current report extends these findings by examining the relationships between *in vivo* activity, plasma drug concentrations, and inhibition of the pharmacodynamic marker, phospho-ERK (pERK), in both tumor and whole blood samples.
Methods: Nude mice with established BxPC3 tumors were treated with ARRY-142886(AZD6244) at 1, 3 and 10 mg/kg, po, bid for 14 days. Effects on tumor growth were assessed. Mice were euthanized at 1, 3, 6 and 9 hours after the last dose. Tumors were analyzed for pERK by Western blot analysis. Whole blood was analyzed for drug concentrations by mass spectrometry and TPA-induced pERK by flow cytometry.
Results: ARRY-142886(AZD6244) inhibited tumor growth by 50% at the 1 mg/kg dose and >90% at the higher doses. Mean plasma concentrations of ARRY-142886(AZD6244) at the last time point (9 hours) were 0.18, 0.34 and 0.95 ug/ml for the 1, 3 and 10 mg/kg doses, respectively. Inhibition of pERK in tumors from both the 3 and 10 mg/kg groups was marked and also sustained throughout the sampling period, whereas the effects at the 1 mg/kg dose were not sustained at the later time point. Inhibition of pERK in *ex vivo* blood was weak but consistent with the EC_{50} (~3 ug/ml) for exogenously added drug. This value in mouse blood is higher than that seen in human blood (EC_{50} ~0.5ug/ml).
Conclusions: Whole blood pERK is not a predictive biomarker in mice, although, due to increased sensitivity, it may be better in humans. These results demonstrate that near complete inhibition of BxPC3 tumor growth corresponds to C_{min} drug concentrations of greater than 0.3ug/ml and sustained inhibition of pERK in tumors.

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Novel role of fumarate in antagonizing VHL function

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The fumarate hydratase (FH) gene product plays an essential role in the Krebs cycle by catalyzing the conversion of fumarate to malate. Germline mutations in FH predispose to dominantly inherited uterine fibroids and papillary renal cell cancer, although the pathway contributing to tumorigenesis is unknown. Hypoxia-inducible factors HIF-1 and HIF-2 promote survival and are required for tumorigenesis in many types of primary tumors and metastases. Under normal oxygen tension, the HIFs are unstable due to the activity of VHL, a protein that targets HIF for proteasome-dependent degradation. The stabilization of HIF proteins under hypoxia results from inactivation of HIF prolyl hydoxylase (HPH) enzymes that hydroxylate HIF, thus preventing an essential prerequisite for VHL recognition of HIF. In this study, we examined whether FH inactivation influences HIF expression. We demonstrate that inhibition of FH, either by siRNA or pharmacologic means, is correlated with an upregulation of HIF-1 and HIF-2 proteins, which reflects the upregulation of these proteins in FH renal tumors. Treatment of cells with fumarate also increased HIF expression. The most potent HIF upregulation was elicited by the combination of a pharmacologic FH inhibitor and fumarate, the latter exhibiting a dose-dependent effect upon HIF. Elevated HIF levels correlated with increased transcription and expression of VEGF, which was corroborated by CD31 staining of FH renal tumors. The mechanism for increased HIF expression was posttranscriptional, due to protein stabilization in a VHL-dependent manner. In vitro binding assays revealed that fumarate prevented the association of VHL with HIF in a dose-dependent fashion that was reversed by exogenous addition of 2-ketoglutarate. Collectively, these data suggest that the mechanism for FH-mediated HIF upregulation depends upon the accumulation of fumarate, which acts as a competitive inhibitor of 2-ketoglutarate, an essential cofactor for HPH. Thus, fumarate impairs the activity of HPH and prevents VHL from recognizing HIF, culminating in elevated levels of transcriptionally active HIF protein. Our results delineate a novel fumarate-dependent pathway for regulation of HIF expression and they highlight a previously unrecognized relationship between dysregulated metabolic pathway intermediates and tumorigenesis. Our data highlight FH as an important molecular target whose function is compromised in a subset of cancers.

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JANEX-1, a novel anti-cancer agent with anti-thrombotic properties

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Background: Here we provide experimental evidence that identifies JAK3 as one of the regulators of platelet function.
Methods: To study the effects of JANEX-1 on platelet function, platelets were subjected to aggregation and functionality assays, immunoprecipitation and western blot analysis, cytoskeletal fractionation, high-resolution low-voltage scanning electron microscopy and transmission electron microscopy. In vivo anticoagulant activity was assessed by measuring bleeding and clotting times in mice as well as improved event-free survival in a mouse model of thromboplastin-induced generalized and invariably fatal thromboembolism.
Results: Treatment of platelets with thrombin induced tyrosine phosphorylation of the JAK3 target substrates STAT1 and STAT3. Platelets from JAK3-deficient mice displayed a decrease in tyrosine phosphorylation of STAT1 and STAT3. In accordance with these data, pretreatment of human platelets with the JAK3 inhibitor JANEX-1 markedly decreased the base-line enzymatic activity of constitutively active JAK3 and abolished the thrombin-induced tyrosine phosphorylation of STAT1 and STAT3. Following thrombin stimulation, JANEX-1-treated platelets did not undergo shape changes indicative of activation such as pseudopod formation. JANEX-1 inhibited thrombin-induced degranulation/serotonin release as well as platelet aggregation. Highly effective platelet inhibitory plasma concentrations of JANEX-1 were achieved in mice without toxicity. JANEX-1 prolonged the bleeding time of mice in a dose-dependent manner and improved event-free survival in a mouse model of thromboplastin-induced generalized and invariably fatal thromboembolism.
Conclusion: To our knowledge, JANEX-1 is the first anti-cancer agent with anti-thrombotic properties that prevents platelet aggregation by inhibiting JAK3.

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The HIV protease inhibitor Amprenavir as a radiation sensitizers

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Background: We have shownt that PI3K activation both *in vitro* and *in vivo* is a critical step regulating tumor cell radiosensitivity in multiple human tumors. There are, however, currently no clinically useful inhibitors of PI3K. Akt is an immediate downstream target of PI3K. There are a number of reports documenting insulin resistance and diabetes in patients on HIV protease inhibitors (HPIs). Since we know that Akt signaling plays a role in insulin signaling, we speculated that these side effects of HPIs might be due to interference with Akt signaling.
Material and Methods: We obtained the HIV protease inhibitor amprenavir from the patient pharmacy. We tested our panel of cell lines with increased signaling thru PI3K that is either Ras or EGFR dependent. The concentration and time course required to inhibit Akt phosphorylation was determined by Western blot analysis. Clonogenic survival curves were carried out.
Results: Akt is a serine/threonine kinase that is phosphorylated at two sites, Thr 308 (kinase domain) and Ser 473 (C-terminal regulatory region). It is the Ser 473 site that appears to be necessary for maximal activation of Akt. We initially tested the human head and neck cancer cell line SQ20B with a constitutively active EGFR receptor and thus increased signaling through PI3K. We found that concentrations of 20 μM completely down-regulated Akt phosphorylation at Ser 473. There was no change in phosphorylation at the Thr 308 site. Clonogenic assays in SQ20B cells showed radiosensitization after treatment with 20 μM amprenavir with the surviving fraction after 2 Gy going from 70% to 51% after 2 Gy and amprenavir. Similar Western blot and survival data was also obtained in the human bladder cancer cell line T24 which has a mutated H-Ras and thus increased signaling thru the PI3K pathway.
Conclusions: HPIs may be useful as radiosensitizers in cells with activation of Akt. Because Akt is constitutively active only in tumors, this approach may be specific for tumors. Further, since HPIs can be given chronically with tolerable to minimal side-effects, this approach could be clinically applicable.