RESEARCH

ERCC1 and CYP1B1 polymorphisms as predictors of response to neoadjuvant chemotherapy in estrogen positive breast tumors

Aurélie Dumont¹*, Diane Pannier², Agnès Ducoulombier², Emmanuelle Tresch³, Jinying Chen¹, Andrew Kramar³, Françoise Révillion¹, Jean-Philippe Peyrat¹ and Jacques Bonneterre²

Abstract

Purpose: Neoadjuvant chemotherapy (NCT) using anthracyclines and taxanes is a standard treatment for locally advanced breast cancer. Efficacy of NCT is however variable among patients and predictive markers are expected to guide the selection of patients who will benefit from NCT. A promising approach stand with polymorphisms located in genes encoding drug transporters, drug metabolizing enzymes and target genes which can affect drug efficacy. Our study investigated the potential of 37 polymorphisms to predict response to NCT in breast cancer.

Methods: 118 women with breast adenocarcinoma were treated with FEC100 and taxotere. Genotyping was performed on germline DNA using the BioMark platform (Fluidigm). Pathological complete response (pCR) according to Sataloff criteria was correlated to clinical characteristics and genotypes using univariate and multivariate analyses.

Results: 25 patients (21.2%) reached complete pathologic response. pCR rate is increased in SBRIII (p = 0.009), ER negative (p = 0.005) and triple negative (p = 0.006) tumors. pCR rate is significantly increased for patients carrying at least one variant allele for BRCA1, ERCC1 or SLCO1B3, and for patients homozygous for CYP1B1. The combination of ERCC1 and CYP1B1 polymorphisms is a potential predictor of NCT response in breast cancer (pCR rate reached 50 vs 21.2% for unselected patients), and particularly in ER+ breast cancer subtype where pCR rate reached 41.2 vs 13.5% for unselected patients.

Conclusions: This study is the first to report ERCC1, BRCA1 and SLCO1B3 as markers of response to NCT in breast cancer. ERCC1/CYP1B1 combination might be of particular interest to predict response to NCT in breast cancer and particularly to help NCT indication for ER+ breast tumors.

Keywords: Breast cancer, Neoadjuvant chemotherapy, ERCC1, CYP1B1, Single nucleotide polymorphism, Predictive factor

Background

Neoadjuvant chemotherapy (NCT) improves clinical outcome as patients whose tumors respond to NCT have superior disease-free and overall survival than patients whose tumors do not (Rastogi et al. 2008). Thus, predicting the chance of response before starting NCT is crucial to identify patients who will benefit from NCT and help to select for the appropriate drugs. Up to now, several markers such as tumor size, histological type, hormone receptor status, and HER2 expression are used to predict efficiency of NCT. However, despite the consideration of these tumor characteristics, heterogeneity in therapy efficacy still subsists and accurate predictive markers for NCT are lacking.
Genetic variations such as single nucleotide polymorphisms (SNPs) are present across individuals and might affect pharmacokinetics and pharmacodynamics of drugs. SNPs in genes encoding drug transporters, drug metabolizing enzymes and target genes can directly affect the drug uptake, its activation and its excretion, accessibility of the target and also related pathways that can potentiate its action. Consequently, SNPs might lead to therapeutic failures and/or adverse drug reactions. Currently, UDP glucuronosyltransferase 1A1 polymorphism \( UGT1A1^{*28} \) is used to predict toxicity of irinotecan in colorectal cancer treatment. Genotyping allows individuals homozygous for the variant allele of \( UGT1A1 \) to be treated with reduced doses of irinotecan and to avoid severe toxicity.

The aim of our study was to identify SNPs as predictive markers for breast cancer NCT. In our institution, breast cancer women treated with NCT receive 5-FU, cyclophosphamide, anthracyclines and/or taxanes. Thus, we selected SNPs previously reported in the literature to be associated with response to these chemotherapeutic agents. Thirty-seven SNPs, located in genes encoding influx and efflux transporters such as \( SLCO1B3 \), \( MDR1 \) and \( ABCC4 \), in genes belonging to drug metabolism (\( CYP1B1 \), \( CYP2B6 \), \( CYP3A4 \), \( MTHFR \) and \( GSTP1 \)) and also in genes of DNA repair pathway such as \( XRCC1 \), \( ERCC1 \), \( BRCA1 \) and \( p53 \) (Additional file 1: Table S1) were investigated in breast cancer patients receiving NCT.

### Patients and methods

#### Patients

191 women with histological proven breast adenocarcinoma were enrolled in the study between November 2007 and January 2012 (ClinicalTrials.gov identifier: NCT00959556). Patients were treated with anthracyclines and/or taxanes based NCT in our institution (Centre Oscar Lambret, Lille, France). Among them, 118 received FEC100-Taxotere, 46 patients with HER2+ tumors were treated with FEC100-Taxotere-Herceptin, 16 patients were treated with FEC100 and 11 patients received diverse chemotherapy regimens.

Patient characteristics are summarized in Table 1 for the two main groups. Median age at diagnosis was 46.5 years for patients treated with FEC100-taxotere and 45.5 years for patients treated with FEC100-taxotere-herceptin. 16 patients were treated with FEC100 and 11 patients received diverse chemotherapy regimens.

Patient characteristics are summarized in Table 1 for the two main groups. Median age at diagnosis was 46.5 years for patients treated with FEC100-taxotere and 45.5 years for patients treated with FEC100-taxotere-herceptin. The median size of the tumors was 30 mm for patients treated with FEC100-taxotere and 30.5 mm for patients treated with FEC100-taxotere-herceptin. The histoprognostic grading was established according to Scarff Bloom and Richardson modified by Elston and Ellis (1998). FEC100-Taxotere was mostly administrated during 6 cycles (84.7%) and the majority of patients (82.2%) received 3 cycles of FEC100 followed by 3 cycles of Taxotere. The median number

| Tumor type       | FEC100-taxotere | FEC100-taxotere-herceptin |
|------------------|-----------------|---------------------------|
| N (\( N = 118 \))| 106             |                           |
| Ductal carcinoma | 89.9            | 84.6                      |
| Lobular carcinoma| 5.9             | 6.2                       |
| Other            | 5.2             | 4.9                       |
| Tumor size       |                 |                           |
| T0-T1            | 5.3             | 4.3                       |
| T2               | 60.1            | 51.7                      |
| T3               | 24.0            | 20.7                      |
| T4               | 27.3            | 23.3                      |
| NA               | 2.3             | 2.2                       |
| Histoprognostic grade |            |                           |
| SBRI             | 7.6             | 6.7                       |
| SBRII            | 54.1            | 51.9                      |
| SBRIII           | 43.3            | 41.3                      |
| NA               | 14.2            | 11.9                      |
| Nodal status     |                 |                           |
| Positive         | 63.4            | 53.4                      |
| Negative         | 36.6            | 46.6                      |
| NA               | 2.0             | 0.0                       |
| Hormone receptor status |        |                           |
| Positive         | 77.0            | 66.3                      |
| Negative         | 39.2            | 33.6                      |
| NA               | 2.0             | 0.0                       |
| HER-2 status     |                 |                           |
| Positive         | 5.0             | 4.7                       |
| Negative         | 102.0           | 95.3                      |
| NA               | 11.0            | 9.3                       |
| Clinical response|                 |                           |
| CR               | 11.0            | 12.2                      |
| PR               | 62.0            | 68.9                      |
| SD               | 15.0            | 16.7                      |
| PD               | 2.0             | 2.2                       |
| NA               | 28.0            | 23.7                      |
| pCR              | Yes             | 25.0                      |
| No               | 93.0            | 78.8                      |
| NA               | 0.0             | 0.0                       |

\( \text{CR} \) complete response, \( \text{PR} \) partial response, \( \text{SD} \) stable disease, \( \text{PD} \) progressive disease, \( \text{NA} \) not applicable, \( \text{pCR} \) pathological complete response.

\( ^{a} \) Percentages of evaluable patients (except for NA: percentage of all patients).

\( ^{b} \) Estrogen receptor or progesterone receptor positive.

\( ^{c} \) Estrogen receptor and progesterone receptor negative.

\( ^{d} \) IHC3+ or IHC2+/FISH+ or CISH+.
of treatment cycles of Herceptin per patient was 18 (3–18).

Our study only focused on patients treated with FEC100-Taxotere (n = 118), in order to identify potential predictive polymorphisms in a homogeneous group according to treatment.

Clinical response assessment
Clinical evaluation of response to therapy was assessed by measuring tumor size (physical examination and ultrasonography) before NCT, and after 3 and 6 cycles. Change in tumor size was determined by comparing the tumor size before and after NCT according to RECIST guidelines, version 1.1 (Eisenhauer et al. 2009).

Pathological response assessment
Tumor response was assessed by a pathologist and graded according Sataloff et al. (1995). Pathological tumor response was used as the gold standard to evaluate treatment response. On the basis of patients’ pathological response in primary breast site and/or in the lymph nodes, patients were designated having a pCR when surgical samples showed total or near-total effect and absence of nodal involvement (TA and NA or NB).

Hormone receptors and HER2 assessment
Immunohistochemistry (IHC) was used for evaluation of oestrogen receptors (ER) (1D5 Dako clone, then SP1 Ventana clone) and progesterone receptors (PR) (1A6 Dako clone, then PR88 Biogenex clone); to be positive, more than 10% of the tumor nucleus cells had to be stained. Evaluation of HER2 was performed with IHC (CB11 Biogenex clone, then HER 485 Dako clone); only tumors classified 3+ were considered positive. All tumors HER2 2+ with IHC were tested by FISH. Triple negative tumors were defined as ER and PR negative and HER2 negative by immune histochemistry (IHC) or by FISH in case of HER2 2+.

DNA extraction
Genomic DNA was extracted from EDTA-treated blood samples (300 µl) using the MagNA Pure Compact instrument (Roche Diagnostics, Meylan, France) and the MagNA Pure Compact Nucleic Acid Isolation Kit according to the manufacturer’s instructions.

Genotyping
SNP genotyping was assessed using allelic discrimination with SNPType assays ordered from Fluidigm. When SNPType assays were not available, TaqMan assays (Life Technologies) were used instead. Because DNA concentration available was below supplier recommendation, a Specific Target Amplification was performed to enrich targeted SNP sequences according to the supplier’s instructions.

Instrumentation and nanofluidic chips
48.48 Dynamic Array used in the present study are nanofluidic chips able to analyze 48 samples with 48 SNP assays on the BioMark platform (Fluidigm). The BioMark system is used to thermal cycle these nanofluidic chips and image the data in real time.

SNP genotyping using SNPType assays
3 µL of SNPType ASP1/ASP2 (allele specific primer 1 and 2) and 8 µL of SNPType LSP were premixed and dilute with 29 µL of DNA suspension buffer to prepare SNPType assay mix. Each assay (5 µL) comprised 1 µL SNPType assay mix, 2.5 µL assay loading reagent 2X (fluidigm) and 1.5 µL DNA-free water. Each sample (6 µL) comprised 3 µL Biotium fast probe master mix 2X (Biotium), 0.3 µL sample loading reagent 20X (fluidigm), 0.1 µL SNPType reagent 60X (Fluidigm), 0.036 µL ROX 50X (Invitrogen), 0.064 µL DNA-free water and 2.5 µL amplified genomic DNA. Each of the assays (4 µL) and samples (5 µL) was pipetted into separate inlets in the chip. Amplification was carried out under the following conditions: 95°C for 5 min, 38 cycles of 95°C for 15 s, 60°C for 45 s, 72°C for 15 s followed by 30 s at 20°C for fluorescence measurement.

SNP genotyping using TaqMan assays
Each assay (5 µL) comprised 2.5 µL assay loading reagent 2X (fluidigm), 0.25 µL ROX 50X (Invitrogen), 1.25 µL SNP Genotyping assay mix 40X (Applied Biosystems) and 1 µL DNA-free water. Each sample (6 µL) comprised 3 µL TaqMan universal PCR master mix 2X (Applied Biosystems), 0.3 µL sample loading reagent 20X (fluidigm), 0.3 U AmpliTaq gold polymerase (Applied Biosystems), 0.12 µL DNA-free water and 2.52 µL amplified genomic DNA. Each of the assays (4 µL) and samples (5 µL) was pipetted into separate inlets in the chip. Amplification was carried out under the following conditions: 50°C for 2 min, 98°C for 10 min, 40 cycles of 95°C for 15 s, 60°C for 60 s. Fluorescence was measured at each slice.

Software
The data were analyzed using the BioMark SNP Genotyping Analysis software version 3.1.2 to obtain genotype. Briefly, the software calculates the FAM, VIC or HEX fluorescence intensities relative to ROX fluorescence background, and then automatically classifies the samples into three possible genotypes.
Statistical analysis
Clinical and histopathological characteristics were presented as frequencies and percentages for categorical variables and as medians and range for continuous variables. Associations between pCR and clinicopathological characteristics were assessed using Khi-2 test for qualitative variables or Fisher exact test in the case of small counts. Associations between pCR and continuous variables were performed using Wilcoxon Mann–Whitney test.

After ensuring that Hardy–Weinberg equilibrium was respected, the search of SNP correlated to pCR was performed by univariate logistic regression (R SNPassoc package). For each SNP, analyses were done considering genotypes separately or grouped to compare each homozygous or heterozygous genotype to other ones (only significant results are shown). Univariate analyses were repeated on subgroups of the population according to ER tumor status.

Multivariate analysis was performed on whole population combining significant SNP in a stepwise multivariate logistic regression selecting variables according to the Akaike Information Criterion (AIC). The model was validated internally on 1,000 random samples with replacement on the whole dataset. The percentage of times each variable was selected was extracted. Only those variables which were selected in >80% of models were retained. The final model was adjusted for ER status.

Results of statistical tests were considered significant at the 5% level. Analyses were performed using R11.2 Stata software (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX, USA) and R2.15.2 R software (R Core Team 2012).

Results
Association between pCR and clinicopathological characteristics
25 patients (21.2%, CI 95%: 14.2–29.7%) reached complete pathologic response according to Sataloff criteria. Median age at diagnosis was 46 years for responder patients, 47 years for non-responder patients and was not significantly correlated to pCR.

The associations between pCR and clinicopathological features are summarized in Table 2. No statistically significant correlations were observed between pCR and tumor size, nodal status or K167. Patients with higher tumor grade were significantly more likely to achieve a pCR than those with lower tumor grade (p = 0.009). We also observed different pCR rates according to Hormone Receptors (HR) status: patients whose tumors were positive for at least one HR had a pCR rate of 15.6% whereas patients who were HR negative had a pCR rate of 33.3% (p = 0.003). Contrary to PR negativity, ER negativity is a significant marker of pCR (p = 0.005). pCR rate is higher for ER− tumors (35.7%) than for ER+ tumors (13.5%; p = 0.005). Tumors with triple negative phenotype achieved significantly higher pathologic response rate than those with non-triple negative phenotype (40.6% vs 16.0%, p = 0.006).

Association between pCR and the 37 selected SNPs
Among the 37 SNPs evaluated, genotyping data were available for 114 of the 118 patients enrolled in the study. The distribution of the genotypes and allelotypes indicated that all frequencies were in Hardy–Weinberg equilibrium and were consistent with previously reported Caucasian populations (data not shown).

Univariate analysis revealed that 4 SNPs are significantly associated with pCR (Table 3).

Patients carrying at least one variant allele for BRCA1 (T), ERCC1 (C) and SLCO1B3 (G) have about a threefold higher likelihood to achieve a pCR to NCT than other patients. Patients homozygous for CYP1B1 Leu432Val showed increased pCR rate compared to heterozygous patients (respectively pCR = 29% and pCR = 11.1%, p = 0.020).

Subsequently, assessment of associations between combinations of these SNPs and pCR showed that a model containing only ERCC1 and CYP1B1 polymorphisms performed as well as more complex models. Stepwise logistic regression combining the four significant SNPs identified the model containing CYP1B1 and ERCC1 as the best: these SNPs were validated respectively in 81.0 and 80.8% of the models whereas BRCA1 and SLCO1B3 were selected in 69.2 and 72.3% of the models. The ERCC1 CT genotype associated with CYP1B1 CC genotype had the highest OR of 8.5 (p = 0.013; Table 4). pCR rate reached 50% for patients harboring these genotypes whereas the pCR rate is 21.2% in the whole population.

Association between pCR and SNPs considering ER status
In breast cancer, tumor subtypes are associated with different responses to neoadjuvant therapies. For example, highly proliferative ER− breast tumors are more sensitive to NCT than ER+ breast tumors: pCR rates are respectively 28–32 and 2–10% (von Minckwitz 2013; Colleoni et al. 2004; Kaufmann et al. 2012).

As pCR can be achieved only in a minority of patients with ER+ breast tumors, it is of particular interest to identify patients who can avoid neoadjuvant chemotherapy or who are candidates for a very high probability of pCR after chemotherapy. Therefore, among the four SNPs previously described as significantly associated to pCR, we searched for potential predictive marker for ER+ breast tumors. pCR is significantly increased for patients carrying variant alleles of BRCA1, SLCO1B3.
and CYP1B1 (Table 5, genotyping data obtained for 71 patients).

Considering patients with ER− tumors, only ERCC1 (Asn118Asn) is significantly associated with response to NCT (Table 6, genotyping data obtained for 41 patients). Patients carrying at least one variant allele for ERCC1 (C) have a fourfold higher likelihood to achieve a pCR after NCT than patients with other genotypes. Moreover, combined ERCC1-CT and CYP1B1-CC genotypes remain significant when adjusted on ER status.

### Table 2  Association between clinicopathological features and pCR in FEC-Taxotere treated group (N = 118)

|                          | Total (N = 118) | pCR (N = 25) | No pCR (N = 93) | P value |
|--------------------------|----------------|--------------|----------------|---------|
|                          | n             | %a           | n             | %a     |
| Tumor size               |               |              |               |         |
| T0-T1                    | 5             | 20.0         | 4             | 80.0    |
| T2                       | 60            | 26.7         | 44            | 73.3    |
| T3                       | 24            | 12.5         | 21            | 87.5    |
| T4                       | 27            | 14.8         | 23            | 85.2    | NS†     |
| NA                       | 2             |              | 1             |         |
| Histopronostic grade     |               |              |               |         |
| SBRI–SBRII               | 61            | 14.8         | 52            | 85.2    |
| SBRIII                   | 43            | 37.2         | 27            | 62.8    | 0.009*  |
| NA                       | 14            |              | 14            |         |
| Nodal status             |               |              |               |         |
| Positive                 | 63            | 19.1         | 51            | 80.9    |
| Negative                 | 53            | 22.6         | 41            | 77.4    | NS†     |
| NA                       | 2             |              | 1             |         |
| Ki67                     |               |              |               |         |
| Positive                 | 42            | 28.6         | 30            | 71.4    |
| Negative                 | 6             | 0.0          | 6             | 100.0   | NS†     |
| NA                       | 70            | 57           |               |         |
| Hormone receptor status  |               |              |               |         |
| RH                       |               |              |               |         |
| Positiveb                | 77            | 15.6         | 65            | 84.4    |
| Negativec                | 39            | 33.3         | 26            | 66.7    | 0.003*  |
| NA                       | 2             |              | 2             |         |
| RE                       |               |              |               |         |
| Positive                 | 74            | 13.5         | 64            | 86.5    |
| Negative                 | 42            | 35.7         | 27            | 64.3    | 0.005*  |
| NA                       | 2             |              | 2             |         |
| RP                       |               |              |               |         |
| Positive                 | 57            | 17.5         | 47            | 82.5    |
| Negative                 | 58            | 25.9         | 43            | 74.1    | NS†     |
| NA                       | 3             |              | 3             |         |
| Triple negative          |               |              |               |         |
| Yes                      | 32            | 40.6         | 19            | 59.4    | 0.006*  |
| No                       | 75            | 16.0         | 63            | 84.0    |         |
| NA                       | 11            |              | 11            |         |

NA not applicable, pCR pathological complete response.

* Khi-2 test.
† Fisher exact test.
‡ Wilcoxon Mann–Whitney test.
§ Percentages of evaluable patients.
b Estrogen receptor or progesterone receptor positive.
c Estrogen receptor and progesterone receptor negative.
(p = 0.005; Table 7). In consequence, pCR rate reached 41.2% for patients with ER+ tumors harboring these genotypes whereas the pCR rate is only 13.5% for unselected patients with ER+ tumors. pCR rate is twice higher for patients with ER- tumors harboring combined ERCC1-CT and CYP1B1-CC genotypes than for unselected patients (80 vs 37.5%).

**Discussion**

In order to identify breast cancer patients who will benefit from anthracyclines and/or taxanes based NCT, we analyzed the relationship between 37 SNPs and the response to these chemotherapeutic agents.

First overall, our data confirmed that response to NCT is related to clinicopathological features such as histoprognostic tumor grade and hormone receptor status. According to previous published data (von Minckwitz 2013), we observed that pCR rate is increased in SBRIII and HR negative tumors and that ER- tumors respond better to NCT than ER+ tumors. Our data also indicated that patients with triple negative tumors achieve significantly higher pathologic complete response rate than those with non-triple negative tumors. Unexpectedly, pCR was not significantly associated to KI67 expression in our study. Nevertheless, this observation may be explained in part by the fact that data were missing for more than half of patients.

Secondly, considering the predictive value of the 37 studied SNPs, we demonstrated using univariate analyses that BRCA1 (Pro871Leu) and ERCC1 (Asn118Asn), two polymorphisms located in DNA repair genes, and also CYP1B1 (Leu432Val) and SLCO1B3 (IVS12-5676), two polymorphisms in genes involved in drug metabolism, are linked to response to taxanes and anthracyclines. Multivariate analysis revealed that ERCC1 (Asn118Asn) combined to CYP1B1 (Leu432Val) might be of particular

---

**Table 3** pCR is related to 4 SNPs (N = 114)

| Gene      | SNP        | Genotype | N  | pCR (N = 25) | OR   | 95% CI     | P value |
|-----------|------------|----------|----|--------------|------|------------|---------|
|           | rs799917   | C CC     | 44 | 5            | 11.4 | 1          |         |
| BRCA1     | Pro871Leu  | CT TT    | 66 | 19           | 28.8 | 3.15       | (1.08–9.2) 0.025 |
| SLCO1B3   | rs11045585 | A AA     | 79 | 13           | 16.5 | 1          |         |
|           | IVS12-5676 | AG GG    | 34 | 12           | 35.3 | 2.77       | (1.10–6.7) 0.031 |
|           | NA         |          | 4  | 1            |      |            |         |
| CYP1B1    | rs1056836  | C CG     | 45 | 5            | 11.1 | 1          | 0.020   |
|           | Leu432Val  | CC GG    | 69 | 20           | 29.0 | 3.26       | (1.12–9.5) |
| ERCC1     | rs11615    | T TT     | 40 | 4            | 10.0 | 1          |         |
|           | Asn118Asn  | CT CC    | 73 | 21           | 28.8 | 3.63       | (1.15–11.5) 0.016 |

NA not applicable, pCR pathological complete response.

* Percentages of evaluable patients.

---

**Table 4** pCR is related to combination of CYP1B1 and ERCC1 polymorphisms

| CYP1B1 (Leu432Val) | ERCC1 (Asn118Asn) | N  | pCR | OR | 95% CI | P value |
|-------------------|-------------------|----|-----|----|--------|---------|
|                   |                   |    |     |    |        |         |
| CC                | TT                | 19 | 2   | 10.5 | 1 | Reference |
| CC                | CT                | 22 | 11  | 50.0 | 8.5 | (1.6–46) 0.013 |
| CC                | CC                | 6  | 1   | 16.7 | 1.7 | (0.13–23) 0.69 |
| CG                | TT                | 15 | 1   | 6.7  | 0.61| (0.05–74) 0.70 |
| CG                | CT                | 20 | 2   | 10.0 | 0.94| (0.12–7.5) 0.96 |
| CG                | CC                | 10 | 2   | 20.0 | 2.1 | (0.25–18) 0.49 |
| GG                | TT                | 6  | 1   | 16.7 | 1.7 | (0.13–23) 0.69 |
| GG                | CT                | 8  | 3   | 37.5 | 5.1 | (0.66–39) 0.12 |
| GG                | CC                | 7  | 2   | 28.6 | 3.4 | (0.38–31) 0.28 |

Significant results are shown in italic.

pCR pathologic complete response.

* Percentages of evaluable patients.
interest as predictive markers for NCT in breast cancer. Indeed, we observed that patients exhibiting ERCC1-CT genotype combined to CYP1B1-CC genotype presented a doubling of pCR rate compared with unselected patients (50 vs 21.2%).

Our results are in agreement with previous published data. The BRCA1 variant allele (T) is associated with better overall survival and longer progression-free survival compared to the reference allele in patients with advanced gastric cancer treated with taxanes and cisplatin (Shim et al. 2010).

Although considered as neutral, the functional significance of Pro871Leu (C > T) polymorphism of BRCA1 is still unknown. Pro871Leu might affect expression, activity or interaction of BRCA1 with its partners. As a consequence, Pro871Leu might impair DNA repairing function of BRCA1 and might contribute to potentiate efficacy of DNA damaging agents.

**Table 5** BRCA1, SLCO1B3 and CYP1B1 are significantly associated with pCR in ER+ tumors (N = 71)

| Gene  | SNP         | Genotype | N   | pCR (N = 10) | OR | 95% CI | P value |
|-------|-------------|----------|-----|-------------|----|--------|---------|
|       | rs799917    | C        | CC  | 26          | 0  | 0      |         |
|       | Pro871Leu   | CT-TT    | 42  | 9           | 21.4 |        |
|       |             | NA       | 3   | 1           |     |        |         |
| SLCO1B3| rs11045585  | A        | AA  | 49          | 4  | 8.2    | 1       |
|       | IVS12-5676  | AG-GG    | 21  | 6           | 28.6 | 4.50 (1.12–18.1) | 0.032 |
|       |             | NA       | 1   | 0           |     |        |         |
| CYP1B1| rs1056836   | C        | CG  | 26          | 0  | 0      |         |
|       | Leu432Val   | CC-GG    | 45  | 10          | 22.2 |        |
| ERCC1 | rs11615     | T        | TT  | 22          | 1  | 1.5    |         |
|       | Asn118Asn   | CT-CC    | 48  | 9           | 18.8 |        |
|       |             | NA       | 1   | 0           |     |        |         |

Significant results are shown in italic.
NA not applicable, pCR pathological complete response.
* Percentages of evaluable patients.

**Table 6** ERCC1 is significantly associated with pCR in ER- tumors (N = 41)

| Gene  | SNP         | Genotype | N   | pCR (N = 15) | OR | 95% CI | P value |
|-------|-------------|----------|-----|-------------|----|--------|---------|
|       | rs799917    | C        | CC  | 17          | 5  | 29.4   |         |
|       | Pro871Leu   | CT-TT    | 23  | 10          | 43.5 |        |
|       |             | NA       | 1   | 0           |     |        |         |
| SLCO1B3| rs11045585  | A        | AA  | 28          | 9  | 32.1   |         |
|       | IVS12-5676  | AG-GG    | 13  | 6           | 46.2 |        |
| CYP1B1| rs1056836   | C        | CG  | 18          | 5  | 27.8   |         |
|       | Leu432Val   | CC-GG    | 23  | 10          | 43.5 |        |
| ERCC1 | rs11615     | T        | TT  | 17          | 3  | 17.6   | 1       |
|       | Asn118Asn   | CT-CC    | 24  | 12          | 50.0 | 4.7 (1.06–20) | 0.030 |

Significant results are shown in italic.
NA not applicable, pCR pathological complete response.
* Percentages of evaluable patients.
demonstrated no difference in \textbf{ERCC1} transcription nor expression between cells stably expressing \textbf{ERCC1}-T allele or \textbf{ERCC1}-C allele (Gao et al. 2011). However, data obtained on lymphocytes of prostate cancer patients revealed that carriers of CC genotype showed lower \textbf{ERCC1} mRNA levels (Woelfelschneider et al. 2008). Thus, by decreasing \textbf{ERCC1} mRNA levels, C genotype might decrease \textbf{ERCC1} mediated DNA repair and might contribute to potentiate efficacy of DNA damaging drugs. This hypothesis is in agreement with clinical data which present that low levels of \textbf{ERCC1} are favorable for sensitivity to platinium compounds (Li et al. 2000; Shirota et al. 2001; Lord et al. 2002; Wang et al. 2008).

\textbf{CYP1B1} Val genotype is associated with lower response rate, shorter progression-free-survival and decrease overall survival in breast and prostate cancer patients treated with taxanes based chemotherapy (Marsh et al. 2007; Sissung et al. 2008; Pastina et al. 2010). However, in our study, only Leu/Val genotype provided the best data fit for the SNP Leu432Val. The hypothesis of an over-dominant model, previously described in bladder cancer (Salinas-Sanchez et al. 2012), might be explained by a higher enzyme activity in heterozygous than in homozygous. CYP1B1 binds and sequestrates docetaxel reducing its available concentration and influencing its activity (McFadyen et al. 2001). CYP1B1 also produces oestrogen metabolites that limit docetaxel efficacy by inhibition of tubulin polymerization (Sissung et al. 2008). Thus, by increasing CYP1B1 mRNA expression and catalytic activity (Shimada et al. 1999; Hanna et al. 2000; Li et al. 2000; Landi et al. 2005), Leu432Val substitution appears to modulate response to taxanes.

\textbf{SLCO1B3} is an influx transporter involved in the uptake of a broad range of drug substrates including docetaxel (Konig et al. 2000; Smith et al. 2005). The functional impact of rs11045585 polymorphism is yet unknown. However, asian nasopharyngeal cancer patients homozygous for the variant allele had higher area under curve and less plasma clearance of docetaxel compared to patients carrying at least one reference allele (Chew et al. 2011). It is to be noted that rs11045585 polymorphism is proposed to exert its functional effect by being in haplotypic combination with 3 other polymorphism in the \textbf{SLCO1B3} gene (Chew et al. 2012).

Finally, as pCR is rarely achieved by NCT in ER+ breast tumors, we searched if \textbf{BRCA1} (Pro871Leu), \textbf{ERCC1} (Asn118Asn), \textbf{CYP1B1} (Leu432Val), and \textbf{SLCO1B3} (IVS12-5676) could stand for predictive markers of NCT for patients with this subtype of tumor. Our results indicated that \textbf{BRCA1} (Pro871Leu), \textbf{CYP1B1} (Leu432Val) and \textbf{SLCO1B3} are significantly associated with response to NCT in \textbf{ER}+ tumors. \textbf{ERCC1}/\textbf{CYP1B1} combination remains significant for \textbf{ER}+ tumors and might be useful to help indication of NCT in this particular type of tumors as patients exhibiting \textbf{ERCC1}-CT genotype combined to \textbf{CYP1B1}-CC genotype presented a threefold higher pCR rate compared with unselected patients: 41.2 vs 13.5%.

Our data also indicated that \textbf{ERCC1} Asn118Asn might be a potential predictive marker for NCT in \textbf{ER}− tumors, as pCR is increased to 50% for patients carrying at least one variant allele (C).

In conclusion, \textbf{BRCA1} (Pro871Leu), \textbf{ERCC1} (Asn118Asn), \textbf{CYP1B1} (Leu432Val), and \textbf{SLCO1B3} (rs11045585) are associated with response to NCT in our cohort of breast cancer

### Table 7 pCR is related to combination of \textbf{CYP1B1} and \textbf{ERCC1} polymorphisms when adjusted on \textbf{ER} status

| CYP1B1 (Leu432Val) | ERCC1 (Asn118Asn) | N   | pCR   | OR   | 95% CI | P value |
|---------------------|-------------------|-----|-------|------|--------|---------|
|                     |                   |     | n     | %a   |        |         |
| CC                 | TT                | 19  | 2     | 10.5 | 1      | Reference |
| CC                 | CT                | 22  | 11    | 50.0 | 16.1 (2.3–112) | 0.005  |
| CC                 | CC                | 6   | 1     | 16.7 | 2.8 (0.16–47)  | 0.48   |
| CG                 | TT                | 15  | 1     | 6.7  | 0.41 (0.03–5.6) | 0.51   |
| CG                 | CT                | 20  | 2     | 10.0 | 0.77 (0.09–6.8) | 0.81   |
| CG                 | CC                | 10  | 2     | 20.0 | 2.4 (0.24–25)  | 0.45   |
| GG                 | TT                | 6   | 1     | 16.7 | 0.89 (0.06–13.5) | 0.93   |
| GG                 | CT                | 8   | 3     | 37.5 | 4.4 (0.46–41)  | 0.20   |
| GG                 | CC                | 7   | 2     | 28.6 | 3.1 (0.28–34)  | 0.35   |
| ER−                |                   | 42  | 15    | 1    | 1      | Reference |
| ER+                |                   | 71  | 10    | 0.12 | (0.03–0.43)  | 0.001  |

*pCR is related to combination of \textbf{CYP1B1} and \textbf{ERCC1} polymorphisms when adjusted on \textbf{ER} status.**

**CC TT 19 2 10.5 1 Reference**

**CC CT 22 11 50.0 16.1 (2.3–112) 0.005**

**CC CC 6 1 16.7 2.8 (0.16–47) 0.48**

**CG TT 15 1 6.7 0.41 (0.03–5.6) 0.51**

**CG CT 20 2 10.0 0.77 (0.09–6.8) 0.81**

**CG CC 10 2 20.0 2.4 (0.24–25) 0.45**

**GG TT 6 1 16.7 0.89 (0.06–13.5) 0.93**

**GG CT 8 3 37.5 4.4 (0.46–41) 0.20**

**GG CC 7 2 28.6 3.1 (0.28–34) 0.35**

**ER−**

**ER+**

**Significant results are shown in italic.**

**pCR pathological complete response.**

**% Percentages of evaluable patients.**
patients. To our knowledge, this study is the first to report ERCC1, BRCA1 and SLCO1B3 as markers of taxanes and/or anthracyclines response in breast cancer NCT.

Moreover ERCC1/CYP1B1 combination appeared to be a potential predictive marker to guide NCT indication in breast cancer and particularly for ER+ breast tumors.

Additional file

Additional file 1. Table S1: List of polymorphisms selected for the study.

Authors’ contributions
AD, FR, JPP and JB planned and designed the study. The development of methodology was assessed by AD, FR, JPP, ET, AK and JB. JC, DR, ADuc performed acquisition of the data. Analysis and interpretation of the data were carried out by JC, ET, AD, FR, JPP and JB. AD, FR, JPP, JB, ET and AK wrote or reviewed the manuscript. All authors read and approved the final manuscript.

Author details
1 Laboratoire d’Oncologie Moléculaire Humaine, Centre Oscar Lambret, 3 rue Frédéric Combemale, BP 307, 59020 Lille Cedex, France. 2 Département de Sénologie, Centre Oscar Lambret, 3 rue Frédéric Combemale, BP 307, 59020 Lille Cedex, France. 3 Unité de Méthodologie et Biostatistique, Centre Oscar Lambret, 3 rue Frédéric Combemale, BP 307, 59020 Lille Cedex, France.

Compliance with ethical guidelines

Competing interests
The authors declare that they have no competing interests.

Consent for publication
All patients gave written informed consent for genetic testing. This study was approved by the Comité de Protection des Personnes Nord Ouest IV (Lille) and by the Agence Française de Sécurité Sanitaire des Produits de Santé (Paris), N°IDRCB: 2007‑A00908‑45.

Received: 19 May 2015 Accepted: 21 May 2015 Published online: 07 July 2015

References

Chang PM, Tseng CH, Chen PM, Lin JK, Lin TC, Chen WS et al (2009) ERCC1 codon 118 C>T polymorphism associated with ERCC1 expression and outcome of FOLFOX-4 treatment in Asian patients with metastatic colorectal carcinoma. Cancer Sci 100:278–283

Chew SC, Singh O, Chen X, Ramasamy RD, Kulkarni T, Lee EJ et al (2011) The effects of CYP3A4, CYP3A5, ABCB1, ABC2, ABCG2 and SLCO1B3 single nucleotide polymorphisms on the pharmacokinetics and pharmacodynamics of docetaxel in nasopharyngeal carcinoma patients. Cancer Chemother Pharmacol 67:1471–1478

Chew SC, Sandanaraj E, Singh O, Chen X, Tan EH, Lim WT et al (2012) Influence of SLCO1B3 haplotype-tag SNPs on docetaxel disposition in Chinese nasopharyngeal carcinoma patients. Br J Clin Pharmacol 73:606–618

Colleoni M, Wale G, Zahndieh G, Prunet G, Gentilini O, Veronesi P et al (2004) Chemotherapy is more effective in patients with breast cancer not expressing steroid hormone receptors: a study of preoperative treatment. Clin Cancer Res 10:662–6628

Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R et al (2000) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 45:228–247

Elston CW, Ellis IO (1998) Assessment of histological grade. The Breast, 3rd edn. Churchill Livingstone, Edinburgh, pp 360–384

Gao R, Reece K, Sissung T, Reed E, Price DK, Figg WD (2011) The ERCC1 N118 N polymorphism does not change cellular ERCC1 protein expression or platinum sensitivity. Mutat Res 708:21–27

Hanna IH, Dawling S, Roodi N, Guengerich FP, Parl FF (2000) Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. Cancer Res 60:3440–3444

Isla D, Samies C, Rosell R, Alonso G, Domíne M, Taron M et al (2004) Single nucleotide polymorphisms and outcome in docetaxel-cisplatin-treated advanced non-small-cell lung cancer. Ann Oncol 15:1194–1203

Kalikai A, Kanaki M, Vassalou H, Souglakos J, Voutsina A, Georgoulas V et al (2009) DNA repair gene polymorphisms predict favorable clinical outcome in advanced non-small-cell lung cancer. Clin Lung Cancer 10:118–123

Kamikozuru H, Kurouchi H, Hayashi K, Nakajima G, Yamamoto M (2008) ERCC1 codon 118 polymorphism is a useful prognostic marker in patients with pancreatic cancer treated with platinum-based chemotherapy. Int J Oncol 32:1091–1096

Kaufmann M, von Minckwitz G, Mamounas EP, Cameron D, Carey LA, Cristofanilli M et al (2012) Recommendations from an international consensus conference on the current status and future of neoadjuvant systemic therapy in primary breast cancer. Ann Surg Oncol 19:1508–1516

Konig J, Cui Y, Nies AT, Keppler D (2000) Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. J Biol Chem 275:21361–21368

Landi MT, Beigenn AW, Baccarell A, Patterson DG Jr, Grassman J, Ter-Minassian M et al (2005) CYP1A1 and CYP1B1 genotypes, haplotypes, and TCDD-induced gene expression in subjects from Seveso, Italy. Toxicology 207:191–202

Li Q, Yu J, Mu C, Yunmbam MK, Slavsky D, Cross CL et al (2000a) Association between the level of ERCC1-1 expression and the repair of cisplatin-induced DNA damage in human ovarian cancer cells. Anticancer Res 20:645–653

Li DN, Seidel A, Pritchard MP, Wolf CR, Friedberg TD (2000b) Polymorphisms in ERCC1/CYP1B1 affect the conversion of estradiol to the potentially carcinogenic metabolite 4-hydroxyestradiol. Pharmacogenomics 10:343–353

Lord RV, Brabender J, Gandara D, Alberola V, Camps D, Domíne M et al (2002) Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. Clin Cancer Res 8:2286–2291

Marsh S, Somlo G, Li X, Frankie P, King CR, Shannon WD et al (2007) Pharmacogenetic analysis of paclitaxel transport and metabolism genes in breast cancer. Pharmacogenomics J 7:362–365

McFadyen MC, McLeod HL, Jackson FC, Melvin WT, Doehmer J, Murray GI (2001) Cytochrome P450 CYP1B1 protein expression: a novel mechanism of anticancer drug resistance. Biochem Pharmacol 62:207–212

Metzger R, Warnede-ebzer U, Alakus H, Kutting F, Brabender J, Vallbohmer D et al (2012) Neoadjuvant radiochemotherapy in adenocarcinoma of the esophagus: ERCC1 gene polymorphisms for prediction of response and prognosis. J Gastrointest Surg 16:26–34

Pastina L, Giovanniatti E, Chioni A, Sissung TM, Crea F, Orlandini C et al (2010) Cytochrome P450 1B1 (CYP1B1) polymorphism: association with response to docetaxel in Castration-Resistant Prostate Cancer (CRPC) patients. BMC Cancer 10:511

R Core team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org/

Rastogi P, Anderson SJ, Bear HD, Geyer CE, Kahnberg MS, Robidoux A et al (2008) Preoperative chemotheraphy: updates of National Surgical Adjuvant Breast and Bowel Project Protocols B-18 and B-27. J Clin Oncol 26:778–785

Ren S, Zhou S, Wu F, Zhang L, Li X, Zhang J et al (2012) Association between polymorphisms of DNA repair genes and survival of advanced NSCLC patients treated with platinum-based chemotherapy. Lung Cancer 75:102–109

SALINAS-SANCHEZ AS, DONATE-MORENO MJ, GOMEZ-GARRIDO PM, GIMENEZ-BACHM JM, ESBRICANO J (2012) Role of CYP1B1 gene polymorphisms in bladder cancer susceptibility. J Urol 187:700–706

Sataloff DM, Mason BA, Prestipino AJ, Seinige UL, Lieder CP, Baloch Z (1995) Pathologic response to induction chemotherapy in locally advanced carcinoma of the breast: a determinant of outcome. J Am Coll Surg 180:297–306

Shim HJ, Yun JY, Hwang JE, Bae WK, Cho SH, Lee JH et al (2010) BRCA1 and XRCC1 polymorphisms associated with survival in advanced gastric cancer treated with taxane and cisplatin. Cancer Sci 101:1247–1254
Shimada T, Watanabe J, Kawajiri K, Sutter TR, Guengerich FP, Gillam EM et al (1999) Catalytic properties of polymorphic human cytochrome P450 1B1 variants. Carcinogenesis 20:1607–1613
Shirota Y, Stoehlmacher J, Brabender J, Xiong YP, Uetake H, Danenberg KD et al (2001) ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. J Clin Oncol 19:4298–4304
Sissung TM, Danesi R, Price DK, Steinberg SM, de Wit R, Zahid M et al (2008) Association of the CYP1B1*5 allele with survival in patients with prostate cancer receiving docetaxel. Mol Cancer Ther 7:19–26
Smith NF, Acharya MR, Desai N, Figg WD, Sparreboom A (2005) Identification of OATP1B3 as a high-affinity hepatocellular transporter of paclitaxel. Cancer Biol Ther 4:815–818
Viguier J, Boige V, Miquel C, Pocard M, Giraudseau B, Sabourin JC et al (2005) ERCC1 codon 118 polymorphism is a predictive factor for the tumor response to oxaliplatin/5-fluorouracil combination chemotherapy in patients with advanced colorectal cancer. Clin Cancer Res 11:6212–6217
von Minckwitz G (2013) Neoadjuvant therapy: what are the lessons so far? Hematol Oncol Clin North Am 27:767–784
Wang L, Wei J, Qian X, Yin H, Zhao Y, Yu L et al (2008) ERCC1 and BRCA1 mRNA expression levels in metastatic malignant effusions is associated with chemosensitivity to cisplatin and/or docetaxel. BMC Cancer 8:97
Warnecke‑Eberz U, Vallbohmer D, Nakus H, Kutting F, Lurje G, Bollschweiler E et al (2009) ERCC1 and XRCC1 gene polymorphisms predict response to neoadjuvant radiochemotherapy in esophageal cancer. J Gastrointest Surg 13:1411–1421
Woelfelschneider A, Popanda O, Lilla C, Linseisen J, Mayer C, Celebi O et al (2008) A distinct ERCC1 haplotype is associated with mRNA expression levels in prostate cancer patients. Carcinogenesis 29:1758–1764