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

Vehicle  AZD3965
X  X  Vehicle  AZD3965
X  X  Vehicle  AZD3965
X  X  Vehicle  AZD3965
X  X

MCT1

GAPDH

X = unrelated sample

Vehicle  AZD3965
X  X
Vehicle  AZD3965
X  X
Vehicle  AZD3965
X  X

MCT4

CD147

Vehicle  AZD3965
X  X
Vehicle  AZD3965
X  X
Vehicle  AZD3965
X  X

(MCT4)  GAPDH

GAPDH
Related to Figure 2B:

X = unrelated sample

MCT1 - AZD3965

MCT2 - AZD3965

GAPDH - AZD3965

- CD147

- GAPDH

S5
Related to Figure 2C:

X = unrelated sample

**MCT1**

**GAPDH**

**MCT4**

**GAPDH**

**Vehicle**

AZD3965

AZD3965

AZD3965

AZD3965

S6
Related to Figure 2D:

- MCT1
- GAPDH
- MCT2
- GAPDH
- CD147
- GAPDH

Vehicle, AZD3965, Vehicle, AZD3965, Vehicle, AZD3965, Vehicle, AZD3965, Vehicle, AZD3965

X = unrelated sample
Related to Figure 5A:

X = unrelated sample

MCT1 - GAPDH - MCT2 - GAPDH

MCT4 - GAPDH - CD147 - GAPDH

Vehicle AZD3965 Vehicle AZD3965 Vehicle AZD3965 Vehicle AZD3965

Vehicle AZD3965 Vehicle AZD3965 Vehicle AZD3965 Vehicle AZD3965
Related to Figure 5B:

MCT1 - 

- MCT2

GAPDH - 

- GAPDH

Same membrane rehybridized

S9
Related to Figure 5C:

- MCT1
- GAPDH

Vehicle Vehicle AZD3965 AZD3965 Vehicle Vehicle

- MCT2

Vehicle Vehicle AZD3965 AZD3965 Vehicle Vehicle

- GAPDH

+ ctrl (SiHa cells)

Vehicle Vehicle AZD3965 AZD3965 AZD3965 AZD3965

- CD147

Same membrane rehybridized

GAPDH

Vehicle Vehicle AZD3965 AZD3965 AZD3965 AZD3965

X = unrelated sample
Related to Figure 5D:

[X = unrelated sample]

**MCT1** -

- **MCT2**

**GAPDH** -

*Same membrane rehybridized*

**MCT4** -

- **CD147**

**GAPDH** -

- **GAPDH**
Related to Figure S1B:

- MCT1
- GAPDH
- MCT2
- GAPDH
- MCT4
- GAPDH
Related to Figure S2C: