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