it has been demonstrated that the stress can influence the development and the growth of some tumors, although It's in the microenvironment its not yet understood. So we decided to study the role of stress in the tumor initiation and the progression in an orthotopic model of melanoma murine cell line not hormone-dependent B16.
Materials and Methods: Thirty eight-week-old male C57BL/6 and ten eNOS−/− mice were injected subcutaneously (s.c.) with the suspension of B16F1 5.10^5/mouse in the hind right footpad. One week before the tumor injections, we placed the animals in a conic tube (Falcon) two hours daily for a total of 21. A group of animals not stressed in which we injected B16F1, was used as positive control. S-propranolol hydrochloride, a non-selective β-adrenoreceptor, (Sigma; 2 mg/kg/d) was given form 7 days before initiation of restraint stress. The tumor growth and progression was monitored by caliper measurement and subjected to Magnetic Resonance Imaging (MRI) with a 1.5 Tesla system (Magnetom Symphony, Syngo MR 2002B, Siemens, Erlangen, Germany) and a phased array coil.
Results: Our data show that chronic stress induce thymic atrophy, infact the weight of thymus in stressed animals was significantly lower than what was observed in control animals (P = 0.04 data not shown). and increased tumor burden (443%) respect to control group and 224% respect to group treated with propranolol, and angiogenesis by upregulation of VEGF and eNos levels in stressed mice.
Conclusion: Our work shows that inhibition of eNOS pathways has a crucial role in both tumorigenesis and tumor growth stress related in melanoma murine cell line not hormone-dependent and improve the prognosis of cancer of patients stressed.

9330 POSTER

Electrochemotherapy with intravenous bleomycin in the treatment of cutaneous and subcutaneous metastases: results of a prospective single centre trial

P. Quaglino[1], C. Mortera[1], F. Marenco[1], T. Nardò[1], M.G. Bernengo[1]. [1]*Section of Dermatology 1st Dermatologic Division University of Turin, Department of Biomedical Sciences and Human Oncology, Torino, Italy*

Background: Electrochemotherapy (ECT) is an emerging treatment modality for cutaneous and subcutaneous metastases from different tumour types; it combines electroporation with injection of a chemotherapic agent, resulting in an enhanced drug uptake by tumour cells.
The aim of the study was to evaluate clinical activity and tolerability of ECT with i.v. bleomycin and to prospectively analyse the response increase associated to repeated sessions, in a large series of cutaneous metastases treated at a single institution (n = 936).
Patients and Methods: According to the ESOPE (European Standard Operating Procedures of Electrochemotherapy) guidelines, a total of 36 patients with cutaneous/subcutaneous metastases were enrolled: 27 suffered from melanoma, 6 from Kaposi's sarcoma, 1 patient respectively from squamous cell carcinoma, breast cancer and angiosarcoma. All patients were treated under general sedation with i.v. bleomycin using Cliniporator™.
Results: Overall, a response was obtained in 32/36 patients (88%). Among melanoma patients, a response was obtained in 24/27 (88%) after the 1st ECT, with a complete regression (CR) in 11 (40%). Two patients obtained a CR after the 2nd ECT course, while 10 underwent 3 to 5 treatments to achieve local tumour control. A response was obtained in 93% metastases, with lower response rates in >1 cm^2 lesions. After a median follow-up of 24 months, none of the CR nodules relapsed. The repeated ECT sessions determined a new response in 29/42 (69%) re-treated lesions, with responses obtained also in >1 cm^2 lesions. The local tumour control rate was 68% at 2 years. A response was achieved in all patients with Kaposi's sarcoma, with response duration ranging between 9 to 26 months. A good clinical response was also obtained in patients with metastatic squamous cell carcinoma and breast cancer. Treatment was well tolerated and no systemic side effects were recorded.
Conclusions: ECT represents a new therapeutic tool in treatment of cutaneous/subcutaneous metastases, thanks to its impressive clinical activity and good tolerability, coupled with an adequate cost/effectiveness ratio. Its clinical activity indicates a specific role in the treatment strategies of relapsed/refractory cutaneous melanoma patients, who show a low response rate to standard chemotherapy. Repeated ECT sessions are associated with a response increase in re-treated lesions which could allow to overcome the reduced activity in >1 cm^2 sized metastases.

9331 POSTER

Primary mucosal melanomas: a difficult diagnosis and a bad prognosis

C. Vieira[1], A. Teixeira[1], A. Ferreira[1], A. Raimundo[1], P. Ferreira[1], P. Antunes[2], C. Castro[3], M. Ribeiro[2]. [1]*Oporto Oncology Portuguese Institute, Medical Oncology Department, Oporto, Portugal;* [2]*Oporto Oncology Portuguese Institute, Department of Surgery, Oporto, Portugal;* [3]*Oporto Oncology Portuguese Institute, Department of Radiotherapy, Oporto, Portugal*

Background: Primary mucosal melanomas are rare, biologically aggressive neoplasms. The authors evaluated primary mucosal melanomas diagnoses between January 2000 and December 2006.
Material and Methods: Retrospective study based on clinical data, collected from 33 patients' records, including demographics, histology, stage, treatment and survival. Data were analyzed using SPSS software (version 16.0; SPSS, Inc., Chicago, IL). Survival analyses were calculated using the Kaplan Meier method and compared using the log-rank test. Overall survival (OS) was defined as the interval between diagnosis and last follow-up visit or death from any cause. Progression free survival (PFS) was defined as the interval between surgery or the last adjuvant treatment and date of relapse or death from any cause.
Results: Median age was 71 years (34−89), 42% had their diagnosis made in the seventh decade. Women were more affected than men (1.5:1). The more frequent presenting symptoms were haemorrhage and nodule. The distribution of head and neck, female genital tract, anal/rectal and oesophagus was 36.4%, 36.4%, 24.2%, and 3.0%, respectively. Fifteen cases (45%) were diagnosed with locoregional advanced clinical stage and 6 (18%) with distant dissemination. Surgery was performed in 75% pts, radiotherapy 30.3%, chemotherapy 15.2% and IFN-gamma 4%; Six patients had the best supportive care. Local control was achieved in 55%, but the rates of locoregional and distant recurrences were 33% and 58% respectively. The more frequent metastatic sites were lymph nodes, lungs and brain. Median OS was 20 months (95% CI 4.2−35.7) and PFS 24 months (95% CI 3.5−44.5), with median follow-up 25 months. A statistical significant difference in median OS was identified according to age ($p < 0.001$); OS in the younger group (<50 years) was higher. Lymph node metastasis and primary site didn't affect survival. The 5-year OS was 28%.
Conclusions: The aggressiveness of this entity and the difficulty in its management make the prognosis dismal. Clinical trials should be encouraged.

9332 POSTER

Non-cutaneous melanomas: not so rare entity – our experience at a centre in India

K. Sharma[1], B.K. Mohanti[1], G.K. Rath[1], P.K. Julka[1], D.N. Sharma[1], S. Mazumder[1]. [1]*All India Institute of Medical Sciences, Radiation Oncology, Delhi, India*

Background: Non cutaneous melanoma (NCM) has been described as a rare disease in the literature with its incidence upto eight percent of all malignant melanomas (MMs). There is an acute paucity of data regarding this entity in the literature. The aim of this study was to assess the incidence, treatment characteristics, and overall outcome of NCMs in patients attending a tertiary cancer care centre in India.
Materials and Methods: It was a retrospective analysis done in the patients who were treated at radiotherapy department, All India Institute of Medical Sciences, New Delhi, between 1995 and 2005. The patient's details were retrieved from their individual departmental record.
Results: Out of the total 69 patients of MM, 42 (60%) cases of NCMs were found. There were 22 (52%) mucosal lesions and 20 (48%) ocular lesions. The median age of patients was 48 years with equal incidence in both sexes. Maximum cases of mucosal lesions were from head and neck region (50%) followed by gastrointestinal (36%) and genito-urinary (9%) tract. Overall, 93% of the cases presented as single lesion with majority in the advanced stage (III or IV) at presentation. In one-fourth of cases, the malignant lesion was preceeded by a long standing pre-malignant lesion. Regional lymphadenopathy and distant metastases were seen in 12% and 26% cases respectively. There was a significantly less incidence of lymphadenopathy in NCMs in comparison with its cutaneous counterpart. Of all the treated patients, 45% achieved complete response, 19% partial response, and others had either stable or progressive disease. The median duration of follow up (DOFU) was 9.7 months in NCMs Vs 19.3 months in cutaneous melanomas. Likewise, median recurrence free survival was 10.7 months in NCMs Vs 24.4 months in cutaneous melanomas. Operability at presentation was the only factor that infuenced DOFU.
Conclusion: Although sample error may be contributory to some extent due to the present study being retrospective in nature and a peculiar referral

trend in our centre, NCMs does not seem to be an uncommon malignancy in India. The prognostic indicators showed inferior results in NCMs in comparison with cutaneous melanomas. The optimum management of NCMs is still not clear regarding the optimum use, doses and schedules of the treatment modalities. More prospective studies in future are required to come to any definite conclusion regarding their management.

9333 POSTER

Fluorescence diagnostics of skin tumors using 5-aminolevulinic acid and its methyl ester

J. Liutkeviciute Navickiene[1], L. Bloznelyte-Plesniene[1], A. Mordas[1], L. Rutkovskiene[1]. [1]*Vilnius University Oncology Institute, Laboratory of Laser and Photodynamic Treatment, Vilnius, Lithuania*

Background: The incidence of malignant skin tumours is rapidly increasing. Early diagnosis, determining the margins of the tumour, is extremely important to achieve good treatment results. We investigated the fluorescence of 5-aminolevulinic acid (ALA) or its methyl ester (MAL)-induced protoporphyrin IX (PpIX) in skin carcinomas. The study aimed to compare the effectiveness of topical ALA and methyl-aminolevulinate in determining exact margins of skin tumours.
Materials and Methods: Fluorescence diagnostics measurements were performed in 132 patients with malignant, premalignant and benign skin lesions for detection of the margins of squamous cell carcinoma and basal cell carcinoma. 5-aminolaevulinic acid or its methyl ester was applied to the skin lesion for 2−4h, and the evaluated PpIX fluorescence data were correlated with morphological tissue examination data. As fluorescence excitation system we used the light system based on blue light emitting diodes.
Results: Malignant tissue shows a specific red fluorescence when illuminated with blue-violet light, whereas no fluorescence was observed in normal skin. In 30% of cases the delineation of neoplastic lesions excited by ALA, was slightly weaker than using MAL. Sensitivity of 94.3%, specificity of 90.8% as well as positive and negative predictive values of 87.7% and 90.8%, respectively, were obtained for 342 lesions FD. The sensitivity, specificity, positive predictive value and negative predictive value for fluorescence diagnosis using MAL were 88.6; 95.4; 96.3 and 86.1, respectively, and for ALA-FD were 92.9; 85.7; 88.1 and 85.7, respectively.
Conclusions: Fluorescence diagnostics can be used for complete visualization of malignant skin lesions after topical 5-aminolaevulinic acid or methyl aminolevulinate application. It has been shown to be highly effective in malignant superficial skin lesion diagnostics. This method is applicable for detecting early superficial tumours, margins of tumours and follow-up after therapy. Topical application of methyl aminolevulinate is slightly superior to ALA in detection of lesion margins.

9334 POSTER

Melanoma metastases to the neck nodes: role of adjuvant irradiation

P. Strojan[1], B. Jancar[2], M. Hocevar[3]. [1]*University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia;* [2]*Institue of Oncology Ljubljana, Department of Radiotherapy, Ljubljana, Slovenia;* [3]*Institue of Oncology Ljubljana, Department of Surgical Oncology, Ljubljana, Slovenia*

Background: In melanoma, the opinion on the value of adjuvant radiotherapy (RT) following therapeutic neck surgery is not uniform. The aim of the study was to review experiences on the treatment of regionally advanced melanoma to the neck and/or parotid with the emphasis on the role of adjuvant RT.
Patients and Methods: Clinical and histopathological data, treatment details and outcomes of pts treated during the period 2000−2006 at the Institute of Oncology Ljubljana, Slovenia were reviewed. The sum of the risk factors present ($\geqslant$3 involved nodes, diameter of positive node $\geqslant$30 mm, extracapsular nodal spread, close/positive margins, satellitosis, disease recurrence) was termed the risk factor score.
Results: 40 pts with 42 dissections had surgery and 43 pts with 45 dissections had RT postoperatively to a median equivalent dose ($eqTD_2$: 2 Gy/fx, 1 fx/d, 5 fx/wk) of 60 Gy (range, 47.8−78.8). Compared to surgical group, irradiated patients had more advanced pN-stage (P = 0.010) and extensive surgery (involving superficial parotidectomy, P = 0.003); higher median number of involved nodes (P = 0.010); higher frequency of extracapsular tumor spread (P = 0.026) and non-radical surgery (P = 0.059) which, altogether, resulted in higher risk factor scores (P < 0.0001). Regional control at 2 yrs after surgery was 56% (95% confidence interval [CI] 40−72%) and after postoperative RT 78% (CI 63−92%) (P = 0.015). On multivariate analysis, postoperative RT (Yes vs. No: hazard ratio [HR] 6.3, CI 2.0−20.6) and risk factor score (HR 1.7 per score point, CI 1.2−2.6) were predictive for regional control. On logistic regression testing, the number of involved nodes was associated with the probability of distant metastases (P = 0.021; with 10−15 involved nodes the risk was $\geqslant$80%). The incidence of late toxicity did not correlate with the mode of therapy, $eqTD_2$ or fractionation pattern.
Conclusions: Adjuvant RT has potential to compensate effectively for the negative impact of adverse histopatological features to disease control in a dissected nodal basin. Bearing in mind the potentially detrimental effect of high fraction doses, more conventionally fractionated RT regimens (2−2.5 Gy/fx), with cumulative $eqTD_2$ $\geqslant$60 Gy are recommended. To spare pts at significant risk of distant metastases (and of dying of disease) from potentially harmful, although effective regional therapy, the number of involved lymph nodes is proposed as an additional criterion for limiting the implementation of adjuvant RT.

9335 POSTER

Changes in metabolism and metastatic properties of melanoma cells after X-ray irradiation

B. Mosch[1], K. Mueller[1], J. Steinbach[1], J. Pietzsch[1]. [1]*Forschungszentrum Dresden-Rossendorf e.V. Institute of Radiopharmacy, Department of Radiopharmaceutical Biology, Dresden, Germany*

Background: Malignant melanoma has the ability to form metastases at very early stages and in addition to surgical resection treatment involves immunotherapy, chemotherapy and also radiotherapy. As it is known that irradiation can influence cellular metabolism it is conceivable that it can induce metabolic changes which lead to a predisposition of certain cells to show enhanced survival, migratory activity and metastasis. The aim of this study was to investigate short term and long term irradiation effects on metabolism and proliferation of irradiated melanoma cells *in vitro* and their ability to form metastases *in vivo*.
Material and Methods: B16-F10 melanoma cells were irradiated with different doses of X-ray irradiation in the range of 1 to 20 Gy. One, two, and three days (short term effects) and, furthermore, 7, 14 and 21 days (long term effects) after treatment cells were analyzed concerning cell growth, viability, proliferation, cell cycle distribution, glucose and amino acid transport. Additionally, we performed *in vivo* studies in a syngeneic mouse model to analyze the capability of irradiated melanoma cells to form lung metastases.
Results: The analysis of short term effects showed decreased cell growth, viability and arrest in the G2/M phase of the cell cycle. Long term effects involve increase in proliferation, cell growth and glucose uptake but still decreased viability and amino acid transport. Our *in vivo* studies showed no formation of lung metastases when cells were irradiated before injection. If irradiated cells were allowed to recover for 2 weeks before injection, mice again developed lung metastases although to a lesser extent than control mice.
Conclusions: We conclude that melanoma cells as short term response to irradiation show cell cycle arrest and decrease in cell viability, growth and metabolic properties. One to three weeks after irradiation, the re-start of proliferation and recurrence of metabolic properties such as glucose uptake indicate that a subpopulation of surviving melanoma cells compensate for the initial irradiation-dependent damage possibly by metabolic modulations such as increase in glycolysis. Furthermore, *in vivo* studies reveal that irradiated melanoma cells are able to resume their metastatic potential within two weeks. As lung metastasis is lower when using recovered cells versus untreated cells, the role of additional mechanisms is strongly suggested.

9336 POSTER

Absence of detectable tumoral cells in the blood or bone marrow of ocular melanoma patients operated for liver metastasis

P. Mariani[1], T. Dorval[2], M. Neel[3], N. Kavian[3], S. Piperno-Neuman[2], B. Asselain[4], V. Servois[5], R. Salmon[1], L. Desjardin[1], O. Lantz[3]. [1]*Institut Curie, Surgery, Paris Cedex 05, France;* [2]*Institut Curie, Medical Oncology, Paris Cedex 05, France;* [3]*Institut Curie, Tumor Biology, Paris Cedex 05, France;* [4]*Institut Curie, Statistics, Paris Cedex 05, France;* [5]*Institut Curie, Imaging, Paris Cedex 05, France*

Disseminated tumor cells have been found in the blood and bone-marrow (BM) of many cancer patients, including those with small tumors at early stages. A few studies have found circulating tumor cells in ocular melanoma patients at the time of diagnosis of either primary tumor or liver metastasis. The presence of disseminated tumor cells in the blood/bone marrow at the time of primary treatment may be indicative of poor prognosis.
Before embarking into a prospective study to assess the prognosis value of detecting disseminated tumor cells at the time of primary treatment, we evaluated the feasibility of detecting tumor cells in the blood and BM of ocular melanoma patients with liver metastasis. Our hypothesis was most of these patients would have disseminated tumor cells in the blood, BM or both.