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Chromophilic renal cell carcinoma: Characterization of four permanent cell lines

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Purpose: We are the first to report on the establishment of permanent cell lines, derived from chromophilic renal cell carcinoma (RCC).

Methods and Results: Immunocytochemically, all cell lines co-expressed vimentin and cytokeratins. Karyotypic analysis revealed the numerical aberrations typical for chromophilic RCC, i.e. loss of Y and tri- or tetrasomy 7 and 17. Moreover, all cell lines exhibited a complex pattern of autocrine and paracrine growth regulation: 1. All cell lines expressed mRNA of TGF-beta type I and II receptors but not type III-R. ELISA analysis demonstrated secretion of inactive TGF-beta, protein in all cell lines. Exposure to TGF-beta, resulted in a significant ($p < 0.05$) inhibition of proliferation only in chromphi-2 and -3. 2. PDGF-AA, PDGF-BB and IGF-1 as well as their corresponding receptors were not present in any cell line. 3. All cell lines exhibited different levels of FGF-4-receptor whereas aFGF mRNA was not detected. Exposure to aFGF resulted in a significant ($p < 0.05$) stimulation of growth in chromphi-1 and -2.

Conclusions: The characteristic morphological and cytogenetic features of chromophilic RCCs are preserved in the cell lines chromphi-1 to -4. Moreover, the heterogeneous expression pattern for various growth factors and their receptors suggests complex mechanisms of autocrine and paracrine growth regulation.

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Cisplatin, etoposide and bleomycin infusion (PEBI regimen) in good-risk patients with germ cell tumors

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Patients with good-risk germ cell tumors have an approximately 85–95% chance of cure with standard chemotherapy. However, acute and late toxicity may be severe and negatively influence the quality of life. In an attempt to reduce toxicity, we evaluated a new schedule including bleomycin administered as a continuous infusion in patients with low and intermediate volume metastatic disease. Patients were treated as follows: cisplatin, 100 mg/m² day 4; etoposide, 100 mg/m² day 1 through 5; bleomycin 15 unit bolus day 1 followed by 30 mg as a continuous infusion for 72 hours, with cycles repeated every 21 days. Between 1992 and 1996, 25 patients entered the study and were assessable for response and side effects. Major patients characteristics were: performance status ECOG 0–1; minimal disease 15, intermediate disease 10; median age, 33 (range 15–50). Twenty-one of 25 patients (84%) achieved a complete remission, 2 patients achieved a partial remission, and 2 patients did not respond to the regimen. At a median follow-up of 24 months, 24/25 patients were alive, 21 (87%) without evidence of disease and 3 had persistent disease. Grade III/IV side effects included leuko-neutropenia (8 patients), anemia (3 patients), and nausea/vomiting (3 patients). No drug related deaths were observed, and no evidence of pulmonary toxicity was registered. In conclusion, the PEBI regimen is an effective and well tolerated regimen in patients with good-risk germ cell tumors and may be considered as a front-line chemotherapy.

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Phase II study of Interferon gamma (rIFN-G) and vinblastine (VBL) in patients with advanced renal cell carcinoma (RCC)

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Purpose: The evaluation of activity and toxicity of combination rIFN-g and VBL in advanced RCC.

Methods: Twenty nine patients entered the study and twenty seven were evaluable. Twenty three male and four female with median age 64 (44–74) years, median ECOG performance status I (0–2), without previous chemioimmunotherapy. Eighteen patients had received nephrectomy. The rIFN-g was administered at a dose of 100 mcg subcutaneously three times weekly and VBL was administered at a dose of 0.15 mg/kg I.V. bolus (max. dose 10 mg) every two weeks.

Results: The response rate was: CR 2/27 pts, PR 3/27 pts, SD 4/27 pts and PD 18/27 pts. Median survival for responders was 11+ months and for non responders was 5 months. Lung was the commonest site of disease with the highest response rate. The toxicity according to WHO criteria was: Anaemia (G1–G3) 3/27 pts, Neutropenia (G1–G3) 4/27 pts, Neurotoxicity G1 5/27 pts and Flu-like symptoms (G1–G2) 22/27 pts. No toxic deaths.

Conclusion: rIFN-g combined with VBL is moderately active with mild toxicity in patients with advanced RCC.

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PUBLICATION

Paclitaxel-induced growth suppression and morphological alterations in different types of human renal cell carcinoma

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Purpose: In clinical trials, the antineoplastic agent paclitaxel has shown promising activity against ovarian and breast cancer, but not yet proved its efficacy against renal cell carcinoma (RCC).

Methods: The growth inhibitory effects of paclitaxel (Taxol®) were analyzed by MTT-assay in 34 newly established RCC cell lines of different types (28 clear cell, 4 chromophilic and 2 chromophobe RCCs). Paclitaxel-induced microtubule alterations were visualized by indirect immunofluorescence using anti-alpha-tubulin. The frequency of micronuclei was determined by light microscopy.

Results: In 32 out of 34 cell lines a marked dose-dependent ($p < 0.05$) growth inhibition became evident after exposure to paclitaxel. Paclitaxel dissolved in Cremophor EL/ethanol (= Taxol®) proved to be more effective than paclitaxel dissolved in DMSO. In all cell lines, a dose-dependent increase in the frequency of micronuclei could be detected. After paclitaxel treatment, microtubule bundles or irregular coarse meshworks of microtubules were observed in clear cell and chromophobe RCCs, whereas exclusively irregular microtubule meshworks but no microtubule bundles were seen in the chromophilic RCCs.

Conclusion: Our study indicates that paclitaxel (Taxol®) significantly inhibits the proliferation of human RCC in vitro, irrespective of the histological type. In chromophilic RCCs, paclitaxel-induced microtubule alterations differed from those observed in clear cell and chromophobe RCCs.

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Radiotherapy in stage I and II seminoma

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Purpose: In seminoma patients, radiotherapy of the paraaortic and pelvic nodes results in excellent survival for stage I and II. In this study we report our results of treatment as well as the acute and late treatment morbidity and a incidence of second cancer.

Methods: From 1982 to 1994, 324 pts. were irradiated at the Institute for Oncology and Radiology in Belgrade: 279 in stage I and 45 in stage II of the disease. Med. age was 35 years (22–69). Radiotherapy was delivered by linear accelerator (10 MeV) in all except in 8 pts (they were treated by Cobalt 60 unit). The doses were 30 Gy/18 fr. at midplane for both stages (in the majority of pts.).

Results: In 87 pts acute gastrointestinal toxicity was registered. The med. follow-up was 7 years (range 2–13) and the overall survival rate 98% and 91% for stage I and II, respectively. Relapse occurred in 14 pts. 10 pts were cured by radiotherapy and or chemotherapy; 4 pts died from seminoma. Late sequelae were registered in 18 pts and second cancer in 5 (2-lung, bladder, 2-opposite testis).

Conclusion: Radiotherapy is still a treatment of choice in st. I and II seminoma with high probability for cure and a low rate of late irradiation induced complications and relapses.

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PUBLICATION

Testis cancer: Endocrinological disturbances

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Men with testicular cancer are often not fertile showing disturbances in spermiogram and the sexual hormone levels. The kinetic of the hormone levels following the tumour ablation are evaluated.

Sera of 37 consecutive patients with germ cell tumour (15 SEM and 22 NSGCT) were used from testicular vein blood and peripheral blood at

operation and 24 hours and 4 to 7 days later for β -HCG, AFP, LDH, PLAP, LH, FSH, testosterone, estradiol and prolactin. Relevant serial counts and relations were only shown in this series by β -HCG, estradiol, testosterone and LH.

In all patients we found increased levels of estradiol in the testicular vein blood. It is 30 times higher than in the cubital blood. If estradiol is also increased in the cubital blood there is a strong correlation to testosterone. There are no increased testosterone levels in patients with normal peripheral estradiol. We found eight patients with increased estradiol and testosterone levels in the cubital blood. These patients show high HCG levels and low levels of LH.

The estradiol-increases are depending on the HCG production of the tumour. The gonadal-pituitary feedback leads to the suppression of LH-expression. After tumour ablation the high estradiol and testosterone levels return to normal ranges parallel to the decrease of HCG. The LH level is increasing to normal in the same interval.

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Testicular Cancer: Human Placental Alkaline Phosphatase (hPLAP)

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The Human Placental Alkaline Phosphatase (hPLAP) is a cell-surface associated sialo-protein which is physiologically concentrated at the syncytiotrophoblastic membranes of the terminal placenta. Different entities produce hPLAP with measurable serum-levels.

The data of 114 consecutive patients with germ cell tumours of the testes are presented: Of 47 seminoma 26 (55%) had a hPLAP of >100 mIU/l. There were 18/34 (53%) in clinical stage (cS) I, 4/10 (40%) in cS II A/B, 2/2 in cS IIC, one cS III was negative. The levels ranged from 101 to 1486 mIU/l. Of 67 non-seminoma (NSGCT) 22 (33%) had a hPLAP of >100 mIU/l. In pathological stage (pS) I there were 8/28 (29%), in pS II A/B 6/19 (32%), in cS II C 4/10 (40%) and in cS III 4/10 (40%). These ranged from 103 to 2553 mIU/l.

The mean half-life in cS I was 2.04 days, the median 1.30 days (range 0.52–7.30). Of the smoking patients 59% were above the norm, of the non-smokers 30% ($p = 0.006$). At complete remission (CR) the mean level of smokers was 46.3 mIU/l (0.1–251) compared to 8.5 mIU/l (0.1–49) of non-smokers ($p = 0.017$).

hPLAP is a marker for diagnosis, control of therapy and follow-up in patients with seminoma and NSGCT. The raised levels of smokers have to be recognized. Important in follow-up is a constant level. Raising hPLAP leads to search of progress.

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PUBLICATION

Evaluation of the role of a single cycle of carboplatin as an adjuvant treatment in stage I seminoma and as a neo-adjuvant prior to radiotherapy in stage II

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Purpose: The treatment options for stage I seminoma include adjuvant radiotherapy and surveillance. However, radiotherapy carries acute morbidity and an increased risk of second malignancies, while surveillance may be difficult in the absence of reliable tumour markers and with an indolent pattern of relapse. Radiotherapy for stage II is well established and effective, but the risk of mediastinal relapse may be as high as 20%.

We therefore evaluated the role of a single cycle of carboplatin in forty-one patients with stage I&II seminoma treated post orchidectomy at Velindre Hospital, Cardiff between 1993–1996.

Methods: The dose of carboplatin was AUC $\approx$ 7 and the dose of radiotherapy for stage II was 35 Gy/17 fractions over three and half weeks using dog-leg technique. Thirty-two patients had stage I disease (age range 21 to 57 years, mean of 34.7 years). The median duration of follow-up is 24.5 months and the mean is 25.7 months. Of nine patients with stage II, three had stage IIA and six had stage IIB disease (age range 21 to 40 years, mean of 29 years). The median duration of follow-up is 23 months and the mean is 26 months.

To date all patients are in complete remission and well. Minimal side effects were recorded through one patient experienced G4 neutropenia & thrombocytopenia, otherwise treatment was well tolerated.

Conclusion: Adjuvant treatment with an out patient single cycle of carboplatin is a simple well-tolerated adjuvant treatment for stage I seminoma.

Combined modality treatment for stage II seminoma is rational, simple and effective. However, larger number of patients with a longer period of follow-up is needed to determine whether a single cycle of carboplatin reduces radiation relapse rate in stage II disease. The efficacy of a single agent carboplatin is currently being tested in a phase III trial (MRC TE 19).

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PUBLICATION

Prognostic factors in renal carcinoma

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In a prospective study, the prognostic value of Ki-67, Her-2, ER, PR, EGFR, Cath-D, P-53, the DNA index and the S phase, as well as the value of established prognostic factors (TNM classification, grading), was investigated in a univariate analysis involving 67 renal-cancer patients.

Grading ($p < 0.0001$), nodal status ($p < 0.0001$), tumor stage ($p < 0.0002$), metastatic status ($p < 0.0004$), the DNA index ($p < 0.005$) and the S phase fraction ($p < 0.045$) as nuclear factors, as well as Ki-67 ($p < 0.040$), all showed statistically significant prognostic value. The remaining factors were not prognostically significant.

Differentiation between G1/2 and G3/4 was the most distinctive prognostic factor, with the total number of survivors differing by 60% during the observation period. In the collective comparing G1/2 patients with G3/4 patients, risk ratio was 8.901; that is, mortality risk for patients with G3/4 was nearly nine times higher than for G1/2 patients. As to T1/2 vs. T3/4, the risk ratio was 4.075; and 4.691 when comparing a DNA index of <1.0 vs. >1.0.

We also found that in the low-risk group with values of G1/2, T1/2, and a DNA index of <1.0 ($n = 11$), only 1 patient had died after 25 months. In the group with values of G1/2, T3/4, and a DNA index of >1.0, 7 of 9 patients were still alive after 25 months. However, in the high-risk group with G3/4, T3/4, and a DNA index of >1.0 ($n = 9$), no patients were alive after a 4-month observation period.

In summary, our study found that the following parameters are suitable for evaluating renal-cell cancer prognosis: TNM stage, grading, the DNA index, the S phase fraction, and Ki-67.

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PUBLICATION

Prognostic factors in the management of clinical stage I (CS I) nonseminomatous germ cell tumors of the testis (NSGCTT)

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Purpose: Surveillance after orchiectomy alone has gained great popularity in the management of CS I NSGCTT. But in an effort to identify pts at high risk of relapse, there has been a search for adverse prognostic factors. The aim of the study was to identify pts in whom a surveillance policy is less likely to be successful.

Methods and Results: 63 pts in CS I NSGCTT were stratified to different risk-adapted therapeutic approaches according to histopathologic findings of primary tumor. 15 pts with vascular invasion and majority of embryonal carcinoma component were treated with adjuvant chemotherapy. None of them experienced disease progression. Five pts with vascular invasion and majority of teratoma elements in the primary tumor were treated with primary RPLND. Two of them had pathologic stage II and underwent subsequent chemotherapy. One of them died due to disease progression 29 months after orchiectomy. 43 pts without vascular invasion were kept under close surveillance, which consisted of regular follow-up with tumor markers chest x-rays and CT of the retroperitoneum. Disease progression was observed in 5 (11.6%) pts 7–10 months following orchiectomy. They were treated with BEP chemotherapy and live with NED. The overall survival rate of all 63 pts was 98.4%.

Conclusions: The authors recommend risk-adapted treatment procedures in CS I NSGCTT pts. Only pts without vascular invasion in the primary tumor may be subjected to a surveillance policy.