Pharmacodynamic modelling reveals synergistic interaction between docetaxel and SCO-101 in a docetaxel-resistant triple negative breast cancer cell line

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Pharmacodynamic modelling reveals synergistic interaction between docetaxel and SCO-101 in a docetaxel-resistant triple negative breast cancer cell line

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A B S T R A C T

One of the primary barriers in treating cancer patients is the development of resistance to the available treatments. This is the case for treatment of triple negative breast cancer (TNBC) with docetaxel, which is part of the neoadjuvant treatment for TNBC. The novel compound SCO-101 is under investigation for its potential treatment effect in several types of drug resistant cancer. The aim of this study was to establish a pharmacodynamic model that captures the effect of docetaxel, SCO-101, and the combination on cell survival in docetaxel resistant MDA-MB-231 TNBC cells. Several combination models were compared and a recently published combination model, the general pharmacodynamic interaction model (GPDI), provided the best fit. The model allowed for description and quantification of the interaction between docetaxel and SCO-101 with respects to both maximal effect and potency. Based on this model, SCO-101 has a synergistic effect with docetaxel. This synergy is not present in the maximal effect, but the combination of SCO-101 and docetaxel showed an approximately 60% increase in potency compared to docetaxel alone. Furthermore, the predicted model surface for the combination provided key information regarding promising dose ratios and dose levels for further studies of the combination. Lastly, the study presents a use case for the GPDI model, which provides a way to quantify and interpret drug-drug interactions.

1. Introduction

1.1. Combination therapies in cancer

Drug combination therapy is widely applied for the treatment of cancers. A major advantage of this treatment strategy, as opposed to conventional monotherapies, is a reduction in the systemic cytotoxicity as multiple pathways are targeted simultaneously (Mokhtari et al., 2017). Furthermore, one of the primary barriers in current cancer treatment is the development of drug resistance, which is the main cause of cancer-related death (Housman et al., 2014, Wang et al., 2019). Drug resistance occurs for a multitude of reasons, ranging from drug target alteration and alterations in drug efflux to inherent cell heterogeneity (Housman et al., 2014, Wang et al., 2019). Currently, the best approach to combat drug resistance in cancer patients is the use of combination therapies (Housman et al., 2014). Therefore, the development of treatment combinations to combat cancers is of great

interest. Thus far, the strategy has not been particularly successful and there is therefore a large unmet medical need for treatment options to patients with resistant cancers.

Docetaxel is a chemotherapeutic agent used in the treatment of several types of cancer, including breast cancer. Of particular note, it is a key component of the neoadjuvant treatment for the aggressive triple-negative breast cancer (TNBC) subtype, which comprises 15–20% of breast cancer cases (Santonja et al., 2018). The mechanism of action of docetaxel is to stabilize microtubules, which then inhibits the normal reorganization of the microtubule network, leading to failures during cells division and eventual apoptosis (Hwang, 2012). Docetaxel is considered by WHO to be one of the Essential Medicines (Organization, 2018). However, half of patients do not respond to treatment with docetaxel due to resistance and additionally the patients who do initially respond will later develop resistance (Hwang, 2012, Gómez-Miragaya et al., 2017). This presents a clinical issue with the effective use of docetaxel and therefore identifying drug combinations

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that synergize with docetaxel in resistant breast cancer would greatly increase the clinical value of docetaxel.

1.2. Modelling combinations in cancer

Characterizing and quantifying the effect of a given pharmaceutical drug combination *in vitro* through pharmacokinetic and pharmacodynamics modelling has been the focus of several studies within the field of cancer (Frances et al., 2011, Goteti et al., 2010, Rocchetti et al., 2009). Particularly, classifying a combination as either additive or synergistic is of great importance, as it supports the validity of the combination. Several criteria exist for attaining either additivity or synergy effects of a combination, which contributes to making these definitions vague. Two effect-based examples of this would be response additivity and Bliss Independence (Foucquier and Guedj, 2015, Greco et al., 1995, Bliss, 1939). For response additivity, simple addition of the effect term for a combination would correspond to additivity and any observed effect above that would be considered synergistic. Bliss Independence is based on probabilities, causing the effect terms to be constrained between 0 and 1, and assumes that the drugs have different sites of action. The combined effect is the product of the two effect terms subtracted from the sum of the two. This results in the combined effect approaching 1 as the effect of the combined compounds increase.

Finally, some implementations are based upon differential equations for tumor growth rates, which provide a more mechanistic approach to describing the drug combination (Frances et al., 2011). However, while inherently empirical, assessing drug combination effect through response additivity or Bliss Independence is more readily applicable, as these do not require time course data. A semi-mechanistic approach can be achieved by combining either response additivity or Bliss Independence with the general pharmacodynamic interaction (GPDI) model (Wicha et al., 2017). The GPDI model considers interactions between the compounds in either the maximal response or the potency, thereby providing a way of interpreting the potential synergistic effect.

1.3. SCO-101 and docetaxel combination

SCO-101 is a novel compound under investigation for its potential treatment effect in several types of drug resistant cancer (Bagger et al., 2018). It was originally found to acts as a modulator of the volume regulated anion channel complex (Hélix et al., 2003). It has also now been demonstrated to have additional mechanisms of action in drug resistant cancer cells and therefore seemingly has a different site of action than docetaxel (Bagger et al., 2018). The drug is taken orally and, as shown by four phase I clinical trials, has an advantageous pharmacokinetic profile with $T_{1/2}$ of 15 h and limited toxicity (Bagger et al., 2018). In this study, the aim was to assess the efficacy of the combination of docetaxel and SCO-101 using docetaxel-resistant MDA-MB-231 human TNBC cells. The potential effect that SCO-101 contributes to the combined effect in these cells was determined by establishing a pharmacodynamic model to describe the effect of SCO-101, docetaxel, and combinations of the two. Lastly, it was investigated whether this effect was mediated through an interaction with docetaxel and further, the potential interaction between SCO-101 and docetaxel was quantified.

2. Method

2.1. MTT assay

Cell viability was assessed based on the metabolic activity using the tetrazolium-based semiautomated colorimetric (MTT) assay as previously described (Carmichael et al., 1987). In brief, cells (4000 cells/

well) were plated in 96-well and allowed to adhere for 24 h prior to addition of drug(s). Medium was discarded and docetaxel and/or SCO-101 was added to the plates in triplicates and the cells were incubated for 72 h. 0.5 mg/ml MTT (Sigma-Aldrich, Broendby, Denmark) was added to each well and incubated for 4 h before addition of 20% sodium dodecyl sulfate in 0.02 M hydrochloric acid to dissolve the formazan crystals overnight. The optical density at 570 nm (690 nm for background) was measured spectrophotometrically and cell viability calculated in percent compared to untreated control cells. A minimum of three independent experiments was performed for each drug.

2.2. Cell line and culturing

The human breast cancer cell line MDA-MB-231 was obtained from American Type Culture Collection (Rockville, MD). Cells were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) including L-glutamine, supplemented with 10% fetal calf serum (FCS). Cells were kept at 37 °C in a humidified atmosphere containing 5% carbon dioxide. The docetaxel-resistant MDA-MB-231 was established by continuous culturing in medium containing gradually increasing concentrations of docetaxel (Hansen et al., 2015). Resistance of the cells to docetaxel is shown in supplementary material 1.

2.3. Data

The optical density measurements, corresponding to cell survival, were collected in replicates of 3–4 following 72 h of treatment with SCO-101, docetaxel, or both. The data available was the concentration achieved in the growth medium following dosing with SCO-101, docetaxel, or both. Docetaxel was dosed at 0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, and 10 μM, while SCO-101 was dosed at 1, 10, 20, 30, 40, 50, 60, 80, and 100 μM, corresponding to 11 docetaxel monotherapies, 9 SCO-101 monotherapies, and 99 combinations of the two. The final dataset contained 20, 27, 33, and 297 observations for untreated, SCO-110, docetaxel, and the combination, respectively, totaling 377 observations.

2.4. Pharmacodynamic modelling

The data was investigated using effect-based models, which consider the concentration C_{drug}, where *drug* is either SCO-101 or docetaxel. The following four models (Linear, log-linear, I_{max} and Hill) were explored for describing the concentration-effect relationship of either SCO-101 or docetaxel monotherapies:

$$I_{drug} = \alpha \cdot C_{drug} + \beta \tag{1}$$

$$I_{drug} = \alpha \cdot \log(C_{drug}) + \beta \tag{2}$$

$$I_{drug} = \frac{I_{max,drug} \cdot C_{drug}}{IC_{50,drug} + C_{drug}} \tag{3}$$

$$I_{drug} = \frac{I_{max,drug} \cdot C_{drug}^{H_{drug}}}{IC_{50,drug}^{H_{drug}} + C_{drug}^{H_{drug}}} \tag{4}$$

In Eqs. 1 and 2, α corresponds to the estimated slope for the linear or log-linear concentration-effect relationship, while the β parameter is the estimated intercept. For Eq. 3 $I_{max, drug}$ is the maximal effect of *drug*, where *drug* is either SCO-101 or docetaxel, while $IC_{50, drug}$ is the half-maximal inhibitory concentration. Lastly, in Eq. 4 in addition to the parameters from Eq. 3, a Hill coefficient H_{drug} is included, which describe the sigmoidicity of the curve.

For the combined effect, response additivity and Bliss Independence presented in Eq. 5 and Eq. 6 were considered. A baseline cell survival parameter I_0 was estimated and the effects of I_{drug} was expressed as

proportional reduction from the estimated baseline $I_{drug, prop} = I_{drug}/I_0$, constraining the effects between 0 and 1. Similarly, the post-treatment effect was expressed as a proportional reduction from baseline $I_{treated} = I_0 \cdot (1 - I_{comb})$. Importantly, both the response additivity and Bliss independence model is nested within the GPDI model.

$$I_{comb} = I_{Doce,prop} + I_{SCO,prop} \tag{5}$$

$$I_{comb} = I_{Doce,prop} + I_{SCO,prop} - I_{Doce,prop} \cdot I_{SCO,prop} \tag{6}$$

In addition, the GPDI model (Wicha et al., 2017) was considered with either 1- or 2-way interaction in the $IC_{50, \; drug}$ or $IC_{50,drug}^{H}$ value. In Eq. 7, this is illustrated for 1-way interaction of SCO-101 on the $IC_{50, \; Doce}$ value of docetaxel. Here $INT_{max, \; SCO \to Doce}$ corresponds to the maximal interaction of SCO-101 on the docetaxel IC_{50} value and $INT_{50, \; SCO \to Doce}$ corresponds to the half-maximal effect of that interaction.

$$I_{Doce} = \frac{I_{max,Doce} \cdot C_{Doce}}{IC_{50,Doce} \cdot \left(1 + \frac{INT_{max,SCO \to Doce} \cdot C_{SCO}}{INT_{50,SCO \to Doce} + C_{SCO}}\right) + C_{Doce}} \tag{7}$$

2.5. Analysis

Cell survival data measured as optical density was analyzed using NONMEM 7.4 (Beal et al., 2009). In the analysis a population approach was used and the population parameters was estimated using the first-order conditional estimation method with interaction (FOCE-I). Any variability in the data was considered as residual variability and therefore did not include any inter-individual variability. Residual error models were considered as either additive, proportional or a combination of both with a mean of zero and a variance σ^2. Model selection was based on objective function value (OFV) as well as graphical summaries of the observed vs. the predicted cell survival. Graphical representation of the data was produced using R 3.6.1 (R Core Team 2016) and the ggplot2 package (Wickham, 2009) as well as the plot3D package (Soetaert, 2017). The 95% confidence interval was computed as:

$$mean \pm 1.96 \cdot \frac{sd}{\sqrt{n}}$$

where sd and n is the standard deviation and the number of samples, respectively.

For identifying optimal dose pairs, a reduction in viable cells of 85% was considered the target, which corresponds to 0.05 OD in this study. Dose pairs, denoted $C_{Doce \to 85\%}$ and $C_{SCO \to 85\%}$, which resulted in reaching the prespecified target of 85% where scaled to their own maximal dose and summed, to identify the lowest total dose combination, Eq. 8.

$$\min(C_{Doce \to 85\%,lt}, \; C_{SCO \to 85\%,lt})$$
$$= \min\left(\frac{C_{Doce \to 85\%}}{\max(C_{Doce})} \cdot WP_{Doce} + \frac{C_{SCO \to 85\%}}{\max(C_{SCO})} \cdot WP_{SCO}\right) \tag{8}$$

Furthermore, the possibility for adding weighted penalties (WP) for either drug was included, but was initially set to 1 for both compounds. Lastly, a similar approach based on Pythagoras' theorem was used to identify the dose pair that minimizes the exposure to both drugs, Eq. 9.

$$\min(C_{Doce \to 85\%,me}, \; C_{SCO \to 85\%,me})$$
$$= \min\left(\sqrt{\left(\frac{C_{Doce \to 85\%}}{\max(C_{Doce})} \cdot WP_{Doce}\right)^2 + \left(\frac{C_{SCO \to 85\%}}{\max(C_{SCO})} \cdot WP_{SCO}\right)^2}\right) \tag{9}$$

3. Results

3.1. Monotherapy model structure

The MDA-MB-231 cells displayed resistance to treatment with

docetaxel in the lower dose range from 0.0001–0.1 μM, however, despite the induced resistance to docetaxel, there is a response to the treatment for the higher doses between 0.3 μM and 10 μM, Fig. 1A + B. Single-agent parameters were estimated using the monotherapy data for each compound by fitting the four models described in Section 2.4 to the data. For docetaxel the I_{max} and sigmoidal I_{max} model described the data equally well. The sigmoidal I_{max} model had a significantly better fit with a drop in OFV of 4.2, however, the hill coefficient included in the model was close to one (mean 0.816 and [0.685–0.947] CI95) and subsequently when fitting the model to the full data set, the parameter became statistically insignificant. Thus, the I_{max} model without hill coefficient was selected, Fig. 1A + B.

The data for SCO-101 shows that despite being given as a monotherapy there is a clear effect of the compound on the survival of the docetaxel resistant MDA-MD-231 cells, Fig. 1C + D. However, within the tested dose range SCO-101 does not seem to reach the same maximal effect as docetaxel. For SCO-101, the data was best described by a sigmoidal I_{max} model, showing a drop in OFV of 4 compared to the second-best model. For this model, the hill coefficient was 3.09 and was significant even when the full data set was analyzed. Model parameters for the monotherapy models are listed in Table 1.

Both model fits capture the data well, especially in the tail of the data; however, there is a noticeable upswing in OD for the lowest doses of both docetaxel and SCO-101, which is unexplained by the models.

3.2. Docetaxel and SCO-101 combination data

Cell survival was assessed following 72 h treatment with the 99 combinations of docetaxel and SCO-101 as well as the untreated controls, Fig. 2. In agreement with the monotherapy data, the data shows that SCO-101 affects cell survival, even when docetaxel is not present. In addition, the breaking point for a treatment effect of docetaxel remain at around 0.1 μM. However, a noticeable change occurs with SCO-101 doses of 50 μM and higher, where the breakpoint is less clear and an approximately linear relationship arises between docetaxel concentration and the effect on cell survival. Across all doses of SCO-101, it is evident that the maximal effect of docetaxel remains the same, essentially eliminating the cancer cells.

The combinatory effect is further explored with each dose treated as a factor in a surface plot, Fig. 3. Each of the visualized docetaxel and SCO-101 doses are log-transformed to provide a better overview of the data. Here it is evident that within a given dose of docetaxel there is an increase in efficacy as the dose of SCO-101 is increased. This trend wears off in the higher docetaxel doses were little difference in efficacy is seen with increasing SCO-101 doses. This indicates that in terms of evaluating the combination, it is uninformative to include very high doses of docetaxel. However, it is not clear whether the added effect from SCO-101 is mediated through additivity or synergistic interactions. Lastly, the surface reveals that in order to reach the overall maximal observed effect, SCO-101 alone is not enough, as it is only at high doses of docetaxel that the maximum is reached.

3.3. Combination model structure

Combination models were explored in order to discern whether the drug combination of docetaxel and SCO-101 have a synergistic or additive effect on docetaxel-resistant MDA-MB-231 cell survival. The monotherapy models where combined as either response additivity (Eq. 5) or Bliss Independence (Eq. 6) and compared using the OFV.

The Bliss Independence model showed a significantly better fit than response additivity with a ΔOFV of 319.6, while maintaining the same number of parameters in the base model. However, the best error model for response additivity was an additive error model, while Bliss Independence incorporated a combined error model of with both additive and proportional error, causing a difference of one parameter between the two. Regardless of the one parameter difference, the Bliss
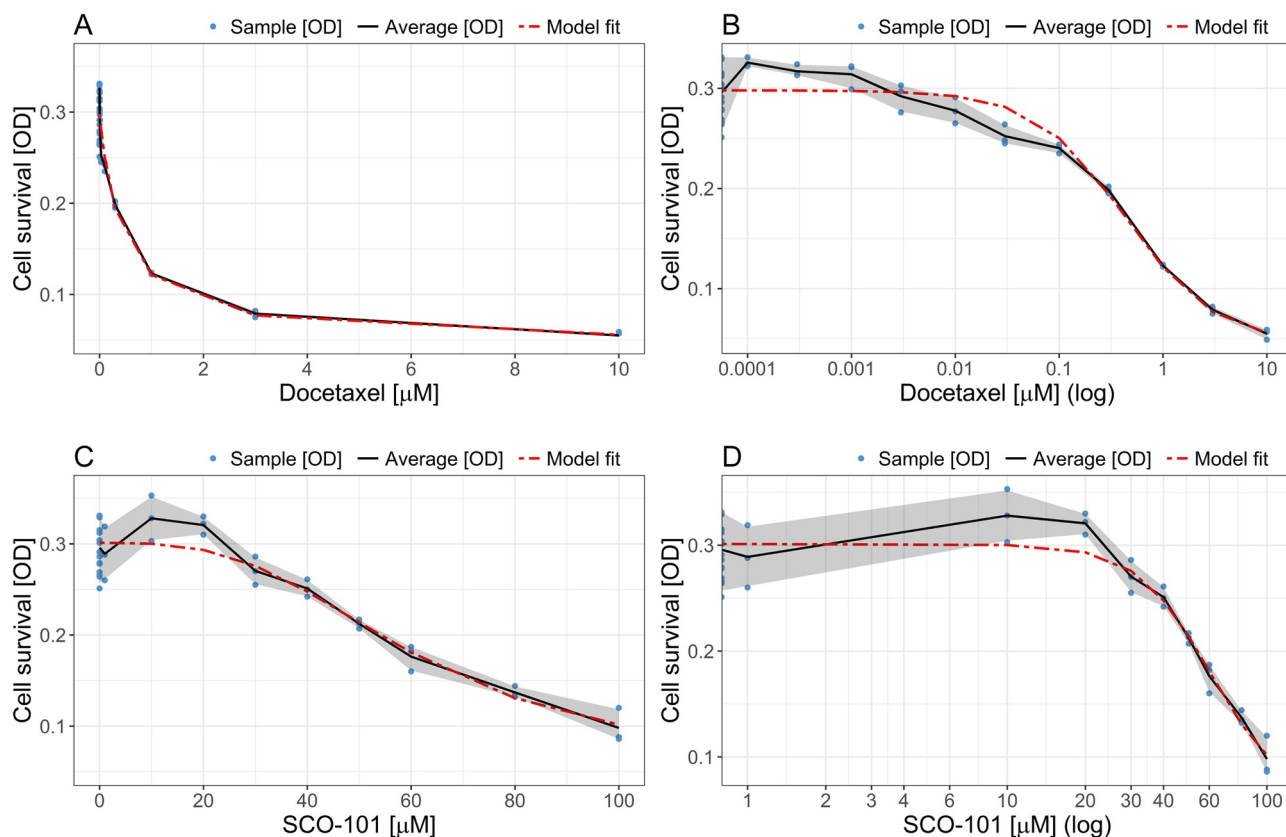
Fig. 1. MDA-MD-231 survival following monotherapy with docetaxel (A + B) or SCO-101 (C + D). The blue dots represent the samples from the experiment, while the black line corresponds to average cell survival. The red dashed line represents the fitted curve for docetaxel (Imax model) and SCO-101 (sigmoid Imax model). B and D contains the same data as A and C, respectively, but on a logarithmic scale. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Table 1
Model parameters for the monotherapy models of docetaxel (Imax) and SCO-101 (Imax with Hill coefficient).

Parameter	Estimate	Lower CI95	Upper CI95
$I_{0,\ Doce}$	0.301 [OD]	0.293 [OD]	0.309 [OD]
$I_{max,\ Doce}$	0.252 [OD]	0.244 [OD]	0.26 [OD]
$IC_{50,\ Doce}$	0.431 [µM]	0.382 [µM]	0.48 [µM]
$I_{0,\ SCO}$	0.301 [OD]	0.291 [OD]	0.311 [OD]
$I_{max,\ SCO}$	0.241 [OD]	0.17 [OD]	0.312 [OD]
$IC_{50,\ SCO}$	60 [µM]	46.2 [µM]	73.8 [µM]
H_{SCO}	3.09	2.12	4.06

I_0: Baseline; I_{max}: Maximal effect; IC_{50}: Half maximal inhibitory concentration; H: Hill coefficient; CI95: 95% confidence interval.

independence model provided a significantly better fit, and was therefore used as the basis for further exploration using the GPDI model.

Three GPDI models were formulated, two models included one-way interaction in the IC_{50} parameter and one model included two-way interactions in the IC_{50} parameter of the compounds. Model 1 considered SCO-101 as the perpetrator affecting the potency of docetaxel, while model 2 considered the reverse scenario. Model 3 included the 2-way interaction and both compounds were therefore considered perpetrators and victims. All three model used a combined error model and no parameters were fixed in the estimation.

$$I_{Doce} = \frac{I_{max,Doce} \cdot C_{Doce}}{IC_{50,Doce} \cdot \left(1 + \frac{INT_{max,SCO \to Doce} \cdot C_{SCO}}{INT_{50,SCO \to Doce} + C_{SCO}}\right) + C_{Doce}}$$

$$I_{SCO} = \frac{I_{max,SCO} \cdot C_{drug}^{H_{drug}}}{IC_{50,drug}^{H_{drug}} + C_{drug}^{H_{drug}}}$$

(Model 1)

$$I_{Doce} = \frac{I_{max,Doce} \cdot C_{Doce}}{IC_{50,Doce} + C_{Doce}}$$

$$I_{SCO} = \frac{I_{max,SCO} \cdot C_{SCO}}{IC_{50,SCO} \cdot \left(1 + \frac{INT_{max,Doce \to SCO} \cdot C_{Doce}}{INT_{50,Doce \to SCO} + C_{Doce}}\right) + C_{SCO}}$$

(Model 2)

$$I_{Doce} = \frac{I_{max,Doce} \cdot C_{Doce}}{IC_{50,Doce} \cdot \left(1 + \frac{INT_{max,SCO \to Doce} \cdot C_{SCO}}{INT_{50,SCO \to Doce} + C_{SCO}}\right) + C_{Doce}}$$

$$I_{SCO} = \frac{I_{max,SCO} \cdot C_{SCO}}{IC_{50,SCO} \cdot \left(1 + \frac{INT_{max,Doce \to SCO} \cdot C_{Doce}}{INT_{50,Doce \to SCO} + C_{Doce}}\right) + C_{SCO}}$$

(Model 3)

Of the 3 models investigated, only model 1 lead to successful convergence and provided reliable parameter estimates, as both model 2 and model 3 encountered problems with minimization and boundary issues in the interaction parameters. Another attempt to estimate model 2 and model 3 was performed using fixed parameters with values from the monotherapy models; however, problems with successful minimization and boundary issues still occurred. Therefore, model 1 was selected as the best candidate of the three GPDI models. Model 1 included two more parameters than the Bliss Independence model, and
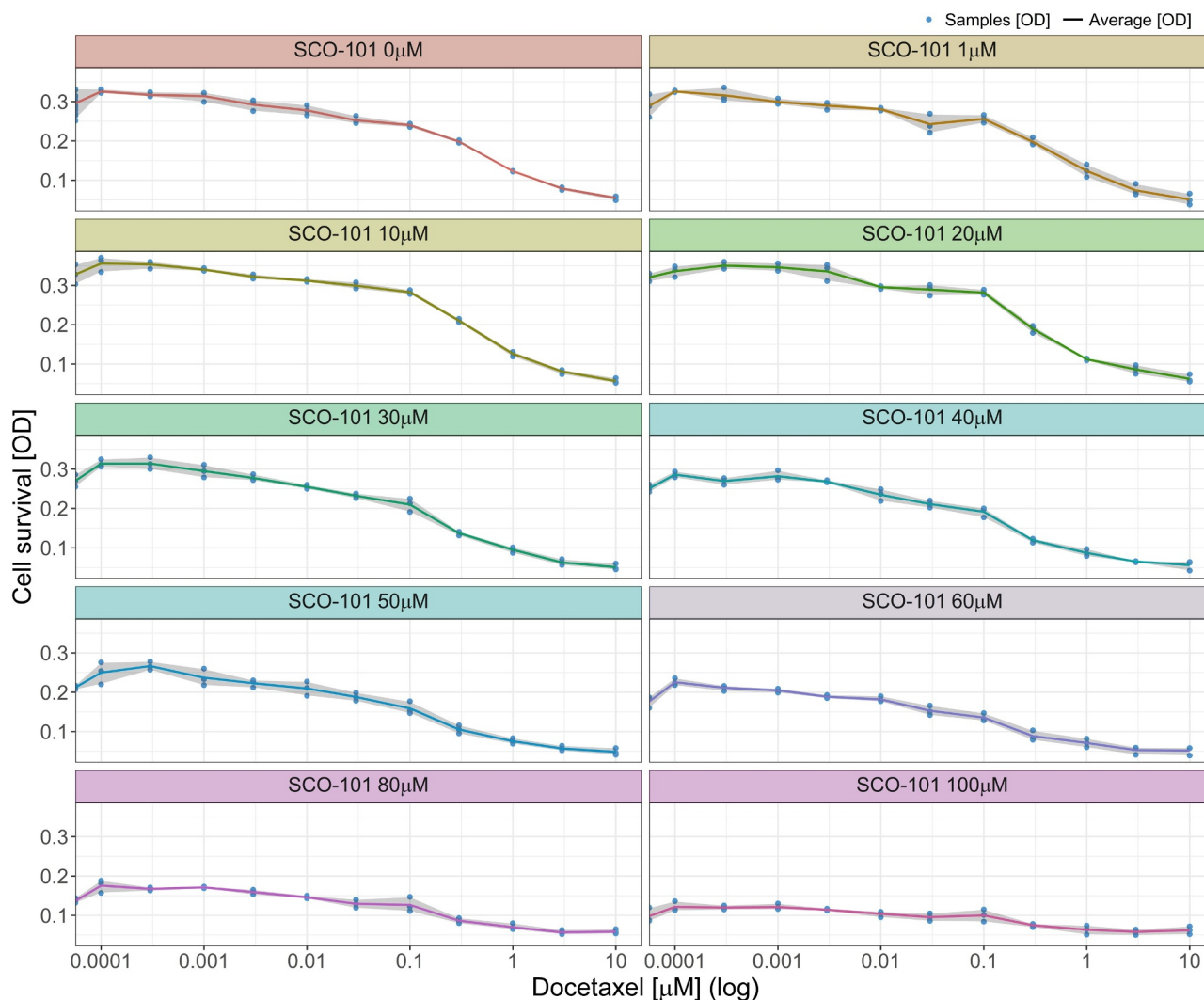
Fig. 2. MDA-MD-231 survival following combination therapy with docetaxel and SCO-101, stratified by the SCO-101 concentration. The blue dots represent the samples from the experiment, while the colored lines correspond to average cell survival within the given SCO-101 concentration. Docetaxel concentration are plotted on a logarithmic scale. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

provided a significantly better fit with a ΔOFV of 13.4.

In general, the parameter estimates in model 1 remain the same as the estimates for the monotherapy models, Table 2. The $INT_{max, SCO \rightarrow Doce}$ parameter was estimated to -0.604. When SCO-101 is administered at concentrations that reach its maximal interaction effect, this correspond to an approximate reduction of 60% in the half-maximal inhibitory concentration for docetaxel when in combination with SCO-101 compared to docetaxel alone. This change in half-maximal inhibitory concentration of docetaxel in the combination is also evident from Fig. 4 where the vertical half-maximal concentration line shifts to the left, as the SCO-101 concentrations increase. Thus, in principle at the concentration of maximal SCO-101 interaction, it is possible to attain the same level of efficacy for the combination as for docetaxel alone, but with less than half the dose of docetaxel in the combination. The $INT_{50, SCO \rightarrow Doce}$ parameter shows that this interaction occurs at lower concentrations than the individual effect of SCO-101. Specifically, the half-maximal effect for the interaction is reached at approximately 31 μM corresponding to half the concentration required to reach the half-maximal effect for the individual effect.

The model prediction was plotted on the observed data and stratified by SCO-101 dose, for visual inspection of the goodness of fit, Fig. 4. Overall, the model captures the shape of the data well, with the prediction interval covering most of the observed data points. In addition,

the weighted residuals vs. predicted plot show an even spread around zero, indicating proper model specification, supplementary material 2. However, towards tail of the data i.e. the lowest predicted OD values, the residuals indicate a slight under-prediction of the cell survival.

Lastly, the predicted response surface and the accompanying contour plot is seen in Figs. 5 and 6, respectively. Here the response surface provides an overview of the entire drug–drug interaction space, while the contour plot allows for investigation into optimal dosing pairs for specific effect targets. Visually comparing the response surface of the data, Fig. 3, with that of the prediction, Fig. 5, it is evident that the prediction captures the shape of the data surface well. However, at the highest doses of the combination the data shows an upswing in cell survival. This upswing could be an experimental artefact, which leads to the under-prediction of survival by the model prediction as was seen in the residuals vs. predicted plot.

Given the prespecified target of 85%, which corresponds to the 0.05 OD line, a range of dose pairs is viable for reaching the target. Based on Eq. 8 the lowest total dose combination was identified as 1.78 μM docetaxel and 66.3 μM SCO-101, while minimizing the exposure to both using Eq. 9 identified the dose pair of 2.85 μM docetaxel and 59.1 μM SCO-101. Both dose pairs are visualized in Fig. 6 as the red and green dot, respectively. In addition, both were considered with a weighting penalty factor of 2 on the docetaxel concentration, as docetaxel
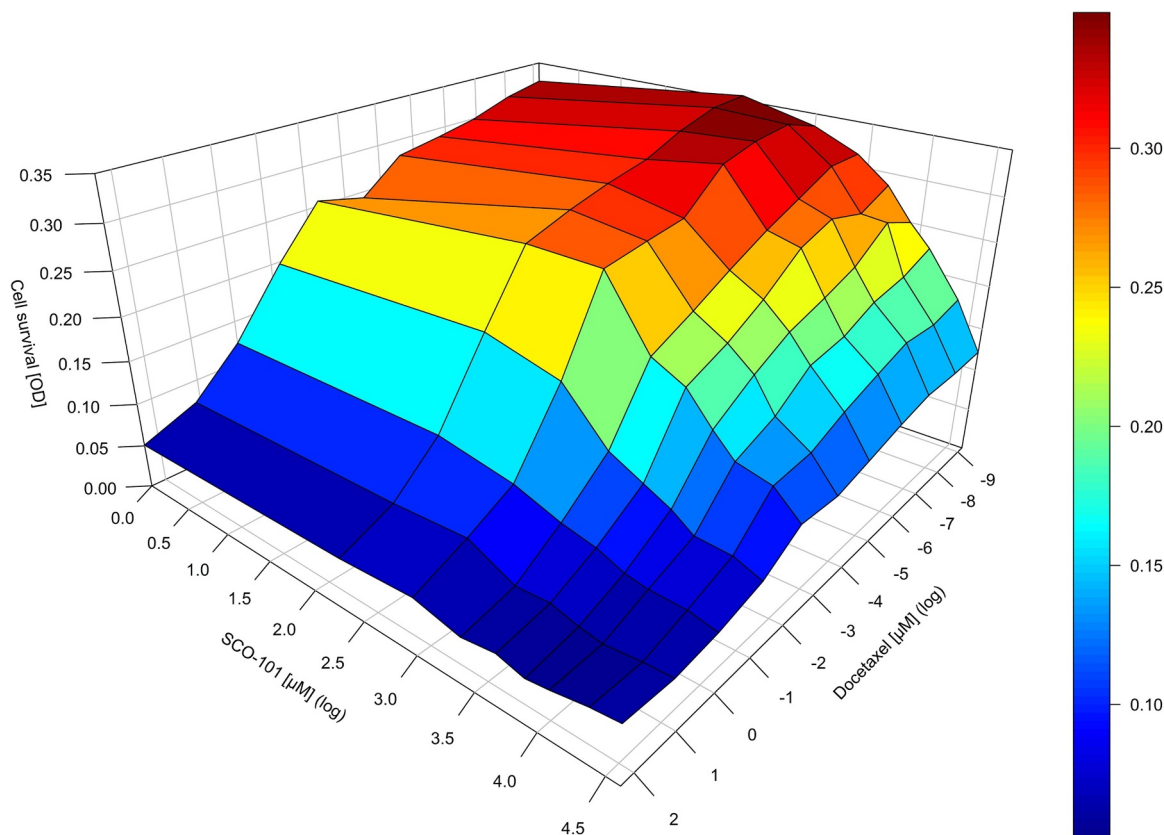
Fig. 3. Response surface of MDA-MD-231 survival following combination therapy with docetaxel and SCO-101. The doses of both docetaxel and SCO-101 are log transformed and the colors correspond to the observed OD.

Table 2
Model parameters for the GPDI interaction model with SCO-101 as the perpetrator affecting the potency of docetaxel.

Parameter	Estimate	Lower CI95	Upper CI95
I_0	0.315 [OD]	0.309 [OD]	0.321 [OD]
$I_{max, Doce}$	0.253 [OD]	0.246 [OD]	0.26 [OD]
$IC_{50, Doce}$	0.325 [μM]	0.282 [μM]	0.368 [μM]
$I_{max, SCO}$	0.227 [OD]	0.2 [OD]	0.254 [OD]
$IC_{50, SCO}$	59.4 [μM]	53.1 [μM]	65.7 [μM]
H_{SCO}	3.09	2.61	3.45
$INT_{max, SCO \rightarrow Doce}$	−0.604	−0.855	−0.353
$INT_{50, SCO \rightarrow Doce}$	30.9 [μM]	16.2 [μM]	45.6 [μM]

I_0: Baseline; I_{max}: Maximal inhibition; IC_{50}: Half maximal inhibitory concentration; H: Hill coefficient; $INT_{max, SCO \rightarrow Doce}$: Maximal interaction effect; $INT_{50, SCO \rightarrow Doce}$: Half maximal interaction concentration; CI95: 95% confidence interval

represents the more toxic of the two compounds. This approach identified the dose pair 1.17 μM docetaxel and 74.7 μM SCO-101 as providing the lowest total dose and 1.68 μM docetaxel and 67.3 μM SCO-101 the minimized exposure to both. These pairs are visualized in Fig. 6 as the orange and yellow dot, respectively.

4. Discussion

4.1. Modelling drug interactions

From a clinical perspective, assessing combination treatment in cancer is not only about defining combinations as additive or synergistic. Quantifying synergy or additivity is important for identifying promising treatment combinations, where the clinical issue is lack of efficacy. However, if the lack of efficacy is driven by emergence of resistance, it is essential that the synergistic effect exists even in the presence of drug resistance. Firmly understanding the underlying biological mechanisms for both the cancer and the pharmaceuticals is an important aspect in determining the potential for cross-resistance to compounds in combinations. However, this approach is not only time consuming but also does not necessarily quantify the interactions in interpretable ways. Proxies for understanding these mechanisms and quantification of the interactions can be achieved through modelling.

Modelling of drug interactions in cancer chemotherapeuticals has previously been done on preclinical data (Frances et al., 2011, Goteti et al., 2010, Rocchetti et al., 2009, Koch et al., 2009). In these studies, different approaches were taken to the modelling, but they all originated from considering differential equations describing the tumuor growth. The advantage of this approach is the semi-physiological nature of the model, which attempts to capture the growth cycle of the tumuor. The drawback is however, that the drug effect is often described through kill rates, which can be difficult to interpret and the models can be parameter intensive, necessitating a large amount of data.

A core issue of evaluating drug interactions is the selection of an additivity criterion. Several such criterion exists amongst the most common are Loewe additivity (Greco et al., 1995, LOEWE, 1953) and Bliss Independence (Greco et al., 1995, Bliss, 1939). The quantification of synergy or antagonism is here dependent on base assumptions surrounding the underlying mechanism for the combined drugs. Modelling drug interactions with the GPDI model addresses the issues with the additivity criterions in a model-based framework that unifies the interpretation of these additivity criteria (Wicha et al., 2017).

4.2. SCO-101 and Docetaxel

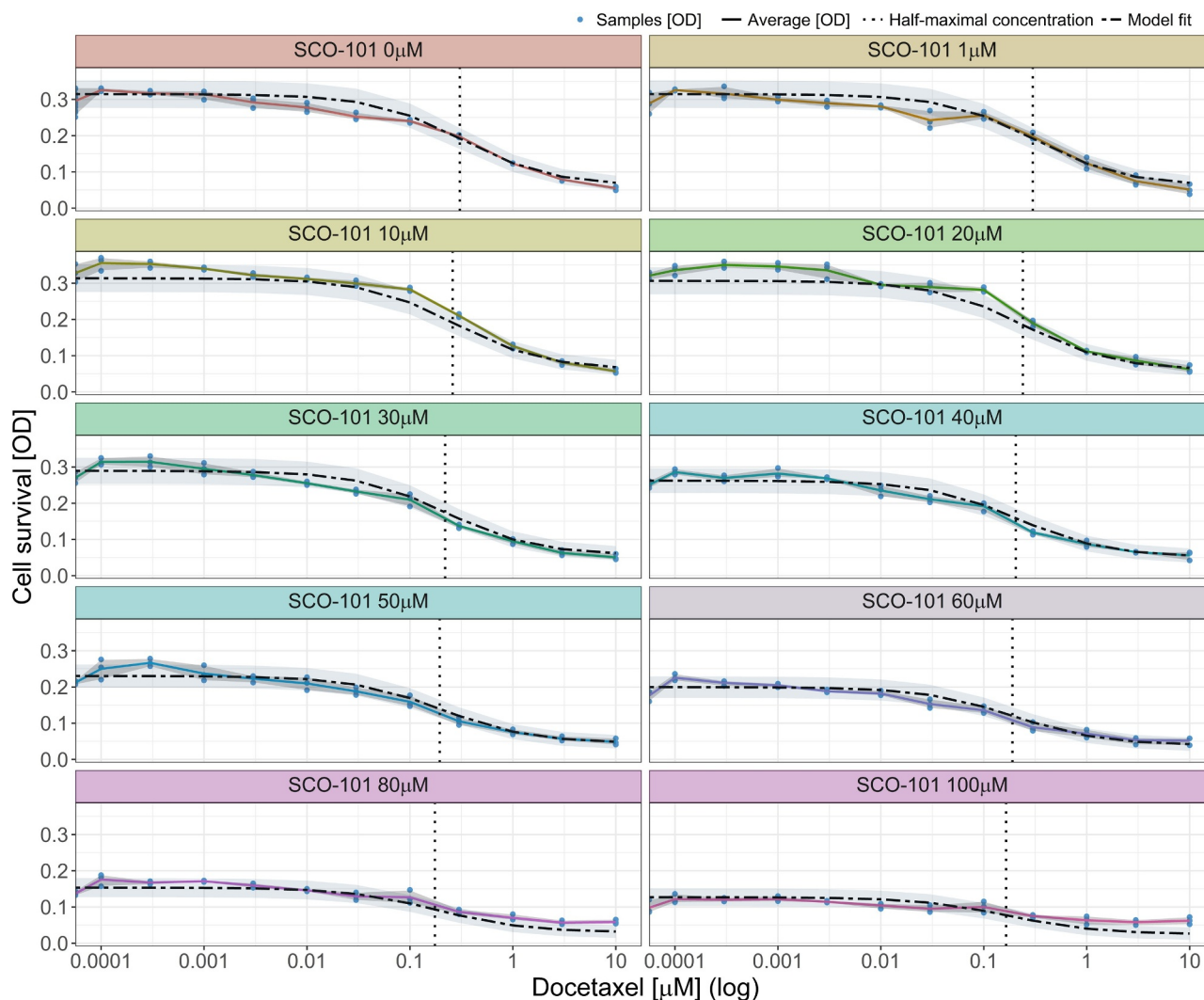Triple-negative breast cancer (TNBC) is cancer that tests negative

Fig. 4. MDA-MD-231 survival following combination therapy with docetaxel and SCO-101, stratified by the SCO-101 concentration. The blue dots represent the samples from the experiment, while the coloured lines correspond to average cell survival within the given SCO-101 concentration. Docetaxel concentration are plotted on a logarithmic scale. The dot-dashed line represents the model fit and the grey area around the model fit represent the residual error in the model. The vertical dotted line shows the half-maximal concentration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

for estrogen receptors, progesterone receptors, and excess HER2 protein. In the present study, a model for TNBC was employed as a simulation of the clinical issue of docetaxel-resistant breast cancer, namely MDA-MB-293 cells with induced docetaxel-resistance. In this setting, the parameters describing the effect of docetaxel and the novel compound SCO-101 was estimated (Table 1). Despite the resistance to docetaxel treatment, an effect of the compound is still observed at higher doses. For docetaxel, a maximum effect of approximately 80% reduction in cell survival and an IC_{50} value of 0.431 μM was observed. These values correspond well with another study using docetaxel resistant MDA-MB-231 cells (Dey et al., 2017). One difference is that the IC_{50} value of the referenced study relates to 50% cell survival, whereas in the present study the IC_{50} value relates to half-maximal effect for the compound.

The main goal of this study was characterizing the novel compound SCO-101 and its pharmacodynamic interaction with docetaxel. To this end, the model for SCO-101 monotherapy showed that it elicited an effect on cell survival, which is not mediated through an interaction with docetaxel. Interestingly, the maximal effect of SCO-101 is comparable to that of docetaxel, with a maximal reduction in cell survival of 0.227 and 0.253 OD, respectively. The combined effect of docetaxel

and SCO-101 was estimated using the GPDI model, which rely on effect-based estimation of the combination as opposed to growth models previously discussed (Frances et al., 2011, Goteti et al., 2010, Rocchetti et al., 2009, Koch et al., 2009). The most significant parameters from this model (Table 2) are the interaction parameters, as the other estimates remained the same as their monotherapy counterparts. $INT_{50, \, SCO \rightarrow Doce}$ was estimated to 30.9 μM, corresponding to half the concentration of that required to reach its half-maximal effect on cell survival (i.e. IC_{50} of 59.4 μM). Based on these estimates, the benefit of SCO-101 for the treatment of breast cancer is even higher when administered in conjunction with docetaxel, than when given as a monotherapy. Furthermore, depending on tolerability, the administered dose of SCO-101 might not reach concentrations where the individual effect is significant, thus, the most important aspect of SCO-101's treatment capacity in breast cancer could be its pharmacodynamic interaction with docetaxel.

Estimating the potency of the interaction is another advantage of the modelling approach compared to the traditional biological evaluation. The implicit underlying assumption being that the potency of the compound is the same for its effect on cell survival and for the interaction (Dey et al., 2017). As opposed to the biological studies, this
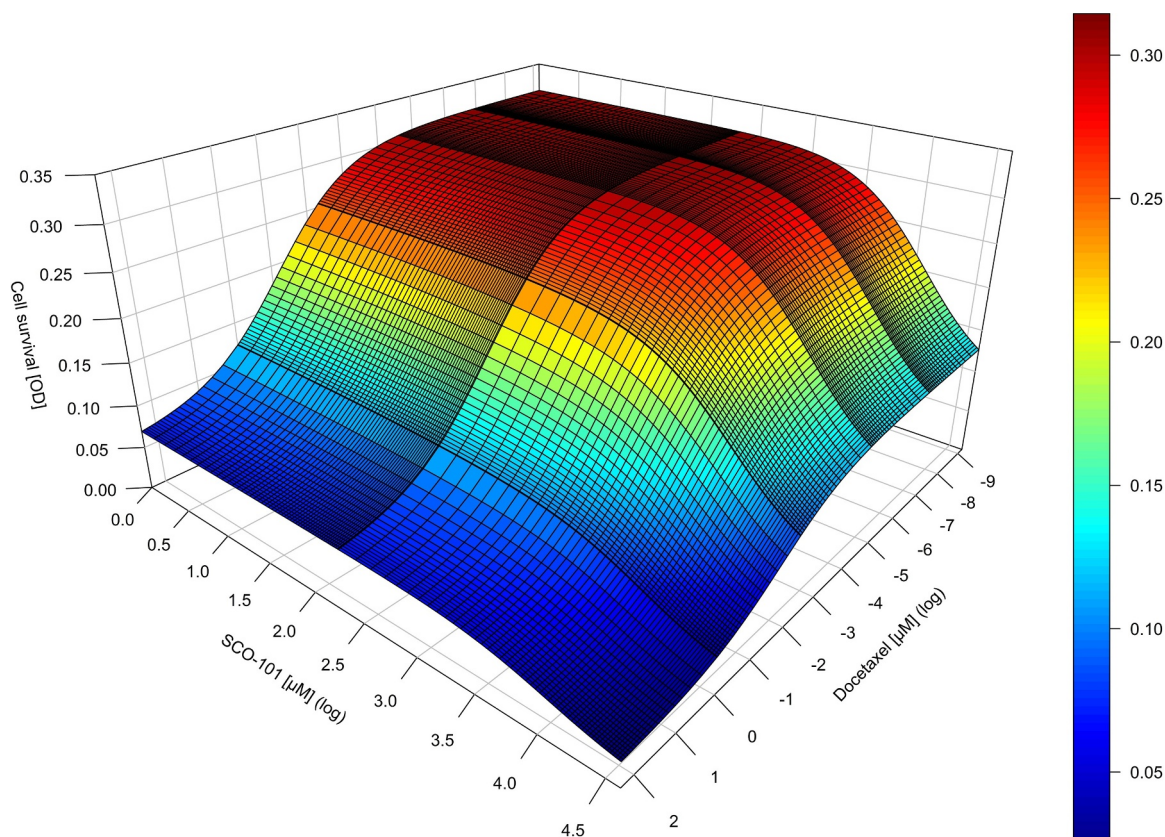
Fig. 5. Predicted response surface of the final general pharmacodynamics interaction model. The resolution of the predicted surface is 100 × 100 dose pairs on a logarithmic scale with the colours corresponding to the resulting effect.

type of model will elucidate whether there is a difference in the potency of the perpetrator compound on the cell survival and on the interaction with the victim compound, which can provide valuable information.

The second parameter of interest is $INT_{max, \; SCO \; \to \; Doce}$. This parameter represents the estimated maximal change in the potency of the victim drug (docetaxel) in the presence of the perpetrator drug (SCO-101). This was estimated to -0.604, which correspond to an approximately 60% decrease in the half-maximal inhibitory concentration of docetaxel when in the combination compared to docetaxel alone. Thus, given that the maximal interaction effect from SCO-101 can be reached safely, the combination of it and docetaxel leads to an expected increase in potency of 60% compared to docetaxel alone.

While the GPDI model structure in itself is empirical, the interpretation of the interaction parameters leads to semi-mechanistic understanding of the interaction. Clinically this interpretation of the interaction parameters means that either the docetaxel dose can be reduced, thereby maintaining the same efficacy but reducing toxicity or the dose can be maintained, thereby achieving a higher efficacy without increasing the side effects from docetaxel. Furthermore, the GPDI model allows for identification of victim and perpetrator drugs, which can be essential when performing large analyses to map interaction networks (Wicha et al., 2017).

4.3. Future studies and extrapolation

The data in the present study is from *in vitro* cell experiments and the results thereof are not considered directly translatable to that of *in vivo* or even human data. One key limitation of modelling *in vitro* experiments is that for the very controlled *in vitro* setting, it is possible to obtain low uncertainty and variability in the estimated parameters, such as IC_{50}, which is infeasible in an *in vivo* setting, as it is an inherently more variable setting. However, the data from this study can

be used to inform further studies and guide the selection of promising drug candidates. One method for supporting further studies *in vivo* is the use of *in vitro in vivo* extrapolation (IVIVE). IVIVE often makes use of physiology-based pharmacokinetic modelling and through modelling and simulation of the physiological parameters attempts to perform a quantitative extrapolation of the drug exposure (Cho et al., 2014, Darwich et al., 2017). Via these methods, it is possible to approximate a dose to carry forward to *in vivo* studies.

Lastly, based on the *in vitro* data some overall considerations and an approximation of the dose ratio can be made. In order to investigate the interaction, it is essential that docetaxel doses are not approaching the dose resulting in maximal effect, as there is no interaction to observe at this level. Furthermore, depending on the factors that govern what the most desirable drug-drug combination is, a ratio between the compounds can be identified for further studies. In the present study, docetaxel presents the more toxic of the compounds, a penalty for the docetaxel concentration is therefore appropriate when identifying optimal dose pairs. Thus, dose ratios between 1:40 and 1:64 (docetaxel:SCO-101) will be of interest, as these were identified as the lowest total drug combination and the minimized exposure to both compounds, respectively, when docetaxel had a weighted penalty factor of 2.

In conclusion, a pharmacodynamic model to describe the effect of SCO-101, docetaxel, and the combination was established. SCO-101 is shown to be a promising compound for the treatment of TNBC and provides a synergistic interaction with docetaxel, showing an increase in potency of approximately 60% of the combination compared to docetaxel alone. Furthermore, modelling the *in vitro* studies have provided key information regarding dose ratios and potentially dose level for carrying out future studies. Lastly, the study displays a practical application of the GPDI model, which provides a modelling framework with significant advantages by allowing for identification of victim and
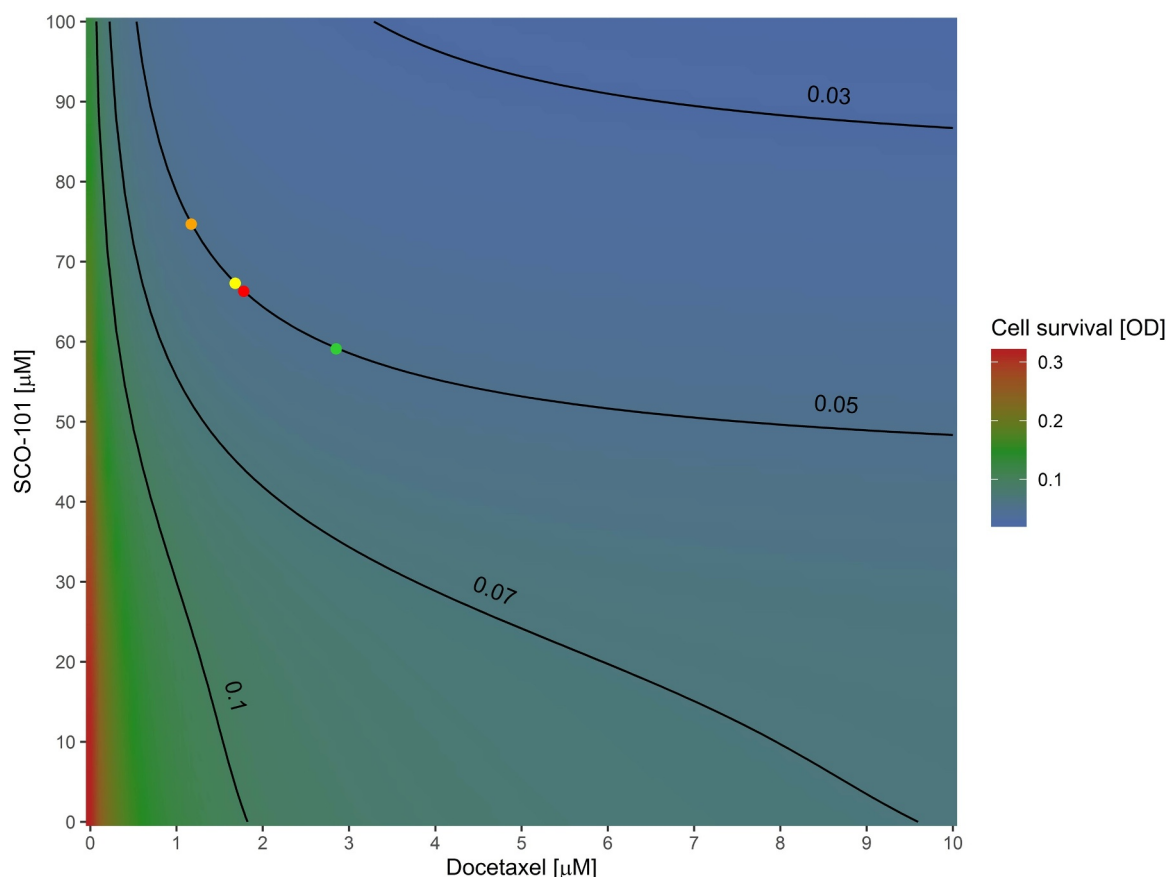
Fig. 6. Contour plot of the final general pharmacodynamics interaction model. The lines indicate four separate response levels of 0.1 OD, 0.07 OD, 0.05 OD, and 0.03 OD across the dose combination space. 0.05 OD corresponds to the 85% reduction in cell viability target. The four dots highlight dose pairs that result in meeting this target, through lowest total dose combination (red), minimized exposure to both compounds (green), and with a weighted penalty factor of 2 on lowest total dose combination (orange) and minimized exposure (yellow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

perpetrator drugs as well as interpretation of the pharmacodynamic interaction parameters, as opposed to the traditional approach of classification as either synergistic, additive or antagonistic drug-drug combinations.

Credit author statement

ANN, SOB, NB, JS and TML contributed to the conceptual design. SOB performed the cell viability study and ANN performed the modelling analysis while TML supervised. ANN wrote the first draft and all authors contributed to the interpretation of the data. All authors were involved in the writing, reviewing, and editing of the manuscript and approved the final manuscript.

Declaration of Competing Interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2020.105315.

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