Protein expression	Relative risk ratio (within 5 years from surgery)	
	Recurrence	Death
Ki67	2.22	0.58
P53	5.99*	2.44
P21	3.8*	5.99*
Mdm2	2.4	2.33
Bcl-2	0.72	2.31
C-jun	6.66*	2.44
C-myc	10.53*	5.49*
CD44	5.0*	15.87*

*$p < 0.05$

1208 POSTER

In vitro sensitivity assay-directed chemotherapy as first-line treatment in metastatic melanoma: a phase-II trial of the DeCOG

S. Ugurel[1], D. Schadendorf[1], C. Pföhler[2], K. Neuber[3], R. Figl[1], J. Ulrich[4], A. Hauschild[5], K. Spieth[6], W. Tilgen[2], U. Reinhold[7]. [1]*German Cancer Research Center, Skin Cancer Unit, Mannheim, Germany;* [2]*The Saarland University Hospital, Department of Dermatology, Homburg/Saar, Germany;* [3]*University Hospital Hamburg, Department of Dermatology, Hamburg, Germany;* [4]*University Hospital Magdeburg, Department of Dermatology, Magdeburg, Germany;* [5]*University Hospital Kiel, Department of Dermatology, Kiel, Germany;* [6]*University Hospital Frankfurt, Department of Dermatology, Frankfurt, Germany;* [7]*Praxisklinik Bonn, Dermato-Onkologie, Bonn, Germany*

This multicenter phase-II trial aimed at investigating the safety and efficacy of a sensitivity-directed chemotherapy in correlation to pretherapeutically tested in vitro chemosensitivity in metastasized melanoma patients. The primary study endpoint was objective response (OR), secondary endpoints were safety, overall (OS) and progression-free survival (PFS).
Viable tumour cells obtained from metastatic lesions were tested for in vitro chemosensitivity to 7 single anticancer drugs and 5 drug combinations using an ATP-based luminescence assay. 13 patients with locoregional (Stage III) and 82 patients with distant (Stage IV) metastases were enrolled (intention to treat, ITT). 2/13 stage III and 57/82 stage IV patients received assay-directed chemotherapy using the individual drug or drug combination showing the highest in vitro sensitivity (best test index). 1/13 stage III and 53/82 stage IV patients were evaluable for all study endpoints (per protocol, PP). The drug combinations revealing the highest in vitro sensitivity results were treosulfan+gemcitabine, paclitaxel+cisplatin, paclitaxel+doxorubicin and gemcitabine+cisplatin.
Patients enrolled at stage IV showed 13 OR (15.9%/24.5%, ITT/PP); median OS was 7.9/8.8 months (ITT/PP), median PFS was 3.6/3.6 months. 22/53 PP patients revealed high in vitro chemosensitivity (best test index $\leqslant 100$) for one of the investigated drugs/drug combinations. This subgroup showed an increased OS of 14.6 months compared to patients revealing low in vitro chemosensitivity (best test index > 100; 31/53 patients; OS 7.4 months), $p = 0.041$. An OR was achieved in 8/22 (36.4%) high sensitivity patients compared to 5/31 (16.1%) low sensitivity patients, $p = 0.032$.
Our study results indicate in vitro chemosensitivity as a surrogate marker for response and survival of melanoma patients treated with sensitivity-directed chemotherapy. These preliminary results need to be confirmed by future prospective trials in a randomized, standard-regimen controlled setting.

1209 POSTER

Heparanase expressions and its clinical significance in osteosarcoma

H. Kim, J. Kim, K. Kim, J. Oh, M. Lee, S. Seol, H. Kang. *Seoul National University College of Medicine, Orthopaedic Surgery, Seoul, Korea*

Background: Heparanase is an ECM degradative enzyme, which cleaves heparan sulfate. Heparanase activity has been implicated in cancer cell invasion, metastasis and angiogenesis. Its up-regulation has been documented in a variety of primary human tumors, correlating with poor prognosis, suggesting that heparanase may be considered as a therapeutic target. This study was designed to determine the expression of heparanase in osteosarcoma and to evaluate its clinical significance.
Material and methods: The immunohistochemical expression of heparanase from 51 osteosarcoma tissues was examined, and the correlations with clinicopathologic factors were evaluated according to the heparanase expression. Methylation-Specific PCR (MSP) of 4 standard cell lines (MG-63, HOS, U-2OS, Saos-2) was analyzed in order to evaluate its methylation status of CpG island.
Results: Overexpression of heparanase was observed in 37 tissues (73%). The heparanase expression correlated with poor response to neoadjuvant chemotherapy, metastasis and poor survival rate. The multivariate analyses revealed that heparanase over-expression was a significant independent risk factor for distant metastasis in osteosarcoma. Among 46 patients who underwent adequate wide resection, the heparanase expression correlated with a high recurrence rate. The 5-year survival rate was 83.8% for patients with heparanase negative tumours, and 46.9% for those with heparanase over-expression ($p < 0.001$). In the multivariate analysis using the Cox regression model, the heparanase expression emerged as an independent prognostic indicator. Methylation-Specific PCR (MSP) screening of 4 cell lines (MG-63, HOS, U-2OS, Saos-2) representing at least one unmethylated allele, as indicated by a PCR product obtained with primers specific to the originally methylated sequence.
Conclusions: These results indicated that the heparanase expression may play an important role in local recurrence, metastasis and poor survival in osteosarcoma patients, and may be a biologic marker with prognostic significance in osteosarcoma.

1210 POSTER

Phase I trial of sorafenib (BAY 43–9006) combined with dacarbazine (DTIC) in patients with metastatic melanoma

T. Eisen[1], T. Ahmad[1], R. Marais[1], I. Gibbens[1], M. James[1], A. Affolter[1], D. Chao[2], L. Bergamini[3], B. Schwartz[4], M.E. Gore[1]. [1]*Royal Marsden Hospital/Institute of Cancer Research, Urology, Skin and Lung Units, London, United Kingdom;* [2]*The Royal Free Hospital, London, United Kingdom;* [3]*BayerSpA, Milan, Italy;* [4]*Bayer Pharmaceuticals Corporation, West Haven, CT, USA*

Background: B-Raf mutations occur in ~70% of melanomas, and are associated with hyperactive Raf/MEK/ERK signalling activity. Sorafenib (BAY 43–9006) inhibits the Raf/MEK/ERK pathway at the level of Raf kinase (Raf-1, wild-type B-Raf, V599E B-Raf) and the receptor tyrosine kinases VEGFR-2 and PDGFR-β, to mediate effects on both the tumor and vasculature. In Phase I/II trials, sorafenib was generally well tolerated as a single agent or with concomitant chemotherapy. Sorafenib, in combination with carboplatin/paclitaxel, has shown preliminary anti-tumor activity against melanoma.
Patients and methods: This single-centre, open-label, Phase I, dose-escalation study was performed to determine the safety profile and maximum tolerated dose (MTD) of sorafenib administered at 200 (cohort 1) or 400 mg bid (cohort 2) in combination with repeated 21-day cycles of DTIC 1000 mg/m^2. In an extension phase (cohort 3), patients received the MTD of sorafenib plus DTIC 1000 mg/m^2.
Results: Patients with metastatic melanoma (ECOG PS 0–1) were enrolled into cohorts 1 (n = 3), 2 (n = 6) and 3 (n = 9). One patient in cohort 2 experienced dose-limiting grade 3 hand–foot skin reaction. The MTD of sorafenib in combination with DTIC was defined as 400 mg bid. Common drug-related adverse events in cohort 1 and cohorts 2–3, generally grade 1–2 in severity, included nausea (100% and 40% of patients), fatigue (67% and 60%), constipation (33% and 67%), alopecia (67% and 13%) and rash (67% and 53%). Grade 3–4 adverse events were rare, and mostly resolved. Frequent grade 3–4 AEs included abnormal lipase (1 patient in cohort 1 and 2 patients in cohorts 2–3), fatigue (2 patients in cohort 2–3) and febrile neutropenia (3 patients in cohorts 2–3). One patient died due to progressive disease after Cycle 1. Of the 10 patients evaluable for change from baseline in tumor diameter at 12 weeks, 3 patients had a >30% reduction, 5 patients remained within 20% and 2 patients had a >40% increase. Two patients are ongoing. B-Raf mutation status did not predict response. Ras mutations were not found.
Conclusions: The MTD of this combination is continuous oral sorafenib 400 mg bid plus DTIC 1000 mg/m^2. This combination is safe and well tolerated, and shows preliminary anti-tumor activity in patients with metastatic melanoma.

1211 POSTER

Local administration of Granulocyte/Macrophage Colony-Stimulating Factor and tumour specific cytotoxic T cell reactivity in the Sentinel Lymph Node of early-stage melanoma

B.G. Molenkamp[1], R.J.C.L.M. Vuylsteke[1], T.D. de Gruijl[2], P.G.J.T.B. Wijnands[3], W. Vos[3], R.J. Scheper[3], S. Meijer[1], P.A.M. van Leeuwen[1]. [1]*VU University Medical Center, Surgical Oncology, Amsterdam, The Netherlands;* [2]*VU University Medical Center, Medical Oncology, Amsterdam, The Netherlands;* [3]*VU University Medical Center, Pathology, Amsterdam, The Netherlands*

Background: In melanoma patients T cells reactive to tumour-associated antigens are detectable both in blood and tumour-draining lymph nodes.