Impacts of clinicopathological factors on efficacy of trastuzumab deruxtecan in patients with HER2-positive metastatic breast cancer

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ABSTRACT

Background: The previous second-line treatment for HER2-positive metastatic breast cancer were ado-trastuzumab emtansine (T-DM1); however, its activity is decreased in tumors with heterogenous, reduced, or loss of HER2 expression. Trastuzumab deruxtecan (T-DXd) has recently been developed as a novel antibody-drug conjugate to overcome resistance to T-DM1. However, clinical evidence on its ability to overcome this resistance is limited.
Materials and methods: We retrospectively analyzed data for patients with HER2-positive metastatic breast cancer who received T-DXd at our institution from April 2020 to March 2021. We evaluated the associations between clinicopathological and molecular biomarkers and the efficacy of T-DXd.
Results: Twenty-two patients were enrolled in this study. The median progression-free survival (PFS) was 9.7 months (95% confidence interval [CI], 7.0–not reached [NR]), and the objective response rate (ORR) was 61.9%. The ORR and PFS were comparable between patients with HER2 immunohistochemistry scores of 3+ and 2+/1+ at initial diagnosis (ORR: 50.0% vs. 72.7%, $p = 0.39$; median PFS, 9.7 months [95%CI, 2.6–NR] vs. 8.3 months [95%CI, 7.1–NR]; hazard ratio, 1.86 [95%CI, 0.53–6.57], $p = 0.34$). Two patients with heterogenous HER2 expression had a partial response or long stable disease ($\geq$6 months). Three of four patients with re-biopsy samples after anti-HER2 targeted therapy and with latest HER2 immunohistochemistry scores of 1+ experienced partial responses (75.0%) to T-DXd, but none had responded to prior T-DM1.
Conclusions: T-DXd demonstrated favorable activity in clinical practice. Moreover, T-DXd showed meaningful benefit in patients with heterogeneity, reduction, or loss of HER2 expression.

1. Introduction

Breast cancer is one of the most commonly diagnosed cancers worldwide, with an estimated 2.3 million new cases and 685,000 deaths in 2020 [1]. Approximately 15%–20% of breast cancers show overexpression or amplification of human epidermal growth factor receptor 2 (HER2) [2–4]. Systemic treatment of HER2-positive metastatic breast cancer centers on HER2-targeted therapy, which targets the HER2 receptor and its downstream signaling pathways.

The standard first-line treatment for metastatic disease is a taxane combined with trastuzumab plus pertuzumab; previous second-line or later-line treatments were ado-trastuzumab emtansine (T-DM1). T-DM1 is an antibody-drug conjugate of trastuzumab and the cytotoxic agent emtansine, which has demonstrated significant survival improvement compared with capecitabine plus lapatinib or treatment of the physician's choice in patients with HER2-positive metastatic breast cancer who progressed to taxane plus trasutzumab [5—8].

Trastuzumab deruxtecan (T-DXd; DS-8201a) is a recently developed antibody-drug conjugate that combines trastuzumab with the topoisomerase I inhibitor, deruxutecan. The single-arm phase 2 DESTINY-Breast01 trial assessed the efficacy and safety of T-DXd in 184 patients with HER2-positive metastatic breast cancer who had received previous treatment with T-DM1, and showed an objective response rate (ORR) of 60.9% and a median progression-free survival (PFS) of 16.4 months [9]. Based on these results, T-DXd has been approved for the treatment of patients with HER2-positive metastatic breast cancer who have previously received treatment with T-DM1 in the United States, Europe, and Japan. The first report of the DESTINY-Breas03 trial, a randomized Phase III trial comparing T-DXd with T-DM1 as a second-line treatment, was recently presented at the 2021 ESMO Congress [10]. T-DXd showed a statistically significant improvement in PFS compared with T-DM1, supporting T-DXd as the new standard second-line treatment for HER2-positive metastatic breast cancer.

The primary mechanism of action of T-DXd is HER2-mediated internalization of T-DXd in HER2-positive tumor cells, followed by the release of DXd via the cleavage of the linker [11]. T-DXd is designed to have several advantages compared with T-DM1, including greater stability in plasma and selective cleavage via the unique linker, a higher drug-to-antibody ratio (approximately 8 *vs.* 3—4), and increased membrane permeability compared with Lys-SMCC-DM1 released from T-DM1, allowing antitumor activity against neighboring cells via the so-called "bystander effect." [11—14] Several biomarkers associated with resistance to T-DM1 have been identified to date [15]. For example, reduced *HER2* amplification or expression [16—20], intra-tumoral heterogeneity of HER2 expression [21,22], and high neutrophil-to-lymphocyte ratio [23] were associated with poor treatment outcomes following T-DM1 in previous studies. However, there is currently no evidence for the relationships between these biomarkers and the efficacy of T-DXd. Moreover, the ability of T-DXd to overcome these resistance mechanisms of T-DM1 in a clinical context, based on its novel drug design, remains unclear.

In this study, we addressed these clinical questions by examining the associations between the efficacy of T-DXd and clinicopathological factors, especially prior T-DM1 treatment, in patients with HER2-positive metastatic breast cancer who had received both T-DM1 and T-DXd.

2. Materials and methods

2.1. Patients and clinical data

We retrospectively evaluated the associations between clinicopathological and molecular biomarkers and the efficacy of T-DXd in patients with HER2-positive metastatic breast cancer at our institution. The inclusion criteria were as follows: (i) pathologically diagnosed metastatic breast cancer; (ii) HER2-positive breast cancer diagnosed according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) HER2 testing in breast cancer guidelines available at the time [24—26] or *HER2* amplification confirmed by FoundationOne® CDx; (iii) an Eastern Cooperative Oncology Group (ECOG) performance status

(PS) of 0—2; (iv) received T-DM1 prior to T-DXd; and (v) received T-DXd between April 2020 and March 2021. The study protocol was approved by the Institutional Review Board of the National Cancer Center Hospital East (Kashiwa, Chiba, Japan), and the study was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

The following clinical data were collected from electronic medical records: patient demographics (age, sex, menopausal status), ECOG PS at the start of T-DXd, *de novo* stage IV or recurrent disease, number of metastatic sites, location of metastatic sites, surgery of primary disease, prior treatment history of chemotherapy and hormonal therapy for early and metastatic disease, number of prior treatments for metastatic disease, and toxicity according to the Common Terminology Criteria for Adverse Events version 5.0. We collected laboratory data including peripheral blood neutrophil, lymphocyte, and platelet counts for each patient at baseline of T-DXd treatment. Neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte (PLR) ratios were calculated as the absolute counts of neutrophils and platelets divided by the absolute counts of lymphocytes, respectively. The start dates of T-DM1 and T-DXd treatments, the best overall response following Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, the date of progression or death, and the reason for discontinuation were recorded.

2.2. Pathological assessment

The following pathological data were referenced from pathological reports: histology, estrogen receptor status, progesterone receptor status, and HER2 status determined by local pathologists. HER2 status was assessed by immunohistochemical score (IHC) and/or fluorescence *in situ* hybridization (ISH) test, following the ASCO/CAP HER2 testing in breast cancer guidelines available at the time [24—26]. We also collected the HER2 IHC scores for patients who underwent re-biopsy after the initiation of anti-HER2 targeted therapy and before the initiation of T-DXd.

We assessed HER2 heterogeneity in patients with HER2 IHC scores of 3+ or 2+ at initial diagnosis using archival pathological specimens. HER2 heterogeneity categories were based on the percentage of cells that were stained positive for HER2, defined as the sum of cells with complete membrane staining with 2+ or 3+ intensity, as described previously [21,22]. Briefly, positive staining of <30% of cells was categorized as HER2 focal; staining of ≥30% and ≤79% of cells was categorized as HER2 heterogeneous; and staining of ≥80% of cells was categorized as HER2 homogeneous. HER2 heterogeneity in this study was assessed by a pathologist (T. Nakai) specialized in breast cancer.

2.3. Genomic profiling

Genomic data were also collected for patients who underwent tissue-based next-generation sequencing (NGS)-targeted gene panel analysis (FoundationOne® CDx [Chugai, Japan], or Onco-Guide™ NCC Oncopanel System [Sysmex, Japan]).

2.4. Statistical analysis

We analyzed the associations between clinicopathological and molecular factors and the efficacy of T-DXd. The endpoints were ORR, disease-control rate (DCR; rate of complete, or partial response [PR] or stable disease [SD]), clinical-benefit rate (CBR, DCR with SD lasting ≥6 months), and PFS (defined as the time from initiation of T-DXd to disease progression or death from any cause). ORRs according to the biomarkers were compared using Fisher's exact test. PFS was estimated using the Kaplan—Meier method and

compared according to each biomarker. Groups were compared using the log-rank test, and the relationships between PFS and potential biomarkers were estimated using Cox's proportional hazard models. All statistical analyses were performed using the statistical program R version 4.1.0 (The R Foundation for Statistical Computing). In all cases, *p* values < 0.05 were considered to indicate statistical significance.

3. Results

3.1. Patient overview

A total of 22 patients were enrolled in this study. All 22 patients were available for efficacy and safety analysis. The patients' clinicopathological and molecular characteristics are described in Table 1. In this cohort, 16 patients (72.7%) had a PS of 1 or higher, and seven patients (31.8%) did not have adequate organ function to meet the eligibility criteria of the DESTINY-Breast01 trial. All patients received trastuzumab, pertuzumab, and T-DM1, and five patients (22.7%) received lapatinib. The best overall response to

Table 1
Baseline patient characteristics.

Characteristics	Patients (N = 22)	
Clinical factors		
Age		
Median, years [range]	59.5	[42–78]
≥65, n (%)	7	31.8
Sex, n (%)		
Female	22	100.0
BMI		
Median [range]	20.5	[16.3 −33.0]
≥25, n (%)	3	13.6
Stage IV or recurrent, n (%)		
De novo Stage IV or locally advanced	6	27.3
Recurrent	16	72.7
ECOG PS, n (%)		
0	6	27.3
1	14	63.6
2	2	9.1
Organ function[a], n (%)		
Any organ dysfunction	7	31.8
Inadequate bone marrow function	5	22.7
Inadequate renal function	0	0
Inadequate hepatic function	1	4.5
Inadequate blood clotting function	0	0
Inadequate cardiac function	1	4.5
Location of metastatic site, n (%)		
Liver	8	36.4
Lung	13	59.1
Bone	15	68.2
Brain	9	40.9
Number of metastatic sites		
Median [range]	3	[1–6]
Prior endocrine therapy, n (%)		
Yes	8	36.4
None	14	63.6
Type of prior cytotoxic agents, n (%)		
Taxane	20	90.9
Anthracycline	17	77.3
Fluoropyrimidine	12	54.5
Eribulin	12	54.5
Vinorelbine	11	50.0
Gemcitabine	2	9.1
Prior anti-HER2 targeted therapy, n (%)		
Trastuzumab	22	100.0
Pertuzumab	22	100.0
T-DM1	22	100.0
Lapatinib	5	22.7

Table 1 (*continued*)

Characteristics	Patients (N = 22)	
Others	2	9.1
Best response to prior T-DM1, n (%)	9	40.9
PR	3	13.6
SD	10	45.5
PD		
T-DXd immediately after T-DM1, n (%)		
Yes	10	45.5
None	12	54.5
Prior lines of chemotherapy for metastatic disease, n (%)		
Median [range]	3.5	[2–8]
NLR		
Median [range]	2.15	[0.8 −12.9]
PLR		
Median (range)	1.80	[0.70 −4.90]
Pathological factors		
Histology, n (%)		
Invasive ductal carcinoma	21	95.5
Mucinous carcinoma with micropapillary pattern	1	4.5
Hormone-receptor status, n (%)		
Positive	15	68.2
Negative		
HER2 status at initial diagnosis, n (%)		
IHC 3+	11	50.0
IHC 2+/1+ and ISH/NGS positive	11	50.0
HER2 heterogeneity at initial diagnosis, n (%)	9	40.9
Homogenous	2	9.1
Heterogenous	11	50.0
NA		
Latest HER2 IHC score at re-biopsy, n (%)		
IHC 3+	3	13.6
IHC 2+	1	4.5
IHC 1+	4	18.2
NA	14	63.6
Molecular factors		
Underwent NGS-targeted gene panel analysis, n (%)		
Yes	5	22.7
None	17	77.3
Detected alterations in patients who underwent NGS-targeted gene panel analysis, n (%)		
TP53 mutation	5	100.0
HER2 amplification	5	100.0
PIK3CA mutation	3	60.0
HER2 mutation	1	20.0
AKT1 mutation	1	20.0

Abbreviations: BMI, body mass index; CR, complete response; ECOG PS, Eastern Cooperative Oncology Group Performance Status; IHC, immunohistochemical; n, number; NA, not available; NGS, next-generation sequencing; NLR, neutrophil-to-lymphocyte ratio; PD, progressive disease; PLR, platelet-to-lymphocyte ratio; PR, partial response; SD, stable disease.
[a] The organ function criteria definition was based on the eligibility of the DESTINY-Breast01 trial.

prior T-DM1 therapy was PR in nine patients (40.9%), SD in three patients (13.6%), and progressive disease (PD) in 10 patients (45.5%). Ten (45.5%) patients received T-DXd immediately after T-DM1 treatment. The median number of prior treatment regimens in patients with metastatic disease was 3.5 (range, 2–8). The HER2 status at initial diagnosis was IHC 3+ in 11 patients (50%) and IHC 1+/2+ and ISH positive/NGS positive (*HER2* amplification) in 11 patients (50%). The latest HER2 IHC scores in eight patients who underwent re-biopsy after exposure to HER2-targeted therapy were 3+ in three patients, 2+ in one patient, and 1+ in four patients. Only five patients underwent tissue-based NGS-targeted gene panel analysis. All the patients harbored *HER2* amplification and *TP53* mutations. In addition, *HER2* and *AKT1* mutations were found in one patient each. *PTEN* loss was not observed in this study.

Other genomic alterations are listed in Supplementary Table 1.

The median follow-up at the time of the analysis was 10.1 months (95% confidence interval [CI], 8.4—12.0). Twelve of the 22 patients progressed on T-DXd and one patient discontinued treatment due to adverse events. Three patients died, including two after disease progression. The median PFS in the overall population was 9.7 months (95%CI, 7.0—not reached [NR]) (Fig. 1A). The ORR, DCR, and CBR in 21 patients (95.5%) with measurable lesions were 61.9%, 90.5%, and 76.2%, respectively (Fig. 1B). Tumor response dynamics during T-DXd are shown in Supplementary Fig. 1. The associations between the comprehensive clinicopathological and molecular characteristics and the efficacy of T-DXd are shown in Fig. 2, and the associations between the efficacy of T-DXd and biomarkers are shown in Tables 2 and 3.

3.2. Associations with clinical factors

We examined the effect of metastatic site on treatment outcomes. Patients with liver metastasis had lower ORRs and significantly shorter PFS than patients without liver metastasis (ORR: 76.9% *vs.* 37.5%, $p = 0.16$; median PFS, 6.3 months [95%CI, 2.1—NR] *vs.* NR [95%CI, 7.1—NR]; hazard ratio [HR], 4.40 [95%CI, 1.35—14.29], $p = 0.01$) (Tables 2 and 3). In contrast, patients with lung metastasis had higher ORRs and significantly longer PFS than those without lung metastasis (ORR: 37.5% *vs.* 76.9%, $p = 0.16$; median PFS, NR [95%CI, 8.0—NR] *vs.* 4.9 months [95%CI, 1.8—NR]; HR, 0.17 [95%CI, 0.05—0.59], $p < 0.01$) (Tables 2 and 3). The presence of metastatic sites other than the liver or lung and number of metastases were not associated with the efficacy of T-DXd (Tables 2 and 3).

We also evaluated the association between the best response to prior T-DM1 therapy and the efficacy of T-DXd. The ORRs were
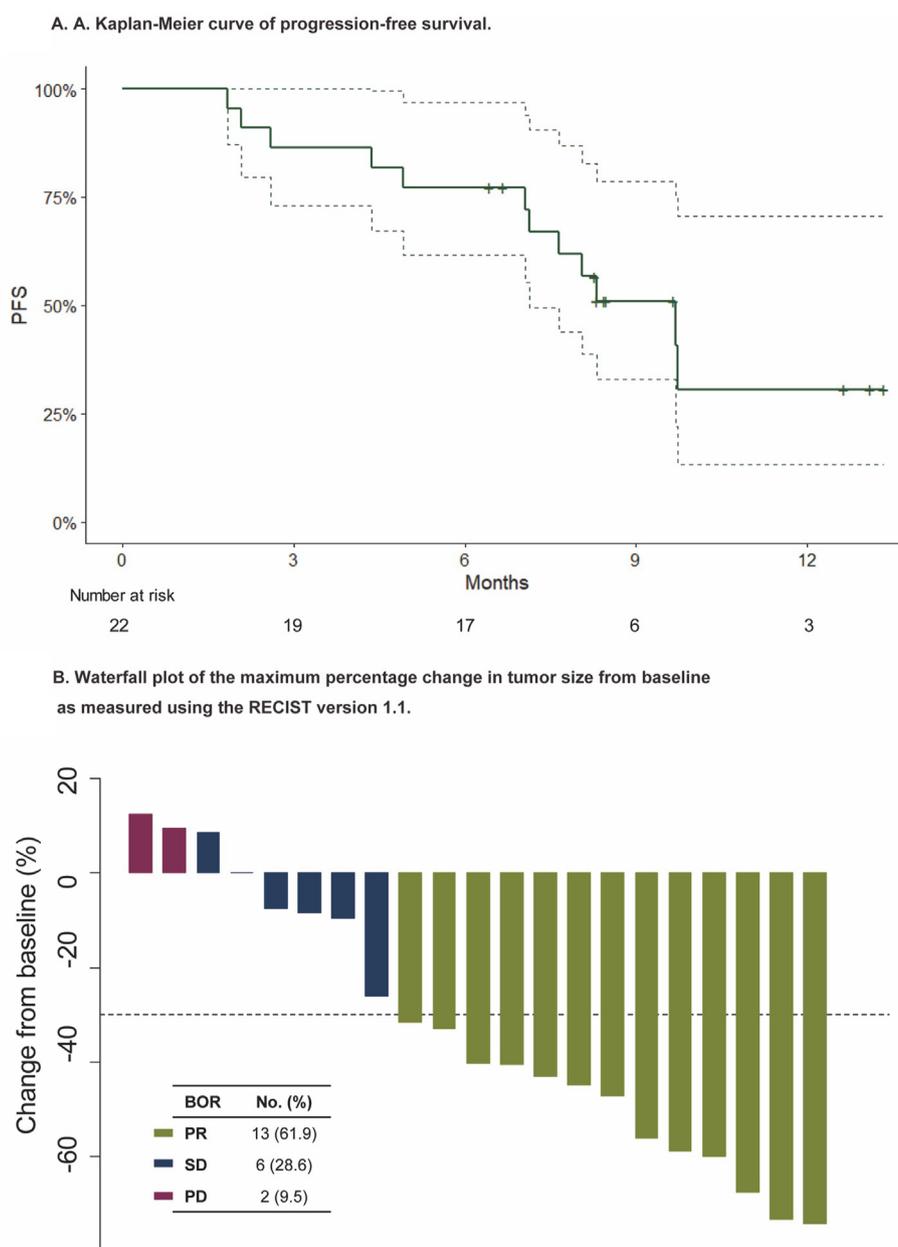

Fig. 1. Efficacy of trastuzumab deruxtecan. A. Kaplan—Meier curve of progression-free survival. Dashed line indicates 95% confidence interval. B. Waterfall plot of maximum percentage change in tumor size from baseline measured using RECIST version 1.1. One patient was excluded due to no target lesion.
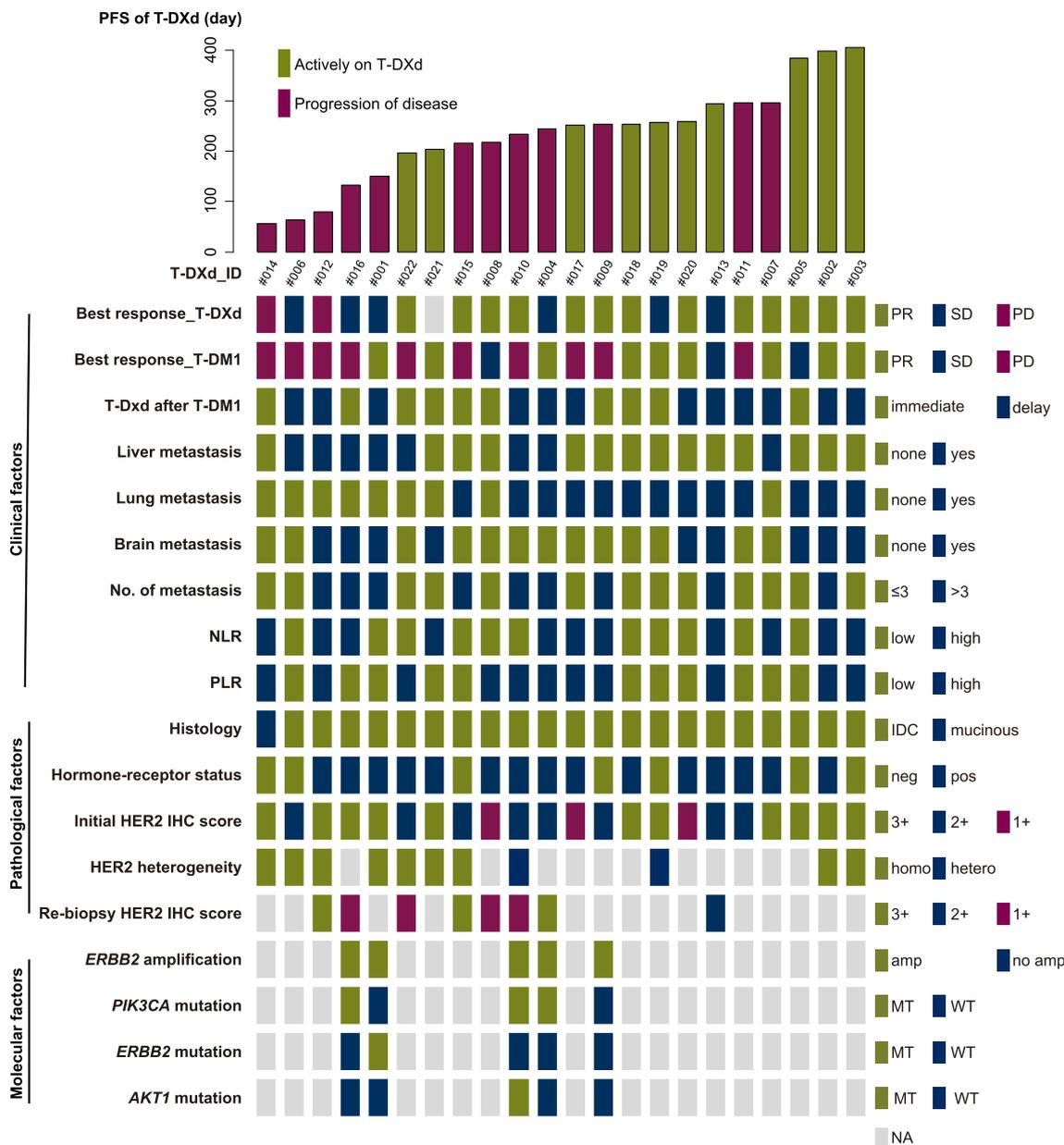
Fig. 2. Comprehensive clinicopathological and molecular factors in patients with HER2-positive metastatic breast cancer treated with trastuzumab deruxtecan. PFS for each patient shown at the top. Clinicopathological and molecular factors for each patient are shown. Each column represents one patient.

comparable regardless of the best response to prior T-DM1 (Table 2). Six of 10 patients who had PD with prior T-DM1 had PR with T-DXd (ORR: 60.0%) (Table 2 and Fig. 2). In contrast, four of five patients without clinical benefit from T-DXd (PD or SD with short PFS [<6 months]) had experienced PD with prior T-DM1 (Fig. 2). Patients who had PD with prior T-DM1 had significantly longer PFS following T-DXd than patients who had PR or SD with prior T-DM1 (median PFS, 7.1 months [95%CI, 1.8–NR] vs. NR [7.1–NR]; HR, 0.17 [0.04–0.65], $p < 0.01$) (Table 3).

There was no significant difference in ORR or PFS between patients treated with T-DXd immediately after prior T-DM1 or not (Tables 2 and 3). NLR and PLR were not significantly associated with the ORR and PFS of T-DXd.

3.3. Associations with pathological factors

We also assessed the clinical significance of HER2 status on the efficacy of T-DXd. The ORR and PFS were comparable between patients with HER2 IHC scores of 3+ and 2+/1+ at initial diagnosis (ORR: 50.0% vs. 72.7%, $p = 0.39$; median PFS, 9.7 months [95%CI, 2.6–NR] vs. 8.3 months [95%CI, 7.1–NR]; HR, 1.86 [95%CI, 0.53–6.57], $p = 0.34$) (Tables 2 and 3). Two patients with heterogenous HER2 expression had PR or long SD ($\geq$6 months) with T-DXd, including one who had PD as a best response with prior T-DM1. Three of four patients with re-biopsy samples after anti-HER2 targeted therapy and with latest HER2 IHC scores of 1+ had PR (75.0%) to T-DXd (Table 2 and Fig. 2), but none had responded to prior T-DM1.

Table 2
Objective tumor responses according to clinicopathological and molecular factors.

		n	Non-responder (SD or PD)	Responder (CR or PR)	ORR (%)	p value (Fisher)
Clinical factors						
Age	<Median	14	6	8	57.1%	0.66
	≥Meidan	7	2	5	71.4%	
Liver metastasis	None	13	3	10	76.9%	0.16
	Yes	8	5	3	37.5%	
Lung metastasis	None	8	5	3	37.5%	0.16
	Yes	13	3	10	76.9%	
Bone metastasis	None	7	2	5	71.4%	0.66
	Yes	14	6	8	57.1%	
Brain metastasis	None	13	4	9	69.2%	0.65
	Yes	8	4	4	50.0%	
Number of metastases	≤3	12	3	9	75.0%	0.20
	>3	9	5	4	44.4%	
Prior endocrine therapy	None	14	7	7	50.0%	0.17
	Yes	7	1	6	85.7%	
Best response to prior T-DM1	PD	10	4	6	60.0%	1.00
	PR/SD	11	4	7	63.6%	
T-DXd immediately after T-DM1	None	12	5	7	58.3%	1.00
	Yes	9	3	6	66.7%	
NLR	<Median	11	3	8	72.7%	0.39
	≥Median	10	5	5	50.0%	
PLR	<Median	10	4	6	60.0%	1.00
	≥Median	11	4	7	63.6%	
Pathological factors						
Hormone-receptor status	Negative	7	3	4	57.1%	1.00
	Positive	14	5	9	64.3%	
HER2 IHC score at initial diagnosis	3+	10	5	5	50.0%	0.39
	2+/1+	11	3	8	72.7%	
HER2 heterogeneity	Homogenous	8	4	4	50.0%	1.00
	Heterogenous	2	1	1	50.0%	
Latest HER2 IHC status at re-biopsy	3+/2+	4	3	1	25.0%	0.49
	1+	4	1	3	75.0%	

Abbreviations: CR, complete response; IHC, immunohistochemical score; n, number; NA, not available; NGS, next-generation sequencing; NLR, neutrophil-to-lymphocyte ratio; ORR, objective response rate; PD, progressive disease; PLR, platelet-to-lymphocyte ratio; PR, partial response; SD, stable disease.

One patient (Fig. 2; #014) with mucinous carcinoma with micropapillary pattern (MPMC) and a HER2 IHC score of 3+ had PD and short PFS (0.7 months and 1.9 months, respectively) with T-DM1 and T-DXd treatment. Hormone-receptor status was not associated with the ORR and PFS of T-DXd.

3.4. Association with genomic profiling

We examined the association between genomic profiling and the efficacy of T-DXd. Only five patients underwent tissue-based NGS-targeted gene panel analysis. All results of genomic alterations and responses to T-DXd in these patients are described in Supplementary Table 1. One patient, who harbored both HER2 amplification and L755S mutation in the primary lesion without exposure to chemotherapy, showed a modest shrinkage of the sum of target lesions (change from baseline −7.5%). We previously reported the detailed clinical course of this patient [27]. Among three patients with PIK3CA mutation, one with AKT1 co-mutation had PR, and the other two had SD.

3.5. Safety

The treatment-related adverse events of grade ≥3 or higher were neutropenia in six patients (27.2%), anemia in four patients (18.2%), and nausea in one patient (4.5%). No patients had febrile neutropenia, thrombocytopenia, or aspartate aminotransaminase/alanine aminotransferase increased (grade ≥3).

Four patients (18.2%) experienced drug-related interstitial lung disease (ILD); one had grade 1, two had grade 2, and one had grade 3, with T-DXd initiation to onset being 8.7, 4.4, 8.1, and 8.9 months, respectively. All the patients were treated with systemic steroids and recovered from drug-related ILD. T-DXd was not re-administered after recovery. None of the patients had a treatment-related decrease in the left ventricular ejection fraction (LVEF). One patient with an LVEF of 41% at baseline received T-DXd with close monitoring by cardiologists and continued treatment without a treatment-related decrease in LVEF.

4. Discussion

In the present study, we investigated the efficacy of T-DXd in patients with HER2-positive metastatic breast cancer in relation to their biomarker profiles. To the best of our knowledge, this study provides the first report of the comprehensive clinicopathological and molecular landscape in patients with HER2-positive metastatic breast cancer treated with T-DXd, and the impact of these factors on their treatment outcomes.

The ORR of T-DXd in this study was 61.9%, in line with the results of the DESTINY-Breast01 trial (61.1%), while the PFS was relatively shorter (9.7 months in this study vs. 19.4 months in the latest report of the DESTINY-Breast01 trial) [28]. The reason for the shorter PFS might be because the patients in this study had a poorer PS and impaired organ function. Seven of the 22 patients in this study failed to meet the eligibility criteria for PS and organ function as defined in the DESTINY-Breast01 trial. Another potential reason is that the prior pertuzumab therapy might have affected the results. A retrospective study showed that patients previously treated with pertuzumab had shorter PFS for T-DM1 than those who did not

Table 3
Progression-free survival according to clinicopathological and molecular factors.

Clinical factors		n	mPFS (months, 95%CI)	Univariate	
				HR (95%CI)	*p* value
Age	<Median	11	7.7 (4.4–9NR)	Ref	
	≥Median	11	NR (2.6–NR)	0.25 (0.06–0.98)	0.05
Liver metastasis	None	14	NR (7.1–NR)	Ref	
	Yes	8	6.3 (2.1–NR)	4.40 (1.35–14.29)	0.01
Lung metastasis	None	9	4.9 (1.8–NR)	Ref	
	Yes	13	NR (8.0–NR)	0.17 (0.05–0.59)	<0.01
Bone metastasis	None	7	8.3 (1.8–NR)	Ref	
	Yes	15	9.7 (4.4–NR)	1.07 (0.32–3.59)	0.91
Brain metastasis	None	13	8.3 (7.1–NR)	Ref	
	Yes	9	NR (2.6–NR)	0.38 (0.10–1.46)	0.16
Number of metastases	≤3	13	9.7 (7.1–NR)	Ref	
	>3	9	7.7 (2.6–NR)	2.55 (0.80–8.17)	0.12
Prior endocrine therapy	None	14	8.2 (4.4–NR)	Ref	
	Yes	8	9.7 (2.6–NR)	0.76 (0.23–2.57)	0.66
Best response to prior T-DM1	PD	10	7.1 (1.8–NR)	Ref	
	PR/SD	12	NR (7.1–NR)	0.17 (0.04–0.65)	<0.01
T-DXd immediately after T-DM1	None	12	9.7 (2.6–NR)Ref		
	Yes	10	8.3 (1.8–NR)	1.20 (0.37–3.84)	0.76
NLR	<Median	11	9.7 (4.9–NR)	Ref	
	≥Median	11	8.3 (2.6–NR)	0.89 (0.28–2.81)	0.85
PLR	<Median	11	9.7 (4.4–NR)	Ref	
	≥Median	11	8.3 (2.6–NR)	1.04 (0.33–3.23)	0.95
Pathological factors					
Hormone-receptor status	Negative	7	8.3 (1.8–NR)	Ref	
	Positive	15	9.7 (4.9–NR)	0.98 (0.29–3.31)	0.98
HER2 IHC score at initial diagnosis	3+	11	9.7 (2.6–NR)	Ref	
	2+/1+	11	8.3 (7.1–NR)	1.86 (0.53–6.57)	0.34
HER2 heterogeneity	Homogenous	9	7.1 (1.8–NR)	Ref	
	Heterogenous	2	7.7 (7.7–NR)	0.46 (0.05–4.18)	0.49
Latest HER2 IHC score at re-biopsy	3+/2+	4	7.6 (2.6–NR)Ref		
	1+	4	7.1 (4.4–NR)	1.78 (0.29–10.95)	0.53

Abbreviations: HR, hazard ratio; IHC, immunohistochemical; n, number; NA, not available; NLR, neutrophil-to-lymphocyte ratio; NR, not reached; PD, progressive disease; PFS, progression-free survival; PLR, platelet-to-lymphocyte ratio; PR, partial response; SD, stable disease.

receive the drug [29]. All patients in this study received pertuzumab previously, while only 65.8% of patients in the DESTINY-Breast01 trial received pertuzumab. Although the effect of prior pertuzumab treatment on T-DXd efficacy is currently unknown, pertuzumab may have reduced the efficacy of T-DXd. Further research is needed to test this hypothesis. Nonetheless, the clinical benefits of T-DXd in this study appeared to be superior to those of chemotherapy plus anti-HER2 targeted agents administered after T-DM1, before the approval of T-DXd [30], and to those of recent phase 3 trials of new agents such as tucatinib and margetuximab [31,32]. Although cross-study comparisons must be interpreted with caution, the current results suggest that T-DXd administered after T-DM1 may have substantial benefits in clinical practice.

Various mechanisms of resistance to T-DM1 have been reported to date. Several studies found that the clinical benefits of T-DM1 were decreased in HER2 IHC 2+/ISH-positive compared with HER2 IHC 3+ patients [16,22]. *Post hoc* analyses of some phase 2 trials showed limited activity of T-DM1 in HER2-negative patients [19,20]. Moreover, intra-tumoral HER2 heterogeneity and loss of HER2 expression in re-biopsy specimens or *HER2* amplification in circulating tumor DNA were associated with poor outcomes following T-DM1 [17,18,21,22,33,34]. Indeed, in the current cohort, one of two patients with heterogenous HER2 expression had PD, and none of the four patients with a HER2 IHC score of 1+ at re-biopsy demonstrated a response to T-DM1. These results emphasize that T-DM1 may not be sufficient to eradicate tumors with reduced expression or heterogeneity of HER2. In contrast, the present study showed that the ORR and PFS of T-DXd were not influenced by the HER2 IHC score at initial diagnosis (3+ vs. 2+/1+). Moreover, patients with heterogenous HER2 expression also derived clinical benefit from T-DXd (one PR, one SD with long PFS

[≥6 months]), and three of the above four patients with a HER2 IHC score of 1+ at re-biopsy responded to T-DXd. Notably, six of the 10 patients whose best response to T-DM1 was PD responded to T-DXd. These results suggest that T-DXd might overcome the resistance mechanism of T-DM1 due to its novel drug design, including a high drug-to-antibody ratio and bystander effect. This clinical activity of T-DXd was also supported by the results of a recent phase 1 b trial, which evaluated the efficacy and safety of T-DXd in patients with HER2-low metastatic breast cancer [35].

The current results showed that the ORR and PFS in patients treated with T-DXd tended differ according to the metastatic site, suggesting that T-DXd might be less effective in patients with liver metastasis. Although this result could simply reflect the prognostic effect of liver metastasis in patients with HER2-positive breast cancer, several hypotheses should be considered. First, the drug delivery of trastuzumab to liver metastases might be insufficient, or HER2 expression levels in liver metastases might be lower than in other metastatic sites. However, there are conflicting data regarding this hypothesis. In the ZEPHIR trial,[35] HER2-positron emission tomography/computed tomography revealed organ-based heterogeneity of tumor uptake of ^{89}Zr-trastuzumab in patients who received T-DM1, with the highest uptake in liver metastases [36]. Other hypotheses include poor internalization of T-DXd or reduced membrane permeability of DXd in the liver. However, evidence to support these hypotheses is currently lacking, and further studies are needed to clarify the association between the efficacy of T-DXd and metastatic sites.

In the present study, one patient with MPMC did not respond to T-DM1 or T-DXd. MPMC is a rare histological type with intermediate characteristics between invasive micropapillary breast cancer and mucinous carcinoma [37]. Mucin 4 (MUC4) is a membrane

glycoprotein that is frequently overexpressed in invasive micro-papillary breast cancer [38], and has been associated with poor treatment outcomes following trastuzumab [38,39]. Although the association between the clinical efficacy of T-DXd and MUC4 has not been studied, preclinical studies found that MUC4 masked the trastuzumab-binding epitope of HER2, thereby unbinding trastuzumab, and this mechanism has also been confirmed for T-DM1 [39–41]. It has been suggested that tumor necrosis factor-alpha inhibitors and acetylcysteine may overcome this resistance mechanism, and further investigations are expected [39,41].

This study had some limitations. First, it was a single-center study with a limited sample size, and HER2 heterogeneity could not be assessed in some patients because the archival specimens were too old to be available. Moreover, a limited number of patients underwent NGS-targeted gene panel analysis and re-biopsy, and the timings of the re-biopsies varied. Furthermore, the timing of the radiographic evaluations was not specified due to the retrospective nature of the study. Finally, we did not correct for possible confounders for the determination of PFS in relation to different treatments, due to the small simple size.

5. Conclusions

T-DXd demonstrated favorable clinical activity in patients with HER2-positive metastatic breast cancer. T-DXd also showed activity in patients with heterogeneity, reduction, or loss of HER2 expression, which have been reported as negative predictive factors for T-DM1 treatment.

Disclosure of potential conflict of interests

HN received consulting fee from Terumo Corporation, and honoraria from Chugai Pharmaceutical and Takeda Pharmaceutical, outside the submitted work. HN also reports an immediate family member employed by Otsuka Pharmaceutical Co., Ltd. KH received research funding from Merck Serono and Daiichi-Sankyo, and honoraria from Astra Zeneca, MSD, Takeda and Chugai, and advisory roles for Astra Zeneca, Chugai, Takeda and Eli Lilly. NM received research funding from Janssen, AstraZeneca, Takeda, Lilly, Amgen, Astellas, Chugai, Bayer, MSD, Ono, Taiho and Pfizer, and honoraria from Janssen and Sanofi, and served in advisory roles for Janssen, Sanofi, AstraZeneca, Roche, Amgen and Pfizer. YN received research funding from Roche Diagnostics, and honoraria from Pfizer, Taiho, Nippon Kayaku, Eli Lilly, AstraZeneca, Merck Serono, Bayer, Meiji Seika, Roche Diagnostics, Novartis, Eisai, Chugai Pharmaceutical and Fuji Film Toyama Chemistry, outside the submitted work. TM received research funding from Daiichi-Sankyo, Sysmex, Eisai, MSD, Pfizer, Novartis, Sanofi, Chugai and Astra Zeneca, and honoraria from Eisai, Pfizer, Novartis, Chugai, Eli Lilly, Astra Zeneca, Kyowa-kirin and Taiho outside the submitted work. The other authors have no conflicts of interest to disclose.

Author contributions

Conception and design: HN, KH, and TM. Acquisition of data: HN, and T. Nakai. Analysis and/or interpretation of data: HN, KH, and TM. Writing, review, and/or revision of the manuscript: HN, KH, T. Nakai, SK, T. Nakao, CF, CK, NM, YN, AH, SM, GI, and TM. Drafting the manuscript: HN, and KH. All authors contributed to the article and approved the submitted version.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2022.01.002.

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