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/m^2) and oxaliplatin (130 mg/m^2) was administered intravenously on day 1. The dose of S-1 was escalated as follows: level 1, 30 mg/m^2; level 2, 40 mg/m^2; level 3, 45 mg/m^2; level 4, 50 mg/m^2. 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 occured 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/