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

D. Meco[1], A.M. Di Francesco[1], A. Torella[1], P. Ubezio[2], C. Pisano[3], L. Vesci[3], P. Carminati[3], M. D'Incalci[2], R. Riccardi[1]. [1]*Catholic University of Rome, Paediatric Oncology, Rome, Italy;* [2]*Istituto di Ricerche Farmacologiche "Mario Negri", Oncology, Milan, Italy;* [3]*Sigma-Tau, Research & Development, Rome, Italy*

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

C.H.A. Cheung, J. Kanwar, G.W. Krissansen. *The University of Auckland, Department of Molecular Medicine and Pathology, Auckland, New Zealand*

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

N. Khanna[1], G. Thomas[2], J. Stern[3], N. Frazer[3]. [1]*Greenebaum Cancer Center, University of Maryland, Department of Family Medicine, Experimental Therapy, Baltimore, USA;* [2]*Gifford OB/GYN, Randolph, USA;* [3]*Erimos Pharmaceuticals, Raleigh, USA*

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

E.W. Duiker[1], E.G.E. de Vries[1], A.G.J. van der Zee[2], G.M. van Dam[3], N. Kooi[1], G.J. Meersma[1], S. de Jong[1]. [1]*University Medical Center Groningen, Dept. of Medical Oncology, Groningen, The Netherlands;* [2]*University Medical Center Groningen, Dept. of Gynecological Oncology, Groningen, The Netherlands;* [3]*University Medical Center Groningen, Dept. of Surgery, Groningen, The Netherlands*

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

cisplatin and rhTRAIL. No differences in protein expression levels of the major constituents of the TRAIL pathway were seen. Membrane expression levels of the TRAIL receptors showed similar results: DR4 and DcR1 were not expressed at both cell lines. DR5 was expressed, whereas low levels of DcR2 were detected. To develop an in vivo BLI-model, we inoculated nude mice ip with different concentrations of A2780-luc (resp. 1×10^7 and 2×10^6 cells). In both groups the bioluminescent signal correlated well with the ip tumor load as assessed by visual inspection of the peritoneal cavity at necropsy. Progressive tumor growth could be monitored by repeated imaging of a defined group at several time points.
Conclusion: This study shows that in vivo BLI is a reliable and feasible method to monitor noninvasively ip tumor growth. We are currently performing the experiments with iv and ip rhTRAIL therapy, cisplatin and the combinatory regimen. The results of these drug modulation studies will be presented.

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Targeting XIAP in paediatric cancers

S.V. Holt[1], K.E. Brookes[1], D. Hussein[1], K. Welsh[2], J. Reed[2], R.A. Houghten[3], C. Pinilla[3], C. Dive[1,4], G.W.J. Makin[1,4]. *[1]Paterson Institute for Cancer Research, Clinical and Experimental Pharmacology, Manchester, United Kingdom; [2]Burnham Institute, La Jolla, USA; [3]Torrey Pines Institute for Molecular Studies, San Diego, USA; [4]University of Manchester, Faculty of Medical and Human Sciences, Manchester, United Kingdom*

Cancer remains one of the commonest causes of death in children in the UK. In selected types of childhood tumours de novo drug resistance is a major problem. One potential cause of pleiotropic drug resistance is a failure to engage apoptosis after cytotoxic drug-induced damage. Endogenous inhibitor of apoptosis proteins (IAPs) prevent apoptosis by inhibiting both initiator (caspase-9) and effector (caspases-3 and 7) caspases. Down-regulating X-linked IAP (XIAP), the most potent endogenous inhibitor of caspases, sensitises adult tumour cells to drug-induced apoptosis. A novel XIAP antisense oligonucleotide is currently in adult phase I trial.
Although little is known about the function of XIAP in paediatric tumours, high levels of XIAP expression correlate with poor survival in childhood AML. We have screened a panel of paediatric tumour cell lines for expression of XIAP and its endogenous inhibitor XAF-1, and found near universal expression of XIAP. The small molecule XIAP inhibitor TPI-1396−11 (xiapuradamib) was effective against neuroblastoma, osteosarcoma, and Ewing's sarcoma cell lines in short term growth assays (SRB) and long term clonogenic assays, with IC50 values ranging from 2.1 to 7.25 μM. The combination index equation was used to define synergistic interactions between xiapuradamib and clinically relevant cytotoxic agents. Clear synergy was seen between xiapuradamib and etoposide in 791T osteosarcoma cells. NGP neuroblastoma cells with stable shRNA repressed XIAP were sensitised to etoposide in clonogenic assay. We are extending these studies into rhabdomyosarcoma, medulloblastoma and lymphoma cell lines and aim to take forward promising combinations into xenograft and ultimately clinical studies.

Differentiation

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Induction of myeloid differentiation by a novel sterol mesylate compound (NSC 67657)

R. Shoemaker[1], C. Glover[1], J. Adelsberger[2], M. Hollingshead[1], A. Monks[3]. *[1]National Cancer Institute, Developmental Therapeutics Program, Frederick, USA; [2]AIDS Monitoring Lab, SAIC-Frederick, Frederick, USA; [3]Laboratory of Functional Genomics, SAIC-Frederick, Frederick, USA*

Background: Inducers of differentiation can offer a relatively non-toxic means of chemotherapy and are of proven value in settings such as acute promyelocytic leukemia. The CEBPa transcription factor plays a key role in the regulation of normal myeloid cell differentiation and thus constitutes a target for discovery of novel differentiation inducing agents.
Materials and Methods: We conducted a high-throughput screening campaign to identify activators of CEBPa signaling using a clone of U937 cells transfected with a luciferase reporter driven by four copies of the CEBPa response element.
Results: Screening of more than 135,000 samples from the National Cancer Institute's repository of chemical compounds identified a novel sterol mesylate (NSC 67657) as a potent activator of CEBPa signaling. Secondary testing in U937 and HL60 cell lines demonstrated that this compound could induce myeloid differentiation manifest as increased expression of CD11b and CD14 cell surface markers, increased NBT activity, and morphologic evidence of differentiation. Transcriptional profiling demonstrated a distinctly different pattern from that produced by retinoic acid and suggested a predominantly monocytic mode of differentiation. Initial studies with cryopreserved leukemic blasts from patients with AML have demonstrated induction of CD11b and CD14 in two FAB type M5 samples by flow cytometry.
Conclusions: Xenograft studies as well as additional ex vivo studies using AML patient samples will be pursued to establish a case for clinical development of NSC 67657 or an optimized derivative.
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Down regulation of topoisomerase IIβ in myeloid leukemia cell lines leads to activation of apoptosis following all-trans retinoic acid-induced differentiation/growth arrest

M. Ganapathi[1], K. Chikamori[1], J. Hill[1], D. Grabowski[1], E. Zarkhin[1], A. Grozav[1], S. Vaziri[1], R. Bukowski[1], A. Yen[2], R. Ganapathi[1]. *[1]Cleveland Clinic Foundation, Taussig Cancer Center, Cleveland, USA; [2]Cornell University, College of Veterinary Medicine, Ithaca, USA*

Background: Among the topoisomerase (topo) II isozymes (α and β), topo IIβ has been suggested to regulate differentiation. In this study we examined the functional role of topo IIβ in all-trans retinoic acid (ATRA)-induced differentiation/growth arrest and apoptosis of myeloid leukemia cells.
Materials and Methods: Topo IIβ was inhibited with ICRF-193 or stably down-regulated with an si-RNA in the myeloid leukemia cell lines HL-60, KG-1 and AP-1060, to determine the role of this enzyme in ATRA-induced differentiation/growth arrest and apoptosis. Differentiation was assessed by microscopy based on reduction of nitroblue tetrazolium. Apoptosis was determined by fluorescent microscopy of cells stained with Hoechst 33342 + propidium iodide. mRNA and protein expression was determined by real-time RT-PCR and Western blot analysis, respectively. Gene expression profiles in topo IIβ-expressing and topo IIβ-deficient cells were compared by cDNA microarray analysis. Reactive oxygen species (ROS) was measured by flow cytometry using the dye dihydroethidium.
Results: Inhibition of topo IIβ activity with ICRF-193 in HL-60, KG-1 or AP-1060 cells or si-RNA mediated down-regulation of topo IIβ protein in HL-60 or KG-1 cells, significantly ($p < 0.05$) enhanced ATRA-induced differentiation/growth arrest and apoptosis. In contrast, down-regulation of topo IIα did not alter ATRA-induced differentiation or apoptosis. ATRA-induced apoptosis in topo IIβ-deficient cells led to activation of caspase 3 and was rescued by ectopic expression of topo IIβ. Gene expression profiling of topo IIβ-expressing and topo IIβ-deficient cells led to the identification of peroxiredoxin 2 (PRDX2) as a candidate gene that was down-regulated in topo IIβ-deficient cells. Reduced expression of PRDX2, validated at the mRNA and protein level, correlated with increased accumulation of ROS following ATRA-induced differentiation and apoptosis. Overexpression of PRDX2 in topo IIβ-deficient cells, prevented accumulation of ROS and partially reversed ATRA-induced apoptosis.
Conclusions: These results support a role for topo IIβ in survival of myeloid leukemia cells following ATRA-induced differentiation/growth arrest. Reduced expression of topo IIβ induces apoptosis in part by impairing the anti-oxidant capacity of the cell due to down-regulation of PRDX2. Thus, suppression of topo IIβ and/or PRDX2 levels in myeloid leukemia cells provides a novel approach for improving ATRA-based differentiation therapy.

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MMTV-RANK transgenic mice show increased mammary epithelial proliferation and impaired alveolar differentiation during pregnancy and a higher incidence of chemically induced mammary tumors

E. Gonzalez Suarez[1], D. Branstetter[2], J. Jones[1], K. Stocking[3], W. Saxbe[2], A. Armstrong[1], H. Dinh[4], H. Blumberg[4], W. Dougall[1]. *[1]Amgen Inc, Oncology, Seattle, USA; [2]Amgen Inc, Pathology, Seattle, USA; [3]Amgen Inc, Lab Animal Resources, Seattle, USA; [4]Amgen Inc, Inflammation, Seattle, USA*

Background: RANK and RANKL, the key regulators of osteoclasts differentiation and activation, also have an important role in the control of proliferation, differentiation and survival of mammary epithelial cells.
Materials and Methods: We have generated transgenic mice that overexpress RANK under the mouse mammary tumor virus (MMTV) promoter, and characterized their mammary gland development during pregnancy and their susceptibility to mammary tumors induced by medroxiprogesterone acetate (MPA) and DMBA. We have also characterized the