Using Pharmacokinetic and Pharmacodynamic Data in Early Decision Making Regarding Drug Development: A Phase I Clinical Trial Evaluating Tyrosine Kinase Inhibitor, AEE788

José Baselga1, Alain C. Mita², Patrick Schöffski³, Herlinde Dumez³, Frederico Rojo⁴, Josep Tabernero⁴, Clifford DiLea⁵, William Mietlowski⁵, Christie Low⁵, Jerry Huang⁵, Margaret Dugan⁵, Kathryn Parker⁵, Eric Walk⁶, Allan van Oosterom³, Erika Martinelli⁷, and Chris H. Takimoto²

Abstract

Purpose: In this first-in-human study of AEE788, a tyrosine kinase inhibitor of epidermal growth factor receptor (EGFR), HER-2, and VEGFR-2, a comprehensive pharmacodynamic program was implemented in addition to the evaluation of safety, pharmacokinetics, and preliminary efficacy of AEE788 in cancer patients.

Experimental design: Patients with advanced, solid tumors received escalating doses of oral AEE788 once daily. Primary endpoints were to determine dose-limiting toxicities (DLTs) and maximum-tolerated dose (MTD). A nonlinear model (Emax model) was used to describe the relationship between AEE788 exposure and target-pathway modulation in skin and tumor tissues.

Results: Overall, 111 patients were treated (25 to 550 mg/day). DLTs included rash and diarrhea; MTD was 450 mg/day. Effects on biomarkers correlated to serum AEE788 concentrations. The concentration at 50% inhibition (IC50) for EGFR in skin (0.033 μmol/L) and tumor (0.0125 μmol/L) were similar to IC50 in vitro suggesting skin may be surrogate tissue for estimating tumor EGFR inhibition. No inhibition of p-MAPK and Ki67 was observed in skin vessels at MTD. Hence, AEE788 inhibited EGFR, but not VEGFR, at doses MTD. A total of 16 of 96 evaluable patients showed a >10% shrinkage of tumor size; one partial response was observed.

Conclusion: Our pharmacodynamic-based study showed effective inhibition of EGFR, but not of VEGFR at tolerable AEE788 doses. Emax modeling integrated with biomarker data effectively guided real-time decision making in the early development of AEE788. Despite clinical activity, target inhibition of only EGFR led to discontinuation of further AEE788 development.

Clin Cancer Res; 18(22); 6364–72. ©2012 AACR.

Introduction

Targeting cellular processes essential to tumor cell proliferation, growth, and survival is a major focus of cancer therapy research. Among them, the ErbB tyrosine kinase (TK) receptors (e.g., epidermal growth factor receptors [EGFR]) and VEGFRs are the ones most actively pursued as potential targets for cancer therapy. The ErbB family of TK receptors [EGFR/ErbB-1, HER-2/neu [ErbB-2], HER-3 [ErbB-3], and HER-4 [ErbB-4]] plays an important role in cancer cell proliferation and differentiation as well as angiogenesis and has been implicated in malignant transformation (1, 2). Tumor angiogenesis is regulated by an intricate balance of various growth factors, of which VEGF is considered to be the most potent and critical (3–5). EGFR/ErbB-2 receptor stimulation increases tumor cell expression of VEGF; blocking EGFR and/or ErbB-2 function by using monoclonal antibodies or small molecule TK inhibitors (TKI) decreases tumor cell production of proangiogenic molecules, such as VEGF, and inhibits tumor-associated angiogenesis (3, 6–10). Several agents designed to inhibit EGFR (e.g., gefitinib, erlotinib, cetuximab, panitumumab) or VEGF/VEGFR (e.g., sorafenib, sunitinib, bevacizumab, ranibizumab) are commercially available and/or under investigation (10). However, because tumor cell proliferation and angiogenesis are dependent on numerous complex biochemical pathways, targeting a single step in a single pathway is unlikely to provide optimal therapeutic outcomes, and may even cause tumor cells to divert to
Pharmacodynamic Modeling for Decisions on AEE788 Development

Translational Relevance

Compared with traditional cytotoxic therapies, the clinical development of targeted anticancer therapies, such as AEE788, warrants fundamental changes in conventional clinical trial design and endpoints to accommodate their unique mechanisms of action. In addition to assessing traditional phase I study endpoints (i.e., maximum-tolerated dose [MTD], dose-limiting toxicities [DLT], pharmacokinetics, preliminary efficacy), the effects of AEE788 on its in vitro targets (EGFR, HER-2, VEGFR-2) was evaluated in this first-in-human, phase I study using a pharmacodynamic model that integrates serum AEE788 exposure and pharmacodynamic data to determine AEE788’s effect on the EGFR and VEGF pathways in adults with advanced solid tumors. AEE788 was found to inhibit phosphorylated EGFR (p-EGFR), but not pVEGFR-2 at doses at or below the MTD. Although preliminary efficacy results were favorable, further clinical development of AEE788 was discontinued since studies indicate that interrupting a single target/pathway (vs. multiple targets) is unlikely to provide optimal therapeutic outcomes and is vulnerable to the development of tumor resistance.

Patients and Methods

Patient population

Patients ≥18 years old with histologically confirmed, advanced solid tumors whose disease progressed despite standard therapy or for whom no standard therapy existed were eligible for enrollment. Main inclusion criteria included measurable or nonmeasurable lesion(s) as defined by the modified Response Evaluation Criteria in Solid Tumor (mRECIST) criteria; life expectancy ≥12 weeks; Karnofsky Performance Status (KPS) of ≥70; and adequate bone marrow, hepatic, and renal function (16). Patients were excluded if they had certain preexisting or concomitant malignancies or other severe and/or uncontrolled medical conditions or received prior VEGF or VEGFR or EGFR/ErbB-2 targeted therapies. Use of chemotherapy, investigational drugs, or wide-field radiation therapy had to be discontinued ≥4 weeks, and immunotherapy, major surgery or hematopoietic colony-stimulating factors discontinued ≥2 weeks before study entry. All patients gave informed consent, and approval was obtained from the ethics committees at the participating institutions and regulatory authorities. The study followed the Declaration of Helsinki and good clinical practice guidelines.

Dose escalation

Patients received once daily, continuous dosing AEE788 in 28-day cycles. The starting dose of 25 mg was selected based on 4-week chronic dosing toxicity studies conducted in rats (1/10th of the nonlethal dose) and monkeys. Using a modified Fibonacci design, doses were escalated in increments of 100%, 67%, 50%, 40%, and 33% of the previous dose until dose limiting toxicities (DLTs) were reached. A DLT was defined as an adverse event (AE) or abnormal laboratory value assessed as unrelated to disease progression, concurrent illness, or concomitant medication that occurred during the 28 days following the first dose of AEE788 in cycle 1 and met predefined criteria (grade ≥ 3 hematologic, hepatic, cardiac, or other AE; grade ≥ 2 renal AE or hypertension; grade > 1 neurologic AE; and/or skin AE requiring AEE788 interruption longer than 7 days). The maximum tolerated dose (MTD) was determined based on a "3 + 3" algorithm using the DLT data from cycle 1. After the completion of the dose-escalation phase, a dose expansion phase further evaluated the safety and efficacy of AEE788; the doses evaluated in the dose-expansion phase were chosen based on the safety and clinical data obtained from the dose-escalation phase.

Study endpoints

The primary study endpoint was to define MTD based on the DLTs of AEE788 in patients with advanced solid tumors. Secondary endpoints included (1) determining the safety and tolerability of AEE788; (2) characterizing the single- and repeated-dose PK profile of AEE788 and its primary metabolite, AQM674; (3) identifying potential biomarkers indicative of target inhibition and efficacy; and (4) evaluating preliminary antitumor activity of AEE788.
Specimen collection
Venous blood samples were collected before each dose on days 1, 8, 15, 22, and 28 of cycle 1 and days 15 and 28 of cycle 2. Postdose venous blood samples were collected at 1, 2, 3, 4, 7, 10, and 24 hours postdose on days 1 and 15, and 1, 3, 5, 9, and 24 hours postdose on day 28 of cycle 1. Trough levels were measured immediately before drug administration on days 8 and 22 of cycle 1, day 15 of cycle 2, and day 1 of cycle 3.

A pair of 4-mm-skin biopsies was obtained from the right upper back before treatment was initiated on days –8 and –1. A second pair of 4-mm-skin biopsies was obtained from the left upper back after treatment initiation on days 22 and 29 of cycle 1. The second in each pair of skin biopsies (i.e., those obtained on days –1 and 29 of cycle 1) was obtained from an area partially overlapping the wound resulting from the corresponding first biopsy in each pair (i.e., those obtained on days –8 and 22 of cycle 1) to collect neovascularized granulation tissue for the purpose of assessing VEGF pathway inhibition in endothelial cells. Incisional or core biopsies were used to collect tumor tissue before (i.e., on days –14 to –1) and after (i.e., day 28 of cycle 1) treatment was initiated; additional posttreatment biopsies were collected when possible.

Immunohistochemistry analysis of skin and tumor biopsies
Tissue biomarker studies were used to determine whether AEE788 inhibits EGFR and VEGFR2 activation in skin and tumor cells. Changes in expression of the receptors, activation level of receptors, relevant downstream signaling network components, and markers of apoptosis, cell cycle control, and proliferation were assessed using immunohistochemistry (IHC) performed on formalin-fixed, paraffin-embedded skin and tumor biopsies, pre- and posttreatment. Phosphorylated (i.e., activated) EGFR (p-EGFR), VEGFR-2 (p-VEGFR2), and mitogen-activated protein kinase (p-MAPK) levels and Ki67 proliferation index were measured in both tumor and basal skin tissues; tumor tissue was also assessed for cyclin D1 and phosphorylated v-akt murine thymoma viral oncogene homolog 1 (p-Akt). Phosphorylated signal transducer and activator of transcription 3 (p-STAT3) expression was measured in skin tissue only. Ki67 and p-MAPK expression were measured in endothelial cells present in skin granulation tissue. IHC analysis of EGFR-related ligands, including EGF, TGF-α, heregulin, and neuregulin were also performed in tumor biopsies.

Total forms of the phosphorylated protein biomarkers were also assayed as controls of stability of the respective proteins. Expression of biomarkers was calculated in a blind fashion using an H-score, as previously described (refs. 17, 18; Supplementary Table 1)

Pharmacokinetics
AEE788 and AQM674 (N-de-ethylated AEE788) concentrations in serum were quantified using a validated liquid chromatography-tandem mass spectrometry assay (Novartis Pharmaceuticals Corporation). PK parameters, including maximum concentration (C_{max}), time to maximum concentration (T_{max}), area under the concentration time curve (AUC), and average steady-state concentration (C_{avss}) were determined for both AEE788 and AQM674. Predose (zero) AUC to C_{last} (AUC_{0-C_{last}}), where C_{last} is the concentration of the last quantifiable serum level, and predose to 24 hours (AUC_{0-24}) were computed by the linear trapezoidal rule. The C_{avss} was defined as AUC_{0-24}/24 and the C_{avss} sum was computed as C_{avss} AEE788 + C_{avss} AQM674.

Pharmacodynamics
PD modeling was used to explore the relationship between the drug exposure and AEE788 PD in skin and tumor tissue. Percentage change in biomarkers from predose was computed by %dE = 100 × (Eo – Ed)/Eo (for inhibition), where Eo and Ed are measured pre- and posttreatment values, respectively. An inhibitory E_{max} model, a logistic model derived from pharmacologic theory of drug-receptor interactions used to describe the relationship between a drug concentration and drug effect, was fit to %dE and sum C_{avss} data (%dE = [E_{max} × C_{avss}]/[IC_{50} + C_{avss}]); zero and negative values were excluded (19-21). Maximum inhibition (E_{max}) of biomarkers was achieved when all target receptors were occupied (efficacy and or intrinsic efficacy equal to 0); the serum drug concentration for 50% inhibition (IC_{50}) and 80% inhibition (IC_{80}) were determined. Modeling procedures were carried out by fitting a mathematical inhibitory E_{max} model to observed concentration-effect data with SAS software and model parameters (E_{max}; IC_{50}) estimated by nonlinear, least-squares regression.

Statistical analyses of PD markers
An exploratory analysis was performed to identify target biomarkers that correlated with progression-free survival (PFS); prognostic patterns were characterized for biomarker pairs (i.e., baseline only, postbaseline only, percentage change from baseline). Because these analyses were completed for exploratory and hypothesis-generating purposes only, no corrections for multiplicity were performed. All biomarker data (both pre- and posttreatment) were standardized by subtracting the pretreatment mean and dividing by the pretreatment standard deviation (SD; linear or log scale) making the pretreatment SD the unit of measurement for all biomarker data. Cox proportional hazards models were applied to these standardized biomarker pairs, adjusting for AEE788 dose; statistical significance (P < 0.05) was determined using likelihood ratio tests. To facilitate clinical interpretation of the findings, Kaplan–Meier estimates of PFS were generated for relevant biomarker subgroups formed by dichotomizing biomarkers at the median. Statistical analyses were performed using SAS Version 8.2 and S-plus version 6.2.

Results
Patients
Between July 2003 and August 2005, 111 patients were enrolled. Three to six evaluable patients were enrolled to
each dose levels: 25, 50, 100, 150, 225, 300, 400, 450, 500, and 550 mg. Baseline patient characteristics are summarized in Table 1. DLTs were reported in 1 of 6, 2 of 6, and 2 of 6 MTD evaluable patients in the 450 mg (skin rash), 500 mg (diarrhea) and 550 mg (diarrhea) dose groups, respectively. The MTD was therefore defined as 450 mg. The most frequently reported drug-related AEs in all groups (reported in >30% of patients) were diarrhea, nausea, anorexia, rash, fatigue, and vomiting (Supplementary Table 2). Grade 3 AST/ALT abnormalities were reported in 13.5% (n = 15) of patients, with grade 4 abnormalities reported in 1 patient (0.9%). Five patients (4.5%) experienced grade 3/4 AST/ALT with a concomitant grade ≥ 2 rise in total bilirubin. The median time to grade 3/4 AST/ALT abnormalities was 91.5 days (range, 8 to 163 days). The incidence of grade 3 or 4 AST/ALT abnormalities did not appear to be dose dependent. Median duration of therapy for all patients (n = 111) was 56 days and was 2 times longer for the 400 to 450 mg cohort (95 days; range, 7 to 393) compared to the other dose groups (25 to 250 mg: 44 days [range 14 to 441]; 250 to 300 mg, 50 days [1 to 153]; 500 to 550 mg, 54 days [7 to 231]). One confirmed (confirmed against RECIST and by an independent radiologist) partial response of 4-month duration was observed in a patient with a heavily pretreated angiosarcoma receiving 400 mg daily. Stable disease for ≥2 cycles across all doses was observed in 43 of 95 patients (45%). Other patients also benefited from AEE788 treatment as shown in Fig. 1, which shows a waterfall plot of maximal tumor percentage change from baseline for those patients with measurable disease; 17 of 94 (18%) showed a >10% shrinkage of tumor size.

Approximately 20% of patients initially receiving 225 to 300 mg experienced grade 3 AST/ALT; therefore, additional 250 mg cohorts were enrolled in the dose-expansion phase to further evaluate AEE788 safety. Likewise, because the MTD was found to be 450 mg and 1 patient experienced a partial response at 400 mg, additional 400 mg cohorts were evaluated in the dose-expansion phase to further evaluate AEE788 safety and clinical activity.

Pharmacokinetics

Serum concentrations of AEE788 and AQM674 were highly variable. The mean coefficients of variation (SD/ mean) for Cmax and AUC0-24 were on average 70%. Cmax for AEE788 and AQM674 was achieved at 2 to 7 and 3 to 8 hours postdose, respectively, and fluctuated 2-fold (Cmax/ Cmin ratio) during the 24-hour dosing interval (Supplementary Table 3).

Serum concentrations of AEE788 and AQM674 increased with increasing dose and dose duration (number of doses) (Fig. 2). AEE788 exposure (a factor of both drug dose and elimination) increased overproportionately with the dose while AQM674 exposure increased proportionately with the dose. After 15 and 28 days of dosing, parent drug and metabolite exposure were 2- to 8- and 2- to 5-fold higher, respectively, than the first dose exposure. Although the apparent terminal disposition (first order) rate constant

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enrolled, no. (%)b</td>
<td>111 (100%)</td>
</tr>
<tr>
<td>Female</td>
<td>51 (45.9)</td>
</tr>
<tr>
<td>Male</td>
<td>60 (54.1)</td>
</tr>
<tr>
<td>Treated per dose level (mg/day)</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>150</td>
<td>5</td>
</tr>
<tr>
<td>225</td>
<td>6</td>
</tr>
<tr>
<td>250</td>
<td>23c</td>
</tr>
<tr>
<td>300</td>
<td>8</td>
</tr>
<tr>
<td>400</td>
<td>31c</td>
</tr>
<tr>
<td>450</td>
<td>7</td>
</tr>
<tr>
<td>500</td>
<td>6</td>
</tr>
<tr>
<td>550</td>
<td>9</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>57</td>
</tr>
<tr>
<td>Range</td>
<td>20–78</td>
</tr>
<tr>
<td>Diagnosis, n (%)</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>14 (12.6)</td>
</tr>
<tr>
<td>Colorectal</td>
<td>14 (12.6)</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>11 (9.9)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>10 (9.0)</td>
</tr>
<tr>
<td>Lung</td>
<td>8 (7.2)</td>
</tr>
<tr>
<td>Thyroid</td>
<td>7 (6.3)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>4 (3.6)</td>
</tr>
<tr>
<td>Esophagus</td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>Prostate</td>
<td>2 (1.8)</td>
</tr>
<tr>
<td>Other</td>
<td>39 (35.1)</td>
</tr>
<tr>
<td>Karnofsky performance status, n (%)</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>4 (3.6)</td>
</tr>
<tr>
<td>80</td>
<td>30 (27.0)</td>
</tr>
<tr>
<td>90</td>
<td>53 (47.7)</td>
</tr>
<tr>
<td>100</td>
<td>24 (21.6)</td>
</tr>
<tr>
<td>Previous therapy, n (%)</td>
<td></td>
</tr>
<tr>
<td>Radiation therapy</td>
<td>47 (49)</td>
</tr>
<tr>
<td>Systemic therapy</td>
<td>96 (100)</td>
</tr>
<tr>
<td>0 regimens</td>
<td>31 (32.3)</td>
</tr>
<tr>
<td>1 regimen</td>
<td>14 (14.6)</td>
</tr>
<tr>
<td>2 regimens</td>
<td>21 (21.9)</td>
</tr>
<tr>
<td>≥3 regimens</td>
<td>30 (31.3)</td>
</tr>
<tr>
<td>Prior anti-EGFR/VEGF</td>
<td>5a (5.2)</td>
</tr>
</tbody>
</table>

aOn the basis of all enrolled patients.
bIncludes only patients enrolled in the continuous once-daily dosing group.
cThirty-nine patients were enrolled at doses of 250 mg (n = 14) and 400 mg (n = 25) for further safety (hepatotoxicity) efficacy (response rate) evaluations; these patients were not considered to be a part of the MTD-determining cohorts. dException for exclusion criteria granted to 5 patients.
could not be accurately estimated, the observed accumulation did not appear to be caused by a decrease in the elimination process. Exposure of parent drug and metabolite after 15 and 28 days of dosing were similar (except for the 25 mg dose), suggesting that steady state is reached on or before day 15.

AQM674 serum concentrations reflect relative changes to the parent drug, suggesting rapid metabolite formation with elimination equal to or faster than the parent drug. The ratio of metabolite to parent drug ranged from 0.2 to 2 and declined with dose and dose duration to a range of 0.3 to 0.4 (225 to 400 mg), an indication that metabolite formation pathway is saturable. Exposure-demographic (age, weight, body surface area, gender) relationships were explored, but not observed.

**Pharmacodynamics**

Cycle 1 day 28 (C1D28) AEE788 and AQM674 exposure in serum and corresponding biomarker response data in skin and tumor tissue was available for 46 to 53 and 10 to 13 patients, respectively, depending on the tissue and biomarker (Supplementary Table 4). Biochemical and functional effects correlate with serum AEE788 and AQM674 concentrations in a pattern that could be characterized by an inhibitory $E_{\text{max}}$ model.

A statistically significant inhibition of p-EGFR and downstream biomarkers was observed, both in basal skin and vascular granulation tissue, comparing pre- and posttreatment expression levels (basal skin: $P < 0.001$ for p-EGFR, p-MAPK, p-STAT3, and Ki67; endothelial cells: $P = 0.019$ for p-VEGFR2; $P < 0.001$ for p-MAPK; $P = 0.023$ for Ki67).
tumor, a significant inhibition of EGFR activation was shown after AEE788 treatment \( (P = 0.001) \), but no downstream effects were observed. Supplementary Fig. 1 shows the pre- and posttreatment IHC results for p-MAPK and Ki67 in tumor, basal skin, and vascular granulation tissues, p-EGFR in tumor and basal skin tissues, and p-Akt in tumor tissue in 2 selected patients treated at 25 and 550 mg dose levels.

The percentage inhibition in skin EGFR phosphorylation and Ki67 (Fig. 3) and tumor p-EGFR and p-MAPK (Fig. 3) levels increased with increasing drug dose and serum concentration of both AEE788 and AQM674. Inhibitory \( E_{\text{max}} \) model fits of the effects-concentration data, however, showed large variability as shown by the wide 95% confidence intervals around model predicted (fitted) \( E_{\text{max}} \) and \( IC_{50} \) values (see Table 2). The 95% confidence intervals around the \( IC_{50} \) values for p-MAPK (\( IC_{50}, 0.00372 \mu\text{mol/L} \)), p-Akt (\( IC_{50}, 0.00128 \mu\text{mol/L} \)), and Ki67 (\( IC_{50}, 0.0225 \mu\text{mol/L} \)) in tumor tissue included zero and were, therefore, not considered good estimates. The \( IC_{50} \) values for p-EGFR in skin (0.033 \( \mu\text{mol/L} \)) and tumor (0.0125 \( \mu\text{mol/L} \)) were in relatively good agreement and suggested the skin may be a potential surrogate tissue for p-EGFR inhibition in tumor tissue.

Conversely, inhibition of p-VEGFR-2 and cyclin D1 was not observed in tumor tissue, most likely because serum concentrations necessary to inhibit these markers were higher than serum concentrations achieved with the MTD (i.e., toxicity occurred before concentrations required to inhibit these biomarkers was achieved). In terms of effects of AEE7888 on vasculature, no effect was seen on p-MAPK and Ki67 levels in skin vascular granulation tissue at doses below MTD; inhibition of vasculature p-MAPK and Ki67 was observed at doses >400 mg. Table 2 summarizes the estimated \( E_{\text{max}} \) model parameters for selected biomarkers in skin and tumor tissue.

**Exploratory statistical analysis of biomarker data**

**Tumor biomarkers.** Because of the limited number of patients in whom fully evaluable paired tumor samples...
were available (n = 24), tumor biomarker analyses were exploratory only. The Ki67 biomarker was identified as a potential predictive factor for PFS (likelihood ratio $\chi^2 = 16.999; 2$ degrees of freedom [DF]; $P = 0.0002);$ Table 2 summarizes the Cox proportional hazard model estimates for the Ki67 biomarker pairs). In particular, the relative hazard for PFS depends strongly on the posttreatment Ki67 tumor value and is virtually independent of the baseline Ki67 tumor value. Interestingly, the percentage change from baseline in Ki67 expression does not appear to be meaningful in terms of prognosis, whereas the C1D28 Ki67 H-score appears to contain essentially all prognostic information. Consequently, a patient with a higher C1D28 Ki67 value has a higher risk of disease progression after C1D28 relative to the patient with the smaller C1D28 Ki67 value; a risk that is independent of baseline value. For example, a Kaplan–Meier plot depicts the PFS of patients with a C1D28 Ki67 value at the median value or less (<30) verses those with a C1D28 Ki67 value greater than the median (>30; Fig. 4). A strong association between the baseline Ki67 value in tumor tissue and the C1D28 Ki67 value was also observed ($n = 24;$ Spearman correlation $= 0.6399; P = 0.0008$).

**Basal skin biomarkers.** AEE788 dosing was a significant prognostic factor for PFS in the subgroup of patients from whom basal skin samples were obtained. The log p-STAT3 biomarker pair was identified as a potential predictive factor for AEE788 treatment (likelihood ratio $\chi^2 = 6.705; 2$ degrees of freedom; $P = 0.035$) in this subgroup. Table 2 summarizes the Cox proportional hazard model estimates for the log p-STAT3 biomarker pair. Higher baseline log p-STAT3 levels in basal skin tissue appears to be a potential adverse predictive factor for PFS in patients treated with AEE788. For example, the PFS rates of patients with a baseline basal skin p-STAT3 value at the median value or less (≤10) verses those greater than the median value (>10) are depicted in the Kaplan–Meier plot in Fig. 4.

**Discussion**

Validated methods to identify optimal dose–response relationships, clinically monitor drug activity, predict therapy outcome, or identify patients and/or tumors most likely to respond to anti-VEGF and anti-EGFR therapies are lacking (22). Identifying PD surrogate markers, such as molecular (circulating VEGF), cellular (circulating endothelial cells), or functional (imaging) parameters that reveal the genetic or functional status of a tumor and whether the tumor is responsive to multitargeted treatments, such as AEE788, may help optimize drug development, clinical trial design, and clinical use of these therapies. Although others have suggested that biomarker analyses in early drug development may only add to cost and erroneous decision making, we found biomarker data to be essential to our decision to terminate further development of AEE788 beyond phase I trials (23). Our approach was to determine the relationship of AEE788 PK and PD using the $E_{\text{max}}$ model and to couple the results with biomarker, efficacy, and safety data to facilitate real-time decisions regarding the clinical development of AEE788.

Although variability in drug exposure and biochemical responses were observed, the PD profiles of EGFR-pathway abrogation in skin and tumor can be considered similar ($E_{\text{max}}$ and IC$_{50}$ values in the same range), suggesting that EGFR inhibition in skin tissue may be a useful surrogate for EGFR inhibition in tumor tissue. A dose–response curve was used to identify AEE788 doses (150 to 225 mg po daily) that corresponded with near maximum inhibition (80%) of p-EGFR in skin and tumor providing consideration of pharmacologic effect of AEE788 for dose escalation. Furthermore, these dose–response curves helped identify AEE788 doses associated with the greatest inhibition of certain biochemical pathways relative to safety and efficacy findings at the same dose. In preclinical studies, AEE788 was found to inhibit EGFR and VEGFR-2 (6). Although the effects of EGFR and VEGFR pathway biomarkers in skin and tumor tissue correlated well with serum concentrations of both AEE788 and AQM674, when the $E_{\text{max}}$ model was applied AEE788 was found to only inhibit activation of EGFR, but not VEGFR-2 in skin and tumor tissue at serum

---

**Figure 4.** Kaplan–Meier plot depicting PFS in patients with (A) a day 28 tumor Ki67 value at the median value or less (≤30) verses those with a day 28 Ki67 value greater than the median value (>30) and (B) a baseline pSTAT3 value at the median value or less (≤10) verses those with a baseline pSTAT3 value greater than the median value (>10). PD, progressive disease.
concentrations at or below the MTD (450 mg). Concentrations to achieve near maximum (80%) p-EGFR inhibition occurred between 0.05 and 0.13 μmol/L, which corresponded with an AEE788 dose of 150 to 225 mg daily. IC50 values of the functional downstream markers p-MAPK (0.02 μmol/L) and Ki67 (0.0076 μmol/L) in skin were similar the IC50 for p-EGFR, suggesting the effects are likely driven by p-EGFR inhibition. At doses achievable in this clinical trial, AEE788 did not, however, inhibit VEGFR-2 in tumor tissue as was hypothesized. Further supporting these findings was the lack of MAPK and Ki67 inhibition in endothelial cells from the skin. Moreover, a large variability in serum AEE788/AQM674 exposure and observed change in biomarker expression was observed, which was likely caused by the small sample size of tumor tissue and/or that most tumor tissue samples came from patients treated at doses less than the MTD. Nevertheless, modeling the PK/PD data made it apparent that AEE788 did not meet the predefined criteria for further clinical development. Based on our findings it was concluded that AEE788 would not be able to provide optimal VEGFR inhibition at tolerable doses and, therefore, would provide no additional benefit from currently available EGFR inhibitors.

Although the exploratory statistical biomarker analysis was not a primary outcome of this study, the analysis was conducted to identify tumor and/or skin tissue biomarkers potentially predictive for PFS for further evaluation in future clinical trials. In this analysis, posttreatment tumor tissue Ki67 and basal skin pSTAT3 levels were found to be significant predictors of PFS. Patients with C1D28 Ki67 (biomarker indicative of cellular proliferation) levels less than the median level observed for the study population experienced a longer PFS. Whether these findings were related to AEE788’s effect on tumor cell proliferation or suggest that slowly proliferating tumors respond to AEE788 more than rapidly proliferating tumors is not known; however, the existence of a correlation between Day 28 Ki67 levels and PFS despite baseline Ki67 levels suggests a direct effect of AEE788 on tumor cell proliferation. The extrapolation of these observations to other populations or settings is questionable; the tumor tissue dataset was small and obtained from patients treated at lower doses (i.e., <400 mg) for shorter durations.

Although the association between basal skin p-STAT3 level changes and PFS was somewhat weaker, p-STAT3 appears to be a tumor progression marker and was up-regulated by AEE788 in basal skin and higher baseline levels were associated with shorter PFS times. Basela and colleagues and Vanhoef and colleagues reported similar findings with other EGFR inhibitors, gefitinib and EMD 72000, respectively [17, 18]. These findings suggest that STAT3 may be a valuable surrogate marker for identifying tumors or patients most likely to benefit from anti-EGFR therapy and may be associated with anti-EGFR therapy resistance development; however, further study is needed.

Conclusion

According to our PD model, which integrated serum exposure of AEE788 and AQM674 and PD data to determine the effects of AEE788 on various targets in the EGFR and VEGF pathways, AEE788 inhibits pEGFR, but not pVEGFR-2 at doses below the MTD. These findings suggest that AEE788, administered at doses at or below the MTD, inhibits one (EGFR) rather than multiple tyrosine kinase receptor targets. Furthermore, biomarker analyses were useful for identifying drug activity in the absence of efficacy. These findings, coupled with the delayed hepatotoxicity and limited clinical efficacy observed with once-daily continuous AEE788 dosing, led to the termination of the AEE788 drug development program. In conclusion, Fmax modeling integrated with biomarker data effectively guides real-time decision making in the early stages of drug development.

Disclosure of Potential Conflicts of Interest

J. Baselga and P. Schoffski are consultants/members of an advisory board for Novartis Pharmaceuticals Corporation; P. Schoffski and C.H. Takimoto received research grants/support from Novartis Pharmaceutical Corporation; P. Schoffski received honoraria for speakers bureau from Novartis Pharmaceuticals Corporation; W. Mietelowski, M. Dugan, and J. Huang have ownership interest in and are employees of Novartis Pharmaceuticals Corporation; C. Low and K. Parker are employees of Novartis Pharmaceuticals Corporation, H. Dumez, E. Martinelli, A.C. Mita, J. Tabernero, A. van Oosterom, and E. Walk have nothing to disclose.

Authors’ Contributions

Conception and design: J. Baselga, F. Rojo, J. Tabernero, J. Huang, M. Dugan, E. Walk, A. van Oosterom, C.H. Takimoto

Development of methodology: J. Baselga, F. Rojo, J. Tabernero, C. DiLea, M. Dugan, E. Martinelli

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Baselga, A.C. Mita, P. Schoffski, H. Dumez, F. Rojo, J. Tabernero, C. Low, M. Dugan, A. van Oosterom, E. Martinelli, C.H. Takimoto

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Baselga, A.C. Mita, H. Dumez, F. Rojo, J. Tabernero, C. DiLea, W. Mietelowski, K. Parker, E. Walk, C.H. Takimoto

Writing, review, and/or revision of the manuscript: J. Baselga, A.C. Mita, P. Schoffski, H. Dumez, F. Rojo, J. Tabernero, C. DiLea, C. Low, J. Huang, M. Dugan, E. Walk, A. van Oosterom, C.H. Takimoto

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): P. Schoffski, H. Dumez, C. Low, K. Parker

Study supervision: J. Baselga, F. Rojo, J. Tabernero, C. Low, J. Huang, M. Dugan, K. Parker, C.H. Takimoto

Acknowledgments

We are indebted to the patients who participated in this study and the study coordinators and research nurses who executed the study. We thank Haobo Ren and Ping Yao for contributions to biomarker analysis, and Syntaxx Communications, Inc. for assistance with manuscript development and editing.

Grant Support

Novartis Pharmaceutical Corporation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 10, 2012; revised August 14, 2012; accepted August 28, 2012; published OnlineFirst September 26, 2012.
References

Using Pharmacokinetic and Pharmacodynamic Data in Early Decision Making Regarding Drug Development: A Phase I Clinical Trial Evaluating Tyrosine Kinase Inhibitor, AEE788

José Baselga, Alain C. Mita, Patrick Schöffski, et al.