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.

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

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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.

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

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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.

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

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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.