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Trastuzumab emtansine (T-DM1) and concurrent radiotherapy for treatment of HER2-positive breast cancer: Review of literature

Muto M[1]*, Amaturo G[1], Sgambato A[2], Colantuoni G[2], Iannace C[1] and Gridelli C[2]

[1]Breast Unit, "S. G. Moscati" Hospital, Avellino, Italy

[2]Division of Medical Oncology, "S. G. Moscati" Hospital, Avellino, Italy

Abstract

Human epidermal growth factor receptor 2 (HER2)-overexpression emerged as a factor of radioresistance in preclinical studies *in vivo* and *in vitro* in breast cancer cells. However, radiosensitivity of these cells can be restored through down-regulation of HER2 by anti-HER2 therapy, suggesting HER2 as an ideal target for sensitizing breast cancer cells to ionizing radiation. Trastuzumab emtansine (T-DM1) is a HER2-targeted antibody and microtubule inhibitor conjugate indicated for the treatment of pretreated patients with HER2-positive metastatic breast cancer and for the adjuvant treatment of patients with HER2-positive early breast cancer who have residual invasive disease after neoadjuvant taxane-based and standard trastuzumab-based treatment.

Given patients with early breast cancer often need curative-intent radiotherapy (such as after breast conservative surgery and/or in presence of positive axilla lymph nodes), as well as those with metastatic breast cancer often need palliative radiotherapy, the extension of T-DM1 treatment indications, either adjuvant or metastatic settings, raised safety concerns about combination of this anti-HER2 agent and radiation therapy. Yet, little is known about the safety of this strategy.

This review of literature will focus on the use of T-DM1 combined with radiotherapy. It aims at listing every published data about such combination so as to understand its possible resulting toxicity in early and metastatic breast cancer.

Introduction

Human epidermal growth factor receptor 2 (HER2) is a protein involved in normal cell growth. HER2 (encoded by ERBB2, formerly known as *neu*) is a member of HER family of receptor tyrosine kinases consisting of three other receptors, designated HER1 (also known as epidermal growth factor receptor [EGFR]), HER3, and HER4 [1,2]. Several ligands bind EGFR, HER3 and HER4 and induce homo- and heterodimerization among the family members resulting in the activation of cancer-driving pathways that are involved in cell proliferation, invasion, migration, or survival. HER2 has no ligand-binding domain but appears to be a preferred dimerization partner of the other three receptors [1,2].

The gene encoding HER2 is amplified and overexpressed in approximately 15-20% of breast cancer. HER2-positive breast cancers tend to be biologically more aggressive (high rates of cell proliferation and metastasis in the absence of targeted therapies) and to be associated with a worse prognosis than HER2-negative breast cancers [1,2]. At the same time, HER2 amplification or overexpression is a predictor of response to HER2-targeting agents (trastuzumab, pertuzumab, lapatinib and trastuzumab emtansine [T-DM1]), which have dramatically changed the natural history of HER2-positive breast cancer [3,4]. Given the lack of data on this topic, the main purpose of this review is to dissect published results on the anti-HER2 therapies, focusing on T-DM1 and starting from the preclinical bases, in order to deepen the topic on the toxicity and efficacy of its administration concomitantly with radiotherapy.

Anti-HER2 therapy: trastuzumab emtansine (T-DM1): Trastuzumab, pertuzumab, and TDM-1 bind to the extracellular domain of HER2. The first two either alter normal tyrosine kinase signaling, through blocking the proteolytic cleavage and dimerization of receptor, so blocking the ligand-independent and downstream signaling (inhibition of PI3K/Akt/MAP pathways) or induce antibody-dependent complement-mediated cytotoxicity (ADCC). Trastuzumab emtansine is an antibody-drug conjugate containing the humanized anti-HER2 IgG1, trastuzumab, covalently linked to the microtubule inhibitor DM1 [5]. This conjugation confers selectivity of the cytotoxic agent for HER2-overexpressing tumour cells, thereby increasing intracellular delivery of DM1 directly to malignant cells and reducing the toxicity on the off-target tissue. T-DM1 is internalized and the chemotherapeutic agent is

Correspondence to: Matteo Muto, MD, Division of Radiotherapy, "S. G. Moscati" Hospital, Avellino, Italy, E-mail: mattomuto@gmail.com

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enzymatically cleaved, resulting in release of DM1-containing cytotoxic catabolites [5,6]. T-DM1 has a dual mechanism of action related to its two active components: like trastuzumab, it binds to the extracellular domain of HER2, inhibits its detachment, inhibits signaling through the metabolic pathway of PI3K, and mediates ADCC in HER2 breast cancer cells; the cytotoxic component DM1, once entered into the cancer cell, becomes active and binds to tubulin, which is important in the formation of the internal "skeleton" that cells need to assemble when they divide, stops the formation of this skeleton, thus preventing the division and growth of the cancer cells. Both DM1 and trastuzumab emtansine cause cells to arrest in the G2/M phase of the cell cycle, ultimately leading to apoptotic cell death [5-7]. The tyrosine kinase inhibitors lapatinib, neratinib, and pazopanib cross the cell membrane and inhibit the intracellular tyrosine kinase domain activities.

Anti-HER2 treatments have revolutionized the therapeutic landscape of both HER2 positive advanced and early-breast cancer. Focusing on metastatic setting, the double-block combination of trastuzumab and pertuzumab plus docetaxel chemotherapy achieved an unprecedented median overall survival (mOS) advantage of 16.3 months (mOS 57.1 vs. 40.8 month with pertuzumab and placebo, respectively) that was maintained after a median follow-up of more than 8 years, confirming this combination as standard first-line treatment [8].

After first-line trastuzumab-based therapy, current guidelines recommend treatment with T-DM1 due to its superior efficacy relative to other HER2-based therapies in the second line (versus lapatinib plus capecitabine) and beyond (versus treatment of physician's choice). Given the OS benefit, T-DM1 should be preferred in patients who have progressed through at least one line of trastuzumab-based therapy. These statements come from available pivotal phase III trials. Among these, the randomized phase III EMILIA trial compared the combination of lapatinib and capecitabine with T-DM1 in 991 patients with HER2-positive breast cancer (naïve to lapatinib and/or capecitabine) previously treated with trastuzumab and a taxane in the adjuvant, unresectable, locally advanced, or metastatic setting whose progression disease occurred during or after most recent treatment or within 6 months after completing of adjuvant therapy [9]. After a median follow-up of 47.8 months, T-DM1 led to an increase in median OS with a gain of 4 months (29.9 vs. 25.9 months in the control group, hazard ratio [HR] 0.75) even in the presence of crossover treatment (in control group, 27% of patients crossed to TDM1; in TDM-1 group, 51% received capecitabine and 49% lapatinib, separately or in combination, after study drug discontinuation). T-DM1 was associated with a lower incidence of grade 3 and 4 adverse events (AEs, 48% vs 60%) [9].

In 602 more pretreated patients with HER2-positive metastatic breast cancer previously exposed to taxane as well as to both trastuzumab and lapatinib and progressed on ≥2 anti-HER2 therapies in the advanced setting (TH3RESA trial), treatment with T-DM1 was associated with a 32% reduction in risk of death (22.7 vs. 15.8 months; HR 0.68, P = 0.0007) versus treatment of physician's choice (47% of the physician's choice group crossed over to TDM-1). A lower incidence of grade ≥3 AEs occurred in T-DM1 group (40% vs 47%) [10].

KAMILLA is a single-arm, phase IIIb study, including the largest population of locally advanced or metastatic HER2-positive breast cancer patients and the closest to real world life studied so far (higher proportion of patients aged ≥ 65 years, with symptomatic disease, visceral disease, hormone-receptor positive, central nervous system [CNS] metastases, heavily pretreated patients) in order to evaluate the safety and efficacy of T-DM1 [11]. In a total of 2002 patients, whose disease progressed to prior HER2-targeted therapy and chemotherapy for metastatic breast cancer or within six months of completing adjuvant therapy, the primary analysis confirmed that T-DM1, across multiple lines of therapy, was well-tolerated and effective, consistently with previous studies. Anaemia, thrombocytopaenia, fatigue, gamma-glutamyltransferase elevation and asthenia were the most common grade 3 AEs (incidence lower than 3% for each). In intent-to-treat population (ITT), T-DM1 treatment showed a median PFS of 6.9 months and OS of 27.2 months; according to treatment line, the longest PFS and OS were reported in second-line setting and decrease numerically with increasing numbers of prior treatment lines (mPFS from 8.3 to 5.6 months and median OS from 31.3 to 22.5 months in 0-1 line and ≥ 4 lines, respectively), consistent with the natural evolution of the disease [11]. Of note, in a post hoc exploratory analysis, a meaningful anti-tumor activity has been reported in 126, of a total of 398, patients with measurable baseline asymptomatic CNS metastases, who had and had not undergone prior radiotherapy. The best overall response rate [ORR] was 21.4% and the clinical benefit rate was 42.9%. A ≥30% reduction in the sum of the largest diameters of target brain lesions occurred in 42.9% of patients, of which 49.3% had not received prior radiotherapy. Among the total of 398 patients, mPFS and mOS were 5.5 and 18.9 months, respectively, with an overall safety profile generally comparable to those without CNS metastases [12].

Concerning the controversy regarding the potential reduced efficacy of T-DM1 after pertuzumab exposure [13-16] unfortunately, the KAMILLA trial did not add information because most patients enrolled did not receive the current standard-of-care for first-line treatment. However, an exploratory analysis of CLEOPATRA trial (median duration of therapy of 7.1 months at any time after discontinuing pertuzumab) [17] and observational evidence [14-16], including an Italian Multicenter Observational Study (median PFS of 6.3 months, ORR of 27.1% with 40% of patients achieving durable disease control) [15], supported T-DM1 activity in this population.

T-DM1 efficacy in pertuzumab-resistant disease was confirmed in the recent publication of KATHERINE trial, in which adjuvant T-DM1 showed a reduced the risk of recurrence even in the subgroup of patients pretreated with neoadjuvant pertuzumab (nearly 20% of study population) [18]. In the phase III KATHRINE trial, adjuvant treatment with T-DM1 resulted in a 50% lower risk of recurrence of invasive disease or death (HR 0.50; P<0.001) than standard adjuvant trastuzumab among 1486 patients with HER2-positive early breast cancer and residual disease after standard neoadjuvant taxane-based chemotherapy (with or without anthracyclines) plus trastuzumab (with or without pertuzumab). T-DM1 achieved an absolute improvement of 11.3% in term of 3-year invasive disease-free survival (IDFS, 88.3% vs. 77.0% with trastuzumab, HR 0.50), that was consistent across several key subgroups (operability status at diagnosis, hormone receptor status, single or dual HER2-targeted therapy in neoadjuvant setting, lymph node status after neoadjuvant treatment). Safety profile of T-DM1 was consistent with previous studies and, as expected, a higher percentage of AEs was observed in the T-DM1 group (grade ≥3: 25.7% vs. 15.4% in the trastuzumab group) [18].

Therefore, T-DM1 should now be considered the standard adjuvant anti-HER2 treatment in patients not achieving a pathologic complete response (pCR) after neoadjuvant chemotherapy plus anti-HER2 therapy.

Among different strategies under evaluation in the HER2-positive adjuvant setting, replacing adjuvant taxane and trastuzumab with T-DM1 in combination with pertuzumab did not improve efficacy or

overall safety in patients with adequately excised breast cancer, either node-positive or node-negative, hormone receptor-negative, and tumor size >2.0 cm in the phase III KAITLIN study [19]. A total of 1846 patients were randomly assigned to receive three to four cycles of anthracycline-based chemotherapy followed by 18 cycles of T-DM1 and pertuzumab combination or taxane plus concurrent trastuzumab and pertuzumab. No significant difference in 3-year IDFS rates have been reported between the two treatments in either patients who were lymph node positive (92.8% vs. 94.1% in taxane plus trastuzumab group; HR 0.97) or in the full ITT population (93.1% vs. 94.2% in taxane plus trastuzumab group; HR 0.98). Therefore, the standard of care for patients with high-risk HER2 positive early breast cancer remains adjuvant trastuzumab and pertuzumab plus chemotherapy [19].

Aiming to identify a regimen with fewer side effects for patients with low-risk HER2-positive early breast cancer, the phase II ATEMPT trial tried to determine whether adjuvant T-DM1 (for 17 cycles) was more tolerable and associated with a clinically acceptable disease-free-survival than the paclitaxel and trastuzumab combination (for 12 weeks followed by trastuzumab for a total of 1 year of treatment) in 512 patients with stage I disease [20]. After a median follow-up of 3 years, 97.5% of patients treated with T-DM1 survived without evidence of cancer recurrence compared to 93.2% of those treated with paclitaxel and trastuzumab, suggesting that these patients may be able to avoid treatment with chemotherapy altogether. However, a longer follow-up is needed before strong conclusions can be drawn on this study [20].

In HER2-neoadjuvant setting (KRISTINE trial), "chemotherapy-free" regimen with T-DM1 plus pertuzumab combination compared to dual anti-HER2 blockade plus chemotherapy (docetaxel, carboplatin, trastuzumab, pertuzumab) resulted in a significantly lower rate of pCR (44% vs. 56% in chemotherapy group, respectively); a higher probability to achieve a pCR, regardless of the therapy performed, was observed in hormone-receptor negative disease (73% vs. 54% in "chemotherapy-free" group, respectively) than hormone-receptor positive tumors (44% vs. 35% in "chemotherapy-free" group, respectively) [21]. At a median follow-up of 37 months, compared with chemotherapy plus dual anti-HER2 blockade, "chemotherapy free" strategy led to a greater risk of an event-free survival event (more locoregional progression events before surgery; HR 2.61), a similar risk of an IDFS event (HR 1.11), fewer grade ≥3 AEs during neoadjuvant treatment, and more AEs leading to treatment discontinuation during adjuvant treatment [21].

Based on available clinical results, the management of patients with HER2-positive early breast cancer has radically changed: all patients candidate to neoadjuvant taxane-based chemotherapy plus anti HER2 targeted therapy (trastuzumab with or without pertuzumab, if available) will receive adjuvant trastuzumab alone (or plus pertuzumab, if available, in the presence of high-risk features at diagnosis) in case of pCR or adjuvant T-DM1 in presence of residual invasive disease at surgery (breast and/or lymph nodes). In metastatic setting, T-DM1, as a single agent, is indicated for the treatment of patients with HER2-positive, unresectable locally advanced or metastatic breast cancer pretreated with trastuzumab and a taxane, separately or in combination, who had either received prior therapy or developed disease recurrence during or within six months of completing adjuvant therapy.

Concurrent administration of T-DM1 and radiotherapy

Patients with early breast cancer often need curative-intent radiotherapy (such as after breast conservative surgery and/or in presence of positive axilla lymph nodes), as well as, those with metastatic breast cancer often need palliative radiotherapy. The extension of indications for treatment with T-DM1, either adjuvant or metastatic settings, raised the question of the safety of this anti-HER2 agent in combination with radiotherapy. Yet, little is known about the safety of this strategy.

HER2-overexpression emerged as a factor of radioresistance in preclinical studies *in vivo* and *in vitro* in breast cancer [22-26]. Liang *et al*. demonstrated that experimental elevation of HER2 levels in the MCF7 breast cancer cell line (which normally expresses low levels of HER2) by tranfection (MCF7HER2) caused increased resistance of the cells to ionizing radiation-induced apoptosis and increased clonogenic survival after radiation. Furthermore, the radiosensitivity of these cells was restored through down-regulation of HER2 by trastuzumab suggesting HER2 as an ideal target for sensitizing breast cancer cells to ionizing radiation. This might be attributed primarily to the inhibition of the PI3K/Akt downstream signal pathway rather than to that of another important downstream signal pathway, the MEK/MAPK-mediated signal transduction [22]. Indeed, PI3K/Akt pathway activation led to resistance to apoptosis, deregulation of the cell cycle and acceleration of DNA repair mechanisms. Other mechanisms of radioresistance of HER2-positive breast cancers include: radiation-induced increase of anti-apoptotic transcription factors (NF-KB and c-myc), the Fak protein or STAT3-survivin signaling [22-26]. Similar conclusions were reported by Pietras *et al*. who, focusing on the cell cycle, observed that breast cancer cell damage by radiation may be particularly vulnerable to injury when lacking the essential HER2-mediated signaling pathways [25].

Beside trastuzumab, different radiosensitizing effects have been reported also with lapatinib treatment, such as downregulation of pDNAPK involved in NHEJ repair, thus preventing repair of radiation-induced DNA damage, and Akt or MEK/ERK inhibition [26-29].

Of note, the restored radiosensitivity of the cells achieved by ErbB signal inhibition is limited by parallel signaling pathways circumventing the blockade. To overcome this, Adams *et al.* studied an alternative ErbB mediated radiosensitization paradigm based on ErbB direct antibody drug conjugates (ADC), that selectively radiosensitized tumours based on surface receptor expression [30]. They found that two class of anti-tubulins, auristatins and maytansinoids, were potent radiosensitizers either as free drug or conjugated to anti-ErbB antibodies (such as T-DM1). However, as free agent, ant-tubulin drugs indiscriminately radiosensitizeb tumor cells while, when conjugated to anti-ErbB antibodies (T-DM1), restricted theirs radiosensitizing capacity directing delivery of highly potent anti-tubulin drugs in a receptor-restricted manner to selectively radiosensitize tumours. The reduced risk of tumor resistance emerging (thanks to multiple mechanisms of attacks against cancer cells), the possibility of reduction of the dose of each individual modality (thus decreasing the toxicities intrinsically associated with each therapy), the attacking against both local disease and potential micrometastases, the potential synergistic effect targeting tumors and not normal tissue, and the definition of temporal window(s) of maxima radiosensitization in which delivers radiation, support the combination of ADC and ionizing radiation. Adams *et al.* suggested that trastuzumab but, especially T-DM1 (due to the coupled mertansines), provided a potent and tumor selective radiosensitization in gastric/esophagus HER-2 positive tumors. Trying to optimize dosing of the combination of the two treatment, they reported that the administration of T-DM1 at day 0 with concomitant radiotherapy at dose of 2.5 Gy on days 1, 2, 3 in mice bearing xenografts gastric or esophagus HER2-positive cells significantly reduced tumor volume compared to control and trastuzumab alone [30].

In a vitro study, Mignot *et al*. have irradiated, at different dose levels, five human breast cancer cell lines presenting various levels of HER2 expression after exposure to T-DM1 to determine *in vitro* the effects of concurrent irradiation and T-DM1 [31]. There was a linear correlation between intrinsic HER2 expression and radioresistance, however, in combination with irradiation, T-DM1 does not induce a radiosensitivity using conditions that allow cell survival. However, further investigations are needed to reach a final conclusion [31].

Therefore, the association of the anti-HER2 agent T-DM1 with radiotherapy is driven by a strong biological rationale. However, a careful analysis of every clinical data is required before greenlighting the simultaneous radiotherapy-T-DM1 combination. Unfortunately, data about toxicity of this strategy are rare. This review of literature aims to expose the clinical studies evaluating the efficacy and toxicity of the radiosensitizing effects of concurrent T-DM1 and radiotherapy.

Materials and methods

Published data useful for this review were identified by searching in PubMed Medline, until January 20, 2020. Only articles English written studies were considered. The research was performed using the following search terms "breast cancer", "radiotherapy", "irradiation", "radiation therapy" and combinations with "anti-HER2 targeted therapy", "T-DM1", and "trastuzumab emtansine". Title and abstracts presented between 2000 and 2020 at the main international meetings also were screened to determine eligibility in the review. Case report were included. The search has been updated for this article with the proceedings of 2020 European Society for Medical Oncology (ESMO) meeting.

Results

While several retrospective and prospective clinical trials have evaluated concomitant treatment with trastuzumab and radiotherapy or lapatinib and radiotherapy, data about efficacy and toxicity of the combination of TDM-1 with radiotherapy are rare.

Concurrent T-DM1 and radiotherapy for CNS and/or leptomeningeal metastases: Breast cancer represents, after lung cancer, the second most common solid malignancy that metastatizes to CNS. HER2-positive breast cancer is more likely than other subtypes to develop brain metastases. It is estimated that over 50% of patients with metastatic HER2-positive breast cancer may develop brain metastases during the natural history of the disease [32]. Of note, the success of anti-HER2 therapies in extracranial systemic management of these patients achieved with trastuzumab and pertuzumab in addition to chemotherapy has contributed to the increased incidence of brain metastases in this group. In addition to HER-2 positivity, other risk factors of developing brain metastases are: young age at diagnosis, lymph node involvement, a short time interval between the diagnosis of primary tumor and the finding of distant metastases, a high burden metastatic disease (more than two metastatic sites), the presence of lung metastases, high grading, and hormone receptor negativity [33]. Treatment options for those patients are limited and consists of a multimodal approach that integrates local (neurosurgery, stereotactic radiotherapy, panencephalic radiotherapy) and systemic therapies based on the use of anti-HER2 agents [32]. Although the appearance of brain metastases continues to represent a prognostically unfavorable event, systemic treatment including HER2-targeted agents have improved clinical outcomes reaching a median survival of approximately 11-30 months. In addition to brain metastases, CNS involvement may also be associated with leptomeningeal metastases (about 5% of breast cancer) with a very poor prognosis (2-4 months) [34]. While trastuzumab is less effective in controlling CNS metastases, targeted tyrosine kinase inhibitors lapatinib and neratinib (alone or in combination with capecitabine) and combination of trastuzumab plus capecitabine and tucatinib (HER2-specific tyrosine kinase inhibitor) have been shown to be active in patients with brain metastases from HER2-positive breast cancer [35-37]. Among other potentially active drugs, T-DM1 appeared to be effective in these patients. A delayed growth of brain metastases with a longer survival benefit and a significant superior ADCC response in brain microenviroment has been reported in murine models of brain lesions from HER2 positive breast cancer treated with T-DM1 compared to trastuzumab, due to the cytotoxicity of the DM1 component [38].

In a post-hoc analysis done on the subgroup of patients with asymptomatic brain metastases at baseline previously treated with radiotherapy in the EMILIA trial, treatment with T-DM1 provided a substantial improvement in OS compared to lapatinib plus capecitabine, with a gain of 13.9 months (median OS: 26.8 vs. 12.9 months, HR 0.38, P = 0.008) [39].

A significant improvement in OS versus treatment of physician's choice was observed with T-DM1 treatment even in the subgroup of 72 HER2-positive heavily pretreated patients with baseline brain metastases enrolled in the phase III TH3RESA trial (17.3 vs. 12.6 months; HR 0.62); of note, patients with symptomatic or untreated CNS metastases or those whose CNS metastases were treated within 1 month of randomization were excluded from this study [39].

Additionally, a handful of small trials and case studies provided signal of clinical activity for T-DM1 supporting its efficacy in this setting, with no unexpected toxicities [40-45].

Perhaps, the most encouraging results are drawn from the exploratory analysis of the phase IIIb KAMILLA study in which T-DM1 treatment was associated with a clinically meaningful antitumor activity in patients pretreated or not with radiotherapy. A reduction in the sum of the major diameters of brain lesions ≥30% occurred in 42.9% of patients with measurable CNS lesions, including 49.3% of 67 patients who had not undergone prior radiotherapy for brain metastases [12].

Concerning the issue of efficacy and tolerance of concurrent administration of T-DM1 and cerebral irradiation, the few available data come from case report or small series of patients (Table 1) [46-51]. Borges *et al.* described a case report of a 62-year-old patients who showed isolated progression in the CNS associated with controlled illness outside the CNS months after the start of treatment with T-DM1. Patients underwent treatment with total cerebral radiotherapy (30 Gy, 3 Gy/fraction) concomitantly with administration of T-DM1 without apparent toxicity. After 6 months of follow-up, there was a good tolerance for radiotherapy, as no adverse reactions or clinically significant toxicity were observed associated with lack of progression in the CNS and partial response outside the CNS [45]. An interesting antitumor activity of T-DM1 and concomitant whole brain radiotherapy (WBRT) was described for the first time in a young patient with extensive brain and leptomeningeal involvement [46]. As known, the presence of leptomeningeal metastases represents a major issue in clinical practice due to its significant morbidity and dismal prognosis. The concomitant treatment provided in this patient a complete response after three course of therapy, with complete resolution of neurological symptoms and no relevant toxicities (confirmed after 13 months of T-DM1 treatment). Of course, further prospective trials are need to confirm these findings [47].

Table 1. Summary of main retrospective review, small series and case reports on concurrent T-DM1 and brain radiotherapy

First Author	Design	N. of pts	Combination	RT	Best response	Radiation-induced toxicity
Borges *et al*. [46]	Case report	1	Concurrent	WBRT (30 Gy/10 fractions)	PR	No AEs
Ricciardi *et al.* [47]	Case report	1	Concurrent	WBRT (20 Gy/5 fractions)	CR	No AEs
Carlson *et al.* [48]	Small series	7	Concurrent	SRS (16-24 Gy)	Not PD	CSRN: 57%
Geraud *et al.* [49]	Small series	12	Concurrently: 4 pts Sequentially: 8 pts	SRS	ORR Concurrently: 75%	RN Concurrently: 50% Sequentially: 28.6% Edema Concurrently: 25% Sequentially: 28.6%
Mitsuya *et al.* [50]	Small series	2	Sequentially	SRS	MRI: heterogeneous mass - No neoplastic cell at pathologic assessment	RN
Stumpf *et al.* [51]	Retrospective review	23 T-DM1 /SRS (of 45 pts on SRS)	Among T-DM1/SRS group: Concurrently: 16 pts Sequentially: 7 pts	SRS	-	CSRN Overall: 22.2% (10/45 pts) T-DM1/SRS group: 39.1% (9/23 pts) of which 6 pts concurrently No T-DM1 /SRS group: 4.5% (1/22)

AEs: adverse events; CR: complete response; CSRN: clinically significant radiation necrosis; MRI: magnetic resonance imaging; PR: partial response; SRS: stereotactic radiosurgery; PD: progressive-disease; RN: radiation necrosis; RT: radiotherapy; WBRT: whole brain radiation therapy

However, several cases of complication due to concurrent administration of radiotherapy, especially stereotactic radiosurgery (SRS), and T-DM1 treatment have been reported in literature. Carlson *et al.* published a series of 13 patients of whom 7 patients received T-DM1 and SRS reporting an overall rate of clinically significant radiation necrosis (CSRN) of 57% [48]. The four patients who developed CSRN presented a clinically significant increased edema as evidenced by neurologic changes and magnetic resonance imaging (MRI) findings shortly after a T-DM1 infusion (SRS to one or more lesions was administered at a median of 8.5 days prior to a T-DM1 infusion). Steroids were administered in all four patients and three of them stopped T-DM1 due to neurologic symptoms. The patient who continued developed worsening symptoms, required steroid resumption and, later, underwent to resection of this symptomatic metastasis: severe radionecrosis with no viable tumor cells identified was observed at pathological assessment [48]. A subsequent publication retrospectively studied twelve patients treated with T-DM1 and SRS concurrently (four patients) or sequentially (eight patients) for brain metastases [49]. After concurrent treatment, a response rate was observed in 75% of patients (with one complete response, one partial response, one stable disease and one progression). Concomitant treatment appeared to be well tolerated (no patients experienced interruption of treatment due to side effect) although there has been observed a higher risk of radiation necrosis (50% vs. 28.6% in the concurrent and the sequential group, respectively) with a similar rate of oedema (25% vs. 28.6%, respectively) [49].

A delayed cerebral radiation necrosis with rapid symptomatic growing mass sign associated with T-DM1 treatment was reported in two heavily treated patients with brain metastases from HER2-positive breast cancer [50]. When these patients received T-DM1 therapy several years after SRS (6 and 8 years), a small radiation necrosis area was recognized on brain magnetic resonance imaging of each patient that increased in size and became symptomatic during 13 or 14 months of T-DM1 treatment. After surgery, necrosis, hematoma, granulation tissue and telangiectasia without neoplastic cells was proven at pathological examination [50].

Recently, Stumpf *et al.* tried to define better the mechanism and the risk of CSRN related to T-DM1 and SRS combination starting from their experience on 45 patients with intracranial metastases from breast cancer [51]. When administration of T-DM1 was combined with SRS, there was a 13.5-fold increase in risk developing CSRN ($P = 0.02$). Twenty-three patients received T-DM1 treatment, of which 16 concurrently with SRS. Of the entire cohort (45 patients), CSRN occurred in ten patients, nine of whom treated with T-DM1 (9/23 patients, 39.1%) and only one case was reported in the group not treated with T-DM1 (1/22 patients, 4.5%). Among nine patients with CSRN in T-DM1 and SRS combining group, 6 of those had received T-DM1 concurrently. Increasing total number of SRS course delivered ($P < 0.01$) and older age (>45 years; $P = 0.04$) were associated with increased risk of developing CSRN. On contrary, CSRN incidence rate was not correlated with the number of treated lesions ($P = 0.57$) or receipt of WBRT ($P = 0.80$) [51]. Furthermore, they explored the mechanism underlying radionecrosis and relative significant cerebral edema due to T-DM1/SRS treatment. Human astrocytes are modulators of the neuroinflammatory response and regulators of water flow across the blood-brain barrier via Aquaporin-4 (water transporter); astrocytes express normal levels of HER2 and thus are targeted both by T-DM1 and trastuzumab. When human astrocytes have been treated with increasing doses of radiation therapy alone or combined to T-DM1 or trastuzumab, T-DM1 targeted reactive astrocytes and increased radiation-induced cytotoxicity and astrocytic swelling via upregulation of Aquaporin-4. This enhanced Aquaporin-4 expression and astrocytic swelling was specific to T-DM1 but not trastuzumab or other chemotherapeutic agents, potentially due to the uptake of emtansine in HER2-positive astrocytes. Furthermore,

higher doses of radiotherapy (but not at lower doses) were associate to most significant changes in Aquaporin-4 upregulation and astrocytic swelling, suggesting that toxic effect on astrocytes and risk of CSRN and radionecrosis may be reduced at lower dose fractionated SRS, as opposed to higher-dose single fraction SRS schedules. Thus, the author suggest that mechanism underlying the significant toxicity associated with T-DM1/SRS was a critical targeting of astrocytes and induction of cytotoxic edema. However, further studies are needed to clarify if Aquaporin-4 and astrocytic swelling are sufficient events to explain this toxicity and to define timing of T-DM1 and radiation dosing to further stratify risk of CSRN and mitigate toxicity [51].

Although it is difficult drawn a clear conclusion due to heterogeneity and overall small number of patients, these results collectively suggest caution for concurrent administration of T-DM1 ad radiotherapy (WBRT and SRS) either as sequential or concomitant therapy. Prospective trials are needed to define the safety of this combination. A phase I study of T-DM1 in combination with sequential WBRT has been completed, however no results have been posted yet (NCT02135159).

Concurrent T-DM1 and curative-intent breast radiotherapy: After the remarkable finding of a 50% relative reduction in the risk of recurrence or death, adjuvant T-DM1 has been adopted as a standard of care for patients with HER2-positive early breast cancer not achieving a pCR after neoadjuvant chemotherapy plus anti-HER2 therapy [18]. Generally, T-DM1 was well tolerated, although treatment toxicities were higher with TDM-1 as compared with trastuzumab. In clinical practice, adjuvant radiotherapy is strongly recommended after conservative surgery as well as after mastectomy in patients with involved resection margins, involvement of more than four axillary nodes and T3–T4 breast cancer. Thus, the possible concurrent use of adjuvant radiotherapy during administration of systemic treatment is a practical issue to be considered of high relevance. While prior studies have suggested the safety of administering radiotherapy during trastuzumab [52], limited data are available on the integration of T-DM1 to standard adjuvant radiation therapy (Table 2). Given the risk of cardiotoxicity related to trastuzumab, the targeting antibody of T-DM1, a phase II trial investigated on the cardiac safety, efficacy, and overall feasibility of

Table 2. Summary of main studies, small series and case reports on concurrent T-DM1 and breast radiotherapy

First Author	Design	N. of pts	Combination	Radiation-induced toxicity	Note
Krop *et al.* [53]	Phase II	116	Concurrently: 39 Sequentially: 77	No protocol prespecified cardiac events G2 AEs: Concurrently: 7.7% Sequentially: 2.6% G3 AEs: Concurrently: 2.6% Sequentially: 2.6% No G4 AEs	Completed ≥95% planned RT dose with delay 5 days Concurrently: 94.7% Sequentially: 96.1%
Von Minckwitz *et al.* [18]	Phase III KATHERINE trial	1486 (743 treated with T-DM1 743 treated with trastuzumab)	Concurrent	Low rate radiation pneumonitis - T-DM1: 1.5% - Trastuzumab: 0.7% Radiation related skin injury - T-DM1: 25.4% - Trastuzumab: 27.6% G3 related skin injury - T-DM1: 1.4% - Trastuzumab: 1%	3-yrs IDFS TDM1: 88.3% Trastuzumab: 77.0% (HR 0.50)
Loibl *et al.* [54]	KATHERINE subgroup analysis	ADJ RT - plus T-DM1: 624 - plus trastuzumab: 597 No ADJ RT - T-DM1: 146 - Trastuzumab: 119	Concurrent	G≥3 AEs ADJ RT - plus T-DM1: 27.4% - plus trastuzumab: 16.2% No ADJ RT - T-DM1: 88.2% - Trastuzumab: 77.5%	3-yrs IDFS ADJ RT - plus T-DM1: 88.3% - plus trastuzumab: 77.4% (HR 0.50) No ADJ RT - T-DM1: 88.2% - Trastuzumab: 75.5% (HR 0.50)
Corbin *et al.* [55]	Case report	1	Concurrent	G3 radiation dermatitis	-
Zolcsak *et al.* [56]	Small series	14	Concurrent	One G1 radiation dermatitis Two G2 left ventricular ejection fraction decline Three transaminases increase (one G1, one G2, and one G3)	-

ADJ: adjuvant; AEs: adverse events; HR: hazard ratio; IDFS: invasive disease-free survival; RT: radiotherapy

T-DM1 treatment, including the concurrent administration of T-DM1 and adjuvant radiotherapy, after administration of anthracycline-based chemotherapy in the (neo)adjuvant setting [53]. Patients with HER2-positive early-breast cancer, with pre-chemotherapy left ventricular ejection fraction (LVEF) ≥55%, received (neo)adjuvant anthracycline-based chemotherapy followed by four cycles of T-DM1. After surgery, patients continued T-DM1 for approximately 1 year; three to four cycles of optional docetaxel with or without trastuzumab were allowed before resuming T-DM1. T-DM1 treatment was administered sequentially or concurrently with adjuvant radiotherapy (sequential radiotherapy could be administered with or without trastuzumab). A total of 153 patients were enrolled, of whom 116 (75.8%) received radiotherapy (concurrent, n= 39; sequential, n = 77). A similar proportion of patients completed ≥ 95% of the planned radiotherapy dose with delay 5 days in the concurrent and the sequential group: 94.7% among those received concurrent radiotherapy (36 of 38 patients of whom radiotherapy dose information was available) and 96.1% (74 of 77 patients) among those received sequential radiotherapy. No protocol-prespecified cardiac events or symptomatic congestive heart failure events were reported after T-DM1 treatment. Cardiac AEs suspected related to T-DM1 occurred in five patients (3.4%). Four patients (2.7%) experienced asymptomatic declines in LVEF (≥ 10 percentage points from baseline to LVEF < 50%) and one patient discontinued T-DM1 with an LVEF of 45% (not reported whether in the concomitant or sequential group). There was not observed LVEF < 40% reductions and mean LVEF was stable during T-DM1 treatment. During concurrent T-DM1-radiotherapy treatment, three patients experienced grade 3 AEs (7.7%; one each: neutropenia, asthenia, erythema) versus two patients treated sequentially (2.6%; neutropenia, radiotherapy pneumonitis); grade 2 radiotherapy associated pneumonitis occurred in one patient (2.6%) for each group. No grade 4 AEs were reported during concurrent or sequential radiotherapy [53].

Like other microtubule inhibitors, including taxanes and vinca alkaloids, T-DM1 is known radiation sensitizers and, thus a potential increase in the risk radiation-related toxicities, including dermatitis and pneumonitis, is possible. In the phase III KATHERINE trial, patients received radiation therapy according to institutional standards and the trial protocol [18]. Whole breast irradiation was performed in patients undergoing breast-conserving surgery (tumor bed boost administered according to local policy). Patients with clinical T3 node-positive disease or T4 disease and/or with clinical N2 or N3 disease received regional node irradiation with either whole breast irradiation (if the patient had undergone breast conserving surgery) or chest wall and regional node irradiation (if the patient had undergone mastectomy). Radiotherapy had been initiated within 60 days of surgery in the absence of complications requiring delay and was given concurrently with T-DM1. The safety profile of T-DM1 was consistent with that in previous studies and, as expected, adjuvant T-DM1 was associated to more AEs than adjuvant trastuzumab (any grade AEs: 98.8% vs. 93.3% with T-DM1 and trastuzumab, respectively). The most common AEs of any grade were fatigue, nausea, increased transaminases, musculoskeletal pain, hemorrhage, thrombocytopenia, headache, peripheral neuropathy, and arthralgia. Grade 3 or 4 AEs occurred in 25.7% versus 15.4% of patients, with the most common in the T-DM1 group (> 2%) being thrombocytopenia (5.7%) and hypertension (2.0%), while hypertension (1.2%) and radiation-related skin injury (1.0%) in the trastuzumab group. Higher incidence of peripheral sensory neuropathy of any grade (18.6% vs. 6.9%) as well as increased aminotransferase levels of any grade (elevated alanine aminotransferase level in 23.1% vs. 5.7% and elevated aspartate aminotransferase level in 28.4% vs. 5.6%) were reported in patients who received T-DM1 than trastuzumab. Pneumonitis of any grade occurred in 19 patients in the T-DM1 group (2.6%) and in 6 patients in the trastuzumab group (0.8%). Although there were only five events (0.3%) overall, there were fewer adjudicated cardiac events in the T-DM1 group (one patient; 0.1%) than in the trastuzumab group (four patients; 0.6%) [18]. Reviewing toxicities relevant to radiation, a numerical increase in the low rate of radiation pneumonitis was noted in patients receiving T-DM1, at 1.5% (11 patients) compared with 0.7% (five patients) in those receiving trastuzumab; all cases were resolved. Radiation related skin injury of any grade was reported in 25.4% and 27.6% of patients on the T-DM1 and the trastuzumab arm, respectively. Grade 1 and 2 radiation-related skin injury was significantly more common than grade 3 toxicity, which occurred in ten patients (1.4%) in T-DM1 arm versus seven (1%) in trastuzumab arm [18].

In a recently published subgroup analysis of the KATHERINE trial, data on three common subgroups (concomitant T-DM1 and adjuvant radiotherapy; concomitant T-DM1 and adjuvant hormonal therapy; conversion from HER2-positive disease prior to neoadjuvant therapy to HER2-negative disease in the surgical specimen) have been presented [54]. Focusing on adjuvant radiotherapy, it was administered in the majority of patients (82% of patients). The 3-years IDFS benefit with T-DM1 was consistent regardless administration of radiotherapy (3-yrs IDFS in adjuvant radiotherapy group: 77.4% vs. 88.3%, HR=0.50; no radiotherapy: 75.5% vs. 88.2%, HR 0.50, with trastuzumab and T-DM,1 respectively), as well as, fewer recurrences occurred with T-DM1 irrespective of radiotherapy (in adjuvant radiotherapy group: 22.8% vs. 12.2%; no radiotherapy: 19.9% vs. 12.6%, with trastuzumab and T-DM1, respectively). There were not reported new safety signals with concomitant treatment. Grade≥3 AEs (27.4% vs. 16.2%) and serious AEs (13.2% vs 10.3%) with T-DM1 were more common in the adjuvant radiotherapy group. In this subgroup, the increase in grade ≥3 AEs with T-DM1 included higher frequencies of decreased platelet count (6.1% vs 3.4%), radiation skin injury (1.6% vs 0%), hypokalemia (1.4% vs 0%), anemia (1.3% vs 0%), diarrhea (1.0% vs 0%) and mastitis (0.8% vs 0%) compared with no-adjuvant radiotherapy group. There was more (mostly low-grade) all-grade hemorrhage, pulmonary toxicity, and cardiac dysfunction in patients receiving T-DM1 plus adjuvant radiotherapy versus T-DM1 alone. Two patients treated with the concurrent treatment had grade 3 radiation pneumonitis; both cases resolved [54].

Within their growing experiences, Corbin KS *et al.* have noted what appears to be heightened skin toxicity in several patients when TDM-1 was administered concurrently with radiotherapy [55]. The authors reported a case of grade 3 radiation dermatitis, with skin findings felt more significant than expected based on skin dose, appeared during the second week of treatment in a patient receiving adjuvant TDM-1 along with radiotherapy to the chest wall and regional lymph nodes at dose of 50 Gy delivered in 25 fractions over 32 total days, without a boost. During desquamation phase, the patient was treated with topical steroids and after that, when developed dry desquamation, was managed with vinegar soaks and silver sulfadiazine cream with completely resolution [55]. Going forward with the use of adjuvant T-DM1, it important continue to monitor skin reactions in patients who receive concurrent T-DM1 and radiotherapy when indicated and try to mitigate these toxicities.

Recently, there were present preliminary results of a single-centre experience on concurrently administration of T-DM1 and radiotherapy in 14 patients with residual invasive HER2-positive breast cancer [56].

Adjuvant radiotherapy was delivered at the dose of 50 Gy for breast/chest wall: ten patients underwent lymph node radiation and four got also tumor bed boost. The acute toxicity rate was assumed acceptable, especially focusing on skin and cardiac toxicity: grade 1 radiodermatitis was the most common side effect. Two patients experienced reversible grade 2 left ventricular ejection fraction decline; increase in alanine aminotransferases occurred in three patients (one grade 1, one grade 2, and one grade 3) [56]. Despite the limited data, the concurrent use of radiotherapy during T-DM1 treatment can be considered feasible.

Concurrent T-DM1 and radiotherapy for bone metastases: A high percentage of the patients with HER2-positive breast cancer will develop bone symptomatic metastases. Radiation therapy has a well-defined role in both the palliation of pain and the prevention of morbidity of bone disease. Radiotherapy allows a reduction of the pain symptom until a complete remission that can be achieved in 20% of cases; it can also induce bone recalcification although it cannot guarantee fracture prevention. It is therefore strongly indicated in cases of pain, risk of fracture, spinal cord compression and after stabilization surgery. As previously explained, yet, very little is known about the association T-DM1 and radiotherapy. Preliminary results of the concurrent use of radiotherapy for bone metastases and T-DM1 have been reported in three heavily pretreated patients with HER2-positive metastatic breast cancer in 2015 by Géraud A *et al*. [57]. During T-DM1 treatment, these patients presented symptomatic bone metastatic disease and thus received hypofractionated radiotherapy (15 Gy/5 fractions in two cases and 8 Gy/1 fraction in one case). While all of the experienced a good relief of the symptoms, there was not observed an increase in toxicity. Of course, this is a very small series of concurrent use and larger prospective studies are needed [57].

Conclusion

With the approval of newer systemic treatments (such as pertuzumab, T-DM1, cyclin-dependent kinases 4 and 6 inhibitors, and the immunotherapies such as pembrolizumab and atezolizumab) for breast cancer and their expanding indications, it seems essential to understand whether these drugs can be safely and effectively paired with radiation therapy. Generally, if combining radiation and systemic therapy is better than either modalities alone, if relapse rates are higher when treatments are combined rather than when given sequentially, if the toxicity of one treatment is enhanced by the other when used concomitantly, what is more convenient and practical between concurrent or sequential strategy, are still questions waiting to be answered. Concerning concurrent anti-HER2 therapy and radiotherapy, HER2-overexpression emerged as a factor of radioresistance in preclinical studies *in vivo* and *in vitro* in breast cancer. However, radiosensitivity of the cells was restored through down-regulation of HER2 by anti-HER2 treatments, suggesting HER2 as an ideal target for sensitizing breast cancer cells to ionizing radiation. Among these, it was shown that T-DM1 induced significant lethality causing cells to arrest in the G2/M phase of the cell cycle.

We aimed at bringing together every published data about the issue of safe combined use of treatment with T-DM1 and palliative or curative radiotherapy. To date, while prior studies have suggested the safety of administering radiotherapy during trastuzumab, combining T-DM1 with radiation therapy has only evaluated through limited retrospective data, small series and case reports. A slightly more common radiation pneumonitis has been reported when T-DM1 was administered with breast radiation (with similar radiation skin injury), as well as, development of radiation brain necrosis when it was administered concurrently with radiosurgery. Based on the available data, it is possible to deduce that T-DM1 plus radiotherapy can be considered feasible, but, generally, it should be avoided the concurrent administration of T-DM1 and brain radiation or T-DM1 and breast regional nodal radiation, due to the proximity of the radiation to the brachial plexus and the increased amount of the lung in the radiation field, which could increase the risk of pneumonitis.

However, as explained, the current data often concern small numbers of patients, many are retrospective, or do not directly compare the concomitant association between anti-HER2 therapy and radiation. Going forward, it is necessary to continue to monitor side effect in patients who receive concurrent T-DM1 and develop new trials in order to better understand the interactions with radiotherapy.

Competing interest

Muto M.: honoraria as speaker bureau for Astra Zeneca; Amaturo G.: no conflict of interest; Sgambato A.: no conflict of interest; Colantuoni G.: Istituto Gentili, Pfizer, Novartis, Eli Lilly; Iannace C.: no conflict of interest; Gridelli C.: honoraria as speaker bureau, advisory board member or consultant for MSD, BMS, Roche, Astra Zeneca, Pfizer, Novartis, Menarini.

Authorship

*Muto M, Amaturo G, Sgambato A, Colantuoni G, Iannace C, Gridelli C.

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References

1. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, et al. (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235: 177-182. [Crossref]

2. Olayioye MA, Neve RM, Lane HA, Hynes NE (2000) The ErbB signaling network: receptor heterodimerization in development and cancer. *The EMBO Journal* 19: 3159-3167. [Crossref]

3. Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, et al. (2009) TheHER-2 receptor and breast cancer: 10 years of targeted anti-HER-2 therapy andpersonalized medicine. *Oncologist* 14: 320-368.

4. Dawood S, Broglio K, Buzdar AU, Hortobagyi GN, Giordano SH, et al. (2010) Prognosis of women with metastatic breast cancer by HER2 status and trastuzumab treatment: an institutional-based review. *Journal of Clinical Oncology* 28: 92-98. [Crossref]

5. Lewis Phillips GD, Li G, Dugger DL, Crocker LM, Parsons KL, et al. (2008) Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibodycytotoxic drug conjugate. *Cancer Research* 68: 9280-9290. [Crossref]

6. Erickson HK, Park PU, Widdison WC, Kovtun YV, Garrett LM, et al. (2006) Antibody-maytansinoid conjugates are activated in targeted cancer cells by lysosomal degradation and linker-dependent intracellular processing. *Cancer Research* 66: 4426-4433.

7. Junttila TT, Li G, Parsons K, Phillips GL, Sliwkowski MX, et al. (2011) Trastuzumab-DM1 (T-DM1) retains all the mechanisms of action of trastuzumab and efficiently inhibits growth of lapatinib insensitive breast cancer. *Breast Cancer Research and Treatment* 128: 347-356. [Crossref]

8. Swain SM, Miles D, Kim SB, Im YH, Im SA, et al. & CLEOPATRA study group (2020) Pertuzumab, trastuzumab, and docetaxel for HER2-positive metastatic breast cancer (CLEOPATRA): end-of-study results from a double-blind, randomised, placebo-controlled, phase 3 study. *The Lancet Oncology* 21: 519-530. [Crossref]

9. Diéras V, Miles D, Verma S, Pegram M, Welslau M, et al. (2017) Trastuzumab emtansine versus capecitabine plus lapatinib in patients with previously treated HER2-positive advanced breast cancer (EMILIA): a descriptive analysis of final overall survival results from a randomised, open-label, phase 3 trial. *The Lancet Oncology* 18: 732-742.

10. Krop IE, Kim SB, Martin AG, LoRusso PM, Ferrero JM, et al. (2017) Trastuzumab emtansine versus treatment of physician's choice in patients with previously treated HER2-positive metastatic breast cancer (TH3RESA): final overall survival results from a randomised open-label phase 3 trial. *The Lancet Oncology* 18: 743-754. [Crossref]

11. Montemurro F, Ellis P, Anton A, Wuerstlein R, Delaloge S, et al. (2019) Safety of trastuzumab emtansine (T-DM1) in patients with HER2-positive advanced breast cancer: Primary results from the KAMILLA study cohort 1. *European Journal of Cancer* 109: 92-102.

12. Montemurro F, Delaloge S, Barrios CH, Wuerstlein R, Anton A, et al. (2020) Trastuzumab emtansine (T-DM1) in patients with HER2-positive metastatic breast cancer and brain metastases: exploratory final analysis of cohort 1 from KAMILLA, a single-arm phase IIIb clinical trial. *Annals of Oncology* 31: 1350-1358.

13. Dzimitrowicz H, Berger M, Vargo C, Hood A, Abdelghany O, et al. (2016) T-DM1 activity in metastatic human epidermal growth factor receptor 2-positive breast cancers that received prior therapy with trastuzumab and pertuzumab. *Journal of Clinical Oncology* 34: 3511e3517. [Crossref]

14. Fabi A, Giannarelli D, Moscetti L, Santini D, Zambelli A, et al. (2017) Ado-trastuzumab emtansine (TDM1) in HER2þ advanced breast cancer patients: does pretreatment with pertuzumab matter? *Future Oncology* 13: 2791e2797.

15. Conte B, Fabi A, Poggio F, Blondeaux E, Dellepiane C, et al. & Gruppo Italiano Mammella (GIM) study group (2020) T-DM1 efficacy in patients with HER2- positive metastatic breast cancer progressing after a taxane plus pertuzumab and trastuzumab: an Italian multicenter observational study. *Clinical Breast Cancer* 20: e181ee187.

16. Battisti NML, Rogerson F, Lee K, Shepherd S, Mohammed K, et al. (2020) Safety and efficacy of T-DM1 in patients with advanced HER2-positive breast cancer The Royal Marsden experience. *Cancer Treatment and Research communications* 24: 100188.

17. Urruticoechea A, Seok-Ah I, Montserrat M, Baselga J, Yardley DA, et al. (2017) Efficacy of trastuzumab emtansine (T-DM1) in patients (pts) with HER2þ metastatic breast cancer (MBC) previously treated with pertuzumab (P). *Journal of Clinical Oncology* 35: 1023.

18. Von Minckwitz G, Huang CS, Mano MS, Loibl S, Mamounas EP, et al. & KATHERINE Investigators (2019) Trastuzumab emtansine for residual invasive HER2-positive breast cancer. *The New England Journal of Medicine* 380: 617-628. [Crossref]

19. Harbeck N, Im S-A, Barrios CH, Bonnefoi HR, Gralow J, et al. (2020) Primary analysis of KAITLIN: A phase III study of trastuzumab emtansine (T-DM1) + pertuzumab versus trastuzumab + pertuzumab + taxane, after anthracyclines as adjuvant therapy for high-risk HER2-positive early breast cancer (EBC). *Journal of Clinical Oncology* 38: 500.

20. Tolaney SM, Trippa L, Barry W, Hu J, Dang C, et al. (2020) Abstract GS1-05: TBREAST CANCERRC 033: A randomized phase II study of adjuvant trastuzumab emtansine (T-DM1) vs paclitaxel (T) in combination with trastuzumab (H) for stage I HER2-positive breast cancer (BC) (ATEMPT). *Cancer Research* 80: GS1-G05.

21. Hurvitz SA, Martin M, Jung KH, Huang CS, Harbeck N, et al. (2019) Neoadjuvant Trastuzumab Emtansine and Pertuzumab in Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer: Three-Year Outcomes from the Phase III KRISTINE Study. *Journal of Clinical Oncology* 37: 2206-2216. [Crossref]

22. Liang K, Lu Y, Jin W, Ang KK, Milas L, et al. (2003) Sensitization of breast cancer cells to radiation by trastuzumab. *Molecular Cancer Therapeutics* 2: 1113-1120.

23. Papanikolaou V, Iliopoulos D, Dimou I, Dubos S, Kappas C, et al. (2011) Survivin regulation by HER2 through NF-jB and c-myc in irradiated breast cancer cells. *Journal of Cellular and Molecular Medicine* 15: 1542-1550.

24. Kim JS, Kim HA, Seong MK, Seol H, Oh JS, et al. (2016) STAT3-survivin signaling mediates a poor response to radiotherapy in HER2-positive breast cancers. *Oncotarget* 7: 7055-7065. [Crossref]

25. Pietras RJ, Poen JC, Gallardo D, Wongvipat PN, Lee HJ, et al. (1999) Monoclonal antibody to HER-2/neureceptor modulates repair of radiation-induced DNA damage and enhances radiosensitivity of human breast cancer cells overexpressing this oncogene. *Cancer Research* 59: 1347-55.

26. Mignot F, Ajgal Z, Xu H, Geraud A, Chen JY, et al. (2017) Concurrent administration of anti-HER2 therapy and radiotherapy: Systematic review. *Radiotherapy and Oncology* 124: 190-199. [Crossref]

27. Yu T, Cho BJ, Choi EF, Park JM, Kim DH, et al. (2016) Radiosensitizing effect of lapatinib in human epidermal growth factor receptor 2-positive breast cancer cells. *Oncotarget* 7: 79089- 79100.

28. Sambade MJ, Kimple RJ, Camp JT, Peters E, Livasy CA, et al. (2010) Lapatinib in combinationison with radiation diminishes tumor regrowth in HER2+ and basal-like/EGFR+ breast tumor xenografts. *International Journal of Radiation Oncology, Biology, Physics* 77: 575-581.

29. Sambade MJ, Camp JT, Kimple RJ, Sartor CI, Shields JM, et al. (2009) Mechanism of lapatinib-mediated radiosensitization of breast cancer cells is primarily by inhibition of the Raf>MEK>ERK mitogen-activated protein kinase cascade and radiosensitization of lapatinib-resistant cells restored by direct inhibition ok MEK. *Radiotherapy and Oncology* 93: 639-644. [Crossref]

30. Adams SR, Yang HC, Savariar EN, Aguilera J, Crisp JL, et al. (2016) Antitubulin drugs conjugated to anti-ErbB antibodies selectively radiosensitize. *Nature Communications* 7: 13019.

31. Mignot F, Kirova Y, Verrelle P, Teulade-Fichou MP, Megnin-Chanet F, et al. (2021) In vitro effects of Trastuzumab Emtansine (T-DM1) and concurrent irradiation on HER2-positive breast cancer cells. *Cancer Radiotherapie* 8: S1278-3218(20)30328-0.

32. Brufsky AM, Mayer M, Rugo HS, Kaufman PA, Tan-Chiu E, et al. (2011) Central nervous system metastases in patients with HER2-positive metastatic breast cancer: incidence, treatment, and survival in patients from registHER. *Clinical Cancer Research* 17: 4834-4843. [Crossref]

33. Graesslin O, Abdulkarim BS, Coutant C, Huguet F, Gabos Z, et al. (2010) Nomogram to predict subsequent brain metastasis in patients with metastatic breast cancer. *Journal of Clinical Oncology* 28: 2032-2037.

34. Morikawa A, Jordan L, Rozner R, Patil S, Boire A, et al. (2017) Characteristics and outcomes of patients with breast cancer with Leptomeningeal metastasis. *Clinical Breast Cancer* 17: 23-28.

35. Bachelot T, Romieu G, Campone M, Diéras V, Cropet C, et al. (2013) Lapatinib plus capecitabine in patients with previously untreated brain metastases from HER2-positive metastatic breast cancer (LANDSCAPE): a single-group phase 2 study. The Lancet. *Oncology* 14: 64-71. [Crossref]

36. Awada AH, Brufsky AM, Saura C, Freedman RA, Lin NU, et al. (2020) Abstract P2-20-01: Impact of neratinib on development and progression of central nervous system (CNS) metastases in patients with HER2-positive metastatic breast cancer (MBC): Findings from the NALA, NEfERT-T, and TBCRC 022 trials. *Cancer Research* 80: P2-20-01.

37. Murthy RK, Loi S, Okines A, Paplomata E, Hamilton E, et al. (2020) Tucatinib, trastuzumab, and capecitabine for HER2-positive metastatic breast cancer. *The New England journal of medicine* 382: 597-609. [Crossref]

38. Askoxylakis V, Ferraro GB, Kodack DP, Badeaux M, Shankaraiah RC, et al. (2015) Preclinical Efficacy of Ado-trastuzumab Emtansine in the Brain Microenvironment. *Journal of the National Cancer Institute* 108: djv313.

39. Krop IE, Lin NU, Blackwell K, Guardino E, Huober J, et al. (2015) Trastuzumab emtansine (T-DM1) versus lapatinib plus capecitabine in patients with HER2-positive metastatic breast cancer and central nervous system metastases: a retrospective, exploratory analysis in EMILIA. *Annals of Oncology* 26: 113-119.

40. Bartsch R, Berghoff AS, Preusser M (2014) Breast cancer brain metastases responding to primary systemic therapy with T-DM1. *Journal of Neuro-oncology* 116: 205-206. [Crossref]

41. Torres S, Maralani P, Verma S (2014) Activity of T-DM1 in HER-2 positive central nervous system breast cancer metastases. *BMJ Case Report*: bcr2014205680.

42. Kalsi R, Feigenberg S, Kwok Y, Tkaczuk K, Mehta M, et al. (2015) Brain metastasis and response to ado-trastuzumab emtansine: a case report and literature review. *Clinical Breast Cancer* 15: e163-e166.

43. Keith KC, Lee Y, Ewend MG, Zagar TM, Anders CK (2016) Activity of Trastuzumab Emtansine (TDM1) in HER2-positive breast cancer brain metastases: a case series. *Cancer Treatment Communications* 7: 43-46. [Crossref]

44. Bartsch R, Berghoff AS, Vogl U, Rudas M, Bergen E, et al. (2015) Activity of T-DM1 in Her2-positive breast cancer brain metastases. *Clinical & Experimental Metastasis* 32: 729-737.

45. Jacot W, Pons E, Frenel JS, Guiu S, Levy C, et al. (2016) Efficacy and safety of trastuzumab emtansine (T-DM1) in patients with HER2-positive breast cancer with brain metastases. *Breast Cancer Research and Treatment* 157: 307-318. [Crossref]

46. Borges GS, Rovere RK, Dias SMK, Chong FH, Morais MDS, et al. (2015) Safety and efficacy of the combination of T-DM1 with radiotherapy of the central nervous system in a patient with HER2-positive metastatic breast cancer: case study and review of the literature. *E cancer medical science* 9: 586.

47. Ricciardi GRR, Russo A, Franchina T, Schifano S, Mastroeni G, et al. (2018) Efficacy of T-DM1 for leptomeningeal and brain metastases in a HER2 positive metastatic breast cancer patient: new directions for systemic therapy - a case report and literature review. *BMC Cancer* 18: 97. [Crossref]

48. Carlson JA, Nooruddin Z, Rusthoven C, Elias A, Borges VF, et al. (2014) Trastuzumab emtansine and stereotactic radiosurgery: an unexpected increase in clinically significant brain edema. *Neuro-Oncology* 16: 1006-1009.

49. Geraud A, Xu HP, Beuzeboc P, Kirova YM (2017) Preliminary experience of the concurrent use of radiosurgery and TDM1 for brain metastases in HER2 positive metastatic breast cancer. *Journal of Neuro-oncology* 131: 69-72.

50. Mitsuya K, Watanabe J, Nakasu Y, Hayashi N, Harada H, et al. (2016) Expansive hematoma in delayed cerebral radiation necrosis in patients treated with TDM1 a report of two cases. *BMC Cancer* 16: 391. [Crossref]

51. Stumpf PK, Cittelly DM, Robin TP, Carlson JA, Stuhr KA, et al. (2019) Combination of Trastuzumab Emtansine and Stereotactic Radiosurgery Results in High Rates of Clinically Significant Radionecrosis and Dysregulation of Aquaporin-4. *Clinical Cancer Research* 25: 3946-3953.

52. Kirova YM, Loap P, Fourquet A (2020) Benefit of Post Mastectomy Radiation Therapy (PMRT) in Node-Positive, HER2-Positive Patients with Breast Cancer Receiving Anti-HER2 Treatments. *International Journal of Radiation Oncology, Biology, Physics* 106: 511-513. [Crossref]

53. Krop IE, Suter TM, Dang CT, Dirix L, Romieu G, et al. (2015) Feasibility and cardiac safety of trastuzumab emtansine after anthracycline-based chemotherapy as (neo) adjuvant therapy for human epidermal growth factor receptor 2-positive early-stage breast cancer. *Journal of Clinical Oncology* 33: 1136-1142.

54. Loibl S, Huang CS, Mano MS, Mamounas TP, Geyer CE, et al. (2020) 96O Adjuvant trastuzumab emtansine (T-DM1) vs trastuzumab (T) in patients (pts) with residual invasive disease after neoadjuvant therapy for HER2+ breast cancer: Subgroup analysis from KATHERINE. *Annals of Oncology* 31: S48.

55. Corbin KS, Breen WG, Strauss JB (2020) Radiation dermatitis in patients treated with concurrent trastuzumab emtansine (T-DM1). *Clinical and Translational Radiation Oncology* 24: 99-101.

56. Zolcsák Z, Loirat D, Fourquet A, Kirova YM (2020) Adjuvant Trastuzumab Emtansine (T-DM1) and Concurrent Radiotherapy for Residual Invasive HER2-positive Breast Cancer: Single-center Preliminary Results. *American journal of clinical oncology* 43: 895-901. [Crossref]

57. Géraud A, Xu HP, Beuzeboc P, Kirova YM (2016) Preliminary results of the concurrent use of radiotherapy for bone metastases and trastuzumab emtansine in patients with HER2-positive metastatic breast cancer. *Cancer Radiotherapie* 20: 312-313. [Crossref]