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"Translation of HDAC6 PET imaging using [18F]EKZ-001 – cGMP production and measurement of HDAC6 target occupancy in NHPs" – A Review

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"Translation of HDAC6 PET imaging using [^{18}F]EKZ-001 – cGMP production and measurement of HDAC6 target occupancy in NHPs" – A Review

An Honors Thesis submitted in partial fulfillment of the requirements of Honors Studies in Chemistry with Biochemistry Concentration

By

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Abstract:

The inhibition of histone deacetylase 6 (HDAC6) has been reported to alleviate the effects of neurodegenerative diseases such as Alzheimer's disease. The brain-penetrant PET radioligand [^{18}F]EKZ-001 has high affinity and selectivity towards HDAC6 and therefore suggests great promise in therapeutic treatment studies and development for neurodegenerative diseases. "*Translation of HDAC6 PET imaging using [^{18}F]EKZ-001 – cGMP production and measurement of HDAC6 target occupancy in NHPs"* has achieved an effective, fully automated method of producing [^{18}F]EKZ-001 in compliance with current good manufacturing practices (cGMP) to support the translation of [^{18}F]EKZ-001 PET for first-in-human studies. This cGMP compliantly produced radioligand was utilized in PET studies in non-human primates (NHPs), where it was determined that the HDAC6 inhibitor EKZ-317 achieves greater target occupancy than the HDAC6 inhibitor ACY-775. The developments made by this research have had significant impact on the progression of therapeutic treatment studies for neurodegenerative diseases. The success of a cGMP compliant method of [^{18}F]EKZ-001 production has enabled the first-in-human [^{18}F]EKZ-001 PET study, further paving the way to a potential treatment for neurodegenerative diseases. This editorial aims to overview "*Translation of HDAC6 PET imaging using [^{18}F]EKZ-001 – cGMP production and measurement of HDAC6 target occupancy in NHPs"*, to analyze and highlight the impact and relevance of this research, and propose future considerations for research in this area.

1. Background:

As defined by the National Institute of Environmental Health Sciences (NIEHS), a neurodegenerative disease is the result of nerve cells in the brain or peripheral nervous system losing their function over time, ultimately dying [1]. Commonly known neurodegenerative diseases include Alzheimer's disease and Parkinson's disease. A 2021 report by the Alzheimer's Association found that 6.2 million Americans aged 65 or older suffer from the condition [2]. Furthermore, the Parkinson's Foundation has found that about 60,000 Americans are diagnosed with Parkinson's disease annually [3]. As of 2015, an estimated 50 million Americans suffer from a disorder impacting the nervous system [4]. Given these statistics, it is imperative that medical strides be taken to address the impact neurodegenerative diseases have on individuals and healthcare systems at large.

Despite the considerable number of individuals affected by neurodegenerative diseases, there remains critical gaps in the understanding of these diseases which makes the development of therapeutic treatments still difficult at this stage. The FDA describes some of these current shortcomings, including unknown molecular defects or pathways that give rise to neurodegenerative diseases [5]. Current research suggests the involvement of reactive oxygen species (ROS) and inflammation in the onset and progression of neurodegenerative diseases [6]. For example, the extracellular accumulation of beta-amyloid plaques that occurs in individuals with Alzheimer's disease leads to increased levels of ROS, and thus oxidative stress [7]. Furthermore, Alzheimer's disease is associated with increased levels of histone deacetylase 6 (HDAC6) in the brain [7]. HDAC6, a cytoplasmic enzyme, is involved in cellular processes such as deacetylating cytosolic proteins (protein quality control) and intracellular transport [8,9].

Considering these connections between ROS, HDAC6, and neurodegenerative diseases (especially Alzheimer's disease), it is notable to consider additional research pointing to a potential crosstalk between HDAC6 and HIV-1 Tat-induced NADPH oxidase activity, an enzymatic source of ROS [8]. In connection with this idea, a study conducted in 2017 determined that the reduction or inhibition of HDAC6 has proven to rescue memory in mice models of Alzheimer's disease- a promising stride in the cure of neurodegenerative diseases [7].

Although research points to HDAC6 being a strong contender and therapeutic target for the treatment of neurodegenerative diseases, a major obstacle to the development of HDAC6-inhibiting therapeutic treatment is the development of an HDAC6 inhibitor able to effectively penetrate the brain. Human positron emission tomography (PET) studies are necessary in the testing of HDAC6 inhibitors for therapeutic treatment. [^{18}F]EKZ-001 is a brain-penetrant PET radioligand that has a high affinity and selectivity for HDAC6 [9], and its binding ability has been evaluated preclinically. Given [^{18}F]EKZ-001's promise in PET studies involving HDAC6, the next step is the development of an efficient, robust, and fully automated current good manufacturing practices (cGMP) compliant production method for [^{18}F]EKZ-001, described by the research "*Translation of HDAC6 PET imaging using [^{18}F]EKZ-001 – cGMP production and measurement of HDAC6 target occupancy in NHPs*" [9].

2. Methods and Results

Positron emission tomography (PET) is a non-invasive way to quantify HDAC6 target engagement in the brain [9]. Therefore, it is a valuable technique in the process of drug development. The structure of the brain-penetrant PET radioligand [^{18}F]EKZ-001 is shown below.

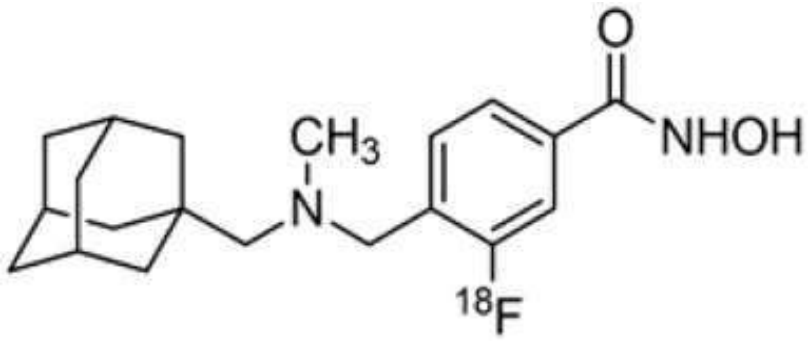

Figure 1- [^{18}F]EKZ-001 Structure [9][1]

[^{18}F]EKZ-001 was synthesized following the route described by described by Strebl *et al* [10]. To make the production process cGMP compliant, the modifications described within "*Supporting Information: Translation of HDAC6 PET imaging using [^{18}F]EKZ-001 – cGMP production and measurement of HDAC6 target occupancy in NHPs"* were made to the procedure [9]. The radiosynthesis of [^{18}F]EKZ-001 utilized the ruthenium complex (CpRu(COD)Cl), which was prepared following a procedure developed by Beyzavi *et al* [11].

1. Reprinted (adapted) with permission from {Celen, S.; Rokka, J.; Gilbert, T. M.; Koole, M.; Vermeulen, I.; Serdons, K.; Schroeder, F. A.; Wagner, F. F.; Bleeser, T.; Hightower, B. G.; Hu, J.; Rahal, D.; Beyzavi, H.; Vanduffel, W.; Van Laere, K.; Kranz, J. E.; Hooker, J. M.; Bormans, G.; Cawthorne, C. J. Translation of HDAC6 PET Imaging Using [18F]EKZ-001-CGMP Production and Measurement of HDAC6 Target Occupancy in Nonhuman Primates. *ACS Chem. Neurosci.* **2020**, *11* (7), 1093–1101. DOI: 10.1021/acschemneuro.0c00074}. Copyright {2020} American Chemical Society.

Figure 2 represents the production of (CpRu(COD)Cl), displayed and described in "*Supporting Information: 18F-Deoxyfluorination of Phenols via Ru π-Complexes"* [11].

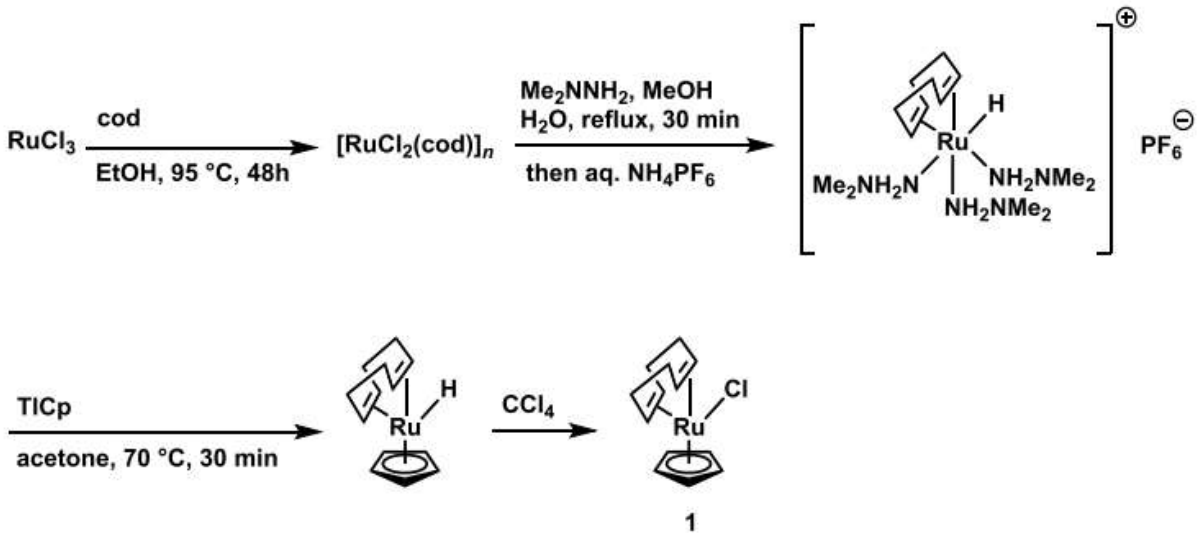

Figure 2- "(CpRu(COD)Cl) (1)" [11]

Using these methods, three cGMP-compliant productions of [^{18}F]EKZ001 were performed. A fully automated cGMP-compliant method of [^{18}F]EKZ-001 production was achieved with a radiochemical purity greater than 98% in a total synthesis time of two hours [9]. The radioligand was then used in PET studies in non-human primates (NHPs). A PET dose-occupancy study performed in a male macaque assessed the target engagement of the HDAC6 inhibitors ACY-775 (an existing HDAC6 inhibitor tool compound) and EKZ-317 (a novel candidate) [9]. The study determined that the novel inhibitor, EKZ-317, had a higher target occupancy compared to ACY-775. These results are represented by Figure 3 [9]. Given

the results achieved by this study, the next logical step in this research involves the translation of [^{18}F]EKZ-001 PET for use in human PET studies.

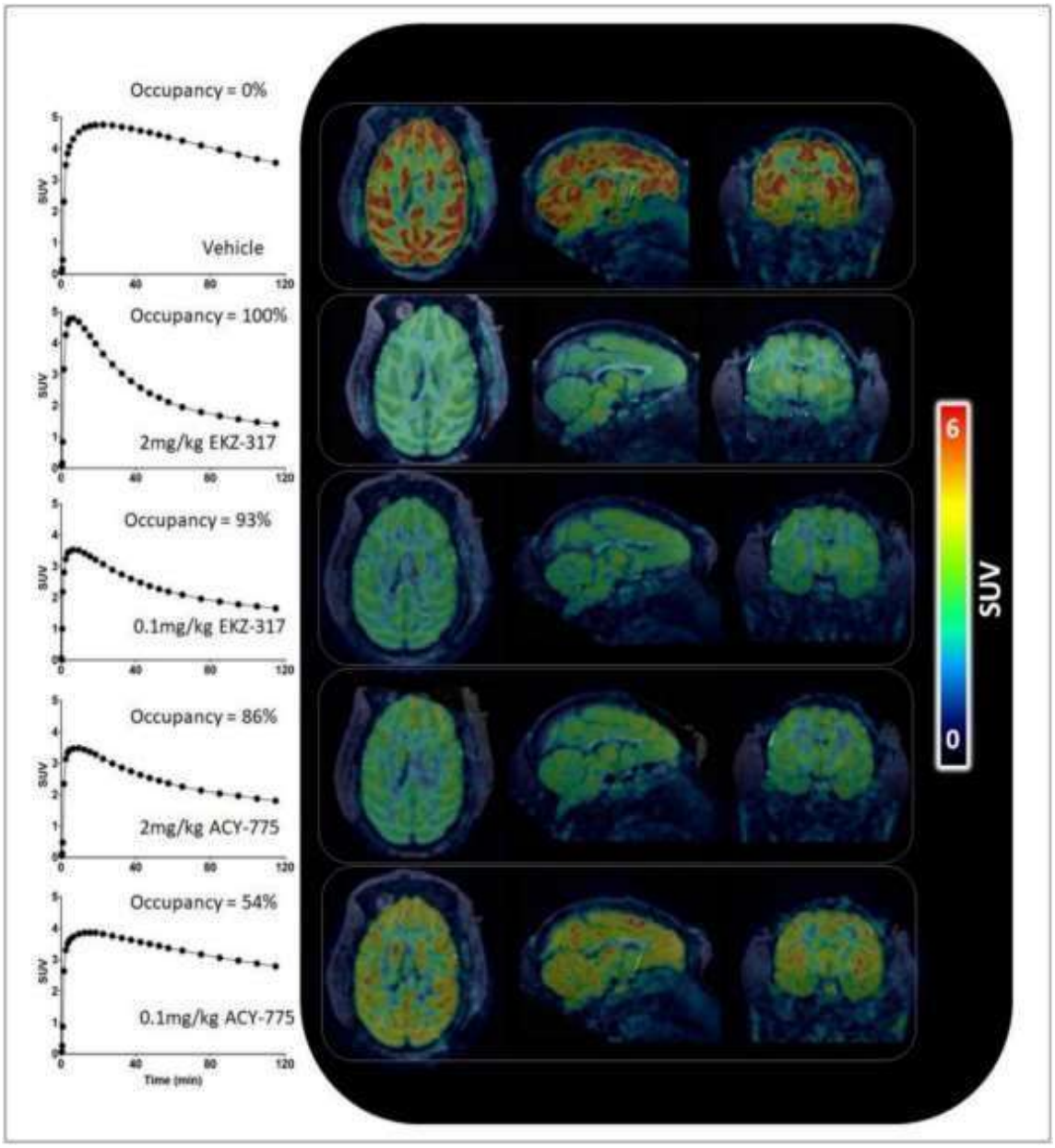

Figure 3- SUV is proportional to radioligand concentration measured via PET in region of interest. Lower SUVs correlate to wider distribution of radioligand in body, indicating extent of target occupancy of the drug. [9][2]

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Since this research was published in 2020, a first-in-human [^{18}F]EKZ-001 PET study has been conducted and has determined that [^{18}F]EKZ-001 is both safe and appropriate to quantify HDAC6 expression in the human brain [12]. This study involved the investigation of the brain binding ability of [^{18}F]EKZ-001 in healthy adult subjects. The greatest uptake of [^{18}F]EKZ-001 was observed in the hippocampus and the entorhinal cortex- two regions with significant relevance to neurodegeneration- with males showing higher HDAC6 expression across the brain in relation to females [12]. These results are displayed in Figure 4 below. This research supports the use of [^{18}F]EKZ-001 PET in quantifying HDAC6 along disease trajectory for neurodegenerative disorders, with the potential to reveal HDAC6's role in neurodegenerative disease onset and progression [12]. Furthermore, it has since been determined that the HDAC6 inhibitor ACY-738 has a greater beneficial effect on the survival of male mice with amyotrophic lateral sclerosis (ALS) compared to female mice [13], further illustrating observed sex-based differences regarding HDAC6 and its inhibitors.

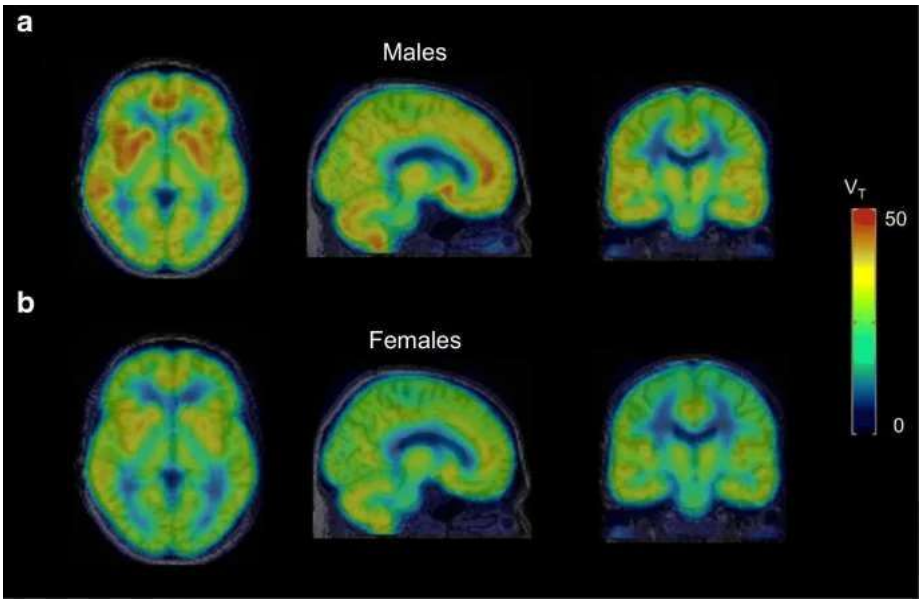

Figure 4- "Average parametric Logan graphical analysis (LGA) VT datasets for a 120-min [18F]EKZ-001 PET scan (n = 6 subjects per group, cohorts B–C, only the first scan was considered for subjects with 2 scans) for males (a) and females (b)" [12]

3. Summary and Future Considerations

HDAC6 is a promising therapeutic target for the treatment of neurodegenerative diseases. The research established by "*Translation of HDAC6 PET imaging using [^{18}F]EKZ-001 – cGMP production and measurement of HDAC6 target occupancy in NHPs"* has described a fully automated cGMP-compliant method of [^{18}F]EKZ-001 production to be translated into use for human studies. Given the observed effectiveness of [^{18}F]EKZ-001 for use in human PET studies regarding neurodegenerative diseases, future [^{18}F]EKZ-001 PET studies have the potential to reveal the underlying mechanisms and factors that contribute to the onset and progression of different neurodegenerative diseases in the brain.

The different performance results of ACY-738 in male vs. female mice with ALS [13], as well as differing HDAC6 expression observed across the brain in human males compared to females [12], suggests that sex may influence the activity of HDAC6- an important consideration for [^{18}F]EKZ-001 PET studies to make going forward to relieve the effects of neurodegenerative diseases. This is an especially relevant consideration given the differences observed between males and females in several neurodegenerative diseases. For example, Huntington's disease progresses faster in females, whereas multiple sclerosis has greater progression in males despite higher incidence and early onset in females, while Alzheimer's disease has significant gender incidence differences after age 65, with more women being diagnosed [14]. Such statistics demonstrate that the underlying mechanisms for the onset and progression of neurodegenerative diseases may also differ between males and females. Given that a major challenge to therapeutic treatment development for neurodegenerative diseases is the current lack of understanding surrounding these mechanisms, the role sex may play is an important factor that following PET studies in this field should account for. This is an

especially relevant consideration given that there still exist gaps in the analyses and reporting of data by sex for many biological disciplines [15].

Given the association of elevated HDAC6 levels in the brain with Alzheimer's disease, it is interesting to highlight the contrast between the finding that healthy adult males show higher HDAC6 expression across the brain in relation to females [12] with the fact that it is women over 65 who are more commonly diagnosed with Alzheimer's disease compared to men over 65 [14]. To better understand the relationship between HDAC6 and Alzheimer's disease in males compared to females, a proposed next step in this research involves utilizing the cGMP-compliantly produced [^{18}F]EKZ-001, given that [^{18}F]EKZ-001 PET imaging of HDAC6 is now determined as safe and appropriate for human studies. This proposed step aims to quantify HDAC6 expression in males and females with Alzheimer's disease over the age of 65 using [^{18}F]EKZ-001 PET, similar to the work of Koole *et al* [12] . By quantifying HDAC6 via [^{18}F]EKZ-001 PET within this sample, comparisons of HDAC6 expression between males and females with Alzheimer's disease can be drawn. Additional comparisons can be drawn between these study subjects and the healthy adult subjects ages 50-64 studied in the first-in-human [^{18}F]EKZ-001 PET study [12]. Doing so may not only lead to a better understanding of the potential differences involving HDAC6 between males and females with Alzheimer's disease, but it would also help paint a clearer picture of how HDAC6 may be involved in the trajectory of the disorder. The conclusions gathered from this research may be used to support future studies that aim to better understand the underlying mechanisms of Alzheimer's disease and the role played by HDAC6, contributing to efforts to develop effective therapeutic treatment for Alzheimer's disease, and potentially neurodegenerative diseases at large.

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