5030 POSTER DISCUSSION

High resolution array Comparative Genomic Hybridization (aCGH) of breast carcinoma identifies Mouse double minutes 4 (Mdm4) as one of the early genetic changes in breast cancer development – Mdm4 is a new independent prognostic and predictive marker

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Background: Genome-wide aCGH identified recurrent amplification/gain of chromosome 1:202,752,134–202,862,753 in 86% and 7% of low (LGBC) and high (HGBC) grade breast cancers, respectively. MDM4 gene maps to this locus.
We hypothesised that it may be a candidate oncogene and tested this hypothesis by determining its association with clinical outcome and biological features in BC.
Methods: MDM4-mRNA expression levels were assessed in 2 independent sets of gene expression arrays. Protein expression levels were assessed using immunohistochemistry in series of 1081 BCs with long term follow up and series of 140 cases of LGBCs with matched normal terminal ductal lobular units (TDLUs) and precursor lesions including columnar cell lesions, atypical ductal hyperplasia, ductal carcinoma *in situ* and lobular neoplasia.
Results: MDM4 mRNA expression levels significantly correlated with copy number (Pearson's correlation = 0.55, $p = 0.0001$) and this gene is overexpressed when amplified (Mann-Whitney U test $p = 0.0018$). Mdm4 was overexpressed in 17% of BC and was associated with low grade, ER+ and normal expressions of p53, ATM and BRCA1. In cases showing coexistent precursors with invasive component, MDM4 expression was identical in both lesions. On multivariate analysis that included NPI, MDM4-overexpression was an independent prognostic marker for patients survival outcomes [HR, 0.4; $p < 0.0001$]. In high risk patients who had received systemic adjuvant therapy, MDM4-overexpression predicted better response to both hormone- [HR, 2.7; $p < 0.0001$] and chemo-therapies [HR, 6.7; $p = 0.008$].
Conclusion: Mdm4 is an independent prognostic and predictor of BC and its overexpression could represent a novel molecular mechanism by which a subset of BC escapes p53-dependent growth control, providing new avenues for therapeutic intervention.

5031 POSTER DISCUSSION

Overall response rate (ORR) and clinical benefit (CB) as clinical indicators for the efficacy of sequential Aromatase Inhibitors (AIs) in 3rd line hormonal treatment (HT) for advanced breast cancer (ABC): pooled analysis of phase II studies

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Background: Although AIs have demonstrated to be active for hormone-sensitive ABC pts after tamoxifen (TAM), their sequential strategies have not been extensively prospectively compared. In order to establish benchmarks for ORR and CB as references for future planned phase II trials with AIs and new HTs, a pooled analysis of prospective, phase II studies in advanced breast cancer patients, exploring the role of the sequential approach with AIs was accomplished.
Methods: Analysis was conducted in order to find eventual significant differences in both ORR and CB. A literature-based pooled-analysis was performed using the pooled estimated event rates (Random-Effect Model) with 95% confidence intervals (CI); a heterogeneity test was applied. To test for trial interaction of pooled results coming from studies, a sensitivity analysis according to the setting was scheduled, by considering either 1) nS-S: non-steroidal followed by steroidal, and 2) S-nS: steroidal followed by non-steroidal. In order to find eventual correlations between outcome effect and predictive factors (ER+, soft tissue, bone, visceral, prior TAM, prior chemo), a meta-regression approach was accomplished as well (i.e. regression of the selected predictor on the Logit of the event rate of the corresponding outcome). Data were analyzed by using CMA version 2.0 software.
Results: Seventeen studies (1,926 pts, nS-S/S-nS: 15/2) were gathered. Results are shown in the following table.
The meta-regression analysis (performed on 14 studies, with 1,098 pts) identified the rate of ER+ ($p = 0.021$), visceral involvement ($p = 0.00013$) and prior TAM ($p = 0.0023$), and visceral involvement ($p = 0.00006$) and prior TAM ($p = 0.014$), as clinical predictors for ORR and CB improvement, respectively.
Conclusions: In absence of a large series of randomized trials addressing the optimal hormonal sequential approach for TAM-pretreated ABC pts, the choice of both ORR and CB as clinical parameters to evaluate the activity of upcoming new hormonal treatments seems appropriate. An ORR of around 10% and a CB of 40% should be considered as expected reasonable standard benchmarks for future phase II trials.

Outcome	Strategy	Pts (Studies)	Rates (%, 95% CI)	Het. (p)
ORR	nS-S	1,221 (15)	10.4 (7.0−15.2)	*<0.001*
	S-nS	39 (2)	16.3 (7.0−33.5)	*0.285*
	Overall	1,260 (17)	11.3 (7.9−15.9)	*<0.001*
CB	nS-S	1,221 (15)	42.3 (36.3−48.7)	*<0.001*
	S-nS	39 (2)	51.3 (35.8−66.5)	*0.83*
	Overall	1,260 (17)	43.6 (37.9−49.5)	*<0.001*

5032 POSTER DISCUSSION

Effects of G-CSF on circulating tumour cells (CTC) and CA 27.29 in breast cancer patients

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Background: Some recent publications indicated that the use of G-CSF could be connected to an increase in CTC as well as elevated levels of tumor markers such as CA 27.29.
In the SUCCESS-B Trial CTC and CA27.29 are examined before and after adjuvant chemotherapy (CHT) in 3754 breast cancer patients (pts).
Materials and Methods: The SUCCESS-B Trial is a phase III trial comparing FEC-Docetaxel vs. FEC-Doc-Gemcitabine regime and 2 vs. 5 years of treatment with zoledronate in patients with primary breast cancer (BC) (N+ or high risk). Blood samples are taken before and after CHT. CTC were assessed with the CellSearchSystem (Veridex, Warren, USA). After immunomagnetic enrichment with an anti-Epcam-antibody, cells were labeled with anti-cytokeratin (8, 18, 19) and anti-CD45 antibodies to distinguish epithelial cells and leukocytes. CA27.29 has been measured with ST AIA-PACK Ca27.29 reagent using MUC-1 for AIA-600II (Tosoh Bioscience, Tessenderlo, Belgium). The cutoff for CA27.29 is 32 U/ml and >1 cell for the CTC analysis. Patients were grouped to CTC/CA27.29 raise or no raise and 1 to 6 cycles with G-CSF or no G-CSF at all.
Results: Data on 1510 pts are available for CTC analysis. 745 pts (49%) received at least one course of G-CSF. 117 pts (8%) showed an increase in CTC after CHT. In this group 52 (3%) pts received G-CSF and 65 (4%) did not. 693 pts with stable or decreased CTC received G-CSF (46%) and 700 did not (46%). There was no significant difference ($p = 0.29$).
The analysis of CA27.29 is based on the data of 2556 pts. 1252 pts (49%) received at least one course of G-CSF. 338 pts (13%) exceeded the threshold for CA27.29 only after CHT. In this group 209 pts (8%) received G-CSF and 129 (5%) did not. 1043 pts with stable or decreased CA27.29 received G-CSF (41%) and 1175 did not (46%). This difference was highly significant ($p < 0.0001$).
Conclusions: No evidence can be provided for a significant correlation between an increase in the number of CTC and the application of G-CSF over CHT. Nevertheless the results on CA27.29 showed a highly significant correlation between the administration of G-CSF and elevated CA27.29 levels directly after CHT. This could be a possible explanation for the often observed increase of tumor markers after CHT.