systolic and diastolic pressures, although not meeting clinical criteria for hypertension. Growth perturbation over a one month term of therapy was not detected. Encouraging disease stabilization was observed in subset of patients with bone and soft-tissue sarcomas. Limited pharmacokinetic data support bevacizumab dosing schedules for children similar to those used in adults. Analysis of apoptotic and viable circulating endothelial cells was feasible in children, and results are promising for mechanistic validation and potential surrogate clinical application. Further evaluation of VEGF blockade therapy in the pediatric population is warranted. Combination studies of bevacizumab with epidermal growth factor receptor inhibition or cytotoxic chemotherapy, and assessment of oral multitargeted small molecular receptor kinase inhibitors BAY 43–9006 and SU11248 are planned or ongoing through the COG.

248/249 INVITED

Clinical phase I-II and pharmacokinetic study of plitidepsin in children with malignant tumors

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. *On behalf of ITCC. [1]Institut Gustave Roussy, Department of Pediatrics and "Pharmacology and new treatments in cancer", Villejuif, France; [2]Institut Curie, Department of Pediatrics, Paris, France; [3]Royal Children's Hospital, Oncology, Manchester, UK; [4]Royal Hospital for Children, Oncology, Bristol, UK; [5]PharmaMar SAU, Clinical R&D, Madrid, Spain*

Background: Plitidepsin is a cyclic depsipeptide isolated from the tunicate *Aplidium albicans*, nowadays manufactured by synthesis. It is a rapid and potent inducer of apoptosis and preliminary clinical activity has been observed in several adult malignancies. Phase I trials in adults explored 5 different schedules. Muscle and liver toxicities were dose-limiting; haematological toxicity was not observed at the recommended dose (RD). Preclinical data suggest activity in pediatric leukemia.
Methods: This is a multicentre, open-label, non-randomized phase I-II study with a dose finding stage in children with solid tumors, and subsequently two expanded cohorts in leukemia and solid tumors at the RD. Plitidepsin was administered as a 3 h iv infusion every 2 weeks (= 1 cycle). The initial dose level was 4 mg/m^2 (80% of the RD in adults) with a classic escalation to 5 and 6 mg/m^2.
Results: 22 patients have been entered to date with median age 7.5 years (range 2–17). 70 cycles in 21 patients were evaluable for toxicity. Eight patients were treated at 4 mg/m^2, five at 5 mg/m^2, seven at 6 mg/m^2, and two in the extension phase at 5 mg/m^2. One patient presented dose-limiting G2 myalgia lasting more than 2 weeks at 4 mg/m^2. One patient experienced asymptomatic G4 CPK elevation and one other G3 non-transient transaminitis at 6 mg/m^2, suggesting 5 mg/m^2 as the RD in children. Non-hematological toxicities included muscle side effects (G1–2 myalgia, muscle weakness, muscle cramps and G1–4 CPK elevation), G1–3 fatigue, G1–3 transaminitis, G3 vomiting, G3 hypersensitivity reaction. Pharmacokinetic data are similar to those reported in adult (extensive tissue distribution, a long half-life); if any, clearance was slightly higher and half-life shorter. Partial tumor response was observed in a refractory neuroblastoma and some evidence of activity in a medulloblastoma and a pancreatoblastoma.
Conclusions: Plitidepsin was well tolerated in children with muscle side effects being the most relevant toxicity observed. The RD for the pediatric population is equivalent to the RD in adults. The extension phase in solid tumors and leukemia is ongoing.

Thursday 9 November 14:45–16:15

PLENARY SESSION 6

Proffered Papers

250 ORAL

Presurgical treatment of metastatic renal cell carcinoma patients with bevacizumab and erlotinib: preliminary efficacy and biomaker data

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Background: A number of new targeted therapies are being developed for metastatic renal cell carcinoma (mRCC) The role and timing of cytoreductive nephrectomy in patients receiving targeted therapies has not been defined. We have initiated a study investigating the role of presurgical treatment of mRCC patients with bevacizumab and erlotinib, and present data on tumor response and biomarker readouts in the first 19 patients in this protocol.
Materials and Methods: Patients were previously untreated, did not have brain metastases, had a performance status of 0 or 1, predominant clear cell histology, and had not undergone cytoreductive nephrectomy. Patients received bevacizumab 10 mg/kg IV every 2 weeks for 4 doses, and erlotinib 150 mg daily for 8 weeks. Two weeks after the last dose of erlotinib, and 4 weeks after the last dose of bevacizumab, patients underwent cytoreductive nephrectomy. Patients who demonstrated disease stability or response were restarted on treatment one month post-surgery and continued until disease progression. Phospho and total EGFR, AKT, S6, FAK and ERK were evaluated by Western blots and by immunohistochemistry on tissue microarrays, and compared to control untreated RCC tissue.
Results: Between 3/23/2005 and 6/04/06, 27 patients were enrolled out of a total planned accrual of 50 patients As of June 4, 2006, 19 patients were evaluable. One patient had a CR in target lesion and stability in his nontarget lesion (bone). There were 3 PRs, 13 patients with stable disease and 3 patients with progressive disease.
Evaluation of protein expression of key signaling molecules controlling proliferation, survival and migration (phospho-AKT, total AKT, PTEN, phospho-FAK and phospho-ERK) did not reveal any statistically significant change between the treated and the untreated groups, with the exception of a modest increase in overall AKT expression in the treated group (p value <0.02). p-ERK and p-AKT (and p-FAK less strongly, p value <0.02) correlate with disease grade, regardless of treatment status (increase in grade IV, p value <0.002).
Conclusions: Early data suggest presurgical treatment with bevacizumab and erlotinib is safe and efficacious in patients with previously unresected, untreated mRCC, with shrinkage of both metastatic disease and primary tumors. There is previous (pre-clinical) evidence for target inhibition of both VEGFR and EGFR, but key downstream signaling molecules were not affected in this study. Further investigation is required to elucidate the pathways involved in mediating the therapeutic effect of these drugs.

251 ORAL

The phosphatidylinositide 3-kinase (PI3-kinase) inhibitor PI103 sensitises some ovarian carcinoma (OC) cell lines to paclitaxel or carboplatin

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PI3-kinase, through phosphorylation of its downstream substrate, AKT, is an important mediator of proliferation and survival signals. Activation of this pathway has been linked to cytotoxic drug resistance and to the ability of PI3-kinase inhibitors, such as LY294002, to sensitise some tumour cell lines to cytotoxic drugs. PI103 is a novel potent inhibitor of the catalytic p110α/β catalytic subunits of PI3-kinase and of mTOR. The PI3-kinase/AKT pathway is frequently activated in OC due to aberrations at different points in the pathway. SKOV-3 cells overexpress ErbB2,

have low PTEN levels and an activating *PIK3CA* mutation, and IGROV-1 cells are null PTEN. Both cell lines express a high basal level of phosphorylated AKT (p-AKT). Exposure to IC50 concentrations of PI103 (0.47 μM, 0.14 μM respectively) led to a reduction in p-AKT, p-GSK3β and p-S6 ribosomal protein at 2h and/or 12h. Paclitaxel or carboplatin, which are commonly used drugs in OC, were combined with PI103 at a fixed ratio of their IC50 and growth inhibition measured after continuous exposure using an MTT assay, and the data analysed using the median-effect equation of Chou and Talalay. In SKOV-3 cells, PI103 was synergistic when combined with paclitaxel with a combination index (CI) @ fraction unaffected (fu)0.5 of 0.42 ± 0.16, demonstrating that <50% of each drug is required to give the same effect as that predicted from additivity. In contrast, PI103 was strongly antagonistic when combined with carboplatin (CI@fu0.5 = 2.8 ± 1.1). Reverse results were obtained with IGROV-1 cells with CI@fu0.5 values of 2.4 ± 0.37 and 0.67 ± 0.11 for paclitaxel and carboplatin respectively. Investigations were extended to the CHI and HX62 OC lines (PI103 IC50 = 1.4 and 0.71 μM respectively) in which the PI3K/AKT pathway appears less deregulated. CH1 is the most sensitive of all the lines to the cytotoxic agents and synergy was observed when PI103 was combined with paclitaxel (CI@fu0.5 = 0.72 ± 0.16) and particularly with carboplatin (CI@fu0.5 = 0.48 ± 0.08). In contrast, the combinations in HX62 cells were close to additive, consistent with their independent drug actions. The two examples of antagonism were investigated further and sequencing the cytotoxic agent 24h prior to PI103 led to additivity. These data suggest that it may be useful to combine inhibitors of PI3K/AKT/mTOR signalling with paclitaxel or carboplatin in patients with ovarian cancer although the preferred molecular context for synergy requires further analysis.

252 ORAL

Identification of responders and non-responders to imatinib prior to treatment

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Background: Chronic myelogenous leukemia (CML) is characterized by a chromosomal translocation resulting in a shortened chromosome 22 termed the Philadelphia chromosome. This aberration leads to the fusion of the *BCR* and *ABL* genes and the production of a chimeric protein with a deregulated tyrosine kinase (TK) activity. Imatinib, a potent and specific TK inhibitor, has emerged as the first line treatment for CML. While imatinib induces complete cytogenetic remission (CCR) in the majority of patients, some individuals do not respond and would benefit from alternative therapies earlier in the course of disease. The goal of this work was to develop an *in vitro* assay to reliably predict response to imatinib therapy prior to treatment.
Human aspartyl (asparaginyl) β-hydroxylase (*ASPH*) is a highly specific biomarker for cancer. Increased *ASPH* expression has been detected at the protein and mRNA levels specifically in tumor cells. Over-expression of ASPH results in its translocation to the cellular surface where it is a potential target for antibody-based cancer therapy.
Methods: Leukocytes from 39 patients were isolated from fresh whole blood prior to the initiation of therapy and cultured for 24 hours in the presence or absence of 1 μM imatinib. *ASPH* and *BCR*/*ABL* transcript levels were determined by real-time quantitative polymerase chain reaction (RQ-PCR) analysis. Patients were treated with imatinib and there response status was assessed vis-a-vis complete molecular remission (CMR) by RQ-PCR of the *BCR*/*ABL* fusion gene.
Results: Prior to treatment, all patient samples had increased expression of the *ASPH* transcript (~5-fold). The leukocytes of 27 patients displayed a 30–75% decrease in *ASPH* expression after culture in the presence of imatinib. All of the corresponding patients achieved CMR after drug therapy. The leukocytes of the 12 other patients displayed less than a 25% reduction in *ASPH* transcript levels and these patients proved to be non-responders to drug treatment. Transcript levels of either the *BCR*/*ABL* gene itself or a control gene, *Ki67*, did not correlate with drug response.
Conclusions: Decreased levels of expression of the *ASPH* transcript after a 24 hour *in vitro* exposure of primary leukocytes to imatinib is a simple and sensitive assay for the determination of likely response to imatinib prior to the initiation of treatment. As well, this assay may represent a quick and simple approach to high throughput screening for new drug candidates against CML.

252A ORAL

Relapse in children with acute lymphoblastic leukaemia is associated with selection of a pre-existing drug resistance subclone

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Acute Lymphoblastic Leukaemia (ALL) is the most common cancer in children. Although virtually all patients achieve clinical remission following induction chemotherapy, one in four patients will subsequently relapse. We have previously shown that these relapses are due to small numbers of surviving and highly drug refractory cells (J Clin Oncol 21:704–9, 2003). It is unclear, however, whether relapse results from acquisition of a therapy-induced resistant phenotype or from selection of an intrinsically resistant subpopulation of cells. To investigate this process, 25 matched diagnosis and relapse ALL samples were analysed for the presence of clonal populations based on rearrangements of over 30 antigen receptor chain genes (Blood 103:3905−14, 2004). These unique clonal markers revealed the emergence or evolution of new clonal populations at the time of relapse in 13 patients. In eight of these samples, highly sensitive clone-specific PCR demonstrated the presence of this 'new' relapse clone at low levels in the matched diagnosis sample, indicating selection of a pre-existing subpopulation of cells as mechanism of relapse. Kaplan-Meier survival analysis demonstrated a significant relationship between the presence of the relapse clone at diagnosis and the length of first clinical remission (CR1). Furthermore, the quantity of the relapse clone at diagnosis was strongly inversely correlated with CR1. In particular, this pattern of clonal evolution could be replicated following engraftment and passaging of primary leukaemia samples in an in vivo model of ALL, using non-obese diabetic/severe combined immunodeficient mice. These data therefore indicate that early relapse in ALL patients commonly results from the selection of an intrinsically resistant subclone that is present at diagnosis but undetectable using routine methods. Using current treatment protocols, relapse appears inevitable for these patients and this research highlights the need to identify such patients early during treatment and to explore alternate therapies for them.

Thursday 9 November 16:30–18:15

PLENARY SESSION 7

The tumour microenvironment and metastasis

253 INVITED

The KISS1 metastasis suppressor regulates tumor cell response to microenvironmental signals and growth at secondary sites

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The primary obstacle to cancer cure is development of distant metastases. At diagnosis, tumors have already shed many cells into the circulation rendering anti-metastatic therapies moot since the cells have already seeded other tissues. Recent findings with metastasis suppressors indicate that tumor cells expressing KISS1 are able to complete every step of the metastatic cascade except proliferation at the secondary site. Tumor cells inoculated at orthotopic sites (i.e., intradermal) form progressively growing tumors that shed cells which remain dormant in the lungs. Tumor cells inoculated intravenously seed the lungs, but fail to establish macroscopic lesions. The nascent KISS1 protein (~17 kDa) has never been detected while a secreted internal 54 amino acid polypeptide [termed metastin or kisspeptin-54 (KP54)] had previously been isolated from placenta. To assess whether KISS1 or KP54 secretion is necessary for metastasis suppression, an internally FLAG-tagged KISS1 was constructed with (designated KFM) or without (designated ΔSS) the putative signal peptide, transfected into metastatic human C8161.9 melanoma cells and evaluated for tumor growth and metastasis following orthotopic or intravascular injection. ΔSS expressing cells no longer produced detectable KISS1 or KP54 in culture media. Moreover, ΔSS-transfected cells were as metastatic as parental cells, while KFM-transfected cells were suppressed for metastasis. Both still allowed local tumor growth. Media was isolated from cells expressing KFM, immunoprecipitated using anti-KISS1 and anti-FLAG antibodies, analyzed by mass spectrometry and internal sequencing and verified to be polypeptides derived from KISS1. Therefore, secretion of KISS1 is necessary for its anti-metastatic effects. As a secreted molecule