obvious pattern emerged to account for these differences. There was no apparent correlation for bortezomid sensitization with the tissue of origin of the tumor cells, their sensitivity to TRAIL as a single agent, or their p53 status. A more detailed analysis of 7 human renal cancer cell lines demonstrated a clear increase in the sensitivity of 3 renal lines to TRAIL following bortezomib treatment. For 2 of 3 sensitized renal lines, c-FLIP levels were significantly reduced by bortezomib as assessed by western blotting. No changes in c-FLIP were seen in the remaining 4 renal cancer lines. In contrast, cycloheximide (CHX) sensitized all 7 human renal cancers to TRAIL, and dramatically reduced the levels of c-FLIP in all cases. Also CHX did not affect cell surface levels of the TRAIL death receptors. In addition, siRNA to c-FLIP reduced concentrations of the protein by western blotting and sensitized the human renal cancers to TRAIL This data suggests that can sensitize some human tumor cells to TRAIL-mediated apoptosis, and reduction in the levels of c-FLIP may be an important component of the molecular mechanism. Funded in part by DHSS #NO1-CO-12400

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Modulation of DNA damage induced apoptosis by the proteasome inhibitor bortezomib (PS) in human colorectal and non-small cell lung cancer cells is p53-dependent and NF-kB-independent

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Inhibition of anti-apoptotic signaling via NF-κB activation is a major mechanism of action of proteasome inhibition. However, our data in human non-small cell lung carcinoma cells (NSCLC-3) suggest that synergistic enhancement of DNA damage induced apoptosis by the proteasome inhibitors, MG-132 (J. Biol. Chem. 276: 8029, 2001) and PS is NF-κB independent. To determine the mechanism by which PS modulates DNA damage (γ-radiation or topoisomerase I inhibitor, SN-38) induced apoptosis, we evaluated the role of p53, another important signaling target of the 26S proteasome. Treatment of human colorectal carcinoma cells, either wild type, HCT-116 (p53 +/+), or p53 null, HCT-116 (p53 −/−), with 40–200 nM SN-38 for 60 min followed by 1 μM PS for 30 min (SN-38 → PS) led to a synergistic increase in SN-38-induced apoptosis only in HCT-116 (p53 +/+) cells. This result suggests that enhancement of SN-38 induced apoptosis by PS involves a p53-dependent pathway. The functional role of p53 was confirmed in HCT-116 (p53 +/+) cells or NSCLC-3 cells transfected with p53 targeted siRNA in which SN-38 → PS treatment did not lead to enhanced apoptosis. Interestingly, treatment with PS followed by SN-38 (PS → SN-38) led to an antagonistic effect. Enhanced apoptosis was accompanied by increased accumulation of p53, including higher molecular weight ubiquitinated species, in SN-38 → PS compared to PS → SN-38 treated cells. Analysis of sub-cellular distribution revealed significantly higher levels of p53 in the cytosolic fraction in SN-38 → PS treated cells. In contrast, p53 was primarily localized to the nucleus in PS → SN-38 treated cells. Increased accumulation of p53 in SN-38 → PS treated cells correlated with persistent inhibition of proteasome activity (up to 16 h) in these cells compared to PS → SN-38 treated cells in which proteasome inhibition was observed only for 3h. Analysis of p53 dependent downstream effectors of the synergistic apoptotic response revealed the down-regulation of survivin transcript and protein in HCT-116 or NSCLC-3 cells, only following SN-38 → PS treatment. The role of survivin in the apoptotic response was confirmed in cells transfected with dominant-negative threonine34alanine mutant survivin or survivin targeted siRNA in which significantly ($P<0.05$) higher apoptosis was observed with SN-38 → PS compared to PS → SN-38 treatment. These results demonstrate that p53 is a key regulator in NF-κB independent sensitization of DNA damage-induced apoptosis by bortezomib.

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HGS-TR2J, a human, agonistic, TRAIL receptor 2 monoclonal antibody, induces apoptosis, tumor regression and growth inhibition as a single agent in diverse human solid tumor cell lines

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Introduction: Tumor necrosis factor related apoptosis-inducing ligand, (TRAIL) death receptors are expressed on the cell surface of many human tumor cells and activation by the ligand, TRAIL, induces programmed cell death. We have developed, in collaboration with Kirin Brewery Co., HGS-TR2J (also known as KMTR-2), a human, agonistic, TRAIL receptor 2 (TRAIL-R2) monoclonal antibody (mAb), for use as therapeutic treatment for human cancer.

Methods: We assessed the in vitro and in vivo efficacy of this human mAb in NSCLC (non-small cell lung cancer), ovarian and colon tumor cell lines in cytotoxicity assays and xenograft tumor models. Several NSCLC, ovarian and colon tumor cell lines were examined for TRAIL receptor expression by flow cytometry and sensitivity to HGS-TR2J in vitro. Multiple in vivo xenograft experiments were used to evaluate the activity of HGS-TR2J as a single agent in these diverse solid tumor types. Immunohistochemical analysis for apoptotic cells was performed on tumors from HGS-TR2J treated animals to confirm the induction of programmed cell death in xenograft tumors.
Results: A majority of the cell lines in each tumor type expressed high levels of TRAIL-R2 on the cell surface and displayed moderate to significant sensitivity (50–90% cell death) to HGS-TR2J in vitro. Two NSCLC (H2122 and H460), one ovarian (A2780) and one colon (COLO205) cell line, that all possessed equivalent TRAIL-R2 cell surface expression, were used to evaluate efficacy of HGS-TR2J in xenograft tumor models. In subcutaneous COLO205 and H2122 xenograft models, HGS-TR2J induced significant ($p<0.0001$) and rapid tumor regression (~80% decrease in tumor volume in 4 days) after a single 2.5 mg/kg IV dose, and persistent inhibition of tumor growth with continual weekly treatment. In contrast, in H460 and A2780 xenograft models, HGS-TR2J significantly ($p<0.001$) inhibited tumor growth but did not induce tumor regression. The difference in observed in vivo responsiveness did not correlate with in vitro TRAIL-R2 expression. In the COLO205 xenograft model the rapid decrease in tumor volume after HGS-TR2J administration was associated with a dramatic increase in intra-tumor apoptosis within 12 hours of treatment.
Conclusions: These data reveal that HGS-TR2J has significant anti-tumor activity that is associated with increased apoptosis in a range of human tumor types and settings. This demonstrates the potential of HGS-TR2J as a therapeutic for the treatment of human cancer.

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Erucylphosphocholine: molecular requirements for apoptosis induction by a membrane targeted drug

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Alkylphosphocholines (APC) have been identified as promising membrane targeted drugs with antineoplastic activity *in vitro* and in animal models. We have shown earlier that erucylphosphocholine (ErPC), the prototype of intravenously applicable APC derivatives induces apoptosis independently from caspase-8 and FADD via a mitochondrial pathway that is inhibited by over-expression of Bcl-2.
The aim of the present study was to analyse further molecular requirements for ErPC induced apoptosis and to define the role of the subcellular localisation of Bcl-2 for its inhibitory action in this process. To this end, caspase activation, alteration of mitochondrial functions and apoptosis induction was tested in cellular systems with dysfunctions of the mitochondrial death pathway and deficiency of Bax as well as in Jurkat T-lymphoma cells with over-expression of Bcl-2 in defined subcellular compartments. Expression of Bcl-2 was restricted to the outer membrane of the mitochondria or the ER by replacing its membrane anchor with the mitochondrial insertion sequence of ActA (Bcl-2/MT) or the ER-specific sequence of cytochrome b5 (Bcl-2/ER), respectively. Additionally, Jurkat cells expressing wild-type Bcl-2 (Bcl-2/WT) or a transmembrane domain-lacking mutant (Bcl-2/deltaTM) were used.
Our results show that apart from the requirement of caspase-9 ErPC-induced apoptosis was dependent on the expression of Apaf-1 and Bax. Furthermore, over-expression of Bcl-2 in the outer membranes of the ER or in the outer membranes of the mitochondria, as well as over-expression of Bcl-2 in the outer membranes of both compartments strongly inhibited ErPC-induced mitochondrial alterations, caspase activation, and apoptosis, while cytosolic Bcl-2/deltaTM was inactive. However, for efficient long-term inhibition Bcl-2 had to be present in both, the outer membrane of the ER as well as of the mitochondria.
In conclusion, our data support our previous findings that ErPC induces apoptosis via a mitochondrial death pathway and demonstrate that membrane localisation of Bcl-2 either in the mitochondria or the ER is a prerequisite for its inhibitory action on ErPC-induced apoptosis. The finding that ER-targeted Bcl-2 can strongly interfere with the ErPC-induced mitochondrial death pathway points to a cross-talk between mitochondria and ER during apoptosis signaling which is reminiscent of our recent results obtained with radiation-induced apoptosis.
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