

# INCYTE CORPORATION



## **Selective ADAM Enzyme Inhibitor Program**

### ***Orally-Administered Therapeutics for Solid Tumors***

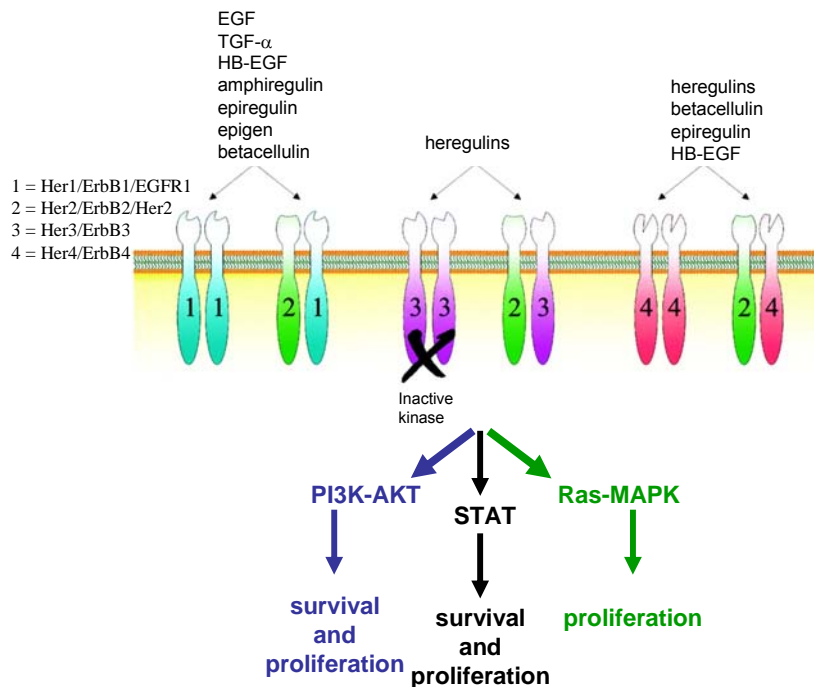
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## Therapeutic Rationale

The epidermal growth factor (ErbB) family of receptor tyrosine kinases (RTKs) play important physiologic roles in the regulation of cell growth, differentiation and survival (1-3). The ErbB family is composed of four distinct members, including EGFR1 (Her-1 or ErbB1), Her-2 (neu or ErbB2), Her-3 (ErbB3) and Her-4 (ErbB4). There are eleven ligands reported for the ErbB receptor family, including epidermal growth factor (EGF), transforming growth factor  $\alpha$  (TGF- $\alpha$ ), heparin-binding EGF-like ligand (HB-EGF), amphiregulin (AR), betacellulin (BTC), epiregulin (EPR), epigen (EPG), and four heregulin (HRG)/neuregulin (NRG) family members. These ligands bind directly to and subsequently activate EGFR1, Her-3 and Her-4 as depicted in Figure 1.

Figure 1 Ligand activation of the ErbB family of receptor tyrosine kinases





Physiologic receptor activation is mediated by ligand-induced receptor homo- or hetero-dimerization which leads to phosphorylation of the intracellular domains of the RTKs and subsequent activation of multiple downstream signaling cascades, including Ras/Raf/MAPK, PI3K/Akt and STATs that are essential for promoting cell growth, survival and resistance to apoptosis. Her-2 is the only receptor without a known ligand, but it can be activated as a result of gene amplification, mutations or hetero-dimerization with other ErbB family members that have bound ligand (1, 3-4). While Her-3 can bind ligand, it lacks kinase activity. Thus, following ligand binding, Her-3 signals by hetero-dimerization with other ErbB family members.

It has become increasingly clear that signaling through the ErbB family is a critical driver of tumor cell growth and survival, and the pathway represents a proven molecular target for anti-cancer agents. Dysregulation of the ErbB signaling pathways has been observed in numerous common solid tumors, including breast, lung, colon and prostate. The increased ErbB signaling observed in these tumors usually results from receptor overexpression, gene amplification, mutation and/or elevated levels of ErbB ligands (1, 5). In certain cancers, activated EGF receptors together with increased level of ligands have been correlated with disease progression and poor prognosis. For example, increased expression of EGFR1 and multiple EGF-like ligands in breast cancer have been linked to resistance to anti-hormone therapies, metastatic progression and poor prognosis (6). In addition, Her-2 is overexpressed in a significant portion of human breast cancers (20~30 %) as well as ovarian, non-small cell lung, colon and pancreatic tumors (1-2). In breast cancer, Her-2 overexpression is a marker of poor clinical outcome in patients with lymph node positive disease (7-8).

For these reasons, the ErbB pathways have been attractive targets for therapeutic intervention. To date, these therapeutics include humanized antibodies like trastuzumab and cetuximab, which directly target Her-2 and EGFR1, respectively, and small molecule inhibitors like gefitinib, which target the kinase activity of EGFR1. The



humanized monoclonal antibody, trastuzumab, which is directed against the extracellular domain (ECD) of Her-2, has proven to be an effective therapy for Her-2 positive breast cancer patients who express the highest levels of Her2 (FISH 3<sup>+</sup>), suggesting that Her-2 is a critical driver of tumor growth in that patient population. The EGFR tyrosine kinase inhibitor gefitinib has also shown efficacy in patients with non-small cell lung carcinomas, especially in a subgroup of patients who have mutations in the EGFR1 kinase domain (9-10). Mechanistically, these agents have been shown to block activation of the ERK and Akt signaling pathways and this inhibition has been shown to correlate with efficacy (11). Despite these advances, the majority of patients do not show long term benefit from these therapies and drug-related resistance is a common consequence of treatment. In addition, the kinase inhibitors are associated with dose-limiting toxicities that may preclude tumor exposures that would optimally inhibit EGFR signaling. Thus, there remains a need to develop additional therapies which target the ErbB pathways, ideally at distinct points of intervention, such that they can be used in combination with existing therapies in to achieve an additive or synergistic tumor response.

To approach this problem, we took advantage of the emerging biology of EGFR receptor and ligand function. The ErbB ligands, described above, are structurally and functionally related membrane proteins that can be proteolytically cleaved and released from cells (5-6, 12-13). Members of the ADAM (a disintegrin and metalloproteinase) family of metalloproteases are thought to mediate the cleavage of ErbB ligands and this event is critical for the production of soluble functional ErbB ligands (6, 13). For example, ADAM17-deficient cells are defective in the shedding of TGF- $\alpha$ , HB-EGF and amphiregulin (14-16). ErbB-driven proliferation of a mammary epithelial cell line can be inhibited by the metalloprotease inhibitor batimastat, and this inhibition is rescued by soluble EGF (17). Moreover, the metalloprotease-dependent release of HB-EGF and amphiregulin from cells has been shown to mediate the transactivation of the EGFR1 by G protein-coupled receptors (18). Although membrane-bound ErbB ligands can also engage in juxtacrine signaling, a recent report suggests that even juxtacrine activation



of the EGFR1 by TGF- $\alpha$  on an adjacent cell requires metalloprotease activity (19). To date, the ADAM family members that have been suggested to effect ErbB ligand shedding include ADAM10, ADAM17, ADAM9, and ADAM12 (13).

While ADAM metalloproteases clearly control the availability of active ErbB ligands, they may also affect ErbB signaling pathways by directly regulating the cleavage of ErbB receptors, in particular Her-2. Studies of blood samples from cancer patients have detected the presence of the extracellular domain (ECD) of Her-2 as well as a soluble form of EGFR1, and further suggest that the serum levels of these fragments, particularly Her-2 ECD, are indicators of poor prognosis and poor responsiveness to EGFR and Her-2-targeted therapies (7, 20-21). While the basis for poor patient outcome and elevated levels of soluble EGFR and Her-2 ECD is not proven, it is known that, in Her-2 over expressing cancer cells the ECD of Her-2 is frequently cleaved, rendering the remaining membrane-bound portion of Her-2 (p95) constitutively active (7, 22). The presence of the p95 portion of active Her-2 kinase in tumor specimens has been correlated with increased metastasis and decreased survival in breast cancer patients, suggesting that the signaling via p95 is clinically relevant (23-24). Consistent with this clinical observation, *in vitro* studies demonstrate that the truncated Her-2 receptor, p95, is ~ 100 times more effective at cellular transformation compared to the full length Her-2 molecule. Additionally, it has also been shown that very high serum levels of soluble ECD can markedly reduce the half-life of trastuzumab in patients with Her-2 over expressing metastatic breast cancer (25).

Published studies have shown that, *in vitro*, the shedding of Her-2 ECD from human cancer cells can be inhibited by broad-spectrum metalloprotease inhibitors (22). Unfortunately, the clinical utility of broad spectrum metalloprotease inhibitors has been severely limited by the development of a musculoskeletal syndrome of painful fibroplasia, and the resulting dose limitations preclude inhibition of this processing event. Studies performed at Incyte have demonstrated that inhibition of ADAM10 protein expression in Her-2 over expressing human cancer cells by siRNA techniques prevents



Her-2 cleavage *in vitro* (26). This result has been further supported by studies correlating Her-2 shedding inhibition with the enzyme inhibitory potency of an extensive library of selective, small molecule metalloprotease inhibitors. These studies suggest that Her-2 ECD shedding is mediated by ADAM 10 *in vivo*.

Additional support for the role of these proteases in cancer stems from data on the expression of TIMP-3, the endogenous inhibitor of several ADAM and MMP family members. Overexpression of TIMP-3, which would be expected to reduce ErbB ligand and receptor shedding, was shown to suppress tumor growth *in vivo* (27). Further, a significant proportion of primary tumors, including gastric, pancreatic, renal, lung, breast, colon and brain, lack detectable levels of TIMP-3 protein due to aberrant DNA hypermethylation and this reduced expression correlates with disease progression (28-30). These results suggest that regulation of ADAM protease activity is required for normal growth and that loss of this control may be a critical step in tumorigenesis. An ADAM inhibitor would, in essence, restore this regulation.

In summary, inhibition of ErbB pathway activity by selective inhibition of ADAM family metalloproteases may represent a novel and effective approach for treating human cancers where ErbB pathway signaling plays a causal role. A potent and selective ADAM inhibitor would function both to limit the availability of ErbB ligands as well as to prevent the formation of activated ErbB receptors in the tumor cells that over express them, most notably Her-2 over expressing breast cancer.

Based on this rationale, Incyte has discovered and advanced into clinical development INCB7839, a potent and selective inhibitor of ADAM10 and ADAM17 with potency in the low nanomolar range. *In vitro*, INCB7839 effectively blocks the cleavage of Her-2 ECD from the surface of Her-2 over expressing human breast cancer cell lines, as well as the production of soluble EGFR ligands, TGF- $\alpha$  and HB-HGF, by model tumor cell lines. In Her-2 over expressing breast cancer cell lines, INCB7839 markedly potentates the antiproliferative activity of trastuzumab by enhancing the inhibition of signaling pathways



downstream of Her-2, MAPK and Akt activation. *In vitro*, INCB7839, in combination with trastuzumab, enhances the cytotoxic and anti-proliferative effects of the chemotherapeutic agents we have assayed, paclitaxel and gefitinib.

These *in vitro* anti-tumor effects were also observed in a number of xenograft models. As a single agent, INCB7839 can inhibit the growth of EGFR-expressing xenografts, independent of the Her-2 level expressed by the tumor. The effect on tumor growth was equivalent to that achieved with the EGFR kinase inhibitor, gefitinib. Tumor specimens from INCB7839 treated animals show reduced Ki67 staining, a marker of proliferation, and decreased Akt activity, indicating decreased anti-apoptotic signaling. These results are similar to those observed following treatment with agents that directly target ErbB receptors. In combination studies, INCB7839 enhanced the *in vivo* anti-tumor effects of paclitaxel. In a Her-2 over expressing xenograft model, a structurally related inhibitor, INCB3619, that exhibits a similar *in vitro* potency and selectivity profile to INCB7839, effectively inhibited the shedding of Her-2 ECD from the tumor cell surface and markedly enhanced the anti-tumor effects of trastuzumab.

These preclinical findings indicate that INCB7839 is an attractive, novel therapeutic approach for treating human cancers where the activation of ErbB family members plays an important role. Used in concert with anti-EGFR antibodies, tyrosine kinase inhibitors, and/or cytotoxic agents, INCB7839 should provide a third, unique, point of attack on these cancers.

The preclinical safety profile of INCB7839 allowed the first Phase I single and multiple dose study to be conducted in normal healthy volunteers. In preclinical studies, it should be noted that INCB7839 did not cause fibroplasia in a rodent model of the musculoskeletal syndrome induced by broad spectrum metalloprotease inhibitors. INCB7839 is now in a dose escalating, safety and tolerability Phase I/II study in cancer patients with solid tumors in which EGFR receptor signaling is known to be of clinical



significance: Her-2 over expressing breast cancer, non-small cell lung, colon, and squamous cell carcinoma of the head and neck.

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