with IC_{50} values in low nanomolar range. Anticancer effect of our new derivatives was investigated in several cancer cell lines of hematological and solid tumor origin where the compounds induced cell death with low micromolar ED_{50} values. Additionally, synergistic toxic effect was observed in combination with standard therapeutics. Biomarker analysis of Pim kinase downstream targets confirmed Pim-dependent mechanism of action of tested compounds. We have observed a potent inhibition of 4EBP1 and S6 phosphorylation, as well as downregulation of c-Myc levels already after 4h of the treatment with our Pim kinase inhibitors. Following the results obtained *in vitro* in cell culture, compounds were further profiled for their ADMET properties like permeability, metabolic stability and bioavailability. Oral administration of our small molecule Pim kinases inhibitors in a subcutaneous leukemia *in vivo* xenograft model revealed strong inhibition of tumor growth with almost 90% TGI. Moreover, histological analysis of the organs from the animals dosed sub-chronic for over 2 weeks with the compounds, did not reveal any significant organ damaging effects.

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PDL192, a humanized antibody to TweakR exhibits potent anti-tumor activity in non-small cell lung cancer models

B.L. Hylander[1], M. Sho[2], S. Tanlimco[3], D. Choi[2], H. Kim[3], D. Chao[2], E.A. Repasky[1], K. Wilson[3], G.C. Starling[2], P.A. Culp[2]. [1]*Roswell Park Cancer Institute, Immunology, Buffalo NY, USA;* [2]*Facet Biotech, Preclinical Program Research, Redwood City CA, USA;* [3]*Facet Biotech, Research Discovery, Redwood City CA, USA*

Background: Approximately 1.3 million new cases of lung cancer are diagnosed worldwide every year, with non-small cell lung cancer (NSCLC) comprising 80−85% of those cases. The 5-year survival rate for NSCLC is 10−15%, highlighting the need for novel effective therapies. We have generated PDL192, a humanized IgG1 antibody to TweakR (Fn14, TNFRSF12A, CD266), a cell surface protein and member of the TNF receptor superfamily. PDL192 has been shown to exhibit anti-tumor activity in xenograft models on a range of solid tumor types via both direct tumor cell growth inhibition and by antibody-dependent cellular cytotoxicity.
Materials and Methods: In this study, we examined the expression of TweakR in NSCLC patients and explored the activity of PDL192 on lung cancer cell lines *in vitro* and in lung cancer xenograft models derived from cell lines, as well as in primary tumor xenograft models.
Results: TweakR protein was found to be expressed at high levels in all four major subtypes of NSCLC: in 59% of lung adenocarcinomas, 42% of squamous cell carcinomas, 66% of large cell carcinomas, and 75% of bronchioalveolar carcinomas (BAC). In *in vitro* growth assays, PDL192 inhibited the growth of 10 of 19 NSCLC cell lines, which included cell lines derived from each of the four major subtypes. *In vivo*, PDL192 exhibited potent anti-tumor activity (68% inhibition) in the H358 BAC model and exhibited moderate tumor growth inhibition (25%) in the Calu6 adenocarcinoma model. In the H358 model, PDL192 treatment resulted in a decrease in the proliferation marker Ki67 and significantly enhanced the activity of both erlotinib and pemetrexed. PDL192 was also tested for its ability to inhibit the growth of primary tumor xenografts. PDL192 inhibited the growth of 1 of 4 adenocarcinomas tested and 2 of 4 large cell carcinomas. Studies exploring the activity of PDL192 on primary squamous cell carcinomas are currently ongoing.
Conclusions: The *in vitro* and *in vivo* activity of PDL192 demonstrate the potential of this antibody as a therapeutic in NSCLC, both as monotherapy and in combination with erlotinib or pemetrexed, agents currently used as standards-of-care in this disease. PDL192 is currently being evaluated in a Phase 1 safety study in patients with solid tumors.

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Targeting Ras-mutated tumors with novel multiplex PI3K inhibitors through inhibition of eIF-4E-mediated protein translation

M. Hamilton[1], A.K. Bernardino[1], Y. Liu[1], K. Provoncha[1], D. Paul[1], Y. Rotshteyn[1], A. Han[1], D. Qian[1]. [1]*Progenics Pharmaceuticals, Research & Development, Tarrytown NY, USA*

Background: The simultaneous dysregulation of both PI3K and Ras-MAPK pathways is characteristic of some of the most aggressive forms of human cancer (e.g. tumors with Ras mutations). It has been demonstrated in preclinical studies that effective treatment of Ras-mutated tumors requires blockade of both pathways. Therefore, the ability to achieve this outcome with a single agent holds great clinical promise.
One key function of PI3K and Ras-MAPK pathways is to converge at eIF-4E, a critical factor in cap-dependent translation of critical proteins involved in tumorigenesis and tumor cell survival (e.g., c-Myc and Mcl-1). The PI3K pathway activates eIF-4E via mTOR-mediated phosphorylation and suppression of 4E-BP1, a negative regulator of eIF-4E; the Ras-MAPK pathway modulates eIF4E function through phosphorylation by MNK, a downstream kinase of MAPK. A combination of rational drug design and conditional lethal screening led to the identification of a novel lead series of small molecule inhibitors with multiplex activities against PI3K, mTOR and MNK which have been characterized preclinically.
Methods and Results: The anti-tumor activity of novel multiplex PI3K inhibitors was assessed against a panel of 30 human tumor cell lines comprised of various genetic backgrounds and histotypes. Multiplex PI3K inhibitors demonstrate broad and potent anti-proliferative activity (EC_{50}: 10 nM − 500 nM) and induce cell death (i.e. >100% inhibition of cell proliferation) in all cell lines tested, including those harboring Ras mutations (e.g., PANC-1, HCT116 and A549). Mechanistically, multiplex PI3K inhibitors induce caspase activity, inhibit the phosphorylation of AKT, 4E-BP1, ribosomal S6 and eIF-4E, and inhibit the expression of c-Myc and Mcl-1 proteins. In contrast, the dual PI3K-mTOR inhibitor NVP-BEZ235, and the PI3K-selective inhibitor GDC-0941, were partially cytostatic in Ras-mutated tumor cell lines, inhibiting cell proliferation by 60−90%. NVP-BEZ235 and GDC-0941 failed to induce significant increases in caspase activity, inhibit eIF-4E phosphorylation, or inhibit c-Myc and Mcl-1 expression. Multiplex PI3K inhibitors display favorable drug-like properties. In human tumor xenografts *in vivo*, multiplex PI3K inhibitors demonstrate a robust anti-tumor effect and inhibition of pharmacodynamic endpoints (i.e. phosphorylation of AKT, 4E-BP1, ribosomal S6 and eIF-4E).
Conclusion: Newly-discovered novel multiplex PI3K inhibitors are capable of simultaneously targeting both PI3K and Ras-MAPK pathways and exhibit potent anti-tumor activity in Ras-mutated tumors through the inhibition of eIF-4E mediated protein translation. These inhibitors warrant further investigation for development as a potentially novel class of anti-cancer drugs.

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Tigatuzumab, a novel anti-human death receptor 5 antibody, shows synergistic efficacy against colon cancer in vitro and in vivo in combination with chemotherapy

A. Yada[1], Y. Ogitani[1], S. Ishida[1], K. Ichikawa[1], K. Fujiwara[1]. [1]*Daiichi Sankyo Co. Ltd., Oncology Research Laboratories, Tokyo, Japan*

Background: Tigatuzumab is a humanized anti-human death receptor 5 (DR5) antibody and induces apoptosis in several human tumor cell lines. The enhanced effect of anti-DR5 antibodies when combined with chemotherapy or radiotherapy has been reported in tests on several cancers. In this study, we investigated the anti-tumor effect of tigatuzumab combined with irinotecan or 5-fluorouracil (5-FU) in human colon carcinoma HCT 116.
Material and Methods: The effect of tigatuzumab alone or in combination with camptothecin or 5-FU on the proliferation of HCT 116 cells was evaluated. The apoptosis pathway, cell-cycle arrest and changes in DR5 expression induced by each single agent or in combination were analyzed *in vitro*. To study *in vivo* efficacy, HCT 116 cells were inoculated into the flanks of Balb/c nude mice. The mice were treated with tigatuzumab (6 mg/kg, weekly), irinotecan (65 mg/kg, single dose) and 5-FU (90 mg/kg, single dose).
Results: Both camptothecin and 5-FU arrested the proliferation of HCT 116 cells at G2/M phase and G1 phase, respectively. However, addition of tigatuzumab to each chemotherapeutic agent almost completely killed HCT 116 cells with apoptosis induction. These combination treatments were determined as synergism when evaluated by combination index, suggesting that both irinotecan and 5-FU in combination with tigatuzumab may augment the apoptosis induction. Both camptothecin and 5-FU upregulated p53 expression in the HCT 116 cells and induced phosphorylation of p53 at the site of Ser^{15} and Ser^{392}, with increased expression of p21/Cip1 and cell surface DR5. The addition of tigatuzumab resulted in the activation of the apoptosis pathway, along with inducing the upregulation of proapoptotic Bax and Bak, and enhanced cleavage of caspases and PARP when compared with those of each single agent treatment. In a xenograft model, tigatuzumab, irinotecan and 5-FU inhibited the growth of HCT 116 tumors by tumor growth inhibitions of 20%, 39% and 35%, respectively (on Day 31). Combined treatment of tigatuzumab with irinotecan and 5-FU significantly enhanced the anti-tumor activity of each single agent, resulting in tumor growth inhibitions of 54% and 56%, respectively. In addition, p53-dependent apoptosis induction was observed in the xenografted HCT 116 tumors treated with tigatuzumab combined with irinotecan and 5-FU.
Conclusions: These results demonstrate that irinotecan and 5-FU affect a synergistic enhancement on the anti-tumor activity of tigatuzumab by activating p53 and the mitochondrial apoptosis pathway. This combination efficacy was further confirmed in a xenograft model. This evidence supports the use of tigatuzumab in combination with FOLFIRI as an effective treatment for patients with CRC.