1200 POSTER

Phase II study of thalidomide in patients with brain metastases from malignant melanoma

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Background: CNS metastases develop in nearly half of patients with advanced melanoma and in 15 to 20% of these patients, CNS is the first site of relapse. Overall median survival is short, ranging from 2 to 4 months, and 1-year survival is no higher than 10 to 15%.
Thalidomide has antiangiogenic and immunomodulatory effects, exhibiting antitumor effects in patients with multiple myeloma and, more rarely, in solid tumours. Results obtained in prior trials indicate that Thalidomide acts as a cytostatic agent in metastatic melanoma. We evaluated single agent antitumour activity and toxicity of Thalidomide in a phase II setting in patients with brain metastases associated with metastatic melanoma.
Methods: Patients with measurable metastatic melanoma in progression and with PS $\leqslant 2$ who signed a written informed consent were enrolled in the study. Thalidomide was given orally, and all patients followed Pharmions Risk Management Program. Dose was escalated over 4 weeks from 100 mg/day to 400 mg/day. Concomitant treatment with steroids was allowed.
Patients were evaluated every 12 weeks for Efficacy. Primary objective of the study was to determine response rate, according to RECIST. Secondary objectives were to estimate time to progression, overall survival and to evaluate tolerability of the regimen according to common toxicity criteria.
Results: 25 men and 12 women were enrolled in the study, median age 48 years. WHO performance status varied: 12 patients with PS 0, 13 with PS 1 and 12 with PS 2. Among 37 eligible patients 36 were evaluable for response. One patient with brain metastases as the only site of disease obtained a CR, 1 had PR with CR in the lungs and NC in the other sites, and 4 NC. Among the latter, 1 patient had CR of lymph nodes and skin metastases, but NC overall. Grade 3 and 4 toxicities included fatigue, constipation, dry mouth, neuropathy-motor, nausea, anorexia, neuropathy-sensory. 23 were irradiated for brain metastases before and 1 under treatment with Thalidomide. 23 were treated with concomitant steroids. 21 achieved the maximum daily dose of 400 mg, but only 13 patients continued on this dose without dose reduction. Median time to progression and survival time was 1.8 and 3.3 months, respectively.
Discussion: Single agent Thalidomide has activity in melanoma patients with brain metastases. It has encouraged us to investigate Thalidomide in combination with Temozolomide, a very lipophilic agent, in this group of patients.

1201 POSTER

Randomized phase II trial of treosulfan alone vs. gemcitabine plus treosulfan (GeT) in stage IV uveal melanoma patients

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Background: Preclinical studies suggested synergistic activity of treosulfan and gemcitabine against uveal melanoma cell lines. In previous phase I and II studies, dose and schedule for the combination treatment were established. This randomized phase II trial was performed to evaluate the clinical efficacy of treosulfan in combination with gemcitabine vs. treosulfan alone in metastatic uveal melanoma patients in order to investigate the potential clinical relevance of the in-vitro synergy.
Methods: Patients with uveal melanoma liver metastases and performance status (PS) above 50% Karnofsky were randomized to receive treosulfan (3.5 g/m^2) without (arm A) or with (arm B) gemcitabine (1 g/m^2) on days 1 and 8 of a 4 week cycle for a maximum of 6 cycles.
Results: A total of 48 patients were randomized and prognostic factors were evenly distributed between both arms. Arm A vs. B: female patients 48% vs. 48%, serum LDH < 2 x upper normal limits 28% vs. 22%, PS <90% 17% vs. 20%. Toxicity was mild and mainly hematogenous, consistent with the observations in the previous phase I and phase II studies of the combination treatment. At time of evaluation of the initial 9 patients into each arm a futility analysis was performed, revealing 2 patients with at least stable disease at 3 months in the treosulfan only arm and 5 patients with at least stable disease in the combination treatment arm, allowing to continue accrual to 24 patients in each arm. As of May 24, 2005, 21 of the 48 patients have died.
Conclusions: The futility analysis revealed a trend towards higher efficacy of the combination treatment, but allowed to continue both arms to full accrual. Full accrual was achieved in May of 2005 and with the current event-rate, unblinding and final analysis of the results will be performed early July of 2005, allowing presentation of the final analysis at the time of the ECCO meeting.

1202 POSTER

Amplification of the 7q31 locus is a frequent event in malignant melanoma and associated with extra copies of EGFR gene

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Development, progression and metastasis formation of malignant melanoma of the skin involves multiple genetic alterations. There is a growing number of evidence demonstrating that alterations of different oncogenes play important role during the progression of the disease. By chromosomal CGH we and others found frequent gains on chromosome 7p12 and high level amplification on 7q mainly covering the 7q31-qter region.
In an effort to describe the copy number distribution pattern of chromosome 7, EGFR and 7q31 loci, we used interphase FISH on melanoma imprint preparations. In the present study 62 primary tumours were analyzed for EGFR and 42 for both loci by FISH. Based on disease progression tumours were grouped into two subgroups; 30 primary lesions did not developed metastases within 1 year after the surgery of the primary tumour, whereas 32 had metastases within the follow up period. Aneusomy for chromosome 7 was present in both subgroups, however the frequency of polysomy was significantly higher in tumours with metastatic behaviour ($p = 0.03$). EGFR alteration~– in at least 10% of tumour cell~– was seen in both subgroups. Gain of EGFR signals in relation to chromosome 7, which is the measure of relative gene amplification, were seen in 45% of tumours without – and 67% of tumours with metastases. Deletion of the EGFR gene was also observed in 13 samples, however subpopulation of cells with amplification was also noted in 10 of these cases. Two melanomas in which more than 75% of the cells showed relative loss of the EGFR gene were mainly diploid for the 7q31 locus, however the third case with EGFR gene copy loss were amplified for the 7q31 locus. The amplification level of the 7q31 region was much higher (sometimes 50–60 copies/cell) compared to the 7p12 locus (4–20 copies/cell). High level amplification of 7q31 was associated with EGFR gene amplification in ten primary tumours, all of these formed metastases within 1 year.
Based on these FISH results we assume that chromosome 7 aneusomy, simultaneous amplification of the EGFR and the 7q31 loci are associated with metastases formation of malignant melanoma. Quantitative analysis of these loci by FISH may improve prognostic assessments in malignant melanoma, because it allows detection of highly amplified malignant cell subpopulation on a cell by cell basis.

1203 POSTER

Downregulation of MMP by RECK (a novel MMP inhibitor) in osteosarcoma

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Background: RECK, a novel MMP inhibitor, is widely expressed in normal human tissues but is down-regulated in tumor cell lines. RECK suppresses tumor invasion and angiogenesis by regulating MMP-2, MMP-9 and MT1-MMP by unknown mechanism. This suggests that RECK may be repressed during tumor progression, invasion and metastasis. We performed the present study to investigate the expression pattern of RECK gene in human osteosarcoma and to see the relationship between RECK and MMP.
Material and methods: Osteosarcoma cell lines that had been established from tumor samples of 23 patients and 4 standard cell lines were used in this study. RNA was extracted and quantitative real time RT-PCR was done. Activity of MMP-2 and MMP-9 was determined in the conditioned media by zymography. RECK gene transfection was done in 5 patient cell lines and 3 standard cell lines. Downregulatin of MMP activity and invasion ability after transfection of RECK gene was evaluated using zymography and Matrigel assay.
Results: RECK gene expression was markedly low in 22 out of 23 cell lines compared with control. Pro-MMP-2 was expressed in all cell lines including standard cell lines, however, MMP-2 was expressed in 4 cell lines. Pro-MMP-9 was expressed in only 1 patient cell line and U2OS, however, MMP-9 was not detectable in any sample of tested cell lines. The low activity of MMP-2 was correlated with higher expression of RECK ($p = 0.01$). After transfection of RECK gene, HOS cell lines showed decreased expression of MMP-2 and MMP-9, and invasion activity decreased in matrigel invasion assay compared with non-transfected cell lines ($p < 0.01$).

Conclusions: It is suggested that decreased expression of RECK gene have a role in the increase of MMP activity in osteosarcoma. Further study is required to analyze the mechanism of RECK action. It can be a new therapeutic strategy for MMP inhibition in human cancer.

1204 POSTER

Heparanase expression in melanoma: updated clinico-pathologic results.

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Background: No effective systemic treatment exists for advanced melanoma. Identification of new markers involved in the initiation and progression of melanocytic tumorigenesis, that will be the basis for developing new therapeutic tools are still needed. The expression of the heparanase gene and its protein has been associated with metastatic potential of several human tumors. The purpose of the study is to determine the expression of heparanase in nevus and melanoma in different stages of tumor progression and to evaluate the clinico-pathologic significance of these findings.
Methods: 60 formalin-fixed and paraffin-embedded specimens of nevus (15) and melanoma (45) were examined with immunohistochemical staining for heparanase expression. The charts of all melanoma patients were reviewed for clinical correlation.
Results: No (0) heparanase expression in 7 specimens or weak (1+) in 8 specimens was detected in nevus. Heparanase was detected in both the cytoplasm and the nucleus of heparanase positive cells. Weak heparanase expression was confirmed in 11 specimens, weak to intermediate in 2 and intermediate (2+) in 2 specimens of superficial spreading melanoma (Breslow <4 mm). Intermediate intensity staining (2+) was detected in 14 cases of thick melanoma (Breslow >4 mm) and only one case showed weak staining. Strong (3+) heparanase expression predominated in 10 cases from different metastatic sites and intermediate to strong staining in 5 specimens (3 from lymph nodes and 2 from lung metastases). Of 15 patients with superficial spreading melanoma, 2 (13%) developed recurrent disease, at 4 and 5 years from diagnosis. In both patients heparanase expression at the time of diagnosis was weak: 1+. Of 15 patients with thick melanoma, 10 (67%) developed recurrent disease, and 6 (40%) died of melanoma. Heparanse expression at the time of diagnosis in this group of patients was intermediate in all patients who developed recurrence. Six of 15 patients (40%) with metastatic melanoma, who died from the disease, had strong and 3 patients had intermediate-to-strong expression of heparanase in tissue specimens obtained from metastases.
Conclusions: Heparanase expression in melanoma is significantly correlated with tumour stage and metastatic potential. The value of heparanse activity as predictor of clinical course of disease requires further investigation in a larger number of patients

1205 POSTER

Clinical significance of serum 5-S-cysteinyldopa determination in patients with malignant melanoma

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The incidence of malignant melanoma is increasing worldwide and the metastatic ability of the disease is very high. Among circulating tumour markers in melanoma (S-100B protein, neuron specific enolase, LDH, cytokines, tyrosinase, etc.) 5-S-cysteinyldopa (5-SCD) is extensively investigated, and may have potential role in the follow-up of patients, monitoring the therapy, prediction of prognosis and in the early detection of recurrences. 5-SCD is a precursor of pheomelanin produced in melanocytes and melanoma cells during the biosynthesis of melanins by a tyrosinase dependent mechanism. The purpose of this study was to evaluate the significance of this marker in the clinical practice by measuring the serum 5-SCD concentrations in different stages of malignant melanoma and monitoring the patients during the therapy, as well as to analyse the data concerning the progression of disease.
Since 1997, nearly 4500 serum samples originated from 1409 patients suffering from malignant melanoma were investigated. The age of patients (including 677 males and 732 females) ranged from 18 to 86 years (mean 56.7). The diagnosis of malignant melanoma and the presence of metastasis were verified by histology and by various imaging techniques. Serum 5-SCD concentration of healthy individuals and melanoma patients was determined by high pressure liquid chromatography with electrochemical detection. Patients were classified according to their AJCC Stages and data statistically evaluated. In addition, 180 patients (3 in Stage I, 93 in Stage II, and 84 in Stage III) were monitored for years.
Significant differences were revealed between control group and stage III-IV, as well as between stage III and IV patients. In about 25 percent of patients suffering from various types of recurrence the elevated 5-SCD level was the first sign of the progression. The increase of 5-SCD level preceded by 1–3 months the detection of spreading of the disease compared with conventional imaging methods.
Summarising our observations it was confirmed that serum concentration of 5-SCD correlates well with Stages in melanoma patients and progression of the disease. The marker had the greatest clinical significance in stage IV and showed important positive predictive value. According to the results presented here determination of serum 5-SCD concentration proved to be a useful tool in the monitoring of melanoma patients.

1206 POSTER

A study of familial melanoma in Greece and identification of germline mutations in the *CDKN2A* tumour suppressor gene

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Background: The *p16/CDKN2A* tumour suppressor gene has been recognised as an important predisposing factor in the development of melanoma. The primary objective of this study is the identification of mutations in the *CDKN2A* gene among Greek families with at least two first-degree relatives afflicted by melanoma.
Material and methods: Members of such families with histological diagnosis of melanoma are invited to participate in the study. After informed written consent patients provide a blood sample. The study has the approval of the relevant Ethics Committee. Mutation analyses were performed on DNA isolated from peripheral blood and exons 1, 2, and 3 of the *CDKN2A* gene were amplified by PCR. All exons were bidirectionally sequenced.
Results: To date 12 Greek families have been identified who qualify for entry into the study. A total of 11 patients from 7 families and a relative with the *Atypical Naevus Syndrome* (ANS) have provided blood samples. Two or more members from 4 families and one surviving patient from each, of another 3 families have been studied. The Arg24Pro mutation in exon 1 has been identified in 6/11 patients who belong to 4 families. The Ala148Thr polymorphism in exon 2 and C500G in the 3'UTR have been identified in the relative with the ANS and in two patients, one of whom also has the Arg24Pro mutation. The study is ongoing.
Conclusions: The present study is the first systematic investigation of potential mutations in familial melanoma in Greece. The Arg24Pro mutation identified by us is likely to be of importance for melanoma risk, since it has previously been reported in different ethnic populations and been shown to segregate with melanoma. Our results indicate that this alteration may be the predominant *CDKN2A* germline mutation in Greek melanoma kindred.
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1207 POSTER

Oncogenes and tumor-suppressor genes in nodular melanoma. Prognostic value

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The most important factors in prognosis of patients with melanoma are tumour thickness, presence of ulceration, localization, pathological stage, surgical treatment, depth of invasion, gender, and age. The purpose of the present study is to evaluate prognostic value of molecular markers (oncogenes, tumour-suppressor genes, apoptotic and proliferative factors, adhesion molecules) in patients with nodular melanoma. Tissue samples were obtained from 62 patients with nodular melanoma and a presence of ulceration, aged 34–81 years (male/female ratio – 1/1.38). Expression of p53, Bcl-2, Ki67, p21, C-myc, C-jun, Mdm2 and CD44 proteins was investigated immuno-histochemically in of primary melanoma lesion. Disease-free survival and overall survival were assessed. Logistic regression analysis was used to compare prognostic value of the proteins expression.
Results are presented in the table.
Conclusion: These results suggest that expression of p21, C-myc and CD44 in the melanoma cells might be additional prognosticators. Further studies are needed to investigate whether prognostic value of the molecular markers is independent of classical prognostic factors.