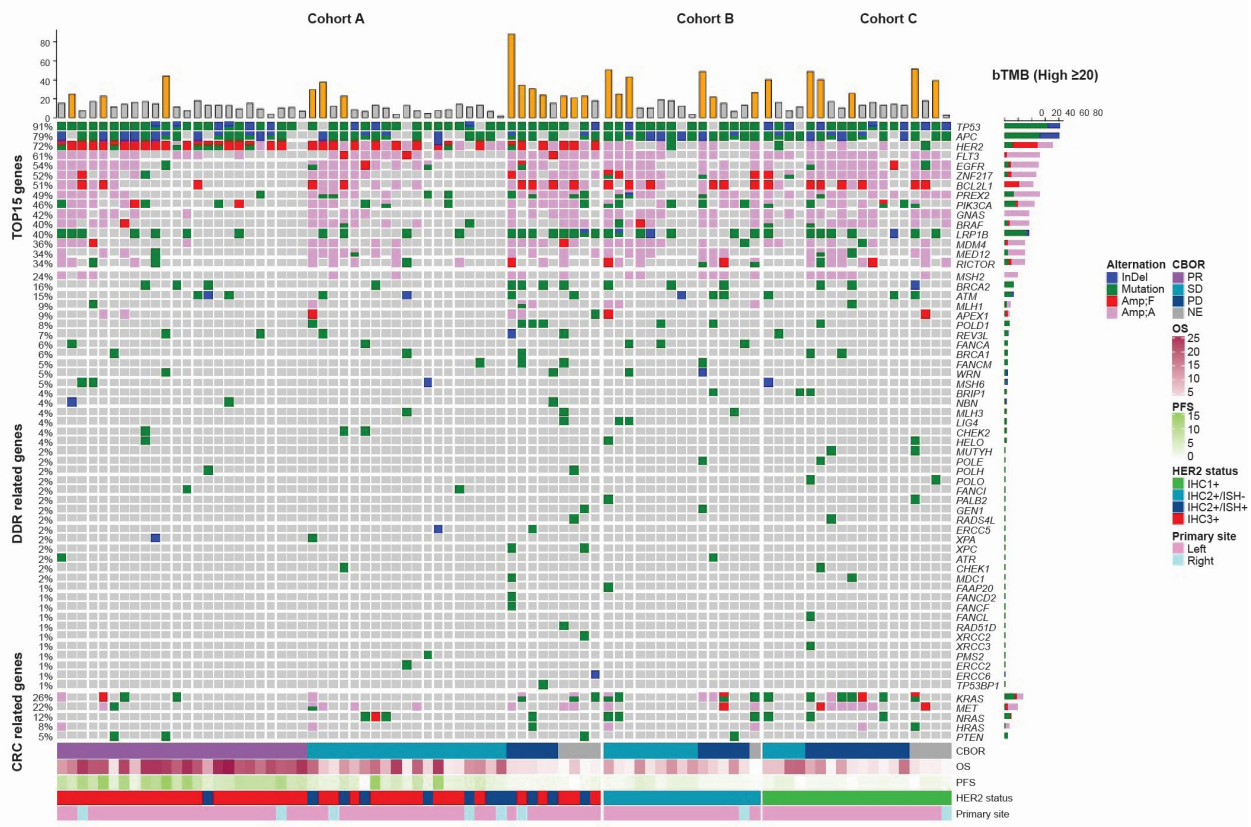
Supplementary Information

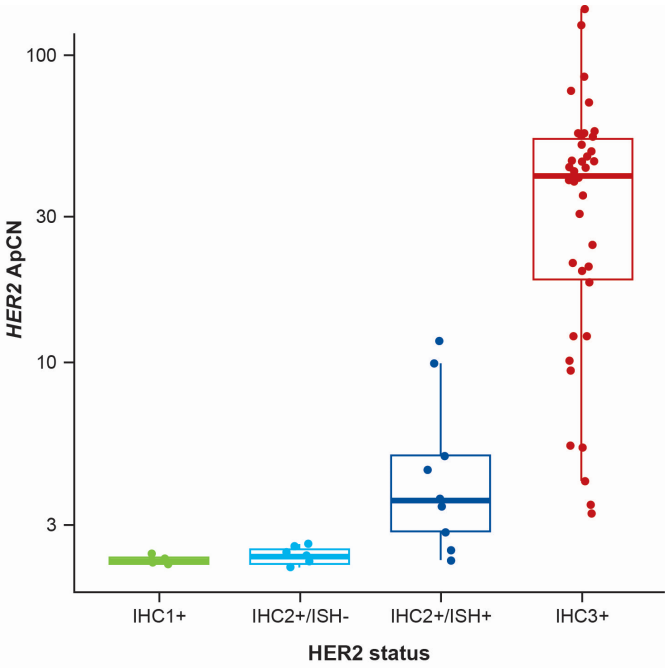
Supplementary Fig. S1. Genomic landscape of ctDNA at baseline.



The top bar plot shows bTMB score; yellow bars represent high bTMB (bTMB ≥20 mut/Mb). Activating and nonactivating mutations are included.
Amp, amplification; CBOR, confirmed best overall response; CRC, colorectal cancer; ctDNA, circulating tumor DNA; DDR, DNA damage response and repair; HER2IHC, human epidermal growth factor receptor immunohistochemistry; IHC, immunohistochemistry; ISH, in situ hybridization; NE, not evaluable; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; bTMB, blood tumor mutational burden.

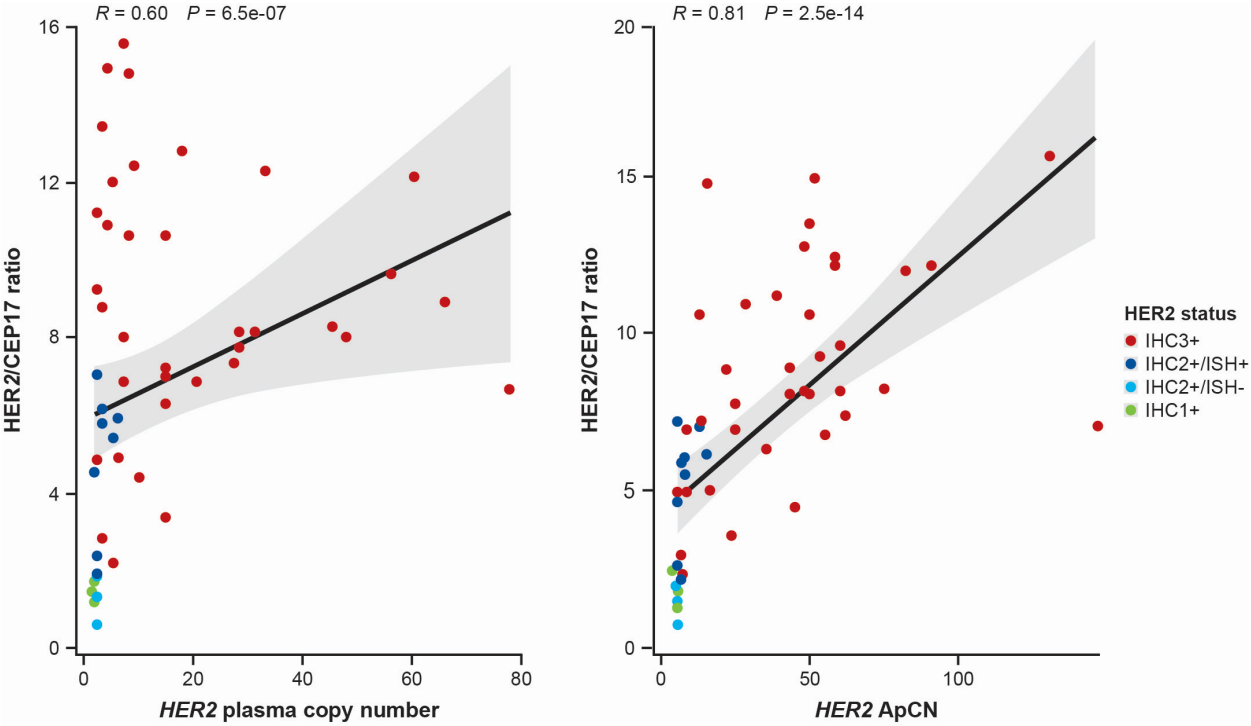
Supplementary Fig. S2. Relationship between HER2 status and plasma *HER2* amplification status/*HER2* ApCN (all cohorts)

		n	Plasma *HER2* amplification status			
			Focal	Aneuploidy	No amp	No data
HER2 status in tissue	IHC3+	40	33	5	2	0
	IHC2+/ISH+	13	3	6	3	1
	IHC2+/ISH-	15	0	7	8	0
	IHC1+	18	0	4	14	0



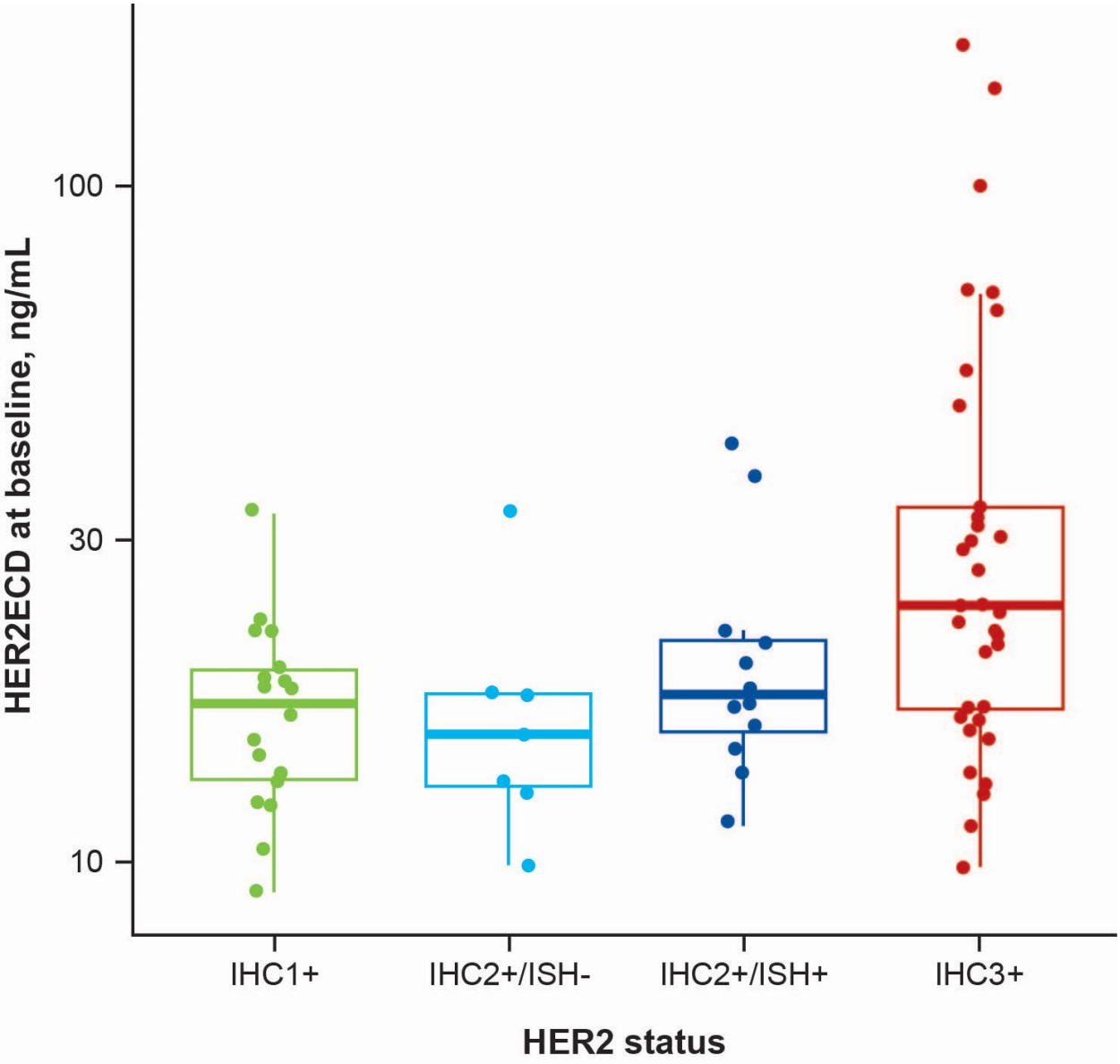
Minimum and maximum, excluding outliers, are represented by the whiskers, the box represents the first and third quarters, the center represents the median, and dots represent individual samples (right panel; n = 58 samples).
Amp, amplification; ApCN, adjusted plasma copy number; ctDNA, circulating tumor DNA; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

Supplementary Fig. S3. Correlation between and HER2/CEP17 ratio and *HER2* plasma copy number/ApCN at baseline



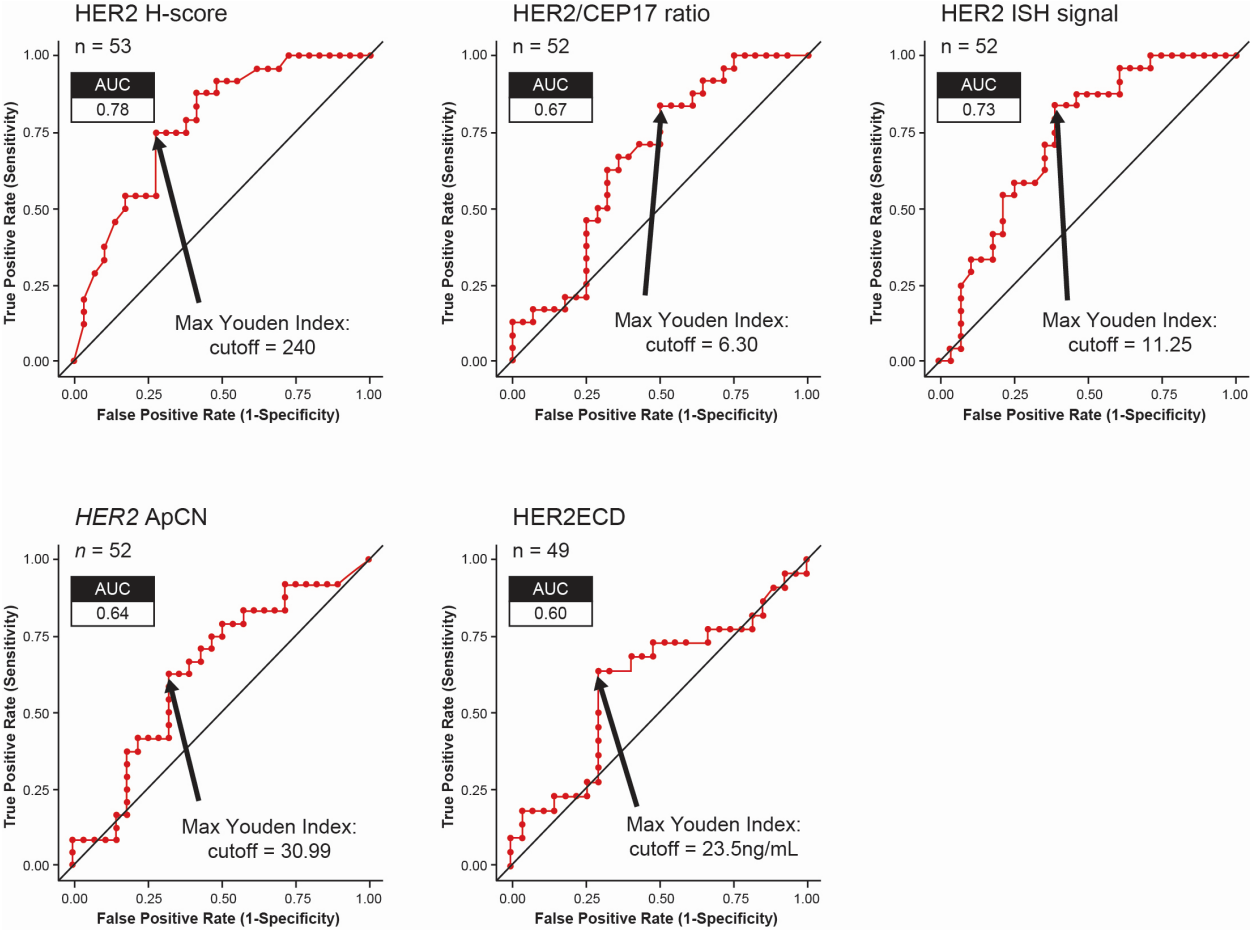
R = Spearman correlation. n = 57 samples.
ApCN, adjusted plasma copy number; CHR, chromosome; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization.

Supplementary Fig. S4. Relationship between tumor HER2 status and plasma HER2ECD at baseline



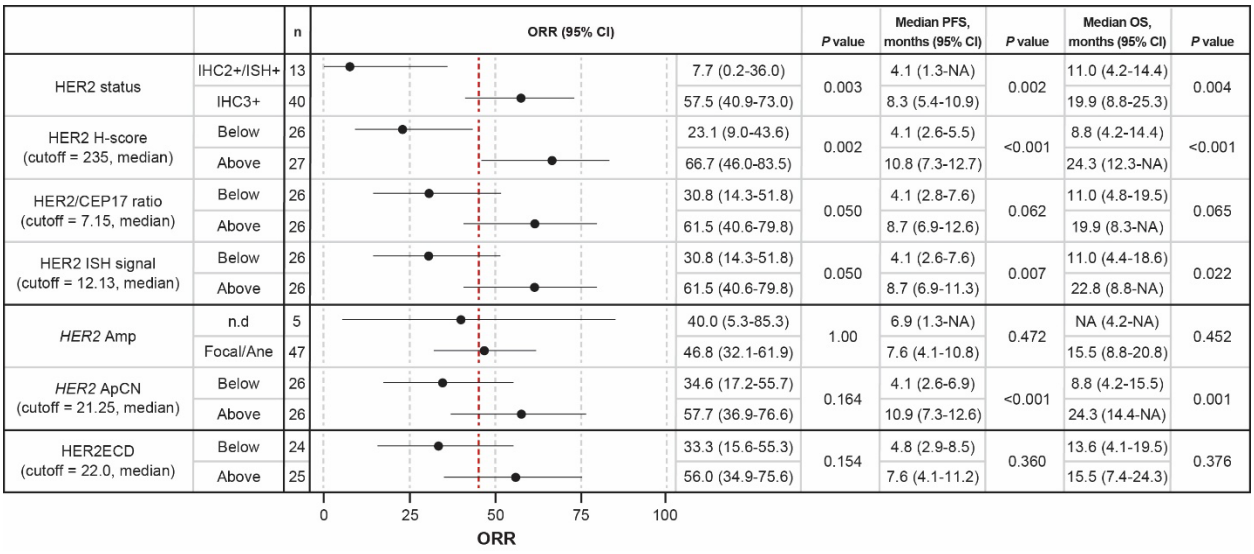
n = 74 samples.
HER2, human epidermal growth factor receptor 2; HER2ECD, human epidermal growth factor receptor 2 extracellular domain; IHC, immunohistochemistry; ISH, in situ hybridization.

Supplementary Fig. S5. ROC analysis of HER2 biomarkers at baseline.



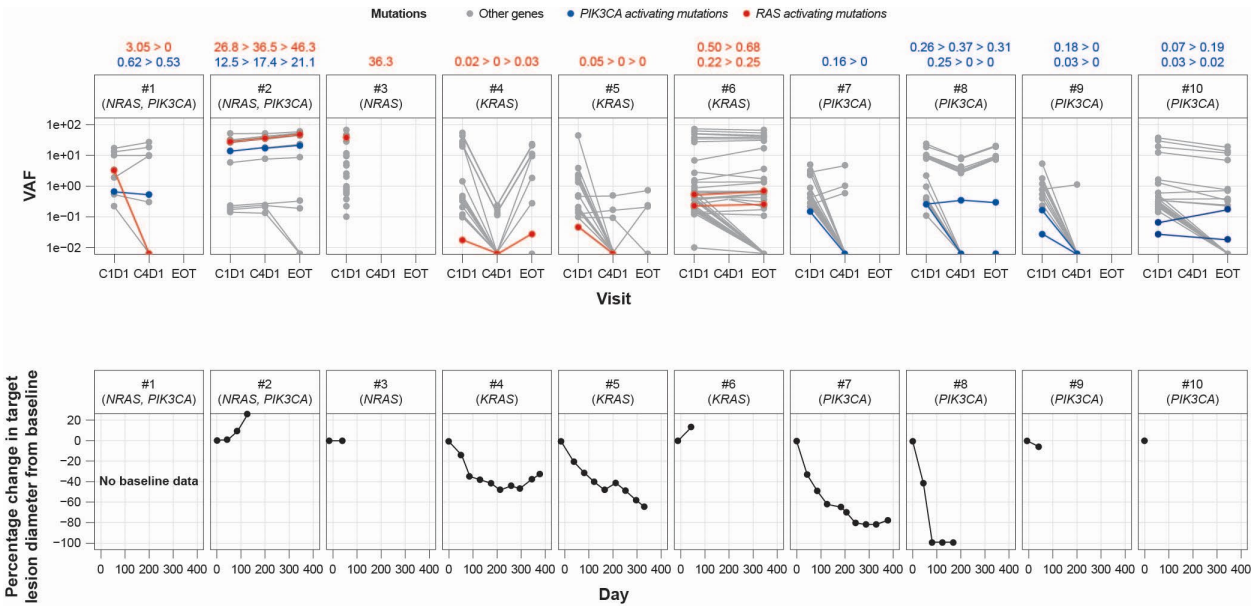
AUC, area under the curve; ApCN, adjusted plasma copy number; ISH, in situ hybridization; HER2, human epidermal growth factor receptor 2; HER2ECD, human epidermal growth factor receptor 2 extracellular domain; pts, patients; ROC, receiver operating characteristics.

Supplementary Fig. S6. Antitumor activity of T-DXd according to baseline HER2 biomarker status using medians as cutoff values



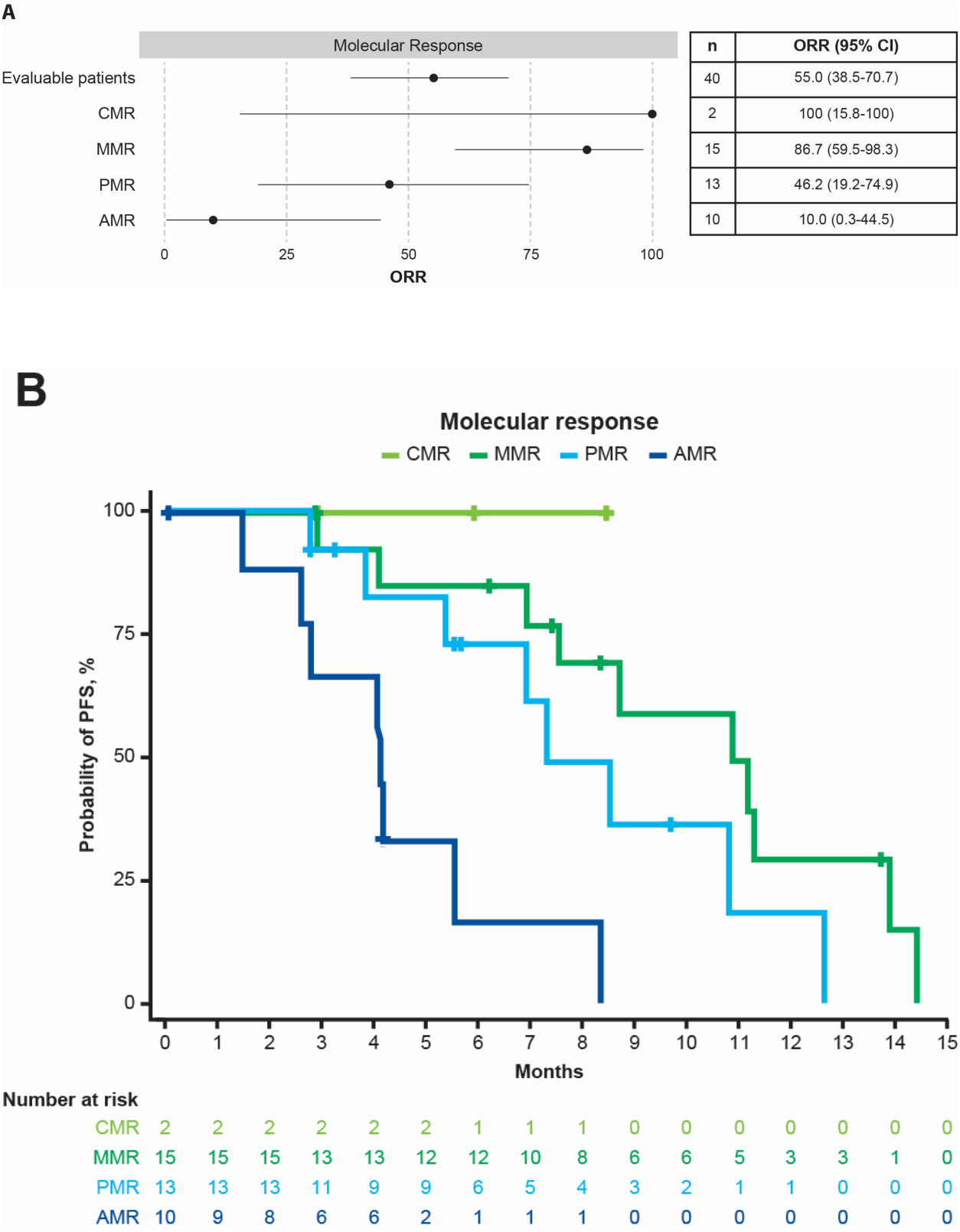
Vertical red dashed line shows the ORR of 45.3% in the overall population for Cohort A. *P* values are based on two-sided Fisher's exact test for ORR and those based on two-sided log-rank test for PFS and OS are shown, without adjustment for multiple comparisons. Error bars represent the 95% CI. The exact *P* values for HER2 H-score for PFS, HER2 H-score for OS, and HER2 ApCN for PFS were 0.000416, 0.000203, and 0.000160, respectively.
Amp, amplification; Ane, aneuploidy; ApCN, adjusted plasma copy number; HER2, human epidermal growth factor receptor 2; HER2ECD, human epidermal growth factor receptor 2 extracellular domain; ISH, in situ hybridization; survival; NA, not applicable; n.d., not determined; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

Supplementary Fig. S7. Change in variant allele fraction during T-DXd treatment in patients with *RAS* or *PIK3CA* mutations



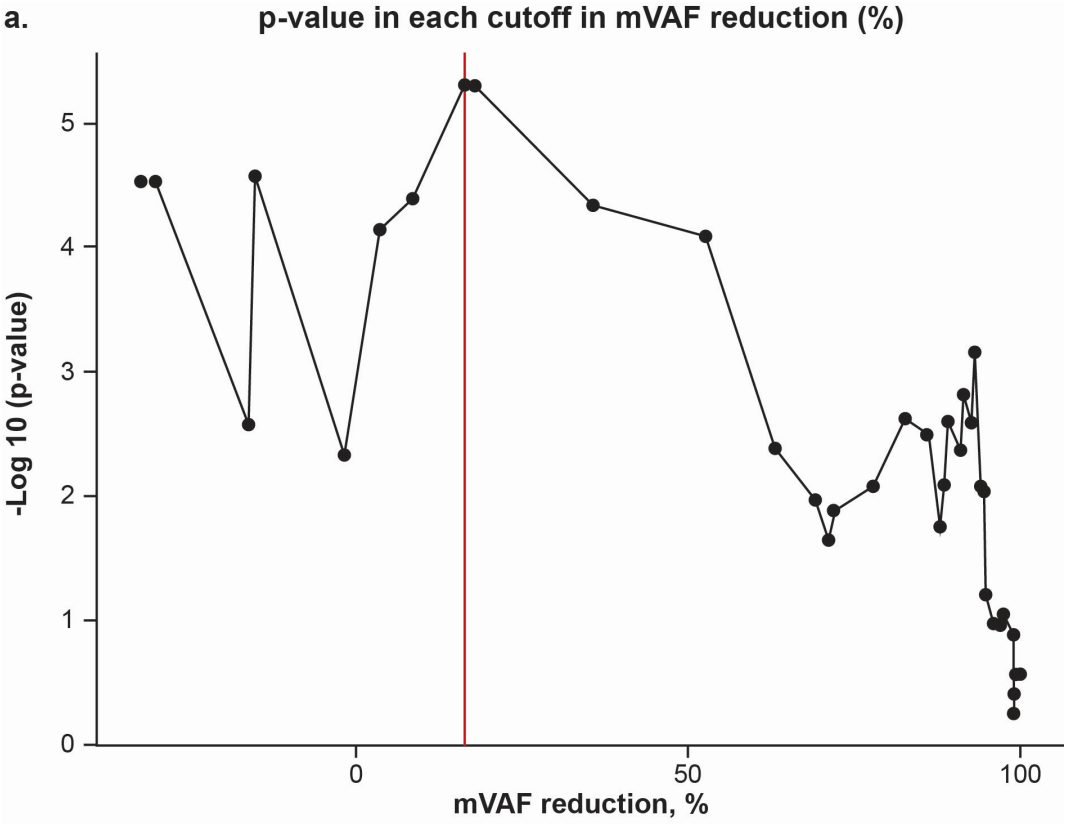
T-DXd, trastuzumab deruxtecan; VAF, variant allele fraction.

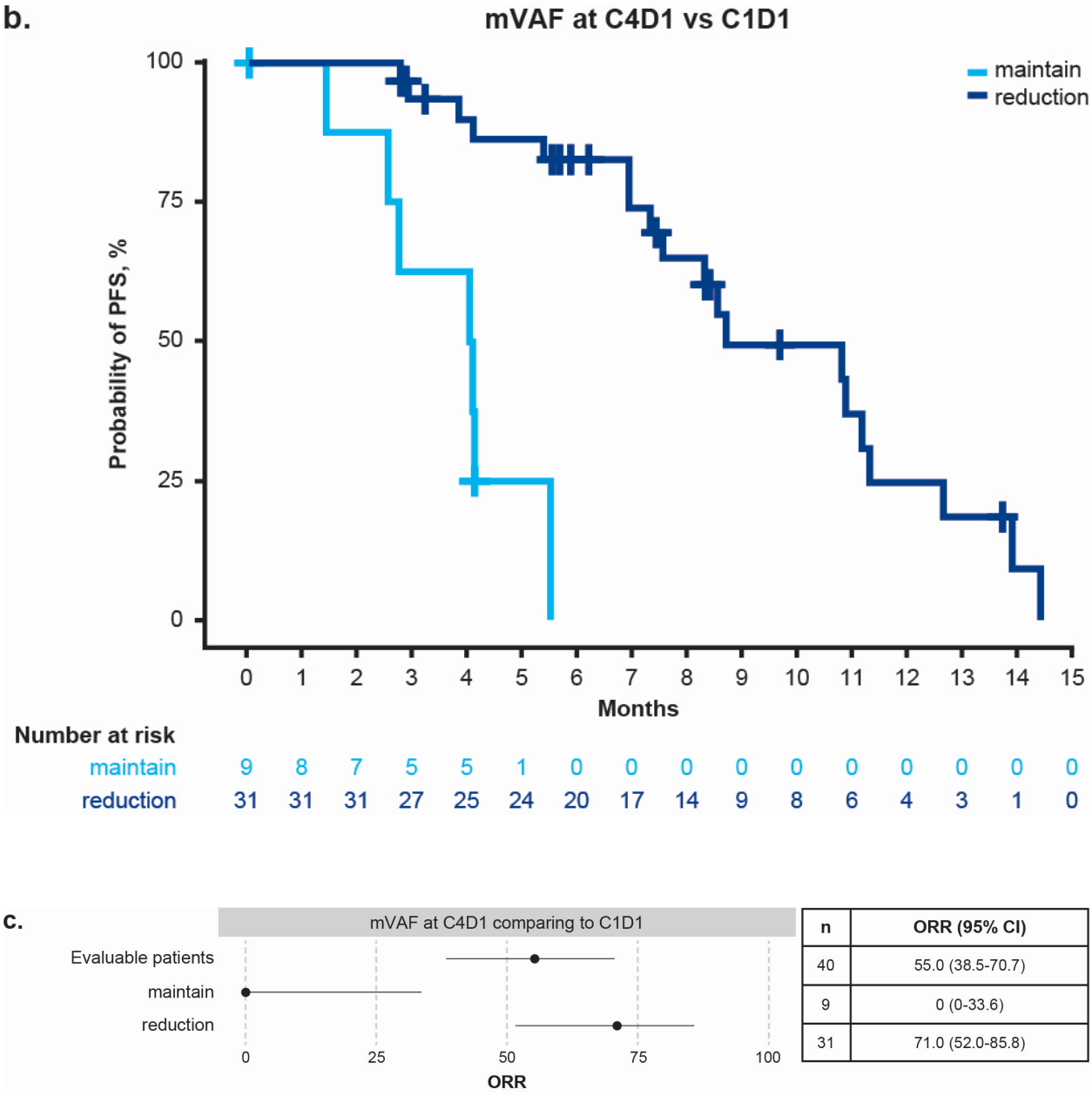
Supplementary Fig. S8. ORR (A) and PFS (B) according to mVAF cutoffs



mVAF cutoffs were determined as CMR, 100% reduction of mVAF (nondetectable ctDNA); MMR, ≥90% to <100% reduction in mVAF reduction; PMR, ≥20% to <90% reduction in mVAF; AMR, <20% reduction in mVAF. Error bars (panel A) represent the 95% CI.
AMR, absent molecular response; CMR, complete molecular response; ctDNA, circulating tumor DNA; MMR, major molecular response; mVAF, mean variant allele fraction; ORR, objective response rate; PFS, progression-free survival; PMR, partial molecular response.

Supplementary Fig. S9. Relationship between change in VAF (a) and outcomes of PFS (b) or ORR (c) at baseline.

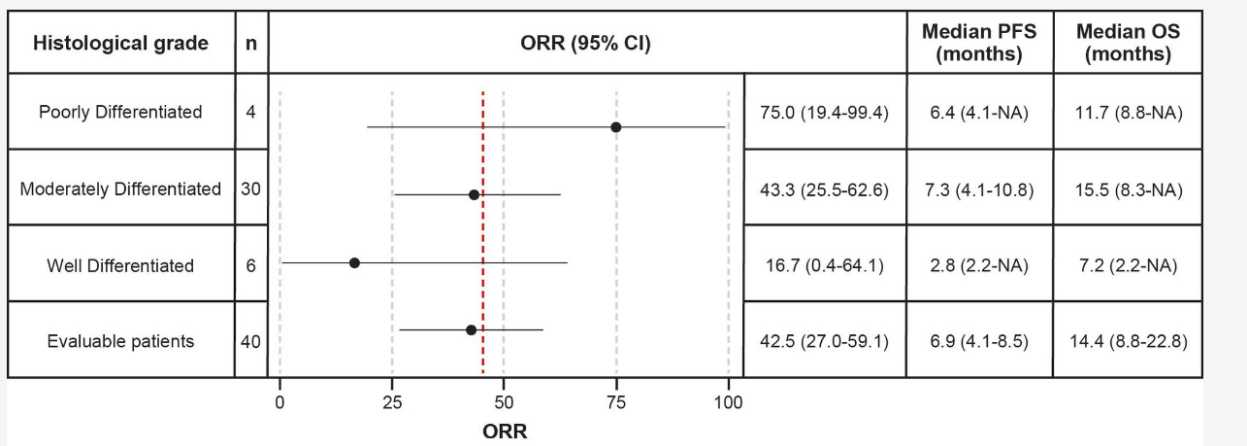




P value is based on log-rank test. PFS and ORR are based on the exploratory cutoff mVAF of ≥16.4%. Maintain represents mVAF reduction <16.4% and reduction represents mVAF reduction ≥16.4%. Vertical red line (panel A) shows the cutoff of 16.4% (n = 38 cutoffs). Error bars (panel C) represent the 95% CI.

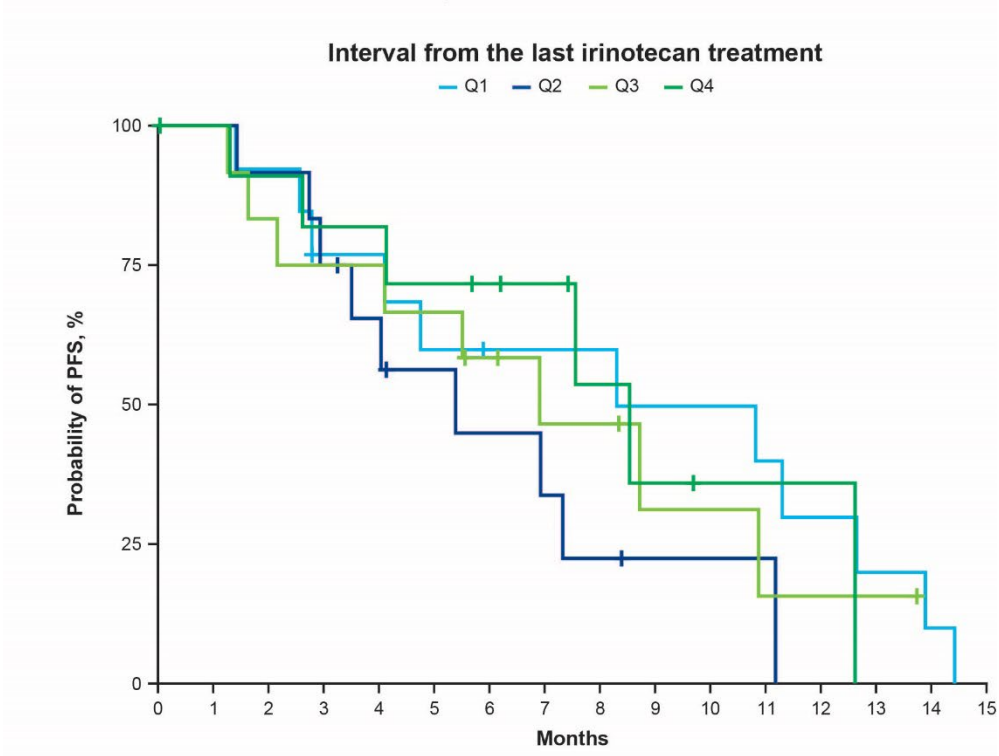
C, cycle; D, day; mVAF, mean variant allele fraction; ORR, objective response rate; PFS, progression-free survival.

Supplementary Fig. S10. Clinical outcomes according to histological grade at baseline (Cohort A)



Vertical red dashed line shows the ORR of 45.3% in the overall population for Cohort A (N = 53). Error bars represent the 95% CI.
Analysis included 40 evaluable patients with HER2-positive mCRC (Cohort A).
NA, not applicable; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

Supplementary Fig. S11. Clinical outcomes according to the interval from the last irinotecan dose in cohort A at baseline



Status (interval)	n	PR	SD	PD	NE	ORR (95%CI)	DCR (95%CI)
Q1 (≤ 98 days)	13	5	7	1	0	38.5 (13.9-68.4)	92.3 (64-99.8)
Q2 (> 98 to ≤ 327 days)	12	3	8	1	0	25.0 (5.5-57.2)	91.7 (61.5-99.8)
Q3 (> 327 to ≤ 663 days)	12	8	1	1	2	66.7 (34.9-90.1)	75.0 (42.8-94.5)
Q4 (> 663 days)	12	7	4	0	1	58.3 (27.7-84.8)	91.7 (61.5-99.8)
Evaluable patients	49	23	20	3	3	46.9 (32.5-61.7)	87.8 (75.2-95.4)

DCR, disease control rate; NE, not evaluable; ORR, objective response rate; PD, progressive disease; PFS, progression-free survival; PR, partial response; Q, quarter; SD, stable disease.

Supplementary Table S1. Baseline characteristics for the biomarker datasets and all patients (cohort A)

	HER2ECD Evaluable Dataset n = 49	**ctDNA Evaluable Dataset n = 52**	**ISH Evaluable Dataset n = 52**	**All Patients N = 53**
Sex, n (%)				
Female	25 (51.0)	28 (53.8)	27 (51.9)	28 (52.8)
Male	24 (49.0)	24 (46.2)	25 (48.1)	25 (47.2)
Age, years				
Mean	57.3	57.4	57.4	57.5
Median	57.0	56.5	56.5	57.0
Region, n (%)				
Asia	15 (30.6)	15 (28.8)	14 (26.9)	15 (28.3)
Europe	28 (57.1)	27 (51.9)	28 (53.8)	28 (52.8)
United States	6 (12.2)	10 (19.2)	10 (19.2)	10 (18.9)
ECOG PS, n (%)				
0	34 (69.4)	37 (71.2)	36 (69.2)	37 (69.8)
1	15 (30.6)	15 (28.8)	16 (30.8)	16 (30.2)
Primary tumor site, n (%)				
Left side	43 (87.8)	46 (88.5)	46 (88.5)	47 (88.7)
Right side	6 (12.2)	6 (11.5)	6 (11.5)	6 (11.3)

ctDNA, circulating tumor DNA; ECOG PS, Eastern Cooperative Oncology Group performance status; HER2ECD, human epidermal growth factor receptor 2 extracellular domain; ISH, in situ hybridization.

Supplementary Table S2. *NRAS/KRAS*, *PIK3CA*, and *HER2* mutations and corresponding VAFs in Cohort A at baseline

Patient	***RAS* (VAF)**	***PIK3CA* (VAF)**	***HER2* (VAF)**	**HER2 Status**	**CBOR**	**Amplification Type**	**ApCN**
1	*NRAS*_G12D (36.34)	NA	NA	IHC 2+/ISH+	PD	No Amp	NA
2	*NRAS*_Q61R (3.05)	C420R (0.62)	NA	IHC 2+/ISH+	SD	Aneuploidy	3.52
3	*NRAS*_Q61R (26.81)	E542K (12.49)	NA	IHC 3+	SD	Aneuploidy	3.37
4	*KRAS*_G12S (0.02)	NA	NA	IHC 3+	PR	Focal	56.25
5	*KRAS*_Q61H (0.05)	NA	T862A (43.64)	IHC 3+	PR	Aneuploidy	3.59
6	*KRAS*_Q61H (0.50), *KRAS*_Q61H (0.22)	NA	NA	IHC 3+	PD	Focal	4.29
7	NA	E542K (0.03), E545A (0.07)	NA	IHC 2+/ISH+	NE	Aneuploidy	2.90
8	NA	E542K (0.03), E545A (0.18)	L755S (0.40)	IHC 3+	SD	Focal	24.64
9	NA	E545K (0.16)	NA	IHC 3+	PR	Focal	140.96
10	NA	E545K (0.26), Q46K (0.25)	L755S (0.11)	IHC 3+	PR	Focal	30.99
11	NA	NA	S310F (0.04)	IHC 3+	PR	Focal	12.49
12	NA	NA	D769Y (81.02), V777L (79.91)	IHC 2+/ISH+	PD	Focal	4.59
13	NA	NA	G776V (79.24)	IHC3+	PR	Focal	5.62
14	NA	NA	V777L (51.14)	IHC 2+/ISH+	PR	Focal	3.68
15	NA	NA	L823del (0.01)	IHC3+	SD	Focal	77.46

Amp, amplification; ApCN, adjusted plasma copy number; CBOR, confirmed best overall response; del, deletion; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ISH, in situ hybridization; NA, not applicable; NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease; VAF, variant allele fraction.

Supplementary Table S3. List of investigators and study sites.

Country, Study Site	**Principal Investigator**
Japan	
Hokkaido University Hospital	Yoshito Komatsu
National Cancer Center Hospital East	Takayuki Yoshino
The Cancer Institute Hospital of JFCR	Kensei Yamaguchi
Aichi Cancer Center Hospital	Toshiki Masuishi
Kindai University Hospital	Hisato Kawakami
Shikoku Cancer Center Hospital	Tomohiro Nishina
Kyushu Cancer Center	Taito Esaki
United States of America	
Mayo Clinic-Jacksonville (Mayo Florida)	Jason Starr
MD Anderson Cancer Center, University of Texas	Kanwal Raghav
City of Hope Medical Center	Marwan Fakih
Vanderbilt University Medical Center	Kristen Ciombor
University of Southern California	Heinz-Josef Lenz
Karmanos Cancer Institute	Anthony Shields
Greenville Health System Cancer Institute	Ki Chung
UCLA Medical Center	Zev Wainberg
West Cancer Center	Axel Grothey

Country, Study Site	Principal Investigator
Italy	
Fondazione IRCCS Istituto Nazionale dei Tumori	Maria Di Bartolomeo
Oncology Institute Veneto IOV-IRCCS	Fotios Loupakis
ASST Grande Ospedale Metropolitano Niguarda	Salvatore Siena
Università degli studi della Campania L. Vanvitelli	Fortunato Ciardiello
Spain	
Hospital Universitari Clinic de Barcelona	Joan Maurel Santasusana
Hospital Universitari Vall d'Hebron	Elena Elez Fernandez
Clinica Universidad de Navarra	Javier Rodriguez
United Kingdom	
Royal Marsden Institute (Sutton)	Ian Chau
Royal Marsden Institute (Chelsea)	Ian Chau

Supplementary Table S4. Additional data sharing information.

Data Sharing Questions	Answers
Will individual participant data be available (including data dictionaries)?	Yes
What data in particular will be made available?	Anonymized individual participant data (IPD) on completed studies and applicable supporting clinical trial documents may be available upon request at https://vivli.org/. In cases where clinical trial data and supporting documents are provided pursuant to our company policies and procedures, Daiichi Sankyo, Inc., will continue to protect the privacy of our clinical trial participants. Details on data sharing criteria and the procedure for requesting access can be found at this web address: https://vivli.org/ourmember/daiichi-sankyo/.
What other documents will be available?	Clinical Trial Protocol, Statistical Analysis Plan, Informed Consent Form, and Clinical Study Report. In cases where clinical trial data and supporting documents are provided pursuant to our company policies and procedures, Daiichi Sankyo will continue to protect the privacy of our clinical trial participants.
When will data be available (start and end dates)?	Anonymized IPD will be available when indication supported receives marketing approvals and study results are published.
To whom will data be available?	Qualified science and medical researchers upon formal request and submission of research proposal detailing planned analyses.
For what type of analyses?	De-identified IPD and relevant clinical trial documents will be shared for the purpose of conducting legitimate research as specified in an approved formal research proposal.
By what mechanism will data be made available?	De-identified IPD will be available upon request at https://vivli.org/.