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Defective Bim induction, downstream of glucocorticoid receptor nuclear translocation, in dexamethasone-resistant childhood acute lymphoblastic leukemia xenografts

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Glucocorticoids are critical components of chemotherapy regimens used in the treatment of many haematological malignancies. However, the development of tumor cell resistance remains a significant impediment to achieving cure in many cases. Clinical response to glucocorticoid therapy is a major prognostic factor in childhood acute lymphoblastic leukemia (ALL). The aim of this study was to develop an experimental model system to define clinically relevant mechanisms of glucocorticoid resistance in childhood ALL. An in vivo model of childhood ALL has been developed in our laboratory, using patient biopsies established as xenografts in immune-deficient NOD/SCID mice (Lock et al., Blood, 99: 4100–4108, 2002). The in vivo responses of these xenografts to the glucocorticoid dexamethasone (DEX) correlated significantly with patient outcome ($p<0.05$), which was also confirmed using short-term in vitro cytotoxicity assays (Lock et al., Blood, 103: 3905–3914, 2004). High-level DEX resistance (IC50 >1 μM) was exhibited by xenografts from 6 patients, 5 of who suffered aggressive and fatal relapses, in contrast to the exquisite sensitivity of 3 xenografts (IC50 <10 nM) derived from patients who are disease-free at least 9 years from diagnosis. DEX resistance was not associated with down-regulation of the glucocorticoid receptor (GR), or with defective ligand-receptor binding. These results contradict studies using leukemia cell lines, in which resistance is almost invariably associated with defects in the GR and impaired ligand binding. DEX resistance could not be attributed to cytoplasmic tethering of the GR, since DEX-induced nuclear translocation of the GR was comparable in all xenografts when assessed by independent methods (immunofluorescence and subcellular fractionation/immunoblotting). However, while expression of the 3 main isoforms of the BH3-only pro-apoptotic protein, Bim, was increased >5-fold within 8 h of exposure of cells from sensitive xenografts to DEX (1 μM), Bim induction was significantly attenuated in 5/6 highly resistant xenografts. Furthermore, activation of caspase-3/7 was also dramatically inhibited in resistant compared with sensitive xenografts. These results indicate that we have identified a novel and clinically relevant mechanism of glucocorticoid resistance in childhood ALL, which occurs downstream of nuclear translocation of the GR, but upstream of the signalling pathway resulting in Bim induction, caspase activation, and apoptosis.

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Activation of the P73-P53AIP1 apoptotic pathway in leukemia cells by combining arsenic trioxide (ATO) with MEK1 inhibitor

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Whereas the role of p53 in stress responses is well established, recent advances strongly support a pivotal role for the p53 paralog p73 in the execution of drug-induced cell death and chemosensitivity of cancer cells in both p53 wild type and p53 null tumors. p73 is sufficient to trigger cell death independently of the status of p53 and, conversely, p53 requires p73 to induce apoptosis. We recently demonstrated that downmodulation of ERK activity inhibits the proliferation and induces the apoptosis of primary acute myelogenous leukemia (AML) blasts. Furthermore, we showed that combination of MEK/ERK pathway inhibitors with Arsenic Trioxide (ATO) enhances ATO induced apoptosis not only in primary acute promyelocytic leukemia (APL) blasts but also in primary blasts of other AML subtypes. To better understanding the mechanisms of this successful combination, we studied the behaviour of p73-p53AIP1 pathway in NB4 (promyelocytic) and K562 (Ph+) leukemic cell lines, both of them carrying an inactive p53. Leukemic cell lines were pre-treated with PD98059 (Cell Signaling Technology, Beverly, MA) or PD184352 (kindly provided to us by Dr J. S. Sebolt-Leopold, Cancer Molecular Sciences, Pfizer Global Research & Development, Ann Arbor, MI), and then treated with ATO 1 microM (NB4) or 2 microM (K562). We observed that the combined treatment significantly increased the amount of apoptotic cells, as compared to ATO alone, in both cell lines. Molecular analysis indicated that the treatment with PD98059 or PD184352 promoted the accumulation of endogenous TAp73α (transactivation competent, pro-apoptotic and anti-proliferative isoform) and the reduction of ΔNp73 (dominant negative, antiapoptotic and pro-proliferative isoform); TAp73α transcriptional activation and its tyrosine phosphorylation, resulted in p21 up-regulation, and significant cell growth inhibition. ATO reduced ΔNp73 levels and increased p300 acetyltranferase-mediated acetylation of endogenous TAp73; TAp73 acetylation correlated well with its recruitment to the apoptotic target genes Bax and p53AIP1. The combined treatment with MEK1 inhibitors and ATO enhanced the affinity of phospho-acetylated p73 for the p53AIP1 promoter *in vivo*, as determined by chromatin immunoprecipitation experiments, leading to p53AIP1 up-regulation and further increase of apoptosis. Finally, the percentage of sub-G1 apoptotic NB4 and K562 cells after 72 hours of treatment with MEK1 inhibitor PD184352 (1 microM) and ATO was significantly diminished in cells transfected with TAp73 siRNA relative to cells transfected with control siRNA. These findings indicate that p73 is a major determinant of PD+ATO efficacy in leukemia cells carrying an inactive p53, and suggest that modulation of p73 proteins expression and/or function might represent in the future a new molecular target for leukemia treatment.

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HGS-ETR1, a fully human monoclonal antibody to the tumor necrosis factor-related apoptosis-inducing ligand death receptor 1 (TRAIL-R1) in patients with advanced solid cancer: results of a phase I trial

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Introduction: HGS-ETR1 (TRM-1) is a fully human monoclonal antibody that is agonistic to the R1 (TRAIL-R1 or DR4) receptors for TRAIL that are expressed on the surface of multiple cancer cell types. A member of the TNF ligand superfamily, TRAIL has been shown to be an important mediator of apoptosis in cancer cell lines. Promising preclinical activity of HGS-ETR1 has been observed in multiple studies.
Methods: This phase I, open-label, dose-escalation study aimed to evaluate the tolerability and toxicity profile of ⩾2 doses of HGS-ETR1 administered IV in patients (pts) with advanced solid tumors or NHL. Secondary objectives were to evaluate the pharmacokinetic (PK) profile and immunogenicity of HGS-ETR1. Pts received HGS-ETR1 every 28 days until progression or unacceptable toxicity and were evaluated weekly for toxicity. Tumor measurements were repeated after each second cycle.
Results: The 6 planned escalation levels (in mg/kg) are: 0.01; 0.03; 0.3; 3.0; 10.0; and 20.0. To date, 20 pts, 8 of them male, with a median age of 56 yrs (range, 29–81 yrs) have been entered onto the first 4 cohorts. Thirteen of 20 pts had PS of 1 and most pts had colorectal (7 pts) or ovarian cancer (5 pts). Pts received a median of 2 cycles (range, 1–8+) cycles. HGS-ETR1 has been very well tolerated with no clearly attributable toxicity other than 1 episode of gr 3 thrombocytopenia, and no dose limiting toxicity has been observed. Preliminary PK for doses up to 0.3 mg/kg are consistent with a two compartment model with first order elimination from the central compartment. At the 0.3 mg/kg dose, the mean PK results are: CL, 3.35 mL/day/kg (range, 2.31–5.02 mL/day/kg); V_1, 42 mL/kg (range, 37–52 mL/kg); V_{ss}, 70 mL/kg (52–109 mL/kg); and, $t_{1/2}$ β, 17 days (range, 9–31 days). No antibodies to HGS-ETR1 have yet been detected. No responses have yet been observed but a number of patients have had prolonged stable disease (1pt – 4 cycles; 1pt – 6 cycles; 2pts – 8+ cycles).
Conclusions: HGS-ETR1 has been well tolerated and dose escalation and accrual continues. Updated results will be presented at the meeting.

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Phase I clinical trial in patients with refractory solid tumors: the weekly 24 hour intravenous infusion of aviscumine, a recombinant ribosome-inactivating protein

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Background: Aviscumine is a recombinant E.coli-derived type II ribosome-inactivating protein targeting terminal alpha 2–6-sialylated structures (CD75s). The drug exhibits potent antitumor activity in vitro and in vivo. In a previous Phase I trial (EORTC 16002), a short t1/2 after 1 h i.v. infusion was observed.
Material and Methods: In the current Phase I study, aviscumine was administered weekly as a 24 h central i.v. infusion in patients (pts) with histologically or cytologically verified refractory solid tumors. Endpoints were safety, dose-limiting toxicity (DLT), maximum tolerated dose (MTD) and PK. Pts were at least 18 yrs old, had an ECOG PS 0–2 and adequate bone marrow, liver and renal function. DLT was any non-hematological gr 3–4 toxicity (CTC version 2.0), ANC below 500/ml for ⩾7 days, febrile neutropenia or thrombocytopenia º4. MTD was defined as dose at which >1 of maximal 6 pts had DLT in cycle 1.
Results: From February 2003 until February 2004, 14 pts (11 male, 3 female) were enrolled. Median age was 61 yrs (41–77). Tumor types

included colorectal cancer (7), soft tissue sarcoma (3), neuroendocrine tumor (1), urothelial (1) and pancreatic cancer (2). Median no. of cycles (i.e. 3 weeks) was 2 (1–8). Based on previous experience the starting dose was 6 μg/kg. DLTs were observed in 2 pts at the starting dose and consisted of an increase in AST and alkaline phosphatase in 1 pt, and elevation of gamma-GT and ALT, hypokalemia and fatigue in 1 pt. The protocol was amended and re-opened at 4 μg/kg for another 3 pts. No DLTs were observed with this dose. 5 μg/kg were tolerated well in 6 of 8 pts. 2 patients, previously exposed to mistletoe extracts, had allergic reactions on day 1 of cycle 1 and were replaced. At doses below 6 μg/kg increases of gamma-GT >50 U/l > baseline were observed in 2 pts (already gr 3 at baseline). The only other toxicity > gr 1 in >1 pt was pollakisurea (4 pts). Best response (RECIST) was stable disease in 4 pts for 4–7 cycles. PK analysis revealed plasma levels in the theoretically active range (>2 ng/ml) during the 24 h infusion.
Conclusions: The prolonged i.v. administration of aviscumine was well tolerated and feasible. The recommended dose for further clinical trials is 5 μg/kg weekly for 24 h.

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Induction of apoptosis in chronic lymphocytic leukaemia by inhibition of NF-kB and novel sulfasalazine analogues

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Chronic lymphocytic leukaemia (CLL) is caused by the relentless accumulation of B-cells through a failure to undergo apoptosis. However, the factors controlling cell survival in CLL are poorly defined. NF-kB is a transcription factor which plays a critical role in controlling cell survival in B-cells. Here we have used molecular and chemical biology approaches to investigate the role of NF-kB in CLL.
Using electrophoretic mobility shift assays, we confirmed that CLL cells contained NF-kB and demonstrated that 66% (n=18) of CLL samples underwent accelerated apoptosis following treatment with various small molecule inhibitors of NF-kB, including sulfasalazine, a drug used in the treatment of inflammatory diseases. Surprisingly, the expression of several "classical" NF-kB target genes was unaltered in cells treated with inhibitors and we used microarray analysis to identify novel candidate NF-kB target genes in CLL, including cytokines and genes involved in apoptosis control and regulation of NF-kB.
To further investigate the role of NF-kB in CLL, we have generated a series of novel derivatives of sulfasalazine. We have identified a number of compounds with significantly improved (up to 8-fold) ability to interfere with NF-kB activity, and demonstrated that these are more effective at inducing cell death in CLL cells. The compounds were also more effective at killing multiple myeloma cells, which are also dependant on NF-kB for survival.
Therefore, NF-kB is required for cell survival in the majority of CLL, but the gene targets of NF-kB in CLL may be novel. NF-kB inhibitors, including newer derivatives of sulfasalazine, may be attractive therapeutic agents for CLL.

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BRCA1 functions as a differential modulator of chemotherapy induced apoptosis

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The BRCA1 tumour suppressor gene is mutated in 5–10% of familial breast cancers and is down regulated in one third of sporadic breast cancers. We have data to suggest that BRCA1 acts as a differential transcriptional regulator of pro and anti apoptotic signalling pathways depending on the nature of cellular insult. Wildtype BRCA1 expression resulted in a 10–100 fold resistance to a range of DNA damaging agents including those that give rise to double strand breaks such as cisplatin, etoposide and bleomycin. In contrast BRCA1 induced a greater than 1000-fold increase in sensitivity to the spindle poisons paclitaxel and vinorelbine. BRCA1 had an anti apoptotic role in response to DNA damaging agents and induced apoptosis in response to spindle poisons.
In this study, three breast cancer cell models were used: the BRCA1 inducible MBR62-bcl2 cell line, the HCC1937 cell line stably reconstituted with a wildtype BRCA1 construct and the BRCA1 siRNA knockout T47D cell line model. Chemotherapy response was quantified using dose response curves, PARP and Caspase 3 apoptotic assays. In addition flow cytometry demonstrated that BRCA1 mediated a G2/M cell cycle arrest in response to both spindle poisons and DNA damaging agents. In order to further investigate these differential BRCA1 effects in response to chemotherapy, we have carried out microarray expression profiling and present our preliminary data.
We believe that this study may have implications for the management of breast cancer in those who carry the BRCA1 mutation or in those with sporadic breast cancer exhibiting low BRCA1 expression.

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Nuclear factor-kB activation in human gastric cancer: its correlation with clinicopathologic features and prognosis

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Background: Although gastric cancer is major cause mortality in Asia, genetic alterations of gastric cancer to understand the behaviors of malignant tumors are largely unknown. High level of basal nuclear factor-κB (NF-κB) activity has been suggested to be related to tumor progression in various cancers. However, little information is available on the biological significance of constitutive NF-κB activation in human gastric cancer. The purpose of this study is to clarify the clinical significance and prognostic value of NF-κB in human gastric cancer.
Material and Methods: With the nuclear staining of RelA as a marker of NF-κB activation, we sought to investigate clinicopathologic significance of NF-κB activation in 290 human gastric carcinomas placed on tissue array slides. In addition, the possible correlation of Akt activation, tumor suppressor gene expression, and Bcl-2 expression with NF-κB activation was analyzed.
Results: Increased nuclear expression of RelA was found in 18% of the tumors. The nuclear expression of RelA was higher in early stage pTNM (P = 0.019). We also found that there is a negative correlation between NF-κB activation and lymphatic invasion (P = 0.034) or lymph node metastasis (P = 0.055). NF-κB activation was positively correlated with overall survival rate of patients with gastric carcinomas. In addition, NF-κB activation was highly correlated with pAkt (P = 0.047), p16 (P = 0.004), APC (adenomatous polyposis coli; P<0.001), Smad4 (P = 0.002), and KAI1 (kangai 1; P<0.001) expression. A combined evaluation of nuclear RelA expression and pAkt expression revealed that the survival rate of patients with either a nuclear RelA-positive and/or pAkt-positive pattern was better than that of patients with a nuclear RelA-negative and pAkt-negative phenotype pattern (P<0.0001).
Conclusions: NF-κB activation, which is frequently observed at the early stage of gastric carcinoma, strongly correlated with Akt activation and a better prognosis. These findings suggest that NF-κB activation is a valuable prognostic parameter in cases of gastric carcinoma.

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von Hippel-Lindau (VHL) and p53 dependent cytotoxic effects of the proteasome inhibitor bortezomib (PS) in human renal cancer cells

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Renal carcinoma with a wild type (wt) or mutant (mt) VHL gene differ in their response to clinical treatment. Since VHL is the substrate recognition unit of a multiprotein E3 ubiquitin ligase complex, we determined the cytotoxic effect of PS, an inhibitor of the 26S proteasome, in renal cell carcinoma (RCC) cell lines with either wt VHL (ACHN and RC-13) or mt VHL (RC-26 and RC-28). Following treatment with 0.05–1 μM PS for 30 minutes and re-incubation in drug free medium for 7 days, RC-26 and RC-28 cell lines with mt VHL were significantly (p<0.05) more susceptible to the cytotoxic effects of PS as compared to ACHN and RC-13 cell lines with wt VHL. The increased cytotoxic response of RC-26 cells to PS (0.25–1 μM) correlated with induction of apoptosis (4–19%), which in the non-responsive RC-13 cell line was minimal (0.5–2%). Cell cycle traverse analysis of RCC cells treated with PS revealed that PS led to a >3-fold increase in the accumulation of cells in the G_2 + M phase (40%) in RC-26 cells, but not in RC-13 cells (9%). The increased accumulation of cells in the G_2 + M phase of the cell cycle was correlated with increased expression of the stress-response protein, p21, at 24 h in RC-26 cells. No significant increase in p21 levels was observed in RC-13 cells. Since, the cellular levels of p21 are regulated by the tumor suppressor protein, p53, we next determined whether the apoptotic response of RC-26 cells to PS was mediated via a p53-dependent pathway. For these studies the effect of down regulation of p53 by stable expression of p53 targeted si-RNA on the apoptotic response of PS was examined in RC-13 and RC-26 cells. Although >80% down-regulation of p53 was achieved in both RC-13 and