(range 48–64), transitional cell carcinoma histologically confirmed, stage T3/T4, performance status ECOG 0–2. A chemo-radiotherapy regimen consisting of weekly gemcitabine (350 mg/m^2) with 45 Gy of external beam radiotherapy (1.8 Gy/fraction, 5 days/week) was delivered in five weeks on extended fields as appropriate and a boost on the bladder to a median total dose of 65 Gy. Patients were evaluated 4–6 weeks after combined treatment with cystoscopy and CT scans.
Results: 23 patients completed chemo-radiotherapy schedule. 4 patients interrupted the treatment, 3 because of grade 4 toxicity and 1 because of progressive disease. Clinical benefit was found in 19 of the 23 patients (7 complete response, 11 partial response and 1 stable disease). Progressive disease was found in the four remained patients. Adverse effects, especially haematological, were common but manageable. No chemoradiation-associated deaths were observed with this gemcitabine based regimen. Grade 3–4 haematological toxicity (neutropenia and/or trombocitopenia) occurred in 7 and 4 patients respectively. Grade 3 gastrointestinal toxicity (diarrhoea) occurred in 8 patients. Grade 3 cystitis occurred in 6 patients. Median follow-up period was 18 months; at this time 20 patients are still alive and 17 patients remain disease-free.
Conclusions: This schedule of Gemcitabine and radiation therapy is relatively well tolerated and has shown to provide prolonged clinical benefit response and disease stabilization in patients with locally advanced bladder carcinoma. These promising results should be further investigated.

876 POSTER

Combined treatment with Bicalutamide and ZD-1839 may be advisable in human prostate cancer in the early phases

M. Bologna[1], C. Festuccia[1], G.L. Gravina[2], P. Muzi[1], R. Pomante[3], L. Ventura[4], S. Speca[1], D. Millimaggi[1], A. Angelucci[2], C. Vicentini[2]. *[1]University of L'Aquila, Department Experimental Medicine and Basic Applied, L'Aquila, Italy; [2]University of L'Aquila, Department of Surgery, L'aquila, Italy; [3]G. Mazzini Hospital, Department of Pathology, Teramo, Italy; [4]S. Salvatore Hospital, Department of Pathology, L'Aquila, Italy*

Background: Combined treatment with Bicalutamide (Casodex) and ZD-1839 (Gefitinib, Iressa) in human Prostate Cancer (PCa) cell lines proved to be effective and hyperadditive (Festuccia et al., Int. J. Cancer, 2005). Relapses after androgen withdrawal in PCa are a significant cause of morbidity and mortality and pose the question of the ideal initial treatment of this very prevalent tumor.
Material and methods: We analyzed by immuno-histochemistry the expression of EGF receptor (EGFR), Erb-B2 (Her2) and PTEN (a tumor-suppressor) in a 50 patient cohort with localized tumors, treated by radical prostatectomy. Among these patients, 21 (group 1) received prostatectomy as initial treatment, whereas the other 29 (group 2) received neo-adjuvant androgen-ablation therapy for 3–6 months based on Casodex (150 mg/die) treatment before surgery. We also obtained primary cultures from 37/50 cases (17 of group 1 and 20 of group 2 patients) to test the Gefitinib antiproliferative/pro-apoptotic effects alone or in combination with Casodex.
Results: We observed a significant increase of EGFR and Her2 in tissues from group 2 patients. This indicates that EGFR/Her2 expression can be regulated in vivo by antiandrogens, as previously observed in cell lines. PTEN expression was lost after Casodex therapy. All PCa primary cultures were sensitive to both Gefitinib and Casodex (IC50 0.2 to 2.0 mM and 0.7 to >4.0 mM, respectively). We observed no differences between the IC50 values calculated in the two groups for Gefitinib indicating that increased EGFR expression was not a pre-requisite for effectiveness. In addition, Gefitinib (0.1 mM) increased the anti-tumour effects of Casodex of 10 fold and, Casodex (0.5 mM) increased the effects of Gefitinib of 2.5-fold. However correlating the IC50 values of single cases with the Her2 and PTEN expression we found that Her2 increase and PTEN decrease can be negative biomarkers of Gefitinib effectiveness.
Conclusions: Our findings favour the clinical development of combination therapies, by early association of Casodex and Gefitinib in newly diagnosed PCa patients by targeting simultaneously EGFR and AR in androgen-dependent/sensitive PCa, since the dual inhibition of AR and EGFR pathways could be useful in naive patients in order to extend the androgen-dependent phase and to delay the onset of EGFR-driven androgen-independence phase of PCa. 'Casodex', 'Gefitinib' and 'Iressa' are trademarks of the AstraZeneca group of companies.

877 POSTER

Apoptosis in urothelial bladder carcinomas and its relation to the expression of caspase 3 and apoptosis regulating proteins bax and bcl-2: prognostic implications

V. Theodoropoulos[1], A.C. Lazaris[2], I. Ghikonti[3], V. Tsoukala[4], E. Chamilos[1], G. Tamvakos[1], K. Aleksandrakis[1], I. Gerzelis[1], F. Sofras[5], I. Kastriotis[6]. *[1]Agia Olga General Hospital, Department of Urology, Athens, Greece; [2]School of Medicine, National and Kapodistrian University, Department of Pathology, Athens, Greece; [3]Agia Olga General Hospital, Department of Pathology, Athens, Greece; [4]School of Medicine, National and Kapodistrian University, Department of Pathology, Athens, Greece; [5]University General Hospital, Department of Urology, Heraklion, Greece; [6]Sismanoglio Hospital, School of Medicine, National and Kapodistrian University, Department of Urology, Athens, Greece*

Background: Apoptosis is the most significant component of programmed cell death that complements cell proliferation in maintaining normal tissue homeostasis. Bax protein accelerates apoptosis by antagonizing the apoptosis repressor bcl-2. Caspase 3 is the final step of the apoptosis-inducing protease pathway. We determined the association of apoptosis with the apoptosis related proteins bax, caspase 3 and bcl-2, as well as their interaction with prognosis in urothelial carcinoma (UC) of the urinary bladder.
Material and methods: Using immunochemistry we investigated the expression of bax, caspase-3 and bcl-2 in 88 primary UC bladder specimens. Apoptosis was detected by staining with a MoAb recognizing exposed single-stranded regions in the DNA of apoptotic cells (anti-ssDNA) and the apoptotic index (AI) was expressed as the percentage of the immunoreactive neoplastic nuclei. Kaplan-Meier survival curves were compared in order to define their possible prognostic role in disease-free survival (DFS).
Results: Positive staining for bax, caspase 3 and bcl-2 was noted in 50%, 90.8% and 55% of cases, respectively. Well differentiated UCs showed overexpression of bax and caspase 3 ($p < 0.05$), as well as a trend for strong expression of bcl-2 ($p > 0.05$). We noted a positive relation between bax and caspase 3 ($p < 0.05$), but no statistical association could be detected between the above proteins and bcl-2. AI increased with increasing grade and stage ($p < 0.05$), but was unrelated to the expression of the apoptosis related proteins. Log-rank test showed that high grade, T stage and increased AI had an adverse impact on DFS ($p < 0.05$), while patients with bax overexpression exhibited significantly longer DFS times ($p < 0.001$).
Conclusions: Apoptosis increases along with progression of the neoplastic lesions of the bladder epithelium. Although bax and bcl-2 are strongly expressed in urothelial bladder carcinomas, they don't seem to be the major regulators of apoptotic activity. The lack of relation of caspase 3 expression with degree of apoptosis may be due to the inability of immunohistochemistry to discriminate between the active and inactive forms of caspase. The adverse prognostic role of apoptotic rate is possibly the result of the loss of normal mechanisms controlling cell death, facilitating the survival of cells with increased ability to resist in unfavorable growth conditions. With regard to disease-free survival, Bax protein emerges as a promising favorable indicator.

878 POSTER

The expression of pentaspan membrane glycoprotein Prominin-1/CD133 is not limited to prostatic stem cells and is down-regulated in prostate cancer

E. Missol-Kolka[1], M. Haase[2], C. Liebers[1], S. Arl[1], C. Lorra[1], W.B. Huttner[1], D. Corbeil[3]. *[1]Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany; [2]Technical University of Dresden, Department of Pathology, Dresden, Germany; [3]Technical University of Dresden, Tissue Engineering Laboratories, BIOTEC, Dresden, Germany*

Background: Prominin-1 (CD133) is known as a cell surface marker of neural and hematopoietic stem/progenitor cells. One report has shown that Prominin-1 carrying the AC133 epitope can also be used to identify the prostatic basal stem cells (Richardson et al., J. Cell Sci. 2004, 117:3539). Furthermore, the expression of Prominin-1 is up-regulated in malignant hematopoietic diseases as well as in certain types of solid tumors such as those derived from the brain and kidney, which prompted us to evaluate whether Prominin-1 can be used as a prognostic and/or predictive clinical marker of prostate cancer.
Methods: The expression of Prominin-1 in normal adult human prostate as well as in 25 prostate cancer samples was monitored by immunohisto-chemistry.
Results: The analysis of human prostate revealed hE2, but not AC133, immunoreactivity on the apical side of prostatic epithelial cells whereas the AC133 immunoreactivity is restricted to a small population of cells