In vitro and in vivo characterization of MCT1 inhibitor AZD3965 confirms preclinical safety compatible with breast cancer treatment

--- Supplementary data ---

Figure S1. MCTs and CD147/basigin expression in breast-associated cells. (A-C) T47D, MCF7, MCF10A cells and BJ fibroblasts were assayed in medium containing 25 mmol/L glucose with 10 mmol/L GlutaMAX, 10% FBS and without added lactate. (A) Relative basal mRNA expression of MCT1, MCT2 and MCT4. Cumulated expression served for normalization (100%) (n = 3). (B) Relative basal protein expression of MCT1, MCT2 and MCT4. Representative western blots are shown with GAPDH as a loading control. Cumulated expression served for normalization (100%) (n = 3). (C) Representative pictures of immunocytochemical staining of MCT1 (red), MCT2 (red), MCT4 (red) and CD147/basigin (green) on T47D, MCF7, MCF10A cells and BJ fibroblasts. Cell nuclei are stained in blue with DAPI. Bar = 20 µm. All data are show as means ± SEM.
Figure S2. Long-term culture with lactate as only exogenous resource induces breast-associated cell necrosis with limited impact of additional MCT1 inhibition by AZD3965. T47D, MCF7, MCF10A cells and BJ fibroblast density was assayed in medium containing 10 mmol/L sodium L-lactate, no glucose, no glutamine, and 1 % FBS. On day 0, cells were treated ± 10 µmol/L of AZD3965. Graphs show the percentage of viable cells, necrotic cells and apoptotic cells over time determined using flow cytometry after Annexin V and propidium iodide labeling (n = 4-6). All data are show as means ± SEM. *** P < 0.05, ns P > 0.05 comparing whole curves; by two-way ANOVA.
Figure S3. A chronic treatment with AZD3965 does not alter the expression of MCTs and CD147/basigin in mouse skeletal muscles, heart and brain. (A-E) Mouse tissues were collected on the day of sacrifice of Group 2 depicted in Figure 7A. (A) Relative mRNA expression of MCT1, MCT2 and MCT4 in the muscles, heart and brain of vehicle-treated mice. Cumulated expression served for normalization (100%) (n = 4-6). (B) Relative protein expression of MCT1, MCT2 and MCT4 in the muscles, heart and brain of vehicle-treated mice. Representative western blots are shown with GAPDH as a loading control. Cumulated expression served for normalization (100%) (n = 4-6). (C) mRNA (left panel) and protein (middle and right panels) expression of MCT1, MCT2, MCT4 and CD147/basigin in the gastrocnemius muscles of mice treated ± 100 mg/Kg AZD3965 (n = 4-24 for RT-qPCR, n = 5-6 for WB). (D) As in (C) but in whole mouse hearts (n = 3-6 for RT-qPCR, n = 5-6 for WB). (E) As in (C) but in whole mouse brains (n = 5-6 for RT-qPCR, n = 5-6 for WB). All data are show as means ± SEM. ** P < 0.01, ns P > 0.05 compared to corresponding tissues from vehicle-treated animals; by Student’s t test (C-E).
Figure S4. Uncropped western blots

Related to Figure 2A:

- MCT2
- GAPDH
- CD147
- GAPDH

Vehicle
AZD3965
Vehicle
AZD3965
Vehicle
AZD3965
Vehicle
AZD3965
Vehicle
AZD3965

X = unrelated sample
Related to Figure 2B:

- MCT1
- GAPDH

- MCT2
- GAPDH

- MCT4
- CD147

- GAPDH

X = unrelated sample
Related to Figure 2C:

- MCT1
- MCT2
- GAPDH
- CD147
- GAPDH

X = unrelated sample
Related to Figure 2D:

|          | Vehicle | AZD3965 | Vehicle | AZD3965 |
|----------|---------|---------|---------|---------|
| MCT1     | -       | -       | -       | -       |
| GAPDH    | -       | -       | -       | -       |
| MCT4     | -       | -       | -       | -       |
| CD147    | -       | -       | -       | -       |

X = unrelated sample
Related to Figure 5A:

- MCT1 - GAPDH - MCT2
- GAPDH

- MCT4 - GAPDH

- CD147
- GAPDH

Vehicle
AZD3965
Vehicle
AZD3965
Vehicle
AZD3965
Vehicle
AZD3965

X = unrelated sample
Related to Figure 5B:

X = unrelated sample

**MCT1**

- **GAPDH**

**MCT2**

- **GAPDH**

Same membrane rehybridized

**MCT4**

- **CD147**

- **GAPDH**

+ ctrl (SiHa cells)

Vehicle  AZD3965  Vehicle  AZD3965  AZD3965  AZD3965  X  X  X  X  X
Related to Figure 5C:

X = unrelated sample

MCT1 -

GAPDH -

Vehicle
AZD3965
Vehicle
AZD3965
Vehicle
AZD3965
Vehicle
AZD3965

-MCT2

-GAPDH

Vehicle
AZD3965
Vehicle
AZD3965
Vehicle
AZD3965
Vehicle
AZD3965

+ ctrl (SIHa cells)

MCT4 -

-CD147

Same membrane
rehybridized

GAPDH -
Related to Figure 5D:

- MCT1
- MCT2

Same membrane rehybridized
Related to Figure S2C: