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In vivo pharmacokinetics of [11C]docetaxel in lung cancer patients

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Background: Although docetaxel is an effective drug for the treatment of lung cancer, a number of patients does not benefit from this therapy due to tumour resistance. Positron emission tomography (PET) is a non-invasive imaging technique that allows for quantification of radiolabelled docetaxel ([^{11}C]docetaxel) kinetics in tumours and might be useful for predicting tumour response to docetaxel treatment. The aim of the present study was to determine the feasibility and reproducibility of [^{11}C]docetaxel PET scans in lung cancer and to investigate whether [^{11}C]docetaxel uptake was related to tumour perfusion.
Patients and Methods: Twenty-five patients with advanced lung cancer underwent a dynamic PET-CT scan with [^{11}C]docetaxel (60 min) and $H_2{}^{15}O$ (10 min). In addition, 8 patients underwent a second [^{11}C]docetaxel PET scan to assess test-retest reproducibility. Arterial and venous blood samples were collected to measure blood and plasma radioactivity concentrations and to assess the presence of radiolabelled metabolites. Lesions were delineated on the CT scan and projected onto the dynamic PET frames. [^{11}C]docetaxel uptake in tumours was quantified using the Patlak method, giving the net influx rate (K_i). Tumour perfusion was quantified by applying the standard single tissue compartment model to the $H_2{}^{15}O$ data.
Results: Clearance of [^{11}C]docetaxel from plasma was rapid and later PET frames suffered from high liver uptake. Therefore, only the first 10 min of data were used for further analysis. In total, 47 lesions were defined, including both primary tumours and metastases. The median net influx rate of [^{11}C]docetaxel was 0.0103 min^{-1} (range 0.0023–0.0358 min^{-1}). Test-retest [^{11}C]docetaxel PET scans showed good reproducibility with an intraclass correlation coefficient of 0.95. The inter-individual and intra-individual variability of [^{11}C]docetaxel uptake in lung cancer was high. [^{11}C]docetaxel uptake was not associated with tumour size, but correlated with tumour perfusion (Spearman's $\rho = 0.846$, $p < 0.001$).
Conclusions: Measurement of [^{11}C]docetaxel uptake in lung cancer is feasible with good reproducibility. [^{11}C]docetaxel uptake depends on tumour perfusion. The variability of [^{11}C]docetaxel uptake in lung cancer may reflect differential sensitivity to docetaxel treatment, suggesting that PET scans with [^{11}C]docetaxel may be useful for personalized treatment planning.

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Berubicin, a novel mechanistically altered anthracycline potently inhibits cell growth and induces apoptosis in mantle cell lymphoma

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Background: Patients with mantle cell lymphoma (MCL) inevitably relapse despite initial effective therapies, and the disease eventually becomes resistant to the currently existing drugs. Therefore, novel anticancer agents are urgently needed for MCL. Doxorubicin (DOX), an anthracycline, topoisomerase II poison, has demonstrated clinical usefulness in MCL; however, its efficacy is limited by drug resistance. To overcome these limitations, we tested berubicin (BRN), a clinically evaluated representative of a novel class of mechanistically altered anthracycline analogs, as a single agent to treat MCL in vitro.
Materials and Methods: We compared the effects of BRN with DOX and another clinically used agent bortezomib. Four human MCL cell lines, Mino, JeKo-1, SP53, and Granta 519, and fresh primary tumor cells isolated from patients with MCL were treated with BRN, DOX, and bortezomib. The effects of these compounds on cell proliferation, apoptosis, and the cell cycle were analyzed using ^{3}H-thymidine incorporation assay, MTS assay, flow cytometry, and Western blot analysis.
Results: BRN potently inhibited the growth of the established MCL cell lines, as well as the fresh primary tumor cells isolated from patients with MCL in a dose-dependent manner. BRN also potently induced caspase 3−mediated apoptosis in both the established and primary CD20^{+} MCL cells in a dose-dependent manner. Notably, BRN induced G2/M cell cycle arrest in all MCL cell lines tested. BRN was significantly and consistently more potent as a cell growth inhibitor and inducer of apoptosis in MCL than either DOX or bortezomib. Most remarkably, BRN did not inhibit cell growth or induce apoptosis in normal resting bone marrow-derived mononuclear cells at the concentrations that were lethal to MCL cells.
Conclusions: BRN, a novel, mechanistically altered, and clinically evaluated DOX analog that targets topoisomerase II, blocks the transcriptional activity of HIF-1a, and circumvents ABC transporter-mediated efflux, appears to be a promising new agent against MCL that could replace DOX as single agent and/or be used in combination with other drugs.

Apoptosis, necrosis, autophagy

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Induction of acute apoptosis by cisplatin is not associated with damage to nuclear DNA and is likely to be an "off-target effect"

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Apoptosis has been widely proposed as a major mechanism of the anti-proliferative effects of cisplatin and this compound is widely used in experimental studies on DNA damage-induced apoptosis. A problem with these studies is that cisplatin concentrations that are at least one order of magnitude higher than the IC50 (i.e. 20−100 μM) are used to induce apoptosis in short-term experiments (24−48 hr). We find that at these concentrations, cisplatin induces formation of cellular superoxide and that apoptosis is inhibited by superoxide scavengers. Importantly, cisplatin induces caspase activation in enucleated cells (cytoplasts) with the same concentration limits as observed with intact cells − showing that cisplatin-induced apoptosis occurs independently of nuclear DNA damage. Even when cisplatin is used at high concentrations, caspase-3 activation is restricted to the peripheral cell layer of multicellular spheroids, showing that apoptosis is likely not an important outcome in 3-D tumor tissue. At IC50 doses, the antiproliferative effects of cisplatin involve premature senescence and secondary, nonstress-induced apoptosis. We propose that the high cisplatin doses currently used in *in vitro* studies are unphysiological and lead to acute, stress-induced apoptosis that is largely DNA damage-independent and represents an "off-target" effect.
Mandicet al., *J Biol Chem.* 278, 9100−6; Berndtssonet al., *Int J Cancer,* 120:175–180; Fayad et al., *IntJ Cancer* 125, 2450–2455.

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Tumor necrosis factor and CCR5 gene associations with cancer risk

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Genetic polymorphisms of cytokine encoding genes are known to predispose to malignant disease. The cytokine *TNF-α* is a central mediator of inflammation and apoptosis and may possess both pro-tumor and anti-tumor activities. *CCR5*, are believed to play a role in anti-tumor immunity through immune-cell recruitment. It has been suggested from earlier reports that the *TNF-α* can also influence the expression of chemokine receptors and causes *CCR5* down-modulation. It has also been reported that *TNF-α* decreases the *CCR5* expression in peripheral blood monocytes and alveolar macrophages by the production of *RANTES*. The objective of this study was to investigate whether single nucleotide polymorphisms with known functional significance in the genes that are involved in immunoregulatory functions such as, *CCR5, TNF-α* are associated with susceptibility to malignant disease.
In this study, *CCR5Δ32* deletion and polymorphism in the promoter region (position −308) in *TNF-α* gene were determined using polymerase chain reaction-(PCR) restriction fragment length polymorphism (RFLP) methods in patients with cancer and healthy controls. We compared the allelic distribution of these genes adjusted for age and sex between cancer patients and healthy subjects to study the possible association with susceptibility to disease. We also assessed potential interactions between these polymorphisms and different types of cancers.
We report two important findings (1) *TNF 2* allele as genetic risk factor for cancer showing strong association with a significance of $p > 0.00232$ (including all cases typed for *CCR5* mutation) and (2) The significance of *TNF-α* is observed only in the presence of functioning *CCR5* (wild type) with $p > 0.0008$, but not in carriers (deletion) $p > 0.547$ indicating a possible gene interaction involved in immunoregulatory function. In conclusion, our data suggest an important role for *TNF-α* and *CCR5* polymorphisms in cancer. The present findings suggest that the inflammatory process may constitute an important step in the initiation and promotion of solid tumors/cancer. To better understand the interaction of these genes

and their significant role in cancer progression, *CCR5* and *TNF* gene expression studies are being investigated.

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Reolysin induces endoplasmic reticular stress in multiple myeloma and enhances the activity of bortezomib

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Background: While the approvals of bortezomib (BZ) and lenalidomide have significantly improved the treatment of multiple myeloma (MM), it remains an incurable disease. MM cells have high protein synthesis rates due to their heavy engagement in immunoglobulin production, which renders them hypersensitive to endoplasmic reticulum (ER) stress-mediated apoptosis. Reolysin is a proprietary formulation of the human reovirus that has shown anticancer efficacy in clinical studies. We hypothesized that Reolysin treatment would lead to the accumulation of viral products in MM cells, stimulate ER stress, and significantly enhance BZ-induced apoptosis.
Materials and Methods: The antimyeloma activity of Reolysin was assessed by MTT assay, propidium iodide staining followed by flow cytometry, and measuring active caspase-3 levels in a panel of MM cell lines. Dual reovirus and ubiquitin accumulation were visualized by confocal microscopy. Intracellular Ca2+ levels were quantified by flow cytometry and increases in ER stress-related genes were determined by quantitative real-time PCR and immunoblotting. Xenograft (RPMI-8226) and syngeneic (5TGM1) models of MM were used to evaluate the effects of Reolysin and bortezomib in vivo.
Results: Reolysin induced viral replication and apoptosis selectively in MM cell lines and not in normal peripheral blood mononuclear cells (PBMCs). Reolysin treatment stimulated ER stress as measured by increased expression of GADD153, GADD34, and XBP-1s, ER swelling visualized by electron microscopy, and an elevation of intracellular Ca2+ levels. Co-treatment with Reolysin and BZ promoted the simultaneous accumulation of viral and ubiquitin-conjugated proteins, which resulted in enhanced levels of ER stress and cell death. Importantly, the Reolysin and bortezomib combination significantly reduced tumor burden in both xenograft and syngeneic MM mouse models.
Conclusion: Reovirus replication in MM cells induces an accumulation of viral products that stimulates ER stress and apoptosis. Co-treatment with Reolysin and bortezomib stimulated a simultaneous accrual of viral and ubiquitinated proteins leading to enhanced ER stress-mediated apoptosis. Reolysin is a promising anticancer agent that displays activity against MM alone and in combination with bortezomib and warrants further investigation for the treatment of MM and other malignancies.

581 POSTER DISCUSSION

Use of functional human cancer cell line mitochondria to explore the mechanisms of ABT-737-induced mitochondrial membrane permeabilization

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Current limitations of chemotherapy include toxicity on healthy tissues and multidrug resistance of malignant cells. A number of recent anti-cancer strategies aim at targeting the mitochondrial apoptotic machinery to induce tumor cell death. In this study, we set up protocols to purify functional mitochondria from various human cell lines to analyze the effect of peptidic and xenobiotic compounds described to harbour either Bcl-2 inhibition properties or toxic effects related to mitochondria. Mitochondrial inner and outer membrane permeabilization were systematically investigated in cancer cell mitochondria *versus* non-cancerous mitochondria. The truncated (t-) Bid protein, synthetic BH3 peptides from Bim and Bak, and the small molecule ABT-737 induced a tumor-specific and OMP-restricted mitochondrio-toxicity, while compounds like HA-14.1, YC-137, Chelerythrine, Gossypol, TW-37 or EM20-25 did not. We found that ABT-737 can induce the Bax-dependent release of apoptotic proteins (cytochrome c, Smac/Diablo and Omi/HtrA2 but not AIF) from various but not all cancer cell mitochondria. Furthermore, ABT-737 addition to isolated cancer cell mitochondria induced oligomerization of Bax and/or Bak monomers already inserted in the mitochondrial membrane. Finally immunoprecipatations indicated that ABT-737 induces Bax, Bak and Bim desequestration from Bcl-2 and Bcl-xL but not from Mcl-1L. This study investigates for the first time the mechanism of action of ABT-737 as a single agent on isolated cancer cell mitochondria. Hence, this method based on MOMP (mitochondrial outer membrane permeabilization) is an interesting screening tool, tailored for identifying Bcl-2 antagonists with selective toxicity profile against cancer cell mitochondria but devoid of toxicity against healthy mitochondria.

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Disruption of autophagic and autolysosomal signaling pathways leads to synergistic augmentation of erlotinib-induced apoptosis in wild type EGFR human non-small cell lung cancer cell lines

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Background: autophagy is a tightly regulated lysosomal self-digestion process that may promote cell survival and/or be involved in type II cell death. Erlotinib, an orally bioavailable EGFR TK inhibitor, has modest but real efficacy in wild type EGFR NSCLC tumors. The potential role of autophagy in erlotinib-induced cytotoxicity has not been previously investigated.
Materials and Methods: four wild type EGFR NSCLC cell lines were used: erlotinib-sensitive H322 and H358, and erlotinib-resistant H460 and A549.
Results: erlotinib at a clinically achievable concentration (2 mM) induced autophagic features including an increase in the formation of AVO and MDM staining, conversion of LC3-I (the cytosolic form) to LC3-II (the lapidated form associated with autophagosome), and the formation of autophagic vacuoles in both erlotinib-sensitive and -resistant cell lines. The combination of erlotinib with chloroquine, an autophagy inhibitor, was synergistic in all tested human NSCLC cell lines. Co-treatment with 3-methyladenine (3-MA), an inhibitor of early stage autophagy or with bafilomycin A1, an inhibitor of late stage autophagy, and down-regulation of Atg-5 and Beclin-1 gene expression by siRNA resulted in enhanced apoptotic cell death and cytotoxicity as compared with erlotinib alone in H460 and A549 cells. Moreover, co-treatment with lysosomal inhibitors, ammonium chloride, E-64, Pepstain-A and Z-LA-fmk also resulted in the enhancement of erlotinib-induced cytotoxicity. The combined treatment with chloroquine did not alter erlotinib-induced EGFR pathway inhibition or G1 cell cycle arrest, but significantly induced apoptosis associated with the activation of caspase-9 and caspase-3, and increase in cleavage of PARP protein in all tested cells. Interestingly, the combination of erlotinib and chloroquine induced up-regulation of Bim protein expression, and down-regulation of Bim gene expression by siRNA attenuated cell death induced by the drug combination. In addition, inhibition of autophagy by chloroquine treatment enhanced erlotinib-induced ROS generation, and activation of p38 but not JNK signaling. Inhibition of ROS generation by NAC, or inhibition of p38 by SB202190 resulted in the attenuation of the potency of the combination, suggesting that ROS generation and p38 activation are potential mediators of the effect of this combination.
Conclusion: inhibition of autophagy by chloroquine represents a novel strategy to enhance erlotinib efficacy in wild type EGFR NSCLC tumors.
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PI3-kinase inhibition enhances ABT-737 induced apoptosis in colorectal cancer cell lines

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Background: Although context dependent, the PI3-kinase signalling pathway is widely reported to promote cell survival, proliferation and migration. This pathway is up-regulated in several solid tumours via mechanisms including PTEN deletion, RAS or PIK3CA mutation and aberrant activation of receptor tyrosine kinases. As such, inhibitors of PI3-kinase and its down-stream effectors are of great interest as tractable anti-cancer agents. In colorectal cancer (CRC) cells inhibition of the PI3-kinase pathway did not induce apoptotic cell death. Moreover, CRC cells were relatively resistant to the pro-apoptotic BH-3 mimetic ABT-737. This study examined whether PI-3K inhibition and ABT-737 combined reduced the threshold of apoptosis in CRC cells.
Methods: The PI3-kinase pathway was inhibited with PI-103 (PI3-kinase inhibitor), AKTi1/2 (AKT inhibitor) or rapamycin (mTORC1 inhibitor). Apoptosis was assessed by activating conformational changes in BAK, caspase 3 cleavage, and phosphatidylserine exposure. Interactions between Bcl-2 BH3 family members were assessed by co-immunoprecipitation. Expression of the ABT-737 resistance factor Mcl-1 was knocked down by transfection of siRNA.
Results: PI3-kinase inhibition enhanced apoptosis induced by ABT-737 upstream of cytochrome *c* release in CRC cells. PI3-kinase inhibition reduced the levels of Mcl-1. However, PI3-kinase inhibition further enhanced ABT-737 induced apoptosis in CRC cells were Mcl-1 was