cells were harvested in lysis buffer, RNA from each plate was extracted and analyzed using the Human Cancer PathwayFinderTM RT2 Profiler PCR Array (SuperArray Bioscience Corp). This array profiles the expression of 84 genes representative of 6 biological pathways involved in transformation and tumorigenesis.
Results: A total of 84 genes were analyzed, representing key signaling molecules in (1) apoptosis and cell senescence, (2) adhesion, (3) signal transduction, (4) angiogenesis, (5) invasion and metastasis, and (6) cell cycle control and DNA damage repair. While a number of genes in each category were modulated by SylA and/or GlbA, the strongest response to SylA was a 13-fold upregulation of IL-8 and a 6-fold downregulation of S100A4, while the strongest response to GlbA was a 19-fold upregulation of TEK and a 62-fold downregulation of E2F1 (Table 1). Interestingly, no effects on E2F1 were observed with SylA. Overall, GlbA induced stronger changes in gene expression than SylA.

Table 1

Gene name	Fold change	
	Syringolin A (SylA)	Glidobactin A (GlbA)
IL-8	+13	+15
S100A4	−6	−10
TEK	+7	+19
E2F1	0	−62

Conclusions: The recently discovered molecules SylA and GlbA, which form a new structural class of proteasome inhibitors (syrbactins), induce biologically important pathways in human neuroblastoma and regulate key molecules in all 6 functional cancer gene groupings, with strongest effects on genes that play a role in angiogenesis as well as cell cycle control and DNA repair.

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Combined therapeutic effects of bortezomib and fenretinide on Neuroblastoma cell growth, apoptosis, and angiogenesis

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Background: The proteasome inhibitor bortezomib inhibited cell growth and angiogenesis in neuroblastoma. Bortezomib has been shown to induce synergistic activity when combined with other antineoplastic agents. Here, we assayed a putative increased antitumour activity of bortezomib if delivered to neuroblastoma cells together with fenretinide, a synthetic retinoid used as potential therapeutic agent in a variety of cancers, including neuroblastoma.
Methods: Different neuroblastoma cell lines were tested for sensitivity to bortezomib and fenretinide, given alone or in different dose-and time-dependent combination schedules. Cell proliferation, cell viability and apoptosis were evaluated by measuring 3H-thymidine incorporation, trypan blue staining, DNA fragmentation and western-blot analysis. Angiogenesis was assessed by the chick embryo chorioallantoic membrane (CAM) assay. An orthotopic neuroblastoma mouse model was used to examine in vivo sensitivity.
Results: Isobologram analysis showed that treatment of neuroblastoma cells with bortezomib plus fenretinide caused a synergistic inhibition of cell growth. This inhibition was associated to marked G1 and G2/M cell cycle arrest with nearly complete depletion of S phase by the combined treatment. Neuroblastoma cell death occurs with apoptosis features via ER stress by the activation of specific genes. Tumour-bearing mice treated with fenretinide plus bortezomib lived statistically significantly longer than mice treated with each single drug. Histological evaluation and CAM analysis of primary tumors evidenced that the combined therapeutic effects were due to both increased antitumour and antiangiogenic activities.
Conclusions: Our findings provide the rationale for design a new therapeutic strategy to treat neuroblastoma based on this pharmacological combination.

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Bortezomib and Flavopiridol combination induces down regulation of Mcl-1, mitochondrial permeabilization and cell death in ALCL cells

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Bortezomib is the first proteasome inhibitor to have shown anti-tumour activity in both solid and haematological malignancies and to be approved for clinical use. Anaplastic Large Cell Lymphoma (ALCL) is a high-grade T-cell non Hodgkin lymphoma, characterized by high mitotic index. We previously demonstrated that Bortezomib (BZ), in a concentration range between 20 and 100 nM, leads ALCL cells to apoptosis, despite the up-regulation of the anti-apoptotic protein Mcl-1.
Here we demonstrated that citostatic concentrations of the pan-cyclin-dependent-kinase (cdk) Flavopiridol (FP), in combination with non toxic concentrations of BZ (3.75 to 5 nM) induces cell death in all three ALCL cell lines examined.
Changes in cell cycle progression were measured by FACS and immunoblotting analyses. Apoptosis was measured by assessing mitochondrial activity at a biochemical level (MTT test, annexinV, DiOC6), and homeostasis by protein regulation at a transcriptional (QRT-PCR) and post-transcriptional levels (immunoblotting, immunofluorescence).
We found that when used alone, FP inhibited cdk-dependent phosphorylation of retinoblastoma protein and induced G1 cell cycle arrest at 24 h, together with p21WAF and cyclin D3 up-regulation, while BZ downregulated S phase and increased expression of p27KIP cdk-inhibitor. However, only when used in combination, FP and BZ, induced apoptosis in ALCL cells, which increased over time ($\geqslant$60% at 48 h) together with reduced DiOC6 up-take into mitochondria (~60% of control cells). This was confirmed by the release of cytochrome-C from mitochondrial membrane inner space and the activation of pro-apoptotic Bax protein 24 h after co-administration of FP and BZ. Loss of mitochondria membrane potential correlated with reduction of Mcl-1 protein expression and transcription, most likely because of FP-dependent inhibition of RNA Polimerase II. Furthermore, Mcl-1 down-regulation caused activation of pro-apoptotic Bak, normally bound to Mcl-1 in inactive state.
In conclusion, we demonstrated that combined administration of FP and BZ in ALCL cells, at subtoxic concentrations, caused cell cycle arrest with a significant reduction of cells in S phase at early time points, followed by induction of intrinsic apoptosis through activation of Bax, release of cytochrome-C and down-regulation of anti-apoptotic protein, Mcl-1.

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NPI-0052 (a 2nd generation proteasome inhibitor) Phase 1 study in patients with lymphoma and solid tumors

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Background: NPI-0052 is a novel 20S proteasome inhibitor that is being evaluated in clinical trials as a single agent and in combination with other oncology therapies in patients with solid tumors, leukemia, lymphoma and myeloma. NPI-0052 has a novel structure and preclinical data suggest the following:
– unique proteasome inhibition profile
– unique signal transduction profile
– improved toxicology profile
– improved therapeutic ratio
Materials and Methods: Patients with solid tumor or lymphoma were treated with NPI-0052 administered weekly, for 3 weeks in 4-week cycles in this 3+3 design dose escalation study. The dose of NPI-0052 was escalated in 50–100% increments. In addition to regular safety monitoring, proteasome inhibition (PI) (baseline, D1−2, D8, D15−17, D22 and D29) and PK (D1 and D15) were assayed in blood. Once a Recommended Phase 2 Dose (RP2D) is identified, an RP2D cohort of up to 20 patients (10 lymphoma and 10 solid tumor) will be enrolled.
Results: A total of 29 patients have been enrolled at doses ranging from 0.0125 mg/m^2 to 0.375 mg/m^2 for up to 12 cycles without reaching an MTD. The most common adverse events include fatigue, nausea, constipation and back pain. SAEs reported as potentially related include MRSA sepsis