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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