stimulation of downstream effectors ROCK, LIMK2 and ADF/destrin. Furthermore, dominant negative Rho/Cdc42 or pharmacological inhibitors of ROCK inhibited both actin organization and apoptosis in DU145 cells. Interestingly, RhoA/B and ROCK were also implicated in mAR-dependent actin polymerization and apoptosis in LNCaP cells, acting most probably downstream of FAK/PI-3K/Rac signaling.
Conclusions: Rho GTPases are major mAR effectors controlling actin reorganization and apoptosis in prostate cancer cells.

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IGF-1 receptor stimulation overrides microenvironment-derived tumour cell quiescence

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Purpose: To study the regulation of cellular proliferation, quiescence and necrosis in the context of the tumour microenvironment.
Background: Most conventional chemotherapy-based therapies rely on the elevated proliferation status of cancer cells as a means of achieving a therapeutic index. However, the microenvironment in solid tumours often limits the efficacy of anti-proliferative therapies by generating a quiescent tumour cell subpopulation. This study investigates the factors driving tumour microenvironment derived cellular quiescence, employing tumour mapping techniques and a 3-D cell culture model, multilayered cell culture (MCC), in which tumour cells are grown into discs of tissue.
Methods: *Tissue mapping studies*: Solid tumours and MCC-discs were grown using HCT-116 and HT29 tumour cells under a range of conditions and then sectioned and immunostained to examine the variation of proliferation, apoptosis and hypoxia with depth into tissue. *Nutrient flux studies*: MCC-discs were used to separate two reservoirs of a flux apparatus and the tissue penetration of a panel of 15 amino acids was evaluated.
Results: Proliferating cells in HCT-116 tumour xenografts cluster around micro-vessels and are significantly reduced at a distance of 50 μm away from vessels while the hypoxia marker pimonidazole reaches maximal intensity at a distance of 100–150 μm. The flux of a panel of 15 amino acids through MCC-discs indicated a similar flux of all amino acids with the exception of glutamine, which passed through at 20% the rate of the others. However, supplementation of glutamine did not alter the proliferation profile in MCC-discs. 95% oxygen induced uniform proliferation throughout MCCs, indicating that under elevated oxygenation the supply of nutrients and glucose and the removal of waste products weren't limiting the depth of proliferation. Stimulation of the IGF-1 receptor was able to more than double the depth of proliferation in MCC-discs, pushing proliferation out to the border of hypoxia.
Conclusions: At intermediate distances into tissue (0–100 μm) the supply and removal of nutrients and waste products were not rate limiting factors in the determination of proliferation status; stimulation of the IGF-1 receptor alone was able to induce proliferation in quiescent cells. At greater distances (100–150 μm) hypoxia, and possibly the subsequent build-up of secreted factors, appeared to be the dominant factors which limited proliferation.

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A novel arsenical, darinaparsin, induces apoptosis in arsenic trioxide-resistant and MRP1/ABCC1-overexpressing cell lines

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Inorganic arsenic trioxide (As_2O_3) has been proven to be a highly effective treatment for acute promyelocytic leukemia (APL). However, other cancers do not respond well to this form of arsenic at clinically achievable doses. We tested a novel organic arsenical, S-dimethylarsino-glutathione (darinaparsin, ZIO-101), for efficacy in various malignancies *in vitro*. We find that darinaparsin is significantly more potent than As_2O_3 at mediating apoptosis *in vitro* in a variety of malignant cell lines and is highly active against APL cells selected for As_2O_3 resistance. We provide evidence that darinaparsin triggers apoptosis by inducing signaling pathways that do not completely overlap with As_2O_3. Darinaparsin induces apoptosis and oxidative stress to a greater extent than As_2O_3, although, like As_2O_3, darinaparsin-induced toxicity is dependent on JNK activation. However, darinaparsin does not induce PML-RARα degradation or rearrange PML nuclear bodies in the APL NB4 cell line, nor is its toxicity increased by depletion of GSH. Treatment with darinaparsin results in higher intracellular accumulation of arsenic when compared to treatment with As_2O_3. This may be explained by our finding that As_2O_3, but not darinaparsin, is efficiently exported by ABCC1. These results suggest darinaparsin might have greater therapeutic efficacy than As_2O_3 in tumors that overexpress ABCC1. Overall our studies indicate that darinaparsin efficiently kills tumor cells with increased antioxidant capacity and drug exporters and suggest that darinaparsin may have a broader therapeutic spectrum than As_2O_3. To test this hypothesis, we have initiated a Phase I clinical trial of oral darinaparsin. Early biomarker and safety data will be discussed.

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A MEK1/2 inhibitor, AZD6244 (ARRY-142886), shows beneficial effects when combined with standards of care or novel therapies – mechanistic characterisation suggests a role for apoptosis

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The Ras/Raf/MEK/ERK signalling cascade is used by growth factors to transmit signals from their receptors which regulate gene expression and survival. In a number of human cancers members of this pathway, in particular Ras and B-Raf, are observed to be mutated or aberrantly expressed. Furthermore, it has been demonstrated that abnormal activation of this pathway plays a role in chemotherapeutic drug resistance. Thus, members of this pathway present themselves as attractive anti-cancer drug targets. AZD6244, (ARRY-142886), is a novel, selective ATP uncompetitive inhibitor of MEK1/2 and is currently in phase II clinical trials. In order to support the clinical progression of AZD6244 we have used in vivo xenograft models from mKRAS human tumour cell lines (HCT-116, CaLu-6, SW620) to investigate the potential benefit of combination therapies. AZD6244 in concurrent combination with either docetaxel, irrinotecan, gemcitabine or temozolomide, was shown to have enhanced anti-tumour efficacy compared to single agent treatments. In addition, AZD6244 was analysed in combination with novel, molecularly targeted, agents (e.g. gefitinib) and shown to be efficacious. All the above AZD6244 combinations appear to be well tolerated. In order to determine if dose sequencing could enhance this anti-tumour effect, we looked at a variety of dosing regimes of AZD6244 in combination with docetaxel in HCT116 xenografts. We demonstrated that docetaxel followed by AZD6244 was more efficacious than AZD6244 dosed prior to docetaxel. Ex-vivo studies were undertaken to investigate the contribution of the apoptotic pathway/s. It has been previously shown that activation of the ERK pathway results in the phosphorylation of the pro-apoptotic BH3 only protein Bim (Weston et al, 2003). In AZD6244 treated xenograft models we have demonstrated that preventing ERK phosphorylation results in a 3.5 fold increase in Bim levels compared to controls. Furthermore, the downstream apoptotic markers, cleaved caspase 3 and cleaved PARP, were also upregulated after exposure to AZD6244. It can be rationalised that AZD6244 in combination with other agents may stimulate the apoptosis pathway by upregulating other pro-apoptotic proteins as well as Bim. Therefore, we are currently extending our mechanistic analysis in our combination studies to determine if the anti-tumour responses we have observed are a result of an upregulation in apoptosis.

References

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Apoptosis induction in acute myeloid leukemia by inhibition of MEK and MDM2 is strongly associated with the BH3-only proteins Puma and Bim

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Background: Constitutive activation of the Ras/Raf/MEK/ERK signaling pathway contributes to a series of molecular events that have been observed in more than 50% of primary acute myeloid leukemias (AML) samples and is an independent prognostic factor for the survival of patients with AML. MDM2 (Murine double minute) overexpression inactivates p53