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Institute for Molecular Medicine Research Program

Dr. Michael Van Dam (UCLA)

Project 1: Chemistry platforms for PET Molecular Imaging Probes

Executive summary:

The lack of availability of desired positron emission tomography (PET) probes is a formidable roadblock to more widespread use of PET in research and clinical settings. The goal of Project 1 was to develop new low-cost, flexible, technology platforms for synthesizing PET probes to alleviate this bottleneck.

Several new approaches were successfully demonstrated that address current limitations in the development and production of PET probes. One technology leverages microscale liquid handling to enable reactions to be performed at extremely small volume scales. This new platform facilitates the development and optimization of novel PET probes that use very expensive precursors (such as engineered antibodies). It does so by increasing the reaction yield, and by increasing number of experiments that can be done with a single batch of precursor. A second technology was developed that uses robotics to enable chemical reactions to be performed under significantly higher pressures than is possible with existing synthesizers. This platform facilitates the production of certain classes of PET probes (e.g. nucleoside analogs) that require high pressure conditions. It also serves as a valuable probe development and optimization tool by providing a very wide range of conditions that can be explored as well as a simple pathway to transition into routine production once the probe shows promise. Due to the unique hardware and software design, the system can readily produce a wide variety of different PET probes without modification, thus enabling a single system to perform the same function as a collection of probe-specific synthesizers.

These technologies are both inexpensive and flexible and could facilitate new PET probe development as well as serve as an affordable standardized platform for routine production of these new tracers at multiple sites. The robotic technology is being commercialized by Sofie Biosciences, Inc. to make it more widely available. By increasing access to novel PET probes, these technologies could accelerate research about the mechanisms and treatment of human diseases such as cancer, provide new clinical diagnostic imaging tools to better match the right patient to the right therapy, and accelerate and streamline the drug development process.

Accomplishments and goals:

The original aims of Project 1 were as follows:

- Aim 1: Develop system based on modular microfluidic chips based on unit chemistry processes, and develop tools to visualizing chip operation in situ.
- Aim 2: Develop flexible, modular radiosynthesizer based on unit operations.
- Aim 3: Design and build modules including a high-pressure reactor for each unit function in the modular radiosynthesizer.
- Aim 4: Develop module for drying and concentration of F-18.

During the project period, these aims were successfully accomplished, with only minor changes in anticipated approaches. The differences are described in the following section.

Summary of research activities:

Microfluidic chips, materials, and systems for radiochemistry (Aim 1):

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Microfluidic technology promises several advantages for the production of PET probes, but one of the most significant is simply the small size of microfluidic chips. Instead of requiring installation in a conventional hot cell to provide radiation protection, a microfluidic chip could be minimally shielded and could potentially be operated on a benchtop, greatly decreasing the barriers to ownership and operation of a PET probe synthesizer in the lab. Several groups had previously demonstrated the synthesis of PET probes in various types of microfluidic chips but these approaches suffered from limitations such as chemical incompatibility (i.e. lack of versatility), and the requirement for accompanying bulky apparatus to perform certain steps that could not be performed on-chip. We aimed to develop microfluidic chip modules that could be pieced together, each designed to efficiently perform a certain chemistry operation such as mixing, reaction, or solvent evaporation.

We developed a microfluidic mixer module based on poly-dimethylsiloxane (PDMS) that measured out desired amounts of each reagent, merged them into a single liquid slug with the desired ratio of reagents, and propelled this slug through a channel to cause efficient mixing. The volume per slug is 10-100 nL but the total mixed volume can be scaled up simply by producing a larger number of slugs. A manuscript on the operation and performance of this device was published in *Microfluidics and Nanofluidics*. Because each slug can serve as an independent reaction with programmable composition, this microfluidic device can be used for performing individual reactions in small volumes, and for performing a large number of small-scale reactions under different conditions for reaction optimization. Based on the design, we developed a microfluidic chip for radiolabeling biomolecules (e.g. engineered antibodies) with N-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB). Antibody PET probes are interesting because of their very high specificity and the potential to track the behavior of antibody therapeutic agents. The efficiency of ^{18}F-labeling is strongly dependent on concentrations and pH and differs for different biomolecules. We demonstrated a reaction optimization experiment in which dozens of different mixtures of reagents could be combined, reacted, and analyzed with simple procedures such as iTLC to determine the optimal labeling conditions. Only a tiny fraction of the sample is consumed during optimization; afterwards, the remainder of the batch could be labeled at the optimal conditions. In a manuscript published in *Molecular Imaging*, we demonstrated an improvement in labeling yield from about 1% (typical value reported in the literature) to >25%.

Another microfluidic chip module we developed was a batch reaction chip for harsh organic chemical reactions needed in the synthesis of the vast majority of PET probes (small molecules). The reactor was implemented as a capillary tube made of either Teflon or glass for optimal inertness and thus more generality than many other microfluidic reaction platforms. Mixed reagents are pumped into the capillary between small slugs of a solvent such as dimethylsulfoxide (DMSO). DMSO has a high freezing point and can easily be frozen into a solid by simple thermoelectric coolers. This very simple microfluidic “valve” has the advantage of being able to withstand tremendous pressures compared to nearly all other microvalves, despite the complex fabrication and cost required by these other approaches. Once the DMSO plugs have been frozen, the intervening length of capillary can be heated to react the mixture inside. The valves were found to remain frozen despite this heating, and to prevent the loss of any vapor or liquid during the reaction, even at temperatures where the solvent vapor pressure was ~120 psig. This work has been presented at the *World Molecular Imaging Congress* and a manuscript is in preparation.

An additional microfluidic chip module was developed to evaporate solvent for the purposes of solvent exchange, as needed between reaction steps in the synthesis of a multitude of PET probes. We designed and fabricated a PDMS microfluidic chip with serpentine channel to store the initial mixture and trap it by closing microvalves at either end of the channel. PDMS is highly gas and vapor permeable, and the solvent can be evaporated by raising the temperature. The chip contained a second serpentine channel in close proximity to the sample channel connected to a vacuum pump to accelerate the removal of vapor. After evaporation, a dry residue of the solute remained in the channel, which could be redissolved in the second, desired solvent. Several iterations of this chip were developed, the optimal design ensuring a uniform distribution of dried residue to avoid clogging of the chip and hence increasing its reliability. This chip was used both for the initial

step of drying the [^{18}F]fluoride that is needed for nearly every fluorine-18-labeled probe as well as more general solvent exchange. This work has been presented at several conferences and a manuscript is in preparation.

While the high gas permeability of PDMS is ideal for the solvent evaporation module, the low chemical compatibility of PDMS limits its flexibility. What is needed is a material with similar mechanical properties to PDMS, but with enhanced chemical resistance. Previous work resulted in the development of a perfluoropolyether (PFPE) elastomer with the desired mechanical properties and excellent chemical resistance, but it was found to be susceptible to attack by basic compounds. Frequent failure of the chip was observed during the process of drying [^{18}F]fluoride. In a collaboration with Prof. Zhibin Guan at the University of California, Irvine, we developed a novel crosslinking mechanism (based on click chemistry) that resulted in an elastomer with similar mechanical properties but without the susceptibility to attack. The synthesis of this material, characterization of its inertness, and initial studies of bonding to create microfluidic devices was published in the *Journal of Materials Chemistry*.

In addition to these chip modules and materials, we developed in collaboration with Prof. Arion Chatziioannou at UCLA a new method for quantitatively imaging microfluidic chips. The conventional analytical tools available to the radiochemist do not provide a convenient means to observe or quantify the processes occurring inside microfluidic chips. For example, one technique is to cut the chip into several small pieces, then measure the radioactivity remaining in each portion using a gamma counter, dose calibrator or similar instrument. This destructive process has low spatial resolution and does not permit multiple timepoints to be compared. Other analytical techniques available to the radiochemist are chemical separation and analysis techniques such as high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) which require a liquid sample to be extracted out of the chip and do not permit in situ observation of processes. The Cerenkov imaging method developed enables near real-time quantification of radioactivity within an operating microfluidic chip. As fluorine-18 decays, some of the emitted positrons travel faster than the speed of light in the surrounding medium (either liquid or PDMS) and thus emit an “optical boom” known as Cerenkov radiation. This visible (blue) light can be detected by a sensitive CCD camera to produce an image of the radioactivity within the chip. This work was published in *Physics of Medicine and Biology*.

Flexible, modular, macroscale radiosynthesizer (Aims 2 and 3):

In conventional radiosynthesizers, the reaction vial and the tubing connecting it to other components is fixed. To perform a reaction at high temperatures, the attached tubes must be closed by valves to prevent the loss of solvent vapor or product. Unfortunately, the operating pressure limits of these valves are rather low, thus limiting the temperatures at which reactions can be performed in volatile solvents.

The originally proposed robotic mechanism removes the tubing from the vial after reagent addition and replaces it with a “stopper” prior to heating, enabling significantly higher pressures and temperatures to be used during reaction steps. In many cases, these high temperatures are necessary in order to achieve high yields. Based on this proposed mechanism, we constructed and tested a first-generation modular synthesizer – the Automated Radiochemistry Platform (ARC-P) – and then made several improvements based on initial test results to increase safety and ease of use. This system is designed to perform known radiochemical syntheses that involve multiple reaction steps at high temperatures (up to 200C) and pressures (up to 150 psig), and to facilitate development and optimization of probes over a much wider range of conditions than is possible on any commercial system. Existing commercial systems generally cannot be operated above about 30-50 psig. Mechanical motion to install and remove the “stopper” is achieved via low-cost stepper motors and pneumatic actuators. With a modular design, the overall system can be configured with different numbers of reactor units, reagent-delivery units, and cartridge-purification units depending on the needs of the radiosynthesis. This flexibility combined with the capability of high-pressure conditions enables the synthesis of the vast majority of tracers on a single system.

We validated the first-generation system by synthesizing 1-(2'-deoxy-2'-[18F]fluoroarabinofuranosyl)cytosine ([18F]FAC), a recently described probe of dCK enzyme activity with many exciting applications in immunology and oncology. We developed a robust synthesis protocol for the 4-step, 3-pot synthesis and were able to consistently produce mCi-scale batches, with RCY of 20-30% (decay-corrected) that are sufficient for multiple preclinical studies. The system was used extensively to supply several research groups at UCLA and outside with [18F]FAC analogs for further development of this family of tracers. Two manuscripts describing this system and the syntheses are currently under review.

While successfully demonstrating the advantages of the robotic approach, the first-generation system was not optimized for robustness, ease of setup, or ease of use. To make this a more accessible and useful tool, we thus developed and validated a second-generation prototype, incorporating many critical improvements in robustness, usability, and automation. The prototype has three integrated reaction vessels, an HPLC injection system, and a reagent handling robot. The vessel heater uses a novel three-segment spring-loaded "chuck" to ensure reliable thermal with the glass reaction vessel despite the large tolerances in actual vessel diameter, and also incorporates a flow path for a liquid cooling system. The latter greatly accelerates cooling after reactions and evaporations, and the overall synthesis time of [^{18}F]FAC was reduced from ~4 to ~3 hours as a result. (Starting from the same amount of [^{18}F]fluoride, this time savings results in 46% more product at the end of synthesis.) The reaction vessels each seal upward against a gasket affixed to the bottom of a disposable cassette containing all of the wetted fluid paths (needles, tubing, stopcock valves). The use of disposable cassettes simplifies setup, eliminates the need for cleaning, and greatly increases reliability of the system. The reagent handling robot above the cassettes supplies vacuum and inert gas to ports in the cassette to drive reagents through the system, and has a vial gripper to move reagent vials from storage locations (where they are kept in crimp cap vials) to delivery positions (with needles to pierce the septum). By keeping sensitive reagents sealed until they are actually needed in the synthesis, the system enables most of the synthesis to proceed without manual interventions such as the addition of freshly prepared reagents.

To validate the second-geneartion system, we carried out repeated synthesis of the nucleoside analogs D-[^{18}F]FAC and L-[^{18}F]FMAU, both requiring all three reaction vessels and high-pressure conditions during the fluorination and base coupling steps. Decay-corrected radiochemical yields were 31±5% (n=6) and 46±1% (n=6), respectively. Other tracers, including 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), 3-deoxy-3-[^{18}F]fluoro-L-thymidine ([^{18}F]FLT), and the biomolecule labeling agent N-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) have also been synthesized with yields comparable to other reports. Switching from one tracer to another required only the use of a different set of reagent vials and a different software program; it did not require any modifications to the hardware or plumbing that is needed on many existing systems.

Because the synthesizer provides tremendous flexibility (up to three reaction vessels, capability for high temperatures and pressures), it has great potential as a tracer development platform in addition to routine production. To increase its utility for this application, we integrated a camera into each reactor (for monitoring reactions, assessing evaporation progress during reaction development, and assessing completion of fluid transfers during reaction optimization) as well as radiation sensors. In addition, we developed a novel software package that enables creation of synthesis programs via "unit operations" via a drag-and-drop interface that runs on PCs, Macs, tablets, and smartphones. The interface greatly reduces the learning curve of programming and the complexity of programs (e.g. 17 unit operations for [^{18}F]FDG compared to 227 low-level steps on a "conventional" automated synthesizer).

The second-geneartion synthesizer is beginning to be used routinely in the Crump Institute for Molecular Imaging to produce tracers for research use and *in vivo* imaging. The system and software have been presented at a number of international conferences in molecular imaging and radiochemistry, and manuscripts on the hardware architecture and software design have just been submitted. An additional manuscript describing our experience with the synthesis of a several different compounds is currently in preparation. This novel synthesizer technology can not only increase access to nucleoside analog PET tracers (by providing a platform

for their fully-automated production), but can also serve as a valuable new tool to the radiochemistry community for development and production of new tracers. To make this technology more widely available, this work is currently being commercialized by Sofie Biosciences, Inc. This project remains under active development and we have recently loaned the system to collaborators to assist with challenging syntheses and to gain feedback from outside users. The feedback obtained so far has played a critical factor in planning the commercial prototype, and will be incorporated in the form of numerous improvements to both the hardware and software.

[18F]fluoride drying module (Aim 4):

[^{18}F]fluoride is produced by proton bombardment of [^{18}O]water in a cyclotron. However, fluoride is extremely unreactive in the presence of water and must be dried prior to reaction with most precursors used in radiochemistry. The conventional approach is to evaporate the water, in the presence of acetonitrile (to enhance removal by formation of a low-boiling azeotrope) and a phase-transfer catalyst, a process that can take 15-30 min. We explored trapping of [^{18}F]fluoride in an electrochemical cell as a means to separate it from [^{18}O]water. Previous work in this direction used glassy carbon as the trapping electrode material, but observed significant erosion of the electrode. This erosion generates particulates that can clog and disrupt downstream processes and adversely affect system reliability. We observed the severity of the erosion to increase with voltage. We thus explored as an alternative an all-metal electrochemical cell. The trapping efficiency (percentage of radioactivity trapped as it is flowed through the cell) and release efficiency (percentage of trapped radioactivity that is subsequently released) both depend on a number of parameters including voltage, geometry, electrode materials, temperature, etc. Optimizing these conditions, the final cell consists of a brass trapping electrode, platinum counterelectrode, and a serpentine microchannel made from an inexpensive “tape” between them. Applying 20V potential during trapping and -2V during release, 95±4% (n=7) trapping and 88±3% (n=7) release were obtained. The use of 20V is significantly higher than other reports (likely limited by the severe erosion of the electrode) and results in extremely fast trapping. The entire process took less than 5 min and resulted in [^{18}F]fluoride that was sufficiently anhydrous that it could be directly reacted to form [^{18}F]FDG. A manuscript on this work is current in the review process.

This technology could increase the speed of PET probe synthesis, and simplify systems for the production of PET probes. Electrochemical trapping and release is especially suitable for integration with microfluidic platforms for PET probe synthesis.

Papers & other products delivered:

Microfluidic chips, materials, and systems for radiochemistry (Aim 1):

Patent Application:

1. van Dam, Michael, Liu, Kan, Shen, Clifton Kwang-Fu, Tseng, Hsian-Rong. Accurate and rapid micromixer for integrated microfluidic devices. WO/2009/092106 (PCT/US2009/031582), filed Jan 21, 2009.

Papers:

1. Jennifer S. Cho, Sebastian Olma, Kan Liu, Yi-Chun Chen, Clifton K-F Shen, R. Michael van Dam, Arion F. Chatziioannou. Cerenkov radiation imaging as a method for quantitative measurements of beta particles in a microfluidic chip, Physics in Medicine and Biology, 54(22): 6757-6771 (2009) [PMCID: PMC2794558]
   [Highlighted as Editor’s Choice in Physics in Medicine and Biology, Medical Physics Web, Nov 6, 2009]

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2. Liu, K., Chen, Y-C, Tseng, H-R, Shen, C.K-F, van Dam, R.M. Microfluidic device for robust generation of two-component liquid-in-air slugs with individually-controlled composition. Microfluidics and Nanofluidics, 2010, DOI 10.1007/s10404-010-0617-0. [PMCID: PMC2944379]
3. Liu, K., Lepin, E.J., Wang, M-W, Guo, F., Lin, W-Y, Chen, Y-C, Sirk, S.J., Olma, S., Phelps, M.E., Zhao, X-Z, Tseng, H-R, van Dam, R.M.*, Wu, A.M.*, Shen, C.K-F*. Microfluidic-based 18F-Labeling of Biomolecules for Immuno-Positron Emission Tomography. Molecular Imaging 10(3): 168-176, 2011. [PMCID: PMC3163899]
4. Ying-Wei Yang, Jens Hentschel, Yi-Chun Chen, Mark Lazari, Hanxiang Zeng, R. Michael van Dam, Zhibin Guan. "Clicked" fluoropolymer elastomers as robust materials for potential microfluidic device applications. Journal of Materials Chemistry 22: 1100-1106, 2012.
[Highlighted as "Hot Article" in RSC Publishing Journal of Materials Chemistry Blog, Jan 17, 2012]

Conference Proceedings:

1. Wei-yu Tseng, Jennifer Cho, Xiaoxiao Ma, Andrew Kunihiro, Arion Chatziioannou and R. Michael van Dam. Toward reliable synthesis of radiotracers for positron emission tomography in PDMS microfluidic chips: Study and optimization of the [^{18}F]fluoride drying process. Proceedings of NanoTech Vol 2: 472-475, Anaheim, CA, June 21-24, 2010.
[Oral Presentation]

Conference Presentations:

1. Mark A. Eddings, Jennifer Sung-Eun Cho, Yuliang Deng, Arion Chatziioannou, and R. Michael van Dam. Integrated radiation detection with microfluidics for the radiolabeling of molecular probes for positron emission tomography (PET). Poster. Gordon Research Conference on Physics & Chemistry of Microfluidics, Lucca (Barga), Italy, June 28 – July 3, 2009.
2. S. Olma, S.J. Sirk, E. Lepin, T. Olafsen, A.M. Wu, R.M. van Dam, and C.K. Shen. Convenient N-succinimidyl-4-[F-18]fluorobenzoate ([F-18]SFB) synthesis and labeling of biomolecules. Poster P066. 18th International Symposium on Radiopharmaceutical Sciences, Edmonton, Canada, July 12-17, 2009.
3. S. Olma, K. Liu, Y. Chen, R.M. van Dam, and C.K. Shen. Microfluidic droplet mixer for fluorine-18 labeling of biomolecules. 18th International Symposium on Radiopharmaceutical Sciences, Edmonton, Canada, July 12-17, 2009.
[Oral Presentation]
4. Jennifer S. Cho, Sebastian Olma, Kan Liu, Yi-Chun Chen, Clifton K-F Shen, R. Michael van Dam and Arion F. Chatziioannou. Quantitative measurements of radioactivity in a microfluidic chip during the radiochemical synthesis of PET imaging probes. Presentation. World Molecular Imaging Congress, Montreal, Canada, Sept. 23-26, 2009.
5. Eric J. Lepin, Kan Liu, Ming Wei Wang, Shannon Sirk, R. M. van Dam, Clifton K-F Shen, Anna M. Wu. Engineered antibody fragments for immunoPET: Optimization of F-18 labeled diabodies. 240th ACS National Meeting, Boston, MA, Aug 22-26, 2010.
[Oral Presentation]
6. Xiaoxiao Ma, Wei-Yu Tseng, Mark A. Eddings, R. Michael van Dam. Microreactor with phase-change microvalves for high-pressure batch synthesis of radiotracers. World Molecular Imaging Congress, Dublin, Ireland, Sept. 5-8, 2012.

Other Products:

1. Cerenkov imaging system for microfluidics chips that allows quantitative monitoring of spatial distribution of radioactivity in transparent microfluidic chips.

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Flexible, modular, macroscale radiosynthesizer (Aims 2 and 3):

Patent Application:

1. N. Satyamurthy, J.R. Barrio, B. Amarasekera, R.M. van Dam, S. Olma, D. Williams, M. Eddings, C.K-F Shen. Modular Radiochemistry Synthesis System. WO/2010/021719 (PCT/US2009/004745) filed Aug 19, 2009

Invention Reports:

1. R. Michael van Dam, Melissa Moore, Kevin Quinn, Shane Claggett, Henry Herman, Mark Lazari. Radiosynthesizer for PET Probe Development and Production. UCLA Case No. 2013-366
2. R. Michael van Dam, Shane Claggett, Kevin Quinn, Mark Lazari, Melissa Moore. Software for PET Probe Radiosynthesizer. UCLA Case No. 2013-365

Papers:

1. Bernard Amaraesekera, Phillip D. Marchis, Krzysztof P. Bobinski, Caius G. Radu, Johannes Czernin, Jorge R. Barrio, R. Michael van Dam. High-pressure, compact, modular radiosynthesizer for production of positron emitting biomarkers. Under Review.
2. Henry Herman, Graciela Flores, Kevin Quinn, Mark Eddings, Sebastian Olma, Melissa Esterby, Huijiang Ding, Krzysztof P. Bobinski, Mingwei Wang, Dirk Williams, Darin Williams, Clifton Kwang-Fu Shen, Michael E. Phelps, R. Michael van Dam. Plug-and-play modules for flexible radiosynthesis. Under Review.
3. Shane B. Claggett, Kevin Quinn, Mark Lazari, Melissa Moore, R. Michael van Dam. Simplified programming and control of automated radiosynthesizers through unit operations. Under Review.
4. Two additional manuscripts in preparation

Book chapter:

1. Pei Yuin Keng, Melissa Esterby, R. Michael van Dam. Emerging Technologies for Decentralized Production of PET Tracers. In Positron Emission Tomography – Current Clinical and Research Aspects. Ed. Hsieh C-H. pg 153-182. ISBN 978-953-307-824-3, InTech, 2012.

Conference Presentations:

1. M.A. Eddings, S. Olma, M. Wang, Y. Deng, H. Ding, N. Satyamurthy, K. Shen, R.M. van Dam. Automated radiochemistry platform (ARC-P): plug-and-play radiochemistry modules for reconfigurable radiosynthesis. 18th International Symposium on Radiopharmaceutical Sciences, Edmonton, Canada, July 12-17, 2009.
2. M.A. Eddings, S. Olma, M.W. Wang, Y. Deng, H. Ding, N. Satyamurthy, C.K-F Shen, R.M. van Dam. Reconfigurable radiochemistry with plug-and-play synthesis modules. World Molecular Imaging Congress, Montreal, Canada, Sept. 23-26, 2009.
3. H. Herman, G. Flores, K. Quinn, M. Esterby, G.J. Sah, M.E. Phelps, N. Satyamurthy, R.M. van Dam. Multi-pot radiosynthesizer capable of high-pressure reactions for production of [18F]FAC and analogs. Society of Nuclear Medicine Annual Conference, San Antonio, TX, June 4 -8, 2011.
4. H. Herman, K. Quinn, M. Esterby, G.J. Shah, G. Flores, M.E. Phelps, N. Satyamurthy, R.M. van Dam. Flexible radiosynthesizer capable of multi-pot high temperature and pressure reactions. 19th International Symposium on Radiopharmaceutical Sciences, Amsterdam, The Netherlands, Aug 28 – Sept 2, 2011.
   [Oral Presentation]
5. Kevin Quinn, Melissa Esterby, Gaurav J. Shah, Graciela Flores, Shane Claggett, Mark Lazari, Henry Herman, Michael E. Phelps, Nagichettiar Satyamurthy, R. Michael van Dam. Synthesis of [^{18}F]FAC on

a new, cassette-based radiosynthesizer capable of multi-pot high temperature and pressure reactions. World Molecular Imaging Congress, San Diego, CA, USA, Sept 7-10, 2011.

6. Melissa D. Moore, Kevin Quinn, Shane Claggett, Mark Lazari, Gaurav J. Shah, Nagichettiar Satyamurthy, Michael E. Phelps, R. Michael van Dam. ARC-P^{HS+}: A versatile radiosynthesizer for the production of PET tracers. American Association for Cancer Research (AACR) Annual Meeting, Chicago, IL, USA, Mar 31–Apr 4, 2012.
7. Mark Lazari, Jeffrey Collins, Kevin Quinn, Shane Claggett, Pei Yuin Keng, Michael E. Phelps, Nagichettiar Satyamurthy, Melissa Esterby, R. Michael van Dam. Diverse nucleophilic syntheses on the ELIXYS multi-pot, cassette-based radiosynthesizer. Society of Nuclear Medicine Annual Meeting, Miami Beach, FL, June 9-13, 2012.
8. Shane Claggett, Kevin Quinn, Mark Lazari, Jeff Esterby, Melissa Esterby, Michael van Dam. A new paradigm for programming and controlling automated radiosynthesizers. Society of Nuclear Medicine Annual Meeting, Miami Beach, FL, June 9-13, 2012.
9. Mark S. Lazari, Shane Claggett, Kevin Quinn, Melissa Esterby, R. Michael van Dam. Sequential synthesis of three different ^{18}F-labeled PET tracers on the ELIXYS radiosynthesizer. World Molecular Imaging Congress, Dublin, Ireland, Sept. 5-8, 2012.
10. Kevin Quinn, Shane Claggett, Mark S. Lazari, Jeff Esterby, Melissa Esterby, R. Michael van Dam. Radiosynthesizer software applied to mobile devices for the rapid communication of PET probe synthesis outcomes. World Molecular Imaging Congress, Dublin, Ireland, Sept. 5-8, 2012.

Other Products:

1. Two sets of a first-generation modular radiosynthesizer for semi-automated synthesis of the [^{18}F]FAC family of tracers (or tracer development/optimization), comprising three reaction vessels compatible with high-pressure (150psi), high-temperature (200C) reactions.
2. Second-generation radiosynthesizer for fully automated synthesis of the [^{18}F]FAC family of tracers. The system is not limited to just these tracers, but also has tremendous flexibility for facilitating the development and optimization of novel tracers. The disposable cassette approach facilitates the transition from development to routine production to instantly increase availability of promising tracers.

[18F]fluoride drying module (Aim 4):

Papers:

1. Saman Sadeghi, Vincent Liang, Shilin Cheung, Suh Woo, Curtis Wu, Jimmy Ly, Yuliang Deng, Mark Eddings, R. Michael van Dam. Reusable electrochemical cell for rapid separation of [^{18}F]fluoride from [^{18}O]water for flow-through synthesis of ^{18}F-labeled tracers. Under review.

Conference Proceedings:

1. Saman Sadeghi, Jimmy Ly, Yuliang Deng, R. Michael van Dam. A Robust Platinum-Based Electrochemical Micro Flow Cell for Drying of [^{18}F]Fluoride for PET Tracer Synthesis. Proceedings of the 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), pg. 318-320, Groningen, The Netherlands, Oct 3-7, 2010.

Conference Presentations:

1. Saman Sadeghi, Jimmy Ly, Yuliang Deng, Nagichettiar Satyamurthy, R. Michael van Dam. Electrochemical micro flow cell for rapid PET tracer synthesis. Society of Nuclear Medicine Annual Conference, San Antonio, TX, June 4 -8, 2011.
   [Oral Presentation]
2. Saman Sadeghi, Jimmy Ly, Nagichettiar Satyamurthy, R. Michael van Dam. High-yield electrochemical separation of [18F]fluoride from [^{18}O]water in a transition metal flow cell. Poster. 19th

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International Symposium on Radiopharmaceutical Sciences, Amsterdam, The Netherlands, Aug 28 – Sept 2, 2011.

Other Products:

1. A rapid, automated, electrochemical [^{18}F]fluoride trap and release system and an automated capillary-based radiosynthesizer for the preparation of ^{18}F-labeled PET probes.

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Dr. Arion Chatziioannou PhD, Chris Behrenbruch, PhD, David Stout, PhD (UCLA)

Project 2: A High Throughput Screening microPET Scanner

Executive summary:

Preclinical research serves as a bridge that helps transfer advances in our understanding of the biology of disease from the bench to the bedside. Radionuclide imaging is an exquisitely sensitive technology and has been used extensively in the development, validation and implementation of these advances. Preclinical imaging with microPET, originally developed at UCLA, is now a widely used technology in academia, pharmaceutical industry and biological research institutes. The microPET imaging technology has seen very significant changes from the early development of the first systems that demonstrated the feasibility of the approach, to high performance systems that can image the entire body of a mouse with high frame rates (one second or lower). Today, complicated multimodality preclinical PET systems that include fully tomographic microCT and microMRI are part of the industry norm. At the same time, the cost of these systems has increased dramatically because most of these systems are developed as multifunctional tools that can image subjects ranging in size from small 20g rodents, to 500g rats or even much larger primates. The resulting devices have initial acquisition costs higher than $1M, and significant operational and maintenance costs are typically deployed in large centralized core facilities. A new technology that can dramatically reduce the complexity and total cost of operation while perform at a very high level as a high throughput preclinical PET system has been the central idea of this proposal. This technology, we call the PETbox system. The key goal of this technology has been to unchain preclinical PET from its tether to high end core imaging laboratories, and let it flourish in biology labs as a low cost but high performance quantitative instrument, that will facilitate measurements that can be translated from the bench to the bedside. A number of innovative technologies have been developed over the course of this proposal, advancing the field of preclinical imaging. These technologies have turned the project into a success story, leading to two successive generations of PETbox systems, with a remarkable performance bypassing by far all the commercially available technologies in terms of sensitivity [4], while maintaining the low cost benefits of the core design. All these technologies have now been translated into commercial products, helping disseminate these technological advances, without losing any of their initial design benefits.

Accomplishments and goals:

As detailed in the sections below, we have accomplished all the aims and goals of the original proposal, and have moved beyond the first generation high throughput instrument, that exceeded the performance of the original proposal, into a second generation technology with performance that surpasses the best state of the art instruments commercially available today.

Summary of research activities:

The main goal of this proposal was to develop an instrument suitable for high throughput screening of preclinical mouse models with PET. Anatomical reference imaging would be provided by optical means via a structured light source and a video camera that would acquire the three dimensional surface of each subject and from there would infer the internal organ locations based on our previous work on a mouse reference anatomical atlas. This instrument, which we called PETbox was designed to include two stationary detectors covering a Field Of View (FOV) with a size equal to that of an average mouse (5 x 10 cm) [1]. Tomographic imaging was enabled by limited angle tomography and an iterative Maximum Likelihood reconstruction algorithm, since the coincidence PET data with two stationary detectors sampled up to 60% of the available data in the axial, and up to 45% of the data in the transverse FOV (Figure 1a). Under its basic design geometry, the PETbox provided three dimensional images with spatial resolution FWHM approximately 1.8 mm in the transverse plane, and 3.5 mm in the sagittal plane, the direction between the two detectors (Figure 1c). This

design afforded a system with good absolute sensitivity (~4%, Figure 1d) and provided quantitative tomographic images (Figure 1e) at low cost [2]. Anatomical imaging was initially provided by the optically

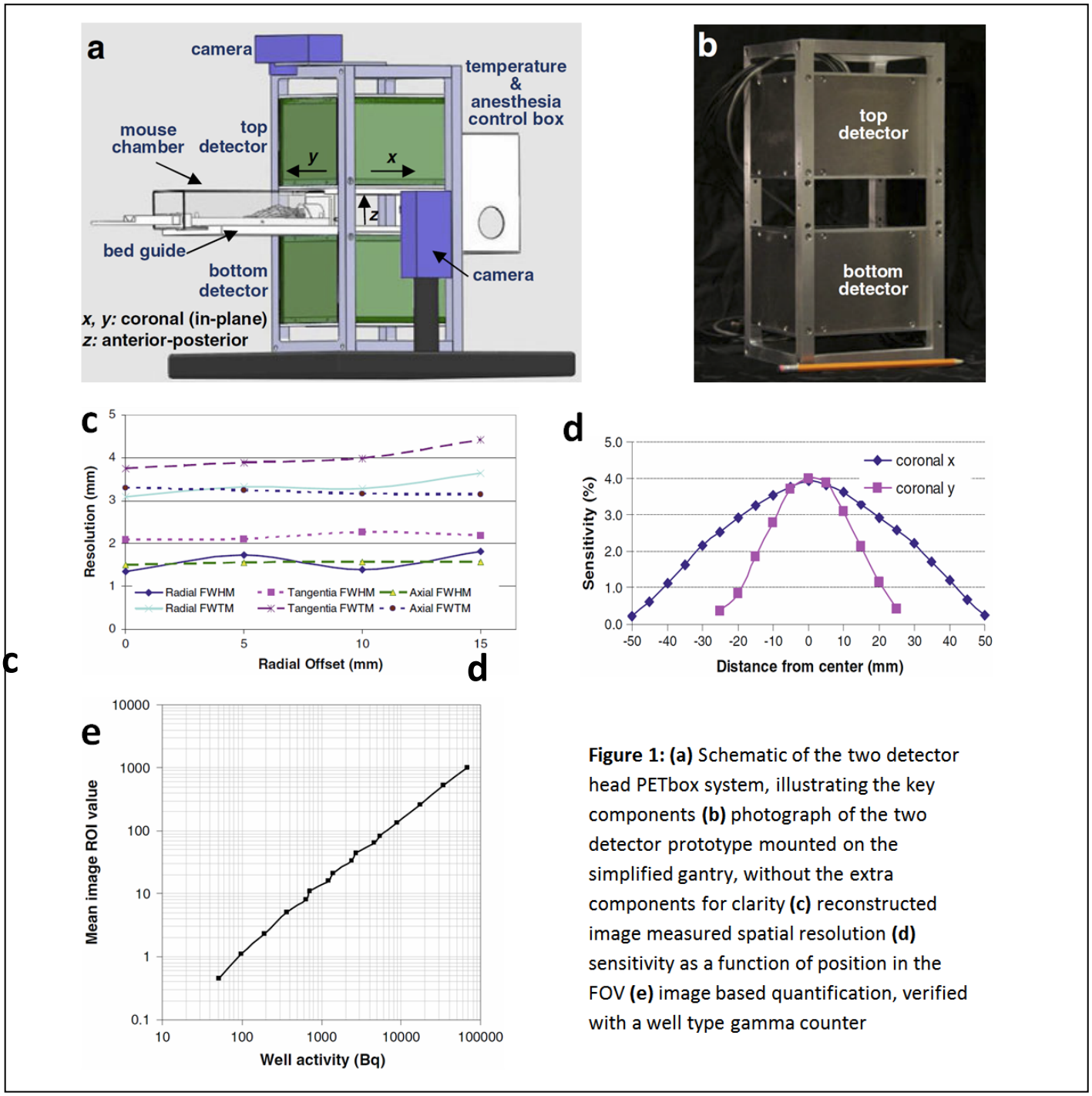

Figure 1: (a) Schematic of the two detector head PETbox system, illustrating the key components **(b)** photograph of the two detector prototype mounted on the simplified gantry, without the extra components for clarity **(c)** reconstructed image measured spatial resolution **(d)** sensitivity as a function of position in the FOV **(e)** image based quantification, verified with a well type gamma counter

determined subject surface which provided a rough first estimate of the location of the mouse's internal organs. A biological support system that provided anesthesia, temperature control, posture support and a pathogen free environment was also developed (Figure 1a). This system was further enhanced by the addition of a non-contact respiration monitor, based on a single video camera and a backlit silhouette view. Routine use of this system at our imaging facility, demonstrated the high significance of the respiration monitor that provided users with a real time visual feedback of the anesthetic status of each subject.

Besides several design elements that were specific to the PETBox, a number of technological innovations that are applicable to high sensitivity imaging in general were devised in that first generation system. Examples of

these are (a) the adaptive energy window that maximized the system sensitivity [3], (b) digital front end electronics [2], (c) model based image reconstruction algorithms and a mathematical model of the mouse geometry based on a principle component analysis [5].

After the success of this first generation system, a number of possible areas of improvements became evident, with two being the most important: (a) limited angle tomography and (b) anatomical information. While the limited angle tomography provided quantitative images, the non-uniform spatial resolution of the first generation system often distracted operators accustomed to the uniformity of spatial resolution of PET. In addition, the external mouse surface did not correlate very accurately with the internal organ anatomy, especially for small volume organs, such as the spleen. Both of these questions were addressed by a second generation system, the PETbox4 that increased the number of detector panels from 2 to 4, completing the angular sampling and also included a projection x-ray source/detector pair. This second generation system retained all the benefits of the first generation system, at a modest increase in the cost of the components (~25%), while at the same time had some other significant advantages. The sensitivity of the second generation system was quite remarkable, measuring in excess of 12% at a 350-650 keV energy window, in close agreement with design specifications. While the actual measured sensitivity of a wide open energy window (150-650kev) exceeded 17%, we are still in the process of optimizing the energy window to reduce the capture of increased levels of gantry backscatter that was introduced by the additional detectors. Still, this performance exceeds by a factor of 2 or more the peak sensitivity of the top of the line commercially available preclinical imaging system [4, 8]. The spatial resolution is now uniform across the FOV, with the use of an accurate system model, and full 3D dynamic images can be acquired with very low levels of injected activities, on the order of 2-25 μCi. Anatomical correlation of the x-ray and optical images of this system with our statistical atlas is at least as good as what can be achieved by expert users with the help of fully tomographic x-ray CT [5,6,7], at least for normal mice. Disease subjects deviating substantially from the statistical mouse model, such as subjects having large tumor xenografts or with significantly enlarged organs are still not very well matched by the statistical atlas and this is an ongoing effort.

Papers & other products delivered:

A total of 8 publications have been generated for this work as well as two technologies have been transferred via the UCLA Office of Intellectual Property to a small company that is taking them to the market. Also, more tangibly, both generations of the PETbox systems are commercially available today, by Sofie Biosciences.

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Dr. Henry Huang (UCLA)

Project 3: Tracer kinetic assays for Cellular and microPET Imaging.

Executive summary:

In this project, we hypothesized that various data/images related to molecular imaging (MI) can be integrated in an informatics environment to facilitate the research activities in MI. The first aim of the project is based on this hypothesis. For the second aim of this project, we further hypothesized that novel strategies and analysis algorithms in couple with new data-acquisition instruments can be developed to assist the interpretation of radioactivity measurements acquired from these instruments in terms of biological information. In the first case, we have developed a now routinely used portal system in an integrated MI environment that strongly support the original hypothesis that modern computation technology could facilitate research activities in MI. In the second case, we have developed novel algorithms and data acquisition strategies in this project that have demonstrated the significant advantages of employing suitable image/data analysis to provide useful biological information from new radio-activity measurement instruments.

Accomplishments and goals:

For the first aim, we have accomplished all aspects of the project, and have now in operation a MI portal system that users are accessing routinely for data acquisition scheduling, image archiving and retrieving, and data/information searching. The system also facilitates the management of the associated data-acquisition devices in terms of data archival and record keeping (for quality control, for usage billing and for satisfying regulation requirements). The developed system is named Molecular Imaging Data Access System (MIDAS) and its website access is at http://midas.nuc.ucla.edu/midap/index.html. In fact, when a separate animal imaging center was set up at the Ahmanson Biological Imaging Division (ABID) at UCLA, the entire system was duplicated to handle all aspects of the operation of the imaging devices in the ABID. Its potential to be transportable to other MI centers in other institutes to help their MI operation and research, however, has yet to be assessed.

For the second aim, we have developed a novel tracer infusion strategy to extract the transport and sequestration rate information from dynamic measurements collected with the microfluidic-PSAPD device developed in Project 2 of this DOE grant. The developed strategy is based on the use of switching on and off the tracer concentration in the perfusion medium of multiple cell-containing wells in a microfluidic chip. The strategy thus made “tracer kinetic assays on a chip” a reality. New methodologies have also been developed and demonstrated in a multiple of mouse PET studies that image quantification provide useful biological information that can be extended to human studies to help improve the value of PET imaging in the nuclear medicine clinics.

Summary of research activities:

For the first aim, we have used the approach of having a central portal system, which consists of, in addition to an Internet-based user interface, a search engine, a meta-database that is extracted from peripheral databases associated with individual data-acquisition device. We further required that all user interfaces be through an Internet browser. The data from associated data-acquisition devices are automatically transferred daily to the central portal system to be extracted into the meta-database, which in turn are available for user searching and/or access. An elaborate, but highly robust, security system has also been implemented that categorizes users into a hierarchical tree of multiple levels of access privilege, and to allow users to set the security level to individual data item that they generated. A difficulty that we have encountered in the development of the system is the understandable reluctance of some investigators who have been in charge of their data-acquisition

devices (e.g., HPLC) to allow us to install a software program in their device-specific computers to connect to the Internet and to transfer their acquired data to the MIDAS for sharing. As a result, the data-acquisition devices associated with MIDAS today are limited mostly to molecular imaging scanners. Even with this limitation, MIDAS is very successful with over 3500 accesses per year by investigators. MIDAS is so successful that, in its entirety, is duplicated for handling all aspects of the operation of a newly setup pre-clinical imaging center (ABID) at UCLA.

For the second aim, the capabilities of this new tracer switching-on-and-off strategy were demonstrated using a microfluidic-PSAPD platform. The figure below shows the measured kinetics from a single well in the microfluidic-PSAPD platform that contained ~100 CaP8 cells (a prostate tumor cell line). The diamond symbols on the figure denote the average radioactivity measured by the PSAPD for the well in alternating intervals (media with or without [18F]FDG). The solid line is the model predicted activity time course after the model parameters have been adjusted to best match the measurements. For this example, the estimated values of k1, k2 and k3 of the FDG model were 0.32+0.08, 0.022+0.003, 0.008+0.002 /min, respectively (k4 was constrained to 0.005 /min). When the glucose level in the medium was increased from 50 mg/dL to 150 mg/dL with the same cell line, the estimated values were 0.15+0.014, 0.025+0.006, 0.011+0.005 /min, respectively. The only parameter with a significant change was k1, reflecting a saturation of the glucose transporter activity from extra-cellular space to the cytosol at 150 mg/dL. The relative stability of the k2 and k3 values indicated that the intracellular glucose level and the glucose metabolic rate were maintained, in spite of the glucose changes in the culture medium, as would be expected if the system was functioning in a well regulated manner. The time course of the tracer exposure and the measurement cycle can also be further optimized to maximize the estimation reliability.

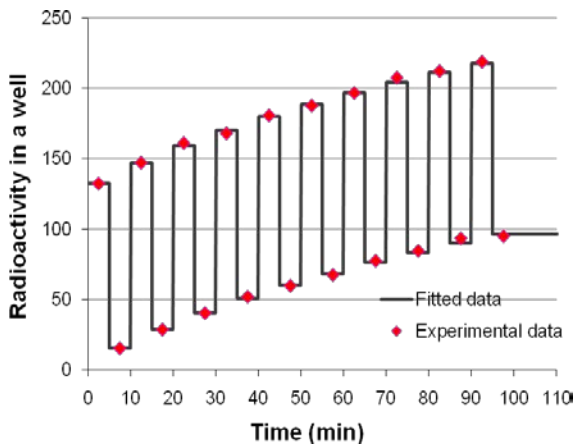

Figure 15. Plot of average measured radioactivity per cell inside a single well (units of pCi/cell). The solid curve is the fitted data after modeling with a tracer kinetic model.

In addition, we have examined ways to improve the reliability of standardized uptake value (SUV) of FDG as a measure of tumor progression in preclinical mouse PET studies, and have found the use of a modified SUV to account for the variability due to animal dependent kidney excretion rate of FDG performed better than regular SUV. We have also developed and validated a modeling approach for deriving the input functions for IV and IP FDG injection. With this approach, we have examined the effect of administrative route, dietary state, and plasma glucose level on FDG kinetics in a set of mouse FDG PET experiments, and have demonstrated that FDG uptake rate constants (Ki) in tissues were independent of the administrative route of the tracer. The study also demonstrated the value of quantitative analysis of mouse PET studies. Many of the developed methodologies or discovered results can be readily applied to human studies to help improve the value of PET imaging in the nuclear medicine clinics.

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Dr. Caius Radu MD, Nagichettiar Satyamurthy, PhD, Johannes Czernin MD (UCLA)

Project 4: Molecular Imaging Probe Discovery

Executive summary:

Novel molecular imaging modalities to measure biochemical and cellular events in living organisms are needed to realize the full potential of molecular medicine. In this project we have developed Positron Emission Tomography (PET) probes for treatment stratification in cancer. Using a differential screening strategy we have identified a series of probes for deoxycytidine kinase (dCK), a protein which plays an important role in providing proliferating cells with nucleotides, which are the building blocks of DNA. The prototype for the new probes is 1-D-(2'-deoxy-2'-[^{18}F]fluoroarabinofuranosyl) cytosine (^{18}F-D-FAC). We have further optimized this probe by developing a series of analogs with improved characteristics. Two of the second generation PET probes for dCK, L-FMAC and L-FAC, along with D-FAC have been translated to the clinic under FDA-granted INDs. These PET probes are being tested for their ability to stratify cancer patients for treatment with widely-used chemotherapeutic agents such as gemcitabine. Since response rates to gemcitabine and related nucleoside analog pro-drugs are low relative to the occurrence of side-effects, and since methods for patient stratification are currently lacking, our approach is addressing an unmet clinical need in the management of malignancies such as ovarian and pancreatic cancer that significantly impact public health.

Accomplishments and goals:
As detailed in Section 5 below, we have accomplished all of our original goals and objectives for Specific Aims 1 and 2, which were the most important aims of our proposal in terms of potential benefit to the public. Work to realize Aim 3 is still in progress.

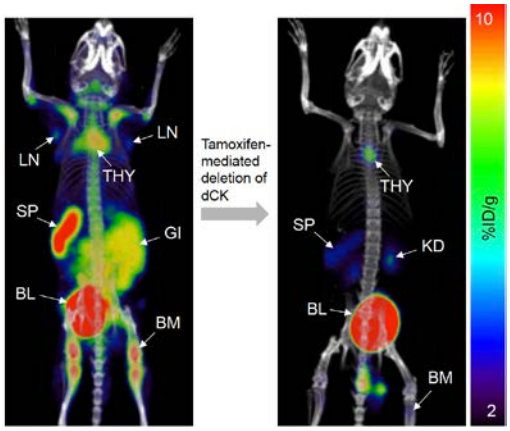

Fig. 1: Effects of Tamoxifen-induced dCK deletion in adult dCK conditional KO mice on ^{18}F-D-FAC biodistribution. Mice were scanned before and after five days of Tamoxifen treatment. Thy – Thymus; SP – Spleen; LN – lymph nodes; GI – Gastrointestinal tract; Bl – Bladder; BM – Bone marrow.

Summary of research activities:

We have demonstrated that three of our original hypotheses are correct. ***First***, by creating genetically modified mice which lack dCK expression we have shown that dCK-mediated phosphorylation is a critical determinant of ^{18}F-D-FAC retention in lymphoid tissues (hypothesis #1). In the dCK conditional KO model, systemic administration of the drug Tamoxifen induces the expression of Cre recombinase followed by dCK deletion throughout the body. We have established that optimal dCK deletion throughout the body can be obtained by injecting the dCK conditional KO mice with Tamoxifen at 20 mg/day for at least five days. This treatment induces a 90% decrease in dCK activity in most tissues, as measured by kinase assay in vitro and by ^{18}F-D-FAC PET imaging in vivo (**Fig. 1**).

Second, we have shown that optimized PET probes for dCK can be developed by synthesizing D-FAC derivatives with increased in vivo stability. ^{18}F-D-FAC (chemical structure and reaction scheme shown in **Fig. 2A**) is a deoxycytidine analog that follows the same transport, phosphorylation and deamination as gemcitabine **(Fig. 2B)**. Thus, ^{18}F-D-FAC is susceptible to extensive deamination by cytidine deaminase (CDA) which is especially a problem in humans, who express significantly more CDA than mice **(Fig. 2C)**. CDA greatly reduces the amount of ^{18}F-D-FAC that can be phosphorylated by dCK, which may limit its clinical utility. To avoid the deamination problem, we have developed, synthesized and evaluated two deamination resistant L-enantiomers (^{18}F-L-FAC and ^{18}F-L-FMAC) (**Fig. 2A**).

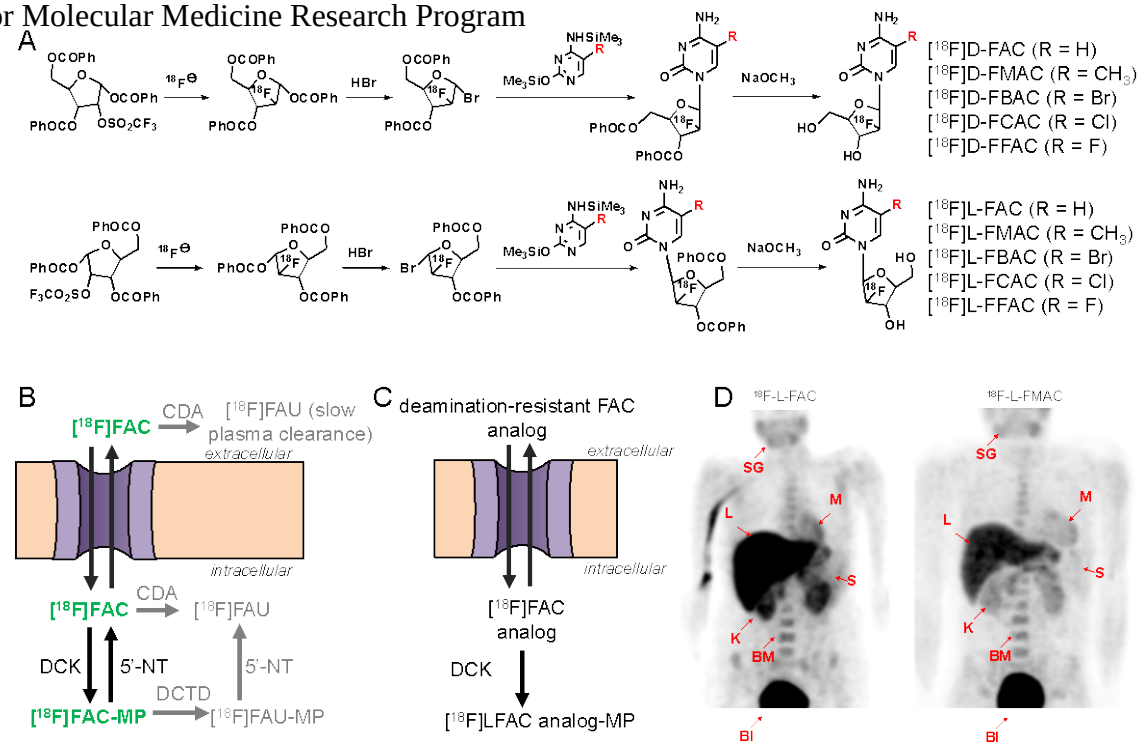

Fig. 2: Synthesis of D-^{18}F-FAC and analogs and their L-enantiomers (A); Comparison of the metabolism of ^{18}F-D-FAC (B) with that of a deamination resistant L-enantiomer (^{18}F-L-FAC (C). MP –monophosphate; DCTD: –cytidylate deaminase. 5'-NT – nucleotidase; The biodistribution of two L-enantiomers in healthy volunteers (D). Organs are indicated by arrows. BM – Bone Marrow; Bl – Bladder; K – Kidney; L – Liver; M – Myocardium; S – Spleen; SG – Salivary gland.

Third, we have shown that PET measurements of dCK activity could enable diagnosis of lymphomas (see publications #**5** and **#6**, in Section **6** below) and selection of therapeutic options by predicting responses to oncolytic pro-drugs activated by dCK.

Clinical evaluation of ^{18}F-L-FAC. The human biodistribution and dosimetry of ^{18}F-FAC, ^{18}F-L-FAC, and ^{18}F-L-FMAC were determined (publication **#5**). As expected (publication **#5, and Fig. 2D**), bone marrow uptake was clearly observed, but no thymic retention was evident, likely due to thymic involution, which is nearly complete by 30 years of age. The whole body biodistribution of candidate probes was favorable, with high target activity and low background. The urinary bladder received the highest radiation dose, Based on these and extensive toxicity and dosimetry data obtained in rodents, the FDA approved investigational new drug (IND) applications covering the candidate deoxycytidine analog PET probes.

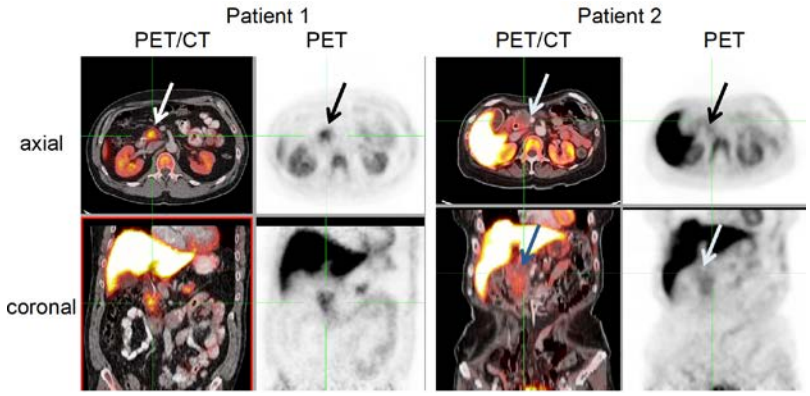

Fig. 3: ^{18}F-labeled deoxycytidine analogue PET/CT studies obtained in two patients with pancreatic adenocarcinoma. Focally increased tumor uptake is noted in the pancreatic cancer of patient 1 (arrow) while tumor uptake is below background for normal tissue in patient 2 suggesting different levels of dCK activity.

Initial studies in patients with pancreatic and ovarian cancer. To determine whether tumor ^{18}F-L-FAC uptake correlates with tumor tissue dCK activity, 9 patients (pancreatic cancer: n=4; ovarian cancer: n=4; cervical cancer: n =1) were imaged one week prior to surgical resection of pancreatic or ovarian cancers. Tumor tissue was examined ex-vivo for dCK activity via kinase assay. **Figure 3** shows one patient with low and one with higher tumor ^{18}F-L-FAC uptake. Initial data suggest a significant correlation between tumor dCK activity and probe uptake.

In summary, we are validating ^{18}F-FAC and analogs as predictive biomarkers for treatment response to gemcitabine response in cancer. This addresses an urgent clinical need, because response rates to dCK-dependent prodrugs such as gemcitabine are low, toxicity is potentially high, and alternative therapies (for instance in pancreatic cancer) have recently become available. In an interdepartmental collaboration with Surgical Oncology (Dr. Timothy Donahue) and Gynecologic Oncology (Dr. Oliver Dorigo), our team is now exploring whether the tumor uptake of ^{18}F-L-FAC correlates with tumor tissue dCK activity. We also are currently recruiting patients with a variety of cancers who are scheduled to undergo surgical tumor excision. If a significant correlation between tumor dCK activity and tumor ^{18}F-FAC uptake is demonstrated, a larger study to corroborate the intriguing preliminary data will be performed to include patients who are non-surgical candidates and who are scheduled to undergo gemcitabine treatment. It will then be determined whether tumor ^{18}F-L-FAC uptake is predictive of treatment responses.

Papers & other products delivered:

Publications:

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Products:

1. A new genetic model to study the function of the deoxyribonucleoside salvage pathway in cancer.
2. New PET imaging probes to image nucleoside metabolism in cancer.
3. Three FDA-granted IND to enable clinical evaluation of ^{18}F-L-FMAC, ^{18}F-L-FAC and ^{18}F-D-FAC in various cancers.