Supplementary Table S1. The set of 45 breast CTC-specific transcripts identified after database and RNAseq search as potential markers for breast CTCs.

AGR2	CXCL13	FAT2	MMP11	PRAME	TFAP2C
AGR3	CXCL14	FOXA1	MUC16	S100A2	TFF12
CADPS2	CYP1A1	GFRA	MUCL1	SCGB2A1	TMPRSS4
CDH11	EFHD1	IRX5	OCLN	SERPINA3	WFDC1
CDH3	ERBB2	MAGED1	PGR	SFRP1	WFDC2
COL11A1	ERBB3	MAGED2	PIP	SFRP2	
COL8A1	ESR1	MCRA5	PKP3	SPP1	
CRABP2	FAT1	MGP	POSTN	TFAP2B	

Supplementary Table S2. Association of clinical characteristics between patients with high versus low CTC-Scores, both at baseline and at 3-4 weeks on-treatment time points.

Categorical variables

	Pretreatment							***3-4 weeks on treatment***						
	All		***CTC Score***				**Fisher's exact p-value**	***All***		***CTC Score (3-4 weeks)***				**Fisher's exact p-values**
			<= 3000		***>3000***					***<= 3000***		***>3000***		
	N	***%***	***N***	***%***	***N***	***%***		***N***	***%***	***N***	***%***	***N***	***%***	
Chemotherapy														
No	79	89.8	46	92	33	86.8	n.s.	57	91.9	34	94.4	23	88.5	n.s.
Yes	9	10.2	4	8	5	13.2		5	8.1	2	5.6	3	11.5	
Anti-HER2														
No	77	87.5	43	86	34	89.5	n.s.	55	88.7	33	91.7	22	84.6	n.s.
Yes	11	12.5	7	14	4	10.5		7	11.3	3	8.3	4	15.4	
Endocrine														
No	50	56.8	25	50	25	65.8	n.s.	35	56.5	19	52.8	16	61.5	n.s.
Yes	38	43.2	25	50	13	34.2		27	43.5	17	47.2	10	38.5	
CDK4/6														
No	61	69.3	33	66	28	73.7	n.s.	40	64.5	23	63.9	17	65.4	n.s.
Yes	27	30.7	17	34	10	26.3		22	35.5	13	36.1	9	34.6	
Status at Diagnosis														
ER	60	68.2	36	72	24	63.2	n.s.	43	69.4	28	77.8	15	57.7	n.s.
HER2	9	10.2	7	14	2	5.3		6	9.7	4	11.1	2	7.7	
TNBC	17	19.3	6	12	11	28.9		12	19.4	4	11.1	8	30.8	
Missing	2	2.3	1	2	1	2.6		1	1.6	-	-	1	3.8	
Type Diagnosis														
Ductal	70	79.5	41	82	29	76.3	n.s.	49	79	29	80.6	20	76.9	n.s.
Lobular	7	8	1	2	6	15.8		5	8.1	1	2.8	4	15.4	
Mixed	5	5.7	4	8	1	2.6		5	8.1	4	11.1	1	3.8	
Unknown	6	6.8	4	8	2	5.2		3	4.8	2	5.6	1	3.8	
Mets visceral														
N	26	29.5	17	34	9	23.7	n.s.	18	29	9	25	9	34.6	n.s.
Y	62	70.5	33	66	29	76.3		44	71	27	75	17	65.4	
Mets bone														
N	31	35.2	15	30	16	42.1	n.s.	22	35.5	13	36.1	9	34.6	n.s.
Y	57	64.8	35	70	22	57.9		40	64.5	23	63.9	17	65.4	
Mets brain														
N	84	95.5	49	98	35	92.1	n.s.	59	95.2	35	97.2	24	92.3	n.s.
Y	4	4.5	1	2	3	7.9		3	4.8	1	2.8	2	7.7	
CA 15-3														
Abnormal (≥ 30)	52	59.1	27	54	25	65.8	n.s.	36	58.1	23	63.9	13	50	n.s.
Missing	15	17	9	18	6	15.8		12	19.4	7	19.4	5	19.2	
Normal (< 30)	21	23.9	14	28	7	18.4		14	22.6	6	16.7	8	30.8	

Continuous variables

		N	*Mean*	*S.D.*	*25th*	*Median*	*75th*		*N*	*Mean*	*S.D.*	*25th*	*Median*	*75th*
Age	*All*	87	59.9	10.7	54	60	68	*All*	61	59.5	10.5	52	60	68
	CTC Score							***CTC Score***						
	<= 3000	50	58.8	11.1	54	59	68	*<= 3000*	35	58.6	12.1	49	59	70
	>3000	37	61.2	10.1	56	62	68	*>3000*	26	60.6	8	54	60	67
Number prior endocrine therapies	*All*	85	1.1	1.2	0	1	2	*All*	61	1	1.1	0	1	2
	CTC Score							***CTC Score***						
	<= 3000	47	1.1	1.1	0	1	2	*<= 3000*	35	1.2	1.2	0	1	2
	>3000	38	1.1	1.3	0	1	2	*>3000*	26	0.8	0.9	0	1	1
Number prior therapies	*All*	85	2.4	2	1	2	4	*All*	61	2.4	1.8	1	2	4
	CTC Score							***CTC Score***						
	<= 3000	47	1.9	1.7	0	2	3	*<= 3000*	35	2.3	1.7	1	2	3
	>3000	38	2.9	2.3	1	2.5	4	*>3000*	26	2.5	1.9	1	2	4

Supplementary Table S3. Significant baseline predictors of overall survival in multivariate Cox proportional hazards model.

Variable	Comparison	Hazard ratio	95% CI	Wald p-value
CTC score at baseline	≤ 3000 vs. > 3000	0.37	0.06 to 0.87	0.02
CDK 4/6	No vs. Yes	4.38	1.35 to 14.2	0.01
ESR1 mutation	No vs. Yes	0.32	0.11 to 0.94	0.04

Supplementary Table S4. Therapies administered to HR+ TRACK patients initiating a new line of endocrine treatment at enrollment.

Therapy	**Number of patients**
AI	1
AI + CDK4/6	17
AI + other combination	2
SERD	5
SERD + CDK4/6	7
SERD + other combination	2
Other	2

Supplementary Table S5. *ESR1* mutations detected by tissue biopsy-based genotyping (SNapShot assay) and RNA-based CTC-ddPCR analysis in women with HR+ breast cancer (receiving endocrine treatment) and in TNBC patients (negative control).

Sample ID	Genotyping site	ESR1 mutation by genotyping	ESR1 mutation by CTC-ddPCR
HR+ patients receiving endocrine therapy			
TRACK03	Metastatic	none	none
TRACK04	Primary	NA	none
TRACK05	Primary	none	none
TRACK06	NA	NA	none
TRACK12	Primary	none	none
TRACK15	Metastatic	**L536Q***	none
TRACK16	NA	NA	none
TRACK19	NA	NA	none
TRACK22	Metastatic	none	**Y537N, D538G**
TRACK23	NA	NA	none
TRACK24	Primary	none	none
TRACK26	Primary	none	none
TRACK33	Metastatic	none	none
TRACK35	Primary	none	**D538G**
TRACK39	NA	NA	none
TRACK42	NA	NA	none
TRACK46	Metastatic	none	none
TRACK47	NA	NA	none
TRACK49	Metastatic	none	none
TRACK51	NA	NA	none
TRACK52	Primary	none	**Y537C**
TRACK64	Metastatic	none	none
TRACK65	Metastatic	none	none
TRACK66	NA	NA	none
TRACK68	NA	NA	none
TRACK70	Metastatic	none	none
TRACK71	Primary	none	none
TRACK73	Metastatic	none	none
TRACK75	Primary	none	none
TRACK76	Primary	none	**D538G**
TRACK84	Metastatic	none	none
TRACK85	NA	NA	none
TRACK86	Primary	none	none
TRACK91	Primary	none	none
TRACK95	Metastatic	**D538G**	none
TRACK101	Metastatic	none	**D538G**
TNBC patients			
TRACK01	Metastatic	none	none
TRACK07	NA	NA	none
TRACK09	Metastatic	none	none
TRACK17	Metastatic	none	none
TRACK20	Primary	none	none
TRACK25	Metastatic	none	none
TRACK32	Metastatic	none	none
TRACK36	Metastatic	NA	none
TRACK38	Metastatic	NA	none
TRACK41	Primary	none	none
TRACK45	Metastatic	none	none
TRACK50	Metastatic	none	none

** Mutation not part of CTC-ddPCR ESR1 mutation panel*

Supplementary Table S6. Patients with both ESR1 mutation and high RS score show faster progression on therapy compared with patients with low RS score or ESR1 mutation alone. Time to progression in HR+ patients with high/low RS score and with/without ESR1 mutations receiving endocrine therapy is shown. Number of patients in each category as well as median TTP are provided.

RS Score	**high**	**high**	**low**	**low**
ESR1 status	**mut**	**WT**	**mut**	**WT**
Number (total)	3	10	2	10
# progressing patients during study (percent)	3 (100%)	7 (70%)	1 (50%)	4 (40%)
Median TTP (days) of progressing patients (range)	56 (24-217)	58 (28-112)	139 (NA)	251.5 (42 - 291)

Supplementary Table S7. Taqman probes used in the breast ddPCR assay.

Gene	IDT Assay ID	Fluorophore
AGR2	Hs.PT.58.38683802	HEX
AGR2	HS.PT.58.20615543	FAM
CXCL13	Hs.PT.58.45801487	FAM
CXCL14	Hs.PT.58.19273291	FAM
EFHD1	Hs.PT..58.27534728	FAM
FAT1	Hs.PT.58.45775110	HEX
FAT1	HS.PT.58.14859907	FAM
FAT2	Hs.PT.58.24846942	HEX
FAT2	HS.PT.58.3832648	FAM
MGP	HS.PT.58.635768	FAM
MUC16	Hs.PT.58.3543722	FAM
PGR	Hs.PT.58.1566542	HEX
PGR	Hs.Pt.58.50458902	FAM
PIP	Hs.PT.58.19165954	FAM
PIP	Hs.PT.58.39868280	HEX
PRAME	Hs.PT.58.45281469	HEX
SCGB2A1	Hs.PT.58.864035	HEX
SCGB2A1	HS.PT.58.25526882	FAM
SERPINA3	Hs.PT.58.15580605	FAM
SFRP1	Hs.PT.58.38429156	FAM
SFRP2	Hs.PT.58.20705989	FAM
TMPRSS4	Hs.PT.58.3161735	FAM
WFDC2	Hs.PT.58.25117187	FAM