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(CD31 staining) in CGP 53716 treated with mice. Collectively, these data suggest that inhibition of tumor stroma formation and angiogenesis following CGP 53716 treatment results in growth inhibition of H226 carcinoma in the lung.

#4246 CGP 57148B, a protein-tyrosine kinase inhibitor with potential for the treatment of Bcr-Abl positive leukemias and diseases involving deregulation of PDGF receptor and c-Kit tyrosine kinases. Buchdunger, E., Zimmermann, J., Mett, H., Müller, M., Law, N., Cioffi, C., Druker, B., and Lydon, N. *Ciba Pharmaceuticals Division, Oncology Research Department, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland, Ciba Pharmaceuticals Division, Ciba-Geigy Ltd., Summit, New Jersey 07901, Oregon Health Sciences University, Portland, Oregon 97201*

CGP 57148B has been identified as a potent protein-tyrosine kinase inhibitor with selectivity for the Abl and PDGF receptor tyrosine kinases. Cellular proliferation and tumor growth of Bcr-Abl or v-Abl expressing cells were specifically inhibited by this compound. CGP 57148B selectively inhibited PDGF-mediated cellular events such as PDGF receptor autophosphorylation, inositol phosphate formation and *c-fos* induction. The compound was a potent inhibitor of PDGF-mediated growth of *v-sis* transformed BALB/c 3T3 cells and PDGFstimulated proliferation of A10 rat aortic smooth muscle cells. *In vivo*, CGP 57148B inhibited growth of a number of PDGF-dependent tumors. Combination of CGP 57148B with cytotoxic agents resulted in tumor regression and cures, with different xenografts being sensitive to different drug combinations. Amongst Type III receptor tyrosine kinases, CGP 57148B was found to potently inhibit the SCF receptor, c-Kit, but not the related c-Fms, Flt-3 and Flt-1 tyrosine kinases. Increasing evidence suggests that inappropriate receptor signaling by c-Kit may be involved in a number of cancers, including small cell lung cancer (SCLC). Interestingly, tumor growth of NCI-H69 and NCI-H209 SCLC lines was sensitive to inhibition by CGP 57148B which is compatible with the inhibition of the c-Kit tyrosine kinase. Taken together, these results suggest that CGP 57148B is a signal transduction inhibitor which might have therapeutic potential for the treatment of Ph+ leukemias as well as diseases which involve abnormal activation of PDGF receptor and c-Kit tyrosine kinases.

#4247 Cytoxicity and phosphotyrosine effects of c-src kinase inhibition by substituted pyridopyrimidine tyrosine kinase inhibitors in human colon carcinoma cell lines. Kraker, A.J., Moore, C.W., Amar, A.M., Shen, C.S., Nelson, J., Slintak, V., Fry, D.W., Lu, G., Panek, R., Klutchko, S., and Hamby, J. *Parke-Davis Pharm Res. Div. of Warner-Lambert Co., Ann Arbor, MI 48105*

Tyrosine kinase inhibition has been identified as a potential therapeutic strategy in neoplastic disease. Potent c-src kinase inhibitors (IC_{50}=9nM in isolated c-src assay for 6-(2,6-dichloro-phenyl)-2-[4-(2-diethylamino-ethoxy)-phenylamino]-8H-pyrido[2,3-d]pyrimidin-7-one dihydrochloride)(PD166285, ***A***) are described. Src family members fyn, lyn, and lck are also potently inhibited in vitro. ***A*** causes growth delay on plastic with IC_{50} values of 140nM for HT-29, 426nM for HCT-8 and 270nM for SW-620 cell lines. In soft agar clonogenic assays with 2 day treatment followed by drug removal, ***A*** gave IC_{50} values of 1.8 μM(HT-29), 780nM(HCT-8), and 330nM(SW-620). Phosphotyrosine(***P***-Y) content of focal adhesion kinase(FAK) and paxillin(known substrates of c-src) is reduced at sub-μM concentrations in HT-29 cells treated with ***A*** for 2 hours and then mitogenically stimulated. In a fibroblast cell line overexpressing epidermal growth factor receptor(EGFr) and c-src, ***A*** is a specific c-src inhibitor(reducing FAK and c-src ***P***-Y) compared to EGFr kinase activity(receptor ***P***-Y). Because of potent kinase inhibition, selectivity, and cellular signaling effects of these compounds, in vivo studies are underway.

#4248 CP-358,774: A selective EGFR kinase inhibitor with potent antiproliferative activity against HN5 head and neck tumor cells. Iwata, K., Miller, P.E., Barbacci, E.G., Arnold, L., Doty, J., DiOrio, C.I., Pustilnik, L.R., Reynolds, M., Thelemann, A., Sloan, D., and Moyer, J.D. *Oncogene Science Inc., Uniondale, NY 11553-3649, Pfizer Central Research, Groton, CT 06340*

CP-358,774 is an inhibitor of the epidermal growth factor receptor (EGFR) tyrosine kinase (TK), an activity which is overexpressed in many carcinomas. CP-358,774 inhibits isolated human EGFR TK with an IC_{50} of 2 nM, and reduces EGFR autophosphorylation in MDA-MB-468 tumor cells in culture with an IC_{50} of 20 nM. EGFR TK is >1000-fold more sensitive to CP-358,774 than other TKs such as human *c-src*, insulin receptor, insulin-like growth factor receptor,or v-abl. As a further indication of selectivity, EGF-induced tyrosine phosphorylation of SHC proteins in HN5 cells is completely blocked by CP-358,774, whereas the insulin-induced phosphorylation of IRS-1 is unaffected. CP-358,774 inhibits EGF-stimulated mitogenesis in Fischer rat embryo cells with an IC_{50} of 70 nM, but does not similarly inhibit mitogenesis stimulated by platelet derived growth factor, insulin-like growth factor, or basic fibroblast growth factor, which also act through transmembrane receptors with TK activity. The proliferation of HN5 cells, which overexpress EGFR, is inhibited by CP-358,774 at 50 nM, and completely blocked at 250 nM. Pretreatment of athymic mice with CP-358,774 (100 mg/kg) completely inhibits EGF-induced autophosphorylation of human EGFR in HN5 tumor xenografts and of EGFR in mouse liver. This inhibitor has potential for the treatment of tumors that are dependent on the EGFR pathway for proliferation.

#4249 Therapy of human carcinomas in athymic mice by inhibition of EGF receptor-mediated signal transduction with CP-358774: Dynamics of receptor inhibition and anti-tumor effects. Pollack, V.A., Savage, D.M., Baker, D.A., Tsaparikos, K.E., Sloan, D.E., Barbacci, E.G., Pustilnik, L.R., Smolarek, T.A., Davis, J.A., Vaidya, M.P., and Iwata, K. *Dept. of Cancer, Pfizer Central Research, Groton, CT 06340, Oncogene Science, Inc., Uniondale, NY 11553*

Tyrosine phosphorylation of EGF receptors is an important early event in signal transduction and tumor cell replication. We devised an ex vivo assay to quantitate EGFr-specific tyrosine phosphorylation in human tumors obtained as s.c. xenografts in athymic mice. Using an enzyme-linked immunosorbent assay (ELISA), we observed reproducible ED_{50}'s and have used this assay to describe the extent and duration of drug action in vivo. CP-358774 is an effective, orally active inhibitor of EGFr tyrosine phosphorylation (ED_{50}= 10 mg/kg po). It has significant duration of action, producing, on average, 70% inhibition of EGFr-PY over a 24-hr period. Most importantly, we observed that inhibition of EGFr-PY in an ex vivo assay effectively correlates with the potency and degree of inhibition of EGFr-dependent human HN5 tumor growth in a xenograft therapy model (ED_{50}= 10 mg/kg qd X 5). These data suggest that CP-358774 may be an important new agent for therapy of EGFr-overexpressing human cancers.

#4250 Pyrido[*d*]pyrimidine inhibitors of the tyrosine kinase activity of the EGF receptor: A binding model and structure-activity relationships for soluble analogues. Denny, W.A., Palmer, B.D., Rewcastle, G.W., Thompson, A.M., Bridges, A.J., Doherty, A.M., Fry, D.W., Nelson, J.M., Rubin, J.R., Showalter, H.D.H., and Trumpp-Kallmeyer, S. *Cancer Society Res. Lab, University of Auckland, Private Bag 92019, Auckland, New Zealand, Parke-Davis Pharmaceutical Res., Division of Warner-Lambert Co., 2800 Plymouth Rd., Ann Arbor, MI 48105*

4-Anilinopyrido[d]pyrimidines are potent, selective inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR), binding at the ATP site with IC_{50} values down to 8 pM. They are of interest as anticancer drugs, since EGFR is over-expressed in many cancers and associated with poor clinical prognosis. A model for their binding to the EGFR was constructed from the catalytic subunit of the related cAMP-dependent protein kinase, and from structure-activity data. This attributes their high selectivity to binding of the 4-anilino ring in a hydrophobic pocket of composition unique to the EGFR. The 6- and 7-positions of the pyrido[*d*]pyrimidines occupy the entrance of the ATP binding pocket, with the 7-position pointing towards the ribose binding site of ATP. In agreement with this, analogues with weakly basic solubilising groups at the 6- or 7-positions retain potent inhibitory activity towards EGFR. They are also potent inhibitors of EGFR auto-phosphorylation in A431 cells. Examples evaluated against murine colon 38 tumors and human tumor xenografts in mice gave substantial growth delays.

#4251 ZD1839, an epidermal growth factor tyrosine kinase inhibitor selected for clinical development. Woodburn, J.R., Barker, A.J., Gibson, K.H., Ashton, S.E., Wakeling, A.E., Curry, B.J., Scarlett, L., and Henthorn, L.R. *Zeneca Pharmacueticals, Alderley Park, Macclesfield, Cheshire, U.K. SK10 4TG*

Epidermal growth factor receptor (EGFR) is overexpressed in a wide variety of solid human cancers including non-small cell lung, breast, head and neck, bladder and ovarian carcinomas. ZD1839, an anilinoquinazoline, is a potent inhibitor (0.02 micromolar) of the EGFR tyrosine kinase *in vitro* and of the EGF-stimulated growth of KB oral carcinoma cells in culture (0.08 micromolar). ZD1839 has excellent oral bioavailability and shows antitumor activity in a broad range of human solid tumor xenografts implanted in nude mice in the dose range 12.5 - 200 mg/kg once per day orally. Sensitive tumors include A431 vulval, KB oral, A549 NSCLC, DU145 prostate, HT29, HCT15, CR10 and LoVo colorectal, and HX62 ovarian. Antitumor effects range from reduced growth rate to stasis; with marked regressions seen in some tumors. Therapy for up 4 months in nude mice is well tolerated. On the basis of these exciting pre-clinical data ZD1839 has been selected for clinical development.

#4252 Novel benzoylacetylenic compounds: Potent and selective EGF receptor tyrosine kinase inhibitors. Suzuki, T., Kitano, Y., Ohya, J., Umeki, H., Inokawa, H., Kawahara, E., Nakamura, H., Takayanagi, H., and Hara, H. *Pharmaceuticals Lab., Yokohama Research Center, Mitsubishi Chemical Co., Yokohama, Japan*

Inhibition of oncogenic tyrosine kinases is a potential approach for the treatment of cancer. We have synthesized a novel series of benzoyl acetylenic compounds as potent and selective inhibitors of EGF receptor tyrosine kinase. We found that one of these compounds, DAB-720, has excellent biological activities in *in vitro* and *in vivo* studies. We show here the structure-activity relationships of these derivatives and biological profiles of DAB-720. Using a panel of protein kinases including EGF receptor, PDGF receptor, c-src, lck and PKC, DAB-720 displayed selective inhibitory activity against EGF receptor with IC_{50} of 0.070 μM and little or no inhibition for the other kinases at 50 μM, except lck for which DAB-720 had an IC_{50} of 16 μM. Autophosphorylation of EGF receptor in A431 cells and of HER2/Erb B2 in BT474 cells were also inhibited over 50% at 5 μM and 10 μM respectively. The IC_{50} for KB cell growth was 0.76 μM. In *in vivo* study significant growth inhibition of KB xenograft in nude mice was demonstrated by 30 mg/kg intraperitoneal administrations of DAB-720.