efficacy can be achieved by inhibiting all Raf isoforms or by selectively inhibiting B-RafV600E. To gain better insight into the role of Raf signaling in tumors and design more effective therapeutic strategies, we have characterized the biochemical and cellular properties of two small molecule Raf inhibitors (Rafi) with different selectivity patterns against wildtype B-Raf and c-Raf versus mutant B-RafV600E. In biochemical kinase assays, Rafi A effectively inhibited only the B-RafV600E protein, while Rafi B was uniformly effective against c-Raf, B-Raf, and B-RafV600E under physiological ATP concentrations. In accordance with these biochemical data, only Rafi B could block basal phospho-ERK levels in wildtype B-Raf melanoma tumor lines, while both were equally effective in B-RafV600E lines. This observation was further supported by the ability of only Rafi B to block phorbol ester-stimulated phospho-Erk levels in human peripheral blood monocytes in vitro and to block epidermal growth factor (EGF)-stimulated pERK levels in EGF receptor-expressing melanoma and colon tumor lines. Despite their distinct abilities to knock down phospho-Erk levels downstream of wildtype Raf versus B-RafV600E signaling, the two inhibitors displayed similar cellular efficacy profiles in in vitro viability studies, being highly selective in blocking the proliferation and survival of B-RafV600E but not wildtype tumor lines. These data suggest that the cellular selectivity of Raf inhibitors against B-Raf mutant tumors is not a function of their biochemical properties but may rather reflect a unique signaling network in B-RafV600E mutant tumors, which renders them dependent on constitutive B-Raf signaling for the maintenance of their transformed phenotype. B-Raf wildtype lines on the other hand may not rely on Raf signaling but rather utilize additional and/or redundant signaling pathways for their survival and proliferation.

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Bench to bedside – Bedside to bench: Preclinical determination of the potential pharmacological activities of vandetanib in the clinic

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Background: Vandetanib (ZACTIMA™) is an orally available inhibitor of VEGFR2, EGFR and RET signalling that has recently completed phase III evaluation in NSCLC and medullary thyroid cancer. In the phase III studies, vandetanib was dosed at 300 mg/day as a monotherapy compared with erlotinib (ZEST) or placebo (ZETA), and at 100 mg in combination with docetaxel (ZODIAC) or pemetrexed (ZEAL) compared with chemotherapy alone. A challenge for multi-targeted agents is to understand the contribution of each pharmacological activity of the agent to the anti-tumour effects observed in the clinic.
Methods: In our current work, we have performed a detailed preclinical evaluation of the relative effects of vandetanib on VEGFR2 and EGFR activity in vivo in mouse models. Importantly, we have used drug doses selected to reflect the vandetanib plasma levels observed in the clinic.
Results: In mice, we demonstrated a substantial reduction in both pVEGFR2 and pEGFR in human tumour xenografts and a surrogate normal tissue (lung) at vandetanib plasma concentrations similar to the steady-state drug levels achieved in patients receiving vandetanib at 300 mg/day, with little additional effect when vandetanib doses were markedly increased. At lower doses, where vandetanib plasma concentrations were broadly similar to those achieved in patients receiving 100 mg/day, there was a significant reduction in both pVEGFR2 and pEGFR, but this was not maximal.
Conclusions: Based on these preclinical data, we consider that the vandetanib plasma exposures achieved in patients at 300 mg/day dosing could produce near-maximal reduction of both pVEGFR2 and pEGFR in tumours, with little additional benefit of increasing vandetanib exposure further. The vandetanib plasma levels in patients receiving vandetanib at 100 mg/day would be expected to substantially reduce both pEGFR and pVEGFR2, though this would be less than maximal. Sub-maximal inhibition of pEGFR and pVEGFR2 may have benefits in terms of increased potential to combine with established or novel therapies. Additionally, the preclinical data suggest that the steady-state drug plasma levels in patients receiving vandetanib 100 mg/day could be sufficient to induce regressions of tumours bearing activating EGFR mutations, though the anti-tumour effects may be more sustained at higher doses, consistent with increased inhibition of both VEGFR2 and EGFR signalling pathways.

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Functional evaluation of members of the LIV-1 family of proteins and their role in breast cancer

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All nine members of the LIV-1 family of proteins belongs to the ZIP superfamily of zinc transporters. Our Affymetrix Human Genome U133A GeneChips® analysis of ZIP7/HKE4/LZT-Hs1, LIV-1, ZIP14/LZT-Hs4, ZIP4/LZT-Hs5 and pS2 in MCF-7 breast cancer cells confirmed that ZIP7/HKE4/LZT-Hs1, LIV-1 and ZIP14/LZT-Hs4 were regulated by estradiol. This study was extended to investigate the expression of all nine LIV-1 family members in breast cancer cells treated with oestradiol, tamoxifen and faslodex. Additionally, we investigated the expression of the LIV-1 family members in our MCF-7 based models of tamoxifen and faslodex resistance using semi-quantitative PCR. Differential expression of these family members was seen with some members constitutively expressed whilst others were either elevated or reduced in the different conditions. This analysis demonstrated that ZIP7/HKE4/LZT-Hs1 was considerably elevated in tamoxifen resistance. In an effort to investigate a possible role for ZIP7/HKE4/LZT-Hs1 in tamoxifen resistant cells siRNA was used to reduced the expression of ZIP7/HKE4/LZT-Hs1. Interestingly, in the presence of siRNA for LZT-Hs1, it was not possible to demonstrate the activation of EGFR or Src as previously observed in the tamoxifen resistant phenotype using Western blotting analysis. This is an exciting result, which suggests a role for ZIP7/HKE4/LZT-Hs1 in driving the growth of tamoxifen resistant breast cancer cells.

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Molecular and antiproliferative effects of inhibitors of fatty acid synthase and of ErbB receptors in ovarian cancer cells

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Treatment of ovarian cancer (OC) is still suboptimal necessitating the search for novel therapies. In normal tissue, the key lipogenic enzyme fatty acid synthase (FASN) converts dietary carbohydrates to triglycerides, whereas in cancer, FASN represents a metabolic oncogene and produces phospholipids for membrane microdomains (lipid rafts) that accommodate clusters of receptor tyrosine kinases including Epidermal Growth Factor Receptor (EGFR, ErbB1) and ErbB2 (HER-2/neu) thus setting the stage for signal initiation. Importantly, both FASN and ErbBs are overexpressed in tumors including OC and represent drugable targets. Recent data suggest a link between FAS and ErbB2 in breast cancer. In OC, the relationship between FAS and ErbB is still elusive. Therefore, we examined the effect of FAS and ErbB inhibition on A2780 ovarian cancer cells (OCC). A FASN inhibitor (C75) and 2 irreversible ErbB inhibitors (EKB-569, Wyeth; CI-1033, Pfizer) inhibit growth of OCC (MTT assay – IC_{50}: C75 = 22 μM; EKB-569 = 5.1 μM; CI-1033 = 3.7 μM). Interestingly, C75 synergizes with EKB-569 or CI-1033 in cell growth inhibition ($p < 0.01$) suggesting cooperation between FAS and ErbB pathways during OCC growth. RT-PCR, real-time analysis and Western blotting revealed that C75 slowly and concordantly reduces EGFR mRNA, protein and activity in OCC. Thus, C75 silences EGFR gene expression at transcriptional levels without directly affecting EGFR signaling. C75 caused deprivation of overall and phosphorylated ErbB2 protein, but failed to diminish ErbB2 mRNA. Although C75 post-transcriptionally represses ErbB2, it does not directly disrupt ErbB2 activity. C75 also caused shut-down of FAS mRNA and protein. On the other hand, EKB-569 abolishes EGFR and ErbB2 protein expression and phosphorylation, but only weakly depresses mRNA levels. Strikingly, EKB-569 also represses FAS mRNA and protein. CI-1033 also failed to affect EGFR and ErbB2 transcript levels, but compromised EGFR activity (but not EGFR protein expression) and ErbB2 protein expression and function. Generally, CI-1033 reduced ErbB function rather than ErbB protein expression. Moreover, CI-1033 correspondingly down-regulated FAS mRNA and protein. Our data indicate that ErbB and FAS pathways mutually interact with each other in OCC. Thus, interference with the FAS and the ErbB systems effectively abrogates their oncogenic activities and may be exploited for OCC treatment.
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