respectively, reverted anti-apoptotic or anti-senescent Vit.D properties. SirT1 protein expression levels were up-regulated by Vit.D. ERKs inhibition blocked Vit.D-induced SirT1 protein up-regulation in proliferating cells. In quiescent HUVEC cells, p38 inhibition counteracted the IR-induced SirT1 protein down-regulation, while MKK6 transfection abrogated the Vit.D positive effects on SirT1 protein levels after irradiation. SirT1 inhibition by sirtinol blocked the Vit.D radioprotective effects.

Conclusion: Vit.D protects HUVEC from IR induced/oxidative stress by positively regulating the MAPKs/SirT1 axis.

EP-2042

Meta-analysis: can amifostine reduce chemoradiotherapy and radiotherapy toxicity in advanced NSCLC?

A. Devine[1], L. Marignol[1]
[1]*Applied Radiation Therapy Trinity, Discipline of Radiation Therapy, Dublin, Ireland Republic of*

Purpose or Objective: Trials of amifostine in patients with advanced non-small cell lung cancer (NSCLC) receiving chemoradiotherapy (CRT) or radiotherapy (RT) alone report varying treatment-related toxicity. A review and meta-analysis was conducted to examine amifostine's effect on toxicity and efficacy of CRT or RT alone in such patients.

Material and Methods: Searches of electronic databases yielded 16 eligible trials comprising 1057 patients. Data extracted from randomised and non-randomised trials were compiled in a review; results of randomised trials were pooled and using meta-analyses to estimate the effect of amifostine on treatment toxicity and efficacy.

Results: Amifostine reduced the risk of >Grade 2 acute oesophagitis by 26% (risk ratio [RR], 0.74; 95% confidence interval [CI], 0.65-0.86; p<0.0001) and the risk of acute pulmonary toxicity by 44% (RR, 0.56; 95%CI, 0.41-0.75; p=0.0001). Amifostine did not alter risk of late pulmonary toxicity (RR, 0.84; 95% CI, 0.65-1.08; p=0.17). Risk of complete response was unchanged (RR, 1.64; 95% CI, 0.99-2.73; p=0.06), partial response was unchanged (RR, 0.92; 95% CI, 0.73-1.16; p=0.48). Statistical heterogeneity was high for toxicity but low for response. Non-randomised trials reported varying incidence of toxicities and survival/response. Studies were medium-high quality.

Conclusion: Statistical heterogeneity casts doubt over amifostine's efficacy in this setting, despite evidence of decreased incidence of acute oesophageal and pulmonary toxicity but not late pulmonary toxicity. Amifostine did not compromise CRT or RT efficacy.

EP-2043

The ANDANTE project: a re-evaluation of the risk from scattered neutrons during proton therapy

A. Ottolenghi[1], V. Smyth[1], K. Trott[1]
[1]*Universita degli Studi di Pavia, Dipartimento di Fisica, Pavia, Italy*

Purpose or Objective: It is well known that proton therapy generates a small but significant exposure to scattered neutrons. The success of paediatric proton treatments leads to a concern about second cancers arising in later life from the neutron exposure. However there are difficulties involved with estimating the risk from exposure to neutrons. The usual approach is through the concept of relative biological effectiveness (RBE) of neutrons compared to photons, since the risk from photon exposure is much better known (ICRP Publication 103. Ann. ICRP 37 (2-4), 2007) The RBE for neutrons has been evaluated using cellular and animal models. But this causes uncertainty when applying the method to humans. The ANDANTE project (http://www.andanteproject.eu/) has investigated the relative risk of cancer from neutrons compared to photons in the context of proton therapy, using three different disciplines in parallel.

Material and Methods: Physics: Charged particle spectra generated by both neutron and photon beams were characterised using Monte Carlo simulation and measurements. A track structure model was used to model the formation of complex lesions in DNA from the different spectra as an indicator of relative likelihood of cancer induction. A method was developed for reconstructing the scattered neutron doses outside the treatment volume during proton therapy, using available clinical data, in order to be able to predict second cancer risks. Stem cell radiobiology: Stem cells from thyroid, salivary gland, and breast tissue were given well characterised exposures to both broad- and narrow-spectrum neutron beams, and to 200 kV X-rays. The relative risk of damage from neutrons compared to photons was estimated using a number of endpoints. Part of the cell population was transplanted into mice. Detailed histopathological and molecular investigations were performed looking for pre-malignant lesions and signs of malignancy. Epidemiology: The results from the track structure modelling and stem cell experiments were combined to generate a relative risk model. Dose reconstruction and data analysis tools were developed for a multi-centre prospective epidemiological study using data from paediatric proton therapy treatments, which will test the relative risk model. The project has made initial plans for the study as a collaboration between centres in Europe and the USA.

Results: The track structure model reproduced the peak in relative risk between neutrons and photons at a neutron energy of around 1 MeV, similar to the ICRP model. The stem cell experiments successfully demonstrated a new methodology, but did not provide conclusive evidence to contradict the ICRP model. The feasibility of a prospective epidemiological study was demonstrated.

Conclusion: The results from the ANDANTE project do not contradict ICRP. In the longer term, the prospective study will provide greater certainty on the RBE for neutrons and how this applies to humans receiving proton therapy.

EP-2044

Radiation-induced lung fibrosis is associated with M2 interstitial and hybrid alveolar macrophages

L. Meziani[1], M. Mondini[1], B. Petit[2], M.C. Vozenin[2], E. Deutsch[1]
[1]*Institut Gustave Roussy, INSERM U1030, Villejuif, France*
[2]*Centre Hospitalier Universitaire Vaudois, Radio-Oncologie/Radiothérapie, Lausanne, Switzerland*

Purpose or Objective: Radiation-induced fibrosis is a delayed complication of radiotherapy often associated with chronic inflammatory process and macrophage infiltration. Nowadays, macrophages are suggested to be important cellular contributors to fibrogenic process, but their implication in the context of RIF is not well known.

Material and Methods: To investigate the role of macrophages in RIF we have used a classical experimental model of lung fibrosis developed in C57Bl/6 mice after 16Gy thorax-IR. We then profiled both alveolar macrophages (AM) and interstitial macrophages (IM) during the various steps of the fibrogenic process.

Results: We confirmed the fact that total lung irradiation at 16Gy (IR) induces an interstitial fibrosis associated with delayed recruitment of pulmonary macrophages.
We found a transient depletion of AM associated with cytokine secretion during the acute post-IR phase (15 days), followed by an active repopulation and an enhanced number of AM during the late post-IR phase (20 weeks). Interestingly, AM were mostly recruited from the bone marrow and exhibit a hybrid polarization (M1/M2) associated with up-regulation of Th1 and Th2 cytokines. The number of M2-polarized IM significantly increased during the late time points after irradiation and a down-regulation of Th1 cytokine was measured in tissue lysate. These results suggest a differential contribution of hybrid AM *vs* M2-IM to fibrogenesis. Interestingly, in contrast to activated hybrid AM, activated

M2-IM were able to induce fibroblast activation *in vitro* mediated by an enhanced TGF-β1 expression suggesting a profibrotic role of M2-IM. Specific depletion of hybrid AM using intranasal administration of clodrosome increased radiation-induced fibrosis score and enhanced M2-IM infiltration suggesting a protective role of hybrid AM.

Conclusion: These present study shows a dual and opposite contribution of alevolar *versus* intertitial macrophages in radiation-induced fibrosis and identify M2-IM as a potential therapeutic target to treat radiation-induced fibrosis.

EP-2045

In vivo monitoring of skin collagen state by multiphoton microscopy in the course of irradiation

N.D. Gladkova[1], V.V. Dudenkova[1,2], V.V. Elagin[1], K.V. Babak[3], A.V. Maslennikova[3,4]

[1]*State Medical Academy, Research institute of biomedical technologies, Nizhny Novgorod, Russian Federation*

[2]*Lobachevsky State University, General physics, Nizhny Novgorod, Russian Federation*

[3]*Lobachevsky State University, Biophysics, Nizhny Novgorod, Russian Federation*

[4]*State Medical Academy, Oncology and Radiotherapy beam diagnostics, Nizhny Novgorod, Russian Federation*

Purpose or Objective: Adverse events in normal tissues during and after a course of cancer radiation treatment are one of the most pressing problems of modern radiation oncology. From among numerous works in this field, there are but a few concerned with the radiation-induced alterations of collagen, the processes of its degradation and subsequent remodeling. A new imaging technique – multiphoton microscopy (MPM) allows studying tissue collagen state on fibers and bundles level without additional staining due to second harmonic generation (SHG) phenomenon. The method has the key advantage of a potential in vivo application. This study's objective was in vivo evaluation of changes occurring at rat's skin collagen upon the exposure of conventional irradiation.

Material and Methods: Rat's ear was chosen as a model for detecting collagen changes. Experiments were carried out under Nizhny Novgorod Medical Academy ethical committee permission. Three male animals, 2 months old at the time of experiment, were used. Rat's ear was irradiated under general anesthesia (Zoletyl, 50 mg/kg, Virbac Sante Animale, France) by a Co60 unit Terabalt (UJP, Czech Republic) by a local field with single dose of 2 Gy up to the total dose of 24 Gy. The 3D imaging of collagen structure was performed by MPTflex (JenaLab, Germany) – a system for in vivo optical biopsies based on near infrared femtosecond laser technology. MPM imaging was carried out two times a week beginning from the first day of irradiation and once a week for three months after its completion. Cross-sectional images were obtained beginning from the horny layer with the step of 5 μm up to the total depth of 100 μm. Excitation was implemented with a pulsed (200 fs) titanium-sapphire laser at a wavelength of 740 nm and a pulse repetition frequency of 80 MHz; SHG collagen imaging was performed at 373-387 nm. Cross-sectional images of 512x512 pixels were obtained; the field size was 130x130 μm. Numerical processing of the images was performed by ImageJ program. Mean fluorescence intensity and its standard deviation was calculated for all images. Coefficient S (a ratio of standard deviation/mean fluorescence intensity) was used for evaluation of collagen state.

Results:

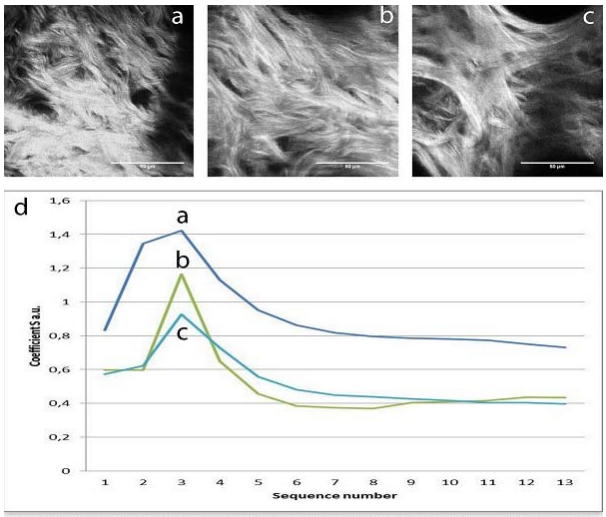

Fig.1. Examples of the MPM images of rat's ear: a - before irradiation, b - 24 Gy, c - 24 Gy, 3 month after irradiation, d – correspondent coefficient S.

Visual evaluation of MPM images demonstrated no noticeable changes of collagen packing and structure independent on the dose and time from radiation beginning (Fig.1, a, b, c). Numerical processing revealed subtle, but clear differences of coefficient S between intact and irradiated collagen. After radiation beginning, a decrease of magnitude of coefficient S and the decrease of title angle of the graph was observed (Fig.1 d). In a month after radiation completion, a magnitude remained decreased, but tilt angle of the graph returned to the initial level (Fig.1 d).

Conclusion: Numerical processing of MPM-images demonstrated changes of optical properties of collagen upon expose of clinically relevant doses of gamma-irradiation. The radiobiological interpretation of these changes require further study.

EP-2046

Modulation of radiation-induced oral mucositis (mouse) by dermatan sulfate

S. Gruber[1], E. Bozsaky[1], K. Frings[1], M. Arnold[1], V. Gernedl[1], S. Hetzendorfer[1], J. Mayer[1], S. Morava[1], S. Pfaffinger[1], P. Kuess[2], W. Dörr[1]

[1]*Medical University of Vienna, Department of Radiotherapy-ATRAB - Applied and Translational Radiobiology and Christian Doppler Laboratory for Medical Radiation Research for Radiation Oncology, Vienna, Austria*

[2]*Medical University of Vienna, Department of Radiation Oncology and Christian Doppler Laboratory for Medical Radiation Research for Radiation Oncology, Vienna, Austria*

Purpose or Objective: Oral mucositis is the most frequently occurring, dose limiting early adverse event of head-and-neck cancer radio(chemo)therapy. The purpose of the present study was to quantify the mucoprotective effect of dermatan sulfate (DS), and to characterise the associated changes in the expression of markers for epithelial proliferation, cell junctions, inflammation and hypoxia.

Material and Methods: The study comprises a functional and a histological arm. For the functional investigations, mice were irradiated with 5x3 Gy/week over one (days 0-4) or two weeks (days 0-4, 7-11). Each protocol was concluded by irradiation with graded top-up doses (day 7/14), to generate complete dose-effect curves. Daily doses of DS (4 mg/kg subcutaneously) were applied over varying time intervals. Mucosal ulceration, was analysed as clinically relevant endpoint during the functional studies. In the histological study, groups of three mice were sacrificed every second day, the tongues were excised and subjected to histological/immunohistochemical processing.

Results: DS significantly increased isoeffective doses for the induction of oral mucositis in almost all protocols, and