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Novel small molecule inhibitors of Jak2/STAT3 pathway: design, structure-activity relationship, and oral bioavailability

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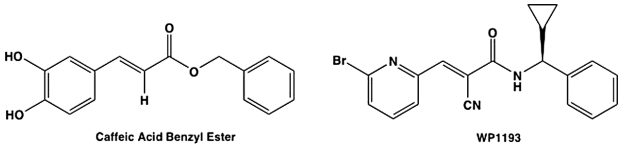
Signal transducer and activator of transcription-3 (STAT3) is constitutively activated in many types of cancer, including pancreatic carcinoma, glioblastoma multiforme, and squamous cell carcinoma of the head and neck. STAT3 activation can promote tumor cell proliferation and survival as well as VEGF expression, angiogenesis, and metastasis in vivo. Because EGF- and IL-6 receptor–mediated signaling induces phosphorylation of Jak2 and then STAT3, both proteins seem excellent targets for drug development. Currently available inhibitors of Jak2/STAT3 activation (e.g., AG490) can inhibit Jak2/STAT3 phosphorylation but only at 50–100 μM concentrations and thus are not active in vivo. Because AG490 shows striking structural similarity to the natural products of the caffeic acid family, we compared the in vitro activity of caffeic acid benzyl ester to AG490 in the Colo357 cell line. Both compounds demonstrated similar activity, which led us to design and synthesize novel inhibitors with far greater potency. This new class of compounds inhibits Jak2 and STAT3 phosphorylation and the related downstream transcription of pro-survival proteins, thereby inhibiting tumor growth in vitro and in vivo. For these studies, all analogs were tested against Colo357-FG pancreatic and U87-MG glioblastoma multiforme cancer cell lines. The most potent compounds were shown to inhibit not only constitutively activated Jak2/STAT3 but also IL-6–stimulated STAT3 activation, suppressing the expression of Stat3 upregulated proteins c-myc, Bcl-2, Bcl-X_L, survivin, and Mcl-1 and inducing apoptosis, all at low micromolar concentrations. To select analogs suitable for more comprehensive preclinical evaluations, we evaluated the pharmaceutical characteristics of the most potent analogs and identified WP1193 as a compound displaying excellent activity and also good oral bioavailability. Preclinical examinations of the in vivo activity, drug biodistribution, and metabolism of WP1193 are currently underway.



Caffeic Acid Benzyl Ester WP1193

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Effect of surgical tumor resection methods on expression of signaling molecules in mouse models

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Surgical stress is thought to have an effect on the mortality and residual tumor progression after surgery; therefore diminishing surgical stress is important. Effect of the surgical stress on the signaling molecules has not been explored. The purpose of this study was to assess in a murine model whether the location of tumor or the resection method has an effect on signalling molecules such as pERK (MAP kinase pathway), pAKT (AKT pathway) or pp38 (MEK pathway). We have evaluated the levels of signaling molecules in subcutaneous and orthotopic MCF-7 human breast tumor xenografts in nude mice as well as in hollow fibers implanted intraperitoneally (IP) and subcutaneously (SC) in these mice. We also compared several methods of tumor tissue collection using cryobiopsy, needle aspiration and classical resection of Colo 829 melanoma tumors implanted subcutaneously into nude mice. The levels of phosphoproteins and total proteins from these tumors were evaluated using western blot analysis. We observed an increase in pERK and total ERK as well as PARP in orthotopic tumors as compared to SC tumors in MCF-7 tumors implanted in nude mice. Quantitatively the levels of pERK, pAKT and pp38 were higher in hollow fibers implanted IP compared to fibers implanted SC. A significant difference was observed in the expression of phosphoproteins and other signaling molecules in tumor tissues collected by cryobiopsy compared to standard resection. In conclusion, the resection method, tumor growth location as well tumor model can affect the signaling molecules. Therefore, one must be cautious during development of experimental protocols to assure that the selected models and methods optimally support the desired experimental goals.

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Epithelial mesenchymal transition (EMT) and hMena expression as determinants of sensitivity of pancreatic adenocarcinoma (PDAC) cell lines to EGFR tyrosine kinase inhibitors (TKI)

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Erlotinib (Tarceva™) is an ATP-competitive EGFR (TKI), that has demonstrated to modestly, but significantly, extend survival in advanced PDAC patients. Here, we sought to identify biomarkers that differentiate erlotinib-responsive from non-responsive PDAC cells. Nine wild-type EGFR human PDAC cell lines were characterized for the expression of EMT markers (E-cadherin, N-cadherin, and vimentin) and hMena, a member of the Ena/VASP family of proteins, involved in the regulation of cell motility and adhesion through actin cytoskeleton remodeling. The growth-inhibitory activity of erlotinib *in vitro*, measured by MTT assay, varied widely among the different cell lines: the four cell lines expressing the epithelial marker E-cadherin (L3.6pl, BxPC3, T3M4, PACA44) showed striking sensitivity (IC_{50}: 0.1–1 μM), while the four cell lines (MiaPaca2, Panc1, HS766T, PT45) expressing N-cadherin and/or vimentin (phenotypic hallmarks of EMT) and the cell line with concurrent expression of epithelial and mesenchymal markers (CfPac1) were relatively resistant (IC_{50} > 10 μM). Interestingly, 2/4 E-cadherin positive and N-cadherin and/or vimentin negative cell lines showed an high level of TGF-α production and constitutive EGFR phosphorylation, whereas a modest TGF-α production and no constitutive EGFR phoshorylation was observed in the N-cadherin and/or vimentin-expressing, erlotinib-resistant cell lines, suggesting the presence of an autocrine, EGFR-mediated, signalling loop in cells with an epithelial phenotype. The analysis of the expression of hMena and its splice variants by western blot and RT-PCR demonstrated a phenotypic correlation between EMT markers, erlotinib sensitivity, and a differential hMena isoform expression pattern. Preliminary data also suggest that the phenotype correlates with functional hMena activity. In fact hMena knockdown with a human ENA-specific siRNA construct reduced the *in vitro* growth rate of the mesenchymal Panc1 cell line as compared to a non-specific siRNA construct. Overall, our data suggest that the EMT status and the differential expression of hMena isoforms are closely related in preclinical models of PDAC and might mark the transition from an epithelial, EGFR signalling-dependent, to a mesenchymal, TKI-resistant, phenotype. These findings may have important implications for the development of novel, molecularly-targeted approaches for the treatment of PDAC.

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AV-412, a potent EGFR/HER2 TK inhibitor causes tumor regression in novel genetically engineered EGFRL858R and EGFRL858R&T790M lung tumor models

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Background: AV-412 (formerly known as MP-412) is a potent and selective inhibitor of the tyrosine kinase (TK) activities of the receptor for epidermal growth factor (EGFR) and HER2 that exerts potent tumor inhibitory activity against wild type and mutant forms of EGFR-TK. AV-412 is scheduled to enter clinical development in 2006.
Material and Methods: AVEO's platform for making complex inducible tumor models uses genetically modified ES cells to generate tumor bearing chimeric mice. Using this platform, multiple lung adenocarcinoma models driven by EGFR mutants, Kras, and HER2 genes known to be altered in human lung cancers have been generated. Furthermore, tumors originally driven by inducible expression of HER2 or Kras have been functionally complemented *in vivo* by expression constructs of EGFR mutants, which provided precise control of the genetic context of the tumor. Tumors from these models have been propagated *in vivo* and tumor inhibitory activity of AV-412 has been examined.
Results: AV-412 is highly active against tumors from the chimeric model carrying $EGFR^{L858R}$ mutation. One mg/kg daily dosing, which is 1/100th of