COGNITIVE DYSFUNCTION IN CANCER:

NEUROIMAGING AND GENETIC APPROACHES TO IDENTIFY

BIOLOGICAL MECHANISMS

Kelly N. H. Nudelman

Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the Department of Medical and Molecular Genetics,
Indiana University

July 2015

Accepted by the Graduate Faculty of Indiana University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

_____

Andrew J. Saykin, PsyD, Chair

_____

Tatiana M. Foroud, PhD

Doctoral Committee

_____

Brenna C. McDonald, PsyD

April 22, 2015

_____

Bryan P. Schneider, MD

_____

Li Shen, PhD

ACKNOWLEDGEMENTS

I would like to recognize the members of my committee, Drs. Andrew Saykin, Brenna McDonald, Li Shen, Bryan Schneider, and Tatiana Foroud, for their continuous training, guidance, and support. I would also like to thank my internal and external collaborators, as well as the researchers, students, and staff of the Indiana University Center for Neuroimaging and the Department of Medical and Molecular Genetics for their work, advice, and encouragement. The NIH R25 Training in Research for Behavioral Oncology and Cancer Control Program at Indiana University provided training and funding to support this work; I would like to thank all of the students and faculty involved in this program for their collaboration and guidance. Finally, I would like to thank my family for their love and support, particularly my husband, Ross Nudelman, whose patient encouragement lends me strength when mine is gone.

Kelly N. H. Nudelman

COGNITIVE DYSFUNCTION IN CANCER: NEUROIMAGING AND GENETIC APPROACHES TO IDENTIFY BIOLOGICAL MECHANISMS

Although cancer and treatment-associated cognitive dysfunction has been well-documented in the literature, much work remains to elucidate the biological mechanisms driving this effect, hampering current therapeutic efforts. To address this gap, we first reviewed studies utilizing neuroimaging to characterize cognitive dysfunction in cancer, as studies of neurodegenerative diseases point to neuroimaging as a sensitive measure of cognitive dysfunction. This review highlighted the need for longitudinal imaging studies of cancer and treatment-related changes in cerebral structure and function. Subsequently, we utilized multimodal neuroimaging techniques in a female breast cancer cohort to investigate the longitudinal impact of cancer and chemotherapy treatment on cerebral perfusion and gray matter. Our findings indicate that chemotherapy is associated with elevated perfusion, primarily in posterior brain regions, as well as depressed frontal perfusion associated with decreased frontal gray matter density. This pattern of results suggests the involvement of multiple mechanisms of chemotherapy-induced cognitive dysfunction. We also investigated the relationship of cognitive dysfunction and chemotherapy-induced peripheral neuropathy (CIPN), another type of chemotherapy-related nervous system sequelae, again utilizing multimodal, longitudinal neuroimaging, and found that peripheral neuropathy symptoms following chemotherapy were associated with changes in cerebral perfusion and gray matter density. Together, these findings support the hypothesis that multiple biological mechanisms drive cancer and

treatment-related cognitive dysfunction. Interestingly, although cancer is associated with cognitive dysfunction, epidemiological studies have shown that cancer and Alzheimer's disease (AD) are inversely correlated. To extend our imaging analysis beyond breast cancer, we leveraged the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort to investigate the inverse relationship of cancer and AD and investigate the impact of both of these diseases on gray matter density. We found that though the inverse relationship of these diseases was replicated in the ADNI cohort, cancer history was associated with lower gray matter density, similar to findings from breast cancer studies, independent of AD diagnostic group. Finally, we reviewed microRNA studies, as microRNAs are important regulators of many cell signaling pathways and have been actively investigated in relation to both diseases. This review suggests several pathways that may be driving the inverse association and may contribute to cognitive dysfunction.

Andrew J. Saykin, PsyD, Chair

TABLE OF CONTENTS

LIST OF TABLES ix

LIST OF FIGURES xi

LIST OF ABBREVIATIONS xiii

I. Introduction 1

A. Cancer and Cognitive Dysfunction 1

B. Posited Mechanisms of Cancer and Treatment-Related Cognitive Dysfunction 2

C. Cognitive Dysfunction in Cancer and Alzheimer's Disease 6

D. Statement of Purpose 8

II. Neuroimaging, Cancer, and Cognition: State of the Knowledge 10

A. Introduction 10

B. Overview of Findings 11

C. Breast Cancer Survivor Studies 12

D. Pre-Chemotherapy Breast Cancer Studies 16

E. Longitudinal Breast Cancer Treatment Studies 18

F. Non-Breast Cancer Studies 20

G. Clinical Implications and Future Research 22

H. Conclusion 23

III. Altered Cerebral Blood Flow One Month after Systemic Chemotherapy for Breast Cancer: A Prospective Study Using Pulsed Arterial Spin Labeling MRI Perfusion 24

A. Introduction 24

B. Methods 25
C. Results 32
D. Discussion 53
IV. Cerebral Perfusion and Gray Matter Changes Associated with Chemotherapy-Induced Peripheral Neuropathy (CIPN) 59
A. Introduction 59
B. Methods 59
C. Results 65
D. Discussion 78
V. Association of Cancer History with Alzheimer's Disease Onset and Structural Brain Changes 82
A. Introduction 82
B. Methods 85
C. Results 95
D. Discussion 110
VI. Functional MicroRNAs in Alzheimer's Disease and Cancer: Differential Regulation of Common Mechanisms and Pathways 117
A. Introduction 117
B. MiR-9 126
C. MiR-29 130
D. MiR-101 134
E. MiR-107 135
F. MiR-125b 138

G. MiR-146 141
H. MiR-153 144
I. MiR-195 146
J. Pathways 148
K. Conclusions 158
VII. Conclusions and Future Directions 161
References 168
Curriculum Vitae

LIST OF TABLES

1. Breast Cancer Survivor Neuroimaging Studies 13

2. Pre-Chemotherapy Breast Cancer Neuroimaging Studies 17

3. Longitudinal Breast Cancer Neuroimaging Studies 19

4. Non-CNS, Non-Breast Cancer Neuroimaging Studies 21

5. Breast Cancer Cohort Demographics 34

6. Regional Perfusion Changes for the Overall F Test, All Groups by Times 38

7. Regional Perfusion Changes for Ctx+ Post-Treatment Increase 42

8. Regional Perfusion Changes for Ctx+ Increase Relative to Controls 45

9. Right Precentral Gyrus Perfusion Group Means 46

10. Global Cognitive Performance 48

11. Regional Perfusion Changes for Ctx+ Perfusion and GMD Positive Association 52

12. Cohort Demographics and Treatment Data 66

13. Chemotherapy-Induced Peripheral Neuropathy Symptom (CIPN-sx) Change over Time 69

14. Clusters of Significant Cerebral Blood Flow (CBF) 72

15. ADNI Study Design 87

16. ADNI Total Cohort Demographics (N=1609) 97

17. Cancer Categories Count and Percentage by Diagnostic Group 100

18. Cancer History by Baseline AD Diagnostic Group 104

19. Age of AD Onset (AoO) by Cancer History 107

20. MiRNA Expression in Cancer and AD 160

LIST OF FIGURES

1. Biological Mechanisms Posited to Drive Cancer and Treatment-Related Cognitive Dysfunction....4

2. Group-by-Time Analysis (F Test)....37

3. Maximum Perfusion Change....39

4. Ctx+ Group Post-Treatment Hyperperfusion....41

5. Ctx+ Perfusion Increase Compared to HC....44

6. Ctx+ Baseline Cognitive Performance and Perfusion Change Correlation....49

7. Perfusion and GMD Positive Association....51

8. Chemotherapy-Induced Peripheral Neuropathy Symptoms (CIPN-sx) Positively Associated with Perfusion....71

9. Chemotherapy-Induced Peripheral Neuropathy Symptoms (CIPN-sx) Associated with Perfusion Increase....74

10. Gray Matter Density (GMD), Perfusion, and Chemotherapy-Induced Peripheral Neuropathy Symptom (CIPN-sx) Change Correlations....76

11. Cerebral Perfusion Decrease Associated with Gray Matter Density Decrease in Regions of Interest....77

12. Categorized Cancer Types Count out of 593 Total Incidences....99

13. Percent of Individuals with Cancer History (CA+) per Diagnostic Group....103

14. Survival Analysis of Age of AD Onset by Cancer History....106

15. Lower Gray Matter Density (GMD) with Cancer History across Diagnostic Groups 109
16. MiRNA Generation and Function 119
17. MiRNA Involvement in the Amyloid Pathway Appears to Contribute to AD 122
18. Molecular and Cellular Pathways Important to AD and Cancer 125
19. Summary of MiRNA Pathway Relationships in Cancer and AD 150
20. MicroRNA Involvement in the Inflammation, Innate Immunity, and Oxidative Stress Pathways in AD and Cancer 153
21. Redundant MicroRNA Mechanisms Regulating Proliferation and Survival Pathways in Cancer 156

LIST OF ABBREVIATIONS

1H-MRS	Proton MR spectroscopy
1M	One month post-treatment study time point
1Y	One year post-treatment study time point
Aβ	Amyloid-beta peptide fragment
Aβ42	Amyloid-beta peptide fragment containing 42 residues
AD	Alzheimer's disease
ADNI	Alzheimer's Disease Neuroimaging Initiative
APOBEC3G	Apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G
APOE	Apolipoprotein E gene
APP	Amyloid protein precursor gene
AML	Acute myelogenous leukemia
ATC	Anaplastic thyroid carcinoma
BACE1	Beta-site APP cleaving enzyme 1 gene
BL	Baseline, post-surgery, pre-treatment study time point
BMF	BCL-2 modifying factor gene
BRMS1	Breast cancer metastasis suppressor 1 gene
CDK6	Cyclin-dependent kinase 6 gene
CDKN1A	Cyclin-dependent kinase inhibitor 1 gene
CDR	Clinical Dementia Rating
CES-D	Center for Epidemiologic Studies-Depression Scale
CFH	Complement factor H gene
CIPN	Chemotherapy-induced peripheral neuropathy
CIPN-sx	Chemotherapy-induced peripheral neuropathy symptoms

CN	Cognitively normal older adults
CNS	Central nervous system
COX2	Cytochrome c oxidase subunit II gene
CRC	Colorectal cancer
CSF	Cerebrospinal fluid
Ctx+	Breast cancer patients treated with chemotherapy
Ctx-	Breast cancer patients treated without chemotherapy
DICER1	Dicer 1, ribonuclease type III gene
DTI	Diffusion tensor imaging
E2F3	E2F transcription factor 3 gene
EGFR	Epidermal growth factor receptor gene
EIF2C2	Eukaryotic translation initiation factor 2C, 2
EZH2	Enhancer of zeste homolog 2 gene
FA	Fractional anisotropy
FDG	Fluorodeoxyglucose
FLAIR	Fluid attenuated inversion recovery
fMRI	Functional magnetic resonance imaging
FOXO1	Forkhead box O1 gene
GI	Gastrointestinal cancer
GLUT3	Glucose transporter type 3 gene
GM	Gray matter
GMD	Gray matter density
GRN	Granulin gene
HC	Healthy controls
HCC	Hepatocellular carcinoma

HIF1b	Hypoxia inducible factor-1beta gene
HL	Hodgkin lymphoma
HNSCC	Head and neck squamous cell carcinoma
HuR	Hu antigen R gene (also ELAVL1)
ICV	Intracranial volume
IL-1β	Interleukin-1β
IRAK1	Interleukin (IL)-1 receptor-associated kinase 1 gene
IRAK2	Interleukin (IL)-1 receptor-associated kinase 2 gene
IRS2	Insulin receptor substrate 2 gene
k	Minimum cluster extent threshold
KDR	Kinase insert domain receptor gene (also *VEGFR2*)
MCI	Mild cognitive impairment
MCL1	Myeloid cell leukemia sequence 1 gene
MEG3	Maternally expressed gene 3
miRNA	MicroRNA
MMP2	Matrix metalloproteinase 2
MMP9	Matrix metalloproteinase 9
MMSE	Mini-Mental State Exam
MNI	Montréal Neurological Institute
MPRAGE	Magnetization prepared rapid gradient echo
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
NF-kappaB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
NMSC	Non-melanoma skin cancer

NSCLC	Non-small cell lung cancer
PACE	Prospective acquisition correction
PASL	Pulsed arterial spin labeling
P_{crit}	Voxel-wise critical significance threshold
PD	Parkinson's disease
PET	Positron emission tomography
PIGF	Placenta growth factor gene
PMI	Post-mortem interval
PKCδ	protein Kinase Cδ
RISC	RNA-induced silencing complex
SMC	Significant memory concerns
SNP	Single nucleotide polymorphism
STAI-S	State Trait Anxiety Inventory-State subscale
TRAF6	TNF receptor-associated factor 6
UTR	Untranslated region
VBM	Voxel-based morphometry
VE-cadherin	Vascular endothelial cadherin
VEGF	Vascular endothelial growth factor gene
WM	White matter

I. Introduction

A. Cancer and Cognitive Dysfunction

Cognitive dysfunction in breast cancer, also referred to as 'chemobrain' in common parlance, has become a research focus of increasing concern given the high five-year survival rate of approximately 89% for the U.S. population from 2004 to 2010 [1]. Although there has been some research on cognitive dysfunction in other types of cancer, breast cancer has become the apparent 'model system' for cancer and treatment-related cognitive dysfunction given the high survival rate, availability of subjects, and subsequent focus on survivor quality of life. To date, the majority of this research has been conducted on the effects of chemotherapy treatment, although there is also evidence that breast cancer may have some impact on cerebral function independent of treatment as well [2-5]. Research has indicated that chemotherapy treatment is associated with more cognitive complaints, lower neuropsychological test scores, and a number of altered neurophysiological measures obtained via neuroimaging, which can persist for years following treatment [6-19].

Increasingly, studies of cancer and chemotherapy-induced cognitive dysfunction are incorporating neuroimaging methodologies. Studies of Alzheimer's disease (AD) have suggested that various neuroimaging methodologies may be more sensitive than clinical neuropsychological testing, as neuroimaging can detect biological changes years before an individual would receive a clinical diagnosis [20]. Neuroimaging studies of cancer and treatment-related cognitive dysfunction were initially conducted in cancer survivors. These

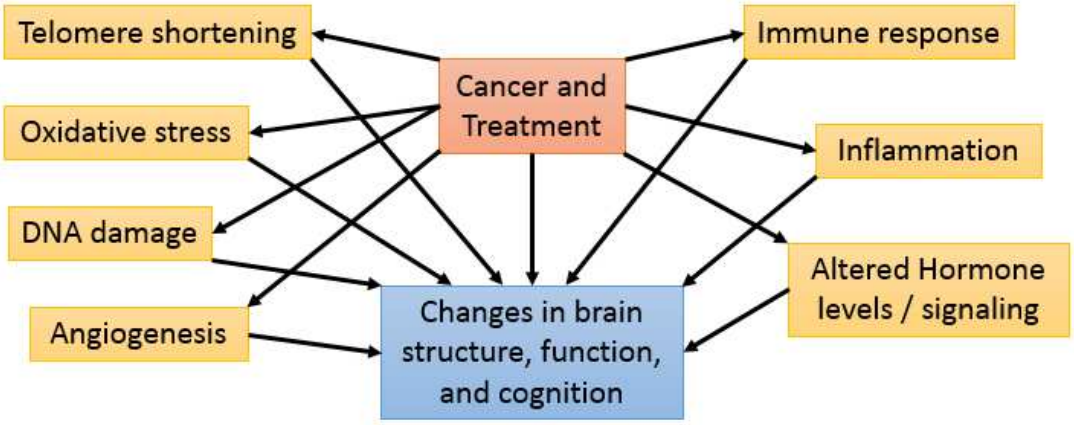
studies showed significant structural and functional changes; however, there were a number of limitations to these study designs, including in many instances the lack of control groups of cancer survivors not treated with chemotherapy, as well as the inability to track change over time to better understand the short and long-term consequences of cancer and treatment. To address this gap, more recent studies have investigated cognitive function in pre-treatment cancer patients, and there have also been a handful of reported longitudinal neuroimaging studies measuring pre- to post-treatment cognitive changes. Significant findings from these studies, discussed in more detail in Chapter II, highlight the utility of these methodologies and the importance of continuing to employ new methodological advances in this field. The cerebral structural and functional information provided by neuroimaging studies may better elucidate the biological mechanisms of cancer and treatment-related cognitive dysfunction, and help direct future research and therapeutic efforts.

B. Posited Mechanisms of Cancer and Treatment-Related Cognitive Dysfunction

There are a number of biological mechanisms which have been proposed to contribute to the development and persistence of cancer and treatment-related cognitive dysfunction [21-23]. Although direct neurotoxicity was initially dismissed given the known interference of the blood brain barrier with chemotherapy delivery to the brain, studies have shown higher than expected levels of some chemotherapeutic agents in brain tissues, reviving this hypothesis [24-28]. Indirect mechanisms leading to cognitive dysfunction have been posited to

include activation or perturbation of inflammatory and immune responses, oxidative stress, DNA repair, telomere shortening, and angiogenic pathways, as well as perturbations in hormone levels and/or signaling by cancer and/or associated treatment(s), particularly chemotherapy and hormone therapies (Figure 1). Furthermore, some of these pathways have been suggested to feed back on themselves; for example, cancer and chemotherapy-related telomere shortening may also be exacerbated by cancer and treatment-related increases in oxidative stress [21]. It is reasonable to posit that more than one mechanism is likely to contribute to cancer and treatment-related cognitive dysfunction, and furthermore that these contributions may vary across individuals; however, at the time that this work was conceptualized, there were very few studies equipped to address this question. Multimodal imaging studies might begin to do so, by investigating whether results observed with different types of imaging modalities, including gray and white matter changes and cerebral function, are correlated or independent.

Figure 1. Biological Mechanisms Posited to Drive Cancer and Treatment-Related Cognitive Dysfunction. Diagram arrows show directionality of hypothesized mechanisms from cancer and treatment (initiation, orange box) to posited mechanisms (yellow boxes) to cerebral structure and functional alterations and cognitive dysfunction (outcomes, blue box). A final arrow from cancer and treatment to outcome represents the hypothesis of direct neurotoxicity resulting from chemotherapy treatment.



We hypothesized that investigation of biological mechanisms driving cancer and treatment-related cognitive dysfunction may be more successful if expanded to encompass more types of cancer and treatment-related sequelae. Of particular interest, although chemotherapy treatment has been shown to cause both peripheral and central nervous system sequelae, there is a lack of investigation of the possible correlation of these sequelae. Boland and colleagues (2014) provided evidence that study participants with multiple myeloma experiencing chemotherapy-induced peripheral neuropathy (CIPN) demonstrated altered cerebral activation during painful stimulation as measured with functional MRI (fMRI) compared to healthy controls, suggesting that these patients experienced a different mechanism of central pain processing [29]. More evidence is needed to show whether this peripheral nervous system sequela relates to cerebral blood flow and structure, or to previously observed neuroimaging measures of chemotherapy treatment-related effects. Given that genetic factors such as variants affecting chemotherapy drug clearance (and toxicity) or DNA repair could theoretically predispose to both sequelae, it is possible that there exists a subgroup of patients at greater risk to develop both. Determining whether there is a correlation between these sequelae could provide direction for future therapeutic efforts.

Similarly, it has been suggested in previous literature that only a subgroup of patients treated with chemotherapy experience significant cognitive effects; identification of this more vulnerable subgroup could be a useful factor in treatment decision-making [21,22,30,31]. Genetics have been repeatedly posited

to impact cognitive sensitivity to treatment; however, to date, there is a paucity of research on this topic [18,21,22]. Only two studies on breast cancer survivors have found genetic association with neuropsychological performance; both tested specific hypotheses involving one candidate gene apiece [32,33]. No studies have been performed using results from neuroimaging analyses as quantitative traits, though these phenotypes might be more sensitive, given the subclinical range of neuropsychological change typically observed in cancer survivors, indicating a significant gap in the literature.

C. Cognitive Dysfunction in Cancer and Alzheimer's Disease

Although findings from neuroimaging studies have provided a number of useful endophenotypes for genetic analyses, these studies have not yet been performed. Genetic analysis is limited by the relatively small number of participants typically collected by these studies. To begin to overcome this limitation, it was posited that larger neuroimaging datasets of other types of cognitive dysfunction might be leveraged. The Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort includes over sixteen hundred participants, approximately a third of whom are cancer survivors, with extensive data including a number of different types of neuroimaging, neuropsychological testing, self-reported cognitive function, genotyping, and medical and demographic information.

This cohort provided a unique opportunity to address questions raised by earlier studies of cancer and treatment-related cognitive dysfunction. The many types of cancer included in the sample permitted analysis to determine whether

findings from cognitive dysfunction studies in breast cancer survivors are more broadly applicable across other cancer types. If this is so, this cohort may in the future provide an opportunity for future research to utilize neuroimaging measures of cancer and treatment-related cognitive dysfunction as quantitative traits in genome-wide association studies, providing the first genome-wide investigation of cancer and treatment-related cognitive dysfunction. More immediately, this cohort also presented a chance to investigate the impact of cancer and treatment-related cognitive dysfunction on the neurodegenerative process of AD.

The impact of cancer and treatment-related cognitive dysfunction on AD is particularly interesting in light of apparently conflicting observations and hypotheses. On the one hand, it has been suggested that there are common biological mechanisms underlying the development of cancer, related cognitive dysfunction, and aging, and that there are some similar cerebral structural and function changes observed in neuroimaging studies of cancer compared to those of healthy aging and AD [34]. On the other hand, there are a growing number of epidemiological studies that indicate that cancer and AD are inversely associated, suggesting that cancer history may actually protect individuals from developing AD [35-42]. Because most studies of cancer and treatment-related cognitive dysfunction to date have been conducted in adults aged less than sixty-five years, there is currently insufficient evidence to directly address the impact of cancer and treatment-related cognitive dysfunction on the aging process and neurodegenerative disease. Determining the impact of cancer and treatment on

cerebral structure and function in older patients, particularly in a cohort of patients spanning the neurodegenerative disease spectrum from cognitively normal individuals to those with AD, would aid in clarifying this apparent conflict of evidence, as well as potentially elucidating some of the biological mechanisms driving these changes.

Another tactic toward this goal is to synthesize and compare the current literature examining the genetic mechanisms driving cancer and AD. An excellent example of this is the review by Tabares-Seisdedos and colleagues (2011), which highlighted a number of shared genes identified independently in studies of cancer and AD [43]. As intriguing as this overlap is, there is still much work to be done describing the biological mechanisms driving the inverse association of these diseases. We hypothesized that a useful extension of this work might similarly compare studies of cancer and AD to identify microRNAs (miRNAs) which were involved in both disease processes. Since one miRNA may regulate many genes, and be involved in many cell signaling pathways, we posited that identifying miRNAs important to both disease processes might shed more light on biological mechanisms driving both diseases, and provide more information to address the mechanisms driving the inverse association of cancer and AD.

D. Statement of Purpose

With the large and increasing number of cancer survivors, it is becoming increasingly important to better understand cancer- and treatment-related cognitive dysfunction, with the eventual goal of developing more effective therapies and interventions. Additionally, considering the growing elderly

population, it is important to better comprehend how cancer survivorship and potential cognitive dysfunction may impact the aging process and the development of AD.

Accordingly, the goals of this work were as follows:

1. Critically review existing neuroimaging studies of cancer and treatment-related cognitive dysfunction, and utilize available longitudinal neuroimaging breast cancer cohort data to address gaps in the literature.

2. Investigate the impact of cancer history on AD age of onset and cerebral gray matter density.

3. Critically review studies of miRNAs in cancer and AD to elucidate biological mechanisms potentially contributing to the inverse association of these diseases.

We hypothesized that applying advanced multimodal neuroimaging methodology to the study of cancer and treatment-related nervous system sequelae would aid in clarifying the relationship of results observed in previous studies, and addressing the question of whether multiple biological mechanisms drive cognitive dysfunction. Additionally, we hypothesized that results similar to those observed in previous cancer neuroimaging studies would be observed in the ADNI cohort, extending these findings beyond breast cancer. Finally, we hypothesized that a review of miRNAs identified independently in cancer and AD would yield common biological pathways, which might prove key to the inverse association of these diseases and provide direction to future therapeutic efforts for both diseases.

II. Neuroimaging, Cancer, and Cognition: State of the Knowledge

A. Introduction

Multiple neuroimaging techniques have been applied in studies of cancer- and chemotherapy treatment-related cognitive dysfunction, with promising results. Magnetic resonance imaging (MRI) most commonly is employed. MRI uses radio frequencies to manipulate magnetization of various types of nuclei in the body, with the resulting signatures used to produce detailed 2- or 3-dimensional images [44,45]. This technology has been adapted to measure a number of different factors, including brain gray matter (GM), white matter (WM), and neural activity using functional MRI (fMRI). WM structure and directional diffusion, or 'fractional anisotropy' (FA), is measured through diffusion tensor imaging (DTI) of magnetized cerebral water flow [46,47]. Brain activation is obtained using magnetized hemoglobin to observe oxygenated blood flow; increased blood flow to active areas is measured during tasks, and fMRI has been reliably correlated with neural activity [48-50]. Proton MR spectroscopy (1H-MRS) also uses magnetic resonance technology to measure levels of brain metabolites and neurochemical changes [51]. MRI techniques have the advantage of being non-invasive and do not require ionizing radiation, permitting multiple measurements and longitudinal studies. Positron emission tomography (PET) is another technique that has been employed to measure brain activity and metabolism using an injected radioactive tracer coupled to a bioactive molecule; two common tracers which will be discussed are [O-15], which measures blood flow, and [F-18] Fluorodeoxyglucose (FDG), which measures metabolism [52-

54]. These techniques can be used to investigate neurophysiological changes and may help explain the mechanisms of cognitive dysfunction in cancer patients.

The purpose of this research brief is to review the current literature on neuroimaging studies of cancer and chemotherapy-induced cerebral alterations, and to provide perspective on the state of research and future directions. Our primary goal is to review and synthesize the evidence regarding the impact of non-central nervous system cancer and related treatment on brain structure and function. Treatments administered for cancers in the central nervous system (CNS) and lymphatic systems operate under different parameters and goals and are beyond the scope of this review [55]. Findings from imaging studies have the potential to identify causal mechanisms and possible therapeutic directions for cancer and treatment-related cognitive dysfunction.

B. Overview of Findings

We reviewed 35 neuroimaging studies. The overwhelming majority of the work in this area has been focused on breast cancer patients, with 27 breast cancer studies [2,4-17,23,56-67] and only eight studies in other cancers [68-75]. In the breast cancer studies, we noted that 18 studies were focused on survivors [6-11,13,14,23,56,57,59-63,66,67], three were pre-treatment cancer studies [2,4,5], and six were prospective, longitudinal studies in which women were followed pre- and post-treatment [12,15-17,58,64]. These studies are grouped by methodology and information is provided regarding authors, cohorts, methods, and results in Tables 1-3. The majority of non-breast cancer studies (see Table

4) were focused on the association of metabolism with psychological factors or cancer. In summary, research to date has been focused on the cognitive effects of breast cancer treatment, likely due to the large pool of survivors with cognitive concerns [18,23,65,76]. This brief provides an overview including all types of neuroimaging studies on multiple types of cancer.

C. Breast Cancer Survivor Studies

Neuroimaging studies began with a focus on survivors treated with chemotherapy. Initial findings on this topic presented in 2003 indicated that chemotherapy treatment was associated with structural changes in gray matter (GM), white matter (WM) loss, and abnormal regional cerebral metabolism measured by PET [23,77,78]. The focus of these breast cancer studies (Table 1) was on patients treated with chemotherapy (Ctx+). All studies included a Ctx+ category, and 15 included healthy controls (HC) [6-8,11,13,14,23,56,57,59-63,66]. However, only seven studies included non-chemotherapy-treated survivors (Ctx-), so possible interpretations are limited since in most studies differentiation between changes caused by chemotherapy treatment versus cancer was not possible [9-11,13,61,66,67].

Table 1. Breast Cancer Survivor Neuroimaging Studies

Study	Cohort	PCI[a]	Method	Results
Saykin et al. [23]	12 Ctx+[b], 12 HC	>5Y	sMRI	Ctx+: ↓ WM and GM
Yoshikawa et al. [67]	44 Ctx+, 31 Ctx-	>3Y	sMRI, NP	Ctx+: No treatment associations
Ferguson et al. [59]	1 Ctx+, 1 HC	22M	s/fMRI, NP, SR	Ctx+: ↑ SR, WM damage, WMem activation
Silverman et al. [66]	16 Ctx+, 5 Ctx-, 13 HC	5-10Y	O-15 & FDG PET, NP	Ctx+: altered CBF during memory task; resting metabolism correlated with task performance
Inagaki et al. [61]	1Y: 51 Ctx+, 54 Ctx-, 55 HC; 3Y: 73 Ctx+, 59 Ctx-, 37 HC	1Y and 3Y	sMRI, NP	1Y Ctx+: ↓ GM and WM vs. Ctx-, not vs. H. 3Y Ctx+: no treatment association with GM/WM
Abraham et al. [56]	10 Ctx+, 9 HC	22M	DTI, NP	Ctx+: ↓ PS, FA
Kesler et al. [62]	14 Ctx+, 14 HC	>6M	fMRI	Ctx+: activation ↓ encoding, ↑ recall in VDM task
Kesler et al. [13]	25 Ctx+, 19 Ctx-, 18 HC	5Y	fMRI, NP, SR	Ctx+ & Ctx-: ↓ activation for EF task Ctx+: ↑ SR complaints, NP errors, ↓ PS, ↓ activation correlated with SR, disease severity
de Ruiter et al. [10]	19 Ctx+, 15 Ctx-	>9Y	fMRI, NP	HD Ctx+: ↓ activation for EF and EMem tasks, NP
Deprez et al. [11]	17 Ctx+, 10 Ctx-, 18 HC	2-4M	DTI, NP, SR	Ctx+: ↓ FA, ↑ MD vs. Ctx- and HC; FA correlated with attention, PS, SR
Bergouignan et al. [6]	16 Ctx+, 21 HC	18-36M	sMRI, NP	Ctx+: ↓ GM, ↓ NP; GM correlated with NP
Kesler et al. [57]	42 Ctx+, 35 HC	4.8Y	sMRI, INF, NP	Ctx+: ↓ GM, NP, ↑ INF; ↓ GM correlated with INF
Koppelmans et al. [14]	184 Ctx+, 368 HC	21Y	sMRI	Ctx+: ↓ TBV, GM
Koppelmans et al. [63]	187Ctx+, 374 HC	21Y	DTI	Ctx+: WM integrity correlated with time since treatment; no change vs. HC
Hosseini et al. [60]	37 Ctx+, 38 HC	4.5Y	sMRI	Ctx+: ↓ GM connectivity, organization, integration
Bruno et al. [7]	34 Ctx+, 27 HC	5.35Y	fMRI, SR	Ctx+: ↑ SR, ↓ global cluster, nodal degree, hubs

de Ruiter et al. [9]	17 HD Ctx+, 15 Ctx-	>9Y	DTI, sMRI, NP, SR	Ctx+: ↑ SR, ↓ NP, GM, focal FA;↑ MD correlated with ↓ neural markers
Conroy et al. [8]	24 Ctx+, 23 HC	3-10Y	s/fMRI, NP, SR, Comet	Ctx+: ↓ GM, WMem activation, NP; GM correlated with PCI, NP, activation correlated with PCI, SR, OD

Ctx+=survivors treated with chemotherapy, Ctx-=survivors not treated with chemotherapy, HC=healthy controls, Y=year, M=month, HD=high dose, sMRI=structural MRI, fMRI=functional MRI, DTI=diffusion tensor imaging, O-15 PET=Oxygen-15 positron emission tomography, FDG-PET=18F Fluorodeoxyglucose positron emission tomography, Comet=assay of oxidative DNA damage, OD=Oxidative DNA damage, NP=neuropsychological testing, SR=self-report cognitive complaints assessment, ↓=decrease, ↑=increase, GM=gray matter, WM=white matter, WMem=working memory, CBF=cerebral blood flow, PS=processing speed, FA=fractional anisotropy, MD=mean diffusivity, VDM=verbal declarative memory task, EF=executive function, EMem=episodic memory, TBV=total brain volume, INF=Serum inflammatory cytokine levels
a. PCI=post-chemotherapy interval; when one number is listed, this is the average length of time post-treatment. b. 10 breast cancer, 2 lymphoma survivors

The major endpoints of these studies were cerebral structural and activation changes. GM and WM damage consistently were reported in survivors except for Yoshikawa et al. (2005). This discrepancy may be explained by unique cohort characteristics such as ethnicity, since the majority of studies were conducted with Caucasian patients and this study only included Japanese individuals [67]. Authors of three studies reported association of these changes with increased cognitive complaints or decreased neuropsychological test performance [6,8,11]. Results from all six functional studies demonstrated activation or metabolic changes in survivors [8,10,13,59,62,66]. The direction of activation change seems to be task-dependent. In two studies activation change was found to be correlated with increased cognitive complaints [8,13]. Importantly, in two studies de Ruiter and colleagues found treatment-related cognitive alterations almost a decade after treatment, accompanied by lower neuropsychological test performance and increased cognitive complaints [9,10]. Koppelmans et al. (2012, 2014) conducted two studies with a very large survivor cohort over 20 years post-treatment. Findings included decreased brain volume, GM, and decreased WM integrity with increasing time since treatment, supporting the idea that breast cancer, treatment, or both are responsible for long-term possibly deleterious cognitive changes [14,63]. Results from all but one of these studies support the association of chemotherapy treatment with some measure of cognitive structural or function alteration which could lead to cognitive dysfunction. The majority of these findings are accompanied by neuropsychological testing deficits, increased self-reported cognitive complaints, or both, indicating the functional relevance of

these measures. However, more work is needed to discern which measures are specific to treatment and which to cancer.

D. Pre-Chemotherapy Breast Cancer Studies

Prompted by the need to discriminate between effects of breast cancer and treatment, three imaging studies were designed specifically to examine the influence of breast cancer on cognition (Table 2) [2,4,5]. Breast cancer patients were examined before treatment and compared to HC with fMRI during neuropsychological tasks. Activation decrease was observed for patients during response inhibition and working memory tasks, while activation increase was observed during a visuospatial task. Interestingly, two studies by Scherling et al. (2011, 2012) were performed in the same cohort using different tasks and found evidence that activation increase or decrease may be dependent on the type of task. These activation changes were not associated with test performance changes, suggesting that they may be compensatory [4,5]. This lack of association suggests that while breast cancer does appear to influence cognitive activation, the effects may vary depending on the cognitive process being assessed. Activation also may be a more sensitive measure of change than test performance.

Table 2. Pre-Chemotherapy Breast Cancer Neuroimaging Studies

Study	Cohort	Method	Results
Cimprich et al. [2]	10 PC, 9 HC	fMRI	PC: ↓ speed, accuracy for verbal WMem task, ↑ activation
Scherling et al. [4]	23 PC, 23 HC	fMRI	PC: ↑ activation during VS task, ↓ reaction time, errors
Scherling et al. [5]	23 PC, 23 HC	fMRI	PC: ↓ activation during RI task, no performance change

PC=cancer patients who have not yet received chemotherapy, HC=healthy controls, fMRI=functional MRI, ↓=decrease, ↑=increase, WMem=working memory, VS=visuospatial, RI=response inhibition

E. Longitudinal Breast Cancer Treatment Studies

The existing longitudinal studies particularly are helpful in differentiating cancer and chemotherapy effects, especially as four of the six studies reviewed included Ctx- and HC (Table 3) [15-17,58,64]. Pre-treatment measures for all patients also allow discrimination of cancer and chemotherapy effects over time. Results of all six studies demonstrated some cerebral changes in cancer patients compared to controls, and results of the five studies with Ctx- controls indicated that some of these changes are specifically attributable to chemotherapy, while others appear to occur in cancer patients regardless of treatment. These findings suggest that while cancer patients experience cognitive alterations, chemotherapy may have independent effects. Thus Ctx+ patients may experience increased alterations compared to Ctx- patients, and may be at increased risk for cognitive sequelae. Two studies were designed to investigate effects of cancer and chemotherapy more than four months post-treatment with Ctx+, Ctx-, and HC groups. Findings included independent cancer and treatment-related activation and GM changes post-treatment [16,17]. At one year post-treatment, some activation changes still were observed in Ctx+ and Ctx-, and GM decrease was not fully recovered in Ctx+ patients, indicating that structural and functional changes can persist for significant periods of time.

Table 3. Longitudinal Breast Cancer Neuroimaging Studies

Study	Cohort	Measures	Method	Results
McDonald et al. [15]	17 Ctx+, 12 Ctx-, 18 HC	BL, 1M, 1Y	sMRI	Ctx+ & Ctx-: ↓ GM from BL to 1M Ctx+: some changes persist at 1Y
McDonald et al. [16]	16 Ctx+, 12 Ctx-, 15 HC	BL, 1M, 1Y	fMRI	Ctx+ & Ctx-: ↑ frontal, ↓ left parietal BL WMem activation, 1M ↓ frontal activation, 1Y partial recovery Ctx+: ↑ frontal activation at BL, 1M, 1Y
McDonald et al. [17]	27 Ctx+, 28 Ctx-, 24 HC	BL, 1M	sMRI, SR	Ctx+: ↓ GM at 1M; ↑ SR correlated with ↓ GM
Ganz et al. [12]	49 Ctx+, 44 Ctx-	8.7MD, 14.7MD, 20.7MD	SR, NP, FDG-PET, INF	Ctx+: 8.7MD ↑ SR, ↑ INF, INF correlated to inferior frontal metabolism; longitudinal ↓ INF correlated to ↑ SR
Deprez et al. [58]	34 Cxt+, 16 Ctx-, 19 HC	BL, 3-4M	DTI, NP	Ctx+: ↓ NP at 1M vs. BL; NP correlated with ↓ FA
Lopez Zunini et al. [64]	21 Ctx+, 21 HC	BL, 1M	fMRI, NP	Ctx+: ↓ VMem activation at BL, 1M vs. BL

Ctx+=survivors treated with chemotherapy, Ctx-=survivors not treated with chemotherapy, HC=healthy controls, BL=baseline (pre-chemotherapy), M=month post-treatment, MD=month post-diagnosis, Y=year post-treatment, sMRI=structural MRI, fMRI=functional MRI, SR=self-report cognitive assessment, FDG-PET=[F-18] Fluorodeoxyglucose positron emission tomography, INF=inflammatory markers, DTI=diffusion tensor imaging, NP=neuropsychological testing, ↓=decrease, ↑=increase, GM=gray matter, WMem=working memory, FA=fractional anisotropy, VMem=verbal memory

F. Non-Breast Cancer Studies

As stated previously, there is a dearth of research on this topic in non-breast cancer cancers. Additionally, of the eight studies found (Table 4), three studies were focused on the correlation of metabolism with psychological factors instead of cognitive factors [68-75]. However, these studies are still informative given that depression and cognitive complaints previously have been linked [79]. These findings provide indirect evidence for association of brain metabolism with cognitive complaints. Interestingly, results of a study in a lung cancer cohort indicated that patients had increased metabolism pre-treatment, suggesting that cancer may have a transitory metabolic effect on the brain in lung cancer [69]. Results from another study of lung cancer indicated that patients had altered neurochemistry pre-treatment, further supporting the hypothesis that lung cancer may alter cerebral activity [68].

Table 4. Non-CNS, Non-Breast Cancer Neuroimaging Studies

Study	Cancer	Cohort	Design	Method	Results
Tashiro et al. [75]	Various	19 PC, 17 HC	CrS	FDG-PET	Cancer: ↓ metabolism
Tashiro et al. [73]	Various	1 PC, 19 Ctx+/-, 10 HC	CrS	FDG-PET, SR	Cancer: ↓ metabolism
Tashiro et al. [74]	Various	2 PC, 7 Ctx+, 12 Ctx-, 10 HC	CrS	FDG-PET, SR	Cancer: ↓ metabolism; metabolism correlated with depression Ctx+: ↓ posterior metabolism
Tashiro et al. [71]	Various	4 PC, 3 Ctx-, 1 Ctx+	CrS	FDG-PET, NKA, SR	Cancer: Metabolism, NKA, and anxiety correlated
Tashiro et al. [72]	Various	11 Ctx-, 5 Ctx+	CrS	FDG-PET, SR	Cancer: Metabolism correlated with social desirability
Kumano et al. [70]	Various	6 Ctx+, 13 Ctx-	Longitu-dinal	FDG-PET, SR	Cancer: BL metabolism associated with depression change over time
Golan et al. [69]	lung	18 PC, 8CS, 11 L	PC CrS	FDG-PET	PC: ↑ metabolism
Benveniste et al. [68]	lung	17 PC, 15 HC	PC CrS	1H-MRS	PC: ↓ Neural markers

PC=cancer patients who have not yet received chemotherapy, Ctx+=cancer patients treated with chemotherapy, Ctx-=cancer patients not treated with chemotherapy, HC=healthy controls, CS=cancer survivors, L=individuals with benign lesions, CrS=cross sectional study design, FDG-PET=[F-18] Fluorodeoxyglucose positron emission tomography, SR=self-reported cognitive/psychological measures, NKA=natural killer cell activity, 1H-MRS=Proton magnetic resonance spectroscopy, ↓=decrease, ↑=increase, BL=baseline, Cancer=individuals with cancer, regardless of treatment status or time

Six studies were conducted in a mixed cancer population. All were limited by the assumption that the cancer types included in the studies affect the brain in a similar manner, which may not be true [70-75]. Only one study was designed to investigate the effect of chemotherapy treatment on metabolism. Decreased metabolism in Ctx+ patients was found, demonstrating that other cancer populations do experience treatment changes [74]. Three studies in mixed cohorts of treated and untreated cancer patients found decreased metabolism associated with cancer, supporting the possibility of cancer-induced cerebral alterations in non-breast cancer patients [73-75]. However, in seven of the eight studies only FDG-PET imaging was used. Clearly, more research is needed to investigate other imaging types in these populations. Future work also should include longitudinal studies with Ctx+, Ctx-, and HC groups to identify cancer and chemotherapy-specific changes, and should control for cancer type, or focus on one cancer.

G. Clinical Implications and Future Research

Oncology nurses and other healthcare providers should understand the role neuroimaging can play in identifying cognitive changes associated with cancer and cancer treatment, as well as the impact of these changes on social relationships, everyday functioning and work ability [80]. Directions for future neuroimaging research are: (1) to elucidate cancer and treatment-related changes in more diverse cohorts; (2) to utilize a range of imaging methodologies, as most studies to date have been focused solely on MRI and fMRI; and (3) to

utilize neuroimaging in interventional cognitive research to establish efficacy as well as elucidate therapeutic mechanisms of action.

H. Conclusion

Results from neuroimaging studies in breast cancer cohorts have provided solid evidence supporting a variety of cerebral structural and functional alterations associated with cancer and chemotherapy treatment. Evidence from breast cancer survivors suggests that some of these changes persist for years. Little imaging research has been conducted in other cancer types; however, preliminary studies support cancer-related cerebral metabolic changes. More research is needed to clarify the individual roles of cancer and treatment-related changes, especially in non-breast cancer populations.

This chapter was modified from research printed in the journal *Seminars in Oncology Nursing*.

Holohan KN, Von Ah D, McDonald BC, Saykin AJ (2013) Neuroimaging, Cancer, and Cognition: State of the Knowledge. *Semin Oncol Nurs* 29: 280-287.

III. Altered Cerebral Blood Flow One Month after Systemic Chemotherapy for Breast Cancer: A Prospective Study Using Pulsed Arterial Spin Labeling MRI Perfusion

A. Introduction

Breast cancer treatment has made great strides, leading to an overall five-year survival rate of 89% according to SEER [81]. Given this high survival rate, resulting side effects are an area of increasing concern. Cognitive dysfunction in particular has been associated with breast cancer, chemotherapy, radiation, and anti-estrogen treatments, underscoring the need to clarify the mechanisms of disease and treatment effects on cognition [3,82-94].

Neuroimaging has been used to measure structural and functional effects of cancer and chemotherapy treatment, including treatment-associated frontal gray matter density (GMD) decrease in breast cancer patients [15,17,61]. However, characterization of the full spectrum of cerebral alterations associated with chemotherapy is far from complete; one novel area of investigation is the effect of chemotherapy on resting cerebral perfusion using pulsed arterial spin labeling (PASL) magnetic resonance imaging (MRI). PASL is a noninvasive advanced technique capable of measuring blood flow by using magnetically labeled arterial blood water as an endogenous contrast tracer. This technique can provide quantitative, stable, and physiologically meaningful images [95]. It has been shown to be well-suited to longitudinal studies of cerebral perfusion in healthy and diseased individuals, or as a surrogate marker of metabolism [96]. Based on previous prospective breast cancer studies, observed GMD decrease post-

treatment suggests the likelihood of accompanying hypoperfusion; however, patterns of increased and decreased activation observed during cognitive task performance post-treatment might indicate the presence of hyperperfusion as well [15-17,61,66]. To test these competing hypotheses, we conducted the first controlled prospective study of cerebral perfusion in breast cancer patients treated with (Ctx+) and without (Ctx-) standard-dose chemotherapy and healthy controls (HC) using PASL MRI. We predicted that the Ctx+ group would evidence statistically significant changes in brain perfusion compared to the HC and Ctx- control groups post-treatment, in response to or in order to compensate for chemotherapy-induced cellular, vascular, or other tissue damage, and that this change would correlate with cognitive performance. Additionally, we analyzed perfusion changes for associations with previously reported frontal GMD change to investigate the functional independence of these measures and underlying mechanisms [17].

B. Methods

Ethics Statement

All participants gave written informed consent according to the Declaration of Helsinki under a protocol approved by the Indiana University Institutional Review Board.

Overall Study Design

This manuscript focuses on a subset of data from a prospective, longitudinal investigation of cancer and treatment-related cognitive effects which includes a comprehensive study protocol of neuroimaging, neurocognitive, and behavioral

assessments as well as biomarker investigation. Other aspects of this and a related cohort have previously been reported [15-17,97]. In brief, study participants include Ctx+ and Ctx- female breast cancer patients and demographically matched HC. For Ctx+ patients, all study measures were acquired at baseline (after surgery but before radiation, chemotherapy, and/or anti-estrogen treatment), approximately one month after chemotherapy treatment completion, and approximately one year later. These time points were chosen to allow examination of both sub-acute and longer-term effects of treatment, with a particular focus on the effects of chemotherapy. Ctx- patients and HC were studied at yoked intervals (see Table 5). In addition to the PASL and T1 sequences examined for this report, the detailed MRI examination included PD/T2 and FLAIR structural sequences, working and episodic memory functional MRI tasks, magnetic resonance spectroscopy, and diffusion tensor imaging. Neurocognitive assessment included a targeted research battery with emphasis on episodic memory and executive functioning, as well as measures briefly assessing other neuropsychological domains (e.g., general intellectual ability, language, attention, processing speed, visuospatial and motor skills, etc.). Self-report measures included demographic and medical information, treatment sequelae, self-perceived cognition, mood and anxiety, and diet and lifestyle factors. Finally, blood samples were acquired for routine labs, genetic analyses, and banked for future investigation of potential biomarkers. Blood work was typically acquired in the early morning, while other study measures were acquired over the course of one or two study visits depending on participant

preference. MRI scanning was conducted prior to cognitive assessment whenever possible, though timing of study measures varied depending on scheduling availability.

Participants

The study cohort consisted of 27 Ctx+ patients, 26 Ctx- patients, and 26 HC. Only patients with non-invasive (stage 0) or non-metastatic invasive (stages I, II, or III) disease were included; additional exclusion criteria for all participants included prior cancer, substance abuse, and other medical, neurological, and psychiatric risk factors which might affect cerebral structure or function, as described in McDonald et al. (2013) [17]. Based on these criteria, 213 individuals were determined to be ineligible during phone screening. Another 15 individuals were excluded after enrolling in the study due to subsequent determination of ineligibility (e.g., tissue expanders, claustrophobia, etc.), withdrawal, or loss to follow-up, and 18 more individuals were excluded from this analysis due to lack of relevant data or scan quality, yielding 79 eligible participants with required data.

All Ctx+ patients were treated with common standard-dose chemotherapy regimens. Eight neoadjuvant chemotherapy patients' baseline measures were completed before surgery as well as chemotherapy; these individuals did not differ significantly from the other Ctx+ patients for any demographic factors, depression, or anxiety. Patients were recruited via the Indiana University Melvin and Bren Simon Cancer Center recruitment core and affiliated clinical practices.

Demographically matched healthy controls were recruited via community advertisements.

Demographic and treatment characteristics are summarized in Table 5. The Center for Epidemiologic Studies-Depression Scale (CES-D) and the State Trait Anxiety Inventory-State subscale (STAI-S) were used to measure depressive symptoms and anxiety at each visit [98,99]. Self-reported caffeine consumption on scan days was also obtained. Demographic and treatment variables were assessed for statistical significance using SPSS 19 (SPSS Statistics 19, IBM Corporation, Somers, NY), using ANOVA, general linear models, and Chi-square analyses as appropriate.

MRI Acquisition

Scans were acquired on a Siemens Tim Trio 3T whole-body MRI scanner using a 12-channel receiver-only head coil. Subjects were scanned in a conscious resting state with closed eyes. Cerebral perfusion measurements were obtained using a previously published scan protocol [95]. Briefly, a Q2TIPS PASL sequence using the PICORE labeling scheme was applied. Utilizing a 10 cm labeling region with 25 mm spacing from the distal edge of the labeled region to the image section, an adiabatic inversion pulse for labeling was followed by optimized inversion time delays TI1 = 700 ms and TI2 = 1800 ms, chosen so as to minimize intravascular signal intensity at 3T. Images were acquired using a gradient-echo single shot EPI readout, with acquisition parameters: TR/TE = 3000/13 ms, FOV = 224 mm, and matrix = 64x64. The imaging region consists of 16 contiguous ascending axial slices of 7 mm thickness. Each perfusion

measurement consists of 100 dynamic (50 control and label image pairs) plus one M0 image (the equilibrium brain tissue magnetization used to normalize the difference perfusion map) with a scan time of approximately 5 minutes. The scanner's built-in 3D online prospective acquisition correction (PACE) was used to minimize head motion artifact during acquisition. A high resolution T1-weighted magnetization prepared rapid gradient echo (MPRAGE) image and a high resolution EPI whole brain scan were acquired for subsequent reference and normalization; T2-weighted and fluid attenuated inversion recovery (FLAIR) sequences were also acquired to examine for incidental pathology.

Image Analysis

PASL scan processing was performed using previously published methods [92]. In brief, labeled images were subtracted from matched control images to create a perfusion-weighted time series; these were used to create quantitative regional perfusion maps for each scan, which were normalized to Montréal Neurological Institute (MNI) space in SPM8 (Wellcome Department of Cognitive Neuroscience, London, UK), resampled to 2 mm3 voxels, and smoothed with a FWHM kernel of 6x6x8 mm. Image analyses in SPM8 were run with and without age at baseline and scan-day caffeine consumption as covariates given previous findings of perfusion variance [100,101]. However, inclusion of these variables is not shown as groups were balanced and these variables did not significantly alter any results.

A general linear model approach was utilized to conduct statistical parametric mapping on a voxel-by-voxel basis in SPM8. To specifically examine perfusion in

gray matter, the Pick Atlas gray matter mask was used as an explicit mask in all analyses [102]. MRI image acquisition and analysis of GMD was performed as described in McDonald et al. (2013) [17].

Baseline analyses using a random effects model were performed to identify voxels with initial perfusion differences between groups. Weighted contrast vectors were entered for each group in the design matrix as described in McDonald et al. (2013) [17]. To test for areas in which cancer patients showed hypoperfusion relative to controls at baseline, -1 was entered in the Ctx+ and Ctx- baseline columns, while 2 was entered in the control baseline column. Alternate directionality and other interactions were similarly tested. The voxel-wise critical significance threshold (P_{crit}) was set to 0.001 uncorrected, with a minimum cluster extent (k) of 100 voxels for this and subsequent tests to reduce noise.

An unbiased F test of overall effects was performed to identify any statistically significant perfusion differences between groups and times. The cluster of voxels with the most significant perfusion difference was used to graph group-by-time contrast estimates and 90% confidence intervals. All groups were tested alone for perfusion change over time using weighted contrast vectors. Based on these results, the Ctx+ group was analyzed for perfusion increase over time relative to HC in an interaction model, also using weighted contrast vectors.

Perfusion and Cognition Analysis

Due to the small cohort size, we chose to investigate cognition utilizing an overall neuropsychological performance test score (global score), generated by

averaging scores for eight neuropsychological domains in a similar fashion as in Conroy et al. (2013) [8] and Ahles et al. (2008, 2010) [84,85]. To discern whether chemotherapy was associated with a change in cognition, global score change was assessed for association with treatment group in SPSS 19 using a one-way ANOVA. Based on the results, global baseline score was chosen for testing with Ctx+ perfusion change. We extracted baseline and post-treatment cluster scores for the right precentral gyrus (area of significant increase in Ctx+ compared to HC) using MarsBar in SPM8, and subtracted baseline from post-treatment scores to obtain a change score for each Ctx+ individual [103]. Ctx+ perfusion change scores were tested for Pearson correlation with global baseline scores in SPSS.

Perfusion and GMD Change Association Analysis

To test for association of GMD and perfusion, we compared the previously identified frontal GMD change clusters with the more regionally diffuse pattern of change observed for perfusion. Association was only tested in the Ctx+ group, since only this group had statistically significant changes in both measures. To analyze this association, we extracted the two frontal clusters where significant GMD decreases occurred after chemotherapy using MarsBar in SPM8 and averaged them to obtain one GMD measure for each subject at each time point [103]. The measures at baseline were subtracted from the post-treatment measures, yielding a change score for each individual. These GMD change scores were used as the covariate of interest in a multiple regression testing for association with whole brain perfusion change. Perfusion change was measured by subtracting PASL baseline scans from post-treatment scans using the ImCalc

utility in SPM8 to create new scans only including regions of change. After multiple regression association testing of frontal GMD change with whole brain perfusion change in SPM8, again using the Pick Atlas gray matter mask, positive and negative associations were analyzed. Covarying for age did not significantly alter the results and is not reported. To graph GMD and perfusion, statistically significant perfusion change clusters from the positive association were extracted using MarsBar and averaged to one change score per individual; these were plotted against the GMD change measure. One individual's perfusion and GMD scores were more than three standard deviations below the mean. Inclusion/ exclusion of this individual's scores did not alter the statistical significance of the findings, so these data points are excluded from the graphical presentation of the results.

C. Results

Demographic and Baseline Comparisons

Ctx+ patients had significantly higher stage disease than Ctx- patients (X^2 =18.82, df = 3, $P < 0.001$), as expected given current treatment protocols, and most had not received either radiotherapy or hormone therapy by one month post-chemotherapy treatment. There were no other statistically significant demographic differences or group-by-time interactions with depression or anxiety symptoms (CES-D, STAI-S, Table 5). The second scan occurred on average six months after the baseline visit; interscan intervals did not differ significantly between groups (Table 5). Eight patients received neoadjuvant treatment; these individuals did not demonstrate statistically significant demographic differences

or group-by-time interactions with depression or anxiety symptoms compared to adjuvant Ctx+, Ctx-, or HC groups.

Baseline comparisons indicated no significant between-group differences in perfusion.

Table 5. Breast Cancer Cohort Demographics

Variable	Ctx+ (N = 27)	Ctx- (N = 26)	HC (N = 26)	*P*[a]
Age at Baseline (yrs.)	49.9 (7.6), (36-69)	52.0 (8.9), (31-68)	48.4 (10.1), (32-69)	0.350
Education (yrs.)	15.4 (2.8), (10-20)	15.4 (2.5), (11-20)	15.6 (2.1), (12-20)	0.954
Estimated Full Scale IQ (Barona Index) [104]	110.1 (6.7), (89-117)	111.1 (6.2), (98-117)	111.2 (5.2), (99-116)	0.760
Handedness (R,L/Amb)	26,1	24,2	24,2	0.662
White, Non-Hispanic	77.8%	88.5%	80.8%	0.588
CES-D raw score: Baseline	10.9 (9.7), (0-38)	9.0 (8.8), (0-34)	7.2 (7.6), (0-28)	
CES-D raw score: 1M	14.6 (9.3), (2-33)	9.8 (9.7), (0-40)	6.9 (7.0), (0-25)	0.216
STAI-S raw score: Baseline	35.1 (15.0), (20-78)	28.9 (8.0), (20-47)	30.9 (10.2), (20-55)	
STAI-S raw score: 1M	35.4 (12.4), (20-62)	32.2 (13.0), (20-64)	32.4 (12.4), (20-62)	0.601
Inter-scan interval (days)	158.7 (68.9), (73-387)	177.2 (72.2), (106,480)	159.6 (28.0), (116-253)	0.465
Cancer stage: 0 (DCIS)	0	6		
Cancer stage: I	11	18		0.000
Cancer stage: II	12	2		
Cancer stage: III	4	0		
Received radiotherapy	2	16		0.000
On anti-estrogen therapy[b]: Baseline	0	1 TAM		
On anti-estrogen therapy: 1M	1 ANA, 1 TAM, 1 LET	11 TAM, 1 TAM/LEU, 4 LET, 2 ANA, 1 EXE, 1 RAL		
Chemotherapy Regimen[c,d]				

DOX/ CYC/ paclitaxel	8			
DOC/ CYC	8			
DOC/ carboplatin	6			
DOC/ DOX/ CYC	2			
DOC/ cisplatin	1			
DOX/ CYC	1			
paclitaxel	1			

Values are Mean (Standard Deviation), (Range). Ctx+=breast cancer patients treated with chemotherapy; Ctx-=breast cancer patients not treated with chemotherapy; BL=baseline (post-surgery, pre-treatment); 1M=one month post-chemotherapy treatment completion; CESD=Center for Epidemiologic Studies-Depression Scale; STAI=State Trait Anxiety Inventory-State subscale.
a. P value for one-way ANOVA with treatment groups for age, education, IQ, handedness, ethnicity, interscan interval, and radiotherapy; group-by-time analysis with treatment group for CESD and STAI, Chi-square with treatment group for cancer stage. b. ANA = anastrozole; TAM = tamoxifen, LET=letrozole; EXE=exemestane; RAL=raloxifene; LEU=lueprolide acetate.
c. Nine Ctx+ patients were also treated with trastuzumab, one was also treated with sunitinib, and one was also treated with bevacizumab. d. DOX=doxorubicin, CYC=cyclophosphamide, DOC=docetaxel.

Perfusion Change: Chemotherapy Group Hyperperfusion

An F test across all groups and both time points indicated statistically significant perfusion change over time, primarily in the right hemisphere (Figure 2, Table 6). The cluster of maximal change, located in the right postcentral gyrus, is presented for all groups and both time points (Figure 3). In this region, the Ctx+ group showed hyperperfusion at one month post-treatment relative to both comparison groups.

Figure 2. Group-by-Time Analysis (F Test). Surface rendering of perfusion differences from baseline to one month post-treatment for all groups (P_{crit}<0.001 uncorrected, k=100). Colored areas indicate statistically significant changes between groups and/or times; red to yellow color scale.

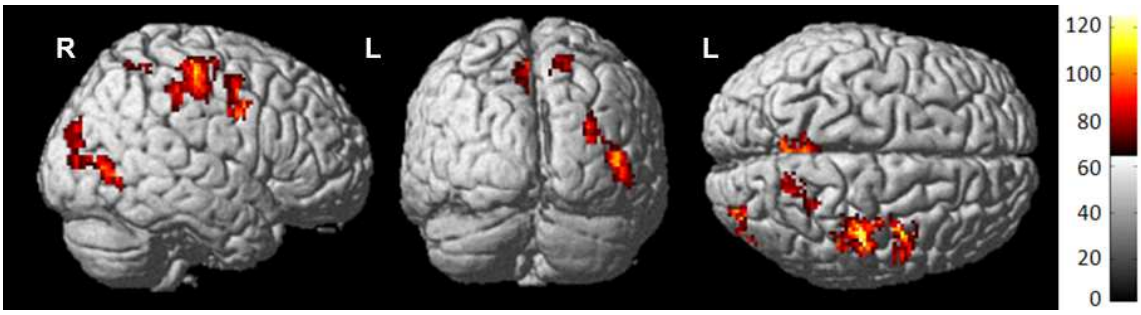
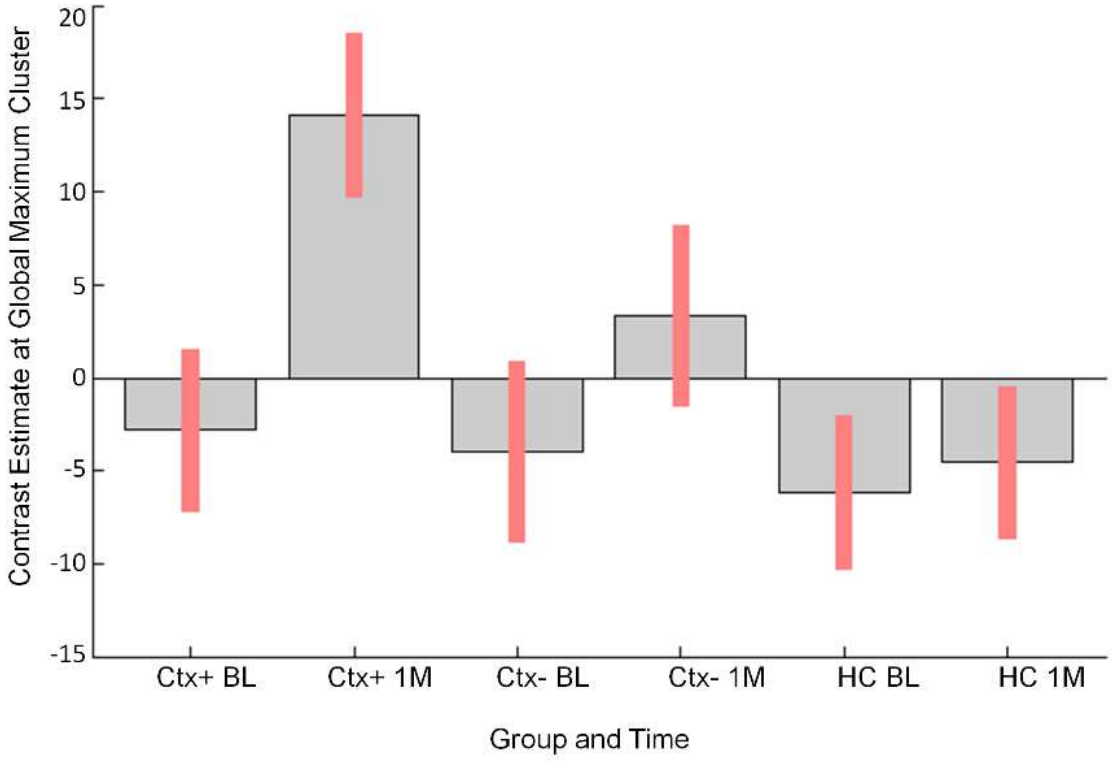


Table 6. Regional Perfusion Changes for the Overall F Test, All Groups by Times.

x[a]	y[a]	z[a]	k[b]	T	Z	$P_{FWE\text{-}corr}$[c]	Region description (for cluster peak)
44	−24	56	437	8.32	4.87	0.009	R Postcentral G (BA3)
16	−58	54	112	7.91	4.72	0.018	R Precuneus (BA7)
48	−70	2	268	7.78	4.67	0.022	R M Temporal G (BA37)
42	0	48	183	7.74	4.65	0.024	R Precentral G (BA6)
-10	-46	50	264	6.39	4.10	0.217	L Precuneus (BA7)

R=Right, L=Left, G=Gyrus, BA=Brodmann Area, I=Inferior, M=Middle, S=Superior
a. MNI coordinates. b. Cluster extent. c. Peak-level p value

Figure 3. Maximum Perfusion Change. Right postcentral gyrus perfusion signal from overall F test graphed by group and time (Red bars indicate 90% confidence intervals (C.I.)). Key: BL=baseline, 1M=one month post-treatment.



To further elucidate group differences in change over time, each group was tested individually. Only the Ctx+ group had statistically significant perfusion change, demonstrating an increase in perfusion from baseline to one month post-treatment, primarily in superior and posterior brain regions (Figure 4, Table 7).

Figure 4. Ctx+ Group Post-Treatment Hyperperfusion. Surface rendering of Ctx+ increase from baseline to one month post-treatment indicates that bilateral superior and posterior brain regions demonstrate hyperperfusion post-treatment.

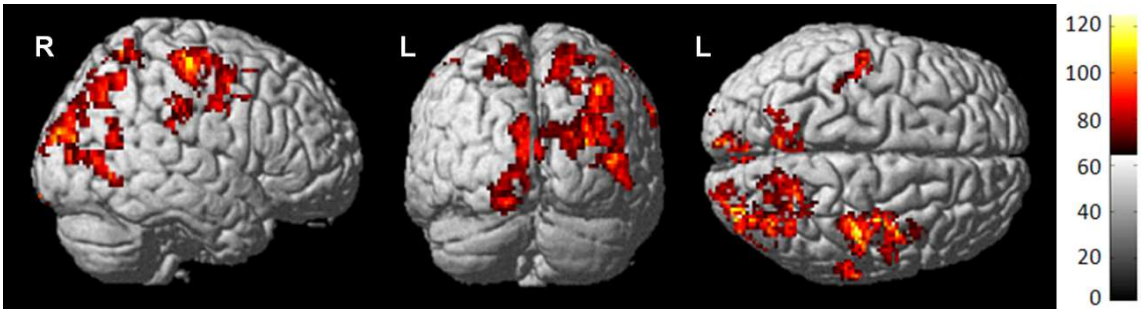


Table 7. Regional Perfusion Changes for Ctx+ Post-Treatment Increase.

x^a	y^a	z^a	k^b	T	Z	$P_{FWE\text{-}corr}{}^c$	Region description (for cluster peak)
46	-22	56	673	5.32	5.09	0.012	R Postcentral G (BA3)
32	-84	24	1464	5.20	4.98	0.000	R S Occipital G (BA19)
66	-24	34	116	5.03	4.83	0.552	R Postcentral G (BA2)
-14	12	-10	119	4.27	4.14	0.540	L Lentiform Nucleus
-6	-86	26	425	4.26	4.14	0.059	L Cuneus (BA19)
-6	-64	56	207	4.22	4.10	0.282	L Precuneus (BA7)
-16	-94	-16	101	4.20	4.08	0.612	L I Occipital G (BA17)
-54	-18	54	128	4.07	3.96	0.507	L Postcentral G (BA3)

R=Right, L=Left, G=Gyrus, BA=Brodmann Area, I=Inferior, M=Middle, S=Superior
a. MNI coordinates. b. Cluster extent. c. Peak-level p value

To determine how much of this increase was statistically significantly different than controls, we analyzed group-by-time interactions; results indicated that the Ctx+ group perfusion increased relative to HC specifically in the right precentral gyrus after treatment, indicating that these patients had resting state hyperperfusion discernible by MRI compared to controls (Figure 5, Table 8). Table 9 lists mean perfusion for both time points as well as perfusion change for the right precentral gyrus cluster.

Figure 5. Ctx+ Perfusion Increase Compared to HC. Surface rendering of Ctx+ increase compared to HC over time indicates statistically significant perfusion increase in Ctx+ in the right precentral gyrus post-treatment.

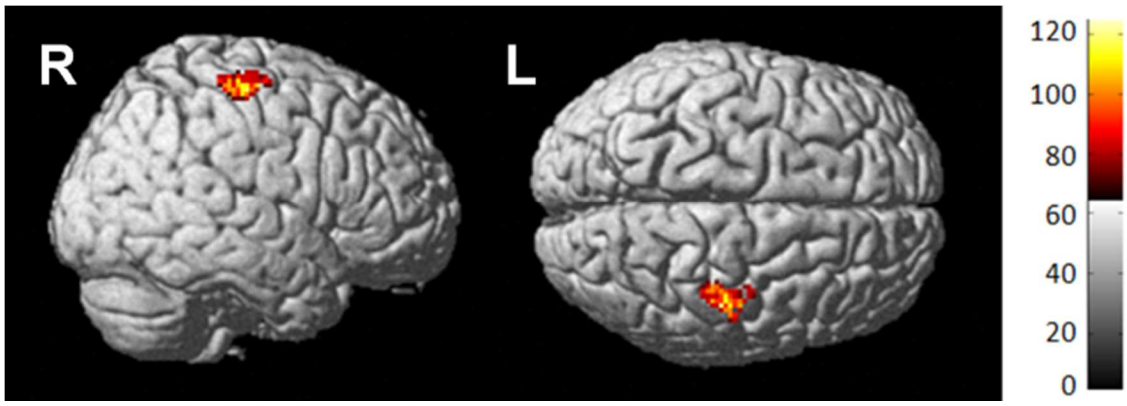


Table 8. Regional Perfusion Changes for Ctx+ Increase Relative to Controls.

x^a	y^a	z^a	k^b	T	Z	$P_{FWE\text{-}corr}{}^c$	Region description (for cluster peak)
40	-24	60	151	4.22	4.10	0.429	R Precentral G (BA4)

R=Right, L=Left, G=Gyrus, BA=Brodmann Area, I=Inferior, M=Middle, S=Superior
a. MNI coordinates. b. Cluster extent. c. Peak-level p value

Table 9. Right Precentral Gyrus Perfusion Group Means

Variable	Ctx+ (N=27)	Ctx- (N=26)	HC (N=26)	*P*[a]
Cluster Perfusion: Baseline	35.0 (10.7), (18.1-53.7)	33.9 (13.3), (2.5-57.5)	35.9 (10.4), (18.1-57.7)	0.821
Cluster Perfusion: 1M	49.7 (18.9), (21.5-96.6)	38.8 (16.5), (-2.6-81.7)	33.9 (10.6), (12.8-56.8)	0.002
Cluster Perfusion: Change[b]	15.0 (16.7), (-9.6-57.2)	4.9 (14.7), (-22.1-33.9)	-2.0 (9.0), (-20.9-11.5)	0.000

Values are Mean (Standard Deviation), (Range). 1M=one month post-treatment
a. Significance, one way ANOVA with treatment group. b. Change=1M – Baseline.

The eight neoadjuvant patients were not analyzed separately, as the study design was not powered to detect subgroup effects. However, in the neoadjuvant-treated participants the mean perfusion increase in the right postcentral gyrus was almost identical to that observed for the adjuvant Ctx+ participants and Ctx+ group as a whole. The three Ctx+ patients who received anti-estrogen treatment in this interval also displayed a post-chemotherapy perfusion increase, though there was insufficient power for a formal assessment of hormonal effects or interactions. Given that only two Ctx+ patients received radiotherapy treatment during this interval, this factor is also unlikely to contribute significantly to the observed variation.

Chemotherapy-Associated Hyperperfusion and Cognition

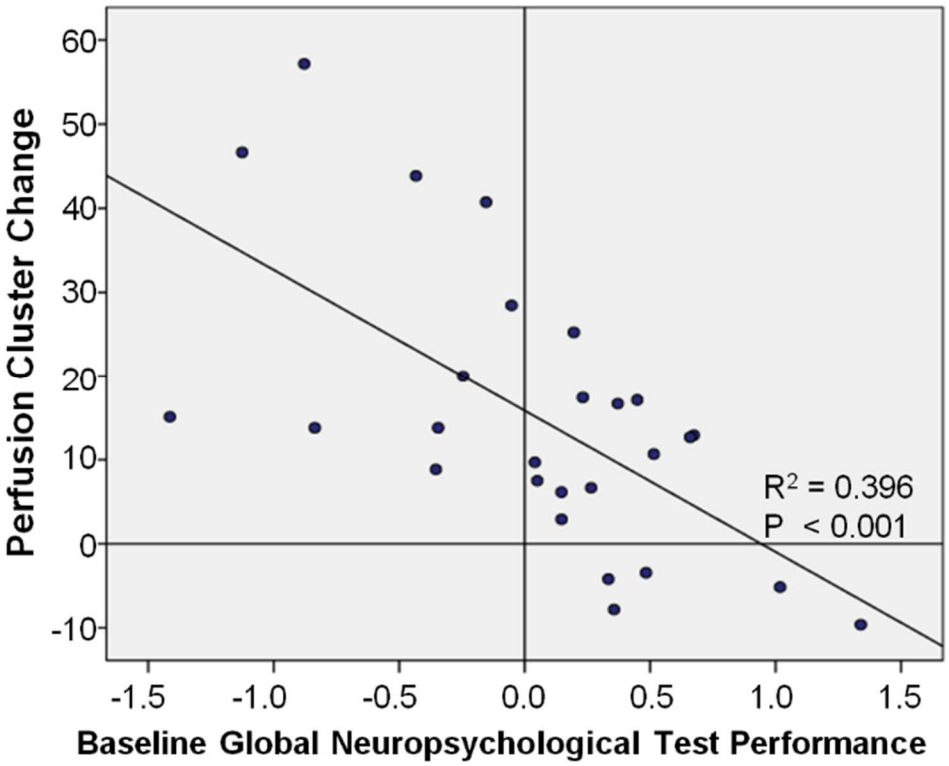
To clarify the clinical significance of this perfusion increase, we assessed perfusion change for correlation with cognitive performance. Change in the global neuropsychological test score from baseline to post-treatment was not statistically significantly different between groups, although all groups showed an increase over time likely attributable to practice effects (Table 10). In the Ctx+ group, baseline global score was negatively correlated ($r = -0.629$, $P < 0.001$) with right precentral gyrus perfusion change from baseline to one month (Figure 6), indicating that individuals with lower baseline cognitive performance showed greater perfusion increase over time.

Table 10. Global Cognitive Performance

Variable	Ctx+ (N=27)[a]	Ctx- (N=25)[a]	HC (N=26)	*P*[b]
Global score: Baseline	0.1 (0.6), (-1.-1.3)	0.2 (0.6), (-1.6-1.0)	0.0 (0.7), (-1.7-1.0)	0.8
Global score: 1M	0.2 (0.6), (-1.0-1.4)	0.3 (0.6), (-1.3-1.3)	0.2 (0.7), (-1.8-1.3)	0.8
Global score change[c]	0.1 (0.2), (-0.2-0.9)	0.2 (0.2), (-0.2-0.5)	0.2 (0.2), (-0.1-0.8)	0.6

Values are Mean (Standard Deviation), (Range). 1M=one month post-treatment
a. Due to missing neuropsychological data, a global score could not be calculated for one Ctx+ individual at 1M (1M Ctx+ N=26), as well as one Ctx- individual at either time point. b. Significance, one way ANOVA with treatment group c. Change=1M - Baseline

Figure 6. Ctx+ Baseline Cognitive Performance and Perfusion Change Correlation. Individual baseline global neuropsychological test performance (x-axis) graphed with right precentral gyrus perfusion cluster (statistically significant increase in Ctx+ vs. HC from baseline to post-treatment) change from baseline to one month post-treatment, indicating a significant negative correlation of baseline cognition with perfusion change.



Perfusion and GMD Association

Because the chemotherapy-associated perfusion increase was not located in the frontal regions where GMD decrease was observed by McDonald et al. (2013) [17], we hypothesized that these effects might be independent. When the frontal GMD decrease was analyzed for association with whole brain perfusion change, we observed that there were statistically significant regions of bilateral frontal and parietal perfusion change positively correlated with GMD change (Figure 7, Table 11), indicating that decreased frontal GMD was associated with lower perfusion in these regions ($r = 0.553$, $P = 0.003$). However, if the frontal GMD decrease was associated with the hyperperfusion observed in Ctx+ patients, we would expect to see significant negative association in the superior and posterior regions. In this analysis there were no statistically significant regions of negative association, supporting our hypothesis that these are independent effects.

Figure 7. Perfusion and GMD Positive Association. A) Surface rendering of positive association between frontal GMD decrease and bilateral frontal and parietal perfusion change; association does not overlap with regions of Ctx+ hyperperfusion. B) Frontal GMD decrease (x-axis) graphed with average of all positively associated clusters of perfusion change (y-axis); decreased GMD is associated with decreased primarily frontal perfusion.

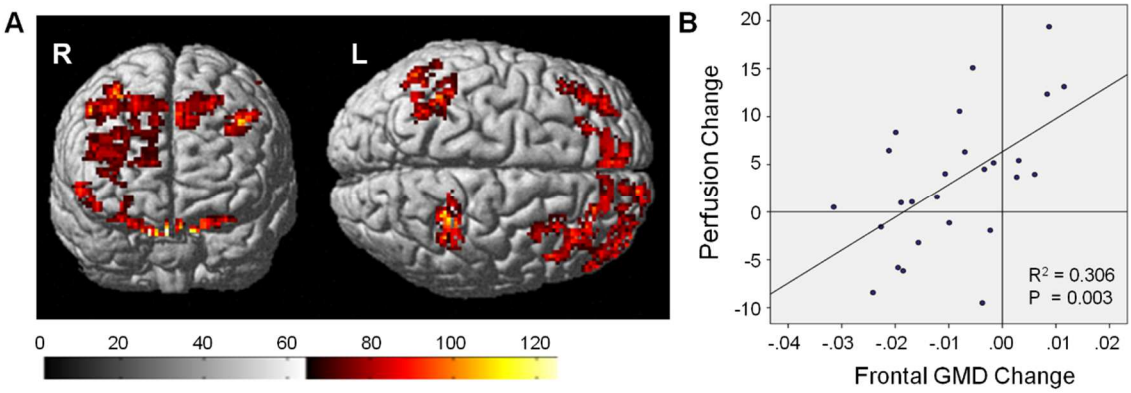


Table 11. Regional Perfusion Changes for Ctx+ Perfusion and GMD Positive Association.

x^a	y^a	z^a	k^b	T	Z	$P_{FWE\text{-}corr}{}^c$	Region description (for cluster peak)
12	18	-24	578	10.13	6.33	0.006	R Rectal G (BA11)
-50	-60	-22	153	9.71	6.20	0.317	L Fusiform G (BA37)
44	48	-10	136	8.26	5.68	0.377	R M Frontal G (BA11)
-4	50	-24	285	7.61	5.43	0.084	L Rectal G (BA11)
22	44	50	513	7.34	5.31	0.011	R S Frontal G (BA8)
-42	-58	44	248	7.20	5.25	0.120	L I Parietal Lobule (BA40)
34	-40	56	264	6.85	5.09	0.103	R Postcentral G (BA40)
-8	58	40	255	6.71	5.03	0.112	L S Frontal G (BA9)
-38	42	38	189	6.52	4.49	0.219	L S Frontal G (BA9)
-48	-58	44	114	6.10	4.73	0.470	L I Parietal Lobule (BA40)
38	64	12	608	5.92	4.64	0.005	R M Frontal G (BA10)
24	-10	-12	121	5.74	4.54	0.439	R Amygdala
-22	-8	0	159	5.07	4.17	0.298	L Lentiform Nucleus

R=Right, L=Left, G=Gyrus, BA=Brodmann Area, I=Inferior, M=Middle, S=Superior
a. MNI coordinates. b. Cluster extent. c. Peak-level p value

D. Discussion

This is the first prospective, longitudinal study documenting the effects of chemotherapy on resting cerebral perfusion in breast cancer patients. Given conflicting evidence from other measures of cerebral effects of chemotherapy, we investigated two competing hypotheses, chemotherapy-induced hypo or hyperperfusion. Although previous evidence suggests that aspects of the cancer disease process and/or host factors in cancer patient may induce cerebral structural and functional alterations, in the present study there were no statistically significant between-group perfusion differences at baseline [2,4,5,8,16,17]. This initial observation suggests that breast cancer itself is not associated with changes in cerebral perfusion. Although requiring replication and possibly specific to the present cohort, the lack of baseline differences suggests that treatment-associated findings are unlikely to be confounded by cancer-induced changes. This is important to note, as it may suggest that perfusion is a more specific indicator of cerebral alterations after chemotherapy, as other functional neuroimaging measures have shown differential alterations both prior to and after chemotherapy.

Although perfusion and metabolism are normally coupled, a previous cerebral resting state metabolic study using fluorodeoxyglucose [^{18}F] positron emission tomography (PET) by Silverman et al. (2007) did not find significant differences between control and adjuvant chemotherapy-treated groups, though they did identify a significant correlation between metabolism and the Rey-Osterrieth Complex Figure delayed recall performance in chemotherapy-treated subjects

[66]. They also found differential perfusion in Ctx+ group patients compared to controls during a functional [^{15}O] PET study. Using functional MRI, McDonald and colleagues found differential activation in Ctx+ group patients, further supporting the idea that chemotherapy may alter perfusion and activation [16]. Thus it appears that PET and MRI may be complementary detection methods for perfusion and metabolic changes in response to chemotherapy. This new PASL data adds another perspective to prior findings, indicating that perfusion is altered in the resting state as well as during tasks, and may be related to previously identified metabolic, cognitive, and structural changes in breast cancer patients.

To date, the precise mechanisms underlying chemotherapy-induced cognitive alterations are unknown. In addition to possible direct neurotoxic injury, several pathways that could influence cerebral structure and function have been posited as potentially affected by chemotherapy, including immune response, DNA repair, oxidative stress, and altered hormone levels or signaling, all of which may be genetically modulated [21,23,105]. Although only small amounts of chemotherapeutic agents have been measured in the brain, this may still be enough to provoke various cellular responses including inflammation and oxidative stress, less effective DNA repair, and possibly cell death, leading to structural and functional alterations and cognitive dysfunction [24-28]. All chemotherapy drugs utilized in this cohort are alkylating agents, anthracyclines, platinating agents, or taxanes; these antineoplastic agents target various aspects of DNA repair and cell cycle division, and cause apoptosis. A systemic response

to cell death could include several of the mechanisms listed above, and have widespread indirect cerebral effects, such as hyperperfusion. Interestingly, paclitaxel and docetaxel have been shown to have peripheral anti-angiogenic effects, and doxorubicin and paclitaxel have been associated with vascular toxicity [106-108]. Since all but one patient received paclitaxel or docetaxel, and only one patient in the Ctx+ group was on bevacizumab during the study, we could not draw any conclusions on the effects of anti-angiogenesis on perfusion; however, the individual receiving bevacizumab did experience increased perfusion post-treatment, consistent with other Ctx+ patients. The study was also not powered to examine any specific vascular toxicity related to doxorubicin or paclitaxel; this will be an important factor to consider in future studies. Although chemotherapy-altered estrogen signaling is still a possible causal mechanism, changes were likely not related to anti-estrogen treatments or aromatase inhibitors, since only three individuals out of 27 in the Ctx+ group were taking these types of medications at one month post-treatment [109]. Although this study was not powered to assess these individuals as a separate group, they did display hyperperfusion that was greater than that observed for the Ctx- or HC groups, though not as large as the Ctx+ group increase as a whole. Based on this evidence, we conclude that anti-estrogen or aromatase inhibitors are unlikely to contribute significantly to the observed hyperperfusion. Radiotherapy was similarly different between groups, with nearly all Ctx+ patients receiving this treatment following the one month post-chemotherapy time point, whereas the majority of Ctx- received radiotherapy before this time point. This emphasizes the

specificity of the chemotherapy effect on cerebral perfusion, as radiotherapy is unlikely to confound these results. Finally, caffeine consumption is known to influence cerebral blood flow. We only obtained general self-reported caffeine consumption on scan days, and so were not powered to do a detailed analysis of the variance accounted for by this measure; however, basic self-reported caffeine consumption was not significantly different between groups, suggesting that this was not a confounding factor for this analysis.

Our observation that the statistically significant Ctx+ increase in cerebral perfusion was negatively correlated with baseline neuropsychological test performance suggests that baseline cognitive reserve may indicate an indirect protective mechanism; perhaps the Ctx+ post-treatment perfusion increase is an unsuccessful compensatory mechanism that is most pronounced in individuals with lower cognitive ability [85]. Future research should focus on identifying the biological mechanism driving the potential protective effect of baseline cognitive performance/reserve.

The statistically significant pre- and postcentral gyri regions of chemotherapy-associated cerebral perfusion increase reported here were independent of frontal GMD decreases noted in this cohort. However, bilateral frontal and parietal decreases in perfusion were observed that correlated with GMD decreases. These regional perfusion alterations may be influenced by different mechanisms. This is supported by previous investigations in AD suggesting that PASL and MRI GMD measures are complementary and have similar sensitivity; consequently if these measures were associated we would likely have detected

this in our study [110]. Assuming different mechanisms are involved, one reason for the lack of correlation could be the influence of individual demographic and risk factors on susceptibility; for instance, it may be that some individuals may have genetic polymorphisms impairing drug clearance, leading to toxicity, cell death, and hypoperfusion, while others are physiologically predisposed to inflammation, which might lead to hyperperfusion. The observed lack of association indicates that there are some individuals who experience both hyperperfusion and decreased GMD post-treatment; future studies should examine whether these two measures have additive relationships with cognitive performance or complaints, which may perhaps explain why only a subgroup of breast cancer survivors appear to experience long-term cognitive dysfunction.

Further studies should also examine possible correlations of perfusion with other imaging methods, in order to further clarify the mechanistic basis of chemotherapy-induced cognitive alterations, with the long-term goal of developing preventative measures or treatments targeting these effects. While some cognitive alterations have been observed in survivors of breast cancer years after treatment, other studies show patient improvement by one year post-treatment [15,16,30,85]. If brain changes such as those observed in this study persist, they may be contributing to long-term cognitive sequelae, prioritizing perfusion as a target for therapeutic measures [10]. Future studies should utilize multimodal imaging and long-term prospective designs to clarify the mechanistic basis and persistence of this adverse effect, to help determine the focus of therapeutic efforts and pharmaceutical intervention in this patient population.

Additional research could also use animal models to investigate the association of mechanisms such as direct neurotoxicity and immune response with neuroimaging measures; findings from such studies could provide important cues for therapeutic efforts.

This first study of cerebral perfusion in a prospective cohort of breast cancer patients and healthy controls provides evidence that chemotherapy is associated with alterations in cerebral perfusion, independent of cancer effects. We found statistically significant hyperperfusion in superior and posterior regions after chemotherapy, which was not seen in patients who did not receive chemotherapy or controls. While this hyperperfusion was independent of frontal GMD decrease after chemotherapy, regional frontal and parietal hypoperfusion post-treatment did correlate with GMD decreases in these patients. The regional dissociation between hyperperfusion and GMD reduction suggests the involvement of independent functional mechanisms, as well as potential influence of individual risk factors, providing important information to guide future investigation towards therapeutic and preventative strategies.

This chapter was modified from research printed in the journal *PLoS One*. Nudelman KNH*, Wang Y*, McDonald BC, Conroy SK, Smith DJ, West JD, O'Neill DP, Schneider BP, Saykin AJ (2014) Altered Cerebral Blood Flow One Month after Systemic Chemotherapy for Breast Cancer: A Prospective Study Using Pulsed Arterial Spin Labeling MRI Perfusion. *PLoS One* 9: e96713. *These authors contributed equally to this work.

IV. Cerebral Perfusion and Gray Matter Changes Associated with Chemotherapy-Induced Peripheral Neuropathy (CIPN)

A. Introduction

Chemotherapy-Induced Peripheral Neuropathy (CIPN) is a common, potentially permanent side-effect of breast cancer treatment [111,112]. However, despite various studies reporting changes in brain structure and function associated with other types of pain [113-118], the impact of CIPN on brain structure and function has not been well studied. To date, there has been only one publication on this topic, which compared multiple myeloma patients with CIPN to controls and found significant differences in CIPN-associated brain activation during pain processing [29]. The relationship between CIPN and cerebral perfusion has not been specifically studied to date but could be valuable in identifying future CIPN treatment targets. We hypothesized that CIPN symptoms (CIPN-sx) in breast cancer patients would be longitudinally associated with altered resting state cerebral perfusion. Additionally, in view of our previous observations of altered cerebral perfusion and gray matter density following breast cancer chemotherapy treatment [3,17,119], we investigated how these changes might relate to any CIPN-associated cerebral perfusion change.

B. Methods

Participants

Written informed consent was obtained from all study participants using a protocol approved by the Indiana University Institutional Review Board in accordance with the Declaration of Helsinki. The female breast cancer cohort

used for this study has been extensively described in previous publications [17,119]. For the current analyses, the sample consisted of patients treated with (Ctx+, N=24) and without (Ctx-, N=23) common, standard-dose chemotherapy regimens. Data was collected at baseline, post-surgery and pre-chemotherapy treatment for most individuals. Eight patients received neoadjuvant chemotherapy; however, these individuals did not differ significantly from the other Ctx+ patients on demographic factors, depression, or anxiety. The same assessments were also collected one month (1M) post-chemotherapy treatment and one year (1Y) later (13 months post-treatment), with yoked intervals for Ctx-. Group sizes were smaller (Ctx+ n=18, Ctx- n=19) for 1Y analyses due to missing CIPN-sx data and participant exclusion (see below). Only patients with non-invasive (stage 0) or non-metastatic invasive (stages I, II, or III) disease were included. Besides metastatic disease, additional exclusion criteria for all participants included prior cancer, substance abuse, and other medical, neurological, and psychiatric risk factors which might affect cerebral structure or function, as described in McDonald et al. (2013) [17].

Demographic and treatment characteristics are summarized in Table 12. The Center for Epidemiologic Studies-Depression Scale (CES-D) and the State Trait Anxiety Inventory-State subscale (STAI-S) were used to measure depressive symptoms and anxiety [98,99]. Between-group comparisons including ANOVA, general linear models, and Chi-square tests were run with SPSS 21 (SPSS Statistics 21, IBM Corporation, Somers, NY).

CIPN-sx were assessed using the self-report, validated FACT/GOG-Ntx 11-item subscale [120]. Summed scores for each time point were generated. Given the specific nature of each included question, missing data was not imputed; if any question was unanswered, CIPN-sx total score was counted as missing. Exclusions from the cohort presented in Nudelman et al. (2014) comprised the following: for Ctx+, two individuals were missing baseline CIPN-sx, and an additional six individuals were missing 1Y CIPN-sx; for Ctx- two individuals were missing baseline or 1M CIPN-sx, and an additional four individuals were missing 1Y CIPN-sx. Two additional individuals were excluded based on high baseline CIPN-sx: one adjuvant Ctx+ had CIPN-sx >4 standard deviations from the group mean at baseline, while one Ctx- with the highest CIPN-sx baseline score was noted to have a pinched nerve, requiring pain medication. Exclusion of these individuals yielded the final group sizes, presented above. Repeated measures ANOVA in SPSS were used to analyze CIPN-sx change over time. Additionally, ANOVA analyses at each time point contrasted CIPN-sx means between treatment groups.

MRI Acquisition

Cerebral perfusion scans were acquired with a Siemens Tim Trio 3T whole-body MRI scanner using a 12-channel receiver-only head coil. During scan acquisition, subjects were in a conscious resting state with closed eyes. Details of the scanning protocol used to obtain cerebral perfusion measurements have been previously published [95]. Briefly, a Q2TIPS pulsed arterial spin labeling sequence was applied using the PICORE labeling scheme. Labeling was

performed with an adiabatic inversion pulse with a 10 cm labeling region and 25 mm spacing from the distal edge of the labeled region to the image section, followed by optimized inversion time delays TI_1 = 700 ms and TI_2 = 1800 ms. These time delays were chosen to minimize 3T intravascular signal intensity. Images were acquired using a gradient-echo single shot EPI readout with acquisition parameters: TR/TE = 3000/13 ms, FOV = 224 mm, and matrix = 64x64. The imaging region consisted of 16 contiguous ascending axial slices of 7 mm thickness. Each perfusion measurement consists of 100 dynamic (50 control and label image pairs) plus one M_0 image (the equilibrium brain tissue magnetization used to normalize the difference perfusion map), requiring a scan time of about 5 minutes. Head motion artifact was minimized using the scanner's built-in 3D online prospective acquisition correction (PACE). High resolution T1-weighted magnetization prepared rapid gradient echo (MPRAGE) images and high resolution EPI whole brain scans were acquired for subsequent reference and normalization. T2-weighted and fluid attenuated inversion recovery (FLAIR) sequences were acquired to check for incidental pathology. MRI scan acquisition for the analysis of gray matter density was conducted as previously described [17].

Image Processing

Pulsed arterial spin labeling MRI scan processing was performed using previously described methods [95]. Briefly, matched control images were subtracted from labeled images to create a perfusion-weighted time series; the results were used to create quantitative regional perfusion maps for each scan,

which were normalized to Montréal Neurological Institute (MNI) space using SPM8 (Wellcome Department of Cognitive Neuroscience, London, UK). Scans were resampled to 2 mm^3 voxels and smoothed with a 6x6x8 mm FWHM kernel. Image processing for MRI scans of gray matter density was performed using a previously described method [17].

Image Analysis

To examine the potential roles of cerebral perfusion and gray matter in CIPN-related pain processing, all imaging analyses were limited to the Ctx+ group.

First, time point analysis was performed to test for association of cerebral perfusion at 1M and 1Y with CIPN-sx at 1M and 1Y, respectively. For each time point, whole brain perfusion scans were tested for association with the CIPN-sx variable using voxel-wise multiple regression imaging analysis in SPM8. A mask of regions of CIPN-sx-related perfusion at 1M was created for later use.

Second, baseline to 1M longitudinal analysis was performed to test for association of cerebral perfusion changes with CIPN-sx. Perfusion changes (1M – baseline scan images, obtained using the ImCalc utility in SPM8) were tested for association with CIPN-sx at 1M covarying for baseline, using voxel-wise multiple regression imaging analysis in SPM8. CIPN-sx scores at 1M covaried for baseline were utilized to examine the relationship between perfusion change and symptom severity, controlling for variance related to higher baseline symptom report unrelated to chemotherapy treatment. Mean values for the cluster identified in this analysis were extracted using MarsBar, and 1M – baseline

values were computed in SPSS to obtain CIPN-sx associated perfusion cluster value changes for later use [102].

Third, correlation analysis was performed for CIPN-sx and related perfusion changes with gray matter density frontal changes, reported in McDonald et al. (2013). Gray matter density frontal cluster values were obtained using MarsBar in SPM8, and 1M – baseline values were computed in SPSS to obtain cluster value changes. Gray matter density cluster value changes were tested using SPSS for Pearson correlation with baseline to 1M changes in both CIPN-sx scores and perfusion cluster values (obtained in the second imaging analysis).

Fourth, image masking of the previously reported gray matter density-associated frontal perfusion decrease observed in this cohort [119] was performed to determine whether it occurred in CIPN-sx-related regions identified in the first imaging analysis. Multiple regression analysis of perfusion changes (1M – baseline scans, as in the second imaging analysis) with gray matter density cluster value baseline to 1M changes in SPM8 was masked with 1M CIPN-sx-related perfusion (from the first imaging analysis).

For the first three imaging analyses, to determine statistical significance and reduce noise, the voxel-wise critical significance threshold (P_{crit}) was set to 0.001 uncorrected, with a minimum cluster extent (k) of 25 voxels. For the fourth analysis, the voxel-wise critical significance threshold was relaxed to $P_{unc.} < 0.01$, k=25, to identify any changes within the masked area. As presented in Nudelman et al. (2014), demographic and other confounding variables such as caffeine consumption were previously considered for their potential impact on imaging

analyses. These variables were not shown to be significant, and are not included in the current analyses.

C. Results

Demographic Analysis

Comparison of treatment factors, including radiation, anti-estrogen, and chemotherapy, as well as cancer stage, identified significant group differences (Table 12); given current treatment protocols, these differences are typical for this type of study. At baseline, there were no significant differences between groups in terms of age, education, IQ, and race/ethnicity. Additionally, though a trend was observed for increased post-treatment depression in the Ctx+ group, there were no significant between-group differences for both baseline and 1M measures of depression and anxiety. Interscan intervals (approximately 5.5 months for baseline to 1M, and 1 year for 1M to 1Y) did not differ significantly between groups. Finally, compared to adjuvant Ctx+ and Ctx-, the eight neoadjuvant Ctx+ did not demonstrate any significant demographic differences.

Table 12. Cohort Demographics and Treatment Data

Variable	Ctx+ (1M N=24; 1Y N=18)	Ctx- (1M N=23; 1Y N=19)	P-value[a]
BL Age	49.4 (7.9)	52.0 (9.1)	0.312
BL Education	15.3 (2.9)	15.4 (2.3)	0.985
BL Estimated Full Scale IQ (Barona Index) [104]	109.9 (6.9)	111.2 (5.7)	0.501
% white non-Hispanic	79%	91%	0.226
BL CESD	9.2 (7.8)	7.3 (7.7)	0.392
1M CESD	13.9 (8.4)	9.4 (9.6)	0.088
1Y CESD	10.9 (7.5)	8.3 (9.9)	0.401
BL STAI	33.4 (13.9)	27.8 (7.7)	0.098
1M STAI	34.8 (12.0)	31.2 (12.7)	0.332
1Y STAI	30.8 (8.2)	28.8 (9.4)	0.498
BL to 1M interscan interval (days)	152.2 (63.1)	178.7 (74.1)	0.192
1M to 1Y interscan interval (days)	379.5 (20.7)	371.8 (11.8)	0.170
Cancer stage: 0 (DCIS)	0	5	0.002
Cancer stage: I	11	16	
Cancer stage: II	9	2	
Cancer stage: III	4	0	
Received radiotherapy by 1M	2	15	<0.001
Received radiotherapy by 1Y	11	12	0.020
On anti-estrogen therapy at BL[b]	0	1	0.498
On anti-estrogen therapy at 1M[b]	3	16	<0.001
On anti-estrogen therapy at 1Y[b]	8	15	0.033
Chemotherapy regimen:[c,d]			
DOX/CYC/paclitaxel	7		
DOC/CYC	6		

DOC/carboplatin	6		
DOC/DOX/CYC	2		
DOC/cisplatin	1		
DOX/CYC	1		
paclitaxel	1		

Values are mean (standard deviation) unless otherwise indicated. Ctx+=breast cancer patients treated with chemotherapy; Ctx-=breast cancer patients not treated with chemotherapy; BL=baseline (post-surgery, pre-treatment); 1M=one month post-chemotherapy treatment completion; 1Y=one year post-treatment completion (yoked interval for Ctx-); CESD=Center for Epidemiologic Studies-Depression Scale; STAI=State Trait Anxiety Inventory-State subscale. a. P value calculated for ANOVA or Chi-square analyses as appropriate. b. Anti-estrogen therapies included tamoxifen (majority), anastrozole, letrozole, exemestane, raloxifene, and lueprolide acetate. c. DOX = doxorubicin, CYC = cyclophosphamide, DOC = docetaxel. d. Eight patients were also treated with trastazumab, one was also treated with sunitinib, and one was also treated with bevacizumab.

Treatment Group CIPN-sx Analysis

At baseline, CIPN-sx for Ctx+ and Ctx- were not significantly different (Table 13). At 1M, Ctx+ reported significantly more CIPN-sx than Ctx- (P=0.006); however, at 1Y CIPN-sx levels did not differ significantly between groups (P=0.264). Repeated measures analysis of all three time points for the Ctx+ group showed that these patients experienced significantly increased CIPN-sx over time (P<0.001). In contrast, Ctx- CIPN-sx showed a non-significant increase from baseline to 1M (P=0.302), but repeated measures analysis over all three time points within this group demonstrated a significant increase (P=0.018). Finally, analysis of group differences in change of baseline to 1M CIPN-sx showed that Ctx+ increased significantly compared to Ctx- (P=0.001).

Table 13. Chemotherapy-Induced Peripheral Neuropathy Symptom (CIPN-sx) Change over Time

Variable	Ctx+ (1M N=24; 1Y N=18)	Ctx- (1M N=23; 1Y N=19)	P[a]
BL CIPN-sx	2.04 (2.6)	3.17 (3.5)	0.212
1M CIPN-sx	9.75 (9.2)	3.87 (3.2)	0.006
1Y CIPN-sx	8.89 (7.3)	6.47 (5.6)	0.264
P[b]	<0.001	0.018	

CIPN-sx values are mean (standard deviation). Ctx+=breast cancer patients treated with chemotherapy; Ctx-=breast cancer patients not treated with chemotherapy; BL=baseline (post-surgery, pre-treatment); 1M=one month post-chemotherapy treatment completion and 1Y=one year post-treatment completion (yoked interval for Ctx-).
a. P value for ANOVA, between-groups at each time; b. P value for repeated measures ANOVA, within-group change over 3 time points.

Ctx+ CIPN-sx-associated Perfusion

Time point analysis at 1M demonstrated that more CIPN-sx were associated with higher perfusion in several frontal region clusters, including the bilateral superior frontal gyri, bilateral medial frontal gyri, and left cingulate gyrus ($P_{unc.}$<0.001; Figure 8, Table 14). However, at 1Y, analysis of CIPN-sx with perfusion did not demonstrate any significant associations.

Figure 8. Chemotherapy-Induced Peripheral Neuropathy Symptoms (CIPN-sx) Positively Associated with Perfusion. Surface rendering of the positive association of one month post-treatment perfusion with level of CIPN-sx reported at one month post-treatment ($P_{unc.}$<0.001, k=25); colored regions indicate increasing statistical significance, as shown on the color scale to the right.

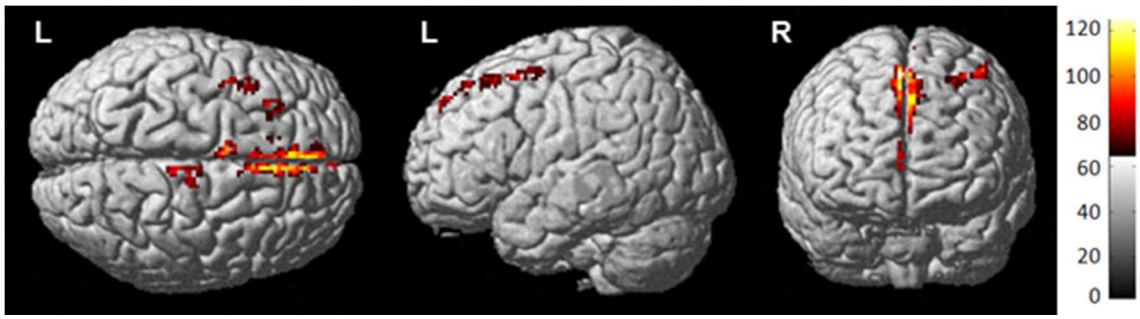


Table 14. Clusters of Significant* Cerebral Blood Flow (CBF)

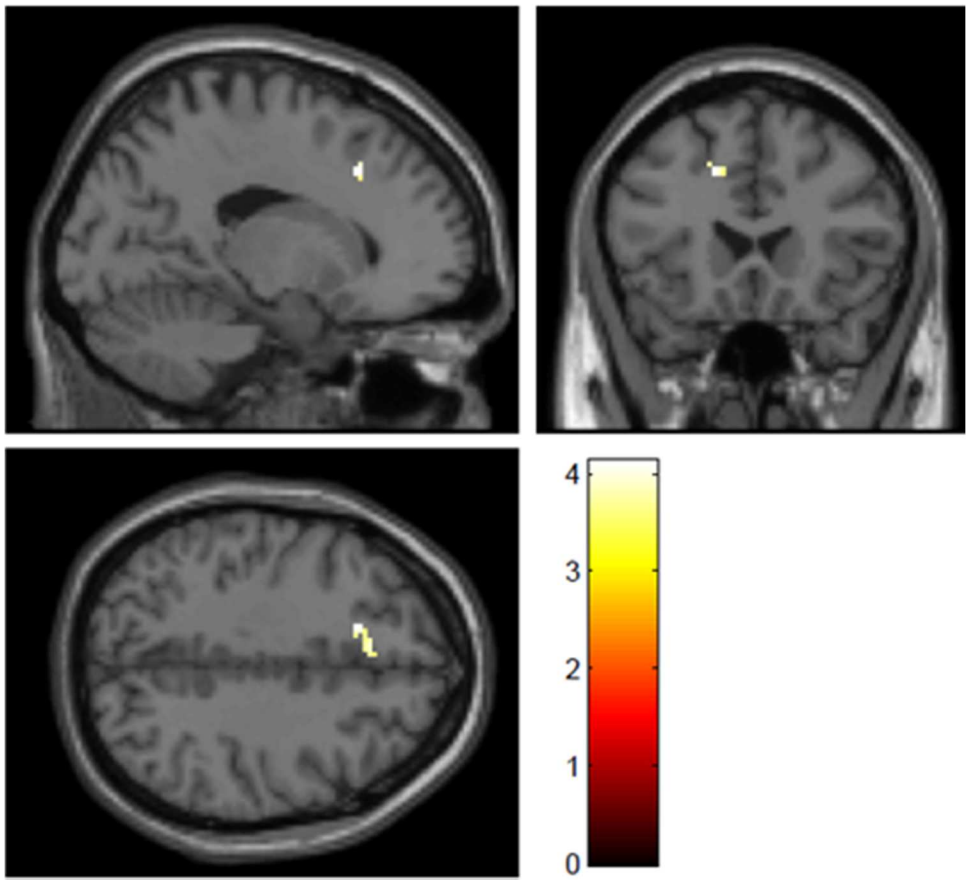
Test	x[a]	y[a]	z[a]	k[b]	T	Z	P[c]	BA[d]	Region
1M CBF, 1M CIPN-sx	4	18	56	231	6.21	4.67	0.031	8	R SFG
1M CBF, 1M CIPN-sx	4	-28	70	73	5.02	4.05	0.199	6	R MFG
1M CBF, 1M CIPN-sx	-4	48	40	430	4.88	3.98	0.005	6	L MFG
1M CBF, 1M CIPN-sx	-6	0	62	60	4.79	3.92	0.243	6	L MFG
1M CBF, 1M CIPN-sx	-40	12	56	50	4.76	3.91	0.286	6	L MiFG
1M CBF, 1M CIPN-sx	-4	-14	32	40	4.18	3.55	0.340	23	L CG
1M CBF, 1M CIPN-sx	-30	22	54	37	4.07	3.48	0.359	8	L SFG
1M CBF, 1M CIPN-sx	2	56	10	33	3.95	3.40	0.387	10	R MFG
1M-BL CBF, 1M c BL CIPN-sx	-16	22	40	37	4.12	3.49	0.329	32	L CG
1M-BL CBF, 1M – BL GMD	-40	10	58	38	5.06	4.13	0.566	4	L PCG
1M-BL CBF, 1M – BL GMD	-6	-22	40	90	3.22	2.91	0.364	32	L CG
1M-BL CBF, 1M – BL GMD	4	16	54	64	2.92	2.67	0.447	8	R SFG

*Chemotherapy-induced peripheral neuropathy symptom (CIPN-sx)-associated perfusion tests used voxel-wise $P_{unc.}<0.001$, k=25; gray matter density (GMD)-associated perfusion tests used $P_{unc.}<0.01$, k=25. BL=baseline (pre-surgery, post-treatment); 1M=one month post-chemotherapy treatment completion; 1M c BL = 1M covarying for BL; R=right; L=left; SFG=superior frontal gyrus; MFG=medial frontal gyrus; MiFG=middle frontal gyrus; CG=cingulate gyrus; PCG=precentral gyrus.

a. MNI coordinates. b. k=cluster extent. c. Cluster-level uncorrected P values. c. BA=Brodmann area.

Longitudinal analysis indicated that more CIPN-sx at 1M, covaried for baseline, were also associated with increased perfusion from baseline to 1M. This increase was observed in part of a cluster identified in the 1M analysis, in the left anterior cingulate (Brodmann area 32; Figure 9, Table 14).

Figure 9. Chemotherapy-Induced Peripheral Neuropathy Symptoms (CIPN-sx) Associated with Perfusion Increase. Colored regions of brain sections show areas with statistically significant association of baseline (post-surgery, pre-treatment) to one month post-treatment perfusion increase and one month post-treatment CIPN-sx covarying for baseline ($P_{unc.}$<0.001, k=25).



Ctx+ Gray Matter Density Correlation

Baseline to 1M gray matter density change was positively associated with both baseline to 1M CIPN-sx change and perfusion cluster change (Figure 10), indicating that individuals with more baseline to 1M gray matter density decrease did not tend to show increased perfusion and report increased CIPN-sx. Furthermore, gray matter density-associated perfusion decrease was observed in the right superior frontal gyrus and left cingulate gyrus, which were both observed in the previous CIPN-sx perfusion analyses (Figure 11, Table 14).

Figure 10. Gray Matter Density (GMD), Perfusion, and Chemotherapy-Induced Peripheral Neuropathy Symptom (CIPN-sx) Change Correlations. A) Scatter plot of baseline (post-surgery, pre-treatment) to one month post-treatment (1M) change in GMD frontal clusters (as seen in McDonald et al. (2013); x-axis) and CIPN-sx-associated perfusion cluster in the left cingulate gyrus (L CG). These variables showed a Pearson correlation r=0.582, P=0.004, as labeled on the fit line. B) Scatter plot of baseline to 1M change in GMD frontal clusters (x-axis) and CIPN-sx. These variables showed a Pearson correlation r=0.612, P=0.001, as labeled on the fit line.

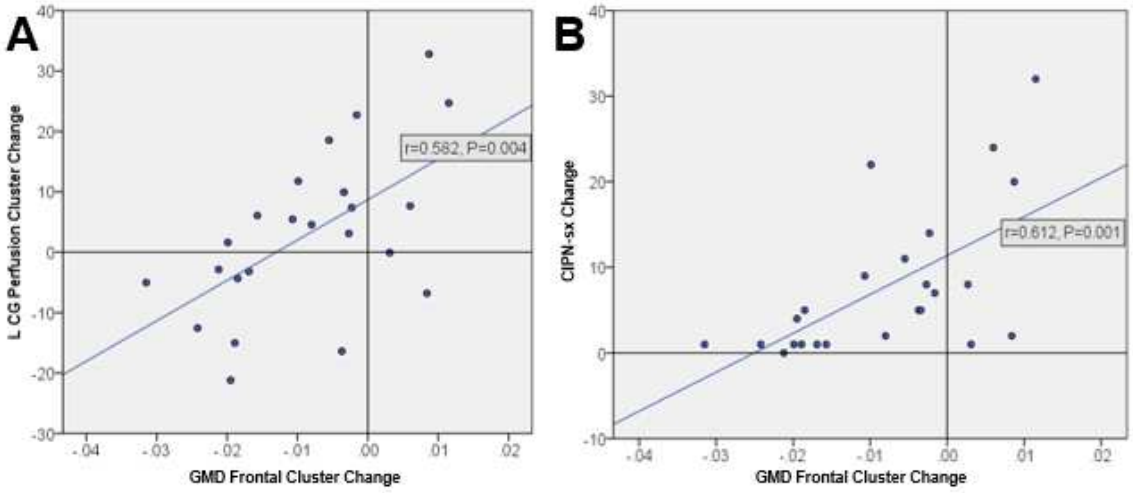
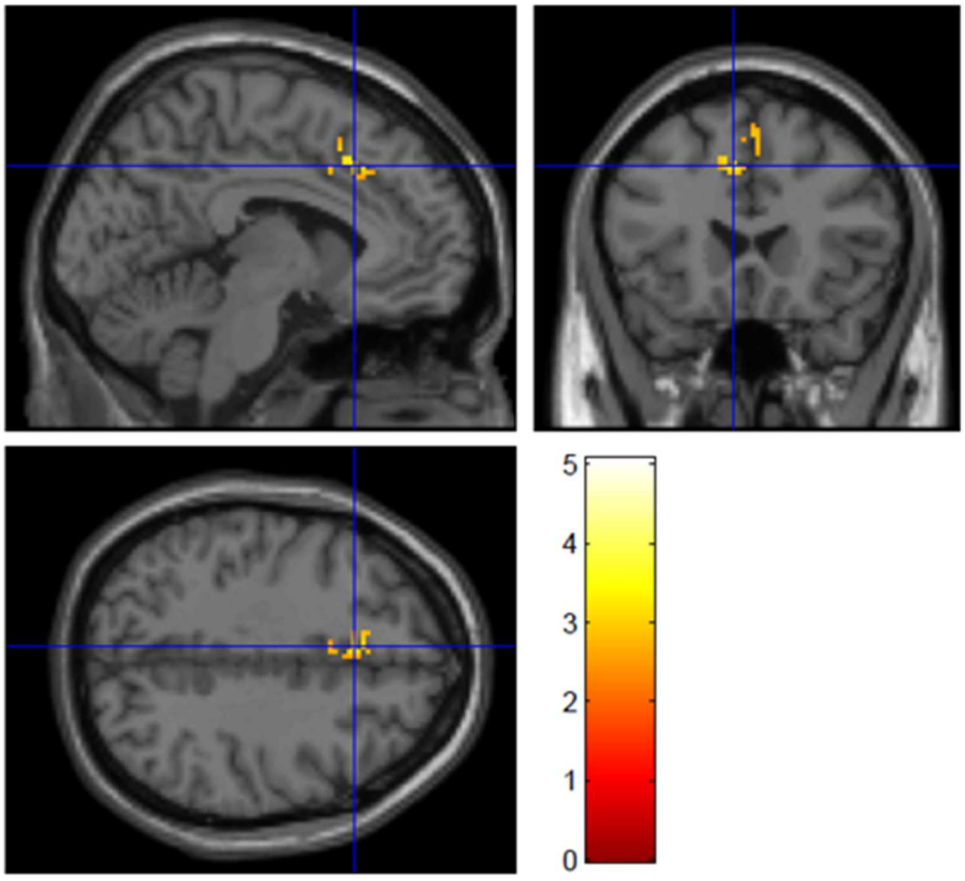


Figure 11. Cerebral Perfusion Decrease Associated with Gray Matter Density Decrease in Regions of Interest. A mask of chemotherapy-induced peripheral neuropathy regions of interest superimposed on the analysis presented in Nudelman et al. (2014) of gray matter density frontal cluster change (baseline to one month post-treatment) with perfusion change, identifies clusters of gray matter density-associated perfusion decrease (P<0.01, k=25) in the cingulate gyrus (cross-hairs), and superior frontal gyrus. Colored areas of brain sections indicate areas of increasing statistical significance.



D. Discussion

Compared to Ctx- patients, breast cancer patients treated with standard dose chemotherapy exhibited significant post-treatment CIPN-sx. Interestingly, the Ctx- group also displayed an increase in peripheral neuropathy symptoms over time, with the most significant symptoms observed 1Y post-treatment – perhaps in part due to post-treatment effects of other cancer drug types, including aromatase inhibitors, which can also produce neuropathy-like symptoms [121,122]. However, the specific increase of Ctx+ CIPN-sx immediately post-treatment, when the majority of Ctx+ were not receiving anti-estrogen therapy (Table 12), suggests that the FACT/GOG-Ntx scale was specifically measuring chemotherapy-related symptoms at this time point. Additionally, the lack of increase in CIPN-sx from 1M to 1Y among women receiving chemotherapy suggests that anti-estrogen treatments did not contribute significantly to symptoms in this group. It should be noted that the majority of Ctx+ were not on anti-estrogen therapy at 1M, while the majority of Ctx- were; the longer average anti-estrogen treatment time for Ctx- might explain why this group showed an increase from 1M to 1Y in peripheral neuropathy symptoms, while the Ctx+ group did not.

This is the first study to identify an association between cerebral resting state perfusion and CIPN-sx. In the Ctx+ group, a significant association was observed between post-treatment CIPN-sx and perfusion in brain regions previously associated with pain processing in other populations. Specifically, the anterior cingulate region is known to be involved in pain processing, and this effect has

been demonstrated in other pain populations and animal models [117,118,123-128]. Preliminary evidence in the literature shows that therapies targeting this region may alleviate pain, suggesting a potential future intervention [129-131].

Although a significant association between cerebral perfusion and CIPN-sx was identified at 1M, a similar association was not observed at 1Y, which may be partly due to a trend towards decreased CIPN-sx at this time. Alternatively, the changes in cerebral perfusion at 1M may reflect an acute pain processing mechanism, perhaps distinctly different from long-term chronic pain processing mechanisms.

Given the previously reported results of frontal gray matter density decrease and associated perfusion decrease observed in this cohort [119], the impact of gray matter density change on CIPN-associated perfusion was also investigated. The association among gray matter density change, CIPN-sx, and perfusion change indicates that individuals showing gray matter density decrease may report less severe CIPN-sx while showing less CIPN-sx related perfusion change. This finding aligns closely with the recent finding that decreased gray matter density was associated with decreased perfusion in the left cingulate gyrus and right superior frontal gyrus [119], two regions where more CIPN-sx are associated with higher perfusion. This suggests that decreases in gray matter density may potentially interfere with CIPN-associated perfusion increase and reduce patient symptom report. This is particularly important because symptoms such as pain, numbness, and tingling are just one component of CIPN; deficits in motor reflexes, loss of dexterity, and issues with balance are also common. If

Ctx+ with gray matter density decrease do experience (and report) fewer symptoms compared to their non- gray matter density affected counterparts, it may mask identification and preclude intervention for potential threats to patient quality of life, occupational concerns, and CIPN-related safety concerns such as falls and accidents in the home, particularly in the elderly population.

Several limitations to this study should be noted. First, although the study protocol asked participants to self-report CIPN-sx using a validated tool, the protocol did not include CIPN evaluation and grading by a clinician. While this information would have aided in clinical interpretation of the results and their implications, the FACT/GOG-Ntx has been shown to be sensitive to treatment differences and change over time [120], and is comparable to other currently used scales [132]. The association of CIPN-sx with perfusion change in known pain processing regions reported here provides some convergent validation for the FACT/GOG-Ntx. A related limitation of this study is the absence of objective peripheral assessments of CIPN (e.g., neurophysiological testing, skin biopsy); filling this knowledge gap will be vital to a more comprehensive understanding of CIPN-related neuroanatomical and functional changes.

In summary, these results identify an objective neuroimaging perfusion measure associated with CIPN-sx. Identifying functional brain regions important to pain processing in this population may suggest future intervention targets for CIPN. Furthermore, these results suggest that gray matter density decrease and associated perfusion decrease may interfere with CIPN-sx-associated perfusion change and patient symptom perception – potentially clinically relevant findings,

as this could result in patient under-reporting of CIPN-sx. Future research should utilize objective peripheral measures of CIPN along with neuroimaging to further elucidate this mechanism and explore potential CIPN diagnostic and clinical implications.

V. Association of Cancer History with Alzheimer's Disease Onset and Structural Brain Changes

A. Introduction

Multiple epidemiological studies have identified a significant inverse association between cancer and Alzheimer's disease (AD), primarily in white non-Hispanic cohorts [37,38,40-42,133-135]. These studies provide convincing evidence that cancer history reduces the risk of AD in the white non-Hispanic population, with effect sizes ranging from 0.4 to greater than 0.6 [35,37-40]. Supporting the validity and specificity of this effect, a study by Roe et al. (2010) found the inverse association of cancer specific to AD as compared to vascular dementia. Another study by Musicco et al. (2013) identified the inverse association of cancer and AD in a very large population-based Italian sample accounting for physician and survival bias. This study of invasive cancer types found reduced relative risk of AD in subpopulations of breast, lung, bladder, prostate, and colorectal cancer survivors, though only the colorectal cancer subpopulation risk reduction was statistically significant. Interestingly, the cancers represented in most of these study populations were highly heterogeneous, suggesting that rather than specific cancer effects, such as estrogen deprivation in breast cancer, the inverse association between cancer and AD is likely due to strong underlying biological mechanisms. Identification of these biological mechanisms may provide direction to future therapeutic efforts, particularly for AD, as there is currently a significant lack of effective treatments for this disease.

There are many proposed mechanisms that may explain the inverse association of AD and cancer [36,136,137]; a common theory posits that it is primarily driven by genetic predisposition and molecular mechanisms either promoting or suppressing metabolic survival or apoptotic cellular pathways. This metabolic survival theory is supported by a recent paper by Ibanez et al. (2014), that identified genes differentially expressed in AD and several types of cancer concentrated in metabolic and genetic information processing pathways essential for cell survival and apoptotic regulation [138]. As regional neurodegeneration, including loss of gray matter density (GMD), is a hallmark of AD, it was hypothesized that if this theory is correct, older individuals with a history of cancer (CA+) would exhibit preserved GMD compared to those without cancer history (CA-), and that lower GMD in CA- would be related to earlier age of AD onset in contrast to CA+ individuals.

However, cognitive and neuroimaging studies of breast cancer patients provide convincing evidence that CA+ survivors treated with chemotherapy have decreased GMD, more memory concerns, and worse neuropsychological test performance than CA-, up to 20 years post-treatment [14,15,17,30,139,140]. There is some evidence to support the negative impact of hormone therapies on perceived and objective cognitive function [92,141,142], and that radiotherapy may also be associated with cognitive dysfunction [143]. Furthermore, although the focus of this research to date has been on the effects of cancer treatments on brain structure and function, several studies of breast cancer patients have also found pre-treatment deficits in neuropsychological performance and brain

activation, suggesting that CA+ may be associated with cognitive dysfunction, independent of treatment effects [2,4,144]. Finally, as previously reviewed [3], there have been several imaging studies in heterogeneous cancer populations which have shown differences in brain activation compared to CA-, suggesting that these effects are not limited to breast cancer [68,69,73-75]. It has been suggested based on this evidence that cancer and treatment-related changes may be responsible for an accelerated aging process, particularly in subgroups of more vulnerable patients [83]. These results and line of reasoning predict that CA+ should experience greater cognitive dysfunction and neurodegeneration compared to CA-, which may actually worsen over time for some individuals.

This growing body of cancer and cognition literature appears to be in conflict with the metabolic survival theory posited to underlie the inverse association of cancer and AD. To investigate this apparent contradiction, this cohort study utilized the Alzheimer's Disease Neuroimaging Initiative dataset, comprising cognitively normal older adults (CN), participants with significant memory concern (SMC) in the absence of psychometric evidence of cognitive decline, older adults diagnosed with early and late mild cognitive impairment (MCI), and patients with mild clinical AD, to investigate the effect of cancer history on AD-related neurodegeneration. We hypothesized that our findings in this independent sample would be consistent with previous research showing an inverse relationship of cancer and AD. However, also based on previous research, we expected to observe cognitive dysfunction and brain structural changes in cancer patients.

B. Methods

Alzheimer's Disease Neuroimaging Initiative (ADNI)

Data used in the preparation of this article were obtained from the ADNI database (adni.loni.usc.edu). ADNI was launched in 2004 as a collaboration including the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), pharmaceutical companies, and non-profit organizations. It was framed as a multi-year, public-private partnership, headed by Principal Investigator Michael W. Weiner, MD, VA Medical Center and UCSF. Many co-investigators from over 50 sites across the United States (U.S.) and Canada have contributed to this longitudinal study, recruiting more than 1700 participants (aged 50-90) in three phases, ADNI-1, ADNI-GO, and ADNI-2.

The ADNI study design is described briefly as follows. Participants were collected from across North American in three phases, ADNI-1, ADNI-GO, and ADNI-2; target participant numbers are listed in Table 15. This is not a population study, as the focus was on recruiting participants with specific AD-spectrum diagnoses. ADNI-GO and ADNI-2 added recruitment of early (EMCI) and late MCI (LMCI) to study the full spectrum of AD progression; these participants were all counted as MCI for the purposes of this analysis. As seen in Table 15, while ADNI-1 collected MRI, fluorodeoxyglucose (FDG) positron emission tomography (PET), and Pittsburgh compound B (PiB) PET, later phases of ADNI collected several additional types of neuroimaging data. All data phases collected neuropsychological and self-reported cognitive data, biological samples such as

blood for genetic analysis, and demographic and medical history data. Longitudinal protocols included data collection for each participant every six months for the first two years, and every twelve months after this point. Further information on ADNI study design, protocols, diagnostic criteria, and all measurements utilized in this analysis can be found at http://adni.loni.usc.edu/ and in previous reports [145-152]. Institutional Review Board approval was obtained by each ADNI site, and informed consent was obtained from each study participant or authorized representative.

Table 15. ADNI Study Design

	Participants*					Data Collection						
	CN	EMCI	MCI	LMCI	AD	MRI	fMRI[a]	DTI[b]	FDG	AV45[c]	PiB	Bios[d]
ADNI-1	200	-	400	-	200	X			X		X	X
ADNI-GO	↓	200	↓	-	-	X	X	X	X	X		X
ADNI-2	150	150	↓	150	200	X	X	X	X	X		X

Arrows indicate that study participants continued longitudinally in later phases of ADNI.
a. fMRI=functional MRI. b. DTI=diffusion tensor imaging. c. AV45=florbetapir PET amyloid imaging. d. Bios=biological samples. *Numbers are study targets, not final statistics.

Participants

Self-reported demographic information for all three ADNI phases included baseline age, education, sex, race, ethnicity, and handedness. These factors have all been previously associated with AD diagnosis [153-158], and as such were considered potential confounders; participants were excluded from this analysis if they were missing any of this information. Additionally, participants were genotyped for apolipoprotein E (*APOE*) ε2/3/4 alleles as described previously; since *APOE* ε4 is the major known genetic risk factor for late-onset AD and a potential confounder, participants were also excluded if they were missing this data [148,159]. All participants included in this analysis met ADNI inclusion and exclusion criteria, which have been described previously, and can be found at http://www.adni-info.org/ [150]. A general exclusion rule, as stated in the Procedures Manual, was that a history of any cancer other than non-melanoma skin cancer (NMSC) within five years of screening was exclusionary. However, the manual also states that exceptions may be made on a case by case basis. Review of qualitative medical data indicated that there were exceptions made to this rule, primarily for individuals with prostate cancer, but also for individuals with other types of cancer which had been successfully treated and were in remission at the time of study enrollment.

Participants were categorized at baseline as CN, SMC, MCI, or mild AD. More information on measures utilized in diagnosis is available on the ADNI website; basic diagnostic criteria are also briefly summarized as follows. Criteria considered include: subject, informant, and clinician report of memory concerns,

memory function documented by neuropsychological testing scores compared to education-adjusted cutoffs on the Logical Memory II subscale (Delayed Paragraph Recall, Paragraph A only) from the Wechsler Memory Scale - Revised (maximum score=25), Mini-Mental State Exam score out of 30 [160], Clinical Dementia Rating (CDR, range 0-1) [161], and qualitative assessment by a physician of cognitive function and functional performance, guided by the NINCDS/ADRDA criteria [162]. CN participants show no signs of depression, memory complaints, MCI, or dementia; neuropsychological memory testing is within the normal range (>8 for 16 or more years of education, >4 for 8-15 years of education, or >2 for 0-7 years of education), they have a Mini-Mental State Exam score between 24 and 30, and have a clinical dementia rating (CDR) of 0. SMC individuals exhibit some forgetfulness; however, their informant does not indicate that they are consistently forgetful or experiencing progressive memory impairment. They score within the normal cognitive range for memory function, have MMSEs between 24 and 30, and have a CDR of 0. MCI individuals report subjective memory concerns, show abnormal memory function documented by neuropsychological testing (<9 for 16 or more years of education, <5 for 8-15 years of education, or <3 for 0-7 years of education), have MMSEs between 24 and 30, and have a CDR of 0.5; however, their general cognition and functional performance are sufficiently preserved such that a diagnosis of AD cannot be made by the site physician at the time of the visit. Finally, individuals with AD exhibit memory concerns, abnormal memory function documented by

neuropsychological testing, have MMSEs between 20 and 26, have CDRs of 0.5 or 1.0, and meet NINCDS/ADRDA criteria for probable AD.

As described in Saykin et al. (2010), *APOE* was genotyped using the two single nucleotide polymorphisms (SNPs) rs429358 and rs7412. A 3 mL sample of blood was taken in ethylenediaminetetraacetic acid (EDTA)-containing vacutainer tubes from all participants. Genomic DNA was extracted at Cogenics (now Beckman Coulter Genomics) utilizing the QIAamp DNA Blood Maxi Kit (Qiagen, Inc., Valencia, CA), following the manufacturer's protocol. Polymerase chain reactions were used to amplify participant DNA, followed by HhaI restriction enzyme digestion, resolution on 4% Metaphor Gel, and visualization by ethidium bromide staining.

Baseline age, education, sex, race and ethnicity (white non-Hispanic vs. all other reported races/ethnicities), handedness, and *APOE* ε4 status (0 ε4 alleles vs. at least 1 ε4 allele) were all analyzed for significant differences between cancer and AD diagnostic groups using Pearson Chi-square and ANOVA methods in SPSS 21 (SPSS Statistics 21, IBM Corporation, Somers, NY), to determine whether these potential confounders should be included in further analyses.

Qualitative and quantitative self-reported medical history data was also obtained for all ADNI study participants. For the purposes of this analysis, all qualitative medical history data was manually curated to obtain a complete, more accurate account of each individual's cancer history than was available based on quantitative data. All cancer types were considered for this analysis, including

NMSC. Medical information regarding cancer was broken down into pre-baseline cancer history (yes, 1, CA+; no, 0, CA-), as well as a count of prior cancer incidences. Reports of multiple NMSC were only counted as one cancer incidence, given the benign, prevalent nature of this cancer, as well as the lower quality of documentation regarding exact number of incidences. Cancer types were recorded and divided into 14 categories for analysis. Cancer types with only one incidence that did not fit any other categories were categorized as 'Other'; notably, there were only seven of these cancer types, showing that the other 13 categories represent the majority of observed cancer. Chi-square analysis of cancer categories by AD diagnostic group was performed to test for potential sample bias. Post-baseline cancer incidents were not utilized in this study due to the small number (43 total), which were distributed evenly between groups (Chi-square χ^2=4.054, p=0.256).

MRI Acquisition

MRI scans acquisition varied as part of the three ADNI initiatives. ADNI-1 participants' structural magnetic resonance imaging (MRI) baseline scans were acquired using 1.5 Tesla field strength; ADNI-2 and ADNI-GO both utilized 3 Tesla field strength. All available baseline structural MRI scans were downloaded from LONI (http://adni.loni.usc.edu/) for included ADNI participants. Scans were corrected prior to download as previously described [145,163].

Comorbidity Association Analysis

Cancer history (CA+/CA-; prior to baseline) was analyzed for association with baseline AD diagnosis (four groups) using the Chi-square test. Post-hoc analysis

was also performed analyzing three types of cancer history, NMSC, prostate cancer, and breast cancer.

Following these results, survival analysis was performed to analyze age of AD onset by cancer history. A time variable was created utilizing age of AD onset for participants diagnosed with AD before or during the study, and age at most current visit for all other study participants. To address potential sources of bias, this time variable was pre-adjusted for the following confounding variables identified in the demographic analysis: sex, education, handedness, race/ethnicity, and *APOE* ε4 allele status. A censor variable was used to denote AD (1) and non-AD (0) participants. Cox regression and Kaplan-Meier survival analyses were conducted utilizing these time and censor variables with CA+/- status as the factor of interest. Median age of AD onset and 95% confidence intervals (CI) were estimated using the Kaplan-Meier method, and Cox regression forward Wald tests were used to generate Chi-square statistics, significance, and odds ratios (OR), as well as graphical representations. A similar analysis using number of prior cancer incidences as the factor of interest was also conducted. Finally, post-hoc analysis investigated the association of the two most common cancer types, NMSC and prostate cancer, with pre-adjusted age of AD onset, using similar methods. Analyses were conducted using SPSS 21.

Of the 1609 included study participants, 257 individuals converted to AD post-baseline, bringing the AD group sizes for this analysis to 160 CA+ AD and 410 CA- AD (total N=570), while CA+ (N=343) and CA- (N=696) included in other diagnostic groups were censored. For the AD cancer history number of

incidences analysis, 23 individuals out of the total 570 had a history of two cancers; no individuals with AD at baseline or individuals who converted to AD during the study had more than two prior cancers.

Image Analysis

Scans were processed for voxel-based morphometry (VBM) analyses in Statistical Parametric Mapping 8 (SPM8; Wellcome Department of Cognitive Neuroscience, London, UK), using an updated version of procedures described in previous reports [44,159,164,165]. The majority of participants had at least two scans from the baseline visit; the first acquired scan of acceptable quality was used. Briefly, scans were co-registered to a T1-weighted template, segmented into gray matter, white matter, and CSF compartments with bias correction, and normalized unmodulated to Montreal Neurologic Institute (MNI) space as 1 x 1 x 1 mm voxels. Smoothing was performed with an 8 mm Gaussian kernel. Extensive quality control was performed on all scans. 1609 ADNI participants had all baseline demographic and medical data and baseline scans that passed all quality control measures.

VBM analysis of GMD was performed in SPM8 to analyze differences between AD/cancer groups. The 1609 included participants were divided into eight groups based on AD diagnostic group and CA+/- status, and baseline corrected scans were analyzed for group differences using a full factorial model, covarying for potential confounding variables including study phase, field strength (1.5 Tesla or 3 Tesla), total intracranial volume (ICV), age, sex, education, handedness, race/ethnicity (white non-Hispanic vs. all else), and *APOE* ε4 allele

status. The SPM8 standard gray matter explicit mask was included in the model. Initial results suggested that this mask may not exclude some differences in white matter regions within the brain stem and cerebellum, likely noise caused by atrophy in the AD group; the SPM8 white matter exclusive mask was used to confirm that these changes occurred in white matter.

Weighted contrast vectors were entered for each group in the design matrix to test hypotheses regarding differences in neurodegeneration across the eight AD/cancer groups. AD and MCI groups were expected to show greater neurodegeneration across large regions of the brain compared to other groups; to confirm this, a linear model of less GMD for each group further along the AD spectrum was applied (-2 for AD CA+/CA-, -1 for MCI CA+/CA-, 1 for SMC CA+/CA-, and 2 for CN CA+/CA-). The critical significance voxel-wise threshold (Pcrit) was set to 0.001 uncorrected, and the minimum cluster extent (k) for this contrast was set to 0; given the extensive GMD loss observed in AD and MCI, there was no correction for cluster size included. To test the hypothesis that cancer history was inversely associated with neurodegeneration, the model included weights of +1 for each CA+ group and -1 for each CA- group. CA+/CA- changes within each AD diagnostic group were considered in a similar fashion, and AD CA+/CA- were also contrasted with CN CA+/CA-. For each hypothesis, inverse models were also tested to confirm the specificity of the findings. The critical significance voxel-wise threshold (Pcrit) was set to 0.001 uncorrected, with a minimum cluster extent (k) of $P \leq 0.1$ uncorrected voxels for these contrasts.

For the significant cluster identified in the voxel-wise model of CA+ lower GMD across diagnostic groups, mean cluster GMD value was extracted for all individuals using MarsBar in SPM8 [103]. These values were analyzed and graphed in SPSS 21 to further investigate diagnostic group differences in GMD change. One outlier from the MCI CA- group with GMD greater than three standard deviations from the mean was excluded. These values were also analyzed with the General Linear Model Univariate ANOVA method, testing for association with types of cancer, covarying for demographic variables previously listed as well as baseline AD diagnostic group. Types of cancer tested included the four largest categories (NMSC, prostate, breast, and melanoma), as well as a category including all cancer types except NMSC. To test specifically whether non-malignant, non-invasive NMSC was associated with this effect, this cancer category was modified for this analysis to exclude individuals who had also had any other type of cancer (57 individuals excluded from 246).

C. Results

Demographic Analysis

The 1609 individuals analyzed in this study were obtained as follows. 1818 individuals had ADNI medical history files. Of these, 1780 also had pertinent demographic information (as listed in Table 16). Out of these individuals, 1609 had quality-controlled MRI scans available for analysis.

Demographic and disease characteristics of the cohort are summarized in Table 16. Age, sex, education, handedness, race/ethnicity, and *APOE* ε4 allele status were all significantly different between AD/cancer groups. There was a

significant, expected association between cancer history and smoking (Chi-square χ^2=4.2, P=0.024), but smoking only showed a trend for association with AD diagnostic groups (χ^2=6.8, P=0.078), with a higher portion of SMC individuals reporting they had ever smoked. Since a higher portion of individuals with cancer were also SMC, the trend for smoking association is likely confounded by cancer history. Given this result, smoking was not included as a covariate in subsequent analyses.

Table 16. ADNI Total Cohort Demographics (N=1609)

	CA+ CN	CA- CN	CA+ SMC	CA- SMC	CA+ MCI	CA- MCI	CA+ AD	CA- AD	P[a]
ADNI-1	75	135	0	0	108	258	45	133	
ADNI-GO	0	1	0	0	50	78	0	0	
ADNI-2	54	126	34	44	99	234	38	97	
ADNI Total	129	262	34	44	257	570	83	230	<0.001*
Age	76(5.4)	74(5.9)	73(6.4)	71(4.8)	75(6.9)	72(7.8)	77(7.7)	74(7.8)	<0.001^{+}
Education	15(3.3)	15(2.9)	16(2.8)	16(2.8)	17(2.5)	17(2.3)	17(2.5)	16(2.7)	<0.001^{+}
% Male	68%	48%	68%	55%	53%	48%	61%	45%	<0.001*
% R-Hand	97%	89%	88%	87%	88%	91%	92%	95%	0.037*
% White, NH	94%	81%	94%	87%	95%	88%	94%	88%	<0.001*
% *APOE* ε4+	29%	28%	38%	27%	49%	49%	60%	68%	<0.001*
% Smoked	49%	35%	59%	50%	39%	40%	45%	36%	0.021*

All included values collected at baseline; participants from ADNI-1 who continued in ADNI-GO or 2 are reported as ADNI-1. Age and education values in years are mean (standard deviation). % R-Hand=% right handed. % White NH=% white non-Hispanic individuals. % *APOE* ε4+=% individuals with at least one *APOE* ε4 allele. % Smoked=% individuals reported ever smoking.
a. P value for Pearson Chi-square(*) or ANOVA($^{+}$) with dependent variables listed and independent variable for treatment group/cancer history (eight groups, as shown).

Given that previous studies used highly heterogeneous cancer populations, the distribution of cancer types in ADNI was further examined. Out of the 1609 individuals utilized in this analysis, there were 421 individuals with a history of one prior cancer, and 82 individuals with a history of multiple cancers, yielding 593 total recorded cancer incidences. Cancer types were classified into 14 categories, as shown in Figure 12 and Table 17. Although there are some differences in cancer distribution among groups, overall, cancer category percentages were not significantly different between diagnostic groups (Chi-square χ^2=31.2, P=0.8). Subsequent analyses investigating the inverse association of cancer and AD were therefore performed using all types of cancer unless otherwise stated.

Figure 12. Categorized Cancer Types Count out of 593 Total Incidences. 14 categories were created from the original 40 cancer types. The “Other” category contained seven types of cancer with one reported case, which did not fit into any other category. GI, gastrointestinal cancer (including colorectal cancer); NMSC, non-melanoma skin cancer.

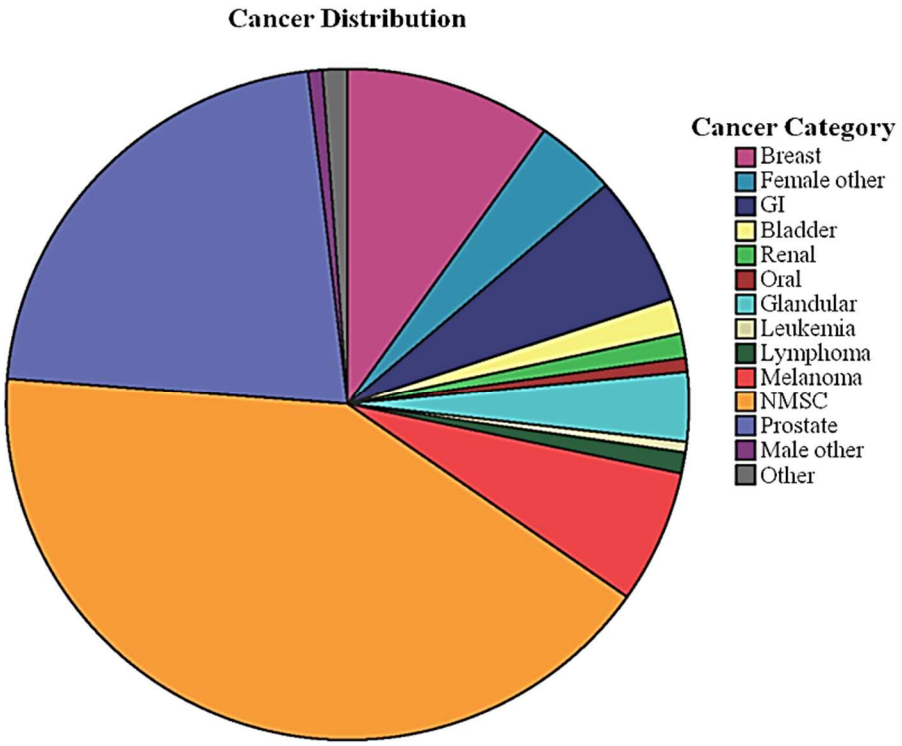


Table 17. Cancer Categories Count and Percentage by Diagnostic Group

Cancer Category	CN	SMC	MCI	AD	Total
NMSC	74 (46.0%)	19 (48.7%)	123 (40.6%)	30 (33.3%)	246 (41.5%)
Prostate	29 (18.0%)	4 (10.3%)	73 (24.1%)	24 (26.7%)	130 (21.9%)
Breast	19 (11.8%)	5 (12.8%)	25 (8.3%)	9 (10.0%)	58 (9.8%)
Melanoma	10 (6.2%)	5 (12.8%)	16 (5.3%)	7 (7.8%)	38 (6.4%)
GI	9 (5.6%)	2 (5.1%)	22 (7.3%)	4 (4.4%)	37 (6.2%)
Female Other	8 (5.0%)	1 (2.6%)	8 (2.6%)	6 (6.7%)	23 (3.9%)
Glandular	3 (1.9%)	2 (5.1%)	12 (4.0%)	3 (3.3%)	20 (3.4%)
Bladder	2 (1.2%)	1 (2.6%)	4 (1.3%)	3 (3.3%)	10 (1.7%)
Renal	1 (0.6%)	0 (0.0%)	4 (1.3%)	2 (2.2%)	7 (1.2%)
Lymphoma	2 (1.2%)	0 (0.0%)	3 (1.0%)	1 (1.1%)	6 (1.0%)
Male Other	1 (0.6%)	0 (0.0%)	3 (1.0%)	0 (0.0%)	4 (0.7%)
Oral	1 (0.6%)	0 (0.0%)	3 (1.0%)	0 (0.0%)	4 (0.7%)
Leukemia	1 (0.6%)	0 (0.0%)	1 (0.3%)	1 (1.1%)	5 (0.5%)
Other	1 (0.6%)	0 (0.0%)	6 (2.0%)	0 (0.0%)	7 (1.2%)
Total	161 (100%)	39 (100%)	303 (100%)	90 (100%)	593 (100%)

Values are expressed as count (percentage of cancer category out of total within each diagnostic group). “Other” category consists of cancer types that do not fit within another category; these included one each of the following cancer types: bone, chondrosarcoma, gallbladder, lung, meningioma, liposarcoma, and a foot tumor of unspecified origin. NMSC=non-melanoma skin cancer; GI=gastrointestinal.

In addition to baseline data collection, participants were also assessed at a number of follow-up visits, including visits at month(M)6, M12, M18, M24, M36, M48, M60, M72, M84, and M96. Though all other analyses concern data collected at baseline, longitudinal information on participant age and diagnosis at most current (latest) visit was downloaded on July 29, 2014 from the ADNI website (http://adni.loni.usc.edu/) for use in age of AD onset analyses discussed below. The numbers of participants at each most current visit were as follows: baseline (N=115), M6 (N=132), M12 (N=292), M18 (N=25), M24 (N=533), M36 (N=223), M48 (N=49), M60 (N=26), M72 (N=52), M84 (N=101), and M96 (N=61). Because ADNI-GO and ADNI-2 are newer initiatives, participants in these phases of the study do not yet have visits beyond M48; data collection is ongoing. Of the 115 individuals with no visits beyond baseline, most participants withdrew voluntarily after this visit, for reasons including scheduling, discomfort or unwillingness to comply with protocols (particularly lumbar puncture), or partner/caregiver burden. There were 12 participants for whom there was no available data on reason for loss to follow-up, and an additional five participants who could not be contacted after the initial visit. Among the remaining participants with only baseline data, there were four participant deaths (three AD and one MCI), and seven participants who withdrew due to stated medical issues (two CN, four MCI, and one AD). Given the small number of participant withdrawals attributable to medical issues and death, it is unlikely that this is a source of bias for the longitudinal data analysis.

AD and Cancer Inverse Association

Chi-square analysis indicated that CA+ was significantly associated with AD diagnostic group at baseline (χ^2=9.4, P=0.025). As seen in Figure 13 and Table 18, fewer study participants with AD are CA+ compared to other diagnostic groups. Interestingly, individuals with SMC are more evenly divided between CA+ and CA- than other groups.

Post-hoc analysis examined the largest cancer category, NMSC, for association with AD diagnostic group. Chi-square analysis indicated that there were significantly fewer individuals with a history of NMSC in the AD diagnostic group (10%) compared to 15% or greater for all other diagnostic groups (χ^2=16.9, P=0.001; Table 18), supporting inclusion of this cancer type in analyses. Interestingly, no such trend was observed for prostate cancer (χ^2=1.8, P=0.61; Table 18). Breast cancer showed a trend for fewer individuals in the AD and MCI groups compared to SMC and CN, but this trend did not reach statistical significance (χ^2=4.8, P=0.19; Table 18). Other cancer types were not examined due to insufficient power.

Figure 13. Percent of Individuals with Cancer History (CA+) per Diagnostic Group. There are significant differences in CA+ (blue striped bars) compared to individuals without cancer history (CA-, red dotted bars) between Alzheimer's disease (AD) diagnostic groups (P=0.025), including cognitively normal controls (CN), and individuals with significant memory concerns (SMC), mild cognitive impairment (MCI), and AD. There is a smaller percentage of AD CA+, and a larger percentage of SMC CA+, compared to the CN CA+ percentage, while the MCI CA+/CA− ratio does not appear to be significantly different than CN CA+/CA−.

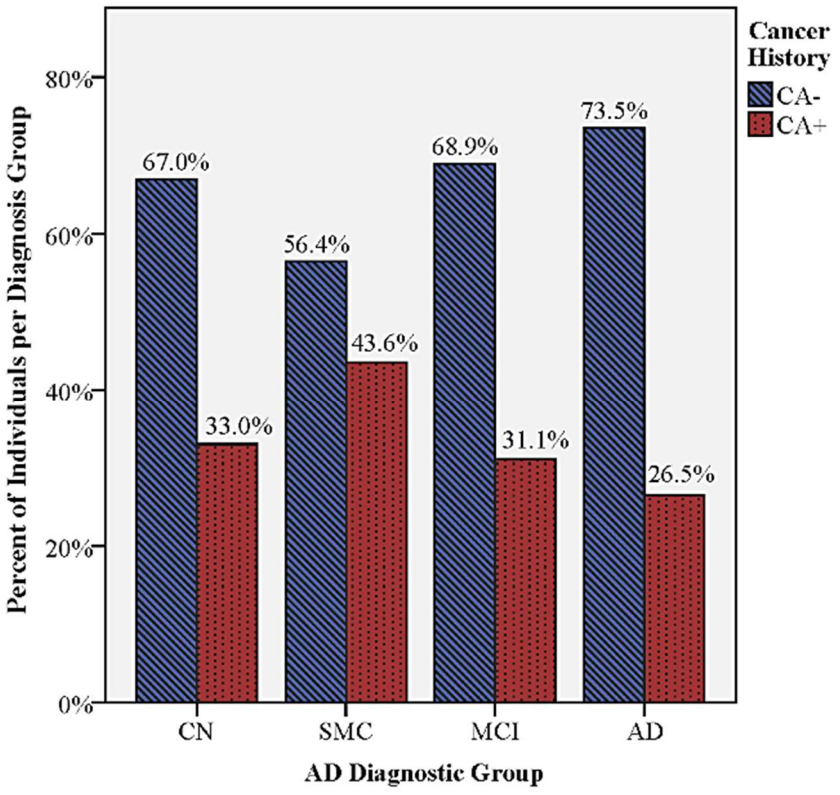


Table 18. Cancer History by Baseline AD Diagnostic Group

	All Cancer Types		NMSC		Prostate Cancer		Breast Cancer	
	CA+	CA-	CA+	CA-	CA+	CA-	CA+	CA-
CN	129 (33%)	262 (67%)	74 (19%)	317 (81%)	29 (7%)	362 (93%)	19 (5%)	372 (95%)
SMC	34 (44%)	44 (56%)	19 (24%)	59 (76%)	4 (5%)	74 (95%)	5 (6%)	73 (94%)
MCI	257 (31%)	570 (69%)	123 (15%)	704 (85%)	73 (9%)	754 (91%)	25 (3%)	802 (97%)
AD	83 (26%)	230 (74%)	30 (10%)	283 (90%)	24 (8%)	289 (92.3%)	9 (3%)	304 (97%)
P[a]	0.025		0.001		0.610		0.190	

Values are expressed as count (percentage within diagnostic group). NMSC=non-melanoma skin cancer.

a. P values for Chi-square analyses of each listed cancer type (or all) by diagnostic group.

Survival Analysis of Age of AD Onset

Kaplan-Meier survival analysis of age of AD onset with cancer history indicated that those with CA- history had significantly earlier median age of AD onset, as seen in Figure 14A and Table 19; Cox regression shows that cancer history is protective against AD, with CA- 1.5 times more likely to develop AD compared to CA+ ($P<0.001$). Importantly, because this analysis was adjusted for *APOE* ε4, these results also suggest that cancer history-associated later age of AD onset is independent of this risk factor. Furthermore, this effect appears to be additive, as CA+ with one prior cancer are still 1.3 times more likely to develop AD compared to CA+ with two prior cancers ($P<0.001$; Figure 14B, Table 19).

Post-hoc analysis of the two largest cancer categories indicated that CA+ NMSC showed the cancer protective effect against AD; CA- were 1.6 times more likely to develop AD compared to individuals with a history of NMSC ($P<0.001$; Table 19). A similar protective effect was also observed for prostate cancer, though this effect was not as significant ($P=0.037$; Table 19), possibly due to the smaller number of individuals with this cancer (see Table 17).

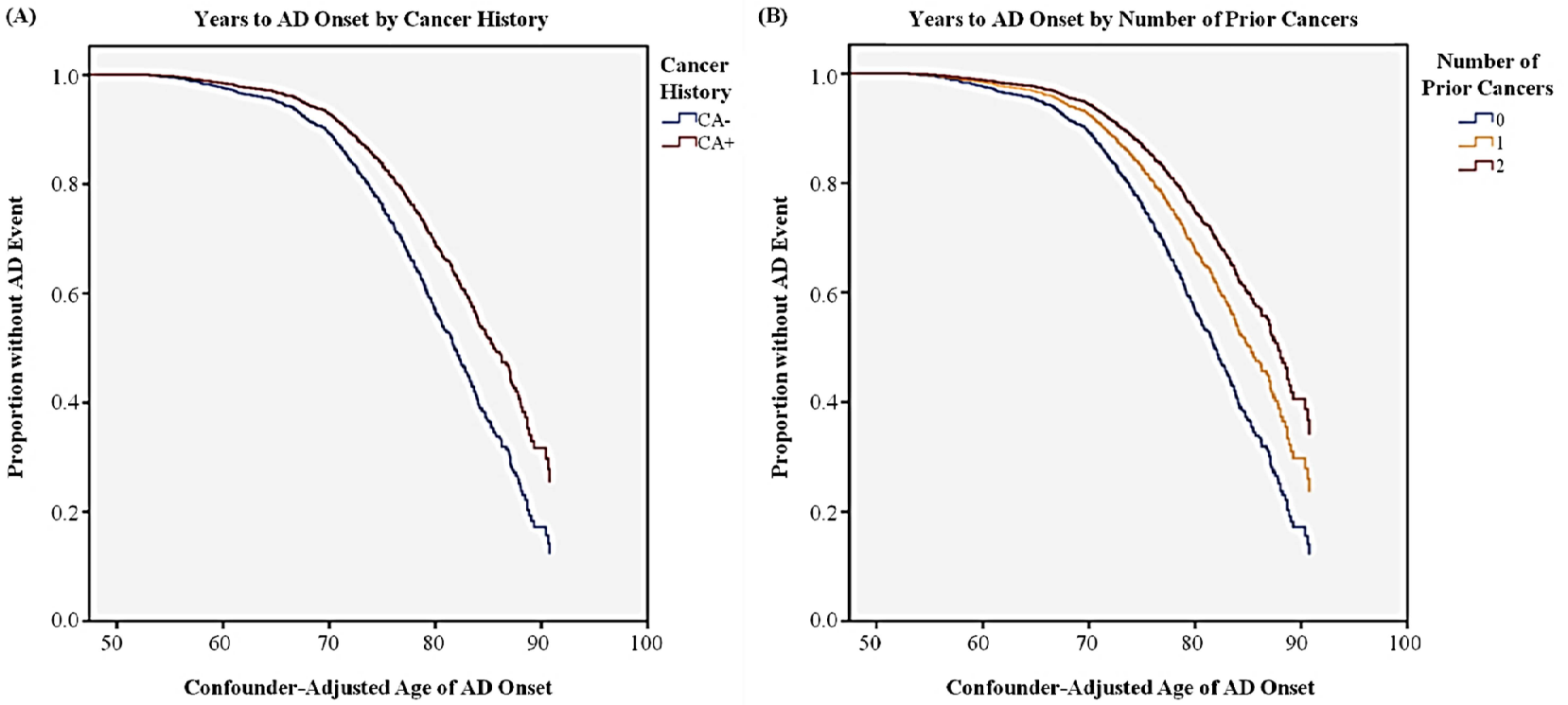


Figure 14. Survival Analysis of Age of AD Onset by Cancer History. (A) Cox regression of confounder-adjusted age (years) of AD onset for individuals with (CA+, red line) or without (CA−, blue line) cancer history, indicating that CA+ have later age of AD onset compared to CA− ($P < 0.001$). (B) Cox regression of confounder-adjusted age (years) of AD onset for individuals with 2 (dark red line), 1 (orange line), or 0 (blue line) prior cancer incidences, indicating that individuals with 2 prior cancer incidences have later age of AD onset compared to individuals with 1 or 0 incidences ($P < 0.001$).

Table 19. Age of AD Onset (AoO) by Cancer History

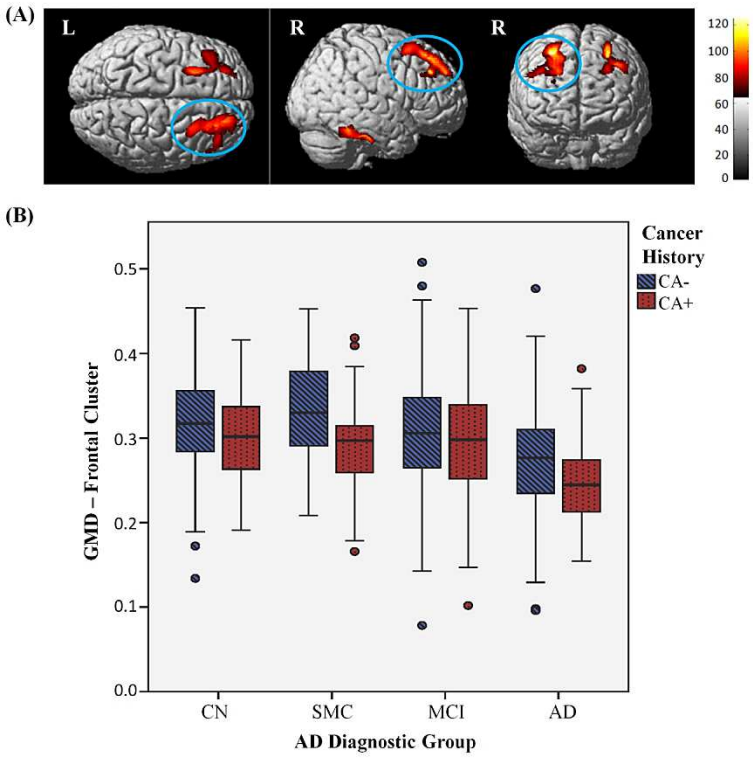
Method:	Kaplan-Meier		Cox Regression			
Measure:	Median AoO	95% CI	χ^2	P	AD OR	
All: CA-	81.7	80.7-82.8	20.9	0.000	1.5	1.3-1.8
All: CA+	84.7	83.4-86.0			*Ref	
All: 0 CA-	81.7	80.7-82.8	22.2	0.000	2.0	1.3-3.0
All: 1 CA+	84.3	83.2-85.4			1.3	0.9-2.1
All: 2 CA+	85.7	82.4-88.9			Ref	
NMSC: CA-	82.4	81.5-83.3	13.3	0.000	1.6	1.2-2.1
NMSC: CA+	85.7	82.7-88.6			Ref*	
Prostate: CA-	82.8	82.0-83.7	4.4	0.037	1.4	1.0-1.8
Prostate: CA+	84.7	82.6-86.9			Ref	

Values for Median AoO and 95% CI are given in years. AoO=age of onset; CI=confidence interval; AD OR=odds ratio of developing Alzheimer's disease. *Ref=reference variable for odds ratio calculation.

GMD Differences between Groups

As noted above, a linear model of GMD deficits in AD, MCI, and SMC groups compared to CN was used to confirm that groups further along the AD spectrum display lower GMD. As expected, lower GMD was observed for affected groups throughout the brain, consistent with prior work [164,165]. Modeling the opposite relationship, with higher GMD in the AD group compared to other groups, showed no significant regions of greater GMD in the AD group. A second VBM analysis examined all groups irrespective of AD diagnosis, to identify regions that were increased or decreased in CA+ compared to CA-. This model showed that CA+ had lower GMD in the right superior frontal gyrus compared to CA- (peak-level $P_{unc.}<0.001$, cluster-level $P_{unc.}\leq 0.1$; Figure 15A). There were no regions of significantly greater GMD in CA+ at this threshold. As seen in Figure 15B, CA+ showed significantly lower GMD in the right superior frontal gyrus cluster across diagnostic groups.

Figure 15. Lower Gray Matter Density (GMD) with Cancer History across Diagnostic Groups. **(A)** Surface rendering shows individuals with a history of cancer (CA+) display lower GMD than individuals without cancer history (CA−), across diagnostic groups, in the right superior frontal gyrus (cluster maximum MNI coordinates 28, 32, 54), shown circled (P_{unc} < 0.001, cluster threshold P_{unc} ≤ 0.1); this effect is observed to be bilateral at a more lenient threshold (P_{unc} < 0.01, cluster threshold P_{unc} ≤ 0.1) as pictured. Colored areas indicate regions where CA+ gray matter density was less than CA− across groups at this threshold; red to yellow color scale indicates increasing statistical significance, with yellow areas indicating the most significant regions. **(B)** GMD values for right superior frontal gyrus cluster graphed by CA+ (red dotted bars) and CA− (blue striped bars) across AD diagnostic groups; CA+ have lower GMD across diagnostic groups.



This finding did not appear to be influenced by disease progression, as AD individuals did not display any significant differences compared to CN. Furthermore, comparing CA+ vs. CA- within each group did not yield any significant regions at this threshold. A lack of significant cortical and subcortical GMD differences between CA+/CA- within groups suggests that the lower CA+ frontal GMD is not being driven by any particular group, but rather is an underlying difference common to all CA+ in this study cohort.

To further investigate this finding, GMD cluster values were tested for association with different types of cancer, controlling for AD diagnostic group and demographic variables. As expected, GMD was significantly associated with all cancer types ($F=10.0$, $P=0.002$), which was still significant after excluding individuals with NMSC ($F=4.9$, $P=0.027$). GMD was associated with prostate cancer ($F=4.3$, $P=0.039$), and showed a trend for association with breast cancer ($F=3.7$, $P=0.055$). Interestingly, GMD only showed a trend for association with NMSC ($F=3.1$, $P=0.081$), and showed no association with melanoma ($F=0.0$, $P=0.916$).

D. Discussion

These findings show a significant inverse association between cancer and subsequent development of AD in the ADNI cohort, in concordance with previous epidemiological studies. Importantly, while previous studies have indicated that this inverse association is mediated by age, our results are the first to quantify the later age of AD onset associated with cancer history, as well as to suggest that this effect may be additive, as the small group of individuals with a history of

multiple cancers showed later age of AD onset compared to individuals with a history of one or no cancers. Furthermore, these data demonstrated that NMSC, which has not been included in most other studies, was a significant driver of this effect. This suggests that the malignancy of the cancer may not be an important factor driving the inverse association with AD. Alternatively, it may highlight potential environmental mechanisms, such as sun exposure and subsequent increase in vitamin D.

In order to obtain a more complete context for this analysis, cancer history data was compared to U.S. population-level cancer data using the SEER Cancer Statistics Review, 1975-2011 data for cancer incidence and 36-year limited duration prevalence. SEER data indicates that breast and prostate cancer are the most common cancer types (with very similar incidence and prevalence), followed by colorectal cancer and melanoma. However, this report did not include NMSC, which would be expected to have a higher incidence and prevalence (given that squamous and basal cell carcinomas are largely benign, noninvasive cancer types), as observed in the ADNI cancer history data. In ADNI, history of prostate cancer is more common than breast cancer, contrary to SEER incidence and first cancer prevalence. This is perhaps not surprising in this context, as prostate cancer has a later onset and thus may have a higher prevalence as a second or third cancer than other cancer types. Additionally, there may be more prostate cancer in the ADNI sample because there are more males with cancer participating in this study than females (65% male CA+). Accounting for these

demographic differences, the cancer history data in the ADNI cohort appears to be relatively similar to national incidence and prevalence estimates.

There was one notable exception to the correspondence of ADNI data with the SEER national incidence data. Interestingly, there was only one reported case of an individual with a prior incidence of lung cancer in the 1609 individuals included in this study. This may be due to survival bias; although lung cancer incidence is quite common (comparable to SEER prostate cancer age-adjusted incidence in white individuals), lung cancer patients have very low SEER five-year survival compared to other types of cancer (less than 20% for white men and women) [166].

There are a few other limitations of this study that are important to consider. Across diagnostic groups, CA+ individuals were, on average, older than CA-. This may represent an inherent study bias; given that CA+ individuals have a later age of AD onset, older CA+ than CA- would be expected in the AD group. However, it is interesting that this trend was also observed in other diagnostic groups, including CN. One possible explanation may be that in addition to later age of AD onset, CA+ individuals also experience later onset of age- or neurodegeneration-related memory concerns, which may be a motivator to enroll in this type of study. While this is an important caveat to keep in mind when interpreting the current results, the Kaplan-Meier survival analysis method, used to examine age of AD onset with all study participants, was chosen to minimize this limitation, and as noted above all neuroimaging analyses covaried for age. There were several other demographic differences between CA+/CA- groups;

CA+ had a higher percentage of white non-Hispanic individuals, likely due to the inclusion of all types of skin cancer in this category, and CA+ also had more males, likely due to the prevalence of prostate cancer in older men, as observed here. Again, all neuroimaging analyses covaried for these demographic confounds. The potential contribution of *APOE* ε4 to cancer was also considered; though *APOE* ε4 alleles are significantly different between diagnostic groups as noted in the demographics table, individuals with *APOE* ε4 alleles were not more likely to have cancer history than those without at least one *APOE* ε4 allele, and this factor was also covaried for in all neuroimaging analyses to account for its impact on neurodegenerative processes in AD. Age of AD onset analysis stratifying for *APOE* ε4 still found CA+ associated with later age of AD onset, supporting the assertion that *APOE* ε4 does not appear to be driving the inverse association of cancer and AD.

The observed lower GMD in CA+ compared to CA- is not predicted by the common theory of the inverse association of cancer and AD, which suggests that CA- would have lower GMD. However, this finding does fit with studies in cancer patients, which have found gray matter reductions in patients undergoing treatment as well as long-term survivors up to 20 years post-treatment [14,15,17]. Additionally, in the present study an increased percentage of CA+ was observed in the SMC group compared to other groups, suggesting that while cancer may delay age of AD onset, CA+ individuals may have increased cognitive concerns, consistent with the cancer and cognition literature [17,21,31,34,167]. Interestingly, the frontal pattern of lower CA+ GMD occurs in

regions similar to those reported in neuroimaging studies of breast cancer and chemotherapy-associated gray matter changes [15,17]. 63% of cancers reported in ADNI were either NMSC or prostate; chemotherapy is not a common treatment for either of these, and chemotherapy is not administered for all patients afflicted with other types of cancer reported in this study, including breast cancer, the next most common cancer type. Therefore, while comprehensive treatment data were not available for these patients, it is probable that the majority did not receive chemotherapy. The CA+ effect observed here could be a synergistic result of cancer-specific changes in addition to a subgroup of patients experiencing chemotherapy treatment-related gray matter effects. The finding that lower GMD is significantly associated with invasive cancer types, and only showed a trend for association with NMSC, further supports this idea. These results highlight the need for more long-term studies of cancer and treatment effects on neuroimaging measures and cognitive dysfunction, particularly in older patients where these medical factors may predispose to neurodegeneration or pose an additional risk for cognitive dysfunction.

Given the finding of lower GMD in CA+, which would be predicted in the context of cancer and cognition literature but is unexpected in the context of cancer and neurodegenerative disease literature, we posit that the inverse association of cancer and AD is more complex than the metabolic survival theory would suggest. As reviewed by Holohan et al. (2012), there are many potential pathways driving this effect, which may have synergistic interactions as well. Considering this information, we propose several alternate biological

mechanisms and highlight important directions for future research of this effect. First, while baseline imaging indicates lower GMD in CA+ patients, potentially as a long-term result of cancer and related treatments, this study does not capture the rate at which gray matter is changing over time in these patients. Examining this data will be an important step to confirm the neurodegenerative profile of CA+ compared to CA-. Second, analyzing the impact of cancer history on the amyloid pathway and associated biomarkers may demonstrate an alternate mechanism through which cancer could protect against AD. Given that high levels of inflammatory markers have been associated with poorer survival in cancer, cancer survivors may be selected for lower cytokine genetic load or expression, which may be protective against neurotoxic inflammation pathways linked to amyloid plaque accumulation in AD when compared to unselected individuals with no cancer history. It is also possible that some polymorphisms in the amyloid pathway may be inversely associated with cancer and AD; peptidylprolyl cis/trans isomerase, NIMA-interacting 1 (*PIN1*) has been proposed as one such candidate [168,169]. Finally, a common theory in the cancer and cognition literature posits that cancer patients and survivors have gray matter reductions and significantly increased subjective cognitive concerns, which are not well correlated with objective neuropsychological performance, potentially due to compensatory activation, wherein the brain recruits additional resources to deal with cognitive challenges. In the ADNI data there was a higher portion of cancer survivors in the SMC group, and cancer survivors had lower GMD than CA-; it is possible that these individuals experience compensatory activation,

similar to that previously shown in a functional MRI study of breast cancer patients, which may delay cognitive performance decline and AD diagnosis [16]. There are a multitude of pathways which have been implicated in AD and cancer, as discussed in Holohan et al. (2012), which require further functional investigation as well. Future research should investigate other biomarkers of AD, including longitudinal gray matter change, amyloid pathway-specific markers, and inflammatory markers, as well as measures of brain activation in cancer and AD diagnostic groups, to further elucidate the biological mechanisms underlying the inverse association of cancer and AD, with the goal of identifying preventative and therapeutic targets.

This chapter was modified from research printed in the journal *Frontiers in Physiology*.

Nudelman KNH, Risacher SL, West JD, McDonald BC, Gao S, Saykin AJ (2014) Association of Cancer History with Alzheimer's Disease Onset and Structural Brain Changes. *Front Physiol* 5:423.

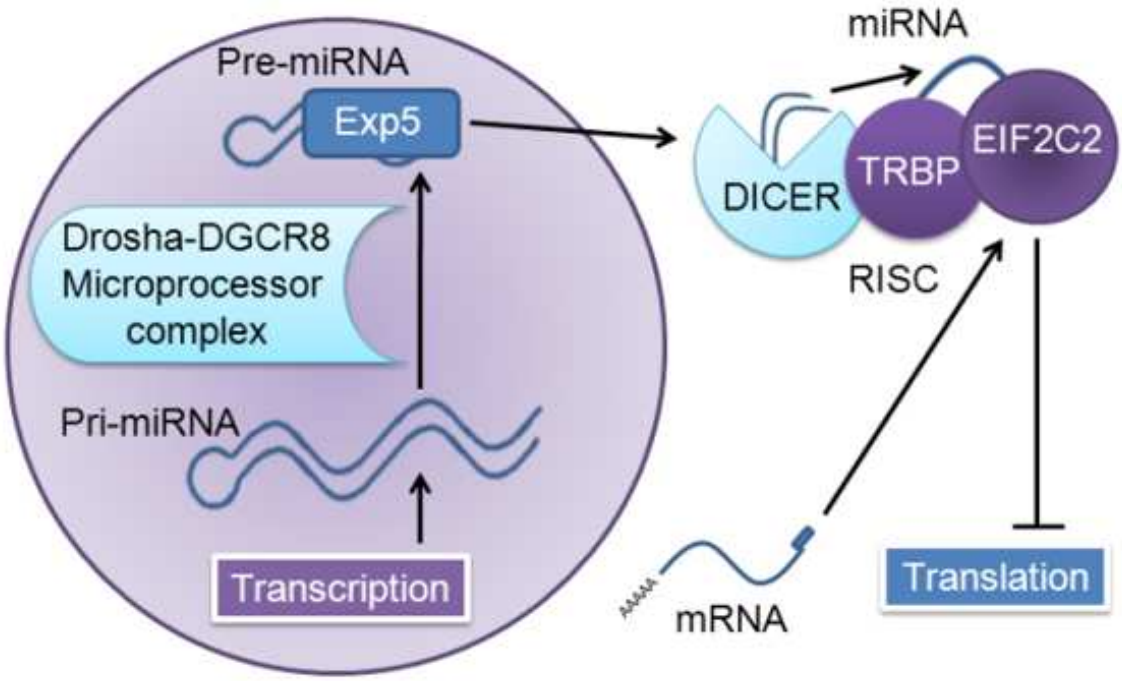
VI. Functional MicroRNAs in Alzheimer's Disease and Cancer: Differential Regulation of Common Mechanisms and Pathways

A. Introduction

Cancer and neurodegenerative disease have become prominent areas of medical research in the United States, as these diseases afflict millions of Americans each year. Since age is a major risk factor for each disease area, our aging population makes progress in treatment a high priority [81,170-172]. Fundamental hallmarks of cancer include uncontrolled proliferation and disruption of apoptosis; conversely neurodegeneration is associated with increased cellular death [173,174]. Therefore, both diseases may potentially result from differential regulation of the same cellular pathways. Supporting this hypothesis, negative epidemiological correlations have been demonstrated between cancer and neurodegenerative diseases including Down syndrome, Parkinson's disease (PD), Alzheimer's disease (AD), schizophrenia, and multiple sclerosis [43]. Interestingly, to date, although the rest of these neurodegenerative diseases appear to be associated with either increased or decreased comorbidity depending on the type of cancer, AD has been associated with a decreased co-occurrence of all types of cancer. A recent large scale report indicated a significant negative correlation of cancer and AD in the Framingham Heart Study [37]. In a longitudinal study, patients with AD also had a lower risk of developing cancer after adjusting for demographic factors [40]. Further, Caucasian participants in another prospective study displayed a negative association between AD incidence and cancer risk [41].

MicroRNAs (miRNAs) comprise one major post-transcriptional regulatory mechanism that has been implicated in a variety of cancers and neurodegenerative diseases [175-180]. As illustrated in Figure 16, miRNA is generated as a long precursor sequence in the nucleus, where it is cleaved to form a shorter stem-loop precursor, transported to the cytoplasm, and further processed in the RNA-induced silencing complex (RISC) by the ribonuclease Dicer 1, ribonuclease type III (DICER1) into a very short (~22 nucleotide) double strand sequence; this is then unwound and one strand is loaded onto Eukaryotic translation initiation factor 2C, 2 (EIF2C2), which consequently inhibits translation or results in cleavage of target messenger RNAs (mRNAs) [181-187].

Figure 16. MiRNA Generation and Function. The nuclear transcript pri-miRNA is several kilobases in length; this transcript is cleaved by the microprocessor complex (DCGR8 and Drosha), which yields a short (~65 nucleotide) stem-loop pre-miRNA. This is transported out of the nucleus by Exportin 5 (Exp5) to the RNA-induced silencing complex (RISC) and cleaved by DICER1 (targeting the hairpin loop), generating a ~22 nucleotide miRNA duplex; one strand is degraded, and the remaining strand is loaded into human immunodeficiency virus-1 transactivating response RNA-binding protein (TRBP)-recruited EIF2C2, also known as Argonaute 2, which then inhibits translation of target messenger RNAs (mRNAs) [181-187].

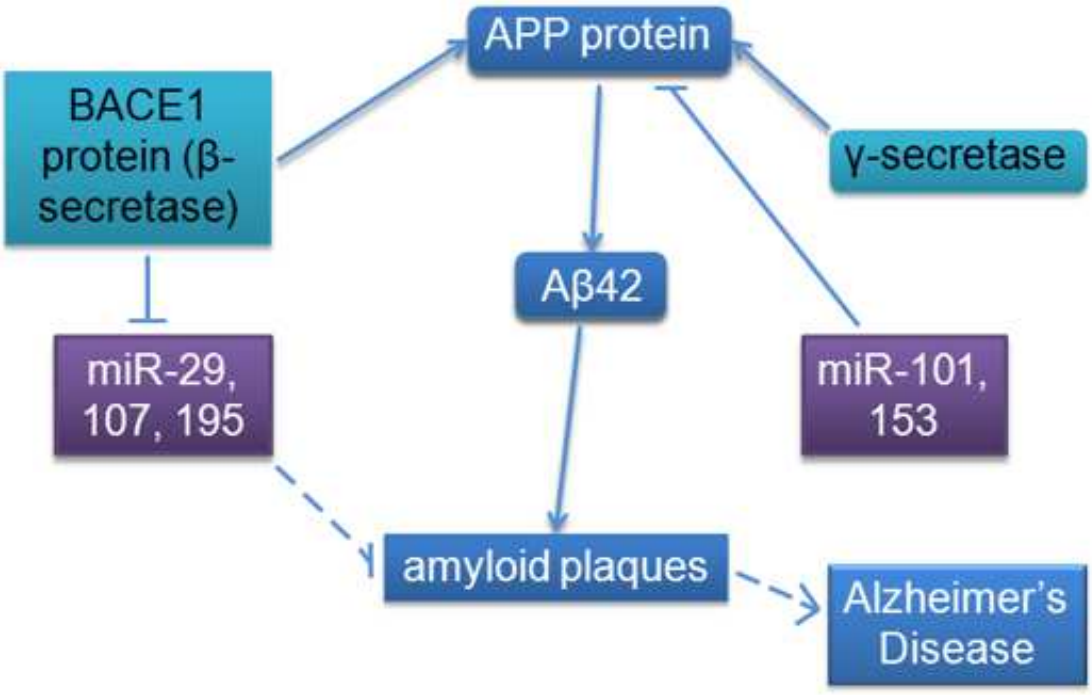


The same miRNAs can be important to both types of disease; a few examples include miR-34b/c downregulation in PD and small cell lung cancer, upregulation of miR-206 as a protective effect against disease progression in a mouse model of amyotrophic lateral sclerosis and miR-206 downregulation in laryngeal squamous cell carcinoma, and miR-132 downregulation in frontotemporal dementia and methylation in prostate cancer [188-193]. To date, there has been a focus on miRNAs in cancer. Although the roles of miRNAs in neurodegenerative diseases have not been as thoroughly investigated, these are currently under increasing scrutiny [194]. Given the plethora of information on cancer regulatory mechanisms including miRNA, as well as the possible involvement of some of the same miRNAs in cancer and neurodegeneration, consideration of the roles of miRNA functions in cancer might help elucidate corresponding or opposing functions in neurodegenerative disease. A more comprehensive review of the roles of miRNAs in cancer and neurodegeneration might yield insights into the underlying pathways involved in these diseases.

The full spectrum of roles of miRNAs in different types of cancer and neurodegenerative diseases is a rich topic but beyond the scope of this targeted review; we decided to focus our analysis on select miRNAs implicated in both AD and cancer. AD pathology is characterized by an accumulation of extracellular amyloid plaques composed of amyloid-beta peptide fragment (Aβ) and intracellular neurofibrillary tangles composed of hyperphosphorylated protein tau, as well as neuronal loss in the hippocampus, temporal, and frontal lobes, increased inflammation, and oxidative stress [195-201]. We will review a number

of studies linking miRNAs with differential expression and pathology in AD such as deposition of amyloid plaques and neurofibrillary tangles, as well as more specific pathway interactions and regulatory functions of the amyloid pathway, including regulation of amyloid protein precursor (*APP*) and beta-site *APP* cleaving enzyme 1 (*BACE1*), encoding a protease (β-secretase) that cleaves amyloid beta precursor protein to generate Aβ. The larger form of Aβ peptide containing 42 amino acid residues (Aβ42) mostly aggregates to form the previously mentioned amyloid plaques (see Figure 17).

Figure 17. MiRNA Involvement in the Amyloid Pathway Appears to Contribute to AD. *BACE1* mRNA expression appears to be redundantly regulated by multiple miRNAs (including predicted miR-9, not shown); *APP* mRNA expression is also regulated by miRNA. More miRNA binding sites have been bioinformatically predicted for both mRNAs, indicating that this regulatory mechanism is most likely very tightly regulated [202]. Solid lines indicate known interactions; dashed lines indicate measured correlations.



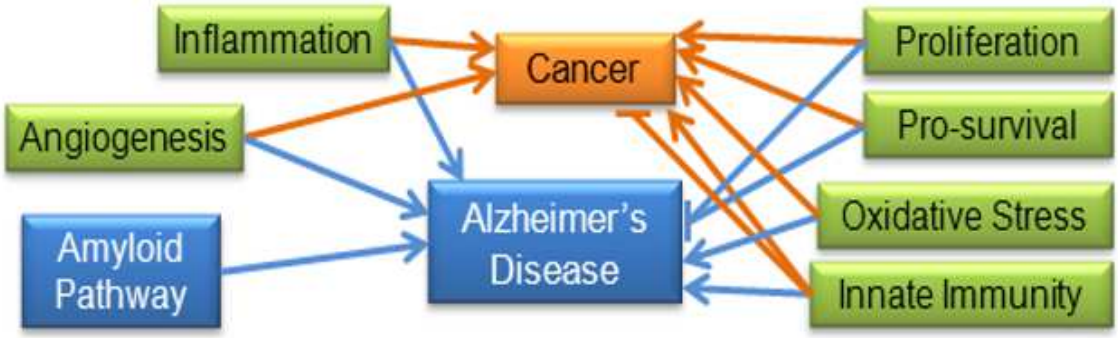
We will focus on eight miRNAs (miR-9, -29a/b, -101, -107, -125b, -146a, -153, and -195) with differential expression in AD, evidence of correlation with disease pathology, and evidence of specific functional interactions, in order to investigate whether the large body of cancer research on these molecules may shed additional light on their function in AD, and whether it is also possible to use existing knowledge of these miRNAs in AD to further elucidate their functions in cancer. Serving as a case study of the potential utility of this type of cross-disease comparison, we suggest that miRNA research in cancer may lead to hypotheses for novel roles of these miRNAs in AD, and highlight important directions for future research.

We expect to observe miRNA involvement in cancer and AD in pathways that may be contributing to both pathologies, as well as activity in disease-specific pathways, and anticipate that the differential expression and or regulation of these miRNAs may contribute to the differences in disease pathology. In addition to the aforementioned proliferative / anti-apoptotic pathway, we expect to observe miRNAs involved in invasion, metastasis, inflammation, oxidative stress, and angiogenesis in cancer; since many of these pathways have also been implicated in neurodegeneration, we expect that these miRNAs will also be implicated in the same or related pathways in AD [43,203-211].

For some pathways, including proliferation and pro-survival mechanisms, we expect to find evidence of inverse relationships [37,169,212]; however, for inflammation, oxidative stress, and angiogenesis it is reasonable to expect similar activity since these are known factors in both areas of pathology

[200,205,206,213-216]. Although there is evidence that the neuro-immune and inflammatory pathways are upregulated in AD, in cancer there is evidence that immunity can both suppress tumor growth and promote tumor progression, a process referred to as ‘immunoediting’ [205,217,218]. Figure 18 summarizes the documented and hypothesized roles of these pathways in cancer and AD. A comparison of miRNA expression and activity may yield more information on the contribution of these pathways to disease initiation and progression.

Figure 18. Molecular and Cellular Pathways Important to AD and Cancer. Increased levels of inflammation and oxidative stress have been positively associated with cancer and AD. Angiogenesis is considered one of the hallmarks of cancer, and has also been shown to be triggered by amyloid in AD [173,213]. Proliferation and pro-survival pathways have been shown to be positively associated with cancer and negatively associated with AD. Immunity has been shown to be both positively and negatively correlated with cancer ('immunoediting'), in addition to being positively correlated with AD. The amyloid pathway does not appear to be involved in cancer pathways, so for the purposes of this review is specific to AD. Blue lines indicate interactions found in AD research; orange lines indicate interactions found in cancer research.



B. MiR-9

Three studies found that miR-9 was significantly upregulated in the hippocampus and temporal lobe neocortex of AD brains compared to normal aging brains, indicating that miR-9 may play a pathological role in AD [219-221]. Because these brain regions show extensive pathology during the progression from health to AD [222-226] upregulation of miR-9 may play a role in degeneration. However, other studies found miR-9 was downregulated in the hippocampus, anterior temporal cortex and medial frontal gyrus in AD patients [227,228]. Therefore, at present there is conflicting data regarding the specific role of miR-9 in AD and its expression pattern in key cerebral regions and differences in sample characteristics and methods may be a factor in directionality of changes. It should be noted that miR-9 has a half-life of 30-60 minutes; the post-mortem interval (PMI) for the three studies finding upregulation of miR-9 was 3 hours or less, while the other two studies had PMIs of 3-10 hours and 24 hours [220]. It seems possible that this variability is enough to explain the measurement discrepancies, in which case miR-9 is upregulated in AD brains, but decays at a rapid rate post-mortem, causing it to appear downregulated at later time points.

In terms of mechanism, miR-9 has been predicted to target the 3' untranslated region (UTR) of *BACE1*, a key element of the amyloid pathway, though to our knowledge there is currently no functional evidence of this interaction. If confirmed, this would support miR-9 downregulation, presumably resulting in increased β-secretase activity and increased Aβ42 production [228].

Finally, there is some initial evidence that miR-9 may be upregulated in response to interleukin-1B (Il-1β) and Aβ42-induced Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NF-kappaB), implying that miR-9 may be involved in the AD inflammatory and oxidative stress pathways [229]. Brain-enriched miR-9 has also been implicated in a wide variety of functions in different life stages and organisms; overexpression in adult mouse neural progenitor cells has been shown to facilitate neuronal differentiation, although inhibiting miR-9 does not inhibit differentiation [230,231].

MiR-9 is active in a variety of pathways, with both tumor suppressive and oncogenic functions for different types of cancer. Downregulation has been observed in metastatic melanoma and head and neck squamous cell carcinoma (HNSCC) [232,233], while upregulation has been observed in glioma, gastric cancer, biliary cancer, Hodgkin lymphoma (HL), colorectal cancer (CRC), breast cancer, and cervical cancer [234-241], indicating that this regulatory miRNA is likely involved in multiple pathways, some of which are differentially regulated in different cancer types.

There is functional evidence that this miRNA may act as a tumor suppressor in some cancers. Cell proliferation was significantly inhibited in a HNSCC cell line by overexpression of miR-9, indicating that it may be a negative regulator of this pathway [233]. MiR-9 was also shown to downregulate proliferation and metastasis pathways in melanoma via the NF-kappaB1 molecular pathway; it was shown to directly target the *NFKB1* 3’ UTR. Since *NFKB1* is known to be activated by inflammatory and oxidative stress signals, this indicates that miR-9

may play an important role in these pathways in cancer as well as AD [200,232,242]. MiR-9 is upregulated by NF-kappaB in AD, and downregulates *NFKB1* in melanoma, suggesting that there may be a negative feedback loop in AD or differential regulation of this mechanism in AD and cancer. Additional research will be required to determine which of these explanations is correct.

In contrast to the role of miR-9 in suppressing proliferation and metastasis, and possible involvement in the NF-kappaB inflammatory and oxidative stress pathways, other data indicate that miR-9 upregulates oncogenic pathways in cancer including proliferation, metastasis, and invasion. An investigation of glioma found that inhibition of miR-9 reduced tumor cell stemness and led to cellular differentiation [238]. It was also shown to increase cell motility, and overexpression downregulated α-catenin in CRC, indicating involvement in metastasis [241]. In breast cancer, miR-9 expression was upregulated in higher grade tumors, indicating that it may be involved in proliferation and or metastasis [235]. Involvement in these pathways is also supported by research in cervical cancer, where upregulation of miR-9 was associated with increased cellular viability, anchorage-independent growth, and migration in vitro [240]. In an endometrial cancer cell line, miR-9 was shown to significantly reduce expression of Forkhead box O1 (*FOXO1*), a transcription factor, while in another endometrial cancer cell line inhibition of this and several other miRNAs resulted in re-expression of *FOXO1*, cell cycle arrest, and apoptosis [243]. Upregulation of miR-9 in HL results in repression of Hu antigen R (*HUR*; responsible for stabilizing messenger RNAs (mRNAs) to regulate gene expression) and *DICER1*

(a major regulator of mRNA, see Figure 16), leading to increased cytokine production by HL cells and attraction of normal inflammatory cells, indicating that miR-9 may be involved in large-scale translational regulation as well as inflammation pathways [236,237].

There is conflicting evidence regarding the function and expression of this miRNA in AD and cancer, making interpretation of its roles challenging. MiR-9 has been found both up- and down-regulated in affected tissue from AD brains and evidence suggests it may play a role in *BACE1* regulation. In cancer, there is evidence that miR-9 can upregulate oncogenic pathways including proliferation, metastasis, and invasion. MiR-9 promotes stemness and prevents differentiation in glioma, increases cell motility and metastasis in CRC, promotes proliferation and or metastasis in breast, endometrial and cervical cancer, and is involved in translational regulation and inflammation pathways in HL.

The evidence suggests that miR-9 may be important in AD, as it has been shown to be differentially expressed in cerebral regions which are significantly associated with pathological progression, yet more research is needed to clarify the molecular causative functions. Cancer research suggests a wide variety of possible functions that could be tested for parallel associations in AD, from transcriptional regulation, proliferation, inflammation, and high-impact translational regulation, to differentiation. Of note, miR-9 appears to have opposite influence on differentiation in neuronal development and glioma. If miR-9 does upregulate differentiation, as suggested by the study of neuronal development, we might expect this miRNA to be upregulated in AD, resulting in a

decrease in neuronal precursors, and gradual neurodegeneration as a consequence of reduced neuronal renewal. This is supported by the reports of miR-9 upregulation in the brains of AD patients.

C. MiR-29

The majority of evidence suggests that miR-29 is downregulated in AD, though, similar to miR-9, there is some conflicting evidence regarding expression pattern. MiR-29 was inversely correlated with the density of amyloid plaques in adjacent tissue in brains from individuals with AD [244]. MiR-29b was also observed to be downregulated in the parietal lobe cortex of individuals with AD compared to age-matched controls [245]. Additionally, loss of miR-29a/b in sporadic AD was correlated with increased *BACE1* expression, supporting downregulation of miR-29 in AD and also providing evidence that miR-29 may be directly involved in the amyloid pathway [228]. By contrast, in another study miR-29a/b expression was increased in the medial frontal gyrus in AD patients [227]. This finding may reflect region-specific tissue expression; however, it is also important to consider methodological differences in this case. While the three papers documenting down-regulation of this miRNA utilized in situ hybridization by microarrays to measure expression, Cogswell et al. (2008) used real-time PCR. This method could introduce additional bias, since it requires primer amplification instead of direct measurement; therefore the consensus of the three other papers on miR-29a/b downregulation may be more credible in this instance.

MiR-29 also has been shown to inhibit neuronal apoptosis during development via inhibition of genes in the pro-apoptotic BH3-only family, which would otherwise inhibit pro-survival proteins in the BCL-2 family [246]. If one of the primary functions of miR-29 is inhibition of apoptosis, one might expect to observe downregulation in AD associated with an increased rate of neurodegeneration caused by members of the pro-apoptotic BH3-only family inhibiting expression of the BCL-2 family. For the majority of studies just mentioned, miR-29 was in fact downregulated in association with disease pathology as predicted by this hypothesis. Most current evidence suggests that miR-29 downregulation is associated with AD pathology. The upregulation observed by Cogswell et al. (2008) may reflect measurement in the medial frontal gyrus, a region which was not included in other studies.

In cancer, miR-29 appears to interact directly with the BCL-2 family pro-survival proteins. It was downregulated in malignant cholangiocarcinoma cells with corresponding upregulation of anti-apoptotic Myeloid cell leukemia sequence 1 (*MCL1*, a member of the BCL-2 anti-apoptotic family). Transfection of miR-29 decreased *MCL1* expression and increased sensitivity to drug-induced cytotoxicity [247]. Supporting the validity of these results and suggesting that this function may be common in cancer, miR-29 was observed to be downregulated in melanoma, mantle cell lymphoma, acute myelogenous leukemia (AML), hepatocellular carcinoma (HCC), cervical cancer, endometrial serous adenocarcinoma, and non-small cell lung cancer (NSCLC), and transfection of miR-29 was correlated with decreased tumorigenicity [248-254]. In AML, miR-29

expression is negatively correlated with *MCL1* mRNA, while in HCC *BCL2* and *MCL1* were both direct targets of miR-29; both of these findings support the theory that miR-29 functions as a tumor suppressor in cancer by promoting apoptosis [249,253].

In addition to this apoptotic pathway, previous cancer research also contains information linking miR-29 to other pathways. In mantle cell lymphoma, miR-29 was shown to regulate Cyclin-dependent kinase 6 (*CDK6*) mRNA to influence cell division; downregulation of miR-29 was correlated with increased CDK6 and decreased survival [254]. This is supported by research in cervical cancer, which indicated that miR-29 level was negatively correlated with CDK6; addition of miR-29 to human papillomavirus-infected cells was shown to retard cell cycle progression and increase the frequency of apoptosis [251]. In cholangiocarcinoma cells miR-29 was transcriptionally repressed by NF-kappaB protein, a key molecule in the inflammatory pathway, as well as c-Myc and hedgehog proteins, implicating this miRNA in proliferation downregulation [247,255]. In HCC, miR-29 was found to downregulate Matrix metalloproteinase-2 (*MMP2*), leading to anti-angiogenesis via subsequent suppression of Kinase insert domain receptor (*KDR*, also called *VEGFR2*) signaling, as well as anti-invasion effects [256]. Supporting this, in CRC, Apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G (APOBEC3G) promotes metastasis via inhibition of miR-29 and subsequent upregulation of *MMP2* [257]. In HCC miR-29 was also implicated in upregulation of the long non-coding RNA Maternally expressed gene 3 (*MEG3*), accompanied by inhibited anchorage-dependent and

-independent cell growth and increased apoptosis [258]. Finally, miR-29 has been associated with transcriptional regulation in cancer via regulation of DNA methylation genes. In melanoma, miR-29 expression was inversely correlated with protein levels of DNA methyltransferases DNMT3A and DNMT3B, which affected overall survival [252]; miR-29 expression was also negatively correlated with DNMT3A and B in lung cancer, and transfection of miR-29 restored normal methylation patterns and expression of various tumor suppressor genes, as well as inhibiting tumorigenicity in vitro and in vivo [248]. From this evidence, miR-29 appears to act as a tumor suppressor within a large variety of pathways involved in cancer including apoptosis, tumorigenicity, cell cycle regulation, proliferation, invasion, metastasis, angiogenesis, inflammation, and transcriptional regulation.

This analysis of miR-29 implicates involvement in a variety of pathways in cancer, only one of which has been observed in AD. In addition to its activity in the amyloid pathway in AD, downregulation of miR-29 may allow BH3-only family proteins to inhibit BCL-2 family proteins, leading to apoptosis, while in cancer action of BH3-only family proteins must be blocked somehow, allowing BCL-2 family proteins to promote survival since miR-29 is also downregulated. The additional interactions of miR-29 in cancer should also be investigated in AD; for example, NF-kappaB, which is known to be upregulated in AD, was shown to target miR-29 in cholangiocarcinoma, but to date has not been shown to target this miRNA in AD [259]. Given the findings in cancer, it would be useful to determine whether miR-29 regulates DNA methyltransferases in the brain, and, if so, whether these interactions influence AD pathology.

D. MiR-101

Previous studies have demonstrated downregulation of miR-101 in the temporal and parietal cortex [228,245]. Subsequently, miR-101 was shown to downregulate APP; additionally, lentiviral-mediated overexpression of miR-101 was observed to reduce fibrillar Aβ and another known target, Cytochrome c oxidase subunit II (*COX2*), in hippocampal neurons [260]. Another study replicated miR-101 downregulation of APP, and also demonstrated that miR-101 is highly expressed in neural cells compared to HeLa and neuroblastoma cells [261]. These studies indicate that miR-101 downregulation results in increased Aβ and COX2, which has previously been shown to induce the innate immune complement component C1qB, and has been associated with inflammation [262,263]. Further, COX2 is elevated in hippocampal tissue in AD cases, and is correlated with amyloid plaque density [264]. MiR-101 therefore may play an important regulatory role in the amyloid, inflammatory, and immune pathways, and downregulation may exacerbate disease pathology.

There is an extensive body of research on the multiple roles of miR-101 in cancer. MiR-101 is consistently downregulated in many cancers including acute lymphoblastic leukemia, HCC, glioblastoma, lung, gastric, colon, renal, prostate, ovarian, bladder, and pancreatic cancer [242,265-273]. Downregulation was associated with many oncogenic characteristics including increased proliferation, invasion, advanced tumor stage, and decreased survival; cellular transfection with miR-101 resulted in increased apoptosis and inhibited proliferation, invasion, and angiogenesis, via inhibition of expression of primary target molecules such

as the histone methyltransferase Enhancer of zeste homolog 2 (*EZH2*), *COX2*, and *MCL1*. EZH2 is a transcriptional regulatory molecule which can potentially regulate many different pathways, greatly increasing the impact of miR-101 activity or loss. Redundancy is reflected in miR-29 and miR-101 regulation of the anti-apoptotic *MCL1*, indicating that this is an important pathway in cancer and highlighting the potential for future AD research, given that molecules involved in cell survival could play an important role in neurodegeneration. As discussed earlier, COX2 has been implicated in inflammatory and immune pathways; this functional interaction is seen in both AD and cancer, reflecting the importance of these pathways in both diseases and indicating that miR-101 may be a key negative regulator of both diseases.

E. MiR-107

MiR-107 downregulation has been reported in AD and has been related to amyloid plaque density, neuritic plaque counts, and neurofibrillary tangle counts in adjacent tissue [244,274]. Additionally, miR-107 has been correlated with *BACE1* mRNA level, and BACE1 has been shown to have miR-107 sequence target sites. Thus in AD, miR-107 may be a regulator of BACE1 activity and consequently affect APP cleavage [274,275]. These results support the role of miR-107 downregulation in AD and suggest a mechanism related to plaque burden.

Regulation in cancer is more of a mixed picture; there is conflicting evidence for miR-107 regulation in prostate cancer and gastric cancer, indicating that more research on this topic is needed. In prostate cancer, there is evidence that

downregulation of miR-107 is correlated with upregulation of the mitogen and growth factor Granulin (*GRN*); other members of this miRNA family have also been shown to display this pattern of downregulation with associated upregulation of *GRN* in 11 different types of cancer [276]. In contrast, a significantly higher level of miR-107 was found in the urine of men with prostate cancer, suggesting that this miRNA may be upregulated [277]. Additional research is needed to resolve this apparent discrepancy. In gastric cancer, there is also conflicting evidence for regulation of miR-107; expression was significantly elevated in gastric tumor tissue compared to normal tissue, was associated with depth of invasion, lymph node metastasis, and stage, decreased overall survival and disease-free survival, and was inversely correlated with *DICER1* mRNA, indicating possible involvement in translational regulation [234,278]. However, miR-107 was also observed to be silenced in gastric cancer cells, and transfection of miR-107 was correlated with downregulation of *CDK6* mRNA and reduced protein expression, cell cycle arrest, and decreased proliferation and invasion [279]. At this time, more research is needed to clarify the roles of miR-107 in prostate and gastric cancer, and whether it is ultimately oncogenic or tumor suppressive.

MiR-107 has also been shown to be upregulated in breast cancer, and downregulated or silenced in HNSCC, colon cancer, and pancreatic cancer. In breast cancer, miR-107 was found to be upregulated, was shown to silence the tumor suppressive miRNA let-7, and was associated with increased tumorigenic potential and metastases in mice [188]. In HNSCC, low expression of miR-107

was correlated with increased Protein Kinase Cδ (PKCδ), and miR-107 inhibited proliferation, DNA replication, colony formation, and invasion [280]. MiR-107 has been implicated in angiogenesis in colon cancer; the tumor suppressive transcription factor p53 can mediate transcription of miR-107, and forced expression of miR-107 suppresses expression of Hypoxia inducible factor-1beta (*HIF1b*), suppressing angiogenesis, tumor growth, and Vascular endothelial growth factor (*VEGF*) expression [281]. Finally, in pancreatic cancer cell lines, miR-107 was reported to be epigenetically silenced, and re-expression by a demethylating agent in tumor cell lines inhibited CDK6 and decreased growth in vitro, indicating that miR-107 is also involved in a cell cycle regulatory pathway [282]. It is possible that the regulation of let-7 by miR-107 in breast cancer overrides other interactions since let-7 has been shown to affect a large number of targets, and that this level of regulation may be blocked in other cancers, allowing other miR-107 interactions to take precedence and produce tumor suppressive effects.

A number of different pathways appear to be regulated by miR-107 in cancer including proliferation, invasion metastasis, cell cycle regulation, additive translational regulation (mediated by let-7), and angiogenesis. More information on the potential interaction of miR-107 with *DICER1*, *CDK6*, *HIF1b*, and *GRN* could further elucidate the molecular pathways involved in AD, as well as the regulatory process of disease initiation and progression. GRN is a particularly interesting association, given that it has been found to cause one form of frontotemporal lobar degeneration [283]. The altered expression patterns for

various types of cancer and AD may indicate that there is another level of regulation that differs across tissues. This in turn may be modulated by other miRNAs, which could alter the methylation status of miR-107 via interaction with histone methyltransferases.

F. MiR-125b

Small increases in miR-125b have been observed in the hippocampal region of AD brains post-mortem. Because synapsin II mRNA has been identified as a target for this miRNA, it has been postulated that it may be responsible for synapsin protein deficits observed in the brains of patients with AD [219,229,284-287]. MiR-125b has been shown to be significantly upregulated in the temporal lobe neocortex of AD patients [220,221]. MiR-125b was also found to be upregulated in the hippocampus, medial frontal gyrus, and cerebellum [227]. Further, miR-125b was positively correlated with gray matter neurofibrillary tangles in post-mortem AD patients [244]. Finally, in cultured human neuronal-glial cells, miR-125b has been shown to be upregulated by NF-kappaB in response to IL-1β and Aβ42-peptide-induced stress; in this model, miR-125b was also shown to target the mRNA of Complement factor-H (*CFH*), known to be an important suppressor of immune and inflammatory pathways in the brain, and lead to decreased expression [259,288]. From these reports, miR-125b is implicated in neuropathological change, and appears to function in the inflammatory and oxidative stress NF-kappaB pathways, innate immunity via CFH, and cellular transport via synapsin.

In cancer, miR-125b appears to function mostly as a tumor suppressor. The majority of research to date has been done in HCC; downregulation of miR-125b has been linked to tumor progression and metastasis, tumorigenicity, proliferation, migration, invasion, angiogenesis, and cell cycle de-regulation in HCC or HCC cell lines [289-291]. In this disease, miR-125b was shown to be silenced by methylation; miR-125b was also shown to decrease angiogenesis via regulation of Placenta growth factor (*PIGF*), a member of the VEGF family, as well as *MMP2* and *MMP9*, which may be involved in miR-125b effects on invasion [289]. Investigation of the effects of miR-125b on angiogenesis in more depth in epithelial cells reveal contradictory evidence that VEGF can induce miR-125b, which in turn inhibits translation of Vascular endothelial cadherin (VE-cadherin) and in vitro tube formation; overexpression of miR-125b was shown to result in nonfunctional blood vessel formation, indicating that miR-125b could promote angiogenesis [292]. It is possible that this contradiction is caused by the model; miR-125b may act through different pathways in cancerous tissue. MiR-125b was also shown to upregulate Cyclin dependent kinase inhibitor 1 (*CDKN1A*) expression, which arrested cell cycle transition and silenced Lin-28 homolog B; these effects were linked to inhibited growth, migration, and invasion (Liang et al., 2010). The histone methyltransferase EZH2 was shown to downregulate miR-125b in HCC, resulting in progression and metastasis [290]. In bladder cancer, miR-125b also appears to function as a tumor suppressor; miR-125b inhibits E2F transcription factor 3 (*E2F3*), which is involved in the G1/S phase cell transition, and transfection of miR-125b in bladder cancer cell lines

decreased E2F3 protein and Cyclin A2, depressing colony formation in vitro and tumor development in mice [293]. Contrary to these results, a study in glioma found that miR-125b promotes glioma cell proliferation and inhibits drug-induced apoptosis, indicating that it may play an oncogenic role in this particular disease; the authors further found that miR-125b may target and downregulate apoptosis-related protein BCL-2 modifying factor, and apoptotic activator (*BMF*) [294]. Aside from glioma, miR-125b seems to play a tumor suppressive role in a variety of molecular pathways, though miR-125b should be investigated to determine whether this is also true in other cancer types.

Given the largely tumor suppressive function of miR-125b, which inhibits growth and is downregulated in cancer, we would expect miR-125b to be upregulated in AD with resulting neurodegeneration, which has been observed as noted above. However, the specific interactions of miR-125b with molecules in a large variety of pathways have not been investigated in AD; research to determine whether miR-125b directly interacts with the molecules genes above including angiogenic *VEGF*, cell cycle regulatory *E2F3*, and apoptotic activator *BMF* could shed more light on the molecular mechanisms involved in AD pathology and further elucidate the pathways involved in this disease. Interestingly, the role of miR-125b in innate immunity does not appear to have been specifically investigated in cancer at this point; this could be an important factor in cancer etiology which should be further investigated as well.

G. MiR-146

Similarly to miR-125b, miR-146a was found to be significantly upregulated in the temporal cortices of patients with AD [220,221]. It was shown to function similarly to miR-125b in the AD inflammatory and oxidative stress pathways, as it was shown to be upregulated by NF-kappaB in response to IL-1β and Aβ42 or oxidative stress in cultured human neuronal-glial cells, and to decrease expression of *CFH* [259,288,295]. Consistent with these results, miR-146a was observed to be upregulated in AD neocortices, and in IL-1β and Aβ42 stressed human astroglial cells; in the astroglial model miR-146a downregulates the Toll-like receptor signaling molecule Interleukin (IL)-1 receptor-associated kinase 1 (*IRAK1*) in tandem with *IRAK2* upregulation by NF-kappaB to promote the innate immune and inflammatory responses [295]. Interestingly, unlike miR-146a, miR-146b was downregulated in both the hippocampus and medial frontal gyrus in individuals with AD; the researchers postulate that downregulation of miR-146b relieves inhibition of IRAK1 and another toll-like receptor signaling molecule, TNF receptor-associated factor 6 (TRAF6), triggering the innate immunity pathway which contributes to the activation of microglia and neurodegeneration [227]. More work is needed to clarify these apparently contradictory roles of miR-146a/b in AD.

Upregulation of miR-146a was observed in anaplastic thyroid carcinoma (ATC) and cervical cancer, while downregulation was observed for miR-146a in pancreatic cancer, and miR-146a and b in prostate and breast cancer, indicating that these miRNAs may play a variety of oncogenic or tumor suppressive roles in

different cancers [296-302]. MiR-146a has been characterized as oncogenic in anaplastic thyroid carcinoma, activated by the inflammatory molecule NF-kappaB; inhibition of miR-146a decreased oncogenic potential in ATC-derived cells and increased susceptibility to chemotherapy-induced cytotoxicity [301]. Additional oncogenic function was observed in cervical cancer, wherein miR-146a was significantly upregulated in cervical cancer and was tied to cellular proliferation [302]. In contrast, miR-146a and b have also been shown to have a variety of tumor suppressive functions in breast, prostate, and pancreatic cancer. Functional interaction research in breast cancer indicates that Breast cancer metastasis suppressor 1 (*BRMS1*) upregulates miR-146a and b; expression of either miRNA resulted in downregulation of Epidermal growth factor receptor (*EGFR*) and reduced invasion, migration, and metastasis [297].

Also in breast cancer, miR-146a and miR-146b expression suppressed *IRAK1* and *TRAF6*, which was reported to impair NF-kappaB activity, resulting in reduced invasion and migration [296]. This negative feedback loop was also observed previously in an acute monocytic leukemia cell line, indicating that miR-146a and b appear to be involved in negatively as well as positively regulating oxidative, immune, and inflammatory responses [303]. Transfection of miR-146a in prostate cancer cell lines led to significantly reduced expression of Rho-associated, coiled-coil containing protein kinase 1, and reduced proliferation, invasion, and metastasis; downregulation of either miR-146a or b in prostate cancer was also linked to focal basal cell layer disruptions, which have been correlated with invasion [299,300]. Similarly, re-expression of miR-146a induced

by isoflavone in pancreatic cancer was also correlated with reduced invasion and downregulation of *IRAK1* and *EGFR*, indicating that this molecular pathway is involved in multiple cancers [298]. Finally, genetic studies have linked polymorphisms in miR-146a to increased risk of gastric cancer, breast cancer, and papillary thyroid carcinoma, while meta-analyses have also linked risk to more generalized cancer susceptibility [304-308]. These various disparate pieces of evidence indicate that miR-146a plays a variety of roles in different cancer types, though there seems to be a commonality to the interactions observed for the tumor suppressive roles miR-146a plays in prostate, pancreatic, and breast cancer. More research would be helpful in clarifying the mediatory mechanisms which determine whether miR-146a is tumor suppressive or oncogenic.

Comparing the evidence for miR-146a function in AD and cancer, there seems to be more overlap than for the miRNAs previously reviewed, though results from cancer may still inform additional interactions in AD. For instance, an investigation of miR-146a regulation of *EGFR* in AD may tie this miRNA to a proliferative pathway; logically, if these molecules are negatively regulated and miR-146a is upregulated in AD, one would expect a decrease in EGFR, with possible subsequent neurodegenerative effects. For example, in rodents with traumatic brain injury, treatment with epidermal growth factor reduced hippocampal neuronal cell loss and improved cognitive function [309]. Interestingly, modulation of the inflammatory/innate immune pathway by miR-146a appears to be play a large role common to both diseases. The evidence that miR-146a and b inhibit *IRAK1* and *TRAF6* in breast cancer may contradict

the hypothesis of Cogswell et al. (2008) that miR-146b relieves inhibition of these molecules in AD, though a functional study of this interaction, as well as the subsequent NF-kappaB modulation, would be required for clarification. Finally, it is important that future studies make an effort to clarify the role of miR-146b in cancer, as many results to date have concerned miR-146a; since there seems to be functional overlap based on current research, it would be interesting to investigate further whether these two miRNAs may be differentially regulated in AD.

H. MiR-153

This miRNA has not been studied as extensively as others mentioned in this review, but is discussed because it has been very recently implicated in regulation of APP. Long and colleagues found that delivery of miR-153 in human fetal brain cultures reduced expression of *APP*, as well as an APP paralogue, Amyloid beta (A4) precursor-like protein 2, via direct interaction with a target site on the *APP* 3' UTR, while inhibition of miR-153 resulted in increased expression of *APP*; supporting this, decreased miR-153 and increased APP were observed in a subset of AD patients with moderate pathology [310]. Given this important functional evidence for miR-153 interaction in the amyloid pathway, it seems important to review current research of this miRNA in cancer to see if we can extrapolate any further roles or further elucidate molecular function for miR-153.

As with the results for AD, a review of cancer research does not yield much information on miR-153; however, the few studies published may shed additional light of the function of this regulatory molecule. In ovarian cancer, miR-153 was

downregulated, and expression was different for four histopathological types of ovarian cancer; the researchers subsequently found that miR-153 was significantly correlated with tumor grade and clinical stage [311]. A key study found that miR-153 is downregulated in glioblastoma, with re-expression in cell lines associated with decreased proliferation and increased apoptosis; the researchers further demonstrated that miR-153 directly inhibited anti-apoptosis molecules *BCL2* and *MCL1* [312]. An additional study in the same model found that miR-153 also inhibits the insulin receptor substrate 2 (*IRS2*), further implicating it in growth regulation [313]. These studies characterize the tumor suppressive role of miR-153, as well as specific interactions which appear to mediate this function. Interestingly, the same endometrial cancer study mentioned previously for miR-9 also found that miR-153 has similar oncogenic properties, appearing to downregulate *FOXO1* and apoptosis [243]. This oncogenic function seems to contradict other tumor suppressive functions listed above and indicates that this miRNA would benefit from more intense scrutiny.

Mediation of *BCL2* and *MCL1* by miR-153 indicates that it may have overlapping functionality with miR-29 and 101; however, the majority of evidence indicates that these miRNAs are downregulated in AD, whereas if they were inhibiting anti-apoptotic molecules, we would expect them to be upregulated in AD leading to neurodegeneration. Additional research is needed to determine if miR-153 can also inhibit anti-apoptosis molecules in the brain, as well as why this interaction would be suppressed in a neurodegenerative disease like AD. Interestingly, if miR-153 does in fact interact with *IRS2*, that would tie this miRNA

to the insulin regulatory pathway; in addition to the known association of diabetes with increased dementia risk, there is some evidence that insulin may be directly involved in AD, so this association should be a focus of further investigation [314-317].

I. MiR-195

There is comparatively little evidence for the involvement of miR-195 in AD; however, miR-195 has been shown to directly target the 3' UTR of *BACE1*, and to decrease protein expression in mouse neuroblastoma cells, with a corresponding drop in Aβ levels [318]. Additionally, miR-195 was decreased in the cerebrospinal fluid (CSF) of AD patients compared with controls [227], while another group found that miR-195 was downregulated and was negatively correlated with diffuse amyloid plaques in the gray matter of post-mortem AD patients [244]. Given these limited but interesting results, it seems important to review the roles of miR-195 in cancer to see if additional information about miR-195 in the brain can be extrapolated for further investigation. Since downregulation could theoretically be tied to increased amyloid beta levels, establishing expression patterns of this miRNA in AD brain tissue should be a research priority, along with more functional investigation.

Importantly, miR-195 has been extensively studied in cancer, with current research indicating that it plays tumor suppressive or oncogenic roles in different cancer types. Downregulation was observed in a number of different cancer types, and was associated with poorer overall survival, increased metastasis incidence, and higher tumor stage; however, upregulation was also observed in

several cancer types [319-330]. In breast cancer and glioma, conflicting studies indicate that more research is needed to determine the impact of this miRNA [331-335]. Research in the tumor suppressive functions of miR-195 has shown that it interacts with a number of molecules involved in cell cycle regulation, as well as metabolic and anti-apoptotic molecules. MiR-195 was shown to inhibit Cyclin D1 in HCC and breast cancer [328,334], Cyclin D3 in glioblastoma [330], Cyclin dependent kinase 4 in bladder cancer [322], *CDK6* in HCC [328], *E2F3* in HCC and glioblastoma [328,330], *BCL2* in breast and colorectal cancer [323,336], Raf-1 proto-oncogene, serine/threonine kinase in breast cancer [334], and Glucose transporter type 3 (*GLUT3*) in bladder cancer [337]. The role of miR-195 in suppression of anti-apoptotic *BCL2* lends support to the evidence that miR-195 is downregulated in breast cancer, promoting cell survival. Unsurprisingly given these genes' known roles in cell cycle regulation, metabolism, and anti-apoptosis, inhibition via miR-195 was variously associated with cell cycle arrest, decreased proliferation, reduced invasion, increased apoptosis, and reduced colony formation and tumor formation. Interestingly, given that the only clinical measure associated with upregulation at this point is increased resistance to Temozolomide [335], it is possible that the tumor suppressive functions of miR-195 early in cancer development are overridden by oncogenic interactions favoring survival in the context of drug resistance; further investigation of this is merited in cancers associated with miR-195 upregulation as listed above. The dearth of functional and clinical investigation of miR-195 upregulation, as well as the contradiction between research indicating that it may

be up or downregulated in breast cancer and glioblastoma, should be addressed to further clarify the mechanisms of miR-195 in cancer.

From the current literature detailing the tumor suppressive role of miR-195 in various cancers, it is possible to hypothesize that this miRNA has functional overlap with miR-29 and miR-153, both of which have been shown to interact with *BCL2*, which would lead to the additional hypothesis that miR-195 is similarly downregulated in AD. This is a logical expectation given the functional evidence that decreases in miR-195 could be tied to increased amyloid beta and decreased levels were observed in CSF and gray matter, however, it requires further investigation. Researching these premises might also point to the possibility that all three miRNAs are regulated via a common mechanism in AD given their apparent redundancy. As suggested earlier, further investigation in AD of the functional interactions ascribed to this miRNA in cancer might help elucidate additional mechanisms by which it influences AD pathology. Given the evidence in cancer, it is possible that this miRNA is also important in proliferative pathways in the brain.

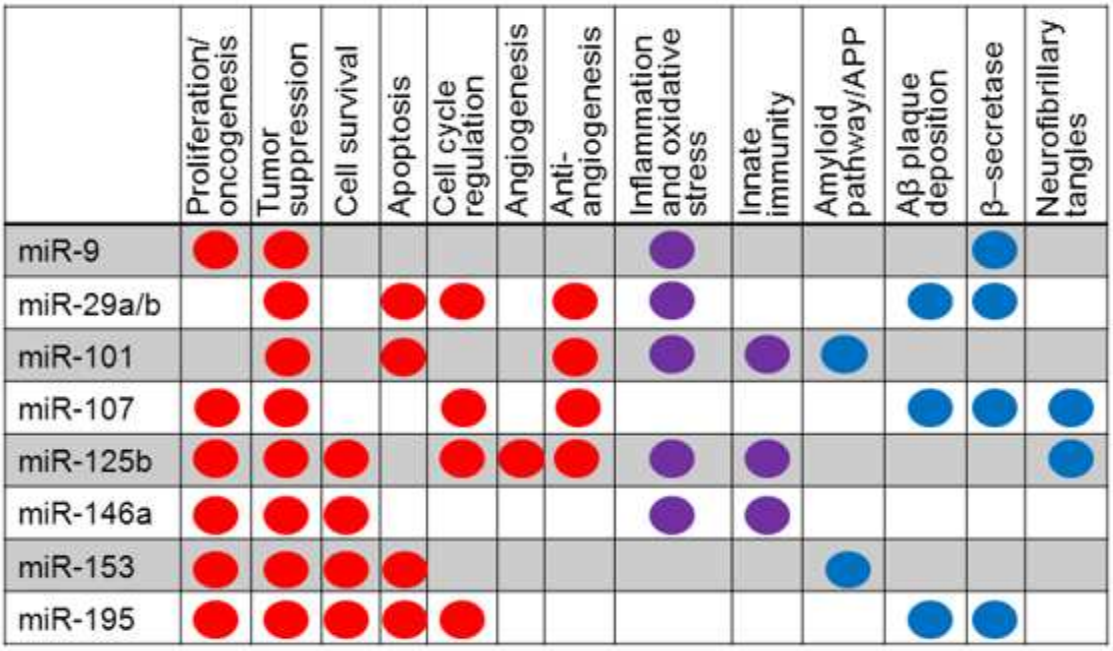
J. Pathways

The overall involvement of miRNAs under consideration in this review in specific biological pathways is summarized in Figure 19, including oncogenic and tumor suppressive functions in cancer and amyloid-specific mechanisms. The amyloid pathway has clearly been a focus of AD research to date. We have reviewed evidence that multiple miRNAs regulate expression of molecules in this pathway; miR9, 29a/b, 107, and 195 have been predicted or shown to target, or

negatively correlated with, *BACE1* mRNA or protein levels, suggesting that these miRNAs may have functional overlap or redundancy, while miR-101 and 153 have been shown to inhibit *APP* protein directly (Figure 17).

Figure 19. Summary of MiRNA Pathway Relationships in Cancer and AD.

Major pathways are listed; dots indicate evidence that a specific miRNA is involved in a particular pathway. Red indicates evidence from cancer research, blue from AD research, and purple from both cancer and AD.

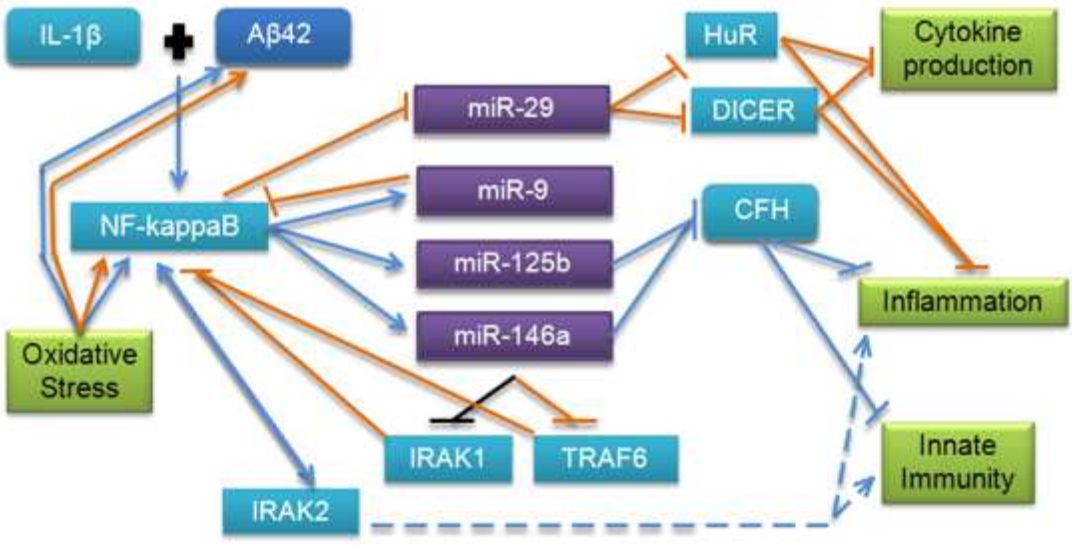


In addition, miR-9, 125b, and 146a are upregulated by the inflammatory and oxidative stress responses, suggesting that the amyloid pathway may have wide-reaching regulatory impact via miRNA.

The inflammatory and innate immune pathways have been extensively investigated in both cancer and AD. MiRNAs appear to be important in these pathways in both diseases and may function using some of the same mechanisms (Figure 20). Regulation involving the major inflammatory and oxidative stress transcription factor NF-kappaB is a common theme observed for many of the miRNAs reviewed here. This molecule is a key signaling molecule in AD as it appears to tie together the amyloid, inflammatory, oxidative stress, and innate immunity pathways, but it has also been shown to play an important role in cancer. As discussed earlier in this review, in cancer, miR-9 downregulates *NFKB1* and NF-kappaB appears to directly downregulate miR-29a and upregulate miR-146a, while in AD, NF-kappaB appears to upregulate miR-9, miR-125b, and miR-146a. MiR-125b and 146a regulate several other molecules in the NF-kappaB pathway and indirectly modulate NF-kappaB; miR-146a has been shown to downregulate Toll-like receptor signaling molecules *IRAK1* and *TRAF6* in cancer, with a corresponding drop in NF-kappaB level. Interestingly, miR-146a was also shown to downregulate *IRAK1* in AD, but did not affect *TRAF6* in the Aβ42 and IL-1β-stressed human cultured astroglial cell model [295]. This process has been postulated to result in ‘fine-tuning’ of these pathways, and may be aided by miR-9 downregulation of *NFKB1*, though this remains to be verified in AD. Given the similar expression and activation of miR-

125b compared with miR-146a, it would also be interesting to investigate whether this miRNA may also regulate downstream toll-like receptor signaling via a similar mechanism.

Figure 20. MicroRNA Involvement in the Inflammation, Innate Immunity, and Oxidative Stress Pathways in AD and Cancer. This pathway has been validated by several studies in AD; however, additional evidence from cancer research points to more molecules which may be involved. Blue lines indicate interactions and effects found in AD research, orange lines indicate interactions and effects found in cancer research, and black lines indicate findings common to both diseases. Dashed lines indicate downstream effects.



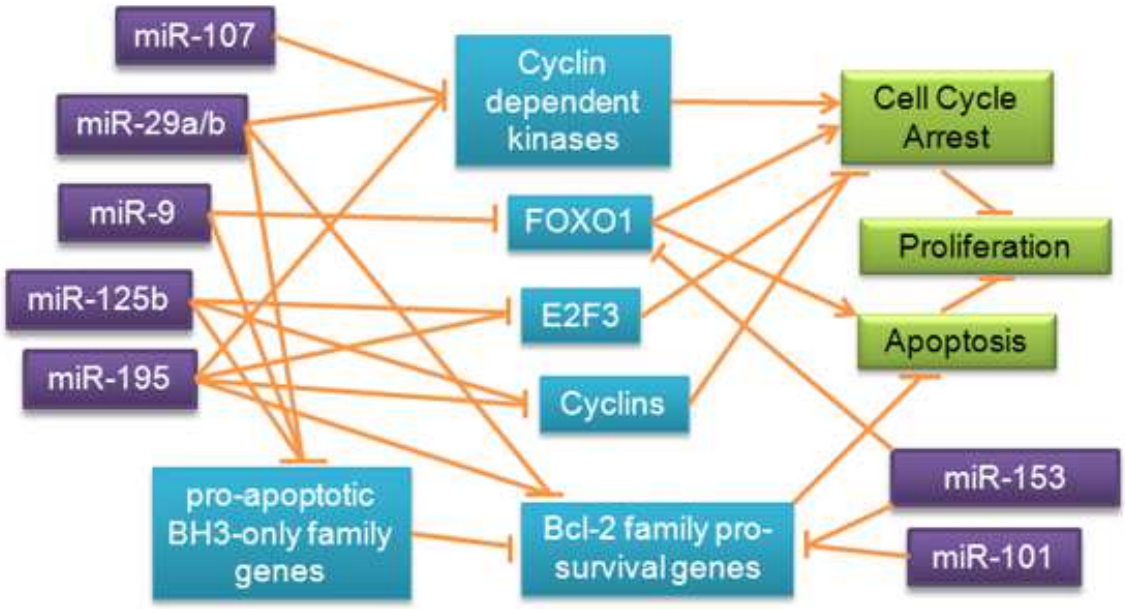
Additionally, in AD, miR-125b and 146a have been shown to regulate *CFH*, which normally represses the cerebral inflammation response. CFH has also been shown to play an important regulatory role in the complement cascade, which is a major component of innate immunity. Dysregulation has also been observed in Down syndrome, which shares some neuropathological characteristics of AD [338]. Interestingly, *CFH* polymorphisms have been previously associated with increased risk of AD in Apolipoprotein E ε4 carriers, indicating that this miRNA regulatory mechanism could be an important factor in pathology, and should be further investigated [339]. Based on the evidence in cancer research, an investigation of the interaction of miR-9 and 29b with NF-kappaB in AD and possible downstream targets in these pathways may further clarify the molecular mechanisms involved. MiR-101 regulation of *COX2*, which has been associated with innate immunity and inflammation, adds complexity to this regulatory pathway. We conclude that the innate immune, oxidative stress, and inflammatory pathways regulated by miRNAs are important in cancer and AD, and utilize some overlapping functional mechanisms; further research into each area based on previous research presented here could help to advance knowledge in both disease areas.

To date, most miRNA research on functional molecular interactions in AD has focused on the amyloid and inflammation pathways since these have been directly implicated in neuropathology. However, a number of other pathways have been postulated or demonstrated to play a role in AD including proliferation

and angiogenesis. Metastasis and invasion, key topics in cancer research, have been shown to be effectively regulated by miRNAs.

Given that some of the underlying functions of extracellular matrix degradation, stemness, and differentiation could affect neurophysiology and be involved in AD, miRNAs regulation of matrix metalloproteinases, cyclin-dependent kinases, and cyclins, all of which have been observed in cancer, should also be investigated in AD. Proliferative molecules including GLUT3 and IRS2, which are specifically involved in cell metabolism and appear to be differentially regulated by miRNAs in cancer, could also contribute to risk for AD and increased rates of neurodegeneration via impaired metabolism. Pro-survival genes such as *BCL2* and *MCL1* have been observed to be redundantly regulated by multiple miRNAs in cancer; inappropriate silencing of these molecules by miRNAs in AD could exacerbate neurodegeneration. Cell cycle regulatory molecules such as cyclin-dependent kinases, cyclins, and E2F3, are shown to be targets of several of the miRNAs discussed, indicating that these miRNAs can inhibit the cell cycle (Figure 21).

Figure 21. Redundant MicroRNA Mechanisms Regulating Proliferation and Survival Pathways in Cancer. Expression of transcription factors, BCL-2 family genes and inhibitors, and cell cycle regulatory molecules is redundantly regulated by miRNAs in cancer, prompting speculation about possible regulatory roles of these miRNAs in a similar pathway in AD.



Amyloid beta appears to upregulate angiogenesis, suggesting that this may be an important area for future miRNAs research in AD [340]. As discussed earlier, various miRNAs have been shown to play important roles in angiogenesis in cancer. MiR-29 downregulates *MMP2*, indirectly reducing VEGFR2 signaling and angiogenesis, miR-107 reduces *HIF1b*, with subsequently reduced angiogenesis, tumor growth, and *VEGF* expression, and miR-125b was shown to inhibit *PIGF* and VE-cadherin in different cell lines, inhibiting angiogenesis.

Interestingly, all instances of angiogenic regulation observed here were tumor suppressive, indicating a degree of functional redundancy, but one of the four miRNAs implicated was upregulated in AD. MiR-125b may be differentially expressed because it has been shown to be upregulated by amyloid beta. TRAF6, the innate immune molecule downregulated by miR-146a in breast cancer, has also been shown to negatively regulate *VEGF* in epithelial cells, leading to the question of whether the miR-146a regulatory pathway may result in increased VEGF signaling and angiogenesis [341]. This pathway, which has been implicated in AD, should be further investigated for miRNA regulation to elucidate function and disease risk factors.

Transcriptional and translational regulation of miRNAs and target genes is also a key process in both diseases, with multiple hierarchical levels of control and feedback mechanisms playing important roles in effect size and specificity. While miRNAs are inherently involved in translational regulation via the RISC silencing complex (Figure 16), several miRNAs in this review have may affect translational regulation at a higher level; both miR-9 and 107 have been shown to

inhibit *DICER1*, and miR-107 has been shown to inhibit another miRNA, let-7. Interestingly, miR-29 and 101 have been implicated in transcription control via inhibition of methyltransferases. An investigation of these properties could elucidate additional functions of these miRNAs and provide information on this important regulatory pathway in AD.

K. Conclusions

MiRNAs are clearly a relevant topic for both cancer and neurodegenerative disease. Many of the miRNAs discussed in this review have differential expression in AD and cancer, suggesting that they play multiple regulatory roles in pathways active across both cancer and AD (Table 20). While miRNA has been actively researched in cancer for a number of years, this is a relatively new area for research in AD and other neurodegenerative diseases. From a review of the literature on miRNAs in AD, much work remains to be accomplished to elucidate all roles of miRNAs in pathological pathways. Reviewing a number of miRNAs with functional evidence of involvement in AD indicates that other than the amyloid pathway and inflammation, possible regulation of other pathways by these molecules has been largely ignored. This review of miRNA regulatory mechanisms in cancer and AD has covered a variety of important molecular mechanisms observed in cancer that could potentially also contribute to pathology in AD. Further investigation of these pathways and mechanisms appears warranted and will hopefully be a focus of future research. Studies addressing other biological pathways such as proliferation and angiogenesis

would be important and help fill the gaps in our understanding of the regulatory mechanisms influencing this very complex disease.

A comparison of research on miRNAs in cancer and AD highlighted areas which could benefit from future research in both diseases. Though the primary focus was on increasing knowledge of AD from previous research in cancer, this case study also demonstrates that a similar strategy could be useful in studying other neurodegenerative diseases, which are starting to experience increasing attention with regard to the roles of miRNAs in disease pathophysiology.

Table 20. MiRNA Expression in Cancer and AD. MiRNAs are significantly differentially expressed, indicating different roles in disease.

	Types of Cancer (see text for citations)		Alzheimer's disease affected tissue	
MiRNA	Downregulated	Upregulated	Downregulated	Upregulated
miR-9	Melanoma and HNSCC	Glioma, GC, biliary, Hodgkin lymphoma, CRC, BC, cervical	Hippocampus and medial frontal gyrus, and ATC	Hippocampus, TL
miR-29	Cholangiocarcinoma, melanoma, MCL, AML, HCC, cervical, non-small cell lung		SMTG, parietal lobe cortex, and ATC	Medial frontal gyrus
miR-101	ALL, HCC, glioma, lung, GC, colon, renal, PC, ovarian, bladder, and pancreatic		Parietal lobe cortex, and ATC	
miR-107	GC, PC, HNSCC, colon, and pancreatic	GC, PC, BC	SMTG (replicated)	
miR-125b	HCC, bladder	glioma		TL, hippocampus, medial frontal gyrus, and cerebellum, and HNG
miR-146a	anaplastic thyroid, cervical	pancreatic, PC, BC		TL, HNG, and human astroglial cells
miR-153	ovarian, glioma	endometrial	Frontal cortex	
miR-195	BC, glioma, CRC, bladder, adrenocortical, HCC, primary peritoneal	BC, glioma, astrocytoma, CLL, malignant mesothelioma	CSF, and SMTG	

HNSCC=head and neck squamous cell carcinoma, GC=gastric cancer, CRC=colorectal cancer, BC=breast cancer, ATC=anterior temporal cortex, TL=temporal lobe neocortex, MCL=mantle cell lymphoma, AML=acute myelogeneous leukemia, HCC=hepatocellular carcinoma, SMTG= Superior and middle temporal gyri, ALL=acute lymphoblastic leukemia, PC=prostate cancer, HNG=human neuronal-glial cells in culture, CLL=chronic lymphocytic leukemia

This chapter was modified from research printed in the journal *Frontiers in Genetics*.

Holohan KN, Lahiri DK, Schneider BP, Foroud TM, Saykin AJ (2013) Functional MicroRNAs in Alzheimer's Disease and Cancer: Differential Regulation of Common Mechanisms and Pathways. *Front Genet* 3:323.

VII. Conclusions and Future Directions

In this work we have identified several important directions for research on cancer and treatment-related cognitive dysfunction, and have taken the first steps towards addressing these questions.

First, our review of neuroimaging studies of cancer and treatment-related cognitive dysfunction (Chapter II, Holohan et al. (2013)) suggested that additional multimodal longitudinal neuroimaging studies would aid in clarifying the course and duration of cognitive dysfunction, as well as potentially contributing to our knowledge of biological mechanisms driving this effect. To begin to address this question, we conducted a longitudinal, multimodal neuroimaging study of blood flow and gray matter density in breast cancer patients (Chapter III, Nudelman et al. (2014)). This work answered a question which had been raised by previous studies, which showed activation alterations (suggesting hyper- and hypoperfusion) [16], and others which showed frontal gray matter density decrease (suggesting hypoperfusion); our results showed a significant cluster of chemotherapy treatment-associated hyperperfusion in the precentral gyrus compared to health controls, but also showed other areas of hypoperfusion associated with frontal gray matter density decrease [17,119]. Importantly, these results support the hypothesis that multiple biological mechanisms may be driving chemotherapy treatment-associated cognitive dysfunction.

To continue to examine the question of whether multiple biological mechanisms drive cancer and treatment-related cognitive dysfunction, we utilized other data collected in this breast cancer cohort on chemotherapy-induced

peripheral neuropathy symptoms (CIPN-sx). Again using a multimodal, longitudinal design examining blood flow and gray matter density, we were able to show that CIPN-sx were associated with increased blood flow in regions of the brain known to be associated with pain processing, and also to show that this result was positively correlated with the previously observed gray matter density decrease (Chapter IV, Nudelman et al. (*under review*)). This indicates that individuals who did not experience significant gray matter density decrease did show increased blood flow in regions associated with pain processing. Again, this supports the hypothesis that multiple mechanisms are driving cancer and treatment-related sequelae.

To continue to address the question of mechanisms driving cancer and treatment-related cognitive dysfunction and other sequelae, it will be important to extend these findings beyond breast cancer. To begin to address this question, we analyzed gray matter density, comparing cancer survivors (CA+) and those who had not reported any cancer incidence (CA-) across diagnostic groups in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort (Chapter V, Nudelman et al. (2014)), and were able to demonstrate lower frontal gray matter density in CA+, across diagnostic groups, in regions similar to those observed in other imaging studies of cancer and treatment-related cognitive dysfunction [15,17,342]. This finding is particularly interesting in light of the high prevalence of skin and prostate cancer in this cohort, as both of these cancer types are not typically treated with chemotherapy. Future research will be needed to clarify the driving mechanism behind this finding, by separately investigating gray matter

density in different types of cancer and accounting for treatment effects. However, these initial results do imply that other types of cancer aside from breast may also exhibit gray matter density alterations, suggesting that other findings from breast cancer studies may be more widely applicable to other types of cancer and highlighting the need for further study in this area.

Interestingly, in the ADNI cohort study, cancer history was also shown to delay Alzheimer's disease (AD) age of onset, suggesting that the mechanism driving the inverse association of cancer and AD may be independent of cancer and treatment-related cognitive dysfunction. This highlights the need for more investigation of other pathways potentially driving this inverse association.

To further advance the understanding of potential mechanisms driving this effect, we have reviewed microRNAs (miRNAs) identified separately in studies of cancer and AD and analyzed potential overlap (Chapter VI, Holohan et al. (2012)), as we hypothesized that a greater understanding of the biological mechanisms driving the inverse association of cancer and AD would be informative towards the study of cognitive dysfunction as well. Our review highlights eight miRNAs with evidence of differential regulation in cancer and AD of a number of important biological pathways, including proliferation, survival, cell cycle regulation, tumor suppression, apoptosis, angiogenesis, inflammation, innate immunity, and AD-specific pathways such as amyloid-beta plaque deposition. Importantly, seven of the eight miRNAs reviewed were shown to be involved in AD-specific pathways, and were also separately shown to be involved in pathways promoting or inhibiting cancer. This supports the hypothesis that

differential expression or regulation of microRNAs may have wide-ranging impacts on a number of different disease pathways in cancer and AD, and that the same microRNAs may be influencing the risk and/or trajectory of both diseases. Additionally, studies of five of the eight miRNAs reviewed showed evidence of involvement in inflammatory and immune pathways in cancer and AD, supporting the hypothesis that common pathways might be differentially regulated to promote each disease.

Our findings from the studies of cancer and cognitive dysfunction (Chapters II-V) highlight the importance of future study in this area. Our review (Chapter II, Holohan et al. (2013)) and the novel findings presented in Chapter III (Nudelman et al. (2014)), highlight the importance of continuing to apply the latest advances in neuroimaging methodologies to study of this research topic, as well as the need for more prospective, longitudinal, and multimodal analyses with appropriate controls, in order to integrate findings into a coherent picture of cancer and treatment-related cognitive change over time. Based on our results from such analyses as presented in Chapters III-IV (Nudelman et al. (2014); Nudelman et al. (*under review*)), we suggest that future studies including functional and metabolic data (fMRI and PET) would contribute significantly to this area of research, as identifying the number and type of independent versus correlated imaging results would significantly aid in our attempt to quantify the number and type of contributing biological mechanisms. Additionally, based on results presented in Chapter IV (Nudelman et al. (*under review*)), it will be very

important for future studies to consider other types of cancer and treatment-related sequelae in relation to cognitive dysfunction.

We suggest two research programs which might address some of the questions raised in the study presented in Chapter IV (Nudelman et al. (*under review*)). The first study would collect a sample (N>50) of breast cancer patients treated with and without chemotherapy and measure self-reported CIPN symptoms (using the FACT/GOG-Ntx), clinical grade of CIPN, and self-reported cognitive dysfunction (using the Multiple Ability Self-Report Questionnaire (MASQ)). This study would enable us to establish the relationship between the clinical level of CIPN and cognitive complaints. The second study would allow a comprehensive, integrated examination of objective and subjective forms of CIPN and cognitive complaints following cancer chemotherapy. This study would include self-reported CIPN symptoms (using the FACT/GOG-Ntx), clinical grade of CIPN, self-reported cognitive dysfunction (using the Multiple Ability Self-Report Questionnaire), neuroimaging (MRI-based perfusion and gray matter density measures), and an objective measure of peripheral neuropathy such as a skin biopsy or neurophysiological measurement. This study, while much more expensive than the first, would permit analysis of association between all measures, as well as investigation of the impact of the positive association between CIPN symptoms/perfusion and gray matter density on perceived versus actual CIPN. These findings would be able to demonstrate whether lower frontal gray matter density in chemotherapy-treated breast cancer patients was associated with a difference in perceived compared to actual (objective) CIPN,

which could have important clinical implications, as treatment needs for CIPN are typically assessed via patient self-report.

Another future direction involves our observation of lower gray matter density in cancer survivors in the ADNI cohort, which highlights the importance of further investigation of the impact of cancer and treatment on aging and neurodegenerative processes. One recommendation to pursue this objective is to obtain cohorts of older cancer survivors, as most studies of cancer and treatment-related cognitive dysfunction are currently conducted on participants less than 65 years old [34]. Another recommendation is to study cohorts of individuals for longer periods of time after treatment; to date, longitudinal studies have only followed survivors for less than two years, though cross-sectional studies have identified cognitive dysfunction in survivors more than 20 years post-treatment [12,14-17,58,64,139]. Finally, it is vital that future studies continue to collect larger cohorts to permit genetic analysis, which will advance understanding of risk factors for cancer and treatment-related cognitive dysfunction. A future direction of the ADNI cohort analysis includes performing genome-wide association analysis of the frontal region of lower gray matter density in cancer survivors as an endophenotype of cancer/treatment-related cognitive dysfunction. However, the ADNI dataset, while much larger than those typically acquired in cancer neuroimaging studies, is still limited in sample size for this type of analysis; future research must therefore focus on identifying additional sample sets with appropriate data to continue these analyses, as well as collecting additional large genetic studies to enable progress in this area.

Identification of the driving biological mechanisms behind this effect may also provide direction towards the study of the inverse effect of cancer and AD, and the impact of cancer survivorship on the neurodegenerative process, highlighting the importance of this research program. Given the current lack of effective treatments for AD, this knowledge could have a vital impact on future therapeutic research.

References

1. (2014) SEER Cancer Statistics Factsheets: Breast Cancer. In: Health NIo, editor. Bethesda, MD: National Cancer Institute.

2. Cimprich B, Reuter-Lorenz P, Nelson J, Clark PM, Therrien B, et al. (2010) Prechemotherapy alterations in brain function in women with breast cancer. J Clin Exp Neuropsychol 32: 324-331.

3. Holohan KN, Von Ah D, McDonald BC, Saykin AJ (2013) Neuroimaging, cancer, and cognition: state of the knowledge. Semin Oncol Nurs 29: 280-287.

4. Scherling C, Collins B, Mackenzie J, Bielajew C, Smith A (2011) Pre-chemotherapy differences in visuospatial working memory in breast cancer patients compared to controls: an FMRI study. Front Hum Neurosci 5: 122.

5. Scherling C, Collins B, Mackenzie J, Bielajew C, Smith A (2012) Prechemotherapy differences in response inhibition in breast cancer patients compared to controls: a functional magnetic resonance imaging study. J Clin Exp Neuropsychol 34: 543-560.

6. Bergouignan L, Lefranc JP, Chupin M, Morel N, Spano JP, et al. (2011) Breast cancer affects both the hippocampus volume and the episodic autobiographical memory retrieval. PLoS One 6: e25349.

7. Bruno J, Hosseini SM, Kesler S (2012) Altered resting state functional brain network topology in chemotherapy-treated breast cancer survivors. Neurobiol Dis 48: 329-338.

8. Conroy SK, McDonald BC, Smith DJ, Moser LR, West JD, et al. (2013) Alterations in brain structure and function in breast cancer survivors: effect of post-chemotherapy interval and relation to oxidative DNA damage. Breast Cancer Res Treat 137: 493-502.

9. de Ruiter MB, Reneman L, Boogerd W, Veltman DJ, Caan M, et al. (2012) Late effects of high-dose adjuvant chemotherapy on white and gray matter in breast cancer survivors: converging results from multimodal magnetic resonance imaging. Hum Brain Mapp 33: 2971-2983.

10. de Ruiter MB, Reneman L, Boogerd W, Veltman DJ, van Dam FS, et al. (2011) Cerebral hyporesponsiveness and cognitive impairment 10 years after chemotherapy for breast cancer. Hum Brain Mapp 32: 1206-1219.

11. Deprez S, Amant F, Yigit R, Porke K, Verhoeven J, et al. (2011) Chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning in breast cancer patients. Hum Brain Mapp 32: 480-493.

12. Ganz PA, Bower JE, Kwan L, Castellon SA, Silverman DH, et al. (2013) Does tumor necrosis factor-alpha (TNF-alpha) play a role in post-chemotherapy cerebral dysfunction? Brain Behav Immun 30 Suppl: S99-108.

13. Kesler SR, Kent JS, O'Hara R (2011) Prefrontal cortex and executive function impairments in primary breast cancer. Arch Neurol 68: 1447-1453.

14. Koppelmans V, de Ruiter MB, van der Lijn F, Boogerd W, Seynaeve C, et al. (2012) Global and focal brain volume in long-term breast cancer survivors

exposed to adjuvant chemotherapy. Breast Cancer Res Treat 132: 1099-1106.

15. McDonald BC, Conroy SK, Ahles TA, West JD, Saykin AJ (2010) Gray matter reduction associated with systemic chemotherapy for breast cancer: a prospective MRI study. Breast Cancer Res Treat 123: 819-828.
16. McDonald BC, Conroy SK, Ahles TA, West JD, Saykin AJ (2012) Alterations in brain activation during working memory processing associated with breast cancer and treatment: a prospective functional magnetic resonance imaging study. J Clin Oncol 30: 2500-2508.
17. McDonald BC, Conroy SK, Smith DJ, West JD, Saykin AJ (2013) Frontal gray matter reduction after breast cancer chemotherapy and association with executive symptoms: a replication and extension study. Brain Behav Immun 30 Suppl: S117-125.
18. McDonald BC, Saykin AJ (2011) Neurocognitive dimensions of breast cancer and its treatment. Neuropsychopharmacology 36: 355-356.
19. Ono M, Ogilvie JM, Wilson JS, Green HJ, Chambers SK, et al. (2015) A meta-analysis of cognitive impairment and decline asssociated with adjuvant chemotherapy in women with breast cancer. Frontiers in Oncology 5.
20. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, et al. (2011) Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups

on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7: 280-292.

21. Ahles TA, Saykin AJ (2007) Candidate mechanisms for chemotherapy-induced cognitive changes. Nat Rev Cancer 7: 192-201.
22. McAllister TW, Ahles TA, Saykin AJ, Ferguson RJ, McDonald BC, et al. (2004) Cognitive effects of cytotoxic cancer chemotherapy: predisposing risk factors and potential treatments. Curr Psychiatry Rep 6: 364-371.
23. Saykin AJ, Ahles TA, McDonald BC (2003) Mechanisms of chemotherapy-induced cognitive disorders: neuropsychological, pathophysiological, and neuroimaging perspectives. Semin Clin Neuropsychiatry 8: 201-216.
24. Dietrich J, Han R, Yang Y, Mayer-Proschel M, Noble M (2006) CNS progenitor cells and oligodendrocytes are targets of chemotherapeutic agents in vitro and in vivo. J Biol 5: 22.
25. Gangloff A, Hsueh WA, Kesner AL, Kiesewetter DO, Pio BS, et al. (2005) Estimation of paclitaxel biodistribution and uptake in human-derived xenografts in vivo with (18)F-fluoropaclitaxel. J Nucl Med 46: 1866-1871.
26. Ginos JZ, Cooper AJ, Dhawan V, Lai JC, Strother SC, et al. (1987) [13N]cisplatin PET to assess pharmacokinetics of intra-arterial versus intravenous chemotherapy for malignant brain tumors. J Nucl Med 28: 1844-1852.
27. Troy L, McFarland K, Littman-Power S, Kelly BJ, Walpole ET, et al. (2000) Cisplatin-based therapy: a neurological and neuropsychological review. Psychooncology 9: 29-39.

28. Verstappen CC, Heimans JJ, Hoekman K, Postma TJ (2003) Neurotoxic complications of chemotherapy in patients with cancer: clinical signs and optimal management. Drugs 63: 1549-1563.

29. Boland EG, Selvarajah D, Hunter M, Ezaydi Y, Tesfaye S, et al. (2014) Central pain processing in chronic chemotherapy-induced peripheral neuropathy: a functional magnetic resonance imaging study. PLoS One 9: e96474.

30. Ahles TA, Saykin AJ, Furstenberg CT, Cole B, Mott LA, et al. (2002) Neuropsychologic impact of standard-dose systemic chemotherapy in long-term survivors of breast cancer and lymphoma. J Clin Oncol 20: 485-493.

31. Mehnert A, Scherwath A, Schirmer L, Schleimer B, Petersen C, et al. (2007) The association between neuropsychological impairment, self-perceived cognitive deficits, fatigue and health related quality of life in breast cancer survivors following standard adjuvant versus high-dose chemotherapy. Patient Educ Couns 66: 108-118.

32. Ahles TA, Saykin AJ, Noll WW, Furstenberg CT, Guerin S, et al. (2003) The relationship of APOE genotype to neuropsychological performance in long-term cancer survivors treated with standard dose chemotherapy. Psychooncology 12: 612-619.

33. Small BJ, Rawson KS, Walsh E, Jim HS, Hughes TF, et al. (2011) Catechol-O-methyltransferase genotype modulates cancer treatment-related cognitive deficits in breast cancer survivors. Cancer 117: 1369-1376.

34. Mandelblatt JS, Hurria A, McDonald BC, Saykin AJ, Stern RA, et al. (2013) Cognitive effects of cancer and its treatments at the intersection of aging: what do we know; what do we need to know? Semin Oncol 40: 709-725.

35. Catala-Lopez F, Crespo-Facorro B, Vieta E, Valderas JM, Valencia A, et al. (2014) Alzheimer's disease and cancer: current epidemiological evidence for a mutual protection. Neuroepidemiology 42: 121-122.

36. Driver JA (2014) Inverse association between cancer and neurodegenerative disease: review of the epidemiologic and biological evidence. Biogerontology 15: 547-557.

37. Driver JA, Beiser A, Au R, Kreger BE, Splansky GL, et al. (2012) Inverse association between cancer and Alzheimer's disease: results from the Framingham Heart Study. BMJ 344: e1442.

38. Musicco M, Adorni F, Di Santo S, Prinelli F, Pettenati C, et al. (2013) Inverse occurrence of cancer and Alzheimer disease: a population-based incidence study. Neurology 81: 322-328.

39. Roe CM, Behrens MI (2013) AD and cancer: epidemiology makes for strange bedfellows. Neurology 81: 310-311.

40. Roe CM, Behrens MI, Xiong C, Miller JP, Morris JC (2005) Alzheimer disease and cancer. Neurology 64: 895-898.

41. Roe CM, Fitzpatrick AL, Xiong C, Sieh W, Kuller L, et al. (2010) Cancer linked to Alzheimer disease but not vascular dementia. Neurology 74: 106-112.

42. Tirumalasetti F, Han L, Birkett DP (1991) The relationship between cancer and Alzheimer's disease. J Am Geriatr Soc 39: 840.

43. Tabares-Seisdedos R, Dumont N, Baudot A, Valderas JM, Climent J, et al. (2011) No paradox, no progress: inverse cancer comorbidity in people with other complex diseases. Lancet Oncol 12: 604-608.

44. Ashburner J, Friston KJ (2000) Voxel-based morphometry--the methods. Neuroimage 11: 805-821.

45. Silk TJ, Wood AG (2011) Lessons about neurodevelopment from anatomical magnetic resonance imaging. J Dev Behav Pediatr 32: 158-168.

46. Assaf Y, Pasternak O (2008) Diffusion tensor imaging (DTI)-based white matter mapping in brain research: a review. J Mol Neurosci 34: 51-61.

47. Basser PJ, Mattiello J, LeBihan D (1994) MR diffusion tensor spectroscopy and imaging. Biophys J 66: 259-267.

48. Christen T, Bolar DS, Zaharchuk G (2013) Imaging brain oxygenation with MRI using blood oxygenation approaches: methods, validation, and clinical applications. AJNR Am J Neuroradiol 34: 1113-1123.

49. Huettel SASAWMG (2009) Functional Magnetic Resonance Imaging. Sunderland, Massachusetts: Sinauer Associates.

50. Lee MH, Smyser CD, Shimony JS (2013) Resting-state fMRI: a review of methods and clinical applications. AJNR Am J Neuroradiol 34: 1866-1872.

51. Posse S, Otazo R, Dager SR, Alger J (2013) MR spectroscopic imaging: principles and recent advances. J Magn Reson Imaging 37: 1301-1325.

52. Pike VW (2009) PET radiotracers: crossing the blood-brain barrier and surviving metabolism. Trends Pharmacol Sci 30: 431-440.

53. Silverman DH, Alavi A (2005) PET imaging in the assessment of normal and impaired cognitive function. Radiol Clin North Am 43: 67-77, x.

54. Silverman DH, Mosconi L, Ercoli L, Chen W, Small GW (2008) Positron emission tomography scans obtained for the evaluation of cognitive dysfunction. Semin Nucl Med 38: 251-261.

55. Butler RW, Haser JK (2006) Neurocognitive effects of treatment for childhood cancer. Ment Retard Dev Disabil Res Rev 12: 184-191.

56. Abraham J, Haut MW, Moran MT, Filburn S, Lemiuex S, et al. (2008) Adjuvant chemotherapy for breast cancer: effects on cerebral white matter seen in diffusion tensor imaging. Clin Breast Cancer 8: 88-91.

57. Kesler S, Janelsins M, Koovakkattu D, Palesh O, Mustian K, et al. (2013) Reduced hippocampal volume and verbal memory performance associated with interleukin-6 and tumor necrosis factor-alpha levels in chemotherapy-treated breast cancer survivors. Brain Behav Immun 30 Suppl: S109-116.

58. Deprez S, Amant F, Smeets A, Peeters R, Leemans A, et al. (2012) Longitudinal assessment of chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning. J Clin Oncol 30: 274-281.

59. Ferguson RJ, McDonald BC, Saykin AJ, Ahles TA (2007) Brain structure and function differences in monozygotic twins: possible effects of breast cancer chemotherapy. J Clin Oncol 25: 3866-3870.

60. Hosseini SM, Koovakkattu D, Kesler SR (2012) Altered small-world properties of gray matter networks in breast cancer. BMC Neurol 12: 28.

61. Inagaki M, Yoshikawa E, Matsuoka Y, Sugawara Y, Nakano T, et al. (2007) Smaller regional volumes of brain gray and white matter demonstrated in breast cancer survivors exposed to adjuvant chemotherapy. Cancer 109: 146-156.

62. Kesler SR, Bennett FC, Mahaffey ML, Spiegel D (2009) Regional brain activation during verbal declarative memory in metastatic breast cancer. Clin Cancer Res 15: 6665-6673.

63. Koppelmans V, de Groot M, de Ruiter MB, Boogerd W, Seynaeve C, et al. (2014) Global and focal white matter integrity in breast cancer survivors 20 years after adjuvant chemotherapy. Hum Brain Mapp 35: 889-899.

64. Lopez Zunini RA, Scherling C, Wallis N, Collins B, MacKenzie J, et al. (2013) Differences in verbal memory retrieval in breast cancer chemotherapy patients compared to healthy controls: a prospective fMRI study. Brain Imaging Behav 7: 460-477.

65. Reuter-Lorenz PA, Cimprich B (2013) Cognitive function and breast cancer: promise and potential insights from functional brain imaging. Breast Cancer Res Treat 137: 33-43.

66. Silverman DH, Dy CJ, Castellon SA, Lai J, Pio BS, et al. (2007) Altered frontocortical, cerebellar, and basal ganglia activity in adjuvant-treated breast cancer survivors 5-10 years after chemotherapy. Breast Cancer Res Treat 103: 303-311.

67. Yoshikawa E, Matsuoka Y, Inagaki M, Nakano T, Akechi T, et al. (2005) No adverse effects of adjuvant chemotherapy on hippocampal volume in Japanese breast cancer survivors. Breast Cancer Res Treat 92: 81-84.

68. Benveniste H, Zhang S, Reinsel RA, Li H, Lee H, et al. (2012) Brain metabolomic profiles of lung cancer patients prior to treatment characterized by proton magnetic resonance spectroscopy. Int J Clin Exp Med 5: 154-164.

69. Golan H, Kennedy JA, Frenkel A, Parmet Y, Feintuch A, et al. (2009) Brain mapping of patients with lung cancer and controls: inquiry into tumor-to-brain communication. J Nucl Med 50: 1072-1075.

70. Kumano H, Ida I, Oshima A, Takahashi K, Yuuki N, et al. (2007) Brain metabolic changes associated with predispotion to onset of major depressive disorder and adjustment disorder in cancer patients--a preliminary PET study. J Psychiatr Res 41: 591-599.

71. Tashiro M, Itoh M, Kubota K, Kumano H, Masud MM, et al. (2001) Relationship between trait anxiety, brain activity and natural killer cell activity in cancer patients: a preliminary PET study. Psychooncology 10: 541-546.

72. Tashiro M, Juengling FD, Moser E, Reinhardt MJ, Kubota K, et al. (2003) High social desirability and prefrontal cortical activity in cancer patients: a preliminary study. Med Sci Monit 9: CR119-124.

73. Tashiro M, Juengling FD, Reinhardt MJ, Brink I, Hoegerle S, et al. (2000) Reproducibility of PET brain mapping of cancer patients. Psychooncology 9: 157-163.

74. Tashiro M, Juengling FD, Reinhardt MJ, Mix M, Kumano H, et al. (2001) Depressive state and regional cerebral activity in cancer patients - a preliminary study. Med Sci Monit 7: 687-695.

75. Tashiro M, Kubota K, Itoh M, Yoshioka T, Yoshida M, et al. (1999) Hypometabolism in the limbic system of cancer patients observed by positron emission tomography. Psychooncology 8: 283-286.

76. Deprez S, Billiet T, Sunaert S, Leemans A (2013) Diffusion tensor MRI of chemotherapy-induced cognitive impairment in non-CNS cancer patients: a review. Brain Imaging Behav 7: 409-435.

77. Saykin AJ, Ahles TA, Schoenfeld JD, Wishart HA, Pietras CG, et al. Gray matter reduction on voxel-based morphometry in chemotherapy-treated cancer survivors; 2003; Honolulu, Hawaii. Journal of the International Neuropsychological Society. pp. 246.

78. Silverman DH, Castellon SA, Abraham L, Dy CJ, Pio BS, et al. Abnormal regional brain metabolism in breast cancer survivors after adjuvant chemotherapy is associated with cognitive changes; 2003.

79. Hermelink K, Kuchenhoff H, Untch M, Bauerfeind I, Lux MP, et al. (2010) Two different sides of 'chemobrain': determinants and nondeterminants of self-perceived cognitive dysfunction in a prospective, randomized, multicenter study. Psychooncology 19: 1321-1328.

80. Von Ah D, Habermann B, Carpenter JS, Schneider BL (2013) Impact of perceived cognitive impairment in breast cancer survivors. Eur J Oncol Nurs 17: 236-241.

81. Howlader N, Noone AM, Krapcho M, Neyman N, Aminou R, et al. (2012) SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations). November 2011 ed. Bethesda, MD: National Cancer Institute.

82. Agrawal K, Onami S, Mortimer JE, Pal SK (2010) Cognitive changes associated with endocrine therapy for breast cancer. Maturitas 67: 209-214.

83. Ahles TA, Root JC, Ryan EL (2012) Cancer- and cancer treatment-associated cognitive change: an update on the state of the science. J Clin Oncol 30: 3675-3686.

84. Ahles TA, Saykin AJ, McDonald BC, Furstenberg CT, Cole BF, et al. (2008) Cognitive function in breast cancer patients prior to adjuvant treatment. Breast Cancer Res Treat 110: 143-152.

85. Ahles TA, Saykin AJ, McDonald BC, Li Y, Furstenberg CT, et al. (2010) Longitudinal assessment of cognitive changes associated with adjuvant treatment for breast cancer: impact of age and cognitive reserve. J Clin Oncol 28: 4434-4440.

86. Bender CM, Sereika SM, Berga SL, Vogel VG, Brufsky AM, et al. (2006) Cognitive impairment associated with adjuvant therapy in breast cancer. Psychooncology 15: 422-430.

87. Collins B, Mackenzie J, Stewart A, Bielajew C, Verma S (2009) Cognitive effects of chemotherapy in post-menopausal breast cancer patients 1 year after treatment. Psychooncology 18: 134-143.

88. Falleti MG, Sanfilippo A, Maruff P, Weih L, Phillips KA (2005) The nature and severity of cognitive impairment associated with adjuvant chemotherapy in women with breast cancer: a meta-analysis of the current literature. Brain Cogn 59: 60-70.

89. Fan HG, Houede-Tchen N, Yi QL, Chemerynsky I, Downie FP, et al. (2005) Fatigue, menopausal symptoms, and cognitive function in women after adjuvant chemotherapy for breast cancer: 1- and 2-year follow-up of a prospective controlled study. J Clin Oncol 23: 8025-8032.

90. Jim HS, Donovan KA, Small BJ, Andrykowski MA, Munster PN, et al. (2009) Cognitive functioning in breast cancer survivors: a controlled comparison. Cancer 115: 1776-1783.

91. Quesnel C, Savard J, Ivers H (2009) Cognitive impairments associated with breast cancer treatments: results from a longitudinal study. Breast Cancer Res Treat 116: 113-123.

92. Schilder CM, Eggens PC, Seynaeve C, Linn SC, Boogerd W, et al. (2009) Neuropsychological functioning in postmenopausal breast cancer patients treated with tamoxifen or exemestane after AC-chemotherapy: cross-

sectional findings from the neuropsychological TEAM-side study. Acta Oncol 48: 76-85.

93. Wefel JS, Lenzi R, Theriault R, Buzdar AU, Cruickshank S, et al. (2004) 'Chemobrain' in breast carcinoma?: a prologue. Cancer 101: 466-475.

94. Wefel JS, Lenzi R, Theriault RL, Davis RN, Meyers CA (2004) The cognitive sequelae of standard-dose adjuvant chemotherapy in women with breast carcinoma: results of a prospective, randomized, longitudinal trial. Cancer 100: 2292-2299.

95. Wang Y, Saykin AJ, Pfeuffer J, Lin C, Mosier KM, et al. (2011) Regional reproducibility of pulsed arterial spin labeling perfusion imaging at 3T. Neuroimage 54: 1188-1195.

96. Detre JA, Wang J, Wang Z, Rao H (2009) Arterial spin-labeled perfusion MRI in basic and clinical neuroscience. Curr Opin Neurol 22: 348-355.

97. Conroy SK, McDonald BC, Ahles TA, West JD, Saykin AJ (2013) Chemotherapy-induced amenorrhea: a prospective study of brain activation changes and neurocognitive correlates. Brain Imaging Behav 7: 491-500.

98. Radloff LS (1977) The CES-D Scale: A self-report depression scale for research in the general population. Applied Psychological Measurement 1: 385-401.

99. Spielberger CD (1983) State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press, Inc.

100. Wang DJ, Chen Y, Fernandez-Seara MA, Detre JA (2011) Potentials and challenges for arterial spin labeling in pharmacological magnetic resonance imaging. J Pharmacol Exp Ther 337: 359-366.

101. Wierenga CE, Clark LR, Dev SI, Shin DD, Jurick SM, et al. (2013) Interaction of age and APOE genotype on cerebral blood flow at rest. J Alzheimers Dis 34: 921-935.

102. Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH (2003) An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. Neuroimage 19: 1233-1239.

103. Brett M, J.-L. A, Valabregue R, Poline J-B (2002) Region of interest analysis using an SPM toolbox. Sendai, Japan.

104. Barona A, Reynolds C, Chastain R (1984) A demographically based index of pre-morbid intelligence for the WAIS-R. Journal of Consulting and Clinical Psychology 52: 885-887.

105. Walker CH, Drew BA, Antoon JW, Kalueff AV, Beckman BS (2012) Neurocognitive effects of chemotherapy and endocrine therapies in the treatment of breast cancer: recent perspectives. Cancer Invest 30: 135-148.

106. Bar-Joseph H, Ben-Aharon I, Tzabari M, Tsarfaty G, Stemmer SM, et al. (2011) In vivo bioimaging as a novel strategy to detect doxorubicin-induced damage to gonadal blood vessels. PLoS One 6: e23492.

107. Vacca A, Ribatti D, Iurlaro M, Merchionne F, Nico B, et al. (2002) Docetaxel versus paclitaxel for antiangiogenesis. J Hematother Stem Cell Res 11: 103-118.

108. Wang J, Lou P, Lesniewski R, Henkin J (2003) Paclitaxel at ultra low concentrations inhibits angiogenesis without affecting cellular microtubule assembly. Anticancer Drugs 14: 13-19.

109. Pachnicki JP, Czeczko NG, Tuon F, Cavalcanti TS, Malafaia AB, et al. (2012) Immunohistochemical evaluation of estrogen and progesterone receptors of pre and post-neoadjuvant chemotherapy for breast cancer. Rev Col Bras Cir 39: 86-92.

110. Dashjamts T, Yoshiura T, Hiwatashi A, Yamashita K, Monji A, et al. (2011) Simultaneous arterial spin labeling cerebral blood flow and morphological assessments for detection of Alzheimer's disease. Acad Radiol 18: 1492-1499.

111. Han Y, Smith MT (2013) Pathobiology of cancer chemotherapy-induced peripheral neuropathy (CIPN). Front Pharmacol 4: 156.

112. Wickham R (2007) Chemotherapy-induced peripheral neuropathy: a review and implications for oncology nursing practice. Clin J Oncol Nurs 11: 361-376.

113. Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, et al. (2004) Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. J Neurosci 24: 10410-10415.

114. Burgmer M, Gaubitz M, Konrad C, Wrenger M, Hilgart S, et al. (2009) Decreased gray matter volumes in the cingulo-frontal cortex and the amygdala in patients with fibromyalgia. Psychosom Med 71: 566-573.

115. Gustin SM, Peck CC, Wilcox SL, Nash PG, Murray GM, et al. (2011) Different pain, different brain: thalamic anatomy in neuropathic and non-neuropathic chronic pain syndromes. J Neurosci 31: 5956-5964.

116. Lin CS (2014) Brain signature of chronic orofacial pain: a systematic review and meta-analysis on neuroimaging research of trigeminal neuropathic pain and temporomandibular joint disorders. PLoS One 9: e94300.

117. Mao CP, Zhang QL, Bao FX, Liao X, Yang XL, et al. (2014) Decreased activation of cingulo-frontal-parietal cognitive/attention network during an attention-demanding task in patients with chronic low back pain. Neuroradiology 56: 903-912.

118. Schmidt-Wilcke T, Kairys A, Ichesco E, Fernandez-Sanchez ML, Barjola P, et al. (2014) Changes in clinical pain in fibromyalgia patients correlate with changes in brain activation in the cingulate cortex in a response inhibition task. Pain Med 15: 1346-1358.

119. Nudelman KN, Wang Y, McDonald BC, Conroy SK, Smith DJ, et al. (2014) Altered cerebral blood flow one month after systemic chemotherapy for breast cancer: a prospective study using pulsed arterial spin labeling MRI perfusion. PLoS One 9: e96713.

120. Huang HQ, Brady MF, Cella D, Fleming G (2007) Validation and reduction of FACT/GOG-Ntx subscale for platinum/paclitaxel-induced neurologic

symptoms: a gynecologic oncology group study. Int J Gynecol Cancer 17: 387-393.

121. Grisold W, Cavaletti G, Windebank AJ (2012) Peripheral neuropathies from chemotherapeutics and targeted agents: diagnosis, treatment, and prevention. Neuro Oncol 14 Suppl 4: iv45-54.

122. Nishihori T, Choi J, DiGiovanna MP, Thomson JG, Kohler PC, et al. (2008) Carpal tunnel syndrome associated with the use of aromatase inhibitors in breast cancer. Clin Breast Cancer 8: 362-365.

123. Peyron R, Laurent B, Garcia-Larrea L (2000) Functional imaging of brain responses to pain. A review and meta-analysis (2000). Neurophysiol Clin 30: 263-288.

124. Toyoda H, Zhao MG, Zhuo M (2009) Enhanced quantal release of excitatory transmitter in anterior cingulate cortex of adult mice with chronic pain. Mol Pain 5: 4.

125. Xu H, Wu LJ, Wang H, Zhang X, Vadakkan KI, et al. (2008) Presynaptic and postsynaptic amplifications of neuropathic pain in the anterior cingulate cortex. J Neurosci 28: 7445-7453.

126. Yamashita A, Hamada A, Suhara Y, Kawabe R, Yanase M, et al. (2014) Astrocytic activation in the anterior cingulate cortex is critical for sleep disorder under neuropathic pain. Synapse 68: 235-247.

127. Yi J, Zheng JY, Zhang W, Wang S, Yang ZF, et al. (2014) Decreased pain threshold and enhanced synaptic transmission in the anterior cingulate cortex of experimental hypothyroidism mice. Mol Pain 10: 38.

128. Zhuo M (2014) Long-term potentiation in the anterior cingulate cortex and chronic pain. Philos Trans R Soc Lond B Biol Sci 369: 20130146.

129. Hasan M, Whiteley J, Bresnahan R, MacIver K, Sacco P, et al. (2014) Somatosensory change and pain relief induced by repetitive transcranial magnetic stimulation in patients with central poststroke pain. Neuromodulation 17: 731-736; discussion 736.

130. Li XY, Ko HG, Chen T, Collingridge GL, Kaang BK, et al. (2011) Erasing injury-related cortical synaptic potentiation as a new treatment for chronic pain. J Mol Med (Berl) 89: 847-855.

131. Tzabazis A, Aparici CM, Rowbotham MC, Schneider MB, Etkin A, et al. (2013) Shaped magnetic field pulses by multi-coil repetitive transcranial magnetic stimulation (rTMS) differentially modulate anterior cingulate cortex responses and pain in volunteers and fibromyalgia patients. Mol Pain 9: 33.

132. Griffith KA, Merkies IS, Hill EE, Cornblath DR (2010) Measures of chemotherapy-induced peripheral neuropathy: a systematic review of psychometric properties. J Peripher Nerv Syst 15: 314-325.

133. DeSouky AL (1992) The relationship between cancer and Alzheimer's disease. J Am Geriatr Soc 40: 1075.

134. Realmuto S, Cinturino A, Arnao V, Mazzola MA, Cupidi C, et al. (2012) Tumor diagnosis preceding Alzheimer's disease onset: is there a link between cancer and Alzheimer's disease? J Alzheimers Dis 31: 177-182.

135. Yamada M, Sasaki H, Mimori Y, Kasagi F, Sudoh S, et al. (1999) Prevalence and risks of dementia in the Japanese population: RERF's adult health study Hiroshima subjects. Radiation Effects Research Foundation. J Am Geriatr Soc 47: 189-195.

136. Behrens MI, Lendon C, Roe CM (2009) A common biological mechanism in cancer and Alzheimer's disease? Curr Alzheimer Res 6: 196-204.

137. Holohan KN, Lahiri DK, Schneider BP, Foroud T, Saykin AJ (2012) Functional microRNAs in Alzheimer's disease and cancer: differential regulation of common mechanisms and pathways. Front Genet 3: 323.

138. Ibanez K, Boullosa C, Tabares-Seisdedos R, Baudot A, Valencia A (2014) Molecular evidence for the inverse comorbidity between central nervous system disorders and cancers detected by transcriptomic meta-analyses. PLoS Genet 10: e1004173.

139. Koppelmans V, Breteler MM, Boogerd W, Seynaeve C, Gundy C, et al. (2012) Neuropsychological performance in survivors of breast cancer more than 20 years after adjuvant chemotherapy. J Clin Oncol 30: 1080-1086.

140. Stouten-Kemperman MM, de Ruiter MB, Koppelmans V, Boogerd W, Reneman L, et al. (2014) Neurotoxicity in breast cancer survivors >/=10 years post-treatment is dependent on treatment type. Brain Imaging Behav.

141. Boele FW, Schilder CM, de Roode ML, Deijen JB, Schagen SB (2015) Cognitive functioning during long-term tamoxifen treatment in postmenopausal women with breast cancer. Menopause 22: 17-25.

142. Schilder CM, Seynaeve C, Beex LV, Boogerd W, Linn SC, et al. (2010) Effects of tamoxifen and exemestane on cognitive functioning of postmenopausal patients with breast cancer: results from the neuropsychological side study of the tamoxifen and exemestane adjuvant multinational trial. J Clin Oncol 28: 1294-1300.

143. Shibayama O, Yoshiuchi K, Inagaki M, Matsuoka Y, Yoshikawa E, et al. (2014) Association between adjuvant regional radiotherapy and cognitive function in breast cancer patients treated with conservation therapy. Cancer Med 3: 702-709.

144. Schilder CM, Seynaeve C, Linn SC, Boogerd W, Beex LV, et al. (2010) Cognitive functioning of postmenopausal breast cancer patients before adjuvant systemic therapy, and its association with medical and psychological factors. Crit Rev Oncol Hematol 76: 133-141.

145. Jack CR, Jr., Bernstein MA, Borowski BJ, Gunter JL, Fox NC, et al. (2010) Update on the magnetic resonance imaging core of the Alzheimer's disease neuroimaging initiative. Alzheimers Dement 6: 212-220.

146. Jagust WJ, Bandy D, Chen K, Foster NL, Landau SM, et al. (2010) The Alzheimer's Disease Neuroimaging Initiative positron emission tomography core. Alzheimers Dement 6: 221-229.

147. Petersen RC, Aisen PS, Beckett LA, Donohue MC, Gamst AC, et al. (2010) Alzheimer's Disease Neuroimaging Initiative (ADNI): clinical characterization. Neurology 74: 201-209.

148. Saykin AJ, Shen L, Foroud TM, Potkin SG, Swaminathan S, et al. (2010) Alzheimer's Disease Neuroimaging Initiative biomarkers as quantitative phenotypes: Genetics core aims, progress, and plans. Alzheimers Dement 6: 265-273.

149. Trojanowski JQ, Vandeerstichele H, Korecka M, Clark CM, Aisen PS, et al. (2010) Update on the biomarker core of the Alzheimer's Disease Neuroimaging Initiative subjects. Alzheimers Dement 6: 230-238.

150. Weiner MW, Aisen PS, Jack CR, Jr., Jagust WJ, Trojanowski JQ, et al. (2010) The Alzheimer's disease neuroimaging initiative: progress report and future plans. Alzheimers Dement 6: 202-211 e207.

151. Weiner MW, Veitch DP, Aisen PS, Beckett LA, Cairns NJ, et al. (2013) The Alzheimer's Disease Neuroimaging Initiative: a review of papers published since its inception. Alzheimers Dement 9: e111-194.

152. Weiner MW, Veitch DP, Aisen PS, Beckett LA, Cairns NJ, et al. (2012) The Alzheimer's Disease Neuroimaging Initiative: a review of papers published since its inception. Alzheimers Dement 8: S1-68.

153. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, et al. (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis.

APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 278: 1349-1356.

154. Fitten LJ, Ortiz F, Ponton M (2001) Frequency of Alzheimer's disease and other dementias in a community outreach sample of Hispanics. J Am Geriatr Soc 49: 1301-1308.

155. Meng X, D'Arcy C (2012) Education and dementia in the context of the cognitive reserve hypothesis: a systematic review with meta-analyses and qualitative analyses. PLoS One 7: e38268.

156. Salmon DP, Ferris SH, Thomas RG, Sano M, Cummings JL, et al. (2013) Age and apolipoprotein E genotype influence rate of cognitive decline in nondemented elderly. Neuropsychology 27: 391-401.

157. Shadlen MF, Siscovick D, Fitzpatrick AL, Dulberg C, Kuller LH, et al. (2006) Education, cognitive test scores, and black-white differences in dementia risk. J Am Geriatr Soc 54: 898-905.

158. Yang YH, Wang H, Lam L, Chan WC, Yu X, et al. (2014) Characteristics of Alzheimer's disease among patients in Taiwan, Hong Kong, and Beijing. J Alzheimers Dis 42: 193-200.

159. Risacher SL, Kim S, Shen L, Nho K, Foroud T, et al. (2013) The role of apolipoprotein E (APOE) genotype in early mild cognitive impairment (E-MCI). Front Aging Neurosci 5: 11.

160. Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12: 189-198.

161. Morris JC (1993) The Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 43: 2412-2414.

162. McKhann G, Drachman D, Folstein M, Katzman R, Price D, et al. (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34: 939-944.

163. Jack CR, Jr., Bernstein MA, Fox NC, Thompson P, Alexander G, et al. (2008) The Alzheimer's Disease Neuroimaging Initiative (ADNI): MRI methods. J Magn Reson Imaging 27: 685-691.

164. Risacher SL, Saykin AJ, West JD, Shen L, Firpi HA, et al. (2009) Baseline MRI predictors of conversion from MCI to probable AD in the ADNI cohort. Curr Alzheimer Res 6: 347-361.

165. Risacher SL, Shen L, West JD, Kim S, McDonald BC, et al. (2010) Longitudinal MRI atrophy biomarkers: relationship to conversion in the ADNI cohort. Neurobiol Aging 31: 1401-1418.

166. Howlader N, Noone AM, Krapcho M, Garshell J, Miller D, et al. (2014) SEER Cancer Statistics Review, 1975-2011. November 2013 ed. Bethesda, MD: National Cancer Institute.

167. Weis J, Poppelreuter M, Bartsch HH (2009) Cognitive deficits as long-term side-effects of adjuvant therapy in breast cancer patients: 'subjective' complaints and 'objective' neuropsychological test results. Psychooncology 18: 775-782.

168. Balastik M, Lim J, Pastorino L, Lu KP (2007) Pin1 in Alzheimer's disease: multiple substrates, one regulatory mechanism? Biochim Biophys Acta 1772: 422-429.

169. Driver JA, Lu KP (2010) Pin1: a new genetic link between Alzheimer's disease, cancer and aging. Curr Aging Sci 3: 158-165.

170. Borgesius NZ, de Waard MC, van der Pluijm I, Omrani A, Zondag GC, et al. (2011) Accelerated age-related cognitive decline and neurodegeneration, caused by deficient DNA repair. J Neurosci 31: 12543-12553.

171. Jeppesen DK, Bohr VA, Stevnsner T (2011) DNA repair deficiency in neurodegeneration. Prog Neurobiol 94: 166-200.

172. Qiu C, Kivipelto M, von Strauss E (2009) Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. Dialogues Clin Neurosci 11: 111-128.

173. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100: 57-70.

174. Leuner K, Muller WE, Reichert AS (2012) From mitochondrial dysfunction to amyloid beta formation: novel insights into the pathogenesis of Alzheimer's disease. Mol Neurobiol 46: 186-193.

175. Bushati N, Cohen SM (2008) MicroRNAs in neurodegeneration. Curr Opin Neurobiol 18: 292-296.

176. Garzon R, Calin GA, Croce CM (2009) MicroRNAs in Cancer. Annu Rev Med 60: 167-179.

177. Lau P, de Strooper B (2010) Dysregulated microRNAs in neurodegenerative disorders. Semin Cell Dev Biol 21: 768-773.

178. Sonntag KC (2010) MicroRNAs and deregulated gene expression networks in neurodegeneration. Brain Res 1338: 48-57.

179. Wu M, Jolicoeur N, Li Z, Zhang L, Fortin Y, et al. (2008) Genetic variations of microRNAs in human cancer and their effects on the expression of miRNAs. Carcinogenesis 29: 1710-1716.

180. Zhang B, Pan X, Cobb GP, Anderson TA (2007) microRNAs as oncogenes and tumor suppressors. Dev Biol 302: 1-12.

181. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116: 281-297.

182. Chendrimada TP, Gregory RI, Kumaraswamy E, Norman J, Cooch N, et al. (2005) TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. Nature 436: 740-744.

183. Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R (2005) Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell 123: 631-640.

184. Gregory RI, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, et al. (2004) The Microprocessor complex mediates the genesis of microRNAs. Nature 432: 235-240.

185. Kim VN, Han J, Siomi MC (2009) Biogenesis of small RNAs in animals. Nat Rev Mol Cell Biol 10: 126-139.

186. Maniataki E, Mourelatos Z (2005) A human, ATP-independent, RISC assembly machine fueled by pre-miRNA. Genes Dev 19: 2979-2990.

187. Rusca N, Monticelli S (2011) MiR-146a in Immunity and Disease. Mol Biol Int 2011: 437301.

188. Chen PS, Su JL, Cha ST, Tarn WY, Wang MY, et al. (2011) miR-107 promotes tumor progression by targeting the let-7 microRNA in mice and humans. J Clin Invest 121: 3442-3455.

189. Formosa A, Lena AM, Markert EK, Cortelli S, Miano R, et al. (2013) DNA methylation silences miR-132 in prostate cancer. Oncogene 32: 127-134.

190. Minones-Moyano E, Porta S, Escaramis G, Rabionet R, Iraola S, et al. (2011) MicroRNA profiling of Parkinson's disease brains identifies early downregulation of miR-34b/c which modulate mitochondrial function. Hum Mol Genet 20: 3067-3078.

191. Tanaka N, Toyooka S, Soh J, Kubo T, Yamamoto H, et al. (2012) Frequent methylation and oncogenic role of microRNA-34b/c in small-cell lung cancer. Lung Cancer 76: 32-38.

192. Williams AH, Valdez G, Moresi V, Qi X, McAnally J, et al. (2009) MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. Science 326: 1549-1554.

193. Zhang T, Liu M, Wang C, Lin C, Sun Y, et al. (2011) Down-regulation of MiR-206 promotes proliferation and invasion of laryngeal cancer by regulating VEGF expression. Anticancer Res 31: 3859-3863.

194. Gascon E, Gao FB (2012) Cause or Effect: Misregulation of microRNA Pathways in Neurodegeneration. Front Neurosci 6: 48.

195. Glenner GG, Wong CW (1984) Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 120: 885-890.

196. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, et al. (1986) Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci U S A 83: 4913-4917.

197. Gustaw-Rothenberg K, Lerner A, Bonda DJ, Lee HG, Zhu X, et al. (2010) Biomarkers in Alzheimer's disease: past, present and future. Biomark Med 4: 15-26.

198. Kosik KS, Joachim CL, Selkoe DJ (1986) Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc Natl Acad Sci U S A 83: 4044-4048.

199. Mohsenzadegan M, Mirshafiey A (2012) The immunopathogenic role of reactive oxygen species in Alzheimer disease. Iran J Allergy Asthma Immunol 11: 203-216.

200. Pavlides S, Tsirigos A, Vera I, Flomenberg N, Frank PG, et al. (2010) Transcriptional evidence for the "Reverse Warburg Effect" in human breast cancer tumor stroma and metastasis: similarities with oxidative stress, inflammation, Alzheimer's disease, and "Neuron-Glia Metabolic Coupling". Aging (Albany NY) 2: 185-199.

201. Small SA, Duff K (2008) Linking Abeta and tau in late-onset Alzheimer's disease: a dual pathway hypothesis. Neuron 60: 534-542.

202. Schonrock N, Matamales M, Ittner LM, Gotz J (2012) MicroRNA networks surrounding APP and amyloid-beta metabolism--implications for Alzheimer's disease. Exp Neurol 235: 447-454.

203. Cole SW, Sood AK (2012) Molecular pathways: beta-adrenergic signaling in cancer. Clin Cancer Res 18: 1201-1206.

204. Lukiw WJ (2004) Gene expression profiling in fetal, aged, and Alzheimer hippocampus: a continuum of stress-related signaling. Neurochem Res 29: 1287-1297.

205. Maccioni RB, Rojo LE, Fernandez JA, Kuljis RO (2009) The role of neuroimmunomodulation in Alzheimer's disease. Ann N Y Acad Sci 1153: 240-246.

206. Rojo LE, Fernandez JA, Maccioni AA, Jimenez JM, Maccioni RB (2008) Neuroinflammation: implications for the pathogenesis and molecular diagnosis of Alzheimer's disease. Arch Med Res 39: 1-16.

207. Segal E, Friedman N, Kaminski N, Regev A, Koller D (2005) From signatures to models: understanding cancer using microarrays. Nat Genet 37 Suppl: S38-45.

208. Segal E, Friedman N, Koller D, Regev A (2004) A module map showing conditional activity of expression modules in cancer. Nat Genet 36: 1090-1098.

209. Streit WJ, Braak H, Xue QS, Bechmann I (2009) Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and

likely precede neurodegeneration in Alzheimer's disease. Acta Neuropathol 118: 475-485.

210. Vadnal J, Houston S, Bhatta S, Freeman E, McDonough J (2012) Transcriptional signatures mediated by acetylation overlap with early-stage Alzheimer's disease. Exp Brain Res 221: 287-297.

211. Vagnucci AH, Jr., Li WW (2003) Alzheimer's disease and angiogenesis. Lancet 361: 605-608.

212. Hedskog L, Zhang S, Ankarcrona M (2012) Strategic role for mitochondria in Alzheimer's disease and cancer. Antioxid Redox Signal 16: 1476-1491.

213. Biron KE, Dickstein DL, Gopaul R, Jefferies WA (2011) Amyloid triggers extensive cerebral angiogenesis causing blood brain barrier permeability and hypervascularity in Alzheimer's disease. PLoS One 6: e23789.

214. Ono M (2008) Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy. Cancer Sci 99: 1501-1506.

215. Sethi G, Shanmugam MK, Ramachandran L, Kumar AP, Tergaonkar V (2012) Multifaceted link between cancer and inflammation. Biosci Rep 32: 1-15.

216. Vendramini-Costa DB, Carvalho JE (2012) Molecular link mechanisms between inflammation and cancer. Curr Pharm Des 18: 3831-3852.

217. Schreiber RD, Old LJ, Smyth MJ (2011) Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science 331: 1565-1570.

218. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ (2011) Natural innate and adaptive immunity to cancer. Annu Rev Immunol 29: 235-271.

219. Lukiw WJ (2007) Micro-RNA speciation in fetal, adult and Alzheimer's disease hippocampus. Neuroreport 18: 297-300.

220. Lukiw WJ, Surjyadipta B, Dua P, Alexandrov PN (2012) Common micro RNAs (miRNAs) target complement factor H (CFH) regulation in Alzheimer's disease (AD) and in age-related macular degeneration (AMD). Int J Biochem Mol Biol 3: 105-116.

221. Sethi P, Lukiw WJ (2009) Micro-RNA abundance and stability in human brain: specific alterations in Alzheimer's disease temporal lobe neocortex. Neurosci Lett 459: 100-104.

222. De Leon MJ, George AE, Golomb J, Tarshish C, Convit A, et al. (1997) Frequency of hippocampal formation atrophy in normal aging and Alzheimer's disease. Neurobiol Aging 18: 1-11.

223. Desikan RS, Sabuncu MR, Schmansky NJ, Reuter M, Cabral HJ, et al. (2010) Selective disruption of the cerebral neocortex in Alzheimer's disease. PLoS One 5: e12853.

224. Jack CR, Jr., Petersen RC, Xu Y, O'Brien PC, Smith GE, et al. (1998) Rate of medial temporal lobe atrophy in typical aging and Alzheimer's disease. Neurology 51: 993-999.

225. Nath S, Agholme L, Kurudenkandy FR, Granseth B, Marcusson J, et al. (2012) Spreading of neurodegenerative pathology via neuron-to-neuron transmission of beta-amyloid. J Neurosci 32: 8767-8777.

226. Spulber G, Niskanen E, Macdonald S, Kivipelto M, Padilla DF, et al. (2012) Evolution of global and local grey matter atrophy on serial MRI scans during the progression from MCI to AD. Curr Alzheimer Res 9: 516-524.

227. Cogswell JP, Ward J, Taylor IA, Waters M, Shi Y, et al. (2008) Identification of miRNA changes in Alzheimer's disease brain and CSF yields putative biomarkers and insights into disease pathways. J Alzheimers Dis 14: 27-41.

228. Hebert SS, Horre K, Nicolai L, Papadopoulou AS, Mandemakers W, et al. (2008) Loss of microRNA cluster miR-29a/b-1 in sporadic Alzheimer's disease correlates with increased BACE1/beta-secretase expression. Proc Natl Acad Sci U S A 105: 6415-6420.

229. Lukiw WJ (2012) NF-small ka, CyrillicB-regulated micro RNAs (miRNAs) in primary human brain cells. Exp Neurol 235: 484-490.

230. Krichevsky AM, Sonntag KC, Isacson O, Kosik KS (2006) Specific microRNAs modulate embryonic stem cell-derived neurogenesis. Stem Cells 24: 857-864.

231. Yuva-Aydemir Y, Simkin A, Gascon E, Gao FB (2011) MicroRNA-9: functional evolution of a conserved small regulatory RNA. RNA Biol 8: 557-564.

232. Liu N, Sun Q, Chen J, Li J, Zeng Y, et al. (2012) MicroRNA-9 suppresses uveal melanoma cell migration and invasion through the NF-kappaB1 pathway. Oncol Rep 28: 961-968.

233. Minor J, Wang X, Zhang F, Song J, Jimeno A, et al. (2012) Methylation of microRNA-9 is a specific and sensitive biomarker for oral and oropharyngeal squamous cell carcinomas. Oral Oncol 48: 73-78.

234. Inoue T, Iinuma H, Ogawa E, Inaba T, Fukushima R (2012) Clinicopathological and prognostic significance of microRNA-107 and its relationship to DICER1 mRNA expression in gastric cancer. Oncol Rep 27: 1759-1764.

235. Krell J, Frampton AE, Jacob J, Pellegrino L, Roca-Alonso L, et al. (2012) The clinico-pathologic role of microRNAs miR-9 and miR-151-5p in breast cancer metastasis. Mol Diagn Ther 16: 167-172.

236. Leucci E, Zriwil A, Gregersen LH, Jensen KT, Obad S, et al. (2012) Inhibition of miR-9 de-represses HuR and DICER1 and impairs Hodgkin lymphoma tumour outgrowth in vivo. Oncogene 31: 5081-5089.

237. Li F, Huang XP, Wang ZQ, Liu FR, Zhou XH, et al. (2011) [Expression of miR-9 in B lymphocytes and B cell lymphomas cell lines and its significance]. Zhonghua Xue Ye Xue Za Zhi 32: 249-253.

238. Schraivogel D, Weinmann L, Beier D, Tabatabai G, Eichner A, et al. (2011) CAMTA1 is a novel tumour suppressor regulated by miR-9/9* in glioblastoma stem cells. EMBO J 30: 4309-4322.

239. Shigehara K, Yokomuro S, Ishibashi O, Mizuguchi Y, Arima Y, et al. (2011) Real-time PCR-based analysis of the human bile microRNAome identifies miR-9 as a potential diagnostic biomarker for biliary tract cancer. PLoS One 6: e23584.

240. Wilting SM, Snijders PJ, Verlaat W, Jaspers A, van de Wiel MA, et al. (2013) Altered microRNA expression associated with chromosomal changes contributes to cervical carcinogenesis. Oncogene 32: 106-116.

241. Zhu L, Chen H, Zhou D, Li D, Bai R, et al. (2012) MicroRNA-9 up-regulation is involved in colorectal cancer metastasis via promoting cell motility. Med Oncol 29: 1037-1043.

242. Liu X, Zou L, Zhu L, Zhang H, Du C, et al. (2012) miRNA mediated up-regulation of cochaperone p23 acts as an anti-apoptotic factor in childhood acute lymphoblastic leukemia. Leuk Res 36: 1098-1104.

243. Myatt SS, Wang J, Monteiro LJ, Christian M, Ho KK, et al. (2010) Definition of microRNAs that repress expression of the tumor suppressor gene FOXO1 in endometrial cancer. Cancer Res 70: 367-377.

244. Wang WX, Huang Q, Hu Y, Stromberg AJ, Nelson PT (2011) Patterns of microRNA expression in normal and early Alzheimer's disease human temporal cortex: white matter versus gray matter. Acta Neuropathol 121: 193-205.

245. Nunez-Iglesias J, Liu CC, Morgan TE, Finch CE, Zhou XJ (2010) Joint genome-wide profiling of miRNA and mRNA expression in Alzheimer's disease cortex reveals altered miRNA regulation. PLoS One 5: e8898.

246. Kole AJ, Swahari V, Hammond SM, Deshmukh M (2011) miR-29b is activated during neuronal maturation and targets BH3-only genes to restrict apoptosis. Genes Dev 25: 125-130.

247. Mott JL, Kobayashi S, Bronk SF, Gores GJ (2007) mir-29 regulates Mcl-1 protein expression and apoptosis. Oncogene 26: 6133-6140.

248. Fabbri M, Garzon R, Cimmino A, Liu Z, Zanesi N, et al. (2007) MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci U S A 104: 15805-15810.

249. Garzon R, Heaphy CE, Havelange V, Fabbri M, Volinia S, et al. (2009) MicroRNA 29b functions in acute myeloid leukemia. Blood 114: 5331-5341.

250. Hiroki E, Akahira J, Suzuki F, Nagase S, Ito K, et al. (2010) Changes in microRNA expression levels correlate with clinicopathological features and prognoses in endometrial serous adenocarcinomas. Cancer Sci 101: 241-249.

251. Li Y, Wang F, Xu J, Ye F, Shen Y, et al. (2011) Progressive miRNA expression profiles in cervical carcinogenesis and identification of HPV-related target genes for miR-29. J Pathol 224: 484-495.

252. Nguyen T, Kuo C, Nicholl MB, Sim MS, Turner RR, et al. (2011) Downregulation of microRNA-29c is associated with hypermethylation of tumor-related genes and disease outcome in cutaneous melanoma. Epigenetics 6: 388-394.

253. Xiong Y, Fang JH, Yun JP, Yang J, Zhang Y, et al. (2010) Effects of microRNA-29 on apoptosis, tumorigenicity, and prognosis of hepatocellular carcinoma. Hepatology 51: 836-845.

254. Zhao JJ, Lin J, Lwin T, Yang H, Guo J, et al. (2010) microRNA expression profile and identification of miR-29 as a prognostic marker and pathogenetic factor by targeting CDK6 in mantle cell lymphoma. Blood 115: 2630-2639.

255. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, et al. (2004) NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 431: 461-466.

256. Fang JH, Zhou HC, Zeng C, Yang J, Liu Y, et al. (2011) MicroRNA-29b suppresses tumor angiogenesis, invasion, and metastasis by regulating matrix metalloproteinase 2 expression. Hepatology 54: 1729-1740.

257. Ding Q, Chang CJ, Xie X, Xia W, Yang JY, et al. (2011) APOBEC3G promotes liver metastasis in an orthotopic mouse model of colorectal cancer and predicts human hepatic metastasis. J Clin Invest 121: 4526-4536.

258. Braconi C, Kogure T, Valeri N, Huang N, Nuovo G, et al. (2011) microRNA-29 can regulate expression of the long non-coding RNA gene MEG3 in hepatocellular cancer. Oncogene 30: 4750-4756.

259. Lukiw WJ, Zhao Y, Cui JG (2008) An NF-kappaB-sensitive micro RNA-146a-mediated inflammatory circuit in Alzheimer disease and in stressed human brain cells. J Biol Chem 283: 31315-31322.

260. Vilardo E, Barbato C, Ciotti M, Cogoni C, Ruberti F (2010) MicroRNA-101 regulates amyloid precursor protein expression in hippocampal neurons. J Biol Chem 285: 18344-18351.

261. Long JM, Lahiri DK (2011) MicroRNA-101 downregulates Alzheimer's amyloid-beta precursor protein levels in human cell cultures and is differentially expressed. Biochem Biophys Res Commun 404: 889-895.

262. Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, et al. (1994) Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. Proc Natl Acad Sci U S A 91: 12013-12017.

263. Spielman L, Winger D, Ho L, Aisen PS, Shohami E, et al. (2002) Induction of the complement component C1qB in brain of transgenic mice with neuronal overexpression of human cyclooxygenase-2. Acta Neuropathol 103: 157-162.

264. Ho L, Pieroni C, Winger D, Purohit DP, Aisen PS, et al. (1999) Regional distribution of cyclooxygenase-2 in the hippocampal formation in Alzheimer's disease. J Neurosci Res 57: 295-303.

265. Luo L, Zhang T, Liu H, Lv T, Yuan D, et al. (2012) MiR-101 and Mcl-1 in non-small-cell lung cancer: expression profile and clinical significance. Med Oncol 29: 1681-1686.

266. Semaan A, Qazi AM, Seward S, Chamala S, Bryant CS, et al. (2011) MicroRNA-101 inhibits growth of epithelial ovarian cancer by relieving chromatin-mediated transcriptional repression of p21(waf(1)/cip(1)). Pharm Res 28: 3079-3090.

267. Smits M, Nilsson J, Mir SE, van der Stoop PM, Hulleman E, et al. (2010) miR-101 is down-regulated in glioblastoma resulting in EZH2-induced proliferation, migration, and angiogenesis. Oncotarget 1: 710-720.

268. Strillacci A, Griffoni C, Sansone P, Paterini P, Piazzi G, et al. (2009) MiR-101 downregulation is involved in cyclooxygenase-2 overexpression in human colon cancer cells. Exp Cell Res 315: 1439-1447.

269. Su H, Yang JR, Xu T, Huang J, Xu L, et al. (2009) MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. Cancer Res 69: 1135-1142.

270. Thu KL, Chari R, Lockwood WW, Lam S, Lam WL (2011) miR-101 DNA copy loss is a prominent subtype specific event in lung cancer. J Thorac Oncol 6: 1594-1598.

271. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, et al. (2008) Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science 322: 1695-1699.

272. Wang HJ, Ruan HJ, He XJ, Ma YY, Jiang XT, et al. (2010) MicroRNA-101 is down-regulated in gastric cancer and involved in cell migration and invasion. Eur J Cancer 46: 2295-2303.

273. Zhang JG, Guo JF, Liu DL, Liu Q, Wang JJ (2011) MicroRNA-101 exerts tumor-suppressive functions in non-small cell lung cancer through directly targeting enhancer of zeste homolog 2. J Thorac Oncol 6: 671-678.

274. Nelson PT, Wang WX (2010) MiR-107 is reduced in Alzheimer's disease brain neocortex: validation study. J Alzheimers Dis 21: 75-79.

275. Wang WX, Rajeev BW, Stromberg AJ, Ren N, Tang G, et al. (2008) The expression of microRNA miR-107 decreases early in Alzheimer's disease and may accelerate disease progression through regulation of beta-site amyloid precursor protein-cleaving enzyme 1. J Neurosci 28: 1213-1223.

276. Wang WX, Kyprianou N, Wang X, Nelson PT (2010) Dysregulation of the mitogen granulin in human cancer through the miR-15/107 microRNA gene group. Cancer Res 70: 9137-9142.

277. Bryant RJ, Pawlowski T, Catto JW, Marsden G, Vessella RL, et al. (2012) Changes in circulating microRNA levels associated with prostate cancer. Br J Cancer 106: 768-774.

278. Li X, Zhang Y, Shi Y, Dong G, Liang J, et al. (2011) MicroRNA-107, an oncogene microRNA that regulates tumour invasion and metastasis by targeting DICER1 in gastric cancer. J Cell Mol Med 15: 1887-1895.

279. Feng L, Xie Y, Zhang H, Wu Y (2012) miR-107 targets cyclin-dependent kinase 6 expression, induces cell cycle G1 arrest and inhibits invasion in gastric cancer cells. Med Oncol 29: 856-863.

280. Datta J, Smith A, Lang JC, Islam M, Dutt D, et al. (2012) microRNA-107 functions as a candidate tumor-suppressor gene in head and neck squamous cell carcinoma by downregulation of protein kinase Cvarepsilon. Oncogene 31: 4045-4053.

281. Yamakuchi M, Lotterman CD, Bao C, Hruban RH, Karim B, et al. (2010) P53-induced microRNA-107 inhibits HIF-1 and tumor angiogenesis. Proc Natl Acad Sci U S A 107: 6334-6339.

282. Lee KH, Lotterman C, Karikari C, Omura N, Feldmann G, et al. (2009) Epigenetic silencing of MicroRNA miR-107 regulates cyclin-dependent kinase 6 expression in pancreatic cancer. Pancreatology 9: 293-301.

283. van Swieten JC, Heutink P (2008) Mutations in progranulin (GRN) within the spectrum of clinical and pathological phenotypes of frontotemporal dementia. Lancet Neurol 7: 965-974.

284. John B, Sander C, Marks DS (2006) Prediction of human microRNA targets. Methods Mol Biol 342: 101-113.

285. Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, et al. (2005) Combinatorial microRNA target predictions. Nat Genet 37: 495-500.

286. Perdahl E, Adolfsson R, Alafuzoff I, Albert KA, Nestler EJ, et al. (1984) Synapsin I (protein I) in different brain regions in senile dementia of Alzheimer type and in multi-infarct dementia. J Neural Transm 60: 133-141.

287. Qin S, Hu XY, Xu H, Zhou JN (2004) Regional alteration of synapsin I in the hippocampal formation of Alzheimer's disease patients. Acta Neuropathol 107: 209-215.

288. Lukiw WJ, Alexandrov PN (2012) Regulation of complement factor H (CFH) by multiple miRNAs in Alzheimer's disease (AD) brain. Mol Neurobiol 46: 11-19.

289. Alpini G, Glaser SS, Zhang JP, Francis H, Han Y, et al. (2011) Regulation of placenta growth factor by microRNA-125b in hepatocellular cancer. J Hepatol 55: 1339-1345.

290. Au SL, Wong CC, Lee JM, Fan DN, Tsang FH, et al. (2012) Enhancer of zeste homolog 2 epigenetically silences multiple tumor suppressor microRNAs to promote liver cancer metastasis. Hepatology 56: 622-631.

291. Liang L, Wong CM, Ying Q, Fan DN, Huang S, et al. (2010) MicroRNA-125b suppressesed human liver cancer cell proliferation and metastasis by directly targeting oncogene LIN28B2. Hepatology 52: 1731-1740.

292. Muramatsu F, Kidoya H, Naito H, Sakimoto S, Takakura N (2013) microRNA-125b inhibits tube formation of blood vessels through translational suppression of VE-cadherin. Oncogene 32: 414-421.

293. Huang L, Luo J, Cai Q, Pan Q, Zeng H, et al. (2011) MicroRNA-125b suppresses the development of bladder cancer by targeting E2F3. Int J Cancer 128: 1758-1769.

294. Xia HF, He TZ, Liu CM, Cui Y, Song PP, et al. (2009) MiR-125b expression affects the proliferation and apoptosis of human glioma cells by targeting Bmf. Cell Physiol Biochem 23: 347-358.

295. Cui JG, Li YY, Zhao Y, Bhattacharjee S, Lukiw WJ (2010) Differential regulation of interleukin-1 receptor-associated kinase-1 (IRAK-1) and IRAK-2 by microRNA-146a and NF-kappaB in stressed human astroglial cells and in Alzheimer disease. J Biol Chem 285: 38951-38960.

296. Bhaumik D, Scott GK, Schokrpur S, Patil CK, Campisi J, et al. (2008) Expression of microRNA-146 suppresses NF-kappaB activity with reduction of metastatic potential in breast cancer cells. Oncogene 27: 5643-5647.

297. Hurst DR, Edmonds MD, Scott GK, Benz CC, Vaidya KS, et al. (2009) Breast cancer metastasis suppressor 1 up-regulates miR-146, which suppresses breast cancer metastasis. Cancer Res 69: 1279-1283.

298. Li Y, Vandenboom TG, 2nd, Wang Z, Kong D, Ali S, et al. (2010) miR-146a suppresses invasion of pancreatic cancer cells. Cancer Res 70: 1486-1495.

299. Lin SL, Chiang A, Chang D, Ying SY (2008) Loss of mir-146a function in hormone-refractory prostate cancer. RNA 14: 417-424.

300. Man YG, Fu SW, Liu AJ, Stojadinovic A, Izadjoo MJ, et al. (2011) Aberrant expression of chromogranin A, miR-146a, and miR-146b-5p in prostate structures with focally disrupted basal cell layers: an early sign of invasion and hormone-refractory cancer? Cancer Genomics Proteomics 8: 235-244.

301. Pacifico F, Crescenzi E, Mellone S, Iannetti A, Porrino N, et al. (2010) Nuclear factor-{kappa}B contributes to anaplastic thyroid carcinomas through up-regulation of miR-146a. J Clin Endocrinol Metab 95: 1421-1430.

302. Wang X, Tang S, Le SY, Lu R, Rader JS, et al. (2008) Aberrant expression of oncogenic and tumor-suppressive microRNAs in cervical cancer is required for cancer cell growth. PLoS One 3: e2557.

303. Taganov KD, Boldin MP, Chang KJ, Baltimore D (2006) NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to

signaling proteins of innate immune responses. Proc Natl Acad Sci U S A 103: 12481-12486.

304. Tian T, Xu Y, Dai J, Wu J, Shen H, et al. (2010) Functional polymorphisms in two pre-microRNAs and cancer risk: a meta-analysis. Int J Mol Epidemiol Genet 1: 358-366.

305. Lian H, Wang L, Zhang J (2012) Increased risk of breast cancer associated with CC genotype of Has-miR-146a Rs2910164 polymorphism in Europeans. PLoS One 7: e31615.

306. Qiu LX, He J, Wang MY, Zhang RX, Shi TY, et al. (2011) The association between common genetic variant of microRNA-146a and cancer susceptibility. Cytokine 56: 695-698.

307. Wang J, Bi J, Liu X, Li K, Di J, et al. (2012) Has-miR-146a polymorphism (rs2910164) and cancer risk: a meta-analysis of 19 case-control studies. Mol Biol Rep 39: 4571-4579.

308. Zeng Y, Sun QM, Liu NN, Dong GH, Chen J, et al. (2010) Correlation between pre-miR-146a C/G polymorphism and gastric cancer risk in Chinese population. World J Gastroenterol 16: 3578-3583.

309. Biessels GJ, Staekenborg S, Brunner E, Brayne C, Scheltens P (2006) Risk of dementia in diabetes mellitus: a systematic review. Lancet Neurol 5: 64-74.

310. Long JM, Ray B, Lahiri DK (2012) MicroRNA-153 physiologically inhibits expression of amyloid-beta precursor protein in cultured human fetal brain

cells and is dysregulated in a subset of Alzheimer disease patients. J Biol Chem 287: 31298-31310.

311. Kim TH, Kim YK, Kwon Y, Heo JH, Kang H, et al. (2010) Deregulation of miR-519a, 153, and 485-5p and its clinicopathological relevance in ovarian epithelial tumours. Histopathology 57: 734-743.

312. Xu J, Liao X, Wong C (2010) Downregulations of B-cell lymphoma 2 and myeloid cell leukemia sequence 1 by microRNA 153 induce apoptosis in a glioblastoma cell line DBTRG-05MG. Int J Cancer 126: 1029-1035.

313. Xu J, Liao X, Lu N, Liu W, Wong CW (2011) Chromatin-modifying drugs induce miRNA-153 expression to suppress Irs-2 in glioblastoma cell lines. Int J Cancer 129: 2527-2531.

314. Biessels GJ, Kappelle LJ, Utrecht Diabetic Encephalopathy Study G (2005) Increased risk of Alzheimer's disease in Type II diabetes: insulin resistance of the brain or insulin-induced amyloid pathology? Biochem Soc Trans 33: 1041-1044.

315. Craft S (2007) Insulin resistance and Alzheimer's disease pathogenesis: potential mechanisms and implications for treatment. Curr Alzheimer Res 4: 147-152.

316. Watson GS, Craft S (2003) The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. CNS Drugs 17: 27-45.

317. Whitmer RA (2007) Type 2 diabetes and risk of cognitive impairment and dementia. Curr Neurol Neurosci Rep 7: 373-380.

318. Zhu HC, Wang LM, Wang M, Song B, Tan S, et al. (2012) MicroRNA-195 downregulates Alzheimer's disease amyloid-beta production by targeting BACE1. Brain Res Bull 88: 596-601.

319. Flavin RJ, Smyth PC, Laios A, O'Toole SA, Barrett C, et al. (2009) Potentially important microRNA cluster on chromosome 17p13.1 in primary peritoneal carcinoma. Mod Pathol 22: 197-205.

320. Guled M, Lahti L, Lindholm PM, Salmenkivi K, Bagwan I, et al. (2009) CDKN2A, NF2, and JUN are dysregulated among other genes by miRNAs in malignant mesothelioma -A miRNA microarray analysis. Genes Chromosomes Cancer 48: 615-623.

321. Guo ST, Jiang CC, Wang GP, Li YP, Wang CY, et al. (2013) MicroRNA-497 targets insulin-like growth factor 1 receptor and has a tumour suppressive role in human colorectal cancer. Oncogene 32: 1910-1920.

322. Lin Y, Wu J, Chen H, Mao Y, Liu Y, et al. (2012) Cyclin-dependent kinase 4 is a novel target in micoRNA-195-mediated cell cycle arrest in bladder cancer cells. FEBS Lett 586: 442-447.

323. Liu L, Chen L, Xu Y, Li R, Du X (2010) microRNA-195 promotes apoptosis and suppresses tumorigenicity of human colorectal cancer cells. Biochem Biophys Res Commun 400: 236-240.

324. Moser JJ, Fritzler MJ (2010) The microRNA and messengerRNA profile of the RNA-induced silencing complex in human primary astrocyte and astrocytoma cells. PLoS One 5: e13445.

325. Ozata DM, Caramuta S, Velazquez-Fernandez D, Akcakaya P, Xie H, et al. (2011) The role of microRNA deregulation in the pathogenesis of adrenocortical carcinoma. Endocr Relat Cancer 18: 643-655.

326. Soon PS, Tacon LJ, Gill AJ, Bambach CP, Sywak MS, et al. (2009) miR-195 and miR-483-5p Identified as Predictors of Poor Prognosis in Adrenocortical Cancer. Clin Cancer Res 15: 7684-7692.

327. Wang X, Wang J, Ma H, Zhang J, Zhou X (2012) Downregulation of miR-195 correlates with lymph node metastasis and poor prognosis in colorectal cancer. Med Oncol 29: 919-927.

328. Xu T, Zhu Y, Xiong Y, Ge YY, Yun JP, et al. (2009) MicroRNA-195 suppresses tumorigenicity and regulates G1/S transition of human hepatocellular carcinoma cells. Hepatology 50: 113-121.

329. Zanette DL, Rivadavia F, Molfetta GA, Barbuzano FG, Proto-Siqueira R, et al. (2007) miRNA expression profiles in chronic lymphocytic and acute lymphocytic leukemia. Braz J Med Biol Res 40: 1435-1440.

330. Zhang QQ, Xu H, Huang MB, Ma LM, Huang QJ, et al. (2012) MicroRNA-195 plays a tumor-suppressor role in human glioblastoma cells by targeting signaling pathways involved in cellular proliferation and invasion. Neuro Oncol 14: 278-287.

331. Heneghan HM, Miller N, Kelly R, Newell J, Kerin MJ (2010) Systemic miRNA-195 differentiates breast cancer from other malignancies and is a potential biomarker for detecting noninvasive and early stage disease. Oncologist 15: 673-682.

332. Heneghan HM, Miller N, Lowery AJ, Sweeney KJ, Newell J, et al. (2010) Circulating microRNAs as novel minimally invasive biomarkers for breast cancer. Ann Surg 251: 499-505.

333. Lakomy R, Sana J, Hankeova S, Fadrus P, Kren L, et al. (2011) MiR-195, miR-196b, miR-181c, miR-21 expression levels and O-6-methylguanine-DNA methyltransferase methylation status are associated with clinical outcome in glioblastoma patients. Cancer Sci 102: 2186-2190.

334. Li D, Zhao Y, Liu C, Chen X, Qi Y, et al. (2011) Analysis of MiR-195 and MiR-497 expression, regulation and role in breast cancer. Clin Cancer Res 17: 1722-1730.

335. Ujifuku K, Mitsutake N, Takakura S, Matsuse M, Saenko V, et al. (2010) miR-195, miR-455-3p and miR-10a(*) are implicated in acquired temozolomide resistance in glioblastoma multiforme cells. Cancer Lett 296: 241-248.

336. Singh R, Saini N (2012) Downregulation of BCL2 by miRNAs augments drug-induced apoptosis--a combined computational and experimental approach. J Cell Sci 125: 1568-1578.

337. Fei X, Qi M, Wu B, Song Y, Wang Y, et al. (2012) MicroRNA-195-5p suppresses glucose uptake and proliferation of human bladder cancer T24 cells by regulating GLUT3 expression. FEBS Lett 586: 392-397.

338. Li YY, Alexandrov PN, Pogue AI, Zhao Y, Bhattacharjee S, et al. (2012) miRNA-155 upregulation and complement factor H deficits in Down's syndrome. Neuroreport 23: 168-173.

339. Zetterberg M, Landgren S, Andersson ME, Palmer MS, Gustafson DR, et al. (2008) Association of complement factor H Y402H gene polymorphism with Alzheimer's disease. Am J Med Genet B Neuropsychiatr Genet 147B: 720-726.

340. Cameron DJ, Galvin C, Alkam T, Sidhu H, Ellison J, et al. (2012) Alzheimer's-related peptide amyloid-beta plays a conserved role in angiogenesis. PLoS One 7: e39598.

341. Bruneau S, Datta D, Flaxenburg JA, Pal S, Briscoe DM (2012) TRAF6 inhibits proangiogenic signals in endothelial cells and regulates the expression of vascular endothelial growth factor. Biochem Biophys Res Commun 419: 66-71.

342. Nudelman KN, Risacher SL, West JD, McDonald BC, Gao S, et al. (2014) Association of cancer history with Alzheimer's disease onset and structural brain changes. Front Physiol 5: 423.

CURRICULUM VITAE

Kelly N. H. Nudelman

Education

2010-2015 Indiana University, Indianapolis, IN
PhD: Medical and Molecular Genetics
GPA: 3.94
Thesis: "Cognitive dysfunction in cancer: neuroimaging and genetic approaches to identify biological mechanisms"
PI: Andrew J. Saykin, PsyD

2002-2006 University of Arizona, Tucson, AZ
BS: Honors, Cum Laude, Molecular and Cellular Biology
Minor: French
GPA: 3.6
Honors Thesis: "Characterization of a Dicer-like gene in maize"
PI: Vicki L. Chandler, PhD

Research Experience

2011-2015 Indiana University School of Medicine, Indianapolis, IN
PhD student (PI: Andrew J. Saykin, PsyD)
1. Neural cell adhesion in Alzheimer's disease
2. Review of microRNA studies as a means to study the inverse association of cancer and Alzheimer's disease
3. Review of neuroimaging studies of cancer and treatment-related cognitive dysfunction
4. Multimodal neuroimaging study of cognitive dysfunction in breast cancer
5. Meta-analysis of candidate neurological SNPs in breast cancer
6. Neuroimaging genetics study of the inverse association of cancer and Alzheimer's disease

2006-2010 Translational Genomics Research Institute (TGen), Phoenix, AZ
Biological Research Associate (PI: Kevin Brown, PhD)
1. High-throughput Sanger and next-generation sequencing and bioinformatic analysis of the 1p22 familial melanoma locus
2. Linkage analysis of familial ocular and melanoma cohort to identify novel loci

2004-2006 University of Arizona, Tucson, AZ
Undergraduate research (PI: Vicki L. Chandler, PhD)
1. Gene sequencing in maize

2005 USDA ARS Western Cotton Research Laboratory, Tempe, AZ
Summer Research Internship (PI: Ben DeRidder, PhD)
1. Gene sequencing and cloning in *A. thaliana*

2004 University of Missouri-Columbia, Columbia, MO
Summer Research Internship (PI: Emmanuel Liscum, PhD)
1. Using FISH to study phototropism

Memberships, Honors, and Awards

2012-2015 Trainee member, American Society of Human Genetics
2014 Merit Award, American Society of Clinical Oncology Conference, Chicago, IL
2014 Travel Award, Indiana University Simon Cancer Center
2012 Poster Award, Cancer Research Day Symposium, Indiana University, IN
2008 Travel Grant, Melanoma Genetics Consortium (GenoMEL): funding for a three month study with collaborator Dr. Nick Hayward at the Queensland Institute for Medical Research (QIMR), Brisbane, Australia
2005 Dean's list
2002-2003 Dean's list
2002 Provost Award, University of Arizona, Tucson, AZ
2001 National Merit Commended Scholar, University of Arizona, Tucson, AZ
2000 President's Award for Academic Excellence, University of Arizona, Tucson, AZ

Abstracts

1. **Nudelman KNH** et al. Variants in mitochondrial intermediate peptidase (MIPEP) gene are associated with gray matter density in the Alzheimer's Disease Neuroimaging Initiative cohort. Poster, Indiana Alzheimer's Disease Center meeting, Indiana University, 03/2015, Indianapolis, IN.
2. **Nudelman KNH** et al. Ephrin receptor genotypes modify chemotherapy-induced peripheral neuropathy symptoms: a candidate gene study in breast cancer patients. Poster, American Society of Human Genetics conference, 10/2014, San Diego, CA.
3. **Nudelman KNH** et al. Nervous system sequelae of chemotherapy treatment: associations and proposed mechanisms. Platform, Grand Rounds, Indiana University School of Medicine, 08/2014, Indianapolis, IN.
4. **Nudelman KNH** et al. Nervous system sequelae of chemotherapy treatment: associations and proposed mechanisms. Poster, American Society of Clinical Oncology conference, 05/2014, Chicago, IL.

5. **Nudelman KNH** et al. Analysis of the inverse association between cancer and Alzheimer's disease: results from the Alzheimer's Disease Neuroimaging Initiative cohort. Poster, Indiana Alzheimer's Disease Center meeting, Indiana University, 03/2014, Indianapolis, IN.
6. **Nudelman KNH** et al. Longitudinal cerebral blood flow increase and recovery after systemic chemotherapy for breast cancer. Platform, International Cancer Control Task Force conference, 02/2014, Seattle, WA.
7. **Holohan KN** et al. Chemotherapy-induced peripheral neuropathy and cognitive dysfunction: role of genetic variation. Poster, American Society of Human Genetics conference, 10/2013, Boston, MA.
8. **Holohan KN** et al. Cerebral perfusion and cognition after breast cancer chemotherapy: a prospective PASL MRI study. Poster, Annual Meeting of the Organization for Human Brain Mapping, 06/2013, Seattle WA.
9. **Holohan KN** et al. Neural cell adhesion gene variation in Alzheimer's disease. Poster, American Society of Human Genetics conference, 11/2012, San Francisco, CA.
10. **Holohan KN** et al. Increased cerebral blood flow one month after systemic chemotherapy for breast cancer: a prospective MRI study using pulsed arterial spin labeled perfusion. Poster, IU Cancer Day Symposium, 05/2012, Indiana University, Indianapolis, IN.
11. **Holohan KN** et al. Investigation of novel ocular melanoma linkage regions with conventional and next-generation sequencing. Platform, GenoMEL conference, 06/2009, Ljubljana, Slovenia.

Publications

1. **Nudelman KNH**, McDonald BC, Wang Y, Smith DJ, West JD, O'Neill DP, Zanville NR, Champion VL, Schneider BP, Saykin AJ. Cerebral perfusion and gray matter changes associated with chemotherapy-induced peripheral neuropathy (CIPN). *Under review at J Clin Oncol.*
2. **Nudelman KNH**, Risacher SL, West JD, McDonald BC, Gao S, Saykin AJ, for the Alzheimer's Disease Neuroimaging Initiative. Association of cancer history with Alzheimer's disease onset and structural brain changes*. Front Physiol*. 2014 Oct 31;5:423. PMCID: PMC4215790
3. **Nudelman KNH**, Wang Y, McDonald BC, Conroy SK, Smith DJ, West JD, O'Neill DP, Schneider BP, Saykin AJ. Altered cerebral blood flow one month after systemic chemotherapy for breast cancer: a prospective study using pulsed arterial spin labeling MRI perfusion. *PLoS One*. 2014 May 9;9(5):e96713. PMCID: PMC4016018.
4. **Holohan KN**, Von Ah D, McDonald BC, Saykin AJ. Neuroimaging, cancer, and cognition: state of the knowledge. *Semin Oncol Nurs*. 2013 Nov;29(4):280-7. PMCID: PMC3821968
5. **Holohan KN**, Lahiri DK, Schneider BP, Foroud T, Saykin AJ. Functional microRNAs in Alzheimer's disease and cancer: differential regulation of

common mechanisms and pathways. *Front Genet*. 2013 Jan 17;3:323. PMCID: PMC3547332

6. Ramanan VK, Kim S, **Holohan KN**, Shen L, Nho K, Risacher SL, Foroud TM, Mukherjee S, Crane PK, Aisen PS, Petersen RC, Weiner MW, Saykin AJ; Alzheimer's Disease Neuroimaging Initiative (ADNI). Genome-wide pathway analysis of memory impairment in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort implicates gene candidates, canonical pathways, and networks. *Brain Imaging Behav*. 2012 Dec;6(4):634-48. PMCID: PMC37136374.
7. Yokoyama S, Woods SL, Boyle GM, Aoude LG, MacGregor S, Zismann V, Gartside M, Cust AE, Haq R, Harland M, Taylor JC, Duffy DL, **Holohan K**, Dutton-Regester K, Palmer JM, Bonazzi V, Stark MS, Symmons J, Law MH, Schmidt C, Lanagan C, O'Connor L, Holland EA, Schmid H, Maskiell JA, Jetann J, Ferguson M, Jenkins MA, Kefford RF, Giles GG, Armstrong BK, Aitken JF, Hopper JL, Whiteman DC, Pharoah PD, Easton DF, Dunning AM, Newton-Bishop JA, Montgomery GW, Martin NG, Mann GJ, Bishop DT, Tsao H, Trent JM, Fisher DE, Hayward NK, Brown KM. A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. *Nature*. 2011 Nov 13;480(7375):99-103. PMCID: PMC3266855

Research Support

2011-2014 R25 CA117865 – Training in Research for Behavioral Oncology and Cancer Control, Indiana University, IN (PI: Victoria Champion, PhD)

Research Skills

- Molecular and cellular biology techniques including cell culture, DNA extraction, cloning, and transformation
- Sanger and next-generation sequencing and chip and array technologies for DNA and RNA
- Familiarity with neuroimaging genetics tools including SAMtools, PLINK, SPM8, SPSS, and web-based biological databases and bioinformatics applications