mortality.

1.2 Metastases

Metastasis is the occurrence of the primary cancer spreading to a secondary site and proliferating. The term “metastasis” was coined by Récamier in 1829 [65]. Although he has been credited with supporting the idea of the spread of metastasis via the blood or lymphatics, he only propagated the idea of the spread into the venous system because of the “continuity of tissues”. He did not support the idea of the veins as a mode of transportation for the metastatic cells [65, 66], a current theory for metastasis. A few theories exist for tumor cell migration. In 1889, Stephen Paget proposed the ‘seed and soil’ or molecular recognition hypothesis, which differed from the existing theory, that metastasis occurred at random. This hypothesis states metastasis does not occur by chance; the tumor spread to all areas but only grew in those organs that were hospitable to those particular tumor cells [67-70]. James Ewing presented the mechanical or hemodynamic hypothesis of metastasis, which proposes that these cells are transported by the blood or lymphatic channels [68, 71]. These hypotheses are not mutually exclusive, particularly in the brain. Metastasis may occur via the blood or lymphatic channels but only proliferate if the tissue is hospitable to those particular tumor cells [68]. Additionally, these two hypotheses may not be the only contributing factors to metastatic growth. More research must be conducted in this area to better predict where and when an individual’s cancer will spread [72].

In order for metastases to develop, all steps of the metastatic cascade must occur (Figure 2) [73, 74]. This cascade of events includes cellular division and growth within

the primary tumor, invasion of the tumor border, intravasation into the bloodstream or lymph vessels, arrest in capillary bed of the secondary site, extravasation, and proliferation of the cancer cells at the site of metastasis [68, 74-76]. If any of these steps are not fully complete, metastasis will not develop [4].

Figure 2: The Metastatic Cascade [77]

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This cascade begins with the cellular division and growth of the primary tumor and then invasion of local tissue [4]. In order for the invasion of the local tissue to occur,

the metastatic cells must have a phenotype that supports detachment, circulation, and invasion [68]. The epithelial cells that comprise most carcinomas generally do not mobilize or invade other tissues. In contrast, mesenchymal cells lack the strength in adhesion that epithelial cells possess which allows for migration. Adding to the differences in migratory capabilities, epithelial cells can only migrate as a sheet, whereas, mesenchymal cells can separate and migrate independently [78]. The opportunity for epithelial cells to transform into mesenchymal cells occurs during embryogenesis, in particular, gastrulation and organ formation [79]. Tumor cells may manipulate this epithelial-mesenchymal transformation process to aid in their migration [68].

After tumor cells proliferate within the primary tumor, have the proper metastatic phenotype, and invade the tumor border, intravasation into the bloodstream or lymphatic channels may occur. Once these cells are in the blood or lymphatic channels, they must travel to the secondary site. The tumor cells attach to platelets and leukocytes for transportation within the blood stream because they are unable to travel in the blood on their own due to elements of the immune system or mechanical damage from velocity [68, 80].

These cancer cells travel until they arrest in a capillary bed of the secondary site. This arrest in a secondary site does not occur at random and is partially determined by the type of tumor and its organ preference [81]. A tumor's organ preference is determined by the tumor's preference in the cells involved in adhesion, invasion and proliferation [82]. The binding capabilities of the tumor cells themselves aid in this arrest as well as the size of the tumor, although this component is debated [68, 83]. Extravasation occurs with the

tumor cells forcing their way into the surrounding tissue [68]. After invasion, cells must proliferate in order to become a metastasis. Otherwise they may resolve to a dormant state of micrometastases [68]. The cells in this dormant state may be multiplying but inhibition of angiogenesis keeps these cells in check and makes the disease appear clinically dormant [84]. This dormant state may explain why metastasis develops years after the initial cancer diagnosis and even after the cancer appears to have gone into remission, particularly with breast cancer [85].

1.3 The Blood-Brain Barrier

The BBB regulates circulation between the periphery and the central nervous system (CNS) selectively allowing the transfer of few compounds to the brain [86, 87]. This barrier modulates the substances traveling to and from the brain, maintaining homeostasis. The endothelial cells permit small hydrophobic molecules such as carbon dioxide, oxygen, and hormones to pass through the BBB to the CNS, while preventing the passage of hydrophilic molecules and bacteria. These molecules must have a molecular weight less than 500 Daltons (Da) [88, 89]. Glucose, amino acids, and other nutrients enter the brain by transporters, while insulin, leptin, iron transferrin, and larger molecules enter by receptor-mediated endocytosis. It can be disrupted by hypertension [90], radiation [91], infection [92-94], multiple sclerosis [95], trauma [96, 97], ischemia [98, 99], inflammation [100], pressure [101], and tumors [102].

1.3.1 Structure

The BBB is comprised of the cerebral microvascular endothelium, neurons, astrocytes, pericytes (PCs), and the extracellular matrix (Figure 3). Between the endothelial cells are tight junctions (TJs) that form a diffusion barrier between the periphery and the brain. Adherens junctions (AJs), which create adhesive contacts between cells, and TJs form the junction complex [86, 87, 103, 104]. This junction complex restricts permeability across the endothelium [86].

Figure 3: Schematic Structure of the Blood-Brain Barrier [105]

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The endothelial cells of the BBB lack fenestrations, have more extensive TJs, and have sparser pinocytotic vesicular transport than the endothelial cells found in the rest of

the human body [87]. Astrocytes control the development and maintenance of the TJ by ensheathing the vessels, but they do not appear to affect the functionality of this barrier [86, 87]. Although PCs are the least understood cellular structure of the BBB, they are thought to be involved in “angiogenesis, structural integrity and differentiation of the vessel, and formation of endothelial TJ” [87].

Tight junctions are composed of membrane proteins and cytoplasmic accessory proteins, while the adherens junctions consist of a cadherin-catenin complex and its associated proteins. Claudin, occludin, and junction adhesion molecules (JAMs) are the integral membrane proteins of the TJs, while the cytoplasmic accessory proteins include zonula occludens proteins (ZO-1, ZO-2, and ZO-3), cingulin, 7H6, and others [87, 104]. To date, ZO-3 has not been found in the BBB [106]. Claudins form the seal of the TJ by binding to other claudins on adjacent endothelial cells, while the permeability is modified by occludin, a regulatory protein. Knowledge regarding JAMs is incomplete, but they are involved in cell-to-cell adhesion and monocyte transmigration through the BBB. Cytoplasmic proteins, another component of the TJ, organize proteins at the plasma membrane and bind to actin, the primary cytoskeleton protein which provides structural support to the endothelium [87, 104].

Several areas of the human brain are not encompassed by the BBB including areas of the brain that regulate the autonomic nervous system and endocrine glands. These areas include area postrema, median eminence, neurohypophysis, pineal gland, subfornical organ, and lamina terminalis. Diffusion of blood-borne molecules occurs through fenestrations in the blood vessels in these areas [87].

1.3.2 Diseases and Other Factors Affecting the BBB

Disruption of the BBB results in the development and progress of neurological disease. These diseases may result from the change in permeability, or they may cause the change in permeability; for example, an ischemic stroke may increase the permeability of the BBB, but multiple sclerosis (MS) may be the result of the increase in permeability [86, 99, 107]. Hypoxia, inflammation, or other insults to the brain are associated with changes in BBB permeability [86, 97, 108-113].

Resulting from a loss of blood flow and, consequently, a decrease in the amount of oxygen and nutrients to the brain, an ischemic stroke increases BBB permeability [86, 114]. This increase in permeability may have resulted from TJ disruption and a decreased expression of occludin. Also with a decrease in occludin expression, diabetes, a known risk factor for ischemic stroke, is associated with an increase in blood-retinal permeability [115].

Neuroinflammatory diseases are known to have compromised BBB TJs, which is supported by the manipulation of BBB permeability by inflammatory mediators. In patients diagnosed with MS, a disruption in the BBB antedates clinical symptoms depicted in magnetic resonance imaging (MRIs) [107]. A similar disruption of the BBB was found in patients with human immunodeficiency virus (HIV) encephalitis, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS) [86, 116-120].

Blood brain barrier TJs were also affected by inflammation in the periphery. A decreased expression of occludin and an increased expression of ZO-1 produced in an

increase in BBB permeability, although the exact mechanism remains unknown. An increase in BBB permeability also occurs during cortical spreading depression, which is associated with migraines, due to the disruption of the TJ and specific loss of ZO-1 [86, 121, 122].

In addition to these diseases, recreational drug use can compromise the BBB. Cocaine may have a direct effect on the endothelium as well as pro-inflammatory effects when it accelerates HIV-associated dementia [123, 124]. Nicotine, a major component in tobacco products, alters the transportation of glucose and ions at the BBB and decreases the expression of ZO-1, which increases BBB permeability [125].

Also increasing permeability, brain tumors allow the passage of nano-sized particles through the BBB [86, 103, 126]. Occurring frequently with brain tumors, edema is affected by TJ abnormalities [102]. This development of edema may be influenced by the loss of occludin. Zagzag *et al.* [126] found Evans blue dye leaked from the brain vasculature in a rabbit model of brain cancer, supporting the hypothesis that brain tumors disrupt the BBB. This permeability correlates with tumor size and increases in a time-dependent manner [126, 127]. An increase in permeability also occurs with brain metastasis and will be discussed further in the next section [127].

1.4 Central Nervous System Metastasis

Brain metastasis is the spread of the primary tumor to the brain and is a complication of systemic cancer [7, 128]. *Solitary brain metastasis* refers to cancer that has metastasized solely to the brain, whereas *single brain metastasis* indicates a single

cerebral lesion as opposed to multiple cerebral lesions [4, 68]. Brain metastasis occurs more frequently than primary brain tumors such as gliomas, meningiomas, astrocytomas, and oligodendrogliomas [129, 130]. Central nervous system metastasis is the spread of the primary tumor to the central nervous system, which includes the brain and leptomeninges.

Survival of patients experiencing CNS metastasis remains limited with the majority of patients surviving an average of 4 to 6 months. Their last months are often filled with seizures, paralysis and cognitive failure [7]. This short survival is compounded by the inability of most traditional chemotherapeutic drugs to cross the BBB to treat the metastasis. Certain cancers are known to spread to the brain more frequently than others, such as lung, breast, melanoma, colorectal and renal cell cancers [4, 7]. Interestingly, melanoma has the highest affinity for brain metastasis and the worst prognosis [5, 131-133].

1.4.1 The Blood-Brain Barrier in CNS Metastases

The exact mechanism for how brain metastases cross the endothelial cells in the blood-brain barrier is unknown. The endothelial cells of the BBB may allow brain metastases to pass through and even help facilitate this process by aiding metastatic growth. Additionally, the cancerous cells are protected from the immune system as they attempt to pass through the BBB [105].

In general, brain tumors cause the loss of tight junctions in the tumor vascular system and an enhanced retention effect of solid tumors [87, 103, 134]. The reason for

this loss, resulting in an increase in permeability, is unknown [87, 135], although abnormalities were found in gap functions, fenestrations of membranes, and open normal junctions [135]. A potential explanation for the increase in permeability was seen in astrocytoma and metastatic adenocarcinoma, where the expression of 55kDa occludin expression was reduced in the microvessels [136]. In previous studies, aquaporin-4 (AQP4), a water channel molecule, has been investigated as a possible explanation for increased permeability, because cerebral edema results from brain tumors. In astrocytoma and metastatic adenocarcinoma, AQP4 was massively up-regulated and correlated with permeability, although the exact mechanism remains uncertain [137]. These brain tumors also produce an overexpression of receptors of folate, insulin and transferrin in the BBB [87, 103, 134]. These changes in the BBB allow the angiogenic vessels to become permeable to nano-sized particles [86, 103, 126, 127, 134].

Possibly affecting the risk of brain metastasis is the drug efflux pump, P-glycoprotein (P-gp) [138, 139]. P-gp is highly expressed in the BBB and modulates multidrug resistance in addition to limiting the penetration into and retention of compounds to the brain [138, 140, 141]. Cancer patients with brain metastasis had lower expression of P-gp than a normal brain; for example, P-gp was undetectable in breast cancer tumors, but it was detected in normal breast tissue [142]. Melanoma metastases expressed only 5% of the P-gp level found in the normal brain [138]. Régina *et al.* [138] suggested that another multidrug resistance mechanism could be involved, which would explain the weak response of brain metastasis to chemotherapy.

1.4.2 Distribution of CNS Metastasis

CNS metastasis transpires in the leptomeninges or brain parenchyma, which is the most common [4, 127]. The dura mater is the outermost layer of the cranial meninges. The inner and middle layer of the cranial meninges, the pia mater and arachnoid mater, respectively, compose the leptomeninges; the cranial meninges surround the brain providing protection [143]. Patients diagnosed with leptomeningeal disease (LMD) have shorter survival times than those diagnosed with metastasis in the parenchyma [127, 144].

Within the parenchyma, the brain tissue, brain metastasis is most common in the cerebral hemispheres [4, 145]. Up to 85% of brain metastases occur in these hemispheres, whereas only 10% to 25% occurs in the cerebellum and 1% to 3% in the brain stem [146, 147]. An autopsy study found the distribution of cerebral metastases to be approximately 20%, 17%, 10%, and 9% to the occipital lobe, frontal lobe, parietal lobe, and temporal lobe, respectively; the same study found slightly more metastases were left-sided (48.9%) in comparison to right-sided (45.2%), and 5.9% were centrally located [147]. This spread of metastasis appears to parallel the amount of blood flow to these areas [4, 68, 139, 148]. The development of these metastases transpires when the cancer cells spread through the internal carotid and vertebrobasilar arteries or the Batson venous plexus and concentrate at the gray-white junction [149]. The concentration in this particular area results from the sudden decrease in blood vessel size which ensnares the cancer cells [4, 139, 148, 149].

This distribution is different for different primary cancers. In contrast to other tumors that metastasize to the brain, melanoma tends to metastasize to the cerebral

cortex, also known as gray matter, instead of the gray-white junction and focuses largely in the temporal and frontal lobes [146, 147, 150]. According to a review article by Hill *et al.* [20], lymphomas metastasize more often to the leptomeninges. Breast cancer tends to spread to the basal ganglia most frequently [147], while different subtypes of breast cancer metastasize to different areas of the brain. Invasive lobular carcinoma of the breast metastasizes to the leptomeninges more frequently than invasive ductal carcinoma, which tends to metastasize to the parenchyma [68, 151, 152]. Leptomeningeal involvement differs by subtype in breast cancer patients, with the majority of the disease occurring in luminal A subtypes followed by HER2 and triple negative cancers. Also differing by subtype, the median parenchymal brain metastases at diagnosis was highest among luminal A subtypes and decreased to luminal B, with the lowest number of parenchymal metastases in the HER2, and triple negative subtypes [153].

These lesions can range from microscopic to several centimeters in size [4]. In breast cancer, this size differs by subtype, with triple negative tumors resulting in the largest median brain metastases [153]. In the parenchyma, these lesions tend to displace the tissue as opposed to permeating it. Edema, cystic degeneration, necrosis, and areas of hemorrhage frequently accompany these metastases [4]. Certain metastatic cancers elicit certain responses more than other cancers; for example, melanoma brain tumors frequently have hemorrhagic lesions [4].

One autopsy study found 60% of brain metastases were multiple lesions, while the other 40% had single lesions [154]. These percents are supported by other autopsy studies, which found 42% of brain metastases of breast cancer patients were single

lesions [145, 147]. One retrospective study found only 15.3% of patients had single lesions [155]. Corroborating the findings in the autopsy investigations, a study in Berlin that found 38% of melanoma patients with brain metastasis had single lesions while the other 62% developed multiple lesions, and 33% of these patients developed more than 4 lesions [156]. A study conducted by Delattre *et al.* [146] contradicts these findings with 56% of breast cancer cases developing as single lesions and 44% as multiple lesions. Differences could exist due to type of cancer and particular subtypes. The majority (70%) of multiple lesions occur in the cerebrum, whereas only about 4% occur exclusively in the cerebellum or brain stem, and the rest (25%) occur in both [157].

1.4.3 Symptoms and Diagnosis

Metastatic CNS tumors should be in the differential diagnosis if a cancer patient reports neurological symptoms such as headaches, nausea, cognitive problems, seizures, dysphasia, motor weakness, or sensory disturbances, since these metastases are usually symptomatic [139, 145, 153, 156-158]. CNS metastasis symptoms can either be generalized or focal. Generalized signs and symptoms include headaches, nausea/vomiting, and cognitive problems resulting from cerebral edema. Focal signs and symptoms include weakness, numbness, speech disturbance, and seizures resulting from brain tissue damage [4].

One of the most common symptoms of brain metastasis is a headache, developing in about 23% to 38% of patients [153, 156-158]. These headaches generally transpire in patients with multiple metastases or metastasis located in the posterior fossa [4].

Occurring as frequently as headaches, altered mental status appeared in almost 25% of patients with brain metastasis [153, 157]. Another study reported motor weakness and convulsion as the most frequent symptoms of brain metastases with almost a quarter of all clinically diagnosed breast cancer cases reporting each of these symptoms [145]. It is important to note that these symptoms occur concurrently in almost 40% of patients [156], although reports of asymptomatic brain metastasis range from 10% of patients to 48% [153, 156, 157, 159].

When these symptoms arise, the diagnosis should be confirmed by radiographic evidence. If the radiographic evidence depicts multiple cerebral lesions, brain metastasis can be assumed, but this assumption cannot be made with as much certainty if a single lesion is found [4]. Patchell *et al.* [160] found the false-positive rate of single lesions to be 11.1%, even with contrast MRI. Upon biopsy or resection, the false-positives included glioblastomas, an astrocytoma, abscesses, and a non-specific anti-inflammatory response [160].

In some patients, brain metastasis may be identified before a primary cancer is diagnosed. Up to 20% of patients reporting brain metastasis have had no prior diagnosis of cancer [4, 68, 150, 157, 161, 162]. Of these patients, 5% to 10% will never receive a definite cancer diagnosis, even with thorough workups [4, 157, 163]. The primary cancer may appear later or a diagnosis can be confirmed by autopsy, and is usually diagnosed as metastatic lung cancer [4, 158]. Identified in a series of patients from 1987 to 1996, patients with unknown primary tumors were primarily male and with a median age of 51.

The lesions were singular and commonly found in the cerebellum and parietal and frontal lobes [163].

1.4.4 CNS Metastasis in Previous Studies

Previous studies of CNS metastasis used data from an array of sources such as death certificates, cancer registries, hospital records, or census data. Unfortunately, since only lung cancer patients get screened systematically for brain metastasis, most studies miss the majority of brain metastasis cases. This problem is compounded by the fact that some of these metastases are asymptomatic and are never diagnosed. Autopsy studies are generally more accurate, and up to a third of all brain metastases were diagnosed upon autopsy, but these studies have significantly declined over recent decades [5].

Interestingly, since the implementation of MRI, brain metastasis found upon autopsy significantly decreased from 53% in the CT era to 15% in the MRI era ($p=0.027$) [164]. To complicate these previous studies, the majority of cancer registries only include the primary tumor code, not a code for metastasis [165].

Additionally, changes in the standard for imaging occurred. Replacing CT scans, MRIs only became the standard for brain imaging in the 1990's, which allows for detection of smaller metastases [148]. For example, asymptomatic small cell lung cancers can now be detected [164]. Although MRI is more useful in identifying brain metastasis, some patients cannot receive MRI due to certain medical conditions, such as a pacemaker; consequently, these patients must undergo CT.

Quality *in vivo* models of brain metastasis are limited, resulting in little knowledge regarding how tumor cells colonize the brain. The exact mechanism of how tumor cells penetrate the BBB is still uncertain [166]. This lack of information contributes to the poor prognosis of brain metastasis patients, and more research is necessary for prevention.

1.4.5 *Epidemiology of CNS Metastasis*

Many believe the cumulative incidence of these metastases is increasing, but this is difficult to quantify since many studies underestimate the true number of cases of brain metastasis [6, 7, 127, 167], because brain metastasis is difficult to identify and many are only identifiable upon autopsy, which are increasingly uncommon [5]. Additionally, conflicting reports exist due to methods of investigation and inclusion of different tumor types [168]. From 1988 to 2000 in a Nationwide Inpatient sample, the number of surgical resections for brain metastasis increased 79% from 3900 to 7000 [3]. An autopsy study demonstrated that almost 25% of cancer patients developed brain metastasis, but only 15% reported neurological signs and symptoms [1]. This increase in cumulative incidence is potentially due to improved therapeutics, increased survival after a primary cancer diagnosis, improved diagnostic and screening mechanisms, an increase in the cumulative incidence of lung cancer and melanoma, and an aging patient population [4-6]. Additionally, determining this cumulative incidence is difficult because surveillance imaging of patients without neurological symptoms is not standard, with the exception of lung cancer [5].

Although these limitations exist, population studies aim to determine the incidence rate of brain metastasis. The incidence rate of brain metastasis ranges from 2.8 per 100,000 in Iceland during the 1950s and 1960s [169] to 14.3 per 100,000 in Scotland in 1989 to 1990 [162]. An increasing trend in incidence rate appears to exist. In Sweden, admissions for brain metastasis increased from 7 per 100,000 in 1987 to 14 per 100,000 in 2006, and van den Hurk *et al.* [2] observed a rise in central nervous system metastasis from 1978 to 2003 in a Munich population [170]. In the United States, Davis *et al.* [6] estimated that 6% of incident primary invasive cancers diagnosed in 2007 will develop brain metastasis.

The incidence of brain metastasis differs between primary tumor types [157, 168]. Lung cancers occur most frequently overall and breast, melanoma, renal cell, and gastrointestinal cancers follow [157, 168]. In a review of 14 studies, CNS metastasis ranged from 2.3% to 10% in NHL patients with an overall CNS relapse of 4.7% [20]. Barnholz-Sloan *et al.* [171] found the cumulative incidence was 6.9% and 5.1% for melanoma and breast cancer, respectively. These results were similar to those reported in other studies [165]. This incidence risk increased as stage increased; for example, the 5-year cumulative incidence of CNS disease increased from 3.1% to 12.6% as stage increased from I to IV [168]. The type of breast cancer also determined the cumulative incidence. Cumulative incidence of brain metastasis in HER-2/*neu* breast cancer patients is 37.3% [172].

Among cancers, striking differences exist among time between initial diagnosis and CNS metastasis. Lymphoma averages 5 to 12 months from initial diagnosis to CNS

metastasis, while melanoma and breast cancer average 1 to 3 and about 2 to 3 years, respectively [5, 155, 173, 174]. These averages differ by subtype; for example, CNS metastasis for triple-negative breast cancers generally develop in 7.4 months and the median time for mantle cell lymphomas can range from 16 to 61 months [20, 153].

Survival times for patients diagnosed with CNS metastasis are short. Breast cancer patients averaged 40 months for survival from primary diagnosis, but only 19 months from time of first metastasis and 4 months from diagnosis of CNS metastasis [174]. Survival depends upon subtype. The median survival from CNS metastasis to death is shortest in luminal A and triple negative patients (median survival of 4.0 and 3.4 months, respectively); ER-, PR-, and HER2-positive patients lived longer than patients with negative receptor statuses [175]. Survival time for patients diagnosed with lymphoma averages 2 to 6.5 months from diagnosis to death [20, 176-184]. Diagnosis of brain metastasis in melanoma patients averaged 2.4 months of survival from time of brain metastasis diagnosis with one-year overall survival of 15% [185], although a study by Nussbaum *et al.* [157] found survival time from diagnosis of brain metastasis to death was 5 months in melanoma patients. Interestingly, brain metastasis can even occur when the original tumor appears under control [186].

Patients with single lesions, and surgery on those lesions, had longer survival than patients with multiple lesions or patients who did not have surgery on their singular lesions [157, 158]. Nussbaum *et al.* [157] conducted a multivariate analysis to determine which variables were important predictors of survival in patients with a single brain

metastasis. Surgery, radiation therapy, and presentation with seizures were associated with a reduction in risk while age increased risk [157].

1.4.6 Risk Factors

Studies on brain metastasis risk factors are scarce, particularly those that are modifiable. Although knowledge regarding modifiable risk factors is scarce, several non-modifiable risk factors are known to influence the cumulative incidence of brain metastasis. Brain metastasis are more common in younger cancer patients than older cancer patients in breast cancer and melanoma [8, 9, 153, 185, 187-194]. The risk of brain metastasis appears to increase until 40 to 49 years of age and then decline thereafter [131]. This difference may be due to a more aggressive cancer in younger patients [5, 57]. The odds ratio for brain metastasis associated with a young age (<50 or 60 years old, depending upon the study) compared to older age ranged from 1.26 (95% CI: 1.03-1.53) to 2.5(95%CI: 1.33-4.76) [189-191]. When older age was the reference in comparison to younger age, it had a protective effect that ranged from 0.5 (95%CI: 0.4-0.7) to 0.98 (95%CI: 0.97-0.99) [9, 185, 187, 192, 195]. A study conducted by Vuong *et al.* [196] found males had a higher prevalence of brain metastasis than females ($p<0.001$), but the frequency of brain metastasis in a study conducted by Pickren *et al.* [131] was not different (8.8% in men and 8.7% in women). Race has not been associated with brain metastasis [131] and was not included as a covariate in the majority of studies [8, 9, 185, 187, 189-195, 197-204].

CNS metastasis differs by subtype of lymphoma. The indolent lymphomas such as follicular lymphoma and mantle cell lymphoma do not metastasize to the brain as frequently as the aggressive lymphomas such as DLBCL and Burkitt lymphoma. The majority of follicular lymphoma cases that did metastasize to the brain had transformed to DLBCL [20]. The Epstein-Barr virus (EBV) has been suggested as having a potential role in CNS disease, cited mostly for primary CNS development than CNS metastasis; unfortunately, research is limited to case reports and small case series with patients only sporadically tested for the virus [205-207].

DLBCL patients with advanced stage, increased IPI, raised LDH, young age, greater than 1 extranodal site, B symptoms, low albumin, and retroperitoneal disease had a greater risk of developing CNS disease [20, 177-184, 208]. Studies of extranodal involvement found an increased risk of brain metastasis from bone marrow, skin, testis, epidural space, retroperitoneum, orbit, nasal/paranasal sinuses, and lung, although this risk did not exist in multivariable analyses [20, 177-181, 183, 209, 210]. Patients with HIV also are at increased risk for brain metastasis development [20]. CNS prophylaxis using high dose IV and intrathecal methotrexate with or without radiation in patients diagnosed with acute lymphoblastic leukemia/lymphoma and Burkitt lymphoma, is successful in reducing CNS relapse [20]. Studies have been less conclusive in the effects of CNS prophylaxis in DLBCL patients [20, 182, 211-215].

Breast cancer is the most common source of brain metastasis for women [5]. Slimane *et al.* [216] found lung metastasis as the first site of relapse and negative hormone receptor status risk factors for brain metastasis in breast cancer patients,

although other studies included young age, tumor size, stage, tumor grade, histology, and human epidermal growth factor receptor 2 (HER2, also known as *neu*) [187, 193, 217-221]. Overexpression of HER2 is repeatedly supported as a risk factor for brain metastasis in breast cancer patients [57, 175, 193, 221, 222], and the risk has increased since the development of trastuzumab (Herceptin; Genentech, San Francisco, CA), a monoclonal antibody therapy [57]. Trastuzumab has significantly reduced progressive systemic disease in HER2 positive patients, but the likelihood of developing brain metastasis is higher [223]. Supporting this increase, 28.6% to 43.1% of stage IV patients treated with trastuzumab developed CNS metastasis [57, 224-227]. This increased risk may result from the improved systemic control and, consequently, longer survival times which may increase the chances of brain metastasis, particularly since the brain is not protected by trastuzumab [228, 229].

Triple-negative tumor status (ER-/PR-/HER2-), ER-, PR-, and basal-like tumors also were associated with an increased risk of brain metastasis [153, 175, 187, 193, 195, 216, 219, 221, 230]. Brain metastasis as the first site of metastasis developed quickly and most frequently in breast cancer patients with HER2 and triple-negative tumors [153, 231]. Patients with lung metastasis also developed brain metastasis more frequently than those patients without lung metastasis [153, 216, 232]. Graesslin *et al.* [233] developed a nomogram to predict brain metastasis in patients with metastatic breast cancer; significant predictors include younger age, higher histological grade, number of metastatic sites, short disease-free survival, and HER2 positivity.

Development of brain metastasis from melanoma was more common in patients with cutaneous melanoma located in the head and neck in comparison to extremity or truncal locations [185]. In addition to location, risk factors included male gender, younger age, mitotic rate, and Breslow thickness greater than 4mm [185, 188, 234-236]. Lung, liver, and bone metastasis did not increase the risk for melanoma [131]. Risk factors for brain metastasis for unresectable stage III/IV melanoma include stage M1b and M1c, visceral tumor metastasis, location of the primary melanoma is at the head, neck, trunk, abdomen, or unknown, depth of primary tumor invasion, elevated lactic acid dehydrogenase (LDH), skin melanoma as opposed to uveal melanoma, and male gender [237]. Also suspected as a risk factor for brain metastasis by Shapovalov et al.[14] is antidepressant use.

1.5 Antidepressant Use

Depression in cancer patients is common. A systematic review reported the median prevalence of depression in advanced cancer patients was 29% using the Hospital Anxiety and Depression Scale [238], while another study found 57% of ambulatory palliative care cancer patients self-reported depression [239]. Depression was documented in as many as 46% of breast cancer patients [240, 241], although this number was not as high in hematologic cancer patients (17.5%) [242]. Stage and treatment status were not correlated with depression among women with breast and gynecological cancers, while a primary diagnosis of breast cancer, higher Karnofsky performance score (KPS), poorer social and family well-being, anxiety, arthritis, and fears about treatment

side effects were [243]. Age was not significantly associated with depression [243, 244]. Interestingly, a study by Sharpe *et al.* [245] found only 49% of patients identified as having major depressive disorder reported speaking to their doctors about depression, and only 15% of all patients identified as having major depressive disorder received a potentially effective evidence-based treatment such as a therapeutic dose of antidepressants or formal psychological treatment.

Depression among cancer patients has also been associated with poor survival [246, 247]. The high percentage of depression may be associated with high suicide among breast cancer patients, with 0.20% cumulative probability 30 years post-diagnosis [248]. Depression among cancer patients is being recognized more frequently as a problem and can be dealt with by antidepressant medications, exercise, or support groups.

Among cancer patients, antidepressants are used not only to treat depression, but they also treat hot flashes, sleep disturbances, pain, and multidrug resistance [11-13]. Preclinical studies indicate that they could also be used to treat 'chemobrain', the chemotherapy-induced alteration in cognition, which occurs in some cancer patients [249-251]. In a study of terminally ill cancer patients, 12.7% were prescribed antidepressants for depression, while an additional 4.2% were prescribed antidepressants for pain treatment [252]. Unfortunately, the side effects of these medications are not fully known. Since cancer patients are prescribed antidepressants, the effect that these medications have on the dissemination of this disease to the brain needs to be understood.

Antidepressants are used more frequently in the cancer population in comparison to the general population [10, 253]. According to a study conducted in Australia,

antidepressant use in the cancer population ranged from 6.2% to 11.7% for the different periods studied, while antidepressant use in individuals without a cancer diagnosis ranged from 4.6% to 7.4% [10]. A Norwegian study found 22% of cancer patients had at least one antidepressant prescription in their last year of life in comparison to 6% of the general population [254].

In the Netherlands, cancer patients were 38% (95% CI: 34%-42%) more likely to use an antidepressant in comparison to the general population when controlling for age, gender, immigrant status, neighborhood socio-economic status, and pre-morbid chronic medical conditions (diabetic, cardiovascular, and pulmonary conditions) [253]. This study also found cancer patients who were prescribed antidepressants were more likely to be younger, female, and have a diabetic, cardiovascular, or pulmonary co-morbid condition [253], while among breast cancer patients in Wisconsin, antidepressant use was more likely among women who were younger, premenopausal, obese, have fewer children, and receive adjuvant therapy in comparison to those women who did not take antidepressants [11]. Interestingly, another study among breast cancer patients found age, education, stage at time of diagnosis, time since diagnosis, or time since last recurrence or metastasis were not associated with antidepressant prescription rates [255]. A decline in antidepressant use in the cancer population was seen after age 49, whereas the antidepressant use in the general population increased with age. It is important to note that this study was not able to access prescription information on hospitalized or nursing home patients, and that these patients are generally older [254]. Although

concern exists with the Norwegian study, this trend in age is supported by other research [256]

Research regarding antidepressant use among cancer patients is sparse and is usually gleaned from self-report or prescription patterns [254]. Among newly diagnosed male and female cancer patients, SSRI use is self-reported in 15.65% [257]; this self-report was substantiated by 18.6% of breast cancer patients' pharmacy records demonstrating SSRI use [258]. Another study found antidepressants were self-reported in 27% of breast cancer patients; this percentage increased to 32% in patients with lymphedema [259]. At a 2-year interview, 15% of breast cancer patients reported taking an antidepressant [260], while another 2-year study found 19.2% of breast cancer patients were prescribed antidepressants [256]. Using a prescription database, among women taking tamoxifen who were older than 66, 30.6% were prescribed at least one SSRI or venlafaxine [261]. Interestingly, among women taking tamoxifen who were 66 years and older, women aged between 76 and 80 were more likely to take antidepressants, with the exception of fluoxetine which was most common among women between the ages of 71 and 75 [261]. A waiting room sample of breast cancer patients revealed that 34% of the population was prescribed antidepressants [255]. Overall, rates of antidepressant use in breast cancer population ranged from 11% to 34% [11, 239, 255-261]. Among patients diagnosed with melanoma, rates ranged from 10.1% to 24.9% [262], and 15% of NHL patients used tricyclic antidepressants [263].

Conflicting results of antidepressant use and *de novo* cancer risk have been reported [264-270]. A case-control study found no association between antidepressant

use and breast cancer risk; a protective effect of paroxetine hydrochloride was observed in subjects who were not overweight [264]. In contrast to this case-control study, another case-control study found a modest association (multivariable OR=1.2, 0.96-1.51) between antidepressant use and breast cancer risk [265]. This variation may be attributed to the types of breast cancer. SSRIs increased the risk of PR- and ER+/PR tumors, but not other types of breast cancer [266]. The association between antidepressants and breast cancer are mixed in the preclinical and clinical data. Lowry *et al.* [263] found long-term use of tricyclic antidepressants was associated with the risk of CLL/SLL (1.5, 1.1-2.0), but this association did not exist with other subtypes of NHL, while another study conducted by Dalton *et al.* [271] found an increased cumulative incidence among all subtypes of NHL (IRR=1.53, 1.06-2.21). A nested case-control study found a protective effect of SSRIs on the risk of lung cancer, but a non-significant elevated risk with tricyclic antidepressants; the authors attribute the potential effects to residual confounding [267]. A reduced risk of colorectal cancer was seen with antidepressant use [268, 269]. The association between antidepressants and breast cancer and antidepressants and ovarian cancer are mixed in the preclinical and clinical data.

Concerns over a possible interaction with tamoxifen also exist. Since SSRIs inhibit cytochrome P-450 2D6 (CYP2D6), the enzyme that metabolizes tamoxifen, they decrease the amount of tamoxifen metabolites in the blood [272]. Some SSRIs inhibit CYP2D6 more than others. For example, fluoxetine is a strong inhibitor of CYP2D6 and paroxetine's effects are irreversible. Citalopram is a weak inhibitor of CYP2D6 activity [273]. Also possibly altering the activity of CYP2D6 are the polymorphic variants.

CYP2D6 genomic variants can be grouped as poor, intermediate, extensive, or ultra-rapid metabolizers, but previous studies have not demonstrated differences in breast cancer recurrence or mortality within these different genomic classifications [274-276]. The idea that the decrease in tamoxifen activity by antidepressants affects breast cancer recurrence has been widely debated [258, 272, 274, 277-279]. The effect of tamoxifen was reduced in patients taking antidepressants resulting in a significantly higher 2-year breast cancer recurrence rate, 13.9% compared to 7.5% in patients not taking antidepressants with tamoxifen (HR 1.92, 95% CI 1.33-2.76, $p < 0.001$) [277]. In contrast to these findings, many studies suggest no association between antidepressants and breast cancer recurrence, even though this contradicts the biology [258, 272, 278, 279]. Reasons for this inconsistency in previous studies include differences in SSRI inhibition ability, genomic differences, differences in data collection, limited information on tamoxifen compliance, and small sample size [274]. Research on genotype and concomitant antidepressant use is also limited by small sample size [280-282], with sample sizes in some studies ranging from $n=4$ to $n=13$, too small to draw any conclusion [281, 282]. More research must be conducted to determine if this interaction truly exists.

Studies exist determining if an association exists between antidepressant use and cancer risk [264-270, 283] and antidepressant use and recurrence of cancer [258, 272, 277, 278], but to our knowledge, no studies exist determining if antidepressant use is associated with an increase in brain metastasis in humans. Shapovalov *et al.* found an association between pretreatment with fluoxetine, a selective serotonin reuptake inhibitor (SSRI), and an increase in brain metastasis from breast cancer in mice [14]. Since cancer

patients are more likely to have a diagnosis of depression and, consequently, be prescribed antidepressant medication [284-286], it is important to determine the effects of antidepressants on disease progression to the brain.

1.5.1 Types of Antidepressants

Modern antidepressants were identified serendipitously in the 1950's when a treatment for tuberculosis was found to improve the mood of patients. This treatment was iproniazid (Marsilid), a monoamine oxidase inhibitor (MAOI), which eventually proved ineffective in the treatment of tuberculosis but would lead to the development of a new class of medication: antidepressants. MAOIs inhibit MAO in various organs instead of directly affecting reuptake or receptor blockers. Currently, these antidepressants are only used when other lines of treatment have failed since the newer antidepressants tend to be safer and more tolerable. Although side effects exist and overdoses of MAOIs can be lethal, some patients respond better to these drugs than other antidepressants. Tricyclics and tetracyclics, another class of antidepressants, were the most commonly used antidepressant until the 1980's; currently in the United States, 8 tricyclics and 2 tetracyclics are available [287].

In 1981 the first serotonergic was introduced, trazodone, but it was not until 1988 with the release of fluoxetine, a SSRI, that another type of antidepressant, would become the most frequently used medication; this occurred because SSRIs have similar effectiveness to MAOIs coupled with a reduction in side effects. SSRIs include fluoxetine, paroxetine, sertraline, fluvoxamine, citalopram, and escitalopram. Beginning

in 1994, the United States market was introduced to a new class of antidepressant: SNRIs [287]. The SNRIs fill the void in patients where SSRIs are not effective and the patient has medical complications or is too fragile for tricyclic antidepressants [288].

Table 3: Antidepressants by Class [289, 290]

Class of Antidepressant	Generic Forms	Brand Name
Selective Serotonin Reuptake Inhibitor (SSRI)	Citalopram	Celexa
	Escitalopram	Lexapro
	Fluoxetine	Prozac, Prozac Weekly, Sarafem, Selfemra
	Fluvoxamine	Luvox, Luvox CR
	Paroxetine	Paxil, Paxil CR, Pexeva, Brisdelle
	Sertraline	Zoloft
	Vilazodone	Viibryd
Serotonin Norepinephrine Reuptake Inhibitor (SNRI)	Venlafaxine	Effexor, Effexor XR
	Desvenlafaxine	Pristiq
	Milnacipran	Savella
	Duloxetine	Cymbalta
Atypical	Bupropion	Aplenzin, Buproban, Budeprion SR, Budeprion XL, Wellbutrin, Wellbutrin SR, Wellbutrin XL, Zyban
	Trazodone	Oleptro
	Mirtazapine	Remeron, Remeron SolTab
	Nefazodone	Serzone
Tricyclic and tetracyclic	Amitriptyline	Elavil
	Clomipramine	Anafranil
	Doxepin	Deptran, Sinequan, Silenor, Zonalon
	Imipramine	Tofranil, Tofranil-PM
	Trimipramine	Surmontil
	Desipramine	Norpramin
	Nortriptyline	Pamelor, Aventyl
	Protriptyline	Vivactil
	Amoxapine	
	Maprotiline	
Monoamine Oxidase Inhibitor (MAOI)	Isocarboxazid	Marplan
	Phenelzine	Nardil
	Tranylcypromine	Parnate
	Selegiline	Emsam, Eldepryl, Zelapar

1.5.2 SSRI Dosing

The most recent dosing and indication information for initial treatment in adults is depicted in Table 4. Indications for antidepressants differ by drug, not just drug category (e.g. SSRI). Indications for Prozac include major depressive disorder (MDD), bulimia nervosa, panic disorder, and obsessive compulsive disorder (OCD), while Luvox is only indicated for OCD. In addition to the indications in Table 4, Prozac may be used in combination with olanzapine to treat depressive episodes associated with bipolar disorder and treatment resistant depression [291].

The optimal dosage for these drugs differs by type of SSRI and indication. Using Prozac as an example, 20 mg/day is the optimal dosage level for adults, taken in the morning. If this dosage is not effective, it can be increased to 80 mg/day. Clinical trials discerned that, in comparison to placebo, 20 mg/day was effective in treating MDD. This trial also compared dosage levels of 20 to 40 and 60 mg/day and found that 20 mg/day was sufficient to obtain a response in MDD. Dosage increases should only be considered after insufficient improvement in MDD after several weeks and may be increased to 80 mg/day. It is important to note that the reasoning for the several week requirement before increasing the dosage is in part from the delay of the full effectiveness of the drug by at least 4 weeks. Additionally, the long half-life of these medications contributes to the several week requirement. The different drugs within the SSRI class have different half-lives, ranging from 21 hours to 3 days for initial treatment and up to 6 days after chronic use. Metabolites of these drugs also have long half-lives; for example, norfluoxetine, the

active metabolite for Prozac, has a half-life ranging from 4 days for initial use to 16 days after chronic use [291].

It is important to note that Brisdelle (paroxetine) was approved by the FDA to treat hot flashes in menopausal women on June 28, 2013. This medication uses a dose of 7.5mg of paroxetine daily, taken at bedtime, which is much lower than the daily dose of Paxil. Brisdelle is the only non-hormonal treatment for hot flashes currently approved by the FDA [291].

Table 4: Initial Treatment Dosing for Adults by Indication [291]

SSRI	Indication						
	Depression	Bulimia	Panic Disorder	PTSD	OCD	SAD/GAD	PMDD
Prozac	20mg/day; 80mg/day max	60 mg/day	10mg/day; after first week 20mg/day		20mg/day; 80mg/day max		
Zoloft	50mg/day		25mg/day; after first week 50mg/day	25mg/day; after first week 50mg/day	50mg/day	25mg/day; after first week 50mg/day	50mg/day (150 mg/day max)
Celexa	20mg/day with an increase to 40mg/day						
Lexapro	10mg/day					10mg/day	
Paxil	20mg/day; increases 10mg/week if needed; max 50 mg/day		10 to 60mg/day; increases 10mg/week if needed	20mg/day	40mg/day (20mg/day initial dose, increases in 10mg/day increments; max 60mg/day	20mg/day	
Luvox					50mg/day (300mg/day max)		
Viibryd	40mg/day (initial dose 10mg/day for 7 days, increased to 20mg/day for 7days, then 40mg/day)						

PMDD - premenstrual dysphoric disorder; GAD - generalized anxiety disorder; SAD - social anxiety disorder; OCD - obsessive compulsive disorder

1.5.3 Antidepressants Affect the Neuroinflammatory Environment

As previously mentioned, small, hydrophobic molecules cross the BBB through endothelial cell tight junctions [87]. Since antidepressants are hydrophobic and small with an average molecular weight of 291 Da (SD=69), they are able to cross the blood brain barrier [89, 288].

In 1993, Mårtensson and Nässberger first demonstrated the evidence of SSRI's anti-inflammatory ability [292]. SSRIs affect the microglial inflammatory process by inhibiting the murine microglia from producing TNF- α and the free radical nitric oxide. Fluoxetine, paroxetine, and sertraline were all found to have this effect in animals [293-300]. In contrast to these findings, mRNA expression of TNF- α , IL-1 α , IL-1 β , and intercellular adhesion molecule-1 (ICAM-1) was increased *in vivo* using polymerase chain reaction (PCR) analysis and may affect the BBB permeability [14]. However, a double-blind study in human subjects found no association between cytokine levels IL-6, IL-8, IL-10, TNF- α , and IL-1ra and escitalopram use [301].

Reasons for this discrepancy include differences in cellular behavior in animals and in humans. Additionally, the majority of studies finding an anti-inflammatory effect failed to examine the baseline inflammatory state and instead examined inflammation due to lipopolysaccharide (LPS) injection or CNS disease [14, 302, 303]. More research should be conducted to confirm the differences in the *in vitro* and *in vivo* setting.

Another potential reason for this difference in cellular behavior is the effect of dose on inflammatory markers. Tynan *et al.* [302] found SSRIs and SNRIs affected TNF- α and free radical nitric oxide (NO) by concentration. Although this study used

LPS to induce inflammation, it's important to note that the authors were comparing concentrations of antidepressants, not just whether or not inflammation exists.

LPS-induced TNF- α and free radical NO were evaluated for their inflammatory properties at 4 hours and 24 hours, although NO was not detectable at 4 hours so it is excluded from Table 5. At 4 hours, fluoxetine, paroxetine, and fluvoxamine all decreased TNF- α production at 5 μ M. Sertraline behaved similarly at 2.5 μ M, while citalopram and venlafaxine exhibited these properties at higher concentrations, 10 μ M and 75 μ M, respectively. At 24 hours, fluoxetine, sertraline, and paroxetine reduced NO production, although no effect was displayed by fluvoxamine, citalopram, and venlafaxine (Table 6). The effects on LPS-induced TNF- α and NO vary depending upon the specific antidepressant [302].

Table 5: The Anti-Inflammatory Effects of Antidepressants on LPS-Induced Microglial TNF- α Production at 4h [302]

μ M	4 h TNF- α					
	FLX	SERT	PARO	FLV	CIT	VEN
0.1	ns	ns	ns	ns	ns	ns
1	ns	ns	ns	ns	ns	ns
2.5	ns	—	ns	ns	ns	ns
5	—	—	—	—	ns	ns
10	—	—	—	—	—	ns
15	—	—	—	—	—	ns
20	—	—	—	—	—	—
25	—	—	—	—	—	ns
35	—	—	—	—	—	ns
50	—	—	—	—	—	ns
75	—	—	—	—	—	—
100					—	—

The table indicates where there were significant positive (+), negative (-) or no significant (ns) deviation in the levels of TNF- α production, when compared to levels of LPS + vehicle treated cells (all significant statistics were $p < 0.05$). As measured using PrestoBlue, the grey boxes indicate where the viability of cells were significantly influenced by antidepressant treatment ($p < 0.05$). FLX - fluoxetine; SERT - sertraline; PARO - paroxetine; FLV - fluvoxamine; CIT - citalopram; VEN – venlafaxine. Printed with permission by Elsevier.

More importantly, at 24 hours, some of these medications both increase and decrease LPS-induced TNF- α production depending upon the concentration (Table 6). Fluoxetine, sertraline, paroxetine, fluvoxamine, and venlafaxine all increase and decrease TNF- α production, depending on the concentration. Lower concentrations of these medications increased TNF- α production, but TNF- α production decreased when concentration increased. At 24 hours, citalopram is the only antidepressant that only decreased TNF- α production. This association may explain the difference between the studies mentioned previously [302].

Table 6: The Pro- and Anti-Inflammatory Effects of Antidepressants on LPS-Induced Microglial TNF- α and Nitric Oxide Production at 24h [302]

μ M	24 h TNF- α						24 h Nitric oxide					
	FLX	SERT	PARO	FLV	CIT	VEN	FLX	SERT	PARO	FLV	CIT	VEN
0.1	+	ns	ns	+	ns	ns	ns	ns	ns	ns	ns	ns
1	+	ns	ns	+	ns	+	ns	ns	ns	ns	ns	ns
2.5	+	+	+	+	ns	ns	ns	ns	ns	ns	ns	ns
5	+	ns	+	+	ns	+	-	ns	ns	ns	ns	ns
10	-	-	-	-	-	-	-	-	-	ns	ns	ns
15	-	-	-	-	-	ns	-	-	-	ns	ns	ns
20	-	-	-	-	-	ns	-	-	-	ns	ns	ns
25	-	-	-	-	-	ns	-	-	-	ns	ns	ns
35	-	-	-	-	-	ns	-	-	-	ns	ns	ns
50	-	-	-	-	-	ns	-	-	-	ns	ns	ns
75	-	-	-	-	-	ns	-	-	-	ns	ns	ns
100	-	-	-	-	-	ns	-	-	-	ns	ns	ns

The table indicates where there was significant positive (+), negative (-) or no significant (ns) deviation in the levels of TNF- α and NO production, when compared to levels of LPS + vehicle treated cells (all significant statistics were $p < 0.05$). As measured using PrestoBlue, the grey boxes indicate where the viability of cells were significantly influenced by antidepressant treatment ($p < 0.05$). FLX - fluoxetine; SERT - sertraline; PARO - paroxetine; FLV - fluvoxamine; CIT - citalopram; VEN – venlafaxine. Printed with permission by Elsevier.

To further support that antidepressants affect the inflammatory environment, antidepressants have been known to alter white blood cell (WBC) count. Yurdakul *et al.* [304] found that WBC counts increased from $7.04 \times 10^9/\text{L}$ ($\pm 2.10 \times 10^9/\text{L}$) to $8.49 \times 10^9/\text{L}$ ($\pm 1.70 \times 10^9/\text{L}$) after patients received an antidepressant for two months ($p < 0.05$). Only 12 patients were included in the study and the patients took different antidepressants and different doses of these antidepressants, which may explain the difference between this study and another study that found a significant decrease in WBC counts in patients who used fluoxetine (20mg/day) and escitalopram (20mg/day) [305]. Patients in this second study were randomized to receive either fluoxetine or escitalopram and no other doses were investigated. Of note, a case report suggested bupropion was associated with eosinophilia and a WBC count of $18.5 \times 10^9/\text{L}$ [306]. A concern would be determining if these effects on WBC counts were due to antidepressants or depression. A study conducted in antidepressant-naïve patients found WBC count was not affected by a diagnosis of major depression ($p = 0.18$) [307]. These findings coupled with the inflammatory studies suggest the effects on WBC counts are due to the antidepressants and not the underlying depression.

1.6 Current Research on Antidepressant Use and CNS Metastasis

To the best of our knowledge, one study analyzed the risk of CNS metastasis associated with antidepressant use [14]. In mice injected with the MDA-MB213BR-GFP breast cancer cell line, Shapovalov *et al.* [14] reported a 52% increase in total number of brain metastases in those mice that received fluoxetine in comparison to controls. These

mice were pretreated for 3 weeks with fluoxetine and 3 weeks after the cancer cell line was injected. Lung metastasis did not significantly increase with fluoxetine treatment in comparison to controls ($1.06 \pm 0.22 \times 10^6$ vs $0.93 \pm 0.10 \times 10^6$ tumors per section, respectively; $p=0.31$). Additionally, growth of glioblastomas or growth of previously established breast cancer brain metastases were not observed in mice pretreated with fluoxetine (growth of glioblastomas: fluoxetine = $47.56 \pm 3.24 \times 10^6$, control = $49.80 \pm 5.98 \times 10^6$, $p=0.76$; growth of previously established brain metastases: fluoxetine = $2.15 \pm 0.49 \times 10^6$, control = $2.15 \pm 0.38 \times 10^6$, $p=0.66$). The authors postulated several mechanisms that may explain this association.

The first potential mechanism suggested was the effect of fluoxetine on brain plasticity and synaptogenesis. Several studies have examined the effect of fluoxetine on brain plasticity and have found fluoxetine associated with both the removal of perineuronal nets (PNNs), a component of the extracellular matrix critical to plasticity, and an increase in plasticity [308, 309]. In their brain metastasis model, Shapolvalov *et al.* [14] found no association between PNN and fluoxetine. Additionally, synaptogenesis was not affected by fluoxetine [14]. These findings suggest that brain metastases may be regulated via a different pathway.

The second potential mechanism hypothesized was an inflammatory process. As previously mentioned (Section 1.5.3: Antidepressants Affect the Neuroinflammatory Environment, page 51-55), antidepressants affect the neuroinflammatory environment. The inflammatory cytokines TNF- α and IL-1 β were upregulated in the brains of those mice that received fluoxetine by 4.96-fold and 3.76-fold, respectively ($p<0.05$) [14]. This

observation along with the findings from Tynan *et al.* [302], previously described, suggests fluoxetine may increase neuroinflammation and increase brain metastases. These associations were not observed in all studies [300, 301], although these differences may result from differences in *in vitro* and *in vivo* settings and lack of baseline measurements, as previously mentioned. In addition to affecting the neuroinflammatory environment [300, 302, 303], antidepressants have peripheral inflammatory effects [310]. Discussed in the previous section, antidepressants alter WBC counts [304-306]. In addition to an effect on systemic inflammation, these findings support a neuroinflammatory process that may increase brain metastases. It is postulated that glia contribute to this inflammatory process by producing cytokines including IL-1 β and TNF- α [14]. Glial cells may also affect metastatic growth within the brain [311, 312] and be used by tumor cells to support its progression and invasion [313, 314].

The third potential mechanism suggested was the direct alteration of the BBB and increased permeability, as opposed to increasing the transportation of tumor cells. Absorption of Evans Blue, a dye used as marker for determining BBB permeability, was 54% higher in mice treated with fluoxetine compared to controls ($p < 0.01$) [14]. Additionally, IL-1 β and TNF- α increased BBB permeability [100, 315], and these pro-inflammatory cytokines were upregulated in the brains of those mice that received fluoxetine [14]. This upregulation of pro-inflammatory cytokines suggests an increase in BBB permeability which may in turn increase brain metastasis.

Finally, Shapovalov *et al.* [14] suggest fluoxetine may impact several steps in the metastatic process, previously described. These impacted processes include improved

arrest of tumor cells in the brain vasculature and enhanced extravasation [316].

Potentially compounding this effect of fluoxetine on the metastatic process is inflammation, which may increase the survival of these tumor cells once they arrest in the brain vasculature. These effects of fluoxetine on the metastatic cascade may influence the development of CNS metastasis.

Fluoxetine may increase the development of CNS metastasis through one or more of these mechanisms. These mechanism studies [100, 300-306, 308-310, 315, 316] and the study by Shapovalov *et al.* [14] were conducted either *in vitro* or *in vivo* using mice. Therefore, in order to determine if this effect occurred in humans, we investigated the development of CNS metastasis associated with antidepressants in cancer patients. Additionally, we examined the effect of fluoxetine on WBC count to assess the effects of fluoxetine on systemic inflammation. Through this thesis, we investigated if fluoxetine increases CNS metastasis development and systemic inflammation.

1.7 Purpose and Specific Aims

The primary purpose of this thesis was to estimate the relative odds of CNS metastasis associated with a history of antidepressant use compared to no antidepressant use in breast cancer, non-Hodgkin lymphoma, and melanoma patients. This association was examined by comparing ‘any antidepressant use’ between subjects with and without CNS metastasis including: selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), tricyclic and tetracyclic antidepressants, monoamine oxidase inhibitors (MAOIs), or atypical antidepressants. We were also

interested in determining whether the risk of CNS metastasis was associated with the use of ‘any SSRI use’ (use of any antidepressant in the class of SSRIs, nonexclusively) and ‘exclusive SSRI use’ (use of an SSRI only, no other antidepressant could have been used).

Additionally, we compared WBC counts between breast cancer control patients who received antidepressants and those breast cancer control patients who did not receive antidepressants. We examined the difference in WBC counts between breast cancer controls who took ‘any antidepressants’, and those who took ‘any SSRIs’, and compared them to those breast cancer controls who did not take antidepressants.

Specific Aim 1: Estimate the relative odds of CNS metastasis associated with a history of ‘any antidepressant use’ compared to no antidepressant use. We repeated this analysis to also estimate the relative odds of CNS metastasis associated with ‘any SSRI use’ and ‘exclusive SSRI use’ compared to no antidepressant use.

Hypothesis 1a: ‘Any antidepressant use’, compared to no antidepressant use, is associated with an increased risk of CNS metastasis.

Hypothesis 1b: ‘Any SSRI use’, compared to no antidepressant use, is associated with increased risk of CNS metastasis.

Hypothesis 1c: ‘Exclusive SSRI use’, compared to no antidepressant use, is associated with increased risk of CNS metastasis.

Specific Aim 2(exploratory): Compare WBC counts between cancer patients who received antidepressants and those cancer patients who did not receive antidepressants.

Hypothesis 2a: ‘Any antidepressant use’, compared to no antidepressant use, is associated with a higher WBC count.

Hypothesis 2b: ‘Any SSRI use’, compared to no antidepressant use, is associated with a higher WBC count.

Chapter 2: Methods

2.1 Study Design

The goal of this study is to estimate the relative odds of CNS metastasis in breast cancer, non-Hodgkin lymphoma, and melanoma patients associated with antidepressant use before and/or after a cancer diagnosis. We used a case-control design, with cases and controls drawn from patients seen at the University of Rochester Medical Center (URMC) from 2005 to 2013. Advantages of this study include 1) the use of medical record data, and not patient recall, to categorize patients with regard to antidepressant use, and 2) sampling cases and controls from the same population ensuring the controls represent that source population from which the cases arose [317], thereby reducing the potential for selection bias. Identifying subjects based upon their CNS metastasis status was more feasible than identifying subjects by their medication use because medication use is not as easy to identify in the medical record, particularly if the medication was not prescribed at URMC. We identified cases of CNS metastasis and controls without CNS metastasis that attended URMC with a breast cancer, non-Hodgkin lymphoma, or melanoma diagnosis from 2005 to 2013. We then retrospectively categorized each case and control with regard to their antidepressant use based on information recorded in their medical record.

Potential study subjects were identified from a cancer patient list compiled from URMC. These patients had inpatient or outpatient visits between January 1st, 2005 and August 31st, 2012. Patients after August 31st, 2012 were identified using the same

method, identifying patients with inpatient and outpatient visits at URM. Since many of these ICD-9 codes were incorrect, pathology reports were also used to identify potential subjects. Incidence density sampling was used to identify controls in the following manner (Figure 4) [318]. Each time a breast cancer patient was diagnosed with CNS metastasis he or she was labeled a ‘case’. One control was randomly selected from the risk set of breast cancer patients alive at the time, who was, at that time, not diagnosed with CNS metastasis. We repeated this control selection procedure again to select controls for each CNS metastasis case with melanoma and each CNS metastasis case with lymphoma in the same manner. Using this method of sampling, controls could become cases at a later date.

Figure 4: Incidence Density Sampling

2.2 Cases - Inclusion and Exclusion

Cases were eligible for selection into the study if they were adults (over the age of 18 years) with inpatient admissions or outpatient visits with an ICD-9 code of breast cancer, lymphoma, or melanoma, and a CNS metastasis diagnosis in the medical record from January 1, 2005 to September 30, 2013 at UPMC. A case of CNS metastasis was defined by the ICD-9 code 198.3 secondary malignant neoplasm of brain and spinal cord. All subtypes of breast cancer, non-Hodgkin lymphoma, and melanoma were included and defined by their respective ICD-9 codes (174.0 to 174.9, 200.0-204.1, and 172.0-172.9, respectively). Males and females were included for cases with a diagnosis of non-Hodgkin lymphoma or melanoma, but only females were included for cases with a diagnosis of breast cancer. Male cases of breast cancer are rare, and male cases of CNS metastasis from breast cancer are even more uncommon. Additional exclusion criteria for the cases included a diagnosis of lung cancer, MS or HIV, a stroke 6 months prior to CNS metastasis, a traumatic brain injury, or any documented drug abuse as these diagnoses have been known to affect the blood-brain barrier and our mechanism.

2.3 Controls - Inclusion and Exclusion

The primary set of controls (Control Group 1) were subjects without any central nervous system (CNS) metastasis. Controls are adults with inpatient admissions or outpatient visits from January 1, 2005 to September 30, 2013 at UPMC diagnosed with non-Hodgkin lymphoma, melanoma, or breast cancer but without a diagnosis of CNS

metastasis. All subtypes of breast cancer, non-Hodgkin lymphoma, and melanoma were included. Males and females were included as controls with a diagnosis of non-Hodgkin lymphoma or melanoma, but only females were included for controls with a diagnosis of breast cancer. As with the cases, only adults (over the age of 18) at the time of the study were included. These controls are representative of the cases without the disease of interest (CNS metastasis), and were selected from the source population that gave rise to the cases, the cancer population seen at UPMC, avoiding selection bias. Exclusion criteria for the controls was the same as that for the cases, with the time period for stroke defined as 6 months prior to the time they were enrolled. The information regarding controls was collected in the same manner as the cases.

Secondary sets of controls

Two secondary sets of controls exist: those cancer patients with metastasis other than CNS metastasis (Control Group 2) and those cancer patients with no metastasis (Control Group 3). Control Group 2 is comprised of patients with Stage IV cancer, similar to our cases. Patients with no metastasis are excluded from this control group. For non-Hodgkin lymphoma, “other metastasis” is defined as other extranodal sites, including bone marrow. The spleen is not counted as an extranodal site for non-Hodgkin lymphoma. Subjects in Control Group 3 have a diagnosis of stage I through III breast cancer, melanoma, or lymphoma. Stage IV cancers are all metastatic in breast cancer and melanoma and, therefore, were excluded from this control group for those two cancers. Non-Hodgkin lymphoma patients could have stage IV disease as long as their disease was not extranodal. These two control groups determined if a different response to anti-

depressants existed between those patients with and without metastasis. If an association CNS metastasis and antidepressant use existed while using the comparison group with metastasis (Control Group 2), the blood-brain barrier mechanism would have additional support.

2.4 Gender, Age, Race of Subjects

Subjects with a diagnosis of non-Hodgkin lymphoma or melanoma are both male and female, with slightly higher percentage of males due to the higher number of male incidence cases of both of these cancers [16]. Through the James P. Wilmot Cancer Institute Registry, we were able to identify that 54% of lymphoma patients and 58% of melanoma patients are male for patients diagnosed at Strong Memorial Hospital in 2011 [319]. All our subjects are 18 years and older at the time of study inclusion.

No subjects were excluded on the basis of race or ethnicity. Based upon patients diagnosed in 2011 at Strong Memorial Hospital, 93% of melanoma patients are white, <1% are black, and 6% are unknown. Non-Hodgkin lymphoma patients were 89%, 6%, and 5% white, black, and unknown, respectively. Similarly, breast cancer patients were 86%, 8% and 6%, white, black, and unknown, respectively. Since we drew our sample from UPMC from 2005 to 2013, we expected our overall racial distribution to be 89% white, 6% black, and 5% unknown[319]. Our actual overall racial distribution is 92% white, 4% black, 1.3% Asian, 2% other, and 0.4% missing. Within the breast cancer subset, 86% were white, 7% were black, 2% were Asian, 3% were other, and 2% were

missing. Of the melanoma subset, 99% were white, 0.3% were other race, and 0.6% were missing. The non-Hodgkin lymphoma subset was 93% white and 7% other race.

2.5 Exposure Definition Assessment

The antidepressant class, ‘any antidepressant use’, is the primary exposure for our Aim 1 and Aim 2 analyses. Each class of antidepressants used (SSRI, SNRI, atypical, tricyclics and tetracyclics, and MAOIs) was coded by ‘any use’. ‘Any antidepressant use’, irrespective of class, was used for the Aim 1a. If a subject used one or more than one type of antidepressant, he or she was grouped as ‘yes’ for taking an antidepressant. Otherwise, the antidepressant use was coded as ‘no’. ‘Any SSRI use’ (Aim 1b) was defined as use of citalopram, escitalopram, fluoxetine, fluvoxamine, paroxetine, sertraline, and/or vilazodone or any of their generic forms (Table 3) [289-291]. ‘Exclusive SSRI use’ was defined as those subjects who have only taken SSRIs and no other class, as recorded in their medical record (Aim 1c). All antidepressant use categories were defined as ‘yes’ or ‘no’ if they were documented any time one year to one month before CNS metastasis was diagnosed (Figure 5). Of note, this one year period could be before or after the patient’s initial cancer diagnosis.

Figure 5: Timeline for Exposure Period of Interest

Antidepressant use information was ascertained from subjects' medical records. After the electronic medical record was established on March 7th, 2011, antidepressant use was systematically collected. In order to examine if documentation of antidepressant use differed before and after the creation of the electronic medical record, we conducted a pilot study. Responses to a telephone survey were compared the subjects' respective medical record. Approximately 94% of the subjects' responses were concordant with their medical records (results shown in Appendix); therefore, we proceeded with our planned medical record review for this thesis.

2.6 Specific Aim 2

For Aim 2, only breast cancer controls were included in the analyses described below, because different cancers and CNS metastasis affect WBC counts differently and the initial study by Shapovalov *et al.* [14] was conducted using a breast cancer cell line. We attempted to collect WBC count data on all 400 breast cancer controls. The main

analysis was conducted on Control Group 1 (cancer patients with and without metastasis). Secondary analyses were conducted on those breast cancer controls who have metastasis (Control Group 2) and those breast cancer controls with no sites of metastasis (Control Group 3). As previously mentioned (Section 2.5: Exposure Definition Assessment; Page 65), ‘any antidepressant use’ compared to no antidepressant use was the primary exposure (Aim 2a). ‘Any SSRI use’ compared to no antidepressant use, was used for the Aim 2b. ‘Exclusive SSRI use’ was excluded due to small sample size. The outcome, WBC count, is a continuous measure collected from tests of complete blood counts in the subjects’ medical records. WBC count was measured using the blood sample collected at the visit closest to the subject’s study inclusion.

If a patient received any cancer treatment one month prior to this collection, that value of white blood cell count was excluded. Surgery, radiation, and chemotherapy have been shown to affect WBC count [291, 320-323], and WBC count takes approximately one month to recover from most treatments [324]. Immunotherapies only affected WBC counts in less than 5% of all patients who received the medication [291]; consequently, patients who received therapies such as tamoxifen, trastuzumab, and anastrozole were included. Additionally, if a subject had a documented infection or second cancer, they were excluded, since WBC count can be affected by these conditions.

2.7 Data Ascertainment from the Electronic Medical Records and Storage

ICD-9 codes were used to identify patients diagnosed with breast cancer (ICD=174.0-174.9), melanoma (ICD=172.0-172.9), and non-Hodgkin lymphoma

(ICD=202.80), and then to determine if they were diagnosed with CNS metastasis (ICD=198.3; a case). These codes were then confirmed in the subject's medical record. A breast cancer, melanoma, or non-Hodgkin lymphoma patient without CNS metastasis, but who was seen at URMH on this same calendar date was included into the dataset and identified as a control (i.e. risk set sampling). This control selection process was conducted for cancer patients with other metastasis (Control Group 2) and with no metastasis (Control Group 3), and then combined into Control Group 1.

Antidepressant use, age, race, and other demographic information were ascertained from the electronic medical record after case and control lists were produced, with the exception of other metastasis which were included in the ICD-9 codes. Antidepressant use was also recorded in the clinician notes, as was type of breast cancer (for the subset analysis), stage, and treatment.

2.8 Statistical Analysis

All analyses for Aim 1 determined the relative odds of CNS metastasis development associated with antidepressant use. The differences between Aim 1a, 1b, and 1c hypotheses are in the definition of our exposure ('any antidepressant use', 'any SSRI use', 'exclusive SSRI use'), which necessitated several models evaluating confounding due to different exposures, control groups, and cancers (all cancers, breast cancer alone, melanoma alone). The primary analysis was conducted using cases and Control Group 1 (all controls regardless of metastasis) for Aim 1. Secondary analyses were conducted using cases and the secondary control groups, those cancer patients who

have other sites of metastasis (Control Group 2) and those cancer patients who have no other sites of metastasis (Control Group 3). Aim 2 was conducted using only the breast cancer controls from each control group (Control Group 1, Control Group 2, and Control Group 3). All statistical analyses were performed using Statistical Analysis Software version 9.3 (SAS Institute, Cary, NC).

2.8.1 Specific Aim 1

Estimate the relative odds of CNS metastasis associated with ‘any antidepressant use’ compared to no antidepressant use. We repeated this analysis to also estimate the relative odds of CNS metastasis associated with ‘any SSRI use’ compared to no antidepressant use and ‘exclusive SSRI use’ compared to no antidepressant use.

In order to build our statistical models, the following variables were considered: age, gender, race, stage, radiation, chemotherapy, immunotherapy, surgery, and type of metastasis, based on previous studies. All potential covariates were recorded at the time of diagnosis, with the exception of treatment, which was documented at any point in the patient’s documented cancer history. Treatment for each patient, chemotherapy, radiation, immunotherapy, or surgery, referred solely to the subject’s treatment between their initial diagnosis and brain metastasis development. All types of treatments were included, but if a subject received more than one chemotherapy, they were only coded as ‘yes’ for having received chemotherapy. Stage was categorized according to the TNM staging criteria for breast cancer and melanoma. If subjects were coded as stage I before

2010, we grouped all stage IA and IB cancers as stage I. Non-Hodgkin lymphoma staging uses the Ann Arbor or Rai staging method and was Stage 0 through IV. Age was continuous, while stage was categorical (0 and I, II, III, IV). The remaining variables were dichotomous, including gender (male, female), race (white, nonwhite), chemotherapy (yes, no), radiation (yes, no), immunotherapy (yes, no), surgery (yes, no), and types of metastases (yes, no). For those continuous and categorical variables, the best functional form was evaluated and is examined in the following paragraphs.

Since age is continuous, it needs to be linear with respect to the logit. In order to determine if the residuals were normally distributed, the residuals were plotted against the logit. Additionally, we needed to determine if the continuous version of the variable was the optimal version. If this was not the case, age was either categorized or dichotomized. The necessity to categorize the variable was determined by ranking age into quintiles and evaluating if they differ. In Control Group 1, age was left as quintiles (Quantile 1: OR=1.0, ref; Quantile 2: OR=0.58, 95% CI=0.35, 0.96; Quantile 3: OR=0.64, 95% CI=0.39, 1.06; Quantile 4: OR=0.75, 95% CI=0.47, 1.21; Quantile 5: OR=0.57, 95% CI=0.35, 0.95). In Control Group 2, cancer patients with other metastasis, age did not differ by quintile (Quantile 1: OR=1.0, ref; Quantile 2: OR=0.77, 95% CI=0.45, 1.33; Quantile 3: OR=0.80, 95% CI=0.46, 1.39; Quantile 4: OR=0.88, 95% CI=0.51, 1.53; Quantile 5: OR=0.81, 95% CI=0.47, 1.48). When the four highest quintiles were grouped, still no difference existed between the age groups (OR=0.82, 95% CI=0.53, 1.25) and, therefore, age remained linear. In Control Group 3, cancer patients with no other sites of metastasis, age did differ (Quantile 1: OR=1.0, ref;

Quantile 2: OR = 0.57, 95% CI = 0.33, 0.98; Quantile 3: OR = 0.52, 95% CI = 0.31, 0.90; Quantile 4: OR = 0.58, 95% CI = 0.33, 1.02; Quantile 5: OR = 0.43, 95% CI = 0.25, 0.75). Since the highest four of the quintiles appeared to be similar they were grouped and this dichotomization of age was used in the analyses.

The ideal form of stage was also evaluated. In Control Group 1, stage remained as four separate levels (0 and I, II, III, IV). In Control Group 2 and 3, the effect estimates for stage were similar for stage II, III, and IV (Control Group 2 – stage 0 and 1: OR=1.0, ref; stage II: OR=1.65, 95% CI=0.86, 3.17; stage III: OR=1.75, 95% CI=0.92, 3.35, stage IV: OR=1.25, 95% CI=0.64, 2.43; Control Group 3- stage 0 and 1: OR=1.0, ref; stage II: OR = 1.84, 95% CI = 1.00, 3.40; stage III: OR = 1.84, 95% CI = 1.01, 3.37; stage IV: OR = 94.03, 95% CI = 20.71, 426.92). Therefore, stage was grouped as 0 or I, II, III, or IV for Control Groups 2 and 3.

For this aim, bivariate analyses determined the relationship between each covariate in Table 7 and CNS metastasis. First, in Control Group 1, in the models with ‘any antidepressant use’ (hypothesis 1a) and ‘any SSRI use’ (hypothesis 1b), none of the variables met the criteria for a confounder. In the model with ‘exclusive SSRI use’ (hypothesis 1c), chemotherapy was a confounder. Stage, age, chemotherapy (except hypothesis 1c), radiation, immunotherapy, liver metastasis, bone metastasis, bone marrow metastasis, lung and pleura metastasis, and other sites of metastasis appeared to be significant predictors of CNS metastasis for these hypotheses. The final model for these hypotheses included stage (0 and I, II, III, IV), age at initial diagnosis (15-42 years, 43-50 years, 51-58 years, 59-67 years, and 68-95 years), chemotherapy (yes, no), radiation (yes,

no), bone marrow metastasis (yes, no), lung and pleura metastasis (yes, no), and other sites of metastasis (yes, no).

Second, using Control Group 2, in all antidepressants (hypotheses 1a), any SSRIs (hypothesis 1b), and exclusive SSRIs (hypothesis 1c), no confounders exist; as a result, chemotherapy, stage, surgery, and bone metastasis appeared to be significant predictors of CNS metastasis. The final model for these hypotheses included stage (0 and I, II, III, IV), chemotherapy (yes, no), surgery (yes, no), and bone metastasis (yes, no).

Last, using Control Group 3, in the model with ‘all antidepressant use’ (hypotheses 1a) and ‘any SSRI use’ (hypothesis 1b), no confounders existed; as a result, chemotherapy, stage, age at initial diagnosis, radiation and immunotherapy appeared to be significant predictors of CNS metastasis. In ‘exclusive SSRI use’ (hypothesis 1c), chemotherapy was a confounder. Stage, age, radiation, and immunotherapy appeared to be significant predictors of CNS metastasis. The final model for these hypotheses included stage (0 and I, versus II, III and IV), age (19-42 years versus 43-95 years), chemotherapy (yes, no), radiation (yes, no), and immunotherapy (yes, no).

Once covariates were identified, we built a final logistic regression model using a manual backward selection method. All covariates were entered into a logistic regression without antidepressant use in the model. Variables that no longer remained statistically significant were then removed individually. The final model was determined once all covariates remained statistically significant. Antidepressant use was then added into the model. This same model building process was used for each of the first three parts of

Aim 1 (i.e. Aim 1a – ‘any antidepressant use’; Aim 1b – ‘any SSRI use’; Aim 1c – ‘exclusive SSRI use’).

Variables were then evaluated for effect modification. Of the variables evaluated as effect modifiers of any antidepressant use/CNS metastasis association included: age, stage, chemotherapy, radiation, immunotherapy, surgery, and sites of metastasis. To evaluate effect modification, we added interaction terms into the models between the exposure of interest (i.e. ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’) and the potential effect modifier (e.g. age, stage, chemotherapy, radiation, immunotherapy, surgery, and sites of metastasis). No variables modified the relationship between antidepressant use and CNS metastasis in all cancers.

One concern with part a) of Aim 1 is reverse causation. Depression may be a potential symptom of CNS metastasis. Since depression is associated with antidepressant use, central nervous system metastasis could lead to depression which would increase antidepressant use. General cognitive dysfunction, which may include depression, is a commonly cited symptom of central nervous system metastasis [139, 145, 153, 156-158]. We planned on conducting a sensitivity analysis using antidepressant indication information and collected indication information whenever possible and planned to compare those with indications for depression to hot flashes, sleep and pain indications. Unfortunately, depression diagnoses were not systematically reported in the patients charts; in the 945 charts, only one mentioned a test for depression, and therefore was excluded from all multivariable analyses. Shown in Table 8 is the final list of variables and how they were used in the statistical analyses including the outcome, exposures (i.e.

‘any antidepressant use’, ‘SSRI use’, ‘exclusive SSRI use’), and those considered as confounders (correlated with antidepressant use and predictor of CNS metastasis), or just predictors of CNS metastasis.

Table 7: Variables to be included in the Statistical Analysis

Variable	Variable Type	References	Control Group 1	Control Group 2	Control Group 3
CNS metastasis	Dichotomous (y/n)	N/A	Outcome	Outcome	Outcome
Antidepressant use	Dichotomous (any or only/none)	N/A	Exposure of interest	Exposure of interest	Exposure of interest
Age	Continuous	[20, 153, 177, 185, 187, 188, 193, 195, 219, 325, 326]	Significant predictor of CNS metastasis		Significant predictor of CNS metastasis
Stage	Ordinal	[20, 177, 178, 180, 185, 187, 188, 221, 326, 327]	Significant predictor of CNS metastasis	Significant predictor of CNS metastasis	Significant predictor of CNS metastasis
Chemotherapy	Dichotomous (y/n)	N/A	Significant predictor of CNS metastasis	Significant predictor of CNS metastasis	Confounder
Radiation	Dichotomous (y/n)	N/A	Significant predictor of CNS metastasis		Significant predictor of CNS metastasis
Surgery	Dichotomous (y/n)	N/A		Significant predictor of CNS metastasis	
Immunotherapy	Dichotomous (y/n)	N/A			Significant predictor of CNS metastasis
Bone Metastasis	Dichotomous (y/n)	N/A		Significant predictor of CNS metastasis	
Bone Marrow Metastasis	Dichotomous (y/n)	N/A	Significant predictor of CNS metastasis		
Lung and Pleura Metastasis	Dichotomous (y/n)	N/A	Significant predictor of CNS metastasis		
Other sites of Metastasis	Dichotomous (y/n)	N/A	Significant predictor of CNS metastasis		

2.8.2 *Subset Analyses*

Subset analyses for Aim 1 were planned *a priori*, creating separate analyses, conducted in the same manner described above, for breast cancer and melanoma. Subset analyses could not be conducted on the non-Hodgkin lymphoma subset due to the small sample size. Additional variables were added and altered in this analysis. For the subset analysis in breast cancer, two categorical variables were added: type of breast cancer (ductal, lobule, and other) and molecular subtype (HER2, triple negative, luminal A, and luminal B). In the melanoma subset analysis a categorical location variable was added (head and neck, trunk, extremity, other, and unknown). All confounders and predictors of CNS metastasis in these subset analyses are presented in Table 8.

Bivariate analyses among only breast cancer cases and controls determined which variables were significant predictors of the relative odds CNS metastasis and antidepressant use (i.e. ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’) in this subset of study subjects (defined as $p < 0.20$) (Table 8). First, in the breast cancer subset using Control Group 1, chemotherapy and molecular subtype were confounders for all hypotheses. Age, radiation, radiation, bone metastasis, bone metastasis, and lung and pleura metastasis were significant predictors of CNS metastasis. The final model for these hypotheses included age at initial diagnosis (15-42 years, 43-50 years, 51-58 years, 59-67 years, and 68-95 years), chemotherapy (yes, no), radiation (yes, no), bone metastasis (yes, no), bone marrow metastasis (yes, no), lung and pleura metastasis (yes, no), and molecular subtype (luminal A, luminal B, triple negative, and HER2).

Second, bivariate analyses using Control Group 2 in breast cancer patients only, determined if the same sets of covariates are included in the models for these multivariable analyses. In the breast cancer subset, chemotherapy was a confounder for all hypotheses, while molecular subtype was a confounder for ‘any SSRI use’ and ‘exclusive SSRI use’ (hypothesis 1b and 1c, respectively). Race and bone metastasis (and molecular subtype for ‘any antidepressant use’ [hypothesis 1a]) were significant predictors of CNS metastasis. The final model for these hypotheses included race (white, nonwhite), chemotherapy (yes, no), bone metastasis (yes, no), and molecular subtype (luminal A, luminal B, triple negative, and HER2).

Last, using Control Group 3 in the breast cancer subset, molecular subtype was a confounder for all hypotheses, while radiation was a confounder in the model using ‘any SSRI use’ (hypothesis 1b). Age and chemotherapy (and radiation for ‘any antidepressant use’ [hypotheses 1a] and ‘exclusive SSRI use’ [hypothesis 1c]) were significant predictors of CNS metastasis. The final model for these hypotheses included age (19-42 years versus 43-95 years), chemotherapy (yes, no), radiation (yes, no), and molecular subtype (luminal A, luminal B, triple negative, and HER2).

Similar bivariate analyses in the melanoma subset determined which variables were significant predictors of CNS metastasis and then antidepressant use (i.e. ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’) (defined as $p < 0.20$) (Table 8). First, in Control Group 1, no variables were associated with the exposure of interest for any of the hypotheses (defined as $p < 0.20$), and, therefore, no confounders existed in Control Group 1. The following variables were significant predictors of CNS

metastasis and included in the model for Control Group 1: chemotherapy (yes, no) and other sites of metastasis (yes, no). Second, in Control Group 2, the final model included chemotherapy (yes, no) as a confounder and skin metastasis (yes, no) as a significant predictor of CNS metastasis. Last, in Control Group 3, the final model for all hypotheses consisted of chemotherapy (yes, no) and location of melanoma (head and neck, trunk, extremity, other, unknown) confounders and stage (0 and I versus II, III and IV), radiation (yes, no), and surgery (yes, no) as significant predictors of CNS metastasis. Additionally, stage (0 and I versus II, III and IV) was a confounder for ‘any SSRI use’ (hypotheses 1b) and ‘exclusive SSRI use’ (hypothesis 1c).

Statistical analyses were the same as the analyses for all cancers. We performed logistic regression using a manual backward selection method. As previously described above (Section 2.8.1: Specific Aim 1, page 76), all variables were evaluated for effect modification through the addition of an interaction term in the model between the exposure of interest (i.e. ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’) and the potential effect modifier (e.g. age, stage, chemotherapy, radiation, immunotherapy, surgery, and sites of metastasis) and evaluation of odds ratios for each stratum. No variables modified the relationship between antidepressant use and CNS metastasis in the breast cancer subset. In the melanoma subset, chemotherapy modified the relationship between ‘any SSRI use’ and CNS metastasis. The results of this interaction are further described in the next section (Section A. Multivariable Analyses, Page 110).

Table 8: Variables Included in the Subset Analyses of Specific Aim 1a

Variable	Variable Type	Group 1	Group 2	Group 3
Breast Cancer Subset Analysis				
Age	Categorical (15-42, 43-50, 51-58, 59-67, 68-95 years)	Significant predictor of CNS metastasis		Significant predictor of CNS metastasis
Race	Dichotomous (white, nonwhite)		Significant predictor of CNS metastasis	
Molecular subtype	Categorical (HER2, triple negative, luminal A, luminal B)	Confounder	Significant predictor of CNS metastasis	Confounder
Chemotherapy	Dichotomous (yes, no)	Confounder	Confounder	Significant predictor of CNS metastasis
Radiation	Dichotomous (yes, no)	Significant predictor of CNS metastasis		Significant predictor of CNS metastasis
Bone metastasis	Dichotomous (yes, no)		Significant predictor of CNS metastasis	
Bone marrow metastasis	Dichotomous (yes, no)	Significant predictor of CNS metastasis		
Lung and pleura metastasis	Dichotomous (yes, no)	Significant predictor of CNS metastasis		
Melanoma Subset Analysis				
Stage	Dichotomous (0 and I versus II, III and IV)			Significant predictor of CNS metastasis
Location of Melanoma	Categorical (extremity, head and neck, trunk, other, and unknown)			Confounder
Chemotherapy	Dichotomous (yes, no)	Significant predictor of CNS metastasis	Confounder	Confounder
Radiation	Dichotomous (yes, no)			Significant predictor of CNS metastasis

Surgery	Dichotomous (yes, no)	Significant predictor of CNS metastasis
Skin metastasis	Dichotomous (yes, no)	Significant predictor of CNS metastasis
Other site of metastasis	Dichotomous (yes, no)	Significant predictor of CNS metastasis

2.8.3 *Specific Aim 2*

Compare white blood cell (WBC) counts between breast cancer controls who received antidepressants and those breast cancer controls who did not receive antidepressants.

We determined the difference in WBC counts in breast cancer controls who did and did not take antidepressants. For part a) of this aim, ‘any antidepressant use’ was any class of antidepressants. Part b) of this aim investigated ‘any SSRI use’. Bivariate analyses used a t-test to determine the difference in WBC count between those with and without any antidepressant use. The t-test was also used to compare the difference between the means of WBC counts of those breast cancer controls who took antidepressants and those breast cancer controls who did not take antidepressants. Since the distribution of WBC count was slightly skewed to the right for all analyses, a Mann-Whitney U test, also known as the Wilcoxon rank sum test, was used to test the null hypothesis that the WBC counts of those breast cancer controls who take antidepressants are similar to those breast cancer controls who do not take antidepressants.

Bivariate analyses among only breast cancer controls determined which variables were significant predictors of the change in WBC count and antidepressant use (i.e. ‘any antidepressant use’ and ‘any SSRI use’) (defined as $p < 0.20$). In all models for Control Groups 1, 2, and 3, bivariate analyses determined radiation was a significant predictor of the change in WBC count but not a confounder. In the model with ‘any SSRI use’ using Control Group 1, immunotherapy was also a significant predictor of the change in WBC count. Since immunotherapy was only a significant predictor in one model, its inclusion

into the model was examined in a sensitivity analysis (page 128). Additionally, in the model with 'any antidepressant use' using Control Group 3, stage was also a significant predictor of the change in WBC count. Since stage was only a significant predictor in this model, its inclusion into the model was examined in a sensitivity analysis (page 138).

Our one multivariable analysis was conducted to estimate the change in WBC count associated with antidepressant use compared to no antidepressant use, adjusting for radiation. The linear regression model was built using a manner similar to Aim 1 by including radiation as a predictor of WBC count, as no confounders existed. The final linear regression model was built using a backwards manual selection method. The assumptions of a linear regression were tested: estimation of the means of the error terms appears to be zero, equal variance, the variance of the error terms appeared to be the standard deviation squared, and the covariance of the error terms appeared to be equal to zero. Of note, we were unable to include molecular subtype as a covariate in the analysis due to the limited sample size (HER2 n=13, triple negative n=25, luminal B n=58, luminal A n=54).

Chapter 3: Results

The results of our study are presented below. First, we described our study population as a whole and then separately by cancer type. Next, we presented our results for Aim 1, and then for Aim 2. Within each Aim, we separately discussed our results for Control Group 1, Control Group 2, and Control Group 3. Within each control group, we discussed our results as a whole population and by cancer type (breast cancer and melanoma). Antidepressant type (i.e. ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’) are also examined separately within each of these groups.

3.1 Study Population Description

The study population was comprised of breast cancer, melanoma, and non-Hodgkin lymphoma patients. We collected data on 100 breast cancer, 64 melanoma, and 25 lymphoma patients with central nervous system metastasis (N=189 cases). With 4 controls per case, we also collected data for 400 breast cancer, 256 melanoma, and 100 non-Hodgkin lymphoma patients without CNS metastasis (N=756 controls; Control Group 1). Of these controls, 200 breast cancer patients, 128 melanoma patients and 50 non-Hodgkin lymphoma patients had another diagnosed metastasis but not central nervous system metastasis (N=378; Control Group 2) including metastasis to the bone (n=204), lung and pleura (n=179), liver (n=104), skin and subcutaneous tissue (n=35), bone marrow (n=13), and several other less common sites of metastasis (n=90) including metastasis to the spleen, adrenal glands, breast, small intestine, pancreas, and testes. An

additional 200 breast cancer patients, 128 melanoma patients and 50 non-Hodgkin lymphoma patients had no metastasis (N=378; Control Group 3). We further describe the characteristics of the subjects in the different control groups in the following sections. Of note, stage was missing for 11% of the subjects. In sensitivity analyses described below, we examined if our results and inference were different if we excluded these study subjects.

The characteristics of the cases, by cancer type, are shown in Table 9. The cases of CNS metastasis differed with regard to cancer type. For example, the median time from initial cancer diagnosis to CNS involvement was 11.1 months (range 2.3 to 149.7 months) in non-Hodgkin lymphoma cases, compared to 49.8 months (range 6.3 to 328.8 months) in breast cancer cases. The majority of both breast cancer (91.0%) and melanoma (95.2%) cases had brain parenchymal involvement, while only 36.0% of lymphoma cases involved the parenchyma. The majority of cases had other metastasis sites at the time of their CNS diagnosis (lymphoma=84.0%, breast cancer=72.0%, melanoma=69.8) (Table 9).

Symptoms also varied across cancer type for cases. Breast cancer cases presented with more headaches (breast cancer=40.0%, melanoma=28.1%, lymphoma=20.0%), nausea and vomiting (breast cancer=25.0%, melanoma=15.9%, lymphoma=12.0%), dizziness (breast cancer=9.0%, melanoma=4.8%, lymphoma=4.0%), back and shoulder pain (breast cancer=12.0%, melanoma=3.2%, lymphoma=8.0%), and gait and coordination disturbances (breast cancer=15.0%, melanoma=9.5%, lymphoma=12.0%) than melanoma and lymphoma cases. Lymphoma cases developed motor symptoms

(breast cancer=29.0%, melanoma=20.6%, lymphoma=44.0%), vision changes (breast cancer=16.0%, melanoma=1.6%, lymphoma=28.0%), and speech disturbances (breast cancer=8.0%, melanoma=9.4%, lymphoma=16.0%) more frequently than breast cancer and melanoma patients. Almost a quarter (23.4%) of melanoma patients were asymptomatic upon brain metastasis diagnosis (Table 9).

Table 9: Characteristics of Case Subjects, by Cancer Type

	Breast Cancer	Melanoma	Lymphoma	Total
	n=100	n=64	n=25	n=189
Characteristics	n (%)	n (%)	n (%)	n (%)
Median time from diagnosis to CNS involvement (months)	49.8 (6.3-328.8)	30.7 (3.2-207.5)	11.1 (2.3-149.7)	34.5 (2.3-328.8)
Type of CNS involvement				
Brain parenchyma	91 (91.0)	60 (95.2)	9 (36.0)	160 (85.1)
Leptomeningeal involvement	6 (6.0)	2 (3.2)	12 (48.0)	20 (10.6)
Both	3 (3.0)	1 (1.6)	4 (16.0)	8 (4.3)
Symptoms				
Headaches	40(40.0)	18 (28.1)	5 (20.0)	63 (33.3)
Nausea and vomiting	25 (25.0)	10 (15.9)	3 (12.0)	38 (20.2)
Cognitive problems	14 (14.0)	16 (25.4)	5 (20.0)	35 (18.5)
Seizures	6 (6.0)	6 (9.4)	2 (8.0)	14 (7.4)
Motor symptoms	29 (29.0)	13 (20.6)	11 (44.0)	53 (28.2)
Vision changes	16 (16.0)	1 (1.6)	7 (28.0)	24 (12.7)
Speech disturbance	8 (8.0)	6 (9.4)	4 (16.0)	18 (9.5)
Dizziness	9 (9.0)	3 (4.8)	1 (4.0)	13 (6.9)
Back and shoulder pain	12 (12.0)	2 (3.2)	2 (8.0)	16 (8.5)
Gait and coordination disturbance	15 (15.0)	6 (9.5)	3 (12.0)	24 (12.7)
Other	6 (6.0)	1 (1.6)	0 (0)	7 (3.7)
Asymptomatic	6 (6.0)	15 (23.4)	1 (4.0)	22 (11.6)
Other Metastasis before CNS Metastasis	72 (72.0)	44 (69.8)	21 (84.0)	137 (72.9)

The characteristics of the controls, by control group, compared to the cases (all cancers types together) are shown in Table 10. Cases were mostly stage II, III, and IV cancers at diagnosis (stage 0 and I=10.0%, stage II=31.2%, stage III=34.7%, and stage IV=24.1%), while controls were diagnosed with more stage 0 and 1 and fewer stage IV cancers (stage 0 and I=18.6%, stage II=32.6%, stage III=35.4%, and stage IV=13.5%). CNS metastasis cases received more therapy than controls, with a higher percent of patients receiving chemotherapy (Cases=81.0%, Control Group 1=58.3%), surgery (Cases=79.9%, Control Group 1=77.5%), radiation (Cases=58.2%, Control Group 1=45.8%), and immunotherapy (Cases=44.4%, Control Group 1=34.8%) (Table 10).

Table 10: Characteristics of Study Population, by Cases and Control Group

Characteristic	Cases	Controls		
	(CNS Metastasis)	Control Group 1 (Other Metastasis and No Metastasis)	Control Group 2 (Other Metastasis)	Control Group 3 (No Metastasis)
	n=189 n (%)	n=756 n (%)	n=378 n (%)	n=378 n (%)
Cancer Type				
Breast Cancer	100 (52.9)	400 (52.9)	200 (52.9)	200 (52.9)
Melanoma	64 (33.9)	256 (33.9)	128 (33.9)	128 (33.9)
Lymphoma	25 (13.2)	100 (13.2)	50 (13.2)	50 (13.2)
Male	56 (29.6)	194(25.7)	103 (27.3)	91 (24.1)
Race				
White	173 (92.0)	692(91.9)	354 (94.2)	338 (89.7)
Nonwhite	15 (8.0)	59 (8.1)	20 (5.8)	39 (10.3)
Missing	1	3	1	2
Ethnicity				
Non-Hispanic	96 (96.0)	529 (96.7)	224 (98.7)	305 (95.3)

Hispanic	4 (4.0)	18 (3.3)	3 (1.3)	15 (4.7)
Missing	89	209	148	61
Median age at initial diagnosis	53 (25-94)	55 (15-95)	53 (15-89)	56 (19-95)
Stage				
0	1 (0.6)	10 (1.5)	4 (1.3)	6 (1.7)
I	16 (9.4)	115 (17.1)	42 (13.4)	72 (20.1)
II	53 (31.2)	219 (32.6)	87 (27.8)	132 (36.8)
III	59 (34.7)	238 (35.4)	91 (29.1)	147 (40.9)
IV	41 (24.1)	91 (13.5)	89 (28.4)	2 (0.6)
Missing	19	84	65	19
Other sites of metastasis	140 (74.0)	378 (50.0)	378 (100.0)	0 (0)
Bone	66 (47.1)	204 (54.0)	204 (54.0)	---
Lung/Pleura	78 (55.7)	170 (45.0)	170 (45.0)	---
Skin/Subcutaneous	14 (10.0)	35 (9.3)	35 (9.3)	---
Liver	47 (33.6)	104 (27.5)	104 (27.5)	---
Bone marrow	11 (7.9)	13 (3.4)	13 (3.4)	---
Other	40 (28.6)	90 (23.8)	90 (23.8)	---
Treatment				
Chemotherapy	153 (81.0)	441 (58.3)	235 (62.2)	206 (54.5)
Radiation	110 (58.2)	346 (45.8)	211 (55.8)	135 (35.7)
Surgery	151 (79.9)	586 (77.5)	275 (72.8)	311 (82.3)
Immunotherapy	84 (44.4)	263 (34.8)	141 (37.3)	122 (32.3)
Median time to study inclusion (months)	35 (2-328)	27 (1-437)	55 (2-437)	15 (1-377)

Cases had more lung and pleura (Cases=55.7%, Control Group 2=45.0%), skin and subcutaneous (Cases=10.0%, Control Group 2=9.3%), liver (Cases= 33.6%, Control Group 2=27.5%), bone marrow (Cases=7.9%, Control Group 2=3.4%) and several other less common sites of metastasis (Cases=28.6%, Control Group 2=23.8%) than controls, while controls had more bone metastasis (Cases=47.1%, Control Group 2=54%). The number of metastases the cases developed was different than the number of metastases Control Group 2 developed (Figure 6). Control Group 2 developed single sites of metastasis more frequently than the cases (Cases=32.1%, Control Group 2 =46.0%),

while the cases had more multiple sites of metastasis (Cases=67.9%, Control Group 2 =54.0%) (Figure 6). Of those cancer patients in Control Group 3 (no metastasis development at the time of study inclusion), 24.3% developed metastasis after study inclusion (Table 10).

Figure 6: Number of Sites of Metastasis per Subject, by Cases and Control Group 2

Antidepressant use, by control group, compared to the cases (all cancers types together) is shown in Table 11. SSRI use was the most commonly used antidepressant among cases and controls (Cases=16.9%, Control Group 1=17.3%). More cases used antidepressants (Cases=28.6%, Control Group 1=27.5%) but fewer SSRIs (Cases=16.9%, Control Group 1= 17.3%) than controls. In comparison to Control Group 2, a higher percentage of cases used antidepressants (Cases=28.6%, Control Group 2=25.7%). In

comparison to Control Group 3, cases had a lower percentage of SSRI use (Cases=16.9%. Control Group 3= 19.1%) (Table 11).

Table 11: Antidepressant Use for Cases and Controls, by Control Group

Characteristic	Cases		Controls					
	(CNS Metastasis)		Control Group 1 (Other Metastasis and No Metastasis)		Control Group 2 (Other Metastasis)		Control Group 3 (No Metastasis)	
	n=189		n=756		n=378		n=378	
	n	%	n	%	n	%	n	%
Any Antidepressant	54	28.6	208	27.5	97	25.7	111	29.4
SSRI	32	16.9	131	17.3	59	15.6	72	19.0
Exclusive SSRI	26	13.8	85	11.2	41	10.8	44	11.6
SNRI	15	7.9	53	7.0	28	7.4	25	6.6
Tricyclic	7	3.7	27	3.6	17	4.5	10	2.6
MAOI	2	1.1	0	0.0	0	0.0	0	0.0
Atypical	6	3.2	64	8.5	26	6.9	38	10.1

3.1.1 Breast Cancer Study Characteristics

The characteristics of the controls, by control group, compared to the cases for breast cancer subjects are shown in Table 12. Breast cancer type included ductal carcinoma, lobular carcinoma and several other less common types including medullary, mixed, inflammatory, mucinous, tubular, and spindle cell cancer. The majority of breast cancer cases and controls were ductal carcinomas (Cases=88.6%, Control Group 1=88.1%). Breast cancer cases had a higher percent of high grade cancer at diagnosis when compared to all breast cancer controls (Cases=62%, Control Group 1=28%),

although grade was missing for 26% of the subjects. The median age at diagnosis was younger in breast cancer patients than in controls (Cases=44.5 years [range 27-83], Control Group 1= 53 years [range22-93]). Cases had more lung and pleura metastasis than controls (Cases=52.7%, Control Group 1=39.5%). Cases were more likely to be triple negative (Cases=29.8%, Control Group 1=16.8%) or HER2 (Cases=21.4%, Control Group 1=7.7%), while controls were more likely to have a luminal A molecular subtype (Cases=11.9%, Control Group 1=37.8%). Grade, ER status, PR status, and HER2 status comprise molecular subtype; consequently, all analyses only included molecular subtype. Cases were more heavily treated (chemotherapy: Cases=96.0%, Control Group 1=77.0%) (Table 12).

Table 12: Characteristics of Breast Cancer Cases and Controls by Control Group

Characteristic	Cases	Controls		
	(CNS Metastasis)	Control Group 1 (Other Metastasis and No Metastasis)	Control Group 2 (Other Metastasis)	Control Group 3 (No Metastasis)
	n=100 n (%)	n=400 n (%)	n=200 n (%)	n=200 n (%)
Cancer Type				
Ductal	78 (88.6)	318 (88.1)	153 (90.0)	165 (86.4)
Lobular	7 (8.0)	35 (9.7)	15 (8.8)	20 (10.5)
Other	3 (3.4)	8 (2.2)	2 (1.2)	6 (3.1)
Missing	12	39	30	9
Race				
White	85 (86.7)	347 (88.1)	182 (91.9)	165 (84.2)
Nonwhite	13 (13.3)	47 (11.9)	16 (8.1)	31 (15.8)
Missing	2	6	2	4
Ethnicity				

Non-Hispanic	58 (93.5)	268 (94.0)	130 (97.7)	168 (94.9)
Hispanic	4 (6.5)	12 (6.0)	3 (2.3)	9 (5.1)
Missing	38	90	67	23
Grade				
1 or 2	27 (38.6)	175 (43.8)	64 (57.7)	111 (63.8)
3	43 (62.3)	110 (27.5)	47 (43.1)	63 (36.2)
Missing	30	115	89	26
Median age at initial diagnosis	44.5 (27-83)	53 (22-93)	49 (22-87)	55 (24-93)
Median age at CNS metastasis or study inclusion	54 (29-84)	60 (25-95)	59.5 (27-92)	60 (25-95)
Stage				
0	1 (1.1)	5 (1.4)	2 (1.2)	3 (1.6)
I	12 (13.2)	78 (20.8)	28 (16.2)	50 (25.9)
II	36 (39.6)	154 (42.1)	62 (35.8)	92 (47.7)
III	26 (28.6)	99 (27.0)	51 (29.5)	48 (25.0)
IV	16 (17.6)	30 (8.2)	30 (17.3)	0 (0)
Missing	9	34	27	7
Other sites of metastasis	74 (74.0)	200 (50.0)	200 (100.0)	0 (0)
Bone	50 (67.6)	145 (72.5)	145 (72.5)	---
Lung/Pleura	39 (52.7)	79 (39.5)	79 (39.5)	---
Skin	7 (9.5)	13 (13.0)	13 (13.0)	---
Liver	27 (36.5)	61 (30.5)	61 (30.5)	---
Bone marrow	4 (5.4)	7 (3.5)	7 (3.5)	---
Other	8 (10.8)	26 (13.0)	26 (13.0)	---
ER+	49 (51.0)	286 (74.9)	143 (74.9)	143 (74.9)
Missing	4	31	22	9
PR+	36 (37.5)	231 (64.7)	112 (66.3)	119 (63.3)
Missing	4	43	31	12
HER2+	36 (41.4)	89 (27.5)	46 (30.3)	43 (25.2)
Missing	13	76	47	29
Molecular Subtype				
Triple Negative	25 (29.8)	48 (16.8)	18 (15.3)	30 (17.9)
HER2	18 (21.4)	22 (7.7)	9 (7.6)	13 (7.7)
Luminal A	10 (11.9)	108 (37.8)	41 (34.7)	67 (39.9)
Luminal B	31 (36.9)	108 (37.8)	50 (42.4)	58 (34.5)
Missing	16	114	82	32
Treatment				

Chemotherapy	96 (96.0)	308 (77.0)	157 (78.5)	151 (75.5)
Radiation	77 (77.0)	257 (64.3)	148 (74.0)	109 (54.5)
Surgery	91 (91.0)	354 (88.5)	171 (85.5)	183 (91.5)
Immunotherapy	32 (32.0)	62 (15.5)	35 (17.5)	27 (13.5)

Antidepressant use, by control group, compared to the cases for breast cancer subjects is shown in Table 13. Overall, the breast cancer subset had a higher rate of antidepressant use than the main analysis using all three cancers (Breast Cancer=34.4%, All Cancers=27.7%). Antidepressant use was similar among cases (33.0%) and controls in Control Group 1 (34.8%). SSRI use was the most commonly used antidepressant among cases and controls (Cases: SSRI=21.0%, Other Classes of Antidepressants=12.0%; Control Group 1: SSRI=21.3%, Other Classes of Antidepressants=13.5%) (Table 12).

Table 13: Antidepressant Use among Breast Cancer Patients, by Cases and Controls

Characteristic	Cases		Controls					
	(CNS Metastasis)		Control Group 1 (Other Metastasis and No Metastasis)		Control Group 2 (Other Metastasis)		Control Group 3 (No Metastasis)	
			n=400		n=200		n=200	
	n	%	n	%	n	%	n	%
Any Antidepressant	33	33.0	139	34.8	71	35.5	68	34.0
SSRI	21	21.0	85	21.3	39	19.5	46	23.0
Exclusive SSRI	17	17.0	62	15.5	33	16.5	29	14.5
Other Classes	12	12.0	54	13.5	32	16.0	22	11.0

3.1.2 Melanoma Study Characteristics

The characteristics of the controls, by control group, compared to the cases for melanoma subjects are shown in Table 14. Among the melanoma subset of cases and controls, location of initial melanoma differed between cases and controls. More cases developed their initial melanoma in their head and neck (Cases=29.7%, Control Group 1=23.4%, Control Group 2=27.6%, Control Group 3=19.2%) or trunk (Cases=32.8%, Control Group 1=24.2%, Control Group 2=16.3%, Control Group 3=31.1%) compared to controls, who more frequently had an unknown primary site (Cases=7.8, Control Group 1=12.1%, Control Group 2=15.5%, Control Group 3=8.8%). Cases were diagnosed with more stage II (Cases=36.8%, Control Group 1=22.9%, Control Group 2=20.4%, Control Group 3=24.8%) disease than controls, who were diagnosed with more stage 0 and I disease (Cases=3.6%, Control Group 1=12.1%, Control Group 2=9.7%, Control Group 3=14.1%). A higher percentage of cases had lung and pleura metastasis (Cases=82.2%, Control Group 1 and 2=61.7%), while Control Group 1 had more bone metastasis (Cases=24.4%, Control Group 1 and 2=35.2%). Cases were more heavily treated with chemotherapy than Control Group 1 (Cases=50.0%, Control Group 1=15.6%, Control Group 2=25.0%, Control Group 3=6.3%) (Table 14).

Table 14: Characteristics of the Melanoma Cases and Controls by Control Group

Characteristic	Cases	Controls		
	(CNS Metastasis)	Control Group 1 (Other Metastasis and No Metastasis)	Control Group 2 (Other Metastasis)	Control Group 3 (No Metastasis)
	n=64	n=256	n=128	n=128
	n (%)	n (%)	n (%)	n (%)
Location				
Head and neck	19 (29.7)	58 (23.4)	34 (27.6)	24 (19.2)
Trunk	21 (32.8)	60 (24.2)	20 (16.3)	40 (31.3)
Extremities	16 (25.0)	81 (32.7)	34 (27.6)	47 (37.6)
Uveal	0 (0)	10 (4.0)	10 (7.8)	0 (0)
Vulvar	1 (1.6)	4 (1.6)	1 (0.8)	3 (2.3)
Anorectal	2 (3.1)	5 (2.0)	5 (3.9)	0 (0)
Unknown primary site	5 (7.8)	30 (12.1)	19 (15.5)	11 (8.8)
Missing	0	8	5	3
Male	39 (60.9)	134 (52.3)	73 (57.0)	61 (47.7)
Race				
White	64 (100)	253 (99.6)	126 (100)	127 (99.2)
Nonwhite	0 (0)	1 (0.4)	0 (0)	1 (0.8)
Missing	0	2	2	0
Ethnicity				
Hispanic	0 (0)	3 (1.8)	0 (0)	3 (2.8)
Non-Hispanic	30 (100)	168 (98.2)	64 (100)	104 (97.2)
Missing	34	85	64	21
Median age at initial diagnosis	60 (25-89)	56.5 (15-95)	59 (15-89)	56 (19-95)
Median age at CNS metastasis or study inclusion	62.5 (25-90)	63 (17-97)	65 (17-90)	60 (22-97)
Stage				
0	0 (0)	3 (1.4)	0 (0)	3 (2.5)
I	2 (3.6)	23 (10.7)	9 (9.7)	14 (11.6)
II	15 (36.8)	49 (22.9)	19 (20.4)	30 (24.8)
III	29 (51.8)	107 (50.0)	33 (35.5)	74 (61.2)

IV	10 (17.9)	32 (15.0)	32 (34.4)	0 (0)
Missing	8	42	35	7
Other sites of metastasis	45 (70.3)	128 (50.0)	128 (100.0)	0 (0)
Bone	11 (24.4)	45 (35.2)	45 (35.2)	---
Lung/Pleura	37 (82.2)	79 (61.7)	79 (61.7)	---
Skin	4 (8.9)	19 (14.8)	19 (14.8)	---
Liver	17 (37.8)	39 (30.5)	39 (30.5)	---
Other	21 (46.7)	38 (29.7)	38 (29.7)	---
Treatment				
Chemotherapy	32 (50.0)	40 (15.6)	32 (25.0)	8 (6.3)
Radiation	24 (37.5)	55 (21.5)	48 (37.5)	7 (5.5)
Surgery	57 (89.1)	224 (87.5)	101 (78.9)	123 (96.1)
Immunotherapy	30 (46.9)	112 (43.8)	61 (47.7)	51 (39.8)

3.1.3 Non-Hodgkin Lymphoma Study Characteristics

The characteristics of the controls, by control group, compared to the cases for non-Hodgkin lymphoma subjects are shown in Table 14. The majority of non-Hodgkin lymphoma subjects, both cases and controls, were diffuse large B-cell lymphoma (DLBCL) (Cases=84.0%, Control Group 1=83.0%, Control Group 2=86.0%, Control Group 3=80.0%), while the remainder had indolent lymphomas that transformed into DLBCL (Cases=16.0%, Control Group 1=17.0%, Control Group 2=14.0%, Control Group 3=20.0%). Compared to controls, cases were more frequently male (Cases=68.0%, Control Group 1, 2, and 3=60.0%) and white (Cases=96.0%, Control Group 1, 2, and 3=92.0%). Cases (median age = 62 years, range 37-94) were generally older than controls (Control Group 1 = median age of 58 years, range 20-91; Control Group 2 = median age of 58 years, range 20-79; Control Group 3 = median age of 56 years, range 30-91). At diagnosis, cases were diagnosed with stage IV disease more

often than controls (Cases=65.2%, Control Group 1=31.5%, Control Group 2=57.5%, Control Group 3=4.4%). Cases developed bone marrow metastasis more frequently than Control Group 1 (Cases=33.3%, Control Group 1 and 2=24.0%). The median lactate dehydrogenase (LDH) was slightly higher in cases (median LDH = 321 U/L, range 188-2487) than in controls (Control Group 1: median LDH = 277 U/L, range 138-1225; Control Group 2: median LDH = 302 U/L, range 138-1225; Control Group 3: median LDH = 227 U/L, range 190-1208) at initial cancer diagnosis (Table 15).

Table 15: Characteristics of Non-Hodgkin Lymphoma Cases and Controls, by Control Group

Characteristic	Cases	Controls		
		Control Group 1	Control Group 2	Control Group 3
	(CNS Metastasis)	(Other Metastasis and No Metastasis)	(Other Metastasis)	(No Metastasis)
	n=25	n=100	n=50	n=50
	n (%)	n (%)	n (%)	n (%)
Cancer Type				
DLBCL	21 (84.0)	83 (83.0)	43 (86.0)	40 (80.0)
Transformed	4 (16.0)	17 (17.0)	7 (14.0)	10 (20.0)
Male	17 (68.0)	60 (60.0)	30 (60.0)	30 (60.0)
Race				
White	24 (96.0)	92 (92.0)	46 (92.0)	46 (92.0)
Nonwhite	1 (4.0)	8 (8.0)	4 (8.0)	4 (8.0)
Ethnicity				
Hispanic	0 (0)	3 (4.5)	0 (0)	3 (8.3)
Non-Hispanic	8 (100)	63 (95.5)	30 (100)	33 (91.7)
Missing	17	34	17	17
Median age at initial diagnosis	62 (37-94)	58 (20-91)	58 (20-79)	56 (30-91)

Median age at CNS metastasis or study inclusion	64 (38-94)	59 (21-92)	60.5 (21-86)	58 (31-92)
Stage				
0	0 (0)	2 (2.2)	2 (4.3)	0 (0)
I	2 (8.7)	13 (14.1)	5 (10.6)	8 (17.8)
II	2 (8.7)	16 (17.4)	6 (12.8)	10 (22.2)
III	4 (17.4)	32 (34.8)	7 (14.9)	25 (55.6)
IV	15 (65.2)	29 (31.5)	27 (57.5)	2 (4.4)
Missing	2	8	3	5
Other sites of disease	21 (84.0)	50 (50.0)	50 (100.0)	0 (0)
Bone	5 (23.8)	14 (28.0)	14 (28.0)	---
Lung/Pleura	2 (9.5)	12 (24.0)	12 (24.0)	---
Skin	3 (14.3)	3 (6.0)	3 (6.0)	---
Liver	3 (14.3)	4 (8.0)	4 (8.0)	---
Bone marrow	7 (33.3)	6 (12.0)	6 (12.0)	---
Other	11 (52.4)	26 (52.0)	26 (52.0)	---
Median LDH	321 (188-2487)	277 (138-1225)	302 (138-1225)	227 (190-1208)
Missing	9	35	13	22
Treatment				
Chemotherapy	25 (100)	93 (93.0)	46 (92.0)	47 (94.0)
Radiation	9 (36.0)	34 (34.0)	15 (30.0)	19 (38.0)
Surgery	3 (12.0)	8 (8.0)	3 (6.0)	5 (10.0)
Immunotherapy	22 (88.0)	89 (89.0)	45 (90.0)	44 (88.0)

3.2 Specific Aim 1

First, the results of each control group (Control Group 1, Control Group 2, and Control Group 3) are presented separately. Second, within each control group, we present the results for all cancers and then separately for the breast cancer and melanoma subsets. Due to sample size constraints, analyses using the non-Hodgkin lymphoma subset were not conducted. Finally, the multivariable analyses and sensitivity analyses, if any were conducted, are presented.

3.2.1 Control Group 1

A. All Cancers

a) Multivariable Analyses

Although not statistically significant, those cancer patients who took ‘any antidepressants’ had a 16% greater odds of developing CNS metastasis (95% CI = 0.78, 1.73) than cancer patients who did not take antidepressants, after controlling for age, stage, chemotherapy, radiation, immunotherapy, liver metastasis, bone metastasis, bone marrow metastasis, lung and pleural metastasis, and other sites of metastasis. Controlling for the same variables, those cancer patients who took ‘any SSRIs’ had a 21% greater odds of developing CNS metastasis (95% CI = 0.74, 1.97) and ‘exclusive SSRIs’ had a 36% greater odds of developing CNS metastasis (95% CI = 0.80, 2.31) compared to those cancer patients who did not take antidepressants.

The unadjusted and adjusted analyses of the relative odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’ (all cancers types together) are shown in Table 16. For ‘any antidepressant use’ (hypothesis 1a), the odds of CNS metastasis associated with antidepressant use were similar to the unadjusted model (Adjusted: OR = 1.17, 95% CI = 0.78, 1.74; Unadjusted OR = 1.10, 95% CI = 0.76, 1.59). Those cancer patients that took antidepressants had 17% greater odds of developing CNS metastasis than those cancer patients who did not take antidepressants, controlling for stage, age, chemotherapy, radiation, bone marrow metastasis, lung and pleura metastasis, and other sites of metastasis. This 95%

confidence interval was fairly wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. ‘Any’ and ‘exclusive SSRI use’ (hypotheses 1b and 1c, respectively) were fairly similar with low effect estimates and wide confidence intervals that cross 1.0. The largest effect estimate was that of ‘exclusive SSRI use’ (hypothesis 1c), which indicates that cancer patients that take ‘SSRIs exclusively’ were 36% more likely to develop CNS metastasis compared to cancer patients who do not take antidepressants controlling for stage, age, chemotherapy, radiation, bone marrow metastasis, lung and pleura metastasis, and other sites of metastasis (Table 16).

Table 16: Unadjusted and Adjusted Analyses: Relative Odds of CNS Metastasis Associated with ‘Any Antidepressant Use’, ‘Any SSRI Use’, and ‘Exclusive SSRI Use’, Using Control Group 1

Models	Control Group #1: All				
	Unadjusted			Adjusted	
	n	OR	95% CI	OR	95% CI
Antidepressants	842	1.10	(0.76, 1.59)	1.16	(0.78, 1.73)
SSRIs	749	1.04	(0.66, 1.64)	1.21	(0.74, 1.97)
Exclusive SSRIs	710	1.30	(0.80, 2.13)	1.36	(0.80, 2.31)

b) Sensitivity Analyses

As previously mentioned, stage was missing for 11% of subjects. We conducted a sensitivity analysis using Control Group 1 in order to determine if our estimates of the odds of CNS metastasis associated with antidepressants use and SSRI use were different with or without stage at diagnosis in the model. The odds of CNS metastasis associated

with ‘any antidepressant use’ (hypothesis 1a) when stage at diagnosis was not included in the model (OR = 1.10, 95% CI = 0.75, 1.61) was very similar to our relative odds estimate when including stage at diagnosis in the model (OR = 1.14, 95% CI = 0.76, 1.70). The odds of CNS metastasis associated with ‘any SSRI use’ (hypothesis 1b) when stage at diagnosis was not included in the model (OR = 1.10, 95% CI = 0.70, 1.75) was very similar to our relative odds estimate when including stage at diagnosis in the model (OR = 1.16, 95% CI = 0.72, 1.89). Therefore, we reported our initial results that include stage for ‘any antidepressant use’ and ‘any SSRI use’ (hypotheses 1a and 1b, respectively).

An additional concern of ours was the exposure definition for ‘any SSRI use’ (hypothesis 1b). We compared those cancer patients who took SSRIs to those who did not take any antidepressants. The reasoning behind this decision was to exclude those patients who took other classes of antidepressants in case the other classes of antidepressants had an effect on CNS metastasis. We conducted a sensitivity analysis in order to determine if our estimates of the odds of CNS metastasis associated with ‘any SSRI use’ compared to either ‘no antidepressant use’ or ‘no SSRI use’ were different. The comparison group of ‘no SSRI use’ could be comprised of subjects who took other classes of antidepressants. The odds of CNS metastasis associated with taking SSRIs compared to ‘no SSRIs’ (OR = 1.14, 95% CI = 0.71, 1.83) were very similar to the relative odds estimate when ‘no antidepressants’ were used as the comparison group (OR = 1.21, 95% CI = 0.74, 1.97); consequently, we reported our initial results that used ‘no antidepressants’ as the comparison group.

c) Summary

Although all confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’, all odds ratios were consistent in direction (greater than 1.0) for Control Group 1. While direction was similar, magnitude increased from ‘any antidepressant use’ (OR = 1.16) to ‘any SSRI use’ (OR = 1.21) to ‘exclusive SSRI use’ (OR = 1.36). SSRI use may be driving this increase in magnitude rather than other classes of antidepressants; we further discuss this explanation in the Discussion section.

B. Breast Cancer

a) Multivariable Analyses

As with the previous analyses, the breast cancer subset underwent multivariable analyses to determine the effect of antidepressant use on the odds of CNS metastasis using Control Group 1. Unadjusted and adjusted relative odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’ and ‘exclusive SSRI use’ are included and presented in Table 17. No effect was observed within any of the hypotheses for the breast cancer subset. All 95% confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. Breast cancer patients who ‘exclusively’ took SSRIs had a 44% greater odds of central nervous

system metastasis compared to breast cancer patients who did not take antidepressants, when controlling for age, chemotherapy, radiation, bone metastasis, bone marrow metastasis, lung and pleura metastasis, and molecular subtype (95% CI = 0.69, 3.00) (Table 17).

Table 17: Adjusted and Unadjusted Analyses: Relative Odds of CNS Metastasis Associated with ‘Any Antidepressant Use’, ‘Any SSRI Use’, and ‘Exclusive SSRI Use’, Using Control Group 1 of Breast Cancer Patients

Models	Control Group #1: All				
	Unadjusted			Adjusted	
	n	OR	95% CI	OR	95% CI
Antidepressants	370	0.97	(0.58, 1.61)	1.12	(0.63, 2.00)
SSRIs	321	0.96	(0.53, 1.76)	1.42	(0.72, 2.79)
Exclusive SSRIs	302	1.07	(0.56, 2.07)	1.44	(0.69, 3.00)

b) Breast Cancer Summary (Control Group 1)

As with all cancers, in the breast cancer subset, confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. Although these odds ratios were not statistically significant, all odds ratios were consistent in direction, and magnitude increased from ‘any antidepressant use’ (OR = 1.12) to ‘any SSRI use’ (OR = 1.42). SSRI use may be driving this increase in the magnitude of relative odds rather than other classes of antidepressants; we will further discuss this explanation in the discussion section. ‘Exclusive SSRI use’ had similar relative odds to ‘any SSRI use’ (‘exclusive SSRI use’: n=302, OR = 1.44; ‘any SSRI

use’: n=321, OR =1.42). The similarity may result from the ‘exclusive SSRI’ group only excluding 19 subjects from the ‘any SSRI’ group.

C. Melanoma

a) Multivariable Analyses

Using Control Group 1, unadjusted and adjusted relative odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’ are presented in Table 18. No effect was seen within any of the hypotheses for the melanoma subsets. All 95% confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. Melanoma patients who ‘exclusively’ took SSRIs had a 37% greater odds of central nervous system metastasis compared to melanoma patients who did not take antidepressants, when controlling for chemotherapy and other sites of metastasis (95% CI = 0.46, 4.07) (Table 18).

Table 18: Adjusted and Unadjusted Analyses: Relative Odds of CNS Metastasis Associated with ‘Any Antidepressant Use’, ‘Any SSRI Use’, and ‘Exclusive SSRI Use’, Using Control Group 1 of Melanoma Cancer Patients

Models	Control Group #1: All				
	Unadjusted			Adjusted	
	n	OR	95% CI	OR	95% CI
Antidepressants	320	1.16	(0.62, 2.20)	1.01	(0.50, 2.04)
SSRIs	295	0.83	(0.36, 1.89)	0.74	(0.30, 1.83)
Exclusive SSRIs	272	1.31	(0.50, 3.46)	1.37	(0.46, 4.07)

b) Melanoma Summary (Control Group 1)

As with all cancers, in the melanoma subset, confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. Odds ratios were different in direction (‘any antidepressant use’: OR = 1.01, 95% CI = 0.50, 2.04; ‘any SSRI use’: OR = 0.74, 95% CI = 0.30, 1.83; ‘exclusive SSRI use’: OR = 1.37, 95% CI = 0.46, 4.07). Differences between SSRIs and other classes of antidepressants may be driving this difference in magnitude. Another possible explanation is that no association between antidepressant use and CNS metastasis exists, as these confidence intervals are wide.

D. Control Group 1 Summary

For all cancers and cancer subsets, no statistically significant association existed between ‘any antidepressant use’, ‘any SSRI use’ or ‘exclusive SSRI use’ and CNS metastasis (Figure 7). Confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. In all cancers and the breast cancer subset, the direction of the odds ratios was similar and magnitudes increased from antidepressant use to SSRI use. In the melanoma subset, the direction and magnitudes differed between antidepressant use and SSRI use. We further discuss these differences in the discussion section.

Figure 7: Adjusted Analyses for Control Group 1 by Cancer Type and Antidepressant Use

3.2.2 Control Group 2

A. All Cancers

a) Multivariable Analyses

Multivariable analyses were repeated using Control Group 2. The adjusted and unadjusted analyses of the relative odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’, using Control Group 2 are presented in Table 19. For ‘any antidepressant use’ (hypothesis 1a), the odds of CNS metastasis associated with antidepressant use were similar to the unadjusted model

(Adjusted: OR = 1.19, 95% CI = 0.77, 1.91; Unadjusted = 1.26 95% CI = 0.83, 1.85).

Those cancer patients that took ‘any antidepressants’ were 19% more likely to develop CNS metastasis than those cancer patients who did not take antidepressants, controlling for stage, chemotherapy, surgery, and bone metastasis. This 95% confidence was fairly wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with any antidepressant use. ‘Any’ and ‘exclusive SSRI use’ (hypotheses 1b and 1c, respectively) were fairly similar with low effect estimates and wide confidence intervals that cross 1.0. The largest effect estimate was that of ‘exclusive SSRI use’ (hypothesis 1c), which indicates that cancer patients that take SSRIs ‘exclusively’ were 43% more likely to develop CNS metastasis compared to cancer patients who do not take antidepressants, controlling for stage, chemotherapy, surgery, and bone metastasis (Table 19).

Table 19: Adjusted and Unadjusted Analyses: Relative Odds of CNS Metastasis Associated with ‘Any Antidepressant Use’, ‘Any SSRI Use’, and ‘Exclusive SSRI Use’, Using Control Group 2

Models	Control Group #2: Other Metastasis				
	Unadjusted			Adjusted	
	n	OR	95% CI	OR	95% CI
Antidepressants	483	1.26	(0.83, 1.91)	1.19	(0.77, 1.85)
SSRIs	429	1.26	(0.75, 2.11)	1.31	(0.76, 2.26)
Exclusive SSRIs	414	1.44	(0.82, 2.52)	1.43	(0.79, 2.57)

b) Sensitivity Analyses

As with Control Group 1, we were concerned with the missing stage data in Control Group 2. We conducted a sensitivity analysis using Control Group 2 in order to determine if our results were robust to the effects of stage at diagnosis. The results of our sensitivity analysis for ‘any antidepressant use’ (hypothesis 1a) (OR = 1.10, 95% CI = 0.73, 1.67) were very similar to our initial results (OR = 1.21, 95% CI = 0.78, 1.88). Similar findings existed for ‘any SSRI use’ (hypothesis 1b). The sensitivity analysis resulted in an odds ratio of 1.17 (95% CI = 0.71, 1.94) and the initial analysis had an odds ratio of 1.31 (95% CI = 0.76, 2.26). Therefore, we report our initial results that include stage for ‘any antidepressant use’ (hypotheses 1a) and ‘any SSRI use’ (hypothesis 1b). As previously mentioned, an additional concern of ours was the exposure definition for ‘any SSRI use’ (hypothesis 1b) (using no antidepressant use as the comparison instead of no SSRI use). In order to determine if this decision affected our results, we conducted a sensitivity analysis comparing those cancer patients who took SSRIs to those cancer patients who did not take SSRIs. The results of our sensitivity analysis for the comparison group of cancer patients who did not take SSRIs (OR = 1.18, 95% CI = 0.72, 1.94) were very similar to our initial results (OR = 1.31, 95% CI = 0.76, 2.26); consequently, we report our initial results.

c) Summary

Although all confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any

antidepressant use', 'any SSRI use', or 'exclusive SSRI use', all odds ratios were consistent in direction (greater than 1.0) for Control Group 2. While direction was similar, magnitude increased from 'any antidepressant use' (OR = 1.19) to 'any SSRI use' (OR = 1.31) to 'exclusive SSRI use' (OR = 1.43). As in Control Group 1, SSRI use may be driving this increase in magnitude rather than other classes of antidepressants; we will further discuss this explanation in the discussion section.

B. Breast Cancer

a) Multivariable Analyses

Using Control Group 2, unadjusted and adjusted analyses determined the relative odds of CNS metastasis associated with 'any antidepressant use', 'any SSRI use', and 'exclusive SSRI use' and are presented in Table 20. In the breast cancer subset, no effect was observed between 'any antidepressant use' and CNS metastasis (OR = 1.11, 95% CI = 0.57, 2.16). When 'any SSRIs' were investigated, the effect estimate increased (OR = 1.73, 95% CI = 0.75, 4.04). Breast cancer patients who took 'any SSRIs' had a 73% greater odds of central nervous system metastasis compared to breast cancer patients who did not take antidepressants, when controlling for race, chemotherapy, bone metastasis, and molecular subtype (95% CI = 0.75, 4.04) (Table 20). However, although neither were statistically significant, this adjusted OR was substantially different from the unadjusted odds ratio (1.73 compared to 1.00, respectively). This difference using 'any SSRIs use' was likely due to the inclusion of two confounders: chemotherapy and molecular subtype. In a model that excluded chemotherapy and molecular subtype, the

odds of CNS metastasis associated with ‘any SSRI use’ was similar to that from the unadjusted model (OR = 1.06, 95% CI = 0.56, 2.01), demonstrating that chemotherapy and molecular subtype are confounding the relationship between ‘any SSRI use’ and the odds of CNS metastasis. As with ‘any SSRI use’, the adjusted odds ratio for ‘exclusive SSRI use’ was substantially different from the unadjusted odds ratio (1.66 compared to 0.95, respectively). In a model that excluded chemotherapy and molecular subtype, the odds of CNS metastasis associated with ‘exclusive SSRI use’ was similar to that from the unadjusted model (OR = 1.08, 95% CI = 0.55, 2.13) demonstrating that chemotherapy and molecular subtype are confounding the relationship between ‘exclusive SSRI use’ and the odds of CNS metastasis.

Table 20: Adjusted and Unadjusted Analyses: Relative Odds of CNS Metastasis Associated with ‘Any Antidepressant Use’, ‘Any SSRI Use’, and ‘Exclusive SSRI Use’, Using the Control Group 2 of Breast Cancer Patients

Models	Control Group #2: Other Metastasis				
	Unadjusted			Adjusted	
	n	OR	95% CI	OR	95% CI
Antidepressants	200	0.88	(0.49, 1.58)	1.11	(0.57, 2.16)
SSRIs	170	1.00	(0.49, 2.01)	1.73	(0.75, 4.04)
Exclusive SSRIs	164	0.95	(0.45, 2.01)	1.66	(0.69, 4.01)

b) Breast Cancer Summary (Control Group 2)

As with all cancers, in the breast cancer subset of Control Group 2, confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. Although these odds ratios were not statistically significant, all

odds ratios were consistent in direction, and magnitude increased from ‘any antidepressant use’ (OR = 1.11) to ‘any SSRI use’ (OR = 1.73). ‘Exclusive SSRI use’ had similar relative odds to ‘any SSRI use’ (‘exclusive SSRI use’: OR = 1.66; ‘any SSRI use’: OR = 1.73). SSRI use may be driving this increase in the magnitude of relative odds rather than other classes of antidepressants; we further examine this explanation in the discussion section.

C. Melanoma

a) Multivariable Analyses

Using Control Group 2, adjusted and unadjusted analyses of the relative odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’ in melanoma patients are presented in Table 21. We did not observe a large effect estimate in ‘any SSRI use’ in the melanoma subset. When controlling for location of melanoma, chemotherapy, and skin and subcutaneous metastasis, the odds of developing central nervous system metastasis in melanoma patients was 24% greater in those patients who took SSRIs compared to those melanoma patients who did not take antidepressants (95% CI: 0.43, 3.55) (Table 21). When the melanoma subset was analyzed using Control Group 2, those subjects who received chemotherapy had greater odds (OR = 6.71, 95% CI = 1.65, 27.36) of developing CNS metastasis than those who did not receive chemotherapy (OR = 0.53, 95% CI = 0.14, 1.98). When the interaction was entered into the model, it was statistically significant ($p < 0.01$) suggesting chemotherapy modifies the relationship between antidepressant use and CNS metastasis.

Table 21: Adjusted and Unadjusted Analyses: Relative Odds of CNS Metastasis Associated with ‘Any Antidepressant Use’, ‘Any SSRI Use’, and ‘Exclusive SSRI Use’, Using Control Group 2 of Melanoma Cancer Patients

Models	Control Group #2: Other Metastasis				
	Unadjusted			Adjusted	
	n	OR	95% CI	OR	95% CI
Antidepressants	192	1.80	(0.86, 3.77)	1.69	(0.77, 3.72)
SSRIs	176	1.08	(0.43, 2.71)	1.24	(0.43, 3.55)
Exclusive SSRIs	168	2.25	(0.69, 7.33)	1.56	(0.46, 5.35)

b) Melanoma Summary (Control Group 2)

As with all cancers using Control Group 2, in the melanoma subset, confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. Odds ratios were similar in direction but different in magnitude (‘any antidepressant use’: OR = 1.69; ‘any SSRI use’: OR = 1.24; ‘exclusive SSRI use’: OR = 1.56). Differences between SSRIs and other classes of antidepressants may be driving this difference in magnitude; we further examine this difference in the discussion section.

D. Control Group 2 Summary

For all cancers and cancer subsets using Control Group 2, no statistically significant association existed between ‘any antidepressant use’, ‘any SSRI use’ or ‘exclusive SSRI use’ and CNS metastasis. Confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. In all

cancers and cancer subsets, the direction of the odds ratios was similar. In all cancers and the breast cancer subset, the magnitudes increased from antidepressant use to SSRI use. While in the melanoma subset, the magnitudes differed between antidepressant use and SSRI use. The possibility exists that classes of antidepressants act differently in distinct cancer types. We further discuss these differences in the discussion section.

Figure 8: Adjusted Analyses for Control Group 2 by Cancer Type and Antidepressant Use

3.2.3 Control Group 3

A. All Cancers

a) Multivariable Analyses

The unadjusted and adjusted analyses of the relative odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’ using Control Group 3 are presented in Table 22. The unadjusted and adjusted analyses using Control Group 3 were similar in each of the hypotheses. As with the Control Group 1, all effect estimates were near 1.0. The confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. For example, cancer patients who took SSRIs ‘exclusively’ (Hypothesis 1c) had a 6% greater odds of developing CNS metastasis than those cancer patients who did not take antidepressants controlling for stage, age, chemotherapy, radiation, and immunotherapy (95% CI = 0.60, 1.88). Additionally, none of the variables were significant effect modifiers of the CNS metastasis/SSRI association using Control Group 3.

Table 22: Adjusted and Unadjusted Analyses: Relative Odds of CNS Metastasis Associated with ‘Any Antidepressant Use’, ‘Any SSRI Use’, and ‘Exclusive SSRI Use’, Using Control Group 3

Models	n	Control Group #3: No Metastasis			
		Unadjusted		Adjusted	
		OR	95% CI	OR	95% CI
Antidepressants	529	0.98	(0.66, 1.46)	0.96	(0.62, 1.47)
SSRIs	469	0.90	(0.55, 1.46)	0.85	(0.51, 1.42)
Exclusive SSRIs	441	1.19	(0.70, 2.04)	1.06	(0.60, 1.88)

b) Sensitivity Analyses

Control Group 3 was comprised of cancer patients with no metastasis at the time of study inclusion. These patients could have developed metastasis after their study inclusion, and this development would make them inherently different than those cancer patients who never go on to develop metastasis. Since this difference was a concern, we went back into the patients' charts to determine which patients from this group went on to develop metastasis. Approximately 24.3% (n= 92/378) of this control group developed metastasis after their study inclusion. We conducted a sensitivity analysis in order to determine if our results were robust to whether we excluded controls that developed metastasis at a later date. The odds of CNS metastasis associated with antidepressant use, when excluding those controls who later developed metastasis (OR = 0.85, 95% CI = 0.54, 1.33), which was similar to our primary analysis where we included these controls (OR = 0.98, 95% CI = 0.64, 1.51). Similar findings existed for SSRI use. When excluding controls that later developed metastasis, we observed a similar odds of CNS metastasis associated with antidepressant use, compared to no antidepressant use (OR=0.79; 95% CI = 0.46, 1.35) as in our main analyses where we included these controls (OR=0.88; 95% CI = 0.53, 1.48). Therefore, we reported our initial results that included these controls in our analyses.

c) Summary

In Control Group 3, all confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. Additionally, all odds ratios were close to 1.0 and suggest no association between antidepressant use and CNS metastasis. Although direction is slightly different (‘any antidepressant use’: OR = 0.96; ‘any SSRI use’: OR = 0.85; ‘exclusive SSRI use’: OR = 1.06), the magnitude of the odds ratios is similar and suggests no difference between ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’. We further examine this similarity in the discussion section.

B. Breast Cancer

a) Multivariable Analyses

Using Control Group 3, adjusted and unadjusted analyses of the relative odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, and ‘exclusive SSRI use’ in the melanoma subset are presented in Table 23. In Control Group 3 of the breast cancer subset, we did not find significantly greater odds of CNS metastasis associated with ‘any SSRI use’ (OR = 1.16, 95% CI = 0.54, 2.47), when controlling for age, chemotherapy, radiation, and molecular subtype. Of note, all odds ratios were of similar direction, suggesting we were underpowered to detect the true association between CNS metastasis and antidepressant use.

Table 23: Adjusted and Unadjusted Analyses: Relative Odds of CNS Metastasis Associated with ‘Any Antidepressant Use’, ‘Any SSRI Use’, and ‘Exclusive SSRI Use’, Using Control Group 3 of Breast Cancer Patients

Models	Control Group #3: No Metastasis				
	Unadjusted			Adjusted	
	n	OR	95% CI	OR	95% CI
Antidepressants	247	0.96	(0.55, 1.67)	1.33	(0.68, 2.61)
SSRIs	219	0.90	(0.47, 1.71)	1.16	(0.54, 2.47)
Exclusive SSRIs	203	1.12	(0.55, 2.29)	1.46	(0.61, 3.47)

b) Breast Cancer Summary (Control Group 3)

As with all cancers using Control Group 3, in the breast cancer subset, confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. Odds ratios were similar in direction but slightly different in magnitude (‘any antidepressant use’: OR = 1.33; ‘any SSRI use’: OR = 1.16; ‘exclusive SSRI use’: OR = 1.46). Differences between SSRIs and other classes of antidepressants may be driving this difference in magnitude or no effect of antidepressant use on CNS metastasis truly exists; we further examine this difference in the discussion section.

C. Melanoma

a) Multivariable Analyses

Using Control Group 3, adjusted and unadjusted analyses of the relative odds of CNS metastasis associated with antidepressant use are presented in Table 24. In the melanoma subset, the odds of developing central nervous system metastasis in melanoma patients was 55% lower in those patients who took ‘any SSRIs’ in comparison to those

melanoma patients who did not take antidepressants (95% CI = 0.12, 1.75), when controlling for stage, site of melanoma, chemotherapy, surgery, and radiation (Table 24).

Table 24: Adjusted and Unadjusted Analyses: Relative Odds of CNS Metastasis Associated with ‘Any Antidepressant Use’, ‘Any SSRI Use’, and ‘Exclusive SSRI Use’, Using Control Group 3 of Melanoma Cancer Patients

Models	Control Group #3: No Metastasis				
	Unadjusted			Adjusted	
	n	OR	95% CI	OR	95% CI
Antidepressants	175	0.81	(0.40, 1.65)	0.76	(0.29, 2.02)
SSRIs	153	0.61	(0.24, 1.54)	0.45	(0.12, 1.74)
Exclusive SSRIs	142	0.92	(0.33, 2.61)	0.39	(0.08, 1.87)

b) Melanoma Summary (Control Group 3)

In the melanoma subset of Control Group 3, confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. Odds ratios were similar in direction but different in magnitude (‘any antidepressant use’: OR = 0.76; ‘any SSRI use’: OR = 0.45; ‘exclusive SSRI use’: OR = 0.39). Differences between SSRIs and other classes of antidepressants may be driving this difference in magnitude or no effect of antidepressant use on CNS metastasis truly exists; we further examine this difference in the discussion section.

D. Control Group 3 Summary

For all cancers and cancer subsets using Control Group 3, no statistically significant association existed between ‘any antidepressant use’, ‘any SSRI use’ or

‘exclusive SSRI use’ and CNS metastasis. Confidence intervals were wide and we did not observe statistically significant increases or decreases in the odds of CNS metastasis associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’. In all cancers the magnitudes of the odds ratios were close to 1.0 (‘any antidepressant use’: OR = 0.96; ‘any SSRI use’: OR = 0.85; ‘exclusive SSRI use’: OR = 1.06). These magnitudes were close to 1.0 because the breast cancer and melanoma subsets had opposite directions of their odds ratios for all hypotheses (breast cancer: ‘any antidepressant use’: OR = 1.33; ‘any SSRI use’: OR = 1.16; ‘exclusive SSRI use’: OR = 1.46; melanoma: ‘any antidepressant use’: OR = 0.76; ‘any SSRI use’: OR = 0.45; ‘exclusive SSRI use’: OR = 0.39). Antidepressants appeared to be acting differently among the different cancer types. We further discuss these differences in the discussion section.

Figure 9: Adjusted Analyses for Control Group 3 by Cancer Type and Antidepressant Use

3.2.4 Summary of Aim 1

A. Comparisons of Control Groups

For all cancers, and each cancer subset (breast cancer and melanoma) we found no statistically significant associations between ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’ and CNS metastasis when using any control group (Table 25). Although we did not observe a statistically significant effect in the odds of CNS metastasis, the relative odds estimates of CNS metastasis associated with ‘any antidepressant use’ in Control Group 1 (OR = 1.16, 95% CI = 0.78, 1.73) and Control Group 2 (OR = 1.19, 95% CI = 0.77, 1.85) were both greater than 1.0. However, the odds ratios of Control group 3 were much closer to 1.0 (‘any antidepressant use’: OR =

0.96; 'any SSRI use': OR = 0.85; 'exclusive SSRI use': OR = 1.06). These differences between control groups and potential interpretation(s) of them, which may be important for planning/designing a follow-up study, are further examined in the discussion section.

Table 25: Adjusted Results for Each Control Group by Cancer Type and Antidepressant Use

		All Cancers		Breast Cancer		Melanoma	
		OR	95% CI	OR	95% CI	OR	95% CI
Control Group 1	Any Antidepressant Use	1.16	(0.78, 1.73)	1.12	(0.63, 2.00)	1.01	(0.50, 2.04)
	Any SSRI Use	1.21	(0.74, 1.97)	1.42	(0.72, 2.79)	0.74	(0.30, 1.83)
	Exclusive SSRI Use	1.36	(0.80, 2.31)	1.44	(0.69, 3.00)	1.37	(0.46, 4.07)
Control Group 2	Any Antidepressant Use	1.19	(0.77, 1.85)	1.06	(0.54, 2.08)	1.69	(0.77, 3.72)
	Any SSRI Use	1.31	(0.76, 2.26)	1.73	(0.75, 4.04)	1.24	(0.43, 3.55)
	Exclusive SSRI Use	1.43	(0.79, 2.57)	1.67	(0.69, 4.01)	1.56	(0.46, 5.35)
Control Group 3	Any Antidepressant Use	0.96	(0.62, 1.47)	1.33	(0.68, 2.61)	0.76	(0.29, 2.02)
	Any SSRI Use	0.85	(0.51, 1.42)	1.16	(0.54, 2.47)	0.45	(0.12, 1.74)
	Exclusive SSRI Use	1.06	(0.60, 1.88)	1.46	(0.61, 3.47)	0.39	(0.08, 1.87)

B. Comparisons of Cancer Types

For all cancers and cancer subsets, we did not observe significantly greater odds of CNS metastasis associated with ‘any antidepressant use’, ‘SSRI use’, or ‘exclusive SSRI use’ (Table 25). In Control Group 2, the direction of the breast cancer and melanoma subsets were similar (‘any antidepressant use’: breast cancer – OR=1.06, melanoma – OR=1.69; ‘any SSRI use’: breast cancer – OR=1.73, melanoma – OR=1.24; ‘exclusive SSRI use’: breast cancer – OR=1.67, melanoma – OR=1.56), but in Control Group 3, the direction of the odds ratios of the cancer subsets were different (‘any antidepressant use’: breast cancer – OR=1.33, melanoma – OR=0.76; ‘any SSRI use’: breast cancer – OR=1.16, melanoma – OR=0.45; ‘exclusive SSRI use’: breast cancer – OR=1.46, melanoma – OR=0.39). In the breast cancer subset, antidepressant use was associated with greater odds of CNS metastasis, while in the melanoma subset antidepressant use was associated with reduced odds of CNS metastasis. The differences between cancer types are further discussed in the discussion section.

C. Comparisons of Drug Types

We did not observe significantly greater odds of CNS metastases associated with ‘any antidepressant use’, ‘any SSRI use’, or ‘exclusive SSRI use’ (Table 25). Although the confidence intervals were wide for each effect estimate, the magnitudes and directions of the odds ratios were similar (Control Group 1: ‘any antidepressant use’ – OR=1.16, ‘any SSRI use’ – OR=1.21, ‘exclusive SSRI use’ – OR=1.36; Control Group 2: ‘any antidepressant use’ – OR=1.19, ‘any SSRI use’ – OR=1.31, ‘exclusive SSRI use’ –

OR=1.43; Control Group 3: ‘any antidepressant use’ – OR=0.96, ‘any SSRI use’ – OR=0.85, ‘exclusive SSRI use’ - OR=1.06). ‘Exclusive SSRI use’ had greater odds of CNS metastasis than ‘any antidepressant use’ in all analyses, except for the melanoma subset using Control Group 3, subjects with no metastasis. We further discuss similarities in relative odds estimates between drug types in the discussion section.

3.3 Specific Aim 2

As previously mentioned (Section 2.6- Specific Aim 2, page 66), Aim 2 was conducted in breast cancer controls only. As with Aim 1, we first presented the results when using each control group (Control Group 1, Control Group 2, and Control Group 3) separately. Within each control group, we presented the bivariate analyses, multivariable analyses and sensitivity analyses, if any were conducted.

Antidepressant use among breast cancer controls by control group is presented in Table 26. Antidepressant use was similar for all control groups (Control Group 1 – use of ‘any antidepressant’ = 33.8%; Control Group 2 – use of ‘any antidepressant’ = 29.5%; Control Group 3 – use of ‘any antidepressant’ = 38.0%).

Table 26: Antidepressant Use among Breast Cancer Controls, by Control Group, in Aim 2

	Control Group 1 (Other Metastasis and No Metastasis) n=195		Control Group 2 (Other Metastasis) n=95		Control Group 3 (No Metastasis) n=100	
	n	%	n	%	n	%
Any Antidepressant	66	33.8	28	29.5	38	38.0
SSRI	45	23.1	18	18.9	27	27.0
Other Classes	21	10.8	10	10.5	11	11.0

I. Control Group 1

A. Bivariate Analyses

Bivariate analyses were conducted to determine if WBC counts differed between those breast cancer control patients who took ‘any antidepressants’ and those breast cancer control patients who did not. The distribution of WBC counts by antidepressant use in Control Group 1 is presented in Figure 10. In the main analysis utilizing all breast cancer controls from Aim 1, the WBC counts of those breast cancer control patients who did not take antidepressants ($n=129$; $5.9 \times 10^9/L$; range: $2.9-12.8 \times 10^9/L$) were statistically significantly ($p=0.04$ from Mann Whitney U Test) lower than the WBC counts of those breast cancer control patients who did take ‘any antidepressants’ ($n=64$; $6.7 \times 10^9/L$; range: $3.7-12.4 \times 10^9/L$).

Figure 10: Distribution of WBC Count by Antidepressant use in Control Group 1 **Figure 11: Distribution of Wilcoxon Scores for WBC Counts for Breast Cancer Patients**

Next, bivariate analyses were conducted to analyze if WBC counts differed between those cancer controls who took ‘any selective serotonin reuptake inhibitors (SSRIs)’ and those cancer controls who did not take antidepressants. The distribution of WBC counts by SSRI use in Control Group 1 is presented in Figure 12. Those breast cancer patients who did not take antidepressants had a significantly lower median WBC count of $5.9 \times 10^9/\text{L}$ ($n=129$; range: $2.9 - 12.8 \times 10^9/\text{L}$) compared to those who did take ‘any SSRIs’ ($7.2 \times 10^9/\text{L}$; $n=45$; range: $3.7 - 12.4 \times 10^9/\text{L}$; $p=0.02$ from Mann Whitney U Test) (Figure 13).

Figure 12: Distribution of WBC Count by SSRI Use in Control Group 1

Figure 13: Distribution of Wilcoxon Scores for WBC Count for Control Group 1 Who Did and Did Not Use SSRIs

B. Multivariable Analyses

The unadjusted and adjusted analyses of the change in WBC count associated with ‘any antidepressant use’ and ‘any SSRI use’ using Control Group 1 are presented in Table 27, and were similar for each of the hypotheses. All changes in WBC count associated with antidepressant use were greater than 0. Breast cancer patients who took ‘any SSRIs’ had WBC counts $0.75 \times 10^9/\text{L}$ higher (95% CI = $0.01 \times 10^9/\text{L}$, $1.49 \times 10^9/\text{L}$) than those patients who did not take antidepressants (Table 27).

Table 27: Unadjusted and Adjusted Analyses: The Estimated Change in WBC Counts Associated with Antidepressant Use Using Control Group 1

Models	Control Group #1: All				
	Unadjusted			Adjusted	
	n	Estimated change in WBC count (x10 ⁹ /L)	95% CI (x10 ⁹ /L)	Estimated change in WBC count (x10 ⁹ /L)	95% CI (x10 ⁹ /L)
Antidepressants	195	0.47	(-0.19, 1.13)	0.49	(-0.15, 1.13)
SSRIs	174	0.76	(-0.00, 1.52)	0.75	(0.01, 1.49)

C. Sensitivity Analysis

We conducted a sensitivity analysis using Control Group 1 in order to determine if our estimates of the change of WBC count associated with ‘any SSRI use’ was different with or without immunotherapy. The change in WBC count associated with ‘any SSRI use’ in the original model excluding immunotherapy (0.75 x10⁹/L, 95% CI = 0.01 x 10⁹/L, 1.49 x 10⁹/L) was similar to our estimate of change when including immunotherapy (0.70 x10⁹/L, 95% CI = -0.04 x 10⁹/L, 1.43 x 10⁹/L) suggesting no difference in the models. As a result, the initial results using the model with radiation only were reported, depicted above.

D. Summary of Control Group 1 in Aim 2

Using Control Group 1, we did not observe a statistically significant change in WBC count associated with ‘any antidepressant use’ among the breast cancer controls. Although not statistically significant, the direction of the estimated change in WBC count

($0.49 \times 10^9/\text{L}$) is similar to the model with ‘any SSRI use’ ($0.75 \times 10^9/\text{L}$), which was statistically significant (95% CI = $0.01 \times 10^9/\text{L}$, $1.49 \times 10^9/\text{L}$). Both are small to moderate increases in WBC count. Our results were robust to the effects of immunotherapy, suggesting no substantial effect of immunotherapy on WBC counts when assessing the effect of ‘any SSRI use’.

II. Control Group 2

A. Bivariate Analyses

The distribution of WBC count by antidepressant use in Control Group 2 is presented in Figure 14. Within this group, those breast cancer controls who did not take antidepressants had a lower median WBC count of $5.8 \times 10^9/\text{L}$ (range: $2.9\text{-}12.8 \times 10^9/\text{L}$), compared to those breast cancer controls who did take ‘any antidepressants’ ($6.8 \times 10^9/\text{L}$; range: $3.1\text{-}12.2 \times 10^9/\text{L}$; $p=0.09$ from Mann Whitney U Test; Figure 15).

Figure 14: Distribution of WBC Count by Antidepressant Use in Control Group 2 **Figure 15: Distribution of Wilcoxon Scores for WBC Counts for Control Group 2 Among Those Who Did and Did Not Take Antidepressants**

The distribution of WBC count by 'SSRI use' in Control Group 2 is presented in Figure 16. Those breast cancer controls who did not take antidepressants had a lower median WBC count of $5.8 \times 10^9/\text{L}$ ($n=67$; range: $2.9 - 12.8 \times 10^9/\text{L}$), compared to those controls who did take SSRIs ($7.5 \times 10^9/\text{L}$; $n=18$; range: $3.8 - 12.2 \times 10^9/\text{L}$; $p=0.05$ from Mann Whitney U test, Figure 17).

Figure 16: Distribution of WBC Count by SSRI Use in Control Group 2

Figure 17: Distribution of Wilcoxon Scores for WBC Count for Control Group 2 Who Did and Did Not Use SSRIs

B. Multivariable Analyses

The unadjusted and adjusted analyses of the change in WBC count associated with ‘any antidepressant use’ and ‘any SSRI use’ using Control Group 2 are presented in Table 28 and were similar in each of hypothesis. All changes in WBC count associated with antidepressant use were greater than 0, although results were not statistically significant. Breast cancer patients who took ‘any antidepressants’ had WBC counts $0.53 \times 10^9/L$ higher (95% CI = $-0.53 \times 10^9/L$, $1.59 \times 10^9/L$) than those patients who did not take antidepressants (Table 28). Breast cancer patients who took ‘any SSRIs’ had WBC

counts $0.89 \times 10^9/\text{L}$ higher (95% CI = $-0.35 \times 10^9/\text{L}$, $2.13 \times 10^9/\text{L}$) than those patients who did not take antidepressants (Table 28). The linearity assumptions of the linear regression models were tested. The residual plot does not display a pattern supporting constant variance.

Table 28: Unadjusted and Adjusted Analyses: The Estimated Change in WBC Count Associated with Any Antidepressant and Any SSRI Use, Using Control Group 2

Models	Control Group #2: Other Metastasis				
	Unadjusted			Adjusted	
	n	Estimated change in WBC count ($\times 10^9/\text{L}$)	95% CI ($\times 10^9/\text{L}$)	Estimated change in WBC count ($\times 10^9/\text{L}$)	95% CI ($\times 10^9/\text{L}$)
Antidepressants	95	0.60	(-0.49, 1.69)	0.53	(-0.53, 1.59)
SSRIs	85	0.91	(-0.37, 2.18)	0.89	(-0.35, 2.13)

C. Summary of Control Group 2 in Aim 2

Using Control Group 2, we did not observe a statistically significant change in WBC count associated with ‘any antidepressant use’ or ‘any SSRI use’ among the breast cancer controls. The directions of the estimated change in WBC counts were similar between the models of ‘any antidepressant use’ ($0.53 \times 10^9/\text{L}$) and ‘any SSRI use’ ($0.89 \times 10^9/\text{L}$). Although not statistically significant, our results suggest a potential association between ‘any antidepressant use’ or ‘any SSRI use’ and WBC counts.

III. Control Group 3

A. Bivariate Analyses

The distribution of WBC counts by antidepressant use in Control Group 3 is presented in Figure 18. Within those breast cancer patients with no other sites of metastasis (Control Group 3), the median WBC count for those patients that did not take antidepressants was $5.9 \times 10^9/\text{L}$ (range: $3.6 - 12.0 \times 10^9/\text{L}$), while the median WBC count for those patients that took ‘any antidepressants’ was $6.3 \times 10^9/\text{L}$ (range: $3.7 - 12.4 \times 10^9/\text{L}$; $p=0.15$ from the Mann Whitney U Test) (Figure 18).

Figure 18: Distribution of WBC Count by Antidepressant Use in Control Group 3

Figure 19: Distribution of Wilcoxon Scores for WBC Counts in Control Group 3

As with antidepressant use, the distribution of WBC count by SSRI use in Control Group 3 (breast cancer patients with no metastasis) is presented in Figure 20. The median WBC count for those subjects who did not take antidepressants was $5.9 \times 10^9/\text{L}$ ($n=62$; range: $3.6 - 12.0 \times 10^9/\text{L}$), while those who did take ‘any SSRIs’ had a WBC count of $6.6 \times 10^9/\text{L}$ ($n=27$; $3.7 - 12.4 \times 10^9/\text{L}$). Although the WBC counts did not differ ($p=0.08$ from the Mann Whitney U Test), the mean WBC count for those cancer patients who took ‘any SSRIs’ was higher than the mean WBC count for those cancer patients who did not take antidepressants.

Figure 20: Distribution of WBC Count by SSRI use in Control Group 3 **Figure 21: Distribution of Wilcoxon Scores for WBC Count for Control Group 3 Who Did and Did Not Use SSRIs**

B. Multivariable Analyses

As previously stated, radiation was included in all models of ‘any antidepressant use’ and ‘any SSRI use’ using Control Group 3. The unadjusted and adjusted analyses of the change in WBC count associated with ‘any antidepressant use’ and ‘any SSRI use’ using Control Group 3 are presented in Table 29 and were similar in each of the hypotheses. All changes in WBC count associated with ‘any antidepressant use’ were greater than 0, although results were not statistically significant. Breast cancer patients who took ‘any antidepressants’ had WBC counts $0.52 \times 10^9/\text{L}$ higher (95% CI = $-0.27 \times 10^9/\text{L}$, $1.31 \times 10^9/\text{L}$) than those patients who did not take antidepressants (Table 28). Breast cancer patients who took ‘any SSRIs’ had WBC counts $0.72 \times 10^9/\text{L}$ higher (95% CI = $-0.19 \times 10^9/\text{L}$, $1.63 \times 10^9/\text{L}$) than those patients who did not take antidepressants (Table 29). The linearity assumptions of the linear regression models were tested. The residual plot does not display a pattern supporting constant variance.

Table 29: Unadjusted and Adjusted Analyses: The Estimated Change in WBC Count Associated with Any Antidepressant and Any SSRI Use

Models	Control Group #3: No Metastasis				
	Unadjusted			Adjusted	
	n	Estimated change in WBC count ($\times 10^9/\text{L}$)	95% CI ($\times 10^9/\text{L}$)	Estimated change in WBC count ($\times 10^9/\text{L}$)	95% CI ($\times 10^9/\text{L}$)
Antidepressants	100	0.38	(-0.44, 1.21)	0.52	(-0.27, 1.31)
SSRIs	89	0.66	(-0.29, 1.61)	0.72	(-0.19, 1.63)

C. Sensitivity Analysis

We conducted a sensitivity analysis using Control Group 3 in order to determine if our estimates of the change of WBC count associated with ‘any SSRI use’ was different with or without stage. The confidence intervals of WBC count associated with ‘any SSRI use’ in the original model excluding stage (95% CI = $-0.45 \times 10^9/\text{L}$, $1.12 \times 10^9/\text{L}$) was similar to our estimate of change when including stage (95% CI = $-0.27 \times 10^9/\text{L}$, $1.31 \times 10^9/\text{L}$) suggesting no difference exists in the models. As a result, the initial results using the model with radiation only was reported, depicted above.

D. Summary of Control Group 3 in Aim 2

Using Control Group 3, we did not observe a statistically significant change in WBC count associated with ‘any antidepressant use’ or ‘any SSRI use’ among the breast cancer controls. The directions of the estimated change in WBC counts were similar between the models of ‘any antidepressant use’ ($0.52 \times 10^9/\text{L}$) and ‘any SSRI use’ ($0.72 \times 10^9/\text{L}$). Although not statistically significant, our results suggest a potential association between ‘any antidepressant use’ or ‘any SSRI use’ and WBC counts. Our results were robust to the effects of stage, suggesting no substantial effect of stage on WBC counts when assessing the effect of ‘any SSRI use’.

IV. Summary of Aim 2

A. Comparisons of Control Groups

Of all the analyses using Control Groups 1, 2, and 3, only analyses using Control Group 1 produced statistically significant results in the direction hypothesized (Table 30). Breast cancer patients who took ‘any SSRIs’ had WBC counts $0.75 \times 10^9/\text{L}$ higher (95% CI = $0.01 \times 10^9/\text{L}$, $1.49 \times 10^9/\text{L}$) than those patients who did not take antidepressants. All analyses showed higher WBC counts associated with ‘any antidepressant use’ or ‘any SSRI use’, even though most were not statistically significant.

Table 30: Adjusted Results for Aim 2 by Control Group and Antidepressant Use

Control Group	Antidepressant Use		SSRI Use	
	Change in WBC count ($\times 10^9/\text{L}$)	95% CI ($\times 10^9/\text{L}$)	Change in WBC count ($\times 10^9/\text{L}$)	95% CI ($\times 10^9/\text{L}$)
Control Group 1	0.49	(-0.15, 1.13)	0.75	(0.01, 1.49)
Control Group 2	0.53	(-0.53, 1.59)	0.89	(-0.35, 2.13)
Control Group 3	0.52	(-0.27, 1.31)	0.72	(-0.19, 1.63)

B. Comparisons between Any Antidepressant Use and Any SSRI Use

Although ‘any antidepressant use’ and ‘any SSRI use’ were both associated with higher WBC counts, only ‘any SSRI use’ had a statistically significant effect estimate (Control Group 1 only). Analyses in all three control groups showed higher WBC counts associated with ‘any SSRI use’ than analyses associated with ‘any antidepressant use’,

although most were not statistically significant. We further discuss this effect in the discussion section.

Chapter 4: Discussion and Conclusion

The purpose of our study was to estimate the odds of central nervous system (CNS) metastasis associated with antidepressant use after cancer diagnoses, and to examine whether antidepressant use was associated with increased systemic inflammation, as estimated by WBC counts. However, we found no significantly greater odds of CNS metastasis associated with ‘any antidepressant’ use, or with a subset of antidepressants, selective serotonin reuptake inhibitors (SSRIs), after adjustment for stage, age, chemotherapy, radiation, surgery, immunotherapy, bone metastasis, bone marrow metastasis, lung and pleura metastasis, and other sites of metastasis, as well as after consideration of race, gender, skin metastasis, and liver metastasis. However, although not statistically significant, the odds of CNS metastases in breast cancer associated with ‘any SSRI use’ was greater than 1.0 (OR=1.73, 95% CI = 0.75, 4.04), suggesting that with a greater sample size, this relative odds estimate would be statistically significant. We also examined the effect of ‘any antidepressant use’ and ‘any SSRI use’ on change in WBC count. A change in WBC count in breast cancer controls was significantly associated with ‘any SSRI use’ (change in WBC count=0.75, 95% CI = 0.01, 1.49) after adjusting for radiation as well as consideration for age, race, stage, immunotherapy, chemotherapy, surgery, and other metastases. Although no other control groups were statistically significant, the change in WBC count associated with ‘any antidepressant use’ and ‘any SSRI use’ was greater than 1.0, suggesting that with a greater sample size, this effect would be statistically significant.

4.1 Differences in Control Groups, Subsets, and Drug Types

Notably, this thesis used three control groups. The primary control group was comprised of all controls (Control Group 1) and two secondary control groups: cancer patients with metastasis other than CNS (Control Group 2) and cancer patients with no metastasis (Control Group 3). Although we did not observe a statistically significant odds of CNS metastasis associated with any class of antidepressant use, our estimates of the relative odds of CNS metastasis associated with antidepressant use were consistently greater than 1.0, which may, in part, be due to a smaller than expected sample size and reduced statistical power. The magnitudes of the odds ratios when Control Group 2 was used ('any antidepressant use': OR = 1.19; 'any SSRI use': OR = 1.31; 'exclusive SSRI use': OR = 1.43) were greater than when Control Group 1 was used ('any antidepressant use': OR = 1.16; 'any SSRI use': OR = 1.21; 'exclusive SSRI use': OR = 1.36) or Control Group 3 ('any antidepressant use': OR = 0.96; 'any SSRI use': OR = 0.85; 'exclusive SSRI use': OR = 1.06). The difference between control groups suggests a possible explanation. Perhaps the metastatic process must be initiated in order for antidepressants to have an effect, because the magnitudes of the odds ratios were greater than 1.0 when the comparison is those patients with other metastasis (Control Group 2), but not those without metastasis (Control Group 3).

Although underpowered, we preplanned subset analyses within the breast cancer and melanoma subsets. As with the main analyses, the breast cancer and melanoma subset analyses found no association between antidepressants or SSRIs and CNS metastasis. In Control Group 2, the direction and magnitudes of the relative odds of CNS

metastasis associated with ‘exclusive SSRI use’ in the breast cancer and melanoma subsets were similar (Breast cancer – OR = 1.66, 95% CI = 0.69, 4.01; Melanoma - OR=1.56, 95% CI = 0.46, 5.35). In the breast cancer subset, ‘any SSRI use’ was associated with a 73% greater odds of CNS metastasis in comparison to those breast cancer patients who did not use antidepressants (95% CI = -25%, 304%). In the melanoma subset, ‘any SSRI use’ was associated with a 24% greater odds of CNS metastasis in comparison to those melanoma patients who did not use antidepressants (95% CI = -57%, 255%). Even though these relative odds were not statistically significant, we believe the magnitude of the effect estimates, particularly in the breast cancer subset, warrants further investigation.

These control groups were then used to assess the change in WBC counts associated with antidepressant use in breast cancer patients. Although not statistically significant, the magnitudes of the effect estimates of antidepressant use on WBC count in each of the three control groups were in the same direction. This consistency suggested antidepressant use was associated with increased systemic inflammation, estimated by WBC counts, when controlling for radiation exposure. We were just underpowered to detect the true effect of antidepressant use on WBC counts.

4.2 Previous Studies

We believe this is the first study to assess the association between antidepressant use and CNS metastasis among breast cancer, melanoma, and non-Hodgkin lymphoma patients. Although not statistically significant, our results suggested an increase in CNS metastasis with those cancer patients who took antidepressants which is consistent with

the findings of Shapovalov *et al.* [14] who found a 52% increase in number of brain metastases in mice associated with fluoxetine. This consistency demonstrates a trend in associations between antidepressants and brain metastasis and suggests future studies are necessary to determine the association between antidepressant use and CNS metastasis.

We then investigated the effect of antidepressant use and SSRI use on systemic inflammation, measured by WBC count. Previously published studies about the effect of antidepressants on WBC count are mixed. For example, Chavda *et al.* [305] found fluoxetine and escitalopram decreased WBC counts $0.8 \times 10^9/L$, while Yurdakul *et al.* [304] found antidepressants increased WBC counts $1.45 \times 10^9/L$. We found a significant increase in WBC count associated with ‘any SSRI use’ (change in WBC count = $0.75 \times 10^9/L$, 95% CI = $0.01 \times 10^9/L$, $1.49 \times 10^9/L$), and a suggestive increase in WBC count associated with ‘any antidepressant use’ (change in WBC count = $0.49 \times 10^9/L$; 95% CI = $-0.15 \times 10^9/L$, $1.13 \times 10^9/L$). Differences in dose of antidepressants could explain this discrepancy. Chavda *et al.* [305] strictly used a dose of 20mg/day, while dose was missing from our data. Tynan *et al.* [302] found SSRIs and SNRIs affected inflammatory markers differently by dose. Since we were using WBC count as a proxy for inflammation, it is possible we could have residual confounding in our antidepressant use due to the missing dose. In designing future studies, dose and duration of antidepressant use should be captured to minimize potential residual confounding.

4.3 Potential Mechanisms

As previously mentioned, although we did not observe a statistically significant odds of CNS metastasis associated with any class of antidepressant use, our estimates of the relative odds of CNS metastasis associated with antidepressant use were consistently greater than 1.0 when using Control Group 2 ('any antidepressant use': OR = 1.19; 'any SSRI use': OR = 1.31; 'exclusive SSRI use': OR = 1.43). Although we did not find any clear association, this increase in the odds of CNS metastasis suggests a role for the blood-brain barrier. However, since these were imprecise estimates due to a smaller than expected sample size, some discussion of potential mechanism(s) is warranted so as to help design a follow-up study to more completely examine this association with adequate statistical power.

Also previously mentioned, a difference existed between the relative odds of CNS metastasis associated with antidepressant use when using Control Group 2 (those with other metastases) and Control Group 3 (those without metastases). We suggest this difference resulted from the control groups being at different points in the metastatic process. Shapovalov *et al.* [14] suggested that fluoxetine may impact several steps in the metastatic process including improved arrest of tumor cells in the brain vasculature and enhanced extravasation [316]. This improvement in these steps of the metastatic process from an antidepressant would only increase CNS metastasis in those cancers that have already begun the metastatic process, such as those of Control Group 2. Whereas, those subjects of Control Group 3 have not begun the metastatic process and would not benefit

from an improvement in any step other than the initial cellular division and growth within the primary tumor.

The third mechanism suggested by Shapovalov *et al.* [14] was inflammation. We used WBC count as a proxy for systemic inflammation. We found that a change in WBC count in breast cancer controls was significantly associated with ‘any SSRI use’ (change in WBC count = $0.75 \times 10^9/L$, 95% CI = $0.01 \times 10^9/L$, $1.49 \times 10^9/L$). Our results are similar to those of Yurdakul *et al.* [304] where WBC counts increased $1.45 \times 10^9/L$ after patients received an antidepressant for two months ($p < 0.05$). As previously mentioned (Section 1.5.3 - Antidepressants Affect the Neuroinflammatory Environment, page 50) findings for the effects of antidepressants on inflammation have been mixed [300-306, 328] and may result from differences between *in vitro* and *in vivo* settings and differences in antidepressants. Additionally, dose may affect inflammation [302]. Increases and decreases in WBC count are listed as side effects for many antidepressants [329]. A possible explanation for this opposite effect is a difference in dose, suggested by Tynan *et al.* [302]. The possibility exists that residual confounding by dose could explain the differences in these studies. Dose of antidepressant use should be considered in future study design to minimize residual confounding and determine if a true association exists between antidepressants and increased inflammation.

4.4 Strengths

There are several strengths and limitations to this thesis, which are described below. First, antidepressant use in our cancer study population (27.7%) was similar to

antidepressant use in previous studies (10% to 34%) [11, 239, 255-262]. Specifically, in breast cancer patients, 34.4% used antidepressants, which is consistent with the maximum reported percentage of breast cancer patients using antidepressants in previous studies (11% to 34%) [11, 239, 255-261]. Additionally, the characteristics of our CNS metastasis cases in our study were similar to what was reported in previous studies [20, 145-147, 150, 153, 156-159, 175, 185, 187, 193, 195, 216, 219, 221, 230, 231, 233, 237]. The similarity of our cases to CNS metastasis subjects in previously reported studies supports that the reason we did not find a clear strong association between CNS metastases and antidepressant use in our study were not due to differences our cases.

Second, we were able to investigate SSRIs in addition to a combined ‘any antidepressants’ category. We examined a population that only developed metastasis and were able to compare that to those cancer patients that developed CNS metastasis. In this study, we preplanned subset analyses, and, within the breast cancer subset using Control Group 2, those cancer patients who took ‘any SSRIs’ had a 73% greater odds of developing CNS metastasis in comparison to those who did not take antidepressants (95% CI = 0.75, 4.04) independent of race, chemotherapy, bone metastasis, and molecular subtype. Although our results were not statistically significant, the magnitude of this effect estimate is suggestive of an increased risk of CNS metastases.

Lastly, this study was designed as a case-control. Although the case-control study design is retrospective in nature, we eliminated some of the problems inherent to retrospective studies. For example, our study eliminated recall bias because the data on antidepressant use was recorded previously. Second, multiple control groups were used to

rule out selection bias as an explanation for study findings. Since effect estimates were generally consistent across control groups, this suggests that the effect estimates were robust to control group.

4.5 Limitations

There were several limitations of the study that should be considered when making inferences. First, our sample size was smaller than originally planned. We initially anticipated we could obtain 447 cases: 239 breast cancer, 117 melanoma, and 94 lymphoma cases, based on the actual number of ICD-9 codes of CNS metastasis (ICD-9 198.3) from January 1st 2005 through September 30, 2012. To address this, we expanded our study time period through September 30, 2013.

Initially, the majority of cases were excluded because the ICD-9 code was incorrect and the cancer patient did not have CNS metastasis. These patients had a spinal bone metastasis, a dural metastasis, or a meningioma, or their oncologist was just checking for CNS metastasis and they were coded incorrectly. Additionally, a case was excluded if they did not have clinical notes or detailed medication information in their medical record. Furthermore, cases were also excluded if they had a diagnosis of lung cancer, if their brain metastasis developed from another cancer other than breast cancer, melanoma, or non-Hodgkin lymphoma, or if their initial cancer was never identified. CNS metastasis cases were excluded if they presented with CNS metastasis or if their first visit to URMH was with their CNS metastasis. Finally, other exclusion reasons included illicit drug use, HIV, and MS. Overall, 496 cancer patients with an ICD-9 code

of CNS metastasis were excluded, resulting in a case ascertainment rate of 27.6% (189 cases included out of 685 total ICD-9 codes of 198.3).

As sample size was a problem with ICD-9 codes, we attempted to identify study subjects using other methods. Since ICD-9 codes did not appear to be accurate in identifying cases, we decided to examine pathology reports. Out of the 212 pathology reports, we were able to add 11 cases to our list. One reason for the low number of pathology reports is that many times biopsy samples of the brain tissue or cerebral spinal fluid are not collected, particularly in those patients with breast cancer and melanoma. Since biopsies are not routine for many of these patients, MRIs were included (ICD-9 code 88.91 - MRI, brain and brain stem). We identified 88 potential cancer patients who received an MRI from 2009 through 2013; data before this date could not be identified. Of these patients, we piloted 47 of them, which resulted in a 0% yield in case abstraction; as a result, we did not feel this was a viable case identification strategy. Additionally, as the majority of brain metastasis patients receive radiosurgery; as a result, we reviewed all breast cancer, melanoma, and non-Hodgkin lymphoma patients with an ICD-9 code for radiosurgery. No additional patients were identified through radiosurgery. After going through each of these newly proposed methods and not substantially increasing our sample size, we are confident that we acquired all the known cases of CNS metastasis at our institution from January 1st, 2005 through September 30th, 2013. Future studies could use ICD-9 codes and be confident they identified the majority of all cases of CNS metastasis at URM. Unfortunately, since we were unable to increase our sample size,

our results were underpowered, and if a true association between the relative odds of CNS metastasis and antidepressant use exists, we were unable to detect it.

We assumed that CNS metastasis occurs similarly and via the same pathway in all cancer types studied. Mechanistically, we have no knowledge if the inflammatory process acts differently across cancers. Additionally, another potential limitation in the grouping of all three cancers into one large group to maximize statistical power is the generalization of our covariates. We grouped lymphoma, a cancer of the blood, with solid tumors: melanoma and breast cancer. Lymphomas cannot be surgically removed, resulting in differences in treatment options between this cancer and breast and melanoma. Additionally, staging was different for these cancers. For example, stage was grouped into I, II, III, and IV, and we could not have stage IA, since that has different meaning for breast cancer, melanoma and lymphoma. We conducted a subset analysis to determine if the effect appears in each cancer separately. However, these analyses were underpowered. This combination of multiple cancers could have induced residual confounding through the mismeasurement of some of these variables.

The study was designed as a case-control, which limited the collection of exposure data to what was recorded in the medical record. This limitation resulted in our primary exposure variable classified as dichotomous (antidepressant use or no antidepressant use). We were unable to collect data on dose or duration of use, which would have more correctly measured antidepressant use as a continuous variable accounting for cumulative exposure. Since antidepressants act differently at different dose levels [302], by using only a binary variable to characterize antidepressant use (Yes

versus No) we may be introducing non-differential exposure misclassification. This misclassification likely biased our results towards the null and underestimated the relative odds of CNS metastasis associated with antidepressant use.

Additionally, the possibility exists that exposure misclassification occurred within our study. Antidepressant use may have not been documented in the patients' charts, particularly if the antidepressant was prescribed outside of oncology. Thus, this non-differential exposure misclassification, which is not likely different for those with and without CNS metastasis, may have biased our results towards the null and underestimated the true odds ratio.

Outcome misclassification could have also occurred. Diagnosis of CNS metastasis was based upon biopsies, MRIs, and CT scans. If a scan or biopsy was misread or incorrect, it would result in outcome misclassification in our study, meaning some controls could have had CNS metastasis or some cases did not. This misclassification would likely be non-differential with regard to antidepressant use (Yes versus No), and thus bias our results towards the null and underestimate the true odds ratio. This misclassification was unlikely since the retrospective nature of this study design relied upon clinical diagnoses. Patients' prognoses and treatments are based upon accurate CNS diagnoses. With the development of the MRI, diagnoses of CNS metastasis decreased upon autopsy from 53% to 15% ($p=0.027$) [164] suggesting an improvement in diagnostic accuracy.

Additionally, uncertainty exists with regards to the latency of CNS metastasis. A lag time likely exists between CNS metastasis onset and CNS metastasis diagnosis.

However, the exact length of time is unknown. We coded those cases and controls who took antidepressants only in the month prior to their study inclusion as not having taking antidepressant use, because we wanted to make sure the exposure occurred before the outcome. Although we proposed that a month was long enough to deal with the impact of this latency, this assumption may not be correct and the actual time may need to be longer. If this assumption is incorrect, we could be creating differential exposure misclassification, which would result in a bias away from the null and an overestimation of the relative odds of CNS metastasis. Additionally, the exact time in which antidepressant use affects CNS metastasis is unknown, in part due to uncertainty with regards to the metastatic process. The exposure must occur once the metastatic cancer cells are in the circulatory system, but this exact time period is difficult to predict. If we were incorrect in our assumption and we included those patients whose only antidepressant use was actually after CNS metastasis, this may have resulted in differential exposure misclassification (i.e. those subjects who were categorized as exposed were really unexposed and this would only occur in those with CNS metastasis) and an overestimation of our effect estimates.

In Aim 2, we used WBC as a surrogate for systemic inflammation. WBC was used because this study is a retrospective study, and WBC is routinely tested. Ideally, we would have conducted a prospective study and collected blood samples or retrospectively conducted serum analysis on samples. Unfortunately due to financial constraints, this study would not have been possible at this time. The possibility exists that the patients WBC count was influenced by other factors, but we attempted to adjust for this issue by

excluding those subjects who recently received treatment or had an infection. If a patient was in a chronic inflammatory state for another reason we were unaware of, we were unable to adjust for that factor. Additionally, we measured systemic inflammation after antidepressant use, making the assumption that antidepressant use may lead to increased systemic inflammation which, in turn, may lead to increased CNS metastasis. The possibility exists that this systemic inflammation led to depression [330] and therefore occurred before antidepressant use.

4.6 Conclusions and Future Directions

This study found no association between antidepressant use or SSRI use and CNS metastasis. Problems with potential exposure misclassification may contribute to this lack of association. Additionally, our analyses may have been underpowered to detect the true relative odds of CNS metastasis associated with antidepressant use, suggested by the relatively large magnitudes and consistent directions of our results.

Although our study resulted in no statistically significant associations between antidepressant use and CNS metastasis, the breast cancer subset had suggestive results in the direction hypothesized (i.e. greater odds of CNS metastasis associated with antidepressant use). We were not powered to detect the odds of CNS metastasis associated with antidepressant use within the subsets, so antidepressants may truly be associated with an increased risk of CNS metastasis. One of the confounders, molecular subtype, was missing in many of the subjects requiring us to exclude those subjects, resulting in decreased sample size and statistical power. Hopefully, since molecular

subtyping is becoming more frequent, future studies can control for this factor in their statistical analyses.

Conversely, when we analyzed the effect of SSRIs on WBC count, we found a statistically significant association. The WBC counts of those breast cancer patients who took ‘any SSRIs’ were $0.75 \times 10^9/L$ higher than those patients who did not take antidepressants while controlling for radiation (95% CI = 0.01, 1.45). These findings suggest that SSRIs increase WBC count, and thus increase the inflammatory state of a cancer patient.

The next steps in this line of research are to repeat this study using a larger sample size, perhaps either adding another cancer center to the current study, or conducting a second study in a single larger cancer center, thereby increasing sample size and statistical power. We would be able to have adequate statistical power for the individual cancer type analyses as well. Ideally, we would be able to link with pharmacy data and identify dose and duration of antidepressant use. It is unknown whether duration or dose of antidepressant affects brain metastasis. Although our study was able to identify different antidepressants and categorize them into their respective classes, the link with pharmacy would improve this process in both speed and accuracy.

Future studies should be conducted in breast cancer patients only, since risk factors and risk for brain metastasis differs by cancer type and our breast cancer results were suggestive of an effect of SSRI use on CNS metastasis. A cohort study would allow an analysis of whether or not antidepressant use affects metastasis in general (e.g. liver

metastasis, lung metastasis, etc.), with what does, and with what duration of use, and then compare those results to CNS metastasis.

Furthermore, we would like to expand our study of antidepressant use on WBC counts. Instead of using WBC count as a surrogate for neuroinflammation, we would like to capture neuroinflammatory markers such as $\text{TNF-}\alpha$ and $\text{IL-1}\beta$. Ideally, we could capture these inflammatory markers at the time of diagnosis and at the time of CNS metastasis, allowing us to examine any differences. Further investigation into the effects of antidepressant use on WBC count and CNS metastasis is required.

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Appendix: Validation Study Results

Table 31: The Number of Patients Taking Antidepressants Before and After Their Cancer Diagnoses

		After Cancer Diagnosis	
Before Cancer Diagnosis	Antidepressant Use	Yes	No
	Yes	11 (37%)	2 (7%)
	No	5 (17%)	12 (40%)

*The patient taking SAME was excluded from this table along with the 4 patients that took antidepressants before our records began.

Table 32: Validation Study Results

Self-Reported Antidepressant Use			
Antidepressant Use in the Medical Record		Yes	No
	Yes	16	2
	No	0	17