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Induction of survivin expression via activation of insulin-like growth factor-1 receptor/epidermal growth factor receptor heterodimer: a novel resistance mechanism of EGFR tyrosine kinase inhibitors in non-small cell lung cancer

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Background: The role of EGFR signaling pathway in tumor progression has long been appreciated. However, the overall response rate to EGFR tyrosine kinases (TKIs) is low and the causes of resistance to these drugs are poorly defined. This study was designed to investigate the mechanisms mediating resistance to the drugs.
Methods: The antitumor activities and action mechanisms of EGFR inhibitors (erlotinib, gefinitib, cetuximab), single or in combination with Insulin-like growth factor-1 receptor (IGF-IR) inhibitors, were assessed *in vitro* in a subset of non-small-cell lung cancer (NSCLC) cell lines by the MTT assay, flow cytometry-based TUNEL assay, anchorage-dependent and -independent colony formation, metabolic labeling, coimmunoprecipitation, and northern and western blot analyses and *in vivo* in animal models. EGFR and IGF-1R expression was assessed in lung tissue samples from patients with NSCLC.
Results: EGFR TKIs inhibited the proliferation and anchorage-dependent and -independent colony-forming abilities of NSCLC cells by inducing apoptosis only when IGF-1R signaling was blocked. Treatment with EGFR TKIs, but not with the EGFR antibody, induced EGFR:IGF-1R heterodimerization on cell membrane and activation of the IGF-1R, resulting in the stimulation of PI3K/Akt/mammalian target of rapamycin (mTOR) pathway, promoting the *de novo* protein synthesis of survivin and EGFR, resulting in the survival of NSCLC cells. Inhibition of IGF-1R activation, suppression of mTOR-mediated protein synthesis, or knockdown of survivin expression abolished resistance to the EGFR TKIs and induced apoptosis in NSCLC cells *in vitro* and *in vivo*. The majority of patients with EGFR-overexpressing NSCLC had correlatively high levels of IGF-1R in tumors compared with those in normal counterparts.
Conclusions: IGF-1R activation interferes with the antitumor activity of EGFR TKIs and IGF-1R expression may serve as a predictor for EGFR TKI resistance in NSCLC. IGF-1R-targeting combination treatment is required when EGFR TKIs are considered as therapeutic strategies for NSCLC patients.

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JG3, a novel heparanase inhibitor simultaneously targeting bFGF, combats tumor angiogenesis and metastasis

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Background: Heparanase has become a tractable and highly attractive target in cancer therapy. A challenge is thus encouraged to develop the appreciable potent heparanase inhibitors with better pharmacological profiles. Here, we report that JG3, a novel marine-derived oligosaccharide, stood out as a potential substrate-based heparanase inhibitor.
Material and Methods: The heparanase activity was determined by FITC-HS-based HPLC chromatography. The binding kinetics profiles and the binding structural motifs were characterized by surface plasmon resonance. The release of bFGF from ECM was determined using ELISA assay. The *in vitro* and *in vivo* angiogenesis was assessed via endothelial cell proliferation and migration, rat aortic ring and chicken chorioallantoic membrane methods. The *in vivo* angiogenesis and metastasis were evaluated in both murine B16F10 experimental lung metastasis model and human breast cancer MDA-MB-435 cells orthotopically xenografted athymic mouse model.
Results: JG3 significantly and concentration-dependently inhibited heparanase enzymatic activity in cell-free system by specifically binding to the KKDC and QPLK epitopes on heparanase, yielding an IC_{50} value of 6.55 ng/ml. In particular, LMW heparin, but not other glycosaminoglycans (GAGs), competitively inhibited the interaction of JG3 with heparanase. Further *in vitro* studies demonstrated that JG3 suppressed heparanse-driven invasion of both NIH-3T3 cells stably expressing heparanase and MDA-MB-435 human breast cancer cells. In addition, JG3 abolished the release of HS-sequestered bFGF from the subendothelial ECM, and repressed its subsequent angiogenesis. Moreover, JG3 was capable of inactivating bFGF-induced FGFR and ERK1/2 phosphorylation, and blocking bFGF-triggered angiogenic events by directly binding to bFGF via heparin-binding domain. Collectively, JG3 combated lung metastasis in a murine B16F10 experimental metastasis model as well as lung metastasis and angiogenesis of MDA-MB-435 orthotopic xenografts in athymic mice, accompanied by a potent suppression of primary tumor growth.
Conclusions: Together, the *in vivo* angiogenesis and metestasis inhibition of JG3 may be the comprehensive reflection of two defined mechanisms but serving the same outcome: namely, JG3 simultaneously blocked heparanase activity as a non-cleavable substrate mimetic of heparan sulfate and limited the availability of HS-binding growth factor bFGF as a competitive inhibitor. These findings favorably suggest that JG3 should be considered as a promising candidate agent for cancer therapy.

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Telomere Damage promotes antitumoral activity of the G-quadruplex ligand RHPS4

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Background: G-quadruplex (G4) ligands were initially designed to counteract telomerase action at telomeres. Surprisingly, their antiproliferative effects can occur in telomerase-negative cells and follow kinetics that cannot be merely explained by telomere shortening, suggesting that these compounds affect other pathways, not necessarily related to telomere biology.
Materials and Methods: TIF (Telomere dysfunction-Induced Foci) index, defined as foci of DNA damage response factors that coincide with TRF1 has been calculated by confocal microscopy using antibodies against endogenous proteins. Antitumoral activity of RHPS4 has been evaluated by i.v. treating xenografted mice with RHPS4 at 15 mg/kg for fifteen consecutive days. *In vivo* pharmacodynamics monitoring of RHPS4 effects has been performed in tumor sections by analysis of telomere length, apoptosis, proliferation and telomere damage response.
Results: We demonstrate that the G4 ligand RHPS4 triggers a rapid and potent DNA damage response at telomeres with the formation of several telomeric foci containing phosphorylated H2AX, Rad17 and 53BP1. This phenomenon is PI3 kinase-dependent, results from delocalization of POT1 and is antagonized by the overexpression of either POT1 or TRF2. *In vivo*, RHPS4 is highly active as a single agent by inducing telomere injury and apoptosis. Tumor inhibition is accompanied by a strong DNA damage response and tumors overexpressing either POT1 or TRF2 are completely resistant to the treatment.
Conclusions: The data reported in this paper provide evidences that the G4 ligand RHPS4 is a telomere damage inducer and that telomere disruption selectively triggered in malignant cells results in a marked anticancer effect. They further validate telomeres as very promising therapeutic targets and identify RHPS4 as a strong candidate for clinical application. The combined use of G4 ligands and TRF2 or POT1 inhibitory molecules may have synergistic effect in tumor response offering new opportunity to cancer therapy.

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Elucidation of additional targets of the thioredoxin inhibitor PMX 464

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4-(Benzothiazol-2-yl)-4-hydroxycyclohexa-2,5-dienone (PMX 464), a novel antitumour agent discovered at the University of Nottingham, UK and licensed to Pharminox Ltd exhibits potent and selective growth inhibitory effects against certain colon, breast and renal carcinoma models *in vitro* and in *vivo*. PMX 464 targets thioredoxin (Trx), binding the active site cysteine residues and inhibiting protein disulphide oxidoreductase activity dose dependently (IC_{50} 3 μM). Herein we describe experiments undertaken to challenge the selectivity of PMX 464, explore downstream consequences of Trx inhibition and aid further molecular target elucidation of PMX 464.
PMX 464 is not a promiscuous cysteine sulfhydryl inhibitor: in the presence of PMX 464 (1 μM, 10 μM) the activity of ficin (a cysteine protease of the papain family) is not compromized. Indeed, the S-S interatomic distance (3.9 Å) in the Trx active site affords sulphur atoms disposed to attack the electrophilic β-carbon atoms of the cyclohexadienone pharmacophore. Trx is a negative regulator of apoptosis signal-regulating kinase (ASK-1); its overexpression in tumors correlates with tumor aggression and resistance to therapy. Active site cysteines (C32 and C35) of reduced Trx bind to

the N-terminal region of ASK-1. When activated in response to various cytotoxic stresses (e.g. H_2O_2, TNF-α, UV light, heat shock) ASK-1, a MAPKKK, can activate both the c-Jun N-terminal kinase (JNK) and p38 MAP kinase pathways leading to a variety of cellular responses including apoptosis. Immunoprecipitation of Trx from lysates of control or treated HCT116 cells has shown that PMX 464 triggers dissociation of ASK-1 from Trx. Futhermore, by using antibodies specific for their phophorylated forms, JNK and P38 activation, are observed.
In order to investigate *in vitro* binding of cellular proteins, a carboxylate analog was immobilized to a solid media leading to identification of peroxiredoxin (Prx) as a molecular target. Prxs act as antioxidants and also regulate H_2O_2-mediated signal transduction, possessing a strictly conserved catalytic cysteine-SH (thiol). Overexpresion of Prx, detected in several cancers correlates with resistance to apoptosis induced by radiation or cisplatin.
In conclusion, perturbation of events downstream of Trx inhibition by PMX 464 has been detected. PMX 464 is not an indiscriminate thiol inhibitor, however, additional molecular targets such as Prx, involved in redox regulation have been identified.

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Phase I study of Amplimexon™ (imexon, inj.) in patients with advanced solid tumors and lymphomas: final report

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Background: Amplimexon (AMP) is an iminopyrrolidone agent, which causes cancer cell kill by inducing mitochondrial injury, cytochrome C leakage and apoptosis. We report here the final results from the phase I trial of Amplimexon in patients with advanced solid tumors and lymphomas.
Methods: The purpose of this trial was to establish safety and maximally tolerated dose of AMP and to investigate pharmacokinetic (PK) and pharmacodynamic (PD) parameters on this schedule. AMP was administered as a 30 min IV infusion, daily X 5, every 14 days. The dose was escalated from 20 up to 1000 mg/m^2.
Results: A total of 49 patients were treated. The MTD was established as 875 mg/m^2 dose. Dose limiting toxicities at 1000 mg/m^2 included grade 3 abdominal pain and grade 4 neutropenia. Common grade 1–2 toxicities included constipation, nausea, fatigue, anemia and anorexia. The systemic clearance of AMP averaged 160mL/min/m^2 at the MTD of 875 mg/m^2. The plasma half life was 95 minutes and the Cmax was 53 ug/mL. This yielded an AUC of 5517 min ug/mL and a Vd ss of 19.1 L/m^2. There were no differences in clearance on day 1 versus day 5, and for the different dose levels of imexon. Pharmacodynamic studies showed that plasma cystine, the Cys-Cys dimer, decreased in a dose-dependant fashion at doses $\geqslant$750 mg/m^2, with a 30% decrease noted 8 hours after the 875 mg/m^2 infusion ended. Other plasma thiols were unchanged by AMP. A patient with a refractory follicular Non-Hodgkin lymphoma achieved a partial response and 10 patients with other solid tumors achieved stable disease for >3 months.
Conclusions: AMP could be safely administered at 875 mg/m^2/d dose and has demonstrated encouraging antitumor activity in this phase I study. Phase II studies of AMP in patients with both epithelial and lymphoid malignancies are warranted.

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A population pharmacokinetic/pharmacodynamic model for the hematological effects of BI 2536 in cancer patients

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Background: Myelosuppression is usually one of the principal dose-limiting toxicities observed in patients treated with anticancer drugs. In this context, the population pharmacokinetic/pharmacodynamic (PK/PD) modelling approach has been shown to be an excellent tool to explore the drug response behaviour under a variety of dosing regimens, making the dose selection process less empirical. BI 2536 is a novel highly potent and specific inhibitor of the serine-threonine Polo-like kinase 1 (Plk1), a key regulator of cell cycle progression. Neutropenia as a mechanism-related toxicity indicates target inhibition in vivo and was the dose limiting toxicity observed in advanced cancer patients. The objective of the population pharmacokinetic/pharmacodynamic analysis was to develop a model that describes the haematological effects of BI 2536 and can serve as a tool to predict the influence of dose and schedule on hematotoxicity.
Methods: BI 2536 was administered as a 60 min intravenous infusion on day 1 of a 21 day treatment cycle (Dose levels 25–250 mg). Blood samples to determine the drug plasma concentration and the neutrophil count were taken at different time points during the 21 day treatment cycle. A semi-mechanistic model of chemotherapy-induced myelosupression (Friberg et al. J Clin Oncol 2002; 20: 4713−21) was used to describe the data. The analysis was performed using NONMEM, version V.
Results: BI 2536 BS plasma concentrations could best be described by a linear three compartment model. A moderate interindividual variability was established on clearance.
The neutrophil counts were adequately described using the semi-mechanistic model. This model allows the discrimination between system and drug related parameters. The estimates of the system related parameters obtained during analysis were similar to those reported previously for other compounds (Friberg et al).
Conclusion: The pharmacokinetics of BI 2536 were best described using a linear three compartment pharmacokinetic model. The time course of the hematological toxicity induced by BI 2536 and measured by the neutrophil cell counts was adequately described using a semi-mechanistic model developed and recently published (Friberg et al). The model developed will serve as a tool to predict hematologic side effects of further dosing schedules of BI 2536 given as a single agent or in combination with other modalities.

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Preclinical pharmacokinetic and comparative biodistribution studies of PX-866, a broad spectrum phosphatidylinositol-3-kinase (PI-3K) inhibitor, in F344 rats

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PX-866, a semisynthetic inhibitor of PI-3K, has antitumor activity as a single agent and in combination with inhibitors of EGF and VEGF signal transduction, and is in late stage pre-clinical development. Constitutive PI-3K activity is found in small cell lung cancer and in 40% of ovarian, head and neck, urinary tract, and cervical cancers. PX-866 is the result of a nucleophilic modification of the furan ring in wortmannin, conferring chemical stability and reduced toxicity. We investigated the plasma pharmacokinetics and tissue distribution in F344 rats following a large single dose of 12.5 mg/kg given IV or PO (0.5 mL of 4 mg/mL PX-866 in NS:DMA, 80:20, v:v). Following sacrifice, plasma and tissue samples were collected (5 animals/timepoint) over a span of 5 minutes to 72 hours following drug administration and immediately processed for analysis. PX-866 was extracted from plasma and tissues using either protein precipitation or tissue disruption followed by liquid-liquid extraction. Samples were quantified using LC/MS/MS in ESI+ mode (LLOQ = 0.1 ng/mL). PK parameters for PX-866 were determined fitting both two-compartment ($r^2 > 0.99$) and non-compartmental ($r^2 = 0.88$) models to the mean measured plasma concentration vs. time data. PX-866 given IV rapidly distributed with a peak plasma concentration of 12.1 ug/mL at 5 minutes and could be measured to 4 hours. Tissue distribution of PX-866 IV bolus was rapid and significant, achieving concentrations 4 times greater than concurrently measured in the plasma in highly perfused organs over the first 30 minutes following injection. IV PK parameters C_{max}, AUC, V_d, Cl, $t_{1/2}$, and MRT were 12.1 ug/mL, 1166.4 ng hr/mL, 3.2 L/kg, 10.7 L/hr/kg, 0.27 hrs, and 0.29 hrs, compared to PO values of 81.7 ng/mL, 19.2 ng hr/mL, 146.4 L/kg, 636.1 L/hr/kg, 0.16 hrs, and 0.25 hrs, respectively. Oral bioavailability of parent PX-866 was 1.64%, similar to the previously reported value of 1.05% in mice. In conclusion, PX-866 biodistribution is extensive, with rapid clearance from plasma and most major organs in F344 rats following 12.5 mg/kg IV bolus delivery. Further investigations of single and multiple dose of PX-866 in these and other species will be conducted to ascertain the drugs behavior in both rodent and non-rodent species.

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Modulation of the activity of tumour associated carbonic anhydrases for therapeutic benefit

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Carbonic anhydrases (CAs) are metalloenzymes involved in the reversible hydration of carbon dioxide to bicarbonate and various physiological