

Methods: Urine was collected from 78 bladder-cancer patients during follow-up, from 20 patients with benign urological disease, and from 20 healthy volunteers. RNA was isolated from exfoliated cells in urine by use of an RNA purification kit, and real-time PCR was performed with specific primers for the amplification of CK20, a marker for TCC urothelium.

Findings: A strong correlation was found between tumour grade and expression of CK20 in urine. All patients with grade III and IV tumours showed positive CK20 expression in the exfoliated cells, with 100% sensitivity. The sensitivity for lower grades was up to 83%. Out of 13 TCC patients, CK20 expression was found in nine patients who were previously diagnosed by biopsy and had a negative biopsy following treatment. These nine patients were followed up for 6 months, and TCC recurred in four patients.

Interpretation: Quantitative detection of CK20 in exfoliated cells of urine is a simple and non-invasive method for monitoring and follow-up of TCC in patients with bladder cancer. However, more information is needed regarding CK20 expression in non-malignant urological diseases to use it as a marker for routine screening.

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P37 ANTHRACYCLINE-BASED NEOADJUVANT CHEMOTHERAPY AND HYPERMETHYLATION OF A TUMOUR-SUPPRESSOR GENE IN LOCALLY ADVANCED BREAST CANCER

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Background: Anthracycline-based neoadjuvant chemotherapy kills cancer cells by inducing DNA double-strand breaks. In-vitro studies have shown that DNA damage leads to localised DNA methylation on CpG-rich sites found in promoter regions. Promoter methylation of some tumour-suppressor genes has been associated with poor prognosis. To determine whether neoadjuvant chemotherapy induces promoter methylation, we evaluated the promoter regions of the SFRP1 and CDH1 genes in locally advanced breast cancer, before and after treatment.

Methods: Paired FFPE blocks of 61 patients with locally advanced breast cancer before and after chemotherapy were collected and confirmed by pathologists. Patients had standard fluorouracil, doxorubicin (adriamycin), and cyclophosphamide (FAC) chemotherapy for three cycles. In a subset of 12 patients, epigenetic therapy (hydralazine and magnesium valproate) was added. DNA isolation and bisulfite conversion were performed to evaluate promoter methylation of SFRP1 and CDH1 genes using methyl-specific PCR (MSP).

Findings: Using SFRP1 and CDH1 as surrogate markers, 13 of 41 (32%) and 18 of 48 (38%) patients showed induction of promoter hypermethylation after chemotherapy ($p = 0.052$ and $p = 0.012$, McNemar test). However, the rate of demethylation of both markers was 10%. To explore the reversibility of chemotherapy-induced promoter hypermethylation, a subset of 12 patients were treated with a combination of epigenetic therapy and chemotherapy. Two of 12 patients (17%) showed hypermethylation and four of 12 (33%) had an increased rate of promoter demethylation. The dynamic status of promoter methylation is not associated with hormone-receptor status, HER2 expression, age, or stage.

Interpretation: Neoadjuvant chemotherapy can induce promoter methylation of tumour suppressor genes in a significant proportion of patients, which may affect long-term clinical outcome. This trend of promoter hypermethylation of tumour-suppressor genes can be reversed using epigenetic therapy.

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P38 PHASE 1 DOSE-FINDING STUDY OF EPIRUBICIN, OXALIPLATIN, AND S-1 IN PATIENTS WITH PREVIOUSLY UNTREATED ADVANCED GASTRIC CANCER

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Background: To determine the recommended dose and dose-limiting toxicity (DLT) of epirubicin, oxaliplatin, and S-1 (EOS) combination in patients with previously untreated advanced gastric cancer (AGC).

Methods: Previously untreated patients with histologically proven metastatic or recurrent AGC and ECOG performance status 0-2 were enrolled. A fixed dose of epirubicin (50 mg/m²) and oxaliplatin (130 mg/m²) was administered intravenously on day 1. The dose of S-1 was escalated as follows: level 1, 30 mg/m²; level 2, 40 mg/m²; level 3, 45 mg/m²; level 4, 50 mg/m². S-1 was administered orally twice a day on days 1-14. Each cycle was repeated every 21 days. DLTs were evaluated during the first two cycles of treatment.

Findings: 19 patients were enrolled: 13 patients in the dose-escalation phase and six patients in the extension at the recommended dose. The median age was 53 years (range, 40-71 years). At dose level 2, one DLT occurred among six patients (grade 4 neutropenia lasting more than 5 days), and at dose level 3, two DLTs were observed among four patients (grade 3 diarrhoea and nausea). Therefore, dose level 2 was determined to be the recommended dose. Cumulative (all cycles) grade 3-4 toxicity included neutropenia (58%), leucopenia (32%), thrombocytopenia (11%), diarrhoea (11%), and nausea (5%). Of 13 patients with measurable lesions, eight achieved a partial response and three had stable disease, and the objective response rate was 62% (95% CI 36-88%). Median progression-free survival was 6.5 months (4.7-8.2).

Interpretation: The recommended dose of the EOS regimen in patients with previously untreated AGC was epirubicin 50 mg/

m² and oxaliplatin 130 mg/m² on day 1, and S-1 40 mg/m² twice a day on days 1-14 of every 21-day cycle. This regimen seems to have promising preliminary activity.

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P39 A20 BINDING AND INHIBITOR OF NF-KAPPAB (ABIN-1) – A POTENTIAL MARKER FOR SURVIVAL IN EARLY STAGE NON-SMALL-CELL LUNG CANCER AFTER LUNG RESECTION

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Background: Currently, the role of A20 binding and inhibitor of NF-kappaB (ABIN-1) in the development of non-small-cell lung cancer remains unknown. This retrospective study investigated expression of ABIN-1 and the association with prognosis in patients with NSCLC after lung resection.

Methods: Quantitative real-time reverse transcriptase (RT)-PCR, and Western blot analyses were used to detect expression of ABIN-1 in 30 samples of NSCLC tissue and paracarcinomatous lung tissue (PCLT), and in four samples of normal lung tissue. In addition, immunohistochemical analysis was done for 80 NSCLC specimens, and follow-up data from these patients were reviewed.

Findings: Both mRNA and protein expression of ABIN-1 were significantly raised in NSCLC tissues compared with normal lung tissues. Patients with NSCLC who had high ABIN-1 expression had shorter overall survival than patients who had low ABIN-1 expression.

Interpretation: The current data revealed that increased expression of ABIN-1 was correlated with survival in patients with NSCLC, indicating that ABIN-1 is a novel prognostic marker for NSCLC.

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P40 CLINICOPATHOLOGICAL PATTERN, CLASSIFICATION, P53 STATUS, AND STAGING OF URINARY BLADDER CARCINOMAS – SIX-YEAR EXPERIENCE AT A TERTIARY CARE HOSPITAL IN CENTRAL PUNJAB

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Background: Transitional-cell carcinoma (TCC) of the urinary bladder is reported as the eighth most common malignancy and the fourth most common among men in Pakistan. This study aimed to assess the clinicopathological pattern, p53 status, and stage distribution of TCC, and to classify bladder carcinomas pre-

senting among the population of central Punjab, including factory workers, according to the revised WHO/ISUP criteria.

Methods: 145 patients, including 52 factory workers (mean age 35.2 years), with newly diagnosed operable primary bladder carcinomas who underwent cystoscopy-associated transurethral resection of bladder tumours from January, 2004, to July, 2006, were included. Relevant clinical and laboratory data of these patients, including age, sex, tumour location, and type of surgical procedure, were recorded in separate proformas. After confirmation of the diagnosis, the tumours were graded separately for each group – first, according to WHO Classification 1972 as papilloma, TCC grade I, II, and III, and later, according to WHO/ISUP Consensus Classification 1998 as papilloma, papillary neoplasm of low malignant potential (PNLMP), low-grade papillary carcinoma (LGPC), and high-grade papillary carcinoma (HGPC). Tumour staging was done according to TNM criteria of the American Joint Commission on Cancer. All tissues were also subjected to immunohistochemistry (IHC) with monoclonal anti-P53 antibody. Patients were followed up for 3 years, from hospital records until July, 2010. Data were entered and analysed using SPSS 17.0.

Findings: About 80% of patients were men and 20% were women (the male-to-female ratio was 5.3:1). Clinical history was similar for both sexes, with most patients (74%) presenting with haematuria with or without altered urinary habits. WHO grading revealed 35.9% grade I, 25.4% grade II, and 38.6% of tumours as grade III. ISUP classification revealed 19.2% PNLMP, 23.6% LGPC, 39.4% HGPC, 9.6% non-papillary urothelial carcinomas (NPUC), and 7.9% as carcinoma in situ (CIS). Tumour staging depicted an overall 11.5% of tumours with stage Ta and 31.5% with stage T3-4. Among 71% invasive carcinomas, 16% were low-grade and 84% were high-grade carcinomas. Immunohistochemical staining of histological tissue sections of 73% of CIS and 84.23% of TCCs were p53 positive. 10.7% of grade I, 44.9% of grade II, and 92.1% of grade III tumours were positive for p53. There were significantly more p53-positive cases seen in grade II-III tumours than in grade I tumours ($p = 0.0036$). Similarly, stage T2-T4 tumours stained more frequently and stronger than stage T1 tumours ($p = 0.021$). No significant association between p53 status and post-operative prognosis was observed in the 3 years of follow-up ($p = 2.131$).

Interpretation: Prolonged follow-up of patients with bladder cancer may indicate an unfavourable prognostic factor linked to histopathological findings, and the presence of p53 mutation, which may also indicate development of aggressive growth characteristics in TCCs.

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P41 EPIDERMAL GROWTH-FACTOR RECEPTOR MUTATIONS AND METASTATIC PRESENTATION IN NON-SMALL-CELL LUNG CANCER

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