

Article

Differential Models of Time-Variant Tumor Growth Trajectories with Sensitive, Persister, Resistant Cell Population in Lung Tumors During Tyrosine Kinase Inhibitor Therapy

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Abstract

Modeling the dynamics of three tumor cell populations, i.e., sensitive, persister, and resistant tumor cells, during molecularly targeted therapy with tyrosine kinase inhibitors (TKIs) would be valuable for adjusting treatment plans for patients with epidermal growth factor receptor-mutated non-small cell lung cancer (EGFR-mt NSCLC). We hypothesized the time-variant tumor growth trajectories (TGTs) of patients with stage IV EGFR-mt NSCLC for the three tumor cell populations could be expressed using differential models after several follow-up computed tomography examinations. We aimed to propose differential models for TGTs in three cell populations from patients with EGFR-mt NSCLC treated with an EGFR-TKI (osimertinib). We selected two differential equations—Bertalanffy–Pütter (BP) and Gompertz—to develop TGT models. The parameters of the models were optimized based on a dual annealing method within parameter ranges determined using synthetic patient data. Using CT examinations that were not employed for model fitting, the mean absolute percentage errors (MAPEs) for BP-based and Gompertz-based models were $36.1 \pm 40.2\%$ and $43.9 \pm 60.1\%$, respectively, for three follow-up computed tomography (FCT) examinations, which indicated no statistically significant difference ($p = 0.61$). This study suggests that the proposed BP-based and Gompertz-based differential models could have the potential to express TGTs in patients with stage IV EGFR-mt NSCLC treated with EGFR-TKIs after three follow-up CT examinations, although MAPEs should be mitigated in future works.

Keywords: differential model; time-variant tumor growth trajectory; tyrosine kinase inhibitors therapy



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1. Introduction

Lung cancer is a leading neoplasm, with new cases and deaths accounting for 11.4% and 18% of all cancers, respectively [1]. The 1-year overall survival rate for patients with stage IV is only 15–19% [2]. Tyrosine kinase inhibitors (TKIs) have been deployed to improve the overall survival rate for stage IV patients with epidermal growth factor receptor (EGFR)-mutated non-small cell lung cancer (EGFR-mt NSCLC), particularly among Asian patients [3]. In the FLAURA study, osimertinib, a third-generation TKI, prolonged the median overall survival by 6.8 months [4]. However, the emergence of resistant tumor cells in EGFR-mt NSCLC with EGFR mutations remains a challenge, even when treated with the third-generation TKI osimertinib [5]. Monitoring the dynamics of resistant tumor cells—whether they are decreasing, remaining stable, or increasing—would be valuable for adjusting treatment plans, such as considering alternative drugs or treatment modalities [6]. Enriquez-Navas et al. reported a novel strategy for adaptive therapy that maintains a stable population of sensitive cells that can suppress the proliferation of resistant cells by controlling tumor volume [7]. By monitoring the dynamics of resistant and sensitive tumor cells before or during treatment, mathematical models can assist physicians in the assessment of treatment efficacy or the decision of treatment strategies.

Modeling TGTs in EGFR-mt NSCLC patients treated with EGFR-TKI therapy after several follow-up computed tomography (FCT) examinations is crucial for TKI therapy during treatment, such as changing the regimens when the tumors turn into progressive disease. However, previous studies have not addressed the modeling of TGTs in EGFR-mt NSCLC patients treated with EGFR-TKI therapy after several FCT examinations.

We hypothesized that the TGTs of patients with stage IV EGFR-mt NSCLC for the three tumor cell populations (sensitive, persister, and resistant cells) could be expressed using differential models after several FCT examinations. This study aimed to propose differential models for TGTs of three tumor cell populations in EGFR-mt NSCLC tumors treated with EGFR-TKIs using the last pretreatment and three FCT examinations during TKI therapy. This study did not aim to identify a universal set of TGT model parameters applicable to general patients. Instead, the model parameters were individually optimized for each patient to understand the dynamics of the three cell populations within tumors. The validation strategy in this study is described as follows. The calibration in this study was used to determine parameter ranges of TGT models for calibration patients, whereas the validation aimed to evaluate the generalization of the parameter ranges of TGT models for validation patients. Mean absolute percentage errors (MAPEs) for validation patients were assessed for all CT examinations, as well as three FCT examinations that were not employed for model fitting. Akaike information criterion (AIC) was deployed for comparison between BP-based and Gompertz-based models.

2. Materials and Methods

The reference number of tumor cells was first computed from CT images at every available time point for each patient. We selected two differential equations—Bertalanffy–Pütter (BP) equation [8] and Gompertz [9]—to develop the TGT models. The parameters of the models were optimized using a dual annealing method [10]. We evaluated the MAPE and AIC using the reference numbers at each CT examination time point and the obtained numbers on the TGTs. Figure 1 illustrates the TGTs of three tumor cells and total tumor cells obtained from BP-based models for a real patient (Patient 146) with the reference numbers for all CT examinations.

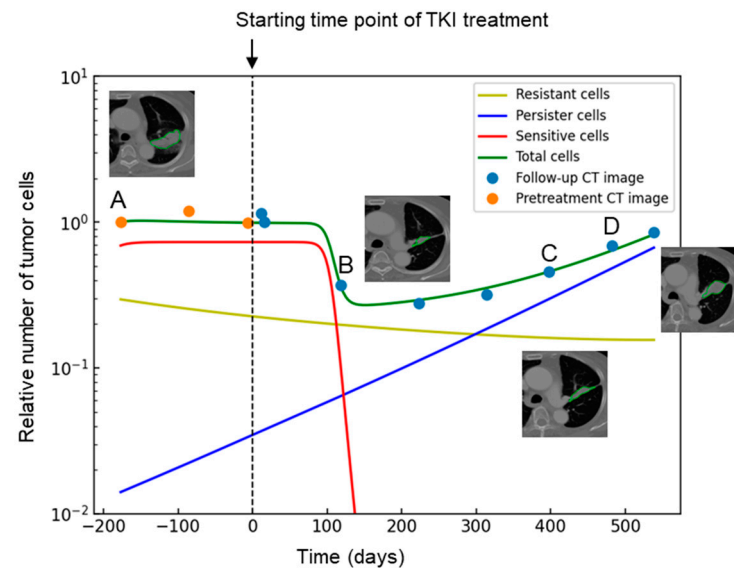


Figure 1. Time-variant tumor growth trajectories (TGTs) of three tumor cells and total tumor cells obtained from the Bertalanffy–Pütter-based models for a real patient (Patient 146) with the reference numbers for all CT examinations of validation patients. ‘A’ indicates the last pretreatment CT examination, and ‘B’, ‘C’, and ‘D’ indicate the first, second, and third follow-up CT examinations, respectively. The green contours in the inset CT images indicate the tumor regions of interest (ROI).

2.1. Patient Data

This retrospective study was performed with ethical approval from the Institutional Review Boards of Kyushu University Hospital. Given the retrospective study design, informed consent was obtained through an opt-out procedure, whereby patients were provided with clear information and the opportunity to withdraw their data from the study. All procedures were performed in accordance with the Declaration of Helsinki.

Sixteen patients with stage IV EGFR-mt NSCLC (adenocarcinoma) treated with osimertinib (Tagrisso), a third-generation EGFR-TKI drug, were selected from 167 patients at Kyushu University Hospital. The EGFR-TKI drug was administered to patients daily. Patients with conditions such as pneumonia, which made it difficult to clearly identify the tumor boundary, were excluded. The patient selection flowchart is summarized in Figure S1. Table S1 presents patient demographics and clinical characteristics for calibration and validation patients. Calibration patients were employed for determination of the parameter ranges of TGT models and validation patients were deployed for the evaluation of TGT models. Regarding EGFR mutations, 6 patients had exon 19 deletion mutations, and 10 patients had an L858R mutation caused by the substitution of leucine (the 858th amino acid in exon 21 of EGFR) with arginine. Two patients had 19 del/T790M and two had L858R/T790M. The duration of TKI treatment was 112–579 days (average, 341 days) between the first day of treatment and the day of disease progression. CT images for each patient were acquired from different scanners to increase operating efficiency (Philips, Brilliance 64, iCT 256, IQon—Spectral CT; TOSHIBA, Aquilion, Aquilion One, Aquilion Precision, Aquilion PRIME; GE Medical Systems, Discovery STE; SIEMENS, SOMATOM Definition AS, Biograph64, Biograph128, Symbia T6; Canon Medical Systems, Aquilion ONE). We delineated 110 regions of interest (ROI) of primary tumors on 24 pretreatment and 86 follow-up CT images from sixteen patients to calculate the reference total number of tumor cells. The interval between consecutive CT examinations ranged from 4 to 196 days (median, 56 days; mean $\pm$ SD, 58.5 $\pm$ 32.5 days) across all patients. Detailed CT examination dates for each patient are provided in Supplementary Table S2.

2.2. Reference Total Number of Tumor Cells Obtained from CT Images

A reference number for the total tumor cells in each tumor was calculated by multiplying the tumor volume by the tumor cell density. The tumor volume was calculated from the contours of the tumor region on the slices of a 3D CT image. The contours of the largest primary tumor volumes on pretreatment and follow-up CT images were delineated as the regions of interest (ROI) in a radiotherapy treatment planning system (Eclipse version 15.0, Varian Medical System Inc., Palo Alto, CA, USA) based on a consensus between a respiratory oncologist (K.T.) and a medical physicist (H.A.) with >6 years of experience. The two observers viewed and discussed all the CT images jointly until they reached an agreement on tumor contours. The tumor lesions on the CT images were viewed at a window width and level of 400 and 50, respectively. An isotropic binary images within an ROI (in-plane pixel size: 0.389–2.0 mm, slice thickness: 1.0–2.0 mm) were converted to binary images with an isovoxel size of $0.625 \times 0.625 \times 0.625 \text{ mm}^3$ using a shape-based interpolation method [11], in which tumor contours on intermediate slices between adjacent CT slices were estimated by performing the distance transform of the binary contours on each slice and linearly interpolating these distance fields, thereby generating isotropic voxels while preserving the 3D shape of the tumor contours. Finally, the total number of tumor cells N_{total} was calculated as follows:

$$N_{total} = n_V V d, \quad (1)$$

where n_V is the number of voxels in an ROI, V is the volume per voxel (cm^3), and d is the tumor cell density ($/\text{cm}^3$). Tumor volumes were calculated by counting the number of voxels within the delineated ROI after isotropic voxelization of the CT images, multiplied by the unit voxel volume. Validation of this volumetric calculation against an independent reference method (e.g., phantom-based or manual segmentation comparison) was not performed in this study. All 16 patients included in this study had pathologically confirmed adenocarcinoma. Therefore, the tumor cell density of $8.56 \times 10^7 / \text{cm}^3$ derived from adenocarcinoma bronchoscopy biopsy specimens [12] was considered applicable across all patients. The relative number of tumor cells was obtained by dividing the original number of tumor cells by that at the first time point for each patient, which was then employed to build the TGT models.

2.3. TGT Models

Grassberger et al. [9] developed mathematical models of time-variant tumor growth trajectories (TGTs) based on the Gompertz equation for three tumor cell populations: sensitive, persister, and resistant cells to EGFR-TKIs. The persister cell population may transition to resistant cells with a compound mutation probability due to genetic mutations [13,14]. These models can temporally quantify resistant and persister cell populations. K  hleitner et al. [15] reported that the BP equation [8] could approximate the TGTs of human breast cancer; however, they did not consider the three tumor cell populations. The BP equation is a generalized tumor growth model with four parameters, including two exponents related to tumor metabolism and morphology. However, there is no evidence on which equation is better for TGT models of three tumor cell populations. Therefore, we clarified the better equation for TGT models in this study.

2.3.1. Initial Values of TGT Models

Each lung cancer region consisted of three tumor cell populations—sensitive cells, persister, and resistant cells to TKIs—denoted by the subscripts S, P, and R, respectively.

Therefore, the total number $N_{total}(t)$ of tumor cells in a lung cancer region was calculated as the sum of the numbers of sensitive N_S , persister N_P , and resistant N_R cells, as follows:

$$N_{total}(t) = N_S(t) + N_P(t) + N_R(t). \quad (2)$$

We defined the initial number of tumor cells at the first pretreatment time t_{1st} for TKI treatment (time A in Figure 1) as

$$N_S(t_{1st}) = (1 - a - b)N_{total}(t_{1st}) \quad (3)$$

$$N_P(t_{1st}) = aN_{total}(t_{1st}) \quad (4)$$

and

$$N_R(t_{1st}) = bN_{total}(t_{1st}), \quad (5)$$

where a and b are the initial fractions of persister and resistant tumor cells, respectively.

2.3.2. BP-Based Models

In the BP differential equation, the growth in the number of tumor cells is deemed to be the remaining difference between anabolic and metabolic processes [16]. The BP differential equation for the number of tumor cells $N(t)$ at time t is defined as

$$\frac{dN(t)}{dt} = uN(t)^j - vN(t)^k, \quad (6)$$

where u and v are the increasing and decreasing rates of tumor cells [9], respectively, and j and k are the parameters related to anabolic and metabolic processes, respectively [16].

Given the flexibility offered by the two exponential parameters expressing tumor proliferation and the decline in response to TKIs, a model for the TGTs of sensitive cells was constructed by combining the BP differential equation with a cell loss model [17], which represents the decrease in tumor cells. The model for the TGTs of sensitive cells is expressed as

$$\frac{dN_S(t)}{dt} = u_S N_S(t)^j - v_S N_S(t)^k - \beta C(t) \left(u_S N_S(t)^j - v_S N_S(t)^k \right), \quad (7)$$

where $C_{daily}(t)$ is the daily dose concentration of TKIs at each time of t (each day) for TKI treatment, given by the following cell loss model:

$$C_{daily}(t) = C_{max} \exp\left(-\frac{\ln(2)}{T_{1/2}} t\right) = C_{max} \left(\frac{1}{2}\right)^{\frac{t}{T_{1/2}}} \quad (8)$$

Here, β is the TKI pharmacological effect parameter, C_{max} represents the maximum concentration at a steady state of the TKI, and $T_{1/2}$ represents the biological half-life of the TKI in vivo. Total dose concentration, administered from a starting time of 0 to a follow-up time of t , $C(t)$, can be defined as

$$C(t) = \sum_{k=0}^{\lfloor t \rfloor} C_{daily}(t-k) = C_{max} \left(\frac{1}{2}\right)^{\frac{t}{T_{1/2}}} \left[\frac{1 - 2^{\frac{1}{T_{1/2}}(\lfloor t \rfloor + 1)}}{1 - 2^{\frac{1}{T_{1/2}}}} \right] \quad (9)$$

The daily dosage, $T_{1/2}$, and C_{max} for osimertinib were 80 (mg/day), 2.03 (days), and 328 ($\mu\text{g/mL}$), respectively. Although these values vary among patients, they were assumed to be common across all patients in this study due to the unavailability of pharmacokinetic

data for each patient. We defined the models for TGTs based on the number of persister cells (N_P) and resistant cells (N_R) as follows:

$$\frac{dN_P(t)}{dt} = u_P N_P(t)^j - v_P N_P(t)^k - \mu \left(u_P N_P(t)^j - v_P N_P(t)^k \right) \quad (10)$$

and

$$\frac{dN_R(t)}{dt} = u_R N_R(t)^j - v_R N_R(t)^k + \mu \left(u_P N_P(t)^j - v_P N_P(t)^k \right), \quad (11)$$

where μ is the compound mutation probability of persister cells to resistant cells. The differential Equations (7), (10) and (11) were solved using the Runge–Kutta method [18] to drive the BP-based TGTs. Table S3 summarizes the parameter descriptions of BP-based models.

2.3.3. Gompertz-Based Models

The Gompertz-based models for the TGTs of three tumor cell populations [9] were defined as follows:

$$\frac{dN_S(t)}{dt} = \lambda_S N_S(t) \ln \left(\frac{K}{N_{total}(t)} \right) - \beta C(t) \lambda_S N_S(t) \ln \left(\frac{K}{N_{total}(t)} \right) \quad (12)$$

$$\frac{dN_P(t)}{dt} = \lambda_P N_P(t) \ln \left(\frac{K}{N_{total}(t)} \right) - \mu \lambda_P N_P(t) \ln \left(\frac{K}{N_{total}(t)} \right) \quad (13)$$

and

$$\frac{dN_R(t)}{dt} = \lambda_R N_R(t) \ln \left(\frac{K}{N_{total}(t)} \right) + \mu \lambda_P N_P(t) \ln \left(\frac{K}{N_{total}(t)} \right), \quad (14)$$

where λ_S , λ_P , and λ_R indicate the growth rates of sensitive, persister, and resistant tumor cells, and K is the carrying capacity, which is the maximum tumor volume depending on a given tumor environment (oxygen and nutrient supply through vascularization, metabolic process, and status of immune surveillance) [19]. The carrying capacity K was set according to a previous study [9] and the following procedure. To estimate the carrying capacity, it must be greater than relative number of $N_{total}(t)$ and scaled appropriately relative to the relative number of $N_{total}(t)$.

Differential Equations (12)–(14) were solved using the Runge–Kutta method to derive the Gompertz-based TGTs. Table S4 summarizes the parameter descriptions of Gompertz-based models.

2.4. Optimization of Parameters for Differential Models

Parameters for models were optimized under bound constraints, which were determined from the parameters of data for 80 patients, including synthetic patients and calibration patients. The TGT parameters were optimized with all FCT time points within randomly assigned parameter ranges and 72 synthetic patients were created from eight real calibration patients. The method for the creation of synthetic patient data is illustrated in Figure S2 with Document S.1 (Supplementary Materials S1). The bound constraints were determined as the mean $\pm$ 3 SD for the optimized parameters of 80 patients' data.

Twelve parameters ($u_S, v_S, u_P, v_P, u_R, v_R, \beta, \mu, a, b, j, k$) in BP-based models and 7 parameters ($\lambda_S, \lambda_P, \lambda_R, \beta, \mu, a, b$) in Gompertz-based models were automatically optimized using a dual annealing method [10]. The dual annealing method features a random exploration of neighboring “worse” candidates around extremum points to avoid being trapped at local extreme points, facilitating the discovery of global optimal solutions.

The MAPE between the reference numbers at all CT examination time points and the obtained numbers on the TGT of each patient at the corresponding time points was minimized as an objective function to optimize the parameters. The MAPE is explained

in Document S.2 (Supplementary Materials S1). The values of model parameters were generated randomly. The initial temperature, minimum temperature, and number of iterations for each case were 10,000, 0.0001, and 100, respectively.

2.5. Evaluation of TGT Models

Statistically significant differences in the optimized parameters of the BP-based and Gompertz-based models between real and synthetic patients were evaluated using the Wilcoxon rank-sum test to investigate whether the synthetic patient data were similar to the real patient data. *p*-values smaller than 0.05 were considered statistically significant.

We evaluated MAPE and AIC for the following comparisons: BP-based and Gompertz-based models, two calibration methods using all examinations and three FCT examinations for both models. The MAPE and AIC for three FCT examinations were calculated using CT examinations that were not employed for the two model fittings. The AIC is detailed in Documents S.3 (Supplementary Materials S1).

Regarding the parameter identifiability, we calculated the coefficient of variance (CV) (standard deviation divided by mean in percentage) and 95% confidence intervals (CIs) in the parameter distributions of 12 parameters. One hundred parameter sets were derived from the models for one real calibration patient's data plus nine synthetic patients' data by selecting 10 parameter sets with the top 10 minimum MAPEs for each patient.

Software specifications are summarized in Document S.4 (Supplementary Material S1). Iso-voxelization was performed using in-house programs in a MATLAB 2023a (The Math-Works Inc., Natick, MA, USA) environment. A python (v3.11) dual annealing package of "Dual annealing" (SciPy v1.15.1) was utilized to run the dual annealing. *p* value of Wilcoxon rank-sum test was calculated using the "scipy" package (SciPy v1.15.1) in python (v3.11). The pseudo-code of construction of TGT models is provided in Document S.5 (Supplementary Material S1).

3. Results

Figure S3 illustrates the TGTs of total tumor cells for nine synthetic patients' data, generated from a real patient (Patient 079) using a BP-based model. Figure S4 illustrates the TGTs of three cell types and total tumor cells for nine synthetic patients' data, generated from a real patient's data (Patient 079) using a Gompertz-based model.

Tables S5 and S6 summarize the optimized parameters of BP-based and Gompertz-based models with all CT examinations for calibration and validation patients. Tables S7 and S8 summarize the optimized parameters of BP-based and Gompertz-based models with the last pretreatment and the first three follow-up CT examinations for validation patients. Figures S5 and S6 present comparisons of the optimized parameters of TGT models using BP-based and Gompertz-based models, respectively, between 8 real calibration patients' data and 72 synthetic patients' data with *p*-values. There were no statistically significant differences in optimized parameters between calibration patients' and synthetic patients' data, excepting the compound mutation probability (μ) of BP models.

Figures S7 and S8 show the TGTs of three cell types and the total tumor cells obtained from BP-based and Gompertz-based models, respectively, with the reference numbers for all CT examinations of calibration patients. Figures S9 and S10 show the TGTs of three cell types and the total tumor cells obtained from BP-based and Gompertz-based models, respectively, with the reference numbers for all CT examinations of validation patients. Tables S9 and S10 indicate MAPE and AIC between the relative total numbers of tumor cells at all CT examinations and TGTs obtained with BP-based and Gompertz-based models, respectively, at the same time points for calibration and validation patients. The means of MAPE and AIC with BP-based models were 3.85% and 265, respectively, for calibration

patients, and 3.73% and 244, respectively, for validation patients. The means of MAPE and AIC with Gompertz-based models were 7.01% and 263, respectively, for calibration patients, and 3.43% and 228, respectively, for validation patients. There were no statistically significant differences in MAPE and AIC for validation patients between BP-based and Gompertz-based models (MAPE: $p = 0.63$, AIC: $p = 0.44$, Wilcoxon signed-rank test).

Figures 2 and 3 demonstrate the TGTs obtained with the last pretreatment and the three FCT examinations using the BP-based and Gompertz-based models, respectively, for the three tumor cell populations of four validation patients (Patients 142, 154, 157, and 159). Figures S11–S14 show the TGTs obtained with the last pretreatment and the three FCT examinations for the three tumor cell populations of calibration and validation patients other than the four patients in Figures 2 and 3.

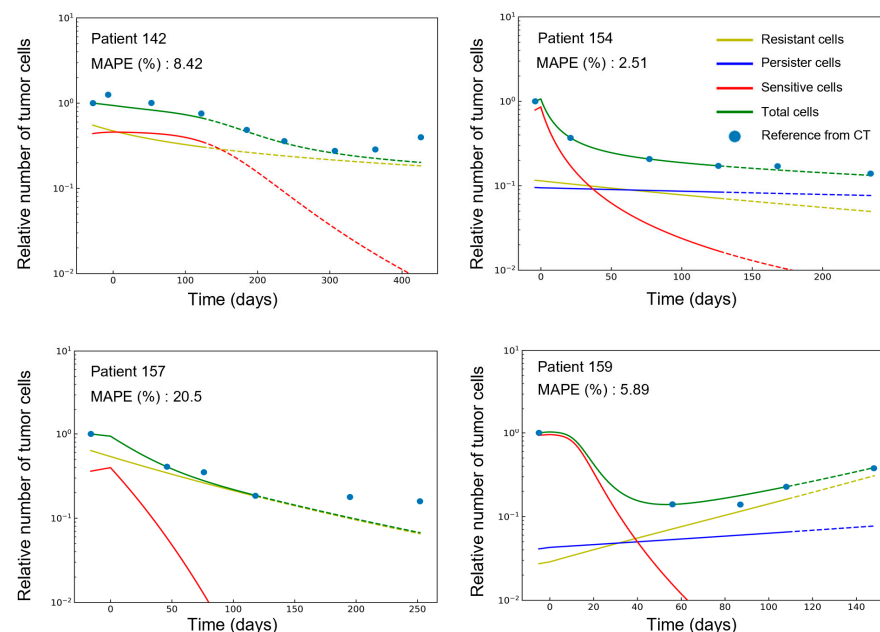


Figure 2. TGTs of three cell types and total tumor cells obtained from BP-based models with the reference numbers at the last pretreatment and the first three follow-up CT examinations of validation patients. Solid lines indicate the fitted trajectories over the last pretreatment and the first three follow-up CT examinations used for model fitting; dashed lines indicate the extrapolated trajectories beyond this fitting window, corresponding to the remaining follow-up CT examinations used for modeling evaluation.

Figure 4 and Table 1 present a comparison of MAPE and AIC values, computed between the relative total tumor cell numbers and the corresponding TGTs at three follow-up CT time points for both BP-based and Gompertz-based models. The two metrics for the three FCT examinations were calculated using CT examinations that were not employed for the two model fittings. Means $\pm$ standard deviations (SDs) of MAPE and AIC with the BP-based models for calibration patients were $40.3 \pm 22.0\%$ and 157 ± 73.4 , respectively, whereas those for the Gompertz-based models were $243.2 \pm 516.6\%$ and 152 ± 73.9 , respectively. For validation patients, means $\pm$ SDs of MAPE and AIC with the BP-based models were $36.1 \pm 40.2\%$ and 118 ± 52.9 , respectively, while those for the Gompertz-based models were $43.9 \pm 60.1\%$ and 109 ± 50.9 , respectively. There were no statistically significant differences in MAPE for calibration or validation patients between the two models ($p = 0.61$, Wilcoxon signed-rank test). For AIC, there was no statistically significant difference for calibration patients, whereas the Gompertz-based models showed a significantly lower AIC than the BP-based models for validation patients ($p = 0.016$, Wilcoxon signed-rank test). The median MAPes for BP-based and Gompertz-based models

were 36.9% and 26.4% for calibration patients, and 15.0% and 21.2% for validation patients, respectively. The median AICs for BP-based and Gompertz-based models were 160 and 158 for calibration patients, and 99 and 93 for validation patients, respectively. The higher mean values relative to the corresponding medians reflect the influence of a small number of patients with large errors.

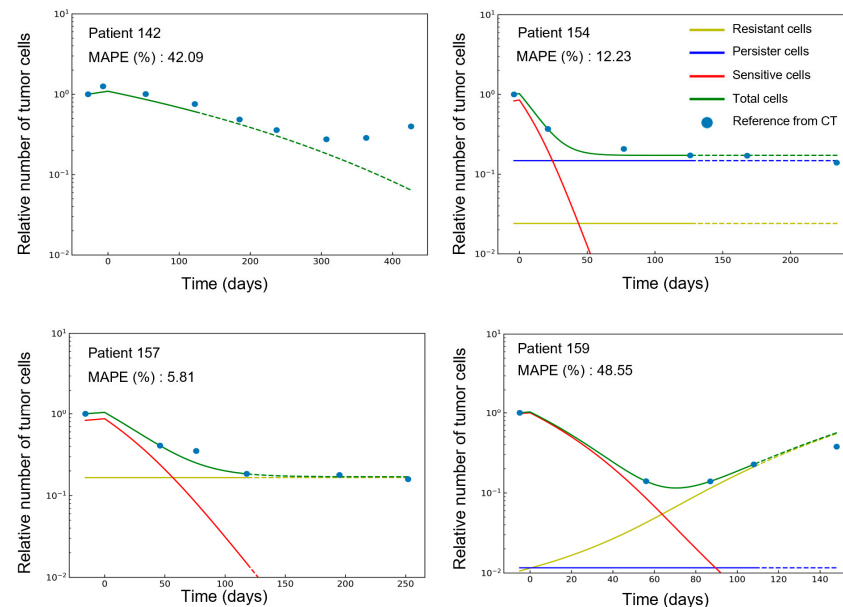


Figure 3. TGTs of three cell types and total tumor cells obtained from Gompertz-based models with the reference numbers at the last pretreatment and the first three follow-up CT examinations of validation patients. Solid lines indicate the fitted trajectories over the last pretreatment and the first three follow-up CT examinations used for model fitting; dashed lines indicate the extrapolated trajectories beyond this fitting window, corresponding to the remaining follow-up CT examinations used for modeling evaluation.

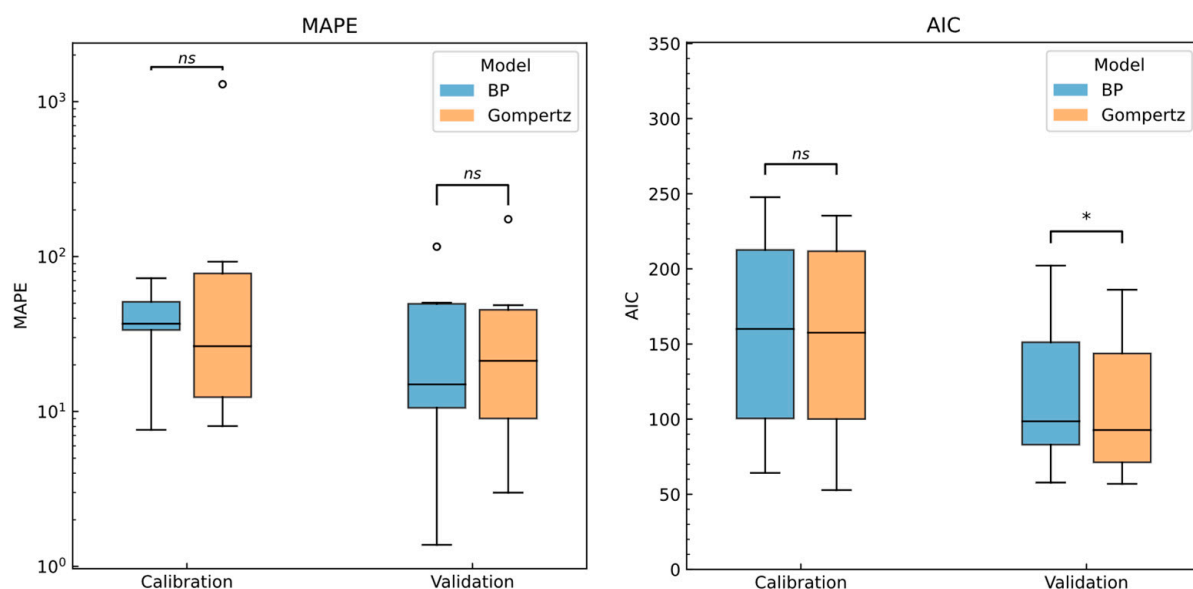


Figure 4. Mean absolute percentage error (MAPE) and Akaike information criteria (AIC) between the relative total number of tumor cells at three follow-up CT examinations and TGTs obtained with BP-based and Gompertz-based models at the same time points for calibration and validation patients.

Table 1. Mean absolute percentage error (MAPE) and Akaike information criteria (AIC) between the relative total number of tumor cells at three follow-up CT examinations and TGTs obtained with BP-based and Gompertz-based models at the same time points for calibration and validation patients. Patients marked with an asterisk * did not have more than three follow-up CT examinations.

		BP-Based Model		Gompertz-Based Model	
	Patient Number	MAPE (%)	AIC	MAPE	AIC
Calibration patients	Patient 079	37.3	248	8.05	227
	Patient 127	72.5	185	19.7	166
	Patient 128 *	-	-	-	-
	Patient 130	55.6	64	9.94	53
	Patient146	36.4	222	92.7	235
	Patient 149 *	-	-	-	-
	Patient 155	32.7	135	1296	149
	Patient 167	7.61	89	33.1	84
Mean		40.3 ± 22.0%	157 ± 73.4	243.2 ± 516.6%	152 ± 73.9
Median		36.9	160	26.4	158
Validation patients	Patient 002	116	68	174	61
	Patient 131 *	-	-	-	-
	Patient 142	15	168	42.1	168
	Patient 154	7.30	98	12.2	93
	Patient 157	50.4	99	5.81	82
	Patient 159	1.38	58	48.5	57
	Patient 161	48.7	202	21.2	186
	Patient 166	13.8	134	2.99	120
Mean		36.1 ± 40.2%	118 ± 52.9	43.9 ± 60.1%	109 ± 50.9
Median		15.0	99	21.2	93

The average CV of all estimated parameters for BP-based and Gompertz-based models was 66% and 98%, respectively (Supplementary Table S11). Supplementary Figures S16 and S17 describe the TGTs of BP-based and Gompertz models with 95% CIs. The average CIs of three cells and total populations for BP-based models were 0.73 (sensitive), 0.40 (persister), 0.52 (resistant), and 0.81 (total), respectively, and those for the Gompertz-based model were 0.38, 0.37, 0.45, and 0.43 (Supplementary Table S12).

4. Discussion

Using CT examinations excluded from model fitting, the MAPEs for BP-based and Gompertz-based models for the validation patients were $36.1 \pm 40.2\%$ and $43.9 \pm 60.1\%$, respectively, for three FCT examinations; the corresponding AICs were 118 ± 52.9 and 109 ± 50.9 , respectively. There was no statistically significant difference in MAPE between the two models ($p = 0.61$, Wilcoxon signed-rank test), whereas the Gompertz-based models showed a significantly lower AIC than the BP-based models ($p = 0.016$, Wilcoxon signed-rank test). The median MAPEs for BP-based and Gompertz-based models were 15.0% and 21.2%, respectively, indicating that the higher mean values reflect the influence of a small number of patients with large modeling errors, consistent with the skewed distributions expected under the non-parametric statistical framework applied in this study. These inter-patient differences in modeling error may be attributable to the variability in tumor growth dynamics under osimertinib. Both BP-based and Gompertz-based models demonstrated comparable potential to describe TGTs after three FCT examinations.

BP-based models can improve the interpretability of TKI-therapeutic outcomes by decomposing the total tumor cells into sensitive, persister, and resistant tumor cells. As

shown in Patients 142, 154, and 157 in Figure 2, the number of sensitive and resistant tumor cells could decrease over the treatment time. Therefore, the total number of tumor cells decreased. On the other hand, for Patient 159, since resistant tumor cells could increase the treatment time, the total number of tumor cells increased after decreasing due to TKI. The loss of the T790M mutation during TKI therapy might lead to the emergence of alternative resistance mechanisms, such as KRAS mutations, MET amplification, and small-cell transformation [20]. On the other hand, it would be hard to interpret the tendencies of three tumor cells in Figure 3 showing Gompertz-based models.

Figure S15 shows mean MAPE and AIC in TGTs obtained from BP-based and Gompertz models based on two and three FCT examinations. For the BP-based model, the mean $\pm$ SD of MAPE for two and three FCT examinations were $117.8 \pm 99.0\%$ and $40.3 \pm 22.0\%$ ($p = 0.094$, Wilcoxon signed-rank test), respectively, and those of AIC for two and three FCT examinations were 202 ± 72 and 157 ± 73 , respectively ($p = 0.031$, Wilcoxon signed-rank test). For the Gompertz-based model, the mean $\pm$ SD of MAPE were $592.6 \pm 928.0\%$ and $243.2 \pm 516.6\%$ for two and three FCT examinations, respectively ($p = 0.031$, Wilcoxon signed-rank test), with corresponding AICs of 205 ± 80 and 152 ± 74 ($p = 0.031$, Wilcoxon signed-rank test). Excepting the MAPEs of the BP-based model, all comparisons showed statistically significant improvements with three FCT examinations. Because both models with three FCT examinations showed better mean MAPEs and AICs as well as smaller SDs, we decided to use three FCT examinations for the evaluation of the models.

The two models did not differ significantly in MAPE, whereas the Gompertz-based model showed a significantly lower AIC, indicating that both models retain potential utility for obtaining TGTs following three FCT examinations. This finding was consistent with, and extends on, prior work on TGT modeling. Grassberger et al. [9] proposed a three-compartment Gompertz-based model to estimate pre-existing and acquired resistance in EGFR-mutant NSCLC patients treated with erlotinib, finding that combining pre-existing resistance with persister-cell evolution was necessary to explain the observed tumor trajectories, with an estimated pre-existing resistant fraction of 10^{-4} to 10^{-1} at treatment initiation. The present study builds on this framework by introducing the BP-based model as an alternative formulation and by restricting model fitting to the last pretreatment scan and the first three FCT examinations, reflecting a clinically realistic scenario in which modeling is required before disease progression is observed, in contrast to Grassberger et al., who fitted models using the full pre- and post-progression trajectory. Other mathematical approaches to predicting tumor response in NSCLC have relied on different modeling strategies and yielded correspondingly different findings. Geng et al. [17] combined a Gompertz growth model with a linear-quadratic radiation response model to predict survival outcomes for chemoradiation in NSCLC, with model parameters estimated from population-level clinical trial databases (SEER, RTOG) rather than from individual patient CT trajectories; this approach predicted improvements in overall survival from concurrent versus sequential chemoradiation that closely matched observed trial outcomes. Sunassee et al. [21] instead used a proliferation saturation index (PSI), a single patient-specific parameter representing the pretreatment tumor volume-to-carrying-capacity ratio, within an adaptive Bayesian framework, and found that daily CT scans acquired during the first two weeks of radiotherapy could predict response to the remaining treatment course with high accuracy (c-index = 0.89). Compared with these single-parameter or population-calibrated approaches, the present study estimates a higher-dimensional, patient-specific parameter set to resolve the dynamics of three biologically distinct cell populations, trading off model parsimony for mechanistic interpretability of treatment response at the level of sensitive, persister, and resistant cell subpopulations.

This study had some limitations. First, the number of internal cases included in this study was limited by the limited number of CT examinations available per patient, raising concerns regarding the statistical reliability of the model comparisons. Thus, more cases with various mutation subtypes should be collected to evaluate the robustness of these models. Furthermore, external validation is required using several external independent datasets to investigate the generalizability and clinical applicability of the differential models in future works.

Second, when calculating the number of tumor cells from the CT images, the same tumor cell density was applied to all the patients. To change the cell density for each patient, a method should be developed to estimate tumor cell density from medical images, such as the association of cell density with the diffusion coefficient calculated from diffusion-weighted magnetic resonance images [22].

Third, the identifiability of model parameters and direct biological validation of the three estimated cell populations (sensitive, persister, and resistant) were substantially important for this study. BP-based and Gompertz-based models indicated a large average CV for all estimated parameters. In addition, the average CIs of three cells and total populations for both models were larger than 0.3. Consequently, since those results indicated a large CV and large CIs for those models, we should improve the parameter identifiability for the models. To address this parameter uncertainty, practical identifiability analysis based on the profile likelihood or Bayesian inference, which explicitly account for the unevenly sampled and censored CT examinations available per patient, could be applied in future studies [23]. In addition, a hierarchical (population) modeling framework that exploits information across patients and structural correlations among model parameters to reduce parameter dimensionality may further improve parameter identifiability [24].

Fourth, although sensitive and resistant cell populations can be directly measured through molecular profiling and resistance-associated genomic alterations [25], persister cell populations cannot yet be directly quantified in patients because no universally accepted biomarker exists [26,27]. Therefore, the persister cell quantity estimated by the proposed models could be needed in an investigation of persister dynamics.

Fifth, osimertinib pharmacokinetic parameters ($T_{1/2}$ and C_{max}) were assumed to be common across all patients based on institutionally measured values due to the unavailability of pharmacokinetic data of each patient. Population pharmacokinetic studies of osimertinib in Japanese patients have reported inter-individual variability in exposure [28]; incorporating patient-specific pharmacokinetics may further improve the accuracy of TGT modeling in future studies.

Sixth, tumor contours were determined through consensus between two observers without independent, blinded delineation; therefore, the inter-observer variability of the ROI contours was not quantitatively assessed in this study.

Finally, the models can be improved by incorporating more data. We assumed that persister tumor cells could be mutated to resistant cells in the proposed models, but we can also assume resistant tumor cells are converted into sensitive cells [29,30] when revising the models in the future.

5. Conclusions

This study suggests that the proposed BP-based and Gompertz-based differential models could have potential to describe TGTs in patients with stage IV EGFR-mt NSCLC treated with EGFR-TKIs after three follow-up CT examinations, although MAPEs should be mitigated. Other limitations should be resolved regarding the model construction, including independent inter-patient validation, in future works.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app16157702/s1>, Figure S1: Selection flowchart of patients with stage IV EGFR-mt NSCLC at Kyushu University Hospital for patient data allocation of calibration and validation; Figure S2: Generation of a synthetic patient datum from an interpolated patient datum between two real patient data; Figure S3: Time-variant tumor growth trajectories of total tumor cells for 9 synthetic patient data generated from a real patient data (Patient 079) using BP-based models; Figure S4: Tumor growth trajectories of three cell types and total tumor cells for 9 synthetic patient data generated from a real patient data (Patient 079) using Gompertz-based models; Figure S5: Comparisons of optimized parameters of TGTs using BP model between 8 real calibration patients and 72 synthetic patient data with p -values; Figure S6: Comparisons of optimized parameters of TGTs using Gompertz model between 8 real calibration patients and 72 synthetic patient data with p -values; Figure S7: TGTs of three cell types and total tumor cells obtained from BP-based models with the reference numbers at all CT examinations for calibration patients; Figure S8: TGTs of three cell types and total tumor cells obtained from Gompertz-based models with the reference numbers at all CT examinations for calibration patients; Figure S9: TGTs of three cell types and total tumor cells obtained from BP-based models with the reference numbers at all CT examinations of validation patients; Figure S10: TGTs of three cell types and total tumor cells obtained from Gompertz-based models with the reference numbers at all CT examinations of validation patients; Figure S11: TGTs of three and total tumor cells obtained from BP-based models with the reference numbers at the last pretreatment and the first three follow-up CT examinations of calibration patients; Figure S12: TGTs of three and total tumor cells obtained from BP-based models with the reference numbers at the last pretreatment and the first three follow-up CT examinations of validation patients; Figure S13: TGTs of three and total tumor cells obtained from Gompertz-based models with the reference numbers at the last pretreatment and the first three follow-up CT examinations of calibration patients; Figure S14: TGTs of three and total tumor cells obtained from Gompertz-based models with the reference numbers at the last pretreatment and the first three follow-up CT examinations of validation patients; Figure S15: Mean values of MAPE and AIC in TGTs with BP-based and Gompertz-based model using the first two and three follow-up CT images of calibration patients; Figure S16: TGTs with 95% confidence intervals (shaded colors) for three and total tumor cells obtained from BP-based models with the reference numbers at the last pretreatment and all follow-up CT examinations of calibration patients; Figure S17: TGTs with 95% confidence intervals (shaded colors) for three and total tumor cells obtained from Gompertz-based models with the reference numbers at the last pretreatment and all follow-up CT examinations of calibration patients; Table S1: Patient demographics and clinical characteristics for calibration and validation patients; Table S2: Time points in days at pretreatment and follow-up CT examinations for all patients; Table S3: Parameter descriptions of BP-based models; Table S4: Parameter descriptions of Gompertz-based models; Table S5: Optimized parameters of BP-based models with all CT examinations; Table S6: Optimized parameters of Gompertz-based models with all CT examinations; Table S7: Optimized parameters of BP-based models with the last pretreatment and the first three follow-up CT examinations; Table S8: Optimized parameters of Gompertz-based models with the last pretreatment and the first three follow-up CT examinations; Table S9: Mean absolute percentage error (MAPE) and Akaike information criterion (AIC) between the relative total numbers of tumor cells at all CT examinations and TGTs obtained with BP-based models at the same time points for calibration and validation patients; Table S10: Mean absolute percentage error (MAPE) and Akaike information criteria (AIC) between the relative total number of tumor cells at all CT examinations and TGTs obtained with Gompertz-based models at the same time points for calibration and validation patients; Table S11: Coefficient of variance (CV) in parameter distributions of model parameters; Table S12: 95% confidence intervals (CIs) of three cell and total populations for BP-based and Gompertz-based models. Document S.1: Production of synthetic patient data. Document S.2: Mean absolute percentage error (MAPE). Document S.3: Akaike information criteria (AIC). Document S.4: Software specifications. Document S.5: Pseudo code of construction of tumor growth trajectory (TGT) model.

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References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [[CrossRef](#)] [[PubMed](#)]
2. Blandin Knight, S.; Crosbie, P.A.; Balata, H.; Chudziak, J.; Hussell, T.; Dive, C. Progress and prospects of early detection in lung cancer. *Open Biol.* **2017**, *7*, 170070. [[CrossRef](#)]
3. Soria, J.C.; Ohe, Y.; Vansteenkiste, J.; Reungwetwattana, T.; Chewaskulyong, B.; Lee, K.H.; Dechaphunkul, A.; Imamura, F.; Nogami, N.; Kurata, T.; et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N. Engl. J. Med.* **2018**, *378*, 113–125. [[CrossRef](#)] [[PubMed](#)]
4. Vaid, A.K.; Gupta, A.; Momi, G. Overall survival in stage IV EGFR mutation positive NSCLC: Comparing first, second and third generation EGFR TKIs. *Int. J. Oncol.* **2021**, *58*, 171–184. [[CrossRef](#)] [[PubMed](#)]
5. Nishihara, S.; Yamaoka, T.; Ishikawa, F.; Ohmori, T.; Ando, K.; Kusumoto, S.; Kishino, Y.; Manabe, R.; Hasebe, Y.; Sagara, H.; et al. Diverse mechanisms of resistance against osimertinib in lung adenocarcinoma cells with an EGFR-activating mutation. *Cells* **2022**, *11*, 2201. [[CrossRef](#)] [[PubMed](#)]
6. Suzuki, M.; Uchibori, K.; Oh-Hara, T.; Nomura, Y.; Suzuki, R.; Takemoto, A.; Araki, M.; Matsumoto, S.; Sagae, Y.; Kukimoto-Niino, M.; et al. A macrocyclic kinase inhibitor overcomes triple resistant mutations in EGFR-positive lung cancer. *npj Precis. Oncol.* **2024**, *8*, 46. [[CrossRef](#)] [[PubMed](#)]
7. Enriquez-Navas, P.M.; Kam, Y.; Das, T.; Hassan, S.; Silva, A.; Foroutan, P.; Ruiz, E.; Martinez, G.; Minton, S.; Gillies, R.J.; et al. Exploiting evolutionary principles to prolong tumor control in preclinical models of breast cancer. *Sci. Transl. Med.* **2016**, *8*, 327ra24. [[CrossRef](#)] [[PubMed](#)]
8. Richards, F.J. A flexible growth function for empirical use. *J. Exp. Bot.* **1959**, *10*, 290–301. [[CrossRef](#)]
9. Grassberger, C.; McClatchy, D.; Geng, C.; Kamran, S.C.; Fintelmann, F.; Maruvka, Y.E.; Piotrowska, Z.; Willers, H.; Sequist, L.V.; Hata, A.N.; et al. Patient-specific tumor growth trajectories determine persistent and resistant cancer cell populations during treatment with targeted therapies. *Cancer Res.* **2019**, *79*, 3776–3788. [[CrossRef](#)] [[PubMed](#)]
10. Xiang, Y.; Sun, D.; Fan, W.; Gong, X. Generalized simulated annealing algorithm and its application to the Thomson model. *Phys. Lett. A* **1997**, *233*, 216–220. [[CrossRef](#)]
11. Herman, G.T.; Zheng, J.; Bucholtz, C.A. Shape-based interpolation. *IEEE Comput. Graph. Appl.* **1992**, *12*, 69–79. [[CrossRef](#)]
12. Katsurada, N.; Tachihara, M.; Jimbo, N.; Yamamoto, M.; Yoshioka, J.; Mimura, C.; Satoh, H.; Furukawa, K.; Otoshi, T.; Kiriu, T.; et al. Yield of tumor samples with a large guide-sheath in endobronchial ultrasound transbronchial biopsy for non-small cell lung cancer. *PLoS ONE* **2021**, *16*, e0259236. [[CrossRef](#)] [[PubMed](#)]
13. Sharma, S.V.; Lee, D.Y.; Li, B.; Quinlan, M.P.; Takahashi, F.; Maheswaran, S.; McDermott, U.; Azizian, N.; Zou, L.; Fischbach, M.A.; et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* **2010**, *141*, 69–80. [[CrossRef](#)] [[PubMed](#)]
14. Lin, J.J.; Shaw, A.T. Resisting resistance: Targeted therapies in lung cancer. *Trends Cancer* **2016**, *2*, 350–364. [[CrossRef](#)] [[PubMed](#)]
15. Kühleitner, M.; Brunner, N.; Nowak, W.G.; Renner-Martin, K.; Scheicher, K. Best fitting tumor growth models of the von Bertalanffy-Pütter type. *BMC Cancer* **2019**, *19*, 683. [[CrossRef](#)] [[PubMed](#)]
16. Taylor, C.C. Growth equations with metabolic parameters. *J. Cons. Int. Explor. Mer.* **1962**, *27*, 270–286. [[CrossRef](#)]
17. Geng, C.; Paganetti, H.; Grassberger, C. Prediction of treatment response for combined chemo- and radiation therapy for non-small cell lung cancer patients using a bio-mathematical model. *Sci. Rep.* **2017**, *7*, 13542. [[CrossRef](#)] [[PubMed](#)]
18. Hairer, E.; Lubich, C.; Roche, M. The Numerical Solution of Differential-Algebraic Systems by Runge-Kutta Methods. In *Lecture Notes in Mathematics*, Vol. 1409; Springer: Berlin/Heidelberg, Germany, 1989. [[CrossRef](#)]

19. Prokopiou, S.; Moros, E.G.; Poleszczuk, J.; Caudell, J.; Torres-Roca, J.F.; Latifi, K.; Lee, J.K.; Myerson, R.; Harrison, L.B.; Enderling, H. A proliferation saturation index to predict radiation response and personalize radiotherapy fractionation. *Radiat. Oncol.* **2015**, *10*, 159. [[CrossRef](#)] [[PubMed](#)]
20. Leonetti, A.; Sharma, S.; Minari, R.; Perego, P.; Giovannetti, E.; Tiseo, M. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. *Br. J. Cancer* **2019**, *121*, 725–737. [[CrossRef](#)] [[PubMed](#)]
21. Sunassee, E.D.; Tan, D.; Ji, N.; Brady, R.; Moros, E.G.; Caudell, J.J.; Yartsev, S.; Enderling, H. Proliferation saturation index in an adaptive Bayesian approach to predict patient-specific radiotherapy responses. *Int. J. Radiat. Biol.* **2019**, *95*, 1421–1426. [[CrossRef](#)] [[PubMed](#)]
22. Yin, Y.; Sedlacek, O.; Muller, B.; Warth, A.; Gonzalez-Vallinas, M.; Lahrmann, B.; Grabe, N.; Kauczor, H.-U.; Breuhahn, K.; Vignon-Clementel, I.E.; et al. Tumor cell load and heterogeneity estimation from diffusion-weighted MRI calibrated with histological data: An example from lung cancer. *IEEE Trans. Med. Imaging* **2018**, *37*, 35–46. [[CrossRef](#)] [[PubMed](#)]
23. Porthiyas, J.; Nussey, D.; Beauchemin, C.A.A.; Warren, D.C.; Quirouette, C.; Wilkie, K.P. Practical parameter identifiability and handling of censored data with Bayesian inference in mathematical tumour models. *npj Syst. Biol. Appl.* **2024**, *10*, 89. [[CrossRef](#)] [[PubMed](#)]
24. Vaghi, C.; Rodallec, A.; Fanciullino, R.; Ciccolini, J.; Mochel, J.P.; Mastri, M.; Poignard, C.; Ebos, J.M.L.; Benzekry, S. Population modeling of tumor growth curves and the reduced Gompertz model improve prediction of the age of experimental tumors. *PLoS Comput. Biol.* **2020**, *16*, e1007178. [[CrossRef](#)] [[PubMed](#)]
25. Parikh, A.R.; Leshchiner, I.; Elagina, L.; Goyal, L.; Levovitz, C.; Siravegna, G.; Livitz, D.; Rhrissorakrai, K.; Martin, E.E.; Van Seventer, E.E.; et al. Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers. *Nat. Med.* **2019**, *25*, 1415–1421. [[CrossRef](#)] [[PubMed](#)]
26. Pu, Y.; Li, L.; Peng, H.; Liu, L.; Heymann, D.; Robert, C.; Vallette, F.; Shen, S. Drug-tolerant persister cells in cancer: The cutting edges and future directions. *Nat. Rev. Clin. Oncol.* **2023**, *20*, 799–813. [[CrossRef](#)] [[PubMed](#)]
27. Russo, M.; Chen, M.; Mariella, E.; Peng, H.; Rehman, S.K.; Sancho, E.; Sogari, A.; Toh, T.S.; Balaban, N.Q.; Battle, E.; et al. Cancer drug-tolerant persister cells: From biological questions to clinical opportunities. *Nat. Rev. Cancer* **2024**, *24*, 694–717. [[CrossRef](#)] [[PubMed](#)]
28. Ishikawa, E.; Yokoyama, Y.; Chishima, H.; Kasai, H.; Kuniyoshi, O.; Kimura, M.; Hakamata, J.; Nakada, H.; Suehiro, N.; Nakaya, N.; et al. Population pharmacokinetics, pharmacogenomics, and adverse events of osimertinib and its two active metabolites, AZ5104 and AZ7550, in Japanese patients with advanced non-small cell lung cancer: A prospective observational study. *Investig. New Drugs* **2023**, *41*, 122–133. [[CrossRef](#)] [[PubMed](#)]
29. Song, X.; Lan, Y.; Zheng, X.; Zhu, Q.; Liao, X.; Liu, K.; Zhang, W.; Peng, Q.; Zhu, Y.; Zhao, L.; et al. Targeting drug-tolerant cells: A promising strategy for overcoming acquired drug resistance in cancer cells. *MedComm* **2023**, *4*, e342. [[CrossRef](#)] [[PubMed](#)]
30. Shlyakhtina, Y.; Moran, K.L.; Portal, M.M. Genetic and non-genetic mechanisms underlying cancer evolution. *Cancers* **2021**, *13*, 1380. [[CrossRef](#)] [[PubMed](#)]

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