Results: A missense mutation (p.D84Y; c.250G>T) was found in exon 2 of the CDKN2A gene. Segregation analysis of the variant was compatible with an association with the disease, since it was present in four affected family members. This mutation was predicted to affect protein function using SIFT and PolyPhen analysis. LOH analysis revealed one tumor with monoallelic loss and another with biallelic loss of the CDKN2A locus. We did not find BRAF and NRAS mutations in the tumors.
Conclusions: We show evidence that the p.D84Y missense mutation predisposes to melanoma since it is localized in a conserved domain and co-segregates with disease in this pedigree. These findings may have important implications for genetic counseling, molecular testing, and clinical management of Portuguese melanoma-prone families.

7012 POSTER

Photodynamic diagnostics of skin and mucosal lesions

J. Liutkeviciute Navickiene, L. Plesniene, A. Mordas, L. Rutkovskiene.
Vilnius University Oncology Institute, Laboratory of Laser and Photodynamic Treatment, Vilnius, Lithuania

Background: Porphyrin-enriched tumor tissue irradiation with fluorescence excitation system leads to emission of pink-red fluorescence. This principle is used as a diagnostic procedure and is called photodynamic diagnosis (PDD). The aim of this work was to investigate the possibilities of PDD in skin and mucosal lesions diagnostics.
Material and Methods: Photodynamic diagnostics measurements were performed in 68 patients with malignant, premalignant and benign skin and mucosal lesions for detection of the foci of squamous cell carcinoma, basal cell carcinoma, primary and metastatic adenocarcinoma, chondrosarcoma. Two different photosensitizers have been used – intravenous injection of hematoporphyrin derivate (HpD) and the topical application of 5-aminolevulinic acid (ALA) induced protoporphyrin IX (PpIX). We used the simple and friendly fluorescence excitation system based on blue light emitting diodes. For the patients with advanced malignant disease, HpD was injected i.v. and 12−24 hours after the injection, malignant lesions were illuminated with violet-blue (405 nm) light for cancerous tissue detection. PDD for patients with T1−2 was carried on 3−6 hours after topical ALA application. The evaluated fluorescence data was correlated with cytological and/or histopathological tissue examination data.
Results: Red or red-pink fluorescence was observed in 72 malignant epithelial tumors; 43 of them fluorescent sharp, 27 – not so intensive. 2 malignant tumors – nasopharyngeal area chondrosarcomas – had no fluorescence. The most intensive red fluorescence was detected in thin superficial malignant lesions. From 165 benign lesions, very slight fluorescence has been detected in a few haemangiomas, paratracheal papillomas, one fragment of herpes zoster and some superficial open wounds with very intensive capillarity. Fluorescence of these benign lesions was different from malignant – not so red, bluer and less intensive. Nevi, papillomas, keratosis, scars and foci of psoriasis had no fluorescention.
Conclusions: Photodynamic diagnostics can be used for complete visualization of malignant lesions after the topical or systemic application of a tumour selective photosensitizer. It has been shown to be high effective in malignant superficial skin and mucosal lesions diagnostics. PDD may be required to optimise the detection of lesions in the post-PDT patients. Fluorescence detection following i.v. injection of HpD or topical application of ALA provides no difference.

7013 POSTER

Multicenter phase II study of chemo-immunotherapy in the treatment of metastatic melanoma

F. Recchia[1], G. Candeloro[1], S. Necozione[2], L. Fumagalli[3], S. Rea[4].
[1]Ospedale Civile, Oncologia, Avezzano, Italy; [2]Università degli Studi de L'Aquila, Epidemiologia Clinica, L'Aquila, Italy; [3]Ospedale San Gerado, Unità Operativa di Chirurgia Generale 3, Monza, Italy; [4]Fondazione "Carlo Ferri", Chirurgia Oncologica, Monterotondo, Italy

Background: Combining chemotherapy and immunotherapeutic agents such as interleukin-2 and interferon alfa-2b may improve treatment results in metastatic melanoma [MM] patients compared with chemotherapy alone. This prospective study evaluated the potential efficacy of a bio-chemotherapy regimen followed by maintenance biotherapy for the treatment of MM.
Materials and Methods: Twenty-two patients with stage IV melanoma were treated for 5 consecutive days with cisplatin 20 mg/m^2, vinblastine 1.6 mg/m^2, and dacarbazine 160 mg/m^2 followed by pegylated ?-interferon 2b 50 ?g every week, subcutaneous interleukin-2 [IL-2] 1.8 M I.U. and oral 13-cis retinoic acid [13-cis-RA] 0.5 mg/kg, both given 5 days/week for 3 weeks each month. To eradicate minimal residual disease, maintenance biotherapy was continued in patients who achieved clinical benefit after 6 courses of bio-chemotherapy. The primary endpoint was response; secondary end-points were the evaluation of the immunological parameters, toxicity, progression-free survival [PFS], and overall survival [OS].
Results: Twelve patients [54.5%] achieved a response, and 7 [31.8%] maintained stable disease for at least 6 months on maintenance biotherapy. The median PFS and OS were 23.3 months and 45.7 months, respectively. The most important toxicities from chemotherapy were grade 3 and 4 neutropenia and thrombocytopenia in 41% and 18% of patients, respectively, while grade 2 autoimmune reactions were observed in 21% of patients after maintenance biotherapy. A prolonged enhancement of immunological function was observed in the 19 patients treated with maintenance therapy.
Conclusions: Six cycles of bio-chemotherapy followed by maintenance immunotherapy is well tolerated and shows significant activity in patients with MM.

7014 POSTER

Thin (<1 mm) and in-situ melanomas during 1989–2004 in Helsinki, Finland – clinical outcomes and prognostic factors

P. Stahlberg[1], S. Virolainen[2], O. Saksela[3], T. Jahkola[1]. *[1]Helsinki University Central Hospital, Department of Plastic Surgery, HUS Helsinki, Finland; [2]Helsinki University Central Hospital, Department of Pathology, HUS Helsinki, Finland; [3]Helsinki University Central Hospital, Department of Dermatology, HUS Helsinki, Finland*

Background: The prognostic factors associated with poor outcome in thin melanomas are not well known. Our purpose was to study the ratio and long term prognosis of thin melanomas in Helsinki region and to find out whether the tumor or patient characteristics that were studied were related with poor outcome.
Materials and Methods: The slides of Breslow thickness <1 mm, in-situ melanomas or lentigo malignae were reviewed, n = 301. The patient registries and the Finnish population registry were studied for the follow-up data.
Results: The mean age of the patients was 54.3 years, 58% were women. The mean follow-up was 6.7 years. There were 246 invasive cases (82%). 60.1% of all cases (n = 301) were of Clark level III, 18.6% were of Clark level II and 3.0% were of Clark level IV. Ten recurrent cases (4.1%) were invasive melanomas (<1 mm), three of which (1.2%) had died of melanoma. Four patients (1.6%) were alive with local recurrence, one with nodal recurrence and one with distant metastasis. One patient had died of coronary disease and was post-mortem diagnosed with metastatic melanoma. The group of in-situ melanomas and lentigo malignae (n = 55) contained one recurrent case with rapid nodal metastasis. The patient was diagnosed with nodal metastasis 13 days after the melanoma diagnosis, and had died of melanoma with distant metastasis one year later. The histological factors that were studied (Breslow thickness, tumor pigment, ulceration, solar elastosis and tumor-infiltrating lymphocytes) did not predict recurrence. The amount of pigment predicted overall survival, but tumor ulceration, the amount of lymphocytes and solar elastosis did not.
Conclusions: The treatment results in invasive melanomas (n = 246) were slightly better than expected (the recurrence rate was 4.1%). Pigment content of the tumor was the only prognostic factor for overall survival of the histological factors studied (pigment, ulceration, solar elastosis and tumor-infiltrating lymphocytes). These histological factors were not associated with disease recurrence. New prognostic factors are needed for staging thin melanomas.

7015 POSTER

The interest of raw data in dynamic contrast-enhanced ultrasonography (DCE-US) for the quantification of perfusion changes in in-transit melanoma metastasis (MM) treated by high doses of chemotherapy: does it predict the response?

L. Chami[1], A. Cavalcanti[2], S. Bidault[1], B. Benatsou[1], S. Koscielny[3], P. Peronneau[1], S. Bonvalot[2], N. Lassau[1]. *[1]Institut Gustave Roussy, Departement d'Imagerie, Villejuif, France; [2]Institut Gustave Roussy, Departement de Médecine, Villejuif, France; [3]Institut Gustave Roussy, Departement de Biostatistique, Villejuif, France*

Background: To evaluate the performances of a new method of quantification of perfusion in DCE-US using the raw data for the prediction of tumoral response.
Materials and Methods: In this prospective study patients suffering from localised MM were included from march 2004. All of them were treated by high doses of chemotherapy (Melphalan +/− TNFa) delivered under isolated limb perfusion. B mode morphological imaging followed by functional examination using contrast agent injection (SonoVue, Bracco, Italy) and a perfusion and quantification software (VRI and CHI-Q, Toshiba, Japan) were performed before treatment and at D+1, D+7 and D+90. Contrast

uptake parameters were provided from the raw data as peak intensity (PI), slope (S), area under curve (AUC), mean transit time (MTT). Volume and contrast uptake changes were compared between the complete (CR) and partial responders (PR) (Kruskal-Wallis test, $p < 0.05$). The response was evaluated from the clinical follow up at 3 months.
Results: At date 55 patients were included and 212 DCE-US were performed. The 3 months follow-up is available for 50 patients and preliminary results are available for 35. The global response rate was 85.7% (30/35): 42.8% (15/35) CR and PR, 11.4% (4/35) stable disease, and 2.8% (1/35) non responder. Volume changes at D+1, D+7 did not predict the CR or PR response. Conversely PI and AUC parameters were significantly higher in CR at D-1 and the ratio values of PI and AUC at D+1 and D+7 /D-1 were significantly lower in CR.
Conclusion: Our preliminary results showed that the use of the raw data in US functional imaging should provide acute parameters of perfusion for early prediction (at D+1 and D+7 after treatment) of tumoral response.

7016 POSTER

Ki67 (MIB1) in differential diagnosis between naevi and melanomas

Y. Vishnevskaya[1], D. Martynkov[2], N. Savelov[1]. *[1]Russian N.N. Blokhin Cancer Research Centre, pathology, Moscow, Russian Federation; [2]Russian N.N. Blokhin Cancer Research Centre, general oncology, Moscow, Russian Federation*

Background: Differentiation between naevi and melanomas sometimes may be difficult on routine histological examination. Ki67 (MIB1) immuno-labeling may be useful in this difficult cases.
Materials and Methods: To evaluate Ki67 labeling 65 patients with primary fast growing melanocytic lesions were selected. 20 from Cancer Research Center (Moscow) and 45 were consultative cases. 46 females (71%), 19 males (29%). Of which 13 patients were children under 14 years (20%). Age from 1y. 10 m. to 90 years. Mean age was 36 years. Skin tumors located on trunk in 31 cases (48%), upper and lower extremity in 28 cases (43%), head and neck region in 6 cases (9%). After routine histological examination the primary diagnosis were as follows: naevus with suspicion to melanoma, dysplastic naevus, cellular blue naevus, spindle cell naevus, Spitz naevus, malignant melanoma. All specimens were studied immunohistochemically. We used monoclonal antibody to Ki67 (clone MIB-1, DakoCytomation, USA). Polymer-based detection system with DAB as chromogene was used for immunostaining. In cases with hyperpigmentation we performed Giemse staining for 5 min to avoid misinterpretation of immunostaining. The usage of AEC instead of DAB gave similar results.
Results: In benigh melanocytic skin lesions Ki67 labeling was 5−9%. In melanomas Ki67 labeling was more than 10%. After immunohistochemical analysis diagnosis “naevi” was in 40 cases (62%), melanoma in 25 (38%). Benign lesions were observed in 26 females (65%), 14 males (35%). All 13 children had benign tumors. Melanomas localized on skin of trunk in 12 cases (48%), extremities in 10 cases (40%), head and neck region in 3 cases (12%).
Conclusions: Ki67 (MIB1) labeling can be used to differentiate benign and malignant melanocytic skin lesions. Especially in difficult cases on routine histological examination.

7017 POSTER

Timing of lymph node involvement is an important prognostic factor in stage III patients with thick (>4.0 mm) lower extremity melanoma

K. Herman[1], W. Wysocki[1], A. Komorowski[1], P. Skotnicki[1], J. Tabor[1], E. Luczynska[2]. *[1]Cracow Cancer Centre, Surgical Oncology, Krakow, Poland; [2]Cracow Cancer Centre, Radiology and Diagnostic Imaging, Krakow, Poland*

Background: The prognosis of stage III melanoma patients is very heterogenic, therefore the new TNM system was verified in the prospective material. Who has a real chance for cure in this subgroup of patients?
Materials and Methods: Between 249 melanoma patients who had selective ilio-inguinal lymphadenectomy 185 patients with thick (>4.00 mm) melanoma with full information were analyzed. The average depth of invasion was 5.85 mm, tumor was ulcerated in 67 of all cases (36.2%) and Clark V was assessed in 82 patients (44.3%). The median interval between primary excision and the time of lymphadenectomy was 11.1 months.
Results: In 150 of 185 patients recurrent disease were reported, including skin (29pts, 15.7%), lymph nodes (25pts, 13.5%) and distant metastases (53pts, 28.7%) as a first site of recurrence. Others (43pts, 23.2%) had multifocal recurrences and 35 pts (18.9%) were disease free. Skip metastases (positive iliac and negative inguinal) were found in 26 patients (14%). In multivariate Cox analysis only the time between first surgery and lymphadenectomy and the number of involved nodes were significant. Relative risk of death was 5.2 times higher for subgroups which had simultaneously lymphadenectomy (compared to lymph dissection performed more than 1 year after primary excision), and circa 2.7 times higher for more advanced N subgroups (pN3v pN1).
Conclusions: The long time before development of lymph node metastases and before node dissection is a favorable prognostic factor independent of other well known parameters. The value of too early lymphadenectomy (including sentinel node procedure) in this group should be reanalyzed very carefully.

7018 POSTER

Evaluation of serum IL-6, DHEA and DHEAS levels in comparison with two conventional metastatic markers in melanoma

M. Boldizsár[1], B. Vincze[1], B. Kapuvári[1], T. Bánfalvi[2], S. Otto[3], K. Gilde[2]. *[1]National Institute of Oncology, Biochemistry, Budapest, Hungary; [2]National Institute of Oncology, Dermatology, Budapest, Hungary; [3]National Institute of Oncology, Central Laboratory, Budapest, Hungary*

Based on our previous studies 5-S-cysteinyldopa (5-SCD) a precursor of pheomelanin and S-100 beta (S-100B), an acidic, low molecular weight calcium-binding protein proved to be metastatic markers in melanoma. Interleukin-6 (IL-6) is a multifunctional inflammatory cytokine secreted by malignant cells appeared to be involved in the progression of the disease. Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) are adrenal hormones with immunostimulating effects. According to the recent reports skin produces DHEA and DHEAS due to the presence of their key enzymes. Data revealed that DHEA, as well as DHEAS had a direct effect on the suppression of IL-6 production, while the circulating DHEAS level has been shown to be correlated negatively with serum IL-6. Our study involved 247 patients (man:127, woman:120) with ($n = 107$) or without ($n = 140$) metastasis of Stage I-IV following surgical intervention. The objectives were to establish the clinical significance of serum levels of IL-6, DHEA and DHEAS measured simultaneously with the melanoma metastasis markers 5-SCD and S-100B. The absence or presence of metastasis was verified by conventional imaging techniques (abdominal UH, X-ray, MR, CT, etc.).
Serum 5-SCD concentration was determined by HPLC with electrochemical detection. IL-6, DHEA, DHEAS and S-100B levels were measured using RIA/IRMA and ILMA methods.
MedCalc Software statistical analysis (Mann Whitney Test, Receiver Operating Characteristic “ROC” curve, logistic regression and multiple regression analysis) was used. Significant increase in the serum concentrations of 5-SCD, S-100B and IL-6 were found in patients with metastasis compared to metastasis-free cases, while a significant decrease in DHEA and DHEAS levels was detected. A significant positive correlation between 5-SCD and S-100B ($P < 0.0001$), 5-SCD and IL-6 ($P < 0.0001$) as well as S-100 and IL-6 ($P < 0.0001$) were found, respectively. In the contrary, a significant negative correlation between IL-6 and DHEAS ($P < 0.0001$) was observed. In order to study the relation of parameters to the localization of metastasis, survival and the progression of the disease further investigations are needed. These results suggest that simultaneous determination of IL-6, DHEA and DHEAS together with 5-SCD and S-100B measured in melanoma patients could be predictive factors of the progression.
This research was supported by the Hungarian Research Fund (OTKA No. T 049814) and NKFP MediChem 2 project.

7019 POSTER

Long-term outcome of patients with advanced melanoma

V. Parolin, R. Nortilli, C. Strina, R. Sabbioni, T. Sava, G.L. Cetto. *Policlinico GB Rossi, Department of Clinical and Experimental Medicine, Verona, Italy*

Background: Advanced melanoma is a devastating disease with a very poor prognosis, an extremely rare long-term survival and limited treatment options. The aim of this study was to evaluate long-term survival and treatment outcomes in a retrospective review of patients with stage IV melanoma.
Methods: Between 1987 and 2006, 452 patients with cutaneous melanoma were followed at our institute. Survival estimates were calculated using the Kaplan-Meier method and multiple logistic regression analysis was performed to assess correlations.
Results: One hundred eighty-eight patients (41.6%) developed distant metastasis. There were 109 males and 79 females, with average age of 54 years at diagnosis. The median survival time was 9 months. Thirty-two patients underwent surgical resection of distant tumor, alone or in combination with other treatments, with a median survival time significantly superior compared with patients not surgically treated ($p < 0.0001$, $HR = 0.3333$, 95% CI : 0.2822–0.5840). The metastatic lesions resected were in the brain (28%), in the gastrointestinal tract (19%), in the lung