Surveillance is attractive option, avoiding unnecessary treatment, but would be even more attractive if a greater proportion of patients with occult metastatic disease could be identified and administered earlier, potentially less toxic, treatment.
A Danish pilot study in CS1 NSGCT showed that FDG PET could identify 70% of patients who subsequently relapsed, and had a negative predictive value of 90%. If confirmed this would suggest that further treatment could be avoided in most patients with CS1 NSGCT and negative PET scans.
Methods: NSGCT patients judged to be CS1 based on markers and CT, and high risk based on vascular invasion, were registered within 8 weeks of orchidectomy, and underwent an 18FDG PET scan. Following a positive scan, patients went off study and could be managed according to local protocols. Patients with negative scans were followed on surveillance. The primary outcome measure was the negative predictive value of the PET scan, defined as the 2-year relapse-free rate in patients with a negative PET scan. This was expected to be approximately 90%, and to exclude rates below 80% with 80% power, at a 5% significance level, approximately 100 PET negative patients were required and we anticipated scanning 135 patients to achieve this.
Results: Patients were registered between May 2002 and January 2005. At this time, when 116 patients were registered and PET scan results were available on 96 patients (78 PET –ve, 18 PET +ve), an independent Data Monitoring Committee review lead to early closure of the trial, due to an unacceptably high relapse rate in the PET-ve patients. PET +ve patients were slightly older than PET –ve patients (35 vs 29 yrs) and more likely to have MTU histology (83% vs 46%) and/or to have normal markers pre-orchidectomy. All PET +ve patients were scheduled for adjuvant BEP chemotherapy. One PET –ve patient requested adjuvant chemotherapy. Of the remaining 77, 23 relapsed leading to a one-year relapse-free rate of 65% 90% CI (53%, 74%). The maximum 2 year relapse-free rate (assuming complete follow-up and no further relapses) would be 70% (60%, 79%).
Conclusions: Though PET identified a proportion of patients with disease not detected by CT scan the relapse rate amongst PET –ve patients remains high. The study results therefore suggest that 18FDG PET scanning is not able to identify patients at sufficiently low risk of relapse to replace other treatment options in this setting.
This study was supported by Cancer Research UK

799 ORAL

Gonadal hormones, sperm counts and post-treatment paternity in long-term survivors of unilateral testicular cancer

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Background: In long-term survivors of unilateral testicular cancer (TC) post-treatment serum follicle-stimulating hormone (s-FSH), serum testosterone (s-tes) and sperm counts were analysed according to previous treatment and associations with post-treatment paternity were assessed.
Material and methods: In 1998–2002 TC patients treated 1980–1994 in Norway were followed-up by a questionnaire, clinical examination and laboratory assessments. Of 1687 eligible men under 65 years without androgen replacement, serum hormones were analysed in 1198 (median follow-up 11 years, age 43 years). 348 delivered a semen sample. Patients were grouped according to treatment: Surgery only (Surg, n = 236), radiotherapy only (RT, n = 487), and two chemotherapy groups, [Cisplatin (Cis) $\leqslant$850 mg, n = 385 and Cis >850 mg, n = 90].
Results: S-FSH was elevated ($\geqslant$12 IU/l) in 42% of the men: Surg, 31% (median 8.8 IU/l); RT, 37% (9.7 IU/l); Cis $\leqslant$850 mg, 47% (11.1 IU/l) and Cis >850 mg, 77% (20.2 IU/l) ($p < 0.001$). In a linear regression model, age, cryptorchism and treatment group were significant factors for logarithmic s-FSH ($p < 0.001$), but with no difference between the RT and Surg group. In a linear regression model including age ($p < 0.001$) and cryptorchism ($p = 0.14$), s-tes was significantly lower in all treatment groups compared to Surg ($p = 0.02$). Sperm counts were <20 mill/ml in 49%, and <10 mill/ml in 36%. The frequency of azoospermia varied from 10% (Surg) to 43% (Cis >850 mg). In a proportional ordinal logistic regression for increasing levels of sperm counts (0, 0.1–1.9, 2.0–9.9, 10.0–19.9 and $\geqslant$20 mill/ml), adjusting for age and cryptorchism, the odds ratios compared to surgery were: RT, 0.74 (95% CI 0.43–1.27); Cis $\leqslant$850 mg, 0.51 (95% CI 0.29–0.89); and Cis > 850 mg, 0.20 (95% CI 0.08–0.52). Overall, 488 had tried to conceive a child following treatment. The median s-FSH value was 8.7 IU/l in those who succeeded (n = 330) vs. 12.8 IU/l in those who failed (n = 157) ($p < 0.001$). Respective s-tes values were 15.2 vs. 14.2 mmol/l (NS) and median sperm counts were 32 vs. 4.2 mill/ml ($p = 0.004$). In a Cox regression model where logarithmic s-FSH, s-tes and cryptorchism were assessed for their association with post-treatment paternity, only s-FSH remained an independent factor ($p < 0.001$). In men whose semen was analysed, both sperm count and s-FSH ($p = 0.03$) were significantly associated with post-treatment paternity.
Conclusions: Post-treatment spermatogenesis evaluated by post-treatment s-FSH and sperm counts was impaired in 42–49% of long-term survivors of TC and was associated with paternity after treatment. RT did not significantly impair long-term spermatogenesis compared to surgery, whereas chemotherapy did, with more severe suppression at the higher doses. Cytotoxic treatment significantly reduced s-tes as compared to surgery alone, but no association was observed between post-treatment paternity and s-tes.

Oral presentations (Tue, 1 Nov, 9.15–11.15)
GU – new frontiers in genitourinary cancers

800 ORAL

Molecular prognostic markers for survival after chemotherapy in advanced bladder cancer

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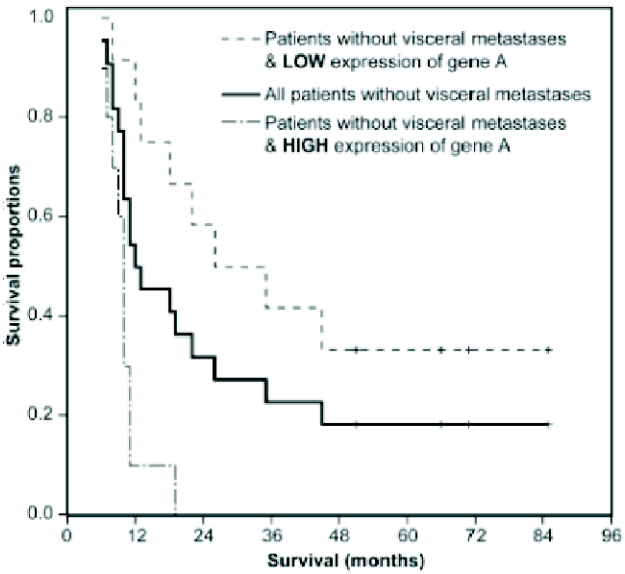
Background: In patients with advanced bladder cancer, cisplatin-containing chemotherapy yields response rates around 50%, with a median survival around 12 months. Poor performance status (PS $\geqslant$ 2) and presence of visceral metastases are identified as independent poor prognostic factors for survival in several studies. However these factors are not strong enough to predict the outcome for the individual patient.
Aim: To identify differentially expressed genes with a prognostic impact on survival after the cisplatin-containing regimens MVAC (methotrexate, vinblastine, doxorubicin, and cisplatin) or GC (gemcitabine and cisplatin).
Material and methods: We identified 31 patients with a follow-up time of more than 15 months following MVAC or GC. Tumor biopsies were sampled less than four months prior to chemotherapy. Gene expression data were generated using Affymetrix GeneChip HU133A. Genes that correlated significant with survival were identified using SAM (Significance Analysis of Microarrays; Stanford University Labs).



Survival of patients with advanced urothelial cancer without visceral metastases according to expression values of gene A.

Results: Thirty-nine genes correlated highly significantly with survival. We selected five genes well annotated and with intelligible biological relevance for further analyses. The genes encode proteins involved in apoptosis regulation, DNA-damage-repair upon chemotherapy, cell-proliferation and angiogeneses. Expression values were dichotomized and analyzed in combination with clinical prognostic factors. Patients with (n = 9) or without

(n = 22) visceral metastases had a median survival of 8 months vs. 12.5 months, respectively (p = 0.014). The group of patients without visceral metastases could be further subdivided by adding the gene expression values. Addition of each single gene expression separated the survival curve for these patients as exemplified in the figure, where patients with low expression values had a median survival time of 30.5 months vs. 10 months for patients with high expression values (p = 0.0001). Addition of further gene expression profiles resulted in further separation of the survival curve.
Conclusions: We have identified five genes with a stronger prognostic impact than the known clinical prognostic factors. Confirmation of results is ongoing.

801 ORAL

Endoglin and CD31 expression in relation to prognosis in conventional renal cell carcinoma

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Background: Quantification of intratumoural microvessel density by endoglin (CD105) or CD31 (PECAM-1) staining has prognostic significance in selected neoplasms. Endoglin is a cell membrane glycoprotein expressed on tumour-associated vascular endothelium and it is a marker of angiogenesis. CD31 is a transmembrane glycoprotein belonging to the immunoglobulin superfamily of cell adhesion molecules presenting on the surface of various blood cells and endothelial cells. In this study, the prognostic information of endoglin and CD31 expression in human renal cell carcinoma (RCC) was evaluated.
Material and methods: Tumour samples from 162 patients with conventional RCC treated between 1982 and 1997 were analysed. The tumour samples were assessed using the tissue microarray technique and immunohistochemically stained for endoglin and CD31. The expression was related to gender, age, TNM stage, nuclear grade, tumour size, and survival data.
Results: The expression of endoglin was inversely associated with TNM stage (p = 0.019), and nuclear grade (p = 0.006). The expression of CD31 was inversely associated to TNM stage (p = 0.03) and to nuclear grade (p = 0.018). Furthermore, a correlation between the expression of endoglin and CD31 was seen (r = 0.439, p < 0.001). No correlation was found between endoglin or CD31 expression and gender, age, or tumour size. The material was subdivided in quartiles depending on the endoglin and CD31 expression. Patients with high CD31 expression (the highest quartile) had better prognosis compared to those with lower expression (p = 0.015). Endoglin showed a similar trend (p = 0.06). A multivariate analysis of prognostic factors showed that TNM stage and nuclear grade were independent predictors of prognosis. Endoglin or CD31 expression did not add further prognostic information.
Conclusion: The expression of endoglin and CD31 in conventional RCC is inversely related to stage and grade. Furthermore a correlation between the expression of endoglin and CD31 was observed. When comparing the endoglin and CD31 expression in conventional RCC, a higher sensitivity of one of the angiogenic factors over the other cannot be suggested. TNM stage and nuclear grade remains the strongest predictors of prognosis in RCC, but the results indicate that angiogenesis is related to prognosis.

802 ORAL

High detection rate of circulating tumor cells in blood of patients with prostate cancer using telomerase activity

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Objectives: To study whether a method using telomerase activity allows to isolate circulating tumor cells (CTC) in patients with prostate cancer.
Material and methods: Peripheral blood mononuclear cells (PBMC) were isolated from blood by using Ficoll/hypaque. Immunomagnetic beads coated with an epithelial-specific antibody (BerEP4) were used to harvest epithelial cells from PBMC. Telomerase activity was detected in harvested epithelial cells using the Telomerase-PCR-ELISA method.
Results: Blood samples from 107 patients with prostate cancer were studied. CTC were detected in 19/24 (75%) patients with advanced prostate cancer. In contrast, CTC were not detected in blood samples from 19 healthy male volunteers. CTC could be identified even in patients with a very low serum PSA (<0.1 ng/mL). CTC were detected in 55/70 (79%) when tested in patients with localized prostate cancer who were planned to be treated by radical prostatectomy (n = 30) or brachytherapy (n = 40). CTC could also be detected in 3/13 patients (23%) with an undetectable prostate specific antigen (PSA) at least 1 year after radical prostatectomy, which is consistent with the expected relapse rate in this setting.
Conclusion: CTC can be detected using telomerase activity in a large majority of patients with prostate cancer, including those with a localized stage. This method appears to be more sensitive than RT-PCR methods to detect CTC. Potential applications include tumor monitoring after definitive local therapy and accessibility to malignant material in the metastatic setting.

803 ORAL

Doppler ultrasonography with perfusion software and contrast agent injection as an early evaluation tool of metastatic renal cancers treated with the RAF kinase and VEGFR inhibitor: a prospective study

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Objectives: The objective of this study was to evaluate Doppler Ultrasonography with perfusion software (Vascular Recognition Imaging, Amplio, Toshiba) and contrast agent injection (Sonovue® –Bracco) (DUSVRI) as a predictor of tumor response to new treatment BAY 43–9006 under investigation in phase III trials for the treatment of metastatic renal cancer.
Material and methods: Tumor vascularization in accessible targets was prospectively studied, (double-blind study with a placebo) with DUSVRI. The examinations were performed before administering BAY 43–9006 (day 1) and at 3 and 6 weeks. The percentage of contrast uptake was evaluated in each tumor by two radiologists. Results were compared to CT scan studies at 6 weeks.
Results: 30 patients were included and a total of 85 examinations were performed: 30 before randomization, 28 at 3 weeks and 27 at 6 weeks. The results showed a decrease in tumor vascularization in 10 patients out of 28 patients at 3 weeks and in 10 patients out of 27 patients at 6 weeks. The final results concerning 30 patients will be presented with a correlation with the CT-scan response at 6 weeks.
Conclusion: DUSVRI is a new cost-effective and simple non invasive imaging technique. Its effectiveness in predicting the efficacy of BAY will be presented.

804 ORAL

Cancer nanotechnology: drug encapsulated nanoparticle-aptamer bioconjugates for targeted delivery to prostate cancer cells

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Introduction: Nucleic acid ligands (aptamers) are potentially well suited for the therapeutic targeting of drug encapsulated controlled release polymer nanoparticles in a cell- or tissue-specific manner. We used Prostate Cancer (PCa) cells as a model to test this hypothesis.
Methods: We synthesized poly(lactic acid)-block-poly(ethylene glycol) controlled release copolymer with a terminal carboxylic acid functional group (PLA-PEG-COOH), and encapsulated rhodamine-labeled dextran (as a model drug) within PLA-PEG-COOH nanoparticles using the double emulsion method. We generated nanoparticle-aptamer bioconjugates using nuclease stabilized RNA aptamers that bind to the Prostate Specific Membrane Antigen (PSMA), a well known PCa tumor-marker which is over-expressed on prostate acinar epithelial cells. These bioconjugates were examined for targeted delivery and uptake by LNCaP (PSMA+) and PC3 (PSMA−) model PCa cells under a range of physiologic shear stress conditions using microfluidic channels.
Results: Nanoparticles had the following desirable characteristics: 1) negative surface charge (−50 mV ± 3 mV, Mean ± SD, N = 3), which may minimize non-specific interaction with the negatively charged nucleic acid aptamers, 2) carboxylic acid groups on the particle surface for potential modification and covalent conjugation to amine-modified aptamers, 3) presence of PEG on particle surface which enhances circulating half-life while contributing to decreased uptake in non-targeted cells. Nanoparticles were conjugated to PSMA aptamers to develop the first example of a