cellular reliance on PI3K signaling. These data are consistent with the hypothesis that mesenchymal-like cells rely more heavily on the PI3K-Akt survival pathway, and that dual inhibition of the MAPK and PI3K signaling networks may drive cellular dependence on Akt-mediated survival signals. Mesenchymal-like cancer cells are further characterized by increased cellular motility which has been linked to the metastatic potential of these cells. OXA-01 inhibited migration of mesenchymal-like cells and inhibited OTC2-mediated regulation of the F-actin cytoskeleton.
Conclusions: These observations suggest that erlotinib may sensitize mesenchymal-like cancer cells to mTOR inhibition. The combination of erlotinib and OXA-01 may be an effective strategy to target heterogeneous tumors, and may inhibit the metastatic potential of mesenchymal tumor cells.

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Dose-finding study of pegylated liposomal doxorubicin (PLD) and the mTOR inhibitor RAD001 (R) in patients (pts) with advanced solid tumors

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Background: R is an orally available mTOR protein kinase inhibitor derivative of rapamycin that has direct effects on tumor cell growth combined with an antiangiogenic mechanism. R enhances the cytotoxicity of cisplatin, paclitaxel, gemcitabine and doxorubicin in preclinical studies.PLD is a pegylated liposomal formulation of doxorubicin with a better tolerability and reduced cardiologic and GI toxicities and alopecia. Aim of the present study is to assess the feasibility of combining R and PLD.
Materials and Methods: A phase Ib study is ongoing in pts with advanced solid tumors. Treatment consists of R given daily continuously at the starting dose of 2.5 mg daily and PLD given at 40 mg/m^2 on day 1 q28 days (1 cycle). The dose of R is escalated according to 3 + 3 cohort design depending on the observed toxicity. Treatment is planned until disease progression or unacceptable toxicity. The dose limiting toxicity (DLT) is assessed on pre-defined criteria during cycle 1. The plasma disposition of PLD is analyzed when given concomitantly (cycle 1) or 48 hrs before R (cycle 2). Tumor response is evaluated every 2 cycles by modified RECIST.
Results: 12 pts were recruited from 2 centers over 6 months, 6 pts in Cohort 1, and 3 in Cohorts 2 and 3, respectively. Median age was 51 (range 27−68 years), ECOG PS was 0−1, the main tumor type was ovarian ca. Preliminary safety data related to cycle 1 are available in the first 9 pts: no DLTs were observed in Cohort 1 (R 2.5 mg/day) but 3 of 6 pts temporarily required R discontinuation due to G2 mucositis (respectively for 5, 8 and 10 days). The administration of R was therefore changed from continuous to intermittent for 21 days q28. Also in Cohort 2 (R 5 mg/day) no DLT were observed and Cohort 3 (7.5 mg/day) is ongoing. The most frequent grade 1−2 treatment related toxicities observed during cycle 1 were: mucositis (78%), skin toxicity (33%), fatigue (22%). No >G1 hematological toxicity was reported. A confirmed partial response was observed in 1 pt with ovarian ca. in Cohort 1. The disposition of D was studied in 6 pts at cycles 1 and 2: total plasma exposure to PLD was unaffected by R.
Conclusions: Preliminary results suggest that the combination of R and PLD is feasible but that a discontinuation interval of R administration is needed to keep an adequate daily dose. No major pharmacokinetic interference of R on PLD disposition was observed.

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The serine 2481-autophosphorylated form of mTOR directly binds the mitotic apparatus to control breast cancer cell proliferation: A new role of mTOR as mitotic checkpoint in cell cycle progression

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Background: The widely accepted role of mTOR is primarily to sense and integrate nutrient and growth factor signals to regulate protein synthesis. Although mTOR is also recognized as a regulator of cell cycle progression and cell proliferation, the molecular mechanisms by which mTOR might mediate these events have been poorly defined. We here sought to analyze whether changes in the sub-cellular compartmentalization of mTOR and of its autophosphorylated form (*i.e.* mTOR^{Ser2481}) occur after acquisition of auto-resistance to the anti-HER2 antibody trastuzumab (Tzb) in breast cancer (BC) cells.
Materials and Methods: Two pools of Tzb-conditioned SKBR3 BC cells optimally growing in the presence of >100 ug/ml Tzb (SKBR3/TzbR POOL1 and POOL2) were obtained by continuously culturing HER2-dependent SKBR3 cells in the presence of high-doses of Tzb for more than 12 months. Changes in the sub-cellular compartmentalization of mTOR/mTOR^{Ser2481} were monitored using a high-resolution, automated confocal imaging system (BD Pathway™ Bioimager).
Results: A homogenous cytoplasmic/perinuclear distribution of total mTOR was observed in Tzb-sensitive BC cells. Perinuclear expression was somewhat increased in a dotted-manner in Tzb-resistant pools. Surprisingly, mTOR^{Ser2481} was found to be massively accumulated within nuclear dots displaying dynamic expression during the M phase. mTOR^{Ser2481} dots showed a close association near and between separating chromosomes and also decorating the contractile ring in BC cells undergoing cytokinesis. In the case of BC cells at the anaphase stage of mitosis, eye-catching mTOR^{Ser2481} dots could be seen symmetrically splitting in the region of the mitotic spindle. The number of immunopositive condensations of mTORSer^{2481} directly related with the percentage of mitotic cells in the absence of Tzb treatment (2-fold higher in Tzb-resistant BC cells). Moreover, the rate of mTOR^{Ser2481}-immunolabeled dividing cells was significantly decreased in Tzb-treated SKBR3 parental cells whereas it remained unaltered in Tzb-treated SKBR3/TzbR POOLs.
Conclusions: mTOR-dependent regulation of the rate of cell cycle progression has been considered a secondary consequence of the mTOR's primary function (*i.e.* to make cycle progression dependent on a sufficient level of cell growth). We now propose that mTOR^{Ser2481} is a novel mitotic checkpoint that directly controls BC cell proliferation through its previously unrecognized capacity to bind the mitotic apparatus.

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Phase II study of MTOR-inhibitor RAD001 and erlotinib for advanced, gemcitabine-refractory pancreatic cancer

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Background: PI3-kinase/Akt pathway is constitutively activated in pancreatic cancer. The sensitivity of pancreatic cancer cell lines to erlotinib may be dependent on inhibition of this pathway. RAD001 selectively inhibits mTOR, a key protein kinase that is activated via the PI3-kinase pathway. Erlotinib and the mTOR inhibitor rapamycin produced a synergistic anti-tumor effect in preclinical studies. Prior phase I study identified both a weekly and daily phase II RAD001 dose when administered with daily erlotinib.
Methods: Forty adult patients with previously treated stage IV pancreatic adenocarcinoma, ECOG PS 0−1 with adequate hematologic, hepatic and renal parameters and measurable disease will be enrolled. Each cycle lasts 28 days and consists of RAD001 30 mg weekly and erlotinib 150 mg once daily. Staging radiological studies are performed every 8 weeks. Pre-treatment tumor biopsy samples are assessed for PTEN, total and activated Erk, Akt, and mTOR expression. Primary study endpoint: 6-month survival. Secondary: Progression-free survival and correlation of biomarkers with outcome.
Results: 13 patients have been enrolled; 10 males, all received prior gemcitabine. A median of one cycle has been administered (range 1−2). There was one grade 5 toxicity, possibly related. Grade 3 toxicities: diarrhea (n = 1), cholangitis (n = 3), fatigue (n = 1). Grade 2 toxicities: pneumonia (n = 2), dehydration (n = 2), nausea (n = 2), mucositis (n = 2), rash (n = 2). Progressive disease occurred in 3, CA 19−9 improvement (<50%) in one. There were 4 hospitalizations, 3 for cholangitis and sepsis. Cholangitis occurred in presence of biliary stents; these patients are now receiving prophylactic quinolones. Interim analysis will be conducted after enrolling 16 patients.
Conclusions: The immunosuppressive properties of RAD001 may predispose patients with biliary stents to cholangitis. These patients can be considered for antibiotic prophylaxis. Updated clinical and correlative data will be presented at meeting.

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Vorinostat significantly enhances the antitumor activity of temsirolimus in renal cancer

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Background: Mammalian target of rapamycin (mTOR) is a critical kinase that is involved in the regulation of protein translation, nutrient uptake and autophagy. mTOR is frequently activated in cancer due to constitutive

signaling by phosphatidylinositol-3-kinase (PI3K) or Akt, two of its important upstream regulators. Considering this, mTOR is a very attractive target for pharmacological inhibition in cancer therapy and several mTOR inhibitors are at various stages in development. One important consequence of mTOR inhibition is the abrogation of HIF1α translation, which leads to the shutdown of the VEGFR and PDGFR signaling cascades and the disruption of angiogenesis. Since HIF1α stabilization frequently occurs in renal cancer, the mTOR inhibitor temsirolimus has exhibited promising anticancer activity for the treatment of this malignancy. Despite this, drug resistance continues to be a major obstacle and there is a major focus on the identification of novel therapeutic strategies to improve clinical outcomes. Here we demonstrate that the histone deacetylase (HDAC) inhibitor vorinostat significantly enhances the anticancer activity of temsirolimus in vitro and in xenograft models of renal cancer.
Materials and Methods: The anticancer efficacy of the temsirolimus and vorinostat combination was determined by MTT and clonogenic assays in a panel of nine renal cancer cell lines. We further investigated the antitumor activity of this therapeutic combination in vivo in two xenograft models of renal cancer. Immunohistochemistry was conducted to evaluate the effects of the drug combination on angiogenesis.
Results: Temsirolimus exhibited varying degrees of in vitro efficacy in the nine renal cancer cell lines tested. In spite of this, vorinostat sensitized all nine renal cancer cell lines to temsirolimus-induced death. Further investigation of a "sensitive" and "resistant" cell line in vivo demonstrated that both tumors were equally sensitive to temsirolimus. This indicates that in vitro models may not best predict the in vivo anticancer activity of this agent. Importantly, vorinostat significantly increased the anticancer activity of temsirolimus in both xenograft models evaluated. The combination regimen potently inhibited tumor cell proliferation and angiogenesis suggesting that these are two key mechanisms of action that underlie the antitumor effects of these agents.
Conclusions: Temsirolimus possessed strong anticancer activity in two different xenograft models of renal cancer. Importantly, vorinostat significantly augmented the efficacy of this agent by blocking angiogenesis and inhibiting tumor cell proliferation. A clinical trial to further investigate the therapeutic potential of this combination regimen for the treatment of renal cancer is planned.

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Preclinical evidence for the effectiveness of mTOR inhibitor, nanoparticle albumin-bound (nab®) rapamycin as an anticancer agent

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Background: The mammalian target of rapamycin (mTOR) is involved in the control of cellular growth and proliferation and is an important target in tumor therapy. Use of rapamycin as an anticancer agent has been hampered because existing oral formulations have previously shown poor solubility, low oral bioavailability, and dose-limiting-intestinal toxicity. We developed a novel albumin-bound nanoparticle form of rapamycin (*nab*-rapamycin) for intravenous administration and describe its preclinical pharmacokinetic (PK) properties and antitumor activity *in vivo*.
Material and Methods: A nanoparticle form of rapamycin was prepared using Abraxis' proprietary *nab*-technology. Repeated-dose toxicity of *nab*-rapamycin was also determined in Sprague-Dawley rats with dose levels of 0 (vehicle), 20, 40, 90, 120, and 180 mg/kg (N = 5M/5F per group) on a q4dx3 schedule. Pharmacokinetics (PK) of *nab*-rapamycin was investigated in Sprague-Dawley rats at dose levels of 1, 15, 30, and 45 mg/kg. Antitumor activity of *nab*-rapamycin was examined against breast (MX-1, N = 4) and colon (HCT-116, N = 10; HT29, N = 8) tumor models in athymic mice at a dose level of 40 mg/kg with a 3× weekly/4 week or 2x weekly/3−4 week schedule respectively.
Results: Intravenous administration of *nab*-rapamycin was well tolerated in rats at dose levels up to 90 mg/kg/dose on a q4dx3 schedule, with no significant clinical signs of toxicity, and no observed hypercholesterolemia and hypertriglyceridemia. *Nab*-rapamycin exhibited linear pharmacokinetics with respect to dose and rapid tissue distribution and was effective against all tumor models tested ($P < 0.005$), achieving a tumor growth inhibition of 71%, 81%, and 88% against HCT-116, HT29, and MX-1 xenografts respectively.
Conclusions: *Nab*-rapamycin was well tolerated at repeated doses up to 90 mg/kg in rats (540 mg/m^2) with no remarkable toxicity, displayed dose-linear PK and demonstrated effective antitumor activity *in vivo*.

New molecular targets

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CYP1A1 activation and pharmacokinetics of a novel chloromethylpyrrollolindoline with potential as a tumour selective prodrug

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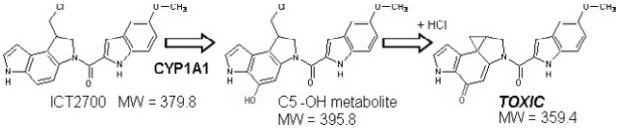
Introduction: The expression in a wide range of cancers of selected isoforms of Cytochrome P450 (CYP) that have drug metabolising activity has important implications for CYP-mediated tumour selective chemotherapy. We are exploring the use of a novel chloromethylpyrolloindoline (ICT2700) that is inactive until metabolised into a highly potent (IC50 < 1 nM) antitumour agent by CYP1A1. Here we describe the activation and pharmacokinetics of ICT2700 using CHO cell transfected with human CYP1A1 and grown as xenografts.
Materials and Methods: Female Balb/C nude mice bearing s.c. CHO xenografts overexpressing hu CYP1A1, were administered ICT2700 at a non-toxic dose of 150 mg/kg (i.p.). The pharmacokinetics of ICT2700 and formation of the active C5 hydroxy metabolite were studied in plasma and major organs including lungs, liver and tumour. Sensitive and specific analytical LC/MS methodology was developed for the analysis of ICT2700 (m/z 379.8) and the C5 hydroxy metabolite (m/z 395.8).



Results: Greater than 95% of ICT2700 was present as parent compound in tissues and plasma indicating the systemic stability of this potential prodrug in normal tissue. The remaining 5% was a complex mixture of metabolites which are non toxic in vitro. ICT2700 AUCs (0−24 h)) and Cmax was 662.8 uM h, 51.3 uM (plasma), 2209 uM h, 72.1 uM (liver), 981.3 uM h, (lung) and 221.5 uM h, 17.2 uM (tumour) respectively demonstrating excellent distribution throughout the host tissue. The C5 hydroxy active metabolite was only detected in xenograft tissue. C5 hydroxylation facilitates conversion of ICT2700 to a cyclopropyl derivative, which is the active species responsible for alkylating DNA and a potent cytotoxin. AUC and Cmax for the C5 metabolite in CHO xenografts were 2.3 uM h and 1.0 uM and are consistent with the concentrations required to produce cytotoxicity in vitro.
Conclusions: The biological stability and CYP1A1 expressing xenograft-selective activation of ICT2700 demonstrates the potential of the chloromethylpyrroloindolines as tumour activated therapies. Structural variants are being explored for activation by a variety of different CYP expressing tumours. In principle these agents could also be used as a biomarkers of CYP functional activity in clinical tumours.

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In vivo activity of SGI-1776, an orally active Pim kinase inhibitor

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A small family of serine/threonine kinases known as Pim-1, Pim-2, and Pim-3 are involved in various signaling pathways in which they act as downstream effectors and potent inhibitors of apoptosis. The Pim kinases are unique in that they are expressed as active kinases and therefore gene expression levels directly correlate to their activity in cells. Pim-1 and Pim-2 are expressed in cells of hematopoietic lineage and Pim-3 appears to be more important in cells of epithelial origin. In concordance with these different patterns of expression, Pim-1 and Pim-2 are commonly overexpressed in hematological malignancies such as leukemias and lymphomas, while Pim-3 overexpression has been noted in melanoma, pancreatic adenocarcinoma, gastric, and other epithelial tumors. Thus, the Pim kinases are interesting targets for drug development, which offer promising potential in the treatment of hematological and solid malignancies.
Utilizing the published Pim-1 crystal structure and our proprietary CLIMB™ process, we identified a subset of leads from a large, virtual library from which a series of optimal analogs were synthesized to produce SGI-1776. The IC50 of this compound in a biochemical enzyme-based assay was 7 nM for Pim-1, 69 nM for Pim-3, and 363 nM for Pim-2. Cell-based activity,