Clinical Cancer Research

Combination of Trastuzumab Emtansine and Stereotactic Radiosurgery Results in High Rates of Clinically Significant Radionecrosis and Dysregulation of Aquaporin-4

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Abstract

Purpose: Patients with human EGFR2-positive ($HER2^+$) breast cancer have a high incidence of brain metastases, and trastuzumab emtansine (T-DM1) is often employed. Stereotactic radiosurgery (SRS) is frequently utilized, and case series report increased toxicity with combination SRS and T-DM1. We provide an update of our experience of T-DM1 and SRS evaluating risk of clinically significant radionecrosis (CSRN) and propose a mechanism for this toxicity.

Experimental Design: Patients with breast cancer who were $\leq$45 years regardless of HER2 status or had $HER2^+$ disease regardless of age and underwent SRS for brain metastases were included. Rates of CSRN, SRS data, and details of T-DM1 administration were recorded. Proliferation and astrocytic swelling studies were performed to elucidate mechanisms of toxicity.

Results: A total of 45 patients were identified; 66.7% were $HER2^+$, and 60.0% were $\leq$ 45 years old. Of the entire cohort, 10 patients (22.2%) developed CSRN, 9 of whom received T-DM1. CSRN was observed in 39.1% of patients who received T-DM1 versus 4.5% of patients who did not. Receipt of T-DM1 was associated with a 13.5-fold ($P = 0.02$) increase in CSRN. Mechanistically, T-DM1 targeted reactive astrocytes and increased radiation-induced cytotoxicity and astrocytic swelling via upregulation of Aquaporin-4 (Aqp4).

Conclusions: The strong correlation between development of CSRN after SRS and T-DM1 warrants prospective studies controlling for variations in timing of T-DM1 and radiation dosing to further stratify risk of CSRN and mitigate toxicity. Until such studies are completed, we advise caution in the combination of SRS and T-DM1.

Introduction

The estimated number of new cases of breast cancer in the United States in 2018 was 266,120 (1) with approximately 20% being HER2-positive ($HER2^+$; 2). Overall, 5%–15% of patients with breast cancer will develop brain metastases in their lifetime (3), although these rates may be 2–4 times higher in patients with $HER2^+$ disease (4). HER2-positivity and young age have both been associated with increased risk of developing intracranial metastases in patients with breast cancer (5). Stereotactic radiosurgery (SRS) plays a critical role in the contemporary management of brain metastases, with excellent local control rates and an improved toxicity profile compared with whole brain radiation (WBRT). Systemic therapies including trastuzumab emtansine (T-DM1) can be effective in treating patients with $HER2^+$ breast cancer with central nervous system (CNS) disease, with T-DM1 showing improved median survival without increased risk for CNS progression compared with capecitabine–lapatinib in the EMILIA trial (6, 7). Jacot and colleagues have also shown T-DM1 to be safe and effective in patients with $HER2^+$ breast cancer with brain metastases (8).

Although both SRS and T-DM1 are important therapies for $HER2^+$ populations with brain metastases, a number of series, including a previous report from our institution, have suggested increased rates of clinically significant radionecrosis (CSRN) with their combination (9, 10). Given clinical findings consistent primarily with edema, and the known role of Aquaporin-4 (Aqp4) in neuroinflammation, we further focused on Aqp4 changes after therapy. In this analysis, we update our institutional series and provide preclinical evidence suggesting that unintended T-DM1 targeting of reactive astrocytes surrounding brain metastases is a mechanism underlying T-DM1/SRS–induced toxicity.

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Translational Relevance

Patients with human EGFR 2-positive ($HER2^+$) breast cancer represent a highly complex group with multiple prior lines of treatment and aggressive management due to prolonged survival. With trastuzumab emtansine (T-DM1) increasing not only overall survival but, specifically, survival in patients with central nervous system disease, the safety and efficacy of stereotactic radiosurgery (SRS) with T-DM1 needs to be reported and the mechanism elucidated. Here, we review the strong correlation of clinically significant radionecrosis (CSRN) with the combination of SRS and T-DM1 and propose a mechanism for this toxicity. This experience provides critical information for providers utilizing this combination and our mechanism, supported by preclinical and clinical data, suggests potential pathways for intervention and mitigation of CSRN.

Materials and Methods

Patient selection

Under Institutional Review Board (IRB) approval, utilizing the MOSAIQ Radiation Oncology software, institutional records were queried to select for female patients with a diagnosis of breast cancer (utilizing ICD-9 and ICD-10 codes) treated with radiosurgery to the brain during the years 2004–2017. Fraction number was limited to five fractions or less to account for fractionated SRS (fSRS). Chart review was then completed to select for patients less than or equal to 45 years of age with any HER2 status as well as patients who had $HER2^+$ tumors regardless of age given the association of increased risk of brain metastases in both of these subgroups (5). These two groups represent a population in our institution who received multiple systemic treatment courses and SRS over their extended lifetime.

Patient demographics and treatment variables

Pertinent patient demographics and treatment characteristics were included in this series. The American Joint Committee on Cancer (AJCC) staging system was utilized to determine stage at diagnosis (11). Age, hormone receptor status (ER/PR status), HER2 status, and systemic therapy were noted in the retrospective review. Dates of both primary diagnosis and diagnosis of brain metastases were recorded. Utilization of T-DM1 in patients' treatment course, including dates of treatment, was documented. Patients were defined as having received concurrent T-DM1 if they received the drug within $\leq$4 weeks of any course of SRS. Charts were queried for diagnosis of CSRN and treatment was captured for each case. CSRN was defined as neurologic symptoms warranting hospital admission and treatment. If craniotomy was a modality of treatment, pathology was recorded. In cases with a diagnosis of CSRN, imaging and prior SRS treatments were reviewed to correlate the lesion associated with CSRN. Dosimetric data from the SRS course resulting in CSRN were recorded including prescription dose, fraction number, prescription isodose line, maximum point dose, volume treated, and planning target volume (PTV) margin for each case of CSRN. For all patients, total number of SRS courses, total lifetime number of lesions treated, and receipt of WBRT were documented.

Mechanism of toxicity

Deidentified samples from $HER2^+$ brain metastases were obtained from archival paraffin embedded tissue under an approved IRB protocol at the University of Colorado (Aurora, CO). Informed written consent was obtained from donors and studies were conducted in accordance with recognized ethical guidelines. Human adult astrocytes immortalized by hTERT and SV40 (referred as THV cells) were a kind gift from Dr. Paul B. Fisher (Virginia Commonwealth University, Richmond, VA). THV cells were maintained in high glucose DMEM (Life Technologies) supplemented with 10% FBS (Life Technologies) at 37°C in 5% CO_2.

Proliferation assays

THV cells were plated at 1,000/cell per well in 96-well plates and treated with indicated doses of radiation in a Rad Source RS2000 irradiator. Following irradiation, cells were treated with vehicle (PBS), 1 μg/mL trastuzumab or 1 μg/mL T-DM1 (Genetech), 10 μmol/L cisplatin, or 0.1 μmol/L paclitaxel as indicated, and cells were imaged over time using Live Cell Incucyte Imaging (Essen Bioscience). Cell confluence per well was calculated in three fields per well in at least four replicates per treatment and three independent experiments.

IHC and digital imaging

Immunostaining was performed using rat anti-GFAP (13-0300, Invitrogen) and rabbit anti-aquaporin 4 (AB3594, Millipore). For immunofluorescence analysis, images were collected using a Nikon Eclipse Ti-S inverted microscope. ROIs were made using Confocal Uniovi 1-51 and an ImageJ bundle, and then digital images were exported as tiff files to Adobe Photoshop. Minor linear adjustments to brightness and contrast were performed identically and in parallel. For quantification of astrocytes cell size, a minimum of 50 cells per treatment in two independent experiments were quantified. Cell area was manually delineated using the drawing tool of NIS-Elements software (version 4.30.01), and cell area was calculated using NIS-Elements software. Western blots were imaged and quantified using Odyssey CLx Imaging System and Image Studio Software v.5.2.5 LI-COR Biosciences.

Statistical analysis

Statistical analyses were performed using SPSS version 24.0 (SPSS Inc.). Logistic regression models were used to estimate odds ratios (OR) for the risk of CSRN associated with receipt of T-DM1 at any point throughout the patient's treatment course, receipt of T-DM1 concurrent with SRS, age, total number of SRS courses, total number of lesions treated, and receipt of WBRT.

For *in vitro* studies, statistics were done using Graphpad Prism 7.3 Software (GraphPad Software Inc). One-way ANOVA or repeated measures ANOVA followed by multiple comparison *post hoc* tests were performed, as appropriate. $P < 0.05$ was considered significant, and test assumptions were checked for all analyses. Adjusted P values are shown in all graphs.

Results

Overall patient and treatment characteristics

A total of 45 patients meeting the aforementioned inclusion criteria were treated with SRS from 2004 to 2017 and were included in this series. Table 1 displays patient, disease, and

Table 1. Patient, disease, and treatment characteristics

Variables	All patients No. (%)	CSRN No. (%)	No CSRN No. (%)
Age at diagnosis (years)			
≤ 45	27 (60.0)	3 (30.0)	24 (68.6)
> 45	18 (40.0)	7 (70.0)	11 (31.4)
AJCC 7th Edition Clinical Stage			
0	2 (4.4)	1 (10.0)	1 (2.9)
1	3 (6.7)	1 (10.0)	2 (5.7)
2	10 (22.2)	1 (10.0)	9 (25.7)
3	11 (24.4)	2 (20.0)	9 (25.7)
4	10 (22.2)	2 (20.0)	8 (22.9)
Unknown	9 (20.0)	3 (30.0)	6 (17.1)
Brain metastases at diagnosis			
No	42 (93.3)	10 (100.0)	32 (91.4)
Yes	1 (2.2)	0 (0.0)	1 (2.9)
Unknown	2 (4.4)	0 (0.0)	2 (5.7)
HER2 status			
Negative	15 (33.3)	0 (0.0)	15 (42.9)
Positive	30 (66.7)	10 (100.0)	20 (57.1)
Receipt of T-DM1			
No	22 (48.9)	1 (10.0)	21 (60.0)
Yes	23 (51.1)	9 (90.0)	14 (40.0)
Timing of T-DM1 with SRS			
Sequential	7 (15.6)	3 (30.0)	4 (11.4)
Concurrent	16 (35.6)	6 (60.0)	10 (28.6)
N/A (no T-DM1)	22 (48.9)	1 (10.0)	21 (60.0)
Total number SRS courses			
1	27 (60.0)	3 (30.0)	24 (68.6)
2	9 (20.0)	1 (10.0)	8 (22.9)
3	4 (8.9)	3 (30.0)	1 (2.9)
4	4 (8.9)	2 (20.0)	2 (5.7)
5	1 (2.2)	1 (10.0)	0 (0.0)
Total number lesions treated			
1–5	26 (57.8)	5 (50.0)	21 (60.0)
> 5	19 (42.2)	5 (50.0)	14 (40.0)
Receipt of WBRT			
No	30 (66.7)	7 (70.0)	23 (65.7)
Yes	15 (33.3)	3 (30.0)	12 (34.3)

treatment characteristics. The median age of the cohort was 45 years (range 28–66). The most common clinical stage at diagnosis was stage III (24.4%), and 30 patients (66.7%) were $HER2^{+}$. Seven patients (15.6%) had triple negative breast cancer (ER/PR/HER2-negative). Only 1 patient (2.2%) had brain metastases at diagnosis. Just over half (23, 51.1%) of patients received T-DM1 as a component of therapy with 16 patients (35.6%) receiving T-DM1 concurrently with SRS. The median number of total SRS courses was 1 (range 1–5). The median number of total intracranial lesions treated from total SRS courses per patient was 5 (range 1–17). One-third of patients also received WBRT as a component of their intracranial radiotherapy during the course of their disease.

CSRN

A total of 10 patients (22.2%) developed CSRN. CSRN was observed in 9 of 23 (39.1%) patients who received T-DM1 compared with only 1 of 22 (4.5%) patients who did not receive T-DM1. Six patients receiving T-DM1 concurrent with any course of SRS, and four receiving concurrent T-DM1 with the course of SRS directed to the lesion, later developed CSRN. Symptoms included seizures, headaches, blurred vision, ataxia, dizziness, altered mental status, and dysarthria. Three patients required only corticosteroid treatment during their admission. One case required hospital admission with supportive care only. In the entire cohort, 6 patients required therapeutic craniotomy with pathologic confirmation of radionecrosis in all cases.

Table 2 displays the dosimetric characteristics of patients with CSRN. The median treated volume was 0.70 cc (corresponding sphere diameter of 1.10 cm) with a range of 0.09–41.55 cc. The median prescription dose and fraction number were 2,000 cGy (range 1,800–2,500 cGy) and 1 (range 1–5), respectively.

Table 3 shows systemic therapies received by patients who had CSRN as well as timing of SRS in relation to the delivery of T-DM1. The median time from SRS of the affected lesion to CSRN was 16 months (range 1–79 months). In those patients who received sequential T-DM1, there was a large range in timing. For those who received T-DM1 prior to SRS, the time range was 77–131 days. The cohort who received T-DM1 after SRS ranged 420–1426 days. Also displayed in Table 3 is the time from T-DM1 to development of CSRN with a range of 8–532 days.

Figure 1 shows a representative example of radiographic changes associated with CSRN. The SRS plan is displayed in the left panel with MRI at the time of CSRN diagnosis in the right panel. In this case, SRS was completed 14 days prior to starting T-DM1. CSRN developed while on T-DM1, 6 months after initiation of the drug.

In analysis of the entire cohort on logistic regression (Table 4), receipt of T-DM1 was associated with a 13.5-fold increased risk of CSRN [$P = 0.02$; 95% confidence interval (CI), 1.5–118.7]. In those who did receive T-DM1, 9 patients developed CSRN and 6 of those had received T-DM1 concurrently. Treatment with increasing number of SRS courses (OR 2.7; 95% CI, 1.3–5.3; $P < 0.01$) and age >45 years (OR 5.1; 95% CI, 1.1–23.5; $P = 0.04$) were both associated with increased risk of developing CSRN. Risk of CSRN was not altered by an increase in the number of treated lesions ($P = 0.57$) or receipt of WBRT ($P = 0.80$). The per-lesion rate of CSRN in the overall cohort was 7.1% (19/268 lesions).

Radiation + T-DM1 mechanism of brain edema

Given clinical findings consistent primarily with edema, we sought to investigate whether T-DM1 in combination with radiation promoted water flow dysfunction in the brain niche. Reactive astrocytes are key modulators of the neuroinflammatory response during brain metastasis and regulate water flow across the blood–brain barrier (BBB) through modulation of the water transporter Aqp4 (12–16). Reactive astrocytes can pose as targets for T-DM1 because human astrocytes (THV) expressed normal levels of HER2 (Fig. 2A). Astrocytic HER2 was downregulated by both T-DM1 and trastuzumab suggesting both HER2-targeting agents are taken up by astrocytes. To assess whether HER2-targeted therapies impacted Aqp4 expression and induced astrocytic swelling (a measurement of water flow impairment), THV cells were treated with increasing doses of radiation (0, 2, 4, 8 Gy) alone or in combination with 1 μg/mL trastuzumab or T-DM1. Western blots showed that while trastuzumab reduced the radiation-induced upregulation in Aqp4, T-DM1 further exacerbated radiation-induced Aqp4 upregulation, and increased PARP activation (Fig. 2A).Consistent with these results, astrocyte survival measured using Incucyte Live Cell Imaging over 5 days showed that radiation alone decreased astrocyte survival by 35%, and trastuzumab did not affect survival of astrocytes or show synergistic effects with 4 Gy (Fig. 2B). By contrast, in the absence of radiation, T-DM1 decreased confluence of astrocytes by 8.8% (91.3 ± 0.8 vs. 99.8% ± 0.2, respectively, $P > 0.01$, at 5 days) as compared with control astrocytes and by 32.1% as

Table 2. Dosimetric characteristics of patients and respective lesions with radionecrosis

Patient	Target volume (cc)	Prescription dose (cGy)	Number of fractions	Prescription isodose line (%)	Maximum point dose (cGy)	PTV margin (mm)	Dose of prior whole brain RT (cGy)	Total SRS courses
1	1.09	2,400	1	80	3,192	0	0	4
2	0.55	200	1	80	2,660	0	0	4
	6.93	2,500	5	88	2,921	1	0	4
3	6.65	1,800	1	64	2,880	0	0	2
4	41.55	2,000	5	81	2,634	5	2000	3
5	0.77	2,000	1	77	2,707	0	3500	1
	0.51	2,000	1	77	2,707	0	3500	1
	0.70	2,000	1	77	2,630	0	3500	1
	0.42	2,000	1	77	2,630	0	3500	1
6	0.89	2,000	1	60	3,380	0	0	5
7	4.77	2,000	1	60	3,072	0	0	3
	0.49	2,000	1	80	2,580	0	0	3
	1.51	2,000	1	60	3,400	0	0	3
8	0.20	2,000	1	80	2,600	0	3000	1
9	20.93	2,400	3	73	3,370	2	0	3
10	0.09	1,800	1	80	2,380	0	0	1
	0.16	2,000	1	80	2,740	0	0	1
	0.22	2,000	1	80	2,780	0	0	1
	0.24	2,000	1	80	2,380	0	0	1

compared with control astrocytes pretreated with 4Gy ($32.8 \pm 3\%$ vs. $64.9 \pm 13.3\%$, respectively, $P < 0.0001$, at 5 days; Fig. 2B). These results suggest that TDM-1 enhances the cytotoxic effects of radiation on reactive astrocytes, concomitant with dysregulation of Aqp4 expression.

Because upregulation of Aqp4 is reported to result in astrocytic swelling (15, 16), the cell size of astrocytes treated with 4 and 8 Gy alone or in combination with trastuzumab or T-DM1 was assessed. Immunofluorescence staining showed that at high dose radiation (8Gy) T-DM1/radiation increased astrocytic cell size in a subpopulation of astrocytes ($P = 0.004$) to a larger extent than equivalent radiation dose in combination with trastuzumab ($P = 0.005$; Fig. 2C). Moreover, immunostaining of brain metastases from patients with $HER2^+$ disease who were treated with T-DM1/SRS showed enlarged reactive astrocytes expressing high levels of Aqp4 (Fig. 2D). Other chemotherapeutic agents (paclitaxel and cisplatin) did not increase Aqp4 protein levels, had no synergistic effects with 4 Gy radiation in reducing astrocytic survival, nor did they result in increased astrocytic swelling (Supplementary Fig. S1A–S1C) *in vitro*. Taken together, these results suggest that uptake of T-DM1 by reactive astrocytes enhances radiation-induced cytotoxic edema in astrocytes during SRS.

Discussion

With promising rates of improved survival with T-DM1 in patients with brain metastases, there remains a paucity of data addressing the safety of combination T-DM1 and SRS. We previously described an unanticipated increase in toxicity with combination of SRS and T-DM1 (9). This article updates our institutional findings and reports a suggested mechanism that the T-DM1 targeting of reactive astrocytes surrounding brain metastases underlies T-DM1/SRS–induced toxicity. To our knowledge, this series represents the largest published experience of the use of SRS in patients with intracranial metastases from breast cancer receiving T-DM1. In this single-institution experience of 45 total patients with brain metastases from breast cancer, receipt of T-DM1 was associated with a 13.5-fold increase in risk of developing CSRN when combined with SRS. CSRN was observed in 39.1% of patients that received T-DM1 compared with 4.5% of those who did not receive T-DM1. Increasing total number of SRS

Table 3. Systemic treatments details in patients with radionecrosis

Patient	Prior systemic treatment	T-DM1 delivered concurrent with SRS	Interval any SRS course from T-DM1 (days)	Interval to CSRN from T-DM1 (days)
1	D, P, T, Ca, L, S, Px, ONT	No	77[a]	221
2	Px, T, A, C, D, P, L, ONT, Ca, Pz	Yes	17	116
		No	131[a]	374
3	A, C, Ev, T, E, Az, Px, Pz, L, Ca	Yes	14	15
4	Px, T, Az, L, ONT, F, Pz, G, S, E	Yes	10	529
5	Px, T, Pz, ONT, S	Yes	16	532
6	A, Px, T, V, X, G, Ca, L	Yes	3	16
7	A, C, Px, T, Tam, Lz, S, Fx, L, Ex, Ca, S with Px, V, ONT	Yes	3	25
8	Az, T, Px, L, Ca, Ex	No	420	92
9	D, P, T, E, A, C, S with Px, Ca	NA (did not receive T-DM1)	NA	NA
10	P, G, T, Ca, L	No	1,426	8

Abbreviations: A, doxorubicin; Az, anstrozole; C, cyclophosphamide; Ca, capecitabine; D, docetaxel; E, eribulin; Ev, everolimus; Ex, exemestane; F, fulvestrant; Fx, fasldoex; G, gemcitabine; L, lapatinib; Lz, letrozole; ONT, ONT-380; P, carboplatin; Px, paclitaxel; Pz, pertuzumab; S, study drug; T, trastuzumab; Tam, tamoxifen; V, vinorelbine; X, abraxane.

[a]In those who did not receive T-DM1 concurrent with SRS, "a" denotes that SRS was delivered after receipt of T-DM1.

Table 4. Logistic regression predicting for CSRN

	Univariate	
Variables	**OR (95% CI)**	***P***
Age at diagnosis (years)		
≤ 45	1	
> 45	5.091 (1.103–23.493)	0.037
HER2 status		
Negative	1	
Positive	—	1
Receipt of T-DM1		
No	1	
Yes	13.500 (1.535–118.692)	0.019
Total number SRS courses		
continuous	2.658 (1.329–5.314)	0.006
Total number lesions treated		
1–5		
>5	1.500 (0.365–6.157)	0.574
Receipt of WBRT		
No	1	
Yes	0.821 (0.179–3.763)	0.800

courses delivered and older age also portended a higher risk of developing CSRN.

Historical published controls suggest much lower rates of significant radionecrosis varying from 5% to 17% in reported studies (17–20). Kondziolka and colleagues published a study of 350 women with breast cancer undergoing SRS for brain metastases (total 1,535 lesions) with symptomatic adverse radiation effects occurring in just 6% of patients with only 3 patients requiring therapeutic resection (17). In 2011, Minniti described patients (18% of total cohort with breast cancer) who underwent SRS for brain metastases (18). Symptomatic radionecrosis was observed in 10% of patients. Severe neurologic complications (RTOG grade 3 or 4) were noted in 5.8% of patients and required surgery or medical treatment. Another study analyzed outcomes of women with breast cancer with 1–3 brain metastases treated with SRS. Pathologic radionecrosis was identified in 8.6% of cases (19). In contrast, in our study, among patients that received T-DM1, 39.1% developed CSRN with 5 patients requiring surgical resection. In patients who did not receive T-DM1, the rate of CSRN (4.5%) was more consistent with published rates of CSRN in historical SRS series (17–20). However, we acknowledge that the incidence of CSRN in patients in our cohort with HER2$^-$ breast cancer (who would not have received T-DM1) may not be accurately captured given historically short survival in this subgroup (21) and short median survival after brain metastases in our series (9.7 months; range 2.9–65.7). Nevertheless, CSRN remains an important clinical outcome in the HER2$^+$ cohort with overall longer survival.

Patients and treatment factors in this series were quite heterogeneous with varying intervals to CSRN, duration of T-DM1, dose and fractionation schemes, volume of treatment, and receipt of WBRT. The interval from SRS to the lesion later presenting with CSRN varied greatly with the shortest interval of 30 days and the longest delay of just over 6.5 years. Most patients did receive only single-fraction SRS; however, some patients did undergo fSRS. Three of the patients who developed CSRN received fSRS. As evidenced in Table 2, the median volume of treated tumors was 0.70cc. Notably, 1 patient had a large volume of 41.55cc treated in a fractionated course to a total dose of 2,000 cGy. This is outside the usual consideration of radiosurgery in terms of dosing and fractionation; however, the patient had CSRN evidenced by new onset seizures and hemorrhage requiring craniotomy and resection with pathology consistent with radionecrosis without any viable tumor. The patient had prior courses of intracranial-directed radiation including WBRT and single-fraction radiosurgery. This case was pertinent to include as this suggests a risk of CSRN at a lower dose threshold and fractionation scheme than may be classically considered. Three patients had also received WBRT as a course of CNS-directed radiation. The concurrent versus sequential delivery of T-DM1 as well as total T-DM1 duration was variable. Total duration of T-DM1 ranged from 22 to 981 days in patients with CSRN. Most patients underwent T-DM1 concurrent with any SRS treatment, though this was not always concurrent with the course of SRS, which later resulted in CSRN. A recent publication by Geraud and colleagues retrospectively reviewed 12 patients treated for brain metastases with T-DM1, four of whom received this concurrently with SRS and eight sequentially (10). In the concurrent group 50% of patients (2 patients) had radionecrosis as compared with only 28.6% in

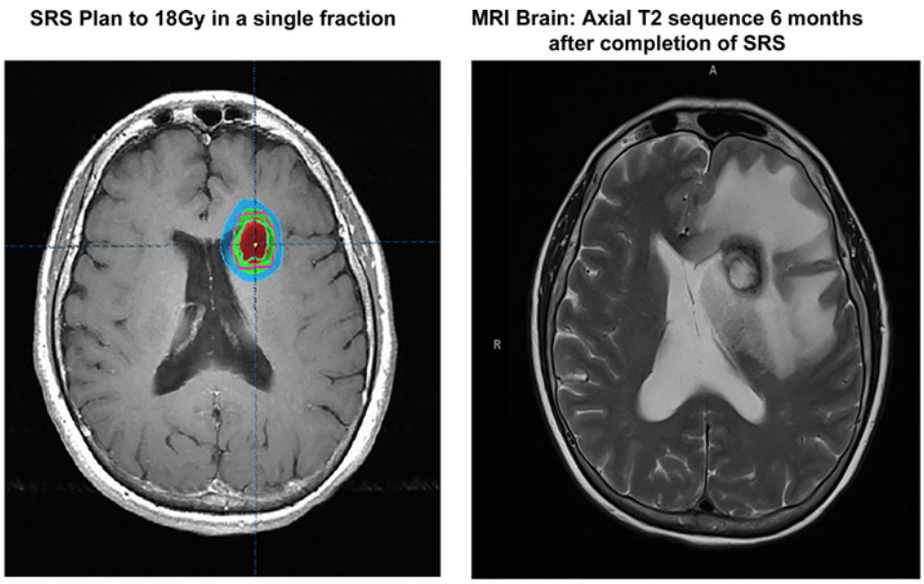

Figure 1.
Left, SRS plan; right, MRI imaging, axial T2 Sequence representing CSRN.

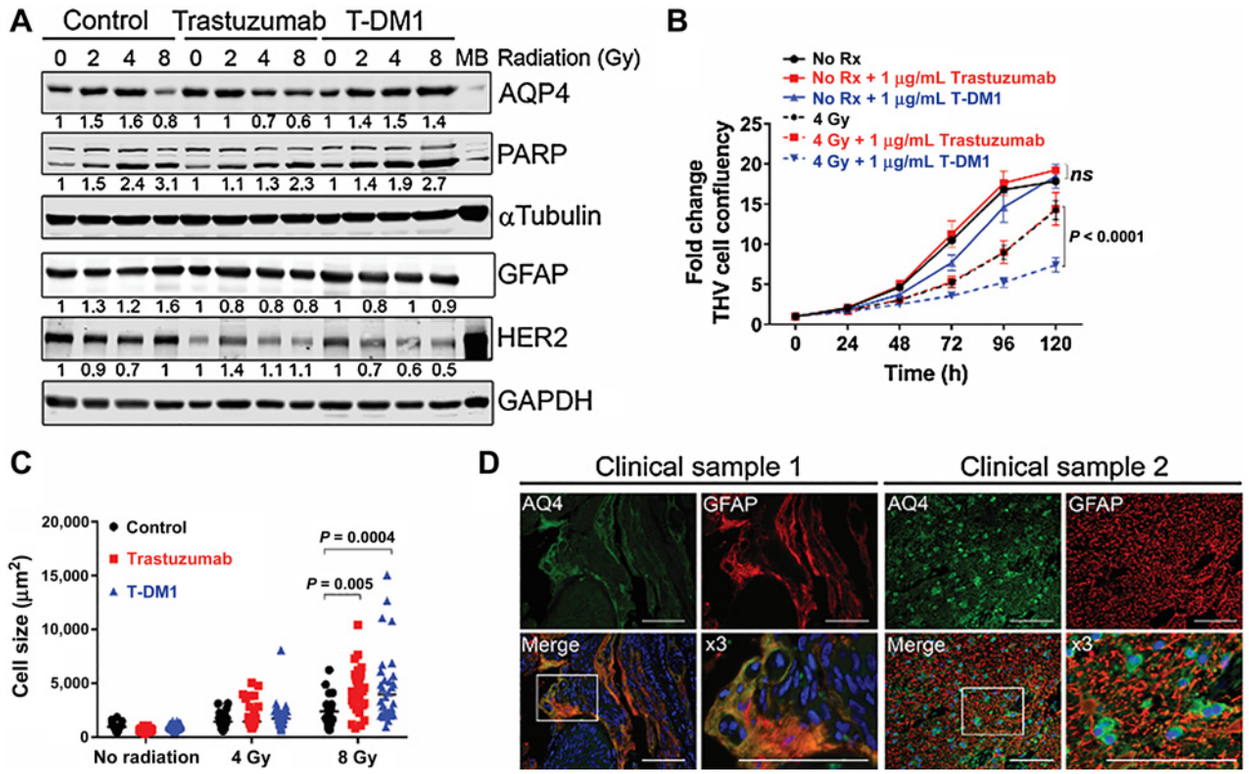

Figure 2.

A, Representative Western blot showing regulation of Aqp4 and PARP in the setting of trastuzumab, T-DM1, and radiation. THV cells were plated in DMEM 10% FBS and treated with 0, 2, 4, 8 Gy as indicated. Cell lysates were analyzed 48 hours later. GAPDH was used as loading control. Numbers represent protein levels normalized to GAPDH and relative to untreated control cells. **B,** Astrocyte survival with varying combinations of trastuzumab, T-DM1, and RT. Cells were treated with 0 (No RT) or 4 Gy RT, and cell confluence was measured over time using live imaging Incucyte system. Graph shows fold change in confluence relative to time 0. Graph shows mean ± SEM for each time point. Data were analyzed using two-way ANOVA followed by Dunn *post hoc* analysis. P_{adj} values are shown. **C,** Astrocyte cell size in the setting of trastuzumab or T-DM1 with or without RT. Cells were plated in coverslips and treated with indicated doses of RT. Cells were stained for Aqp4 and cell size measure in at least 50 individual cells per treatment, in two independent experiments. **D,** Double immunofluorescence staining of reactive astrocytes (GFAP, red) and Aqp4 (green) in brain metastases from 2 patients with HER2$^+$ disease. Dapi, nuclei.

the sequential group. Carlson and colleagues at our institution published a series of 13 patients of whom 7 patients had HER2$^+$ disease and received T-DM1 (9). The rate of CSRN in the T-DM1 treatment group was 57%.

Furthermore, we explored the mechanism for the observed increase in toxicity. Studies have asserted that the integrity of the BBB is compromised by metastatic CNS disease, and T-DM1 has been found to cross the BBB (22). Because the radionecrosis associated with T-DM1/SRS treatment is accompanied by significant cerebral edema, we sought to investigate the mechanism underlying this toxicity. While SRS and brain tumor burden can cause toxicity via upregulation of VEGF and disruption of the BBB (23, 24), our preclinical data suggest that additional unintended targeting of HER2-positive reactive astrocytes (25) by T-DM1, enhances radiation-induced cytotoxic edema, a premorbid cellular process that results in osmotic expansion of cells and leads to necrotic cell death (12, 26). Our data show that enhanced Aqp4 expression and astrocytic swelling is specific to T-DM1 but not trastuzumab or other chemotherapeutic agents. Given that emtansine-related compounds have been reported to enhance irradiation-induced cell death (27), it is possible that T-DMI–induced cytotoxicity results from the uptake of emtansine in HER2$^+$ astrocytes. Taken together, our studies suggest that a critical targeting of astrocytes and induction of cytotoxic edema is a mechanism underlying the significant toxicity associated with T-DM1/SRS. However, it remains to be investigated whether Aqp4 and astrocytic swelling are required and sufficient events that explain T-DM1/radiation–induced toxicity. Further studies are needed to fully decipher molecular and cellular mechanisms resulting in radionecrosis and edema.

The finding that the most significant changes in Aqp4 upregulation and astrocytic swelling occur at higher doses of radiation but not at lower radiation doses suggests that lower dose fSRS, as opposed to higher-dose single-fraction SRS schedules, might be needed to diminish the T-DM1/SRS toxic effect on astrocytes and reduce risk of clinically significant cerebral edema and radionecrosis. However, further testing and observation in these patients is needed given the case of CSRN in a fSRS course reported here. Targeting changes in Aqp4 expression may offer additional options to prevent the observed toxicity. FDA-approved antiepileptic drugs that target Aqp4 might represent rapidly translatable candidates to this end (28).

The patients captured in this series represent a highly complex group with multiple prior lines of treatment and aggressive management due to prolonged survival. With T-DM1 increasing not only overall survival but, specifically, survival in patients with

CNS disease, the safety and efficacy of SRS with T-DM1 should be a focus of future trials. Because of the heterogeneity displayed by this cohort, it is imperative that future prospective trials assessing the risks of combination SRS and T-DM1 consider dose, fractionation, number of SRS courses, treatment volume, and timing of T-DM1. With the delay in development of CSRN, the need for protracted follow-up in future studies remains of utmost importance.

In our series, the combination of T-DM1 and SRS results in alarming rates of CSRN in patients with brain metastases from breast cancer. Given the considerable level of heterogeneity and overall small patient numbers, it is difficult to ascertain which variables may significantly elevate the risk of developing CSRN, though the association between receipt of T-DM1 and SRS is clear. Prospective trials are necessary to determine the safety of the combination of T-DM1 and SRS. However, caution is warranted in this specific cohort until such trials are completed.

Disclosure of Potential Conflicts of Interest

C. M. Fisher is a consultant/advisory board member for Accuray. No potential conflicts of interest were disclosed by the other authors.

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