Conclusion: HDR-BT seems to be a good alternative for treatment of epitheliomas in special locations, above all in elderly patients with comorbidities that preclude surgery. Its ability to treat a wide area with minimal alteration of normal tissues allows a high probability of cure with excellent cosmetic results and without affecting functionality.

We can conclude that HDR-BT could be a valid alternative to surgery with acceptable acute toxicity, good early local control and exceptional cosmetic outcomes in skin lesions.

EP-2022
Compare EBRT and brachytherapy in the treatment children's vaginal rhabdomyosarcoma.

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Purpose or Objective: Rhabdomyosarcoma of the vagina is very rare disease, mainly girls were 1-3 years, only a few patients were 13-15 years old. Early studies have shown the advantage of intracavitary radiation therapy over surgical treatment and EBRT. There are new methods of planning EBRT from CRT moved to VMAT and IMRT. The emergence of new techniques in the EBRT and brachytherapy inspired us to the evaluation of methods of treatment children's vaginal rhabdomyosarcoma.

Material and Methods: From 1980 till 2015 38 patients received intracavitary brachytherapy with source Co-60 and Ir-192. In our cancer center were made special applicators of different designs. The main treatments were applicators for direct 8 mm diameter and a length of about 6-7 cm. Were specially made Co-60 tube source (LDR). Children were immobilized for several days. The active length was 4-5 cm. Since the 90s we switched to using stepping source Ir-192 HDR. Normalisation point changed from 5 mm to 2 mm from the surface of applicator. This made it possible to irradiate the entire vagina.

Planning is optimized for the creation of uniform dose distribution throughout the vagina. Accordingly, it was necessary to calculate dose distribution for these cases. For calculations were chosen CT and MRI and patient anatomy was extended, contoured target and OAR’s. The calculation of CRT / IMRT / VMAT / Brachy. CTV was 6.5 cm3.

Unlike cervical cancer, in OAR’s we added the urethra, which is located close to vagina, and which dose close to 100%. We have calculated % dose to the rectum, bladder, urethra and ovaries. For EBRT, we calculated the mean dose to OAR’s, Brachytherapy for rectum and bladder, we calculated dose to 1 cm3, and the entire volume of urethra and ovaries.

Results: In both cases (EBRT and Brachy) ovaries was about 2% (2.0% - 2.3%) of normalisation dose. However, it is worth considering that brachytherapy is given high dose per fraction, so radiobiological dose above. CRT / IMRT / VMAT / Brachy:
- Rectum: 37.7 / 26.6 / 29.9 / 37.2 %
- Bladder: 58.7 / 39.6 / 37.1 / 30.8 %
- Urethra: 99.0 / 99.2 / 97.2 / 50.2 %

Conclusion: Although improvement in EBRT (from CRT to IMRT and VMAT) and decrease in dose to OAR’s, brachytherapy maintains its position in the treatment of this localization. When less integral dose brachytherapy and dose on OAR’s (not whole body is irradiated, but only part of it), which significantly reduces late effects. In modern time, we should pay attention to other radionuclides, which can give uniform dose distribution (example Yb-169).

Electronic Poster: Radiobiology track: Molecular targeted agents and radiotherapy

EP-2023
Radiation resistance induced immunity evasion by evoking PD-L1 expression

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Purpose or Objective: To characterize PD-L1 expression in non-small-cell lung cancer (NSCLC) cell lines, and explore the relationship between immunology escaping and tumor cell proliferation and apoptosis with receiving radiotherapy.

Material and Methods: Evaluating the PD-L1 protein and CD8+ T cells with immunohistochemistry in tumor tissue from NSCLC patients. In vitro assay, to detect the expression of PD-L1 in different NSCLC cell lines after conventional and hypofractionated radiation therapy by westernblotting and study the difference between A549 and radiation resistance A549 cell line by flow cytometry and westernblotting. To analyze PI3K/Akt and stat3 proliferation pathway and Bcl2 family apoptosis signaling pathway in A549 radiation resistance cell by westernblotting. Small interfering RNA (siRNA) was used to A549 radiation resistance cell, and then to observe the difference in PI3K/Akt and stat3 pathway. As for in vivo study, immunohistochemistry was used to detect the relationship between the expression of PD-L1 and NK-β protein in control group, anti-PD-L1 group, radiation group and radiation plus anti-PD-L1 group.

Results: We found that patients whose tumor expression the higher PD-L1 protein, who had the more radiation resistance and had less CD8+ T cell around tumor microenvironment. PD-L1 protein improved obviously in NSCLC cell lines after receiving conventional radiation, but there is not the same tendency after hypofractionated radiation. We found that A549 radiation resistance cell had activation in PI3K/Akt and stat3 pathway and its’ NK-β protein would be up-regulation. When the A549 acquired radiation resistance, it would be apoptotic less. We observed the activation of the anti-apoptosis protein bcl2 and the inhibition of the pro-apoptosis protein bim in A549 radiation resistance cell. After siRNA interfering to this cell, it’s PD-L1 protein decreased. A549 radiation resistance cell came to be apoptotic. While it’s pAkt, pstat3 and NK-β didn’t change.

Conclusion: Conventional radiation would be easy to induce radiation resistance by overexpressing the PD-L1. When the lung cancer cell express PD-L1 more, the tumor would escape from CD8+ T cell. NK-β protein is the key to up-regulation PD-L1. When PD-L1 overexpression, lung cancer would be apoptosis less and immunity escaping. siRNA interfering PD-L1 can eliminate the radiation resistance of the A549 cell line. It provide the evidence for the combination of the anti-PD-L1 drug and radiation therapy in clinic.

EP-2024
Optimising hyperthermia induced radiosensitisation for treating HPV+ cervical tumours

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Purpose or Objective: To evaluate the radiosensitization effect of hyperthermia and the relationship between the expression of PD-L1 and NF-κB in different cell lines after conventional and hypofractionated radiation therapy. To determine the effects of PD-L1/PD-L1 combination therapy on NSCLC cell lines, and explore the relationship between the expression of PD-L1 and NF-β protein in control group, anti-PD-L1 group, radiation group and radiation plus anti-PD-L1 group.

Results: We found that patients whose tumor expression the higher PD-L1 protein, who had the more radiation resistance and had less CD8+ T cell around tumor microenvironment. PD-L1 protein improved obviously in NSCLC cell lines after receiving conventional radiation, but there is not the same tendency after hypofractionated radiation. We found that A549 radiation resistance cell had activation in PI3K/Akt and stat3 pathway and its’ NK-β protein would be up-regulation. When the A549 acquired radiation resistance, it would be apoptotic less. We observed the activation of the anti-apoptosis protein bcl2 and the inhibition of the pro-apoptosis protein bim in A549 radiation resistance cell. After siRNA interfering to this cell, it’s PD-L1 protein decreased. A549 radiation resistance cell came to be apoptotic. While it’s pAkt, pstat3 and NK-β didn’t change.

Conclusion: Conventional radiation would be easy to induce radiation resistance by overexpressing the PD-L1. When the lung cancer cell express PD-L1 more, the tumor would escape from CD8+ T cell. NK-β protein is the key to up-regulation PD-L1. When PD-L1 overexpression, lung cancer would be apoptosis less and immunity escaping. siRNA interfering PD-L1 can eliminate the radiation resistance of the A549 cell line. It provide the evidence for the combination of the anti-PD-L1 drug and radiation therapy in clinic.
Purpose or Objective: Hyperthermia (raising the tumour temperature to 40-43°C) is an effective treatment in combination with radiotherapy for several tumour sites, including cervical cancer, which is mainly caused by infection with the Human Papillomavirus (HPV). The aim of our study is to improve treatment strategies for cervical carcinoma by (1) unravelling mechanisms of hyperthermia induced radiosensitization, (2) optimization of time interval between hyperthermia and radiotherapy and (3) investigating the benefit of additional treatments.

Material and Methods: HPV-positive cervical cell lines SiHa and HeLa were used. Cells were treated with (1#) hyperthermia alone (42°C for 1h), (2#) hyperthermia and irradiation in different time intervals between the two therapies and (3#) hyperthermia and radiation with additional agents PARP1-inhibitor (i.e. a drug blocking a DNA repair protein) and cisplatin. Clonogenic survival assays and yH2AX stainings (a staining to visualize DNA double strand breaks) were carried out in order to determine the effectiveness of the (combined) treatments. Protein levels of p53 and DNA repair proteins were investigated using western blot. Apoptosis was measured in cell lines using the Nicoletti assay and cell cycle distribution was analyzed using the BrdU-assay.

Results: (1#) The high-risk HPV types 16 and 18 produce the oncoprotein, early protein 6 (E6), which binds to p53 before both proteins get degraded. Therefore, p53 cannot induce cell cycle block nor apoptosis, limiting the radiation effects. Hyperthermia increases the effectiveness by preventing the formation of the E6-p53 complex, rescuing p53 from degradation, resulting into functional p53 causing apoptosis and cell cycle arrest. (2#) Higher levels of p53 are present immediately after hyperthermia and remain up to four hours after treatment. The main therapy, radiotherapy or chemotherapy, should be applied within this time frame to yield a beneficial effect. (3#) Combination treatment of radiotherapy, hyperthermia, cisplatin and PARP1-inhibitors resulted in a lower survival fraction due to an increased number of DNA double strand breaks as compared to radiation alone. Cisplatin and PARP1-inhibition significantly enhanced the combined hyperthermia/radiation treatment.

Conclusion: Our findings provide new insights for patients suffering from HPV-positive cervical cancer. Hyperthermic-radiosensitization, makes radiotherapy significantly more effective by rescuing p53 from getting degraded. Adding PARP1-inhibitor or cisplatin further improves the effectiveness of hyperthermic-radiosensitization, which will increase clinical outcomes substantially.

EP-2026

Effect of PARP-1 inhibition on human soft tissue sarcoma cells radiosensitivity

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Purpose or Objective: Soft-tissue sarcomas (STS) are aggressive tumours with a poor prognosis and there is a major clinical need for novel strategies. Poly-ADP ribose polymerase (PARP)-1 promotes base excision repair and DNA strand break repair. Inhibitors of PARP (PARPi) have shown to enhance the cytotoxic effect of irradiation (IR), and evidences suggest that PARPi could be used to selectively kill cancers defective in DNA repair. Sarcomagenesis is linked to aberrant biological pathways and some STS have a defect in DNA repair systems, so there is a rationale for using PARPi in STS. We investigated the effect of PARP inhibition on STS cell lines survival after IR and on radiation-induced DNA damage foci.

Material and Methods: Nanoparticles range in size from 1 x 10⁻⁹m to 100 x 10⁻⁹m, and exert their effect by either entering the cell, or by attaching to the cell membrane surface. Radiation enhancement by gold nanoparticles (AuNP) is based on the generation of much localized secondary radiation when irradiated. This results in a Dose Enhancement Factor (DEF) and has been well described for photon irradiation and is most pronounced with kV photon energies, but happens also with Mega Voltage (MeV). For protons the DEF obtained with metallic nanoparticles has recently been studied. We took the definition of DEF as being: DEF = (Dpure + DGNP – Dwnp) / Dpure , where Dpure is the dose deposited in pure water.

Results: In vivo studies on tumors in mice have shown a considerable delay in tumor growth for mice receiving AuNPs with protons compared to protons alone. Protons have a high cross-section for gold over a large range of clinical energies, and the interaction produces Auger electrons with a very short range. The sphere of DEF around the AuNP is influenced by its size. For an AuNP of r = 22nm and 80 MeV protons the radius of the sphere of DEF is in the order of 18nm, with dose enhancement factors of up to 2 described. We obtained a value of 1.06 at 1 nm from a nanoparticle with radius 25 nm and taking Dpure as being: Dpure [Gy] = 8.16 x 5w [MeV x cm²/g], where Sw is the stopping power of water. This small radius means that in order to be effective the AuNPs need to be in very close contact with the target. In the treatment of AVMs the prime target is the endothelial cell. Angiogenesis occurring in AVMs is driven by endothelial cells stimulated by vascular angiogenic factors binding on cell membrane receptors. AVM endothelial cells over express these receptors compared to their counterparts in normal brain vessels. IMC-1121B, a human antibody to VEGFR2, when linked with an AuNP has the potential to selectively increase the local AuNP concentration on the membrane of AVM endothelial cells. For conventional dose/fractionation schedules the radiobiological effects are governed by DNA damage in the cell nucleus. Membrane location could also be exploited because a cell membrane initiated effect is described, whereby activation of the acid sphingomyelinnase/ ceramide pathway occurs after doses >10 Gy, leading to endothelial apoptosis.

Conclusion: Successful AVM radiosurgery is amongst others dose dependent. Therapeutic gain in proton radiosurgery is possible with AuNP-VEGFR2ab located on the cell membrane, combined with doses > 10 Gy. This approach needs to be researched further, but offers the possibility for better obliteration rates and/or shorter latent intervals.

EP-2026

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