Exploring the clonal evolution of CD133/aldehyde-dehydrogenase-1 (ALDH1)-positive cancer stem-like cells from primary to recurrent high-grade serous ovarian cancer (HGSOC). A study of the Ovarian Cancer Therapy–Innovative Models Prolong Survival (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

(rOC) OC, has not been elucidated yet and information about the changes in CSC presence within the tumor after relapse is still lacking.

The aim of this study was to investigate the evolution of CSC biomarkers CD133 and ALDH1 expression in a large series of paired primary and recurrent HGSOCs.

MATERIALS AND METHODS

Sample Collection

224 paired samples from 112 HGSOC patients were collected during primary and secondary tumor debulking. Patients were included consecutively and have been treated between 1985 and 2013 through primary cytoreduction followed by platinum-based chemotherapy. Patients, retrospectively selected from the OCTIPS (Ovarian Cancer Therapy–Innovative Models Prolong Survival, Agreement No.279113-2) Consortium database, were treated for both pOC and rOC in one of the European Gynecologic Oncology Referral Centers of the following Institutions: Charité Universitätsmedizin Berlin,Germany; Katholieke Universiteit Leuven,Belgium; Imperial College, London,UK; University of Edinburgh,UK.

Inclusion criteria were: having experienced at least one OC relapse for which having been subjected to at least one palliative surgery. Exclusion criterion was: no cancer tissue available from both pOC and rOC. Approval from each local ethics committee was obtained (EK207/2003,ML2524,05/Q0406/178,EK130113,06/S1101/16). OC tissue samples were collected during primary cytoreduction and at the surgery for relapse. All included samples underwent central histopathological assessment to confirm the diagnosis of HGSOC and to evaluate the tissue quality and tumor contain.

Immunohistochemistry

Immunohistochemical staining was performed on tissue microarrays (TMAs).

Slides were deparaffinized in xylol, rehydrated in graded alcohol and boiled in a pressure cooker for 5 minutes in citrate buffer (pH=6) for ALDH1 staining or in EDTA (pH=9) for CD133 staining. Mouse anti-human ALDH1-antibody (clone 44;BD Transduction Laboratories,Franklin Lakes, NJ,USA) and mouse anti-human CD133/1-antibody (AC133 clone;Miltenyi-Biotech, BergischGladbach,Germany) were diluted 1:500 and incubated on the slides for 60 minutes at room temperature. Bound antibodies were visualized using DAKO Real Detection System and DAB+ (3,3′ -diaminobenzidine;DAKO,Glostrup,Denmark) as a chromogen. Finally, the slides were co-stained with hematoxylin.

CD133 stained samples were assessed basing on the number of stained tumor cells. Samples were classified as "CD133-negative"(<10% CD133 positive tumor cells) and "CD133-positive"(>10% CD133-positive tumor cells)[**19-20**].

For ALDH1 staining evaluation, as previously published[**21-22**], the number of stained tumor cells (0%=0;1-10%=1;11-50%=2;>50%=3) was multiplied with the intensity of staining(negative=0;weak=1;moderate=2;strong=3), resulting in a semiquantitive immunoreactivity score(IRS) that ranged from 0 to 9. For further analysis, samples were classified "ALDH1-negative", for absent or weak focal staining(IRS=0-1), or "ALDH1-positive", for ALDH1-high tumor expression(IRS=2-9).

All samples were evaluated independently by two co-authors (IR and SDE).

Clinical Data and Follow-up

Patients' clinical data and information on 52 patients' germline and/or somatic BRCA status were retrieved from OCTIPS Consortium database[**23-24**]. Platinum-resistance and platinum-sensitivity were defined, according to GCIG, as relapse occurring before or after six months following the last platinum-based chemotherapy,

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

Statistical Analysis

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

RESULTS

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

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

CD133 expression.

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

ALDH1 expression.

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

CD133/ALDH1 co-expression.

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

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

CSCs biomarkers and clinico-pathological factors

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

Survival

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

Median OS for $ALDH1^+$ and $ALDH1^-$ patients was 52 and 64 months, respectively (p=0.402) and median PFI-1 was 9 and 17 months, respectively (p=0.199)(**Fig.5d,5e**).

ALDH1/CD133 co-expression in pOCs was found to significantly affect HGSOC patients' outcome. A significant decrease in OS and PFI has been found in patients co-expressing ALDH1/CD133 in primary tissue (46 and 9 months, respectively) compared to patients without biomarker co-expression (68 and 17 months, respectively) (p=0.019,**Fig.5g**;p=0.015,**Fig.5h**). No significant difference in PFS after relapse was observed between patients who reported CD133/ALDH1 co-expression or no co-expression in rOC(p=0.898,**Fig.5i**).

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

Outliers' sub-analysis

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

CSC biomarker expression and BRCA status

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

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

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

significant shift from higher levels in pOCs to lower levels in the rOCs ($p<0.0001$,Wilcoxon test;**Fig.6a**). In contrast, among mBRCA1/2 patients, no correlation between pOCs and rOCs ($p=0.493$,Spearman coefficient 0.158), or a tendency towards a change in CD133+ expression was observed ($p=0.167$,Wilcoxon test;**Fig.6b**).

Regarding ALDH1 expression, among BRCA-WT patients no correlation between pOCs and rOCs in ADH1 IRS was found ($p=0.986$,Spearman coefficient 0.003), as well as no change in paired testing ($p=0.895$,Wilcoxon test;**Fig.7a**); also for mBRCA1/2 patients no difference was observed in ALDH1-IRS between primary and recurrent patients ($p=0.410$,Spearman coefficient 0.190;$p=0.385$,Wilcoxon test;**Fig.7b**).

Among BRCA-WT patients, only 1/31 patient (3.2%) showed CD133/ALDH1 co-expression in both pOCs and rOCs. In 3/31(9.7%) patients the co-expression was evidenced in rOCs but not in pOCs. 90% of patients (9/10) reporting CD133/ALDH1 co-expression in pOC lost biomarker co-expression at tumor relapse.

Also for mBRCA1/2 patients, only 1/21(4.8%) patient showed CD133/ALDH1 co-expression in both pOC and rOC. Two patients (9.5%) had co-expression at recurrent rather than at primary disease. The difference between BRCA-WT and mBRCA1/2 patients in terms of co-expression loss at rOC was not significant (4/5 vs 9/10,$p=1$, Fisher's exact test).

Considering patients who were CD133+ and/or ALDH1+ at pOC, no significant difference could be detected in PFI and OS among BRCA-WT vs mBRCA1/2 cases(**Fig.8**).

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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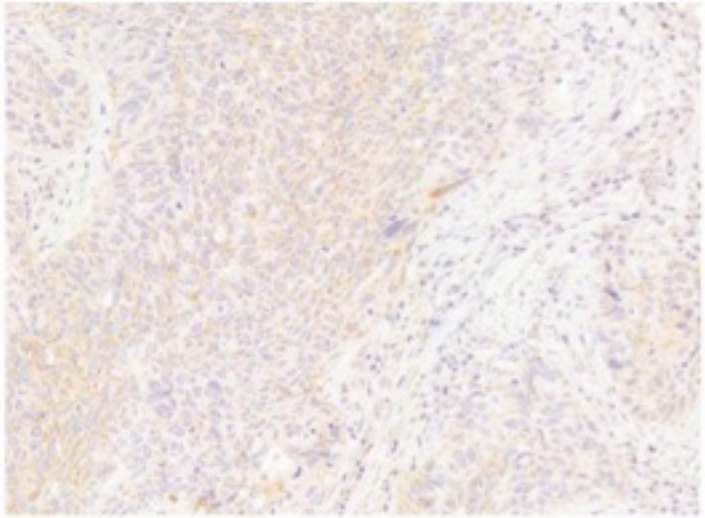
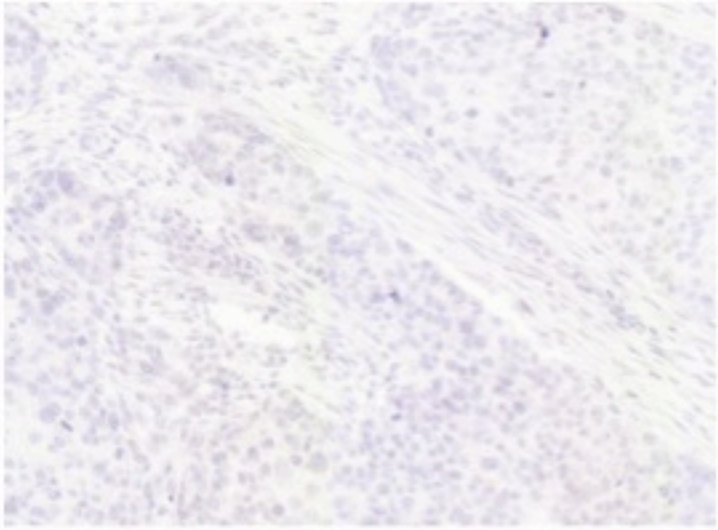
Figure 1

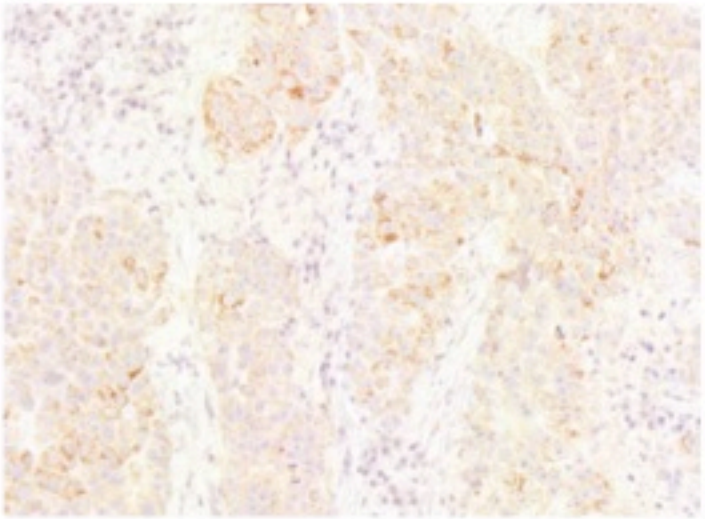
a



b



c



d

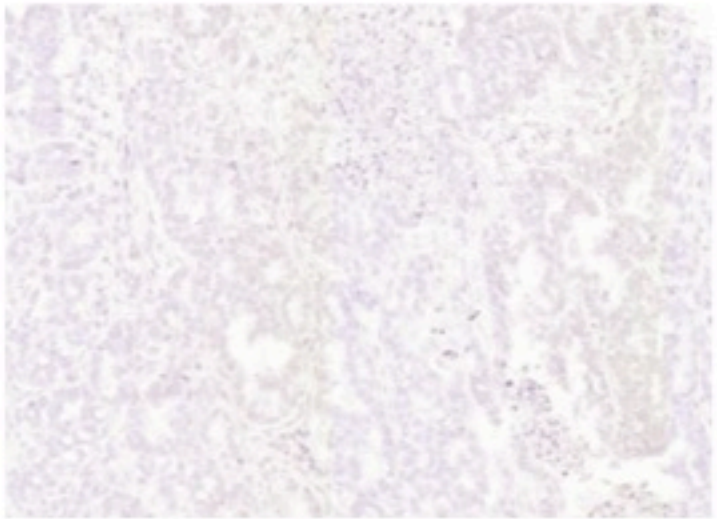


Figure 2

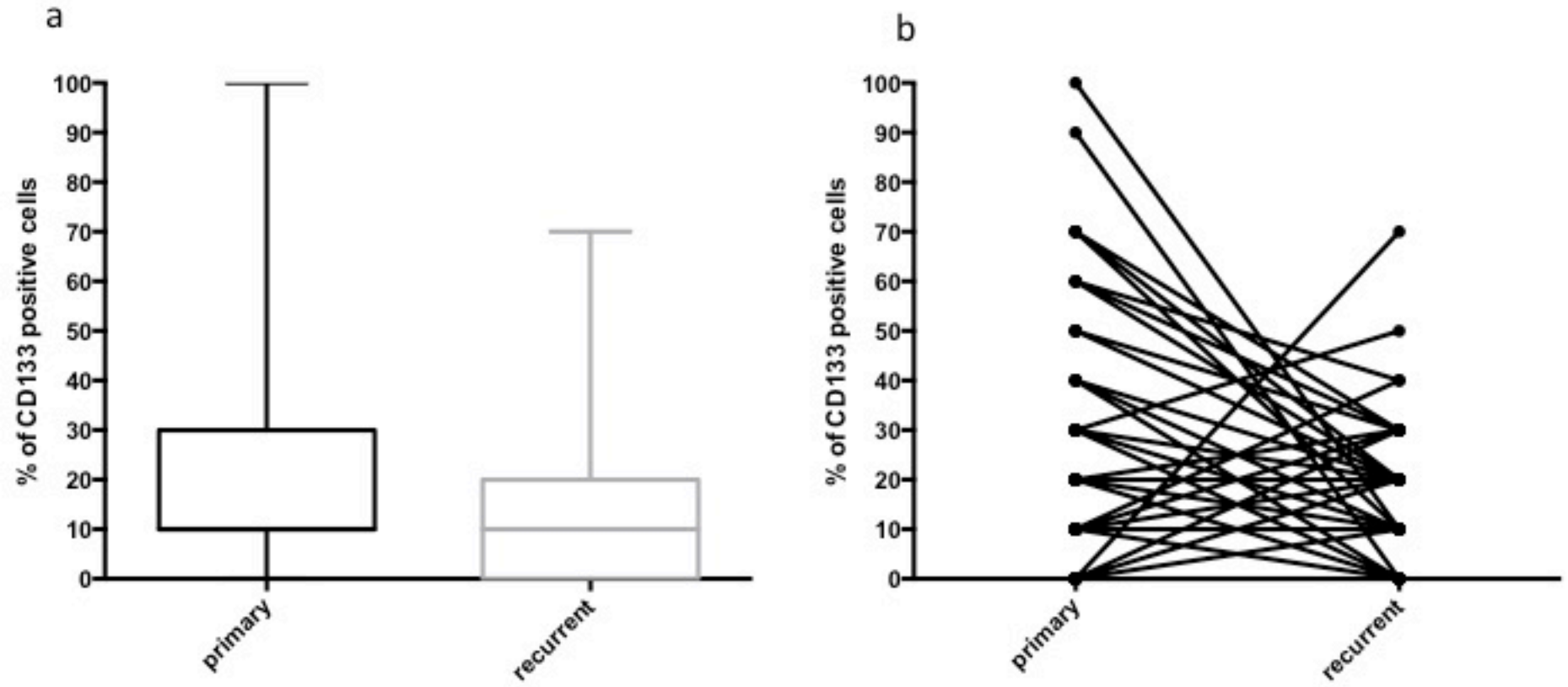
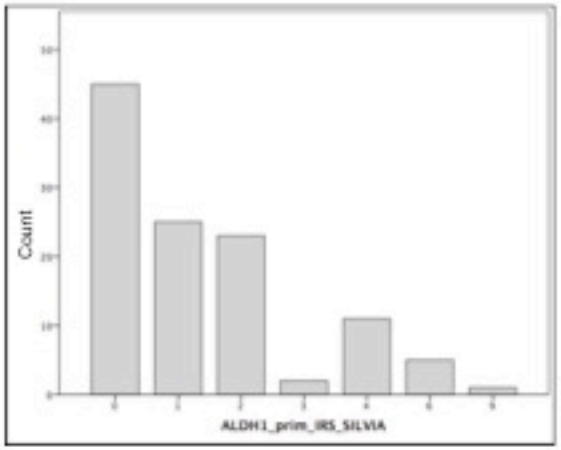

a
b
% of CD133 positive cells
100
90
80
70
60
50
40
30
20
10
0
primary
recurrent

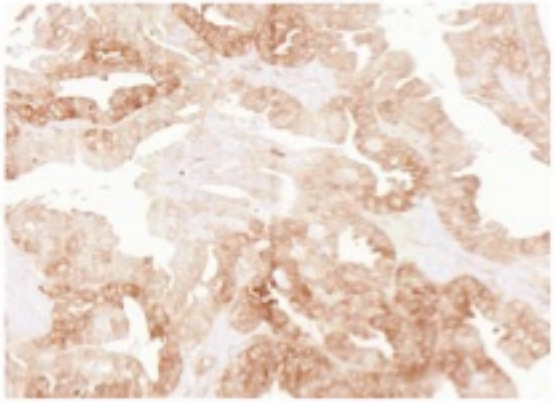
Figure 3

a

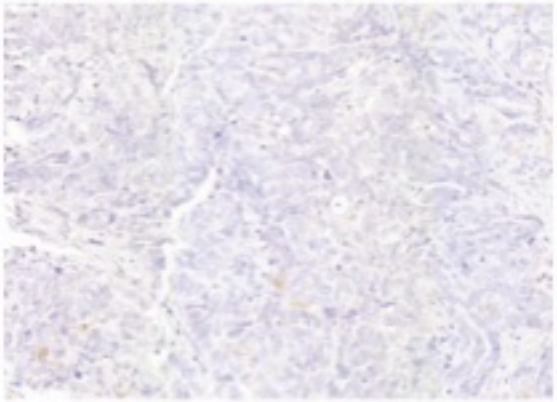


ALDH1 IRS primary

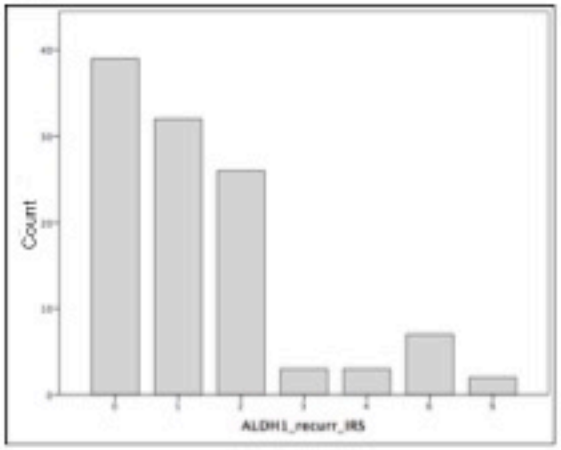
b



c

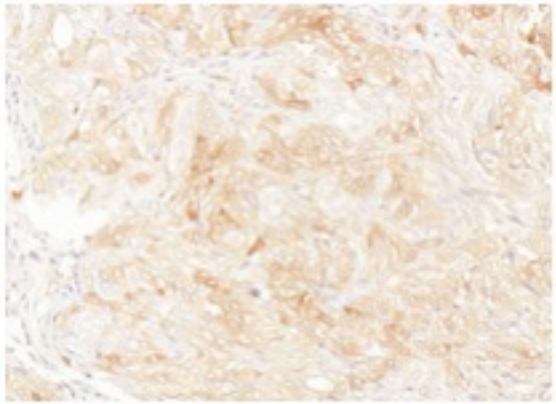


d



ALDH1 IRS recurrent

e



f

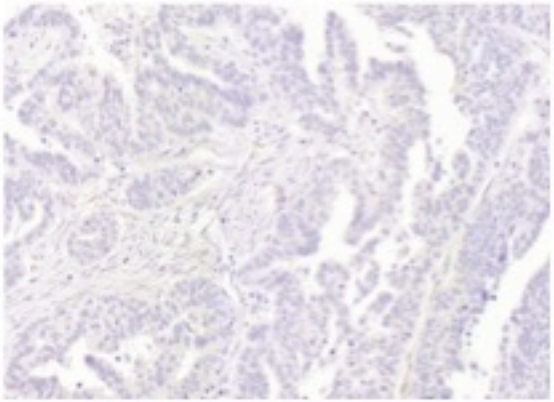
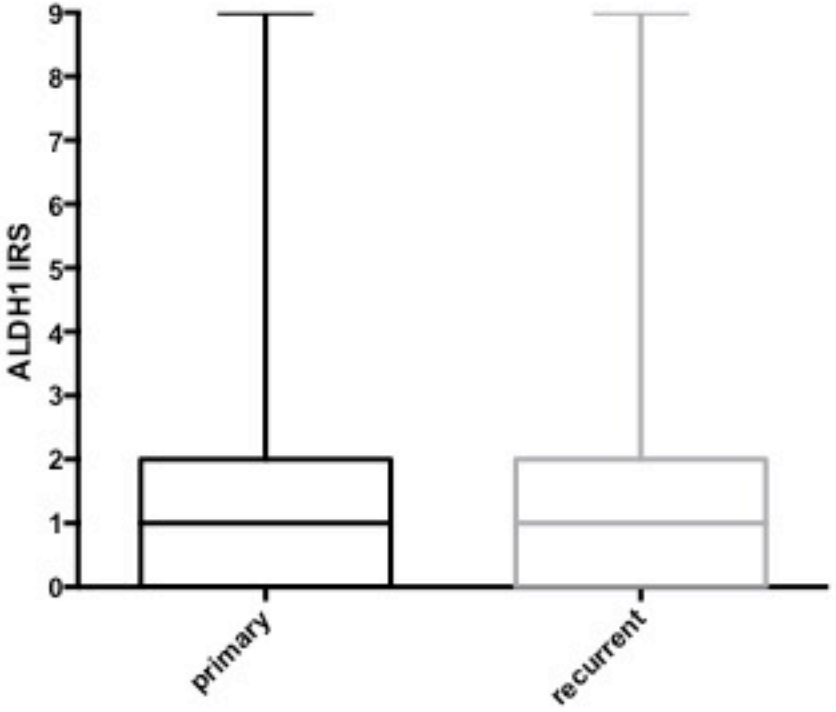


Figure 4

a


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

b

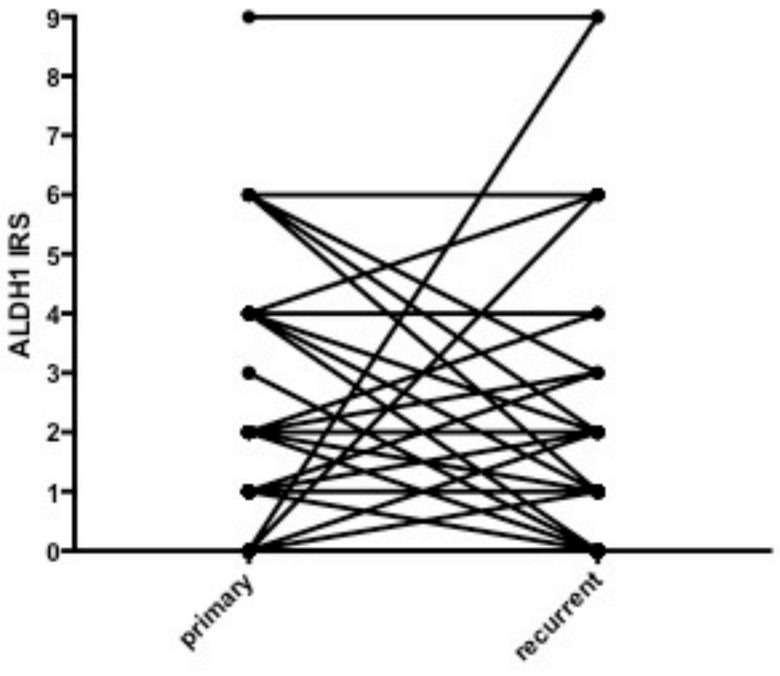

ALDH1 IRS
0
1
2
3
4
5
6
7
8
9
primary
recurrent

Figure 5

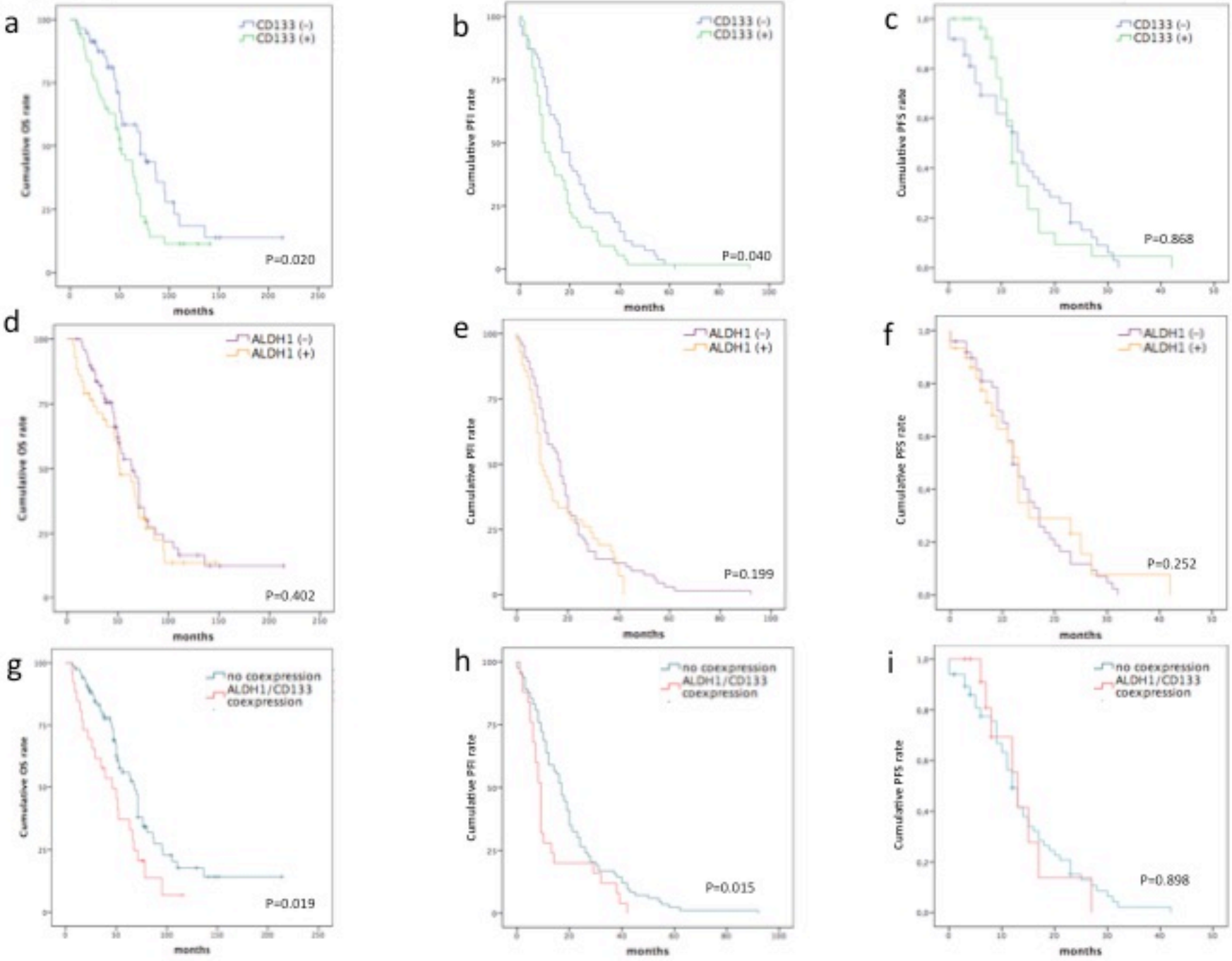

a
CD133 (-)
CD133 (+)
Cumulative OS rate
P=0.020
months
b
CD133 (-)
CD133 (+)
Cumulative PFI rate
P=0.040
months
c
CD133 (-)
CD133 (+)
Cumulative PFS rate
P=0.868
months
d
ALDH1 (-)
ALDH1 (+)
Cumulative OS rate
P=0.402
months
e
ALDH1 (-)
ALDH1 (+)
Cumulative PFI rate
P=0.199
months
f
ALDH1 (-)
ALDH1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

Figure 6

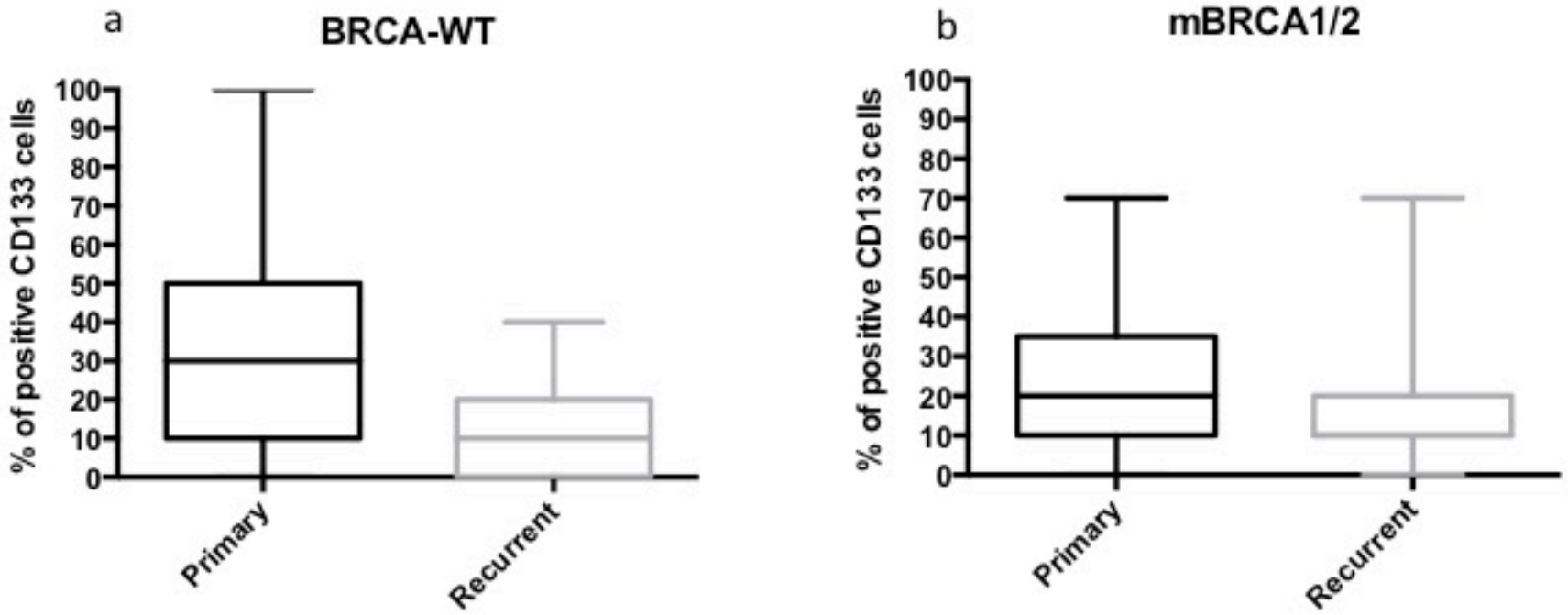

a
BRCA-WT
% of positive CD133 cells
100
90
80
70
60
50
40
30
20
10
0
Primary
Recurrent
b
mBRCA1/2
% of positive CD133 cells
100
90
80
70
60
50
40
30
20
10
0
Primary
Recurrent

Figure 7

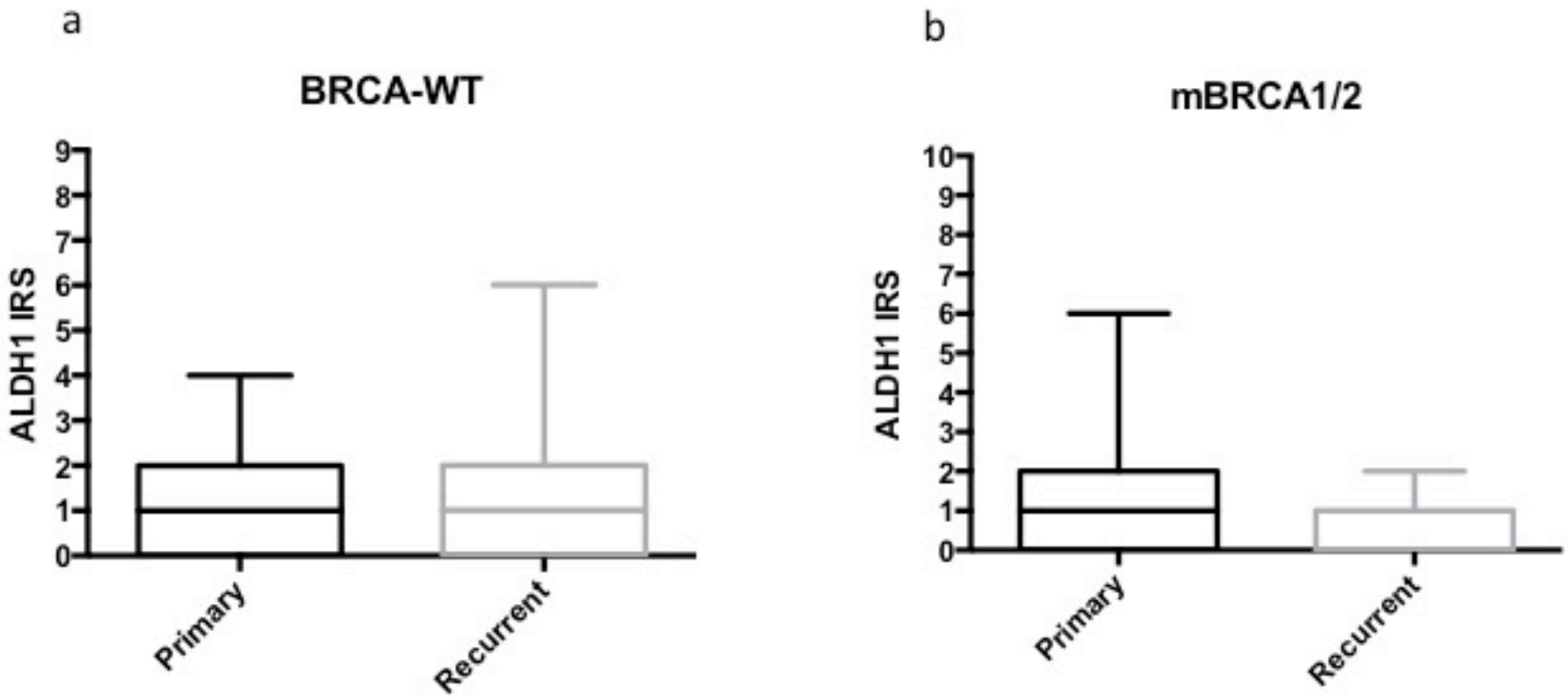
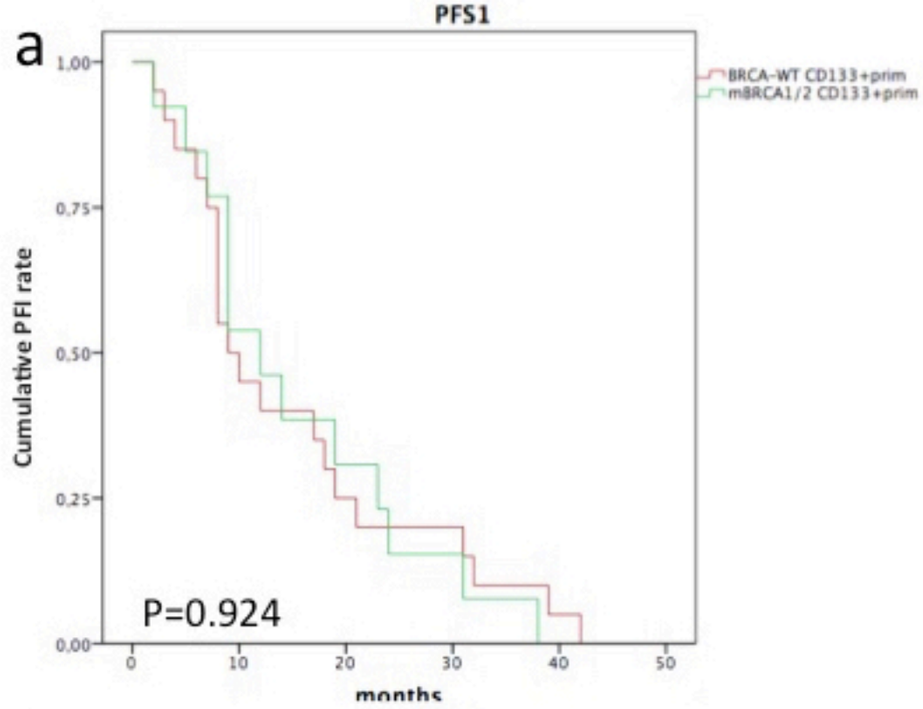
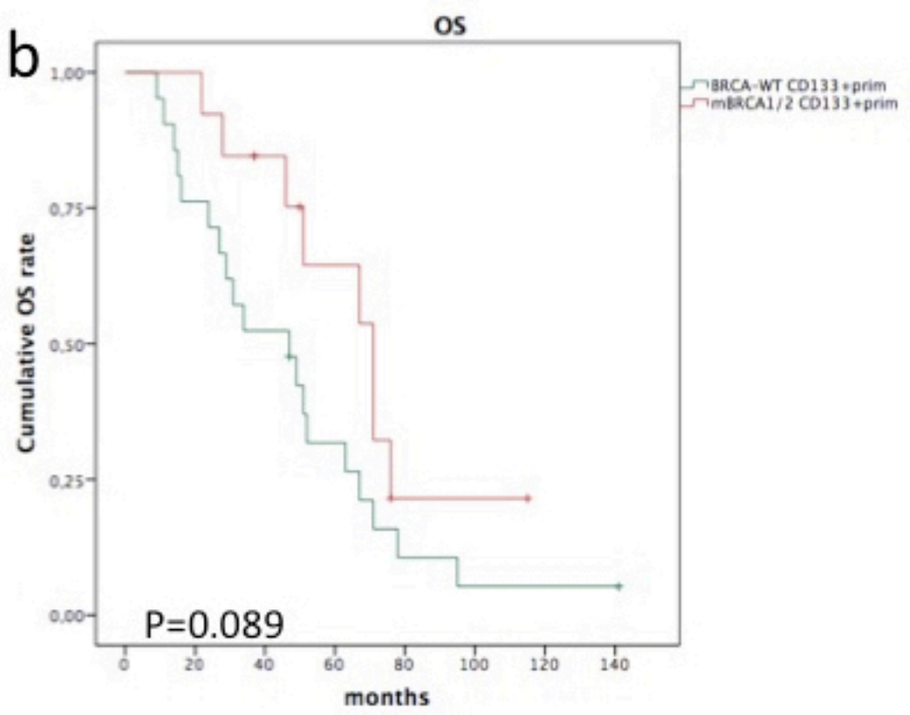
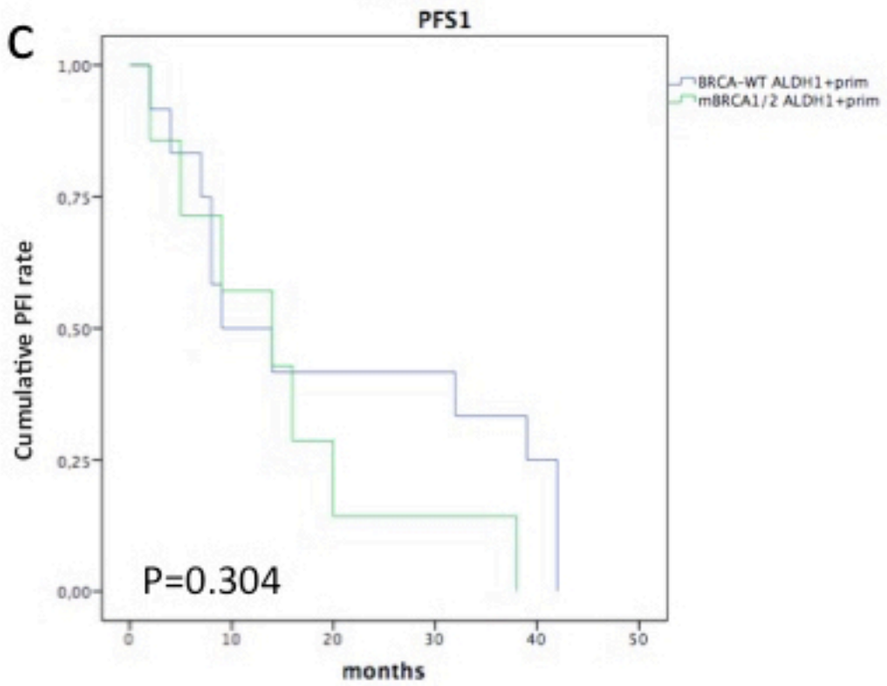
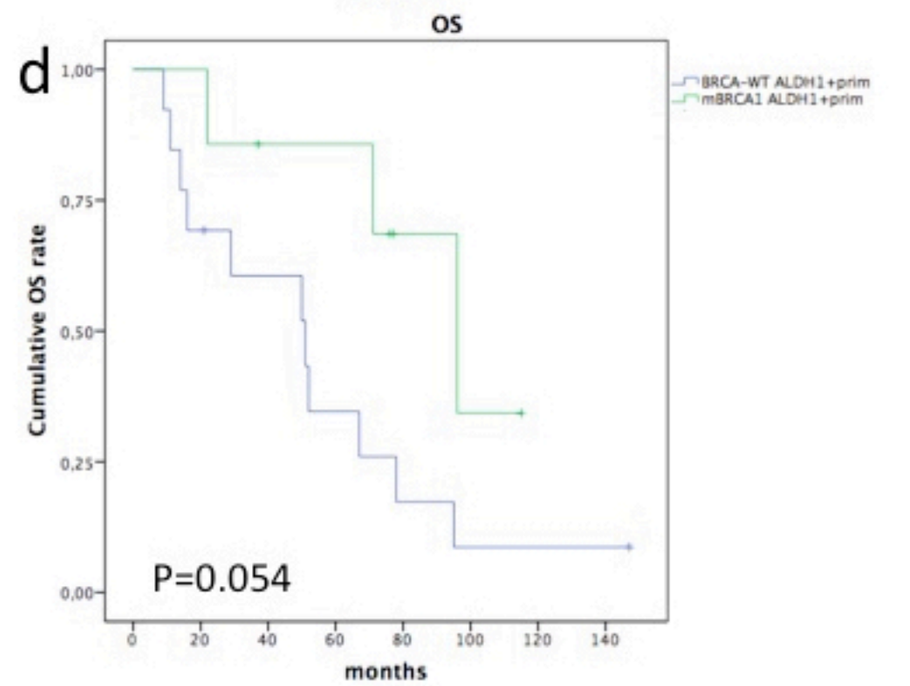

a
BRCA-WT
ALDH1 IRS
0
1
2
3
4
5
6
7
8
9
Primary
Recurrent
b
mBRCA1/2
ALDH1 IRS
0
1
2
3
4
5
6
7
8
9
10
Primary
Recurrent

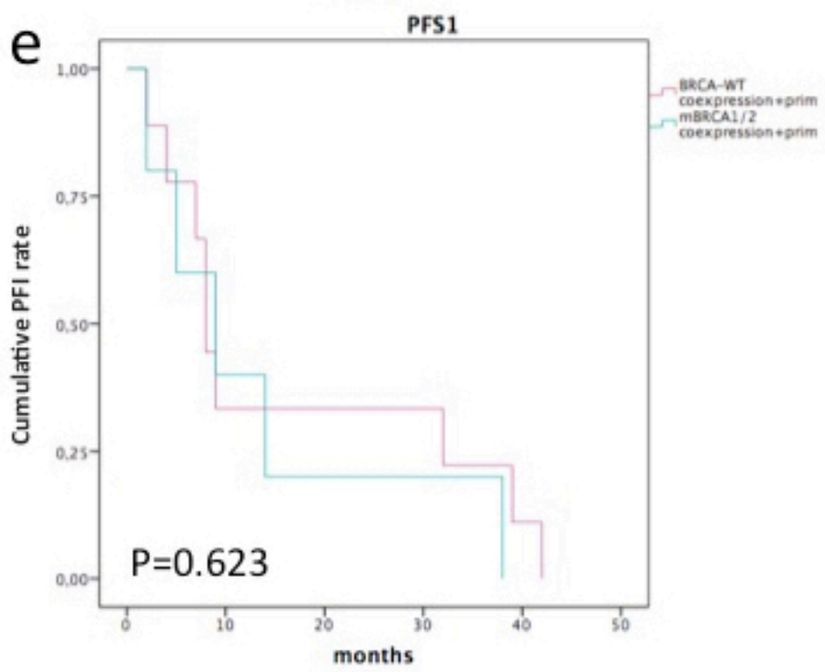
Figure 8


a
PFS1
Cumulative PFI rate
1,00
0,75
0,50
0,25
0,00
0
10
20
30
40
50
months
P=0.924
BRCA-WT CD133+prim
mBRCA1/2 CD133+prim


b
OS
Cumulative OS rate
1,00
0,75
0,50
0,25
0,00
0
20
40
60
80
100
120
140
months
P=0.089
BRCA-WT CD133+prim
mBRCA1/2 CD133+prim


c
PFS1
Cumulative PFI rate
1,00
0,75
0,50
0,25
0,00
0
10
20
30
40
50
months
P=0.304
BRCA-WT ALDH1+prim
mBRCA1/2 ALDH1+prim


d
OS
Cumulative OS rate
1,00
0,75
0,50
0,25
0,00
0
20
40
60
80
100
120
140
months
P=0.054
BRCA-WT ALDH1+prim
mBRCA1 ALDH1+prim


e
PFS1
Cumulative PFI rate
1,00
0,75
0,50
0,25
0,00
0
10
20
30
40
50
months
P=0.623
BRCA-WT coexpression+prim
mBRCA1/2 coexpression+prim

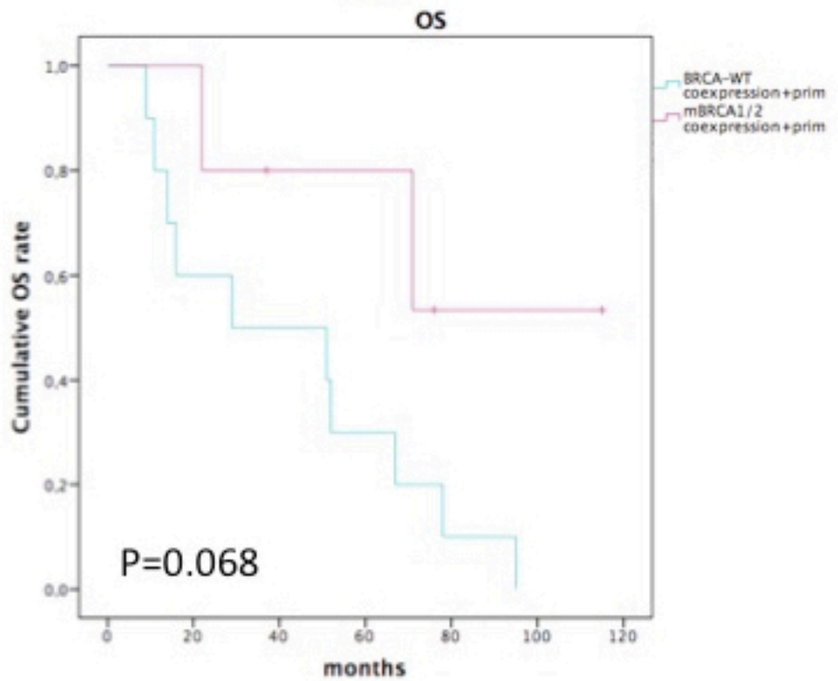

OS
Cumulative OS rate
1,0
0,8
0,6
0,4
0,2
0,0
0
20
40
60
80
100
120
months
P=0.068
BRCA-WT coexpression+prim
mBRCA1/2 coexpression+prim

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%)	