Received: 15 January 2021 | Revised: 2 August 2021 | Accepted: 17 August 2021

DOI: 10.1002/psp4.12710

ARTICLE

Modeling restoration of gefitinib efficacy by co-administration of MET inhibitors in an EGFR inhibitor-resistant NSCLC xenograft model: A tumor-in-host DEB-based approach

Elena M. Tosca[1] | **Glenn Gauderat**[2] | **Sylvain Fouliard**[2] | **Mike Burbridge**[3] | **Marylore Chenel**[2] | **Paolo Magni**[1]

[1]Laboratory of Bioinformatics, Mathematical Modelling and Synthetic Biology, Department of Electrical, Computer and Biomedical Engineering, Università degli Studi di Pavia, Pavia, Italy

[2]Clinical Pharmacokinetics and Pharmacometrics Division, Servier, Suresnes, France

[3]Center for Therapeutic Innovation in Oncology, Servier, Suresnes, France

Correspondence
Paolo Magni, Via Ferrata 5, I-27100 Pavia, Italy.
Email: paolo.magni@unipv.it

Present address
Mike Burbridge, Engitix therapeutics, London, UK

Marylore Chenel, Pharmetheus AB, Uppsala, Sweden

Funding information
No funding was received for this work.

Abstract

MET receptor tyrosine kinase inhibitors (TKIs) can restore sensitivity to gefitinib, a TKI targeting epidermal growth factor receptor (EGFR), and promote apoptosis in non-small cell lung cancer (NSCLC) models resistant to gefitinib treatment in vitro and in vivo. Several novel MET inhibitors are currently under study in different phases of development. In this work, a novel tumor-in-host modeling approach, based on the Dynamic Energy Budget (DEB) theory, was proposed and successfully applied to the context of poly-targeted combination therapies. The population DEB-based tumor growth inhibition (TGI) model well-described the effect of gefitinib and of two MET inhibitors, capmatinib and S49076, on both tumor growth and host body weight when administered alone or in combination in an NSCLC mice model involving the gefitinib-resistant tumor line HCC827ER1. The introduction of a synergistic effect in the combination DEB-TGI model allowed to capture gefitinib anticancer activity enhanced by the co-administered MET inhibitor, providing also a quantitative evaluation of the synergistic drug interaction. The model-based comparison of the two MET inhibitors highlighted that S49076 exhibited a greater anticancer effect as well as a greater ability in restoring sensitivity to gefitinib than the competitor capmatinib. In summary, the DEB-based tumor-in-host framework proposed here can be applied to routine combination xenograft experiments, providing an assessment of drug interactions and contributing to rank investigated compounds and to select the optimal combinations, based on both tumor and host body weight dynamics. Thus, the combination tumor-in-host DEB-TGI model can be considered a useful tool in the preclinical development and a significant advance toward better characterization of combination therapies.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Several pharmacokinetic/pharmacodynamic tumor growth inhibition models describing combination treatments in xenograft studies are available. A tumor-in-host Dynamic Energy Budget (DEB)-based models has been introduced to describe the effect of cytotoxic or anti-angiogenic agents in monotherapy on tumor and host body weight.

WHAT QUESTION DID THIS STUDY ADDRESS?

Can the tumor-in-host DEB-based approach be extended to describe tumor and host body responses to the epidermal growth factor receptor-tyrosine kinase inhibitor gefitinib and the MET inhibitors capmatinib and S49076 in mono- and combination therapies? Can this approach account for their synergism?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The developed model well-described tumor and host response to gefitinib, capmatinib, S49076 and their combination. The introduction of a synergistic interaction allowed to account for the ability of the MET inhibitors in restoring tumor sensitivity to gefitinib providing quantitative estimates of the combination anticancer potency.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

The proposed model can simulate and predict tumor and host body weight response to gefitinib, capmatinib and S49076 or their combinations to guide future experiments. The model can be potentially used to quantitatively evaluate, rank, and compare different combination therapies.

INTRODUCTION

In the past decades, the development of several tyrosine kinase inhibitors (TKIs) has led to important progress in the treatment of non-small cell lung cancer (NSCLC), the most common histological subtype of lung cancer.[1,2] The use of molecular targeted therapies in NSCLC began in the late 1990s when gefitinib (Iressa) entered clinical development. Gefitinib is an epidermal growth factor receptor (EGFR) TKI indicated for the first-line treatment of patients with NSCLC whose tumors harbor activating EGFR mutations. Despite the great success achieved by treatment with gefitinib and other EGFR-TKIs, the development of acquired resistance is inevitable.[3–5] Dysregulations of the hepatocyte growth factor receptor, MET, represent relevant mechanisms underlying acquired resistance to EGFR-TKI therapy[6–10] and is considered a therapeutically tractable target.[4,11] Currently, extensive research focuses on the development of treatment combinations targeting this compensatory MET-pathway and several novel MET inhibitors are under study in different settings.[12] Preclinical studies in EGFR-mutant/MET-activated NSCLC xenograft models of acquired EGFR-TKI resistance have demonstrated the ability of these compounds to restore tumor sensitivity to EGFR-TKI therapy.[13–19] Benefits of co-administration of the MET inhibitors and EGFR-TKIs have been also observed in specific patients with NSLC during early phase clinical studies,[20] although, in some cases, later phase trials failed to demonstrate an adequate efficacy and safety profile.[11,12,21]

In this scenario, characterized by deep gaps between the promising preclinical results and the contradictory clinical outcomes, making the most of the limited information coming from the in vivo preclinical experiments is crucial. Mathematical pharmacokinetic/pharmacodynamic (PK/PD) models represent the most useful tools for leveraging information collected during preclinical studies.[22] In particular, several tumor growth inhibition (TGI) models have been proposed to investigate the effects of multiple drug administration on xenograft tumor models.[23–26]

They are essential to quantitatively evaluate the effect of drug combinations based on efficacy metrics independent from experimental conditions and to rigorously define the nature of possible drug interactions. Indeed, synergistic/antagonistic effect can be defined as an effect greater/lower than some baseline, referred to an additive effect (absence of interaction). Therefore, no-interaction combination TGI models, predicting the responses following the administration of a drug combination under the hypothesis of a null-interaction, provide a theoretical reference to objectively assess synergistic (more effective than expected under the

no-interaction hypothesis) and antagonistic (negative effective) behaviors.

One of the most interesting methods to characterize the PD interactions of drugs given in combination during xenograft studies relies on the well-known Simeoni TGI model.[23,24,27–29] However, this as well as other modeling approaches focuses only on the anticancer activity of the drug and considers the tumor as an independent entity that does not interact with the host organism. In this way, the assessment of drug interactions is based only on tumor response and important effects, like tumor-related cachexia and drug toxicities, that should be carefully evaluated during combination treatments, are not taken into account.

The aim of the present work was the development of a new modeling approach to assess the effect of anticancer drug combinations based on the dynamics of both tumor and host body weight. This new model was developed starting from a tumor-in-host modeling approach recently proposed based on the Dynamic Energy Budget (DEB) theory.[30,31] This unique modeling framework, already successfully applied to describe the effects of cytotoxic or anti-angiogenic agents administered in monotherapy,[32–34] accounts for the different aspects characterizing the in vivo tumor growth studies: the drug anticancer activity on the tumor, the cachexia onset due to the treatment, the tumor effect on the host and, vice versa, the influence of the host condition on tumor dynamics.[35] In addition, the DEB-based model provided quantitative measurements of the anticancer effect of a single-compound treatment and its undesired effect on host body weight. Further, it was found to be extremely useful to characterize the arising of hypoxia-resistance mechanisms during prolonged bevacizumab treatment.[34]

Here, the applicability of the tumor-in-host DEB-based framework was extend to the context of poly-targeted combination therapies. Indeed, a new DEB-TGI model describing the effect of EGFR-TKIs and MET inhibitors on both tumor growth and host body weight when administered alone or in combination in an EGFR-mutant/MET-activated NSCLC human xenograft model in mice was proposed. Data were taken from a combination xenograft study performed on the EGFR-TKI resistant tumor cell line HCC827ER1 with the aim of evaluating the effects of two competitor MET inhibitors, capmatinib (Tabrecta) and S49076, in mono- and combination therapy with gefitinib. Specific modeling efforts were focused on the quantitative evaluation of the synergistic PD interactions between drugs. Based on the developed PK/PD tumor-in-host model, the effects of the two MET inhibitors were quantitatively compared as well as their ability in restoring the tumor sensitivity to the standard of care gefitinib.

METHODS

Experimental methods

Compounds

Gefitinib (Iressa), a TKI indicated for first-line treatment of patients with metastatic NSCLC whose tumors have specific EGFR alterations, was used for the in vivo antitumor assessment alone or combined with two competitor MET inhibitors.

Capmatinib (Tabrecta; Novartis, Basel, Switzerland) is an oral, ATP-competitive, class I MET inhibitor being evaluated in combination with gefitinib in patients with EGFR-mutant NSCLC after progression to gefitinib treatment.[36]

S49076 (Servier, Suresnes, France) is a novel oral ATP-competitive inhibitor of MET, being evaluated in phase I/II studies involving patients with NSCLC.[37,38]

Pharmacokinetic assessment

Results from previous studies with serial blood sampling were used to derive information relative to gefitinib, capmatinib and S49076 PKs in mice.

PKs were more sparsely measured during the in vivo combination TGI study. In particular, blood samples were collected after 2 weeks of treatments (1 and 7 h postdose) in all the treated animals.

The measurement of plasma concentrations was carried out by turbulent flow chromatography combined with tandem mass spectrometry (TFC-LC-MS/MS).

In vivo combination TGI study

Severe combined immunodeficiency (SCID) female mice (5–7 weeks old) were purchased from Charles River. Five million human lung adenocarcinoma cells resistant to EGFR-TKIs due to MET-alteration (HCC827ER1, purchased from ATCC) were injected subcutaneously into the left flank of animals. Eighteen days after inoculation, mice bearing a palpable tumor (167 $mm^3 \pm 3$ mm^3) were randomized into eight groups of eight animals each. Mice then received gefitinib alone or combined with capmatinib or S49076, as detailed in Table 1, whereas control animals received vehicle only.

Mice were kept in the Servier Institute-specified pathogen-free animal area for mouse experimental purpose (facility license n. B78-100–2). All animal studies were approved by the institute's ethical committee and carried out in compliance with current European and national

TABLE 1 Treatment information

Group	Arm	Compound	Route of administration	Dose level (mg/kg)	Regimen
Control	A	Vehicle	p.o.	-	-
Gefitinib single-agent	B	Gefitinib	p.o.	12.5	q.d. × 5[a] for 3 weeks
Capmatinib single-agent	C	Capmatinib	p.o.	17.5	b.i.d. × 5[a] for 2 weeks
S49076 single-agent	D_1	S49076	p.o.	17.5	b.i.d. × 5[a] for 2 weeks
	D_2			35	
Combination 1	E	Gefitinib	p.o.	12.5	q.d. × 5[a] for 3 weeks
		Capmatinib	p.o.	17.5	b.i.d. × 5[a] for 3 weeks
Combination 2a	F_1	Gefitinib	p.o.	12.5	q.d. × 5[a] for 3 weeks
		S49076	p.o.	17.5	b.i.d. × 5[a] for 3 weeks
Combination 2b	F_2	Gefitinib	p.o.	12.5	q.d. × 5[a] for 3 weeks
		S49076	p.o.	17.5	b.i.d. × 5[a] for 3 weeks

[a]The 1: q.d. × 5: daily for 5 days, b.i.d. × 5: bi-daily for 5 days.

regulations for the protection of vertebrate animals used for experimental research. Mice body weight was monitored three times a week and tumor sizes were measured using electronic calipers. Mice were euthanized at the first measurement for which the tumor volume exceeded 2000 mm^3 or at the first signs of animal health deterioration. From measurements, tumor weight (g) was calculated as:

$$\text{Tumor weight}\,(g) = \text{TW} = \rho\frac{\text{length}\,(\text{cm}) \cdot \text{width}^2\,(\text{cm}^2)}{2} \tag{1}$$

assuming unit density 1 g/cm^3.

Pharmacokinetic modeling

Two-compartment disposition models with linear absorption and elimination were used to describe gefitinib and capmatinib PK. Differently, S49076 plasma concentration-time profile was described by a one-compartment disposition model with linear absorption and nonlinear elimination (Michaelis-Menten model).

Pharmacodynamic modeling

Tumor-in-host DEB-based modeling framework

The tumor-in-host modeling framework,[32–34] describing tumor and host dynamics based on the DEB-theory, is here adapted to analyze the in vivo combination TGI study.

Briefly, it is supposed that the host body is composed by two components, the energy reserve, e(t), and the structural biomass, V(t), which dynamics follow from energetic balances between physiological processes, such as assimilation, energetic consumption, and maintenance/growth of structural biomass. To survive and proliferate, the tumor appropriates a fraction, $k_u(t)$, of the host energy. The tumor-host energy partition fraction, $k_u(t)$ Equation 2f, depends on volumes of tumor mass, $V_u(t)$, and of host structural biomass, and on the coefficient of gluttony of tumor cells, μ_u, a measure of tumor aggressiveness. As the tumor exploits host energy resources, the host has to reduce its growth rate and, eventually, to degrade its structural biomass (tumor-related cachexia). The degradation rate increases until a maximum threshold, $\delta_{V_{max}}$, is reached. Finally, tumor-related anorexia is modeled assuming that tumor progression inhibits the host assimilation (Equation 2d).

The model equations are reported in Equations 2a–2g where $W(t)$ and $W_u(t)$ represent the host body and tumor weight, respectively. The complete model formulation is reported in ref. 34 and in Supplementary Material S1, whereas model parameters are defined in Table 2.

$$\begin{cases} \dfrac{de(t)}{dt} = \dfrac{\nu}{V(t)^{1/3}}\left(\rho(t)\left(\dfrac{V_{1\infty}}{V_u(t)+V(t)}\right)^{2/3} - e(t)\right) \\ \dfrac{dV(t)}{dt} = F_V\left(e, V, V_u\right) \\ \dfrac{dV_u(t)}{dt} = F_{V_u}\left(e, V, V_u\right) \\ \rho(t) = \rho_b\left(1 - \dfrac{V_u(t)}{IV_{u,50}+V_u(t)}\right) \\ k_u(t) = \dfrac{\mu_u V_u(t)}{V(t)+\mu_u V_u(t)} \\ W(t) = d_V\left(1+\xi e(t)\right)V(t) \\ W_u(t) = d_{V_u} V_u(t) \end{cases} \tag{2a-g}$$

TABLE 2 Structural parameters of tumor-in-host DEB-TGI models

Parameter	Dimension	Description
		Host-related parameters
N	L/T	Energy conductance
ρ_b	–	Tumor-free food-supply coefficient
$V_{1\infty}$	L^3	Maximum structural biomass volume
G	–	Growth energy-investment ratio
M	$1/T$	Maintenance-growth rate ratio
Ξ	–	Energy reserve weight scaling factor
d_V	W/L^3	Specific weight of structural biomass
		Tumor-related parameters
μ_u	–	Coefficient of gluttony
g_u	–	Tumor growth energy-investment ratio
m_u	$1/T$	Tumor maintenance-growth rate ratio
d_{Vu}	W/L^3	Specific weight of tumor mass
		Cachexia-related parameters
Ω	–	Biomass thermodynamic extraction efficiency coefficient
δ_{Vmax}	L^3/T	Maximum structural biomass degradation rate
IV_{u50}	L^3	Half maximal inhibitory tumor volume
		EGFR-TKI-related parameters
$IC_{50,\ EGFR\text{-}TKI}$	$CONC$	Half maximal inhibitory concentration
I_{max}	–	Maximum inhibitory effect
$K_{2,EGFR\text{-}TKI}$	$CONC^{-1}/T$	EGFR-TKI potency
		MET inhibitor-related parameters
$K_{2,MET\text{-}inhib}$	$CONC^{-1}/T$	MET inhibitor potency
$IC_{50\ MET\text{-}inhib}$	$CONC$	Half maximal inhibitory concentration for toxic effect on BW
		Synergistic factors
φ_1	–	Synergistic factor acting on cytostatic effect of EGFR-TKIs
φ_2	–	Synergistic factor acting on cytotoxic effect of EGFR-TKIs

Abbreviations: BW, body weight; DEB, Dynamic Energy Budget; EGFR, epidermal growth factor receptor; I_{max}, maximum unbound systemic concentration; TGI, tumor growth inhibition; TKI, tyrosine kinase inhibitor.

with $e(t_0) = e_0$, $V(t_0) = V_0$, $V_u(t_0) = V_{u,0}$ and growth functions, $F_V\left(e, V, V_u\right)$ and $F_{V_u}\left(e, V, V_u\right)$, defined by the Equations S2–S3 in Supplementary Material S1.

This tumor-in-host DEB-based framework can be easily extended to describe effects of different anticancer treatments on both tumor mass and host body (Figure 1). Indeed, using drug concentrations as drivers and accounting for drug-specific mode of action, the effect of a given compound can be integrated into the model as exemplified in the following.

Drug cytotoxicity, the ability to damage tumor cells and to trigger apoptosis, is modeled as a direct killing effect on tumor cells that makes some of them nonproliferating and eventually brings them to death through several damage stages.[32,33]

Cytostatic agents stop cancer cells from proliferating without producing cytotoxicity. Independently on the drug-specific mechanism of action, drug effect is described as a reduction of tumor ability to survive and proliferate. Because in the DEB-framework tumor survival and proliferation is dependent on the available energy, cytostatic drug action is modeled as an inhibitory effect on the energy flow to tumor, $k_u(t)$.[34]

Finally, side effects of anticancer treatments related to drug-induced anorexia are incorporated into the model as an inhibition of the host assimilation (food-supply coefficient $\rho(t)$). This leads to a host energy decrease, followed by a loss of structural biomass (drug-related sarcopenia), that causes the body weight loss (BWL) observed during experiments.

Tumor-in-host DEB-TGI model for EGFR-TKI (gefitinib single-agent treated group)

EGFR-TKIs, such as gefitinib, target processes that are involved in tumor growth and progression, including

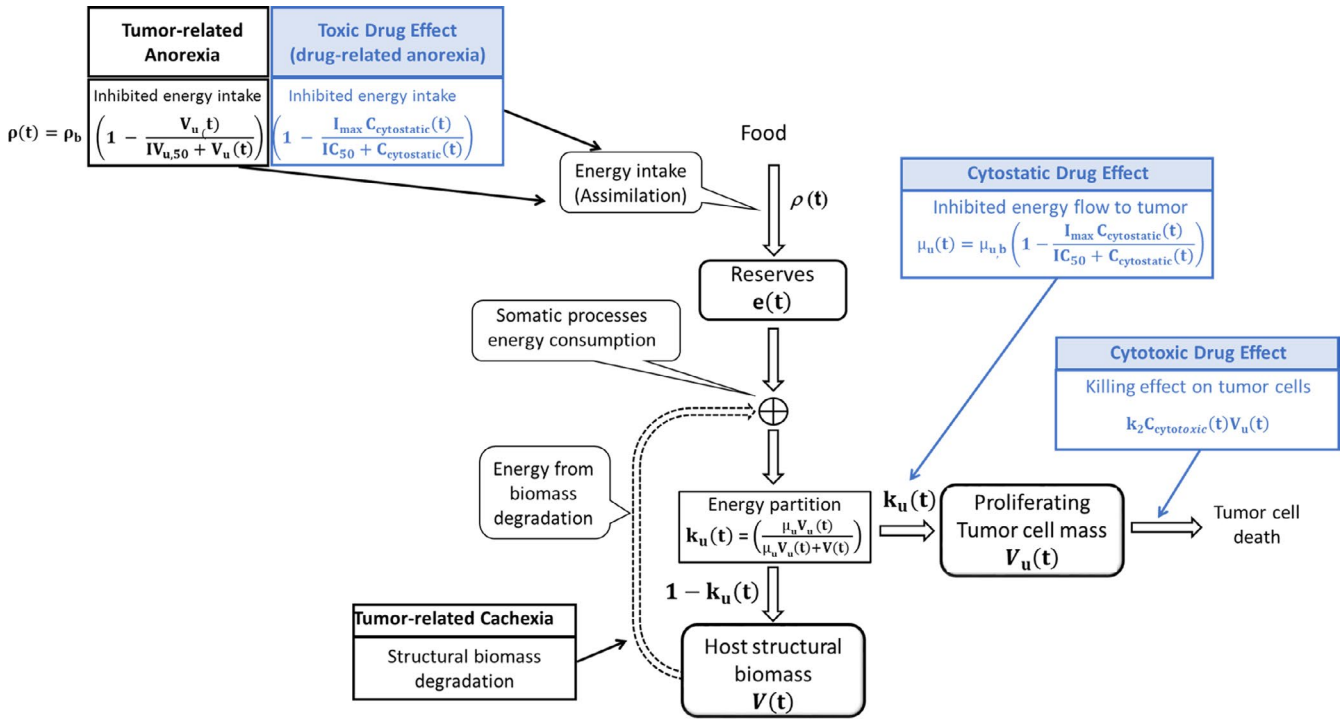

FIGURE 1 Schematic representation of the Dynamic Energy Budget-tumor growth inhibition (DEB-TGI) modeling framework. Energy is taken up from food and delivered to the reserves. Energy required by the somatic processes is obtained from reserves and assigned to host or to tumor through the partition fraction $k_u(t)$ on the basis of the gluttony coefficient μ_u. Due to the tumor energy request, host starts to degrade its structural biomass (tumor-related cachexia). In case of cytostatic treatment, the energy flow to the tumor is reduced. Cytotoxic drug exerts a killing effect on proliferating tumor cells. The presence of tumor mass itself (tumor-related anorexia) or toxic effect of drug treatment (drug-related anorexia) may reduce the host energy intake

proliferation, apoptosis, and angiogenesis.[39,40] Based on this information, the gefitinib single-agent model hypothesis a double anticancer drug effect. First, a direct killing effect on tumor cells (cytotoxic effect) is implemented as a reduction of tumor growth function proportional to drug concentration, $C_{gef}(t)$, and to proliferating tumor cell mass, $V_u(t)$:

$$\frac{dV_u(t)}{dt} = F_{V_u}\left(e, V, V_u\right) - C_{\text{gef}}(t)\, V_u(t)\, k_{2,\text{gef}} \quad (3)$$

where the parameter $k_{2,gef}$ represents the gefitinib cytotoxic potency.

Second, a cytostatic effect, accounting for gefitinib ability in reducing tumor survival and proliferation, is modeled through an inhibitory I_{max} function on tumor aggressiveness, μ_u, governing $k_u(t)$:

$$\mu_u = \mu_{u,b}\left(1 - \frac{I_{\max} C_{\text{gef}}(t)}{IC_{50,\text{gef}} + C_{\text{gef}}(t)}\right) \quad (4)$$

where $\mu_{u,b}$ is the tumor gluttony in absence of treatment and $IC_{50,\text{gef}}$ the gefitinib concentration exerting the 50% of the maximal inhibitory effect, $I_{\max}$. From Equation 2e and Equation 4, this cytostatic effect results in a reduction of tumor energy flow that leads to a tumor growth modulation.

In accordance with experimental mice body weight data, no direct pharmacological effect on host organism is modeled.

Tumor-in-host DEB-TGI model for MET inhibitors (capmatinib or S49076 single-agent treated group)

Single-agent administration of an MET inhibitors induces tumor cytotoxicity, which extent strongly depends on how much the considered cancer line relies on the MET-pathway.[18] Accordingly, the anticancer effect of capmatinib or S49076 is implemented as a killing effect on tumor cells that, similarly to gefitinib case, is described by:

$$\frac{dV_u(t)}{dt} = F_{V_u}\left(e, V, V_u\right) - C_{\text{MET}}(t)\, V_u(t)\, k_{2,\text{MET}} \quad (5)$$

where $k_{2,\mathrm{MET}}$ represents the cytotoxic potency of the MET inhibitor treatment.

Moreover, because BWL were observed during capmatinib or S49076 treatment, a drug toxic effect is included on host assimilation:

$$\rho(t) = \rho_b \left(1 - \frac{V_u(t)}{IV_{u,50} + V_u(t)}\right)\left(1 - \frac{C_{\mathrm{MET}}(t)}{IC_{50,\mathrm{MET}} + C_{\mathrm{MET}}(t)}\right) \quad (6)$$

where $IC_{50,\mathrm{MET}}$ denotes the drug concentration producing the 50% of the maximal caloric restriction.

Null-interaction tumor-in-host DEB-TGI model for the combination of gefitinib and an MET inhibitor

Following the strategy proposed in ref. 24, a tumor-in-host DEB-TGI model predicting tumor and host body responses to the combination of gefitinib and a MET inhibitor in absence of drug interactions is developed. The null-interaction assumption requires that the effects of administered compounds are independent of each other. Thus, based on the single-compound TGI models, the null-interaction combination model includes the inhibition of tumor energy flow due to gefitinib (Equation 4), the killing effect induced by both the agents (Equations 3 and 5), as well as the toxic effect of capmatinib or S49076 on host assimilation (Equation 6). The model equations are reported in Equations S4a–S4h in Supplementary Material S1 where all the parameters are fixed according to the single-compound models.

Synergistic tumor-in-host DEB-TGI model for the combination of gefitinib and MET inhibitors

The inhibition of MET kinase activity is notably able to restore tumor sensitivity to EGFR-TKIs and to promote apoptosis in resistant MET-activated NSCLC models. Therefore, a synergistic tumor-in-host DEB-TGI model for gefitinib combined with an MET inhibitor is developed starting from the null-interaction combination model. The model assumes that the MET-blockage induced by capmatinib or S49076 is able to reverse tumor resistance to EGFR-inhibition and, thus, to re-establish the gefitinib anticancer effect.

To account for this synergism, in the combination groups, the gefitinib anticancer activity is enhanced by the MET inhibitor treatment. The gefitinib-related parameters governing both the cytostatic and cytotoxic effects are thus increased by the interaction factors, φ_1 and φ_2:

$$\mu_u = \mu_{u,b}\left(1 - \frac{I_{\max} C_{\mathrm{gef}}(t)}{IC_{50,\mathrm{gef,combo}} + C_{\mathrm{gef}}(t)}\right) \quad (7)$$

where $IC_{50,\mathrm{gef,combo}}$ is given by:

$$\mathrm{IC}_{50,\mathrm{gef,combo}} = \mathrm{IC}_{50,\mathrm{gef}}\left(\frac{1}{1+\varphi_1}\right) \quad (8)$$

and

$$\frac{dV_u(t)}{dt} = F_{V_u}(e, V, V_u) - V_u(t)\left(C_{\mathrm{MET}}(t)\,k_{2,\mathrm{MET}} + C_{\mathrm{gef}}(t)\,k_{2,\mathrm{gef,combo}}\right) \quad (9)$$

where $k_{2,\mathrm{gef,combo}}$ is given by:

$$k_{2,\mathrm{gef,combo}} = k_{2,\mathrm{gef}}\varphi_2 \quad (10)$$

All the model parameters are fixed according to the monotherapy models, including the drug-related parameters $IC_{50,\mathrm{gef}}$, $k_{2,\mathrm{gef}}$ and $k_{2,\mathrm{MET}}$.

Data analysis

For all the three compounds, PK model structures and population parameters were determined on PK data from previous studies. Individual PK parameters were then estimated on concentrations measured in the TGI study by a post hoc step and used to simulate individual concentration profiles given in input into the tumor-in-host TGI models.

Tumor-in-host DEB-TGI models were identified on tumor weight (TW) and host body weight (BW) data assuming a residual error proportional to the square root of the predicted values. Each experimental arm was separately analyzed, adopting the following sequential strategy to facilitate parameter estimation. Host-related parameters were fixed to the values derived from healthy mice growth data in ref. 32 (Table S1 in Supplementary Material S2); thermodynamic efficiency coefficient, ω, was fixed to 0.75 and the tumor volume density, dV_u, to 1 g/cm^3. Tumor-related (μ_u, g_u, m_u) and cachexia-related ($\delta_{V_{\max}}$, $IV_{u,50}$) parameters, food-supply coefficient ρ_b and the initial conditions, e_0 and $V_{u,0}$ (t_0 = inoculation day) were estimated from control group and, then, fixed in the treated groups. For each arm, a different value of W_0 was estimated and V_0 was derived as $V_0 = W_0/(1 + e_0\xi)$. Parameters related to gefitinib ($IC_{50,\mathrm{gef}}$, $k_{2,\mathrm{gef}}$), capmatinib ($k_{2,\mathrm{cap}}$, $IC_{50,\mathrm{cap}}$) and S49076 ($k_{2,S49}$, $IC_{50,S49}$)

were estimated identifying the corresponding single-compound TGI model against single-agent treated arms B to D.

The parameter estimates were used to compute the predicted tumor and host body growth curves in the combination groups in absence of (PK and) PD interactions using the null-interaction combination model. First, the synergism of combinations was visually assessed comparing observed and predicted individual TW and host BW. In addition, a statistical evaluation was performed based on the Normalized Prediction Distribution Errors (npde)[41–44]: in case of additivity of the effects, the null-interaction model should adequately describe TW and BW of combination arms (H_0 hypothesis) and the correspondent npde should be normally distributed. Npde related to TW and mice BW were computed and analyzed in R ("npde" package) to statistically test the null-interaction hypothesis. To account for typical and individual behavior, a *t*-test for mean and a Fisher test for variance were used.

Once the synergism of combinations had been assessed, the interaction factors, φ_1 and φ_2, for the two combinations were estimated on data from arms E and F.

To limit the model complexity and the related identifiability issues, interindividual variability was considered only for the initial values W_0 and $V_{u,0}$, for the half maximal inhibitory concentration of gefitinib ($IC_{50,gef}$), for the food-supply coefficient ρ_b and for the cytotoxic potency of gefitinib ($k_{2,gef}$) and MET inhibitors ($k_{2,cap}$ and $k_{2,S49}$). This choice was a priori guided considering the most important parameters and has been a posteriori assessed looking to the fitting and estimates main matrixes. Individual parameters were supposed to be log-normally distributed. Because ρ_b takes value in (0,1), it was re-parametrized as $\rho_b = 1/\left(1+R_b\right)$ with R_b in (0,+ ∞), and then R_b was supposed to be log-normally distributed.

Analysis was performed in Monolix (version 2016R1 and 2019R2, http://lixoft.com/products/monolix/). Goodness-of-fit (GOF) plots were produced in R (version 3.6). Model codes and dataset templates based on simulated data are provided as Supplementary Material to facilitate the reproducibility of the results.

RESULTS

Pharmacokinetic modeling

Despite the high interindividual variability, individual concentration-time data of the three compounds were correctly described by the PK models. Obtained population parameter estimates (Table S2 in Supplementary Material S3) were used to derive individual PK parameters for the treated animals of the TGI study.

Tumor-in-host DEB-based models for control and single agent arms

Individual TW and mice BW data of control and single-agent treated groups were analyzed through a nonlinear mixed-effect approach. The obtained parameter estimates are reported in Table 3; examples of individual fit for one individual in each arms A to D are shown in Figure 2 and additional diagnostic plots are included in Supplementary Material S4 (Figures S1–S12).

Importantly, the unperturbed tumor-in-host model was successfully identified for the control group, confirming its ability in simultaneously describing TW and host BW dynamics at both typical and individual level. For the gefitinib single-agent (arm B), the drug-related parameters, $IC_{50,gef}$ and $k_{2,gef}$, were estimated fixing I_{max} to 1, because its estimation did not provide any relevant improvement. The exiguous tumor growth modulation was captured by the DEB-TGI model for EGFR-TKIs that provided estimates of the low gefitinib anticancer potency ($IC_{50,gef}$ and $k_{2,gef}$). The DEB-TGI model for MET inhibitors was separately identified against arm C and arms D_1-D_2 and a different set of drug-related parameters were estimated for capmatinib and S49076. In both the cases, TGI and host BWL following MET inhibitor administration were well-described by the model. For S49076, BW data highlighted a delay between the end of treatment and the BW increase. Thus, an effect compartment was included and the inhibition of energy intake was driven by the effect compartment concentration. Further, because in arms D_1-D_2 BWLs induced by the drug were not completely consistent between the S49076 dose levels, two $IC_{50,eff,S49}$ values (one for each dose) were estimated. Similarly, to account for the higher interindividual variability of tumor response observed in the 35 mg/kg dose arm, two different sd($k_{2,S49}$) were estimated.

Overall, for each experimental arm, the GOF plots visually confirmed that the proposed population DEB-TGI models adequately described the collected data; weighted residuals were randomly distributed around zero, indicating the absence of model bias; finally, visual predictive checks (VPCs) confirmed that the interindividual variability was also well-captured.

Assessment of synergistic interactions between gefitinib and MET inhibitors

Obtained parameter estimates were used, together with individual PK parameters estimated on combination arms, to simulate TW and host BW dynamics following

TABLE 3 Parameter estimates

Parameter	Dimension	Typical value	Interindividual variability (SD)
Host-related parameters			
ρ_b[a]	–	**0.779**	0.047
Tumor-related parameters			
μ_u	–	2.28	–
g_u	–	8.38	–
m_u	1/day	0.028	–
Cachexia-related parameters			
δ_{Vmax}	cm^3/day	0.0148	–
IV_{u50}	cm^3	5.58	–
Initial conditions			
W_0	g	20.2 (arm A), 20.7 (arm B), 19.2 (arm C), 21.4 (arms D), 21.6 (arm E), 22.1 (arms F)	0.078
$V_{u,0}$	cm^3	0.038	0.122
e_0	–		-
Gefitinib-related parameters			
$IC_{50,\ gef}$	µg/L	911	–
$K_{2,\ gef}$	L/ µg day	1.83e−5	0.630
Capmatinib-related parameters			
$K_{2,\ cap}$	L/µg day	1.14e−6	0.363
$IC_{50\ cap}$	µg/L	921	–
S49076-related parameters			
$K_{2,S49}$	L/µg day	4.16e−5	0.194 (arm D_1), 1.280 (arm D_2)
$IC_{50,eff,\ S49}$	µg/L	864 (arm D_1), 25.2 (arm D_2)	–
$K_{eff,\ S49}$	1/day	0.312	–
Synergistic factors			
$\varphi_{1,cap}$	–	30.7	–
$\varphi_{2,cap}$	–	15.8	–
$IC_{50,\ gef,\ combo1}$[b]	µg/L	28.74	–
$K_{2,gef,combo1}$[b]	L/µg day	2.89e−4	0.403
$\varphi_{1,S49}$	–	54.9	–
$\varphi_{2,S49}$	–	16.7	–
$IC_{50,\ gef,\ combo2}$[b]	µg/L	16.30	–
$K_{2,gef,combo2}$[b]	L/µg day	3.06e−4	1.08

Note: Individual parameters are given by $P_i = \theta \exp(\eta_i)$ where θ is the typical value and η a random effect with SD (P).

[a]Values for ρ_b and SD (ρ_b) were approximations from the estimates of $R_b = 0.283$ and SD (R_b) = 0.205.

[b]Typical values for $IC_{50,gef,\ combo}$ and $K_{2,gef,combo}$ were derived from the expression reported in Equations 8 and 10.

combination treatments under the null-interaction hypothesis.

In Figure 3, individual predicted profiles (1000 simulations of the dataset) were superimposed to the experimental data allowing an easy assessment of the drug interactions. In particular, for both compounds, the observed TWs lied below the predicted tumor growth curves confirming the presence of a synergistic interaction that was more relevant at the lower dose level. For what concerned host dynamics, experimental and predicted BWs resulted closed during the treatment period, suggesting that the toxic effect of the MET inhibitors is

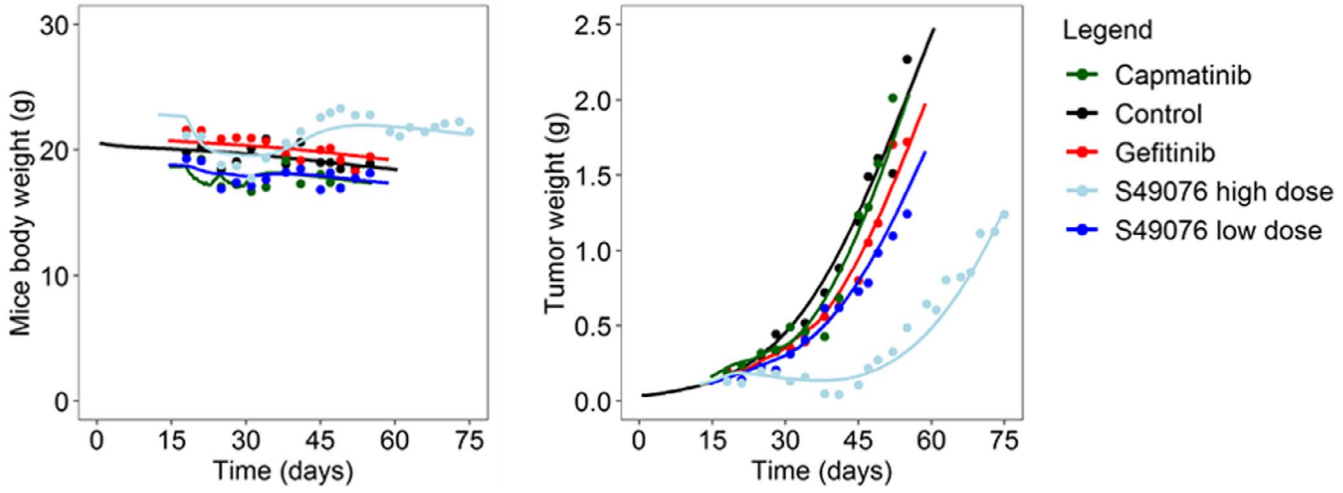

FIGURE 2 Representative individual time courses of the tumor and mice body weight profiles (solid lines) together with the corresponding observed data (dots) for control and single-agent treated arms (arms A–D)

not increased by the co-administered gefitinib. Moreover, observed BWs were above the model predictions at the end of the study. These increased BWLs resulted from the energetic interactions between the host and the higher tumor mass predicted by the model and further confirmed the synergistic nature of drug combination.

Finally, the *p* values of the statistical tests performed on npde are reported in Table S3 in Supplementary S5. Statistical results suggested to reject the no-interaction hypothesis confirming the results from visual inspections.

Synergistic tumor-in-host DEB-TGI model for the combination of gefitinib and MET inhibitors

Once the synergism of the combinations had been assessed, the synergistic tumor-in-host DEB-TGI model here proposed was identified on TW and host BW data from the combination arms. Arm E and arms F_1–F_2 were analyzed separately and different sets of synergistic factors (φ_1, φ_2) were identified for capmatinib and S49076. Model parameters were fixed to the previous estimates, except for the sd($k_{2,gef}$) that was re-estimated to account for the difference in interindividual variability of tumor response to combination treatment. VPC plots for each combination arm and parameter estimates are reported in Figure 4 and Table 3, respectively (see Supplementary Material S4 for additional analyses). For both the combinations, the introduction of the synergistic factors allowed to well capture the enhanced TGI. Moreover, the tumor-host energetic interactions enabled the model to grasp the lower than expected BWLs without the need of further interaction dynamics.

DISCUSSION

Poly-targeted combination therapy represents the cornerstone for overcoming resistant to standard anticancer treatments. A widely studied case is the co-administration of MET inhibitors and EGFR-TKIs in NSCLC treatment. Considering the great interest and the relevant efforts put into the design and assessment of new anticancer compounds targeting the MET axis, we proposed a novel population PK/PD model able to comparatively characterize and quantify the interaction between two MET inhibitors, capmatinib and S49076, and the standard of care, gefitinib, when co-administered in an EGFR-mutant/MET-activated NSCLC xenograft model.

To this aim, the tumor-in-host modeling framework, based on the DEB theory, was extended to describe tumor and host BW responses to mono- and combination therapies of EGFR-TKIs and MET inhibitors. The developed DEB-TGI models well-described TW and BW data from controls and single-agent groups, accounting for the exiguous tumor growth modulation, as well as host BWL due to tumor progression or to the anorexic effect of the MET treatment. Further, the synergism of both the combinations was rigorously assessed using the null-interaction combination tumor-in-host model that highlighted the benefits of the co-administration not only in terms of increased TGI but also in terms of reduced BWLs. Finally, the synergistic behaviors were well-described by the synergistic tumor-in-host model, through the introduction of the interactions.

In addition to an excellent description of the experimental data, quantitative estimates of the anticancer drug potency were provided by the model. In particular, $IC_{50,gef}$ and $k_{2,gef}$ quantify the TGI effect induced by

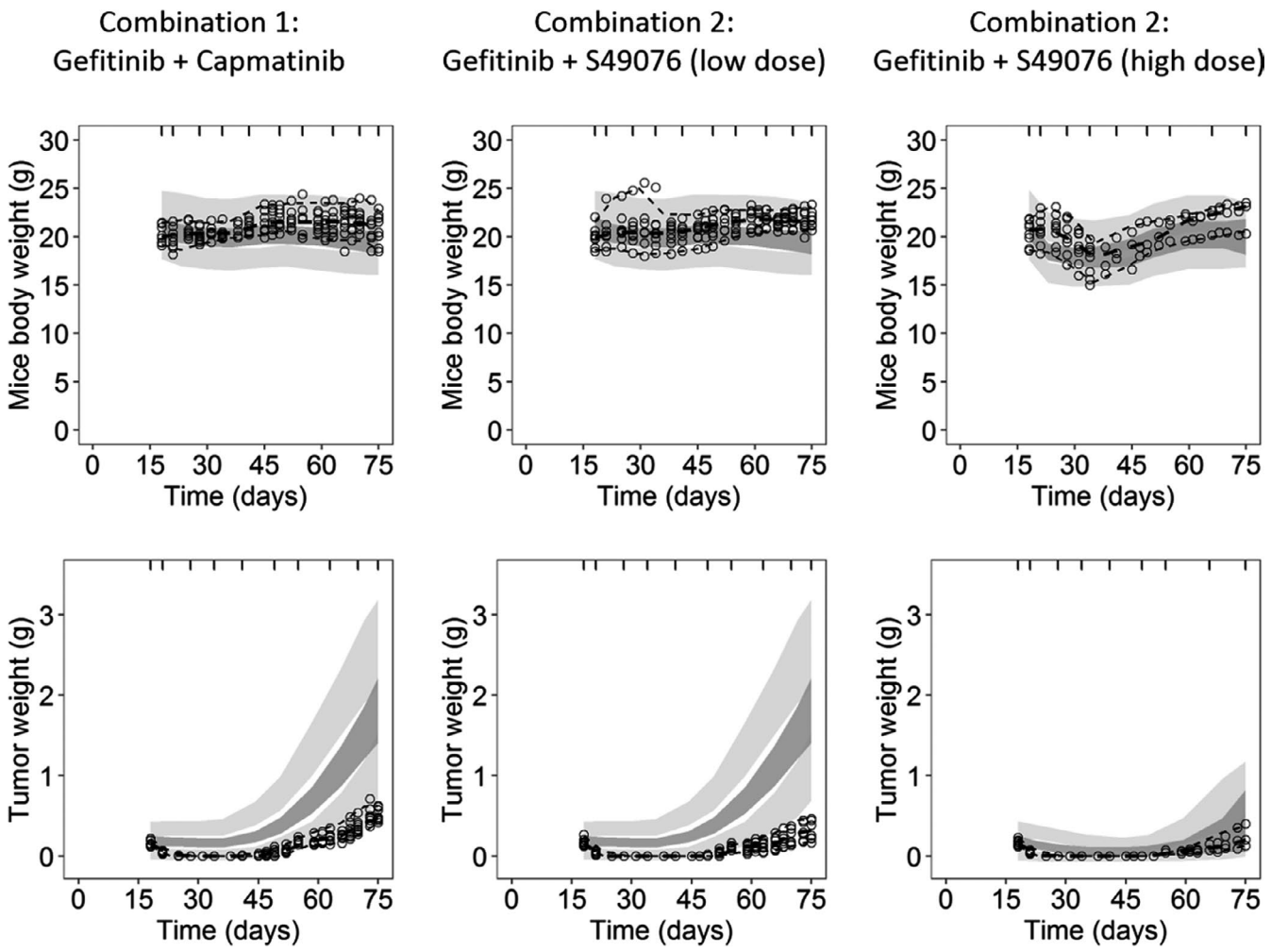

FIGURE 3 External visual predictive check plots stratified by group (1000 replicates of the dataset) relative to combination arms E and F: dashed lines represent the 90% confidence interval for the corresponding percentile predicted by the null-interaction combination model, dots are individual observed data

gefitinib, $k_{2,\mathrm{MET}}$ the cytotoxic potency of capmatinib or S49076, whereas the interaction factors, φ_1 and φ_2, the intensity of the synergism. These metrics of efficacy were used to compare the two MET inhibitors in terms of their direct anticancer activity and their ability to act as synergistic modulators of gefitinib-related TGI. Obtained estimates highlighted that in monotherapy S49076 exerted a greater, even if modest, anticancer effect than capmatinib: $k_{2,s49} = 4.16e-5\,\mathrm{L/\mu g\cdot day} > k_{2,\mathrm{cap}} = 1.14e-6\,\mathrm{L/\mu g\cdot day}$. The interaction factor values ($\varphi_{1,\mathrm{cap}} = 30.7$, $\varphi_{2,\mathrm{cap}} = 15.8$ and $\varphi_{1,S49} = 54.9$, $\varphi_{2,S49} = 16.7$) showed that the strength of the synergism of gefitinib with S49076 is higher than with capmatinib. In order to provide more understandable measures of the interaction, the additional indexes $\mathrm{IC}_{50,\mathrm{gef,combo}}$ and $k_{2,\mathrm{gef,combo}}$ were derived from Equations 8 and 10. Comparing these values with the estimates of anticancer potency of gefitinib in monotherapy ($\mathrm{IC}_{50,\mathrm{gef}} = 911\ \mu\mathrm{g/L} > \mathrm{IC}_{50,\mathrm{gef.combo1}} = 28.74\ \mu\mathrm{g/L} > \mathrm{IC}_{50,\mathrm{gef.combo2}} = 16.7\ \mu\mathrm{g/L}$ and $k_{2,\mathrm{gef}} = 1.83e-5\,\mathrm{L/\mu g\cdot day} < k_{2,\mathrm{gef,combo1}} = 2.89e-4\,\mathrm{L/\mu g\cdot day} < k_{2,\mathrm{gef,combo2}} = 3.06e-4\,\mathrm{L/\mu g\cdot day}$), it was evident that the gefitinib efficacy was greatly strengthened by the co-administration of MET inhibitors and that the restoration of tumor sensitivity to EGFR-TKI was greater for S49076 than for capmatinib. Together with the enhanced gefitinib anticancer activity, the combination model also includes the direct cytotoxic effect on tumor cell of the MET inhibitors. Thus, assuming that $C_{\mathrm{gef}}(t) = C_{\mathrm{MET}}(t)$, the whole cytotoxic effect of the combination is proportional to $k_{2,\mathrm{combo}} = k_{2,\mathrm{gef,combo}} + k_{2,\mathrm{MET}}$. The values of $\mathrm{IC}_{50,\mathrm{gef,combo}}$ and $k_{2,\mathrm{combo}}$ ($k_{2,\mathrm{combo1}} = 2.9e-4\,\mathrm{L/\mu g\cdot day}$ and $k_{2,\mathrm{combo2}} = 3.48e-4\,\mathrm{L/\mu g\cdot day}$) synthesized the anticancer activity of the combinations allowing to rank the considered competitors.

Further, a mathematical analysis of the DEB-TGI models for the single agents and the combination, under both null-interaction and synergistic hypothesis, was conducted. Following the works of refs. 45 and 46, we searched for the possible equilibrium points of the system and

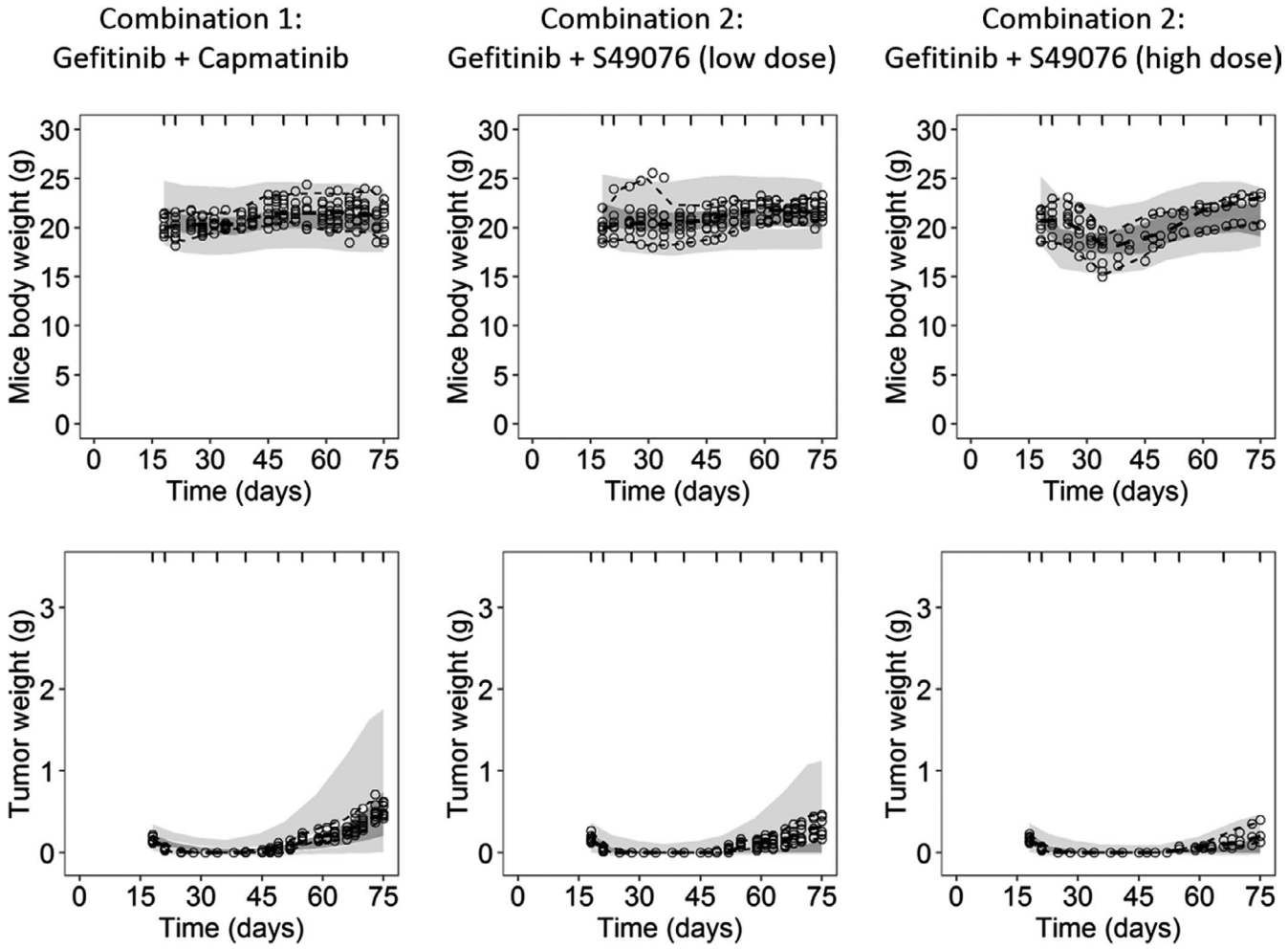

FIGURE 4 External visual predictive check plots stratified by group (1000 replicates of the dataset) relative to combination arms E and F: dashed lines represent the 90% confidence interval for the corresponding percentile predicted by the combination model, dots are individual observed data

studied their stability in case of structural biomass growth or structural biomass degradation with a rate $< \delta_{V_{max}}$ and supposing that both the drugs are administered through an infusion yielding a (steady-state) constant concentration ($C_{\text{gef}}(t) = C_{\text{gef}}$ and $C_{\text{MET}}(t) = C_{\text{MET}}$). The analysis (not shown) demonstrated the existence of an equilibrium point characterized by tumor eradication ($V_u = 0$), which stability is guaranteed for a sufficient high concentration of gefitinib ($C_{\text{gef}} \geq \overline{C_{\text{gef}}}$). The minimum concentration level necessary to eradicate tumor mass, $\overline{C_{\text{gef}}}$, is given by Equations 11a and 11b for monotherapy and combination, respectively. For combination, the minimum effective concentration is a function of the MET inhibitor level ($\overline{C_{\text{gef}}}_{\text{combo}}(C_{\text{MET}})$). Its definition is the same under both the null-interaction and the synergistic hypothesis; however, in the latter case the monotherapy values of $IC50_{gef}$ and $k_{2,gef}$ are replaced by $IC50_{\text{gef, combo}}$ and $k_{2,\text{gef,combo}}$ (Equations 8 and 10).

A quantification of the synergistic co-administration of MET inhibitors can be derived comparing the minimum effective gefitinib concentration obtained for monotherapy ($\overline{C_{\text{gef}}}_{\text{mono}} = 1225$ μg/L) to

$$\overline{C_{gef}}_{\text{mono}} = \frac{-\left(m_u + \text{IC50}_{\text{gef}} k_{2,gef}\right) + \sqrt{\left(m_u + \text{IC50}_{\text{gef}} k_{2,\text{gef}}\right)^2 + 4k_{2,\text{gef}} IC50_{\text{gef}} \left(\frac{mg\mu_u}{g_u} - m_u\right)}}{2k_{2,\text{gef}}} \tag{11a}$$

$$\overline{C_{gef}}_{\text{combo}} = \overline{C_{gef}}_{\text{combo}}(C_{\text{MET}}) = \frac{-\left(m_u + IC50_{\text{gef}} k_{2,\text{gef}} + k_{2,\text{MET}} C_{\text{MET}}\right) + \sqrt{\left(m_u + IC50_{\text{gef}} k_{2,\text{gef}} + k_{2,\text{MET}} C_{\text{MET}}\right)^2 - 4k_{2,\text{gef}} IC50_{gef} \left(k_{2,\text{MET}} C_{\text{MET}} - \frac{mg\mu_u}{g_u} + m_u\right)}}{2k_{2,\text{gef}}} \tag{11b}$$

that obtained in combination for each arbitrary concentration level of MET-inhibitors. For example, for S49076, we could consider the average concentration ($C_{S49,avg} = AUC_{S49,0-24}/24 = 4284\,h \cdot \mu g/L/24\,h = 179 \mu g/L$) observed in patients after administration of the RP2D (600 mg daily p.o.).[37] The corresponding gefitinib thresholds were $\overline{C_{gef_{combo,add}}} = 1055\ \mu g/L$ under the null-interaction hypothesis and $\overline{C_{gef_{combo,syn}}} = 28\ \mu g/L$ considering the synergistic mechanism. Note that only an exiguous gain can be attributable to the direct effect of S49076 on tumor mass ($\overline{C_{GEFcombo,add}} \approx \overline{C_{GEFmono}}$). Differently, the comparison $\overline{C_{GEFcombo,syn}} \ll \overline{C_{GEFmono}}$ quantifies the real benefit provided by the S49076 co-administration, due to the restoration of the gefitinib efficacy. Finally, the obtained estimates of the minimal effective concentration can be compared to the average gefitinib concentrations observed during clinical trials ($C_{gef,avg} = AUC_{Gef,0-24}/24$ was 210 and 238 μg/L after administration of gefitinib at 225 mg daily and 300 mg daily, respectively[47]). Of interest, in a phase I study,[48] gefitinib 250 mg daily was administered in combination with S49076 at 600 mg daily to 14 patients with EGFR TKI-resistant NSCLC resulting in two partial responses and eight patients with stable disease.

In this work, a new TGI model evaluating drug combination in xenograft studies was proposed based on the DEB theory. Even if several mathematical models are already available in the literature,[23–26,28,29] to the best of our knowledge, this is the first modeling exercise in which the assessment of drug interactions was based on a joint analysis of both TGI and cachexia dynamics. Indeed, the simultaneous modeling of tumor and host body weight and of their mutual interaction represents the novelty of the DEB-based framework and its potential of providing additional insights.

First, the tumor-in-host approach allowed a deeper exploiting of the experimental preclinical data, accounting for all the different dynamics characterizing the in vivo TGI studies. Among them, BW decreases due to tumor progression and treatment toxic effect were of particular relevance because they reflect the effects of cachexia observed in patients with cancer. The high impact of tumor-related cachexia was only partially evident from the BWL (>7% from the begin of the treatment) in animals receiving placebo and gefitinib for which no drug toxic effect was observed. Indeed, the natural weight increases due to the young age of mice masked the actual negative effect of tumor-progression on host body growth: at the end of the experiment a difference of 9 g was observed between untreated tumor-bearing mice and tumor-free (equal to −33% of the initial BW). In the MET-inhibitor groups, the tumor-related cachexia was aggravated by the drug toxic effect: during the treatment period BWLs of about 14%, 13%, and 17% were observed in the capmatinib group, in the S49076 low and high doses, respectively. Finally, the reduced BWLs observed in the combination groups were an additional sign of the enhanced anticancer effect of the treatment: lower tumor masses exerted a lower cachectic effect on the host organism. The DEB-based tumor-in-host model, accounting for the energetic interactions between tumor mass and host organism and for the toxic effect of the treatment on the animal BW, was able to grasp all these dynamics discerning the contribution of cachexia induced by tumor progression and by the anticancer therapy. In this way, the benefits of the co-administration were assessed not only in terms of increased TGI but also in term of reduced BWLs allowing to quantify the synergistic effect based on both tumor and host dynamics.

Further, the tumor-in-host approach provided a better characterization of treatment anticancer efficacy. Indeed, the disentangling of the direct effect of the drug or drug combination on tumor cells from the slow-down in tumor growth due to the depletion of host energies prevented an overestimation of the anticancer potency.

Finally, the semimechanistic properties of the DEB-based framework could facilitate translation of the tumor-in-host growth from preclinical to clinical setting. Exploiting the general rules for the interspecies scaling provided by the DEB theory, the model estimates obtained on xenograft studies would be used to predict human tumor volume doubling time (TVDT), a noninvasive assay of disease progression in patients with cancer. For example, estimates obtained on the HCC827ER1 tumor cell line allowed to predict a human TVDT = 297 days, comparable with the 222 days observed in patients with cancer affected by lung adenocarcinoma.[49] Once this approach is validated, similarly it could be applied also to the drug effect on tumor and on the host. Currently, investigations go in this direction.

As in general for (semi)mechanistic models, the high complexity of the DEB-based model and its number of parameters could lead to some identifiability problems, especially on data taken from nonoptimally designed studies. Indeed, the negative impact of a nonoptimal experimental design on the model parameter identification has been demonstrated for the simpler Simeoni model.[50] The xenograft study here analyzed is characterized by a protocol not optimized for the purpose of model identification. Specifically, (i) tumor-related parameter identification was hampered by the no-observation of the plateau phase in the tumor growth (tumor masses lower than 2 g); (ii) precision of the drug-related parameter estimates was hindered by the extremely poor anticancer effect of the drugs in monotherapy; and (iii) population approach was limited by the reduced number of animals per arm. For these reasons, interindividual variability was included on a reduced number of key parameters: the initial host

BW and tumor volume, the food-supply coefficient, and on the k_2 parameters to account for the heterogeneity in the tumor-in-host experimental setting, assimilation and tumor response to treatments. This choice was a posteriori confirmed by typical model checking analysis. Further, a sequential identification strategy was adopted, and the reliability of the parameter estimates was assessed in terms of penalized-likelihood criteria (several runs were performed varying the initial values). Nevertheless, some identifiability issues remained and could limit the use of the obtained point values of the model parameters and of the derived metrics, without affecting the overall results and the comprehensive scenarios predicted by the model. In addition, different experimental protocols, characterized by treated or not-treated groups of tumor-free animals and prolonged sampling after the end of the treatment in which tumor volumes reach higher values due to the regrowth, demonstrated to provide relevant benefits in term of parameter estimations.[33]

In summary, for the first time, a PK/PD tumor-in-host model based on the DEB theory was proposed to describe the effect of the EGFR-TKI gefitinib administered alone or in combination with the MET inhibitors, capmatinib and S49076, in an NSCLC model. In this way, the relevance and the applicability of the tumor-in-host DEB-based framework, until now exploited only in case of cytotoxic or anti-angiogenic agents in monotherapy,[32–34] was further demonstrated. It has been shown that this approach provides a versatile modeling tool enough to account for the effects of several anticancer agents, including different types of targeted therapies. In addition, the null-interaction combination modeling approach could be used to assess the presence of drug interaction based on both tumor and host dynamics, also in absence of a priori strong knowledge on the interaction nature. However, this work is affected by some limitations. As in general for all the (semi)mechanistic models, the high complexity and the large number of parameters led to some identifiability issues. These were enhanced by the nonoptimized experimental settings of the considered xenograft study that also prevented an external validation of the combination model. Albeit with these limitations, the presented approach has practical implications because it can be applied to routinely performed xenograft experiments and may assist on the ranking of investigated compounds and on the selection of the most promising combination able to show an adequate efficacy and a tolerable toxicity.

Further, the physiologic properties and the robustness of the DEB approach recommend its use for translational purposes during the anticancer drug development process and its application for a better characterization of cancer-associated cachexia. Currently research efforts are going in these directions.[51–53]

For all these reasons, this novel combination tumor-in-host DEB-TGI model can be considered a useful tool in the preclinical development for a better characterization of the combination therapies.

CONFLICTS OF INTEREST

G.G., S.F., M.B., and M.C. are employed by Servier. E.M.T. and P.M. are employed by Università degli Studi di Pavia.

AUTHOR CONTRIBUTIONS

E.M.T., G.G., and P.M. wrote the manuscript. E.M.T., P.M., M.C., and G.G. designed the research. E.M.T., S.F., and M.B. performed the research. E.M.T. analyzed the data.

REFERENCES

1. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. *Nature*. 2018;553(7689):446-454.
2. Duma N, Santana-Davila R, Molina JR. Non-small cell lung cancer: epidemiology, screening, diagnosis, and treatment. *Mayo Clin Proc*. 2019;94(8):1623-1640.
3. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2005;352(8):786-792.
4. Lin L, Bivona TG. Mechanisms of resistance to epidermal growth factor receptor inhibitors and novel therapeutic strategies to overcome resistance in NSCLC patients. *Chemother Res Pract*. 2012;2012(817297):1-9.
5. Pao W, Chmielecki J. Rational, biologically based treatment of EGFR-mutant non-small-cell lung cancer. *Nat Rev Cancer*. 2010;10(11):760-774.
6. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science (80-)*. 2007;316(5827):1039-1043.
7. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. *Proc Natl Acad Sci U S A*. 2007;104(52):20932-20937.
8. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med*. 2011;3(75):75ra26.
9. Yu HA, Arcila ME, Rekhtman N, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res*. 2013;19(8):2240-2247.
10. Wang S, Cang S, Liu D. Third-generation inhibitors targeting EGFR T790M mutation in advanced non-small cell lung cancer. *J Hematol Oncol*. 2016;9(1):1-7.
11. Yang Z, Tam KY. Combination strategies using EGFR-TKi in NSCLC therapy: Learning from the gap between pre-clinical results and clinical outcomes. *Int J Biol Sci*. 2018;14(2):204–216.

12. Puccini A, Marín-Ramos NI, Bergamo F, et al. Safety and tolerability of c-MET inhibitors in cancer. *Drug Saf*. 2019;42(2):211-233.
13. Liu X, Wang Q, Yang G, et al. A novel kinase inhibitor, INCB28060, blocks c-MET-dependent signaling, neoplastic activities, and cross-talk with EGFR and HER-3. *Clin Cancer Res*. 2011;17(22):7127-7138.
14. Burbridge MF. P2.09 preclinical antitumour activity of S49076, a novel Met / Axl / Fgfr kinase inhibitor, and molecular stratification for tumour sensitivity. *Ann Oncol*. 2012;23:v24-v26.
15. Burbridge MF, Bossard CJ, Saunier C, et al. S49076 Is a novel kinase inhibitor of MET, AXL, and FGFR with strong preclinical activity alone and in association with bevacizumab. *Mol Cancer Ther*. 2013;12(9):1749-1762.
16. Zhang YW, Staal B, Essenburg C, Lewis S, Kaufman D, Vande Woude GF. Strengthening context-dependent anticancer effects on non-small cell lung carcinoma by inhibition of both MET and EGFR. *Mol Cancer Ther*. 2013;12(8):1429-1441.
17. Stabile LP, Rothstein ME, Keohavong P, et al. Targeting of both the c-Met and EGFR pathways results in additive inhibition of lung tumorigenesis in transgenic mice. *Cancers (Basel)*. 2010;2(4):2153-2170.
18. Lara MS, Holland WS, Chinn D, et al. Preclinical evaluation of MET inhibitor INC-280 with or without the epidermal growth factor receptor inhibitor erlotinib in non–small-cell lung cancer. *Clin Lung Cancer*. 2017;18(3):281-285.
19. Friese-Hamim M, Bladt F, Locatelli G, Stammberger U, Blaukat A. The selective c-Met inhibitor tepotinib can overcome epidermal growth factor receptor inhibitor resistance mediated by aberrant c-Met activation in NSCLC models. *Am J Cancer Res*. 2017;7(4):962.
20. Wu YL, Zhang L, Kim DW, et al. Phase Ib/II study of capmatinib (INC280) plus gefitinib after failure of epidermal growth factor receptor (EGFR) inhibitor therapy in patients with EGFR-mutated, MET factor-dysregulated non-small-cell lung cancer. *J Clin Oncol*. 2018;36(31):3101.
21. Wu YL, Soo RA, Locatelli G, Stammberger U, Scagliotti G, Park K. Does c-Met remain a rational target for therapy in patients with EGFR TKI-resistant non-small cell lung cancer? *Cancer Treat Rev*. 2017;61:70-81.
22. Carrara L, Lavezzi SM, Borella E, De Nicolao G, Magni P, Poggesi I. Current mathematical models for cancer drug discovery. *Expert Opin Drug Discov*. 2017;12(8):785-799.
23. Terranova N, Germani M, Del Bene F, Magni P. A predictive pharmacokinetic-pharmacodynamic model of tumor growth kinetics in xenograft mice after administration of anticancer agents given in combination. *Cancer Chemother Pharmacol*. 2013;72(2):471-482.
24. Rocchetti M, Del Bene F, Germani M, et al. Testing additivity of anticancer agents in pre-clinical studies: a PK/PD modelling approach. *Eur J Cancer*. 2009;45(18):3336–3346.
25. Koch G, Walz A, Lahu G, Schropp J. Modeling of tumor growth and anticancer effects of combination therapy. *J Pharmacokinet Pharmacodyn*. 2009;36(2):179-197.
26. Li JY, Ren YP, Yuan Y, et al. Preclinical PK/PD model for combined administration of erlotinib and sunitinib in the treatment of A549 human NSCLC xenograft mice. *Acta Pharmacol Sin*. 2016;37(7):930-940.
27. Simeoni M, Magni P, Cammia C, et al. Predictive pharmacokinetic-pharmacodynamic modeling of tumor growth kinetics in xenograft models after administration of anticancer agents. *Cancer Res*. 2004;64(3):1094-1101.
28. Magni P, Terranova N, Del Bene F, Germani M, De Nicolao G. A minimal model of tumor growth inhibition in combination regimens under the hypothesis of no interaction between drugs. *IEEE Trans Biomed Eng*. 2012;59(8):2161-2170.
29. Rocchetti M, Germani M, Del Bene F, et al. Predictive pharmacokinetic–pharmacodynamic modeling of tumor growth after administration of an anti-angiogenic agent, bevacizumab, as single-agent and combination therapy in tumor xenografts. *Cancer Chemother Pharmacol*. 2013;71(5):1147-1157.
30. Kooijman SALM. *Dynamic Energy and Mass Budgets in Biological Systems*. Cambridge University Press; 2000.
31. Kooijman SALM. Quantitative aspects of metabolic organization: a discussion of concepts. *Philos Trans R Soc London B Biol Sci*. 2001;356(1407):331-349.
32. Terranova N, Tosca EM, Borella E, Pesenti E, Rocchetti M, Magni P. Modeling tumor growth inhibition and toxicity outcome after administration of anticancer agents in xenograft mice: a dynamic energy budget (DEB) approach. *J Theor Biol*. 2018;450:1-14.
33. Tosca EM, Pigatto MC, Dalla Costa T, Magni P. A population dynamic energy budget-based tumor growth inhibition model for etoposide Effects on Wistar rats. *Pharm Res*. 2019;36(3):38.
34. Tosca EM, Rocchetti M, Pesenti E, Magni P. A tumor-in-host DEB-based approach for modeling cachexia and bevacizumab resistance. *Cancer Res*. 2020;80(4):820-831.
35. Tosca EM, Rocchetti M, Magni P. A Dynamic Energy Budget (DEB) based modeling framework to describe tumor-in-host growth inhibition and cachexia onset during anticancer treatment in in vivo xenograft studies. *Oncotarget*. 2021;12(14):1434.
36. Wu Y-L, Kim D-W, Felip E, et al. Phase (Ph) II safety and efficacy results of a single-arm ph ib/II study of capmatinib (INC280) + gefitinib in patients (pts) with EGFR-mutated (mut), cMET-positive (cMET+) non-small cell lung cancer (NSCLC). *J Clin Oncol*. 2016;34(15):9020.
37. Rodon J, Postel-Vinay S, Hollebecque A, et al. First-in-human phase I study of oral S49076, a unique MET/AXL/FGFR inhibitor, in advanced solid tumours. *Eur J Cancer*. 2017;81:142-150.
38. Chang G, Curigliano G, Lim W, et al. MA 12.02 Phase I/II study of S49076, a MET/AXL/FGFR inhibitor, combined with gefitinib in NSCLC patients progressing on EGFR TKI. *J Thorac Oncol*. 2017;12(11):S1847-S1848.
39. Baselga J. Why the epidermal growth factor receptor? The rationale for cancer therapy. *Oncologist*. 2002;7:2-8.
40. Cragg MS, Kuroda J, Puthalakath H, Huang DCS, Strasser A. Gefitinib-induced killing of NSCLC cell lines expressing mutant EGFR requires BIM and can be enhanced by BH3 mimetics. *PLoS Med*. 2007;4(10):e316.
41. Brendel K, Comets E, Laffont C, Mentré F. Evaluation of different tests based on observations for external model evaluation of population analyses. *J Pharmacokinet Pharmacodyn*. 2010;37(1):49-65.
42. Comets E, Brendel K, Mentré F, Comets E. Model evaluation in nonlinear mixed effect models, with applications to pharmacokinetics. *J la Société Française Stat Rev Stat appliquée*. 2010;151(1):106-128.

43. Nguyen THT, Mouksassi MS, Holford N, et al. Model evaluation of continuous data pharmacometric models: metrics and graphics. *CPT Pharmacometrics Syst Pharmacol*. 2017;6(2):87-109.
44. Comets E, Brendel K, Mentré F. Computing normalised prediction distribution errors to evaluate nonlinear mixed-effect models: the npde add-on package for R. *Comput Methods Programs Biomed*. 2008;90(2):154-166.
45. Magni P, Simeoni M, Poggesi I, Rocchetti M, De Nicolao G. A mathematical model to study the effects of drugs administration on tumor growth dynamics. *Math Biosci*. 2006;200(2):127-151.
46. Cardilin T, Almquist J, Jirstrand M, et al. Tumor static concentration curves in combination therapy. *AAPS J*. 2017;19(2):456-467.
47. Ranson M, Hammond LA, Ferry D, et al. ZD1839, a selective oral epidermal growth factor receptor-tyrosine kinase inhibitor, is well tolerated and active in patients with solid, malignant tumors: results of a phase I trial. *J Clin Oncol*. 2002;20(9):2240-2250.
48. Park K, Chang G-C, Curigliano G, et al. Phase I results of S49076 plus gefitinib in patients with EGFR TKI-resistant non-small cell lung cancer harbouring MET/AXL dysregulation. *Lung Cancer*. 2021;155:127-135.
49. Arai T, Kuroishi T, Saito Y, et al. Tumor doubling time and prognosis in lung cancer patients: evaluation from chest films and clinical follow-up study. *Jpn J Clin Oncol*. 1994;24(4):199-204.
50. Lestini G, Mentré F, Magni P. Optimal design for informative protocols in xenograft tumor growth inhibition experiments in mice. *AAPS J*. 2016;18(5):1233-1243.
51. Tosca EM, Montanaro A, Rocchetti M, Magni P. Cancer cachexia: a Dynamic Energy Budget (DEB)-based model for tumor-related anorexia and sarcopenia. *CPT Pharmacometrics Syst Pharmacol*. 2020;9(S1):S9-S26.
52. Tosca EM, Montanaro A, Rocchetti M, Magni P. Reduced food-intake and sarcopenia in animal models of cancer anorexia-cachexia syndrome: a dynamic energy budget (DEB)-based modeling approach. In: PAGE 29 (2021) Abstract 9879. Available from: www.page-meeting.org/?abstract=9879.
53. Tosca EM, Castellano S, Ronchi D, Rocchetti M, Magni P. A tumor-in-host Dynamic Energy Budget (DEB)-based paradigm for preclinical to clinical translation: predictions of tumor-in-host growth dynamics. In: PAGE 29 (2021) Abstract 9812 [Internet]. Available from: www.page-meeting.org/?abstract=9812.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Tosca EM, Gauderat G, Fouliard S, Burbridge M, Chenel M, Magni P. Modeling restoration of gefitinib efficacy by co-administration of MET inhibitors in an EGFR inhibitor-resistant NSCLC xenograft model: A tumor-in-host DEB-based approach. *CPT Pharmacometrics Syst Pharmacol*. 2021;10:1396–1411. doi:10.1002/psp4.12710