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