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

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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

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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

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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

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

Unfortunately, early melanoma development is accompanied by impaired immune effector functions in the initial tumor-draining lymph node, the sentinel lymph node (SLN) and this may facilitate early metastatic events. Immunopotentation in these early stages may therefore be a valuable adjuvant treatment option. We recently demonstrated that intradermal injections of granulocyte/macrophage colony-stimulating factor (GM-CSF) around the excision site of primary melanoma increase the number and activation state of dendritic cells (DC) in SLN and hypothesized that this may be more conducive to the generation of T cell-mediated anti-tumor immunity. The aim of this study was to investigate the frequency of tumor-specific T cells in the SLN and blood of stage I melanoma patients and the effect of locally administered GM-CSF or saline.
Material and methods: Twelve stage I melanoma patients were randomly assigned to preoperative local administration of either recombinant human GM-CSF or saline. SLN-DC were phenotypically and morphologically characterized by flowcytometry and immuncytochemistry. From both the SLN and peripheral blood CD8+ T cells were isolated and investigated for tumor antigen specificity with IFN-γ ELISPOT-assay and tetramer staining.
Results: Primed tumor-specific T cells can be found as early as stage I melanoma patients. Overall tumor-specific T cell response rates in the SLN were 1/6 for the control group and 4/6 for the GM-CSF-administered group, only one patient had tumor-specific CD8+ T cells in the peripheral blood. All patients with detectable tumor-specific CD8+ T cells had a percentage of SLN-DC above median (0.33%). The association between the percentage of SLN-DC and tumor-specific CD8+ T cells was significant in a two-sided Fisher's Exact Test ($p = 0.015$).
Conclusions: Even in these early stages of melanoma development anti-tumor T cell responses are present and correlate to the myeloid DC content of the SLN, which can be enhanced by GM-CSF. More robust melanoma-specific CTL reactivity was consistently found in the SLN than in peripheral blood. This is consistent with local priming of tumor-specific CTL in the tumor-draining SLN.

Publication
Melanoma and sarcoma

1212 PUBLICATION

Risk factors for the squamous cell carcinoma developing on burn scar

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Background: Squamous cell carcinoma is malignancy of epidermal keratinocytes. After basal cell carcinoma, is the most frequently encountered malignant neoplasm of the skin. The aim of this study was to demonstrated the squamous cell carcinoma (SCC) rate and risk factors for developing on burn scar.
Methods: Retrospectively were followed data of 241 patients with SCC diagnosed in Dermatologic clinic Nis during the 5 years period. Rate of SCC developing on burn scars, risk factors and latent period between burn and carcinoma were evaluated.
Results: The number of SCC that developing on burn scar was 19(7.88%). Seventy-one per cent were male and 29% were female with a mean age of 34.5 years. The most common localization of SCC developing on burn scar was lower extremities 11(57.89%). The period between burn and carcinoma is 8 to 45 years. Cox multivariate analysis of risk factors for the squamous cell carcinoma developing on burn scar showed that degree of burn HR 3.69 (1.50 to 11.72) and localization HR 2.18 (0.90 to 8.26) had statistically significant effect on malignant transformation.
Conclusion: SCG developing on burn scar is seen at early age and more frequently localized on lower extremities. Degree of burn and scar localization were the most important prognostic factors for malignant transformation.

1213 PUBLICATION

A novel method of eye immobilisation and treatment delivery for photon beam irradiation of ocular melanoma

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Introduction: Photon beam radiotherapy using a relocatable stereotactic frame has been described for treatment of ocular melanoma. The relocatable frame must be modified to achieve eye immobilisation. We use a soft-contact lens attached to a rod to provide eye immobilisation in the treatment of retinoblastoma. We have developed an apparatus that combines the Gill-Thomas-Cosman frame with the lens-rod set-up for treatment of adults with ocular melanoma.
Methods: An in-house designed and built lens-rod system is attached to the GTC frame. The position of the lens over the iris is recorded with measurements and a photo. A small amount of pressure is applied to the eye by downward displacement of the rod before fixing the rod position. This limits movement of the eye and gives the patient a physical guide as to the correct position of the eye. It is not necessary for the eye to have vision for the system to work. A horizontal line engraved on the rod is the reference point for treatment set-up. It lies a fixed distance from the cornea and a known distance from the treatment isocentre so stereotactic coordinates are not required. Multiple small fixed photon fields using a multi-leaf collimator with 5 mm wide leaves are used instead of circular collimators. Correction factors for small photon fields have been generated.
Results: The lens-rod system provides a closed system of eye immobilisation and treatment set-up. The position of the treatment volume relative to the globe is not affected by movement of the eye as the set-up point moves with the eye. The GTC frame allows a wide range of beam angles to be used. The technique requires the presence of the treating radiation oncologist for approximately 10 minutes before each treatment fraction. Verification of the lens position over the iris is all that is required for daily treatment verification. The apparatus has been used to treat 5 patients up until now and has been well tolerated. All patients reported that the main discomfort was pressure on the face from the GTC frame. Mild to moderate watering of the eye occurred as the lens was placed on the eye. Acute toxicities are reported in detail.
Conclusion: This hybrid lens-rod-GTC apparatus provides excellent and reproducible eye immobilisation. It enables the delivery of fractionated small-field radiation to the posterior eye.

1214 PUBLICATION

The modified St. Jude hospital protocol in adult Ewing Sarcoma cases

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Background: To test the effectiveness of intensive chemotherapy regimen in adult Ewing sarcoma cases.
Material and methods: 23 patients (22 men, 1 woman) with Ewing sarcoma were administered modified St. Jude hospital protocol for a period of 41 weeks. The chemotherapy protocol was as follows: Induction chemotherapy from week 0 to the end of week 6 [Ifosfamide 2 g/m^2/day on days 1–3; etoposide 150 mg/m^2/day on days 1–3; cyclophosphamide 1.5 g/m^2 on 5th day and doxorubicin 45 mg/m^2 on 5th day]; followed by surgical excision with or without radiotherapy on week 9; then, consolidation chemotherapy were given between weeks 11 to 17 [Vincristin 1.5 mg/m^2, Dactinomycin 1.5 mg/m^2]; and finally, maintenance chemotherapy from week 20 to the end of week 41 [Ifosfamide 2 g/m^2/day on days 1–5, Etoposide 150 mg/m^2/day on days 1–5, Cyclophosphamide 1 g/m^2/day on days 1 and 2, Doxorubicin 60 mg/m^2 given as 24 hours-continuous infusion; with two drugs in combination]. All chemotherapies were given every three weeks.
Results: The median age of patients was 21 (range: 20–55). Initial stage at diagnosis was stage II in 17 patients and Stage IV in 6 patients. Eleven patients were treated by radiotherapy, 3 patients by surgery and 8 patients by combination of surgery and radiotherapy. The other one patient has not reached at the 9th week of treatment, yet. Total therapy duration was 9.08 months (median). Two patients with initial Stage II have relapsed during the administration of the protocol or after the completion of chemotherapy. The median time to disease relapse after the completion of chemotherapy was 129.3 days (median). Ten patients had complete response, 5 had partial response, while 8 patients progressed. All progressed patients have died. Myelotoxicity was the most common side-effect (35%). Transfusion was done in 30% of patients and growth factor was used in 22% of patients, totally. Two patients showed transient nephrotoxicity and neurotoxicity and one patient had transient hepatotoxicity. The median follow-up period was 20.57 months (2.1–57.8). The median survival was 28.8 months. The 36-month overall and disease-free survival rates were 41% and 26%, respectively.
Conclusion: This protocol is tolerable with non-serious side-effects. The prolonged follow-up period of non-metastatic and metastatic cases is henceforth required to see if survival benefit may be procured by this treatment approach.