FONDAZIONERICERCAMOLINETTE ets

STRUCTURE OF APPLICATION - TRANSLATIONAL RESEARCH

Translational investigation on **Poly** (ADP-ribose) polymerase-1 (**PARP**-1) as a target against **neuro**degeneration:a multi-model and multi-tracer approach from bench to bedside (**POLYPARP-NEURO**)

PART 1, Project proposal

A) abstract (in lingua inglese): Alzheimer's disease (AD) is the most frequent neurodegenerative dementia. Oxidative stress, which originates from an unbalanced production of reactive oxygen species, plays a crucial role in AD pathophysiology. Poly(ADP-ribose)polymerases (PARP) is an enzyme family deeply involved in DNA repairing and oxidative stress modulation. Several studies have shown high levels of PARP expression in AD patients. PARP-1 could represent a new interesting pharmacological target in AD. To date, several PARPis are approved or under trial for the treatment of breast, ovarian, prostate, and pancreatic cancers in specific clinical settings and based on the presence of BRCA mutations. Positron Emission Tomography (PET) has a well-established role both in oncological and neurodegenerative diseases. [18F]FDG has a pivotal role in cancers with high glucose metabolism and, given its capability to measure glucose consumption at the synaptic level, it is a validated surrogate to measure neurodegeneration *in vivo*. PET scanning has also the potential to allow non-invasive, whole-body, repeatable visualization of drug delivery including PARP-binding thanks to the development of dedicated radiotracers. This project aims to investigate the link between synaptic and neuronal function/dysfunction and PARP-related expression with a translational approach including PARP-radiolabelling, in vitro cell cultures analyses, and PET imaging in mice models of AD as well as in patients receiving treatment with PARPi as a standard-of-care.

Abstract (in lingua italiano): La malattia di Alzheimer (AD) è la piu' frequente demenza neurodegenerativa. Lo stress ossidativo, che ha origine da una produzione sbilanciata di specie reattive dell'ossigeno, gioca un ruolo cruciale nella fisiopatologia dell'AD. Le poli(ADP-ribosio)polimerasi (PARP) sono una famiglia di enzimi profondamente coinvolta nella riparazione del DNA e nella modulazione dello stress ossidativo. Diversi studi hanno mostrato alti livelli di espressione di PARP nei pazienti con AD. PARP-1 potrebbe quindi rappresentare un nuovo bersaglio farmacologico nell'AD. Ad oggi, i PARP inibitori sono approvati o in fase di sperimentazione per il trattamento dei carcinomi mammario, ovarico, prostatico. La Tomografia ad Emissione di Positroni (PET) ha un ruolo ormai consolidato sia nel campo delle malattie oncologiche che neurodegenerative. In particolare il [18F]FDG ha un ruolo chiave nei tumori con elevato metabolismo del glucosio e, data la sua capacità di misurare il consumo di glucosio a livello sinaptico, è considerato un surrogato per misurare la neurodegenerazione in vivo. La scansione PET ha anche il potenziale per consentire la visualizzazione non invasiva, ripetibile della somministrazione di farmaci, compreso il legame con PARP, grazie allo sviluppo di radiotraccianti dedicati. Questo progetto mira a studiare il legame tra funzione/disfunzione sinaptica e neuronale e l'espressione correlata a PARP con un approccio traslazionale che comprende la radiomarcatura di PARP, analisi di colture cellulari in vitro e imaging PET in modelli murini di AD e in pazienti sottoposti a trattamento con PARPi per indicazioni gia' registrate nella pratica clinica.

B) Background and preliminary results: AD is the leading cause of cognitive impairment and dementia in older individuals (aged ≥ 65 years) throughout the world [1]. The main pathological features of AD in the brain are the extracellular accumulation of senile plaque derived from the aggregation of amyloid beta peptide (Aβ) [2]. These pathways lead to an overproduction of free radicals causing excessive oxidative stress leading to cellular damage, tightly associated with the development and/or progression of AD [3]. Along the different epigenetic mechanisms, poly(ADP-ribosyl)ation, catalyzed by a group of enzymes known as poly(ADP-ribose)polymerases (PARPs) [4], seems to play a central role in the molecular network of interactions responsible for AD pathogenesis [5].PARP-1, the founding member of this enzyme family, catalyzes a NAD+-dependent poly(ADP-ribose) polymerization reaction onto amino acid residues of acceptor proteins. PARP-1 modulates many cellular processes important for the maintenance of cellular functionality and viability such as the ones related to oxidative stress and ageing [6]. The pioneering work of Love and colleagues found an overactivation of PARP-1 in the frontal and temporal lobes of the brains of AD patients.[7] The results were confirmed also from other experiments using both animal and cellular models where PARP activity was evaluated in the presence of Aβ peptide [8].In this framework, a plethora of PARP-1-mediated mechanisms were proposed to promote the neurodegenerative process such as metabolic impairment related to NAD+ depletion and different death pathways triggered by intracellular stress conditions and chronic inflammation [9]. In light of increasing evidence supporting an involvement of PARylation in normal neuronal functions as well as in neurodegeneration and neuropathology [10,11], PARP-1 could represent a new interesting

pharmacological target in AD. PARP-1 inhibitors (PARPis) could become the object of a drug-repurposing evaluation to identify a new beneficial treatment for AD. To date, PARPis are involved in over 448 clinical trials [12] mostly related to the oncology field. To date, several PARPis are approved or under trial for the treatment of breast, ovarian, prostate, and pancreatic cancers in specific clinical settings and based on the presence of BRCA mutations [13]. There's an urgent need to promote preclinical and clinical studies aiming to expand our knowledge on the role of PARP-related DNA repair pathways also in the field of neurodegenerative disease. Positron Emission Tomography (PET) has a well-established role both in the field of oncological and neurodegenerative diseases. In particular [18F]FDG has a pivotal role in cancers characterized by high glucose metabolism and, given its capability to measure glucose consumption at the synaptic level, it is a validated surrogate to measure neurodegeneration in vivo [14]. PET scanning has also the potential to allow non-invasive, whole-body, repeatable visualization of drug delivery including PARP-binding thanks to the development of dedicated radiotracers. Several variants of existing PARPi have been reported for use with fluorescence, SPECT, and PET imaging. Two notable examples are [18F]FTT and [18F]PARPi, which have been evaluated in a clinical trial in oncological patients. [15,16] However, these tracers have no BBB permeability, [11C]NMV was recently evaluated in rat glioblastoma model and non-human primates as the first BBB permeable PET radioligand. [17] In 2022, Schou and co-workers reported the labelling of [11C]AZ3391 where a high binding was observed in organs known to express PARP such as the brain. [18] Along with AZ3391, another PARP inhibitor, AZD-9574,[19] was confirmed to highly cross the BBB, but its radiolabelling has not been reported yet. The multi-tracer, multi-faced nature of PET may thus provide a unique window to investigate the effect of PARP-1 inhibition at the central nervous system (CNS) level. The quantification of PARP in the CNS would be ground-breaking as this would enable the exploration of the PARP-related pathological processes and its link with neurodegeneration which can be assessed in vivo through [18F]FDG. Moreover, the multi-organ and multiple clinical indications to [18F]FDG PET may allow to investigation PARPi effect on brain metabolism (as a surrogate for neural and synaptic function/dysfunction) linking preclinical findings in animal models to observational evidence in patients treated with PARPi for clinical reasons. In this regard, the present project builds naturally on the experience of the PI who, besides, having well-recognized international experience in the use of FDG PET in neurodegenerative diseases, has carried out clinical and preclinical studies aiming to disentangle pathways triggered by oxidative stress both in humans and animal models [20; DOI: 10.2967/jnumed.112.113928 and doi: 10.3390/ijms21218154]. Besides the inclusion of experienced clinicians in the field of oncology and neuropsychology, the team involves an expert in radiochemistry who has previously worked on PARP-radiolabelling with isotopes other than Flourine-18 and has thus the competence to translate his previous work to radiosynthesis a correspondent fluorinated compound. [21 doi: 10.1007/s00259-022-05835-4]

C) Aims: this project aims to investigate the link between synaptic and neuronal function/dysfunction and PARP-related expression with a translational approach including PARP-radiolabelling, in vitro cell cultures analyses, and PET imaging in mice models of AD as well as in patients receiving treatment with PARPi as a registered standard-of-care.

In the **preclinical part** of the project we aim to: 1. To **radiosynthesise [18F]AZD-9574** and its **precursor** for radiofluoriantion as a PET agent for brain imaging *(**Task 1 and Task 2:** **month 1-7**)*.

2. To assess **target engagement**, the pharmacokinetics of the new tracer and its match with the topography of synaptic dysfunction through:

A. Uptake, selectivity and stability measurement of [18F]AZD-9574 *in vitro* in a panel of AD models expressing PARP (***Task 3:*** **month 5-9**);

B. Brain PET imaging with [18F]AZD-9574 *in vivo* in mice model of AD. The same model will be used to assess brain distribution FDG PET, as a surrogate marker of synaptic dysfunction, for comparison of the topographical distribution of the two tracers (***Task 4:*** **month 9-16**).

In the **clinical part** (**Task 5: month 7-21**) of the project, we aim to evaluate the effect of treatment with the PARPi Olaparib on brain metabolism and its relationship to cognition measured through neuropsychological tests in in 30 patients with either BRCA1/2 mutated breast or castration-resistant prostate cancer who will receive treatment with PARPi as a standard-of-care.

D) Project plan: ***Task 1: Synthesis of AZD-9574 precursors for radiofluorination.*** the Gouverneur group developed a robust procedure to forge new Csp2-18F bonds, the copper-mediated radiofluorination, to date, this reaction is largely utilized by the radiochemist community and in the clinic for the production of [18F]FDOPA and [18F]Flumazenil radiotracers. For this reason, the precursor of choice to radiolabel AZD-9574 will be a boronic ester. The synthetic pathway consists of 12 steps, most of which are reported in the AstraZeneca patent (*Task 1, Activity 1*). All the chemical intermediates will be characterized through NMR spectroscopy with proton, carbon and fluorine

spectra. The mass analysis will confirm the identity and purity of every compound. All the steps to carry out the chemical synthesis can be performed in the organic chemistry laboratory present at the Molecular Biotechnology Center (MBC) “Guido Tarone”.

Task 2: Radiosynthesis of [18F]AZD-9574. The radiosynthesis of [18F]AZD-9574 (*Task 2, Activity 1*) consists of a first step related to fluorine-18 installation and a deprotection one yielding the desired product. Copper-mediated radiofluorination is a robust reaction that involves the use of copper salt in the presence of a nucleophilic source of fluoride such as [18F]KF and a solubilising agent like kryptofix. The radioactive steps will be assessed through radio-HPLC analysis for identification and confirmation of yielding the chosen compound. The reaction will be performed on an automatised all-in-one radiosynthesizer.

Task 3: In vitro characterisation of [18F]AZD-9574. The first moment will be dedicated to the set-up and characterization of cell cultures. We have selected as relevant *in vitro* AD models: 7PA2 cells, Aβ25–35-treated human neuroblastoma SH-SY5Y and SK-N-BE neuroblastoma cells. We will assess PARP-1 basal expression levels in these cell lines through western blot as reported in the literature. To assess [18F]AZD-9574 uptake, the aforementioned cells will be exposed to the radiotracer at a chosen concentration, and incubated for increasing lengths of time. To assess [18F]AZD-9574 binding selectivity, unlabelled AZD-9574 and other PARPis will be added in excess before the addition of the radiotracer to the cells and then incubated. Then, in both cases, cells will be lysed and the amount of 18F in the cell lysates will be measured using a Wizard2 Automatic Gamma Counter (PerkinElmer). Those tests will be done in triplicate and repeated twice on different days to have good statistical results (one-way and two-way ANOVA will be performed with GraphPad Prism 7). (*Task 2, Activity 1, 2, 3*)

***Task 4: In vivo characterisation of [18F]AZD-9574 and comparison with brain metabolism in* 5XFAD transgenic mice (model of AD).** [18F]AZD-9574 biodistribution will be investigated through PET at different time points (30, 60 and 120 minutes and 24h) in wild-type control mice (n=3). After imaging, mice will be sacrificed and organs and blood harvested to determine with gamma counter the amount of 18F in each tissue. (*Task 4, Activity 1*). Thanks to 5XFAD mice we can investigate the major pathologies associated with amyloid plaque build-up, gliosis, and neuronal loss along with cognitive and motor deficits. We will scan through dynamic PET imaging different groups of 5XFAD transgenic mice: Group 1: 3 mice before the main symptoms manifestation. Group 2: 3 mice at months where we have the main brain alterations. Group 3: 3 mice with a developed pathology (*Task 4, Activity 2*). We will administer [18F]AZD-9574 (5-10 MBq) intravenously. Images will be quantified using volume-of-interest analysis. After imaging, we will remove the brain and harvest blood and selected tissues to determine the amount of 18F in different regions. Further analysis will be performed on brain and tissue sections by autoradiography, immunohistochemistry staining for various PARP isoforms, and H&E staining to determine the PARP-selectivity of [18F]AZD-9574 uptake (*Task 4, Activity 3*). Additionally for Group 2, following the first acquisition, we will also administer a dose of 4 MBq of FDG soon after the start of a list mode acquisition lasting 50 minutes. The same will be done for other 3 wild-type mice. With these experiments, we aim to correlate brain metabolism (as a marker of neurodegeneration) with [18F]AZD-9574 distribution. Quantitative maps will be generated by mean of Gjedde-Patlak graphical approach PMOD, Zurich, Switzerland).

Task 5: Interplay between PARPi and brain metabolism in patients treated with PARPi.

Patients: we will include 30 patients affected by either BRCA1/2 mutated breast or castration-resistant prostate cancer who will receive treatment with the PARPi Olaparib as a standard-of-care and candidates to FDG PET/CT for a specific clinical question will be recruited. This part of the project will require a submission and approval to the local ethical committee which we will start immediately after the project will be funded (prospective interventional non-pharmacologic study; months 1-6) (*Task 5 Activity 1*). Accordingly, recruitment will start after 6 months and will end after 17 months to allow follow-up examinations in all patients. Exclusion criteria are: presence or history of brain lesions on CT or MRI, previous diagnosis of encephalopathy/encephalitis, cerebrovascular disorders, or any other previous or current neurodegenerative or neuroncological disease. Given the aim of the present project (and the expected features of the included cohort) subjective cognitive impairment will not be considered an exclusion criterion.

Images’ acquisition: Patients will undergo whole-body FDG PET at baseline and 4 months after treatment (*Task 5 Activity 2*) following a modified protocol including a dedicated brain acquisition according to the European Association of Nuclear Medicine (EANM) guidelines for body and brain FDG PET imaging.

Cognitive assessment: Cognitive assessment will be performed twice (within 15 days from each PET examination) and will include the Montreal Cognitive Assessment (MoCA) and a more extended test battery to provide standardized measures of verbal learning and delayed recall, working memory, attention, cognitive flexibility, language, abstraction, and visuospatial abilities (*Task 5 Activity 3*).

Image processing and statistical analysis: Image preprocessing will be conducted using SPM12. After pre-processing baseline and post-therapy scans will be compared on whole-brain voxel-wise analyses using a two-sample t-test design of SPM12. Multiple Regression analysis in SPM will be used to correlate neuropsychological tests’ scores

with brain metabolism before and after treatment (age and sex will be included as confounding variables). Analyses for the clinical task will start after all baseline PET scans are acquired (month 17). Analyses of the follow-up results and dissemination activities will be performed from month 20 to month 24 (*Task 5 Activity 4*).

E) Expected results and contingency plans: The present project will have several translational deliverables:
1.Validation of radiolabelling of AZD-9574 with fluorine-18 which offers crucial advantages over carbon-11-labelling. Fluorine-18 possess a longer half-life that permits the transportation of the radiotracer (of interest in a future clinical trial perspective). 2. We will disclose the relationship between PARPi and neurodegeneration in different pre-clinical and clinical models. 3. All the analyses will take advantage of the intrinsic quantitative nature of radiopharmaceuticals-based analysis to whom we will also associate translational topographical insight (at SNC level) of PARPi effect in mice and humans.
For radiolabelling and in vitro analyses, the **challenges** will include the complex synthesis of suitable drug precursor for radiofluorination and the following fluorine-18 labelling since many motifs on pharmacologically active small molecules hampered this step. To overcome this challenge we also plan to synthesise AZD-9574 precursors for radiofluorination.
F) References: 1) Monfared et al. Neurol Ther. 2022,11, 525-551. 2) Therriault et al. Nat Aging. 2022, 2,526-535. 3) Breijyeh et al. Molecules. 2020, 25, 5789. 4) Ummarino et al. Genes (Basel). 2021, 12, 446. 5) Martire et al. Mechanisms of Ageing and Development. 2015, 146, 53-64. 6) Chaudhuri et al. Nature Reviews Molecular Cell Biology. 2017, 18, 610–621. 7) Gammella et al. Oxid Med Cell Longev. 2016, 8629024. 8) Abeti et al. Brain. 2011, 134, 1658–1672. 9) Hegedus et al. Redox Biology. 2014, 2, 978-982. 10) Zhao et al. Int J Nanomedicine. 2023, 18, 7825–7845. 11) Mao et al. The FEBS Journal. 2022, 289, 2013-2024. 12) www.clinicaltrials.gov, Feb 2024 13) Mateo et al. Annals of Oncology. 2019, 30, 1437-1447.14) Fletcher et al. The Journal of Nuclear Medicine. 2008, 49, 3. 15) Kassubek et al. Curr. Opin. Neurol. 2019, 32, 740-746. 16) Crisan et al. Int. J. Mol. Sci. 2022, 23, 5023. 17) Tong et al. Front Med (Lausanne). 2022, 9, 1062432. 18) Johnstrom et al. Cancer Res (2022) 82 (12_Supplement): 5977. 19) Jamal K. et al. Cancer Res. 2022;82,(12_Supplement):2609. 20) MIceli Int J Mol Sci. 2020 31;21:8154. 21a) Chan et al. J Nucl Med. 2023 https://doi.org/10.2967/jnumed.123.265429. b) G. Destro et al. Nuclear Medicine and Biology. 2023, 116-118. c) Chan et al. EJNMMI. 2022, 49, 3668-3678. d) Gendron et al. EJNMMI radiopharm. Chem. 2022, 7, 5. e) Chen et al. Org. Lett. 2021, 23, 7290-7294. 22)Carruthers et al. CNS Oncol. 2012, 1, 85–97. 23)Picco A EJNMMI. 2014 764-75; 24) Packer et al. WO2021/260092A1. 25) Tredwell et al. Angew Chem Int Ed Engl. 2014, 53, 7751-5. 26) Shah et al. Poly(ADP-ribose) Polymerase. 2011, 3–34. 27) Dhapola et al. Laboratory Animal Research. 2023, 39, 33.

PART 2, Proponent's CV and team

Proponent's CV

A. Positions and Honors: Silvia Daniela Morbelli, born on 22nd December 1976, is a full professor of Nuclear Medicine at the University of Turin. She holds a PhD degree in neuroscience and she is a member of the Academic Board of the PhD School in "Biotechnologies in Translational Medicine" of the University of Genoa. She is a past-chair of the Neuroimaging Committee of the European Association of Nuclear Medicine (EANM; Chair from 2020 to 2023). Leader (2018-2020) of the joint Task Force of the European Association of Nuclear Medicine (EANM) and of the Society of Nuclear Medicine and Molecular Imaging (SNMMI, USA) for preparation of Procedural Guidelines on Dopaminergic Imaging in Parkinsonian Syndromes. She is a member of the Steering Committee of Neurology study Group of the Italian Association of Nuclear Medicine (AIMN). She was a member of the Steering Committee of Oncology study Group of AIMN (2017-2022). She is a formed "Honorary Clinical Fellow" of Wolfson Brain Imaging Center, Addenbrooke's Hospital (Cambridge University) (2004)
B. Publications: **Total Number of publications in the last 5 years:** 157 (peer reviewed publications average IF 5.3). H-index: 43; H-index of the last 5 years: 24

Most relevant Publications in the last 5 years:

1. Lanfranchi F….**Morbelli S**. Different z-score cut-offs for striatal binding ratio (SBR) of DaT SPECT are needed to support the diagnosis of Parkinson's Disease (PD) and dementia with Lewy bodies (DLB). **Eur J Nucl Med Mol Imaging**. 2023 **I.F 9.1**

2.Chételat G, Arbizu J, Barthel H, Garibotto V, Law I, **Morbelli S**, et al. Amyloid-PET and ^{18}F-FDG-PET in the diagnostic investigation of Alzheimer's disease and other dementias. **Lancet Neurol**. 2020 **I.F. 48**

3. Morbelli S et al EANM practice guideline/SNMMI procedure standard for dopaminergic imaging in Parkinsonian syndromes 1.0. **Eur J Nucl Med Mol Imaging**. 2020 **I.F. 9.1**

4. Bauckneht M…. **Morbelli S**. Association among education, age, and the dementia with Lewy body (DLB) metabolic pattern A European-DLB consortium project. **Alzheimers Dement**. 2021 **I.F. 14**

5. Morbelli S et al Metabolic patterns across core features in dementia with Lewy bodies. **Ann Neurol**. 2019 **I.F. 11.27**

C. Conferences: **31** Invited talks in the last 5 years at national and international congresses, including: 1. **Plenary Lecture Annual Congress of European Association of Nuclear Medicine (EANM)** 2022 and **6** further invited talks in 5 EANM congresses; **2** invited talks at the **European Conference on Clinical Neuroimaging** in the last five years. **1** Invited to talk at the annual congress of the **European Academy of Neurology** 2022; **1** invited talk at the **European Congress of Psychiatry** in 2023. **2** invited talks in the last 5 years at National Congress of the Italian Association of Nuclear Medicine (AIMN); **2** Invited talks XVI National Congress of the **Societa' Italiana di Neurologia per le Demenze (SINDEM)** 2021 and 2023.

D. Current and anticipated grant support:
1. **PI** of the project "Brain estrogen receptor expression in the perimenopausal transition**" funded by MUR Bando PRIN 2022.**
2. PI of the project "Predictive tools for precision medicine in prodromal stages of neurodegeneration from Lewy Body to Alzheimer's Diseases" Funded by **Ministero della Salute PNRR-POC.**
3. PI of the project "In vivo assessment of demyelination and remyelination in patients with Multiple Sclerosis" **Funded by Ministero della Salute Ricerca Finalizzata 2018.**
4. Investigator in the project "European Cluster for Imaging Biomarkers – ECIB" **EBRA project Funded by European Union call Horizon 2020**.
E. **Previous experience in collaborative research**: **PI** of 3 initiative of the PET-data sharing project of the **European Alzheimer's disease consortium**; Task Leader of the Molecular Imaging Hub of the **European Consortium for Dementia with Lewy Bodies (E-DLB**); Subinvestigator of 5 clinical trials focusing on potential new d**isease modifying drugs for Alzheimer's Disease** in the last 5 years.

F. Team:
The study arises within a multidisciplinary group with complementary competencies including besides the PI:
1.**Gianluca Destro, Radiochemist** (Dept of Molecular Biotechnology and Health Sciences), with experience and facilities needed to set up the radiolabeling of PARPi and relative preclinical studies. He will lead the preclinical in vitro and in vivo activities.
2. **Alessandra Beano** (**Medical Oncologist**), head of "SSD Oncologia Medica Senologica dell'AOU Città della Salute e della Scienza".
3.**Ilaria Depetris** (**Medical Oncologist**, Oncologia 1 U dell'AOU Città della Salute e della Scienza).
4. **Roberto Filippi** (**Medical Oncologist**, Oncologia 1 U dell'AOU Città della Salute e della Scienza).
The project will involve two teams of oncologists with recognized experience in the care of patients with breast and prostate cancer and with a specific competence in patients' recruitment in the framework of clinical trials. Both oncological teams have a well-defined, pivotal, role in the activities of Citta' della Salute e della Scienza di Torino for breast and prostate cancer respectively.
5. **Aurora Cermelli** (Aging Brain and Memory Clinic, Dept of Neuroscience, University of Torino) **neuropsychologist** with a specific experience in cognitive evaluation of patients with neurodegenerative dementia as well as of patients with subtle cognitive deficits regardless of the underlying etiology.

In compliance with the GDPR (Regulation EU 2016/679) and the Italian Legislative Decree n. 196 (30/06/03), I authorise to use and process my personal details contained in this document.

Turin, The 14th February, 2024 *Prof.ssa Silvia Daniela Morbelli*