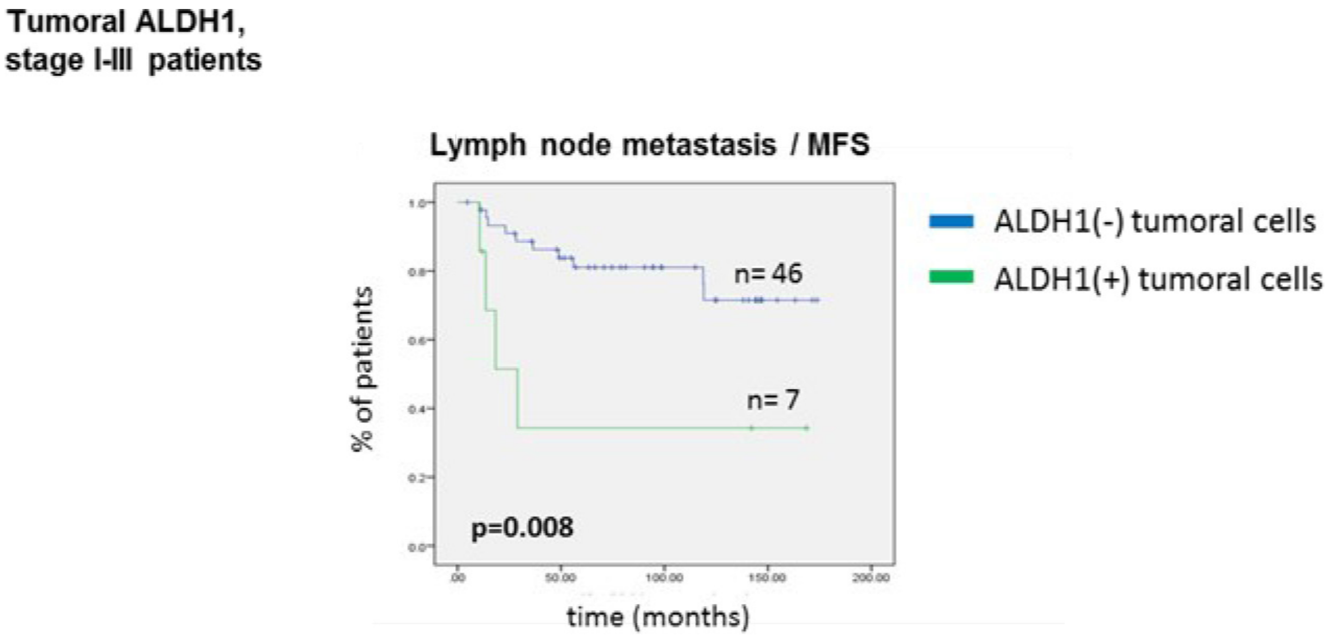
SUPPLEMENTARY INFORMATION

IMMUNOHISTOCHEMICAL DETECTION OF ALDH1/HLA-DR DOUBLE STAINING

Deparaffinized TMA sections were treated for 5 min. in citrate buffer (pH 6.0, Biogenex, USA) at 120°C in steamer and incubated overnight at 4°C with rabbit monoclonal anti-HLA-DR antibody (EPR3692, Abcam) and mouse monoclonal anti-ALDH1 antibody (44/ALDH1, BD Biosciences, US) diluted 1:800 and 1:500, respectively, in Dako REALTM Antibody Diluent (Dako, Denmark). Primary antibodies were detected and envisioned with the usage of secondary anti-mouse antibody labelled with horseradish peroxidase diluted 1:100 followed by 10 min. incubation with DAB substrate diluted 1:50 (Dako, Denmark) and anti-rabbit antibody labelled with alkaline phosphatase diluted 1:40 followed by 20 min. incubation with new fuchsine. The specimens were counterstained with hematoxylin (Merck, Germany).

SUPPLEMENTARY FIGURE AND TABLES



Supplementary Figure S1: Impact of ALDH1 expression in tumor cells of lymph node metastasis on survival of stage I-III breast cancer patients.

Supplementary Table S1. Comparison of stromal and tumoral ALDH1 expression to clinico-pathological data

Clinical, pathological and molecular parameters		ALDH1 stromal				ALDH1 tumoral			
		Neg		Pos		Neg		Pos	
		n	%	*n*	%	*n*	%	*n*	%
Age	**<median**	79	47.3	96	54.9	143	51.3	32	50.8
(years)	**> = median**	88	52.7	79	45.1	136	48.7	31	49.2
	***p*-value**	0.163				0.947			
T status	**T1**	73	43.7	84	48.3	133	47.7	24	38.7
	T2	76	45.5	75	43.1	119	42.7	32	51.6
	T3	8	4.8	9	5.2	14	5.0	3	4.8
	T4	10	6.0	6	3.4	13	4.7	3	4.8
	***p*-value**	0.638				0.613			
N status	**N0**	96	57.8	100	58.1	166	60.4	30	47.6
	N1-3	70	42.2	72	41.9	109	39.6	33	52.4
	***p*-value**	0.954				**0.065**			
M status	**M0**	198	97.5	218	99.5	340	98.3	76	100.0
	M1	5	2.5	1	0.5	6	1.7	0	0.0
	***p*-value**	0.110 (F)				0.597 (F)			
TNM	**IA**	52	31.7	65	38.0	98	35.9	19	30.6
staging	**IIA**	53	32.3	49	28.7	87	31.9	15	24.2
	IIB	27	16.5	31	18.1	43	15.8	15	24.2
	IIIA	14	8.5	16	9.4	23	8.4	7	11.3
	IIIB	10	6.1	5	2.9	12	4.4	3	4.8
	IIIC	3	1.8	4	2.3	4	1.5	3	4.8
	IV	5	3.0	1	0.6	6	2.2	0	0.0
	***p*-value**	0.386				0.234			
Grade	**G1**	17	10.3	30	17.5	36	13.2	11	17.5
	G2	92	55.8	82	48.0	143	52.4	31	49.2
	G3	56	33.9	59	34.5	94	34.4	21	33.3
	***p*-value**	0.126				0.675			
Vascular	**no**	86	71.7	92	78.0	167	81.1	11	34.4
invasion	**yes**	34	28.3	26	22.0	39	18.9	21	65.6
	***p*-value**	0.263				**<0.001**			
Recurrence	**no**	113	71.5	140	80.9	203	75.5	50	80.6

(*Continued*)

Clinical, pathological and molecular parameters		**ALDH1 stromal**				**ALDH1 tumoral**			
		Neg		**Pos**		**Neg**		**Pos**	
		n	**%**	***n***	**%**	***n***	**%**	***n***	**%**
	yes	45	28.5	33	19.1	66	24.5	12	19.4
	***p*-value**	**0.044**				0.386			
Death	**no**	132	81.0	156	89.1	231	84.0	57	90.5
	yes	31	19.0	19	10.9	44	16.0	6	9.5
	***p*-value**	**0.035**				0.239 (F)			
Her2	**neg**	141	92.8	150	95.5	241	94.9	50	90.9
	pos	11	7.2	7	4.5	13	5.1	9	9.1
	***p*-value**	0.297				0.254			
HR	**neg**	38	22.9	35	20.2	59	21.2	14	23.0
	pos	128	77.1	138	79.8	219	78.8	47	77.0
	***p*-value**	0.551				0.766			
Molecular subtype	**Luminal A**	42	31.6	44	31.2	77	33.8	9	19.6
	Luminal B	56	42.1	63	44.7	96	42.1	23	50.0
	Her2-pos	3	2.3	2	1.4	2	0.9	3	6.5
	Basal-like	32	24.1	32	22.7	53	23.2	11	23.9
	***p*-value**	0.935				**0.021**			

F indicates Fisher exact test, otherwise Chi square test.

Supplementary Table S2. Clinico-pathological characteristics of breast cancer patients included in the study

Clinical, pathological and molecular parameters	Status	*n*	%
Age (years)	median=58	58	
	range	27-86	
	<median	284	49.6
	>=median	289	50.4
	total	573	
T status	T1	276	48.3
	T2	241	42.2
	T3	30	5.3
	T4	24	4.2
	total	571	
N status	N0	332	58.3
	N1-3	237	41.7
	total	569	
M status	M0	566	97.6
	M1	14	2.4
	total	580	
TNM staging	IA	200	35.8
	IIA	171	30.6
	IIB	92	16.5
	IIIA	47	8.4
	IIIB	21	3.8
	IIIC	13	2.3
	IV	14	2.5
	total	558	
Grade	well differentiated (G1)	78	13.9
	moderately differentiated (G2)	298	52.9
	poorly differentiated (G3)	187	33.2
	total	563	
Vascular invasion	No	360	82.6
	Yes	76	17.4
	total	436	
Recurrence	No	466	82.5
	Yes	99	17.5
	total	565	

(*Continued*)

Clinical, pathological and molecular parameters	Status	*n*	%
Death	No	496	88
	Yes	68	12
	total	564	
Her2	negative	483	94.3
	positive	29	5.7
	total	512	
HR	negative	117	20.5
	positive	454	79.5
	total	571	
Molecular subtype	Luminal A	158	34.6
	Luminal B	194	42.5
	Her2-postive	6	1.3
	Basal-like	98	21.5
	total	456	

Note that due to the missing values not all numbers sum up to 589 cases.
HR indicates hormone receptor

Supplementary Table S3. Clinico-pathological and treatment characteristics of breast cancer patients included in Hamburg Cohort

	total	***n***	**%**
DTC status	**neg**	297	74.3
in bone marrow	**pos**	103	25.8
	total	400	
Metastatic relapse	**no**	320	85.6
	yes	54	14.4
	total	374	
Chemotherapy	**no**	150	37.8
	yes	247	62.2
	total	397	
Radiotherapy	**no**	81	20.3
	yes	319	79.8
	total	400	
Endocrine therapy	**no**	99	24.9
	yes	298	75.1
	total	397	

Note that due to the missing values not all numbers sum up to 411 cases.

Supplementary Table S4. Molecular and clinico-pathological characteristics of breast cancer patients included in Polish Cohort

	total	*n*	%
Ki-67	**neg**	96	58.9
	pos	67	41.1
	total	163	
	missing		
CK5/6	**neg**	139	91.4
	pos	13	8.6
	total	152	
	missing		
E-cadherin	**neg**	45	30.8
	pos	101	69.2
	total	146	
	missing		
Vimentin	**neg**	143	89.4
	pos	17	10.6
	total	160	
	missing		
EMT	**E-cad(+)Vim(−)**	90	62.5
	E-cad(−)Vim(−)	38	26.4
	E-cad(+)Vim(+)	10	6.9
	E-cad(−)Vim(+)	6	4.2
	total	144	
	missing		

Note that due to the missing values not all numbers sum up to 178 cases.
EMT indicates epithelial-mesenchymal transition, E-cad – E-cadherin, vim - vimentin

Supplementary Table S5. Different cut-offs tested in the current study

Cut-off for ALDH1 in tumor cells based on	**neg vs. pos**
intensity	no vs. weak vs. moderate vs. strong
intensity	no, weak vs. moderate, strong
intensity	no, weak, moderate vs. strong
intensity	no vs. weak, moderate, strong
% of positive cells	≥1%
% of positive cells	≥5%
index score (maximal result for two tumor samples)	**<mean index score vs. > mean index score**
index score (maximal result for two tumor samples)	<median index score vs. > median index score
index score (sum for two tumor samples)	<mean index score vs. > mean index score
index score (sum for two tumor samples)	<median index score vs. > median index score
Cut-off for ALDH1 in stromal cells based on	**neg vs. pos**
index score	no expression vs. expression in <10% of stromal cells vs. expression in 10-50% of stromal cells vs. expression in >50% of stromal cells
index score	no expression vs. expression in >1% of stromal cells
index score	**expression in <10% of stromal cells vs. expression in >10% of stromal cells**
index score	expression in <50% of stromal cells vs. expression in >50% of stromal cells