drugs modulating the tumor-stromal cell interactions. In this study we have reported the results of our screening of the small molecules using microbial cultured broth. We purified the active small molecules and identified them as leucinostatins and atpenins. They significantly inhibited the growth of prostate cancer DU-145 cells in coculture with PrSC, but they only slightly affected that in monoculture. We evaluated their antitumor effects using a mouse xenograft model of coinoculation of DU-145 and PrSC. As a result, leucinostatin A suppressed the growth of DU-145 tumors more significantly than atpenin B did. Leucinostatin A was found to inhibit the expression of IGF-I in PrSC. Since these small molecules are considered to modulate the tumor-stromal cell interactions of prostate, we are now studying the mechanism of their actions.

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Solution structure of a 2:1 C2-(2-naphthyl)pyrrolo[2,1-c][1,4]-benzodiazepine (PBD) DNA adduct: molecular basis for unexpectedly high DNA helix stabilization

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The naturally occurring PBD monomers such as sibiromycin, anthramycin and tomaymycin form stable covalent adducts with duplex DNA at purine-guanine-purine sequences. A correlative relationship exists between DNA-binding affinity (as measured by enhanced thermal denaturation of calf-thymus DNA; ΔTm) and cytotoxicity for these naturally occurring compounds and a range of synthetic analogues. Sibiromycin has the highest ΔTm value (16.3ºC) of the naturally occurring PBDs reflecting a number of favourable hydrogen bonding interactions between the molecule and DNA bases. We report here that, surprisingly, the simple synthetic C2-(2-naphthyl)-substituted pyrrolo[2,1-c][1,4]benzodiazepine monomer SG2313 (DA046) provides a ΔTm value (15.8ºC) of the same order of magnitude as sibiromycin and significantly higher than those for anthramycin (13.0ºC) or tomaymycin (2.6ºC). It is also similar in cytotoxic potency to sibiromycin which is widely regarded as the most potent naturally occurring PBD monomer. Given the structural simplicity of SG2313 compared to sibiromycin, we have investigated its unexpectedly high ΔTm using high-field NMR in conjunction with molecular dynamics to study its interaction with the DNA duplex d(AATCTTTAAAGATT)2. A 2:1 drug/DNA adduct was observed similar to that reported by Hurley et al for tomaymycin. The results show that the high binding affinity of SG2313 is due predominantly to hydrophobic (van der Waals) interactions in contrast to the hydrogen bonds which predominate in the case of sibiromycin, anthramycin and tomaymycin drug/DNA adducts. Using high resolution 2D NOESY experiments, unequivocal determination of the orientation of the SG2313 molecule (i.e., A-ring towards 3′-end of covalently bound strand) and stereochemistry at the C11-position (C11S) could be achieved. In addition, the location of the C2-naphthyl ring could be determined, indicating that it extends along the floor of the minor groove thus optimizing hydrophobic interactions with DNA functional groups and explaining the high ΔTm value. These results provide further opportunities for drug design in terms of extending planar hydrophobic groups at the C2-position of PBDs to maximize DNA binding affinity.

Paediatric – early drug development

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Pediatric Preclinical Testing Program (PPTP) evaluation of rapamycin combined with cytotoxic drugs used frequently in treatment of childhood cancer

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Background: Rapamycin (Rap) is a specific inhibitor of mTOR that has demonstrated broad-spectrum antitumor activity as a single agent against the PPTP in vivo panels of childhood tumors. Here we have extended the studies with Rap to combinations with agents used frequently in the treatment of childhood malignancies.
Methods: Rap was tested against the PPTP in vitro panel of 23 cell lines at a concentration of 10 nM alone or in combination with increasing concentrations of melphalan, cisplatin, vincristine or dexamethasone [acute lymphoblastic leukemia (ALL) models only]. Rap was tested in vivo at a dose of 5 mg/kg i.p. 5 days per week for 6 weeks for solid tumors or 4 weeks for leukemia models. Cytotoxic agents were administered at their maximum tolerated dose (MTD, approximately LD10), and 0.5 $\times$ MTD. Three measures of antitumor activity were used: (1) response criteria modeled after the clinical setting; (2) treated to control (T/C) tumor volume at day 21; and (3) a time to event (4-fold increase in tumor volume) measure based on the median EFS of treated and control lines.
Results: Combining Rap with cytotoxic agents in vitro gave predominantly <-additive or additive effects, except with dexamethasone in ALL models for which the effect was >-additive. In vivo Rap significantly increased the toxicity of cisplatin but not vincristine or cyclophosphamide. Rap combined with vincristine (MTD) was additive or >-additive in 10 of 12 models and with cyclophosphamide (MTD) was additive or >-additive activity in 8 of 9 models and antagonistic in 1 model. Cisplatin (0.63 $\times$ MTD) – Rap combination gave additive or >-additive activity in 9 of 9 models. Against the ALL panel the combination with vincristine was predominantly <-additive, while with cyclophosphamide the effect was additive or <-additive. Rap combined with dexamethasone was >-additive, additive, or antagonistic, respectively, in 3 ALL models.
Conclusions: Rap combined with cyclophosphamide or vincristine appeared superior to either single agent against several tumor models. There was little evidence that rapamycin potentiated the toxicity of these agents. Rap significantly potentiated the toxicity of cisplatin. However, the antitumor activity of Rap combined with either cisplatin administered at 0.63 $\times$ MTD or with vincristine or cyclophosphamide (both at 0.5 $\times$ MTD) was greater than that for each cytotoxic agent alone administered at its MTD in most solid tumor models.
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Pediatric Preclinical Testing Program (PPTP) evaluation of the oncolytic picornavirus, NTX-010 (SVV-001)

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Background: NTX-010 is a novel oncolytic picornavirus with antitumor activity against human cancers expressing neuroendocrine markers. The activity of NTX-010 was evaluated against the in vitro and in vivo panels of the Pediatric Preclinical Testing Program (PPTP).
Methods: The PPTP includes a molecularly characterized in vitro panel of cell lines (n = 27) and in vivo panel of xenografts (n = 61) representing most of the common types of childhood solid tumors and childhood acute lymphoblastic leukemia (ALL). NTX-010 was tested against the PPTP in vitro panel at concentrations ranging from 10^4 virus particles per cell to 10^{-4} virus particles per cell and was tested against the PPTP in vivo panels at a dose of 3×10^{12} virus particles per kg administered as a single dose via intravenous injection. Three measures of antitumor activity were used: (1) response criteria modeled after the clinical setting; (2) treated to control (T/C) tumor volume at day 21; and (3) a time to event (4-fold increase in tumor volume) measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).
Results: NTX-010 was variably active against lines in the in vitro panel with activity focused in the Ewing, neuroblastoma and rhabdomyosarcoma histologies, while no activity was observed against leukemia and lymphoma lines. NTX-010 achieved objective responses in 12 of 35 xenografts tested (34%) with objective responses in the Wilms, rhabdoid, glioblastoma, neuroblastoma and alveolar rhabdomyosarcoma panels. Activity was greatest for the neuroblastoma and alveolar rhabdomyosarcoma panels. In the neuroblastoma panel, there was 1 partial response and 3 maintained complete responses (MCRs) among 5 xenografts tested. Each of the 4 alveolar rhabdomyosarcoma xenografts tested achieved MCRs. NTX-010 was not evaluated against the ALL in vivo panel.
Conclusions: NTX-010 demonstrated activity against the PPTP's in vitro and in vivo solid tumor panels, with activity concentrated in models expressing neuroendocrine markers (e.g., NCAM1). Particularly notable was the high level of in vivo activity observed for the neuroblastoma and alveolar rhabdomyosarcoma panels. Further studies characterizing molecular predictors of response and the activity of combinations of NTX-010 with other anticancer agents are anticipated.
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