CDH1 mutations predict resistance to neoadjuvant taxane therapy

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Background

Despite recent advances in personalized medicine, conventional chemotherapy remains a backbone in breast cancer therapy and resistance to chemotherapy is still a main cause of treatment failure. Thus, identifying markers predicting sensitivity or resistance to individual chemotherapeutics is of great importance. Such biomarkers may be used for selection of optimal treatment strategies, improving the chances of favourable responses and curation as well as allowing omission of treatment that is unlikely to be effective and would result in unnecessary side effects to the individual patient.

Exploring molecular resistance mechanisms in neoadjuvant trials is an attractive approach since the response evaluation is performed within the trial time frame and one is not required to wait for long term follow-up to evaluate the efficacy of treatment.

Patients and Methods

I. In the EpiTax neoadjuvant trial, enrolling patients between 1997-2003, patients with primary breast cancers (T2>4cm, T3/T4 and/or N2/N3) were randomized to epirubicin 90mg/m^2/3W or paclitaxel 200mg/m^2/3W monotherapy, with cross-over in case of inferior response. Pre-treatment snap-frozen tumor biopsies from 223 patients were analyzed by targeted NGS of a 360 gene panel. The endpoint for the comparison was clinical response to the first regimen, since pCR was rare due to the large tumor sizes at inclusion.

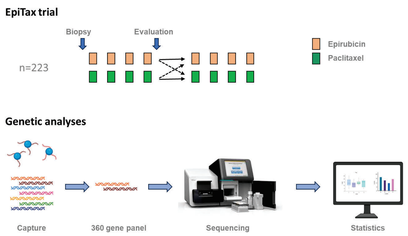


Figure 1. The EpiTax trial.
Top: Trial design. Pretreatment biopsies were collected for genetic analyses and clinical response was evaluated after monotherapy with either epirubicin or Paclitaxel according the RECIST-criteria (1,2).
Bottom: Genetic analyses were performed by targeted capture and sequencing of a panel of 360 cancer related genes. Mutation-distribution was compared across response groups.

II. For validation purposes we performed targeted sequencing of tumor samples from a total of 478 patients included in the Gepar Trio (n=132), Quattro (n=171) and Quinto (n=175) trials, in which patients with >2cm tumors received neoadjuvant anthracycline / taxane combination regimens. Here, the primary endpoint was clinical response to combined treatment, but since these tumors were smaller than in the EpiTax-trial, pCR was included as a secondary endpoint.

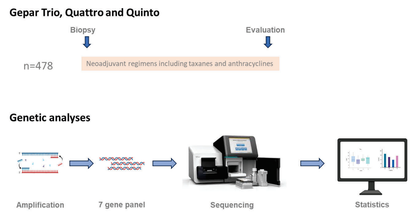


Figure 2. Validation cohort from the Gepar Trio (G3), Gepar Quattro (G4) and Gepar Quinto (G5) trials.
Top: General trial outline. Pretreatment biopsies were collected for genetic analyses and clinical response and pathological response were evaluated after treatment with anthracycline and Taxane containing regimens (3,4,5).
Bottom: Genetic analyses were performed by targeted amplicon based library generation and sequenicng of a 7 gene panel (*CDH1, GATA3, TP53, PIK3CA, TBX3, BRCA1 and ERBB2*). Mutation-distribution was compared across response groups.

III. Experimental validations were performed by *CDH1* knock-down and CRISPR/Cas9 knock-out in cell line models.

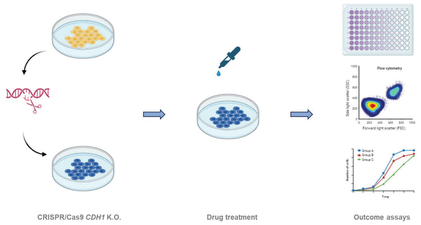


Figure 3. *In vitro* validation experiments.
A *CDH1* knock-out MCF7 cell line model was generated by CRISPR/Cas9 mediated editing. The model was used for comparison with the original, unmodified MCF7 cells.
Cells were subject to treatment with anthracyclins and taxanes and monitored for response.
Response was measured as growth/death rates, apoptosis, metabolic activity, cell cycle distribution and microtubule stabilization.

Results

I. In samples from the EpiTax-trial, *CDH1* mutations predicted an inferior response (trend across response groups; cPD, cSD, cPR and cCR) in the paclitaxel arm (p=0.01) as well as the epirubicin arm (p=0.04). The predictive value was observed within the subgroup of ER-positive cases (both for paclitaxel (p=0.005) and epirubicin (p=0.003)) but not among ER-negative tumors. The majority of *CDH1* mutations (24/34=71%) were observed in lobular cancers. While lobular histology predicted resistance to paclitaxel (but not epirubicin), *CDH1* mutations predicted resistance also within the subgroup of lobular cases (p=0.002), demonstrating *CDH1* mutations to be an independent predictor and not only a co-variate to lobular histology. Assessing functionally linked genes, mutations in *GATA3*, a transcriptional regulator of *CDH1*, were predominantly observed in ductal cancers, and were not predictive of resistance to any compound. Yet, combining *GATA3* and *CDH1* mutations into a composite biomarker predicted resistance to both paclitaxel (p=0.007) and epirubicin (p=0.01), especially in ER-positive cases (p=0.002 and p=0.0004, respectively). While epithelial to mesenchymal transition (EMT)-signatures had predictive value, this effect was largely dominated by *CDH1*, while other EMT-related genes had limited impact on response.

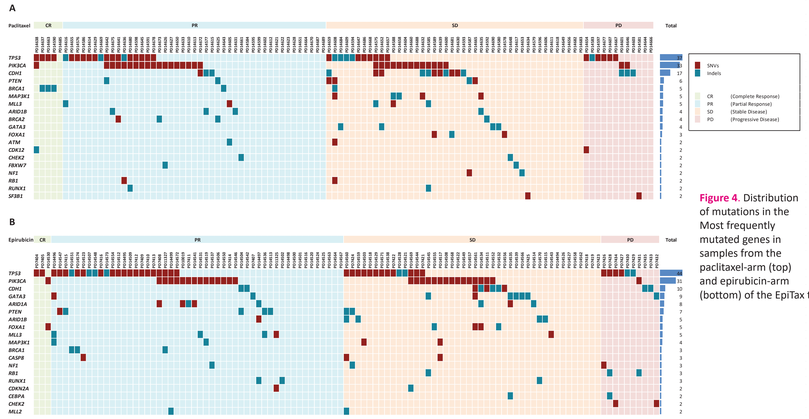


Figure 4. Distribution of mutations in the Most frequently mutated genes in samples from the paclitaxel-arm (top) and epirubicin-arm (bottom) of the EpiTax trial

All cases

Paclitaxel	CR	PR	SD	PD	total	p trend
total	5	45	44	12	106	
CDH1 driver mut	0	3	11	3	17	0.01
GATA3 driver mut	0	0	4	0	4	0.27
CDH1/GATA3 driver mut	0	3	14	3	20	0.007

Epirubicin	CR	PR	SD	PD	total	p trend
total	3	50	44	10	107	
CDH1 driver mut	0	2	6	2	10	0.04
GATA3 driver mut	0	2	6	1	9	0.15
CDH1/GATA3 driver mut	0	4	11	3	18	0.01

Paclitaxel + Epirubicin	CR	PR	SD	PD	total	p trend
total	8	95	88	22	213	
CDH1 driver mut	0	5	17	5	27	0.001
GATA3 driver mut	0	2	10	1	13	0.08
CDH1/GATA3 driver mut	0	7	25	6	38	0.0002

ER+ tumours

Paclitaxel	CR	PR	SD	PD	total	p trend
total	1	29	28	5	63	
CDH1 driver mut	0	3	9	3	15	0.005
GATA3 driver mut	0	0	3	0	3	0.27
CDH1/GATA3 driver mut	0	3	12	3	18	0.002

Epirubicin	CR	PR	SD	PD	total	p trend
total	1	28	26	3	58	
CDH1 driver mut	0	1	5	2	8	0.003
GATA3 driver mut	0	2	6	1	9	0.06
CDH1/GATA3 driver mut	0	3	10	3	16	0.0004

Paclitaxel + Epirubicin	CR	PR	SD	PD	total	p trend
total	2	57	54	8	121	
CDH1 driver mut	0	4	14	5	23	6.3e-05
GATA3 driver mut	0	2	9	1	12	0.04
CDH1/GATA3 driver mut	0	6	22	6	34	2.7e-06

Lobular tumours

Paclitaxel	CR	PR	SD	PD	total	p trend
total	0	2	13	3	18	
CDH1 driver mut	0	2	10	3	15	0.84

Epirubicin	CR	PR	SD	PD	total	p trend
total	0	9	8	2	19	
CDH1 mut	0	1	5	2	8	0.006

Paclitaxel + Epirubicin	CR	PR	SD	PD	total	p trend
total	0	11	21	5	37	
CDH1 mut	0	3	15	5	23	0.002

Table 1. Associations between mutation status and response to neoadjuvant treatment for tumours in the EpiTax trial

II. In an independent validation cohort from the Gepar trials, selected with enrichment for lobular cancers (34%), *CDH1* mutations were not significantly associated with clinical resistance to therapy (p=0.19) although they predicted lack of pCR (p=0.01). Combining *GATA3* and *CDH1* mutations predicted lack of clinical response (p=0.05) and lack of pCR (p=0.0007) respectively in this cohort.

Study	Clinical response				missing	Total
	CR	PR	SD	PD		
G3	44 (33.3)	65 (49.2)	22 (16.7)	1 (0.8)	13	132
G4	36 (21.1)	105 (61.4)	19 (11.1)	11 (6.4)	4	171
G5	32 (18.3)	116 (66.3)	19 (10.9)	8 (4.6)	5	175
	112	286	60	20	22	478
				SD+PD		

Table 2. Clinical response for tumours from the G3,G4 and G5 trials. Due to low number of tumours with PD, the SD and PD groups were merged for statistical analyses.

All cases

G3+4+5	CR	PR	SD+PD	total	p trend
total	112	286	80	478	
CDH1 driver mut	14	63	15	92	0.19
CDH1/GATA3 driver mut	18	78	22	118	0.05

G3+4+5	pCR	non-PCR	total	p trend
total	103	397	500	
CDH1 driver mut	10	84	94	0.01
CDH1/GATA3 driver mut	11	109	120	0.0007

ER+ tumours

G3+4+5	CR	PR	SD+PD	total	p trend
total	66	211	51	328	
CDH1 driver mut	11	59	14	84	0.15
CDH1/GATA3 driver mut	15	73	21	109	0.03

G3+4+5	pCR	non-PCR	total	p trend
total	298	45	343	
CDH1 driver mut	6	79	85	0.06
CDH1/GATA3 driver mut	7	103	110	0.01

Table 3. Associations between mutation status and response to neoadjuvant treatment for tumours in the G3, G4 and G5 trials.

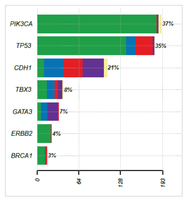


Figure 5. Distribution of mutations in the 7 genes analysed in samples from the G3, G4 and G5 trials.

III. In *in vitro* analyses, resistance to paclitaxel was observed in three different breast cancer cell lines upon siRNA mediated knock-down of *CDH1*, as well as in a CRISPR/Cas9 mediated *CDH1* knock-out model, as measured by growth rate, induction of apoptosis, G2 arrest, mitochondrial respiration and tubulin stability. For anthracyclines, similar effects were observed for mitochondrial respiration.

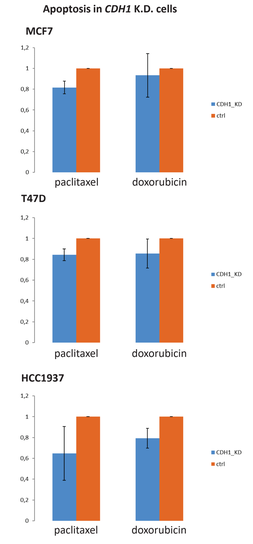


Figure 6. Induction of apoptosis in breast cancer cells after *CDH1* knock-down. Three different breast cancer cell lines (MCF7; top, T47D; middle and HCC1937 bottom) were subjected to siRNA-mediated knockdown of *CDH1* mRNA. Cells were treated with paclitaxel or doxorubicin and assessed for apoptosis via Annexin V staining and flow-cytometry analyses. Bars show the ratio of apoptotic cells over non-apoptotic cells. Blue bars illustrate data for *CDH1* knock-down cells and are normalised to the corresponding data for control cells (transfected with unspecific siRNA), where the latter was set to 1.0

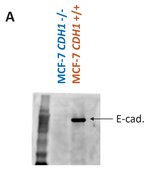


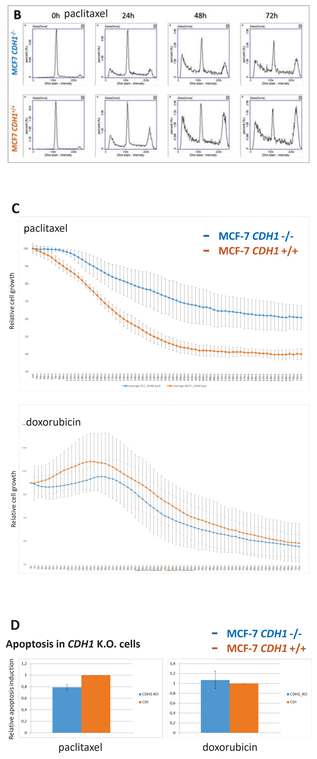
Figure 7. Response to taxane or anthracyclin treatment in MCF7 cells after *CDH1* knock out.

A. Western blot showing lack of E-cadherin expression in MCF7 cells subject to CRISPR/Cas9-mediated knock-out of *CDH1*.

B. Cell cycle phase distribution in MCF7 *CDH1-/-* cells versus control cells during treatment with paclitaxel. Analyses were performed on a Nucleocounter 3000 instrument.

C. Death curves for MCF7 *CDH1-/-* cells versus control cells during treatment with either paclitaxel or doxorubicin. Amounts of living cells were monitored by electric cell-substrate impedance sensing (ECIS). Data for each well was normalised to the starting point at time zero (set to 100%).

D. Cells were treated with paclitaxel or doxorubicin and assessed for apoptosis via Annexin V staining and flow-cytometry analyses. Bars show the ratio of apoptotic cells over non-apoptotic cells. Blue bars illustrate data for *CDH1* knock-out cells and are normalised to the corresponding data for unmodified (*CDH1* wt) MCF7 cells, where the latter was set to 1.0. Analyses were performed on a Nucleocounter 3000 instrument.



Conclusion

In conclusion, mutations in *CDH1* predicted clinical resistance to neoadjuvant paclitaxel and epirubicin monotherapy. The findings were largely validated in an independent sample set where tumours have been treated with taxane- and anthracycline- containing regimens. *In vitro* tests validated resistance to paclitaxel in cells with knock-out of *CDH1*, while the effect of anthracycline treatment seemed similar in *CDH1* knock-out cells as in control cells.

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