both c-myc and cyclin D1. Together, these data show that prostate cancers consistently have elevated free eIF-4E – a consequence of elevated eIF-4E expression and/ or reduced 4EBP1 expression/ function. As such, eIF-4E represents a novel target for prostate cancer therapy. Indeed, treatment of prostate cancer cells with siRNAs or ASOs specific for eIF-4E dramatically reduces eIF-4E expression and suppresses proliferation in concert with markedly diminished expression of cyclin D1 protein. Moreover, systemic treatment of CaP xenografts with eIF4E-specific ASOs or siRNAs suppresses xenograft tumor growth. These data firmly implicate eIF-4E in the development and progression of both human and experimental prostatic adenocarcinomas and indicate the therapeutic utility of targeting eIF4E.

46 POSTER

Biological testing of the tyrphostin tyrosine kinase inhibitor Adaphostin (NSC 680410) as a vascular endothelial growth factor secretion inhibitor in human and murine tumor cells and tumor-activated bone marrow stromal cells

E. Egilegor[1], L. Mendoza[1], N. Gallot[1], M. Valcárcel[2], M. Solaun[1], M. Hollingshead[3], E. Sausville[3], F. Vidal-Vanaclocha[1,2]. [1]*Dominion Pharmakine Ltd., Bizkaia Technology Park, Bizkaia, Spain;* [2]*Basque Country University School of Medicine and Dentistry, Bizkaia Spain;* [3]*Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, USA*

Background: Vascular endothelial growth factor (VEGF) is a proinflammatory and proangiogenic factor whose production by host and tumor cells contributes to cancer progression and metastasis. Drugs decreasing endogenous VEGF secretion may contribute to cancer prevention and inhibition. However, production of VEGF by host and tumor cells has redundant and heterogeneous mechanisms that hamper discovery of the most efficient inhibitors and their targeted mechanisms. Preliminary studies on the tyrphostin tyrosine kinase inhibitor adaphostin (NSC 680410) suggest its potential ability to inhibit VEGF secretion in human T-lymphoblastic leukemia cell lines. Herein, the inhibitory activity of Adaphostin on VEGF secretion was tested in different solid tumor cell lines that secrete VEGF under basal and cytokine-induced conditions and in bone marrow stromal cells (BMSCs) activated by tumor-derived soluble factors.
Materials and Methods: Human (MDA-MB-435 breast carcinoma, PC3 prostate carcinoma, HT-29 colon carcinoma, SK-HEP-1 hepatoma) and a murine tumor cell line (B16 melanoma) characterized by the ability to secrete VEGF were used. Primary cultured BMSCs were obtained from human and murine organ samples. Adaphostin was added to cultured tumor and host cells at a 0.1-to-10 μM concentration range for 24 and 48 hours. Measurement of human and murine VEGF secretion in control and Adaphostin-treated cells was performed by ELISA. Cytotoxic and proapoptotic effects of Adaphostin were assessed in cultured cells by the MTT assay and apoptotic cell markers.
Results: Following 12 hour-culture under basal conditions with 1 μM Adaphostin, the secretion of VEGF from human cancer cells decreased by 40-to-50% in the absence of any evidence of toxicity or proapoptotic signs. Not statistically significant alterations of VEGF secretion were observed in normal human BMSCs cultured under basal conditions with 1 and 5 μM adaphostin for up to 48 hours. Addition of conditioned medium (CM) from MDA-MB-435 breast carcinoma cells, PC3 prostate carcinoma cells, and HT-29 colon carcinoma cells to human BMSCs for 1 hour increased VEGF secretion by 5-folds. Addition of Adaphostin to tumor-activated BMSCs decreased tumor-induced VEGF production by 50-to-100%. Two BMSC-derived cytokines (IL-18 and TNFalpha), given as recombinant murine proteins at 10 ng/ml for 4 hours, also significantly increased (by 50%) VEGF production from cultured B16 melanoma cells. Addition of 1 μM Adaphostin to B16 melanoma cells 30 min prior to cytokine stimulation completely abrogated cytokine-dependent VEGF secretion. Moreover, the VEGF concentration was also significantly increased by 3-fold in the 6 hour-supernatant of primary cultured murine BMSCs given B16 melanoma-CM for 1 hour. Tumor-induced VEGF was abolished by Adaphostin when added to BMSCs 30 min prior to tumor-CM.
Conclusions: Current results demonstrate the ability of Adaphostin to inhibit VEGF secretion by solid tumor cells and tumor-activated BMSCs. Combination of this tyrosine kinase inhibitor with other antiangiogenic drugs are under study to test possible synergistic drug regimens. This work was supported in part by NCI Contract NO1-CO-12400.

47 POSTER

CRx-026: discovery and clinical development of a syncretic anti-mitotic agent with significant anti-cancer activity

P.J. Elliott, M.S. Lee, M. Keegan, Y. Zhang, M.J. Nichols, A.A. Borisy, C.T. Keith. *CombinatoRx, Inc., Product Development, Boston, MA, USA*

Background: A 'syncretic' drug comprises multiple biologically active compounds that interact synergistically to provide an optimal therapeutic effect with minimal side effects. CRx-026, is a novel syncretic anti-cancer agent, comprising chlorpromazine and pentamidine, that achieves its synergistic effects through a dual action in mitosis, selectively inhibiting hsEg5/KSP, a mitotic kinesin essential for centrosome separation, and PRL phosphatases, which play an important role in regulating mitotic progression and proper chromosome separation. By modulating the activity of these two targets in a concerted fashion, CRx-026 achieves a synergistic effect greater than either component agent achieves on its own.
Methods &Results: *In vitro*, CRx-026 exerts anti-proliferative and cytotoxic effects across a spectrum of human cancer cell lines. Additionally, we have tested CRx-026 in pair-wise combination with 48 anti-cancer agents representing all major mechanistic classes. CRx-026 synergized with several chemotherapeutic classes – especially with taxanes and vinca alkaloids. *In vivo*, CRx-026 elicits substantial anti-tumor activity in the xenograft tumors, A549, HCT116 and HT29. Results demonstrate that CRx-026, but not its components, had significant anti-tumor activity which was comparable or superior to paclitaxel or 5-FU. Moreover, CRx-026 has a more favorable side effect profile than paclitaxel and 5-FU, with improved survival and overall lower toxicity effects. Finally, we have confirmed several *in vitro* synergies in xenografts where CRx-026 was synergistic both with paclitaxel and with vinorelbine, enhancing anti-tumor activity without increasing toxicity in xenografts.
Conclusions: CRx-026 is a novel anti-cancer agent with obvious anti-cancer activity in both *in vitro* and *in vivo*. The two components of CRx-026 are now being integrated into a novel pharmaceutical composition, and we are currently advancing this new anti-cancer drug through clinical development. Phase I studies are currently underway in a standard dose escalation schema for patients with solid tumors to determine safety and pharmacokinetics. Multiple treatment regimens are currently being explored to help optimize future clinical development. Data are expected in late 2004.

48 POSTER

In vivo selection of vascular extravasating phage: novel means to isolate prostate tumor-homing peptides

S. Deutscher[1,2], M. Dickerson[1,2], L. Landon[1], C. Illy[1]. [1]*University of Missouri, Biochemistry, Columbia, USA;* [2]*Harry S Truman Memorial Veterans Hospital, Columbia, USA*

Background: New tumor targeting agents are required to advance cancer diagnosis and therapy. Bacteriophage (phage) peptide display technology is an emerging approach to identify and improve peptides as tumor-targeting agents. However, only a few of the peptides identified by *in vitro* phage selections have been clinically useful. This failure may be attributed to poor peptide solubility, affinity, specificity, and stability *in vivo*. It is our hypothesis that the *in vivo* application of phage display would have the advantage over *in vitro* selection strategies in that one can "select" in the environment of the whole animal, peptides that can extravastate tumor vasculature and selectively and stably bind to a given tumor. To this end we developed *in vivo* schemes to select for phage that target prostate tumors in mice.
Material and Methods: First, the biodistribution and pharmacokinetics of a fUSE 5 phage library (15 amino acid peptides displayed on cpIII) in scid mice was determined. Next, the library was cleared through non-tumor bearing mice in an effort to enrich for extravasating phage. The pre-cleared library was injected into human PC-3 prostate carcinoma-bearing scid mice. After one hour, tumors were removed, phage were eluted with acidic or neutral buffers (with 0.5% CHAPs detergent) and amplified. After four rounds of selection phage clones were analyzed. Peptides displayed on phage with the highest titer for the tumors or PC-3 cultured cells were chemically synthesized and their binding properties analyzed.
Results: Biodistribution studies demonstrated that fUSE5 phage half-life in the blood was ~18 min with optimal tumor/tissue localization of ~1 h. Only those phage extracted from the prostate tumors with detergent exhibited specific binding to PC-3 carcinoma cells. The phage and synthesized peptides bound PC-3 cells and PC-3 xenografted tumor tissue but did not bind appreciably to human PC-3M (metastatic form of PC-3), OVCAR3 ovarian, MDA-435 breast, or LS174T colon carcinoma cell lines. The peptides exhibited a K_d of 100pM for binding to live PC-3 cells. While the peptide target(s) remain to be identified, they may represent new classes of prostate tumor biomarkers. Future work will employ the peptides as an affinity ligand for target identification and, once radiolabeled, as potential cancer imaging or therapeutic agents.