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Insulin stimulation of head/neck squamous cancer cells confers resistance to the anti-proliferative effects of an EGFR kinase inhibitor

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Although most squamous cell cancers of head/neck (HNSCC) overexpress epidermal growth factor receptors (EGFRs), these tumors respond infrequently to treatment with EGFR kinase inhibitors (EGFR-TKI). In other tumor types, resistance to EGFR-TKIs has been associated with increased expression of IGF-1 receptors. These receptors may generate EGFR-independent growth factor signals that limit the anti-tumor effects of EGFR-TKIs. To test this hypothesis in HNSCC cells, we studied the SCC-25 cell line. Treatment of serum-deprived cells with the EGFR-TKI PD158780 produced a dose-dependent inhibition of growth (IC50=300nM). This correlated with a decrease in the proportion of cells in S-phase at 24 hours from 0.21 to 0.11, as measured by flow cytometry. Immunoblots with phospho-specific antibodies showed that PD158780 treatment blocked constitutive low-level phosphorylation of the EGFR and the potential downstream effectors Gab1, Shc, and Erk. However, there was little effect on the level of spontaneously phosphorylated Stat3. Thus, EGFR-independent signals constitutively activate stat3 despite the fact that treatment of the cells with exogenous EGF increased phosphorylation of stat3 as well as the other proteins. The SCC-25 cells also expressed moderate levels of IGF-1 receptor protein. We attempted to activate the receptors with high concentrations of insulin rather than IGF-1 since these cells are reported to produce high levels of IGF-1 binding proteins. Insulin (0.1; 1uM) treatment produced a modest increase in cell proliferation (1.5X after 48 hours) but also abrogated the negative effects of PD158780 on cell growth and on cell cycle progression. In contrast, EGF (0.1;1nM), IGF (1;10nM), or VEGF (5;50 ng/ml) had little or no influence on drug sensitivity in these assays. Short-term stimulation with insulin (5 min) substantially increased phosphorylation of AKT1 but had minimal effects on Erk or Stat3 phosphorylation, either in the absence or presence of PD158780. Taken together, these studies show that signaling by the IGF-1 and/or insulin receptors in HNSCC cells can abrogate the growth suppressive effects of EGFR kinase inhibition. The mechanism remains to be determined but the ability of insulin to selectively increase AKT phosphorylation suggests that in SCC-25 cells, activation of the upstream phosphatidyl-3-inositol kinase (PI3K) pathway plays a role. Also, constitutive phosphorylation of stat3 may be required but is not sufficient to confer resistance to EGFR-TKIs.

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Pharmacodynamic evaluation of the mTOR inhibitor AP23573 in phase 1 clinical trials

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Background: AP23573 is a novel non-prodrug analog of rapamycin that inhibits mTOR signaling in tumors, which leads to cell cycle arrest, tumor cell shrinkage and inhibition of angiogenesis. mTOR inhibition results in a decrease in the phosphorylation and activity of a number of critical signaling proteins including 4EBP1. We have developed a pharmacodynamic (PD) marker of AP23573 action in peripheral blood mononuclear cells (PBMCs) that is being utilized in ongoing phase 1 trials in cancer patients (pts).
Materials and Methods: Two phase 1 dose escalation trials are being conducted in pts with advanced malignancies to evaluate the safety, tolerability and maximum tolerated dose of AP23573 administered IV using 2 dosing regimens (weekly, and daily × 5 every 2 weeks [QDx5]). The trials also are designed to evaluate potential PD markers of AP23573 activity in PBMCs and to estimate AP23573 pharmacokinetic (PK) parameters. Whole blood samples are being collected for PD analysis at a subset of time points designated for PK sampling. For PD analysis, protein extracts were prepared from PBMCs and analyzed by Western blot using antibodies specific for 4EBP1 phosphorylated at Ser65/Thr70 (P-4EBP1).
Results: A robust P-4EBP1 signal was reproducibly detected in extracts from PBMCs obtained from volunteers, and ex vivo incubation with AP23573 led to a dose-dependent decrease in phosphorylation with a 50% reduction in signal at ~3 ng/mL. PD analysis has been carried out on samples from 8 patients dosed on the weekly schedule (6.25–100 mg) and 10 patients dosed on the QDx5 schedule (3–28 mg). In all patients, P-4EBP1 levels were reduced by at least 90% within 1 h after infusion of AP23573. In pts dosed on the weekly schedule, P-4EBP1 levels remained reduced by >70% 48 h after dosing, with this level of inhibition persisting in some pts for 7 days. Similarly, in pts dosed on the QDx5 schedule, P-4EBP1 levels remained reduced by >70% 72 h after the last dose, with this level of inhibition persisting in some pts for 10 days. AP23573 levels >10 ng/mL generally correlate with >70% inhibition of P-4EBP1.
Conclusions: We have developed a sensitive PD assay that demonstrates rapid and prolonged P-4EBP1 inhibition in PBMCs of pts administered AP23573. Ongoing analysis of additional pts will help determine the relationship between the dose of AP23573 and the duration of the PD response. This information will be used to guide dosing regimens for future trials.

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A phase I and pharmacokinetic study of AEE788, a novel multi-targeted inhibitor of ErbB and VEGF receptor family tyrosine kinases

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Background: An open label phase I dose escalation study of AEE788 is ongoing which evaluates safety, preliminary efficacy, pharmacokinetics (PK) and pharmacodynamics in patients (pts) with advanced cancer. AEE788 is an orally active, reversible, small molecule multi-targeted kinase inhibitor with potent inhibitory activity against ErbB and VEGF receptor family of tyrosine kinases. It is extensively metabolized in the liver by cytochrome P-450 3A4 and forms significant amounts of an active metabolite, AQM674, in humans. AQM674 has *in vitro* pharmacologic activity similar to parent AEE788. 27 pts with advanced cancer received daily doses (qd) of AEE788 at 25, 50, 100, 150, or 225 mg. No DLT has been observed. Mild skin rash and diarrhea have been reported at ⩾ 100 mg/day.
Methods: A 24-hour PK profile was obtained on days 1, 15 and 28, with trough sampling on days 8 and 22 to determine drug serum concentrations (conc) using a validated LC/MS/MS assay. The PK parameters of AEE788 and AQM674 were computed by non-compartmental methods.
Results: Serum conc of AEE788 and AQM674 were highly variable; coefficients of variation were on average 70% in Cmax and AUC. Serum conc of parent and metabolite increase as dose and dose duration increase. AEE788 exposure increases overproportionately with dose. Cmax was reached 2–5 and 3–7 hours post dose for AEE788 and AQM674, respectively. The metabolite serum conc profile appears to reflect relative changes in parent, suggesting rapid metabolite formation and elimination (⩾ parent). The mean metabolite/parent (M/P) ratio is 0.7 (range 0.2 to 2), and appears to decline with dose and/or dose duration. In patients where the accumulation index could be measured, the mean among dose groups was 3.5 (range 2–6) for parent and 3 (range 2–5) for metabolite. The effective half life, estimated from the accumulation index, exceeds 24 hours. Similar exposure of parent and metabolite was observed on day 15 and 28 (except for the 25 mg dose), indicating PK steady state is reached on or before day 15. The PK profiles of AEE788 in rats and humans are similar, however, substantially less AQM674 is formed by rats (M/P ratio after a single intravenous or oral dose of 0.12 and 0.18, respectively).
Conclusion: After oral administration of AEE788 in pts with advanced cancer a significant amount of AQM674, the pharmacologically active metabolite, is rapidly formed by a saturable pre-systemic metabolic process. The serum concentration profile of AQM674 appears to reflect relative changes in parent, suggesting elimination of AQM674 is equal to or more rapid than that of AEE788.

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Pharmacokinetic/pharmacodynamic analysis of OSI-930, a novel selective tyrosine kinase inhibitor with anti-tumor activity

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We have recently identified a series of 2,3-substituted thiophenes with potent inhibitory activity against the tyrosine kinases Kit, KDR and PDGFRa/b, and OSI-930 has emerged from this series as an IND-track clinical candidate for use in tumor types dependent on these receptor tyrosine kinases including GIST, SCLC, renal cell carcinoma, colon carcinoma and glioblastoma. We have investigated the relationships between compound potency in cell-based assays in vitro, plasma exposure levels following oral dosing of OSI-930, the time course of target inhibition in vivo (KDR and Kit), and anti-tumor activity in tumor xenograft models. Thus, in the HMC-1 model, which expresses a constitutively activated form