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Pharmacodynamic markers for the choline kinase inhibitor MN58b in human breast cancer model by magnetic resonance spectroscopy

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Choline kinase (ChoK) is a cytosolic enzyme that catalyses the phosphorylation of choline to form phosphocholine (PC) which is involved in cell membrane synthesis. Elevated levels of PC and ChoK found in tumours are associated with cell proliferation and malignant transformation. Inhibition of ChoK with MN58b, a novel anticancer drug and putative competitive inhibitor, demonstrated an antiproliferative effect in human tumour xenografts (1).
The aims of this work were: a) to confirm the mechanism of action of MN58b; and b) to develop a robust and non-invasive surrogate marker for tumour response following MN58b treatment. We carried out an *in vivo* ^{31}phosphorus magnetic resonance spectroscopy (^{31}P MRS) study of MN58b (4mg/kg via ip for 5 days) and vehicle in MDA-231-MB human breast cancer xenografts. *In vitro* ^{31}P MRS, assays for ChoK activity and western blots for ChoK expression, were performed on tumour extracts.
A significant growth delay was observed in the MN58b-treated MDA-231-MB xenografts when compared with controls. *In vivo* ^{31}P MRS of the MDA-231-MB xenografts showed a decrease in the phosphomonoester/total phosphorus signals (PME/TotP) ($p<0.05$) and PME/β-NTP ratios ($p<0.05$) after 5 days of MN58b treatment. No significant changes were observed in the control group. *In vitro* ^{31}P MRS of extracts from MN58-treated tumours showed significant decreases in PC ($p<0.03$) when compared with controls. No changes in other phospholipid metabolites (phosphoethanolamine, glycerophosphocholine and glycerophosphoethanolamine) were observed. No significant changes in ChoK activity or expression were found in extracts from MN58b-treated tumours when compared with control. This is consistent with MN58b being a competitive inhibitor of ChoK.
Treatment with MN58b resulted in tumour growth delay and altered phospholipid metabolism *in vivo*. These MRS changes suggest inhibition of ChoK and are consistent with the mechanism of action of MN58b. The decrease of PC and PME may have potential as surrogate non-invasive pharmacodynamic markers for determining tumour response following treatment with MN58b or other ChoK inhibitors.
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References

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Biological evaluation of a novel, synthetic pyrazole class of Hsp90 inhibitors

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The molecular chaperone, Hsp90 is an essential protein for maintaining the ATP-dependent folding and function of a multitude of key oncogenic client proteins, including c-Raf, ErbB2, Cdk4 and mutant p53. The inhibition of ATP binding at the N-terminal of Hsp90 ultimately leads to the degradation of the client proteins via the proteosome pathway. This provided a good strategy for generating novel Hsp90 inhibitors to overcome some of the limitations observed with the current Hsp90 inhibitor, 17AAG (17-allylamino-17-demethoxygeldanamycin), which is being evaluated in Phase I and Phase II clinical trials. The hit pyrazole compound CCT018159, which was discovered by high throughput screening at our Centre (Aherne et al., Proc. AACR 44, Abstract #4002) has led to synthesis of a series of closely related analogues, which have showed improved enzyme activity and cell potency. The ATPase and cellular IC_{50} values for CCT018159 (determined by malachite green assay and sulfohodamine B assay) were 6.4 and 4 μM, respectively. The derivatives of CCT018159 have demonstrated a 4–30 fold increase in enzyme potency and up to a 10-fold improvement in cellular potency in a range of tumour types, including colon and melanoma cell lines. The molecular signature of Hsp90 inhibition has been well defined (Maloney et al, Current Cancer Drug Targets, 3: 331–341, 2003); this includes upregulation of Hsp70 protein and downregulation of c-Raf, ErbB2 and Cdk4. This has been confirmed using western blotting and ELISA. These characteristic pharmacodynamic marker changes have been observed with the active analogues. Cell cycle analysis of these novel inhibitors using the dual-staining BrDU/Hoechst-propidium iodide method showed similar profiles to 17AAG and CCT101859. Apotosis confirmed by PARP cleavage has also been demonstrated in HCT116 treated colon cancer cells. 17AAG activity has been shown to be dependent on the quinone reductase enzyme DT-diaphorase and the multidrug transporter, P-glycoprotein (Kelland et al., J. Natl. Cancer Institute, 91:1940–1949, 1999). Cellular activity of these pyrazole analogues is not significantly altered in a BE colon cell line transfected with the DT-diaphorase gene, *NQO1* or in a P-glycoprotein positive cell line. Pharmacokinetic properties of this class of Hsp90 inhibitors look promising (Smith et al., Clin. Cancer Res., *9:* 239, 2003) and solubility is much favourable than 17AAG. Phase I combination trials of 17AAG and docetaxol are ongoing in the USA, and a phase II clinical trial of 17AAG alone in melanoma is scheduled. *In vitro* combination studies with 17AAG or CCT018159 and temozolomide or dacarbazine in a melanoma cell line, SKMEL5 are underway. In summary, this series of pyrazole compounds has contributed significantly to the lead optimisation program aimed to identify developmental clinical candidates.
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HER2 imaging as a non-invasive pharmacodynamic marker of Hsp90 inhibition

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17-allylamino-geldanamycin (17-AAG) is the first Hsp90 inhibitor to be tested in clinical trial. This agent causes the ubiquitination and proteasomal degradation of HER2 and other Hsp90 client proteins. To date, we have treated 85 patients with various cancers on two clinical trials of 17-AAG alone and in combination with docetaxel. One obstacle in the clinical development of this class of agents is the inability to quantitatively assess as a function of time the effect of the drug on Hsp90 function in patients. While the optimal correlative assay is pre- and post-treatment collection of tumor tissue for analysis of treatment induced changes in relevant Hsp90 client proteins, in only a small fraction of the patients treated on these protocols was tumor tissue accessible for biopsy. As an alternative, we have sought to determine changes in chaperone and client protein expression in surrogate tissues such as skin or peripheral blood lymphocytes. In these studies, post-therapy increases in Hsp70 in peripheral blood lymphocytes (PBL) have been observed in all patients treated with 17-AAG at or above 110 mg/m^2 while declines in Raf-1 and Akt have been seen in a minority of patients treated at doses of 17-AAG above 160 mg/m^2. As an alternative, we have developed a method for the non-invasive quantification of HER2 protein expression in tumors. HER2 is an Hsp90 client protein and 17-AAG inhibits HER2 signaling by inducing its proteasomal degradation. We labeled an F(ab')$_2$ fragment of Herceptin (trastuzumab) with ^{68}Ga, a positron emitter, which allows for the sequential non-invasive quantitation of HER2 expression using Positron Emission Tomography (PET) imaging. The rapid elimination of this radiotracer from the blood pool allowed for serial determinations of HER2 expression pre- and post treatment with 17-AAG. Imaging results correlated with direct determinations of tracer uptake by gamma counter and with changes in HER2 protein expression by immunoblot. This technology has immediate clinical applicability as a pharmacodynamic marker in the ongoing clinical trials of Hsp90 inhibitors. It also highlights the potential utility of molecular imaging technologies in the translation of novel targeted therapies from the laboratory to patients.

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Restoration of PTEN-expression in tumor cells causes them to depend on EGFR for suppression

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Activation of EGFR-mediated signaling pathways in epithelial cells stimulates their proliferation and desensitizes them to apoptotic stimuli. Tumor cells with mutated PTEN are EGFR-independent, however, and are insensitive to selective inhibitors of EGFR. Restoration of PTEN-expression sensitizes these tumors to EGFR inhibitors. We introduced a tet-inducible PTEN into the PTEN-deficient, EGFR-overexpressing breast cancer cell line MDA-468 in order to study the mechanism of this effect. In this cell line, PI3 kinase and Akt kinase are activated, EGFR independent and insensitive to Iressa, an inhibitor of EGFR tyrosine kinase. Induction of PTEN expression inhibits Akt kinase and causes a slowing of growth. Expression of wt PTEN sensitizes these cells to EGFR inhibition by Iressa. Growth inhibition is not due to cell cycle arrest but to synergistic induction of apoptosis. This synergy is not due to further inhibition of Akt kinase