(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

nanoparticle-aptamer bioconjugate. Nanoparticle-aptamer bioconjugates selectively adhered to LNCaP but not PC3 cells at static and low shear (<1 dyne/cm^2) but not higher shear (~4.5 dynes/cm^2) conditions. Using z-axis fluorescent microscopy and 3-D image reconstruction (figure 1), we studied the localization of the nanoparticle-aptamer bioconjugates (red dots) after incubation with LNCaP cells, and confirmed that even at 2 hrs, the particles were largely internalized into cells. In contrast to LNCaP cells, the uptake of these particles is not enhanced in PC3 cells which do not express the PSMA protein.

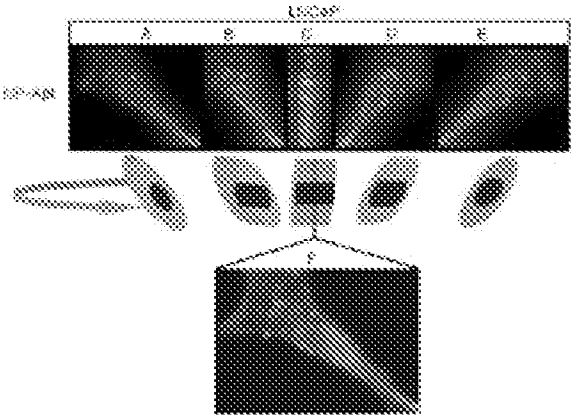

Fig. 1

Discussion: This represents the first example of targeted drug delivery using nanoparticle-aptamer bioconjugates. Through modification of the controlled release polymer system or the aptamer targeting group, similar vehicles can be made to target a myriad of important human cancers.

805 ORAL

Use of ultrasound contrast agent microbubbles for delivery of androgen receptor antisense molecules into prostate cancer cells and tumors

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Background: The androgen receptor (AR) is a key cellular regulator in normal and malignant prostate cells and a prime target for prostate cancer treatment. Whereas its inhibition in the hormone naïve tumors by androgen ablation and antiandrogens is efficient and allows controlling tumor growth for some time, targeting it after development of hormone-refractory disease remains a challenge. A promising approach is the use of antisense molecules for AR knockdown, as successfully demonstrated in vitro and in vivo. The main obstacle is the problem of specific and efficient delivery of antisense drugs into the tumor. We studied the usefulness of ultrasound contrast microbubbles as carriers for antisense molecules and their delivery into tumor cells xenografts by ultrasound triggered bursting.
Material and methods: Antisense molecules were charge-coupled to cationic perfluorocarbon gas-filled microbubbles and added to LNCaP prostate cancer cells cultured in Opticells chambers. In a water bath the loaded microbubble were then bursted with high energy, low frequency ultrasound (1.75 MHz, mechanical index 1.9, 9 min).
For in-vivo testing delivery into the LNCaPabl xenograft tumor model, representing a hormone-refractory tumor stage, was employed. Digoxigenin labeled antisense oligonucleotides were loaded to cationic microbubbles and applied either into the tumor or intravenously and delivered by ultrasound bursting. 24 hours after the last of three treatments the animals were sacrificed, tumors and organs isolated and analyzed by anti-digoxigenin immunohistochemistry.
Results: Delivery of 50 pmol of AR antisense oligonucleotide or siRNA loaded on 1×107 cationic microbubbles resulted in a significant uptake of fluorescence labeled antisense molecules (more than 50% positive cells) and a significant down regulation of AR protein in LNCaP cells. Treatment was also associated with induction of apoptosis and inhibition of cell proliferation when compared to control antisense treatment.
In the xenograft model uptake of labeled antisense oligonucleotides in tumors was confirmed. Oligonucleotides were detected also in Kupffer cells in the liver.
Conclusion: We conclude that ultrasound contrast microbubbles are suitable as carriers for small antisense molecules and can be used in combination with ultrasound bursting for efficient delivery of these drugs into tumor cells in-vitro and in vivo.

Oral presentations (Wed, 2 Nov, 9.15–11.15)
GU – Prostate cancer

806 ORAL

EORTC trial 22911: Immediate post-prostatectomy irradiation improves biochemical and clinical progression-free survival in patients with pathologically high risk prostate cancer

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Background: After radical prostatectomy, the risk of local failure for patients with cancer extending beyond the capsule (pT3) ranges from 10 to 50%. Independent predictors of biochemical relapse are initial PSA level, Gleason score and positive surgical margins. Earlier reports showed postoperative radiotherapy eradicated residual microscopic disease and significantly reduced local relapse and PSA failure rates but showed no impact on clinical disease free survival. We randomly compared immediate external irradiation (RT) with wait-and-see (W&S) after retro-pubic radical prostatectomy for patients with positive surgical margin or pT3 prostate cancer.
Material and methods: Eligible patients had pN0 M0 tumours and ⩾1 pathological risk factor of: capsule perforation, positive surgical margins, invasion of seminal vesicles. Post-operative radiotherapy was conducted on linear accelerators of 5 to 25 MV using a non 3D planning with an isocentric technique. A target volume including the surgical limits from the seminal vesicles to the apex with a security margin received a dose of 50 Gy/25 fr/5 wks. A reduced volume circumscribing the previous landmarks of the prostate with a reduced security margin received a 10 Gy/5 fr/1 wk boost. Biochemical progression was every increase over the lowest postoperative value to a value >0.2 ng/ml confirmed twice at minimum 2-week intervals. Clinical failure was documented by imaging. A 2% significance level 2-sided Logrank test was used.
Results: From late 1992 to end 2001, 1005 patients aged 65 years in median (range: 47–75) entered the study. After 5 years median follow-up, the biochemical progression free survival was significantly improved in the RT arm with 5-year event-free rate of 74.0% compared to 52.6% in the W&S arm ($P < 0.0001$). Clinical progression-free survival was also significantly improved ($P = 0.0009$). The cumulative loco-regional failure rate was significantly lower in the RT arm ($P < 0.0001$, 5.4% versus 15.4% at 5 years). Grade 2–3 late effects were significantly more frequent in the RT arm ($P = 0.0005$), but the events of severe toxicity (grade ⩾3) were rare with a 5-year rate of 4.2% in the RT arm versus 2.6% in the W&S arm ($P = 0.0726$).
Conclusion: Immediate external irradiation after radical prostatectomy improves biochemical progression free survival and local control in patients with positive surgical margin or pT3 prostate cancer who are at high risk of progression. Further follow-up is needed to assess the impact on survival.

807 ORAL

TROG 96.01: first report of the main endpoints

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Background: To determine whether 3 or 6 months maximal androgen deprivation [MAD] administered prior to and during radiotherapy improves treatment outcomes for patients with locally advanced prostate carcinoma [PC], we conducted a large scale randomised controlled trial.
Material and methods: Men with Stage T2bc, T3 and T4 (N0, M0) PC were randomised to radiotherapy alone (66Gy in 2Gy fractions to the prostate and seminal vesicles) [RT], or 3 months MAD (Goserelin 3.6mg im monthly