tumor)/(growth volume of untreated tumor) ×100 (T/C %). HER2 and EGFR tyrosine phosphorylation in the tumor tissue was detected by Western blot analysis.
Results: TAK-285 demonstrated significant tumor growth inhibition in the BT-474-bearing nude mice in a dose dependent manner with T/C values of 64% at 50 mg/kg, bid, and 29% at 100 mg/kg, bid. Also in 4−1ST-bearing mice TAK-285 showed significant antitumor activity with T/C values of 44% at 50 mg/kg, bid, and 11% at 100 mg/kg, bid.
In vivo antitumor effect of TAK-285 was further studied using the rat xenograft model implanted with 4−1ST. In the dose range about 8 times lower than that of the mice models TAK-285 demonstrated antitumor efficacy (T/C% values of 38% at 6.25 mg/kg and 14% at 12.5 mg/kg) without any body weight reduction. In rats, TAK-285 had a good plasma pharmacokinetic profile and concentrations 5 to 7 times higher in the cancer tissue compared with plasma levels, indicating the reason for the stronger activity. In the rat bearing A431, TAK-285 also showed strong antitumor activity with T/C% values of 48% at 6.25 mg/kg and 13% at 12.5 mg/kg, demonstrating that TAK-285 is efficacious not only for HER2-overexpresssing cancers but also for EGFR-overexpressing cancers – an advantage of possessing HER2 and EGFR dual inhibitory activity.
Significant inhibition of HER2 and EGFR phosphorylation by TAK-285 was observed in these tumor xenograft tissues. The reduction of the HER2 phosphorylation level in tumor tissues can be a good biomarker of antitumor efficacy because of the very good correlation between the reduction and antitumor activities. The phosphorylation and activation of Akt and ERK, downstream signaling molecules, were also strongly downregulated after TAK-285 treatment.
Conclusion: TAK-285 showed very good tumor tissue distribution, much higher than that in plasma, and can be efficacious to human cancers with expression not only of HER2 but also of EGFR.

312 POSTER

EZN-3920, an ErbB3-locked nucleic acid-based RNA inhibitor, potently silences target gene expression in tumor cells grown in vitro and in vivo

B. Liao[1], T. Qu[1], J. Kosek[1], S. Castaneda[1], P. Sapra[1], Y. Zhang[1], R. Bandaru[1], L.M. Greenberger[1], I.D. Horak[1]. *[1]Enzon Pharmaceuticals, Research, Piscataway, USA*

Background: Small molecules and antibodies that target ErbB1 and/or ErbB2 have proven anticancer activity in patients. An addition family member, ErbB3, is unique since it has little or no kinase activity, and therefore is not easily druggable by small molecules. However, inhibition of ErbB3 is likely to have antitumor effects since ErbB3 (1) heterodimerizes with ErbB2 and ErbB1, (2) is a key link to the PI3K pro-survival signaling pathway, and (3) is activated in cells resistant to ErbB1 or ErbB2 therapeutics. In this report, a locked nucleic Acid (LNA)-containing antisense oligonucleotide (LNA-ON), designated EZN-3920 and chosen from a panel of ErbB3 LNA-ONs, has been used to inhibit the expression of ErbB3. EZN-3920 is stable in plasma for greater than 72 hours and has very high binding affinity to complementary RNA. The molecule was evaluated in biological models.
Material and Methods: In vitro, the ability of EZN-3920 to knockdown mRNA and inhibit cell growth were evaluated by qRT-PCR and MTS assays, respectively, in several cancer cell lines (derived from prostate, liver, lung, colon and epithelium) after transfection of cells with lipofectamine. In vivo, ErbB3 mRNA silencing in liver and human tumors derived from 15PC-3 (prostate), A549 (lung), and N87 (gastric) cells, which were grown on the flank of nude mice, were evaluated after intravenous administration of EZN-3920 given on multiple regimens. Scrambled LNA-ONs served as controls.
Results: In vitro, EZN-3920 significantly inhibited ErbB3 mRNA expression (IC50 < 4 nM), protein expression, and proliferation (IC50 < 10 nM) in multiple cell lines. These effects were dose-dependent, were not observed with scrambled LNA-ONs, and did not alter levels of an off-target mRNA encoding HIF-1?. In vivo, up to 90% inhibition of ErbB3 mRNA in the liver was observed after administration of as little as 20 mg/kg EZN-3920 (given qdx5). In addition, EZN-3920 inhibited the expression of ErbB3 mRNA in tumors approximately 50% when given at tolerated doses. The negative control LNA-ONs did not exhibit the target gene silencing effects in liver and tumors.
Conclusions: EZN-3920 potently and specifically inhibits ErbB3 mRNA expression both in vitro and in vivo, particularly in tumors after intravenous injection. Further studies will examine the antitumor efficacy of EZN-3920. EZN-3920 represents a new generation of anticancer agents that may be useful in tumors where ErbB3 is a critical mediator of tumor growth.

313 POSTER

Novel inhibitory mechanisms of TAK-285, a new EGFR/ErbB2 dual inhibitor

S.A. Shell[1], K.J. Pry[1], P. Trusk[1], R.L. Wappel[1], Y. Ohta[2], W. Klohs[3], S.S. Bacus[1]. *[1]Targeted Molecular Diagnostics, Research and Development, Westmont, USA; [2]Takeda Pharmaceutical Company, Pharmacology Research Laboratories, Osaka, Japan; [3]Takeda Pharmaceutical Company, T G R D, Deerfield, USA*

Background: Activated mutations downstream of EGFR and ErbB2 receptor tyrosine kinases (RTK), such as in KRAS, often render targeted therapy less effective. Furthermore, inactivated mutations in the LKB1 tumor suppressor gene can result in unchecked tumor growth. Therefore, the existence of secondary mutations requires therapeutic regimens beyond inhibiting just the RTK. We show the dual EGFR/ErbB2 inhibitor TAK-285 can kill EGFR-driven tumor cells with activated RAS mutation. Moreover, TAK-285 also activates the AMPK pathway in LKB1-deficient tumor cells. Kinome analysis of TAK-285 showed that the EGFR/ErbB2 inhibitor also binds MEK1/2, downstream enzymes of RAS, with moderate affinity. Thus TAK-285 may demonstrate increased efficacy alone or in combination therapy across a broad spectrum of solid tumors including those harboring activated RAS or inactivated LKB1 mutations.
Materials and Methods: Breast and lung tumor cell lines were treated in triplicate across a range of concentrations (0.01 to 10.0 μM) with GW2974 (Sigma) or TAK-285 for 72 hours. Changes in cell growth were determined using CellTiter-Glo (Promega) and normalized to DMSO. KinomeScan (Ambit) was performed on TAK-285 at 10.0 μM across 402 human kinases.
Results: TAK-285 effectively inhibited growth of tumor cells with activated RAS mutation better than GW2974. While both TAK-285 and GW2974 could activate AMPK and inhibit cell growth in LKB1-positive cells, TAK-285 could do the same in LKB1-deficient tumor cells where GW2974 could not. Interestingly, kinome analysis demonstrated TAK-285 binds with moderate affinity to MEK1/2, downstream enzymes of RAS. Moreover, TAK285 showed no significant interaction with members of the AMPK pathway, thus providing insight as to how TAK-285 treatment allows AMPK activation to proceed.
Conclusions: We show that the increased killing of TAK-285 treated tumor cells with activated RAS mutation is likely through TAK-285 to also moderately inhibit MEK1/2, downstream enzymes of RAS. By targeting the oncogenic EGFR pathway upstream by direct EGFR inhibition and downstream by inhibiting enzymes distal to activated RAS, TAK-285 demonstrates better efficacy as a mono-therapy than other drugs that solely target RTKs. Furthermore, by combining MEK inhibitors with TAK-285, synergistic results may be achieved at lower doses. TAK-285 also has the ability to activate the catabolic pathway through AMPK in LKB1-deficient tumor cells adding to its potent killing effects.

314 POSTER

Combined antitumor efficacies of TAK-285, a novel ErbB1/ErbB2 dual kinase inhibitor, with other anticancer drugs

T. Tamura[1], S. Takagi[1], K. Horikoshi[1], T. Yusa[1], M. Koyama[1], H. Tojo[1], Y. Ohta[1]. *[1]Takeda Pharmaceutical Company Limited, Pharmacology Research Laboratories II, Tsukuba, Japan*

Background: The HER tyrosine kinase is a critical target for an effective anticancer drug. Recently we have shown TAK-285, a novel HER2/EGFR dual kinase inhibitor, to be a promising orally available anticancer drug. Here, we report the synergistic antitumor effects with other anticancer drugs. We also address the interaction of TAK-285 with the multidrug resistant (MDR) molecule, which plays a key role in drug-drug interactions.
Methods: The combinational dose-effect relationship on the cell growth inhibition was analyzed using the median-effect method. For the distinct efficacy value, the combination index (CI) was calculated (CI < 1 means synergy, CI = 1 additive, and CI > 1 antagonism). The antitumor effect was also investigated in mice xenograft models. MDR activity was examined by rhodamin efflux via flow cytometry and measurement of ATP consumption.
Results: A clear synergistic antitumor effect (CI < 1) was observed in vitro BT-474 growth assay by the combination of TAK-285 with trastuzumab. Here trastuzumab had no effect against phosphorylation of HER2 while TAK-285 strongly downregulated. The synergistic antitumor effect was confirmed by BT-474 mouse xenograft model. The combination of TAK-285 100 mg/kg, bid, with trastuzumab 10 mg/kg, twice a week, caused strong regression of tumor size. Further, in A431 the synergistic antitumor effect of TAK-285 (CI < 1) was clearly observed in combination with the EGFR-selective inhibitor erlotinib. The precise mechanism of this synergy is not clear, because HER2 expression in A431 is very low. We speculate HER2 signals or its heterodimerization with EGFR still play a key role in A431.

With the chemotheraputic anticancer drugs, paclitaxel, doxorubicin, docetaxel and 5-FU, additive antitumor effects (CI = 1) were observed suggesting TAK-285 would not reduce the efficacy of these drugs.
In MES-SA/Dx-5, a human uterine sarcoma overexpressing MDR1, rhodamine efflux showed that TAK-285 had no MDR inhibition and was supposed to be a weak subject of MDR. ATP consumption assay confirmed the results. Many drugs are known to be substrates of MDR, which has a key role in the pharmacokinetic profile. TAK-285 will not affect it and contribute for a safe combination. The results also suggest a lower chance of drug resistance occurring with MDR.
Conclusion: TAK-285 demonstrated the synergistic antitumor effects with other inhibitors against the HER tyrosine kinase family. TAK-285 also has a profile, which would be ideal in combination therapy.

315 POSTER

A novel pan-HER ligand trap based on human EGFR (HER1) and HER3 inhibits tumor growth and metastasis in models of human cancer

Z. Huang[1], H.M. Shepard[1], D. Maneval[1], P. Jin[1], M. Beryt[1], J. Zhang[1], C. Brdlik[1], L. Cousens[1], B. Jorgensen[1], X. Bai[1]. *[1]Receptor BioLogix Inc., Pharmacology, Palo Alto, CA, USA*

The human EGF receptor family (HER1, 2, 3, 4) significantly contributes to the aggressiveness of many human malignancies, and therefore serves as targets of anti-cancer drugs with vast clinical potential. Single-targeted agents, like monoclonal antibodies (MAbs: trastuzumab, cetuximab, or panitumumab) or small molecule tyrosine kinase inhibitors (TKIs: erlotinib and gefitinib), have been approved for cancer treatment. These agents share a narrow focus of clinical activity and suffer in the endeavor of expansion on their clinical application because of their exquisite specificity (MAbs) or lack thereof (TKIs). To extend the activity of anti-HER therapy, we have developed a bispecific (HER1/HER3) ligand binding trap. This domain engineered molecule (hermodulin) is an Fc-mediated dimer of the HER1 and HER3 extracellular domains (ECD). Hermodulin can sequester most growth factors that activate the human HER receptor family, except NRG3 and NRG4, which are specific for HER4. Hermodulin inhibits proliferation of a broad range of cancer cells in vitro, alone or in combination with various approved chemotherapeutics, and suppresses tumorigenesis, tumor growth and metastasis in mouse xenograft models.

316 POSTER

ErbB4 suppresses proteasomal degradation of HIF-1a and promotes survival of cancer cells in hypoxia

I. Paatero[1], S. Heikkinen[1], E. Iivanainen[1], T.T. Junttila[1], P. Heikkinen[2], O. Kallioniemi[3], P. Jaakkola[2], K. Elenius[1]. *[1]University of Turku, Medical Biochemistry and Molecular Biology, Turku, Finland; [2]University of Turku, Turku Centre for Biotechnology, Turku, Finland; [3]VTT Technical Research Centre of Finland, Medical Biotechnology Center, Turku, Finland*

Background: Tumors frequently exhibit hypoxic areas in which insufficient supply of oxygen and other nutrients reduce cell viability. Thus, a selection pressure favors molecular adaptations that enhance survival of tumor cells in hypoxic conditions. One of the key transcription factors that regulate adaptation to hypoxia is hypoxia inducible factor-1a (HIF-1a).
Results: We have identified the receptor tyrosine kinase ErbB4 as protein that promotes signaling via HIF-1a in cancer cells. ErbB4 stimulated hypoxia response element-driven promoter activity and promoted up-regulation of known HIF-1a-regulated genes in cancer cells by a mechanism involving suppressed VHL-independent proteasomal degradation of HIF-1a. In addition, hypoxia reciprocally enhanced signaling via ErbB4, indicating a positive feed-back loop. The kinase domain of ErbB4 physically associated with HIF-1a and the two proteins also co-localized in the nucleus. ErbB4 expression promoted survival of cancer cells under hypoxic conditions in vitro and correlated with expression of hypoxia-regulated genes in vivo in an in silico analysis of microarray samples representing over 6000 human tissue samples.
Conclusions: The results suggest that ErbB4 promotes HIF-1a signaling in cancer. These data may have implications for the use and development for ErbB-based cancer therapeutics, and provide new insights into how hypoxic microenvironment regulates tumor cell behavior.

317 POSTER

Antiproliferative effects of PM02734, a novel marine cyclic peptide compared with currently used Erb-B inhibitors, in a panel of human cancer cell lines characterised for Erb-B expression

M. Serova[1], I. Bieche[2], A. Ghoul[1], M. Vidaud[2], M. Aracil[3], J. Jimeno[3], S. Faivre[1], E. Raymond[1]. *[1]RayLab-INSERM U728 & Department of Medical Oncology, Beaujon University Hospital, Clichy, France; [2]Laboratory of Molecular Genetics, Beaujon University Hospital, Clichy, France; [3]PharmaMar, R&D, Madrid, Spain*

Background: PM02734 is a novel marine-derived cyclic peptide that belongs to Kahalalide F family. PM02734 is under late phase I development with evidence of a positive therapeutic index. Recent preclinical studies have identified Erb-B3 as major determinant of the cytotoxic activity of Kahalalide F and PM002734 in vitro. In this study, we investigated the antiproliferative effects of PM02734 in comparison with that of other Erb-B/HER family inhibitors in a panel of human cancer cell lines.
Materials and Methods: Antiproliferative effects of PM02734, lapatinib, gefitinib, erlotinib, cetuximab and trastuzumab were evaluated in a panel of colon (HT29, HCT116, COLO205, HCC2998), breast (MCF7, MDA-MB-435, SKBR3), ovarian (OVCAR3, IGROV1), lung (Hop62, Hop92), prostate (PC3, DU145), Head&Neck (SCC61, SQ20B, HEP2), and pancreatic (MiaPaCa2, CAPAN1) cancer cell lines after 72 hour exposure using MTT assay. Expression of Erb-B1, 2, 3 and 4 was evaluated using quantitative RT-PCR.
Results: PM02734 displayed antiproliferative effects against cancer cells at concentrations that may be achieved in the clinic with IC50s ranging 0.4−9 μM, two Erb-B2-expressing breast cancer cell lines, ZR-75−1 and SKBR3, being the most sensitive. The cytotoxicity profile of PM02734 was distinct from other Erb-B inhibitors with cross-resistance/sensitivity with lapatinib in only a limited number of cell lines. No clear correlation between PM02734 antiproliferative effects and the levels of Erb-B1−4 expression was detectable, although PM02734 was more potent in cells with higher degree of expression of Erb-B family members especially Erb-B2. We studied the antiproliferative effects of PM02734 in two isogenic cell lines Colo205 and Colo205-R cells with epithelial and mesenchymal phenotypes, respectively. The parental Colo205 cells were at least 8 fold more sensitive to PM02734 than Colo205-R with IC50s of 0.5 and 4 μM, respectively. Interestingly, Colo205-R cell line was also resistant to lapatinib but not to gefitinib. These data strongly suggest that sensitivity to PM02734 may be dependent on the type of differentiation of cancer cells, better results being observed in cancer cells retaining an epithelial phenotype.
Conclusions: PM02734 displayed antiproliferative effects against a broad number of human cancer cell lines at concentrations that may be achieved in the clinic. PM02734 displays an original cytotoxicity profile, being a more potent inhibitor of cell proliferation than other Erb-B inhibitors. EMT appears to play a role in sensitivity to PM02734. Further work is needed to identify predictive markers of activity among several EMT genes.

318 POSTER

Downregulation of thymidylate synthase by lapatinib: blockage of EGF-induced translocation of nuclear EGFR and HER2

H. Kim[1], S. Han[2], K. Lee[3], J. Jung[4], Y. Yoon[1], H. Hur[1], S. Im[2], D. Oh[2], Y. Bang[2], T. Kim[2]. *[1]Cancer Research Institute, Seoul National University College of Medicine, Seoul, South Korea; [2]Department of Internal Medicine, Seoul National University College of Medicine, Seoul, South Korea; [3]Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, South Korea; [4]Department of Internal Medicine, Hallym University College of Medicine, Seoul, South Korea*

Epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) induced downregulation of thymidylate synthase (TS) results in the synergistic antitumor effect of combination treatment with EGFR-TKIs and 5-fluorouracil (5-FU). However, the underlying molecular mechanism of TS downregulation is not fully understood. In this study, we show that dual-inhibition of EGFR and HER2 results in a more prominent inhibition of TS than that seen with single inhibition of either receptor in HER2-positive SNU216, N87, and SKBr3 cells. We did a transcriptional profiling study in SNU216 cell and observed that the pivotal genes related to 5-FU sensitivity [TS, thymidine kinase 1 (TK1), dihydrofolate reductase (DHFR), and ribonucleotide reductase M2 (RRM2)] were downregulated after lapatinib treatment. These genes were more significantly reduced in lapatinib-treated HER2 positive cells compared with gefitinib or trastuzumab-treated cells. Moreover, we identified that activation of TS reporter gene expression by EGFR/HER2. We further demonstrated that nuclear translocation of EGFR/HER2 induced by EGF and its association with TS promoter in vivo analyzed by chromatin immunoprecipitation assay is effectively abolished by lapatinib. These results suggest that the nuclear pathway of EGFR/HER2 is essential in regulating TS and blockage of this pathway