added to the cells in a growth phase and after 16 hrs removed. Adult C57BL mice in groups of 5−6 female mice with measurable SC growing B-16 melanoma nodules were dosed IP daily (150–200 mg/kg) for 5 days with DM-CHOC-PEN and monitored daily until death or moribund and sacrificed. Temozolamide (TMZ) was used as control.
Results: In vitro, DM-CHOC-PEN had an IC50 of 0.5 μg/mL vs. B-16 melanoma cells. Floating heavily melanotic cells that formed were separated, analyzed for DM-CHOC-PEN and found to contain 125% more drug than did the adhered amelanotic cells. For the in vivo studies, T-C for mice bearing B-16 melanoma treated with DM-CHOC-PEN vs. controls was 60−82%; thus supporting the in vitro observations. For TMZ, the T-C was 78%.
Conclusion: Electronic modeling studies support DM-CHOC-PEN's ability to act as a pyridinium co-factor in the transfer of electrons from DOPA to the intermediary metabolism pool. Previously, we reported that dacarbazine inhibited DOPA oxidase and melanin formation in melanoma pts, resulting in amelanotic melanomas; often considered a more aggressive variant (Pigment Cell 2, 327–338, 1976). Although tyrosine-DOPA transport/metabolism is not a target for DM-CHOC-PEN (its MOA is considered to be via alkylation/adduct formation with N7-guanine), the accumulation of intracellular melanin does influence/interfere with cellular metabolism. In the current Phase I study, pts with melanoma lesions will be biopsied for DM-CHOC-PEN content and tumor tissue DOPA oxidase activity/melanin content when possible. Early results from the Phase I trial will be included with this presentation; no toxicity has been observed to date. A possible role of DOPA oxidase in drug selection to treat melanoma will be discussed. Supported by NCI/SBIR grants − CA85021 and CA132257.

PP 73

Prognostic relevance of constitutive expression of γ-H2AX in triple negative breast cancers

A. Nagelkerke, S. van Kuijk, F. Sweep, I. Nagtegaal, N. Hoogerbrugge, J. Martens, M. Timmermans, H. van Laarhoven, J. Bussink, P. Span. *Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands*

Background: Constitutive expression of γ-H2AX, a key indicator of double stranded DNA breaks, is found in a number of cancers but not in normal tissues. The DNA damage repair (DDR) pathway may be disrupted in these cancers, resulting in a higher risk of unrepaired lesions even in the absence of DNA damage inducing therapy such as radiotherapy. Data from recent studies indicate that the endogenous expression of DNA damage response factors may also be associated with damaged telomeres. Here, we quantified constitutive γ-H2AX expression in a large number of breast cancer cell lines (n = 54) and in a cohort of human breast cancers (n = 122) enriched for triple negative tumors.
Materials and Methods: Formalin fixed paraffin embedded breast cancer cell lines and tumors were immunohistochemically analyzed for expression of γ-H2AX and its downstream factor 53BP1.
Results: Expression of γ-H2AX was assessed in 54 different breast cell lines, embedded in triplicate in a cell line microarray and correlated with estrogen receptor (ER), progesterone receptor (PR) and HER2 receptor status, mutation status and breast cancer subtype. We found that triple-negative cell lines, BRCA1 mutated cell lines and basal-like tumors exhibited the most γ-H2AX foci. A borderline significant association with p53 status was found. Next, a tissue microarray of a cohort of 122 node-negative, non-adjuvantly treated breast cancer patients was stained for γ-H2AX. No direct correlation with triple negativity was found in these patients. However, in the triple negative breast cancer patients a high number of γ-H2AX foci had a significantly worse prognosis ($p = 0.006$ for triple negative (n = 42) vs. $p = 0.417$ for ER, PR or HER2 positive (n = 54) patients). A similar association with survival was found for 53BP1 and γ-H2AX and 53BP1 combined.
Conclusion: Enhanced endogenous γ-H2AX and 53BP1 expression, indicative of malfunctioning DNA repair, reveals a subset of patients with triple negative breast tumors that have a significantly poorer prognosis. We are currently assessing whether this specific subset of patients exhibits defects in the DDR pathway, e.g. BRCA1 and/or p53 mutations, or have damaged telomeres, which would explain the prognostic association.

PP 31

INT70/09 Phase II study of Pazopanib (PZP) monotherapy for patients (pts) with relapsed/refractory urothelial cancer (UC): updated results of a proof-of-concept trial

A. Necchi, N. Nicolai, C. Ortega, T. Sava, C. Sacco, F. Crippa, C. Morosi, C. Messina, N. Zaffaroni, R. Salvioni. *Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy*

Background: Discouraging results have been reported in relapsed/refractory UC with the use of salvage therapies (Rx). In 2nd line setting, median PFS and OS approximate 3 and 6 months (mos), respectively. On 10/2010 we reported preliminary, yet encouraging, results of a phase II trial with PZP, a multitargeted drug with distinct anti-angiogenic activity (ESMO 2010, LBA#23). An update of the trial is presented.
Materials and Methods: Eligibility included histologically confirmed UC failing ⩾1 CDDP-based Rx for metastatic disease. PZP 800 mg once daily until disease progression or unacceptable toxicity was planned. Both CT scan and PET/CT scan were set at baseline and q4weeks thereafter. RECIST v.1.1 response-rate (RR) was the primary endpoint. Circulating VEGF, VEGFR1−2, KIT, HGF, IL4−8−12, OPN, TIMP-1 as well as SNPs of 13 candidate genes and circulating tumor cell analysis were planned.
Results: 36 of a total planned of 41 pts were enrolled from 02/10 to 03/11 (28 males, 8 females). Median age was 64 yrs (42−79). 13 pts (36%) had UC of the upper urinary tract and 23 had a bladder primary tumor. 33 pts had multiple disease sites (median 3, range 1−5). Median number of prior cytotoxic agents was 3 (2−8), of prior Rx lines was 2 (1−4). 30 pts (83%) had visceral metastases (hepatic in 17 pts). Median ECOG PS was 1 (0−2). 4 pts (11%) had a confirmed RECIST-defined partial response (PR), 26 had a stable disease (83% clinical benefit). 19 pts (53%) had a clear necrotic evolution of multiple metastases and/or a decreased SUV at PET consistent with PR. Of the 34/36 pts having 2 mos minimum follow up, median PFS and OS were 3 mos (1−11) and 6 mos (2−11), respectively. 5/36 (14%) had a very long-term PFS (> 10 mos). G3 hypertension occurred in 2 pts, G1−2 asthenia in 13, diarrhoea in 5, anemia and hand-foot syndrome in 3 pts each. No discontinuations/dose reductions were needed.
Conclusion: This is the first report of a consistent activity and potential efficacy of a targeted agent in UC. Though the PR-rate by RECIST is low, half of pts had a densitometric/metabolic response, the majority of pts had a clinical benefit and PFS-rate is promising (approaching pure 2nd line results). Results of biomarker analysis will be available in Sept 2011 and may help to personalize treatment and to corroborate new response criteria to angiogenesis inhibitors. This proof-of-concept trial is part of a multi-targeting platform aimed at elucidating the role of microenvironment in UC.

PP 32

Pilot study of cisplatin, 5-fluorouracil and a taxane (TPF) in patients (pts) with advanced squamous-cell carcinoma (SCC) of the penis: results from a single-institution series

A. Necchi, N. Nicolai, M. Colecchia, L. Piva, M. Catanzaro, T. Torelli, D. Biasoni, S. Stagni, A. Milani, R. Salvioni. *Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy*

Background: Few data indicate poor to moderate activity of chemotherapy in advanced penile SCC, and no definitive acquisition is available concerning timing for integrated surgery. Pts with metastatic bilateral or pelvic nodes show an overall survival (OS) of 15% and less than 10%, respectively. We evaluated TPF in either neoadjuvant (NA), adjuvant (A) or metastatic (M) setting in a single-center pilot trial.
Materials and Methods: 3−4 courses of paclitaxel 120 mg/m2 d1 or docetaxel 75 mg/m2 d1 + cisplatin 75 mg/m2 d1 + 5-FU 750 mg/m2 96 hrs continuous infusion from d1, q3wks were provided. Primary endpoint (EP) was progression-free survival (PFS). Safety profile, response rate (RR) and OS were the secondary EPs. Immunostaining for p53, p16, p63, EGFR, HER2/neu and mutational analysis of TP53 were planned on available tissue.
Results: From 7/2004 to 03/2011, 46 consecutive pts were treated, 40/46 evaluable for response and outcome. 8 pts underwent paclitaxel-PF and 32 docetaxel-PF. Grade ⩾ 3 hematologic toxicity was observed in 4 pts, grade ⩾ 3 renal and neurotoxicity occurred in 1 pt each. Median PFS and OS in the whole series were 6 (1−73) and 9.5 mos (1−73) respectively. Positive p53 staining significantly associated with better OS and PFS at univariate analysis (Log-Rank test $p = 0.0421$ and $p = 0.0483$, respectively). Adjuvant setting: 17 pts (4 bilateral pN+ and 11 pelvic pN+) underwent adjuvant TPF. Median PFS and OS were 10 mos (1−73) and 13 mos (1−73). 10 pts (59%) were alive with 17 mos (1−73) of median follow-up (f-u). Neoadjuvant setting: 16 pts with cN2/3 SCC (9 cN3) were treated, either at diagnosis (11) or following recurrence after prior lymphadenectomy (5). Median PFS was 4 mos (1−46). 3 pts achieved a complete response (CR) and 6 pts achieved a partial response (PR, RR = 62%). OS was 5 mos (3−46). 11/16 pts underwent surgery that was radical in 9 (82%). 3 pathologic-CR (27%) have been achieved. 8 pts (50%) were alive with a median f-u of 9 mos (3−46). Metastatic setting: 7 pts were treated. 2 had a PR and 1 a SD that lasted a median of 5 mos (3−8), and all died of disease. Median PFS and OS were 2 mos (1−8) and 5 mos (2−12).
Conclusion: Perioperative TPF was effective in advanced penile SCC, either in A or NA setting. It deserves further investigation including earlier stages (probably all cN+). For the first time a prognostic value of p53 has been reported in penile SCC. Mature results on the predictive role of biomarkers will be available in Sept 2011.