Topiramate decreases radiation cytotoxic edema in Her2+ brain metastases

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Introduction

- 20-40% of HER2+ breast cancer patients will develop brain metastases (BrM)[1].
- HER2+ patients are treated with Trastuzumab or T-DM1[2].
- Most patients with BM are also treated with radiotherapy (RTx), including SRS
- Clinical evidence suggests that a combination of T-DM1 and RTx results in high rates of radionecrosis (CSRN)[3].

Brain metastases/breast cancer patients SRS treatment n=45			
Receipt T-DM1	All patients	CSRN n (%)	No CSRN n (%)
No	22	1 (4.5%)	21 (95.5%)
Yes	23	**9 (39.1%)**	14 (60.9%)
Receipt T-DM1	**OR (95% CI) for development of CSRN**		**P value**
No	1		
Yes	**13.5** (1.535-118.692)		*0.019*

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To identify potential targets for intervention or mitigation of this side effect:

Goal: To define the mechanisms underlying brain edema and CSRN induced by RTx alone or in combination with T-DM1

- Brain edema results from cytotoxic **(cellular swelling)** and vasogenic **(Blood-Brain Barrier (BBB) disruption)** edema.
- RTx is best known to induce vasogenic edema through upregulation of VEGF leading to endothelial cell disruption.
- Cytotoxic edema is the reversible first step in the sequence of events leading to vasogenic edema and necrosis.
- Astrocytes are gatekeepers of water flow in the brain through modulation of the water channel aquaporin 4 (AQP4).

Hypothesis: T-DM1/RTx-induces cytotoxic edema through upregulation of AQP4 in astrocytes

Prediction: Blockage of AQP4 would prevent astrocytic swelling – cytotoxic edema in Her2 Brain mets

Results

RTx could induce endfeet astrocytic swelling as a pre cytotoxic event

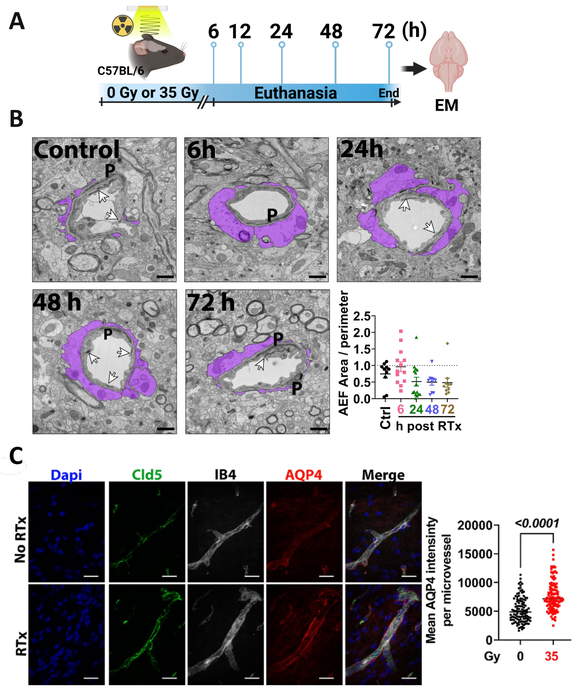


Fig 1 **(A)** Schema of experimental design. **(B)** EM of brain microvessels, non-irradiated mice as control or mice treated with a 35 Gy single doses of WBRT. In purple perivascular endfeet astrocytes, pericytes (P), tight junction (white arrows). Scale bar 1 µm. **(C)** z-stack of brain microvessels. Scale 20 µm. Graph shows mean of AQP4 intensity/ microvessel, n=100 two-tail Mann Whitney test.

RTx increases AQP4 expression and cellular swelling in human astrocytes

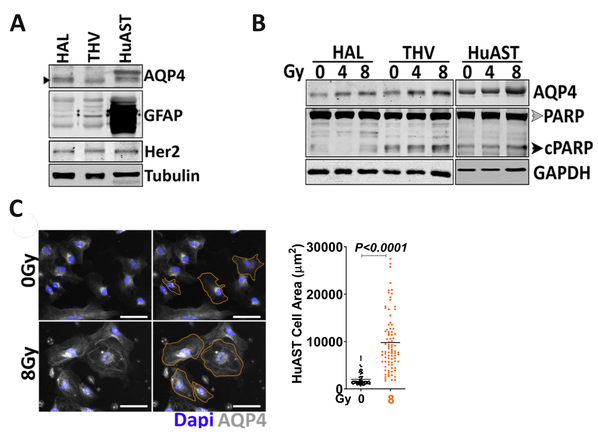


Fig 2 **(A)** AQP4, GFAP, and Her2 expression in human astrocytes **(B)** AQP4 expression and PARP cleavage, 24 h after 4Gy, 8Gy RTx. **(C)** IF of AQP4 in HuAST 24 h after 8Gy RTx. Dots are cell areas of single cells n=100. Two tail Mann-Whitney test. Bar scale 100 µm.

The AQP4 inhibitor Topiramate decreases T-DM1/ RTX induced swelling and loss of transelectric resistance.

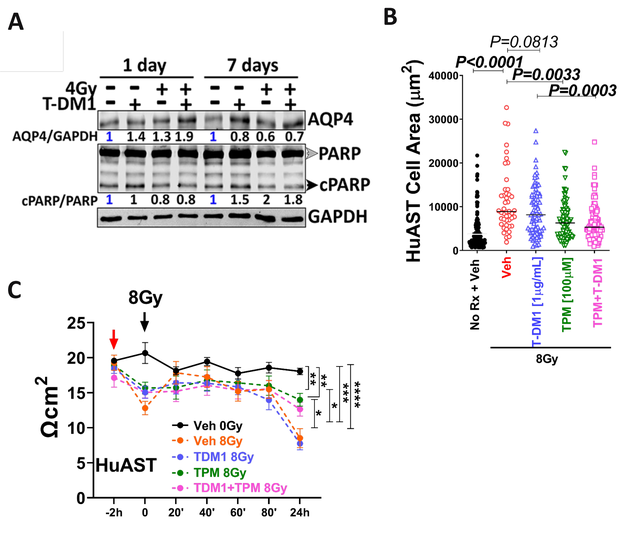


Fig 3. **(A)** WB of AQP4 and PARP cleavage of HuAST treated with RTx alone or combined with T-DM1 [1 µg/mL]. **(B)** *C*ell area in µm2 of HuAST treated with Vehicle (DMSO), 1 µg/mL T-DM1, 100 µM TPM, or the combination TPM+T-DM1, during 48 h after 8 Gy RTx. Two tail Mann-Whitney. **(C)** TEER (Ωcm²) measures of HuAST grown on transwell inserts treated for 2 h as indicated before 8Gy of RTx. Two-way ANOVA Tukey's test.

Topiramate does not alter the response of cancer cells to radiation

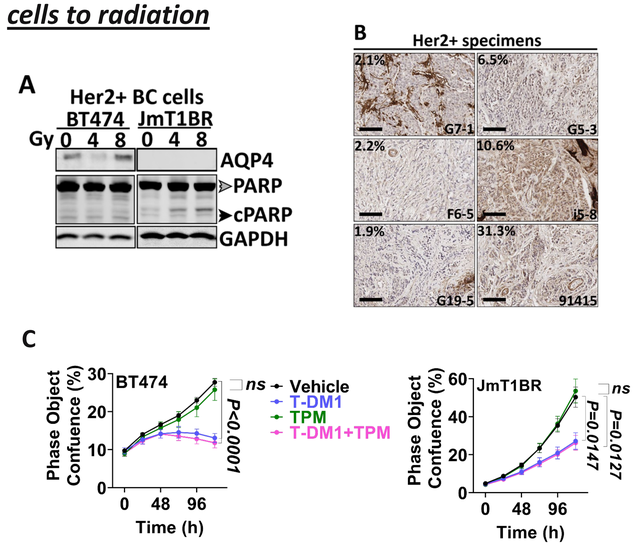


Fig 4 **(A)** WB of AQP4 and PARP of Her2+ breast cancer cells, irradiated with 0 , 4 and 8 Gy. **(B)** IHC shows AQP4 expression in breast metastatic Her2+ brain tumor patient samples. **(C)** Data shows media ± SEM of percentage phase object confluence of HER2+cell BT474 (left) or JmT1BR (right) treated with Vehicle (DMSO), 1 µg/mL T-DM1, 100 µM TPM, or TPM+T-DM1. Until 120 h.

Topiramate pretreatment decreases Brain water content after whole-brain radiation in a Her2+ Brain mets animal model

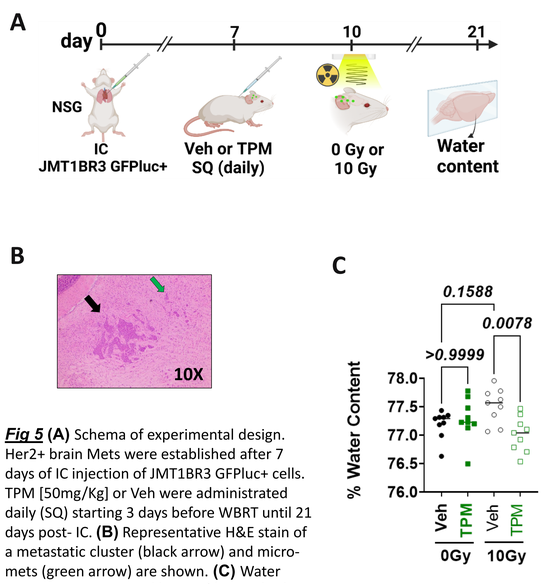
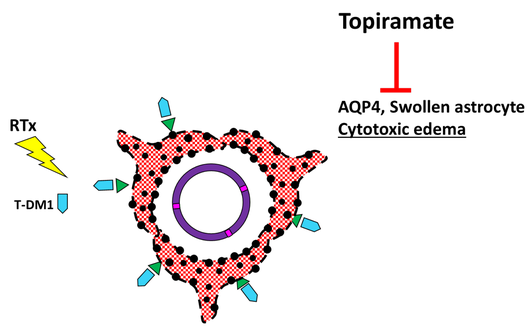


Fig 5 **(A)** Schema of experimental design. Her2+ brain Mets were established after 7 days of IC injection of JMT1BR3 GFPluc+ cells. TPM [50mg/Kg] or Veh were administrated daily (SQ) starting 3 days before WBRT until 21 days post- IC. **(B)** Representative H&E stain of a metastatic cluster (black arrow) and micro-mets (green arrow) are shown. **(C)** Water content was measured by wet/dry method N=10. two-way ANOVA Tukey's test.

Conclusions

- RTx induces cytotoxic edema in astrocytes.
- T-DM1 enhances RTx-induced cytotoxic edema through unintended targeting of Her2+ reactive astrocytes.
- AQP4 upregulation is associated with RTx-induced astrocytic swelling.
- AQP4 inhibition ameliorates Radiation/TDM1-increased cell swelling of astrocytes *in vitro* and water content *in vivo.*



References:

1. Breast Cancer Res. 2016; 18: 8
2. Ther Adv Med Oncol. 2015 Nov; 7(6): 321–339
3. Clin Cancer Res. 2019 Jul 1;25(13):3946-3953

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