

while still detecting 93% of carcinoma and the majority of missed cancer were low grade and low stage.

Conclusions: Measurement of % fPSA can reduce 28% of unnecessary biopsies in patients with PSA level between 4 and 25 ng/ml with a reasonable sensitivity in detecting prostate cancer. The optimal cut off value of 30% fPSA may apply to the cis bio PSA assay for reference.

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POSTER

Cisplatin versus carboplatin based chemotherapy as induction chemotherapy of bladder cancer

G. Aravantinos¹, M.A. Dimopoulos¹, H. Athanasiou¹, G. Fountzilas¹, E. Samantas¹, Ch. Kiamouris¹, D.V. Skarlos¹. ¹Hellenic Co-operative Oncology Group, Athens, Greece

Purpose: To compare combinations of the 2 platinum analogues as induction chemotherapy for invasive or locally advanced bladder cancer.

Methods: Patients (pts) with T2-4 N0/+ M0 transitional cell bladder cancer are randomized to either 3 cycles of combination Cisplatin 70 mg/m² (Day 1), Epirubicin (E) 50 mg/m² (Day 1) and Methotrexate (M) 50 mg/m² (Days 8, 15) (Arm A) or Carboplatin 300 mg/m² (Day 1) plus E and M at the same doses (Arm B) given every 3 weeks. Consequently all complete responders and pts not feasible or refusing cystectomy are treated with local radiotherapy (60 Gy).

Results: A total of 96 pts (52 in arm A, 44 in arm B) entered the study. The median age is 66 years, while the majority of pts (73) had T2-3 disease. Toxicity was mild. Anemia and thrombocytopenia were more frequent in arm B, while nausea/vomiting was more evident in arm A. Relative dose intensity was higher in Cisplatin arm. There was not statistically significant differences in term of overall response rate (75% vs 60%), complete response rate (44% vs 50%), time to progression (19.6 vs 21.7 months) and survival (51.8 vs 58.1 months), between the two arms.

Conclusions: Carboplatin based chemotherapy appears to demonstrate comparable activity to cisplatin as induction chemotherapy for invasive or locally advanced bladder cancer.

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POSTER

Loss of p27Kip1 expression correlates with tumor grade and with reduced disease-free survival in primary superficial bladder cancers

A. Sgambato¹, M. Migaldi², B. Faraglia¹, L. Garagnani², G. Romano¹, C. De Gaetani³, P. Ferrari³, R. Ardito¹, G.P. Trentini², A. Cittadini¹. ¹"Giovanni XXIII" cancer Research Center, Catholic University, Rome; ²Dipartimento di Scienze Morfologiche e Medico Legale, University of Modena, Modena; ³Division of Urology, USL Modena, Modena, Italy

p27Kip1 is a member of the Cip1/Kip1 family of cyclin-dependent kinase inhibitors and is a potential tumor suppressor gene. The expression level of p27Kip1 has been reported as an important prognostic factor in primary lung, breast, colon, gastric and prostate cancers. This study was undertaken to assess the prognostic value of p27Kip1 in human bladder cancer. The expression of p27Kip1 protein was evaluated by immunostaining in a series of 96 superficial (Ta-T1) human bladder carcinomas. p27Kip1 protein was expressed at high (>50% positive cells), moderate (25–50%) and low (<25%) level in 39 (41%), 19 (20%) and 38 (39%) out of the 96 primary superficial bladder cancers, respectively. Decreased p27Kip1 staining correlated with higher tumor grade ($p = 0.0005$). A significant correlation was also observed between high expression of p27Kip1 and increased disease-free survival ($P = 0.003$ by log-rank test) and overall survival ($P = 0.01$ by log-rank test). On multivariate analysis low p27Kip1 protein expression was an independent predictor of reduced disease-free survival second only to tumor stage. These data indicate that p27Kip1 protein is frequently expressed at high level in well differentiated tumors and suggest that this protein might represent a useful prognostic marker for disease recurrence in primary superficial bladder carcinomas.

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POSTER

Prognostic value of growth fraction measurement with MIB-1 in bladder cancer

J.W.A. Oosterhuis¹, R.F.M. Schapers², R.P.E. Pauwels³, M. Janssen-Heijnen⁴. ¹St Maartensgasthuis, Surgery, Venlo; ²St Maartensgasthuis, Pathology, Venlo; ³St Maartensgasthuis, Urology, Venlo; ⁴Comprehensive Cancer Centre, Eindhoven, Netherlands

Introduction: Prognosis in transitional cell cancer (TCC) of the bladder is usually assessed with the TNM staging system and a grading system (e.g. WHO). We evaluated the prognostic value of growth fraction in TCC with the monoclonal antibody MIB-1 (Ki-67), which suffers less from inter-observer variation.

Methods: Histological tumour specimens of 301 patients diagnosed between 1979 and 1991 were stained with monoclonal antibody MIB-1 and the fraction of positively stained cells was counted. All patients were staged and graded by classical methods and the mitotic index was counted on conventionally stained histological material. Follow-up data from a prospective database were used to calculate crude survival, recurrence free survival (for Ta and T1 tumours) and progression free survival.

Results: In univariate analysis crude survival, recurrence free survival and progression free survival were strongly related to all analysed prognostic factors after an median follow-up of 60 months. In multivariate analysis however, crude survival and progression free survival were only determined by stage ($p = 0.0001$) and recurrence free survival was determined by mitotic index ($p = 0.0246$) and MIB-1 index ($p = 0.0319$).

Conclusion: In this series stage was the most important prognostic factor for crude survival and progression free survival in TCC, while in superficial tumours MIB-1 and mitotic index determined recurrence

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POSTER

Bladder preservation with sequential chemotherapy (CT) and radiotherapy (RT)

E. Athanasiou¹, G. Aravantinos¹, M.A. Dimopoulos¹, G. Fountzilas¹, E. Samantas¹, Ch. Kiamouris¹, D.V. Skarlos¹. ¹A.G. Hellenic Co-operative Oncology Group, Athens, Greece

Purpose: To evaluate the effectiveness of combined induction chemotherapy and local radiotherapy for invasive or locally advanced bladder cancer.

Methods: Patients (pts) with T2-4 M0/+ M0 transitional cell bladder cancer are treated initially with platinum based chemotherapy, either Cisplatin 75 mg/m² or Carboplatin 300 mg/m² (day 1) plus Epirubicin 50 mg/m² (Day 1) and Methotrexate 50 mg/m² (Days 8, 15). Consequently complete responders (CR) are treated with RT (60 Gy). Partial responders (PR) were treated with RT in case cystectomy was not feasible or they refused cystectomy.

Results: A total of 96 pts entered the study. The CR rate after chemotherapy was 47% and the overall response rate was 71%. 71 pts (37 CRs, 34 PRs) underwent radiotherapy. The CR rate after RT was increased by 10%. After median follow-up of 33 months, median time to progression for patients treated with sequential CT and RT is 26.5 months and median survival 58 months. The 5-year survival is 49% and the 5-year survival with intact bladder 42.7%.

Conclusions: Comparable results to radical cystectomy in terms of survival can be achieved with the above combination of CT and RT with high rate of bladder preservation.

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POSTER

Technetium-99m labelling of monoclonal antibody, C595 for bladder cancer immunoscintigraphy – Pre-clinical evaluation

A. Murray¹, M.S. Simms², G. Denton³, O.D.M. Hughes², M.R. Price³, M.C. Bishop², A.C. Perkins¹. ¹University of Nottingham, Medical Physics, Nottingham; ²City Hospital, Urology, Nottingham; ³University of Nottingham, Cancer Research Labs, Nottingham, United Kingdom

Introduction: Current radiological techniques for staging bladder cancer are inaccurate in up to 40% of cases. We have previously reported on the results of Indium-111 labelled C595 monoclonal antibody immunoscintigraphy and showed that it may offer additional information to current staging techniques prior to radical therapy for invasive bladder cancer. Technetium-99m offers advantages over 111In in terms of cost, availability and quality of imaging. The direct reduction mediated method is a reliable technique for labelling antibodies with 99mTc.

Methods: C595 was reduced with 2-mercaptoethanol and labelling was performed by adding 200 MBq of ^{99m}Tc and 0.1 mL of methylene diphosphonate from a bone scanning kit. In vitro studies revealed the size, stability and immunoreactivity of the conjugate. The ability of the conjugate to bind to bladder tumours was initially tested in an ex-vivo model in which five fresh cystectomy specimens containing tumour were filled with ^{99m}Tc -C595 for 1 h. Bladders were imaged and biopsies of tumour and normal urothelium taken. The conjugate was then administered intravesically to five patients who had been diagnosed radiologically or endoscopically with bladder cancer. Patients underwent gamma camera imaging post-washout followed by Transurethral Resection of Tumour (TUR) and biopsy 2 h later.

Results: The direct reduction mediated method produced a stable conjugate of ^{99m}Tc -C595 which had an immunoreactivity exceeding 85%. Gel filtration chromatography revealed a single peak of labelled material at the expected 150 kD region. Binding to bladder tumours was confirmed in the ex-vivo model; four out of the five tumours were successfully imaged with a mean tumour:normal ratio of 5.7:1. In the patient studies, four out of the five tumours were imaged with a mean tumour:normal ratio of 22:1.

Discussion: We have successfully labelled the monoclonal antibody C595 with ^{99m}Tc . The conjugate is stable in vitro and retains its high immunoreactivity. It has demonstrated its ability to localise to bladder tumours in an ex-vivo model and in patients when given intravesically. On the basis of these results we have embarked upon a study of intravenous ^{99m}Tc -C595 immunoscintigraphy in patients with invasive bladder cancer. This reagent may also have an application in diagnostic imaging of other intra-cavity tumours such as ovarian carcinoma.

1402

POSTER

Testicular self-examination (TSE) and testicular ultrasound (TUS) assisted follow-up for the early detection of secondary testicular tumors (STT)

L. Géczi, I. Bodrogi. *National Institute of Oncology, Budapest, Hungary*

Purpose: To improve, by an analysis of the results of 3 years' experience, the early detection of STT assisted by monthly TSE and annual TUS.

Methods: Between April 1 1995 and April 1 1998 annually 702, 782, and 761 patients (pts) participated in our new follow-up program. We provided informative materials to our previously treated and controlled testicular cancer patients about the early signs of testicular cancer, the correct method of self-investigation and the importance of the early detection of STT. If any signs of tumor were palpable TUS was applied within a few days. During this period 14 STT cases were detected; 8 other STT pts were managed in our Department in the usual way. The clinical details of the two groups were compared.

Results: In the first group 4 stage I/A and 10 stage I/B tumors were detected; in 10 pts adjuvant chemotherapy was used and in 4 pts a wait and see approach was adopted. In group 2 the stage distribution was 2 I/A, 2 I/B, 1 II/A and 3 III/B. Three pts received adjuvant and 3 curative chemotherapy, 2 were managed by wait and see policies, and 1 received mediastinal irradiation with chemotherapy. Early detection helped to reduce the duration of symptoms, the number of chemotherapy cycles, the side effects and treatment costs.

Conclusion: With regular follow-up the prognosis of STT is good (CR: 100%), and a biopsy to detect CIS is unnecessary. The pts must be followed regularly for up to 5 years, must be prepared to use TSE-techniques and must recognize the importance of the early detection of STT. TUS, in combination with TSE, enhances the chance of early diagnosis; subsequently these tests can provide the basis for a cost-effective treatment with reduced side effects.

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POSTER

Radiotherapy (XRT) for testicular intraepithelial neoplasia (TIN)

J. Classen, K.-P. Dieckmann, M. Bamberg. *University of Tuebingen, Radiooncology, Hoppe-Seyler-Str. 3, 72076 Tuebingen, Germany*

Introduction: TIN, also known as carcinoma in situ of the testis, has been recognized as the common precursor lesion of testicular cancer except for spermatocytic seminoma. In case of TIN in a solitary testis or double sided TIN local XRT with 20 Gy is the treatment of first choice yielding 100% cure rates. However, subsequent to XRT patients are at risk for delayed Leydig-cell insufficiency eventually necessitating testosterone substitution. Therefore new treatment strategies aim at the reduction of the total dose of XRT without compromising local control of TIN.

Methods: A multicenter prospective trial was initiated for XRT of histologically proven TIN. Patients were treated with reduced total doses. A

progressive dose reduction in 2 Gy steps was performed starting with 18 Gy. All patients had complete hormonal evaluation prior to XRT and again during follow-up. At least one control biopsy after XRT was required. Acute and possible late effects of treatment were prospectively documented using the EORTC-score.

Results: To date, 13 patients have been enrolled for the trial. 11 patients were treated with 18 Gy, 8 of which have so far undergone control biopsy showing complete eradication of TIN. 2 patients were treated with 16 Gy. Acute side effects of treatment are negligible. With a median time to follow-up of 6.8 months (range: 0–18.5 mo.) so far no significant decline in serum testosterone after XRT has been observed.

Conclusion: Preliminary data of the study indicate that XRT of TIN with 18 Gy may be sufficient for local control of this premalignant lesion. Further reduction of the XRT dose in the course of the study may provide a less toxic yet highly efficient therapy for TIN.

1404

POSTER

Inhibition of telomerase activity by antisense oligonucleotides in renal cell carcinoma

R. Heicappell¹, J. Sachsinger¹, H. Krause¹, M. Müller¹, K. Müller¹. ¹Freie Universität, Urology, Berlin, Germany

With only a few exceptions, the ribonucleoprotein telomerase is found in malignant, but not in benign tissues. It has been previously shown by us and others that telomerase activity can be found in malignant tumors of bladder and kidney. Telomerase is obviously required for indefinite, malignant growth. Therefore, inhibition of telomerase activity would be a logical approach for therapy of malignant tumors such as renal cell carcinoma. Antisense RNA was chosen for telomerase inhibition in the present study. As tools for this type of therapy are not readily available, vectors were constructed allowing for expression of antisense telomerase RNA in murine RCC cells.

Murine renal cell carcinoma line RENCA, which is highly tumorigenic in mice, displays high telomerase activity, and a mean telomere length comparable to human cells (7–10 kb) was used as a model. After electroporation with eukaryotic expression vectors harboring the RNA template in antisense direction stable transfectants were isolated and tested for telomerase activity (TRAP assay), shortening of telomere length, and proliferative activity. After 35 population doublings 9 out of 13 antisense-transfected RENCA clones showed a reduced replicative potential compared to their non-treated counterparts. Fluorescence-based TRAP assay and measurement of the mean terminal restriction fragment length (TRF) gave evidence for reduced enzyme activity and resumption of telomere shortening in the transfectants. Growth inhibition was, however, of temporary nature only in some cell clones.

Antisense oligonucleotides may effectively inhibit telomerase activity, addition of telomeric DNA, and proliferation of murine renal cell carcinoma.

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POSTER

Gemcitabine/cisplatin in advanced transitional cell carcinoma of the urinary tract (TCC): A phase II multicenter trial

S. Mancarella, V. Lorusso, L. Manzone, M. Antimi, F. De Vita, A. Santoro, L. Maiorino, R. Mattioli, G. Luporini, D. Bilancia, M. De Lena. *Italian Cooperative Study on Bladder cancer Oncology Institute, Via Amendola 209, 70121 Bari, Italy*

Since January 1997, 54 patients (pts) affected by measurable advanced TCC were treated with gemcitabine 1000 mg/m² on days 1, 8, 15 and cisplatin 70 mg/m² on day 2 repeated every 4 weeks. Patients' median age was 67 (range 18 to 80), none had prior chemotherapy for metastatic disease, 7 had adjuvant cisplatin-chemotherapy concluded by >1 year, 52 were male and 2 female, 16 had prior cystectomy, 36 had ECOG 0–1 and 18 had ECOG 2 PS respectively. All patients entered on study have been evaluated for response and toxicity (*intent to treat*). Complete (CR) plus partial (PR) response were observed in 26/54 pts (48%) with 8 pts (15%) attaining CR. Twelve pts (22%) showed stable disease (SD) and 6 progressed (15.5%). G3–4 leukopenia was observed in 21 (39%) pts, and G4 neutropenia in 6 (11%). Grade 3–4 thrombocytopenia was recorded in 11 (20%) pts and G4 thrombocytopenia in 3 pts. The median number of cycles delivered was 4; 28 pts (52%) required dosage reduction of gemcitabine or omission to deliver it, on day 15 for neutropenia or thrombocytopenia. No other relevant side effects were recorded. Median time to progression and survival were 26 weeks and 36 weeks respectively. Out of 32 pts with disease related symptoms (haematuria, pain, weight loss), 16 (50%) improved.