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.

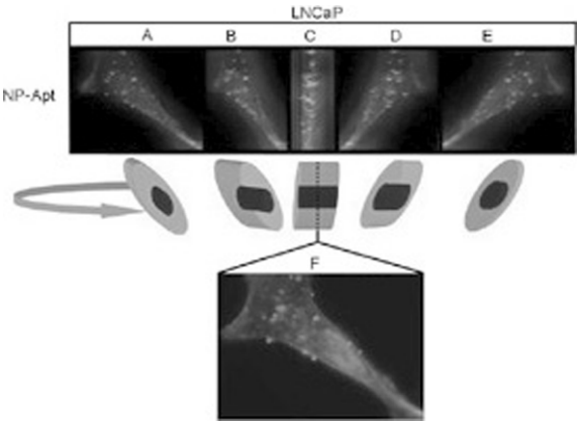

Fig. 1

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

H. Klocker[1], I.E. Eder[1], P. Haag[2], F. Frauscher[1], J.R. Lindner[3], A.L. Klibanov[3], G. Bartsch[1]. [1]*Innsbruck Medical University, Department of Urology, Innsbruck, Austria;* [2]*Innsbruck Medical University, Department of Radiology, Innsbruck, Austria;* [3]*University of Virginia, Cardiovascular Division, Charlottesville, USA*

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

M. Bolla[1], H. Van Poppel[2], L. Collette[3], P. Van Cangh[4], K. Vekemans[5], L. Da Pozzo[6], T.M. de Reijke[7], A. Verbaeys[8], J.F. Bosset[9], R.F.P. Van Velthoven[10]. [1]*Centre Hospitalier Régional de Grenoble – La Tronc, Radiation Oncology Department, Grenoble, France;* [2]*Universitair Ziekenhuis Gasthuisberg, Urology Department, Leuven, Belgium;* [3]*European Organisation for Research and Treatment of Cancer (EORTC), Data Center, Brussels, Belgium;* [4]*Hopital Universitaire Saint Luc, Urology Department, Brussels, Belgium;* [5]*Virga Jesse Ziekenhuis, Urology Department, Hasselt, Belgium;* [6]*Ospedale San Raffaele, Urology Department, Milano, Italy;* [7]*Academisch Medisch Centrum, Urology Department, Amsterdam, The Netherlands;* [8]*Universiteit Gent, Urology Department, Gent, Belgium;* [9]*Centre Hospitalier Régional de Besançon – Hopital Jean Minjoz, Radiation Oncology Department, Besançon, France;* [10]*Institut Jules Bordet, Urology Department, Brussels, Belgium*

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

J.W. Denham, A. Steigler, D.S. Lamb, D. Joseph, S. Turner, J. Matthews, I. Franklin, C. Atkinson, D. Christie, N.A. Spry. *University of Newcastle, Radiation Oncology, Waratah, Australia*

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