Thus, ChoK inhibitors can be potencially useful in the clinic as a new tool for cancer therapy.

References

[1] Ramirez de Molina A, Rodriguez-Gonz‡lez A., Gutierrez R., Martinez-Pi–eiro L., Sanchez J.J., Bonilla F., Rosell R., and Lacal J.C. Overexpression of choline kinase is a frequent feature in human tumor-derived cell lines and in lung, prostate and colorectal human cancers. Biochem. Biophys. Res. Commun. 296:580–583, 2002
[2] Hernandez-Alcoceba R., Fernandez F. and Lacal J.C. (1999). *In vivo* antitumor activity of choline kinase inhibitors: a novel target for anticancer drug discovery. Cancer Research 59:3112–3118
[3] Lacal J.C. (2001). Choline kinase: a novel target to search for antitumor drugs. IDrugs. 4: 419–426, 2001.

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Determinants of the synergistic interaction between TS inhibitors, IFN-gamma and Fas signaling in therapy of colon cancer

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We have demonstrated a Fas-dependent component in thymineless death of human colon carcinoma cell lines (cc). The cytotoxicity of 5-fluorouracil (FUra) + leucovorin (LV), or the pure thymidylate synthase (TS) inhibitor ZD9331, is synergistic when combined with interferon-gamma (IFN-g) in a panel of cc, dependent on the Fas death receptor, DNA damage, independent of p53. Synergism was also demonstrated in HCT116 cells treated with ZD9331 + IFN-g, demonstrating RNA-mediated FUra/LV cytotoxicity not potentiated by IFN-g. In HT29 cells, IFN-g (but not ZD9331) upregulated the expression of caspases −3, −4, −7 and −8, and ZD9331 + IFN-g enhanced caspase activation and PARP cleavage, not prevented by overexpression of Bcl-2. IFN-g increased proteasomal activity, leading to selective downregulation of the IAP protein survivin, as well as increasing Fas expression. The cyclin dependent kinase inhibitor p21Cip1 also regulated thymineless death. HCT116 wt and p53−/− cells underwent apoptosis and loss in clonogenic survival when exposed to ZD9331, while p21Cip1−/− cells were resistant. In contrast, IFN-g induced marked cytotoxicity in p21Cip1−/− cells only. Cell cycle analyses determined that HCT116 wt and p21Cip1−/− cells accumulated in S phase within 24 hr of ZD9331 exposure, however wt cells exited S-phase more rapidly, apoptosis occurring prior to mitosis, either in late S or G2. ZD9331 induced p21Cip1 upregulation in all cc examined, as did dThd deprivation in TS-deficient cells. Selective induction of p21Cip1 in RKO was also sufficient to induce apoptosis. Based on results from preclinical studies, a Phase I trial was conducted. FUra (370 mg/m2) and LV (200 mg/m2), i.v. bolus daily × 5 days, were combined with escalating doses of IFN-g (10–100 mg/m2) s.c. on days 1, 3 and 5, every 28 days. Twenty-five patients with carcinomas were enrolled. MR or SD were observed in 6/21 heavily pretreated patients. Three evaluable chemo-naive patients demonstrated PR (2) or CR (1). Data demonstrate 1) several sites of interaction between the TS inhibitor, IFN-g and Fas signaling pathways including Fas, caspases, and an increased Fas/survivin ratio, independent of the mitochondria, 2) regulation of sensitivity to both TS inhibitors and IFN-g by p21Cip1, and 3) activity in a Phase I trial in colorectal carcinoma when FUra/LV was combined with IFN-g. This combination is to be evaluated in a Phase II trial. Supported by NCI awards CA 32613, CA 21765 and by ALSAC.

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Conjugates of lytic peptides target and destroy prostate cancer metastases

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In previous studies hormone resistant breast, prostate and ovarian xenografts were destroyed by targeting their receptors for chorionic gonadotropin (CG) with membrane disrupting peptide conjugates (Leuschner et al. Prostate 46, 116–125, 2001, Gawronska et al. Gynecol Oncol 85, 45–52, 2002, Leuschner et al Breast Cancer Research and Treatment, 78, 17–27, 2003).
Objective: To test the efficacy of a lytic peptide conjugate, Phor14-betaCG-ala, to destroy human prostate cancer cells and their metastases in vivo.
Methods: Phor14 [$(KFAKFAK)_2$] was conjugated to a modification of the 15-amino acid segment (81–95) of betaCG in which the cysteines were replaced by alanines. Luciferase transfected PC-3. luc prostate cancer cells were injected s.c. in a Matrigel suspension into nude mice. In the first experiment, the efficacy of Phor14-betaCG-ala at concentrations of 0.02, 0.2, 2, 5 or 10 mg/kg was tested in PC-3. luc tumor bearing mice. The mice received single (1× per week) or multiple (3× per week) injections through the lateral tail vein for 3 weeks starting on day 35 after tumor inoculation. In a second experiment the primary tumors were removed and the mice treated with Phor14-betaCG-ala at concentrations of 0.02, 0.2 and 2 mg/kg in single or multiple weekly injections. The tumor weights (in tumor bearing mice) were determined at necropsy. Metastases in lymph nodes (LN) were determined as luciferase positive cells through luciferase assays.
Results: In the single injection group 8 out of 50 tumor bearing mice were tumor free, whereas 16 out of 50 mice were tumor free in the multiple injection group. Tumor weights were reduced from 1.6±0.2 g in control mice to 0.5±0.03 g (0.2 mg/kg), and 0.2±0.08 g (10 mg/kg) in single injection groups, and to 0.3±0.04 g to 0.15±0.02 g in multiple injection groups ($p<0.01$ vs saline control; $p<0.05$ single vs multiple injections). LN metastases were significantly lower at all concentrations in the multiple injection groups than in the single injection groups ($p<0.002$, N=10). Phor14-betaCG-ala (0.02 mg/kg; multiple injections) was highly effective in destroying LN metastases in interscapular, axillary, hepatic and mesenteric LN ($p<0.002$).
Resection of the primary tumor stimulated metastatic progression in axillary interscapular LN (luciferase positive cells/LN) from 210±64 to 929±396 in saline controls and from 164±44 to 1154±405 in interscalular LN ($p<0.05$, N=10). Multiple injections of Phor14-betaCG-ala reduced the metastatic load in axillary and interscapular LN by 99% at concentrations as low as 0.02 mg/kg ($p<0.03$).
Conclusion: Phor14-betaCG-ala destroys primary tumors and lymph node metastases at concentrations as low as 0.02 mg/kg. Multiple injections are more effective than single injections. Lymph node metastases are directly targeted and destroyed by the lytic peptide conjugate.

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ZD4054: assessment of endothelin A receptor specificity following single dose administration in healthy volunteers

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In pre-clinical studies ZD4054 has been shown to be an orally active, potent, specific endothelin A receptor (ET_A) antagonist with potential utility in prostate cancer and metastatic bone disease (Curwen and Wilson. Eur J Cancer 2002;38[Suppl 7]: S102). Specific blockade of the ET_A receptor may be optimal in the oncology arena, since the anti-cancer effects of endothelin antagonists appear to be mediated via blockade of the ET_A receptor while concomitant inhibition of the endothelin B receptor (ET_B) may affect clearance of endothelin 1 (ET-1) and other beneficial processes such as apoptosis. In healthy volunteers, biological activity (objective pharmacodynamic activity and the adverse event profile) consistent with ET_A receptor blockade is seen with ZD4054 doses above 5mg.
Circulating ET-1 concentrations have been established as a biomarker of ET_B blockade *in vivo* in man e.g. Strachan et al (Hypertension 1999;33:581–5), and its measurement was utilised during the initial human studies with ZD4054 to determine its specificity for the ET_A receptor. This initial study comprised a randomised, ascending, double-blind, placebo-controlled design. At each dose level studied, six subjects were randomised to single doses of ZD4054 and two to placebo. Dose escalation was continued based on tolerability until the maximum tolerated dose had been defined. Doses of 2.5mg, 10mg, 20mg, 30mg, 60mg, 120mg, 150mg and 240mg ZD4054 were investigated within this study. Samples were collected for measurement of ET-1 and its precursor, Big-ET-1, at 4 hours and 24 hours post dose to assess the specificity of ZD4054 for ET_A versus ET_B. A rise in ET-1 without an accompanying rise in Big ET-1 would be taken as evidence for ET_B blockade *in vivo*.
Mean values for ET-1 at all doses, for both timepoints, were within the placebo range defined by the 2.5% and 97.5% percentiles of the pre-dose and placebo samples. No consistent profile was observed when comparing the 4 hour and 24 hour timepoints at each dose, and there was no evidence of a dose response based on a rise in mean values or percentage change from baseline.
In conclusion, this clinical study confirms preclinical findings that ZD4054 specifically antagonises ET_A, with no evidence for inhibition of ET_B. As a result of this specificity, ZD4054 has the potential to block the pathological processes in malignancy that are mediated by ET_A while allowing the beneficial processes mediated by ET_B to proceed.