**Clinical-pathological features and BRCA 1\2 mutation in women with epithelial ovarian cancer: is there a molecular landmark?**

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In verde la biblio, in giallo le cose da sistemare

**Riviste papabili**

* Cancer, IF=6.86, <https://acsjournals.onlinelibrary.wiley.com/journal/10970142>
* Gynecologic Oncology, IF=5.842, <https://www.journals.elsevier.com/gynecologic-oncology>
* BMC Cancer, IF=4.43, <https://bmccancer.biomedcentral.com/about?gclid=CjwKCAjw7--KBhAMEiwAxfpkWDslm9T3AqwMfWq4QikGLfy35bQOfHoKS9vjXNP6gkaQh9Qoxt_ubRoCuWcQAvD_BwE>
* International Journal of gynecological cancer, IF=3.44, <https://ijgc.bmj.com/>

**Abstract**

**Background:**…

**Materials and methods:**…

**Results:…**

**Conclusions:….**

**Introduction**

…

The aim of this study is to evaluate the synergism between BRCA status and histopathologic features (i.e., molecular markers), in patients with primary advanced ovarian cancer by considering BRCA status as a modification effect, on overall survival (OS), disease free survival (DFS) and platinum resistance.

**Materials and methods**

…

*Study design*

This observational retrospective cohort study included 202 subjects. In depth, we enrolled patients with high grade serous ovarian cancer admitted to Gynaecologic Oncology Unit of “Policlinico Universitario Agostino Gemelli” (Rome) between 2014 and 2019 with either known BRCA 1/2 germline or somatic mutation status with known histopathologic and molecular features. All patients provided informed consent to the use of their samples for research purposes. In the case of paraffin-embedded samples unavailable for histological analyses, subjects were excluded. Histopathologic features will be reviewed and stratified in an electronic database. The flow-chart of the study is reported in Figure 1.

The study was designed in accordance with 1976 Declaration of Helsinki and its later amendments and was approved by our local Ethics Committee.

**Figure 1. Flow chart of the study**

*Endpoints*

Primary endpoint is to assess the potential correlation between the histopathologic characteristics of the two study populations and the tumour clinical behaviour, in terms of survival outcome. As secondary endpoints, we aimed to assess the potential correlation between the histopathologic characteristics of the two study populations and the tumour clinical behaviour, in terms of Disease Free Survival outcome and platinum resistance.

Primary outcome was the overall survival (1=death, 0=survivor), while the secondary outcomes were the relapse/progression, i.e. DFS (Disease Free Survival) follow-up and platinum resistance. In addition, age, BRCA mutation (both as dicothomic “0 = BRCA wild type, 1-2 = BRCA” and “wild-type, 1 and 2”), and molecular markers such as …. che sono stati testati e valutati sul pezzo operatorio disponibile retrospettivamente, tendenzialmente dopo citoriduzione se citoridotte o dopo laparoscopia diagnostica se mai citoridotte. Missing values were managed by list-wise deletion.

*Statistical analysis*

Preliminary, descriptive statistics were computed both on overall population and strata related to BRCA mutation (wild-type vs. BRCA 1 vs. BRCA 2 or wild-type vs. BRCA mutated). Qualitative data were expressed as absolute and percentage frequencies, whilst quantitative variables either as mean and standard deviation (SD) or as median and interquartile range (IQR), after assessment of distribution by the Shapiro Wilk test. As for quantitative variables, between groups differences were assessed either by ANOVA or Kruskal-Wallis test across the three sub-populations, and by Student t test or Mann Withney U test in case of two sub-populations. Chi-squared test was instead applied on qualitative data.

In order to evaluate the raw effects of each molecular marker and clinical data (predictor) on time-to-event outcomes, a survival analysis was performed by ordinary Proportional hazard Cox Models. The exponential transformation of the regression coefficients associated to the marker allowed more easily interpretable hazard ratio (HR), i.e., the hazard variation per unit increase of predictor. Thus, to evaluate combined effects between molecular markers and BRCA mutations, multivariable interaction Cox models were fitted, and the interaction hazard ratios (IHR) were evaluated. In particular, for each molecular marker, one interaction Cox model was fitted. In this framework, IHR = 1 indicated no synergy between molecular marker and BRCA mutation, IHR <1 expressed a reduction of hazard due to the synergy, while IHR >1 denoted an increased hazard. In summary, the coefficients of the main effects (in exponential terms) were interpreted as HRs of the outcome by considering the BRCA mutations from wild type (HRBRCA), by fixing the values of molecular marker to 0, and HRs of the outcome by considering a unit increase of the molecular marker in the wtBRCA (HRMM). The interaction parameters (IHR) were interpreted as difference (in HR terms) of the MM variations between BRCA conditions (wt as ref­erence category).

Proportionality of the hazard functions was assessed by visual inspection of hazard plots and Schoenfeld residuals. When proportionality was doubtful, weighted Cox regression models (Schemper, 1992; Schemper et al., 2009) were fitted.

Finally, a univariable logistic regression model was implemented to assess factors potentially involved in platinum resistance onset. To evaluate the combined effects between MMs and BRCA mutations, multivariable interaction models were fitted, and the interaction odd ratios (IOR) were evaluated. In particular, for each MM, one interaction logistic regression model was fitted. In summary, the coefficients of the main effects (in exponential terms) were interpreted as ORs of the outcome by considering the BRCA mutations from wild type (ORBRCA), by fixing MM values to 0, and ORs of the outcome by considering a unit increase of the MM in the wtBRCA (ORMM). The interaction parameters (IOR) were interpreted as difference (in OR terms) of the MM variations between BRCA conditions (wt as ref­erence category).

Statistical significance was set at P value < 0.05. P values between 0.05 and 0.10 were also reported as suggestive. All analyses were performed by using R software (v. 4.1.2, R Core Team, 2020) and its packages survival (Therneau, 2015; Therneau et al., 2000), survminer (Kassambara et al., 2019), and coxphw (Dunkler et al., 2018).

**Results**

***BRCA wild-type vs. BRCA mutated (BRCA 1 or BRCA 2)***

202 women were finally included in the study, stratified according to BRCA mutation groups in 132 BRCA wild-type, 44 BRCA 1 and 26 BRCA 2.

Overall mortality was 34.6%, much more prominent in wt-BRCA women (42.4% vs. 18.2% in BRCA 1 and 23.1% in BRCA 2; p=0.006). Likewise, overall relapses set at the 72.8%, lower among BRCA 1 (61.4%) and BRCA2 (69.2%), than in wt-BRCA women (77.3%; p=0.111).

Differences between BRCA mutation groups disclosed significant findings only for age (mean age 60.5 in wt-BRCA vs. 54.7 in BRCA1 and 59.7 in BRCA2; p=0.009), platinum resistance (38.6% in wt vs. 11.4% in BRCA1 and 19.2% in BRCA2, p=0.001) and PFS since last platinum (median 10 months in wt-BRCA vs. 19 in BRCA1 and 17.5 in BRCA2, p=0.006). Indeed, a suggestive finding emerged towards a lower Nucleus ER-β1 score in BRCA2 women (p=0.079) and a higher prevalence of ct-beva in BRCA1 (56.8% vs. 37.1% in WT and 34.6% in BRCA2; p=0.055). Likewise, cytoreduction was slightly lower among WT women (86.4% vs. 97.7% in BRCA1 and 92.3% in BRCA2, p=0.090). No significant difference instead emerged as for MM, baseline BMI and menopausal status. The whole data are reported in Table 1.

Concerning the ordinary Cox regression models, significant results on the overall survival analysis revealed a protective role (HR<1) of BRCA 1 mutation (vs wild type, HR=0.30, p=0.002) and, likewise, cytoreduction (HR 0.09; p<0.001). As well, as for MM, a similar finding emerged on PR score (HR=0.87, p=0.024) and Cytoplasm ER-β5 score (HR 0.84; p=0.049), whilst among clinical data menopausal status (HR 2.31; p=0.008), chir\_ascites (HR 2.23; p=0.002), chir\_pi\_lps\_score (HR 1.13; p<0.001) and platinum resistance (HR 11.42; p<0.001) disclosed a negative prognostic role (HR >>1). A suggestive protective role instead emerged as for BRCA2 (HR 0.44; p=0.060). Similarly, BRCA 1 protective action also persisted on the relapse-related outcomes (HR=0.59; p=0.015) (Table 2).

Table 2 also shows the results obtained by age-adjusted interaction Cox regression models to test the main effects of MM (i.e., within wild-type condition) and clinical conditions. In depth, no significant finding emerged as for MM. Conversely, platinum resistance confirmed as negative prognostic factor of both mortality (HR 8.76; p<0.001) and DFS (16.38; p<0.001), as well as chir\_pi\_lps score (HR 1.12; p=0.001 on OS and HR 1.15; p<0.001 on DFS). Cytoreduction, indeed, confirmed its protective role on both OS (HR 0.11; p<0.001) and DFS (HR 0.02; p<0.001), whilst menopausal status revealed as protective only on DFS (HR 0.52; p=0.029). Of note, in addition, that in the OS models BRCA1 mutation resulted significantly protective in the case of Nucleus AR score (HR 0.38; p=0.034), Cytoplasm ER-β1 Score (HR 0.29; p=0.030) and ER-α/Nucleus ER-β5 ratio (HR 0.27; p=0.042), whilst in the DFS models this finding emerged for nucleus AR score (HR 0.53; p=0.022) and PR score (HR 0.54; p=0.043), as well as in ER-α/Nucleus ER-β1 ratio (HR 0.55; p=0.030), and ER-α/Nucleus ER-β2 ratio (HR 0.53; p=0.033) and ER-α/Nucleus ER-β5 ratio (HR 0.46; p=0.024). Instead, a suggestive protective effect emerged in the OS model related to Cytoplasm ER-β5 Score and in DFS models related to ER-α, Cytoplasm ER-β1 and ER-β2 Score. Indeed, the main effect of the BRCA 2 resulted significant (vs wild type) only in the OS model related to Cytoplasm ER-β5 Score (HR=0.35; p=0.045). Finally, as for the interaction effects, none significant result emerged in both OS and DFS models. All data are reported in Supplementary Table 1.

Finally, we assessed potential predictors of platinum resistance. At univariable analysis, the logistic regression model disclosed BRCA 1 mutation as protective towards platinum resistance onset (OR 0.20; p=0.002), whilst a suggestive protective role emerged as for BRCA 2 mutation (OR 0.38; p=0.066). A similar behavior also emerged as for PR score (OR 0.82; p=0.016), ct\_Beva (OR 0.24; p<0.001) and cytoreduction (OR 0.01; p<0.001). Conversely, ascites (OR 2.44; p=0.006), chir\_pi\_lps\_score (OR 1.16; p=0.001) and menopausal status (OR 2.27; p=0.030) revealed as significantly involved in platinum resistance onset (Table 3). Table 3 further establishes the results obtained by age-adjusted interaction logistic regression models to test the main effects of MM (i.e., within wild-type condition) and clinical conditions. In depth, among MM, only Cytoplasm ER-β1 score (OR 0.83; p=0.043), cytoreduction (OR 0.06; p<0.001) and ct\_beva (OR 0.37; p=0.018) revealed a protective role towards platinum resistance onset, whilst chir\_pi\_lps score disclosed a negative effect on the outcome (OR 1.16; p=0.06). Of note, in addition, that in the OS models BRCA1 mutation resulted significantly protective in the case of all MM, except for Nucleus ER-β1 score and nucleus ER-β2 Score, as well as in ER-α/Nucleus ER-β5 ratio; whilst for PR score, a suggestive protective effect emerged. Indeed, the main effect of the BRCA 2 resulted significant (vs wild type) only related to Cytoplasm ER-β1 Score (OR 0.11; p=0.009) and Cytoplasm ER-β5 Score (OR 0.24; p=0.034). Finally, as for the interaction effects, only Cytoplasm ER-β1 score disclosed a negative effect on platinum resistant in BRCA2 patients (OR 1.61; p=0.023).

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| **Table 1.** General characteristics of the study population (n=202).\* | | | | | |
|  | **Overall** | **BRCA mutation** | | | |
|  |  | **wtBRCA**  **(n=132)** | **BRCA 1**  **(n=44)** | **BRCA 2**  **(n=26)** | **p\*\*** |
| Age (yrs.) | 59.1 (11.5) | 60.5 (11.4) | 54.7 (10.8) | 59.7 (11.2) | **0.009** |
| Baseline BMI | 24.1 (21.5-28) | 24.2 (21.7-27.9) | 24.1 (21.6-28.8) | 23.2 (20-27.5) | 0.518 |
| Menopausa | 144 (71.3) | 100 (75.8) | 26 (59.1) | 18 (69.2) | 0.103 |
| Nucleus AR score | 0 (0-2) | 0 (0-2) | 0 (0-2) | 0 (0–4) | 0.920 |
| PR score | 1 (0-3) | 1 (0-2.5) | 1.5 (0-5) | 1 (0-3) | 0.268 |
| ER-α score | 4 (2-8) | 4 (2-8) | 4 (2-6) | 3 (1-6) | 0.184 |
| Nucleus ER-β1 score | 4 (3-8) | 5 (3-8) | 5 (3-8) | 3 (2-8) | *0.079* |
| Cytoplasm ER-β1 score | 3 (0-3) | 3 (0-3) | 2.5 (0-4) | 1 (0-3) | 0.297 |
| Nucleus ER-β2 score | 8 (4-8) | 7.1 (3.3) | 6.8 (3.4) | 7 (3.3) | 0.811 |
| Cytoplasm ER-β2 score | 2 (0-3) | 2 (0-3) | 2.5 (0-3) | 0 (0-3) | 0.154 |
| Nucleus ER-β5 score | 6 (4-8) | 6.3 (2.8) | 5.9 (3.4) | 5.7 (3.2) | 0.334 |
| Cytoplasm ER-β5 score | 0 (0-3) | 0 (0-3) | 0 (0-3) | 0 (0-0) | 0.662 |
| ER-α/Nucleus ER-β1 ratio | 0.8 (0.4-2.0) | 0.9 (0.3-2.0) | 0.8 (0.4-1.2) | 0.8 (0.3-2.0) | 0.814 |
| ER-α/Nucleus ER-β2 ratio | 0.7 (0.3-1.1) | 0.7 (0.3-1.3) | 0.7 (0.3-1.0) | 0.5 (0.3-0.8) | 0.415 |
| ER-α/Nucleus ER-β5 ratio | 0.8 (0.3-1.3) | 0.8 (0.3-1.3) | 0.8 (0.4-1.5) | 0.5 (0.3-1.0) | 0.637 |
| Chir\_ascite | 109 (54) | 74 (56.1) | 22 (50) | 13 (50) | 0.713 |
| Chir\_pi\_lps\_score | 6 (2-8) | 5.8 (3.8) | 5.5 (3.9) | 6.4 (3.4) | 0.653 |
| Ct\_Beva | 83 (41.1) | 49 (37.1) | 25 (56.8) | 9 (34.6) | *0.055* |
| PFS since last platinum (mths) | 14 (5-26) | 10 (3-24) | 19 (10.5-29) | 17.5 (7-27) | **0.006** |
| Histology stadium  *1*  *2*  *3*  *4* | 4 (2)  14 (6.9)  126 (62.4)  58 (28.7) | 2 (1.5)  10 (7.6)  81 (61.4)  39 (29.6) | 2 (4.6)  4 (9.1)  26 (59.1)  12 (27.3) | -  -  19 (73.1)  7 (26.9) | 0.548 |
| Ca125 at diagnosis | 869.1 (327-2127) | 994.8 (356-2261.8) | 567.6 (210.7-1616.6) | 630 (341.3-2033) | 0.205 |
| Cytoreduction  *Yes*  *No* | 181 (89.6)  21 (10.4) | 114 (86.4)  18 (13.6) | 43 (97.7)  1 (2.3) | 24 (92.3)  2 (7.7) | *0.090* |
| **Outcomes** |  |  |  |  |  |
| *Primary outcome* |  |  |  |  |  |
| Overall Survival | 132 (65.4) | 76 (57.6) | 36 (81.8) | 20 (76.9) | **0.006** |
| OS follow-up (months) | 34 (21-43) | 33 (16.5-42) | 40.5 (32.5-47) | 35.5 (28-44) | **<0.001** |
| *Secondary outcome* |  |  |  |  |  |
| DFS | 147 (72.8) | 102 (77.3) | 27 (61.4) | 18 (69.2) | 0.111 |
| DFS follow-up (mths) | 19.5 (9-30) | 15 (7-29) | 23 (15-35) | 22.5 (9-31) | **0.013** |
| Platinum resistance | 61 (30.2) | 51 (38.6) | 5 (11.4) | 5 (19.2) | **0.001** |
| **Abbreviations**: wt: wild-type; AR: androgen receptor; PR: progesterone receptor; ER: oestrogen receptor; OS: overall survival; DFS: disease free survival; PFS: Progression free Survival  \* Descriptive statistics are expressed as median [interquartile range] or mean (standard deviation) for quantitative variables, as absolute and percentage frequencies for qualitative variables  \*\* p-values were computed, as for qualitative variables, by the Chi-square test. For quantitative variables ANOVA (if normally distributed) or Kruskal-Wallis test were used | | | | | |

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| **Table 2.** Survival Analysis (n=202). | | | |
|  | **Ordinary Cox models** | **Interaction Cox models** | |
|  |  | **Predictor main effect** | **Predictor x BRCA interaction** |
|  | **HR (95% CI); p** | **HR (95% CI); p** | **IHR (95% CI); p** |
| ***Death (primary outcome)*** |  |  |  |
| BRCA (Ref.=wt) | **grp1 0.30 (0.14; 0.64); 0.002**  *grp2 0.44 (0.18; 1.04); 0.060* | - | - |
| Nucleus AR score | 0.92 (0.80; 1.05); 0.215 | 0.89 (0.75; 1.05); 0.166 | grp1 1.05 (0.70; 1.58); 0.810  grp2 1.22 (0.84; 1.79); 0.295 |
| PR score | **0.87 (0.77; 0.98); 0.024** | 0.93 (0.82; 1.06); 0.293 | grp1 0.87 (0.60; 1.25); 0.446  grp2 0.91 (0.49; 1.68); 0.758 |
| ER-α score | 0.98 (0.91; 1.06); 0.631 | 0.95 (0.88; 1.02); 0.181 | grp1 1.07 (0.83; 1.38); 0.620  grp2 1.12 (0.81; 1.56); 0.495 |
| Nucleus ER-β1 score | 1.06 (0.98; 1.14); 0.153 | 1.01 (0.93; 1.10); 0.797 | grp1 1.10 (0.83; 1.46); 0.505  grp2 1.08 (0.84; 1.38); 0.553 |
| Cytoplasm ER-β1 score | 0.99 (0.90; 1.09); 0.786 | 0.96 (0.85; 1.09); 0.505 | grp1 1.12 (0.85; 1.47); 0.414  grp2 1.20 (0.89; 1.62); 0.234 |
| Nucleus ER-β2 score | 1.00 (0.93; 1.08); 0.990 | 0.96 (0.89; 1.04); 0.355 | grp1 1.07 (0.86; 1.34); 0.539  grp2 1.12 (0.85; 1.47); 0.417 |
| Cytoplasm ER-β2 score | 1.05 (0.94; 1.17); 0.370 | 1.03 (0.92; 1.16); 0.598 | grp1 1.10 (0.79; 1.53); 0.562  grp2 1.12 (0.72; 1.73); 0.616 |
| Nucleus ER-β5 score | 0.99 (0.91; 1.07); 0.724 | 0.98 (0.90; 1.07); 0.650 | grp1 0.90 (0.69; 1.15); 0.392  grp2 1.20 (0.90; 1.58); 0.211 |
| Cytoplasm ER-β5 score | **0.84 (0.71; 1.00); 0.049** | 0.89 (0.75; 1.06); 0.195 | grp1 0.69 (0.34; 1.41); 0.310  grp2 1.32 (0.66; 2.65); 0.428 |
| ER-α/Nucleus ER-β1 ratio | 0.97 (0.93; 1.03); 0.340 | 0.98 (0.94; 1.03); 0.443 | grp1 0.83 (0.42; 1.66); 0.596  grp2 0.94 (0.64; 1.38); 0.739 |
| ER-α/Nucleus ER-β2 ratio | 0.97 (0.88; 1.07); 0.552 | 0.97 (0.87; 1.09); 0.602 | grp1 0.81 (0.30; 2.21); 0.688  grp2 0.66 (0.14; 3.19); 0.609 |
| ER-α/Nucleus ER-β5 ratio | 0.99 (0.96; 1.02); 0.580 | 0.86 (0.66; 1.13); 0.286 | grp1 1.19 (0.91; 1.57); 0.209  grp2 0.92 (0.32; 2.68); 0.880 |
| PFS last platinum | **0.77 (0.72; 0.82); <0.001** | **0.78 (0.72; 0.84); <0.001** | grp1 1.07 (0.93; 1.23); 0.364  grp2 0.79 (0.59; 1.07); 0.135 |
| Platinum Resistance | **11.42 (6.76; 19.29); <0.001** | **8.76 (4.76; 16.12); <0.001** | grp10.74 (0.15; 3.56); 0.704  grp2 2.66 (0.40; 17.34); 0.306 |
| Chir\_ascites | **2.23 (1.34; 3.69); 0.002** | *1.74 (1.00; 3.03); 0.051* | grp1 2.00 (0.36; 11.00); 0.427  grp2 2.03 (0.22; 19.20); 0.536 |
| Chir\_pi\_lps score | **1.13 (1.06; 1.21); <0.001** | **1.12 (1.05; 1.20); 0.001** | grp1 1.04 (0.83; 1.31); 0.706  grp2 1.03 (0.78; 1.37); 0.811 |
| BMI at baseline | 1.02 (0.97; 1.06); 0.466 | 1.01 (0.95; 1.08); 0.685 | grp10.93 (0.84; 1.03); 0.191  grp2 1.04 (0.90; 1.20); 0.584 |
| Menopausa | **2.31 (1.24; 4.31); 0.008** | 0.72 (0.30; 1.73); 0.467 | grp1 2.09 (0.33; 13.26); 0.436  grp2 2.92 (0.22; 38.18); 0.415 |
| ct\_beva | **0.42 (0.25; 0.72); 0.002** | 0.68 (0.37; 1.25); 0.215 | grp10.31 (0.06; 1.74); 0.183  grp2 0.87 (0.09; 8.28); 0.903 |
| Cytoreduction | **0.09 (0.05; 0.17); <0.001** | **0.11 (0.06; 0.21); <0.001** | grp1 2.64 (0.27; 25.54); 0.402  grp2 0.27 (0.04; 1.68); 0.159 |
| ***Secondary outcomes*** |  |  |  |
| ***DFS Relapse*** |  |  |  |
| BRCA (Ref.=wt) | **grp1 0.59 (0.38; 0.90); 0.015**  grp2 0.76 (0.46; 1.26); 0.287 | - | - |
| Nucleus AR score | 0.99 (0.90; 1.08); 0.771 | 0.93 (0.82; 1.04); 0.193 | *grp1 1.21 (0.98; 1.49); 0.083*  grp2 1.11 (0.88; 1.41); 0.380 |
| PR score | 0.97 (0.90; 1.04); 0.326 | 0.96 (0.88; 1.05); 0.330 | grp11.10 (0.95; 1.29); 0.215  grp2 0.98 (0.75; 1.29); 0.903 |
| ER-α score | 1.02 (0.97; 1.07); 0.465 | 0.99 (0.93; 1.05); 0.674 | grp1*1.10 (0.94; 1.27); 0.230*  grp2 1.05 (0.88; 1.25); 0.571 |
| Nucleus ER-β1 score | 1.02 (0.97; 1.08); 0.481 | 1.02 (0.95; 1.09); 0.594 | grp10.98 (0.84; 1.14); 0.787  grp2 0.96 (0.82; 1.13); 0.632 |
| Cytoplasm ER-β1 score | 0.99 (0.93; 1.06); 0.851 | 0.96 (0.88; 1.05); 0.384 | grp11.07 (0.91; 1.25); 0.401  grp2 1.18 (0.96; 1.46); 0.123 |
| Nucleus ER-β2 score | 0.99 (0.94; 1.04); 0.754 | 0.97 (0.91; 1.03); 0.290 | grp11.00 (0.88; 1.14); 0.975  grp2 1.07 (0.92; 1.24); 0.400 |
| Cytoplasm ER-β2 score | 0.96 (0.89; 1.04); 0.321 | 0.92 (0.84; 1.02); 0.107 | grp1 1.03 (0.84; 1.26); 0.780  *grp2 1.31 (0.99; 1.73); 0.064* |
| Nucleus ER-β5 score | 0.99 (0.94; 1.04); 0.657 | 1.00 (0.93; 1.07); 0.985 | grp10.99 (0.87; 1.13); 0.877  grp2 0.93 (0.78; 1.11); 0.405 |
| Cytoplasm ER-β5 score | 0.97 (0.87; 1.08); 0.550 | 1.01 (0.90; 1.14); 0.816 | grp10.88 (0.67; 1.16); 0.362  grp2 0.97 (0.63; 1.50); 0.907 |
| ER-α/Nucleus ER-β1 ratio | 1.00 (0.98; 1.01); 0.565 | 0.99 (0.97; 1.01); 0.410 | grp1 1.15 (0.96; 1.38); 0.125  grp2 1.01 (0.97; 1.05); 0.738 |
| ER-α/Nucleus ER-β2 ratio | 1.01 (0.98; 1.03); 0.641 | 1.01 (0.98; 1.03); 0.606 | *grp1 1.02 (1.01; 1.04); 0.057*  grp2 1.38 (0.99; 1.92); 0.801 |
| ER-α/Nucleus ER-β5 ratio | 1.01 (0.99; 1.02); 0.520 | 1.00 (0.98; 1.02); 0.791 | grp1 1.02 (0.98; 1.05); 0.381  grp2 1.08 (0.98; 1.19); 0.118 |
| PFS last platinum | **0.79 (0.76; 0.81); <0.001** | **0.78 (0.76; 0.81); <0.001** | grp10.98 (0.94; 1.03); 0.500  grp2 0.99 (0.94; 1.04); 0.599 |
| Platinum Resistance | **16.83 (10.86; 26.10); <0.001** | **16.38 (9.95; 26.97); <0.001** | grp1 1.26 (0.43; 3.65); 0.675  grp2 1.10 (0.33; 3.66); 0.877 |
| Chir\_ascites | **2.17 (1.55; 3.05); <0.001** | **1.91 (1.28; 2.86); 0.002** | grp1 1.45 (0.60; 3.53); 0.413  grp2 1.05 (0.38; 2.91); 0.926 |
| Chir\_pi\_lps score | **1.13 (1.08; 1.18); <0.001** | **1.15 (1.09; 1.21); <0.001** | grp10.97 (0.87; 1.08); 0.568  grp2 0.91 (0.79; 1.05); 0.216 |
| BMI at baseline | 1.00 (0.97; 1.03); 0.942 | 1.01 (0.97; 1.06); 0.666 | grp10.96 (0.88; 1.04); 0.267  grp2 0.99 (0.92; 1.06); 0.712 |
| Menopausa | 1.27 (0.89; 1.83); 0.194 | **0.52 (0.29; 0.94); 0.029** | grp1 2.07 (0.82; 5.25); 0.124  grp2 2.36 (0.76; 7.27); 0.136 |
| ct\_beva | 0.81 (0.58; 1.14); 0.225 | 1.04 (0.68; 1.58); 0.871 | grp10.75 (0.31; 1.82); 0.523  grp2 0.89 (0.32; 2.49); 0.820 |
| Cytoreduction | **0.02 (0.01; 0.04); <0.001** | **0.02 (0.01; 0.05); <0.001** | grp1^Inf (^Inf; ^Inf); n.a.  grp2 0.25 (0.05; 1.31); 0.101 |
| **Abbreviations:** MM: Molecular Marker; wt: wild type; AR: androgen receptor; PR: progesterone receptor; ER: oestrogen receptor; OS: overall survival; DFS: disease free survival; PFS: Progression Free Survival; HR: Hazard ratio; IHR: Interaction Hazard Ratio; 95%CI: 95% Confidence Interval; Ref.: Reference; ^Inf: Infinite (due to poor or null variability within predictors); n.a.: not applicable  \*In **bold** the significant results (p<0.05), in *italic* the suggestive results (0.05<p<0.10). | | | |

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| **Table 3.** Logistic Regression (n=202). | | | | | |
|  | **Univariable Analysis** | | | **Multivariable Analysis** | |
|  | **Platinum Resistance** | |  | **Predictor main effect** | **Predictor x BRCA interaction** |
|  | **Yes (n=61)** | **No (n=141)** | **OR (95% CI); p** | **OR (95% CI); p** | **IOR (95% CI); p** |
| Age | 65 (59-71) | 56 (49-65) | **1.05 (1.02; 1.08); 0.002** | - | - |
| BRCA (Ref=wt)  *BRCA 1*  *BRCA 2* | 5 (8.2)  5 (8.2) | 39 (27.7)  21 (14.9) | **0.20 (0.08; 0.55); 0.002**  *0.38 (0.13; 1.07); 0.066* | **-** | - |
| BMI at baseline | 23.8 (21.2-27.9) | 24.1 (21.6-28) | 0.99 (0.94; 1.04); 0.687 | 0.97 (0.90; 1.05); 0.504 | grp1 0.95 (0.82; 1.10); 0.511  grp2 1.03 (0.88; 1.21); 0.682 |
| Menopausa | 43 (81.1) | 92 (68.2) | **2.27 (1.08; 4.77); 0.030** | 0.94 (0.30; 2.95); 0.916 | grp1 0.65 (0.08; 5.35); 0.691  grp2 ^Inf (0.00; ^Inf); 0.986 |
| Nucleus AR score | 0 (0-1) | 0 (0-2) | 0.97 (0.82; 1.14); 0.694 | 0.89 (0.72; 1.10); 0.275 | grp1 1.43 (0.92; 2.21); 0.111  grp2 1.17 (0.75; 1.84); 0.489 |
| PR score | 1 (0-2) | 1 (0-4) | **0.82 (0.70; 0.96); 0.016** | 0.87 (0.73; 1.04); 0.137 | grp1 0.86 (0.49; 1.51); 0.606  grp2 0.92 (0.45; 1.87); 0.821 |
| ER-α score | 4 (2-8) | 4 (2-6) | 1.05 (0.96; 1.15); 0.259 | 1.01 (0.91; 1.12); 0.839 | grp1 1.17 (0.83; 1.65); 0.370  grp2 1.17 (0.77; 1.79); 0.457 |
| Nucleus ER-β1 score | 4 (3-8) | 6 (3-8) | 0.98 (0.89; 1.08); 0.689 | 0.96 (0.85; 1.07); 0.440 | grp1 0.81 (0.54; 1.23); 0.331  grp2 1.10 (0.80; 1.52); 0.568 |
| Cytoplasm ER-β1 score | 2 (0-3) | 3 (0-3) | 0.91 (0.79; 1.04); 0.158 | **0.83 (0.69; 0.99); 0.043** | grp1 0.99 (0.60; 1.63); 0.969  **grp2 1.61 (1.07; 2.44); 0.023** |
| Nucleus ER-β2 score | 6.5 (3.3) | 7.2 (3.3) | 0.94 (0.86; 1.03); 0.184 | *0.91 (0.81; 1.02); 0.090* | grp1 0.98 (0.72; 1.33); 0.889  grp2 1.19 (0.85; 1.65); 0.306 |
| Cytoplasm ER-β2 score | 2 (0-3) | 2 (0-3) | 0.92 (0.80; 1.07); 0.288 | 0.89 (0.75; 1.06); 0.183 | grp1 1.23 (0.76; 1.99); 0.406  grp2 1.13 (0.59; 2.17); 0.717 |
| Nucleus ER-β5 score | 6.1 (2.6) | 6.1 (3.1) | 1.00 (0.91; 1.11); 0.927 | 0.95 (0.83; 1.08); 0.450 | grp1 1.21 (0.88; 1.66); 0.252  grp2 1.24 (0.88; 1.74); 0.221 |
| Cytoplasm ER-β5 score | 0 (0-0) | 0 (0-3) | 0.89 (0.73; 1.08); 0.238 | 0.89 (0.72; 1.12); 0.332 | grp1 0.89 (0.42; 1.88); 0.764  grp2 1.68 (0.80; 3.52); 0.172 |
| ER-α/Nucleus ER-β1 ratio | 1.0 (0.3-2.7) | 0.8 (0.4-1.5) | 0.99 (0.95; 1.03); 0.619 | 0.99 (0.95; 1.03); 0.657 | grp1 1.71 (0.78; 3.76); 0.182  grp2 0.94 (0.74; 1.20); 0.632 |
| ER-α/Nucleus ER-β2 ratio | 0.8 (0.3-1.5) | 0.5 (0.3-1.0) | 1.00 (0.95; 1.05); 0.984 | 1.00 (0.94; 1.06); 0.904 | grp1 2.07 (0.84; 5.06); 0.111  grp2 0.78 (0.15; 4.19); 0.774 |
| ER-α/Nucleus ER-β5 ratio | 0.8 (0.3-1.3) | 0.8 (0.3-1.3) | 0.98 (0.93; 1.03); 0.364 | 0.98 (0.93; 1.02); 0.346 | grp1 0.73 (0.21; 2.56); 0.628  grp2 0.86 (0.32; 2.31); 0.766 |
| Chir\_ascite | 37 (69.8) | 66 (48.9) | **2.44 (1.29; 4.61); 0.006** | 1.68 (0.81; 3.50); 0.165 | grp1 ^Inf (0.00; ^Inf); 0.985  grp2 0.27 (0.23; 32.52); 0.430 |
| Chir\_pi\_lps\_score | 7.2 (3.1) | 5.2 (3.9) | **1.16 (1.07; 1.27); 0.001** | **1.16 (1.04; 1.28); 0.006** | grp1 1.09 (0.78; 1.52); 0.631  grp2 1.14 (0.78, 1.67); 0.501 |
| ct\_Beva | 10 (18.9) | 68 (50.4) | **0.24 (0.12; 0.49); <0.001** | **0.37 (0.16; 0.85); 0.018** | grp1 ^Inf (0.00; ^Inf); 0.984  grp2 1.32 (0.11; 16.12); 0.829 |
| Cytoreduction | 38 (71.7) | 134 (99.3) | **0.05 (0.01; 0.18); <0.001** | **0.06 (0.01; 0.26); <0.001** | grp1 ^Inf (0.00; ^Inf); 0.990  grp2 ^Inf (0.00; ^Inf); 0.990 |
| **Abbreviations:** wt: wild type; AR: androgen receptor; PR: progesterone receptor; ER: oestrogen receptor; OR: Odd ratio; 95%CI: 95% Confidence Interval; Ref.: Reference; ^Inf: Infinite (due to poor or null variability within predictors);  \*In **bold** the significant results (p<0.05), in *italic* the suggestive results (0.05<p<0.10). | | | | | |

***BRCA wild-type vs. BRCA mutated (1 or 2)***

Indeed, looking at BRCA wt vs. BRCA mutated (BRCA 1 or BRCA 2), these latter were significantly younger (p=0.012) and had a lower platinum resistance rate (14.3% vs. 38.6%, p<0.001). As well, they further disclosed a higher overall survival (80% vs. 57.6%, p<0.001). The whole data are reported in Supplementary Table 3.

Concerning age-adjusted interaction Cox regression models, with regard to both OS and DFS, as reported in Supplementary Table 4, we did only find a potential protective effect of PFS (HR 0.78; p<0.001 both related to OS and DFS) and cytoreduction (HR 0.11; p<0.111 related to OS and HR 0.02; p<0.001 related to DFS). Conversely, platinum resistance (HR 8.59; p<0.001 as for OS and HR 16.29; p<0.001 for DFS) and chir\_pi\_lps\_score (HR 1.12; p=0.001 as for OS and HR 1.15; p<0.001 for DFS) seem to exert a negative role, whilst ascites (HR 1.91; p=0.002) and menopausal status only on DFS (HR 0.51; p=0.025).

Of note, in addition, that BRCA mutation resulted in the vast majority of models either in a significant or suggestive protective effect, whilst, as for the interaction effects, none significant finding emerged, except for a negative prognostic role of menopausal status in BRCA mutated (IHR 2.20, p=0.047). All data are reported in Supplementary Table 1.

As for platinum resistance, instead, at univariable analysis, the logistic regression model disclosed BRCA mutation as protective towards platinum resistance onset (OR 0.26; p=0.001). Likewise, also PR score (OR 0.82; p=0.016), ct\_Beva (OR 0.24; p<0.001) and cytoreduction (OR 0.05; p<0.001) disclosed a similar behaviour. Conversely, ascites (OR 2.44; p=0.006), chir\_pi\_lps\_score (OR 1.16; p=0.001) and menopausal status (OR 2.27; p=0.030) revealed as significantly involved in platinum resistance onset (Supplementary Table 5). The age-adjusted interaction logistic regression models further assessed the main effects of MM (i.e., within wild-type condition) and clinical conditions. In depth, among MM, only Cytoplasm ER-β1 score (OR 0.83; p=0.043), cytoreduction (OR 0.06; p<0.001) and ct\_beva (OR 0.37; p=0.018) revealed a protective role towards platinum resistance onset, whilst chir\_pi\_lps score disclosed a negative effect on the outcome (OR 1.16; p=0.06). As for the interaction effects, none molecular marker/clinical predictor revealed a significant effect on platinum resistance (Supplementary Table 5).

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| **Supplementary Table 1.** BRCA main effects in theInteraction Cox Models (n=202). | | |
|  | **Interaction Cox models** | |
|  | **BRCA1 vs. BRCA wt**  **BRCA2 vs. BRCA wt**  **(Ref.=0 [wt])** | **BRCA (1 or 2) vs. BRCA wt**  **(Ref.=0 [wt])** |
|  | **HR (95% CI); p** | **HR (95% CI); p** |
| ***Death (primary outcome)*** |  |  |
| BRCA (Ref.=wt) | - | - |
| Nucleus AR score | **grp1 0.38 (0.16; 0.93); 0.034**  *grp2 0.35 (0.12; 1.02); 0.055* | **0.36 (0.18; 0.74); 0.005** |
| PR score | grp1 0.53 (0.21; 1.35); 0.182  **grp**20.50 (0.15; 1.61); 0.244 | *0.52 (0.25; 1.10); 0.087* |
| ER-α score | grp1 0.29 (0.06; 1.30); 0.106  grp20.28 (0.06; 1.34); 0.110 | **0.30 (0.10; 0.90); 0.032** |
| Nucleus ER-β1 score | grp1 0.23 (0.03; 1.47); 0.119  grp20.32 (0.07; 1.53); 0.152 | *0.27 (0.07; 1.02); 0.052* |
| Cytoplasm ER-β1 score | **grp1 0.29 (0.09; 0.88); 0.030**  *grp2**0.28 (0.08; 1.01); 0.051* | **0.28 (0.12; 0.70); 0.006** |
| Nucleus ER-β2 score | grp1 0.24 (0.04; 1.48); 0.123  grp20.19 (0.02; 1.97); 0.166 | **0.22 (0.05; 0.99); 0.048** |
| Cytoplasm ER-β2 score | *grp1 0.31 (0.09; 1.05); 0.059*  grp2 0.40 (0.12; 1.30); 0.127 | **0.36 (0.15; 0.88); 0.025** |
| Nucleus ER-β5 score | grp1 0.69 (0.16; 3.01); 0.621  *grp2 0.15 (0.02; 1.18); 0.072* | **0.40 (0.11; 1.43); 0.158** |
| Cytoplasm ER-β5 score | *grp1 0.50 (0.23; 1.12); 0.093*  **grp2 0.35 (0.13; 0.98); 0.045** | **0.44 (0.23; 0.85); 0.014** |
| ER-α/Nucleus ER-β1 ratio | grp1 0.45 (0.15; 1.31); 0.141  grp2 0.35 (0.10; 1.28); 0.113 | *0.49 (0.22; 1.05); 0.066* |
| ER-α/Nucleus ER-β2 ratio | grp1 0.47 (0.16; 1.40); 0.176  grp2 0.56 (0.17; 1.87); 0.348 | 0.51 (0.22; 1.19); 0.118 |
| ER-α/Nucleus ER-β5 ratio | **grp1 0.22 (0.10; 0.51); <0.001**  grp2 0.46 (0.15; 1.42); 0.177 | **0.32 (0.16; 0.62); <0.001** |
| PFS last platinum | grp1 0.37 (0.11; 1.23); 0.104  grp2 2.44 (0.40; 14.85); 0.334 | 0.52 (0.18; 1.50); 0.225 |
| Platinum Resistance | grp1 0.62 (0.22; 1.77); 0.368  grp2 0.40 (0.09; 1.88); 0.248 | 0.55 (0.21; 1.42); 0.217 |
| Chir\_ascites | *grp1 0.25 (0.06; 1.10); 0.067*  grp2 0.25 (0.03; 1.89); 0.179 | **0.25 (0.07; 0.86); 0.027** |
| Chir\_pi\_lps score | grp1 0.28 (0.05; 1.73); 0.174  grp2 0.33 (0.03; 3.18); 0.337 | 0.30 (0.07; 1.34); 0.115 |
| BMI at baseline | grp1 2.05 (0.17; 24.61); 0.571  grp2 0.16 (0.01; 7.86); 0.359 | 0.74 (0.07; 7.41); 0.794 |
| Menopausa | *grp1 0.22 (0.04; 1.14); 0.072*  grp2 0.18 (0.02; 2.01); 0.164 | **0.21 (0.05; 0.96); 0.044** |
| ct\_beva | grp1 0.65 (0.27; 1.55); 0.330  *grp2 0.43 (0.16; 1.17); 0.098* | *0.54 (0.27; 1.07); 0.076* |
| Cytoreduction | *grp1 0.17 (0.02; 1.35); 0.093*  grp2 1.43 (0.32; 6.44); 0.640 | 0.44 (0.12; 1.59); 0.209 |
| ***Secondary outcomes*** |  |  |
| ***DFS Relapse*** |  |  |
| BRCA (Ref.=wt) | - | - |
| Nucleus AR score | **grp1 0.53 (0.31; 0.91); 0.022**  grp2 0.72 (0.39; 1.31); 0.279 | **0.60 (0.39; 0.93); 0.022** |
| PR score | **grp1 0.54 (0.29; 0.98); 0.043**  grp2 0.85 (0.42; 1.73); 0.653 | *0.63 (0.39; 1.03); 0.064* |
| ER-α score | *grp1 0.45 (0.19; 1.03); 0.059*  grp20.67 (0.30; 1.53); 0.346 | *0.55 (0.29; 1.03); 0.062* |
| Nucleus ER-β1 score | grp 1 0.76 (0.28; 2.05); 0.590  grp 20.99 (0.41; 2.37); 0.982 | 0.88 (0.43; 1.83); 0.740 |
| Cytoplasm ER-β1 score | *grp 1 0.56 (0.30; 1.04); 0.067*  grp2*0.58 (0.29; 1.17); 0.127* | **0.59 (0.36; 0.98); 0.040** |
| Nucleus ER-β2 score | grp1 0.66 (0.25; 1.79); 0.419  grp20.52 (0.16; 1.69); 0.279 | 0.60 (0.26; 1.38); 0.228 |
| Cytoplasm ER-β2 score | grp1 0.64 (0.34; 1.20); 0.163  *grp2 0.54 (0.27; 1.06); 0.075* | *0.61 (0.37; 1.00); 0.052* |
| Nucleus ER-β5 score | grp1 0.71 (0.29; 1.78); 0.469  grp2 1.20 (0.43; 3.38); 0.725 | 0.88 (0.41; 1.87); 0.738 |
| Cytoplasm ER-β5 score | grp1 0.78 (0.46; 1.31); 0.340  grp2 0.83 (0.48; 1.45); 0.518 | 0.80 (0.53; 1.22); 0.301 |
| ER-α/Nucleus ER-β1 ratio | **grp1 0.57 (0.34; 0.94); 0.028**  grp2 0.80 (0.47; 1.37); 0.420 | *0.70 (0.48; 1.03); 0.070* |
| ER-α/Nucleus ER-β2 ratio | **grp1 0.52 (0.30; 0.91); 0.021**  grp2 0.78 (0.40; 1.52); 0.466 | **0.60 (0.38; 0.95); 0.030** |
| ER-α/Nucleus ER-β5 ratio | *grp1 0.64 (0.40; 1.02); 0.060*  grp2 0.73 (0.43; 1.26); 0.258 | *0.70 (0.48; 1.02); 0.061* |
| PFS last platinum | grp1 1.56 (0.73; 3.32); 0.254  grp2 1.43 (0.57; 3.55); 0.443 | 1.51 (0.81; 2.81); 0.199 |
| Platinum Resistance | grp1 0.87 (0.52; 1.47); 0.611  grp2 1.12 (0.62; 2.05); 0.702 | 0.96 (0.62; 1.50); 0.863 |
| Chir\_ascites | grp1 0.57 (0.28; 1.15); 0.118  grp2 0.83 (0.39; 1.79); 0.635 | 0.66 (0.37; 1.18); 0.159 |
| Chir\_pi\_lps score | grp1 0.77 (0.34; 1.73); 0.523  grp2 1.25 (0.44; 3.61); 0.676 | 0.90 (0.45; 1.79); 0.752 |
| BMI at baseline | grp1 2.06 (0.28; 15.12); 0.476  grp2 1.13 (0.20; 6.37); 0.890 | 1.43 (0.32; 6.30); 0.637 |
| Menopausa | **grp1 0.42 (0.20; 0.89); 0.023**  *grp2 0.44 (0.17; 1.15); 0.093* | **0.43 (0.22; 0.81); 0.010** |
| ct\_beva | grp1 0.80 (0.41; 1.55); 0.507  grp2 0.86 (0.45; 1.64); 0.640 | 0.83 (0.50; 1.37); 0.460 |
| Cytoreduction | grp1 0.77 (0.49; 1.21); 0.251  grp2 3.39 (0.71; 16.18); 0.127 | 3.38 (0.71; 16.16); 0.127 |
| **Abbreviations:** MM: Molecular Marker; wt: wild type; AR: androgen receptor; PR: progesterone receptor; ER: oestrogen receptor; OS: overall survival; DFS: disease free survival; PFS: Progression Free Survival; HR: Hazard ratio; IHR: Interaction Hazard Ratio; 95%CI: 95% Confidence Interval; Ref.: Reference; ^Inf: Infinite (due to poor or null variability within predictors); n.a.: not applicable  \*In **bold** the significant results (p<0.05), in *italic* the suggestive results (0.05<p<0.10). | | |

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| **Supplementary Table 2.** BRCA main effect in thelogistic regression Interaction Models (n=202). | | |
|  | **Logistic Regression Interaction Model** | |
|  | **BRCA1 vs. BRCA wt**  **BRCA2 vs. BRCA wt**  **(Ref.=0 [wt])** | **BRCA (1 or 2) vs BRCA wt**  **(Ref.=0 [wt])** |
|  | **HR (95% CI); p** | **HR (95% CI); p** |
| Age | - | - |
| BRCA (Ref.=wt) | - | - |
| BMI at baseline | grp1 0.80 (0.02; 31.34);0.906  *grp2 0.17 (0.01; 8.82); 0.376* | 0.39 (0.02; 7.60); 0.531 |
| Menopausa | grp1 0.32 (0.06; 1.67); 0.175  grp2 ^Inf (0.00; ^Inf); 0.985 | *0.21 (0.04; 1.09); 0.063* |
| Nucleus AR score | **grp1 0.14 (0.04; 0.54); 0.004**  *grp2 0.31 (0.09; 1.08); 0.066* | **0.21 (0.08; 0.53); 0.001** |
| PR score | *grp1 0.32 (0.09; 1.14); 0.079*  grp20.42 (0.10; 1.71); 0.224 | **0.37 (0.14; 0.98); 0.045** |
| ER-α score | **grp1 0.11 (0.01; 0.98); 0.048**  grp20.21 (0.03; 1.64); 0.135 | **0.16 (0.04; 0.75); 0.020** |
| Nucleus ER-β1 score | grp1 0.66 (0.08; 5.45); 0.699  grp20.24 (0.04; 1.48); 0.124 | 0.35 (0.08; 1.51); 0.159 |
| Cytoplasm ER-β1 score | **grp1 0.24 (0.06; 0.99); 0.048**  **grp2 0.11 (0.02; 0.58); 0.009** | **0.16 (0.05; 0.49); 0.001** |
| Nucleus ER-β2 score | grp1 0.27 (0.03; 2.43); 0.245  grp20.11 (0.01; 1.58); 0.105 | *0.19 (0.03; 1.09); 0.063* |
| Cytoplasm ER-β2 score | **grp1 0.15 (0.03; 0.75); 0.020**  *grp2 0.29 (0.07; 1.14); 0.077* | **0.22 (0.08; 0.65); 0.006** |
| Nucleus ER-β5 score | **grp1 0.08 (0.01; 0.81); 0.033**  *grp2 0.10 (0.01; 1.22); 0.072* | **0.09 (0.01; 0.54); 0.009** |
| Cytoplasm ER-β5 score | **grp1 0.27 (0.09; 0.83); 0.022**  **grp2 0.24 (0.07; 0.90); 0.034** | **0.26 (0.11; 0.64); 0.003** |
| ER-α/Nucleus ER-β1 ratio | **grp1 0.11 (0.02; 0.50); 0.004**  grp2 0.45 (0.14; 1.39); 0.166 | **0.30 (0.13; 0.66); 0.003** |
| ER-α/Nucleus ER-β2 ratio | **grp1 0.11 (0.03; 0.50); 0.003**  grp2 0.44 (0.10; 1.96); 0.283 | **0.19 (0.07; 0.53); 0.001** |
| ER-α/Nucleus ER-β5 ratio | grp1 0.34 (0.08; 1.48); 0.152  grp2 0.42 (0.12; 1.53); 0.189 | *0.38 (0.13; 1.09); 0.071* |
| Chir\_ascite | grp1 ^Inf (0.00; ^Inf); 0.984  grp2 0.19 (0.02; 1.63); 0.131 | **0.07 (0.01; 0.60); 0.014** |
| Chir\_pi\_lps\_score | grp1 0.12 (0.01; 1.97); 0.139  grp2 0.12 (0.01; 3.03); 0.200 | *0.12 (0.01; 1.06); 0.057* |
| ct\_Beva | grp1 0.48 (0.15; 1.50); 0.208  *grp2 0.33 (0.10; 1.12); 0.075* | **0.40 (0.17; 0.97); 0.043** |
| Cytoreduction | grp1 ^Inf (0.00; ^Inf); 0.990  grp2 ^Inf (0.00; ^Inf); 0.990 | 0.16 (0.01; 2.88); 0.217 |
| **Abbreviations:** wt: wild type; AR: androgen receptor; PR: progesterone receptor; ER: oestrogen receptor; OR: Odd ratio; 95%CI: 95% Confidence Interval; Ref.: Reference;  \*In **bold** the significant results (p<0.05), in *italic* the suggestive results (0.05<p<0.10). | | |

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| **Supplementary Table 3.** General characteristics of the study population according to BRCA wt and BRCA mutated (1 or 2) (n=202).\* | | | | |
|  | **Overall** | **BRCA mutation** | | |
|  |  | **wtBRCA**  **(n=132)** | **BRCA 1 or 2**  **(n=70)** | **p\*\*** |
| Age (yrs.) | 59.1 (11.5) | 60.5 (11.4) | 56.5 (11.1) | **0.012** |
| Baseline BMI | 24.1 (21.5-28) | 24.2 (21.7-27.9) | 24 (20.4-28.4) | 0.541 |
| Menopausa | 144 (71.3) | 100 (75.8) | 44 (62.9) | *0.054* |
| Nucleus AR score | 0 (0-2) | 0 (0-2) | 0 (0-2) | 0.821 |
| PR score | 1 (0-3) | 1 (0-2.5) | 1 (0-4) | 0.145 |
| ER-α score | 4 (2-8) | 4 (2-8) | 3 (1-6) | 0.139 |
| Nucleus ER-β1 score | 4 (3-8) | 5 (3-8) | 4 (3-8) | 0.218 |
| Cytoplasm ER-β1 score | 3 (0-3) | 3 (0-3) | 2 (0-3) | 0.519 |
| Nucleus ER-β2 score | 8 (4-8) | 7.1 (3.3) | 6.9 (3.3) | 0.535 |
| Cytoplasm ER-β2 score | 2 (0-3) | 2 (0-3) | 2 (0-3) | 0.509 |
| Nucleus ER-β5 score | 6 (4-8) | 6.3 (2.8) | 5.8 (3.3) | 0.155 |
| Cytoplasm ER-β5 score | 0 (0-3) | 0 (0-3) | 0 (0-3) | 0.799 |
| ER-α/Nucleus ER-β1 ratio | 0.8 (0.4-2.0) | 0.9 (0.3-2.0) | 0.8 (0.4-1.2) | 0.784 |
| ER-α/Nucleus ER-β2 ratio | 0.7 (0.3-1.1) | 0.7 (0.3-1.3) | 0.7 (0.3-1.0) | 0.737 |
| ER-α/Nucleus ER-β5 ratio | 0.8 (0.3-1.3) | 0.8 (0.3-1.3) | 0.8 (0.4-1.5) | 0.505 |
| Chir\_ascite | 109 (54) | 74 (56.1) | 35 (50) | 0.988 |
| Chir\_pi\_lps\_score | 6 (2-8) | 5.8 (3.8) | 5.8 (3.7) | 0.919 |
| Ct\_Beva | 83 (41.1) | 49 (37.1) | 34 (48.6) | 0.115 |
| PFS since last platinum (mths) | 14 (5-26) | 10 (3-24) | 19 (9-27) | **0.002** |
| Histology stadium  *1*  *2*  *3*  *4* | 4 (2)  14 (6.9)  126 (62.4)  58 (28.7) | 2 (1.5)  10 (7.6)  81 (61.4)  39 (29.6) | 2 (2.9)  4 (5.7)  45 (64.3)  19 (27.1) | 0.850 |
| Ca125 at diagnosis | 869.1 (327-2127) | 994.8 (356-2261.8) | 601 (287-1682.4) | *0.085* |
| Cytoreduction  *Yes*  *No* | 181 (89.6)  21 (10.4) | 114 (86.4)  18 (14.6) | 67 (95.7)  3 (4.3) | **0.038** |
| **Outcomes** |  |  |  |  |
| ***Primary outcome*** |  |  |  |  |
| Overall Survival | 132 (65.4) | 76 (57.6) | 56 (80) | **0.001** |
| OS follow-up (mths) | 34 (21-43) | 33 (16.5-42) | 38.5 (32-46) | **<0.001** |
| ***Secondary outcome*** |  |  |  |  |
| DFS | 147 (72.8) | 102 (77.3) | 45 (64.3) | **0.048** |
| DFS follow-up (mths) | 19.5 (9-30) | 15 (7-29) | 23 (13-32) | **0.006** |
| Platinum resistance | 61 (30.2) | 51 (38.6) | 10 (14.3) | **<0.001** |
| **Abbreviations**: wt: wild-type; AR: androgen receptor; PR: progesterone receptor; ER: oestrogen receptor; OS: overall survival; DFS: disease free survival; PFS: Progression Free Survival  \* Descriptive statistics are expressed as median (interquartile range) or mean (standard deviation) for quantitative variables, as absolute and percentage frequencies for qualitative variables  \*\* p-values were computed, as for qualitative variables, by the Chi-square test. For quantitative variables Student t test (if normally distributed) or Mann Whitney U test were used | | | | |

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| --- | --- | --- | --- |
| **Supplementary Table 4.** Survival Analysis on BRCA wt vs. BRCA mutated (n=202). | | | |
|  | **Ordinary Cox models** | **Interaction Cox models** | |
|  |  | **Predictor main effect** | **Predictor x BRCA interaction** |
|  | **HR (95% CI); p** | **HR (95% CI); p** | **IHR (95% CI); p** |
| ***Death (primary outcome)*** |  |  |  |
| BRCA (Ref.=wt) | **0.35 (0.19; 0.64); 0.001** | - | - |
| Nucleus AR score | 0.92 (0.80; 1.05); 0.215 | 0.89 (0.75; 1.05); 0.165 | 1.13 (0.85; 1.52); 0.402 |
| PR score | **0.87 (0.77; 0.98); 0.024** | 0.93 (0.82; 1.06); 0.293 | 0.88 (0.63; 1.22); 0.427 |
| ER-α score | 0.98 (0.92; 1.06); 0.631 | 0.95 (0.88; 1.02); 0.180 | 1.08 (0.88; 1.32); 0.469 |
| Nucleus ER-β1 score | 1.06 (0.98; 1.14); 0.153 | 1.01 (0.93; 1.10); 0.800 | 1.08 (0.89; 1.32); 0.434 |
| Cytoplasm ER-β1 score | 0.99 (0.90; 1.09); 0.786 | 0.96 (0.85; 1.09); 0.512 | 1.15 (0.92; 1.43); 0.210 |
| Nucleus ER-β2 score | 1.00 (0.93; 1.08); 0.990 | 0.96 (0.89; 1.04); 0.355 | 1.09 (0.91; 1.31); 0.343 |
| Cytoplasm ER-β2 score | 1.05 (0.94; 1.17); 0.370 | 1.03 (0.92; 1.16); 0.597 | 1.08 (0.82; 1.44); 0.578 |
| Nucleus ER-β5 score | 0.99 (0.91; 1.07); 0.724 | 0.98 (0.90; 1.07); 0.661 | 1.01 (0.83; 1.22); 0.956 |
| Cytoplasm ER-β5 score | **0.84 (0.71; 1.00); 0.049** | 0.89 (0.75; 1.06); 0.195 | 0.87 (0.53; 1.46); 0.605 |
| ER-α/Nucleus ER-β1 ratio | 0.97 (0.93; 1.03); 0.340 | 0.98 (0.94; 1.03); 0.444 | 0.89 (0.58; 1.36); 0.593 |
| ER-α/Nucleus ER-β2 ratio | 0.97 (0.88; 1.07; 0.552 | 0.97 (0.87; 1.09); 0.603 | 0.76 (0.32; 1.79); 0.524 |
| ER-α/Nucleus ER-β5 ratio | 0.99 (0.96; 1.02); 0.580 | 0.85 (0.65; 1.12); 0.261 | 1.20 (0.91; 1.58); 0.201 |
| PFS last platinum | **0.77 (0.72; 0.82); <0.001** | **0.78 (0.72; 0.84); <0.001** | 1.02 (0.88; 1.18); 0.817 |
| Platinum resistance | **11.42 (6.76; 19.29);<0.001** | **8.59 (4.67; 15.78); <0.001** | 1.25 (0.36; 4.32); 0.728 |
| Chir\_ascite | **2.23 (1.34; 3.69); 0.002** | *1.74 (1.00; 3.03); 0.051* | 2.01 (0.50; 8.11); 0.325 |
| Chir\_pi\_lps\_score | **1.13 (1.06; 1.21); <0.001** | **1.12 (1.05; 1.20); 0.001** | 1.04 (0.87; 1.25); 0.665 |
| BMI at baseline | 1.02 (0.97; 1.06); 0.466 | 1.01 (0.95; 1.08); 0.691 | 0.98 (0.89; 1.07); 0.621 |
| Menopausa | **2.31 (1.24; 4.31); 0.008** | 0.72 (0.30; 1.71); 0.455 | 2.34 (0.45; 12.23); 0.315 |
| ct\_Beva | **0.42 (0.25; 0.72); 0.002** | 0.67 (0.36; 1.25); 0.207 | 0.45 (0.11; 1.84); 0.266 |
| Cytoreduction | **0.09 (0.05; 0.17); <0.001** | **0.11 (0.06; 0.21); <0.001** | 0.95 (0.21; 4.28); 0.951 |
| ***Secondary outcomes*** |  |  |  |
| ***DFS Relapse*** |  |  |  |
| BRCA (Ref.=wt) | **0.57 (0.40; 0.80); 0.001** | - | - |
| Nucleus AR score | 0.997 (0.90; 1.08); 0.771 | 0.93 (0.82; 1.04); 0.191 | *1.17 (0.98; 1.39); 0.089* |
| PR score | 0.97 (0.90; 1.04); 0.326 | 0.96 (0.88; 1.05); 0.333 | 1.07 (0.93; 1.24); 0.346 |
| ER-α score | 1.02 (0.97; 1.07); 0.465 | 0.99 (0.93; 1.05); 0.670 | 1.07 (0.95; 1.21); 0.258 |
| Nucleus ER-β1 score | 1.02 (0.97; 1.08); 0.481 | 1.02 (0.95; 1.09); 0.598 | 0.97 (0.86; 1.09); 0.556 |
| Cytoplasm ER-β1 score | 0.99 (0.93; 1.06); 0.851 | 0.96 (0.88 1.05); 0.389 | 1.09 (0.95; 1.25); 0.224 |
| Nucleus ER-β2 score | 0.99 (0.94; 1.04); 0.754 | 0.97 (0.91; 1.03); 0.290 | 1.03 (0.92; 1.14); 0.616 |
| Cytoplasm ER-β2 score | 0.96 (0.90; 1.04); 0.321 | 0.93 (0.84; 1.02); 0.111 | 1.08 (0.91; 1.28); 0.362 |
| Nucleus ER-β5 score | 0.99 (0.94; 1.04); 0.657 | 1.00 (0.93; 1.07); 0.990 | 0.97 (0.86; 1.08); 0.558 |
| Cytoplasm ER-β5 score | 0.97 (0.87; 1.08); 0.550 | 1.01 (0.90; 1.14); 0.815 | 0.90 (0.71; 1.13); 0.355 |
| ER-α/Nucleus ER-β1 ratio | 1.00 (0.98; 1.01); 0.565 | 0.99 (0.97; 1.01); 0.412 | 1.01 (0.97; 1.05); 0.537 |
| ER-α/Nucleus ER-β2 ratio | 1.01 (0.98; 1.03); 0.641 | 1.01 (0.98; 1.03); 0.600 | 1.28 (0.94; 1.75); 0.111 |
| ER-α/Nucleus ER-β5 ratio | 1.01 (0.99; 1.02); 0.520 | 1.00 (0.98; 1.02); 0.785 | 1.02 (0.99; 1.05); 0.312 |
| PFS last platinum | **0.79 (0.77; 0.81); 0.002** | **0.78 (0.76; 0.81); <0.001** | 0.99 (0.95; 1.02); 0.425 |
| Platinum resistance | **16.83 (10.86; 26.10); <0.001** | **16.29 (9.90; 26.80); <0.001** | 1.20 (0.51; 2.82); 0.671 |
| Chir\_ascite | **2.17 (1.55; 3.05); <0.001** | **1.91 (1.28; 2.86); 0.002** | 1.27 (0.61; 2.63); 0.520 |
| Chir\_pi\_lps\_score | **1.13 (1.08; 1.18); <0.001** | **1.15 (1.09; 1.21); <0.001** | 0.95 (0.87; 1.04); 0.294 |
| BMI at baseline | 1.00 (0.97; 1.03); 0.942 | 1.01 (0.97; 1.06); 0.668 | 0.97 (0.92; 1.03); 0.358 |
| Menopausa | 1.27 (0.89; 1.83); 0.194 | **0.51 (0.29; 0.92); 0.025** | **2.20 (1.01; 4.78); 0.047** |
| ct\_Beva | 0.81 (0.58; 1.14); 0.225 | 1.04 (0.68; 1.58); 0.867 | 0.78 (0.38; 1.60); 0.490 |
| Cytoreduction | **0.02 (0.01; 0.04); <0.001** | **0.02 (0.01; 0.05); <0.001** | *0.24 (0.05; 1.18); 0.079* |
| **Abbreviations:** MM: Molecular Marker; wt: wild type; AR: androgen receptor; PR: progesterone receptor; ER: oestrogen receptor; OS: overall survival; DFS: disease free survival; PFS: Progression free Survival HR: Hazard ratio; IHR: Interaction Hazard Ratio; 95%CI: 95% Confidence Interval; Ref.: Reference; ^Inf: Infinite (due to poor or null variability within predictors)  \*In **bold** the significant results (p<0.05), in *italic* the suggestive results (0.05<p<0.10). | | | |

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| **Supplementary Table 5.** Logistic Regression on BRCA wt vs. BRCA mutated (n=202). | | | | | |
|  | **Univariable Analysis** | | | **Interaction Model** | |
|  | **Platinum Resistance** | |  | **Predictor main effect** | **Predictor x BRCA interaction** |
|  | **Yes (n=61)** | **No (n=141)** | **OR (95% CI); p** | **OR (95% CI); p** | **IOR (95% CI); p** |
| Age | 65 (59-71) | 56 (49-65) | **1.05 (1.02; 1.08); 0.002** | - | - |
| BRCA (Ref.=wt) | 10 (16.4) | 60 (42.6) | **0.26 (0.12; 0.56); 0.001** | - | - |
| BMI at baseline | 23.8 (21.2-27.9) | 24.1 (21.6-28) | 0.99 (0.94; 1.04); 0.687 | 0.97 (0.90; 1.05); 0.498 | 0.99 (0.88; 1.11); 0.848 |
| Menopausa | 43 (81.1) | 92 (68.2) | **2.27 (1.08; 4.77); 0.030** | 0.89 (0.29; 2.80); 0.847 | 1.56 (0.24; 10.01); 0.640 |
| Nucleus AR score | 0 (0-1) | 0 (0-2) | 0.97 (0.82; 1.14); 0.694 | 0.89 (0.72; 1.10); 0.272 | 1.30 (0.92; 1.84); 0.142 |
| PR score | 1 (0-2) | 1 (0-4) | **0.82 (0.70; 0.96); 0.016** | 0.87 (0.73; 1.04); 0.138 | 0.88 (0.56; 1.37); 0.572 |
| ER-α score | 4 (2-8) | 4 (2-6) | 1.05 (0.96; 1.15); 0.259 | 1.01 (0.91; 1.12); 0.839 | 1.14 (0.87; 1.48); 0.340 |
| Nucleus ER-β1 score | 4 (3-8) | 6 (3-8) | 0.98 (0.89; 1.08); 0.689 | 0.96 (0.85; 1.07); 0.441 | 0.96 (0.74; 1.25); 0.760 |
| Cytoplasm ER-β1 score | 2 (0-3) | 3 (0-3) | 0.91 (0.79; 1.04); 0.158 | **0.83 (0.69; 0.99); 0.043** | 1.28 (0.94; 1.73); 0.113 |
| Nucleus ER-β2 score | 6.5 (3.3) | 7.2 (3.3) | 0.94 (0.86; 1.03); 0.184 | *0.91 (0.81; 1.02); 0.090* | 1.07 (0.85; 1.35); 0.576 |
| Cytoplasm ER-β2 score | 2 (0-3) | 2 (0-3) | 0.92 (0.80; 1.07); 0.288 | 0.89 (0.75; 1.06); 0.183 | 1.15 (0.78; 1.70); 0.483 |
| Nucleus ER-β5 score | 6.1 (2.6) | 6.1 (3.1) | 1.00 (0.91; 1.11); 0.927 | 0.95 (0.83; 1.09); 0.454 | 1.22 (0.95; 1.56); 0.124 |
| Cytoplasm ER-β5 score | 0 (0-0) | 0 (0-3) | 0.89 (0.73; 1.08); 0.238 | 0.90 (0.72; 1.12); 0.334 | 1.14 (0.69; 1.88); 0.611 |
| ER-α/Nucleus ER-β1 ratio | 1.0 (0.3-2.7) | 0.8 (0.4-1.5) | *1.23 (0.98; 1.55); 0.080* | 0.99 (0.95; 1.03); 0.659 | 1.00 (0.91; 1.10); 0.972 |
| ER-α/Nucleus ER-β2 ratio | 0.8 (0.3-1.5) | 0.5 (0.3-1.0) | 1.26 (0.94; 1.71); 0.126 | 1.00 (0.94; 1.06); 0.909 | 1.59 (0.83; 3.03); 0.158 |
| ER-α/Nucleus ER-β5 ratio | 0.8 (0.3-1.3) | 0.8 (0.3-1.3) | 1.15 (0.81; 1.63); 0.421 | 0.98 (0.93; 1.02); 0.344 | 0.77 (0.32; 1.89); 0.572 |
| Chir\_ascite | 37 (69.8) | 66 (48.9) | **2.44 (1.29; 4.61); 0.006** | 1.68 (0.81; 3.50); 0.166 | 6.13 (0.64; 58.67); 0.116 |
| Chir\_pi\_lps\_score | 7.2 (3.1) | 5.2 (3.9) | **1.16 (1.07; 1.27); 0.001** | **1.16 (1.04; 1.28); 0.006** | 1.11 (0.85; 1.44); 0.433 |
| ct\_Beva | 10 (18.9) | 68 (50.4) | **0.24 (0.12; 0.49); <0.001** | **0.37 (0.16; 0.84); 0.018** | 0.27 (0.03; 2.60); 0.255 |
| Cytoreduction | 38 (71.7) | 134 (99.3) | **0.05 (0.01; 0.18); <0.001** | **0.06 (0.01; 0.26); <0.001** | 2.12 (0.11; 42.58); 0.625 |
| **Abbreviations:** wt: wild type; AR: androgen receptor; PR: progesterone receptor; ER: oestrogen receptor; OR: Odd ratio; 95%CI: 95% Confidence Interval; Ref.: Reference;  \*In **bold** the significant results (p<0.05), in *italic* the suggestive results (0.05<p<0.10). | | | | | |

**Discussion**

**…**

**Conclusions**

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**Acknowledgments**

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**Author Contributions**

Conceptualization: …

Investigation: …

Data curation: …

Methodology: DG PCP LG

Formal analysis: DG PCP

Project administration: …

Funding acquisition: …

Resources: …

Supervision: …

Writing – original draft: DG PCP …

Writing – review & editing: …

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…

**References**

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

Therneau T (2015). \_A Package for Survival Analysis in S\_. version 2.38, <URL: https://CRAN.R-project.org/package=survival>.

Terry M. Therneau, Patricia M. Grambsch (2000). \_Modeling Survival Data: Extending the Cox Model\_. Springer, New York. ISBN 0-387-98784-3.

Alboukadel Kassambara, Marcin Kosinski and Przemyslaw Biecek (2019). survminer: Drawing Survival Curves using 'ggplot2'. R package version 0.4.6. https://CRAN.R-project.org/package=survminer

Dunkler D, Ploner M, Schemper M, Heinze G (2018). “Weighted Cox Regression Using the R Package coxphw.” Journal of Statistical Software, \*84\*(2), 1-26. doi: 10.18637/jss.v084.i02 (URL: <http://doi.org/10.18637/jss.v084.i02>).