1503 ORAL

Bcl-2 and bcl-2 family genes expression in metastatic cutaneous melanoma

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Melanocytes express bcl-2 constitutively, but there is a considerable discrepancy in the data of its expression in melanoma. It is known that melanomas have low rate of spontaneous apoptosis and are resistant to apoptosis-inducing agents, so overexpression of antiapoptotic proteins is expected in this type of tumours.

In this study the bcl-2 family genes (bcl-2, bax, bak, bcl-x_S and bcl-x_L) expression was evaluated in 20 metastatic cutaneous melanoma specimens and 6 melanoma cell lines by RT-PCR with a subsequent sequencing of the amplified products, and compared with the expression in normal melanocytes. The mRNA was extracted from melanoma tumour cells, previously immunoselected of the whole biopsy. To assure the absence of infiltrating lymphocyte contamination, control CD45 amplification was performed and negative results were obtained. Samples evaluated have shown no alteration in the bcl-2-like genes expression pattern. Proapoptotic genes were expressed with the similar to the antiapoptotic genes frequency. Bcl-2 was expressed in 18 of 20 tumours (90%) and bcl-x_L in 15 (75%). There was no loss of expression of antiapoptotic bax and bcl-x_s genes in tumour samples (70% and 80%, respectively). To the contrary in melanoma cell lines the antiapoptotic bcl-X_L was overexpressed in 5 of 6 lines, while bcl-x_S was lost in 5.

Our data permit to conclude that metastatic melanomas present normal pattern of bcl-2 family gene expression and that melanoma resistance to the apoptosis-inducing stimuli should be attributed to other than bcl-2 family-involved mechanisms.

1504 ORAL

Definition of new tumor progression markers in malignant melanoma (MM)

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Purpose: RT-PCR with multiple markers has been demonstrated to be highly sensitive in detecting metastatic cells in peripheral blood of MM patients. We previously showed that MM circulating cells are significantly correlated with disease stages (Palmieri *et al.; J.C.O.* 17, 304–311, 1999). We further evaluated clinical significance of the presence of specific PCR-positive mRNA markers in both peripheral blood and regional lymph nodes.

Methods: From January 1997, peripheral blood samples from 295 MM patients with either localized (N = 195) or metastatic (N = 100) disease were taken at the time of each follow-up visit. In addition, histologically negative paraffin-embedded lymph nodes were collected from the same series of MM patients. Total cellular RNA was isolated and both qualitatively and quantitatively tested. RT-PCR was performed using tyrosinase, p97, and MelanA/MART1 as mRNA markers. PCR products were analyzed by gel-electrophoresis.

Results: Although detected at various levels among assessable patients, presence of mRNA markers in peripheral blood was significantly correlated with tumor burden. Statistical analysis showed a significant correlation between risk of recurrence (evaluated in stage I–III patients) and increasing number of PCR-positive markers (p = 0.0002). PCR results on histologically negative nodes in MM patients with localized disease as well as on additional peripheral blood samples (taken at different times during follow-up) are being analyzed; presence and/or variation in rates of PCR-positive markers is being correlated to the clinical status.

Conclusion: Existence of significant correlations between presence of micrometastases, detected by RT-PCR, and tumor progression in MM patients could be useful *a*) to assess subsets of patients with higher risk of recurrence, and *b*) to define the role of RT-PCR in monitoring MM patients.

1505 ORAL

Brain metastases of melanoma: Fotemustine compared with its combination to whole brain radiation

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Purpose: Double the brain response rate at eight weeks from treatment onset or obtain prolonged time to brain progression with the combined treatment.

Methods: Randomised phase III study; A total of 106 patients was required. In arm A, Fotemustine was administered at the dose of 100 mg/m^2 IV over 1 hour, days 1, 8, 15, followed by a 5 week rest period and, in case of response or stabilisation, continued at the dose of 100 g/m^2 every 3 weeks. In arm B, the same schedule of Fotemustine as in arm A was used and whole brain radiation was combined from day 1 to day 19 at the total dose of 37.5 Gy (2.5 Gy/day, 5 days/week). Both arms received similar amounts of corticosteroids at treatment initiation.

Results: The study was closed prematurely. Seventy-six patients (arm A: 39, arm B: 37) were included in 16 centres within 7 years. The brain response rates after 8 weeks were not significantly different between the two arms (arm A: 7%; arm B: 10%), nor were the brain control rates (response + stabilisation: arm A: 30%, arm B: 47%) or the delayed brain response rates at or after day 50 (arm A: 11%, arm B: 17%). However there was a significant difference in favour of arm B for time to brain progression (p = 0.028). No significant differences were found for overall survival (arm A: 86 days, arm B: 105 days).

Conclusion: The therapeutic choice in this palliative setting will take into account the tolerance and constraints of each treatment. The current data might orient towards the combined treatment.

1506 ORAL

Clinical relevance of PET scan in stage III & IV melanoma patients

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Purpose: evaluation of additional value of whole body 18FDG-PET scan besides conventional staging procedures (CSP: X-ray, US, CT & MRI) in patients with recurrent, potentially resectable melanoma.

Methods: lesion- and patient-based retrospective analysis of a consecutive series of melanoma recurrences admitted between Nov. 1996 & Sept. 1998, with a follow-up of at least 6 months or till death.

Results: 476 regions were depicted in 98 patients, with a final diagnosis of tumor in 183 sites whereas 262 regions proved to be tumor free and 36 remained undetermined, based on results of additional imaging modalities, histology or follow-up. Sensitivity & specificity reached 82% and 94% for PET vs. 78% and 94% for CSP. Very small deposits in skin, nodes or liver and 6/8 brain metastases were missed. PET could clarify 34/40 inconclusive CSP results.

In 28 patients, PET showed a positive additional value by ruling out 5 true-negative regional & 3 distant metastases, by imaging 5 unknown regional & 11 distant lesions and detecting 2 unrelated tumors. PET incorrectly upstaged 11 patients based on false-positive deposits (Schwannoma, inflammatory lung, soft tissue or bowel conditions) and understaged 6 patients. PET influenced therapy in 6/23 patients with LR, satellites or in transit met., in 12/46 patients with regional nodes, 1/18 with distant met. and 9/11 with presumed recurrence.

Conclusion: PET is more accurate than CSP with a clear impact on therapy in 28% of Stage III & limited Stage IV melanoma. Due to the possibility of false-positive results, any lesion that would lead to therapy change, imaged on the PET scan only, should be confirmed. A careful clinical examination together with a CT/MRI of the brain and a whole body PET are probably the most accurate way to evaluate MM recurrence.

1507 POSTER

Vulvar melanoma are different from cutaneous melanoma

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Purpose: The incidence of vulvar melanoma (MMV) decreased by on

average 3% annually over a 25-year period in Sweden in contrast to cutaneous melanoma (CMM) which increased 5–6% annually during the same time period. We now characterize the MMV in terms of clinical and histopathological features and compare the results both with CMM and within the hairy and glabrous skin compartments of the vulva.

Methods: 219 consecutive cases from the Swedish Cancer Registry were investigated. All clinical records, pathology reports and histological slides were reviewed by us. Uni-and multivariate analyses were also carried out.

Results: Clinical amelanosis was common. The density of melanoma in vulvar glabrous, but not hairy, skin was about 2.5 times more common than in the body skin, on average. Histogenetic types dominated by the mucosal lentiginous melanoma, reversed the order of incidence in CMM, but were similar to those of palms and soles. Pre-existing nevi were found exclusively in the hairy vulvar skin, significantly related to superficial spreading melanoma, but rare in the vulva. Inborn biological aggressiveness in MMV was indicated by thick tumors and ulceration – independent predictors of tumor specific survival.

Conclusion: MMV not exposed to UV light and in several important respects different from CMM should constitute a convenient model for studying pathogenic mechanisms of MM other than UV light exposure.

1508 POSTER

Ectopic expression of allbb3 integrin in human melanoma modulate organ metastasis

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Purpose: Literature data indicated that rodent tumors can express thrombocyte integrin allbb3 ectopically. Therefore we have postulated that human melanoma may also express this ectopic thrombocyte integrin.

Methods: Expression of the allb chain in human melanoma cell lines (7) was studied at genetic as well as protein level using RT-PCR, Western blotting and immunocytochemistry. By stable transfection, high and low allb-expressing melanoma clones were isolated. In human melanoma samples (30) allb and av expressions were studied using double label immunohistochemistry and confocal microscopy.

Results: All the human melanoma cell lines studied, expressed allbb3 to various extent both at genetic as well as protein level. High av expressing clones colonized the lung and liver, while high-allb expressors colonized the brain and bone in SCID mice. On the contrary to the constitutive homogenous expression of the av chain in fresh human melanoma samples, the expression of the allb chain was gradually increased with the Breslow stages.

Conclusion: Our experimental and pathology data indicate that the megakaryocytic cell line-specific allbb3 integrin is expressed ectopically in human melanoma cell lines and skin primary melanomas. Experimental data indicate that av and allbb3 integrins may control the organ-preference of the metastatic process.

1509 POSTER

Phase II trial of 4-hourly temozolomide (TMZ) in advanced malignant melanoma (MM)

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TMZ is a methylating imidazotetrazine that has shown promising activity against MM. Its activity depends on the transfer of a methyl group to guanine in DNA at the O^6 position. The protein O^6-methylguanine-DNA methyltransferase (MGMT) repairs this lesion in a stoichiometric, auto-inactivating reaction, and is a major determinant of cell resistance to TMZ treatment. MGMT levels are depleted after TMZ dosing, but recover by 24 hours – the time of subsequent dosing. Optimizing the schedule of TMZ so that subsequent doses are given at the MGMT nadir (4 hours after the last dose) might enhance the effectiveness of TMZ against MM.

Material and Methods: Patients (pts) with advanced MM were treated with TMZ 1000 mg/m^2 (or 750 mg/m^2 if they had received prior chemotherapy), equally split into 5 doses over a 16-hour period, repeated every 28 days, for up to 6 courses.

Patients: 30 pts were entered into the study (14 M/16 F); median age was 57 yrs (29–83) and median WHO PS 1.20 pts had visceral metastases and 4 pts had received prior treatment.

Results: 28 pts were evaluable for toxicity and 25 for response. Eighty-three courses of TMZ were administered (median 2). Dose reductions were required in 45.7% of cycles and treatment was delayed on 9 occasions. The main toxicities observed were grade 4 thrombocytopenia in 12 pts (42.8%) and grade 4 neutropenia in 11 pts (39.2%), associated with infection in 8 cases. There were no treatment-related deaths. There were 7 responses (1 CR), for an ORR of 24%, with 5 SD (17%) and 13 PD (45%). Median overall survival was 6.1 months and median time to progression 1.8 months.

Conclusion: The 4-hourly schedule of TMZ is likely to enhance methylation in tumour and normal tissue, compared with the standard 5-day regimen. The compressed schedule has activity against MM, but the observed myelosupression, although readily managed, precludes its wider application.

1510 POSTER

Proteolytic enzymes prevent B16 melanoma metastasizing in C57B16 mice

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Purpose: Aim of the study was to examine antimetastatic effect of proteolytic enzyme (trypsin, chymotrypsin, papain) mixture on syngenic B16 melanoma in C57B15 mice. We intended to confirm our previous positive pilot results (Wald et al, Life Sci 1998)

Methods: 140 mice were included into experiment:

Control group 1 (C1) – rectal administration of saline from the day of melanoma B16 cell transplantation (20 animals)

Control group 2 (C2) – rectal administration of saline from the day of primary B16 melanoma extirpation (20 animals)

Enzyme group 1 (E1) – rectal administration of enzyme mixture from the day of B 16 melanoma cell transplantation (50 animals)

Enzyme group 2 (E2) – rectal administration of enzyme mixture from the day of primary B16 melanoma extirpation (50 animals)

Survival of mice and B16 melanoma generalization were observed for 100 days. All animals were dissected and organs were histologically examined.

Results:

(1) primary tumor size was significantly smaller in E1 group than in the groups E2, C1, and C2 (73.8 mm^3 in comparison to 302.4 mm^3, 298.9 mm^3 and 293.8 mm^3, respectively). In 36% of the group E1 animals, primary tumor did not grow at all.

(2) average survival of mice dependent on the B16 melanoma generalization was 27.25 and 28.65 days, respectively, in control groups (C1 and C2), while in the enzyme groups (E1 and E2) it was 72.06 and 53.5 days, respectively

Conclusions: Mixture of proteolytic enzymes (trypsin, chymotrypsin, papain) showed antimetastatic and antiproliferative effect on B16 melanoma in C57Bl6 mice.

1511 POSTER

C-myc expression as a new prognostic factor in melanoma

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Purpose: C-myc gene lies on the long arm of chromosome 8q (24) and encodes a nuclear phosphoprotein of 62 kDa that is known to play a key role in the control of proliferation. Our study was aimed to examine how expression of c-myc oncoprotein influences the average period of patient survival in melanoma, and to check any relationship between c-myc expression and a lesion thickness.

Methods: 49 patients treated in the years of 1990–92, aged 19–82 (average age 57), with different locations of melanoma were taken into the study. C-myc oncogene expression was examined by 'In Situ' hybridisation method.

Results: Patients' ages, lesion localisation, and histopathological parameters were analysed. It was found that there is a correlation between high c-myc expression and a short patient's survival and a short disease-free interval. The study also confirmed the correlation between melanoma lesions > 3 mm thick (Clark IV–V) and high expression of c-myc oncoproteins. Low c-myc expression was related to favourable prognosis for the patients.

Conclusion: The examination of the expression of c-myc oncoprotein is a very good prognostic marker in cutaneous melanoma, especially in thick lesions.