Results: As revealed by cluster analysis the Tam-sensitive and -resistant breast carcinoma line differed regarding their gene expression pattern. When comparing the groups, the expression of relatively few genes was distinctly changed. For example, in the resistant MaCa 3366/TAM 45 genes were up-regulated and 19 genes down-regulated more than two fold compared to the Tam-sensitive MaCa 3366. Among the up-regulated genes are several interferone inducible genes as well as genes that are known to be involved in breast cancer. To further evaluate interesting candidates we developed RT-PCRs for several genes and found confirmation between fold regulation as measured by RT-PCR on unpooled RNAs and in the chip experiments for the different treatment groups.
Conclusion: Recent studies reveal that breast cancer prognosis can be correlated with the gene expression pattern of the primary tumor. Our investigations provide the possibility to derive markers for Tam resistance by differential gene expression profiling in a human breast cancer model of acquired Tam resistance. Genes whose expression is distinctly changed between both lines will be further evaluated as potential targets for diagnostic or therapeutic approaches.

Radiation interactive agents

535

Prospective phase I/II trial of the cyclooxygenase-2 inhibitor celecoxib in patients with carcinoma of the cervix

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Purpose: To evaluate the toxicity and biomarker response of celecoxib as a biologic agent in combination with definitive chemoradiotherapy in women with cervix cancer.
Materials and Methods: Fifteen patients with cervix cancer were entered into the first phase of a study between March 2001 and January 2002. FIGO stages included IB(2), IIB(8), IIIB(4), and IVA(1), and median age was 51 (range 26-62). Celecoxib 400mg orally was given twice daily 2 weeks prior to, and during the course of weekly cisplatin and definitive radiotherapy. Toxicity assessments were performed weekly up to 12 weeks following treatment with analysis of toxicity prior to further accrual. Haematological, genitourinary, gastrointestinal and skin toxicity were recorded using the NCIC-CTC. Hypoxia and interstitial fluid pressure (IFP) assays were performed prior to and 2 weeks after celecoxib (prior to radiation).
Results: Eleven patients completed the prescribed therapy with celecoxib. Only 2 of the 4 remaining patients discontinued celecoxib due to GI toxicity > 3. In total there were 6 patients (40%) with grade 3/4 acute toxicity. Four were related to nausea and vomiting, one to perineal reaction and one to neutropenic sepsis. Although it was difficult to distinguish toxicity from chemoradiotherapy from that of celecoxib, the proportion of patients with severe acute toxicity was similar to our previously reported study with chemoradiotherapy alone (8/24 or 33%, Rodrigues, Int J Radiat Oncol Biol Phys 2001:Vol 51:(3): (Supp 1): 334). GI toxicity was more common in this study whereas haematologic effects were more frequent in the previous study. Four of 10 measurable patients showed a decrease in hypoxic fraction of 3.8-89.7% while 7 of 9 measurable patients had a reduction in IFP of 2.7 to 59%. Biomarker correlation with response to treatment or outcome is not yet available.
Conclusions: Celecoxib is tolerated by patients receiving definitive chemoradiotherapy for cervix cancer. The proportion of grade 3 toxicity did not differ from our previous study but further follow-up will be required to determine late toxicity. Biomarker response to celecoxib can be seen in individual patients. Accrual continues to a planned sample size of 45 patients.

536

Pifithrin alpha and beta do not inhibit ionising radiation dependent p53 responses in human wild type p53 ovarian and colon cell lines

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Many toxic side effects of conventional chemotherapy are induced via p53-dependent apoptosis. Inhibitor of p53+/+ function may therefore have utility in protecting normal tissue and allowing increased doses of chemotherapy. The compound pifithrin alpha (PFT alpha, Science 285:1733-1737, 1999) has been reported to be an inhibitor of p53 and shown to abrogates p53+/+ dependent cell cycle arrest, cytotoxicity and apoptosis in rodent model tumour cell lines following ionising radiation (IR) and UV exposure. In view of the possible utility and drug development potential of such compounds we have carried out a detailed study of PFT alpha and its ring-closed product PFT beta in human p53+/+ tumour cell lines. Both PFT alpha and beta were synthesised and the structures confirmed by NMR-MS (accurate mass and FAB), microanalysis and crystal structure of the beta-form. PFT alpha converted rapidly to the beta-form in tissue culture medium, with a half-life of 4.9 min and 82% conversion within 30 min. Consequently drugs were made up immediately prior to use. Initial studies were carried out to determine if PFT alpha and beta would inhibit p53-dependent responses in the human colon tumour cell line HCT116 and the human ovarian tumour lines A2780 and CH1. Cells were treated with various doses of PFT alpha and beta (1-30uM) for 1h prior to IR (5Gy) and harvested 24h following exposure. In both A2780 and HCT116, p53 protein induction and G1/S cell cycle arrest were unaltered as measured by western blotting and flow cytometry, respectively. Clonogenic assays showed that there was no protective effect of PFT alpha when combined with an IC_{50} dose of IR in A2780 cells. Mice bearing CH1 ovarian tumour xenografts (p53+/+) were administered a non-toxic dose of PFT alpha (4mg kg-1 ip) 24h prior to IR (5Gy) and tumours removed 4h following irradiation. There was no evidence of inhibition of p21 or MDM-2 mRNA induction as measured by northern blotting. Studies on the mechanism of action of these compounds are in progress. These data suggest that PFT alpha and beta are not offering marked protection from p53 dependent events following ionising radiation treatment in human ovarian and colon tumour cell lines.

537

Cassette dosing iv and ip of novel DNA dependent protein kinase inhibitors

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DNA-dependent protein kinase (DNA-PK) is involved in the repair of double strand breaks, thus inhibition of this enzyme could potentiate the effects of radiotherapy or chemotherapy by DNA-damaging agents. The aim of this study was to evaluate cassette dosing, the simultaneous administration of several compounds to a single animal, as a method to predict the pharmacokinetics of a series of novel DNA-PK inhibitors in high throughput. NU7053, NU7059, NU7062, NU7119 and NU7163 were administered intravenously to Balb C- mice alone at 5mg/kg and in combination at 1mg/kg each. NU7026, NU7031, NU7046, NU7048 and LY294002 were administered ip alone at 20mg/kg and in a cassette with 10mg/kg of each drug. Pharmacokinetic parameters were evaluated by non-compartmental analysis. When administered iv, the compounds displayed a linear increase in maximum concentration (Cmax) and area under the curve (AUC) with a 5-fold increase in dose from 1mg/kg cassette administration to 5mg/kg single administration. The clearance and half-lives of the compounds were similar following cassette and single dosing. For example, the clearance and half-life of NU7059 following cassette and single dosing was 0.091L/hr and 0.17hr, and 0.081L/hr and 0.16hr respectively. The rank order of the compounds from lowest to highest clearance was similar whether they were dosed as single agents. In contrast, when administered ip, the clearances varied up to 50%. For example the clearance for NU7026 decreased from 0.44L/hr after single dosing to 0.20L/hr after cassette dosing or as single agent. Cmax also varied with more than 3 fold variation for NU7046. As a result, ranking of the compounds was different after single and cassette dosing. Although the compounds used in the cassettes iv and ip were different, this suggests that absorption ip may be limiting for cassette dosing. Cassette dosing iv, however will be used to assess a larger number of compounds from this series in order to increase throughput, thus reducing the number of animals used.