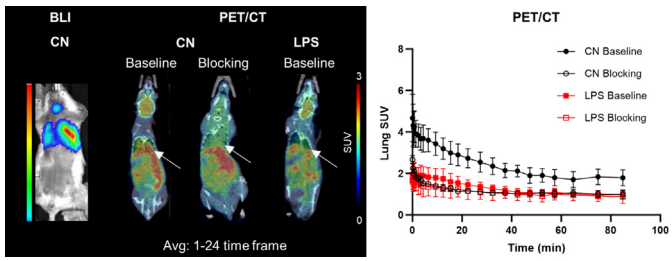


brain static μPET/MR and whole-body dynamic μPET/CT imaging studies of [$^{11}$C]HSP990 were conducted in Balb/C mice intracranially injected in the right striatum with 10,000 CN H99 cells or PBS (negative control). Additionally, whole-body dynamic μPET/CT studies were performed in Balb/cAnNCrl mice following intratracheal instillation of 250,000 CN kn99α NE1270 cells (Vanherp et al., 2019) (a bioluminescent strain of H99), PBS (negative control), or LPS (positive control). All μPET studies were performed under baseline conditions and after pre-treatment with HSP990 (5 mg/kg, ip, 60 min before tracer injection) to evaluate in vivo Hsp90 binding specificity of the tracer. Post-mortem in vitro Hsp90-specific binding of [$^{11}$C] HSP990 to tissue slices of lungs infected with CN kn99α NE1270 lesions was evaluated using autoradiography following pre-incubation with HSP990 or Onalespib. Pathogenesis and lesion formation of cerebral cryptococcosis was confirmed by MRI (9.4T Biospec) and for pulmonary cryptococcosis by BLI (IVIS Spectrum) and high resolution CT (Bruker Skyscan 1278).

**Results:** In vitro, [$^{11}$C]HSP990 binding to CN H99 cells was significantly higher at 37°C compared to 30°C and RT, indicating temperature-dependent binding. Tracer binding was confirmed to be Hsp90-specific, as significant reductions were observed after blocking with Hsp90 inhibitors. In vivo μPET imaging of CN H99-infected mouse brains demonstrated high, Hsp90-specific [$^{11}$C] HSP990 uptake, but the uptake was homogeneous, with no discernible contrast in lesion-affected regions vs healthy brain. In contrast, in vivo μPET imaging of CN kn99α NE1270-infected mouse lungs showed significantly higher Hsp90-specific tracer retention in infected lungs compared to LPS-positive and PBS-negative controls. Autoradiography of lung tissue slices revealed substantial binding of [$^{11}$C]HSP990 to CN kn99α NE1270 lesions, with significant reductions observed after pre-incubation with Hsp90 inhibitors. Binding was also confirmed to be CN-specific, as significantly lower binding was observed in LPS-positive control lung tissue samples.

**Conclusions:** [$^{11}$C]HSP990 is a suitable tracer for in vivo visualization of Hsp90 in *CN* lesions, demonstrating good contrast in lung infections, highlighting potential for disease characterization. Although no contrast was observed in CN brain lesions, the tracer's good blood-brain barrier permeability and high uptake in infected brain regions highlight its potential as a tool for assessing Hsp90 expression. This could aid in predicting responsiveness to Hsp90-targeted therapy and monitoring in vivo Hsp90 occupancy as a function of dosing with Hsp90-targeting therapeutics.

**Figure Caption for Publication:** BLI and [$^{11}$C]HSP990 PET/CT images of lung CN and LPS mice under baseline and blocking (HSP990 5mg/kg) conditions with corresponding TACs.

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## P-250
## STUDY OF ANTITUMOR ACTIVITY OF MCP *IN VIVO* ADMINISTERED ORALLY AND INTRAVENOUSLY USING MOLECULAR IMAGING

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**Introduction:** Cancer remains a significant public health challenge and a leading cause of mortality worldwide. Modified citrus pectin (MCP), a polysaccharide, has demonstrated anti-tumorigenic properties, the ability to prevent chemoresistance, and modulation of the immune system. The mechanism of action of MCP has been related to the direct effect of MCP binding and inhibition of Gal-3 proteins, as well as indirect effects such as systemic immune modulation. This study explores the biological activity of MCP in an in vivo cancer model using molecular imaging techniques.

**Methods:** In this study, tumor xenografts of SKOV-3 and MDA-MD-231 cells were produced, through which a tumor reduction study was performed using oral and intravenous routes of administration. In addition, we radiolabeled MCP with 99mTc and verified its biodistribution, absorption, bioavailability, pharmacokinetics, binding in blood components, by molecular imaging.

**Results:** The results showed that intravenous (IV) administration of MCP (10 mg/kg) effectively reduced tumor growth by 50% in an in vivo SKOV-3 cancer model. In contrast, oral administration of MCP (200 mg/kg) did not produce significant effects. Kinetic studies revealed that orally administered [99mTc]MCP is absorbed in the gastrointestinal tract at low concentrations, with a Tmax of 60 minutes and a bioavailability of 4.3 x 10^-5%. Biodistribution and μSPECT/CT imaging indicated that low concentrations of IV [99mTc] MCP reached tumors and bound to areas of cell death, with renal/hepatobiliary elimination for IV administration and renal/gastrointestinal elimination for oral administration. Hemagglutination and sepharose/Gal-3 assays showed that MCP3 inhibits Gal-3 at high concentrations (25 mg/ml) and that approximately 37.5% of MCP3 binds to Gal-3. However, SPECT/CT imaging did not show tumor absorption of MCP3 in vivo. Blood compartment distribution studies revealed that [99mTc]MCP has an affinity for plasma proteins and cells in C57BL/6Lgals3+/+ mice, which is lost in Gal3 knockout animals. Pharmacokinetic analysis indicated that the elimination rate of MCP is higher in C57BL/6Lgals3-/- mice, suggesting that galectin-3 deficiency accelerates MCP elimination.

**Conclusions:** Our findings reveal, for the first time, that MCP can be radiolabeled with 99mTc, and this method can serve as a valuable tool for studying MCP's biological activity in vivo. We demonstrated that MCP shows anticancer activity in a tumor xenograft model when administered intravenously, but not orally, highlighting the low absorption of MCP through oral administration. Additionally, we found that intravenously administered MCP reaches tumors at low

concentrations and binds to regions of cell death. We characterized its monosaccharide composition, partial affinity, and inhibitory capacity against galectin-3 using low molecular weight MCP. Furthermore, we examined the influence of Gal-3 on the biodistribution, blood compartmentalization, and intravenous pharmacokinetics of [99mTc]MCP in vivo. These results elucidate the pharmacological behavior of MCP in vivo and may enhance our understanding of the mechanisms underlying its anticancer effects.

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**P-251**

**An Improved Automated Synthesis for [$^{18}$F]SynVesT-1, an SV2A Imaging Agent, Using Trasis AllInOne**

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**Introduction:** Synaptic vesicle proteins 2 (SV2s) are a family of proteins found on the membrane of presynaptic vesicles and are involved in a variety of functions. Among the SV2 family, SV2A is the most expressed and is used as target for epilepsy treatment.[1] [$^{18}$F] SynVesT-1 is a small molecule that selectively binds to SV2A and has potential for imaging several neurological conditions.[2] [$^{18}$F]SynVesT-1 synthesis occurs via copper-mediated radiofluorination of organotin precursor in the presence of pyridine and is obtained with moderate RCY (12%).[3] Given the versatility of [$^{18}$F]SynVesT-1 for the imaging of neurological conditions, there is an increasing interest for its use in clinical trials. The purpose of this study is to find the best conditions for production of [$^{18}$F]SynVesT-1 using a Trasis AllInOne module, to develop a robust QC method and to validate the procedure for GMP use, unlocking its use in clinical trials.

**Methods:** Whilst the radiolabelling conditions were kept as per literature,[2] the synthesis sequence and cassette layout were developed and optimised in-house. The design of the cassette consists of three areas: synthesis, purification and reformulation, each with a dedicated syringe to avoid contamination of final product (**Figure**). Optimization studies focused on lowering the activity loss between steps, lowering operator's input and shortening the synthesis time. Several aspects of the sequence, such as reactor shape and volume, gas flow and vacuum levels, order of reagents introduction, sequence automation and length of each step were tested to try increasing the RCY. Alongside the production method, several conditions for HPLC, TLC and GC were tested to develop a robust QC method to be used for routine analysis of the product.

**Results:** Initial results returned a RCY (non-decay corrected) of 8.90% within 80 minutes from end of delivery (EOD). After optimisation of the sequence and cassette layout, [$^{18}$F]SynVesT-1 was obtained with a RCY (non-decay corrected) of up to 21% within 63 minutes from EOD. In regards to the developed QC analysis procedure, the HPLC method showed radiochemical and chemical purity ≥ 99%, the GC method confirmed that neither the solvent nor the pyridine are present in the formulated product and the TLC method showed a good separation between [$^{18}$F]SynVesT-1 and [$^{18}$F] fluoride.

**Conclusions:** The proposed method produced [$^{18}$F]SynVesT-1 with a RCY (non-decay corrected) of 19.67 ± 2.43% and $A_m$ of 274.44 ± 28.64 GBq/μmol within 60 min from EOD (n = 6). Moreover, the developed methods for HPLC, TLC and GC analysis showed good separation of the analytes and consistent results, hence being suitable for routine analysis of the product. Future work will focus on validating the production and QC methods to allow in-human production of [$^{18}$F]SynVesT-1.

**Figure Caption for Publication:** Cassette layout and radio HPLC of the purification showing the radiochemical conversion obtained.

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**P-252**

**Performance Evaluation of the KAERI Cryo RPT Container for Ultra-Low Temperature Transportation of I-131 mIBG in International Shipping**

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**Introduction:** Transporting radiopharmaceuticals, particularly I-131 metaiodobenzylguanidine(mIBG), requires strict temperature control to ensure stability and safety during international shipping. The KAERI Cryo RPT Container was developed to meet these needs, utilizing vacuum insulation panels and a dry ice-based cooling system to maintain ultra-low temperatures during long-distance transport. Previous solutions encountered challenges in maintaining consistent temperatures, but this container addresses those limitations. This study evaluates the KAERI Cryo RPT Container's performance under real-world shipping conditions, including shipments to Poland, India, and the United States.

**Methods:** The KAERI Cryo RPT Container consists of vacuum insulation panels, polypropylene structure, and dry ice blocks designed to maintain temperatures below -60°C during transportation. The performance of the container was first tested in a climate chamber under ISTA 7E standards to simulate extreme temperature fluctuations. This test verified the container's ability to maintain stable internal temperatures despite external environmental changes. Following this, an international shipping trial was conducted to three countries (Poland, India, and the United States). Each country received two KAERI Cryo RPT Containers, with temperature data loggers placed both inside and outside the containers to monitor temperature throughout the shipping process. The trials lasted approximately 144 hours, evaluating the container's temperature maintenance capacity under various environmental conditions such as high temperatures, humidity, and fluctuating climates. Poland's route represented seasonal changes in inland Europe, India's route simulated tropical conditions, and the United States route crossed multiple climate zones, providing a robust test of the container's durability and reliability.