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BCIRG 001 Molecular Analysis : Identification of prognostic factors in patients (pts) receiving adjuvant therapy for node-positive (N+) Breast Cancer (BC)

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ABSTRACT *

Background : BCIRG 001 (1,491 patients) demonstrated significant superiority of docetaxel / doxorubicin / cyclophosphamide (TAC) over fluorouracil / doxorubicin / cyclophosphamide (FAC) given as adjuvant therapy for N+ operable BC in terms of disease-free survival (DFS) and overall survival (OS) [Martin et al. N Eng J Med, 2005]. This ancillary study was aimed to identify tumor-associated factors related to DFS and OS.

Methods : Formalin-fixed primary tumors from patients in BCIRG 001 were analysed by immunohistochemistry. Protocol-specified assessment of histological grade (G), tumor size (TS), estrogen (ER) and progesterone receptors (PR), lymph node status (LN), HER2, MUC1, Mib, p53, Bcl-2, Bax, Bcl-X, Bag-1, tubulin β isotypes II, III and IV, Tau protein and detyrosinated α tubulin was performed. Parameters were scored as the percentage of positive cells and analysed as lower or greater than median values. The samples were randomly split into training (2/3) and validation (1/3) sets. Associations between selected parameters and DFS or OS were tested through univariate analyses using the Kaplan-Meier method (log-rank test) on the training set. A backward stepwise Cox regression analysis was performed to identify the final model of prognostic factors on the training set. Multivariate analyses were applied to the validation set.

Results : 1,350 samples were split into a training (n = 906) and a validation (n = 444) set. In univariate GR, TS, LN, ER and PR, Mib, Tubulin III, Tau protein and HER2 were correlated with DFS in both sets. In multivariate GR, ER, PR, TS, LN, p53 and Tau (all $p < 0.05$) were significantly associated with DFS in the training set. In univariate GR, TS, LN, ER and PR, Mib, MUC1, Bcl-2, Tubulin III and Tau were correlated with OS in both sets. In multivariate GR, TS, LN, ER and PR, p53 and Tau (all $p < 0.05$) were independently correlated with OS in the training set.

Conclusions : These data suggest that Tau and p53 are independent markers of DFS and OS in patients receiving these forms of adjuvant chemotherapy for N+ BC. Complementary analyses will be presented.

*** Data updated since initial abstract submission**

SETTING

- There are presently no validated immunohistochemical parameters reliably predicting patient outcomes in patients with node positive breast cancer receiving docetaxel-containing regimens. Preclinical studies have identified a number of biological parameters which might be correlated with disease aggressivity and or sensitivity to docetaxel including apoptosis-related, proliferation-related and microtubule-related parameters.
- The BCIRG 001 trial compared FAC and TAC adjuvant chemotherapy in 1,491 with axillary node positive breast cancer. Six cycles of chemotherapy were administered after surgery. The randomization was stratified by center, hormone receptors status and number of axillary lymph nodes (1-3 versus 4+). Treatment regimens were as follows:
  - **TAC:** docetaxel 75 mg/m^2 as 1 hour iv infusion on day 1 every 3 weeks in combination with doxorubicin 50 mg/m^2 as an iv bolus and cyclophosphamide 500 mg/m^2 as iv bolus on day 1 every 3 weeks,
  - **FAC:** 5-fluorouracil 500 mg/m^2 as an iv bolus on day 1 every 3 weeks in combination with doxorubicin 50 mg/m^2 as an iv bolus and cyclophosphamide 500 mg/m^2 as an iv bolus on day 1 every 3 weeks.
- In this trial the primary end-point was Disease-Free Survival (DFS), defined as the time from randomization until the first to occur among breast cancer relapse, appearance of a secondary primary malignancy or death. The second interim analysis, mandated by the IDMC, was performed once 400 DFS events were reported. With a median follow-up of 55 months the estimated rates of DFS at 5 years were 75% in the TAC group and 68% in the FAC group. The overall survival at 5 years was 87% in the TAC group and 81% in the FAC group [M Martin, T Pienkowski, M Mackey et al. Adjuvant Docetaxel for Node Positive Breast Cancer. N Engl J Med 2005; 352: 2302-13].

MATERIALS & METHODS

Immunohistochemistry of primary tumors

- Formalin-fixed primary tumors were collected for 1,350 patients among the 1,491 included in the trial (91 %). Immunohistochemical analyses and central review of tumor characteristics were performed by three participating laboratories.
- **Cross Cancer Center (Edmonton, Canada)**
  - **Markers :** Estrogen and Progesteron receptors, MIB-1, p 53, HER2 neu status, MUC1.
  - **Tumor characteristics :** Uni or multifocal disease, Tumor size, Histologic grade, Histologic type, Vascular invasion, Lymph nodes status.
- **Burnham Institute for Medical Research (La Jolla, CA)**
  - Bcl-2, Bax, Bcl-X, Bag-1 cytosolic and nuclear.
- **Centre Léon Bérard (Lyon, France)**
  - β Tubulin type II, β Tubulin type III, β Tubulin type IV, Tau protein, Glu Tubulin.
- Results of analysis included intensity and percentage of positive cells. The data were entered in the study data base maintained by the CIRG. Patients characteristics (age, race, menopausal status and Karnofsky index for performance status) were obtained from the CIRG central data base.

Statistical analysis

- The analysis was conducted in two steps
  - 1- Hypotheses generation on 2/3 of the data.
  - 2- Validation of hypotheses on 1/3 of the data.
- The 1,350 samples were randomly split into training (n = 906) and validation (n = 444) sets. In order to ensure that each dataset is representative of the whole study, the randomization was stratified by 4 factors: completeness of tumor marker data (complete versus incomplete), treatment group (TAC versus FAC), actual nodal status (1-3 versus 4+), and DFS event observed (yes versus no).
- In the first step, correlations between tumors markers, tumor characteristics, patient characteristics and Disease-Free Survival (DFS) and Overall Survival (OS) were investigated through univariate analyses using the Kaplan-Meier method (log-rank test).
- Then, the univariety significant covariates ($p < 0.10$) were included in a multivariate Cox model with backward elimination procedure on the training dataset to identify the final model of prognostic factors.
- Finally, multivariate analyses were performed on the validation dataset to validate the previous findings.

RESULTS

Patient characteristics

- Patient characteristics at baseline were comparable between treatment arms. Patient characteristics on training dataset were not different compared to patient characteristics on validation dataset. However, the distribution of maximal size of axillary node metastases (less than 2 mm or not) differed slightly between training and validation datasets there were 32.3% of patients with micromets only in training set whereas there were 24.3% on validation set.

Tumor marker characteristics

- The distributions of MIB-1, p53, MUC 1 cytoplasmic diffuse, MUC 1 cytoplasmic luminal, MUC 1 membrane intercellular, MUC 1 membrane peri-aggregate, CEP17 count, C-erbB-2 (CB11) and C-erbB-2 (DAKO), Bcl-X, Bax, Bag-1 nuclear, tubulin type II and IV, Tau protein and glu tubulin were comparable between training and validation datasets.
- However, the median values of MUC 1 total, MUC 1 cytoplasmic granular, HER2/CEP17 ratio, Bcl-2 and Bag-1 cytoplasmic on the training dataset were higher than these median values on validation dataset. The median values of HER2 count, and tubulin type III on training dataset were lower than these median values on validation dataset.

Disease-Free Survival (DFS)

► In the training dataset, there were a total of 243 DFS events. In the validation dataset, there were a total of 118 DFS events with 66 and 52 in FAC and TAC arms, respectively.

Table 1. Univariate analyses : DFS Hazard Ratio - Whole training dataset

PARAMETERS			DFS (n = 906)		
	TITLE	CRITERION	n	HR * [95 % CI **]	p
PATIENT CHARACTERISTICS	Age (years)	< 49 versus ≥ 49	906	0.915 [0.711 - 1.177]	0.4899
	Tumor Size (cm)	< 2.3 versus ≥ 2.3	906	1.707 [1.320 - 2.208]	< 0.0001
	Race	No caucasian vs caucasian	879	0.678 [0.420 - 1.096]	0.1130
	Menopause	Pre versus post	906	1.224 [0.953 - 1.573]	0.1138
	Multifocal disease	Single versus multi	781	1.113 [0.838 - 1.480]	0.4589
	Histological type	Other vs mixed ductal/lobular	887	0.496 [0.307 - 0.801]	**0.0042**
	Macromets/Micromets	Micro versus macro	903	0.890 [0.678 - 1.167]	0.3993
	Estrogen receptor	Other vs > 80% positive	901	0.443 [0.344 - 0.571]	**< 0.0001**
	Progesterone receptor	Other vs > 80% positive	901	0.262 [0.158 - 0.435]	**< 0.0001**
	NG grade	< 3 versus 3	896	1.889 [1.460 - 2.445]	**< 0.0001**
	AG grade	< 3 versus 3	896	1.830 [1.268 - 2.640]	**0.0012**
	MG grade	< 3 versus 3	896	2.003 [1.531 - 2.620]	**< 0.0001**
	OHG grade	< 3 versus 3	896	2.134 [1.659 - 2.745]	**< 0.0001**
	Vascular invasion	None vs involved, extensive	854	1.250 [0.958 - 1.632]	0.1008
	Extra nodal disease	Absent versus present	654	1.527 [1.172 - 2.107]	**0.0025**
	Nodes (CRF)	< 3 versus ≥ 3	892	2.017 [1.547 - 2.630]	**< 0.0001**
	Karnofsky PS	< 100 versus 100	906	1.572 [0.657 - 1.193]	0.4236
TUMOR MARKER CHARACTERISTICS	MIB-1 positive	< 30% versus ≥ 30%	902	2.228 [1.705 - 2.910]	**< 0.0001**
	p53 positive	≤ 5% versus > 5%	898	1.305 [1.014 - 1.680]	**0.0387**
	MUC 1 total positive	< 90% versus ≥ 90%	854	0.859 [0.660 - 1.118]	0.2583
	MUC 1 cyto. granular positive	≤ 60% versus > 60%	853	0.944 [0.725 - 1.229]	0.6691
	MUC 1 cyto. diffuse positive	≤ 0% versus > 0%	853	0.952 [0.602 - 1.507]	0.8350
	MUC 1 cyto. luminal positive	≤ 0% versus > 0%	853	0.657 [0.499 - 0.866]	**0.0029**
	MUC 1 memb. intercell. positive	≤ 0% versus > 0%	853	1.138 [0.874 - 1.482]	0.3368
	MUC 1 memb. peri-agg. positive	≤ 0% versus > 0%	853	1.017 [0.782 - 1.324]	0.8976
	HER 2	≤ 123 count vs > 123 count	840	1.089 [0.837 - 1.416]	0.5260
	CEP 17	< 75 count vs ≥ 75 count	839	0.965 [0.742 - 1.256]	0.7922
	HER 2/CEP 17 ratio	< 1.15 versus ≥ 1.15	839	1.020 [0.784 - 1.327]	0.8848
	C-erbB-2 (CB11)	≤ 0% versus > 0%	845	1.199 [0.922 - 1.561]	0.1756
	C-erbB-2 (DAKO)	< 5% versus ≥ 5%	441	1.388 [0.952 - 2.025]	**0.0884**
	HER 2 status	Negative versus positive	839	1.624 [1.230 - 2.144]	**0.0006**
	Bcl-2 positive	< 80% versus ≥ 80%	881	0.640 [0.493 - 0.830]	**0.0008**
	Bax positive	< 80% versus ≥ 80%	880	0.935 [0.724 - 1.209]	0.6096
	Bcl-X positive	< 80% versus ≥ 80%	883	0.917 [0.711 - 1.183]	0.5039
	Bag-1 cyto. positive	< 60% versus ≥ 60%	852	0.952 [0.734 - 1.235]	0.7122
	Bag-1 nuclear positive	≤ 20% versus > 20%	852	1.062 [0.819 - 1.377]	0.6498
	Tubulin II positive	≤ 40% versus > 40%	850	0.879 [0.677 - 1.140]	0.3297
	Tubulin III positive	< 40% versus ≥ 40%	808	1.280 [0.979 - 1.673]	**0.0714**
	Tubulin IV positive	< 90% versus ≥ 90%	854	0.796 [0.608 - 1.044]	**0.0989**
	Tau protein	≤ 80% versus > 80%	854	0.465 [0.352 - 0.616]	**< 0.0001**
	Glu Tubulin positive	< 60% versus ≥ 60%	830	1.295 [0.995 - 1.684]	**0.0544**

Table 2. Univariate analyses : DFS Hazard Ratio - Whole validation dataset

PARAMETERS			DFS (n = 444)		
	TITLE	CRITERION	n	HR * [95 % CI **]	p
PATIENT CHARACTERISTICS	Age (years)	< 49 versus ≥ 49	444	0.895 [0.624 - 1.284]	0.5480
	Tumor Size (cm)	< 2.3 versus ≥ 2.3	444	1.575 [1.091 - 2.275]	**0.0153**
	Race	No caucasian vs caucasian	429	1.070 [0.542 - 2.111]	0.8456
	Menopause	Pre versus post	444	0.826 [0.564 - 1.209]	0.3255
	Multifocal disease	Single versus multi	405	1.174 [0.777 - 1.774]	0.4474
	Histological type	Other vs mixed ductal/lobular	439	0.847 [0.485 - 1.480]	0.5598
	Macromets/Micromets	Micro versus macro	444	0.588 [0.364 - 0.951]	**0.0304**
	Estrogen receptor	Other vs > 80% positive	438	0.442 [0.304 - 0.642]	**< 0.0001**
	Progesterone receptor	Other vs > 80% positive	438	0.256 [0.119 - 0.549]	**0.0005**
	NG grade	< 3 versus 3	441	1.735 [1.198 - 2.512]	**< 0.0035**
	AG grade	< 3 versus 3	441	1.760 [1.040 - 2.980]	**0.0353**
	MG grade	< 3 versus 3	441	2.671 [1.849 - 3.859]	**< 0.0001**
	OHG grade	< 3 versus 3	441	2.055 [1.431 - 2.951]	**< 0.0001**
	Vascular invasion	None vs involved, extensive	422	1.639 [1.111 - 2.417]	**0.0128**
	Extra nodal disease	Absent versus present	353	1.451 [0.974 - 2.161]	**0.0669**
	Nodes (CRF)	< 3 versus ≥ 3	434	2.287 [1.543 - 3.389]	**< 0.0001**
	Karnofsky PS	< 100 versus 100	444	2.024 [1.178 - 3.480]	**0.0107**
TUMOR MARKER CHARACTERISTICS	MIB-1 positive	< 30% versus ≥ 30%	439	2.232 [1.506 - 3.309]	**0.0001**
	p53 positive	≤ 5% versus > 5%	436	1.191 [0.826 - 1.716]	0.3494
	MUC 1 total positive	< 80% versus ≥ 80%	417	0.567 [0.381 - 0.844]	**0.0052**
	MUC 1 cyto. granular positive	≤ 50% versus > 50%	417	1.000 [0.684 - 1.461]	0.9998
	MUC 1 cyto. diffuse positive	0% versus > 0%	416	0.811 [0.410 - 1.606]	0.5485
	MUC 1 cyto. luminal positive	0% versus > 0%	417	0.852 [0.579 - 1.252]	0.4140
	MUC 1 memb. intercell. positive	0% versus > 0%	417	1.214 [0.828 - 1.778]	0.3206
	MUC 1 memb. peri-agg. positive	0% versus > 0%	417	0.577 [0.389 - 0.856]	**0.0062**
	HER 2	≤ 124 count vs > 124 count	411	1.415 [0.964 - 2.078]	**0.0764**
	CEP 17	< 75 count vs ≥ 75 count	410	1.204 [0.822 - 1.764]	0.3414
	HER 2/CEP 17 ratio	< 1.14 versus ≥ 1.14	410	1.440 [0.981 - 2.115]	**0.0628**
	C-erbB-2 (CB11)	0% versus > 0%	411	1.210 [0.825 - 1.775]	0.3281
	C-erbB-2 (DAKO)	< 5% versus ≥ 5%	201	0.903 [0.530 - 1.539]	0.7072
	HER 2 status	Negative versus positive	410	1.568 [1.049 - 2.343]	**0.0284**
	Bcl-2 positive	< 70% versus ≥ 70%	432	0.736 [0.507 - 1.067]	0.1055
	Bax positive	< 80% versus ≥ 80%	432	1.314 [0.907 - 1.902]	0.1487
	Bcl-X positive	< 80% versus ≥ 80%	432	0.919 [0.637 - 1.324]	0.6495
	Bag-1 cyto. positive	< 50% versus ≥ 50%	419	0.884 [0.607 - 1.287]	0.5185
	Bag-1 nuclear positive	≤ 20% versus > 20%	419	0.673 [0.457 - 0.989]	**0.0441**
	Tubulin II positive	≤ 40% versus > 40%	417	1.352 [0.928 - 1.972]	0.1165
	Tubulin III positive	< 50% versus ≥ 50%	397	1.394 [0.951 - 2.045]	**0.0887**
	Tubulin IV positive	< 90% versus ≥ 90%	418	0.957 [0.637 - 1.437]	0.8311
	Tau protein	≤ 80% versus > 80%	416	0.535 [0.362 - 0.791]	**0.0017**
	Glu Tubulin positive	< 60% versus ≥ 60%	413	0.941 [0.643 - 1.376]	0.7521

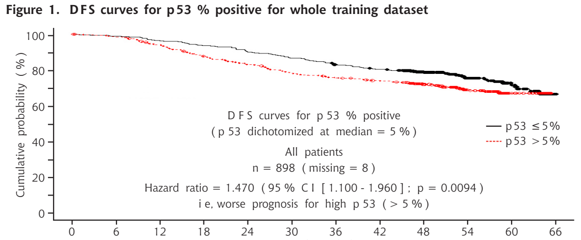
Table 3. Multivariate analyses: DFS final Cox model - Whole training dataset

		ADJUSTED TREATMENT EFFECT ON COVARIATES SELECTED IN TRAINING SET	
COVARIATES	CRITERION	HR [95 % CI] [lower - upper]	SCORE TEST p
Randomization group	TAC/FAC	0.730 [0.550 - 0.960]	0.0245
Estrogen receptor	Other versus > 80% positive	0.610 [0.450 - 0.840]	0.0022
Progesterone receptor	Other versus > 80% positive	0.370 [0.200 - 0.660]	0.0008
MG grade	< 3 versus 3	1.490 [1.080 - 2.050]	0.0147
Tumor size (cm)	< 2.3 versus ≥ 2.3	1.450 [1.090 - 1.930]	0.0110
Nodes (CRF)	< 3 versus ≥ 3	1.780 [1.340 - 2.360]	< 0.0001
p53 positive	≤ 5% versus > 5%	1.470 [1.100 - 1.960]	0.0094
Tau positive	≤ 80% versus > 80%	0.670 [0.500 - 0.900]	0.0074

Table 4. Multivariate analyses: DFS final Cox model - Whole validation dataset

		ADJUSTED TREATMENT EFFECT ON COVARIATES SELECTED IN TRAINING SET	
COVARIATES	CRITERION	HR [95 % CI] [lower - upper]	SCORE TEST p
Randomization group	TAC/FAC	0.690 [0.460 - 1.030]	0.0713
Estrogen receptor	Other versus > 80% positive	0.600 [0.380 - 0.940]	0.0274
Progesterone receptor	Other versus > 80% positive	0.350 [0.150 - 0.800]	0.0124
MG grade	< 3 versus 3	1.700 [1.070 - 2.710]	0.0241
Tumor size (cm)	< 2.3 versus ≥ 2.3	1.160 [0.780 - 1.730]	0.4553
Nodes (CRF)	< 3 versus ≥ 3	2.410 [1.580 - 3.670]	< 0.0001
p53 positive	≤ 5% versus > 5%	1.160 [0.770 - 1.760]	0.4815
Tau positive	≤ 80% versus > 80%	0.810 [0.550 - 1.210]	0.3058

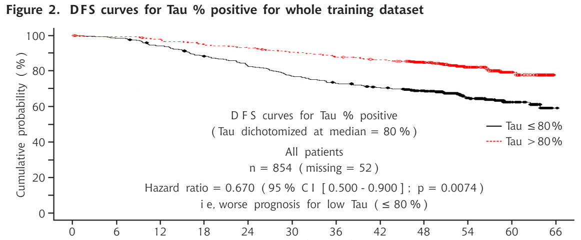
Figure 1. DFS curves for p53 % positive for whole training dataset



Number of subjects still at risk

≤ 5%	488	483	471	458	441	425	405	392	313	178	74	8
> 5%	410	402	382	354	336	316	305	295	241	139	49	-

Figure 2. DFS curves for Tau % positive for whole training dataset



Number of subjects still at risk

≤ 80%	452	445	424	396	371	346	328	314	254	147	57	5
> 80%	402	398	390	379	372	361	350	342	277	152	54	-

Relation between tumor marker and DFS

- Univariate analyses have shown that MIB-1, HER2 status, tubulin type III and Tau protein were associated with DFS in both training and validation datasets as well as estrogen and progesterone receptors, tumor size, number of positive nodes, NG, AG, MG and OHG grades.
- Multivariate analyses taking into account both the biological and classical prognostic variables showed that overall, **p53** (HR = 1.47) and **Tau protein (HR = 0.67) were independently associated with DFS on the training dataset. Similar trends** (HR = 1.16 and 0.81, respectively) **were seen but were not statistically significant with the validation dataset.** In these analyses, MG grade, nodes CRF and estrogen and progesterone receptors were independently associated with DFS on the both training and validation datasets.

Overall Survival (OS)

► In the training dataset, there were a total of 131 deaths. In the validation dataset, there were a total of 68 deaths.

Table 5. Univariate analyses : OS Hazard Ratio - Whole training dataset

PARAMETERS			OS (n = 906)		
	TITLE	CRITERION	n	HR * [95 % CI **]	p
PATIENT CHARACTERISTICS	Age (year)	< 49 versus ≥ 49	906	0.863 [0.611 - 1.217]	0.3998
	Tumor Size (cm)	< 2.3 versus ≥ 2.3	906	2.015 [1.407 - 2.884]	**0.0001**
	Race	No caucasian vs caucasian	879	0.983 [0.459 - 2.106]	0.9649
	Menopause	Pre versus post	906	1.183 [0.837 - 1.671]	0.3411
	Multifocal disease	Single versus multi	781	0.969 [0.650 - 1.445]	0.8785
	Histological type	Other vs mixed ductal/lobular	887	0.479 [0.243 - 0.942]	**0.0330**
	Macromets/Micromets	Micro versus macro	903	0.665 [0.446 - 0.991]	**0.0448**
	Estrogen receptor	Other vs > 80% positive	901	0.301 [0.208 - 0.436]	**< 0.0001**
	Progesterone receptor	Other vs > 80% positive	901	0.159 [0.065 - 0.388]	**0.0001**
	NG grade	< 3 versus 3	896	2.623 [1.801 - 3.819]	**< 0.0001**
	AG grade	< 3 versus 3	896	2.494 [1.406 - 4.424]	**0.0018**
	MG grade	< 3 versus 3	896	2.934 [2.068 - 4.164]	**< 0.0001**
	OHG grade	< 3 versus 3	896	3.020 [2.124 - 4.294]	**< 0.0001**
	Vascular invasion	None vs involved, extensive	854	1.369 [0.946 - 1.980]	**0.0955**
	Extra nodal disease	Absent versus present	654	1.377 [0.930 - 2.037]	0.1098
	Nodes (CRF)	< 3 versus ≥ 3	892	2.243 [1.550 - 3.245]	**< 0.0001**
	Karnofsky PS	< 100 versus 100	906	0.745 [0.504 - 1.102]	0.1406
TUMOR MARKER CHARACTERISTICS	MIB-1 positive	< 30% versus ≥ 30%	902	3.764 [2.492 - 5.687]	**< 0.0001**
	p53 positive	≤ 5% versus > 5%	898	1.860 [1.311 - 2.639]	**0.0005**
	MUC 1 total positive	< 90% versus ≥ 90%	854	0.857 [0.600 - 1.226]	0.3994
	MUC 1 cyto. granular positive	≤ 60% versus > 60%	853	0.992 [0.693 - 1.420]	0.9634
	MUC 1 cyto. diffuse positive	0% versus > 0%	853	1.254 [0.718 - 2.190]	0.4261
	MUC 1 cyto. luminal positive	0% versus > 0%	853	0.573 [0.390 - 0.842]	**0.0046**
	MUC 1 memb. intercell. positive	0% versus > 0%	853	1.164 [0.813 - 1.666]	0.4070
	MUC 1 memb. peri-agg. positive	0% versus > 0%	853	1.014 [0.709 - 1.451]	0.9390
	HER 2	≤ 123 count vs > 123 count	840	1.173 [0.824 - 1.670]	0.3752
	CEP 17	< 75 count vs ≥ 75 count	839	0.912 [0.640 - 1.299]	0.6105
	HER 2/CEP 17 ratio	< 1.15 versus ≥ 1.15	839	1.012 [0.710 - 1.441]	0.9486
	C-erbB-2 (CB11)	≤ 0% versus > 0%	845	1.174 [0.821 - 1.680]	0.3795
	C-erbB-2 (DAKO)	< 5% versus ≥ 5%	441	2.041 [1.185 - 3.518]	**0.0101**
	HER 2 status	Negative versus positive	839	1.852 [1.283 - 2.674]	**0.0010**
	Bcl-2 positive	< 80% versus ≥ 80%	881	0.567 [0.395 - 0.815]	**0.0021**
	Bax positive	< 80% versus ≥ 80%	880	0.911 [0.641 - 1.296]	0.6051
	Bcl-X positive	< 80% versus ≥ 80%	883	0.832 [0.587 - 1.179]	0.3007
	Bag-1 cyto. positive	< 60% versus ≥ 60%	852	0.814 [0.569 - 1.164]	0.2588
	Bag-1 nuclear positive	≤ 20% versus > 20%	852	1.069 [0.747 - 1.529]	0.7165
	Tubulin II positive	≤ 40% versus > 40%	850	0.770 [0.537 - 1.104]	0.1548
	Tubulin III positive	< 40% versus ≥ 40%	808	1.849 [1.270 - 2.693]	**0.0014**
	Tubulin IV positive	< 90% versus ≥ 90%	854	0.671 [0.468 - 0.962]	**0.0300**
	Tau protein	≤ 80% versus > 80%	854	0.382 [0.255 - 0.573]	**< 0.0001**
	Glu Tubulin positive	< 60% versus ≥ 60%	830	1.273 [0.887 - 1.826]	0.1899

Table 6. Univariate analyses : OS Hazard Ratio - Whole validation dataset

PARAMETERS			OS (n = 444)		
	TITLE	CRITERION	n	HR [95 % CI]	p
PATIENT CHARACTERISTICS	Age (year)	< 49 versus ≥ 49	444	0.941 [0.584 - 1.514]	0.8013
	Tumor Size (cm)	< 2.3 versus ≥ 2.3	444	2.269 [1.354 - 3.803]	**0.0019**
	Race	No caucasian vs caucasian	429	1.590 [0.577 - 4.383]	0.3696
	Menopause	Pre versus post	444	0.937 [0.572 - 1.534]	0.7958
	Multifocal disease	Single versus multi	405	1.120 [0.647 - 1.938]	0.6864
	Histological type	Other vs mixed ductal/lobular	439	0.882 [0.421 - 1.846]	0.7392
	Macromets/Micromets	Micro versus macro	444	0.537 [0.280 - 1.028]	**0.0606**
	Estrogen receptor	Other vs > 80% positive	438	0.255 [0.147 - 0.444]	**< 0.0001**
	Progesterone receptor	Other vs > 80% positive	438	0.281 [0.102 - 0.773]	**0.0139**
	NG grade	< 3 versus 3	441	2.108 [1.275 - 3.486]	**0.0037**
	AG grade	< 3 versus 3	441	2.769 [1.197 - 6.406]	**0.0173**
	MG grade	< 3 versus 3	441	3.813 [2.360 - 6.158]	**< 0.0001**
	OHG grade	< 3 versus 3	441	2.590 [1.593 - 4.211]	**0.0001**
	Vascular invasion	None vs involved, extensive	422	1.394 [0.840 - 2.313]	0.1985
	Extra nodal disease	Absent versus present	353	1.760 [1.039 - 2.982]	**0.0355**
	Nodes (CRF)	< 3 versus ≥ 3	434	2.927 [1.664 - 5.147]	**0.0002**
	Karnofsky PS	< 100 versus 100	444	1.454 [0.762 - 2.773]	0.2559
TUMOR MARKER CHARACTERISTICS	MIB-1 positive	< 30% versus ≥ 30%	439	2.762 [1.590 - 4.797]	**0.0003**
	p53 positive	≤ 5% versus > 5%	436	1.493 [0.913 - 2.441]	0.1101
	MUC 1 total positive	< 80% versus ≥ 80%	417	0.572 [0.338 - 0.968]	**0.0375**
	MUC 1 cyto. granular positive	≤ 50% versus > 50%	417	1.247 [0.759 - 2.048]	0.3836
	MUC 1 cyto. diffuse positive	0% versus > 0%	416	0.419 [0.131 - 1.336]	0.1413
	MUC 1 cyto. luminal positive	0% versus > 0%	417	0.571 [0.335 - 0.973]	**0.0395**
	MUC 1 memb. intercell. positive	0% versus > 0%	417	1.017 [0.612 - 1.690]	0.9480
	MUC 1 memb. peri-agg. positive	0% versus > 0%	417	0.522 [0.309 - 0.882]	**0.0150**
	HER 2	≤ 124 count vs > 124 count	411	1.129 [0.681 - 1.874]	0.6376
	CEP 17	< 75 count vs ≥ 75 count	410	1.094 [0.659 - 1.816]	0.7282
	HER 2/CEP 17 ratio	< 1.14 versus ≥ 1.14	410	1.293 [0.778 - 2.151]	0.3217
	C-erbB-2 (CB11)	0% versus > 0%	411	0.903 [0.534 - 1.527]	0.7040
	C-erbB-2 (DAKO)	< 5% versus ≥ 5%	201	0.824 [0.376 - 1.807]	0.6292
	HER 2 status	Negative versus positive	410	1.354 [1.786 - 2.333]	0.2752
	Bcl-2 positive	< 70% versus ≥ 70%	432	0.550 [0.330 - 0.918]	**0.0223**
	Bax positive	< 80% versus ≥ 80%	432	1.176 [0.722 - 1.916]	0.5154
	Bcl-X positive	< 80% versus ≥ 80%	432	0.631 [0.387 - 1.028]	**0.0645**
	Bag-1 cyto. positive	< 50% versus ≥ 50%	419	0.849 [0.517 - 1.394]	0.5177
	Bag-1 nuclear positive	≤ 20% versus > 20%	419	0.680 [0.409 - 1.132]	0.1378
	Tubulin II positive	≤ 40% versus > 40%	417	1.581 [0.945 - 2.643]	**0.0810**
	Tubulin III positive	< 50% versus ≥ 50%	397	1.943 [1.135 - 3.327]	**0.0155**
	Tubulin IV positive	< 90% versus ≥ 90%	418	0.962 [0.555 - 1.669]	0.8916
	Tau protein	≤ 80% versus > 80%	416	0.399 [0.228 - 0.699]	**0.0013**
	Glu Tubulin positive	< 60% versus ≥ 60%	413	1.009 [0.609 - 1.672]	0.9708

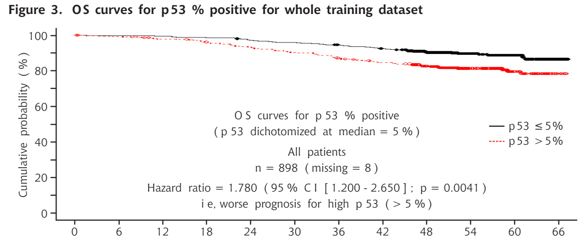
Table 7. Multivariate analyses: OS final Cox model - Whole training dataset

		ADJUSTED TREATMENT EFFECT ON COVARIATES SELECTED IN TRAINING SET	
COVARIATES	CRITERION	HR [95 % CI] [lower - upper]	SCORE TEST p
Randomization group	TAC/FAC	0.730 [0.500 - 1.070]	0.1090
Estrogen receptor	Other versus > 80% positive	0.470 [0.300 - 0.740]	0.0012
Progesterone receptor	Other versus > 80% positive	0.350 [0.140 - 0.900]	0.0297
MG grade	< 3 versus 3	1.910 [1.250 - 2.910]	0.0026
Tumor size (cm)	< 2.3 versus ≥ 2.3	1.800 [1.210 - 2.660]	0.0034
Nodes (CRF)	< 3 versus ≥ 3	2.040 [1.370 - 3.040]	0.0004
p53 positive	≤ 5% versus > 5%	1.780 [1.200 - 2.650]	0.0041
Tau positive	≤ 80% versus > 80%	0.580 [0.390 - 0.850]	0.0049

Table 8. Multivariate analyses: OS final Cox model - Whole validation dataset

		ADJUSTED TREATMENT EFFECT ON COVARIATES SELECTED IN TRAINING SET	
COVARIATES	CRITERION	HR [95 % CI] [lower - upper]	SCORE TEST p
Randomization group	TAC/FAC	0.560 [0.320 - 0.980]	0.0433
Estrogen receptor	Other versus > 80% positive	0.330 [0.170 - 0.660]	0.0017
Progesterone receptor	Other versus > 80% positive	0.540 [0.160 - 1.770]	0.3069
MG grade	< 3 versus ≥ 3	2.000 [1.070 - 3.730]	0.0287
Tumor size (cm)	< 2.3 versus ≥ 2.3	1.500 [0.840 - 2.690]	0.1720
Nodes (CRF)	< 3 versus ≥ 3	3.570 [1.860 - 6.860]	0.0001
p53 positive	≤ 5% versus > 5%	1.150 [0.660 - 1.990]	0.6299
Tau positive	≤ 80% versus > 80%	0.780 [0.450 - 1.330]	0.3589

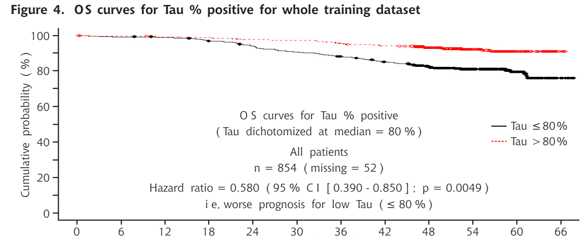
Figure 3. OS curves for p53 % positive for whole training dataset



Number of subjects still at risk

≤ 5%	488	486	485	481	473	467	458	447	383	237	107	15
> 5%	410	405	396	387	376	364	350	337	299	166	70	11

Figure 4. OS curves for Tau % positive for whole training dataset



Number of subjects still at risk

≤ 80%	452	449	445	434	420	406	392	376	327	195	87	15
> 80%	402	399	396	395	390	388	381	375	327	187	75	8

Relation between tumor marker and OS

- Univariate analyses showed that MIB-1, MUC-1 cytoplasmic luminal, Bcl-2, Tubulin type III and Tau protein were associated with OS in both training and validation datasets. p53 was correlated with OS on the training series and associated with a trend (p = 0.11) in the validation series.
- Multivariate analyses taking into account these variables and classical prognostic variables showed that in addition to estrogen and progesterone receptors, tumor size, number of positive nodes and MG grade, **p53** (HR = 1.78) and **Tau** (HR = 0.58) **were independently correlated with OS on the training dataset.** However, p53 and Tau were not confirmed **on the validation dataset,** but **similar trend were seen.**

* Hazard Ratio ; ** 95 % Confidence Interval

CONCLUSION

- These data suggest that **Tau and p53 are independent markers both of DFS and of OS** in patients receiving adjuvant chemotherapy for axillary node-positive breast cancer.
- We suggest that analysis of these parameters by immunohistochemistry could help identify prognostic subgroups and design novel therapeutic approaches in this patient population.

ACKNOWLEDGMENTS

A Allard : Sanofi-aventis statistician,
V Wilson and A Popovic : CIRG data managers,
for their contribution to these analyses

Presented at the 43[rd] Annual Meeting of the American Society of Clinical Oncology, June 1-5, 2007, Chicago, USA