

**SCIENTIFIC SESSION 11
UVEAL MELANOMA 1**

Shilpakalavedika Convention Center
Monday, January 26, 2004
2:00 PM – 4:00 PM

Chair: Tero Kivela
Co-chair: Gordon Klintworth
Moderator: Martine Jager
Session Summary: Martine Jager

	Presenter	Title of Presentation	Time
1	Ralph Eagle	Comparative Loss of Heterozygosity in Uveal Melanocytoma, Uveal melanoma, and Cutaneous Melanoma	2:00 PM
2	Gerasimos Anastassiou	New Insights in Uveal Melanoma Biology and Their Potential Impact on Clinical Practice	2:10 PM
3	J Giannios	Chimeric MAb against EGFR Linked on Pegylated-Liposomal Vinorelbine Exert Antiangiogenic Action and Induce ADCC, CMC, Anoikis and Apoptosis in Chemoresistant Metastatic Choroidal Melanoma	2:20 PM
4	Vera Likhvantseva	Specific Features of Angiogenesis in Uveal Melanoma	2:30 PM
5	Sanjiv Agarwala	Histamine and Interleukin-2 in Ocular Melanoma Metastatic to the Liver	2:40 PM
6	Louise Bergman	A Population-Based Study on Incidence of Additional Cancers in Swedish Uveal Melanoma Patients	2:50 PM
7	Igor Kaiserman	Forecasting the Prognosis of Uveal Melanoma Using an Artificial Neural Network	3:00 PM
8	Stefan Seregard	A Quality of Life Module Specifically Designed for Uveal Melanoma Patients within the Framework of the Ocular Oncology Task Force	3:10 PM
9	Jens Lindegaard	Neuroinvasive Malignant Melanoma of the Uvea	3:20 PM
10	Charlotta All-Ericsson	Clinical Response After Glivec for Metastatic Uveal Melanoma	3:30 PM
11	Gre Luyten	Dose Fractionation Effects in Primary and Metastatic Human Uveal Melanoma Cell Lines	3:40 PM
12	Martine Jager	Session Summary	3:50 PM

COMPARATIVE LOSS OF HETEROZYGOSITY IN UVEAL MELANOCYTOMA, UVEAL MELANOMA AND CUTANEOUS MELANOMA

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PURPOSE: Melanocytomas are rare melanocytic lesions of the uvea and optic nerve. Although melanocytomas are classified as benign magnocellular nevi, transformation to malignant melanoma has been reported. Uveal melanomas resemble cutaneous melanomas histologically, but differ significantly in their clinical behavior. In this study we compared the presence of LOH in uveal melanocytoma and melanoma using markers of loss of heterozygosity (LOH) known to be frequent in cutaneous melanoma. **METHODS:** We evaluated 6 melanocytomas, two of which contained atypical histologic foci, 11 uveal melanomas, 4 cutaneous melanomas and two lymph node metastases from cutaneous melanoma. Laser capture microdissection was used to sample malignant and atypical areas of the tumors; surrounding normal tissue served as controls. The specimens were analyzed for LOH at chromosomes 1, 6, 9, 10 and 11. **RESULTS:** No LOH was found in any of the melanocytomas. LOH at chromosome 1 was observed in 4 of 11 (36%) uveal melanomas and 3 of 4 (75%) cutaneous melanomas ($p < 0.05$ vs melanocytomas). One additional cutaneous melanoma demonstrated LOH at chromosome 9 (9p16). No LOH was observed in the two metastatic foci of cutaneous melanoma. **CONCLUSION:** We conclude that melanocytomas, including atypical cellular areas within melanocytomas, demonstrate significantly less LOH compared to uveal or cutaneous malignant melanomas.

NEW INSIGHTS IN UVEAL MELANOMA BIOLOGY AND THEIR POTENTIAL IMPACT ON CLINICAL PRACTICE

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According to the results of microarray analyses it could be shown that based on the gene expression profiles two distinct uveal melanoma entities exist. These two entities correlated almost perfectly with the chromosome 3 status. As already known from many independent studies metastatic disease almost exclusively develops from tumors, which have a loss of one copy of chromosome 3 (monosomy 3). None of the investigated clinical and histopathological features showed such a correlation with the two entities. According to these results, to date many patients with uveal melanoma are treated though the tumor is not threatening their lives. The status of chromosome 3 can only be detected in tumor tissue. Shouldn't we perform that routinely in any patient who is treated either by enucleation or tumor excision and plan adjuvant therapy according to the results? Shouldn't we even for small-medium size tumors, which are treated by irradiation, perform more diagnostic (e.g., biopsy for chromosome 3 status evaluation) and as a result less treatment? Alternative non-invasive procedures to identify the two entities based on the results of the microarray analyses will be also discussed.

CHIMERIC MABS AGAINST EGFR LINKED ON PEGYLATED-LIPOSOMAL VINORELBINE EXERT ANTIANGIOGENIC ACTION AND INDUCE ADCC, CMC, ANOIKIS AND APOPTOSIS IN CHEMORESISTANT METASTATIC CHOROIDAL MELANOMA

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PURPOSE: Eighty-five percent of metastatic choroidal

melanomas will be initially found in the liver. Standard chemotherapy drugs usually do not cure metastatic choroidal melanoma. We aimed to analyze the molecular basis.

METHODS: Needle biopsies were used to aspirate metastatic choroidal melanoma cells from multifocal liver tumors of a chemoresistant patient. EGFR overexpression was detected by immunocytochemistry. Tumor cells were treated with a pegylated colloidal complex with encapsulated vinorelbine with linked anti-EGFR chimeric human-mouse MAb. **RESULTS:** Post-treatment, the liposomal complex fused onto the plasma membranes where the chimeric human-mouse Mab IgG1 was bound to the ECD of EGFR consisting of 622 AA, blocking binding of EGFR and TGF- α with subsequent inhibition of the whole signal transduction cascade and internalization of the receptor-antibody complex leading to protein downregulation. The anti-EGFR Mabs inhibited VEGF and bFGF, while they induced complement-mediated cytotoxicity and antibody-dependent cellular cytotoxicity. The released liposomal vinorelbine in the cytoplasm of tumor cells circumvented biological milieu interactions such as RES elimination enhancing its therapeutic index with subsequent reduction of its systemic toxicity. Vinorelbine triggered the phosphorylation of bcl-2 at two different serine residues at G2/M phase with mitotic arrest due to microtubular polymerization of tumor and endothelial cells leading to inhibition of cell proliferation, motility and invasiveness. Also, we observed morphologically withdrawal of anchorage dependent epithelial tumor cells from their association with substratum (ECM) resulting in apoptotic cell death or anoikis. Furthermore, the downregulation of EGF and bcl-2 allowed vinorelbine to exert its irreversible apoptotic activity in endothelial cells and metastatic tumor cells, where there was interruption of mitochondrial transmembrane potential releasing cytochrome-c. Fluorometric caspase protease assays exhibited activation of caspase-9 protease (initiator) after association of Apaf-1 with cytochrome-c forming an Apaf-1/cyt-c apoptosome complex. Caspase-9 directly cleaved and activated caspase-3 (effector). TEM showed that caspase-3 cleaved the cytoarchitecture leading to blebbing and the nuclear lamina leading to nuclear breakdown, while it activated DNAase causing breakdown of DNA and laddering. All these resulted in irreversible D2 apoptotic signs where cellular and nuclear fragmentation formed membrane bound apoptotic bodies which were rapidly recognized due to external phosphatidyl-serine residues (Annexin-V) and phagocytosed by adjacent tumor cells leading to a bystander killing effect. MTT and BrdU analysis exhibited a great reduction of metabolic activity and DNA synthesis, respectively, of treated tumor and endothelial cells compared to untreated controls. The combined immunochemotherapy reduced expression and release of angiogenic factors inhibiting tumor induced neovascularization as determined by microvessel count compared with untreated controls. **CONCLUSION:** Combined immunochemotherapy consisting of colloidal and antiEGFR Mab with encapsulated vinorelbine exerted synergistic antiangiogenic and antitumor activity against metastatic choroidal melanoma.

SPECIFIC FEATURES OF ANGIOGENESIS IN UVEAL MELANOMA

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PURPOSE: The aim was to study specific features of angiogenesis in uveal melanoma (UM). **METHODS:** 87 UM patients were studied. 48 patients lived for more than 25 years and were still alive by the end of the study. 49 people died during the first 3 years after the surgery. The UM tissue was studied in a two-step research. Step 1 included immunohistochemical analysis of the protein expression VEGF, an angiogenesis regulator, and Bax, an apoptosis regulator ($n=87$). Step 2 included histomorphological light microscopy of the structure of the newly formed vessels in the tissue of UM ($n=35$). **RESULTS:** The most aggressive melanomas,

almost all of ciliochoroidal localization and epitheloid structure, were characterized by a special type of angiogenesis. It was closely connected to apoptosis. Due to active apoptosis, developing simultaneously in 10-20 tumor cells, wide intercellular tunnels were formed, which connected the main arterioles during the lysis of the adjacent vessel walls. The walls of these tunnels was represented by tightly closed basal membranes of tumor cells, set in a row and actively expressing VEGF, an endothelial growth factor. It is well known that VEGF protects tumor cells from cytotoxic peptides, which are produced by blood cells in close contact, for example tumor necrosis factor, TNF. The presence of erythrocytes within these tunnels ensured accurate identification. **CONCLUSION:** A close direct correlation is found between the expression of VEGF and Bax. The most aggressive melanomas were distinguished with hyperexpression of both peptides ($p < 0,02$).

HISTAMINE AND INTERLEUKIN-2 IN OCULAR MELANOMA METASTATIC TO THE LIVER

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PURPOSE: Ocular melanoma (OM) is the most common intra-ocular malignancy, and leads to liver metastases (LM) in about two-thirds of cases. Immunotherapy with interleukin-2 (IL-2) has a 16% response rate in metastatic melanoma but its role in OM-LM has not been formally evaluated. **METHODS:** Histamine dihydrochloride (HDC; Ceplene & #61652) enhances the clinical efficacy of IL-2 through modulation of the inhibitory effects of reactive oxygen species produced by monocytes/macrophages in the tumor microenvironment. This has led to the investigation of this combination (IL-2, 9x10⁶ IU/m² twice daily on days 1 and 2 of weeks 1 and 3, and 2x10⁶ IU/m² twice daily on days 1 through 5 in weeks 2 and 4 + HDC 1 mg SC) in two large phase II and III trials in the United States involving more than 450 patients with metastatic melanoma. A separate analysis of the 39 patients with OM with LM enrolled in these trials was performed. **RESULTS:** Twenty-two patients received IL-2 alone and 17 patients received both IL-2 and HDC. Both groups were well matched for prognostic factors including age, gender and LDH level. The median survival for OM-LM patients was 370 days (12.1 months) for the HDC/IL-2 arm compared to 144 days (4.8 months) for the IL-2 alone arm and overall survival was significantly improved ($p = 0.0503$). **CONCLUSION:** These are the first data indicating a potential benefit for IL-2 in patients with OM-LM.

A POPULATION-BASED STUDY ON INCIDENCE OF ADDITIONAL CANCERS IN SWEDISH UVEAL MELANOMA PATIENTS

Louise Bergman, Bo Nilsson, Boel Ragnarsson-Olding, Stefan Seregard

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PURPOSE: A population-based investigation of the risk of additional cancers in uveal melanoma patients. **METHODS:** In the Swedish Cancer Registry 2997 uveal melanoma cases were identified during the period 1960-1998. The individual national registration number enabled a linkage to other diagnosis of cancer found in the Cancer Registry. The Standardized Incidence Rates (SIR) with 99% CI were estimated. In the case of two melanoma registrations (uveal and cutaneous) the specimens (when available) were re-evaluated and, to minimize the risk of including misdiagnosed metastases, only patients surviving more than a year after the last diagnosis were put into analysis. **RESULTS:** No elevated overall risk for other cancers was found (SIR 1.0). Of the 38 registrations of cutaneous melanoma only 21 could

be analyzed, with a SIR of 1.58 (99% CI: 0.81-2.62). This figure might be adjusted when the re-evaluation of specimens is completed. The SIR of non-melanoma skin cancer was 0.81 (CI: 0.41-1.37), having omitted the in-situ cases. The SIR 6.09 (2.71-11.67) of secondary liver cancer likely reflects the metastatic pattern of uveal melanoma. Significantly lower risks were found for lung- and stomach cancer. **CONCLUSION:** A slight over-risk of cutaneous melanoma could not be ruled out and requires further investigation.

FORECASTING THE PROGNOSIS OF UVEAL MELANOMA USING AN ARTIFICIAL NEURAL NETWORK

Igor Kaiserman, Jacob Pe'er

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PURPOSE: To evaluate the ability of an artificial neural network (ANN) to forecast the 5-year survival of uveal melanoma. **METHODS:** 153 eyes with uveal melanoma, (mean age 61 years) treated with Ru-106 brachytherapy between 1988-1998 were followed clinically and ultrasonically every 6.7 ± 0.3 months (mean follow-up 9.6 ± 3.7 years). 3-4 layers backpropagation ANNs (2-16 neurons each) were constructed and trained on 75 patients. The information fed to the ANN included patient's age, sex and country of birth; tumor base size, height, internal reflectivity, regularity, vascularity, extra-scleral extension, location in the eye and the initial post-brachytherapy regression rate. The ANN ability to forecast the 5-year survival was tested on a separate group of 78 patients using ROC curves and likelihood ratios (LR). **RESULTS:** 57 patients had small tumors (2-4mm), 66 medium tumors (4-8mm) and 24 large ones (>8 mm). Multivariate Cox regression of all input parameters showed that the most significant prognostic parameter was tumor height followed by tumor regression rate. Large tumors had a 23% 5-year mortality compared to 10% for small tumors. Fast regressing tumors (>0.7mm/month) had 37% 5-year mortality compared to 16% for the slow regressing tumors. The best 3-layers ANN had a diagnostic precision of 84% (LR=31.5) while the best four layers ANN had a diagnostic precision of 82% (LR=15.7). **CONCLUSION:** Three layers ANNs have a very good ability to forecast the 5-year mortality from uveal melanoma. Additional layers do not add forecasting accuracy.

A QUALITY OF LIFE MODULE SPECIFICALLY DESIGNED FOR UVEAL MELANOMA PATIENTS WITHIN THE FRAMEWORK OF THE OCULAR ONCOLOGY TASK FORCE

Stefan Seregard, Bertil Damato, Tero Kivelä, Yvonne Brandberg

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PURPOSE: To develop a questionnaire module to be used, in addition to the European Organization into Research and Treatment of Cancer (EORTC) QLQ-C30, for measurement of quality of life (QL) among patients with uveal melanoma treated with methods such as transpupillary thermotherapy, plaque radiotherapy, proton beam radiotherapy, local resection and enucleation. **METHODS:** Relevant QL issues were generated from literature search and from interviews with ophthalmologists, nurses and patients with uveal melanoma representing three major treatment options: enucleation, plaque brachytherapy and proton beam therapy. **RESULTS:** The provisional module was pre-tested in 61 patients from Finland, Sweden and UK. The EORTC QLQ-OPT30 module consists of 26 items for all patients, and 4 additional items for patient receiving treatments other than enucleation. It measures ocular irritation, vision impairment, headache, worry

about recurrent disease, problems with driving, problems with appearance, functional problems due to vision impairment and problems reading. **CONCLUSION:** Several treatment modalities are available for uveal melanoma. There is limited knowledge of the impact of these treatments on QL in the long and short term. The OPT30 module may be combined with the EORTC QLQ-C30 core questionnaire to estimate this impact.

NEUROINVASIVE MALIGNANT MELANOMA OF THE UVEA

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PURPOSE: To characterize a neuroinvasive phenotype of uveal melanoma (NUM), based on clinical and histopathological findings. **METHODS:** Eighty patients with NUM were ascertained from the register of the Eye Pathology Institute, Denmark. NUM is a uveal melanoma with presence of tumor cells in the optic nerve or its sheaths. Clinical data were collected and all specimens were re-examined. Data were compared with melanomas (n=52) of the optic nerve head (ONHM) and with melanomas (n=66) without neuroinvasion or invasion of the optic nerve head (UM). **RESULTS:** The mean intraocular pressure (41mmHg vs. 20mmHg), the mean largest tumor diameter (15mm vs. 13mm) and the percentage of mixed/epithelioid cell type (78% vs. 44%) all showed significantly higher values in NUM than in UM (p<0.05). Half of the patients with NUM had a blind eye at the time of enucleation, in contrast to the patients with UM (10%). Metastatic spread to the CNS was only found in patients with NUM and death occurred significantly earlier in patients with NUM compared to patients with UM (2-year mortality: NUM 53%, ONHM 24% and UM 14%). **CONCLUSION:** Neuroinvasive melanoma is a subtype of uveal melanomas with a different clinical and histopathological pattern compared to other uveal melanomas.

CLINICAL RESPONSE AFTER GLIVEC FOR METASTATIC UVEAL MELANOMA

Charlotta All-Ericsson, Leonard Girnita, Johan Hansson, Eva Dafgard-Kopp, Olle Larsson, Stefan Seregard

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PURPOSE: Similar to gastrointestinal stromal tumors (GIST), uveal melanoma express the tyrosine kinase receptor c-kit, which can be detected by immunohistochemical staining for CD117. Some 60-70% of patients with c-kit positive GISTs have successfully been treated with Glivec (imatinib or STI 571), which inhibits the tyrosine kinase activity of c-kit. As our in vitro studies indicate that uveal melanoma cell lines are inhibited by low doses of Glivec we evaluated the clinical efficacy of Glivec in patients with metastatic uveal melanoma. **METHODS:** Four patients with c-kit positive liver metastasis from uveal melanoma have been treated with 400 – 800 mg Glivec daily for 4 – 8 months. **RESULTS:** One patient had stable disease for 4 months, 2 patients had progressive disease after 2 and 7 months of Glivec therapy and 1 patient died after 3 months of Glivec. Side effects were limited to moderate nausea in 3 of 4 patients. **CONCLUSION:** The results from this study combined with our in vitro studies suggest that Glivec may have a role in the management of metastatic uveal melanoma. However, the clinical response rate after Glivec therapy in patients with disseminated uveal melanoma appears to be considerably lower than for patients with GIST.

DOSE FRACTIONATION EFFECTS IN PRIMARY AND METASTATIC HUMAN UVEAL MELANOMA CELL LINES

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PURPOSE: To investigate the effects of split dose irradiation on primary and metastatic uveal melanoma cell lines using a clonogenic survival assay. **METHODS:** Appropriate cell concentrations of four primary and four metastatic human uveal melanoma cell lines were cultured for irradiation with single doses and with two equal fractions separated by 5 hr. After irradiation colony formation was allowed for 7-21 days. All survival curves were analyzed using the Linear Quadratic (LQ) model. **RESULTS:** After single dose irradiation a wide range in α - and β -values was obtained for both primary and metastatic uveal melanomas, which resulted in a wide range of α/β ratios. In contrast, calculations based on split dose data with which the β -component could be estimated independent of the α -component, indicated that estimates for the capacity of sublethal DNA damage repair was very similar for all cell lines. This indicated that intrinsic factors dominated the radiosensitivity of these cell lines. Split dose irradiation had little influence on the intrinsic radiosensitivity (α -component), but cell survival increased for all cell lines. **CONCLUSION:** The effects of radiation on primary and metastatic human uveal melanoma cell lines are mainly dominated by the intrinsic radiosensitivities, while the contribution of sublethal DNA damage repair is less.

SESSION SUMMARY

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