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Initial evaluation of Cu-64 labeled PARPi-DOTA PET imaging in mice with mesothelioma

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ABSTRACT

Poly(ADP-ribose) polymerase (PARP) has emerged as an important molecular target for the treatment of several oncological diseases. A couple of molecular probes based on Olaparib scaffold have been developed by incorporation of F-18 or fluorophore for positron emission tomography (PET) or optical imaging in several types of tumor. PARP has been reported overexpressed in mesothelioma. We hereby synthesized an analogue of Olaparib containing DOTA moiety and radiolabeled it with Cu-64 to evaluate its utility of PET tracer for mesothelioma. The Cu-64 labeling was conveniently achieved at 90% yield with final compound at >99% radiochemistry purity. The biodistribution and PET imaging were performed at 0.5, 1, 2 and 18 h to confirm the in vivo tumor targeting. The tumor uptake in study group was significant higher than that in control group (3.45 ± 0.47% ID/g vs 2.26 ± 0.30% ID/g) and tumor were clearly detected by PET imaging. These results suggest the feasibility to develop an Olaparib-based theranostic agent for mesothelioma.

Poly(ADP-ribose) polymerase is a family of nuclear enzymes that sense DNA damage induced by chemical or ionizing radiation, and participate the repair process by binding to the DNA breaks.[1] In cancer cells with the HR repair pathway deficiency due to BRCA1 and BRCA2 mutation, inhibition of PARP will lead to DNA breaks unrepaired and cause cell death eventually.[2] Therefore, PARP has become a novel target for cancer therapy in the past decades and a variety of small molecule inhibitors of PARP have been developed to treat cancers. These PARP inhibitors are used either as a single agent in cancers with BRCA1 and BRCA2 dysfunction or in combination with DNA damaging therapeutics (radiation or chemotherapy) to improve the therapeutic benefits by blocking the repair.[3] Also, due to the well-established role in the DNA repair and the potential value to be a prognostic indicator, and the fact that the expression levels of PARP enzyme are significantly higher in a variety of tumors compared to normal tissues,[4–8] As such, PARP has become an attractive biomarker for non-invasive PET imaging.

Olaparib (AZD2281), a FDA-approved first class drug for ovarian cancer, is a potent and bioavailable small molecule inhibitor for PARP-1 and PARP-2. Among a library of candidate inhibitors, Olaparib was picked up for further clinical trials based on a comprehensive consideration of potency and pharmacokinetic properties. In terms of potency, a number of candidates hold sub nano-molar IC_{50}.[9] Based on the core scaffold of this molecule, several imaging agents have been developed by incorporation of F-18 or/and BODIPY-FL dye into it (Fig. 1A). ^{18}F-BO was radiolabeled via bio-orthogonal reaction between *trans*-cyclooctenes (TCO) and tetrazine (Tz) and can be used as a companion diagnostic PET tracer to monitor therapeutic effect of PARP1 inhibition.[10–12] PARPi-FL was created by replacing the cyclopropane moiety with the green fluorescent BODIPY-FL. It was mainly used for fluorescent imaging although it could be labeled with F-18 via ion exchange to become a dual-modality agent for both PET and fluorescent imaging. The low specific activity after F-18 labeling and the in vivo defluorination which results in high bone uptake limit its application in PET imaging.[12–14] It should be noted that although both modifications introduced bulky substituents to replace the small cyclopropane moiety, the binding affinities were only slightly affected. Combined with observation from the affinity of the library analogues,[9] it turned out the molecule can tolerate a variety of modification and still keep the potency intact or slightly impacted.

Our lab has been studying malignant mesothelioma (MM) treatment for last decade.[15,16] MM is an asbestos-related tumor that forms in the thin layer of tissue that covers the lung, chest wall, or abdomen. Its prognosis remains poor due to the diagnostic challenge and resistance to conventional therapies. A recent report shows that PARP1 is highly expressed in MM cells, and suggests

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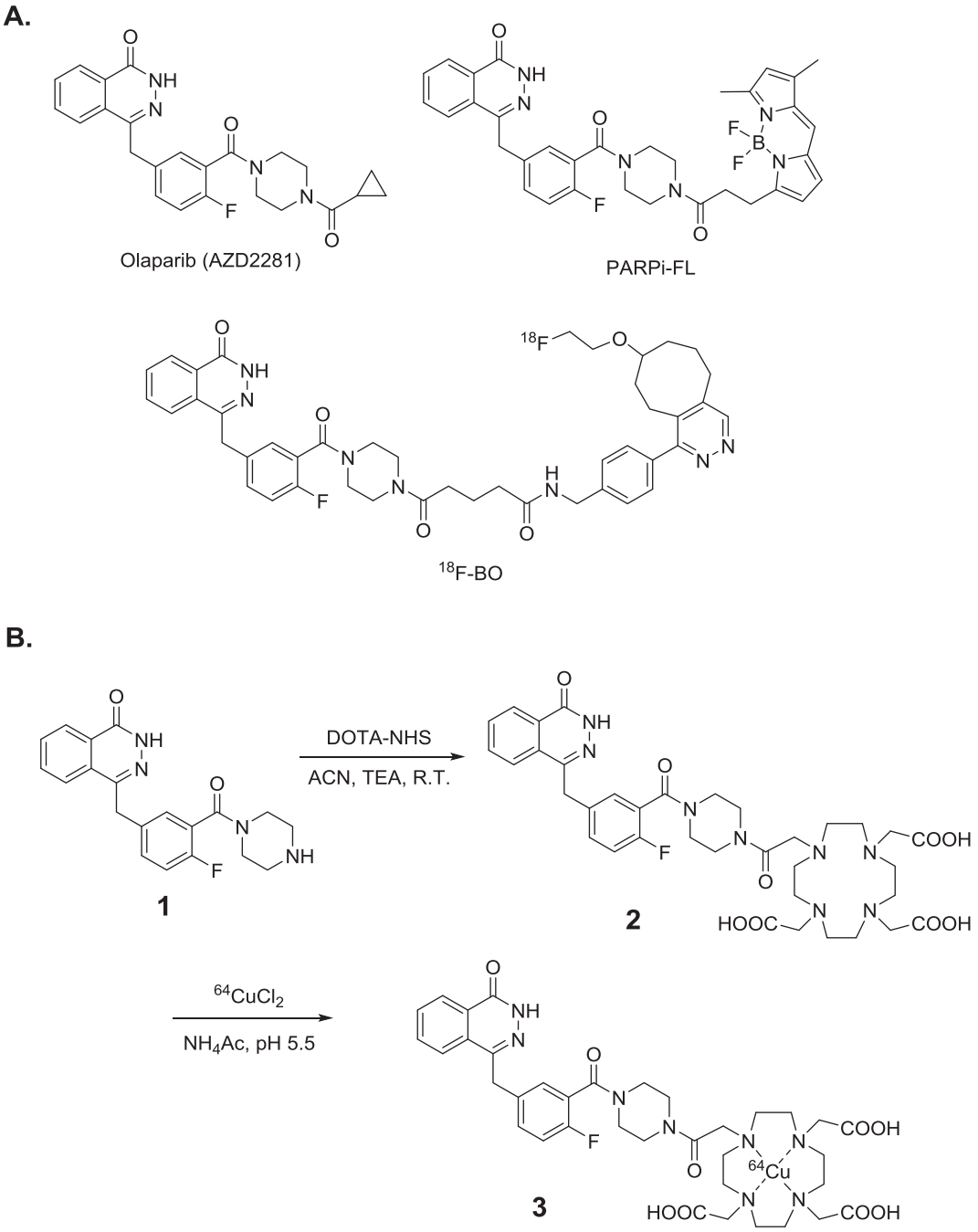

Fig. 1. (A) Structure of Olaparib (AZD2281), PARPi-FL (PET and Fluorescent dual modality probe), and 18F-BO (PET probe); (B) Synthesis of the designed compound **3**.

that chemoresistance to MM treatment may result from a higher level of PARP1-mediated DNA repair.[17] With this regard, we proposed a proof-of-concept study to develop an Olaparib-derived agent targeting PARP for MM imaging or/and therapy. Different from the previous F-18 labeled agents, we would like to modify this molecule with introduction of Cu-64, a PET isotope with favorable characteristics ($T_{1/2}$ = 12.7 h, β^+ 17.4%, E_{max} = 0.656 MeV, β^- 39%, E_{max} = 0.573 MeV).[18] Compared with F-18 ($T_{1/2}$ = 109 min), there is a longer physical half-life for Cu-64 which allow more time for time-sensitive operations. More importantly, Cu-64 or other isotope Cu-67 has therapeutic efficacy[19] by different radiation and therefore, can be used for both PET imaging and radiotherapy. Moreover the radiotherapy effect can be synergistically enhanced by blocking PARP repair pathway.

As outlined in Fig. 1B, the compound **2** was synthesized by incubating a mixture of 4-(4-fluoro-3-(piperazine-1-carbonyl)benzyl) phthalazin-1(2*H*)-one (compound **1**, 73 mg, 0.2 mmol) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono-*N*-hydroxysuccinimide ester (DOTA-NHS, 153 mg, 0.2 mmol) dissolved in 2 mL acetonitrile (ACN) in the presence of triethylamine (TEA) overnight under room temperature, purified with reverse-phase thin lay tomography (RP-TLC) plate and characterized by MALDI-TOF mass spectroscopy (W.M. Keck Biomedical mass spectrometry laboratory at the University of Virginia). The analyzed *m*/*z* results were $[M+H]^+$ 753.3, $[M+K]^+$ 791.3, which confirmed the successful conjugation. The chemical yield was around 55%.

After conjugation with DOTA, the bioactivity of the compound **2** was assessed following a reported assay,[20] which gave a IC50 of