successfully into a clinical setting could potentially help to improve local control and subsequently influence survival.

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RTA 401 (CDDO) and RTA 402 (CDDO-Me), promising new anti-cancer agents that also prevent oral mucositis

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RTA 401 and RTA 402 are novel synthetic triterpenoids that have demonstrated potent anti-cancer and anti-inflammatory activities. Both are in phase 1 clinical trials as anti-cancer agents. We have previously reported that these agents have significant radioprotective activity in a model of oral mucositis induced by acute radiation. This activity results from induction of the phase 2 response via the Keap1/Nrf2/ARE pathway. To further assess the anti-mucositis activity of these compounds in additional clinical settings, we studied RTA 401 and 402 in preclinical models of oral mucositis induced by chemo-radiation-induced and by fractionated radiation. Two studies were conducted in the Golden Syrian Hamster. In the chemo-radiation model, animals received 60 mg/kg of 5-FU IP on Day (D) -4 and D-2 followed by 30 Gy of radiation directed to the left buccal cheek pouch mucosa on D0. The rest of the animal was protected with a lead shield. In both studies, RTA 401 was administered at 5 mg/kg IP BID from D-9 to D15 or D-5 to D-1. RTA 402 was administered *per os* at either 6.5 or 9 mg/kg BID from D-9 to D15. Groups of 8–10 animals were used. Animals were evaluated clinically for mucositis from D6 through D28. Mucositis was scored visually by comparison to a validated photographic scale ranging from 0 for normal to 5 for severe ulceration. Ulceration was defined as a score of $\geqslant 3$. The primary endpoint was the number of days with mucositis scores of $\geqslant 3$ (D3+). Chi-Squared analysis was performed to assess the significance of the difference in D3+ between treated and control groups. Both RTA 401 and RTA 402 demonstrated significant anti-mucositis activity in each study. In the chemo-radiation study, the best results were obtained by RTA 401 dosed D-9 to D15, which reduced D3+ by 79% ($p < 0.001$), RTA 402 dosed orally at 6.5 mg/kg BID reduced D3+ by 59% ($p < 0.001$). In the fractionated radiation study, RTA 401 dosed from D-9 to D15 reduced D3+ by 52% ($p < 0.001$), and RTA 402 dosed at 6.5 mg/kg PO BID reduced D3+ by 17% ($p = 0.016$). These studies indicate that the radio and chemo-protective effects of RTA 401 and RTA 402 cross multiple clinical settings, and that the agents can be dosed in a variety of schedules before, during, or after radiation. Taken together with other studies of ROS-mediated toxicities, it appears these Phase 1 anti-cancer agents also hold considerable promise in protecting against the side effects of standard therapies, including oral mucositis. Phase 1/2 clinical studies examining both anti-cancer and anti-mucositis endpoints with RTA 401 and RTA 402 are expected to begin in late 2006.

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Targeting CTP synthetase by cyclopentenyl cytosine (CPEC) increases the effectiveness of Gemcitabine and radiation in human tumor cells

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Background: Gemcitabine is a potent enhancer of radiosensitivity but the current clinical efficacy of Gemcitabine and radiotherapy in non small cell lung (NSCLC) and pancreatic cancer is hampered by severe side-effects. To improve its therapeutic ratio, the activation of Gemcitabine can be modulated by pre-incubation with cyclopentenyl cytosine (CPEC). The triphosphate form of CPEC specifically targets cytidine triphosphate (CTP) synthetase leading to decreased cellular CTP-levels. Subsequently, the activity of the rate-limiting enzyme in the activation of Gemcitabine, deoxycytidine kinase (dCK), increases. In this study, we determined the influence of CPEC on the anabolism, cytotoxicity and radioenhancement of Gemcitabine in human tumor cells.
Materials and Methods: Human NSCLC and pancreatic tumor cells were exposed to CPEC, Gemcitabine and radiation. The effects of treatment on CTP-levels, dCK-activity and anabolism of Gemcitabine were assessed by HPLC. Effects on cell cycle distributions were analysed by bivariate flowcytometry. Treatment sensitivity was evaluated by clonogenic and cell death assays.
Results: In NSCLC cells, a 24-hour exposure to CPEC reduced CTP-levels to 10−25% and increased dCK-activity about 2-fold. This resulted in a 4 to 6-fold increase of Gemcitabine incorporated into the DNA ($P = 0.01$). CPEC markedly increased the ability of Gemcitabine to enhance radiosensitivity ($P < 0.001$). CPEC alone enhanced radiosensitivity at doses above 4 Gy ($P = 0.02$). Also in human pancreatic tumor cell lines, CPEC markedly increased the effectiveness of Gemcitabine alone as well as in combination with radiation. Again a 2–3 fold increase in dCK-activity was found, but only after longer exposures to CPEC (48−72 hours). CPEC increased the number of S phase cells, but reduced the incorporation of bromodeoxyuridine. The importance of the scheduling of CPEC, Gemcitabine and radiation together with preliminary data on the effects of CPEC in animal tumor models will be presented.
Conclusions: Targeting the synthesis of CTP by CPEC allows an improved efficacy of Gemcitabine and radiation in different human tumor cells. The demonstration that CPEC increases the therapeutic ratio of Gemcitabine combined with radiation in animal tumor models may provide guidelines for future clinical application.

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AZD2171, a highly potent, orally active VEGF signalling inhibitor, enhances the effect of fractionated radiotherapy in human lung tumour xenografts

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Background: There is an increasing body of evidence suggesting that blockade of the vascular endothelial growth factor (VEGF) signalling cascade enhances the therapeutic effect of radiation. AZD2171 is a highly potent, orally active, VEGF signalling inhibitor that prevents physiological and pathological angiogenesis *in vivo* and significantly inhibits the growth of histologically diverse human tumour xenografts.
Materials and Methods: AZD2171 was analyzed in combination with radiation in Calu-6 (non-small-cell lung cancer) human tumour xenografts. Radiation was administered as 5 consecutive daily fractions of 2 Gy. Chronic, once-daily, oral dosing with AZD2171 (3 mg/kg/day) was initiated 2 hours after completion of the radiotherapy course (sequential regimen) or 2 hours prior to each 2 Gy fraction and continued post-radiotherapy (concomitant regimen). Treatments were initiated at a tumour size of 250 mm^3 (n = 7/group). The experimental endpoint was a quadrupling of tumour volume following the initiation of treatment (RTV4). To assess hypoxia and perfusion of the tumours, pimonidazole and Hoechst 33342 were given to additional animals, treated with concomitant radiotherapy ± AZD2171, prior to sacrifice.
Results: Chronic administration of AZD2171 alone slowed Calu-6 tumour growth significantly (Table 1). When combined with 5×2 Gy there was a positive interaction between the two treatment modalities (Table 1). Both sequential and concomitant regimens resulted in larger average growth delays than would have been predicted from simply combining the growth delays produced by AZD2171 and radiation alone (Table 1). The enhanced response to the combined treatment was associated with an increased tumour doubling time (Table 1) when compared with AZD2171 alone. This suggests that in the Calu-6 model the tumour vasculature may be sensitized to treatment with AZD2171 following fractionated radiotherapy. Histological analyses revealed that concomitant AZD2171 and 5×2 Gy reduced vessel density, with a proportional change in perfusion. This was associated with increases in both tumour hypoxia and apoptosis when compared with radiotherapy alone.

Table 1. AZD2171 combined with 5×2 Gy radiation in Calu-6 tumour xenografts

Treatment	Doubling time (days)	Growth delay, $RTV4_{treated} - RTV4_{vehiclecontrol}$ (days)
Vehicle	6	–
$5\times$ 2 Gy	8	16
AZD2171	12	7
AZD2171 + $5\times$ 2 Gy (sequential)	16	39
AZD2171 + $5\times$ 2 Gy (concomitant)	17	42

Conclusions: These data further support inhibition of VEGF signalling as a means to enhance tumour radiation response *in vivo* and suggest that the antitumour activity of AZD2171 is more pronounced against vasculature treated with extended fractions of radiotherapy.