enhances DU145 cell death 4–10-fold. DU145 cells are null for Bax and Bcl2 and have mutated p53.
Material & Methods: We studied increasing sensitivity of DU145 prostate cancer cells using LCL102/LCL204 with AdGFPFasL virus. Cell proliferation assays and apoptosis were evaluated using MTS. Western blots demonstrated the effects of LCL102/204 on the antiapoptotic protein. Reintroduction of FLIPs, Survivin, Bcl2 was by trasfection.
Results: Treatment with subtoxic concentration of LCL102/LCL204 sensitized DU145 to AdGFPFasL mediated apoptosis. At this subtoxic dose, LCL 102 downregulates anti apoptotic genes include FLIPs, Survivin, cIAP1 and RIP within 24 h and was more pronounced at 48 h. XIAP, $cFLIP_L$ BID and caspase 8 were not affected suggesting that lysosomal ceramide elevation acts downstream of caspase 8 to activate the pro-apoptotic phenotype and facilitate viral-induced sensitivity already downstated in our laboratory to act through caspase 8 (previously reported).
Conclusions: This study suggests the pretreatment of DU145 cells with acid ceramidase inhibitors (LCL102/LCL204) sensitizes cells to AdGFPFasL mediated apoptosis. This is likely mediated through downregulation of anti apoptotic genes includes FLIPs, Survivin and cIAP1. It appears that cFLIPs is the most important component of resistance in DU145.

MP-13.12
Androgen receptor regulates tumor angiogenesis and tumor growth in prostate cancer by TSP1
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Introduction & Objectives: Androgen ablation causes degeneration of normal and cancerous prostate tissue. Yet ultimately tumors progress to the androgen-independence. Precise understanding of androgen effects on normal and cancerous prostate cells may help to delay transition to androgen independence and prolong the period where tumors are therapeutically manageable. We developed a model where the wild type and hypersensitive or promiscuous androgen receptor (AR) variants are conditionally expressed in human prostate cancer cell line PC-3. This model allows to study the effects of the wild type AR and mutants on prostate cancer cells in culture and in mouse tumor model on *in vitro* growth, tumor take and tumor associated angiogenesis.
Material & Methods: We used a tetracycline (T-Rex) Tet-on system where AR is expressed under the control of Tet operator and tetracycline binding to the repressor releases expression of the controlled gene. We created constructs expressing WT AR and two mutants, AR 715 (715V → M, hypersensitive) and AR 877 (877T → A, promiscuous). All constructs were transfected into AR-negative PC-3 cell line.
Results: We showed that AR induction caused no differences in the growth of cultured cells in full serum but delayed tumor growth and suppressed tumor vascularity (angiogenesis). AR activation increased the expression of thrombospondin-1 (TSP1), a protein that destroys tumor vasculature, and U19, a tesosterone dependent inducer of cell death. AR (wild type and mutants) regulated TSP1 expression at transcription level. AR 715 and AR 877 showed higher affinity for TSP1 promoter compared to the WT AR.
Conclusions: Thus it appears that the role of androgen in prostate cancer (PrCa) is not straightforward. It is possible that in normal prostate tissue it maintains functional homeostasis between proliferation and apoptosis and that androgen ablation may involve alterations downstream of AR that change the spectrum of its molecular targets from pro-apoptotic to those promoting survival and proliferation. Our results suggest that combined androgen ablation and antiangiogenic therapy may further delay tumor progression.

MP-13.13
Gene transfection efficiency comparison between lipofectomy-mediated versus adenovirus-mediated in suicidal gene therapy of prostate cancer
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Introduction & Objectives: To develop an efficient, safe, and reliable gene delivery system is one of the critical steps for gene therapy as a general and this is special for prostate cancer. Many vectors can be applied to deliver therapeutic genes to target cells or tissues. Each vector system has advantages and disadvantages and a certain application mode. We conduct this study for comparison between adenoviruses-mediated versus liposomes-mediated transduction of the thymidine kinase gene to both human and murine prostate cancer cell lines.
Material & Methods: We used two human prostate cancer cell lines; LNCaP cells, and DU145, and one murine prostate cancer cell line; the RM-1 cells. The prostate cancer cells was transduced by either two systems; (a) the herpes simplex virus/thymidine kinase gene (*tk*-cDNA) recombinant DNA plasmids after coupling with liposome delivery system (Lipofectamine 2000 Plus reagent), or with (b) replication-deficient adenovirus containing the herpes simplex virus thymidine kinase gene (ADV/HSV-*tk*) at a multiplicity of infection (MOI) 50. The cells were incubated with the medium containing 10 mg/ml of ganciclovir (GCV) antiviral drug, 24 h after gene transfection. The number of recovered viable cells over 7 days period was counted by trypan blue exclusion method. Also, the thymidine kinase gene expression was monitored by Western immunoblotting.
Results: The adenovirus-mediated gene expression was more than double the strength of transduction of liposomes-mediated gene delivery system at the same time points. By transfection with adenovirus vector, the average viable cells for all the three prostate cancer cell lines were 63% vs 82% in lipofectomy-mediated gene delivery group in the first day. The viable cells decreased further down every day to reach a bottom at the seventh day to be 3.6% viable cells in adenovirus-mediated transfection, vs 23% in lipofectomy-mediated transfection. Thus adenovirus was able to reduce the average tumor cell population by 37%, versus 18% only in lipofectomy-based transfection. This difference is deepen further on time course, to reach its maximum on day 7 where the adenviral-based transfection has resulted in 96.4% average eradication of the three prostate cancer cell lines, vs 77% in lipofectomy-based group.
Conclusions: The transfection efficiency dominance of adenoviruses over the liposomes-based vector in the gene therapy of prostate cancer was clear as shown in both the western immunoblotting results, and in cytotoxicity parameters. This may direct our selection of vector to be directed towards the adenovirus-based gene therapy vectors rather than liposome-based, for their favorable transfection rate in both the human and murine prostate cancer cell lines.

MP-13.14
Prostatic atrophy: Possible role in PSA elevation
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Introduction & Objectives: Chronic ischemia due to severe local arteriosclerosis may be a cause for prostatic atrophy. Acute ischemia due to prostate infarct and ischemic damage to the prostate during cardiac surgery are well known causes of PSA elevation. The purpose of this study was to find any possible role of chronic ischemia due to prostate atrophy in PSA elevation.
Material & Methods: The study was based on 93 prostatic needle biopsies corresponding to 70 patients, all having a high PSA level not explained by prostate volume. At least six regions were biopsied; and in 13 patients more than one biopsy. All the biopsies showed prostatic atrophy without the presence of carcinoma, high-grade prostatic intraepithelial neoplasia (PIN), atypical lesions, or prostatitis. According to a high, median or low probability of a given patient to have PSA elevation due to atrophy an Índex based on the sum of credits was obtained according to the number of biopsies, extended biopsies, number of fragments showing the lesion, sampling of the transition zone and free/total PSA favorable levels.
Results: Of the total of 70 patients, 11(15.71%), 53 (75.71%), and six (8.57%) had high, median and low probability, respectively, for atrophy to be a possible cause of PSA elevation.
Conclusions: The data show that in approximately 15% of patients who show atrophy as the only lesion on needle biopsies, this lesion may be a possible cause of PSA elevation.

MP-13.15
Role of lycopene as chemopreventive in treatment of high-grade prostate intraepithelial neoplasia (HGPIN)
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Introduction & Objectives: HGPIN is a precursor of prostate cancer. Since prostate cancer has a long latency period, slow growing & high prevalence rate, it is the best model for chemo prevention. This study was undertaken to find the efficacy of lycopene as a

chemopreventive agent in management of HGPIN in preventing this vulnerable group to develop prostate cancer.
Material and Methods: Forty patients of HGPIN (Gr.II-III) were randomized into two groups, one received lycopene 4 mg twice a day for 1 year and other was periodically followed up with prostate specific antigen, digital rectal examination and serum lycopene.
Results: The serum PSA is treated group decreased from a mean 6.07 to 3.5 ng/ml while in the control group it increased from mean 6.55 to 8.06 ng/ml. Serum lycopene increased in the treated group and decreased in the control group. Two out of twenty patients (10%) in the treated group subsequently developed malignancy while six out of two patients (30%) in control group developed malignancy of the gland subsequently. Our result shows that lycopene can prevent HGPIN to develop into occult cancer prostate and being a vegetable carotanoid is a safe drug without any side effects. There exists an inverse relationship between serum lycopene and serum prostate specific antigen.
Conclusions: Lycopene is an effective chemopreventive agent in management of HGPIN with no toxicity and good patient's tolerance.

MP-13.16
Controlled gene delivery of PTEN expression vector conjugated with cationized gelatin in prostate cancer cells
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Introduction & Objectives: Locally advanced prostate cancer has been considered candidates for radical operation or radiotherapy with or without combining androgen deprivation. However, approximately one-fourth of these patients suffer from disease recurrence or progression. At present, androgen independent prostate cancer has an extremely poor prognosis and almost no curative treatment option. Thus, new modal treatments that can help current initial treatment more effective and curative for prostate cancer are needed. We have demonstrated that an adenoviral gene therapy of PTEN can effectively treat bladder and prostate cancers, and can be effectively treated tumors which exhibit drug or radiation resistance associated with expression of phosphorylated Akt in combination with chemotherapy or radiotherapy. PTEN is well known as a tumor suppressor gene and has a phosphatase activity in the phosphatidylinositol 3'-kinase mediated signal transduction pathway and inhibits the activation of Akt, a serine-threonine kinase involved in proliferative and anti-apoptotic pathways. These days, using virus vector for cancer gene therapy is controversial, and nonviral gene transfer is a future promising procedure but several problems need to be cleared, such as transduction efficacy.
Material & Methods: We have developed nonviral compound conjugated with cationized gelatin microsphere and plasmid DNA, which is a new type of gene transfer drug and designed to release plasmid DNA and last the gene expression continuously for a long period *in vivo*. In this study, we originally generated the GelaTen, which is a conjugate with cationized gelatin microsphere (2 mg) and PTEN expression vector (100 μg), and examined the efficacy of GelaTen as a combination therapy with radiation in prostate cancer.
Results: Single direct injection of GelaTen into established subcutaneous bcl-2-overexpressing PC3 prostate cancer tumors (PTEN deleted, up-regulation of phosphorylated Akt and Bcl-2) in nude mice, which reached approximately 5–7 mm in diameter, resulted in significantly decreased growth compared to the conjugate with β-gal plasmid (control) or PBS treated tumors. Immunohistochemical analysis showed that tumors inducted with GelaTen expressed PTEN and exhibited decreased amounts of phosphorylated Akt, whereas tumors treated with CTL or PBS were negative for PTEN and diffusely positive for phosphorylated Akt. Since PTEN down-regulates phosphorylated Akt and Bcl-2 and increases sensitivity to radiation, we explored combination therapy with GelaTen and radiation *in vivo*. Combination therapy with GelaTen and 5 Gy irradiation (5 days after GelaTen injection) improved the *in vivo* efficacy of tumor growth compared with the GelaTen monotherapy alone in these tumors.
Conclusions: These data demonstrate that PTEN gene therapy with gene drug GelaTen can effectively treat prostate cancers that have genomic alterations in PTEN. Furthermore, tumors that exhibit radiation resistance associated with expression of phosphorylated Akt and Bcl-2 can be effectively treated with GelaTen and radiotherapy.

MP-13.17
Antisense oligonucleotide therapy in combination with taxol, cytoxan and mitoxantrone in the treatment of prostate cancer
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Introduction & Objectives: Combination therapy with antisense oligonucleotides (oligos) and traditional chemotherapeutic agents offers potential benefits by increasing the effectiveness of the chemotherapeutics, lowering their effective dosage, and reducing toxicity. Antisense oligonucleotides (oligos) directed against mRNA encoding TGF-alpha (MR1) and EGFR (MR2) have demonstrated *in vitro* and *in vivo* efficacy against prostate tumor models.
Material & Methods: We evaluated the effectiveness of these oligos (3.32, 6.64 μM/l) with the chemotherapeutics paclitaxel (Taxol; 2.5, 5.0 nm), cyclophosphamide (Cytoxan; 40 nm) and mitoxantrone (20 nm) in treating the PC-3 prostate cancer cell line. To determine optimal growth inhibitory combinations or sequencing, oligos and chemotherapeutics were administered: (1) alone; (2) simultaneously (in a combined therapy); or (3) sequentially (combination therapy with both agents administered individually in a series).
Results: When either oligo was given simultaneously with Taxol, no synergistic activity was noted. However, when sequentially administered in a series one day apart, a pretreatment with MR1 (6.64 μM/l) followed by Taxol (5 nm) had significantly greater activity than these agents similarly administered in the reverse order or simultaneously ($P < 0.001$). When Cytoxan was administered in sequence with oligos (6.64 μM/l) significantly greater growth inhibition was obtained compared to Cytoxan administered alone. A 1-day treatment with Cytoxan followed the next day with MR1 was significantly more effective ($P = 0.0001$) than the reverse order, which also resulted in significant additional inhibition ($P < 0.0004$). Comparing these alternative sequences, pretreatment with Cytoxan before MR1 was significantly more inhibitory than the reverse order ($P = 0.0062$). In contrast to the previous oligo, for MR2 combination therapy, pretreatment with Cytoxan was not significantly more effective than Cytoxan treatment alone, whereas the reverse order of treatment was significantly more effective than Cytoxan administered either alone ($P = 0.0014$) or in the reverse order ($P = 0.0002$). Comparing the two most effective treatments, Cytoxan followed by MR1 and MR2 followed by Cytoxan, the differences were not significant. We conclude that the addition of either antisense oligo increased the effectiveness of Cytoxan therapy, however, the sequence in which the two agents are administered makes a significant difference. Combination therapy with mitoxantrone administered simultaneously with MR1 was significantly more inhibitory than the combination of both MR1 and MR2 oligos (6.64 μM/L) ($P = 0.006$) or mitoxantrone administered alone ($P = 0.0012$). The combined treatment of mitoxantrone with MR1 was not significantly different from mitoxantrone in combination with MR2. Although mitoxantrone with MR2 was statistically ($P = 00015$) more inhibitory than mitoxantrone alone, this combination was barely more effective ($P = 0.04$) than the MR1 oligo administered alone.
Conclusions: When suitably timed, combination therapy including antisense oligos directed against TGF-alpha and EGFR can significantly increase the effects produced by Taxol, Cytoxan and mitoxantrone alone in the treatment of prostate cancer.

MP-13.18
The effect of bicalutamide on prostatic epithelium in patients with isolated high grade prostatic intraepithelial neoplasia
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Introduction & Objectives: High grade prostatic intraepithelial neoplasia (HGPIN) is a precursor of prostatic adenocarcinoma. The incidence of isolated HGPIN in patients undergoing prostate biopsies varies from 2 to 16.5%. Approximately 50% of patients with HGPIN are found to have cancer on subsequent biopsies within 2 years. Androgen deprivation seems to be able to significantly reduce the frequency and extension of HGPIN. The aim of the present study was to assess the effect of bicalutamide on prostatic epithelial cells in order to determine whether this treatment could have some potential value in chemoprevention.
Material & Methods: Twenty-one patients with isolated HGPIN, serum PSA concentrations between 4 and 10 ng/ml and free/total PSA ratios.
Results: Average serum PSA was 8.3 ng/ml. TRUS showed suspicious hypoechoic areas in only four patients. There was an average of 1.8 foci of HGPIN. With treatment, all patients had an evident PSA reduction. Modest gynecomastia without mastodinia was observed in four patients (19%). No case of gastrointestinal or hepatic toxicity was observed. At present, eight of 21 (38%) patients have completed 6 months of treatment and have been rebiopsied. No HGPIN or cancer was found. Light microscopy revealed: atrophy of the secretory epithelium of the ducts and acini, focal hyperplasia of the basal cells, absence of mitotic figures and the presence of