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The atypical retinoid ST1926 is synergistic with cisplatin in human neuroblastoma xenografts

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Background: Retinoic acid therapy represents an important component of treatment for residual disease of stage IV neuroblastoma after chemotherapy. The novel related adamantyl retinoid ST1926 (*E*-3-(4-hydroxy-3-adamantylbiphenyl-4-yl)acrylic acid), is a strong inducer of apoptosis with a potent anti-proliferative activity in different tumour models. We already showed that ST1926 is active in neuroblastoma preclinical models as single agent. In the present study, we address the question whether ST1926 is synergistic with other established clinical treatments of paediatric neuroblastoma.
Methods: The evaluation of multiple drug effect was carried out by SRB assay in SK-N-DZ (N-type) and SK-N-AS (S-type) cell lines selected from previous studies. Combination Index and Isobologram Analyses were used to disclose synergism or antagonism in combined treatments of neuroblastoma cells and Cisplatin, Camptothecin, Doxorubicin, Etoposide, Gleevec, ATRA and 13-cis retinoic acid. A 24 hours treatment and pre- or co-treatments, were assayed. *In vivo*, the antitumor efficacy of ST1926 and Cisplatin as single agents and in combination, were evaluated in CD1 nu/nu male mice bearing SK-N-BE(2)c and SK-N-AS xenografts. ST1926 (30 mg/Kg/day) was administered orally for two consecutive days and three consecutive weeks, whereas Cisplatin (4.7 mg/Kg/day) was administered iv once a week for three consecutive weeks. In the combined treatment, Cisplatin was administered one hour before ST1926.
Results: *In vitro*, none of the combined treatments appeared synergistic in the SK-N-AS cell line. In SK-N-DZ cells, only ATRA at low doses appeared synergistic with ST1926. Treatment schedule appeared to play an important role, as synergism was observed only when ST1926 was given before or at the same time than ATRA. *In vivo*, in SK-N-BE(2)c xenografts, Cisplatin and ST1926 did not appear effective as single agents whereas they were synergistic when administered in combination (TWI and LCK at the end of treatment, were 60% and 1, respectively). In SK-N-AS xenografts, on the other hand, the two compounds were moderately active as single agents and the combined treatment appeared significantly synergistic (TWI and LCK at day 10 after the end of treatment, were 95% and >1, respectively).
Conclusion: This study suggests that ST1926 has an interesting clinical potential as combined treatment of neuroblastoma tumors which do not respond to single therapies. These findings warrant further investigation.
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A cell-permeable dominant-negative Survivin protein as a tool to understand how Survivin maintains tumour cell survival

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Introduction: Survivin is a member of the Inhibitors of Apoptosis (IAPs) family and is expressed in most major types of cancer where it inhibits apoptosis, cell death and simultaneously promotes cell growth. In this project, a cell-permeable dominant-negative form of survivin (dNSurR9) was produced as a competitive antagonist to investigate the molecular mechanisms by which wild-type Survivin (WT-Sur) inhibits apoptosis.
Material and Methods: Recombinant dNSurR9 protein was produced in bacteria and purified by glutathione agarose chromatography. DU145 human prostate carcinoma and HUVEC human umbilical vein endothelial cells were treated with various concentrations of dNSurR9, and cell viability was analysed 12 h post-incubation using the MTS assay. Changes in mitochondrial membrane potential and caspase activities were measured, and cellular integrity was analysed by immuno-fluorescent microscopy with an anti-tubulin antibody and DAPI nuclear stain.
Results: dNSurR9 induced the activation of caspase 3, caspase 7, and caspase 9 in survivin-dependent DU145 human prostate cancer cells. In contrast, caspase 8 was not activated. Surprisingly, the addition of caspase inhibitors could not rescue DU145 cells from dNSurR9-induced cell death. Mitochondrial transmembrane potential was disrupted after dNSurR9 treatment, concomitant with an increase in cytoplasmic volume.
Conclusion: Survivin may inhibit apoptosis, in part, by inhibiting the classical intrinsic apoptosis pathway involving mitochondria and activation of caspase 9, however it also appears to inhibit a non-classical caspase-independent apoptosis pathway.

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Effects of EM-1421, a novel transcription inhibitor, on cervical intraepithelial neoplasia: results of a pilot phase I/II study

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EM-1421 is a novel transcription inhibitor that targets HPV genes E6/E7 and induces apoptosis in neoplastic cells by inhibiting the production and activation of key apoptosis inhibitors, including Survivin. This trial was designed to test the hypothesis that intravaginal EM-1421 is safe and effective in patients with cervical squamous intraepithelial lesions. Intravenous EM-1421 is also currently in clinical development for the treatment of solid cancers.
Objectives: To obtain preliminary data on safety and efficacy of intravaginal EM-1421, including lesion size, levels of the biomarkers Survivin and CDC2, pharmacokinetics of intravaginal administration, and reduction in HPV viral load.
Methods: An open label, dose escalation study enrolled women with biopsy confirmed CIN 1, 2 or 3. EM-1421 (45 or 90 mg) was physician administered directly to the cervix uteri in 3 once weekly applications. The pharmacokinetics of administration was examined on day 1 of dosing. Patients underwent colposcopic examinations, HPV testing, and cervical punch biopsy on day 1 and day 71.
Results: Recruitment ended March 30, 2006 and 7 patients were enrolled. Median age was 24 yr. No treatment related AEs were reported and there were no SAEs. Two isolated incidences of Grade 1 vaginitis or vulvar irritation occurred but were not attributed to drug. All AEs were classified as Grade 1 and resolved without sequelae. All patients were HPV positive at baseline and day 71. Histopathologies of lesions were graded as CIN 1 (n = 5), CIN 3 (n = 1) or atypical (n = 1). Analysis of lesions on day 71 by colposcopy showed stable disease in 3 pts (43%), partial response in 3 pts (43%) and complete response in 1 pt (14%). Day 1 biopsies of 5 of 6 evaluable samples showed expression of Survivin and all were positive for CDC2. In 60% of 5 evaluable biopsy pairs, immunohistochemical detection of Survivin was reduced or absent compared to baseline and CDC2 levels were unchanged. EM-1421 was not detectable in serum.
Conclusions: EM-1421 was well tolerated. No SAEs or treatment related AEs occurred. All pts had stable disease or better at the day 71 assessment, and levels of Survivin were decreased in 60% of evaluable biopsy pairs. Absence of changes in CDC2 and HPV load could be due to re-infection by the virus or a need for longer treatment. These results support further Phase II evaluation of intravaginal EM-1421 in CIN using a new higher concentration formulation and a more prolonged dosing regimen.

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In vivo bioluminescent imaging of intraperitoneal disseminated ovarian carcinoma: a quantifiable model for in vivo drug modulation

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Background: For the evaluation of in vivo efficacy of novel therapeutics, orthotopic xenograft models are of greater clinical significance than subcutaneous models. Ovarian carcinoma is presented in 70% of the patients as intraperitoneal (ip) disseminated disease. Therefore models for ip disseminated ovarian cancer are of great importance. Furthermore, based on clinical results, ip treatment for ovarian cancer is considered of major interest. Unfortunately, those models are hampered by the difficulty to monitor disease formation and progression. Visualization and quantification of the tumor by means of bioluminescent imaging (BLI) overcomes these difficulties.
Aim of the study: Firstly, to develop an ovarian carcinoma cell line with stable expression of the firefly luciferase gene and to validate in vivo BLI as a reliable non-invasive method for the assessment of ip tumor burden. Secondly, to apply this model for the in vivo evaluation of ovarian carcinoma therapy with the death ligand rhTRAIL, cisplatin and a combination of cisplatin and rhTRAIL.
Results: The ovarian carcinoma cell line A2780 was transfected with a modified firefly luciferase gene. A2780 and A2780-luc were treated with rhTRAIL, cisplatin and the combination of the two drugs. A2780 was sensitive to cisplatin, moderately sensitive to rhTRAIL, whereas combination of cisplatin and rhTRAIL led to high levels of apoptosis. A2780-luc was slightly less sensitive to rhTRAIL alone or the combination of