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TITLE: Development of [11C]CPPC as a Clinical PET Radioligand Biomarker of Microglial Activation in ALS

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14. ABSTRACT

There are a paucity of reliable biomarkers and validated neuroimaging techniques to aid in amyotrophic lateral sclerosis (ALS) diagnosis, prognosis, or pharmacodynamic insight. Positron emission tomography (PET) imaging is a technique that uses radioactive molecules attached to a ligand of interest allowing for visualization of the 3D distribution of the ligand's target receptor. One of the upstream processes thought to lead to motor neuron degeneration in ALS is microglial dysfunction, resulting in the initiation of neuroinflammatory cascades. Macrophage colony stimulating factor 1 receptor is found on microglia with low levels of expression in neurons and other neural cells, making it a promising target for studying microglial activation. Given CSF1's potential role in ALS disease progression, and that its receptor (CSF1R) can be directly targeted, ligands binding this receptor are an area of interest for imaging in ALS. [^{11}C]CPPC, is a positron-emitting, high-affinity ligand that is specific for CSF1R. [^{11}C]CPPC demonstrates high and specific brain uptake in postmortem human tissue from control patients and those with other neurodegenerative diseases. We will establish the utility of [^{11}C]CPPC PET as a measure of neuroinflammation, specifically microglial activation, and to correlate this activity with other clinical and biofluid measures of disease progression in ALS.

15. SUBJECT TERMS
Amyotrophic lateral sclerosis, Positron Emission Tomography, imaging, neuroinflammation, human, CSF1R, microglia, biomarker.

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TABLE OF CONTENTS

1. INTRODUCTION

There are a paucity of reliable serum and cerebrospinal (CSF) biomarkers and validated neuroimaging techniques to aid in amyotrophic lateral sclerosis (ALS) diagnosis, prognosis, or pharmacodynamic insight. Positron emission tomography (PET) imaging is a technique that uses radioactive molecules attached to a ligand of interest which localizes to the desired target, allowing for visualization of the three dimensional distribution of the ligand's target receptor. PET imaging has been used to examine several different putative disease mechanisms in ALS.

One of the upstream processes that are thought to lead to motor neuron degeneration in ALS is microglial activation, resulting in the initiation of neuroinflammatory cascades. Prior PET studies of microglial mediated inflammation in ALS focused on targeting the 18 kDa translocator protein (TSPO) using [^{11}C] PBR28[3] and $^{(18)}$F-DPA-714 radioligands. In a small but important study, PET imaging with [^{11}C] PBR28 showed increased binding in motor cortices and corticospinal tracts in patients with ALS. Of note, the study was somewhat limited in the number of bulbar-onset patients recruited (3/10) making interpretation of [^{11}C] PBR28 activity in this ALS subtype more challenging. Encouragingly, the binding of [^{11}C] PBR28 correlated with upper motor neuron burden scores and negatively correlated with the ALSFRS-R providing an important framework for further study. However, these studies were limited by lack of cell type specificity due to expression of TSPO on astroglia and endothelial cells among others. Indeed, a recent study examining TSPO activity in another neurodegenerative disease, Alzheimer's disease, showed that TSPO is expressed not only in microglia but also astrocytes, endothelial cells and smooth muscle cells. More confounding were the observations that there was a substantial overlap of TSPO levels between control and AD subjects and no correlation between TSPO and glial responses[7]. Also, one must genotype patients before studying patients with PBR28 due to uptake being dependent on TSPO polymorphisms, which results in smaller sample sizes.

Therefore, while TSPO has provided potentially important insights into ALS disease activity, these emerging studies suggest that there is significant room for improving cell specificity and signal to noise ratio between control and disease. This is especially important given the challenges faced by ALS investigators to hone in on pathway-specific targets and for the development of biomarkers that could be predictive of disease responses to therapeutics.

Macrophage colony stimulating factor 1 receptor (CSF1R) is found on microglia predominately in the brain, with low levels of expression in neurons and other neural cells, making it a promising target for studying microglial activation.[5,8] CSF1R and its ligand, CSF1, have been implicated in the pathogenesis of ALS through mouse and human studies. Elevated levels of CSF1 have been shown in immunohistochemical analyses of postmortem human brain tissue from both patients with ALS and those with Alzheimer's disease.[9] Inhibition of CSF1R in SOD1^{G93A} mice (one model of ALS) is associated with decreased macrophage invasion in the peripheral nervous system, as well as decreased proliferation of microglia, and extended survival of the treated animals.[10]

Given CSF1's potential role in ALS disease progression, and that its receptor (CSF1R) can be directly targeted, ligands binding this receptor are an area of interest for imaging in ALS. [^{11}C]CPPC [5-cyano-N-(4-(4-[^{11}C]methylpiperazin-1-yl)-2-(piperidin-1-yl)phenyl)furan-2-carboxamide], is a positron-emitting, high-affinity ligand that is specific for CSF1R (**Figure 1**). [^{11}C]CPPC can be synthesized in sufficient radiochemical yield, purity, and specific radioactivity.

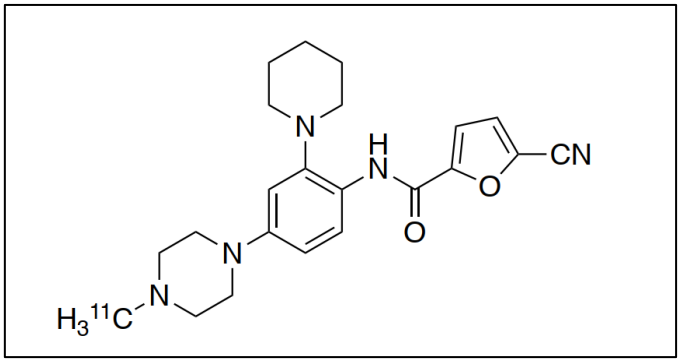

Figure 1: Structural Formula for [^{11}C]CPPC

2. KEYWORDS

Amyotrophic lateral sclerosis, Positron Emission Tomography, imaging, neuroinflammation, human, CSF1R, microglia, biomarker.

3. ACCOMPLISHMENTS

Major Goals--SPECIFIC AIMS

Aim #1: Examine whether [^{11}C]CPPC PET uptake is elevated in brains of ALS patients and whether there is a correlation with clinical phenotype.

Accomplishments

Because this research study was only approved 1 month ago (9/11/2023) by the USAMRDC OHARO OHRO, we have not yet recruited participants for this study. However, our Co-PI, Dr. Pomper, has recently published the following manuscript with relevance to our current study.

COUGHLIN JM, DU Y, LESNIAK WG, HARRINGTON CK, BROSNAN MK, O'TOOLE R, ZANDI A, SWEENEY SE, ABDALLAH R, WU Y, HOLT DP, HALL AW, DANNALS RF, SOLNES L, HORTI AG, POMPER MG. FIRST-IN-HUMAN USE OF ^{11}C-CPPC WITH POSITRON EMISSION TOMOGRAPHY FOR IMAGING THE MACROPHAGE COLONY-STIMULATING FACTOR 1 RECEPTOR. EJNMMI RES. 2022 SEP 30;12(1):64. DOI: 10.1186/S13550-022-00929-4.

Aim #2: Examine longitudinal changes in [^{11}C]CPPC PET imaging during disease course.

Accomplishments

Again, we have just received approval from USAMRDC OHARO OHRO for this study and therefore, the longitudinal element of this study has not yielded data at this time.

Aim #3: Correlate [^{11}C]CPPC PET imaging with ALS clinical outcome measures and biofluid biomarkers

Accomplishments

Biofluid biomarkers will be obtained when obtained from the participants in Aims #1 and #2.

Opportunities for professional development

Nothing to report

Dissemination

Plans

1. Coordinate with our ALS Clinical Trials Unit for the initiation of recruitment of ALS participants as well as age matched controls for this study.

4. IMPACT

There are no well-established diagnostic or prognostic imaging modalities for ALS used in clinical practice. The development of this microglial specific [^{11}C]CPPC PET radioligand for imaging patients with ALS has many applications. From a clinical practice standpoint, a reliable radiographic biomarker could improve clinicians' abilities to diagnose ALS in a timely manner. We hypothesize that this technique could aid in identifying cortical and corticospinal motor neuron (CSMN) burden prior to the onset of much less sophisticated clinical observations of CSMN dysfunction. As a prognostic biomarker, increases in [^{11}C]CPPC PET uptake could correlate with disease progression allowing clinicians, patients, and families to make more informed care decisions. As a predictive biomarker, this ligand could help stratify patients for clinical trials into those who might be most likely to respond to pharmacotherapies targeting neuroinflammatory cascades. Finally, given that there are developing studies targeting these cascades, this technology could be used as a pharmacodynamic marker to assess the pharmacodynamic response to treatment, allowing for more tailored therapeutic regimens.

Impact on other disciplines: Nothing to report
Impact on technology transfer: Nothing to report
Impact on society beyond science and technology: Nothing to report

5. CHANGES/PROBLEMS

The Johns Hopkins University approved the current protocol on May 11, 2023.
The USAMRDC OHARO OHRO approved this greater than minimal risk study for the enrollment of 10 subjects with an ALS diagnosis and 10 healthy subjects. I was notified about this approval on 9/11/2023. Because this is a recent approval, we have not yet been recruiting participants for this study but are not in the process of beginning the recruitment process for participants.

6. PRODUCTS

Nothing to report

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Name	Nicholas J. Maragakis
Project Role:	PI
Researcher ID	0000-0002-7311-9614
Nearest person month worked	0.8 cal mos
Contribution to project	Responsible for obtaining consent on all participants and overseeing the study
Funding support	ALSRP, NIH

Name	Martin Pomper
Project Role:	Co-PI
Researcher ID	0000-0001-6753-3010
Nearest person month worked	0.4 cal mos
Contribution to project	This investigator will be blinded to the results but is responsible for coordinating the PET imaging for the study
Funding support	ALSRP, NIH

Name	JinAe Arneklev
Project Role:	Research Nurse
Researcher ID	
Nearest person month worked	0.2 cal mos
Contribution to project	Will assist in performing participant physical examinations.
Funding support	ALSRP, ALS Association

Name	Betsy Mosmiller
Project Role:	Research Coordinator
Researcher ID	
Nearest person month worked	2.4 cal mos
Contribution to project	Will be responsible for IRB submissions, change in research, continuing review, and data

	management.
Funding support	ALSRP

Name	Ergi Spiro
Project Role:	Study Coordinator
Researcher ID	
Nearest person month worked	2.4 cal mos
Contribution to project	Will schedule imaging studies and coordinate the imaging portion of the study
Funding support	ALSRP

Name:	Amy Tesch
Project Role:	Study Coordinator
Researcher ID:	
Nearest Person Month Worked:	2.4 cal mos
Contribution to project:	She will be responsible for recruiting participants to the study
Funding Support:	ALSRP

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

9. APPENDICES

N/A