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Appendix E1

Reagents for Animal Experiments and Experimental Groups

All data reported for the olaparib and iniparib treatment groups were obtained by analyzing the activity in organs from mice implanted with either SCC1 (seven in the olaparib group and four in the iniparib group) or MDA-MB-231 tumors (seven each in the olaparib and iniparib groups). The median weights of the mice were 22.5 g (IQR: 2.1 g) and 22.8 g (IQR: 1.8 g) for the olaparib and iniparib treatment groups, respectively.

Tumor Implantation Protocol

The HNSCC cell line SCC1 was propagated in Dulbecco's modified eagle's medium supplemented with 10% fetal bovine serum (FBS), 1fbs% penicillin-streptomycin, and 100 ng/mL hydrocortisone (Sigma-Aldrich, St Louis, Mo). The human breast cancer cell line MDA-MB-231 (American Type Culture Collection, Manasses, Va) was propagated in Eagle's minimum essential medium supplemented with 5% FBS, 2% vitamins for minimum essential media, 1% 200 mmol/L L-glutamine, 1% 10 mmol/L nonessential amino acid. All cell culture reagents not otherwise annotated were purchased from Gibco (ThermoFisher Scientific, Carlsbad, Calif). Olaparib (Selleckchem), a known PARP inhibitor, and iniparib (Selleckchem), originally believed to act as a PARP inhibitor but later demonstrated to have no effect on PARP activity (21,22), were used for in vitro and in vivo experiments.

Four- to six-week-old athymic nude mice were implanted with 2×10^6 SCC1 cells in the mammary fat pads or flank subcutaneously or with 1×10^7 MDA-MB-231 cells in the mammary fat pads for the imaging experiments. Tumor volumes were determined as previously described (36). Tumors were imaged when they reached 100 mm^3 (5). However, many of the MDA-MB-231 tumor growth curves did not match the rate of growth we have observed for previous experiments, suggesting an issue either with the tumor implantation protocol or cell line. In the end, only six MDA-MB-231 tumors in three mice in the olaparib group and three tumors in two mice in the iniparib group were evaluable, yielding too few tumors for analysis. Therefore, these results are not reported. Although four evaluable SCC1 tumors in four mice in the iniparib treatment group and 10 evaluable tumors in seven mice in the olaparib group, the SCC1 tumors also had low uptake levels that was just above background; thus, the uptake in these tumors could not be quantified reliably. Therefore, the results in these tumors are also not included in this report. Because the axillary lymph nodes and the spine were easily visible on the micro-PET images, and the nodes could be resected for PARP enzyme assays, the goal of demonstrating differential effects of olaparib and iniparib was achieved by evaluating normal organs with PARP expression instead of focusing on the tumor data.

Animal Biodistribution Studies and Metabolism Studies

Mice were injected intravenously via the tail vein with ^{18}F-FTT and sacrificed by cervical dislocation 5 or 30 minutes after injecting 1.1 MBq, 1 or 2 hours after injecting 1.7 MBq, or 4 hours after injecting 2.2 MBq of ^{18}F-FTT ($n = 4$ at each time point, except $n = 3$ at 2 hours after tracer injection). Blood, heart, lung, muscle, liver, spleen, fat, adrenal glands, kidney, uterus,

ovaries, bone, bone marrow, pancreas, stomach, small intestine, and large intestine were collected from each animal, blotted to remove excess blood, and weighed. Urine and feces were also collected. All organs and excretions were counted in a Beckmann 6000 gamma counter to determine the percentage injected dose per gram.

Mice injected intravenously with approximately 14.8 MBq of ^{18}F-FTT were sacrificed by cervical dislocation 5 or 30 minutes after injection (n = 3 each time point) to collect blood and liver samples for metabolism studies. The inferior vena cava was lacerated for blood collection. The plasma was separated from the red blood cells by centrifugation at 14 000 rpm. Plasma (100 µL) was mixed with acetonitrile at a 1:1.5 ratio and centrifuged. Radioactivity in the red blood cell pellet, whole plasma, and acetonitrile-soluble and insoluble fractions was measured. The liver was frozen on dry ice immediately after harvest and homogenized in 2 mL acetonitrile. Then, a 1 mL aliquot was centrifuged. Radioactivity associated with the supernatant and pellet was measured in a gamma counter. Acetonitrile-soluble plasma or liver supernatant (100 µL) was then mixed at a 1:1 ratio with water and separated by reverse-phase high-performance liquid chromatography (HPLC). The parent compound was also assayed directly by HPLC as a reference. The radioactivity associated with each HPLC fraction was measured. The amount of parent compound in each plasma and liver sample was expressed as a percentage of the total activity in each sample.

Estimation of Human Dosimetry from Murine Biodistribution Results

Initial estimates of the human organ radiation exposure were obtained from the biodistribution data in mice. The mouse organ residence times were calculated using standard Medical Internal Radiation Dose methodology as previously described (37). The residence times calculated from these data were entered into the program OLINDA/EXM to determine the estimated human dosimetry using male and female anthropomorphic models. Based on this estimate, a dose of 10 mCi was selected for the human dosimetry study.

Micro-PET Imaging Protocol and Analysis

Mice were induced with 2% isoflurane followed by 1% isoflurane via nose cone for anesthesia maintenance during imaging. The mice were injected intravenously via a tail vein with 11.4 MBq ± 0.5 of ^{18}F-FTT and underwent a 60-minute dynamic scan with either Focus 220 (Siemens/CTI) or Inveon PET/CT (Siemens) scanners along with CT imaging on the Inveon. Mice were randomly assigned to each scanner and imaged on the same scanner before and after treatment. Posttreatment scans were obtained the next day, with olaparib or iniparib given 30 minutes before ^{18}F-FTT injection. The PET and CT images were coregistered and analyzed by using Integrated Research Workplace software (Siemens). Regions of interest (ROIs) were drawn over all visible organs with uptake (axillary lymph nodes, lungs, left ventricle cavity for blood pool, liver, gallbladder, small intestine, and kidneys) to determine the effects of olaparib and iniparib treatment on the time-activity curves. The time-activity curves were represented as the standard uptake value and then used to determine the AUC, which is the area under the ensemble-averaged time–standardized uptake value curve. The ensemble-averaged time–standardized uptake value curve was created for each organ by averaging the N individual time–standardized uptake value curve values at each time point. The AUC was then computed by means of the trapezoid rule (with NumPy v1.11 in Python 3.5) for the entire 60-minute curve.

Human Image Acquisition Protocol and Image Analysis

PET/CT images from the midskull to the midcalves were obtained in healthy volunteers or participants with cancer at the following times: 0 and 30 minutes (2 minutes per bed position), 60 and 90 minutes (3 minutes per bed position), 120 and 150 minutes (4 minutes per bed position), and 180 and 210 minutes (5 minutes per bed position). Images from two participants with a cancer diagnosis and two healthy volunteers were evaluated for each time point. CT images were obtained with 50 mAs (effective) for attenuation correction. PET images were reconstructed by means of three-dimensional ordered subset expectation maximization with the point spread function correction model (3D-OSEM +PSF) by using two iterations and 21 subsets. A 2-mm Gaussian filter was applied after reconstruction by using a 168 × 168 matrix. The final image pixel size was 4.07 × 4.07 mm. ROIs were placed manually over all visible organs on the CT images (brain, all visible bones including bone marrow, spine including bone marrow, heart, lungs, kidneys, spleen, liver, stomach, gallbladder, small bowel, large bowel, urinary bladder, pancreas, muscle, and fat) as well as the whole body by using Integrated Research Workplace (Siemens) and then transferred to the PET images. The ROIs were then further edited to avoid spillover from adjacent organs into each ROI. Urine excreted between scans was collected, a 1e-mL aliquot counted on a gamma counter, and the activity multiplied by the total measured urine volume to determine the total amount of excreted urinary activity. The percentage injected dose was then determined for each organ and used for determining the residence times for dosimetry calculations.

Human Dosimetry Estimate Calculation

The individual organ residence times were calculated from the percentage injected dose for each organ derived from the human ^{18}F-FTT PET images. The percentage injected dose per organ was plotted versus time and regression fits determined to estimate the residence time by determining the AUC of the regression fit. Given the small number of subjects in this pilot study, we chose to estimate the uncertainty in the radiation dose estimate by using the 95% confidence bounds on the regression parameters used to determine the residence times and then computing the minimal and maximal residence times for each organ. These limits were then entered in OLINDA to generate a minimum and maximum organ radiation dose estimate. These limits represent the range of radiation doses observed for this tracer in the patient population we studied and are mostly driven by interpatient fluctuations.

Detailed Eligibility Criteria for Healthy Volunteers and Participants with Cancer

Inclusion Criteria

Inclusion criteria were as follows: men or women aged 18 years age or older and healthy volunteer or have biopsy-proved diagnosis of head and neck squamous cell cancer or any histopathologic type of lung cancer or any other type of cancer that can be treated with platinum-based chemotherapy as first-line therapy (which includes but is not limited to ovarian, gastric, and pancreatic cancers).

Exclusion Criteria

Exclusion criteria were as follows: history of claustrophobia or other preventing condition that has previously or would interfere with completion of protocol specified imaging sessions; inability to comprehend or unwillingness to follow instructions for study procedures required per protocol; inability to lie in the PET/CT scanner for the time required for scanning, up to 1 hour and 15 minutes at a time and possibly with arms raised above the head for lung imaging; presence of an implanted device that is incompatible with CT scanning. For dosimetry studies (arm only), healthy volunteers must not have a history of cardiopulmonary conditions requiring any treatment or medical intervention or be a current smoker.

References

36. Zhang K, Jones L, Lim S, et al. Loss of Trop2 causes ErbB3 activation through a neuregulin-1-dependent mechanism in the mesenchymal subtype of HNSCC. Oncotarget 2014;5(19):9281–9294.

37. Laforest R, Dehdashti F, Lewis JS, Schwarz SW. Dosimetry of 60/61/62/64Cu-ATSM: a hypoxia imaging agent for PET. Eur J Nucl Med Mol Imaging 2005;32(7):764–770.

Table E1. Mouse Biodistribution Data

Organ	Percentage Injected Dose per Gram				
	5 min (*n* = 4)	30 min (*n* = 4)	60 min (*n* = 4)	120 min (*n* = 3)	240 min (*n* = 4)
Blood	1.6/0.3	2.0/0.09	2.3/0.5	2.5/0.1	1.6/0.03
Heart	6.6/0.4	3.3/0.2	2.8/0.1	2.7/0.1	1.7/0.08
Lung	12/1.1	5.4/0.8	3.9/0.3	3.2/0.3	1.9/0.4
Muscle	2.0/0.4	2.4/0.1	2.2/0.4	2.0/0.1	1.4/0.1
Liver	13/1.1	8.1/0.6	6.4/0.6	4.6/0.3	2.4/0.2
Spleen	10/5.7	26/2.2	25/2.0	18/1.0	9.9/0.7
Fat	1.1/0.6	1.1/0.26	1.0/0.5	1.1/0.1	0.5/0.5
Adrenals	12/0.4	55/1.1	3.6/1.7	2.7/0.4	1.6/0.3
Kidney	35.7/3.0	22/2.4	14/1.5	8.6/0.7	3.7/0.7
Uterus	2.9/1.3	4.7/1.2	5.2/0.5	4.1/0.2	2.1/0.4
Ovaries	4.6/1.9	5.3/1.1	4.6/0.4	3.9/0.5	2.1/0.4
Bone	3.5/0.5	4.3/0.2	5.2/0.6	6.9/0.7	9.4/1.5
Marrow	0.06/0.03	0.10/0.02	0.13/0.06	0.07/0.01	0.04/0.01
Pancreas	9.4/3.0	10.7/0.7	9.1/1.9	5.1/0.6	2.9/0.2
Stomach	3.0/0.4	3.9/0.7	3.5/0.3	2.7/0.3	1.2/0.1
SI	13/0.9	13.00/0.75	8.9/1.7	6.3/0.7	3.0/0.2
ULI	7.7/0.8	14.07/1.72	15/2.1	10/0.6	4.5/0.5
LLI	4.2/0.3	7.43/0.59	11/1.1	13/0.9	7.1/0.5
Thyroid	4.18/1.18*	4.1/0.2	3.3/0.2	3.4/0.1	3.0/0.3
Brain	0.55/0.16	1.0/0.1	1.2/0.2	1.7/0.2	1.2/0.07

Note.—Data are medians/IQRs. LLI = lower large intestine, SI = small intestine, ULI = upper large intestine.

* Data are from three mice owing to one missing sample.

Table E2. Vital Signs and Blood Work Results before and after Scan Sessions

Parameter	Healthy Subjects					Subjects with Cancer				
	Before Imaging		After Imaging		*P* Value*	Before Imaging		After Imaging		*P* Value*
	Median	IQR	Median	IQR		Median	IQR	Median	IQR	
Temperature (°F)	97	0.8	98	0.5	ND	98	1	98	0.3	ND

Heart rate (beats/min)	63	12	60	20	0.48	71	17	67	11	0.09
Systolic blood pressure (mm Hg)	113	10	120	14	0.34	113	33	130	17	0.04
Diastolic blood pressure (mmHg)	70	6	72	10	0.61	73	11	79	8	0.23
Mean arterial pressure (mm Hg)	84	7	88	14	0.53	89	18	99	10	0.11
Respiratory rate (breaths/min)	12	0.5	12	0.3	0.10	13	5	14	2	1.00
% Oxygen saturation	97	4	100	1	0.07	99	1	100	1	0.12
White blood cells (10^3 mm^3)	5.7	1.8	6.8	3.4	0.04	4.1	1.4	4.0	1.6	0.78
Hemoglobin (g/dL)	12.9	1.4	13.4	1.2	0.03	11.5	1.1	11.3	1.5	0.57
Hematocrit	39.6	3.7	41.2	2.3	0.07	34.6	4.7	34.0	5.6	0.94
Platelets (10^3 mm^3)	214	48	212	41	0.48	171	59	168	59	0.62
% Neutrophils	59	13	54	13	0.62	61	15	63	12	0.06
Absolute neutrophils (10^3 mm^3)	3.2	1.8	3.5	3.2	0.29	2.4	1.5	2.7	1.6	0.09
% Lymphocytes	30	13	33	12	0.26	24	14	21	12	0.08
Absolute lymphocyte (10^3 mm^3)	1.8	0.2	2.2	0.4	0.02	1.2	0.8	0.9	0.7	0.21
% Monocytes	7.7	3.7	7.0	2.3	0.11	10.7	4.4	10.4	4.5	0.83
Absolute monocytes (10^3 mm^3)	0.5	0.3	0.5	0.3	1.00	0.5	0.2	0.5	0.2	0.85
% Eosinophils	3.5	3.7	3.3	2.7	0.29	5.2	2.3	4.1	2.0	0.15
% Basophils	0.5	0.3	0.5	0.3	0.18	0.4	0.2	0.4	0.4	0.89
Sodium (mmol/L)	141	1	141	4	0.24	141	3	140	3	0.08
Potassium (mmol/L)	4.0	0.3	3.9	0.4	0.20	4.2	0.7	3.9	0.5	1.00
Chloride (mmol/L)	105	3	103	6	0.68	105	3	103	4	0.57
Carbon dioxide (mmol/L)	27.0	2.8	27.0	4.0	0.60	27.0	2.3	27.0	3.0	0.59
Anion gap (mmol/L)	9.5	2.3	9.5	2.3	0.20	10.5	4.3	9.5	1.5	0.30
Glucose (mg/dL)	84	10	84	12	0.73	83	8	101	29	0.03
Urea nitrogen (mg/dL)	11	3	17	8	0.58	11	4	19	10	0.02
Creatinine (mg/dL)	0.8	0.2	0.8	0.3	0.48	0.7	0.2	0.9	0.4	0.23
Calcium (mg/dL)	9.5	0.6	9.4	1.0	0.45	9.7	0.2	9.2	0.3	0.57
Plasma protein (g/dL)	7.1	0.5	6.9	0.6	0.92	7.2	0.2	7.0	0.6	0.72
Albumin (g/dL)	4.3	0.1	3.9	0.6	0.62	4.4	0.2	4.0	0.4	0.34
Total bilirubin (mg/dL)	0.5	0.3	0.3	0.2	0.20	0.4	0.1	0.3	0.2	0.17
Alkaline phosphatase (U/L)	65	26	78	47	1.00	65	23	87	33	0.15
AST (U/L)	22	4	30	10	0.40	23	4	29	13	0.89
ALT (U/L)	17	6	31	22	0.60	18	7	34	19	0.83

Note.—ALT = alanine transaminase, AST = aspartate aminotransferase.

* Level of significance for *P* value set at .0015 for 33 comparisons with Bonferroni correction.

Table E3. Residence Times Calculated from Human ^{18}F-FTT PET Images

Organ	Residence Time (h)	
	Male	Female
Brain	0.0241	0.0222
Skeleton	0.2030	0.2455
Vertebrae	0.0918	0.1790
Heart	0.0149	0.0152
Lungs	0.0194	0.0215
Kidneys	0.0191	0.0285
Spleen	0.0236	0.0366

Liver	0.2015	0.2353
Stomach	0.0125	0.0165
Gallbladder	0.0016	0.0010
Small.intestine	0.0731	0.0863
Large.bowel	0.0102	0.0092
Muscle	1.1816	1.1322
Fat	0.1128	0.1024
Full.body	2.5205	2.4358
Pancreas	0.0130	0.0172
Thyroid	0.0013	0.0021
Bladder	0.0041	0.0041
Excreted	0.0064	0.0064
Remainder	0.7444	0.5870