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Enhanced anticancer activity of [Tris(1,10-phenanthroline)-lanthanum(III)]-trithiocyanate (KP772; FFC24) against ABC-transporter-overexpressing cells

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Introduction: The aim of the study was to investigate the impact of common multidrug resistance (MDR) mechanisms on the activity of the recently presented (Heffeter et al. *Biochem Pharmacol.* 2006; 71(4):426−40) new anticancer lanthanum drug KP772 (FFC24) and to clarify whether drug resistance might develop during KP772 treatment.
Material and Methods: Sensitivity of P-gp-, MRP1- and BCRP-overexpressing cells to treatment with KP772 was compared with their respective parental cell models by MTT assays. Additionally, the influence of diverse MDR modulators (e.g. verapamil) was investigated. Intracellular amounts of lanthanum were measured by inductively-coupled plasma mass spectroscopy. Moreover, interaction of KP772 with P-gp was analysed using ATPase assay. DAPI, JC-1 and propidium iodide stainings were used to compare the potency of KP772 to induce apoptosis and cell cycle arrest in dependence of P-gp-overexpression. Protein expression was detected by Western blotting. Furthermore, KB 3–1 cells were stepwise selected over one year to develop resistance against the lanthanum drug.
Results: None of the tested ABC-transporter-overexpressing cell lines was found to be resistant against KP772. In contrast, these MDR cell models were hypersensitive against the drug. Consequently, none of the used MDR modulators was able to sensitise MDR cells against KP772. Unexpectedly, verapamil, cyclosporin A and dipyridamole exerted protective effects against KP772-induced cytotoxicity in several MDR cell models. For further analysis, P-gp-overexpressing KBC-1 and its respective parental cell model KB-3–1 were chosen. In KBC-1 cells, apoptosis induction and cell cycle arrest by KP772 were significantly enhanced as compared to KB-3–1 cells. Additionally, uptake studies revealed that the KP772 hypersensitivity of P-gp-overexpressing cells was not based on an enhanced drug uptake. Furthermore, selection against subtoxic concentrations of KP772 did not lead to development of KP772-resistant subclones.
Conclusion: Summarizing, our data suggest that the promising anticancer activity of KP772 is not limited but rather enhanced by the expression of MDR proteins. This implicates that emergence of acquired resistance during KP772 chemotherapy is unlikely and that KP772 might be especially suitable in second line treatment of patients which already failed chemotherapy due to resistance development.
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A Phase Ib safety and pharmacodynamic study of PXD101 alone and in combination with 5-fluorouracil in patients with advanced solid tumors

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Background: PXD101 is a low molecular weight HDAC inhibitor of the hydroxamate class. In preclinical studies, PXD101 has broad anti-neoplastic activity in vitro and in vivo, and has potential utility as monotherapy and in combination with standard chemotherapeutics for treatment of cancer. In vitro, PXD101 down-regulates expression of thymidylate synthase (TS), a target of the chemotherapeutic 5-fluorouracil (5-FU). In vivo, PXD101 plus 5-FU is more effective against human colorectal cancer (CRC) xenografts than either agent alone. High TS levels in colorectal tumors correlate with clinical resistance to 5-FU, providing a rationale for this Phase Ib study of PXD101 in combination with 5-FU.
Methods: Patients with advanced solid tumors were enrolled in sequential cohorts to test escalating doses of combination of PXD101 (300–1000 mg/m^2/d) and 5-FU (250–1000 mg/m^2/d). 5 dose levels are planned, the highest being 1000 mg/m^2/d of each drug. PXD101 was administered in all cycles, as a 30-min IV infusion 5× daily, on Days 1–5 of a 3-wk cycle. 5-FU was administered from cycle 2 on, as a continuous 96-hr IV infusion starting on Day 2 of the same 3-wk cycle. Dose escalation was guided by drug tolerability in cycles 1 and 2. Dose-limiting toxicities (DLTs) were any PXD101-related ⩾ grade (Gr) 3 non-hematologic or Gr 4 hematologic toxicities. In an exploratory analysis, PXD101 effect on TS mRNA expression in peripheral blood mononuclear cells was measured by RTQ-PCR. ECG data were collected and analyzed by a central laboratory.
Results: To date, 10 pts have been treated in 3 dose levels, receiving a median of 2 cycles of therapy (range 1–6). The 3 dose levels completed are 300, 600, and 1000 mg/m^2 PXD101, each in combination with 250 mg/m^2/d 5-FU. One pt at the lowest dose level did not complete cycle 2, and one pt at the intermediate dose level received 1000 mg/m^2/d 5-FU in an unintentional deviation from protocol. No DLTs have occurred. There was one Gr 4 pulmonary embolism; Gr 3 toxicities were fatigue (2 pts), and back pain, hypoxemia, tumor fever, anemia and hypokalemia (all 1 pt). All Gr 3/4 events were assessed as not related to drug. Gr 2 events occurring in more than 2 pts were fatigue (5), dyspnea (3), anorexia (3), dehydration (2) and diarrhea (2). In >750 ECG's collected, there were no QTcF > 500 ms, and no QTcF increase >60 ms above baseline (QTcF = Fridericia's corrected QT interval). One pt with progressive metastatic adenocarcinoma of the colon has had stable disease through 5 cycles.
Conclusions: PXD101 in combination with 5-FU has been well tolerated up to 1000 mg/m^2 PXD101 plus 250 mg/m^2 5-FU. The study is ongoing and updated safety data and TS pharmacodynamic data will be presented at the meeting.

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Differential proteomic analysis of low-level anti-microtubule resistance in acute lymphoblastic leukaemia

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Acute lymphoblastic leukaemia (ALL) is the most common childhood cancer, responsible for 30% of all childhood malignancies. Although complete remission is achievable in most patients, 20−30% will relapse, and further treatments for these patients often fail due to intrinsic or acquired drug resistance. Anti-microtubule agents are amongst the most important drugs used in ALL therapy. These agents bind to β-tubulin, disrupting microtubule dynamics and consequently induce mitotic arrest and cell death. Despite the success of these agents, drug resistance can be a significant clinical problem and better understanding of mechanisms mediating resistance is required to improve treatment outcome for ALL patients. Studies from our group previously identified microtubule alterations in ALL cell lines selected for high-level resistance to *vinca* alkaloids. It is not clear however, which protein changes occur during the early development of resistance.
To identify proteins involved in the early development of resistance, three drug-resistant human T-cell ALL (CCRF-CEM) cell lines have been established in a manner that mimicked clinical treatment, with 6 repeat exposures to the same drug concentration (3 and 6 nM vincristine, and 5 nM paclitaxel). Resulting vincristine resistant cell lines; CEM/VCR3 and CEM/VCR6 have 2.7- and 5.5-fold resistance, respectively, compared to parental CEM cells, while CEM/TAX5 has 3-fold resistance to its selecting agent, paclitaxel. Initial investigations identified distinct alterations in class II and III β-tubulin and microtubule-associated protein 4 expression. Proteomic analysis of whole cell lysates of these cell lines was then performed using pH 4–7 immobiline gradient strips and 2-dimensional difference gel electrophoresis (2-D DIGE) technology followed by LC/MS. Several differentially expressed proteins were detected in the drug resistant cell lines compared to the parental control cell line. Of these, 9 proteins were identified as being involved in cell structure, signalling, growth or defence. These proteins are currently being validated and high resolution narrow range protein separation is also being used to resolve tubulin isoforms.
Identification of novel components and response pathways of drug resistance can be exploited clinically to overcome drug resistance and improve cancer therapy. This study will further assist in accurate targeting of existing therapies and identify novel cellular targets for rational drug development.