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Exploring the clonal evolution of CD133/ALDH1-positive cancer stem-like cells from primary to recurrent high-grade serous ovarian cancer (HGSOC). A study of the OCTIPS Consortium.

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ABSTRACT

Background: High-grade serous ovarian cancer (HGSOC) causes 80% of all OC deaths. In this setting, the role of cancer stem-like cells (CSCs) is still unclear. In particular, the evolution of CSC biomarkers from primary (pOC) to recurrent (rOC) HGSOCs is unknown. Aim of this study was to investigate changes in CD133 and aldehyde dehydrogenase-1(ALDH1) CSC biomarker expression in pOC and rOC HGSOCs.

Methods:224 pOC and rOC intra-patient paired tissue samples derived from 112 HGSOC patients(pts) were evaluated for CD133 and ALDH1 expression using IHC. pOCs and rOCs were compared for CD133 and/or ALDH1 levels. Expression profiles were also correlated with patients´clinico-pathological and survival data.

Results:49.1%(55/112) and 37.5%(42/112) pOCs were CD133+ and ALDH1+, respectively. CD133+ and ALDH1+ samples were detected in 33.9%(38/112) and 36.6%(41/112) rOCs. CD133/ALDH1 coexpression was observed in 23.2%(26/112) and 15.2%(17/112) of pOCs and rOCs, respectively. Pairwise analysis showed a significant shift of CD133 staining from higher (pOCs) to lower expression levels (rOCs)($p<0.0001$). Furthermore, all CD133+pOC pts were FIGO-stage III/IV ($p<0.0001$) and had significantly worse PFI($p=0.04$) and OS($p=0.02$). On multivariate analysis, CD133/ALDH1 coexpression in pOCs was identified as independent prognostic factor for PFI (HR:1.64;95%CI:1.03-2.60;$p=0.036$) and OS (HR:1.71;95%CI:1.01-2.88;$p=0.045$). Analysis on 52 pts with known somatic BRCA status revealed that BRCA mutations did not influence CSC biomarker expression.

Conclusions: The study showed that CD133/ALDH1 expression impacts HGSOC pts' survival and firstly suggests that CSCs might undergo phenotypic change during the

disease course similarly to non stem-like cancer cells, providing also a first evidence that there is no correlation between CSCs and BRCA status.

Key Words: Ovarian Cancer; CD133; ALDH1; Aldehyde dehydrogenase-1; cancer stem-like cell; BRCA; prognosis; survival.

INTRODUCTION

Ovarian cancer (OC) remains the most lethal gynecologic malignancy[**1**]. Advances in cancer genomics, epigenomics and proteomics has led to the understanding that OC is a heterogeneous group of different tumors displaying distinct phenotypes and etiology[**2,3**]. The current dichotomous OC classification[**4,5**]groups these tumors in two distinct categories: Type I (low-grade serous-papillary, low-grade endometrioid, mucinous and clear-cell carcinomas) and Type II (high-grade serous-papillary, high-grade endometroid, carcinosarcomas and undifferentiated tumors). Type II OCs show a more aggressive biological behavior, are diagnosed at advanced stage and are chromosomally highly unstable. Among them, high-grade serous OC (HGSOC) accounts for around 80% of all OC deaths[**3**]. The identification of predictive biomarkers is pivotal for designing new treatment strategies able to reduce HGSOC-related mortality. In this context, the cancer stem-like cell (CSC) theory represents one model to investigate OC heterogeneity. This hypothesis, supported by increased evidence acquired in the last decade, proposes that, within OC tissues, a small population of cells has an increased capacity for self-renewal, tumorigenesis and differentiation[**6**]. In multiple experimental studies CSCs showed to increase potential of tumorigenesis, metastasis/invasion, neoangiogenesis and chemoresistance[**7,8**]and have been often correlated with a poor prognosis[**9-13**].

Several potential CSC markers have been identified in OC samples[**14-15**]. Among them, aldehyde dehydrogenase-1 (ALDH1) and CD133 are currently the best characterized for ovarian CSCs. Their expression on the cell surface is associated with increased tumorigenesis and self-renewal capability [**16-18**]. Nevertheless, the clonal evolution of CSCs throughout the course of disease, from primary (pOC) to recurrent

112 (rOC) OC, has not been elucidated yet and information about the changes in CSC
113 presence within the tumor after relapse is still lacking.
114 The aim of this study was to investigate the evolution of CSC biomarkers CD133 and
115 ALDH1 expression in a large series of paired primary and recurrent HGSOCs.
116
117 **MATERIALS AND METHODS**
118 *Sample Collection*
119 224 paired samples from 112 HGSOC patients were collected during primary and
120 secondary tumor debulking. Patients were included consecutively and have been
121 treated between 1985 and 2013 through primary cytoreduction followed by platinum-
122 based chemotherapy. Patients, retrospectively selected from the OCTIPS (Ovarian
123 Cancer Therapy–Innovative Models Prolong Survival, Agreement No.279113-2)
124 Consortium database, were treated for both pOC and rOC in one of the European
125 Gynecologic Oncology Referral Centers of the following Institutions: Charité
126 Universitätsmedizin Berlin,Germany; Katholieke Universiteit Leuven,Belgium;
127 Imperial College, London,UK; University of Edinburgh,UK.
128 Inclusion criteria were: having experienced at least one OC relapse for which having
129 been subjected to at least one palliative surgery. Exclusion criterion was: no cancer
130 tissue available from both pOC and rOC. Approval from each local ethics committee
131 was obtained (EK207/2003,ML2524,05/Q0406/178,EK130113,06/S1101/16). OC
132 tissue samples were collected during primary cytoreduction and at the surgery for
133 relapse. All included samples underwent central histopathological assessment to
134 confirm the diagnosis of HGSOC and to evaluate the tissue quality and tumor contain.
135 *Immunohistochemistry*
136 Immunohistochemical staining was performed on tissue microarrays (TMAs).

137 Slides were deparaffinized in xylol, rehydrated in graded alcohol and boiled in a
138 pressure cooker for 5 minutes in citrate buffer (pH=6) for ALDH1 staining or in
139 EDTA (pH=9) for CD133 staining. Mouse anti-human ALDH1-antibody (clone
140 44;BD Transduction Laboratories,Franklin Lakes, NJ,USA) and mouse anti-human
141 CD133/1-antibody (AC133 clone;Miltenyi-Biotech, BergischGladbach,Germany)
142 were diluted 1:500 and incubated on the slides for 60 minutes at room temperature.
143 Bound antibodies were visualized using DAKO Real Detection System and DAB+
144 (3,3′ -diaminobenzidine;DAKO,Glostrup,Denmark) as a chromogen. Finally, the
145 slides were co-stained with hematoxylin.
146 CD133 stained samples were assessed basing on the number of stained tumor cells.
147 Samples were classified as "CD133-negative"(<10% CD133 positive tumor cells) and
148 "CD133-positive"(>10% CD133-positive tumor cells)[**19-20**].
149 For ALDH1 staining evaluation, as previously published[**21-22**], the number of
150 stained tumor cells (0%=0;1-10%=1;11-50%=2;>50%=3) was multiplied with the
151 intensity of staining(negative=0;weak=1;moderate=2;strong=3), resulting in a
152 semiquantitive immunoreactivity score(IRS) that ranged from 0 to 9. For further
153 analysis, samples were classified "ALDH1-negative", for absent or weak focal
154 staining(IRS=0-1), or "ALDH1-positive", for ALDH1-high tumor expression(IRS=2-
155 9).
156 All samples were evaluated independently by two co-authors (IR and SDE).
157 *Clinical Data and Follow-up*
158 Patients' clinical data and information on 52 patients' germline and/or somatic BRCA
159 status were retrieved from OCTIPS Consortium database[**23-24**]. Platinum-
160 resistance and platinum-sensitivity were defined, according to GCIG, as relapse
161 occurring before or after six months following the last platinum-based chemotherapy,

162 respectively[25]. Recurrence was defined basing on RECIST Criteria[26]. A sole
163 CA125 serum elevation was not considered relapse[27].

164 *Statistical Analysis*

165 Statistical analysis was performed using SPSS version 22.0(SPSS Inc, Chicago, IL,
166 USA). To assess the difference between pOCs and rOCs in terms of biomarker
167 expression, the correlation test (Spearman coefficient, 2-tailed) and the "Wilcoxon
168 signed rank" non-parametric test for related samples were applied. Correlation of
169 CD133 and ALDH1 tumor expression with patients' clinico-pathological categorical
170 data was assessed using the Fisher's exact test. Patients' progression-free
171 interval(PFI), progression-free survival (PFS) and overall survival(OS) were
172 determined by Kaplan–Meier analysis (Log-Rank test).PFI represented the time
173 interval from the last adjuvant chemotherapy to relapse, whereas progression-free
174 survival (PFS) was the time interval between first recurrence diagnosis and tumor
175 progression. For univariate and multivariate survival analyses, the Cox regression
176 model was used. Multivariable models were performed among variables reporting a
177 p-value≤0.1 in univariate analysis. P values≤0.05 were considered statistically
178 significant.

179

180 **RESULTS**

181 Primary and recurrent intra-patient paired tumor samples derived from 112 HGSOC
182 patients were analyzed for CD133 and ALDH1 expression. Patients' characteristics
183 are listed in **Table 1**.

184 Immunohistochemistry staining showed that ALDH1 and CD133 proteins were
185 localized to the cytoplasm(**Fig1,Fig.3**).

186

187

188 *CD133 expression.*

189 CD133-positive ($CD133^+$) staining was significantly more frequent among
190 pOCs[55/112(49.1%)] compared to rOCs[38/112(33.9%)], p=0.030(Fisher's exact
191 test,**Fig.1a,1c**). Investigation of sequential changes in CD133+ expression in paired
192 tumors, with a correlation test (Spearman coefficient) between pOCs and rOCs,
193 demonstrated a significant correlation (p=0.001,Spearman coefficient 0.306).
194 Furthermore, pairwise testing revealed a significant shift from higher frequency of
195 $CD133^+$ cells in pOCs to lower levels in the paired recurrent samples (p<0.0001,
196 Wilcoxon test;**Fig.2**), thus indicating significantly higher rates of $CD133^+$ cells in
197 pOCs compared to rOCs.

198 *ALDH1 expression.*

199 Distribution of ALDH1 IRS in pOCs and rOCs is shown in **Fig.3a,3d**. ALDH-1
200 positive tumors were found in 37.5%(42/112) and 36.6%(41/112) of primary and
201 recurrent samples, respectively (p=1,Fisher's exact test,**Fig.3b,3e**). A trend for
202 significant correlation between pOCs and rOCs ALDH1-expression levels was seen
203 (p=0.059,Spearman coefficient 0.179). Pairwise analysis showed no tendency towards
204 a change of IRS values to higher or lower levels in recurrences (p=0.988,Wilcoxon
205 test;**Fig.4**).

206 *CD133/ALDH1 co-expression.*

207 Co-expression of both CSCs biomarkers was detected in 23.2%(26/112) of pOCs and
208 in 15.2%(17/112) of rOCs(p=0.174,Fisher's exact test). Among 26 patients reporting
209 CD133/ALDH1 co-expression in pOCs, 22(84.6%) lost this pathological
210 characteristic in relapse situation. Of the 17 patients presenting biomarker co-
211 expression in rOC, 13(76.5%) showed no co-expression in pOC. Consequently, 4/112

212 patients (3.6%) showed CD133/ALDH1 co-expression in both pOC and rOC: two of
213 them were platinum-resistant and two were platinum-sensitive.

214 *CSCs biomarkers and clinico-pathological factors*

215 We analyzed the correlation of ALDH1 and/or CD133 tumor expression patterns in
216 pOCs with patients´ clinico-pathological characteristics. All primary $CD133^{+}$ patients
217 were diagnosed at FIGO III/IV stage (p=0.006). No correlation was observed between
218 other clinico-pathological factors and ALDH1 and/or CD133 tumor
219 expression(**Tab.2**).

220 *Survival*

221 CD133 positivity in pOCs was significantly associated with poor PFI and OS
222 (**Fig.5a,5b**). In particular, $CD133^{+}$ and $CD133^{-}$ patients reported median OS of 51 and
223 71 months (HR:1.713;95%CI:1.076-2.727;p=0.02) and median PFI of 9 and 17
224 months (HR:1.477;95%CI:1.006-2.170;p=0.04). PFS after recurrence was not
225 significantly different (p=0.868,**Fig.5c)** between patients with CD133+ and CD133-
226 or between (p=0.252,**Fig.5f**) patients with ALDH1+ and ALDH1rOC.

227 Median OS for $ALDH1^{+}$ and $ALDH1^{-}$ patients was 52 and 64 months, respectively
228 (p=0.402) and median PFI-1 was 9 and 17 months, respectively (p=0.199)(**Fig.5d,5e**).
229 ALDH1/CD133 co-expression in pOCs was found to significantly affect HGSOC
230 patients' outcome. A significant decrease in OS and PFI has been found in patients
231 co-expressing ALDH1/CD133 in primary tissue (46 and 9 months, respectively)
232 compared to patients without biomarker co-expression (68 and 17 months,
233 respectively) (p=0.019,**Fig.5g**;p=0.015,**Fig.5h**). No significant difference in PFS after
234 relapse was observed between patients who reported CD133/ALDH1 co-expression or
235 no co-expression in rOC(p=0.898,**Fig.5i**).

236 On multivariate analysis, the co-expression of ALDH1 and CD133 in pOC, rather
237 than the single expression of one biomarker, was identified to be an independent
238 prognostic factor for both PFI (HR:1.638;95%CI:1.033-2.598;p=0.036) and OS
239 (HR:1.707;95%CI:1.012-2.881;p=0.045) in HGSOC(**Tab.3,4**).

240 *Outliers' sub-analysis*

241 "Outliers" were considered patients for whom the highest difference between pOC
242 and rOC could be detected in CD133+cell rate. Three patients were identified: two
243 reported a difference in CD133+cell rate of -90%(from 90% of CD133+cells at pOC
244 to 0% at rOC); the first one was a platinum-resistant patient with PFI of 2 months and
245 OS of 14 months; the second one was a platinum-sensitive patient with PFI of 7
246 months and OS of 9 months. The third patient showed a difference in CD133+cell
247 rate of +70%(from 0% of CD133+ at pOC cells to 70% in rOC) with PFI of 15
248 months (platinum-sensitive) and OS of 44 months.

249 *CSC biomarker expression and BRCA status*

250 In order to investigate if BRCA mutations could influence CSC biomarker expression,
251 a subgroup analysis was carried out among 52 patients, whose germline and/or
252 somatic BRCA status (assessed on pOC and rOC) was available [**24**]. 40.4% of tested
253 patients (21/52) had a somatic BRCA mutation in both pOCs and rOCs: 16/52(30.8%)
254 were BRCA1-mutated (mBRCA1) and 5/52(9.6%) were BRCA2-mutated
255 (mBRCA2)(**Tab.5**).

256 No significant difference in CD133 and/or ALDH1 expression was found between
257 BRCA-wild type (BRCA-WT) and BRCA-mutant (mBRCA1/2) tumors(**Tab.6**).

258 Among BRCA-WT patients, no correlation between pOCs and rOCs in CD133+
259 expression was observed (p=0.088,Spearman coefficient 0.312). Furthermore, in
260 accordance with results observed in the whole population, paired testing revealed a

261 significant shift from higher levels in pOCs to lower levels in the rOCs
262 ($p<0.0001$,Wilcoxon test;**Fig.6a**). In contrast, among mBRCA1/2 patients, no
263 correlation between pOCs and rOCs ($p=0.493$,Spearman coefficient 0.158), or a
264 tendency towards a change in CD133+ expression was observed ($p=0.167$,Wilcoxon
265 test;**Fig.6b**).
266 Regarding ALDH1 expression, among BRCA-WT patients no correlation between
267 pOCs and rOCs in ADH1 IRS was found ($p=0.986$,Spearman coefficient 0.003), as
268 well as no change in paired testing ($p=0.895$,Wilcoxon test;**Fig.7a**); also for
269 mBRCA1/2 patients no difference was observed in ALDH1-IRS between primary and
270 recurrent patients ($p=0.410$,Spearman coefficient 0.190;$p=0.385$,Wilcoxon
271 test;**Fig.7b**).
272 Among BRCA-WT patients, only 1/31 patient (3.2%) showed CD133/ALDH1 co-
273 expression in both pOCs and rOCs. In 3/31(9.7%) patients the co-expression was
274 evidenced in rOCs but not in pOCs. 90% of patients (9/10) reporting CD133/ALDH1
275 co-expression in pOC lost biomarker co-expression at tumor relapse.
276 Also for mBRCA1/2 patients, only 1/21(4.8%) patient showed CD133/ALDH1 co-
277 expression in both pOC and rOC. Two patients (9.5%) had co-expression at recurrent
278 rather than at primary disease. The difference between BRCA-WT and mBRCA1/2
279 patients in terms of co-expression loss at rOC was not significant (4/5 vs 9/10,$p=1$,
280 Fisher's exact test).
281 Considering patients who were CD133+ and/or ALDH1+ at pOC, no significant
282 difference could be detected in PFI and OS among BRCA-WT vs mBRCA1/2
283 cases(**Fig.8**).
284
285 **DISCUSSION**

In the Era of Precision Medicine, huge steps have been taken in the understanding of HGSOC biology. In this tumor setting, the role of CSC and its clonal evolution during subsequent disease relapse has been relatively unexplored.

This study investigated the changes in CSC biomarkers CD133 and ALDH1 expression in primary and recurrent HGSOCs and showed that CD133+CSCs are significantly more represented in pOCs rather than rOCs, whereas no significant changes in terms of ALDH1 expression levels occurred at disease relapse. Furthermore, CD133 positivity in pOCs significantly correlates with poor survival, while co-expression of both CD133 and ALDH1 in primary samples independently predicted poor PFI and OS in HGSOC patients.

In 2015, Zhou published a meta-analysis[**28**], which investigated the prognostic value of immunohistochemical CD133 expression in OC. Pooled data derived from 1050 patients from 8 studies showed that CD133 positivity significantly correlates with advanced FIGO stage at diagnosis and with worse OS, in accordance with our findings, although our population was restricted to HGSOC.

Other recent meta-analysis demonstrated that also ALDH1 is a promising prognostic biomarker for breast[**9**], head/neck[**10**], lung[**11**]and colorectal cancer[**12**] but its predictive or prognostic role in OC is still controversial[**13,29-31**]. In contrast to CD133, ALDH1 expression is usually low or negative in serous OC compared to other cancer histotype and more frequent in low FIGO stage tumors[**13,29**].

Previously, Liebscher[**21**] investigated the prognostic impact of ALDH1 expression in a homogeneous group of primary HGSOC patients and demonstrated that ALDH1 was an independent prognostic factor for OS. These results differ from our findings, since in our population ALDH1 did not have an impact on patients' survival. Nevertheless, in Liebscher's population the frequency of FIGO Stage I-II cases was

311 higher than in our population (11.5% vs 7.2%), while the number of optimally
312 cytoreduced patients was lower (66.3% vs 80.4%).
313 Silva[**32**] showed that the co-expression of CD133 and ALDH1 correlated with
314 significant worse PFI and OS in a small cohort of 56 ovarian cancer patient**s**. These
315 results were in accordance with our findings in a larger HGSOC population.
316 To our knowledge, this is the first study analyzing the evolution of CSC markers in
317 the largest cohort of primary and recurrent HGSOC patients. Furthermore, the
318 subanalysis on patients with known BRCA status increases the value of the findings
319 by taking into consideration the genetic influence of BRCA status on patients'
320 survival[**33-34**]and provides a first evidence of the correlation between tumor-
321 initiating cells and homologous recombination deficieny. Limitation of the study was
322 the lack of information regarding BRCA1/2 status on all enrolled patients. The
323 analysis on a cohort of 52 patients could not provide definitive conclusions for this
324 issue.
325 Interestingly, we observed that 84.6% of our patients' cohort reporting
326 CD133/ALDH1 co-expression in pOC lost this pathological characteristic at relapse.
327 Nevertheless, while CSC biomarker expression is significantly correlated with poor
328 prognosis, it is enigmatic why in a recurrent setting, which represents a more
329 aggressive step of the disease compared to primary disease, CSCs are less frequently
330 encountered. Theoretically, CSCs were expected to be much more frequent in rOC
331 than in the pOC. We hypothesize that the reduction in CSC biomarker expression
332 does not represent a reduction in CSC number within the tumor sample, but might be
333 the result of cellular reprogramming occurring in the CSC itself, which might lead to
334 the loss of CSC biomarker expression. Studies on this issue are still lacking.

This study shows that CD133 and ALDH1 as biomarkers can have influence on HGSOC patients' survival and for the first time suggests that they might be caused by a phenotypical change during the course of the disease similarly to non stem-like cancer cells. However, the need for recurrent tumor tissue to be analyzed implied that this cohort of samples might be not the most representative one for ovarian cancer patients, due to the fact that most of patients had a platinum sensitive relapse, and surgical approach at relapse was feasible. For this reason, general conclusion for the whole recurrent ovarian cancer setting cannot currently be drawn.

Another limitation of the study is that these biomarkers, in particular ALDH1, are broadly expressed, not only by CSCs. The identification of CSC is actually sure only based on the capacity to build spheroids, on tumor xenograft assay and on serial transplantation assay, which require fresh tumor tissue**.** Nevertheless, IHC allowed to analyze a large cohort of paired tumor tissues and to observe that there is a change in CSC–associated biomarker expression between primary and relapse disease.

Further investigations on larger cohort of paired pOC and rOC samples are warranted, potentially expanding the scope with inclusion of further candidate CSC markers and with evaluation of CSCs behavior following neoadjuvant chemotherapy[**31,35-36**], in order to reduce mortality of one of the most deadly malignancies of our time.

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Conflict of interest statement.

All Authors declare no conflict of interest.

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516 **LEGEND TO TABLES AND FIGURES**

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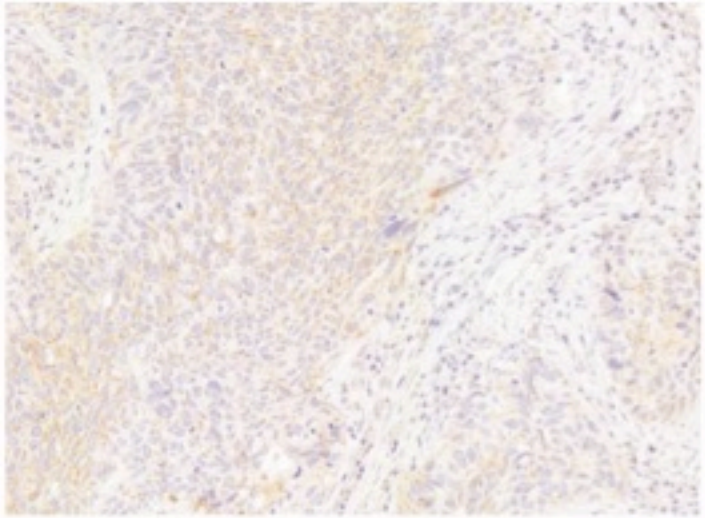
Figure 1

a



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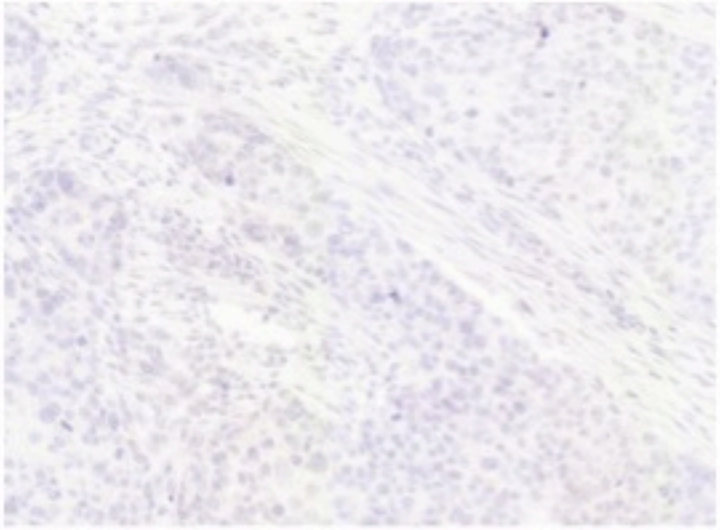

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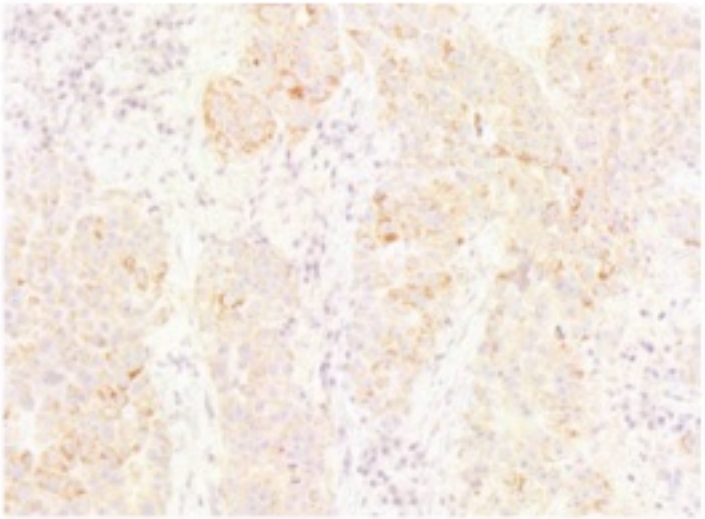


d

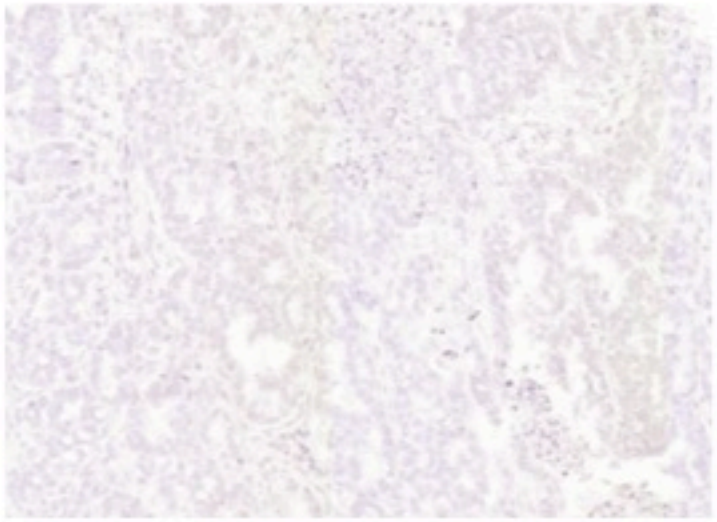
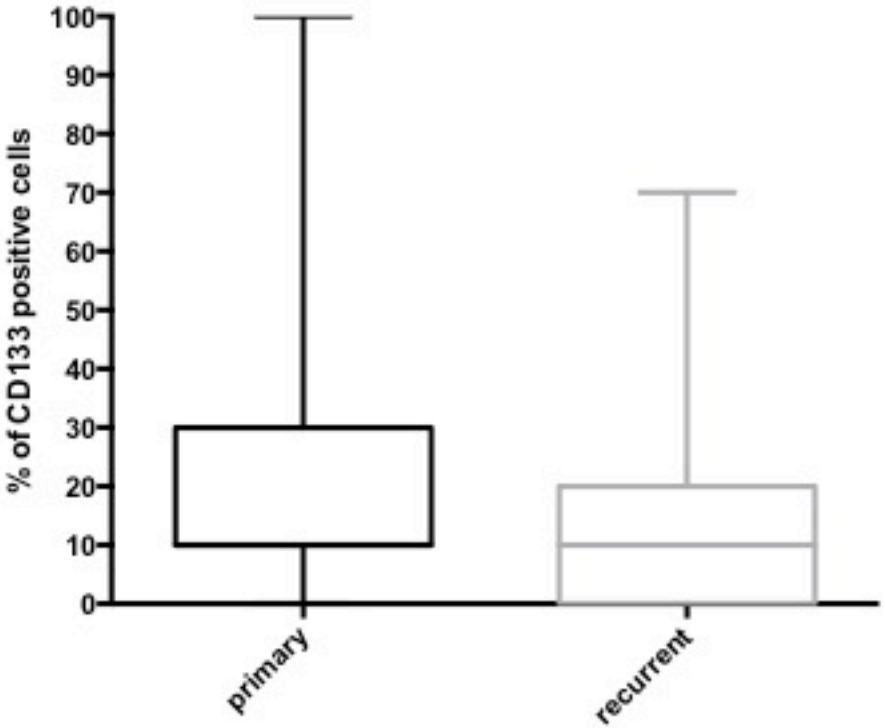


Figure 2

a


% of CD133 positive cells
100
90
80
70
60
50
40
30
20
10
0
primary
recurrent

b

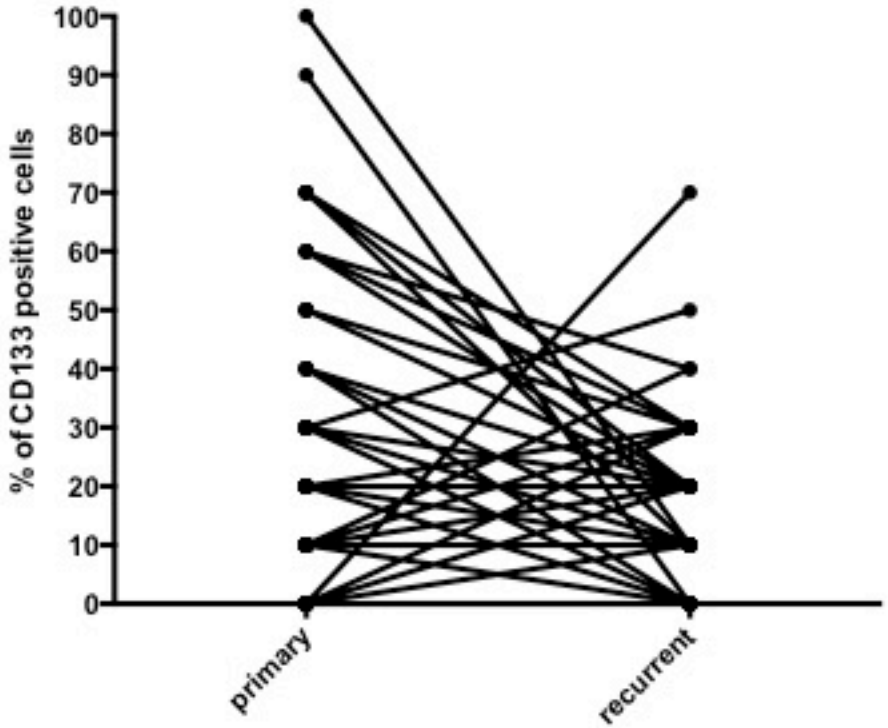
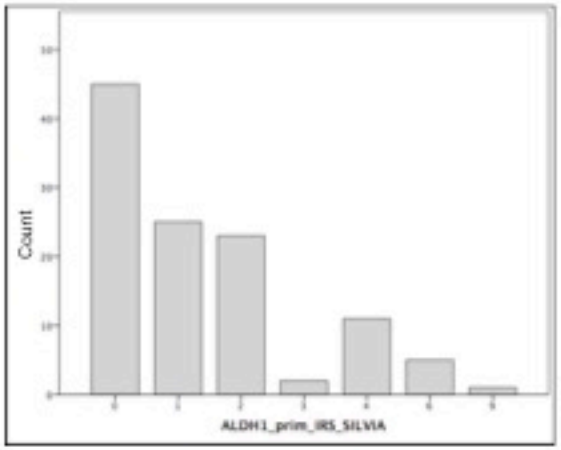

% of CD133 positive cells
100
90
80
70
60
50
40
30
20
10
0
primary
recurrent

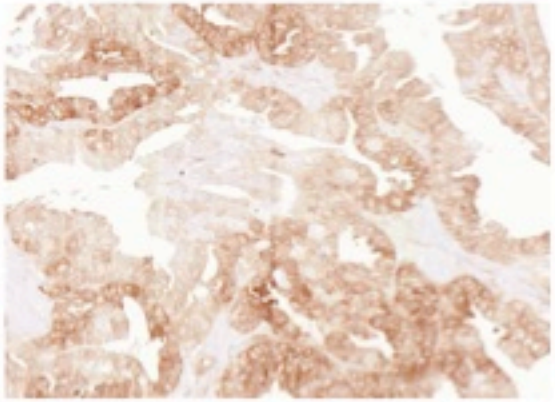
Figure 3

a

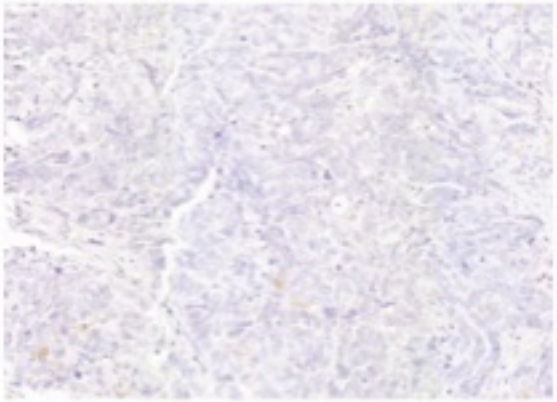


ALDH1 IRS primary

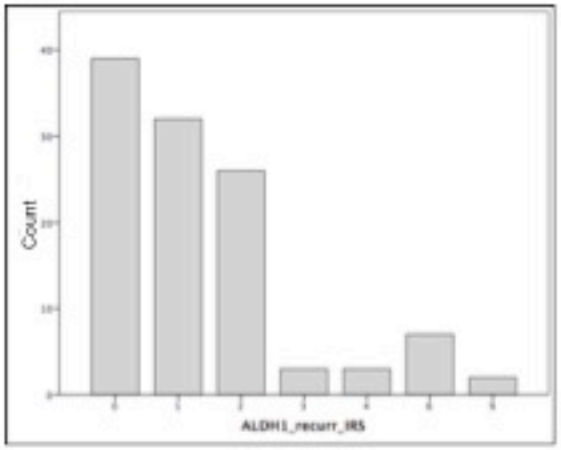
b



c

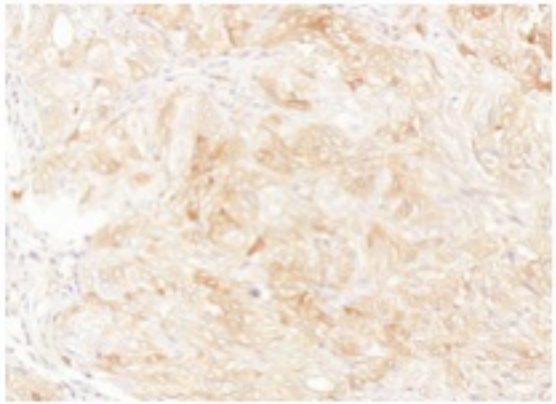


d



ALDH1 IRS recurrent

e



f

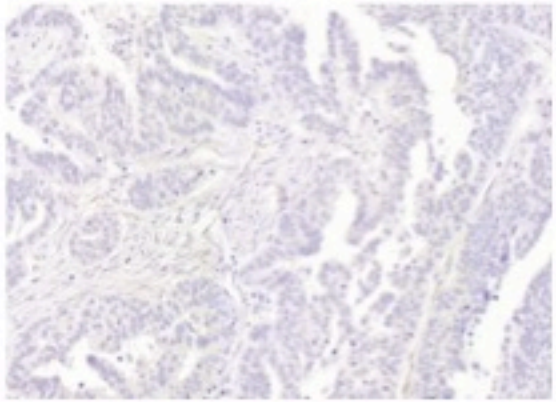
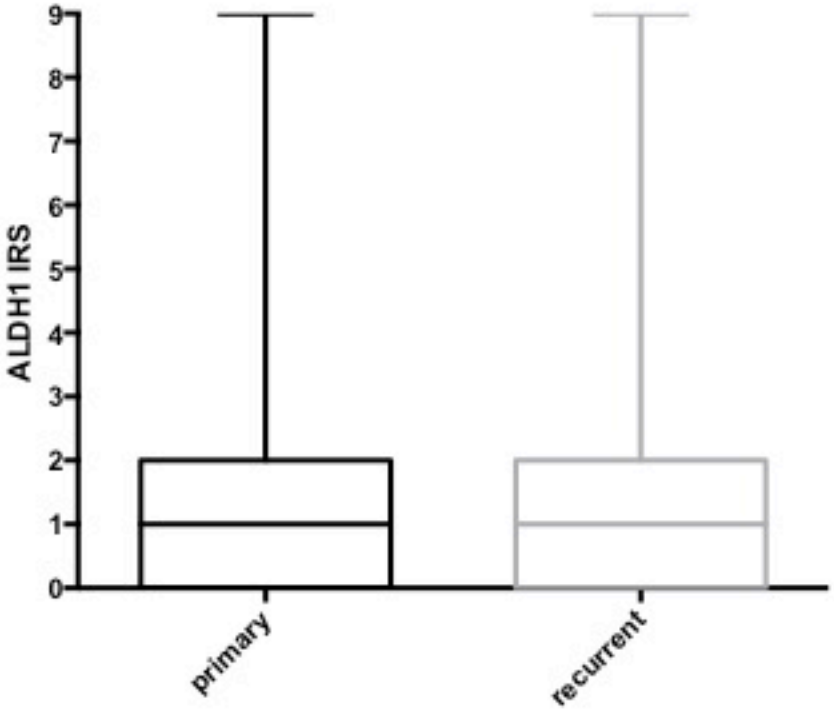


Figure 4

a


ALDH1 IRS
0
1
2
3
4
5
6
7
8
9
primary
recurrent

b

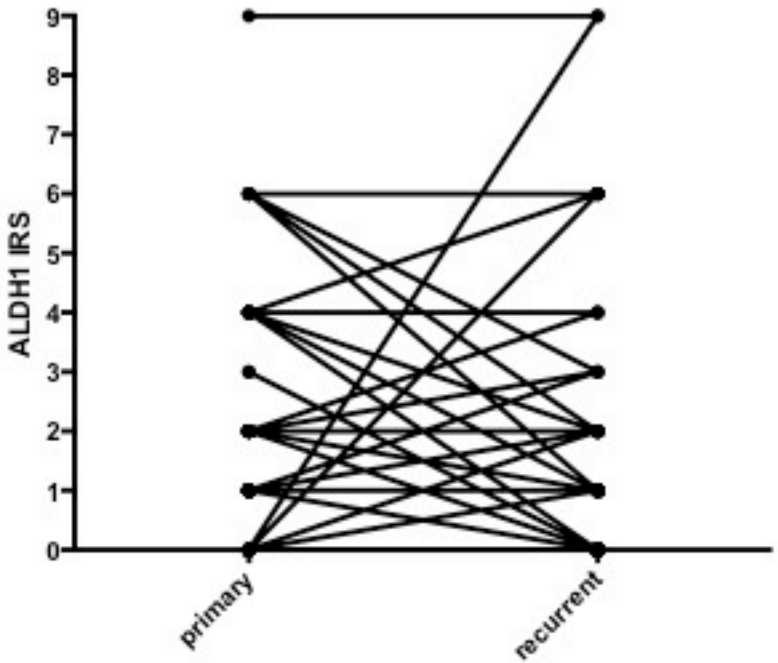

ALDH1 IRS
0
1
2
3
4
5
6
7
8
9
primary
recurrent

Figure 5

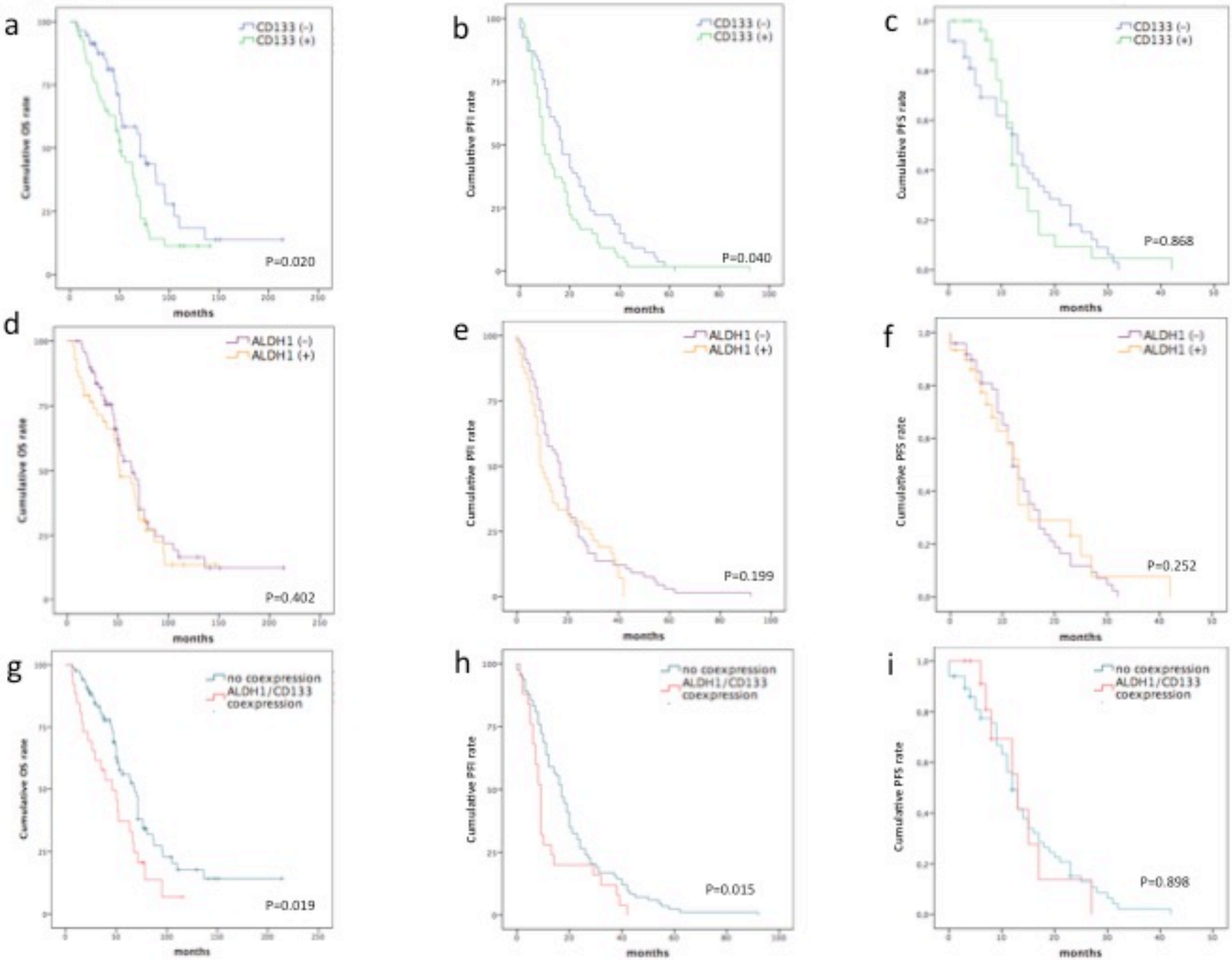

a
CD133 (-)
CD133 (+)
Cumulative OS rate
months
P=0.020
b
CD133 (-)
CD133 (+)
Cumulative PFI rate
months
P=0.040
c
CD133 (-)
CD133 (+)
Cumulative PFS rate
months
P=0.868
d
ALDH1 (-)
ALDH1 (+)
Cumulative OS rate
months
P=0.402
e
ALDH1 (-)
ALDH1 (+)
Cumulative PFI rate
months
P=0.199
f
ALDH1 (-)
ALDH1 (+)
Cumulative PFS rate
months
P=0.252
g
no coexpression
ALDH1/CD133 coexpression
Cumulative OS rate
months
P=0.019
h
no coexpression
ALDH1/CD133 coexpression
Cumulative PFI rate
months
P=0.015
i
no coexpression
ALDH1/CD133 coexpression
Cumulative PFS rate
months
P=0.898

Figure 6

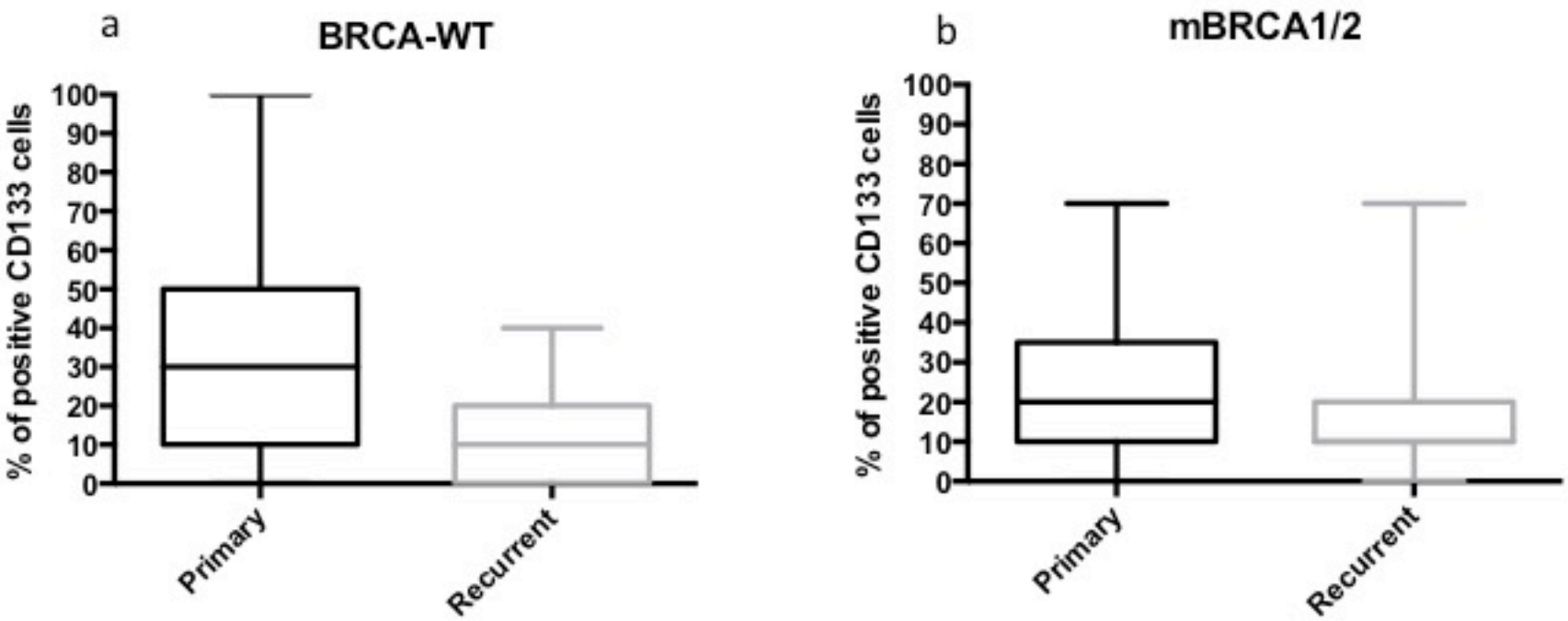

a
BRCA-WT
% of positive CD133 cells
100
90
80
70
60
50
40
30
20
10
0
Primary
Recurrent
b
mBRCA1/2
% of positive CD133 cells
100
90
80
70
60
50
40
30
20
10
0
Primary
Recurrent

Figure 7

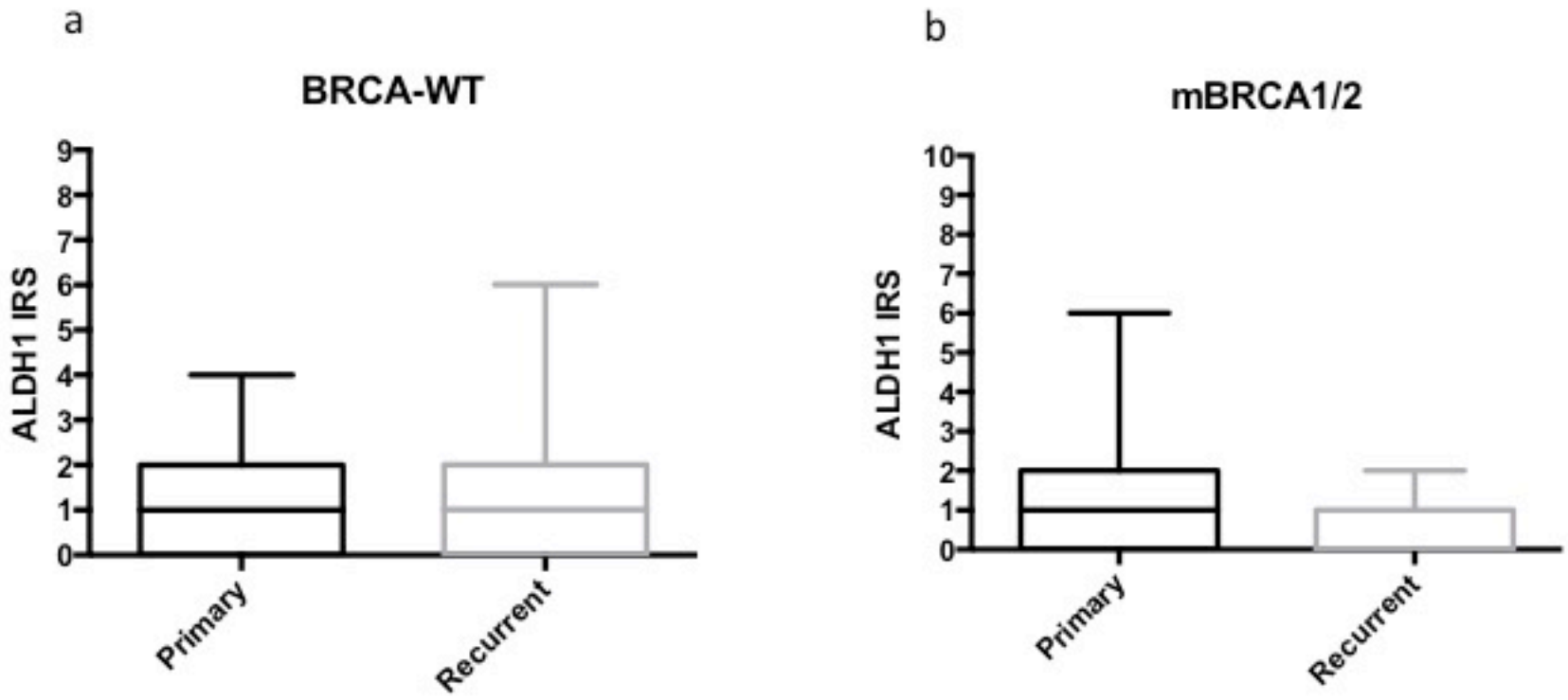

a
BRCA-WT
ALDH1 IRS
0
1
2
3
4
5
6
7
8
9
Primary
Recurrent
b
mBRCA1/2
ALDH1 IRS
0
1
2
3
4
5
6
7
8
9
10
Primary
Recurrent

Figure 8

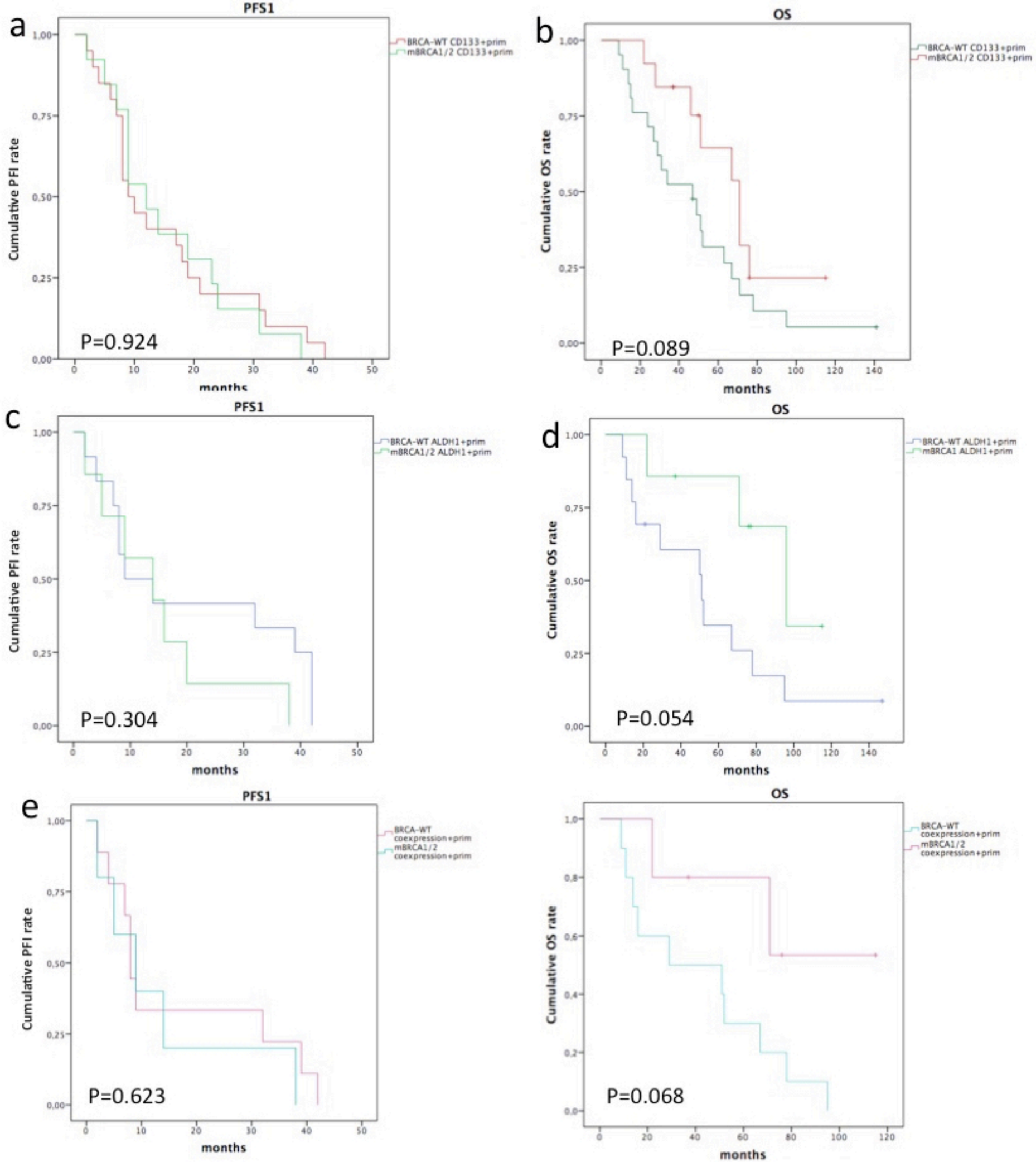

a
PFS1
Cumulative PFI rate
months
BRCA-WT CD133+prim
mBRCA1/2 CD133+prim
P=0.924
b
OS
Cumulative OS rate
months
BRCA-WT CD133+prim
mBRCA1/2 CD133+prim
P=0.089
c
PFS1
Cumulative PFI rate
months
BRCA-WT ALDH1+prim
mBRCA1/2 ALDH1+prim
P=0.304
d
OS
Cumulative OS rate
months
BRCA-WT ALDH1+prim
mBRCA1 ALDH1+prim
P=0.054
e
PFS1
Cumulative PFI rate
months
BRCA-WT coexpression+prim
mBRCA1/2 coexpression+prim
P=0.623
OS
Cumulative OS rate
months
BRCA-WT coexpression+prim
mBRCA1/2 coexpression+prim
P=0.068

Table 1

PARAMETER	
PATIENTS (n.)	112
AGE Median (range)	 56y (33-77y)
FIGO STAGE (%) - I - II - III - IV	 2 (1.8%) 6 (5.4%) 93 (83%) 11 (9.8%)
RESIDUAL TUMOR AFTER PRIMARY DEBULKING SURGERY: - No residual tumor - Residual Tumor	 90 (80.4%) 22 (19.6%)
PLATINUM SENSITIVITY STATUS AFTER PRIMARY TREATMENT - Platinum sensitive - Platinum resistant - Missing	 90 (80.4%) 18 (16.1%) 4 (3.5%)
PLATINUM SENSITIVITY STATUS AFTER TREATMENT FOR DISEASE RELAPSE - Platinum sensitive - Platinum resistant - Missing	 59 (52.7%) 12 (10.7%) 41(36.6%)

Table 2

Clinico-pathological factors	Total N°	CD133			ALDH1			CD133 and ALDH1 coexpression		
		Positive (%)	Negative (%)	P	Positive (%)	Negative (%)	P	Positive (%)	Negative (%)	P
Patients' Age										
< 56y	54	27 (50%)	27 (50%)	0.855	18 (33%)	36 (67%)	0.288	11 (20%)	43 (80%)	0.492
≥ 56y	58	28 (48%)	30 (52%)		25 (43%)	33 (57%)		15 (26%)	43 (74%)	
FIGO STAGE										
I/II	8	0	8 (100%)	**0.006**	3 (38%)	5 (62%)	1.000	0	8 (100%)	0.194
III/IV	104	55 (53%)	49 (47%)		40 (39%)	64 (61%)		26 (25%)	78 (75%)	
RESIDUAL TUMOR AFTER FIRST CYTOREDUCTIVE SURGERY										
No residual	90	42 (47%)	48 (53%)	0.346	35 (39%)	55 (61%)	1.000	20 (22%)	70 (78%)	0.586
Any residual	22	13 (59%)	9 (41%)		8 (36%)	14 (64%)		6 (27%)	16 (73%)	
PLATINUM SENSITIVITY STATUS AFTER PRIMARY TREATMENT										
Platinum sensitive	90	43 (48%)	47 (52%)	0.439	33 (37%)	57 (63%)	0.303	19 (21%)	71 (79%)	0.357
Platinum resistant	18	7 (39%)	11 (61%)		9 (50%)	9 (50%)		6 (33%)	12 (67%)	

Table 3

PROGRESSION FREE INTERVAL

	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
	HR (95% CI)	P	HR (95% CI)	P
Age	1.003 (0.983-1.024)	0.774		
FIGO Stage (III/IV vs I/II)	2.019 (0.907-4.496)	0.085	1.856 (0.826-4.169)	0.134
Residual Tumor (any residual vs no residual)	1.026 (0.625-1.684)	0.919		
CD133/ALDH1 coexpression (positive vs negative)	1.729 (1.093-2.733)	0.019	1.638 (1.033-2.598)	**0.036**

Table 4

OVERALL SURVIVAL

	UNIVARIATE ANALYSIS		MULTIVARIATE ANALYSIS	
	HR (95% CI)	P	HR (95% CI)	P
Age	1.011 (0.985-1.038)	0.404		
FIGO Stage (III/IV vs I/II)	1.465 (0.533-4.020)	0.459		
Residual Tumor (any residual vs no residual)	1.632 (0.973-2.736)	0.063	1.272 (0.725-2.231)	0.401
Platinum sensitivity status after primary treatment (platinum resistant vs platinum sensitive)	3.394 (1.927-5.978)	<0.001	2.907 (1.594-5.302)	**<0.001**
CD133/ALDH1 coexpression (positive vs negative)	1.799 (1.089-2.971)	0.022	1.707 (1.012-2.881)	**0.045**

Table 5

PATIENT ID	GERMLINE BRCA STATUS	SOMATIC BRCA STATUS – PRIMARY TUMOR	SOMATIC BRCA STATUS – RECURRENT TUMOR
B001	mBRCA1	mBRCA1	mBRCA1
B002	WT	WT	WT
B003	WT	WT	WT
B006	N/A	WT	WT
B007	N/A	WT	WT
B009	N/A	WT	WT
B012	N/A	WT	WT
B015	N/A	WT	WT
B019	WT	mBRCA2	mBRCA2
B021	N/A	WT	WT
B022	N/A	WT	WT
B024	mBRCA1	mBRCA1	mBRCA1
B025	N/A	WT	WT
B026	N/A	WT	WT
B028	mBRCA1	mBRCA1	mBRCA1
B029	mBRCA1	mBRCA1	mBRCA1
B030	N/A	WT	WT
B032	WT	WT	WT
B037	N/A	WT	WT
B041	mBRCA1	mBRCA1	mBRCA1
B044	N/A	WT	WT
B045	N/A	mBRCA1	mBRCA1
B048	WT	WT	WT
B050	WT	WT	WT
B051	N/A	mBRCA2	mBRCA2
B052	WT	WT	WT
B053	N/A	WT	WT
B054	N/A	WT	WT
B062	N/A	WT	WT
B063	N/A	mBRCA2	mBRCA2
B065	WT	WT	WT
B068	N/A	mBRCA1	mBRCA1
B069	N/A	WT	WT
B071	N/A	mBRCA1	mBRCA1
B077	mBRCA2	mBRCA2	mBRCA2
B080	mBRCA2	mBRCA2	mBRCA2
B081	WT	mBRCA1	mBRCA1
B082	N/A	mBRCA1	mBRCA1
B085	N/A	mBRCA1	mBRCA1
B087	mBRCA1	mBRCA1	mBRCA1
B088	N/A	WT	WT
B090	N/A	mBRCA1	mBRCA1
B093	N/A	WT	WT
B094	N/A	mBRCA1	mBRCA1
B097	N/A	mBRCA1	mBRCA1
B098	N/A	WT	WT
B099	N/A	WT	WT
B100	N/A	WT	WT
L007	WT	WT	WT
L010	WT	WT	WT
L017	WT	WT	WT
L020	mBRCA1	mBRCA1	mBRCA1

Table 6

BRCA status	Total N°	CD133			ALDH1			CD133 and ALDH1 coexpression		
		Positive (%)	Negative (%)	P	Positive (%)	Negative (%)	P	Positive (%)	Negative (%)	P
BRCA-WT	31	21 (68%)	10 (32%)	0.769	13 (42%)	18 (58%)	0.575	10 (32%)	21 (68%)	0.551
mBRCA1/2	21	13 (62%)	8 (38%)		7 (33%)	14 (67%)		5 (24%)	16 (76%)	