median follow-up of 15 months for this 50 pt, 15 pt have died due to AOC. Median survival has not been reached, and 2-year overall survival is 76%.

Conclusion: In this population of pt with AOC suboptimally debulked, TC-Tp seems to be a very active and safe regimen. Final results of cCR, pCR and toxicity will be available next year.

967 POSTER DISCUSSION

ZD0473 phase II monotherapy trial in second-line ovarian cancer

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Aims: ZD0473 (cis-amminedichloro[2-methylpyridine]platinum [II]) is a new generation platinum drug designed to deliver an extended spectrum of antitumour activity and overcome platinum resistance mechanisms. In this ongoing Phase II open-label, multicentre trial, the efficacy and tolerability of ZD0473 was evaluated in patients (pts) with ovarian cancer who have failed one prior platinum-based therapy.

Methods: Pts were to receive ZD0473 120 mg/m^2 1-h iv infusion on day 1, every 3 weeks. Later the dose was increased to 150 mg/m^2, every 3 weeks. Pts were considered resistant (cohorts 1–3) or sensitive (cohort 4) if they relapsed/progressed ≤26 weeks or >26 weeks, respectively, following completion of prior platinum-based chemotherapy.

Results: To date, 58 pts have been recruited to this study (32 resistant, 26 sensitive; median age 58 years [range 35–75 years]; 57 with performance status 0/1; 45 with distant metastases). Twenty pts received a starting dose of 120 mg/m^2 without escalation, 16 pts received a starting dose of 120 mg/m^2 escalated to 150 mg/m^2, and 22 pts received a starting dose of 150 mg/m^2. Dose reductions and delays occurred primarily in the pts receiving the higher dose of 150 mg/m^2 (58%). Grade 3/4 anaemia, neutropenia or thrombocytopenia was observed in 5, 8, and 7 pts at a dose of 120 mg/m^2; 7, 6 and 9 pts at 120/150 mg/m^2; and 5, 18 and 17 pts at 150 mg/m^2, respectively. The extent of prior exposure to carboplatin appears to be an important factor for haematological toxicity. Three pts were withdrawn from the trial due to drug-related toxicity and no drug-related deaths occurred. No clinically relevant nephro- or neurotoxicity were reported. Grade 3/4 nausea or vomiting was reported in 5 and 6 pts, respectively. Preliminary data have shown that an objective response was observed in 3/21 evaluable resistant pts (2 CR, 1 PR) and 7/22 evaluable sensitive pts (2 CR, 5 PR*). Five of the responses were observed at a dose of 120 mg/m^2, the other 5 responses were observed in pts who started on 120 mg/m^2 and were escalated to 150 mg/m^2. A further 6 resistant pts and 10 sensitive pts had stable disease (2 and 5 pts with some evidence of tumour shrinkage, respectively).

Conclusion: ZD0473 shows encouraging activity in second-line ovarian cancer including resistant disease. ZD0473 has an acceptable safety profile at 120 mg/m^2 and this is the preferred dose in this patient population who have received a high number of prior cycles of carboplatin.

*3/5 PR are currently unconfirmed.

Breast cancer biology

968 POSTER DISCUSSION

Mutation analysis of the CHK2 gene in breast carcinoma and other cancers

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Mutations in the CHK2 gene at chromosome 22q12.1 have been reported in families with Li-Fraumeni syndrome. The Chk2 is an effector kinase that is activated in response to DNA damage and is involved in cell cycle and p53 pathways. We have screened 139 sporadic breast tumours for LOH at chromosome 22q, using 7 microsatellite markers. Seventy four breast tumours (53%) show LOH with at least one marker. These samples and 45 tumours from individuals carrying the BRCA2 999del5 mutation were screened with SSCP and DNA sequencing for mutations in the CHK2 gene. In addition to putative polymorphic regions in short mononucleotide repeats in a noncoding exon and intron 2, a germ line variant (T59K) in the first coding exon was detected. By screening additional 1137 cancer patients for the T59K sequence variant, it was detected in totally 4 breast-, 3 colon-, 1 stomach- and 1 ovary cancer patients, but not in 178 healthy individuals, suggesting that this is a low penetrance allele. A tumour specific 5' splice site mutation at site +3 in intron 8 (TTgt(a->c)atg) was detected in a tumour with extensive LOH in the genome. We conclude that somatic CHK2 mutations are rare in breast cancer, but our results suggest a tumour suppressor function for CHK2 in a minority of breast tumours.

969 POSTER DISCUSSION

The EGFR-selective tyrosine kinase inhibitor ZD1839 ('Iressa') is an effective inhibitor of tamoxifen-resistant breast cancer growth

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Purpose: Many ER+ breast cancer (BC) patients initially respond to antihormone agents, eg tamoxifen ('Nolvadex'); however, acquisition of resistance is often seen. Overexpression of EGFR and/or EGFR ligands (EGF or TGFalpha;) is associated with the antihormone-resistant phase of clinical disease.

Methods: This study investigated the potential of the EGFR-selective tyrosine kinase inhibitor ZD1839 ('Iressa') to treat antihormone-resistant BC using tamoxifen-resistant (R) and tamoxifen-sensitive (wild type [WT]) MCF-7 BC cell lines.

Results: As with tumours from patients with resistance to tamoxifen, R-MCF-7 cells exhibit markedly elevated mRNA and expression of EGFR and c-erbB2 compared with WT-MCF-7 cells. Western blotting and immunocytochemical analysis showed that in R-MCF-7 cells these receptors immunoprecipitated as heterodimers, had increased activity, and were associated with increased levels of the phosphorylated mitogen-activated protein kinases, ERK 1/2. In R-MCF-7 cells treated with EGF and TGFalpha further increases in activation of EGFR-signalling elements and substantial growth responses were observed. Under ligand-stimulated conditions, ERK 1/2 activation was increased in a sustained manner, but ERK 1/2 exhibited only transient activation in WT-MCF-7 cells. ZD1839 blocked activation of EGFR signalling in R-MCF-7 cells under basal and ligand-stimulated conditions, and resulted in profound, long-lasting growth inhibition. WT-MCF-7 cells were much less sensitive to growth inhibition by ZD1839 (15% decrease in WT-MCF-7 cells vs up to 90% for R-MCF-7). These studies show that in BC cells with acquired resistance to tamoxifen, autocrine activation of the EGFR signalling pathway is of critical importance to growth and that these cells are substantially more sensitive to ZD1839 than WT-MCF-7 cells. Finally, co-treating WT-MCF-7 cells with tamoxifen and ZD1839, in anticipation of the switch to EGFR signalling on acquisition of antihormonal resistance, results in synergistic growth inhibition, marked decreases in proliferation, increased apoptosis, and failure to develop resistant growth.

Conclusion: Since the biochemical characteristics of tumours from patients with antihormone-resistant disease parallel those of R-MCF-7 cells, these studies predict that ZD1839 may provide an effective treatment for tamoxifen-resistant BC and prevent the development of this condition.

'Iressa'and 'Nolvadex' are trademarks of the AstraZeneca group of companies

970 POSTER DISCUSSION

Fluorescence in situ hybridization (FISH) may accurately identify patients who obtain survival benefit from herceptin plus chemotherapy

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Background: Women eligible for the pivotal phase III trial of chemotherapy (C) (doxorubicin/epirubicin and cyclophosphamide [AC] or paclitaxel [T]) with or without Herceptin (H) had metastatic breast cancer overexpressing HER2 at the 2+ or 3+ level measured using a standardized, semi-quantitative, immunohistochemistry (IHC) assay. This trial demonstrated that the addition of H to C improved response rate (RR) (50% vs 38%, p=0.003) and survival (25.1 vs 20.3 months, odds ratio, 0.80, p=0.046). These benefits were

observed despite 65% of C-alone patients crossing over to receive H at disease progression. A previous analysis demonstrated that 89% of 3+ tumors and 24% of 2+ tumors show HER2 gene amplification. We sought to determine how the clinical benefit of H relates to HER2 amplification in this trial.

Methods: Histologic material from 458 of 469 enrolled subjects was available for FISH testing. This consisted of archived, unstained tissue sections (44%) or previously immunostained tissue sections (56%). The PathVysion dual probe FISH assay system was used to determine the HER2:CEP17 signal ratio in these samples. Amplification was prospectively defined as a ratio of >2.

Results: FISH results were obtained in 451/469 enrolled patients (96.2%). Amplification was detected in 76% of the study population (89% of 3+ and 31% of 2+ cases). The addition of H to C improved the RR in the FISH-positive subgroup from 30.8% to 54.0% (p<0.0001). There was no improvement in the FISH-negative subgroup (37.5% vs 38.0%, p=NS). Furthermore, the addition of H to C in the FISH-positive group resulted in a survival benefit (odds ratio 0.71, 95% CI: 0.54, 0.92, p=0.009) that was not detected in the FISH-negative subgroup (odds ratio 1.11, 95% CI: 0.70, 1.80, p=NS).

Conclusions: The survival benefit in FISH-positive patients is significant. Patient selection based on HER2 amplification as determined using FISH may accurately identify patients who obtain clinical benefit from H. These data support the use of FISH testing to select patients for H therapy.

971 POSTER DISCUSSION

Over-representation of a polymorphism/missense mutation in the ataxia telangiectasia, mutated (ATM) gene in breast cancer patients versus controls

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Purpose: Mothers of children diagnosed with ataxia telangiectasia have been reported to be at increased risk for breast cancer development. To test whether germline mutations in the ATM gene are associated with breast cancer, we compared the frequency of ATM cDNA sequence changes in breast cancer patients and controls.

Methods: We sequenced ATM cDNA in 91 breast cancer patients and compared sequence changes in these patients to the frequency of these alterations in a control set of 996 individuals with no cancer history. An allele specific oligonucleotide assay was used to study the specific polymorphisms of interest in the ATM cDNA for the control set. The frequency of identified base changes was also tested across ethnic groups and gender.

Results: No mutations that would lead to protein truncation were identified, but several polymorphisms were found in the cDNA of the breast cancer patients. The three polymorphisms that were found in two or more patients cause amino acid substitutions in the ATM protein of the following type: Ser49Cys, Pro1054Arg, and Asp1853Asn. The Ser49Cys polymorphism was found in 6.7% (5/75) of the breast cancer patients compared to 1.6% (12/946) of the control group (P=0.006, Fisher's 2-sided exact). The subgroup of patients with bilateral breast cancer had a frequency rate of 11.8% (2/17) which again was significantly different from the control group (P=0.025, Fisher's 2-sided exact). None of the 9 breast cancer patients that had a normal tissue complication following radiation treatment had the Ser49Cys change. The allelic frequencies of the other two polymorphisms were not different between cases and controls.

Conclusion: Breast cancer patients, particularly those with bilateral disease, are more likely to have a polymorphism in the ATM gene that results in a Ser49Cys change in the protein compared to controls. These data suggest Ser49Cys may be a functional polymorphism that contributes to breast cancer development or a polymorphism that is linked to another causative genetic factor.

972 POSTER DISCUSSION

Comparison of the prognostic significance of occult metastatic cells in the bone marrow (OMC-BM) and HER2-status in patients with stage I-III breast cancer (BC)

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Purpose: Both HER2neu gene amplification and protein over-expression as well as the presence of OMC-BM are significant biological factors for the survival of patients with BC. Recent studies have supported their potential clinical role to stratify patients for adjuvant therapy. In this ongoing study, the prognostic influence of OMC-BM in 378 BC patients (stage I-III) was compared to the prognostic impact of the HER2 status of the primary tumor tissue.

Methods: HER2/neu-status in the paraffin-embedded primary tumor tissue was determined by either immunostaining with antibodies CB11 and A0485, and automated cellular imaging (ACIS>), or FISH and confocal laser scanning microscopy. Occult tumor cells in the bone marrow aspirates were detected with immunocytochemistry, using the anti-cytokeratin antibody A45 B/B3, and screening of 2 x 106 cells per aspirate in bright field microscopy.

Results: OMC-BM were found in 112/378 (30%) patients, HER2 overexpression (2/3) in 62/299 (21%) and amplification (HER2/chromosome-17 ratio greater than 2) in 54/235 (23%) patients. HER2 status was associated with lymph node metastasis (p=0.04 for immunostaining and p=0.033 for FISH), while the presence of OMC-BM was related to an increasing tumor size (p=0.006), but not to HER2 overexpression and amplification. After 40 (12-72) months of median follow-up, OS was significantly reduced in patients with OMC-BM (p<0.0001), while the HER2 status only reported a statistical trend towards poor OS (p=0.052 and p=0.11). The presence of OMC-BM was found to be an independent prognostic factor with a 2.9-fold increased relative risk of cancer-related death (p=0.028) in the multivariate analysis.

Conclusion: The direct identification of metastatic precursor cells in the bone marrow could help to improve current stratification of stage I-III breast cancer patients at high risk of relapse. In contrast to HER2 status of the primary tumor, OMC-BM can also be used to monitor patients during specific adjuvant therapy (e.g. antibody therapy), as previously shown by our group.

Cell biology/Genetics II

973 POSTER DISCUSSION

Pamidronate (P) induces modifications of circulating angiogenetic factors in cancer patients

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Purpose: This study was designed primarily to investigate the potential antiangiogenic role of P in patients with malignancies.

Methods: 16 consecutive patients (11 males, 5 females), aged 49-77 years (median 65), with bone metastases from solid cancer, were included in the study. Exclusion criteria were: a) the presence of acute or chronic inflammatory diseases or infections; b) previous radiotherapy, chemotherapy, immunotherapy or haemopoietic growth factors administration during the 4 weeks before accrual in the study; c) recent or simultaneous administration of steroids. Patients received 90 mg i.v. of P over a 2 hours infusion. Blood samples for cytokines assessment (VEGF, gamma-IFN, IL-6 and IL-8) were collected as follows: before and after 24, 48 hours and 1 week from P administration.

Results: The mean value of basal VEGF was 762,46 pg/ml (Standard Deviation (SD): 291,04). 24 hours after single P infusion the mean value of VEGF decreased to 515,93 pg/ml (SD: 186,91) (p=0.006), and after 48 hours persisted lower with a mean value of 485,57 pg/ml (SD: 237,13) (p=0.001). The effect of P on VEGF persisted after 1 week with a mean value of 596,47 pg/ml (SD: 385,71) (p=0.028). The mean value of basal gamma-IFN was 11,12 pg/ml (SD: 4,34). After 24 hours the P infusion the mean value significantly increased to 21,93 pg/ml (SD: 14,26) (p=0.019). Otherwise, after 48 hours gamma-IFN (12,66 pg/ml; SD: 6,11l) did not significantly differ from the basal value (p= 0.701) and persisted stationary also after 1 week (12,25 pg/ml; SD: 8,34) (p=0.929). The mean basal value of IL-6 was 9,88 pg/ml (SD: 12,1). 24 hours after the P administration the