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

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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

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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

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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

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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.

A magnetic bead enrichment technique using the 9.2.27 mAb was used for the bone-marrow (BM), followed by immuno-staining with anti-HMB45 and -MelanA mAb to verify the specificity of the sorting. RT-QPCR for Melan-A and Tyrosinase on mRNA directly extracted from whole blood was used. In reconstruction experiments, the detection limit of the 2 techniques was 2 cells out of 20 10^6 nucleated cells for the BM and 2 cells in 7 ml of blood, respectively.
Between January and October 2008, 15 (9 F/6 M) patients with ocular melanoma who had a limited number of liver metastasis accessible to surgical resection were included. All patients had liver metastases proved by histological results. Blood samples were successfully tested in 15 patients: only 3 patients harbored barely significant levels of Melan-A or Tyrosinase mRNA. For the BM, the mean number of nucleated cells analyzed cells was 18 $\pm$ 18 millions (m $\pm$ SD, range $3-80\times10^6$). No undisputable tumor cells could be found in either the unseparated or positive/negative 9.2.27 magnetically sorted fractions.
Our negative results could be related either to a technical failure or to a true absence of disseminated tumor cells in ocular melanoma patients. Indeed, the discrepancy between our results and those previously published could be due to the high specificity of our technique given by the final anti-HMB45/melan-A immuno-staining.
Alternatively, this could be related to the selection of patients with limited liver involvement whose tumor cells and micro-environment may differ from those of patients with widespread liver metastases.

9337 POSTER

Study the chromosomal pathology in mononuclears of peripheral blood of patients with eye melanoma

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Background: To study chromosomal aberrations in peripheral blood lymphocytes of patients with eye melanoma.
Material and Methods: 16 patients with eye melanoma have been surveyed. From them men – 10, women – 6. Age of patients varied from 27 till 70 years. In 13 patients tumor was localized in vascular environment, at 3 – in eye conjunctiva. Diagnosis was put on the basis of patients' complaints, anamnesis, clinical and instrumental examinations, cytologic and histologic analysis. For comparison there was lead cytogenetic analysis in practically healthy 20 people of corresponding age and sex. For research of chromosomal aberrations in peripheral blood lymphocytes, there were used their temporary cultivation with fytohemagglutinin, by standard technique. The analysis of as structural and genomic disturbances of chromosomes was investigated according to the International system of cytogenetic nomenclatures. Primary cytogenetic research of patients was spent before their treatment.
Results: At studying of chromosomal pathology in practically healthy people the average level of chromosome aberrations which has made $1.6\pm0.4\%$. These data do not exceed the standard level for healthy people. Cytogenetic analysis lead in patients with eye melanoma has shown, that at 8/10 male patients and in all women chromosomal disturbances (6.0% and 8.4% accordingly) which have been presented with the deletion of short shoulder of 5 chromosomes (5′-), long shoulder of 17 chromosomes (17q-), hypo-, hyperaneuploidy and polyploidy of cells. Except that on the average 2−3 chromosomes had heps.
Conclusion: Thus at tumor process in patients with eye melanoma there is observed increase of chromosomal pathology.

9338 POSTER

Experience in administration of high-dose interleukin-2 immunotherapy in combination with chemotherapy for disseminated skin melanoma management

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Background: In the overwhelming majority of trials evaluating immunotherapy benefit in the treatment of disseminated skin melanoma, recombinant interleukin-2 is used which is produced by E. Coli and is not a complete analogue of human endogenous interleukin-2 molecule. Roncoleukin® (Biotech, Russia) is a complete structural and functional analogue of human endogenous interleukin-2 produced from nonpathogenic Saccharomyces cerevisiae.
Materials and Methods: Our prospective randomized trial evaluated the results of the treatment of 80 disseminated skin melanoma patients. The patients of arm 1 (n = 31) received chemotherapy (dacarbazine 800 mg/m^2, day 1 and cisplatin 20 mg/m^2, days 1−4). The treatment of arm 2 (n = 31) patients was supplemented with high-dose Roncoleukin® (9 mg/m^2, days 1−5), and arm 3 (n = 20) – Decrescendo regimen of Roncoleukin® (18 mg/m^2, day 1; 9 mg/m^2, day 2; 4 mg/m^2, days 3−4). The median follow-up was 8.5 months.
Results: The most common complications in the study arm of chemoimmunotherapy (CTCAE) were grade 1−2 fever (57.0 and 60.0%), grade 3 fever (0.9 and 3.3%), grade 1−2 hypotension (0.9 and 0.0%) in the patients of arms 2 and 3 respectively.
The objective response rate was 19.4%, 29.0% and 33.3%, the median progression-free survival (PFS) was 3.2, 5.3, and 7.8 months ($p = 0.004$), median overall survival was 8.3, 10.0 and 8.7 months ($p = 0.4$) in arms 1, 2 and 3 respectively. The blood serum level of lactate dehydrogenase (LDH) exceeding more than 1.5 fold the upper reference value was an unfavourable prognostic factor with no impact of the treatment type (chemotherapy or chemoimmunotherapy) on its efficacy ($p = 0.7$). At the same time, chemoimmunotherapy for disseminated skin melanoma patients with the LDH level not exceeding more than 1.5-fold the upper reference value increased the median PFS up to 11.2 months ($p = 0.005$). The prognostic factors of positive response to chemoimmunotherapy in this patient group were the number of lymphocytes, CD3+, CD16+, CD25+, CD4+/CD8+ ratio before the treatment start, as well as the change in CD4+/CD8+ ratio and the number of CD3+, CD4+, CD8+ lymphocytes after the completion of the 1st course of chemoimmunotherapy (Cox regression model, $p = 0.0005$).
Conclusion: High-dose Roncoleukin® in the treatment of disseminated skin melanoma is satisfactorily tolerated and may be beneficial in patients with the LDH level not exceeding more than 1.5-fold the upper reference value and with initially decreased rates of cellular immunity.

9339 POSTER

Ocular melanoma: a single-institution experience in a rare disease

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Background: Melanomas are the most common primary intraocular malignancy in adults. Primary ocular melanoma can involve the uveal tract, conjunctiva, eyelid, or orbit, comprising approximately 5% of all melanomas. The majority (85%) of ocular melanomas is uveal in origin; primary conjunctival and orbital melanomas are rare. Our goal was to characterize the population of patients with the diagnosis of ocular melanoma, treatment, response to treatment and survival.
Material and Methods: We present a single-institution retrospective study that reviews all patients diagnosed with ocular melanoma from January 2000 to December 2006. Data on demographics, tumour characteristics, therapeutic modalities and treatment results were analysed.
Results: Our sample included 32 patients (pts), being 21 of them females. Median age at diagnosis was 60 years (range: 38−81). The main complain (75%) in those pts was visual loss. Clinical evidence of metastatic disease at the time of presentation was detected in 3 pts. Up to 84% of ocular melanoma were uveal (primarily choroidal) in origin. Nearly all (94%) of them were managed with primary enucleation or orbital exenteration (in locally advanced disease). Chemotherapy was the first choice in metastatic disease, with regimens including dacarbazine. Median follow up was 42 months. The rate of recurrence was nearly 10%. The 5-year overall survival was 64.5%. Comparison between pts whose tumours had intraocular spread and those with extraocular extension, didn't reveal a statistical difference in overall survival ($p = 0.62$). The difference in survival concerning histological subtypes wasn't statistical significant ($p = 0.35$). The tumour thickness did not influence survival ($p = 0.52$). Pts with tumours with mitotic activity $\leqslant$1/40 high power fields (hpf) had better overall survival than pts whose tumours had an index superior to 1/40 hpf ($p = 0.027$). Lymphocyte infiltration was associated with worse survival, although not reaching statistical significance ($p = 0.064$).
Conclusions: Primary surgical management is not always considered the standard treatment in ocular melanoma, but it allows a histological evaluation. Cytogenetic and molecular factors are increasingly being investigated in order to identify abnormalities that could be related to prognosis and survival: alterations in chromosomes 3, 6 and 8 are strongly related to tumour behaviour.