conclusion, both in vivo imaging methods were able to detect and quantify induction of apoptosis in response to Plk1 inhibition early in the course of treatment and prior to detectable changes in tumor volume. These non-invasive approaches may be useful in further preclinical profiling and clinical development of Plk1 inhibitors.

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Detection of surrogate markers of apoptosis in the peripheral blood of a preclinical tumour model treated with a selective inhibitor of Aurora B kinase (AZD1152)

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AZD1152 is a specific aurora kinase inhibitor with selectivity for Aurora B kinase, targeting proliferating tumour cells. Inhibition of Aurora B reduces histone H3 phosphorylation and inhibits cytokinesis, inducing multi-nucleation and polyploidy, leading to cell death and apoptosis. AZD1152 shows antitumour activity against a number of human tumour cell lines and preclinical xenograft models, and is being evaluated in early clinical studies. It has been suggested that cytokeratins released from dying carcinoma cells into the peripheral blood could serve as useful surrogate markers in the treatment of epithelial malignancies. The neo-epitope M30, revealed after cleavage of cytokeratin 18 (CK18) by activated effector caspases may provide an indirect marker for apoptosis. In contrast, the M65 epitope, present on both cleaved and intact CK18, should provide a measure of overall cell death.
We studied the correlation between cell death in tumour tissue and the level of M30/M65 in peripheral blood of nude rats bearing established human SW620 colon cell xenografts. Rats were dosed with either vehicle or AZD1152 (iv 25 mg/kg/day for 4 days), which leads to significant antitumour effects (maximum tumour growth inhibition >90%). Flow cytometric analysis of disaggregated xenograft tissue indicated a sequence of phenotypic events in tumours treated with AZD1152 including suppression in phosphorylation of histone H3 followed by an increase in polyploidy. Histological analysis in AZD1152-treated tumours confirmed the aberrant cell division phenotype and indicated an increase in apoptosis (by cleaved caspase 3 immunoreactivity) versus control treated tumours.
Dynamic changes in M30 and M65 levels were detected using ELISA. A rapid elevation in M30 plasma levels was observed in the AZD1152-treated group compared with the control group. Compared with M30, higher levels of M65 antigen were observed in both groups. Interestingly, at later timepoints higher M65 levels were observed in the control group, possibly reflecting higher tumour burden associated with more cell death at the core of the larger tumours.
These findings demonstrate that AZD1152 induces apoptotic cell death in a tumour xenograft model and this effect correlates with an increase in M30 and M65 detection in the peripheral blood. We are currently validating these findings with different dosing schedules of AZD1152 to further assess the utility of such non-invasive 'signals of efficacy'.

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Molecular characterization of cell death signaling by mercaptopyridine oxide and its analogs in human tumor cells-evidence for p53-independent growth arrest

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Efficient execution of cell death signaling is the desirable fate of cancer chemotherapy, and therefore the need to identify novel compounds with better apoptotic potential. Over the years, our laboratory has been studying the mechanism(s) underlying resistance to death signaling in tumor cell with the ultimate goal of identifying druggable targets for enhancing the efficacy of cancer chemotherapy. Among the various small molecules under investigation for their growth inhibitory activity is mercaptopyridine oxide (MPO) and its two synthetic analogs, MPO-Na and MPO-Zn. Using conventional biochemical approaches and laser scanning cytometry we investigated the mechanism of action of these small cell permeable compounds. Results show that all three compounds possess promising cell death inducing activity against a host of human tumor cell lines, however interestingly with diverse mechanisms. Whereas MPO and MPO-Na activated classical apoptotic pathway, characterized by robust caspase activation, H2gX phosphorylation (DNA damage), and mitochondrial outer membrane permeablization (MOMP), the mode of action of MPO-Zn appeared distinctly different with minimal involvement of the caspase proteases, but inhibitable by the necro-apoptosis inhibitor, necrostatin. Interestingly, exposure of human colorectal carcinoma cells expressing wild type p53 (HCT116 p53 +/−) to MPO resulted in early cell cycle arrest in the G2/M phase and later in G1 phase, mediated by ser15 phosphorylation of p53 and upregulation of the p53 inducible cell cycle inhibitor p21. In addition, these cells stained positive for senescence associated beta galactosidase (SA-b gal), thus strongly suggesting the acquisition of senescent phenotype. In comparison, p53 −/− variant of the cell line underwent a late S phase arrest and exhibited morphological features consistent with mitotic arrest. Furthermore, MPO compounds were excellent sensitizers when used alongside TRAIL in TRAIL responsive tumor cells. Finally, we tested these compounds against human B cell lymphomas derived from biopsies. Indeed, MPO and its analogs showed excellent growth inhibitory and death inducing activities against clinical lymphoma cells. Taken together, these data highlight the tremendous potential of these compounds as anti-cancer agents with the ability to either induce cell cycle arrest or apoptosis or caspase-independent cell death.

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Functional assessment of Bcl-2 disordered loop through plasmon surface resonance technology

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Bcl-2 family is divided in proapoptotic and antiapoptotic members. Protein function within the family is regulated at several levels, but a prominent mechanism consists in the dimerization of the proteins and the consequent modulation of the apoptotic threshold at the mitochondrial level. Within the family, there are conserved regions referred to as Bcl-2 homology domain (BH1–4). In some antiapoptotic members like Bcl-2 and Bcl-X_L, domains BH4 and BH3 are separated with a disordered loop. In this work, we used surface plasmon resonance technology to characterise the loop as the most important site for the protein-protein interactions within the Bcl-2 family. Bcl-2 strongly interacts with Bcl-2 itself and Bcl-X_L. Kinetics parameters were obtained using the heterogeneous ligand model, since dimers of Bcl-2 (KD 8.1×10^{-7} and 2.7×10^{-6} for Bcl-2 and Bcl-X_L, respectively) revealed minor affinity of binding with respect to the monomers (KD 2.3×10^{-9} and 6.2×10^{-9} for Bcl-2 and Bcl-X_L, respectively). To analyse the relevance of the loop in this binding, we replaced the disordered loop with a linker sequence of 4 alanines (Bcl-2 Δ), thereby demonstrating that without the loop the ability of Bcl-2 to homodimerize is completely abrogated. Computer assisted modelling helped us to design a Bcl-2 mutant in which Pro-39 was replaced with a Gly. As predicted, this mutation disrupted the structure of the loop and consequently the ability to homodimerize. Recently, also tubulin has been reported as a protein able to interact with Bcl-2. Upon the activity of microtubule polymerizing agents, it could occur Bcl-2 sequestering and consequently the induction of apoptosis. Using this technology, we measured the binding of tubulin to Bcl-2. Kinetic analysis showed that heterogeneous ligand model does not fit experimental data, thereby suggesting that both monomers and dimers of Bcl-2 equally bind to tubulin (KD 3.2×10^{-7}). As a first approach with three monoclonal antibodies specific for BH3, N terminal and the loop we performed the epitope mapping upon Bcl-2/tubulin interaction. The results pointed out that only the monoclonal antibody specific for the loop domain was relevantly affected upon binding, thereby indicating the involvement of the loop in this interaction. The same experiments was repeated with two antibodies specific for α and β-tubulin, thereby demonstrating that only this latter was affected in this interaction. These findings point out that the disordered loop plays a prominent role in interactions of Bcl-2 within the family and with tubulin.

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Development of a 300,000 base pair custom sequencing microarray for biomarker discovery and anticancer drug development

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Biomarker discovery is increasingly recognized as an important strategy for improving the efficiency of anticancer drug development and for optimizing cancer therapy in individual patients. For example, the presence of c-Kit in GIST or the Philadelphia chromosome in CML can be thought of as biomarkers of response to imatinib in patients with these diseases [1,2]. Also, specific mutations in the active site of the EGFR receptor were found in tumors from patients that were highly responsive to gefitinib therapy [3,4]. Because of the economy of scale and the immense quantity