Demonstration of the equivalent pharmacokinetic/pharmacodynamic dosing strategy in a multiple-dose study of gefitinib

Shining Wang, Qingyu Zhou, and James M. Gallo

Department of Pharmaceutical Sciences, School of Pharmacy, Temple University, Philadelphia, Pennsylvania

Abstract

The goals of this investigation were to illustrate the use of pharmacokinetic (PK)/pharmacodynamic (PD) modeling strategies in drug development based on a multiple-dose study of gefitinib in a preclinical tumor model. Mice bearing s.c. LN229-wild-type epidermal growth factor receptor or LN229-EGFRvIII mutant (a sensitizing mutation) tumors were administered gefitinib at oral doses of either 55 mg/kg/d p.o. × 15 days or 30 mg/kg/d p.o. × 15 days, respectively, as dictated by the equivalent PK/PD dosing strategy. In each tumor group, gefitinib plasma and tumor concentrations were quantitated, as well as the tumoral amounts of phosphorylated-extracellular signal-regulated kinase 1/2 (pERK), a selected PD end point, and tumor size. The resultant data provided the basis to develop hybrid physiologically based PK/PD/tumor growth models for each tumor type. It was found that the 1.83-fold dose difference administered to the two tumor groups resulted in analogous pERK profiles on both days 1 and 15, and further induced similar antitumor efficacy based on tumor size. In addition, using brain tumor patient PK data linked to the pERK PD model, simulations were conducted to illustrate potential applications of a target tumor model to patients. The simulations provided insight on the relationships between blood-brain barrier penetration, brain tumor gefitinib concentrations, and the extent of inhibition of pERK. The implementation of the PK/PD equivalent dosing strategy offers a new approach to drug development. [Mol Cancer Ther 2009;8(6):1438–47]

Introduction

The epidermal growth factor receptor (EGFR) is an important anticancer drug target based on its role in tumor cell proliferation, and because it is overexpressed or mutated in many cancers, such as malignant gliomas in which it is altered in over 50% of patients. The activation of EGFR, located on the cell membrane, causes activation of key downstream signaling pathways, notably ERK and PI3K-AKT, which are involved with the maintenance of the cancer phenotype of unabated growth and inhibition of apoptosis. Therapeutic intervention of EGFR signaling has included antibodies (i.e., Cetuximab) binding to the extracellular domain, and small molecule inhibitors, such as erlotinib and gefitinib that competitively bind to intracellular kinase domains. The clinical activity of these agents is the subject of numerous trials, mostly as part of combination chemotherapeutic strategies (1,3). In an effort to define a role for anti-EGFR therapy, a number of EGFR gene mutations have been identified that are associated with drug sensitivity or resistance. One mutation in the EGFR gene, known as the VIII mutant, causes constitutive activation and of its signaling partners, is prominent in 27% to 64% of glioma patients with overexpressed EGFR, and causes sensitization to EGFR inhibitors, particularly in the presence of normal phosphatase and tensin homologue. It is likely that anti-EGFR therapy will be integrated into the fabric of cancer chemotherapy in a number of cancer types in the foreseeable future.

There has been a resurgence in drug discovery and development approaches attributed, in part, to the failure of past strategies overly focused on high-throughput screens, to the limited number of new drug approvals by the Food and Drug Administration, and to the explosion of potential new drug targets afforded by the human genome. A recent panel, the Translational Research Working Group for anticancer drug development (6), highlighted many issues confronting the drug development community and considered the importance of preclinical pharmacokinetic (PK) studies. This is not a new idea, and in the Food and Drug Administration Critical Path Initiative and subsequent documents, the desire for model-based drug development was recognized as an important fundamental direction. Although these initiatives are timely and stimulate discussion, the lack of attention to how preclinical PK and pharmacodynamic (PD) investigations should be conducted and integrated into a global translational effort is apparent. Our efforts in this regard have highlighted the importance of examining drug disposition and dynamics in target tissues that allows comprehensive PK/PD models to be derived and extrapolated to predict pharmacologic characteristics in patients (8,9).
The combination of model-based drug development and burgeoning interest in EGFR inhibitors led to our first study on gefitinib (9). This study used LN229 EGFR wild-type and vIII mutant tumors and single doses of gefitinib to contrast the PD response by measuring phosphorylated-extracellular signal-regulated kinase 1/2 (pERK) in tumors. The resultant PK/PD models of gefitinib in each tumor type were used to illustrate the concept of equivalent PK/PD dosing that devised multiple-dose regimens to achieve the same degree of pERK inhibition. To assess the validity of the equivalent PK/PD methodology, the current investigation completed multiple-dose trials in wild-type EGFR and EGFRvIII mutant tumor groups, and extended the associated PK/PD models to a preclinical efficacy model based on tumor size, and finally to brain tumor patients.

Materials and Methods
Chemicals and Reagents
Gefitinib was supplied by Polymed Therapeutics, Inc. LN229-wild-type EGFR cell line was purchased from the American Type Culture Collection. LN229-EGFRvIII mutant cell line was kindly provided by Dr. W.K. Cavenee (University of California, San Diego, CA). The following reagents were purchased from commercial suppliers: DMEM (Mediatech, Inc.), heat-inactivated fetal bovine serum (Invitrogen), penicillin and streptomycin (Life Technologies-Bethesda Research Laboratories), Matrigel matrix (Becton Dickinson), and ELISA kit to measure pERK (R&D systems).

Tissue Culture
Human glioblastoma cell lines LN229-wild-type EGFR and LN229-EGFRvIII mutant were cultured in DMEM.
supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO₂ at 37°C.

Design of Gefitinib Dosing Regimens for EGFR Wild-Type and vIII Mutant Tumor-Bearing Groups

The design of gefitinib multiple-dose regimens for EGFR-wild-type and vIII mutant tumor-bearing mice was done in a series of steps that culminated in the selection of gefitinib doses of 55 mg/kg/d p.o. × 15 d in the wild-type tumor group and 30 mg/kg/d p.o. × 15 d in the vIII mutant tumor group. The goal of these steps that used the PK/PD model derived from our single dose studies (9) was to identify gefitinib doses that produced equivalent pERK profiles in each tumor group based on maximum gefitinib tumor concentrations of 15 μg/g in wild-type EGFR tumors. This concentration was targeted because it was within the range of values reported in brain tumor patients (10). The model-predicted PK inhibition nadirs and area between the effect curves (ABEC) values, a measure of the cumulative degree of inhibition, at the designated equivalent doses were 0.36 versus 0.35 and 132 versus 129 for the EGFR wild-type and the vIII mutant tumor group, respectively. ABEC is determined as the difference between the baseline value of pERK (equal to 1 without drug present) to the pERK values obtained after inhibition due to gefitinib and, as a cumulative measure, in this case, drug response, is complimentary to the area under the drug concentration-time curve or AUC that measures the cumulative drug exposure.

In vivo Experiments of Gefitinib in Xenografts of EGFR-Wild-Type and Mutant Tumors

Animal and tumor implantation methods were analogous to those previously reported (9). All animal studies were approved by the Institutional Animal Care and Use Committee. Male adult (20–25 grams) athymic swiss mice (nu/nu) bearing s.c. xenografts of EGFR-wild-type and vIII mutant tumors of ~0.3 mL volume were randomly assigned to control or gefitinib treatment groups. In the wild-type EGFR tumor group, gefitinib was administered at a dose of 55 mg/kg/d p.o. × 15 d, whereas in the vIII mutant tumor group, the dose was 30 mg/kg/d p.o. × 15 d by oral gavage. Both the wild-type and vIII mutant tumor vehicle-control groups were administered with 1% polysorbate-80 at 0.4 mL/d p.o. × 15 d. Gefitinib PK/PD investigations were conducted in the treatment group on both day 1 and day 15. Model variables of bioavailability (f), volume of distribution of the central compartment (Vc), absorption rate constant (Ka), and tumor blood flow rate (Q) were set equal to those obtained from the single-dose gefitinib PK/PD models (9) in which i.v. and oral drug administrations were used. The model-estimated variables in each tumor group were the elimination rate constant (Ke), intercompartmental rate constants (K12, K21), and tumor to plasma partition coefficient (Kr). An exponential equation describing the actual mean tumor volumes (Vt) in wild-type and mutant EGFR tumor groups during the 15-d treatment was incorporated into the respective PK models to account for the volume changes.

Once the hybrid PK models were finalized, the associated PK variables were held constant and linked to the associated EGFR wild-type and vIII mutant tumor PD models that used the fraction of baseline pERK in the tumor as the PD response. A two-compartment target-response model (9) was applied to the PD observations on both day 1 and day 15, in which the target compartment represented pEGFR, and the response compartment represented pERK (see Fig. 1). The best-fit hybrid PK/PD models were evaluated according to the statistical criteria provided by the SAAM II program including the minimization of the objective function, the Akaike information criteria, and the precision of variable estimation as measured by the coefficient of variations.

Finally, a Gompertz tumor growth model (12) was linked to the final PK model for each tumor group by using pERK as the biomarker. The tumor growth model and link scheme to the PK model (see Fig. 1) was analogous to that used previously for a transforming growth factor-β kinase antagonist, LY2157299, in which phosphorylation of Smad was observed (ref. 11) based on a sequential modeling approach of first defining the PK model, then the PD model, and finally the tumor growth model.

A physiologically based hybrid PK model (see Fig. 1) for each tumor group was developed based on the observed plasma and tumor gefitinib concentrations from both day 1 and day 15. Model variables of bioavailability (f), volume of distribution of the central compartment (Vc), absorption rate constant (Ka), and tumor blood flow rate (Q) were set equal to those obtained from the single-dose gefitinib PK/PD models (9) in which i.v. and oral drug administrations were used. The model-estimated variables in each tumor group were the elimination rate constant (Ke), intercompartmental rate constants (K12, K21), and tumor to plasma partition coefficient (Kr). An exponential equation describing the actual mean tumor volumes (Vt) in wild-type and mutant EGFR tumor groups during the 15-d treatment was incorporated into the respective PK models to account for the volume changes.

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\[
\frac{dTS}{dt} = \frac{K_{gexp} \times (1 - INH2) \times TS}{[1 + \left(\frac{INH0}{K_{gzero}} \times TS\right)]^\gamma}
\]

where TS is the tumor size, K_{gexp} and K_{gzero} are the rate constants for exponential and linear tumor growth, respectively; γ is a variable that mediates the switch from the exponential to linear tumor growth, and was fixed to 0.34 by model simulations. INH2 represents the final growth inhibitory signal due to gefitinib that was transmitted through a series of steps from INH0 (pERK inhibition) and another
signal transduction compartment INH1. The rate equations for INH0, INH1, and INH2 are listed as followed:

\[ \frac{d{INH_0}}{dt} = K_{sp}INH_0 - K_{sp}INH_1 \]
\[ \frac{d{INH_2}}{dt} = K_{sp}INH_1 - K_{sp}INH_2 \]

where \( K_{sp} \) is a first-order rate constant representing the signal propagation between the inhibitory compartments. INH0, INH1, and INH2 are equal to 0 for the baseline condition or control group. The tumor growth models for each tumor group were derived in a sequential manner by estimating values for \( K_{gexp} \), \( K_{gzero} \) in the control group first, and then estimating \( K_{sp} \) in the gefitinib-treated groups.

**Clinical PK/PD Models**

Previously published PK data in cancer (14, 15) and brain tumor (16, 17) patients permitted the development of a compartmental PK model that described gefitinib plasma concentrations (see Supplementary Method S1). Because those studies used noncompartmental analyses, the two studies (14, 15) that provided graphs of gefitinib plasma concentrations were digitized so that compartmental models could be developed. The net result of this exercise yielded a one-compartment model with first-order absorption and elimination to characterize gefitinib plasma concentrations for multiple-dose regimens in brain tumor patients not receiving enzyme-inducing antiepileptic drugs because these agents can alter the PKs of gefitinib (16, 17). This model was then used as input into a physiologically based blood flow–limited model that predicted the brain tumor concentrations of gefitinib. The model for brain tumor was based on patient data reported by Hofer (10) and Lassman (18) in which a small number of tissue samples from surgically resected malignant gliomas were analyzed for gefitinib. In 4 patients (2 from each study), the gefitinib brain tumor/plasma concentration ratio ranged from approximately 2 to 13 supporting appreciable drug penetration in tumor, and the use of a blood flow–limited model in brain tumor. These tumor/plasma concentration ratios also can be used to estimate the range of partition coefficient values likely to be encountered in patients. The blood flow–limited model structure also required estimates of tumor volume (i.e., 50 mL) and blood flow (i.e., 2,400 mL/h) that were taken from previously published work (8). The complete PK model for gefitinib in brain tumor patients consisted of the one-compartment model that generated plasma concentrations of gefitinib for input into the blood flow–limited brain tumor compartment.

The PK model was linked directly to the preclinical PD pERK models for either wild-type EGFR or mutant vIII EGFR tumors. Two simulation exercises were completed; one, to determine the gefitinib dose that predicted equivalent pERK profiles in the two tumor types, and two, to examine the effect of gefitinib brain tumor concentrations as set by the tumor/plasma partition coefficient on the percent inhibition of pERK at the nadir. Both simulations were done in the context of a multiple-dose regimen of gefitinib administered every 24 h for 15 d to ensure steady-state was achieved.

**Results**

**Preclinical PK Model of Gefitinib in Mice Bearing Wild-Type and vIII Mutant EGFR Tumors**

Hybrid physiologically based PK models (see Fig. 1) were applied to describe the multiple dose PK data from both day 1 and day 15. The preclinical hybrid PK models, one for the wild-type EGFR and the other for the mutant vIII tumor groups, were applied simultaneously to observed plasma and tumor gefitinib concentrations collected on days 1 and 15. The best-fit PK models identified by this procedure consisted of two-compartment systemic models linked to single blood flow-limited tumor compartments, which were analogous to those found after single doses of gefitinib (9). Similar to the single-dose PK models, the estimated gefitinib tumor to plasma partition coefficient (\( R_p \)) was much greater than 1, indicating high tumor penetration. It can be seen that the predicted plasma and tumor concentration profiles on day 1 and day 15 generally agreed with the observed values (see Fig. 2), and that the estimated variables were reliably estimated with low coefficients of variation (see Table 1). Specifically, both the plasma and tumor gefitinib model–predicted and observed areas under the concentration-time curves for each tumor group were within 20% of one another. For each tumor group, the plasma and tumor gefitinib concentration profiles were similar between day 1 and day 15, with <16% differences in the observed area under the concentration-time curves, suggesting gefitinib obeyed linear pharmacokinetic properties with no apparent saturable or time-dependent processes altering the PK behavior on days 1 and 15. It was anticipated that higher plasma and tumor concentrations would be obtained in the wild-type tumor group compared with the vIII mutant tumor group given the approximate 1.83-fold dose difference between the two tumor groups. Moreover, and consistent with model linearity, the two tumor types possessed similar model predicted parameters except the intercompartment rate constant \( K_{12} \) (see Table 1), and thus, the dose difference accounts for the major differences in actual gefitinib concentrations.

**PD Model of Gefitinib in Wild-Type and vIII Mutant EGFR Tumors**

The PD model was fitted to the combined day 1 and day 15 pERK observations for both the wild-type EGFR and mutant vIII EGFR tumor groups. Each treatment group showed a relatively rapid decline in pERK with nadirs within 2 to
4 hours after a gefitinib dose, and about 70% to 80% baseline return at 24 hours for each tumor type (see Fig. 3). In both tumor groups, more profound nadirs were observed on day 1 (0.39, 0.37) compared with day 15 (0.50, 0.47), which suggested the development of mild resistance to gefitinib over the 15-day treatment period. The ABEC values further supported mild resistance to suppression of pERK by gefitinib, with observed ABEC values (fraction of baseline pERK × day) decreasing from 11.3 on day 1 to 8.7 on day 15 in the wild-type tumor group, and from 9.8 on day 1 to 7.3 on day 15 in the vIII mutant tumor group. To account for the augmented pERK response over the 15-day treatment period, it was found that a time-dependent exponential function for $K_{out}(\text{day}) = K_{out}(0) e^{-r \times \text{time}}$, in which $K_{out}(0)$ represents the baseline $K_{out}$ value and $r$ is the arbitrary constant) a variable reflecting the dephosphorylation and degradation of pERK best characterized this phenomenon. Alternate approaches of applying time-dependent functions to $K_{in}$ and $K_{tr}$ did not properly characterize the pERK profiles; however, at this time, a specific mechanism for reduced dephosphorylation/degradation of pERK on day 15 is unknown. The drug-resistant phenomenon was not due to altered gefitinib PKs as both plasma and tumor exposures were similar on days 1 and 15. The exponential functions for $K_{out}$ in each tumor group were quite similar (see Table 1), and thus, whatever the underlying mechanism, it is not unique to a particular tumor type.

In the modeling process, the baseline pEGFR and $I_{max}$ were set to 1 according to the assumption that the phosphorylation of pEGFR was not inhibited in the absence of gefitinib treatment, and that it could be fully inactivated if the drug concentration in the tumor is sufficiently high. To reduce the parameter estimation difficulties, the values of $K_{in}$, a variable that represents the formation of pEGFR, were set equal to those obtained from the single-dose gefitinib PK/PD models, based on the assumption that the rate of change in pEGFR is dependent on the tumor cell line, and not a factor in resistance to gefitinib. The 2.5-fold

![Figure 2](image-url)
greater IC₅₀ value in the wild-type tumor group was consistent with the lower drug sensitivity seen in vitro in wild-type tumor cells compared with the mutant VIII EGFR cells (9). The other PD model variable, Kent, reflects the signaling efficiency from pEGFR and pERK, and was estimated to be 2.2 h⁻¹ and 1.4 h⁻¹ for the wild–type tumor and the VIII mutant tumor, respectively. All of the estimated PD variables are listed in Table 1. Overall, the model-predicted pERK profiles agreed with the actual measurements of pERK in tumors for both tumor groups (see Fig. 3) with the differences between model-predicted and observed ABC values being <11% on both days 1 and 15. The greatest discrepancies in singular values were the predose values on day 15 that were near baseline and underpredicted by about 15% to 20%. Unlike day 1, we did not assume the predose pEGFR0 was equal to 1 on day 15.

### Tumor Growth Model

A tumor growth model was applied to characterize the size of the EGFR wild-type and EGFRvIII mutant xenografts, in which the growth inhibitory signal was assumed to be due to the extent of pERK inhibition. In this case, it was assumed that the control and gefitinib treatment groups had equivalent values for Kexp, Kzon, and γ, and the inhibitory action of gefitinib on tumor growth was transmitted through the signalling cascade of INH0, INH1, and INH2. Therefore, the only variable that needed to be estimated for the drug-treated tumor growth models was Ksp, a variable representing the rate of signal propagation between the inhibitory compartments. γ was set equal to 0.34 for both tumor types based on the model simulations, and it was found that this value best described the transition from the exponential tumor growth phase to the linear tumor growth phase. Kvg was estimated as 1.3 day⁻¹ and 1.04 day⁻¹ for the wild-type tumor and the VIII mutant tumor, respectively. The tumor growth model variables are listed in Table 1. The model-predicted tumor growth profiles sufficiently captured the measured tumor size for each tumor type (see Fig. 4). It can be seen that tumor size was reduced in the gefitinib-treated groups compared with the corresponding control group, with ~38% and 30% tumor growth inhibition on day 15 in the wild-type tumor and the VIII mutant tumors, respectively. The nearly equal tumor growth inhibition in the EGFR wild-type and VIII mutant groups supports a fundamental premise of equivalent PK/PD dosing that analogous PD profiles will be equally efficacious.

### Clinical PK/PD Model for Gefitinib

The PK/PD model structure for gefitinib in brain tumor patients was similar to that in mice except systemic disposition in patients required only a single compartment (see Supplementary Fig. S1). The one-compartment model describing the plasma concentrations of gefitinib was based on data obtained in cancer patients who received a 14-day course of 525 mg/day (14), a dose level close to the 500 mg/day dose used in brain tumor patients not receiving enzyme-inducing antiepileptic drugs (16, 17). The model would not be applicable to patients receiving enzyme-inducing antiepileptic drugs, and extension of the systemic model developed at 525 mg/day to much lower or higher gefitinib doses should be done cautiously as the available data suggests gefitinib PKs are not strictly dose proportional (15). The blood flow–limited model for gefitinib’s disposition in brain tumors assumes appreciable drug accumulation and homogeneity, and has no provisions to specifically assess blood-brain barrier (BBB) transport.

### Table 1. PK, PD, and tumor growth model parameters of gefitinib in LN229-wild-type EGFR and LN229-EGFRvIII mutant tumor bearing athymic mice after 55 mg/kg/d p.o. × 15 d and 30 mg/kg/d p.o. × 15 d administrations, respectively

<table>
<thead>
<tr>
<th>Variables</th>
<th>LN229-wild-type EGFR tumor-bearing group</th>
<th>LN229-EGFRvIII mutant tumor-bearing group</th>
</tr>
</thead>
<tbody>
<tr>
<td>PK model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.45</td>
<td>0.52</td>
</tr>
<tr>
<td>K100 (h⁻¹)</td>
<td>0.54 (1.6)</td>
<td>0.40 (1.6)</td>
</tr>
<tr>
<td>K12 (h⁻¹)</td>
<td>1.65 (6.7)</td>
<td>0.74 (8.3)</td>
</tr>
<tr>
<td>K22 (h⁻¹)</td>
<td>0.55 (6.3)</td>
<td>0.50 (7.2)</td>
</tr>
<tr>
<td>Ksp (h⁻¹)</td>
<td>0.88</td>
<td>0.82</td>
</tr>
<tr>
<td>Q (mL h⁻¹)</td>
<td>4.5</td>
<td>4.4</td>
</tr>
<tr>
<td>R1</td>
<td>13.7 (2.1)</td>
<td>10.7 (2.0)</td>
</tr>
<tr>
<td>Vc (mL)</td>
<td>75.0</td>
<td>103.8</td>
</tr>
<tr>
<td>V1 (mL)</td>
<td>0.2891e0.0024-time⁻¹</td>
<td>0.2693e0.0006-time⁻¹</td>
</tr>
<tr>
<td>PD model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC₅₀ (µg/g)</td>
<td>25.5 (20.2)</td>
<td>9.8 (22.6)</td>
</tr>
<tr>
<td>Km (h⁻¹)</td>
<td>4.5</td>
<td>5.3</td>
</tr>
<tr>
<td>Kout(0) (h⁻¹)</td>
<td>6.4 (7.3)</td>
<td>6.7 (9.6)</td>
</tr>
<tr>
<td>r</td>
<td>0.0006 (30.5)</td>
<td>0.0005 (42.8)</td>
</tr>
<tr>
<td>Kvg (h⁻¹)</td>
<td>2.2 (20.0)</td>
<td>1.4 (24.7)</td>
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<td>Kvg0 (h⁻¹)</td>
<td>6.1 (147.7)</td>
<td>27.6 (52.3)</td>
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<td>Kvgp (h⁻¹)</td>
<td>0.14 (342.9)</td>
<td>0.034 (46.0)</td>
</tr>
<tr>
<td>Kg (h⁻¹)</td>
<td>1.3 (3.45)</td>
<td>1.0 (4.7)</td>
</tr>
<tr>
<td>γ</td>
<td>0.34</td>
<td>0.34</td>
</tr>
</tbody>
</table>

*Values represent mean (% coefficient of variation) of model-fitted variables.
The relatively high tumor/plasma concentration ratios is an indication that significant efflux at the BBB by ABC transporters, such as P-glycoprotein and ABCG2, is limited; however, these processes require further consideration as gefitinib has been shown to be both a substrate and inhibitor depending on its concentration (19). The simplifying assumption of concentration homogeneity within the tumor is consistent with blood flow-limited tissue compartments, yet does not account for well-known spatial dependencies in anticancer drug concentrations in an individual’s brain tumor (20). However, the simulations conducted by varying the brain tumor partition coefficient ($R_{bt}$) reflect the spatial variations in the tumor accumulation of gefitinib, either within or between tumors as observed in patients (10, 18), which are often attributed to breakdown of the BBB.

The first simulation applied the equivalent PK/PD dosing strategy to brain tumor patients bearing either EGFR wild-type or vIII mutant tumors (see Fig. 5A) assuming the same PK model parameters with the $R_{bt}$ equal to 10. This value decreases within the range of 2 to 13 reported for brain tumor to plasma gefitinib concentration ratios in patients (10, 18), and correspondingly predicted brain tumor concentrations (see Fig. 5A) that coincided with the mean of 9.4 ± 9.3 μg/g ($n = 5$) measured in two patients with malignant gliomas (10). The simulations indicated that to achieve a similar degree of pERK inhibition patients with wild-type EGFR tumors required a 2-fold greater dose of 1,000 mg/day compared with a 500 mg/day dose in patients with the vIII variant tumors. It can also be seen that the fluctuation in pERK levels is minimal in a 24-hour dosing period, suggesting that current schedules of once daily dosing are adequate at least in terms of duration of inhibitory action. Once steady-state is achieved at about day 10, the fluctuation in pERK values are <10% in a 24-hour dosing interval, whereas brain tumor concentrations vary on the order of 30%.

Figure 3. The PK/PD model-predicted (—) and mean ($n = 3–4$) observed (●) fractional inhibition of pERK for wild-type tumor group (A) and vIII mutant tumor group (B), respectively. Points, mean; bars, SD. Once the hybrid PK models were finalized, the associated PK variables were held constant and linked to a two-compartment target-response model, consisting of a target compartment representing pEGFR, and a response compartment representing pERK, which was applied to the PD observations on both day 1 and day 15.

Figure 4. The PK/PD/tumor growth model-predicted (—) gefitinib-treated group; (●), vehicle control group) and mean observed (●, gefitinib-treated group, $n = 23$; ○, vehicle control group, $n = 9$) tumor sizes are presented for wild-type tumor group (A) and vIII mutant tumor group (B), respectively. Points, mean; bars, SD. Once the hybrid PK/PD models were finalized, a Gompertz tumor growth model was linked to the final PD model for each tumor group by using pERK as the biomarker. The tumor growth models for each tumor group were derived in a sequential manner by fitting to the control groups first, and then fitting to the gefitinib-treated groups.
concentrations need to be at least 10 μg/gram to reach 50% inhibition of pERK, which corresponds to a partition coefficient of 12. Based on the small amount of patient data and the known inhomogeneity in drug distribution in tumors, it is unlikely that all regions of the tumor could achieve this degree on inhibition at a dose of 500 mg/day. Achievement of this degree of pERK inhibition throughout mutant vIII tumors is more readily attainable, and according to the model occurs at gefitinib maximum concentrations of between 5 to 6 μg/grams and a partition coefficient of between 6 and 7. Overall, the PK model of gefitinib is consistent with the very limited available data, yet should be applied judiciously given its speculative nature.

Discussion

The inclusion of translational medicine in the NIH Roadmap and Food and Drug Administration Critical Path Initiatives has motivated a reanalysis of how preclinical and clinical research may be bridged to hasten drug development and yield a clearer understanding of the relationships between preclinical and clinical studies. Academic drug discovery and development programs have arisen as a means to foster a “from the bench to the bedside” strategy. These programmatic efforts, although not always explicitly stated, renew the importance of PK/PD principles and investigations throughout the drug development process, in part, due to the quantitative pharmacologic information that can be provided. In this regard, and as highlighted by the Food and Drug Administration,2 model-based drug development is a key component of the translational science effort. However, much of the current focus has been in the clinical domain, and definitive model-based approaches in the preclinical setting have not been forthcoming. The current project was conducted based on the equivalent PK/PD dosing strategy (9) that is representative of model-based drug development, and may prove to be a technique that enhances our ability to select the most efficacious drugs in an era of molecular and targeted chemotherapy.

A new component of the model development approach was to link the PD model to a tumor growth model, and thus, facilitate quantitative comparisons between drugs and tumor types in a preclinical setting. The efficacy model and link to the PD model were based on an approach recently described by Bueno et al. (13) that analyzed a pSMAD inhibitor. The approach of using two intermediate compartments (i.e., INH1 and INH2) seemed applicable to our study because inhibition of pERK is amongst a cascade of events that result in apoptosis and register as a retardation in tumor size. The control and gefitinib-treated efficacy models were constructed to readily determine the drug effect as INH2 is nonzero only for gefitinib treatments, and K_{gexp}, K_{gzero}, and γ were equal in the control and drug-treated models. This attribute of distinguishing the drug-dependent features enables the use of simulations to see...

how different dosing regimens alter tumor size relative to control, and may permit the elimination of costly size-based efficacy studies. It should be appreciated that the complex mechanisms of gefitinib activities involve other signaling pathways, such as PI3K-AKT, which may reveal other useful PD end points. Whether one or multiple PD end points can best be formulated into tumor size models will require further studies.

The equivalent PK/PD dosing strategy was designed to overcome the limitations of semiempirical tumor size–based studies, and place PK/PD investigations at the center of preclinical drug development. The hypothesis that equal degrees of pERK inhibition afforded by gefitinib in the sensitive and resistant tumor variants will produce equal degrees of tumor growth retardation was supported, and suggests further examination of equivalent PK/PD dosing, for instance, to assess relationships between target inhibition and tumor growth for a series of tumors and drug candidates.

The development of a clinical PK/PD model for gefitinib based on patient PK properties and the preclinical pERK model was a novel feature of the investigation, and showed through simulations the types of questions that can be addressed. Although the model is viewed as speculative it is believed that the model-based approach to address pharmacologic questions may generate increased interest to obtain clinical data to further develop and validate tumor-based models. It was shown that patients possessing the vIII EGFR mutation in tumors require about a 50% lower dose than patients with wild-type EGFR, assuming normal phosphatase and tensin homologue and no other genetic differences in the two tumor types. The 24-hour dosing intervals, regardless of tumor type, maintained suppression of pERK over the 24-hour periods once steady-state was achieved, although the extent of inhibition never exceed 80% at doses of 500 mg/day. The simple physiologically based blood flow–limited brain tumor model is able to predict gefitinib concentrations due to changes in tumor volume, blood flow, and drug distribution into tumor. The latter was used as a variable to represent possible changes in drug penetration and associated maximum brain tumor gefitinib concentrations within and between patients due to alterations in the BBB. The simulations showed the limited conditions in which 50% PERK inhibition at the nadir could be achieved, and baits the question of the degree and duration of inhibition that leads to antitumor activity. This type of uncertainty in the meaning of target inhibition (i.e., pEGFR) and downstream effectors (i.e., pERK and pAKT) has been expressed by Lassman and coworkers (18) based on sparse data collected in brain tumor patients. It is believed that through the continued collection of sparse brain tumor samples from patients and the application of predictive models that these uncertainties can be mitigated. The application of the preclinical PD pERK model to patients was done without any variable scaling to account for possible differences between mouse xenograft gliomas and patient tumors. The pertinent PD variables in the target-response model are $K_{dtr}$, $K_{nat}$, $K_{tr}$ and IC$_{50}$, and how these may be related between species will have to be addressed in a broader context with more patient data. It is also appreciated that the complexities of drug resistance, feedback signalling, and crosstalk when multiple drugs are used will require a reassessment of the PD model. In summary, although the clinical PK/PD model is speculative it highlights a future direction of establishing links between preclinical and clinical models based on tumor measurements, sites that cannot be frequently sampled in patients.

In conclusion, this investigation developed a preclinical PK/PD/efficacy model predicated upon a molecular determinant of drug activity that highlighted the use of equivalent PK/PD dosing. Furthermore, a clinical PK/PD model was also devised based on cancer patient data and naive scaling of a PD model that showed target inhibition as a function of drug accumulation and tumor type. It is proposed that these preclinical and clinical modelling approaches can provide a foundation for drug development, and lead to the rational selection and use of anticancer drug candidates in different tumor types.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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Shining Wang, Qingyu Zhou and James M. Gallo

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