DT into a promising new anti-tumor agent of potential interest for further oncological/pharmacological investigation.

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The combination of ET-743 and Irinotecan is active in preclinical models in rhabdomyosarcoma

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Background: ET-743 is a marine natural product that has shown activity in early clinical trials in adults. Response were observed in soft tissue sarcoma and ovarian cancer refractory to previous chemotherapy. This study was designed to investigate the effect of the combination ET-743 with the topoisomerase I inhibitor Irinotecan in the rhabdomyosarcoma cell line TE671 and in xenografts derived from this cell line.
Methods: ET-743 was combined with either Irinotecan or its active metabolite SN38 in the sequential schedule ET-743 (1 h exposure) followed by Irinotecan or SN38 (24 h exposure) and in the reverse sequence. Cell growth inhibition exerted by each drug and by their combination was assessed utilising the clonogenic assay. Dose-effect relationships were analysed by the combination index-isobologram method. We also investigated the antitumoral effects of the combination of ET-743 and Irinotecan in TE671 xenograft bearing mice. ET-743 and Irinotecan were administered at the doses of 0.2 mg/kg/d and 20 mg/kg/d respectively and drug treatments were delivered every 4 days for 3 times.
Results: The sequence ET-743 followed by Irinotecan was synergistic at the IC70 and IC50 doses, additive at lower doses (IC30). The reverse sequence (Irinotecan followed by ET-743) was synergistic at the IC70 dose and additive at lower doses. Averaging the results of independent experiments, the combination of ET-743 followed by Irinotecan resulted slightly more effective than the reverse sequence (combination Index at IC50:0.89 vs 1.07), but both were close to the additivity level. Similar results were obtained when SN38 was used in combination with ET-743. In vivo studies showed that ET-743 and Irinotecan as single agent were only marginally active, whereas the combination produced a significant antitumor effect: tumor weight inhibition (TWI) and Log Cell Kill (LCK) were significantly higher (TWI:85%, LCK: 0.91) after the combination of the two drugs than after each drug given alone (40% and 0.321 for ET743 and 47% and 0.521 for Irinotecan, respectively). No difference in weight loss was observed after treatment with the combination compared with the individual agents.
Conclusions: These results suggest that the combination of ET-743 and Irinotecan should be assessed in clinical trials for the treatment of rhabdomyosarcoma. Supported by AIRC, FOP, CNR, Italian Ministry of Health

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Distinct effects of proteasome inhibition by a novel inhibitor in lymphoid cells

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The 20S proteasome represents a novel target for cancer therapy because it controls the degradation of numerous key substrates including cell cycle regulatory proteins and apoptosis related proteins. Strategies to inhibit the 20S proteasome have been successful in multiple myeloma, using the recently FDA approved compound, bortezomib (Velcade). NPI-0052 is a novel, orally active, non-peptide small molecule proteasome inhibitor, which was discovered during the fermentation of a new marine Gram-positive actinomycete, *Salinospora* sp. Unlike bortezomib which only inhibits the chymotrypsin-like activity of the proteasome, NPI-0052 inhibits the chymotrypsin-like as well as the trypsin-like and caspase-like activities of human erythrocyte derived 20S proteasomes with EC_{50} values in the picomolar to nanomolar range. NPI-0052 is also a potent regulator of NF-kB activation and TNF-α synthesis in a murine macrophage-like cell line. In the present study we examined apoptosis induction by NPI-0052 in leukemia cell lines and patient specimens. NPI-0052 demonstrated varying degrees of apoptosis in three leukemia cell lines representative of AML, (ML-1), ALL, (Jurkat), and CML, (K562). All three cell lines underwent DNA fragmentation as measured by propidium iodide staining and subsequent FACS analysis upon addition of NPI-0052 doses ranging between 1 nM to 10 μM. In addition, treatment of Jurkat cells with NPI-0052 resulted in a dose related activation of caspase-3 and the cleavage of poly ADP-ribose polymerase (PARP). Closer examination of the mechanism of apoptosis induction revealed that lymphocytes may be more susceptible to modulation by caspase-8 and pro-oxidant production. When combined with an inhibitor specific for caspase-8 (IETD-fmk), Jurkat cells were protected against NPI-0052 induced apoptosis whereas ML-1 and K562 cells were not. Similarly, the antioxidant, N-acetyl cysteine (NAC), elicited a cell line dependent protection against apoptosis when combined with NPI-0052. NAC dramatically protected the Jurkat cells from apoptosis in combination with NPI-0052, but surprisingly, ML-1 were not protected by NAC. Direct examination of ROS production revealed increases in peroxide and superoxide production following a four hour exposure to NPI-0052 in cell lines of lymphocytic origin. CLL and Ph+ ALL patient material confirmed that lymphocytes from these patients are sensitive to antioxidant inhibition of NPI induced apoptosis. In the Ph+ ALL patient, differential Ficoll was conducted to separate lymphocytes and polymorphonuclear cells. Subsequent experiments on these cell populations within the same patient revealed that lymphocytes were more sensitive to NPI-0052 induced apoptosis and that apoptosis induction relied on oxidant production, whereas non-lymphocytes did not. These preliminary findings suggest that NPI-0052 induces apoptosis in leukemia cells, however the mechanisms governing apoptosis induction vary in cells of nonlymphoid versus lymphoid lineage.

Natural products

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In vitro cytotoxic activity of NBT-272, a novel quassinoid analog

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Seventy-six proprietary quassinoid analogs were designed and screened for cytotoxic potency in order to improve upon the cytotoxic effects of the quassinoid bruceantin, which selectively inhibits peptidyl transferase, polyribosomes, peptide chains, and protein synthesis. In turn, these effects are associated with decreased expression of C-myc, (Cuendet, M. *et al* Clin Ca Res 2004), diminished DNA synthesis, and reduced cell cycling beyond the G1/S transition point. Bruceantin, paclitaxel, and the quassinoid analogs were screened in the neuroblastoma cell line SK-N-AS which lacks N-myc over-expression, MDR-1, and which permits reproducible drug responses. Twenty-four hours after adding 4000 SK-N-AS cells into each well of a 96 well plate 0.01–1.0 ug/ml of analog was added to sextuplicate wells. After 1 or 24 hours, cells were washed with fresh medium and incubated for 5 days in drug-free medium. Cell viability was assayed with MTT. Cytotoxicity was calculated by comparing the mean O.D.s of sextuplicate wells containing treated or control cells. The most potent analog from the initial screening, NBT-272, was evaluated in other cell lines and in combination at ED_{50} levels with paclitaxel (PTX). NBT-272 was 2 and 10 fold more potent than bruceantin in C-myc positive intestinal cancer (LS174T) cells and in N-myc normal SK-N-AS cells respectively. It exceeded PTX potency in 7 of 10 cell lines including NCI-AR, an MDR-1 adriamycin resistant MCF-7 variant. Subtractive effects of 28–60% were observed with concomitant NBT-272 and PTX exposure. These persisted when taxane exposure preceded NBT-272 by 4 or 24 hours, and vice versa. Cytotoxic effects in both C-myc over-expressing LS174T and N-myc normal SK-N-AS cells suggest that myc inhibition is not the only intracellular target. The subtractive effects of combined NBT-272 and PTX remain unexplained. NBT-272 should have clinical utility in tumors where the cytotoxicity data show that it is more potent than paclitaxel. These include those expressing a neuronal phenotype such as desmoplastic small round cell tumor, neuroblastoma, GI tract, breast, and lung cancers.

Cancer	Cell line	ED_{50} in ug/ml 1 hour exposure		Relative potency NBT-272 vs PTX	Cytotoxicity after co-administration of PTX + NBT-272
		NBT-272	PTX		
Desmoplastic	JPNSRLT	0.03	0.3	10	
Duodenal	HUTU-80	0.04	0.08	2	
Neuroblastoma	SK-N-AS	0.05	0.09	1.8	−60%
Breast	MCF-7	0.07	0.1	1.4	
Squamous cell	SCC-25	0.1	0.09	0.9	−28%
Squamous cell	FADU	0.1	0.05	0.5	
Pancreas	PL-45	0.2	0.1	0.5	−38%
Fibroblast	BNR-11*	0.4	4.0		