**Supporting information for**

**89Zr-anti-γH2AX-TAT but not 18F-FDG allows early monitoring of response to chemotherapy in a mouse model of pancreatic ductal adenocarcinoma**

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**Supplemental Methods**

**PET/CT Imaging**

*General*

PET/CT imaging was performed using an Inveon PET/CT scanner (Siemens Preclinical Solutions) equipped with a custom-built imaging cradle. CT based attenuation correction was performed before each PET emission scan and was also used for anatomical referencing. List-mode data were acquired for 30 minutes using a γ-ray energy window of 350–650 keV and a coincidence timing window of 3.432 ns. Data were histogrammed and global deadtime correction and Fourier rebinning were applied. The histograms were reconstructed using a 2-dimensional filtered back-projection algorithm, a Ramp projection filter, a 0.5/mm Nyquist projection cut-off value, no zoom, and a matrix size of 128×128×159 (sagittal×coronal×transversal). Mice were kept under anaesthesia by inhalation of 2% isofluorane in air and maintained at 37°C. Volume-of-interest analyses were performed using the Inveon Research Workplace software package.

**5-FU therapy**

*89Zr-anti-γH2AX-TAT:* When tumours reached ~3 mm in diameter, mice were administered either 5-FU (40 mg/kg in 0.9% saline) or vehicle control *via* intraperitoneal injection. At 48 h after treatment, mice were then administered 89Zr-anti-γH2AX-TAT or 89Zr-RIgG-TAT (0.5 MBq, 5 µg) in sterile PBS (100 µL) by injection into a lateral tail vein. PET/CT images were then acquired at 24 h post-injection (p.i.) of the imaging agent.

*18F-FDG:* When tumours reached ~3 mm in diameter, mice were administered either 5-FU (40 mg/kg in 0.9% saline) or vehicle control on days 0, 2, and 4 *via* intraperitoneal injection. Mice were then fasted for at least 4 h prior to intraperitoneal administration of 18F-FDG (5 MBq in ~100 µL of 0.9% saline) on day 0 (immediately before treatment), day 3 and day 9. Water was provided ad libitum. After an uptake period of 1 h during which mice were conscious, PET/CT images were then acquired.

**Gemcitabine therapy**

*89Zr-anti-γH2AX-TAT:* When tumours reached ~5 mm in diameter, mice were administered either gemcitabine (200 mg/kg in 0.9% saline) or vehicle control *via* intraperitoneal injection. At 48 h after treatment, mice were then administered 89Zr-anti-γH2AX-TAT or 89Zr-RIgG-TAT (0.5 MBq, 5 µg) in sterile PBS (100 µL) by injection into a lateral tail vein. PET/CT images were then acquired at 24 h p.i. of the imaging agent.

*18F-FDG:* When tumours reached ~5 mm in diameter, mice were administered either gemcitabine (200 mg/kg in 0.9% saline) or vehicle control on days 0 and 5 *via* intraperitoneal injection. Mice were then fasted for at least 4 h prior to intraperitoneal administration of 18F-FDG (5 MBq in ~100 µL of 0.9% saline) on day 3 and day 8. Water was provided ad libitum. After an uptake period of 1 h during which mice were conscious, PET/CT images were then acquired.

**Capecitabine therapy**

*89Zr-anti-γH2AX-TAT:* When tumours reached ~3 mm in diameter, mice were administered either capecitabine (400 mg/kg in 40 mM citrate buffer pH 6.0 and 5% Gum Arabic) or vehicle control *via* oral gavage once daily for 7 days. After the final treatment of day 7, mice were then administered 89Zr-anti-γH2AX-TAT or 89Zr-RIgG-TAT (0.05 MBq, 5 µg) in sterile PBS (100 µL) by injection into a lateral tail vein. PET/CT images were then acquired at 24 h p.i. of the imaging agent.

*18F-FDG:* When tumours reached ~3 mm in diameter, mice were administered either capecitabine (400 mg/kg in 40 mM citrate buffer pH 6.0 and 5% Gum Arabic) or vehicle control *via* oral gavage once daily for 7 days. Mice were then fasted for at least 4 h prior to intraperitoneal administration of 18F-FDG (5 MBq in ~100 µL of 0.9% saline) on day 3 and day 8. Water was provided ad libitum. After an uptake period of 1 h during which mice were conscious, PET/CT images were then acquired.

**Results of Capecitabine Therapy Monitoring Experiments**

*89Zr-anti-γH2AX-TAT*

These experiments differed from 5-FU and gemcitabine experiments as 89Zr-anti-γH2AX-TAT was administered at the lower specific activity of 0.01 MBq µg-1. Consequently, PET images of satisfactory quality could not be obtained. Despite administering a lower specific activity preparation, *ex vivo* biodistribution data obtained on Day 7 (Table S5) (before a difference in RTV was observed between capecitabine-treated and vehicle-treated mice) indicated that 89Zr-anti-γH2AX-TAT achieved an uptake value of 7.79±0.89 %ID/g (Fig. S2A and S2B). Consistent with 5-FU and gemcitabine experiments, ANOVA testing indicated that there was a significant difference (*P*<0.05) between the means of the independent experimental groups. Analysis of confocal microscopy images of harvested allograft tissues revealed significantly higher levels of γH2AX in the tumours of capecitabine-treated versus vehicle-treated mice (39±14 and 26±13 a.u., respectively; *P*<0.001; Figs S2C and S2D).

*18F-FDG*

PET images acquired after three days and after seven days of daily oral gavaging of capecitabine revealed no significant differences in tumour uptake of 18F-FDG between capecitabine-treated and vehicle-treated mice (Fig. S3A-C). Within the timescale of this experiment, no impediment of tumour growth was observed as a result of capecitabine treatment, however the results of the previously described 89Zr-anti-γH2AX-TAT experiments indicated that capecitabine was exerting a therapeutic effect sufficient to activate DDR signalling pathways. Lastly, analysis of confocal microscopy images of sections of tumour tissue acquired on Day 7 revealed that no reduction in GLUT-1 expression levels occurred as a result of capecitabine therapy (Figs. S3D and S3E).

**TABLE S1:** *Ex vivo* biodistribution data acquired at 24 h p.i. of 89Zr-anti-γH2AX-TAT or 89Zr-RIgG-TAT (0.1 MBq µg-1) in 5-FU-treated mice. Values are %ID/g ± SD.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 89Zr-anti-γH2AX-TAT  5-FU-treated (*n*=5) | 89Zr-anti-γH2AX-TAT  vehicle (*n*=5) | 89Zr-RIgG-TAT  5-FU-treated (*n*=5) | 89Zr-RIgG-TAT  vehicle (*n*=5) |
| Blood | 13.00±3.17 | 13.27±1.60 | 13.53±2.29 | 12.95±2.57 |
| Tumour | 8.55±1.06 | 6.12±1.11 | 5.28±1.88 | 5.35±1.49 |
| Heart | 3.84±0.81 | 3.53±0.62 | 3.41±0.38 | 3.35±0.49 |
| Lung | 5.15±1.24 | 5.36±0.78 | 5.34±1.07 | 5.46±0.68 |
| Liver | 5.36±1.71 | 7.83±4.20 | 4.49±0.71 | 5.30±1.26 |
| Spleen | 6.74±0.62 | 6.68±1.36 | 6.41±1.09 | 5.63±1.53 |
| Stomach | 0.83±0.34 | 0.73±0.19 | 0.72±0.15 | 0.73±0.27 |
| Large intestine | 1.35±0.49 | 1.40±0.05 | 1.21±0.17 | 1.23±0.23 |
| Small intestine | 1.67±0.21 | 1.58±0.12 | 1.51±0.10 | 1.21±0.72 |
| Pancreas | 1.64±0.27 | 1.75±0.38 | 1.86±0.26 | 1.71±0.21 |
| Kidney | 3.55±0.51 | 3.67±0.63 | 3.78±0.43 | 3.90±0.37 |
| Muscle | 0.76±0.08 | 0.76±0.16 | 0.83±0.06 | 0.81±0.08 |
| Bone | 2.50±0.38 | 2.10±0.45 | 2.62±0.55 | 2.36±0.27 |
| Skin | 1.72±0.27 | 1.54±0.31 | 1.74±0.23 | 2.30±1.03 |
| Fat | 1.64±0.70 | 1.48±0.49 | 1.73±0.36 | 1.46±0.41 |

**TABLE S2:** *Ex vivo* biodistribution data acquired at 1.5 h p.i. of 18F-FDG in 5-FU-treated mice. Values are %ID/g ± SD.

|  |  |  |
| --- | --- | --- |
|  | 18F-FDG  5-FU-treated  (*n*=5) | 18F-FDG  vehicle (*n*=5) |
| Blood | 0.96±0.24 | 0.83±0.17 |
| Tumour | 7.88±2.14 | 8.31±1.53 |
| Heart | 25.63±5.80 | 30.84±4.79 |
| Lung | 3.45±0.56 | 3.41±0.34 |
| Liver | 1.77±0.24 | 1.62±0.18 |
| Spleen | 4.02±0.75 | 4.14±0.72 |
| Stomach | 1.53±0.30 | 1.88±0.53 |
| Large intestine | 2.85±0.57 | 2.27±0.38 |
| Small intestine | 3.21±0.94 | 3.27±0.45 |
| Pancreas | 3.00±1.01 | 2.23±0.59 |
| Kidney | 3.68±0.68 | 3.38±0.67 |
| Muscle | 1.08±0.50 | 0.96±0.23 |
| Skin | 1.13±0.21 | 2.07±1.52 |
| Fat | 2.12±1.09 | 2.79±1.47 |

**TABLE S3:** *Ex vivo* biodistribution data acquired at 24 h p.i. of 89Zr-anti-γH2AX-TAT or 89Zr-RIgG-TAT (0.1 MBq µg-1) in gemcitabine-treated mice. Values are %ID/g ± SD.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 89Zr-anti-γH2AX-TAT  Gemcitabine-treated (*n*=4) | 89Zr-anti-γH2AX-TAT  vehicle (*n*=4) | 89Zr-RIgG-TAT  Gemcitabine-treated (*n*=3) | 89Zr-RIgG-TAT  vehicle (*n*=4) |
| Blood | 11.75±3.30 | 10.17±2.22 | 12.83±1.64 | 11.14±1.83 |
| Tumour | 6.81±0.62 | 5.06±0.52 | 5.02±0.96 | 4.57±1.08 |
| Heart | 3.08±0.41 | 2.73±0.29 | 3.50±0.59 | 2.83±0.54 |
| Lung | 3.63±0.47 | 3.59±0.18 | 4.07±0.05 | 4.93±1.32 |
| Liver | 7.34±0.89 | 7.35±0.43 | 5.85±0.15 | 5.50±0.86 |
| Spleen | 6.83±1.28 | 8.46±0.91 | 6.39±0.28 | 6.43±1.23 |
| Stomach | 0.80±0.21 | 1.01±0.15 | 0.78±0.21 | 0.93±0.20 |
| Large intestine | 1.60±0.26 | 1.68±0.32 | 0.99±0.20 | 1.24±0.07 |
| Small intestine | 1.32±0.14 | 1.25±0.12 | 1.41±0.04 | 1.25±0.27 |
| Pancreas | 1.81±0.30 | 1.42±0.31 | 1.97±0.19 | 1.40±0.26 |
| Kidney | 6.21±0.45 | 6.18±0.70 | 4.19±0.28 | 4.01±1.00 |
| Muscle | 0.53±0.22 | 0.51±0.16 | 1.06±0.36 | 1.18±0.62 |
| Bone | 4.36±2.25 | 4.33±0.88 | 4.80±0.94 | 4.65±3.10 |
| Skin | 1.99±0.77 | 1.48±0.55 | 4.57±1.93 | 3.07±2.87 |
| Fat | 2.01±0.82 | 1.50±0.26 | 2.57±0.78 | 1.71±0.84 |

**TABLE S4:** *Ex vivo* biodistribution data acquired at 1.5 h p.i. of 18F-FDG in gemcitabine-treated mice. Values are %ID/g ± SD.

|  |  |  |
| --- | --- | --- |
|  | 18F-FDG  Gemcitabine-treated (*n*=3) | 18F-FDG  vehicle (*n*=3) |
| Blood | 0.82±0.45 | 0.62±0.30 |
| Tumour | 7.61±1.50 | 3.31±1.97 |
| Heart | 19.08±11.14 | 8.83±5.22 |
| Lung | 3.42±1.52 | 3.57±1.81 |
| Liver | 1.99±1.15 | 1.36±0.25 |
| Spleen | 4.24±1.33 | 3.60±1.60 |
| Stomach | 2.26±0.85 | 1.99±1.49 |
| Large intestine | 3.24±1.09 | 1.30±1.09 |
| Small intestine | 3.93±1.18 | 0.67±0.24 |
| Pancreas | 4.33±0.32 | 4.53±1.64 |
| Kidney | 2.45±0.89 | 2.75±2.07 |
| Muscle | 1.83±1.34 | 2.81±1.30 |
| Skin | 2.60±1.33 | 2.57±1.20 |
| Fat | 4.41±1.47 | 3.84±1.23 |

**TABLE S5:** *Ex vivo* biodistribution data acquired at 24 h p.i. of 89Zr-anti-γH2AX-TAT or 89Zr-RIgG-TAT (0.01 MBq µg-1) in capecitabine-treated mice. Values are %ID/g ± SD.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 89Zr-anti-γH2AX-TAT  Capecitabine-treated (*n*=4) | 89Zr-anti-γH2AX-TAT  vehicle (*n*=5) | 89Zr-RIgG-TAT  Capecitabine-treated (*n*=3) | 89Zr-RIgG-TAT  vehicle (*n*=4) |
| Blood | 14.89±5.19 | 14.39±4.27 | 14.24±3.56 | 15.23±1.04 |
| Tumour | 7.79±0.89 | 4.28±1.58 | 3.85±0.60 | 5.00±2.30 |
| Heart | 4.17±1.30 | 6.06±6.17 | 3.88±2.08 | 4.77±2.28 |
| Lung | 5.48±1.26 | 5.60±3.48 | 5.63±3.92 | 5.79±1.82 |
| Liver | 7.50±2.07 | 4.95±0.85 | 6.26±0.37 | 7.81±0.51 |
| Spleen | 6.66±2.61 | 9.16±3.92 | 8.88±1.36 | 10.34±5.66 |
| Stomach | 1.77±1.85 | 2.63±2.95 | 1.64±1.34 | 2.78±2.24 |
| Large intestine | 2.15±0.49 | 2.85±3.24 | 3.02±0.95 | 3.77±2.13 |
| Small intestine | 2.35±0.42 | 2.21±1.66 | 1.90±0.27 | 2.44±0.15 |
| Pancreas | 4.00±3.13 | 2.86±1.10 | 2.57±1.08 | 3.67±1.51 |
| Kidney | 8.09±1.05 | 11.63±7.61 | 7.94±1.85 | 10.25±2.62 |
| Muscle | 2.31±1.73 | 2.25±1.32 | 1.10±0.09 | 1.98±0.98 |
| Bone | 12.41±7.63 | 8.41±5.94 | 8.07±1.19 | 9.43±7.14 |
| Skin | 8.12±8.74 | 6.46±9.40 | 6.81±3.11 | 4.15±5.13 |
| Fat | 4.67±4.29 | 7.04±6.44 | 6.21±1.03 | 6.73±6.53 |

**TABLE S6:** *Ex vivo* biodistribution data acquired at 1.5 h p.i. of 18F-FDG in capecitabine-treated mice. Values are %ID/g ± SD.

|  |  |  |
| --- | --- | --- |
|  | 18F-FDG  Capecitabine-treated (*n*=5) | 18F-FDG  vehicle (*n*=4) |
| Blood | 0.51±0.24 | 0.69±0.20 |
| Tumour | 16.68±3.80 | 10.40±2.04 |
| Heart | 27.99±4.67 | 23.59±7.95 |
| Lung | 2.71±0.56 | 2.82±0.50 |
| Liver | 1.68±0.33 | 1.77±0.38 |
| Spleen | 2.57±0.59 | 2.48±0.58 |
| Stomach | 1.99±1.56 | 1.82±0.34 |
| Large intestine | 1.58±0.26 | 2.09±0.33 |
| Small intestine | 2.41±0.73 | 2.36±0.46 |
| Pancreas | 2.16±0.43 | 2.30±0.47 |
| Kidney | 3.73±2.03 | 3.15±1.04 |
| Muscle | 1.52±0.51 | 1.60±0.40 |
| Skin | 3.16±2.42 | 2.01±0.19 |
| Fat | 1.98±1.32 | 3.96±2.19 |

**Fig. S1** Representative iTLCs of crude reaction mixtures upon completion of reaction (left) and quality control of the purified product (right)



**Fig. S2** Monitoring capecitabine therapy with 89Zr-anti-γH2AX-TAT: (A) Tumour growth curve, (B) Tumour uptake values obtained from *ex vivo* biodistribution experiments, (C) Representative confocal microscopy images, 63x (blue: DAPI, green: γH2AX), (D) Quantification of γH2AX signal on confocal microscopy images normalised to DAPI signal.

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**Fig. S3** Monitoring capecitabine therapy with 18F-FDG: (A) PET/CT images showing coronal (upper) and transaxial (lower) sections intersecting the centre of the allograft tumour (white dotted circle), (B) Tumour growth curve, (C) Tumour uptake values obtained from VOI analysis, (D) Representative confocal microscopy images, 20x (blue: DAPI, green: γH2AX), (E) Quantification of GLUT-1 signal on confocal microscopy images normalised to DAPI signal.

