tumor. Attempts of developing tumor targeting drug, which would be capable to deliver necessary amount of high atomic number element into the tumor haven’t succeed yet. Another possible way to deliver such elements into the tumor is to utilize some pathological processes caused by tumorogenesis such as blood barrier disruption in case of brain tumors or high vascularization inherent to some tumors. In this work the efficacy of x-rays irradiation with disodium gadopentetate (Gd-DTPA) administration in treating highly vascularized transplanted tumor in mice was studied.

Material and Methods: C57Bl/6 mice with transplanted adenocarcinoma Ca755 were used in the study. Animals were divided into three groups. 1st group undergo no treatment. 2nd group was irradiated with 10 Gy of x-rays. Animals in 3rd group were administrated with 0.3 ml of 0.5M water solution of Gd-DTPA, containing 23 mg of gadolinium and then irradiated as well. Administration of Gd-DTPA was performed with single systemic injection. Irradiation was performed using x-rays generator with anode voltage of 200 kV. Antineoplastic efficacy was estimated by measuring tumor volume and life span of mice.

Results: Tumor growth rate plots are presented in Figure.

Tumor growth delay for test group was 13 days whereas in irradiated control group tumor growth delay was just 4 days. Median life span was 22 days, 37 days and 46 days for control group, irradiated control group and test group respectively. In test group 25% of animals have full tumor regression whereas in both control groups no tumor regression was observed. Endpoints of antitumor evaluation, i.e. T/C% ratio and gross log10 tumor cell kill are represented in Table.

<table>
<thead>
<tr>
<th>Group</th>
<th>T/C, %</th>
<th>Tumor growth delay, days</th>
<th>Gross tumor cell kill</th>
<th>Antimune activity (Tabelon 2004)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated control group (irradiation only)</td>
<td>37.56</td>
<td>4</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Test group</td>
<td>101.3</td>
<td>13</td>
<td>1.6</td>
<td>+++</td>
</tr>
</tbody>
</table>

Conclusion: Obtained results show that systemic injection of extracellular drug with gadolinium prior irradiation with x-rays provide enough amount of gadolinium in highly vascularized tumors and lead to significant increase of antineoplastic efficacy of x-rays irradiation.

EP-2031
Research on p53 and endostatin gene-radiotherapy induced by EGFR-targeted adenovirus vector in NSCLC

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Purpose or Objective: With the development of molecular biology and gene engineering, more and more attention has been paid to gene-radiotherapy of malignant tumors. The combination of gene therapy and radiotherapy is regarded as one of the effective methods for the treatment of tumors. This research focused on the Egfr-1 promoter with radiation-induced effect, p53 and endostatin genes with function of inducing apoptosis and anti-angiogenesis, and EGFR-targeted adenovirus vector with higher cell infection efficiency. The therapeutic effect of adenovirus vectors Ad.Egr-wtp53-endostatin and Ad.CMV-sCAR-EGF combined with radiotherapy in non-small cell lung cancer is here reported.

Material and Methods: The adenovirus vectors Ad.Egr-wtp53-endostatin containing both wild type p53 and antiangiogenic molecule endostatin genes downstream of early growth response-1 (Egr-1) and Ad.CMV-sCAR-EGF containing coxsackie virus receptor extracellular segment (sCAR) and epidermal growth factor (EGF) were constructed using gene recombination technique. The infection efficiency in non-small cell lung cancer cell lines (A549, LK-2 and Lu65) of Ad.Egr-wtp53-endostatin mediated with Ad.CMV-sCAR-EGF expressed fusion protein sCAR-EGF was detected. The expression of wild type p53 and endostatin genes by the radiation-sensitive promoter Egfr-1 in non-small cell lung cancer cell lines were observed. Immunodeficient mice (NOD/scid) subcutaneously implanted with A549 cells were treated by conventional radiotherapy (2Gy×6) and/or gene therapy (intratumor injection of adenovirus vectors Ad.Egr-wtp53-endostatin and Ad.CMV-sCAR-EGF 24 h before the first and fourth local doses). Immunologic mechanisms were explored.

Results: The fusion protein SCAR-EGF expressed from Ad.CMV-sCAR-EGF significantly increased infection efficiency of Ad.Egr-wtp53-endostatin in human non-small cell lung cancer cell lines. Cancer control was most significantly improved in the group receiving local radiotherapy combined with gene therapy as shown by prolongation of mean survival time by 75.2%, reduction in average tumor weight by 88.7%, decrease in pulmonary metastasis by 76.9% and decrease in intratumor angiogenesis by 80.4% as compared to local radiotherapy alone (P < 0.05). Immunologic studies showed stimulated natural killer (NK) and cytokotoxic T lymphocyte (CTL) activity as well as increased interferon-γ (IFN-γ) and tumor necrosis factor-a (TNF-α) secretion in this combined treatment group as compared with the group receiving local treatment alone (P < 0.05).

Conclusion: The experimental findings in the present study showed that adenovirus vectors Ad.Egr-wtp53-endostatin and Ad.CMV-sCAR-EGF in combination with local radiotherapy could improve the tumor control. These observations may set the stage for developing clinical protocols with recombinant adenovirus-mediated gene-radiotherapy in non-small cell lung cancer.

EP-2032
Radiotherapy gets improved by a nanotechnology based enzyme therapy in glioblastoma primary cultures

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Purpose or Objective: One of the main effects of radiotherapy is the generation of free radicals as a consequence of the incidence of radiation on the aqueous molecules present in the cells. The enzyme D-aminoacid oxidase (DAO) is also able to generate free radicals when converting D-aminoacids in their corresponding cetoacids. Our principal aim is to increase radiotherapy effects, using

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the combination of radiotherapy and DAO, in primary cultures from glioblastoma.

**Material and Methods:** We have used primary cultures and stabilized cell lines from patients with glioblastoma. Recombinant DAO carrying the C-terminal domain of the major lytic amidase (CLyA) specific for binding to choline was immobilized to magnetic nanoparticles having a magnetite core covered with Diethylaminoethyl (DEAE) cellulose. Primary cultures were irradiated at 7 and 15 Gy. After irradiation, cultures were treated in the absence or in the presence of DAO (free or immobilized in nanoparticles) and D-alanine (enzyme substrate). After irradiation, cells were harvested and cell cycle distribution was determined by flow cytometry.

**Results:** We have demonstrated in primary cultures from glioblastoma, that the treatment with DAO after irradiation, potentiates dramatically the effect of the radiation alone, increasing especially the percentage of cells in the sub-G1 phase, an indicator of cell death. Some representative results are included in the attached file. DAO immobilized in magnetic nanoparticles is more effective than free enzyme, since DAO is more stable at 37ºC immobilized in nanoparticles.

**Conclusion:** The combination of radiotherapy and enzymatic therapy with DAO based on the nanotechnology, induce an increase in cell death when it is compared with radiotherapy alone.

**EP-2033 Combining Hedgehog inhibition with metformin to induce radiosensitization in prostate cancer cells**

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**Purpose or Objective:** There are several indications that the Hedgehog (Hh) pathway could be a potential target for radiosensitization. Furthermore, a link between Hh signaling and the cellular energy metabolism has been described recently, more specific at the level of AMP-activated protein kinase (AMPK). Activation of AMPK, in turn, has also been shown to result in radiosensitization. Therefore, it seems worthwhile to explore whether the combination of Hh signaling inhibitors and AMPK activators such as metformin could further increase the response to radiotherapy. This combination strategy is being tested in prostate cancer (PCa) cells, as there is increasing evidence that the Hh pathway plays an important role in the development as well as progression to more advanced disease stages of PCa.

**Material and Methods:** Three PCa cell lines (PC3, DU145, 22Rv1) were treated for 72h with the SM0 inhibitor GDC-0449 (1µM, 10µM) or GLI1/2 inhibitor GANT61 (1µM, 10µM), with or without metformin (5mM). The effects on cell survival, proliferation and radiation sensitivity were investigated by means of Sulforhodamine B (SRB) assays, Bromodeoxyuridine (BrdU) assays and colony assays. The effects on gene and protein expression (qRT-PCR/Western blotting) were also examined, both in the absence and presence of ionizing radiation (4 Gy).

**Results:** GDC-0449 on its own did not significantly affect cell proliferation, survival or radiation sensitivity of any of the PCa cells lines tested. Treatment with 10µM GANT61 on the other hand did result in a significant reduction of cell survival in all cell lines and induced radiosensitization in the 22Rv1 cells (DE50=1.39±0.11, p=0.002) (Fig 1A). The latter could be ascribed to the drug’s effect on apoptosis (Fig 1B). Similar results as for GANT61 were observed after metformin monotherapy (DE50=1.36±0.08, p=0.012). Moreover, metformin induced a significant downregulation of GLI1, both at the gene and protein expression level. While the combination of metformin and GDC-0449 resulted in no additional effects, addition of metformin to GANT61 further enhanced the radiosensitization effects as induced by single agent treatment in the 22Rv1 cells.

**Conclusion:** The GLI1/2 inhibitor GANT61 as well as metformin induced radiosensitization in the 22Rv1 PCa cells. The combination of both agents further enhanced the response to radiotherapy, indicating that this might be a more powerful radiosensitization strategy as compared to either agent alone. Investigations are currently ongoing to explore the underlying working mechanisms.

**EP-2034 Targeting hypoxic cancer cells by inhibition of checkpoint kinases ATR and CHK1**

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**Purpose or Objective:** The checkpoint kinases ATR and CHK1 are considered promising targets for cancer treatment due to their roles in regulation of the S and G2 checkpoints and in the repair of DNA double strand breaks through homologous recombination. Interestingly, severe levels of hypoxia (<0.1% O2) have been shown to activate ATR/CHK1 signaling, which could likely make hypoxic cancer cells sensitive to inhibitors of these kinases. The aim of this project is to explore whether inhibition of ATR or CHK1 could be used to selectively target hypoxic cancer cells, both in combination with ionizing radiation and on its own.

**Material and Methods:** Cancer cell lines U2OS, HCT116, H460, A549 and H1975 were treated with inhibitors of ATR (VE821, VE822) or Chk1 (AZD7762, UCN01) in the absence and presence of hypoxia (InVivo2 hypoxia chamber) and X-ray-irradiation. Cells were analyzed by flow cytometry, immunoblotting and clonogenic survival assays.

**Results:** We previously measured clonogenic survival, cell cycle distribution and activation of DNA damage signaling pathways in U2OS and HCT116 cancer cells at different oxygen concentrations (21%, 0.2% and 0.0% O2) in combination with the CHK1 inhibitors UCN-01 and AZD7762 and ionizing radiation. We found that hypoxia alone did not alter the sensitivity to CHK1 inhibitors, but inhibition of CHK1 after reoxygenation following periods of extreme hypoxia (0.0% O2) did result in decreased clonogenic survival and an increased fraction of γ-H2AX positive cells. Hypoxic cells were also found to be radiosensitized at least to the same extent as normoxic cells by CHK1 inhibition. Currently we are performing similar studies in lung cancer cell lines H460, A549 and H1975 treated with the ATR inhibitors VE821 and VE822. We have found that the number of γ-H2AX positive cells after ATR inhibition was higher in cells incubated at hypoxia (0.0% O2, 20h) compared to normoxia (21% O2). The ATR inhibitors also abrogated the radiation-induced G2 checkpoint. Clonogenic survival assays are ongoing.

**Conclusion:** These studies help determine the potential of using inhibitors of ATR and CHK1 to eradicate radioresistant hypoxic cancer cells.