Dissecting sources of variability in patient response to targeted therapy: anti-HER2 therapies as a case study

Timothy Qi^1 and Yanguang Cao^1

¹The University of North Carolina at Chapel Hill

October 6, 2022

Abstract

Background and Purpose: Despite their use to treat cancers with specific genetic aberrations, targeted therapies elicit heterogeneous responses. Sources of variability are critical to targeted therapy drug development, yet there exists no method to discern their relative contribution to response heterogeneity. Experimental Approach: We use HER2-amplified breast cancer and two agents, neratinib and lapatinib, to develop a platform for dissecting sources of variability in patient response. The platform comprises four components: pharmacokinetics, tumor burden and growth kinetics, clonal composition, and sensitivity to treatment. Pharmacokinetics are simulated using population models to capture variable systemic exposure. Tumor burden and growth kinetics are derived from clinical data comprising over 800,000 women. The fraction of sensitive and resistant tumor cells is informed by HER2 immunohistochemistry. Growth rate-corrected drug potency is used to predict response. We integrate these factors and simulate clinical outcomes for virtual patients. The relative contribution of these factors to response heterogeneity are compared. Key Results: The platform was verified with clinical data, including response rate and progressionfree survival (PFS). For both neratinib and lapatinib, the growth rate of resistant clones influenced PFS to a higher degree than systemic drug exposure. Variability in exposure at labeled doses did not significantly influence response. Drug potency strongly influenced responses to neratinib. Variability in patient HER2 immunohistochemistry scores influenced responses to lapatinib. Exploratory twice daily dosing improved PFS for neratinib but not lapatinib. Conclusion and Implications: The platform can dissect sources of variability in response to target therapy, which may facilitate decision-making during drug development.

- 1 TITLE
- 2 Dissecting sources of variability in patient response to targeted therapy: anti-HER2 therapies as
- 3 a case study

5 RUNNING TITLE

- 6 Dissecting sources of variability in response to targeted therapy

8 AUTHORS

- 9 Timothy Qi, B.S.,¹ and Yanguang Cao, Ph.D.^{1,2*}

11 AFFILIATIONS

- ¹Division of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy,
- 13 The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA
- ¹⁴ ²Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill,
- 15 Chapel Hill, NC 27599, USA

38 DATA AVAILABILITY STATEMENT

- 39 The data that support the findings of this study will be made available from the corresponding
- 40 author upon reasonable request.

42 WORD COUNT (excluding Methods): 2926

- 44 *Lead Contact. Email: yanguang@unc.edu. Address: 301 Pharmacy Ln, Chapel Hill, NC 27599

45 DECLARATIONS OF INTEREST

- 46 T.Q. is a contractor for Hatteras Venture Partners.
- 47
- 48

49 KEYWORDS

- 50 HER2, tyrosine kinase inhibitor, breast cancer, free drug hypothesis, population
- 51 pharmacokinetics, tumor evolution, tumor growth modeling, growth rate metrics
- 52

- 54 ABBREVIATIONS
- 55 AUC, area under the curve
- 56 BID, twice daily
- 57 BOR, best overall response
- 58 CDK, cyclin-dependent kinase
- 59 DNTP, death or non-target progression
- 60 FDA, Food & Drug Administration
- 61 FISH, fluorescence in situ hybridization
- 62 GEC₅₀, concentration at half-maximal growth rate inhibition effect
- 63 GR, growth rate inhibition [metrics]
- 64 GR_{inf}, growth rate inhibition at infinite drug concentration
- 65 HER, human epidermal growth factor receptor
- 66 HMS LINCS, Harvard Medical School Library of Network-based Cell Signatures
- 67 IHC, immunohistochemistry
- 68 ORR, objective response rate
- 69 PD, pharmacodynamics
- 70 PFS, progression-free survival
- 71 PI3Kα, phosphoinositide 3-kinase alpha
- 72 PK, pharmacokinetics
- 73 PPB, plasma protein bound
- 74 QD, once daily
- 75 TKI, tyrosine kinase inhibitor
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89 ABSTRACT

90 Background and Purpose: Despite their use to treat cancers with specific genetic aberrations,

91 targeted therapies elicit heterogeneous responses. Sources of variability are critical to targeted

- 92 therapy drug development, yet there exists no method to discern their relative contribution to 93 response heterogeneity.

Experimental Approach: We use HER2-amplified breast cancer and two agents, neratinib and lapatinib, to develop a platform for dissecting sources of variability in patient response. The platform comprises four components: pharmacokinetics, tumor burden and growth kinetics, clonal composition, and sensitivity to treatment. Pharmacokinetics are simulated using population models to capture variable systemic exposure. Tumor burden and growth kinetics are derived from clinical data comprising over 800,000 women. The fraction of sensitive and resistant tumor cells is informed by HER2 immunohistochemistry. Growth rate-corrected drug potency is used to predict response. We integrate these factors and simulate clinical outcomes for virtual patients. The relative contribution of these factors to response heterogeneity are compared. Key Results: The platform was verified with clinical data, including response rate and progression-free survival (PFS). For both neratinib and lapatinib, the growth rate of resistant clones influenced PFS to a higher degree than systemic drug exposure. Variability in exposure at labeled doses did not significantly influence response. Drug potency strongly influenced responses to neratinib. Variability in patient HER2 immunohistochemistry scores influenced responses to lapatinib. Exploratory twice daily dosing improved PFS for neratinib but not lapatinib. **Conclusion and Implications:** The platform can dissect sources of variability in response to target therapy, which may facilitate decision-making during drug development.

133 INTRODUCTION

Patient response to targeted therapy is highly variable and difficult to predict. Multiple 134 135 factors contribute to diverse patient responses to targeted therapy, including but not limited to drug pharmacokinetics (PK) and biodistribution [1], tumor growth characteristics [2], clonal 136 composition [3], and tumor sensitivity or resistance [4]. Understanding the source of response 137 138 variability can be valuable for decisions made during drug development concerning dose selection, patient stratification, and therapeutic benefit evaluation. However, some of these 139 factors are challenging to characterize during clinical studies. For example, it is implausible to 140 141 measure tumor growth rates without treatment in a metastatic disease setting. As a result, the 142 relative contribution of these factors to discrepancies in drug efficacy within diverse patient populations remains largely undefined. Simulations informed by high volumes of historical 143 144 clinical data and drug potency parameters measured during early-stage drug discovery may be convenient and intuitive tools for dissecting sources of variability in patient response and 145 146 informing decision-making during drug development.

147 Here, we use HER2-amplified metastatic breast cancer as a model system to decompose sources of variability in response to HER2 tyrosine kinase inhibitors (TKI). Given the routine 148 clinical assessment of HER2 amplification in patients with newly diagnosed metastatic breast 149 150 cancer, we assessed whether a pharmacokinetics/pharmacodynamics/tumor growth (PK/PD/TG)-based model could be applied to predict the efficacy of HER2 TKIs in patients with 151 different levels of HER2 amplification. Such a model might also be useful in determining 152 153 whether variability in response to approved HER2 TKIs depend on tumor-intrinsic features, like growth rate and HER2+ fraction, or drug-specific properties, like PK and biodistribution. 154

Toward this end, we have developed a PK/PD/TG model for decomposing sources of variability between responders and non-responders. We simulate the highly variable PK of two HER2 TKIs, neratinib and lapatinib, and their therapeutic effects on tumor growth and progression. We also model a wide range of tumor sensitivity to therapy as informed by drug potency in cell lines with varying HER2 expression and population-level diversity in HER2 immunohistochemistry (IHC) scores. These data are integrated into a virtual patient population to dissect the sources of variability in patient response.

162 There are two key aspects of our approach. One is the use of population PK models to simulate drug PK and variability in patients. Population PK models and parameter estimates for 163 investigational and approved drugs are usually readily available to sponsors. Using the drug 164 sponsors' population PK models ensures our simulations recapitulate a wide range of possible 165 drug exposures. This is of critical importance, as drug exposure can vary dramatically between 166 patients. Second, we explored the therapeutic effect in a tumor comprising mixed 167 168 subpopulations of sensitive (HER2-amplified) and resistant (HER2-negative) cells. The clonal 169 composition of these cells in a given tumor was informed by patient HER2 IHC scores. We considered subpopulation-specific cytostatic and cytotoxic effects using the growth rate 170 inhibition metrics developed by Hafner et al. [4]. These metrics correct for differences in growth 171 rates among cell lines, which can otherwise confound interpretations of drug potency. Recent 172 173 publications showcase the added value of using growth rate metrics for predicting in vivo 174 responses from in vitro data alone [5] and support the further elaboration of this approach for 175 drug development. 176

177 METHODS

178 Modeling framework overview

The framework is shown in Fig. 1A. First, we used population PK models to derive drug exposure in patients. Next, we constructed a virtual HER2-positive patient population from large clinical datasets of HER2 IHC and tumor growth kinetics. We then used the in vitro GR metrics to determine sensitivity and resistance of tumor cellular subpopulations to treatment. Finally, we integrated these sources of variability to model the response of tumors with mixed populations to HER2 TKI treatment.

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186 Variability in systemic drug exposure

For neratinib, a population PK model was used to simulate plasma concentration vs.
 time profiles [6]. Among all significant covariates, we included the effects of normally
 distributed age on k_a and central volume, as well as the effects of lognormally distributed
 weight on clearance and central volume. No transporter-mediated uptake into cells was

191 reported. We also assumed that patients would take the drug with a standard, low-fat

192 breakfast under real-world conditions.

For lapatinib, a structurally similar population PK model was used, the only difference being that drug absorption was modeled as a T_{lag} -delayed zero-order input followed by a firstorder input at the rate k_a [7]. We included the effects of normally distributed age on k_a and excluded the effects of ethnicity and race on other parameters. No transporter-mediated uptake into cells was reported. We also assumed that patients would take the drug with a standard, low-fat breakfast under real-world conditions.

Free drug concentrations were simulated to inform tumor concentrations based on the
Free Drug Hypothesis [8]. Prior to PK/PD simulation, we used the following equation to
estimate the average unbound steady-state concentrations of alpelisib (PI3Kα), abemaciclib
(CDK4/6), palbociclib (CDK4/6), ribociclib (CDK4/6), lapatinib (HER1/2), and neratinib
(HER1/2/4):

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$$C_{ss,free} = \frac{X/\tau}{CL/F} \cdot (1 - PPB)$$

207 Where X is the approved dose, τ is the approved dosing interval, and CL/F is the 208 apparent clearance upon oral administration. PPB is the protein-bound fraction reported in publicly available US Food & Drug Administration (FDA) multidisciplinary review documents for 209 210 each drug; the exception was neratinib, where the PPB in plasma samples from healthy 211 volunteers was reported to be significantly lower than the in vitro PPB. For lapatinib, the 212 reported PPB of "> 99%" was estimated as 99.5%. The following values were used for each 213 drug: abemaciclib (X = 200 mg, τ = 12 hours, CL/F = 38 L/hour, PPB = 96.3%), alpelisib (X = 300 214 mg, τ = 24 hours, CL/F = 9 L/hour, PPB = 89.2%), lapatinib (X = 1250 mg, τ = 24 hours, CL/F = 114 L/hour, PPB = 99.5%), neratinib (X = 240 mg, τ = 24 hours, CL/F = 195 L/hour, in vitro PPB = 99%, 215 in vivo PPB = 88%), palbociclib (X = 125 mg, τ = 24 hours, CL/F = 81 L/hour, PPB = 85%), and 216 ribociclib (X = 600 mg, τ = 24 hours, CL/F = 26 L/hour, PPB = 70%). As it is generally thought that 217 218 only free drug is available to interact with pharmacologically relevant targets in vivo [8], [9],

- 219 GEC₅₀ values of cell lines were corrected for PPB to estimate sensitivity to free drug
- 220 concentrations.
- 221

222 Variability in tumor size and composition

To estimate the baseline tumor burden at diagnosis of metastatic disease, we used data 223 224 from a study of 819,647 women in the Surveillance, Epidemiology and End Results (SEER) 225 registry [10]. To calculate the distribution of baseline tumor burden among patients diagnosed with metastatic disease, we multiplied the baseline tumor burden of all patients at diagnosis by 226 227 the probability of a patient to have metastatic disease given a particular tumor burden (Fig. 1B). 228 For tumor size probabilities reported over size intervals, we assumed a uniform distribution 229 over the interval and calculated probabilities in diameter increments of 1 mm. A lognormal fit 230 to these data yielded a mean log tumor diameter (mm) of 3.624 and a standard deviation of 0.668. Baseline diameters were converted to volumes for simulation, assuming approximately 231 232 spherical tumors (Fig. 1C). Parameters used for generating virtual populations and treatment 233 simulations are provided in Table S1.

As HER2 TKIs are indicated for HER2+ breast cancer patients, we needed to estimate the cellular fraction of each tumor that was HER2 amplified. HER2 positivity is currently defined as IHC scores of IHC3+ or IHC2+ with amplification on orthogonal fluorescence in situ hybridization (FISH) assay. The proportion of IHC3+ and IHC+ patients among all HER2+ patients was calculated from 1,522 samples reported in a 12-year, single-center study [11] (Fig. 1D). For simplicity, we assumed each tumor harbored one sensitive (HER2-amplified) and one resistant (HER2-negative) population of cells with different levels of sensitivity to HER2 TKIs.

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242 Variability in tumor sensitivity and resistance

Once a virtual patient was designated as IHC3+ or IHC2+, a fraction f_{sens} of their tumor cells was designated as sensitive, while $f_{res} = 1 - f_{sens}$ was designated as resistant. By definition, 10 - 30% of cancer cells in IHC2+ tumors are HER2 amplified, compared to 30 - 100% of cells within IHC3+ tumors. Within these two strata, we assumed HER2 amplification was uniformly distributed.

248 To calibrate the effect of drug concentrations on tumor growth, the in vitro growth rate 249 (GR) metrics of 75 human breast cancer cell lines under treatment with alpelisib, abemaciclib, 250 palbociclib, ribociclib, lapatinib, and neratinib were identified from the literature [4], [12]. Data are presented in Fig. 2A. For use in the model, the HER2 status and clinical subtype of each cell 251 252 line was cross-referenced with data from [13]. Cell lines with unknown or inconsistent HER2+ 253 status across publications were excluded. Drug potency (GEC_{50}), efficacy (GR_{inf}), and Hill 254 coefficient parameters of HER2+ cell lines were sampled to generate HER2 TKI-sensitive cell 255 populations, while the parameters for Basal A and Basal B cell lines were sampled to generate resistant populations. GEC₅₀ values were adjusted for PPB as described above. The GR metrics 256 257 for neratinib and lapatinib are presented in Fig. 2B and Fig. S1, respectively.

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259 Modeling responses to HER2 TKI

Tumor growth with and without treatment was modeled using a generalized logistic model with an exponent of 0.25 [14] (Fig. 1C), which has been validated to faithfully characterize the growth kinetics of breast tumors in a study of 395,188 women [2]. The *b* growth parameter estimated in this study was lognormally distributed with a mean of 1.38 and variance of 1.36 for women under the age of 60. This corresponds to a geometric mean k_g = 1.66 x 10⁻⁴ hours, or 2.78 x 10⁻² weeks. The equations used to model changes in the abundance (volume) of sensitive and resistant cell populations over time were:

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$$\frac{dSens}{dt} = k_{g,sens} N_{sens} \left(1 - \frac{N_{sens} + N_{res}}{K}\right)^4 \log_2(1 + Effect_{sens})$$

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270
$$\frac{dRes}{dt} = k_{g,res} N_{res} \left(1 - \frac{N_{sens} + N_{res}}{K}\right)^4 \log_2(1 + Effect_{res})$$
271

272
$$\frac{dTumor}{dt} = \frac{dRes}{dt} + \frac{dSens}{dt}$$

273

Where N represents the volume of the population and K represents the carrying
 capacity (diameter = 128 mm [2]). The effects of drug on tumor growth, Effect_{sens} and Effect_{res},
 were calculated as follows [4]:

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$$Effect = GR_{inf} + \frac{1 - GR_{inf}}{1 + \left(\frac{Free \ drug}{GEC_{50}}\right)^{h}}$$

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280 Where *h* is a Hill coefficient estimated for a given cell line's relationship between drug 281 concentrations, GR_{inf} , and GEC_{50} . Of note, a proportion (27%) of neratinib's therapeutic effect is 282 attributed to active metabolites [6]. We therefore multiplied free neratinib concentrations by 1 283 / (1 – 0.27) when calculating Effect_{sens} and Effect_{res} to adjust for expected metabolite activity. 284

285 Analysis and software

286 Virtual populations were generated in MATLAB R2020b. Treatment simulations were 287 performed in Simulx 2020. Statistical analyses were performed in MATLAB R2020b. Diameters were "measured" by sampling from simulations every 6 or 8 weeks to assess for progression-288 free survival (PFS), mirroring the frequency of radiographic assessment in clinical trials. PFS was 289 evaluated per RECIST v1.1 criteria, which designate target lesion diameter increases of \geq 20% 290 from nadir and at least 5 mm in absolute terms as progressive disease [15]. Importantly, events 291 292 such as the appearance of new metastases, enlargement of non-target lesions, and death from 293 any cause are also classified as progressive disease. We termed these events "DNTP", or Death 294 or Non-target Progression, and modeled the daily probability of patients to progress due to DNTP at the empirically estimated rate of 1.5×10^{-4} events/mm of tumor diameter/day. 295 Objective response rates (ORR) were also evaluated per RECIST v1.1. This required the 296 297 calculation of best overall response (BOR) for each patient. To do so, diameter measurements sampled from simulations every 6 or 8 weeks were compared to baseline tumor diameters. The 298 smallest non-baseline tumor measurement was used to evaluate BOR. Patients with BOR 299

diameter reductions of at least 30% from baseline were considered responders. ORR was

301 calculated by dividing the fraction of responders by the total number of patients.

302 **RESULTS**

303 Tumor sensitivity and resistance to targeted therapies is heterogeneous

304 We initially questioned whether the steady-state unbound concentrations of several targeted therapies at their approved doses for the treatment of metastatic breast cancer were 305 306 above or below their in vitro potency, as measured by PPB-adjusted GEC₅₀. Plotting the PPB-307 adjusted GEC_{50} of breast cancer cell lines [4], [12] against the estimated $C_{ss,free}$ (Fig. 2A, see Methods) of 6 different drugs revealed significant differences in predicted response. The 308 majority of cell lines in the dataset possessed GEC₅₀ below the C_{ss,free} of abemaciclib, neratinib, 309 310 and palbociclib, but not alpelisib, lapatinib, and ribociclib (Fig. 1). While these cell lines may not 311 fully reflect the mixed cellular populations in patients, it is reassuring that HER2-amplified cell types in particular tended to have sensitivities below the predicted C_{ss.free} of HER2 TKIs neratinib

- 312 types in particular tended to have sensitivities below the predicted C_{ss,free} of HER2 TKIs ne 313 and lapatinib. No such enrichment of HER2-amplified cell lines was noted for the PARP
- inhibitors abemaciclib, palbociclib, and ribociclib, nor for the PI3Kα inhibitor alpelisib. These
- 315 data support the fidelity of GR metrics for characterizing and discriminating sensitivity and
- 316 resistance to HER2 TKIs across diverse cell lines.
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Plasma free neratinib is closely associated with patient response and confers therapeutic effect beyond target lesions

We created a virtual HER2+ breast cancer population and simulated patient response to
 neratinib (see Methods). The population PK of neratinib was simulated using a two compartment model with sequential first-order absorption, T_{lag}, and first-order elimination (Fig.
 3A-B) [6], [16], [17]. A generalized logistic growth model was used to describe the growth of a
 two-population tumor with different subpopulation sensitivities to neratinib (Fig. 3C-D) [2],
 [14].

We explored several possibilities for tumor suppressive effects, including total- and free-326 327 drug concentration-driven tumor growth inhibition of target lesions as well as indirect 328 (unobserved) suppression of the growth of non-target and new lesions. When only target lesions were considered, tumor growth inhibition driven by total drug concentrations resulted 329 in an overestimation of PFS and ORR (Fig. S2) [16]. This was not entirely unexpected, as 330 331 progression from target lesions only reflects a subset of patients; many breast cancer 332 progression events are due to death, new metastases, or non-target lesion progression while 333 patients are still under therapy [18]. The use of free neratinib concentrations improved 334 concordance and matched the target lesion response rates reported in [16] (31.6% simulated vs. 30.3% reported), but still overestimated PFS (Fig. 3E-F). 335

We found the indirect tumor suppressive effect on non-target and new lesions to be 336 337 critical for producing clinically consistent outcomes. For tumors that were already large at 338 baseline, modeling drug effect on target lesions alone may overpredict patient response as these patients are at a higher risk of non-target progression and new lesions. Considering (1) 339 the positive correlation between tumor burden and mortality and/or metastasis [10], and (2) 340 341 more patients with metastatic breast cancer progress from non-target sources than target 342 sources [18], we derived an additional time-variant probability of Death or Non-target progression (DNTP) for each patient that increased as a function of tumor burden. DNTP 343 represents the instantaneous daily probability of a patient to die, experience new metastasis, 344

or exhibit non-target lesion growth and was estimated as 1.5 x 10⁻⁴ events/mm tumor
 diameter/day.

With free drug-driven tumor growth inhibition and indirect tumor burden-driven DNTP, the platform generated PFS estimates that matched well with clinical outcomes [16] (Fig. 3G). Interestingly, the risk of progression from non-target, DNTP sources was relatively consistent throughout the course of treatment, whereas the risk of progression from target lesions was highest toward the end of treatment (Fig. 3H). Free drug-driven PD with concomitant DNTP was carried forward for all additional simulations.

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Patient response to neratinib is mostly influenced by tumor characteristics and less by systemic drug exposure

Having established the model's ability to reasonably recapitulate the clinical outcomes of neratinib, we proceeded to simulate 52 weeks of neratinib monotherapy in 1,000 virtual patients in search of tumor characteristics associated with longer PFS. Treatment with 240 mg QD neratinib prolonged median PFS from 15.5 to 18.3 weeks (Fig. 4A) and extended DNTP-free survival in 25.7% of patients (Fig. 4B).

Patients were stratified by tumor characteristics and systemic drug exposure (Fig. 4C). 361 362 As expected, a greater baseline tumor burden correlated with shorter PFS. The growth rate of 363 resistant cells and drug potency on sensitive cell populations were also great sources of variability in PFS (Fig. 4D). Patients with more sensitive cell populations or slower growth of 364 365 resistant populations tended to have longer survival. In contrast, the growth of sensitive cell populations, drug potency on resistant cell populations, and drug PK parameters like clearance, 366 area under the curve (AUC), and C_{ss,trough} at the labeled dose did not significantly influence 367 patient response and PFS (Fig. 4C). Interestingly, differences in HER2+ fraction were also 368 369 insignificant to patient response.

Patients who achieved above-median PFS tended to have greater killing of sensitive cells and better control of resistant cell outgrowth than patients who did not (Fig. 4E). On an individual level, however, a diverse range of response dynamics was observed (Fig. 4F).

373 Sensitive subpopulations could be completely eradicated in some patients, fully controlled in

others, and completely insensitive in still others (Fig. 4F), raising the possibility of post-

treatment changes to HER2+ fraction [19]. Collectively, these data suggest that tumor-intrinsic

376 characteristics and cellular heterogeneity strongly influence patient responses to targeted

therapy, and to a higher degree than inter-individual variability in drug PK at the approveddoses.

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Patient response to lapatinib is also largely explained by variability in tumor characteristics and cellular composition

To investigate the broader applicability of our modeling approach, we adjusted the PK/PD/TG model for another HER2 TKI, lapatinib (Fig. 5A). Simulations using the virtual breast cancer population, publicly available GR metrics [4], [12], and the original sponsor-developed population PK models reproduced the clinically observed PK, PFS, and ORR of 1500 mg QD lapatinib (Fig. 5B, Fig. S3) [7], [20]–[22]. As with neratinib, free plasma lapatinib was assumed to drive tumor suppressive effect while accounting for indirect effects on additional probability of progressive disease from DNTP.

389	Lapatinib monotherapy was predicted to improve median PFS to 17.4 weeks, from 15.1
390	weeks if untreated (Fig 5C). DNTP-free survival was also extended in 11.4% of patients (Fig. 5D).
391	Similar to neratinib, tumor-intrinsic properties such as tumor burden and resistant cell growth
392	rate differed between patients with longer and shorter PFS, while PK parameters did not (Fig.
393	5E). HER2+ fraction also impacted lapatinib outcomes, with the lowest quartile exhibiting
394	markedly shorter PFS (Fig. 5F), while drug sensitivity did not. In contrast to neratinib, there was
395	no difference in the killing of sensitive cells between patients who achieved above- and below-
396	median PFS, although there was a difference in the control of resistant cell outgrowth (Fig. 5G).
397	
398	Dose intensification may improve clinical response for neratinib but may not for lapatinib
399	Although there was only a modest relationship between PK variability and PFS under the
400	approved doses of neratinib and lapatinib, we wondered whether BID dosing might improve
401	tumor control and patient outcomes. To our surprise, doubling the dosing frequency improved
402	outcomes for neratinib significantly more than for lapatinib, with 27% versus 2% deriving longer
403	PFS from BID dosing (Fig. 6A-B). This suggests different levels of susceptibility of neratinib and
404	lapatinib, at their approved doses, to significant changes in PK; 240 mg QD neratinib is perhaps
405	in a steeper portion of its dose-response curve than 1500 mg QD lapatinib. Dose fractionation
406	of neratinib into 120 mg BID to mitigate dose-limiting diarrhea did not significantly influence
407	efficacy (Fig. S4) [6].
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433 DISCUSSION

In this study, we integrated PK, tumor stage and growth characteristics, clonal composition,
and tumor sensitivity and resistance to treatment into a PK/PD/TG modeling platform and
simulated the antitumor efficacy of two HER2 TKIs in a virtual metastatic breast cancer
population. Previous studies conducted to evaluate the interplay between PK and tumor
characteristics and their influence on patient response have found both to be major sources of
variability in patient response [1]. While we also found tumor growth parameters such as
resistant cell growth rate to significantly contribute to PFS, we did not find a strong influence of

- 441 drug PK on patient response to the assessed target therapies.
- The finding that HER2+ positivity influenced clinical outcomes under lapatinib treatment is 442 consistent with clinical observations [23]. It is not clear why a similar magnitude of effect was 443 444 not observed in patients treated with neratinib. We also found the growth rate of resistance clones, analogous to tumor regrowth rate after progression, to be significant to patient 445 446 response. Interestingly, growth rate after progression has been directly correlated with patient 447 survival in non-small cell lung cancer and glioblastoma [24], [25]. A similar analysis has been conducted for HER2-negative breast cancer response to chemotherapy [26], though to our 448 449 knowledge, no such work exists for HER2-positive breast cancer. Our results in the context of 450 accumulating literature highlighting the importance of HER2+ fraction suggests a similar relationship may between regrowth rate and long-term outcomes may exist. 451
- To recapitulate clinically observed PFS curves that account for progression from both target and non-target sources, a tumor burden-based risk of DNTP seems to be critical [10], [18]. Patients with metastatic disease may have discordant responses to therapy across metastases, confounding PFS based on changes in the target lesions [27], [28]. To our knowledge, DNTP rates have not been well characterized previously. As shown in our study, DNTP rate could be informed by the progression difference between target lesions and all lesions that are clinically observed per RECIST 1.1.
- 459 Two points should be kept in mind when interpreting this study. First, nonspecific protein binding is much higher in tumors than in blood for most small molecule therapeutics. For 460 lapatinib specifically, Spector et al. [29] showed that total drug concentrations in xenograft 461 tumors are slower clearing and at least 6-fold higher than in plasma. With substantial total drug 462 accumulation in the tumor due to nonspecific binding, one would expect total drug-driven 463 464 effect to result in even longer PFS than we have simulated here, therefore requiring a higher rate of DNTP to "bring down" the PFS curve. One major assumption of this work is that 465 nonspecific proteins available for drug binding are always in great excess, leading to a constant 466 fup over time [30], [31]. Hypoalbuminemia does occur in breast cancer, although whether this 467 468 meaningfully affects free drug concentrations is not known [32]. 469 Second, the Free Drug Hypothesis is generally taken prima facie when translating drug PD
- from in vitro systems into living organisms [5], [8]. This draws from the assumption that drug
 nonspecifically bound to circulating plasma protein or extracellular parenchymal protein is
 unable to interact with its intended target in this case, the intracellular kinase domain of
 HER2. Violations of the free drug hypothesis are generally driven by mechanisms that disturb
 steady state equilibria assumptions, such as drug transport proteins like P-glycoprotein (Pgp) or
 high tissue clearance rates relative to diffusion [8]. While lapatinib is a substrate for Pgp and
 breast cancer resistance protein (BCRP) [33], the direction of its transport is extracellular, such

that intracellular lapatinib concentrations are unlikely to be higher than what one would expect
from free plasma concentrations. With no evidence to suggest transporter-mediated uptake
into cancer cells, nor increased clearance from the tumor, we consider free drug concentrations

- 480 to be an appropriate driver of PD for lapatinib [6], [9], [29]. Hypothetically, covalent inhibitors
- 481 such as neratinib might cause receptor internalization, leading to longer PD than would be
- expected from free concentrations [34]. If de novo HER2 re-synthesis kinetics are faster than
 neratinib's half-life, however, we would expect this prolonged PD to be negligible [35].

Oncology drug developers may benefit from seriously considering free drug concentrations 484 485 when predicting clinical efficacy from pre- and non-clinical data. With our emphasis on free drug concentrations, it might be tempting to use this study to support optimizing protein 486 binding as a goal during early development. However, this would be misguided, as decreasing 487 488 protein binding does not increase free drug AUC without a concomitant decrease in intrinsic clearance [36]. For drugs such as lapatinib, correcting for protein binding and comparing to GR 489 490 metrics like GEC₅₀ may have alerted investigators to the relatively minimal effect lapatinib 491 would exert as a monotherapy [21]; indeed, lapatinib is only approved for use in combination with capecitabine. Previous studies that estimated brain tumor lapatinib concentrations did not 492 493 consider nonspecific protein binding and used an oversimplified representation of the drug's 494 pharmacokinetic variability [37]. Interestingly, the 0.61 tumor-to-blood ratio that was used is 495 lower than the tumor accumulation ratio reported by Spector et al., though this may be due to 496 differences in brain and peripheral drug distribution [29]. In any case, it may be useful to re-497 evaluate these studies in the context of blood brain barrier-mediated transport of free drug.

498 Developing a well-calibrated model with realistic characterization of inter-patient PK and 499 tumor growth variability allowed us to explore alternative dosing regimens in a semi-realistic population. We were surprised to find substantial differences in the extent of benefit achieved 500 501 with BID neratinib and lapatinib. Hypothetically, this suggests dose titration of lapatinib up to 1500 mg BID would be futile in most cases, whereas 240 mg BID neratinib would be reasonably 502 503 likely to extend PFS. In practice, dose-limiting diarrhea may prevent the administration of more 504 than 240 mg QD neratinib, highlighting that safety and tolerability can impose upper limits to the range of the free drug ER that can be explored [6]. Splitting the 240 mg dose into two 120 505 mg doses did not compromise efficacy of neratinib (Fig. S4), but did lower C_{ss,max} without 506 changing C_{ss,avg}. This may be an alternative to the currently on-label dose adjustment strategy, 507 508 which recommends sequential dose reductions in increments of 40 mg.

HER2 was an unusually apt target on which to demonstrate modeling proof of concept, as 509 HER2 amplification is routinely quantified in the clinic. In addition, widespread breast cancer 510 511 screening efforts have generated volumes of data against which it is possible to calibrate 512 models of tumor growth [2]. Based on these results, it may be useful to consider HER2+ fraction 513 as a predictor of outcomes on HER2 targeted therapy; this may provide greater granularity than HER2 IHC scores. An immediate expansion of this approach would be to predict the efficacy of 514 EGFR TKIs, as several groups have found EGFR mutation fraction to influence outcomes during 515 treatment with EGFR mutation-selective therapies [38], [39]. Overall, our GR metric-based 516 517 population PK/PD/TG modeling approach has potentially broad utility for decision-making 518 during early drug development. 519

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521	AUTHOR CONTRIBUTIONS
522	Conceptualization, T.Q. and Y.C.; Methodology, T.Q.; Software, T.Q.; Validation, T.Q.;
523	Formal Analysis, T.Q.; Investigation, T.Q.; Resources, Y.C.; Data Curation, T.Q.; Writing – Original
524	Draft, T.Q.; Writing – Review & Editing – T.Q. and Y.C.; Visualization – T.Q.; Supervision – Y.C.;
525	Project Administration – T.Q. and Y.C.; Funding Acquisition – Y.C.
526	
527	
528	ACKNOWLEDGEMENTS
529	Figures were prepared in BioRender.
530	
531	
532	FUNDING
533	This work was supported by the National Institute of General Medical Sciences R35GM119661.
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536	SUPPLEMENTAL MATERIALS
537	Table S1. Model parameters
538	Table S2. Patient characteristics of neratinib monotherapy trials
539	Table S3. Patient characteristics of lapatinib monotherapy trials
540	Figure S1. Lapatinib drug sensitivity
541	Figure S2. Neratinib pharmacodynamics using total or free drug
542	Figure S3. Lapatinib efficacy calibration
543	Figure S4. Neratinib does fractionation
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695 FIGURES

- 696 Figure 1. Virtual patients reflect real-world sources of variability
- 697 A. Variability in drug pharmacokinetics, target fraction, subpopulation growth rates, and 698 drug-specific subpopulation sensitivity were considered during model development.
- Lapatinib and neratinib population PK models were adapted from publications by the
 original developers and FDA review documents. Target fraction (subpopulation
 proportions) was determined using HER2 IHC2/IHC3+ frequencies available in the
- literature [11]. Growth rates were derived from a published tumor growth model built
 on breast cancer screening data from 395,188 women [2]. Drug sensitivity for tumor
 subpopulations was obtained from GR metrics reported in the literature [4], [12].
- B. Baseline tumor diameter at diagnosis (left) and the likelihood of metastatic disease at diagnosis based on baseline tumor diameter (center) used to calculate baseline tumor diameter at diagnosis of metastatic disease (right). Baseline tumor diameter frequencies below 20 mm were available in increments of 1 mm; above 20 mm were available in increments of 10 mm [10].
- C. Simulated growth (red) of 15 mm diameter tumors as compared to [2] (black). Dashed
 lines indicate 5th, 25th, 75th, and 95th percentiles of 100 simulated tumors, while solid
 lines indicate medians.
- D. Relative frequency of IHC3+ and FISH-amplified IHC2+ breast cancers at diagnosis
 reported in [11].



Figure 2. Tumor sensitivity and resistance to targeted therapies is heterogenous 716 717 A. Protein binding (PPB)-adjusted sensitivity (log₁₀ nM) of breast cancer cell lines to six 718 drugs approved for the treatment of metastatic breast cancer [4], [12]. Dashed lines 719 indicate estimated average steady-state concentrations of free drug (Css,free). Inset percentages indicate the proportion of cell lines in the dataset with PPB-adjusted GEC₅₀ 720 below the drug's free C_{ss,free}. For visualization purposes, cell lines with no detected 721 sensitivity to a particular drug at tested concentrations were assigned a nominal value 722 of 1 x 10⁹. 723 B. Neratinib GEC₅₀ (left) and GR_{inf} (center) values for breast cancer cell lines reported in 724 [12]. HER2 status was determined by [13]. Cell lines with conflicting HER2 status in the 725 literature were excluded. GR_{expected} (right) values reflect the predicted GR of each cell 726

727 line at the estimated C_{ss,avg} of neratinib.





734 A. Population pharmacokinetic model structure for neratinib reported in [6]. Drug 735 absorption occurs through a first-order (K_a) process with delay (T_{lag}). Drug subsequently 736 distributes (Q) between central/plasma (V_c) and peripheral (V_p) compartments and clears from the central compartment by first-order elimination (CL). 737 B. Simulated pharmacokinetic profiles during the first week of once-daily treatment with 738 240 mg neratinib. Lines represent medians, whereas shaded regions represent 5th and 739 95th percentiles. Black circles represent observations from [17]. 740 741 C. Pharmacodynamic model of tumor growth comprising two competing cell populations with differential sensitivity to a cytostatic drug. 742 D. Growth dynamics of HER2-amplified ("sensitive") and HER2-negative ("resistant") 743 744 subpopulations within an illustrative IHC2+ tumor. Values for either subpopulation are normalized to their abundance at treatment initiation. 745 746 E. Predicted PFS during 52 weeks of treatment with 240 mg QD neratinib using free drug-747 driven pharmacodynamics, compared to [16]. Only progression from target lesions is included. 748 F. Predicted ORR during 5 weeks of treatment with 240 mg QD neratinib using free drug-749 750 driven pharmacodynamics, compared to [16]. G. (E) with an additional daily chance of Death or Non-target Progression (DNTP) estimated 751 as 1.5×10^{-4} events/mm of tumor diameter/day. 752 H. Risk of progression by source during each 6-week scan interval during 52 weeks of 753 754 treatment with 240 mg QD neratinib. NAR, number at risk during the scan interval. 755

Figure 3. PK/PD/TG model recapitulates the clinical efficacy of neratinib



762 A. Simulated PFS of 1000 patients with and without 52 weeks of treatment with 240 mg 763 QD neratinib. 764 B. DNTP-free survival for each patient in untreated (blue) and treated (orange) states. DNTP-free survival is the time on study without a DNTP event. 765 C. Multiplicity-corrected significance of differences in PFS between patients with 766 parameter values above or below the population median (bars). The dashed line 767 represents the Benjamin-Hochberg significance threshold for one-way ANOVA. For 768 769 visualization purposes, the y axis is truncated at 2.5. D. Significant differences in PFS stratified by tumor burden (left), resistant cell growth rate 770 (middle), sensitive cell GEC₅₀ (right) quartiles. 771 E. Normalized tumor diameters (left), sensitive cell volume (middle), and resistant cell 772 773 volume (right) stratified length of PFS relative to median PFS (mPFS). All y axes are on a 774 log scale. Shaded regions represent interguartile ranges. 775 F. Representative profiles of baseline-normalized sensitive and resistant cell abundance from three virtual patients treated with 52 weeks of 240 mg QD neratinib. 776

Figure 4. Tumor-intrinsic characteristics influence neratinib efficacy



789 Figure 5. Different tumor-intrinsic characteristics influence lapatinib efficacy 790 A. Population pharmacokinetic model structure for lapatinib reported in [7]. Drug 791 absorption occurs through a sequential zero-order (Dur) and first-order (K_a) process with 792 delay (T_{lag}). Drug subsequently distributes (Q) between central/plasma (V_c) and peripheral (V_p) compartments and clears from the central compartment by first-order 793 794 elimination (CL). B. Simulated pharmacokinetic profiles during the first week of once-daily treatment with 795 1500 mg lapatinib. Lines represent medians, whereas shaded regions represent 5th and 796 95th percentiles. Black circles represent observations from [22]. 797 C. Simulated PFS of 1000 patients with and without treatment of 1500 mg daily lapatinib. 798 D. DNTP-free survival for each patient in untreated (blue) and treated (orange) states. 799 800 DNTP-free survival is the time on study without a DNTP event. E. Multiplicity-corrected significance of differences in PFS between patients with 801 802 parameter values above or below the population median (bars). The dashed line 803 represents the Benjamin-Hochberg significance threshold for one-way ANOVA. For visualization purposes, the y axis is truncated at 2.5. 804 F. Significant differences in PFS stratified by tumor burden (left), resistant cell growth rate 805 806 (middle), sensitive cell HER2+ fraction (right) quartiles. G. Normalized tumor diameters (left), sensitive cell volume (middle), and resistant cell 807 volume (right) stratified length of PFS relative to median PFS (mPFS). All y axes are on a 808 809 log scale. Shaded regions represent interquartile ranges.



Figure 6. Dose intensification benefits neratinib more than lapatinib

A. PFS benefit from treatment with 240 mg QD or BID neratinib versus no treatment. PFS

- benefit is the positive difference in predicted PFS between two treatment schedules.
 - B. (A) for 1500 mg QD or BID lapatinib.



853 Figure S1. Lapatinib drug sensitivity

A. Lapatinib GEC₅₀ (left) and GR_{inf} (center) values for breast cancer cell lines reported in [4].
 HER2 status was determined by [13]. Cell lines with conflicting HER2 status in the
 literature were excluded. GR_{expected} (right) values reflect the predicted GR of each cell
 line at the estimated C_{ss,avg} of lapatinib.



- **Figure S2.** Neratinib pharmacodynamics using total or free drug
- A. Predicted PFS (top) and ORR (bottom) during treatment with 240 mg daily neratinib
 using total drug-driven pharmacodynamics, compared to [16]. Only progression from
 target lesions is included.
- B. (A) using free drug-driven pharmacodynamics.
- 877 C. (B) with an additional daily chance of Death or Non-target Progression (DNTP) estimated 878 as 1.5×10^{-4} events/mm of tumor diameter/day.



Figure S3. Lapatinib efficacy calibration

A. Predicted PFS (left) and ORR (right) of 1500 mg QD lapatinib using free-driven

- pharmacodynamics and an additional daily chance of Death or Non-target Progression (DNTP) estimated as $1.5 \times 10^{-4} *$ tumor diameter in mm, compared to [20], [21].



925 Figure S4. Neratinib dose fractionation

A. PFS benefit from treatment with 240 mg QD or 120 mg BID neratinib versus no
 treatment. PFS benefit is the positive difference in predicted PFS between two
 treatment schedules. Lack of orange bars indicates PFS equivalence between 240 mg
 QD and 120 mg BID.

