Final Scientific/Technical Report

Principal Investigator (PI): Dr. Johannes Czernin, M.D.

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Executive summary: PET imaging provides multiple types of biomarkers that can be used in personalized cancer care and in drug development. First, PET can be used to measure expression levels for therapeutic targets in tumor tissues and therefore provide predictive biomarkers that improve patient stratification towards the most effective therapy. Second, radiolabeled candidate anti-cancer drugs can be tracked and quantified by PET in tissues throughout the body to enable measurements of drug pharmacokinetics (PK) that are difficult to obtain using conventional approaches. Third, PET can also provide non-invasive pharmacodynamic (PD) biomarkers by enabling in vivo measurements of the magnitude and kinetics of drug-mediated inhibition of therapeutic targets in tumor lesions.

We have developed a novel deoxycytidine (dCK) dependent PET probe that allows to measure non-invasively in humans the activity of dCK (1). At the same time we have developed a small molecule inhibitor of dCK that awaits early clinical testing (2, 3). We have also radiolabeled, with carbon-11, one of our small molecule dCK inhibitors to enable pharmacokinetic measurements in vivo.

The PET probe developed by our group will be tested as a pharmacodynamics biomarker for the dCK inhibitor developed by our group. Thus, we have developed a drug/biomarker pair that will help to stratify patients to the most appropriate management.

Using these projects we have also established a streamlined pathway from probe discovery to clinical translation. For instance, we have now filed for an Investigational New Drug approval of our new dCK dependent PET probe, 18F-clofarabine.

Comparison of the actual accomplishments with the goals and objectives of the project. We have continued to made significant progress towards our goal to develop a clinically applicable PET imaging assay that can guide treatment for pancreatic adenocarcinoma (PDAC). During the second year of funding, our objective has been to develop an improved imaging probe for deoxycytidine kinase (dCK) activity. We have successfully achieved this objective by developing [18F]CFA, a clinical translatable probe for measuring dCK activity, as summarized below. A paper describing these results was recently published in the Proc Natl Acad Sci U S A (1). We are now in the process of submitting an IND application for [18F]CFA, which will enable other sites unrestricted access to this new PET imaging probe. We are also exploring additional applications for this new probe. Accelerating the clinical translation and adoption of PET biomarkers requires the development of generally applicable approaches to i) assist with the identification of relevant therapeutic and imaging targets, ii) enable efficient radiolabeling of candidate probes and drugs, iii) meet FDA regulatory standards for clinical translation, and iv) establish programs to train clinicians, biologists and radiochemists in the development and translation of new imaging biomarkers and therapies. This proposal provides a roadmap for achieving these objectives by developing, applying and translating predictive, PD and PK PET biomarkers to improve the care of cancer patients. Furthermore, we will use the proposed studies and the existing expertise, technology and courses (START, SOMI, the nuclear medicine training program) to build a framework for

training (radio) chemists and nuclear medicine physicians in i) identifying and validating relevant imaging and drug targets, ii) applying chemistry and radiochemistry approaches to synthesize PET imaging biomarkers, and iii) translating the newly developed PET assays to the clinic.
After identifying a new clinically relevant imaging and therapeutic target (e.g., dCK), we have successfully developed and optimized therapeutic approaches (e.g., dCK inhibitors) and have developed and applied new PK and PD PET biomarkers to improve patient stratification and facilitate drug development. As proposed the research under Aims 1-3 served as a training ground for a new generation of translational molecular imaging experts. Upon completion of the program they understood the concept of predictive PET biomarkers in cancer leading to improved patient stratification, ii) and the role of PET based PK and PD biomarkers in optimizing new drugs such as dCK inhibitors. Finally, they learned the regulatory challenges (IND; FDA) and pathways sometimes blocking the successful translation of PET probes to the clinic. We have successfully recruited a radiochemist, a radiologist and a nuclear medicine trainee too be trained in this highly relevant translational diagnostic and therapeutic approach and have thus established, as proposed, the UCLA Translational Biomarker Development Program for integrated Nuclear Medicine Research and Training.

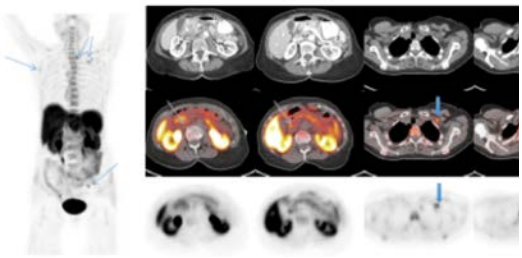
Summary of Project activities for the funding period

Scientific Progress:

A novel PET probe of dCK activity: Our first PET probe described for dCK imaging was 1-(2'-deoxy-2'-[^{18}F]fluoro-β-D-arabinofuranosyl)cytosine ([^{18}F]FAC) (2). While [^{18}F]FAC enables PET imaging of dCK activity in mice , a subsequent study from our group questioned its clinical utility because of rapid catabolism mediated by cytidine deaminase (CDA), an enzyme present at higher levels in humans than in rodents . CDA converts [^{18}F]FAC to [^{18}F]FAU (1-(2'-deoxy-2'-[^{18}F]fluoro-β-D-arabinofuranosyl)uracil), a metabolite that is not phosphorylated by dCK. To overcome this problem, we developed two L-enantiomer analogs of [^{18}F]FAC that resist deamination and retain affinity for dCK were developed . Two of the L-enantiomer analogs, L-[^{18}F]FAC (1-(2'-deoxy-2'-[^{18}F]fluoro-β-L-arabinofuranosyl)cytosine) and L-[^{18}F]FMAC (2′-deoxy-2′-[^{18}F]fluoro-5-methyl-β-L-arabinofuranosylcytosine) were translated to the clinic. These second generation dCK probes had better sensitivity than [^{18}F]FAC in humans as reflected by increased accumulation in the bone marrow, a tissue with high dCK activity. However, both L-FAC analogs are cross-reactive with mitochondrial thymidine kinase 2 (TK2), which, like dCK, lacks enantioselectivity and thereby phosphorylates deoxypyrimidines with both D and L-enantiomeric configurations. In humans, cross-reactivity with TK2 was likely responsible for the observed uptake of these probes into the myocardium, a tissue with high TK2 expression.

Because dCK phosphorylates both pyrimidines and purines, the limitations of current PET probes for dCK could, in theory, be circumvented using fluorinated purine analogs. Two candidate purine PET probes for dCK have been proposed: [^{18}F]CFA ([^{18}F]Clofarabine, 2-chloro-2'-deoxy-2'-[^{18}F]fluoro-9-β-D-arabinofuranosyl-adenine) and [^{18}F]F-AraG (2′-deoxy-2′-[^{18}F]fluoro-9-β-D-arabinofuranosyl-guanine). While neither of these purine analogues are phosphorylated by TK2, both probes may be substrates for mitochondrial deoxyguanosine kinase (dGK). Despite some overlap in their substrate specificities, dCK and dGK have distinct biological functions and different expression patterns. Therefore, to properly interpret the information provided by [^{18}F]CFA and [^{18}F]F-AraG PET scans it is important to delineate the roles played by dCK and dGK in promoting the accumulation of these candidate probes. Towards this goal we compared the selectivity of [^{18}F]CFA and [^{18}F]F-AraG for dCK and dGK. Significant differences in kinase selectivity indicated that PET assays using these probes would likely have distinct applications, with [^{18}F]CFA being the primary choice for imaging dCK activity and with [^{18}F]F-AraG being the primary choice for imaging dGK activity. We also investigated how competition between [^{18}F]CFA and endogenous deoxycytidine affects the sensitivity of [^{18}F]CFA PET imaging and we examined the implications of this phenomenon in the context of the substantial differences in plasma levels of this nucleoside observed in mice compared to humans. As a consequence of these differences, we examined how cytidine deaminase impacts plasma deoxycytidine levels and tumor [^{18}F]CFA accumulation. We have also initiated first-in-human studies of [^{18}F]CFA (Fig. 1). Fifteen patients were scanned with [^{18}F]CFA PET in the second year of funding.

Fig. 1: [^{18}F]CFA scan of a 69 year old female with pancreatic adenocarcinoma.Blue arrows Indicate lymph nodes; Pancreas lesion without clear uptake (green arrow).



Achievements:

1) In summary, during the funding period we showed that [^{18}F]CFA is a highly specific substrate/probe for dCK with minimal cross-reactivity with dGK, a related mitochondrial nucleoside kinase. [^{18}F]CFA accumulation in cancer cells correlates with dCK expression and is competitively inhibited in a concentration-dependent manner by dC, the physiological substrate of dCK. We provided evidence that plasma dC levels vary significantly across species and that these variations correspond to striking differences in the [^{18}F]CFA biodistribution between mice and humans. Accordingly, the [^{18}F]CFA PET assay is more sensitive in humans than in mice, presumably due to greatly reduced competition between the probe and

endogenous dC in primates relative to rodents. We have continued to enroll pancreatic cancer patients in our clinical study (Fig. 1). We are also expanding the use of the probe to other malignancies in which dCK-dependent prodrugs are being used.

2) An application for an Investigational New Drug is being filed with the FDA.

3) A training program, UCLA Translational Biomarker Development Program (UTBD) has been developed. We have recruited a postdoctoral radiochemist, a nuclear medicine and a radiology trainee who were fully integrated in the entire developmental; process from target identification to IND application. These program established the foundation for a successful translational imaging biomarker at the David Geffen School of Medicine at UCLA.

Papers published during the funding period:

1) Structure-guided development of deoxycytidine kinase inhibitors with nanomolar affinity and improved metabolic stability. Nomme J1, Li Z, Gipson RM, Wang J, Armijo AL, Le T, Poddar S, Smith T, Santarsiero BD, Nguyen HA, Czernin J, Alexandrova AN, Jung ME, Radu CG, Lavie A. J Med Chem. 2014;57(22):9480-94. PMID: 25341194 PMCID: PMC4255734
2) [18F]CFA as a clinically translatable probe for PET imaging of deoxycytidine kinase activity. Kim W, Le TM, Wei L, Poddar S, Bazzy J, Wang X, Uong NT, Abt ER, Capri JR, Austin WR, Van Valkenburgh J, Steele D, Gipson RM, Slavik R, Cabebe AE, Taechariyakul T, Yaghoubi SS, Lee JT, Sadeghi S, Lavie A, Faull KF, Witte ON, Donahue TR, Phelps ME, Herschman HR, Herrmann K, Czernin , Radu CG. Proc Natl Acad Sci U S A. 2016;113:4027- PMID: 27035974 PMCID: PMC4839461
3) Human biodistribution and radiation dosimetry of 18F-CFA, a PET probe targeting the deoxyribonucleoside salvage pathway. Barrio M, Spick C, Radu CG, Lassmann M, Eberlein U, Allen-Auerbach MS, Schiepers C, Slavik R, Czernin J, Herrmann K. J Nucl Med 2016