# Preclinical and Clinical Efficacy of Trastuzumab Deruxtecan in Breast Cancer Brain Metastases

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## ABSTRACT

**Purpose:** Brain metastases can occur in up to 50% of patients with metastatic HER2-positive breast cancer. Because patients with active brain metastases were excluded from previous pivotal clinical trials, the central nervous system (CNS) activity of the antibody–drug conjugate trastuzumab deruxtecan (T-DXd) is not well characterized.

**Experimental Design:** We studied how T-DXd affects growth and overall survival in orthotopic patient-derived xenografts (PDX) of HER2-positive and HER2-low breast cancer brain metastases (BCBM). Separately, we evaluated the effects of T-DXd in a retrospective cohort study of 17 patients with stable or active brain metastases.

**Results:** T-DXd inhibited tumor growth and prolonged survival in orthotopic PDX models of HER2-positive (IHC 3+) and HER2-low (IHC 2+/FISH ratio < 2) BCBMs. T-DXd reduced tumor size and prolonged survival in a T-DM1–resistant HER2-positive BCBM PDX model. In a retrospective multi-institutional cohort study of 17 patients with predominantly HER2-positive BCBMs, the CNS objective response rate (ORR) was 73% (11/15) while extracranial response rate was 45% (5/11). In the subset of patients with untreated or progressive BCBM at baseline, the CNS ORR was 70% (7/10). The median time on treatment with T-DXd was 8.9 (1.3–16.2) months, with 42% (7/17) remaining on treatment at data cutoff.

**Conclusions:** T-DXd demonstrates evidence of CNS activity in HER2-positive and HER2-low PDX models of BCBM and preliminary evidence of clinical efficacy in a multi-institution case series of patients with BCBM. Prospective clinical trials to further evaluate CNS activity of T-DXd in patients with active brain metastases are warranted.

*See related commentary by Soffietti and Pellerino, p. 8*

## Introduction

Over time, up to half of patients with HER2-amplified (HER2-positive) metastatic breast cancer will develop central nervous system (CNS) metastases (1). The incidence of CNS metastases in HER2-low tumors (1+ or 2+ by IHC and FISH of HER2/chromosome 17 ratio < 2.0; ref. 2) is not well described in the literature; however, brain metastases are frequent in patients with metastatic triple-negative breast cancer, and occur in about 15% of patients with metastatic estrogen receptor (ER)-positive/HER2-negative breast cancer (3, 4).

Antibody–drug conjugates (ADC) are rapidly altering the therapeutic landscape for both localized and metastatic solid tumors (5). Comprised of a targeting antibody linked to a cytotoxic payload, notable advantages of ADCs compared with naked cytotoxics include a higher therapeutic index, bystander killing due to dispersion of payload, and antitumor immune activity via antibody-dependent cellular cytotoxicity (ADCC; ref. 5).

Trastuzumab deruxtecan (T-DXd) is an ADC comprised of the anti-HER2 antibody trastuzumab and deruxtecan, which is comprised of a topoisomerase I inhibitor and a cleavable tetrapeptide-based linker (6). In the pivotal trial, DESTINY-Breast01, T-DXd demonstrated remarkable efficacy against extracranial metastases in patients with heavily pretreated, HER2-positive, metastatic breast cancer. In the 24 patients with stable, treated brain metastases at study entry, progression-free survival (PFS) and objective response rates (ORR) were similar to the overall study population (7). In a *post hoc* analysis, CNS responses were observed in 50% of patients ($N = 14$) with baseline diameters available; however, patients with active brain metastases were excluded from the trial and time from last CNS-directed radiation was not reported, making it difficult to draw firm conclusions regarding CNS efficacy of T-DXd from this study (8).

It has been assumed that mAbs, and by extension ADCs, would be inactive in the CNS, because of poor penetration across the blood–blood barrier. However, a recent phase II study reported CNS responses and prolonged stable disease associated with use of high-dose intravenous trastuzumab (9). Furthermore, in the phase IIIb KAMILLA study, ado-trastuzumab emtansine (T-DM1) resulted in CNS responses in 42.9% of patients with measurable brain metastases at study baseline (10). Notably, preceding publication of the KAMILLA results, preclinical models (11, 12) and case series (13) strongly suggested that anti-HER2 antibodies and ADCs penetrate the potentially compromised blood–tumor barrier with demonstrated efficacy against HER2-positive breast cancer brain metastases (BCBM; ref. 14).

Emerging evidence suggests that response to ADCs can occur despite low levels of detected target protein expression (5). For this

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## Translational Relevance

Antibody–drug conjugates (ADC), such as trastuzumab deruxtecan (T-DXd), are rapidly altering the therapeutic landscape for both localized and metastatic solid tumors. However, a clinically urgent question remains: Is T-DXd active against brain metastases? The central nervous system (CNS) activity of T-DXd is not well characterized because patients with active breast cancer brain metastases (BCBM) were excluded from pivotal randomized trials. Here we show that T-DXd reduces tumor growth and prolongs survival of HER2-positive, HER2-low, and T-DM1–resistant BCBM patient-derived xenograft models. In addition, T-DXd was associated with a CNS objective response rate of 73% (11/15) in heavily pretreated patients with BCBMs. In 10 participants with untreated or progressive BCBMs, 7 experienced partial response while 3 experienced stable disease. This study demonstrates the validity of using orthotopic patient-derived xenograft models to demonstrate proof of CNS activity of ADCs. Our data also suggest that HER2-altered brain metastases in other diseases may be targetable with T-DXd.

reason, there has been specific interest in how trastuzumab-based ADCs might provide efficacy for treatment of HER2-"low" breast cancers (15). In the phase II DAISY study of T-DXd in metastatic breast cancer, patients were enrolled on the basis of levels of HER2 expression by IHC (16). Cohort 2 included patients in whom ISH was negative and IHC either 1+ or 2+, while cohort 3 included patients in whom ISH was negative and IHC was 0 (<10% of cells stained for HER2 by IHC). Patients in cohort 2 had a response rate of 33.3% while cohort 3 had a response rate of 30.6%, with median PFS of 6.7 and 4.2 months. This compared favorably to a response rate of 69.1% and median PFS of 11.1 months in cohort 1 (HER2 overexpressing).

Patient-derived xenograft (PDX) models derived from human BCBMs provide a robust and high-fidelity model in which to test novel therapeutics for CNS efficacy and to prioritize agents or combinations for clinical trials in patients with BCBMs (11, 12, 17). Here, we use PDX models of HER2-positive (HER2-amplified) BCBMs, as well as BCBMs that have low expression of HER2 by IHC (2+ ISH negative), to evaluate the effects of T-DXd on CNS tumor growth and overall survival. We further tested T-DXd in a HER2-positive T-DM1–resistant BCBM PDX model. Finally, to demonstrate the clinical relevance of these studies, we also undertook a retrospective multicenter cohort study of patients with brain metastases ($n = 17$) treated with T-DXd for refractory, HER2-positive or HER2-low breast cancer and report outcomes to treatment.

# Materials and Methods

## PDX models

### PDXs

PDXs were established as described previously (11, 12, 17). Briefly, informed consent was obtained from patients with breast cancer. Fresh brain metastases tissue samples were acquired from patients undergoing neurosurgery at the Brigham and Women's Hospital, in accordance with Institutional Review Board (IRB)-approved protocols [Dana-Farber Cancer Institute (DFCI) IRB 93-085 and 10-417]. Patient tumor samples were dissociated with Collagenase/Hyaluronidase (Stemcell Technologies) and accutase (Sigma). Approximately $1 \times 10^5$ cells suspended in PBS (1–2 μL) was intracranially injected into 6–10 weeks old ICR-SCID female mice (Taconic, IcrTac:ICR-Prkdcscid) at the right striatum (2.0 mm to the right and 2.5 mm deep relative to bregma). All the animal experiments followed the protocols approved by the DFCI Animal Care and Use Committee in compliance with NIH animal guidelines.

### IHC

IHC staining was performed as described previously (9). Anti-Ki67 antibody (MIB-1) was from DAKO. Anti-Cleaved Caspase 3 antibody was from Cell Signaling Technology. Anti-HER2 (OP15) antibody was purchased from MilliporeSgma. Quantification of Ki67 and Cleaved Caspase 3 was conducted using the Image J software with ImmunoRatio plugin. Sections of BCBM tissue from 2 animals per condition were imaged and 4–6 randomly chosen regions of interest per section were used for quantification.

### *In vivo* drug treatment

T-DM1 was purchased from the DFCI Pharmacy. T-DXd (DS8201a) was provided by Daiichi Sankyo Co., Ltd. After tumors reached uniform bioluminescence, T-DXd was initially given at 6.7 mg/kg to ensure tolerability and subsequently at 10 mg/kg by intravenous injection once per 3 weeks except where otherwise stated. T-DM1 was given at 15 mg/kg once per 3 weeks by intravenous injection. Doses were chosen based on previously reported efficacy against extracranial HER2-positive cell line models *in vivo* (6, 18).

### Bioluminescence imaging

Bioluminescence imaging was performed as described previously (11). Briefly, mice received 80 mg/kg D-luciferin (Gold Biotechnology) by intraperitoneal injection 10–15 minutes before imaging. Bioluminescence signals were then recorded with IVIS Lumina III Imaging System (PerkinElmer) according to manufacturer's protocol. The signals were analyzed with Living Image Software (PerkinElmer).

### Statistical analysis

Statistical analyses were calculated using Mann–Whitney *U* test for two-sample comparison or log-rank (Mantel–Cox) test for mice survival data by Prism 9 (GraphPad Software), except where otherwise stated. Clinical data were analyzed and plotted using R (19). Data are considered significant when *P* values are $< 0.05$. Median and 12-month PFS of 17 patients with predominantly HER2-positive BCBMs were estimated using the Kaplan–Meier estimation method via SAS9.4. PFS figures were plotted using R.

## Patients

This retrospective cohort study was approved by the IRBs at the Dana-Farber/Harvard Cancer Center (DF/HCC), the Duke Cancer Institute, and MD Anderson Cancer Center. Patients provided written informed consent and studies were conducted in accordance with the U.S. common rule. Key inclusion criteria were: (i) age ≥ 18 years; (ii) diagnosis of metastatic breast cancer; (iii) known stable or active/progressive brain metastases at time of initiation of T-DXd; (iv) seen at least once at either DFCI (Boston, MA), Duke Cancer Center (Durham, NC) or MD Anderson Cancer Center, (Houston, TX) after the diagnosis of brain metastases and prior to initiation of T-DXd; (v) initiated T-DXd between January 1, 2020 and November 1, 2020. Data cut-off date was June 1, 2021. Patients enrolled on prospective clinical trials of T-DXd were excluded. To reduce reporting bias, all patients who met inclusion criteria were included in this retrospective study cohort.

### Clinicopathologic data

Age, stage at diagnosis, dates of recurrence, receptor (ER, progesterone receptor, HER2) status, treatment history including chemotherapy, targeted therapy, and CNS-directed local therapy, and survival status were abstracted from the medical record. Dates and number of cycles of T-DXd were collected, as was time on treatment, and reason for discontinuation.

### Definition of clinical endpoints

CNS images before and after initiation of T-DXd were collected and centrally reviewed by a neuroradiologist at each participating site (L. Hsu, C. Lascola, M. Gule-Monroe). CNS responses were recorded according to modified response assessment in neuro-oncology brain metastases (RANO-BM) criteria and reported for the subset of patients with measurable [≥ 1 cm in longest dimension (LD)] CNS disease at baseline. As this was a retrospective study, corticosteroid dose and neurologic status were not available in all patients at each imaging timepoint, and thus, were not included in the evaluation of response as would be the case using standard RANO-BM criteria. Up to five CNS target lesions were recorded for each patient. A CNS partial response (PR) required all of the following: ≥30% decrease in sum LD of CNS target lesions and no new lesions. CNS progressive disease (PD) was defined as ≥ 20% in the sum LD of CNS target lesions, taking as reference the smallest sum on study. Extracranial responses were recorded according to RECIST 1.1 (by P.J. DiPiro). Time on treatment was defined as the time from first to last dose of T-DXd. Reason for treatment discontinuation was recorded as due to toxicity, CNS disease progression, extracranial disease progression, both CNS and extracranial disease progression, or other. PFS was defined as time from first treatment to PD or death prior to data cut-off date (June 1, 2021), whichever occurred first. Patients without a PFS event were censored at the last disease assessment date.

### Data availability

The data generated in this study are available within the article and its Supplementary Data. Raw data, excluding protected health information, are available on request from Dr Nancy U. Lin, nancy_lin@dfci.harvard.edu.

## Results

### T-DXd reduces tumor growth and prolongs survival in a PDX mouse model of HER2-positive BCBM

We previously demonstrated that orthotopic PDX models of HER2-positive BCBMs recapitulate the genomic characteristics of matched patient tissue and can be used to identify and test CNS-specific treatment strategies (11). A HER2-positive, ER-positive, PDX model (DFBM-355) was established to measure tumor response to and animal survival after T-DXd treatment. In DFBM-355, HER2 expression was measured as 3+ by IHC and matched pathologic assessment of parent patient tissue (Supplementary Fig. S1A; ref. 11). Treatment with T-DXd resulted in reductions of tumor size as measured by bioluminescent imaging (**Fig. 1A**, left and middle) and significantly prolonged animal survival compared with vehicle control (77.5 days vs. 155.5 days, $P = 0.0067$). These findings were confirmed using a second HER2-positive (ER-negative) orthotopic BCBM PDX model, DFBM-354 (Supplementary Fig. S1B) where treatment with T-DXd reduced tumor growth and significantly prolonged overall survival (Supplementary Fig. S1B) compared with vehicle control (67 days vs. 154 days, $P = 0.0018$). To determine whether T-DXd prolonged animal survival by a cytotoxic or cytostatic effect, we undertook assessment of PDX tumor tissues 4 days after a single dose of T-DXd (10 mg/kg). As shown in **Fig. 1B**, T-DXd did not significantly reduce proliferation (as measured by Ki67 IHC) in either DFBM-355 or DFBM-354 (Supplementary Fig. S1C), compared with control. In contrast, expression of the apoptosis marker, cleaved caspase 3, was significantly increased in T-DXd–treated compared with control-treated tissues (**Fig. 1B**; Supplementary Fig. S1C). These data suggest that T-DXd administered intravenously induces cell death and tumor shrinkage in HER2-positive BCBM PDXs.

### T-DXd reduces tumor growth and prolongs survival in a HER2-low BCBM PDX model

An ongoing trial of T-DXd (DESTINY-Breast04, NCT03734029) is testing whether T-DXd is active in patients with HER2-low metastatic breast cancer. However, patients with active brain metastases are excluded. Patients treated with T-DXd in DESTINY-Breast01 did not differ in their ORR by HER2 IHC (1+/2+/3+; ref. 20). Therefore, we asked whether treatment with T-DXd would impair tumor growth and prolong survival in a BCBM PDX model that was $ER^+$ HER2-low by IHC (2+) and negative by ISH (ratio <2). Compared with vehicle control, treatment with T-DXd reduced tumor growth and prolonged overall survival in model DFBM-1409 (**Fig. 1C**). These data suggest that T-DXd is also effective in treating BCBM PDX tumors with HER2 IHC 2+.

### T-DXd prolongs survival in a T-DM1–resistant HER2-positive BCBM PDX model

In DESTINY-Breast01, the response rate to T-DXd was similar regardless of previous proximal treatment with trastuzumab emtansine (20). We hypothesized that T-DM1 resistance could be overcome by T-DXd in the CNS. We treated the HER2-positive (HER2 3+ by IHC, ER-positive) BCBM PDX model DFBM-355 with T-DM1 for 4 months until resistance developed. This T-DM1–treated PDX, called DFBM-355-TDM1R, was then treated with either vehicle, T-DM1 or T-DXd (**Fig. 1D**). Treatment of DFBM-355-TDM1R with T-DXd 10 mg/kg every 3 weeks prolonged overall survival compared with T-DM1 or vehicle control (215 days for T-DXd vs. 99 days for T-DM1 vs. 63 days for control, $P = 0.0182$; **Fig. 1D**). These data suggest that T-DM1–resistant HER2-positive BCBMs remain sensitive to T-DXd.

### Clinicopathologic characteristics of patient retrospective study cohort

The study scheme for the retrospective patient study cohort with inclusion criteria is shown in **Fig. 2A**. A total of 18 patients met entry criteria but one was excluded from outcome analysis due to missing follow-up data. Of the 18 patients in the cohort, the majority had a disease-free interval of greater than 2 years (72%) prior to the development of metastatic disease (**Table 1**). A total of 16 of 18 (89%) had confirmed HER2-positive BCBMs by IHC with 1 patient having IHC 1+/FISH-negative and 1 additional patient did not have tissue available for receptor assessment. A total of 17 of 18 (94%) patients had received at least one line of previous HER2-directed therapy in the metastatic setting (median, 4; range, 0–10), and 15 of 18 (83%) had received previous T-DM1, while 13 of 18 (72%) had received a previous HER2 tyrosine kinase inhibitor (TKI; tucatinib or lapatinib). A total of 4 of 18 (22%) patients had previously had

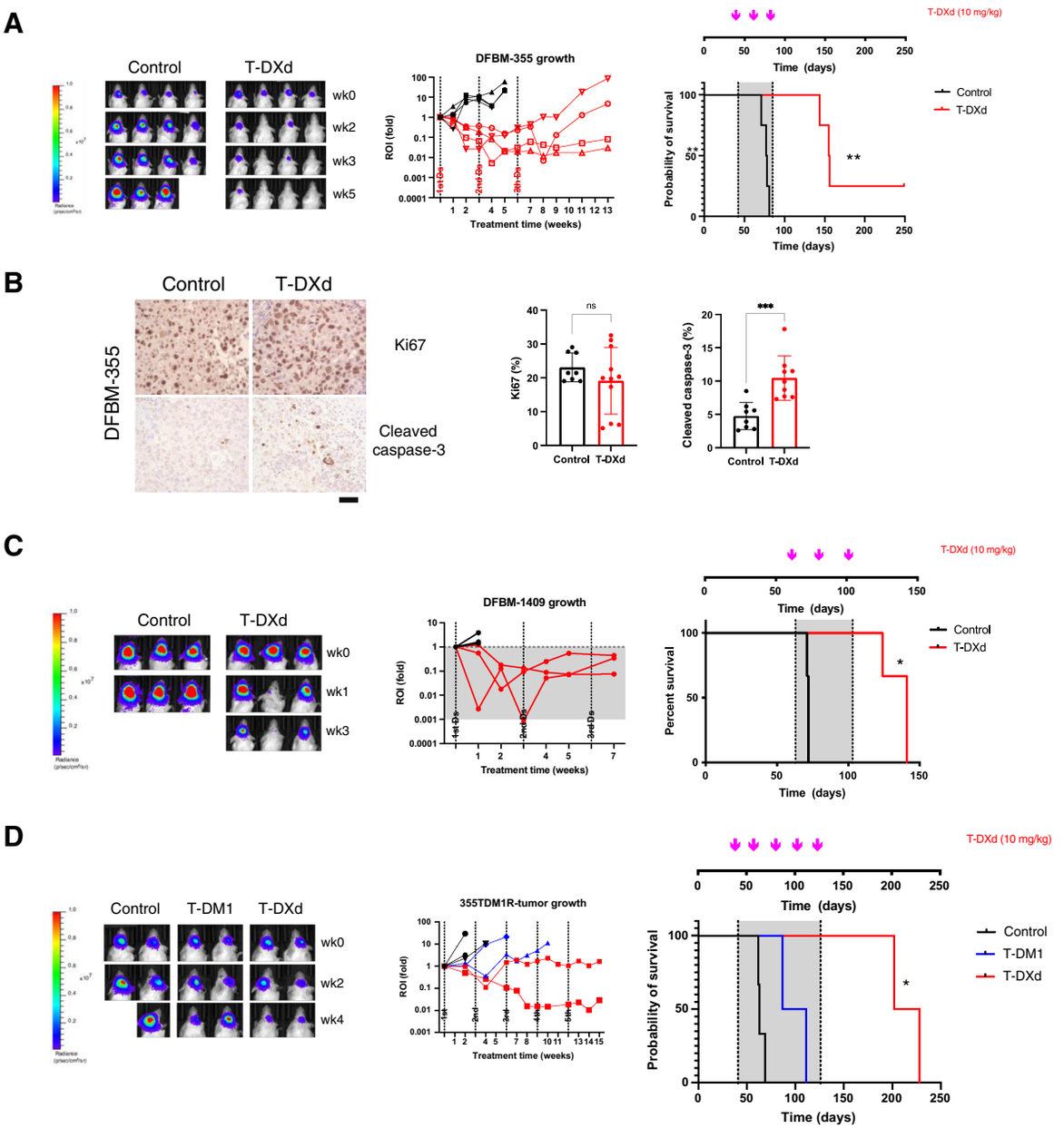


**Figure 1.**

*In vivo* effects of T-DXd on HER2-positive BCBM PDXs. **A,** Representative bioluminescence images (left), spider plots of tumor response (middle), and Kaplan–Meier survival analysis of mice bearing intracranial DFBM-355 cells treated with vehicle control (black line) or T-DXd (red line) as indicated (right). $n = 4$/group. The gray area indicates the treatment period. The treatment schedule and doses are indicated on the graph. **, $P < 0.01$, log-rank (Mantel–Cox) test. **B,** IHC analyses of Ki67 and cleaved caspase-3 of DFBM-355 tumor samples harvested from tumor-bearing mice treated 4 days after one dose of T-DXd (10 mg/kg, i.v.) (Scale bar = 50 μmol/L). Data show mean ± SD of quantification of Ki67 and cleaved caspase-3 in tumors. Mann–Whitney *U* test; ***, $P < 0.001$. **C,** T-DXd effect on intracranial DFBM-1409 (HER2-low) BCBM PDX. Bioluminescence images (left), spider plots of tumor response (middle), and Kaplan–Meier survival analysis of mice bearing DFBM-1409 (right) treated with vehicle control (black line) or T-DXd (red line) as indicated. $n = 3$/group. The treatment schedule and doses were indicated on the graph. *, $P < 0.05$, log-rank (Mantel–Cox) test. **D,** Representative bioluminescence images (left) in each group of mice at indicated imaging timepoints with spider plots of tumor response (middle). Kaplan–Meier survival analysis of mice bearing T-DM1–resistant model DFBM-355TDM1R (right) intracranially, treated with vehicle control (black line), T-DM1 (blue line), or T-DXd (red line) as indicated. $n = 2$–3/group.

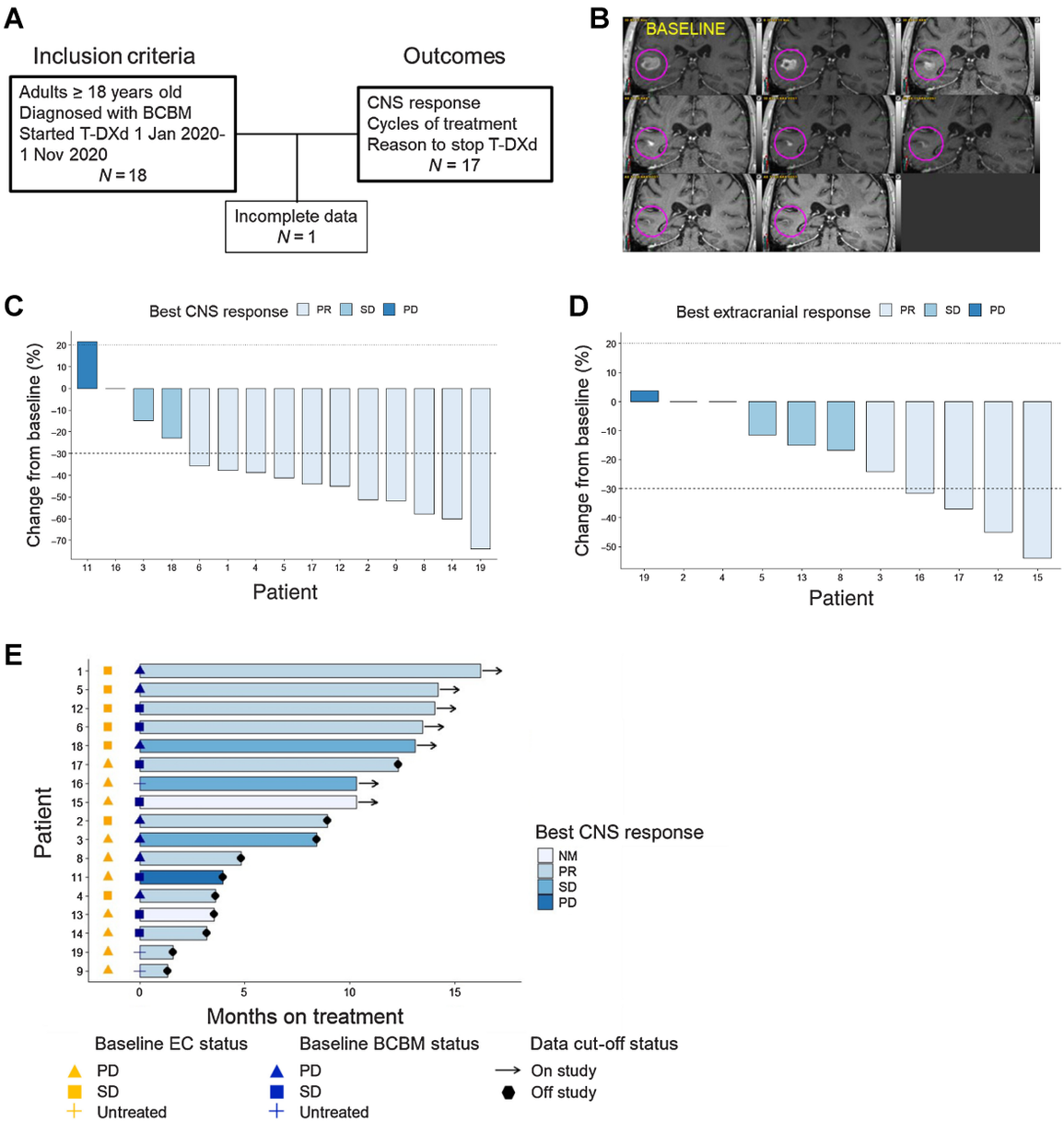


**Figure 2.**

Response to T-DXd in patients with BCBMs. **A,** Study scheme showing study entry criteria and reported outcomes. **B,** Contrast-enhanced brain MRIs of patient response to T-DXd. Baseline image shown at top left and images arranged in serpentine succession at restaging intervals show evidence of durable response. The lesion shown had not received prior stereotactic radiosurgery, and the patient had progressed through prior treatment with T-DM1, neratinib, and tucatinib. **C,** Best CNS response to T-DXd. Waterfall plot of best CNS response in patients with measurable disease ($n = 15$). **D,** Best extracranial response to T-DXd. Waterfall plot of best extracranial response in patients with measurable disease ($n = 11$). **E,** Months on treatment with T-DXd. Swimmer plot showing patient ($n = 17$) cycles on treatment with T-DXd by baseline extracranial (EC) disease (gold shapes) and BCBM status (navy blue shapes). A total of 10 of 17 (59%) had progressive or untreated CNS disease at baseline. NM, nonmeasurable; PR, partial response; SD, stable disease; PD, progressive disease.

resection of a CNS metastasis while 9 of 18 (50%) had received previous whole brain radiation and 11 of 18 (61%) had received previous stereotactic radiosurgery. The median interval from any previous radiation to start of T-DXd was 14.7 (1.3–45.2) months. At study entry, 10 of 17 (59%) had active (either progressive or untreated) BCBMs.

## Response to T-DXd in patients with BCBMs

Baseline characteristics for 15 patients with evaluable CNS disease are shown in **Table 2**. The CNS ORR to T-DXd was 73% (11/15). A total of 11 patients experienced a PR, 2 experienced stable disease while 1 patient had PD (**Tables 2** and **3**; **Fig. 2B** and **C**). ORR in patients with active BCBMs was 70% (7/10; **Table 3**). In the active BCBM subgroup,

**Table 1.** Cohort characteristics.

| | *N* (%) |
|---|---|
| Number of patients | 18 |
| Median age | 46 |
| Patients per site | |
| DFCI | 10 (56) |
| Duke | 7 (39) |
| MDACC | 1 (6) |
| Disease-free interval (years) | |
| DFI <2 | 5 (28) |
| DFI ≥2 | 13 (72) |
| Primary tumor subtypes | |
| HER2$^+$ ER$^-$ | 7 (39) |
| HER2$^+$ ER$^+$ | 8 (44) |
| HER2$^-$ ER$^+$ | 2 (11) |
| Unknown | 1 (6) |
| BCBM subtypes | |
| HER2$^+$ ER$^-$ | 7 (39) |
| HER2$^+$ ER$^+$ | 9 (50) |
| HER2$^-$ ER$^+$ | 1 (6) |
| Unknown | 1 (6) |
| BCBM status per investigator | |
| Untreated | 3 (18) |
| Progressive disease (PD) | 7 (41) |
| Stable disease (SD) | 7 (41) |
| (Neo)adjuvant HER2 therapy | |
| None | 2 (11) |
| Trastuzumab-based | 8 (44) |
| Trastuzumab + lapatinib-based | 1 (6) |
| Trastuzumab + pertuzumab-based | 5 (28) |
| Not available | 2 (11) |
| Metastatic HER2 therapy | |
| Median number of previous HER2 therapies in metastatic setting | 4 (0–10) |
| Trastuzumab | 15 (83) |
| Pertuzumab | 11 (61) |
| Trastuzumab emtansine (T-DM1) | 15 (83) |
| Tyrosine kinase inhibitor | 13 (72) |
| Local therapy | |
| Surgery for brain metastasis | 4 (22) |
| Whole brain radiation (WBRT) | 9 (50) |
| Stereotactic radiosurgery (SRS) | 11 (61) |
| No SRS or WBRT | 4 (22) |
| Median interval from last radiation to C1D1 (months) | 14.7 (1.3–45.2) |

**Table 2.** Characteristics of patients with evaluable CNS disease.

| | *N* (%) |
|---|---|
| Number of patients | 15 |
| Median age | 46 (35–69) |
| Patients per site | |
| DFCI | 9 (60) |
| Duke | 5 (33) |
| MDACC | 1 (7) |
| Disease-free interval (years) | |
| DFI <2 | 4 (27) |
| DFI ≥2 | 11 (73) |
| Primary tumor subtypes | |
| HER2$^+$ ER$^-$ | 6 (40) |
| HER2$^+$ ER$^+$ | 2 (13) |
| HER2$^-$ ER$^+$ | 7 (47) |
| BCBM subtypes | |
| HER2$^+$ ER$^-$ | 5 (33) |
| HER2$^-$ ER$^+$ | 1 (7) |
| HER2$^+$ ER$^+$ | 8 (53) |
| Unknown | 1 (7) |
| BCBM status per investigator | |
| Untreated | 3 (20) |
| Progressive disease (PD) | 7 (47) |
| Stable disease (SD) | 2 (13) |
| (Neo)adjuvant HER2 therapy | |
| None | 1 (7) |
| Trastuzumab-based | 6 (40) |
| Trastuzumab + lapatinib-based | 1 (7) |
| Trastuzumab + pertuzumab-based | 5 (33) |
| Not available | 2 (13) |
| Metastatic HER2 therapy | |
| Median number of previous HER2 therapies in metastatic setting | 4 (0–10) |
| Trastuzumab | 12 (80) |
| Pertuzumab | 8 (53) |
| Trastuzumab emtansine (T-DM1) | 12 (80) |
| Tyrosine kinase inhibitor | 10 (67) |
| Median interval from last radiation to C1D1 (months) | 13.4 (1.3–18.2) |

5 of 7 responders had been previously treated with CNS radiation, and median time from last radiation was 14.2 (range, 8.2–18.2) months. In 11 patients with measurable extracranial disease, the extracranial ORR by RECIST 1.1 was 45% (5/11, **Table 2**): 5 patients experienced an extracranial PR, 5 patients experienced stable disease and 1 had PD (**Fig. 2D**). A total of 2 patients experienced both CNS and extracranial response and their baseline characteristics are shown in Supplementary Table S1. A single patient (Patient 14; **Fig. 2E**) had progressive, HER2-low BCBM (HER2 IHC 1+/FISH negative) and had a PR in the CNS lasting 3.2 months on T-DXd.

Across the cohort, median time on treatment with T-DXd was 8.9 (1.3–16.2) months with 42% (7/17) remaining on treatment at data cutoff (7 months of follow-up; **Fig. 2E**). A total of 3 of 17 (18%) of patients came off of T-DXd due to CNS progression. The most common reason for censoring was extracranial disease progression leading to switch of systemic therapy, noted in 4 of 17 (24%) of patients (**Table 3**). PFS results are shown in Supplementary Fig. S2. There were three CNS progression events and seven CNS or extracranial progression events prior to data cutoff (**Table 3**). Median CNS PFS was not reached [95% confidence interval (CI), 7.0–not reached] 12-month CNS PFS was 74.7% (95% CI, 39.5–91.2). Median overall PFS (CNS and extracranial disease) was not reached (95% CI, 4.4–not reached) while 12-month overall PFS was 57.8% (95% CI, 31.1–77.3).

## Discussion

ADCs have demonstrated extracranial activity, extending PFS in metastatic HER2-positive breast cancer (20–22). Here we present results from a hybrid study of HER2-positive BCBM PDX models and a retrospective cohort of patients with HER2-positive BCBMs treated with T-DXd. We find that T-DXd results in intracranial objective responses in both PDX models and patients with active HER2-positive BCBMs. We demonstrate that T-DXd results in reduced BCBM growth and prolongs BCBM PDX model survival, primarily by inducing tumor cell apoptosis. We observed intracranial radiographic responses in 73% (11/15) of patients with evaluable CNS disease, out of which 67% (10/15) had untreated or PD. Duration of T-DXd treatment was also notably long, with 6 of 18 (33%) patients on treatment 1 year or longer, and 42% still on treatment as of the data cut-off date.

**Table 3.** Outcomes at data cutoff.

| | *N* (%) |
|---|---|
| Treatment status | 17 |
| Patients on treatment | 7 (42) |
| Patients off treatment | 10 (59) |
| Central nervous system (CNS) progressive disease | 2 (12) |
| Extracranial progressive disease | 4 (24) |
| CNS + extracranial progressive disease | 1 (6) |
| Radiation necrosis | 1 (6) |
| Adverse effect | 1 (6) |
| Patient choice | 1 (6) |
| Median months on treatment | 8.9 (1.3–16.2) |
| Best extracranial response | 11 |
| Complete response | 0 (0) |
| Partial response | 5 (45) |
| Stable disease | 5 (45) |
| Progressive disease | 1 (5) |
| Best CNS response | 15 |
| Complete response | 0 (0) |
| Partial response | 11 (73) |
| Stable disease | 3 (20) |
| Progressive disease | 1 (5) |
| Best CNS response in active BCBM | 10 |
| Complete response | 0 (0) |
| Partial response | 7 (70) |
| Stable disease | 3 (30) |
| Progressive disease | 0 (0) |

Our data confirm and extend recently presented results from patients with DESTINY-Breast03, where in a subgroup analysis of patients with stable BCBMs at baseline ($n = 82$), median PFS was 15.0 months (95% CI, 12.5–22.2) for T-DXd versus 3.0 months (95% CI, 2.8–5.8) for T-DM1 [HR, 0.25 (95% CI, 0.31–0.45)]. In DESTINY-Breast03, the confirmed CNS ORR for T-DXd was 79.7% versus 34.2% for T-DM1. Though promising, these CNS overall response rates are difficult to interpret formally given the unknown radiation status and time since radiation of these lesions. In contrast to DESTINY-Breast03, our series included patients with active/progressive brain metastases and demonstrated substantial intracranial activity in this subset. Median time from last CNS-directed radiotherapy was 14.7 months in all patients and 12.4 months in patients with progressive BCBM at baseline. Thus, we believe the activity we observed can be directly attributed to T-DXd therapy and not prior local CNS-directed radiotherapy. Finally, in contrast to DESTINY-Breast03, patients in our series were heavily pretreated, with a median of four prior lines of metastatic systemic therapy. Over 80% of patients had received prior T-DM1 and 72% had been treated with a HER2-targeted TKI in the metastatic setting.

We also showed that response to T-DXd occurs in BCBM PDX models defined as HER2-low based on HER2 IHC1+, or 2+ and negative for HER2 amplification by ISH (15). Intriguingly, 1 patient in our case series with HER2-low BCBM also achieved a CNS PR while extracranial response was not measurable. As we await results of DESTINY-Breast04, which is testing the value of T-DXd versus physician's choice of chemotherapy in patients with HER2-low, metastatic breast cancer, we believe our results justify further exploration of the potential role of T-DXd in patients with HER2-low tumors who have active/progressive BCBM.

Together, these data strongly suggest that T-DXd is an active drug for treating BCBMs with both low and high HER2 expression and support randomized studies of T-DXd in patients with brain metastases. Our real-world data are corroborated by recently published data from two single-arm prospective trials of T-DXd in patients with HER2-positive BCBMs (23, 24). TUXEDO-1 was a prospective, single-center, single-arm, phase II trial investigating T-DXd in patients with active BCBMs (23). The intracranial response rate to T-DXd was 73.3% (11/15) in the intention-to-treat population, identical to the intracranial response rate in our study (73%). The median PFS in TUXEDO-1 was 14 months (95% CI, 11.0 months to not reached). Our data are also consistent with recently published results from cohorts 1–3 of DEBBRAH, a multicenter, single-arm, phase II trial of patients with HER2-positive or HER2-low active brain metastases or leptomeningeal carcinomatosis (24). In patients with active brain metastases (either untreated or progressive) with measurable CNS disease (cohorts 2–3, $n = 13$), the ORR (complete response or PR) was 6/13 (46%) compared with 7/10 (70%) in our cohort.

More definitive data will be available from DESTINY-Breast12, which is a multinational, multicenter, phase IIIb/IV study of T-DXd in patients with or without baseline BCBMs with previously treated advanced/metastatic HER2-positive breast cancer (NCT04739761). In DESTINY-Breast12, up to 250 patients with BCBM at baseline will be enrolled in a separate cohort to assess clinical outcomes to T-DXd. We believe our data also support exploration of T-DXd in patients with HER2-low BCBMs, and potentially even patients with other solid tumors, including HER2-mutated non–small cell lung cancer, and other indications in which T-DXd is being actively studied (25).

Our study had certain limitations. Orthotopic BCBM PDX models recapitulate parent tumor tissue genetics and patterns of response and resistance (11, 17, 26) but may underrepresent the therapeutic efficacy of ADCs because of absent ADCC. In addition, we selected PDX models to demonstrate proof-of-principle efficacy but we did not aim to capture the range of potential BCBM responses to T-DXd. We did not undertake an exhaustive pharmacodynamic assessment in our BCBM PDX models but our data suggest apoptosis as one mechanism by which T-DXd induces tumor shrinkage and prolongs animal survival. This was a retrospective observational study without standardized assessment intervals and RANO-BM criteria could not be used because of lack of information about steroid use and patient neurologic status. However, central radiology review did allow for uniform measurements of change in intracranial and extracranial disease on T-DXd. While we report CNS and overall PFS, this should be interpreted with caution due to lack of standardized assessment intervals and limited sample size in this retrospective study.

More broadly, our work demonstrates the power of a "functional precision medicine" approach for rapidly demonstrating preclinical and clinical efficacy of novel agents in areas of unmet clinical need (27). As such, ADCs may represent an active drug class for patients with brain metastases across solid tumors.

## Authors' Disclosures

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## Authors' Contributions

**S. Kabraji:** Conceptualization, data curation, software, formal analysis, investigation, visualization, methodology, writing–original draft, writing–review and editing. **J. Ni:** Conceptualization, data curation, software, formal analysis, validation, investigation, visualization, methodology, writing–original draft, writing–review and editing. **S. Sammons:** Data curation, writing–review and editing. **T. Li:** Formal analysis, writing–review and editing. **A.E.D. Van Swearingen:** Data curation, formal analysis, writing–review and editing. **Y. Wang:** Data curation. **A. Pereslete:** Data curation, formal analysis, writing–review and editing. **L. Hsu:** Data curation, writing–review and editing. **P.J. DiPiro:** Data curation, writing–review and editing. **C. Lascola:** Data curation, writing–review and editing. **H. Moore:** Data curation, writing–review and editing. **M. Hughes:** Data curation, project administration, writing–review and editing. **A.S. Raghavendra:** Data curation, writing–review and editing. **M. Gule-Monroe:** Data curation, writing–review and editing. **R.K. Murthy:** Data curation, supervision, investigation, writing–review and editing. **E.P. Winer:** Supervision, writing–review and editing. **C.K. Anders:** Supervision, writing–review and editing. **J.J. Zhao:** Conceptualization, resources, supervision, funding acquisition, investigation, project administration, writing–review and editing. **N.U. Lin:** Conceptualization, resources, data curation, supervision, funding acquisition, validation, methodology, writing–original draft, project administration, writing–review and editing.

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## Note

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