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.

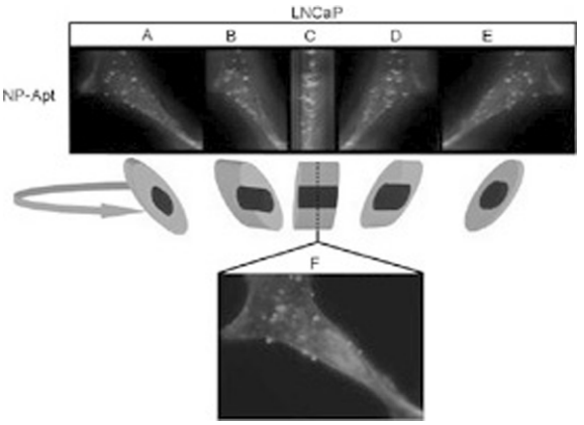

Fig. 1

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 $\geqslant 1$ 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 $\geqslant 3$) 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

and Flutamide 250 mg tds) starting 2 months prior to RT; or 6 months MAD starting 5 months prior to RT.
Results: Between June, 1996 and February, 2000, 818 men were randomised at 19 Australian and New Zealand centres. 802 were eligible for analysis. In comparison to RT alone 3 months MAD reduced local failure [LF]: HR 0.55 ($p = 0.001$), improved biochemical failure free survival (Houston method) [BFS]: HR 0.71($p = 0.003$), clinical disease free survival [DFS]: HR 0.66 ($p < 0.001$) and freedom from salvage therapy [FST] HR 0.73 ($p = 0.024$). In addition to producing even greater improvements in LF: HR 0.41($p < 0.001$), BFS: HR 0.57($p < 0.001$), DFS: HR 0.55 ($p < 0.001$), FST: HR 0.52 ($p < 0.001$) 6 months MAD also reduced distant failure [DF] HR 0.66 ($p = 0.04$) and produced a significant improvement in cause specific survival: HR 0.58 ($p = 0.048$). In this treatment arm patients with "high risk" cancer also experienced a strong trend towards improved overall survival: HR 0.66 ($p = 0.066$).
Conclusions: Six months MAD administered prior to and during RT improves all outcomes in patients with locally advanced P.C.
Further follow-up is necessary now to estimate the size of survival benefits precisely.

808 ORAL

PSA doubling time calculated early on following PSA relapse predicts for subsequent death from prostate cancer

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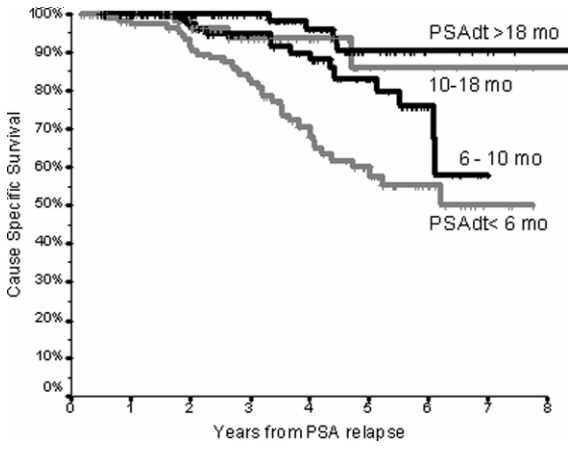
Background: PSA doubling time (PSAdt) has been shown by several groups to be a strong predictor of death from prostate cancer (CSS), when PSA relapse has occurred after radiation therapy. However most reports have calculated the PSAdt using all available data from an initial rise up until the time of secondary intervention. In this study we explore whether an early derived PSAdt, using PSA results from the first PSA to breach a level of 1ng/ml to the PSA level that triggers the PSA relapse definition (trigger PSA) could also predict for survival, and thus help to identify early those men who may potentially benefit from intensified intervention.
Material and methods: From a prospective database of men treated with external beam radiation therapy established in 1994 of over 1850 men, patients were selected for inclusion if they had a biochemical relapse by the new RTOG-ASTRO ('lowest PSA to date plus 2') definition. For each patient the PSAdt was calculated from the first PSA to exceed 1ng/ml post-radiation and the PSA that triggered the relapse definition (trigger PSA). For patients whose first PSA post-nadir was the trigger PSA, the nadir PSA was used. The PSA relapse slope (ln(2)/PSAdt) was split into quartiles and included in Kaplan Meier and Cox regression for cause specific survival, timed from the trigger PSA time point.
Results: 390 men fulfilled the selection criteria. The median time to secondary intervention after trigger PSA was 12 months. The median PSAdt was 9.4 months. The 5 year CSS (timed from trigger PSA) was 77%. In those with PSAdt faster than 6 months the 5 year CSS was 60% ($p < 0.0001$) compared with 83% for 6–10 months ($p = 0.03$), 86% (10–18 months, reference value) and 90% for >18 months (p = ns), see figure. Multivariate analysis showed faster PSAdt, higher T stage, and higher Gleason grade to be independent factors predictive of prostate death. Initial PSA and the use of neoadjuvant or adjuvant androgen ablation were not significant. Earlier intervention in those who have been treated ($n = 256$, 66%) was associated with worse survival ($p = 0.036$).



Conclusions: PSAdt calculated on the basis of early serial results between 1ng/ml and the PSA that triggers relapse predicts for CSS. Patients with PSAdt faster than 6 months have very poor survival, whereas those with PSAdt of slower than10 months do relatively well. Men who received early secondary intervention appear to do worse, presumably due to case selection for intervention of the worst prognosis patients. Those with fast PSAdt may benefit from intensification of therapy such as the early use of chemotherapy. Conversely those with slow PSAdt may not require intervention.

809 ORAL

Longitudinal observations of QOL changes in men receiving intermittant androgen suppression treatment for prostate cancer; an Australian GUOG study

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Objective: Health related quality of life (HQOL) research is a means of broadening the assessment of treatment effects. This longitudinal study investigated the dynamic change to quality of life (QOL) and testosterone dependant physiology in men commencing an intermittent maximal androgen blockade program (MAB).
Patients and methods: Two hundred fifty men were accrued to the multi-centre study of IAB (Eulexin® 250 mg TDS, Lucrin® 22.5 mg depot) ceasing treatment after 9 months if PSA <4 ng/ml, and restarting when PSA >20 ng/ml. QOL was assessed every 3 months for 30 months using the EORTC QLQ-C30 and Prostate 26 module.
Results: Data completion for the whole study was >99%. At baseline, our cohort was less symptomatic and had better function than the EORTC reference cohort, which may be related to a shift in clinical practice over time. Testosterone suppression (AS) lead to a significant reduction in global HQOL and deterioration in most function and symptom scales, maximal in the first 3 months. Thirty one percent (79 men) required adjustment of Eulexin dose at 3 months. Apart from a temporary increase in diarrhoea score (a recognised side effect) this adjustment was not a factor for any other symptom or function change. During the off treatment period, median time for Testosterone recovery was 9.3 months. There was a trend of progressive improvement in HQOL that paralleled testosterone recovery and was slower than the rate of deterioration during the treatment phase. Median time to re-treatment (141 men) from end of treatment was 14.5 months. Maximum recovery of HQOL occurred most frequently by months 9–12.
Conclusion: Whilst the magnitude of mean change to scale scores was small, there was a consistent and simultaneous deterioration during MAB and improvement during androgen recovery over many separate scales. Older men are more likely to show an impaired testosterone recovery, and this was paralleled by a slower HQOL recovery. Newer methods of analysis to describe results in a way that has meaning to the individual patient are warranted.

810 ORAL

Risk and risk factors of renal impairment in hormone refractory prostate cancer (HPRC) patients with bone metastases (BM) treated with Zoledronic Acid (ZA)

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Background: To quantify the risk of renal impairment and identify the associated risk factors in HRPC patients receiving ZA for BM.
Material and methods: A comprehensive medical record review was performed, using both electronic databases and paper records, in a large tertiary oncology center. Results of creatinine tests conducted outside of the center were obtained through patients' community physicians. Patients were included in the study if they were $\geqslant$18 years old, actively treated at the center, had HRPC with BM, received at least one ZA infusion in the period from 12/1999 to 4/2005, and had at least one creatinine reading before and after the first ZA infusion. The observation period began on the date of the first ZA infusion and ended on the last center visit date or last creatinine test date, whichever occurred later. The renal impairment outcome was defined as an increase of $\geqslant$0.5 mg/dL and $\geqslant$1.0 mg/dL over baseline creatinine value (defined as the final creatinine serum test prior to beginning ZA treatment) if the baseline value was <1.4 mg/dL and