Dynamic phenotypic switching and group behavior help non-small cell lung cancer cells evade chemotherapy

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Abstract

Drug resistance, a major challenge in cancer therapy, is typically attributed to mutations and genetic heterogeneity. On the other hand, emerging evidence suggests that dynamic cellular interactions and group behavior also contribute to drug resistance. However, the underlying mechanisms remain poorly understood. Here, we present a new mathematical approach with game theoretical underpinnings that we developed to model real-time growth data of non-small cell lung cancer (NSCLC) cells and discern patterns in response to treatment 68 with cisplatin. We show that the cisplatin-sensitive and cisplatin-tolerant NSCLC cells when co69 cultured in the absence or presence of the drug, display dynamic group behavior strategies. Tolerant cells exhibit a 'persister-like' behavior and are attenuated by sensitive cells; they also appear to 'educate' sensitive cells to evade chemotherapy. Further, tolerant cells can switch phenotypes to become sensitive, especially at low cisplatin concentrations. Finally, switching treatment from continuous to an intermittent regimen can attenuate the emergence of tolerant cells, suggesting that intermittent chemotherapy may improve outcomes in lung cancer.

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conforming to the relevant regulatory standards under protocol IACUC# 18119. The zebrafish larvae used for microinjections were 48 h old i.e., 2 days post fertilization (dpf), and were continuously observed for tumor development until 7 dpf. During this period, no sex differentiation is observed. Ovarian development is the default pathway, which is initiated at 10 dpf and around 21 dpf sexual differentiation into females and males is established. The two cell lines used for microinjections were H2009 and H23. The cell lines were obtained from ATCC. H2009 is an adenocarcinoma cell line derived from a 68-year-old female patient and H23 is another adenocarcinoma cell line derived from a 51-year-old male patient.

Code Availability: The codes used in our data analysis have been deposited in a private repository on Github. This will be made public once the article is accepted. However, to share it with the reviewers, we have prepared a Google Drive folder containing the compressed files of the private GitHub repository (https://drive.google.com/drive/folders/1AcT6gd8pOokq6uTCQlGiGscUNYOnMoBq?usp=sharing). Once unzipped, the README.md file within the master directory will give all code viewers clear directions and guidance for navigating sub-directories and running all relevant codes for models used in the manuscript."

Running title: Modeling chemoresistance

Key Words: chemoresistance, cisplatin, lung cancer, evolutionary game theory, group behavior, persister trait, phenotypic switching

Summary

Drug resistance, a major challenge in cancer therapy, is typically attributed to mutations and genetic heterogeneity. On the other hand, emerging evidence suggests that dynamic cellular interactions and group behavior also contribute to drug resistance. However, the underlying mechanisms remain poorly understood. Here, we present a new mathematical approach with game theoretical underpinnings that we developed to model real-time growth data of non-small cell lung cancer (NSCLC) cells and discern patterns in response to treatment with cisplatin. We show that the cisplatin-sensitive and cisplatin-tolerant NSCLC cells when co-cultured in the absence or presence of the drug, display dynamic group behavior strategies. Tolerant cells exhibit a 'persister-like' behavior and are attenuated by sensitive cells; they also appear to 'educate' sensitive cells to evade chemotherapy. Further, tolerant cells can switch phenotypes to become sensitive, especially at low cisplatin concentrations. Finally, switching treatment from continuous to an intermittent regimen can attenuate the emergence of tolerant cells, suggesting that intermittent chemotherapy may improve outcomes in lung cancer.

Introduction

Drug resistance in cancer is generally believed to arise stochastically through random genetic mutations and the subsequent expansion of mutant clones via Darwinian selection.[1,2] However, emerging evidence suggests that non-genetic and epigenetic mechanisms may also play a critical role[3-5], leading to enhanced adaptability and cooperation under stressful conditions.[3-5] Nonetheless, such mechanisms have not been fully explored and are rarely integrated into clinical trials or in precision oncology initiatives.[6-8] Furthermore, combining conventional therapies with treatment strategies based on cancer ecology could potentially delay or even prevent drug tolerance and eventually, drug resistance.[2,8-10]

Several studies have reported the existence of drug-resistant and tolerant (clones that are weakly or moderately resistant) clones in pre-treatment tumors[11], although the population of these clones is usually low in the presence of drug-sensitive cells [12]. This raises the question, how do drug-sensitive and resistant/tolerant clones in a tumor influence each other's fitness (growth), and whether cooperation and competition (group behavior) between the sensitive and tolerant cells influence the response to drug therapy. Thus, discerning group behavior by monitoring interactions between drug-tolerant and -sensitive cells in real time in absence or presence of the drug is a powerful tool to elucidate the role of group behavior.[13,14]

Here, we have used human non-small cell lung cancer (NSCLC) cells that are sensitive or tolerant to cisplatin, one of the most commonly used chemotherapy, to understand the role of group behavior in emergence of drug-tolerant clones, and eventually resistant clones. The cells expressing red fluorescent protein (RFP) or green fluorescent protein (GFP) were mixed (heterotypic culture) and cultured in different ratios. The proliferation of the two cell types was followed in real time and compared to the same cells grown alone (monotypic culture). The cell counts were used to determine the dynamic behavior of the population, and results were correlated with the cell-autonomous and non-cell-autonomous fitness (growth rate) effects[5]. Since cell-autonomous fitness effects are defined as those inherent to the cell, the growth rates from monotypic cultures provided the necessary information for determining these effects.[4] In contrast, non-cell-autonomous effects are those that allow fitness to depend on a cell's microenvironment including the frequency of other cellular phenotypes as well as diffusible factors in the media.[15]

Since standard models based on evolutionary game theory proved inadequate to analyze the data, we developed a new approach, Phenotypic Switch Model with Stress Response (PSMSR), that incorporates concepts from chemical reaction kinetics and the cooperative behavior of drug-tolerant phenotypes in the community. A distinguishing feature of the PSMSR model is that it considers the ability of cancer cells to switch phenotypes. Employing PSMSR, we showed that quantitatively, the two cell populations when co-cultured in the absence of cisplatin, display dynamic group behavior that can be interpreted using evolutionary game theory as payoffs (benefit or loss of individual players associated with a set of game strategies such as competition or cooperation). Due to phenotypic switching by the cells, the game strategies were dynamically altered based on cell frequencies and stress level while maximizing group survival. However, in presence of cisplatin, the group behavior (cooperation)

118 was attenuated in favor of self-survival. Furthermore, we demonstrate that switching
119 treatments from a continuous to an intermittent regimen can attenuate the emergence of
120 tolerant cells, underscoring a potentially new treatment option that could benefit patients with
121 NSCLC.

122 **Results**

123 *Cisplatin-sensitive and tolerant cells demonstrate different behaviors in monotypic and*
124 *heterotypic cultures*

125 A schematic overview summarizing the experiments and the source of data collection
126 used in developing the theoretical models are presented in **Fig.1A-C**. Fluorescently labeled
127 cisplatin-sensitive H23 and cisplatin-tolerant H2009 NSCLC cells[16] were co-cultured and
128 monitored in real time (**Supplementary Fig. 1A & B**). To discern differences in their behavior,
129 the two cell cultures were grown as monotypic or as heterotypic cultures in a 1:1 ratio. To
130 determine the short-term effects of heterotypic culture, we incubated the cells for 12 h before
131 the start of the experiment (**Supplementary Fig. 2A,** schematic). But to determine the long-
132 term effects, they were co-cultured for 3 weeks (without cisplatin) before the start of the
133 experiment (**Fig. 2A**, schematic).

134 At a 1:1 ratio, there was no significant difference in the fold-change (ratio of the cell
135 population at any time point relative to t = 0 hours) of the sensitive cell counts between the
136 monotypic (grown by themselves) and heterotypic cultures (mixed with tolerant cells for 12 h
137 or 3 weeks prior to counting), and they were equally sensitive to 5 µM cisplatin in all three
138 conditions (**Fig. 2A**, left panel bar graph in red). These monotypic and heterotypic culture
139 experiments were also performed using tolerant cells, and no significant change in cell count or
140 drug tolerance was observed for the cells mixed only for 12 h prior to the experiment (**Fig. 2A**,
141 right panel bar graph in green, bars labeled "Alone" and "Mix before"). However, when tolerant
142 cells were co-cultured with sensitive cells for 3 weeks prior to the experiment, they showed a
143 marked reduction in cell proliferation (**Fig. 2A** green bar graph, dark green bar labeled "3-wk co-
144 culture"). Moreover, when cisplatin was added to this 3-week co-cultured cells, the tolerant
145 cells showed a smaller reduction in cell growth compared to when cultured separately or mixed
146 12 h before the experiment was started (**Fig. 2A**, right panel bar graph in green), and their
147 proliferation was significantly attenuated by long coexistence with the sensitive cells,
148 suggesting that tolerant cells appear to exhibit a 'persister-like' trait (please see the
149 "Discussion" section).[17,18]

150 We further explored the long term 3-week co-culture experiments by seeding the cells
151 at increasing tolerant to sensitive ratios (1:1, 2:1, 4:1, 8:1), and recorded their growth every 2 h
152 using the IncuCyte live cell imaging system (**Supplementary Fig. 1C** schematic). At a seeding
153 ratio of 1:1, the sensitive cells showed an 8-fold increase in cell count within 96 h, and reached
154 a plateau post 96 h, whereas the tolerant cells exhibited a 4-fold increase in cell count within 72
155 hours, followed by a drop and plateau for the rest of the time (**Fig. 2B**). At the end of the
156 experiment i.e. 144 h, the sensitive cell growth was 2.5-fold more than the tolerant cells for 1:1
157 ratio, compared to 1.5, 1.4 and 1.2-fold for the 2:1, 4:1 and 8:1 ratios, respectively (**Fig. 2C**).

158 These data revealed that the tolerant cell proliferation was suppressed in presence of sensitive
159 cells but could be rescued by increasing the fraction of tolerant cells in the co-cultures.

160 Next, we determined the proliferation profile for all the seeding ratios in presence of
161 cisplatin. Cisplatin had a cytostatic effect on the sensitive cells, and the fold change in the cell
162 count remained approximately 1 for all the ratios (**Fig. 2D**, purple bar graph). However, the
163 tolerant cell proliferation was approximately 1.4, 2.09, 1.95, and 2.04 fold at the
164 tolerant:sensitive seeding ratios 1:1, 2:1, 4:1 and 8:1, respectively. Therefore, the
165 administration of cisplatin rescued the tolerant cell growth from the suppressive effect of the
166 sensitive cells by selectively curtailing the sensitive cell growth. In fact, the tolerant cells
167 proliferated better in the presence of cisplatin and increasing the seeding ratio of tolerant cells
168 in the population also favored their growth (**Fig. 2D**).

169 The suppressive effect of the sensitive cells on the tolerant cells in a frequency-
170 dependent manner was also evident by analyzing the change in tolerant to sensitive fraction at
171 the end of 144 hours in the untreated conditions (0.2, 1.4, 6.3, 17.4 at T:S seeding ratios of 1:1,
172 2:1, 4:1 and 8:1, respectively, **Fig. 2E**, black bars). In addition, the tolerant to sensitive fractions
173 in presence of cisplatin were 0.8, 4.9, 16.9 and 38.8 for the same seeding ratios (**Fig. 2E**, orange
174 bars). Thus, there was an increase in the tolerant to sensitive cell fraction in the population
175 treated with cisplatin, suggesting a reduction in the sensitive cell population as well as better
176 fitness of the tolerant cells in presence of cisplatin.

177 *Sensitive cells suppress growth of tolerant cells in absence of drug*

178 To discern the effect of short-term association between the sensitive and the tolerant
179 cells on their group behavior, we repeated the above experiments by incubating the co-culture
180 for only 12 h instead of 3 weeks prior to starting the experiments (**Supplementary Fig. 2A**,
181 schematic). Here, at 1:1 tolerant:sensitive seeding ratio, the fold change in the tolerant cell
182 population at the end of 144 h was 8-fold (**Supplementary Fig. 2B),** whereas after three weeks
183 of co-culture, we observed a 4 fold change in the growth **(Fig. 2C and Supplementary Table 1**).
184 Also, compared to the 3 weeks co-culture, where the tolerant cell fold changes were
185 significantly lower ($p<0.0001$) than that of the sensitive cells for all seeding ratios except 8:1,
186 the 12-hour co-culture showed less difference in fold changes between the two cell types ($p<$
187 0.001) at seeding ratios 2:1 and 4:1, and insignificant at 8:1 (**Supplementary Fig. 2E**). The
188 tolerant to sensitive fraction in the population at 144 hours was also higher than in the 3 weeks
189 co-culture experiments for seeding ratios 1:1 and 2:1 (1.2 and 3.3 vs. 0.2 and 1.4 in the 3 weeks
190 co-culture, **Supplementary Fig. 2G**). Taken together, these data show that the short-term
191 association between the two cell types did not suppress the tolerant cell population as
192 efficiently as the long-term association. However, in presence of cisplatin, the tolerant cell
193 proliferation was significantly higher compared to the sensitive cells ($p<0.0001$, **Supplementary**
194 **Fig. 2C and F**). Next, we compared the change in the tolerant cells to the sensitive cell fraction
195 and observed a similar trend as seen in the 3 weeks co-culture for all seeding ratios. The
196 increase in growth of the tolerant cells was also supported by the increase in their fraction in
197 the population in absence of cisplatin, and further the presence of cisplatin supported their
198 growth. (**Supplementary Fig. 2C, 2D orange line graph and 2G** orange bar graph,).

199 The growth trends indicated a competition between the two cell types which was
200 enhanced by long-term association. In contrast, the behavior of the tolerant cells suggested
201 mutual cooperation to improve survival, which was favored by the higher seeding ratios. To
202 explore the frequency-dependent competition of the sensitive cells towards the tolerant cells,
203 we expanded the seeding ratios to increased proportions of sensitive cells in the population
204 (i.e. sensitive to tolerant ratios of 1:1 to 8:1) (**Supplementary Fig. 3A**, schematic).

205 Consistent with the previous experiments, in the absence of cisplatin, the sensitive cells
206 suppressed the growth of tolerant cells (**Supplementary Fig. 3B** and **E**). Again, in the presence
207 of cisplatin, the tolerant cell growth was dominated in the population by approximately 1.9, 2.2,
208 2.7 or 3.8-fold over sensitive cells for the sensitive to tolerant ratios of 1:1, 2:1, 4:1 or 8:1,
209 respectively (**Supplementary Fig. 3F**). The increase in growth of the tolerant cells was also
210 supported by the presence of cisplatin (**Supplementary Fig. 3C,3D orange line graph and 3G**
211 **orange bar graph**). Together, these experiments indicated that in a heterogeneous population,
212 dynamic competition and cooperation exist between the sensitive and the tolerant cells, and
213 the sensitive cells dominate over the tolerant cells. In contrast, the presence of cisplatin favors
214 the survival and proliferation of the tolerant cells by inducing cell death among the sensitive
215 cells.

216 *Sensitive cells secrete a factor(s) that retards the growth of tolerant cells*

217 To discern whether a physical interaction between the two cell types is necessary for
218 this, or the sensitive cells secrete an 'inhibitory factor' to attenuate tolerant cell growth, the
219 cells were grown in conditioned medium from sensitive or tolerant cell monocultures (**Fig. 2F,**
220 schematic). As seen in the right graph in **Fig. 2G**, conditioned medium from the sensitive cells
221 impeded proliferation of tolerant cells by ~4.5-fold. In contrast, the conditioned medium from
222 the tolerant cells had no significant effect on the growth of the sensitive cells (**Fig. 2G**, left
223 graph), alluding to the presence of one or more inhibitory factors in the conditioned medium
224 from the sensitive cells. Furthermore, at a lower seeding density (**Fig. 2H,** schematic), the
225 conditioned medium had a greater inhibitory effect (approximately 6-fold) on the tolerant cells
226 and reduced in a dose-dependent fashion with an increase in tolerant cell seeding density (**Fig.**
227 **2I**). Thus, the suppressive effect of the sensitive cell-conditioned medium was reduced with a
228 greater number of tolerant cells (**Fig. 2I**).

229 *Intermittent therapy can sustain a population of cisplatin-sensitive tumor cells while attenuating*
230 *the proliferation of resistant cells*

231 Since the proliferation of the tolerant cells was remarkably impeded when co-cultured
232 with sensitive cells for prolonged periods prior to cisplatin treatment, we asked if continuous or
233 intermittent cisplatin treatments would differentially affect a mixed population of the two cell
234 types. Toward this end, we mixed the two cell types at different sensitive to tolerant ratios and
235 treated them as described in **Supplementary Fig. 4,** schematic. Within 10 days, we observed
236 that the ratio of tolerant to sensitive cells increased by 50- to 100-fold for the initial seeding
237 ratios of 1:1, 2:1 and 4:1 (sensitive:tolerant), respectively, under continuous treatment. On the
238 other hand, the tolerant to sensitive (T:S) ratio for the intermittent therapy increased only 3- to

239 8-fold (**Fig. 3A-C**), suggesting that sensitive cells were able to recover from the drug toxicity and
240 proliferate.

241 We prolonged the intermittent therapy by splitting the cells growing in cisplatin-free
242 media post cisplatin treatment into two sets: 'Intermittent 1 cycle' and 'Intermittent 2 cycles' as
243 described **Supplementary Fig. 4**. In 'Intermittent 1', we observed the (initially cisplatin exposed)
244 cells cisplatin free for 25 days, whereas in 'Intermittent 2', we treated the cells with one extra
245 dose of cisplatin for 4 days, before observing them in cisplatin-free media for the rest of the
246 duration. We then asked if the sensitive cells once exposed to cisplatin would outgrow the
247 tolerant cells to recapitulate the data shown in **Fig. 2B** and **Supplementary Fig. 3B**.

248 We continued the culture for 24 days, to let the cells grow and once confluent, passaged
249 1 to 5 every 6-8 days. We observed that the tolerant vs. sensitive ratio fell to approximately 2,
250 1.6 and 0.4 for initial seeding densities of 1:1, 2:1 or 4:1, respectively, for the cells treated only
251 once with cisplatin on Day 3 (Intermittent – 1 cycle) (**Fig. 3D-F**, black line). In contrast, the ratio
252 of cells that received the 2nd dose of cisplatin treatment ('Intermittent – 2 cycles') and were
253 allowed to recover in fresh media, did not show any decrease in the tolerant population and
254 maintained a S:T ratio of 100/800 (**Fig. 3D-F**, red line). To validate the *in vitro* observations *in*
255 *vivo,* we injected zebrafish larvae with fluorescently tagged cells and treated them with
256 cisplatin (see **Supplemental Information** for details). While continuous cisplatin treatment for 5
257 days resulted in a tumor with predominantly tolerant cells, intermittent treatment led to no
258 significant change in the T:S ratio (**Fig. 3G**), indicating that intermittent treatment can ensure a
259 stable disease whereas the continues therapy favors emergence of drug refractory disease.

260 *Epigenetic modulation can distinguish drug sensitivity, tolerance and resistance in lung cancer*
261

262 To test the possibility that drug sensitivity can be regulated at the epigenetic level in a
263 reversible way, as opposed to genetic mutations alone, we used two different epigenetic
264 modulators namely, 5-azacytidine (5-AZA), a DNA methyltransferase inhibitor, and
265 suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, and determined their
266 effects on cisplatin resistance. While SAHA treatment did not enhance the effect of cisplatin on
267 sensitive H23 cells (**Fig. 3H**) or H1993 cells that are resistant to cisplatin (IC50>300μM) (**Fig. 3J**),
268 it had a significant additive effect on the H2009 cells, suggesting that these cells can become
269 sensitive through epigenetic intervention (**Fig. 3I**). However, 5-AZA had no discernable effect
270 (not shown), suggesting that epigenetic regulation of chromatin rather than specific cytosine
271 residues in the DNA modulates cisplatin tolerance in the H2009 cells. Based on these criteria,
272 H2009 qualify as cisplatin-tolerant (reversible) rather than resistant (irreversible) while H1993
273 may represent a truly resistant phenotype. Taken together, these observations suggest that
274 tolerance to cisplatin can be reversed unless the tolerant cells acquire mutations making them
275 irreversibly resistant.

276

Modeling cancer group behavior using experimentally derived growth curves

Typically, group behavior among cancer cell populations is studied using variants of the Lotka-Volterra (LV) model, where the inter-species competition and cooperation depend on the species frequencies [19-21]. We tested one such model (Li et al) which has been successful in explaining the evolutionary dynamics in bacterial co-cultures [22]. The major difference of the Li et al model to LV is the implementation of growth rates that depend on the species frequencies, leading to more complex dynamics than what could be captured by the classical LV model. However, the Li et al model did not quantitatively explain our experimental data (details in the **Supplementary text, Section 1 and Supplementary Fig. 5**) warranting alternative and possibly more complex models. We think the models like LV or Li et al are not suitable for our system partly due to the fact that they treat each cell identity as immutable, therefore ignoring the plastic phenotypes of cancer cells due to phenotypic switching. To address these deficiencies, we have developed a new model (Phenotype Switch Model with Stress Response or PSMSR), incorporating the knowledge about our specific cellular system and the observed growth trends.

The key evidence that motivated the new model PSMSR are as follows:

(1) Sensitive cells suppress the proliferation of the tolerant cells by secreting diffusible factors (can be overcome by increasing the frequency of tolerant cells)

(2) The suppressive effect is only prominent after co-culture of the two cell types for three weeks, not if the cells are mixed and monitored immediately

(3) Competition by the sensitive cells is eliminated in presence of cisplatin, and

(4) Epigenetic modifier SAHA can switch the tolerant cells to be drug-sensitive through non-genetic means, implying that these cells can switch their phenotypes in response to the environment.

Based on the above observations, the following are the key premises involved in PSMSR (**Fig. 4A**):

(1) Sensitive cells generate one or more products that affect the proliferation of the tolerant cells (and possibly their own as well). We call this hypothetical product(s) 'stress' (we explain its significance in **Supplementary Text Section 2**).

(2) Since the cohabitation of the cells appear to change their phenotypes (e.g. stronger suppression of tolerant cells by the sensitive cells after three weeks of co-culture), and we do not have enough information to model this phenotypic change as function of the cohabitation conditions, every system (i.e. monotypic, heterotypic-12 h and heterotypic-3 weeks) must be treated as distinct with their own phenotypic parameters.

(3) Through mutual cooperation, the tolerant cells can mitigate or neutralize the 'stress' generated by the sensitive cells in a frequency-dependent manner.

(4) Due to the stochastic phenotypic switching (sensitive ⇌ tolerant) by the two cellular species, a state of equilibrium exists between the two phenotypes at any point of time, where the equilibrium constant depends on the stress. As stress increases in the system, the equilibrium shifts to the right to increase the fraction of the tolerant phenotype.

The model presented here reflects the following mechanisms: i) cellular growth leads to stress accumulation, ii) accumulated stress reduces growth, iii) tolerant cells are efficient in neutralizing stress, iv) stress accumulation triggers the switching of sensitive cells to the tolerant phenotype. Hence, the growth rates of the sensitive (S) and the tolerant cells (T) can be expressed as,

$$\frac{\mathrm{d}S}{\mathrm{dt}} = -K_a S + K_b T + K_{GS} S \qquad (1)$$

$$\frac{\mathrm{d}T}{\mathrm{dt}} = -K_b T + K_a S + K_{GT} T \qquad (2)$$

Here, K_{GS} and K_{GT} are the stress-dependent effective growth rates (incorporating both proliferation and cell death) of the sensitive and tolerant cells respectively. K_a and K_b are the rate of switching from sensitive to the tolerant phenotype and vice versa and K is the equilibrium constant of phenotypic switching (eqn. 3).[23] We assume K_{GS}, K_{GT}, K to be linearly dependent on stress and K_b to be fixed, although the exact functional forms that map these quantities to stress is less important, as long as a monotonic relationship is maintained. Notably, we also fit the PSMSR model assuming sigmoidal as opposed to linear relationships of the above rate parameters with stress (**Supplementary Fig. 6)**, without significant worsening of fitting error (**Supplementary Fig. 7)**. For details, see **Supplementary section 3**.

$K = \frac{K_a}{K_b}$ (3), where *K* is the equilibrium constant for phenotypic switching.

Next, we assume that stress is predominantly generated by the fast-growing sensitive cells at a rate proportional to the cell population and neutralized by the tolerant cells. The resulting rate equation is given by:

$$\frac{\mathrm{d}C_{Str}}{\mathrm{dt}} = K_{Str} S - K_{Str,d} T \qquad (4)$$

where C_{Str} is a hidden variable representing the stress level, and K_{Str} and $K_{Str,d}$ are the rates of stress generation and removal, respectively.

PSMSR and cisplatin response

To model the effect of cisplatin, we added a cisplatin dose-dependent cellular death rate term to equations 1 and 2 to obtain equations 5 and 6. AUC stands for "area under the curve" (AUC = cisplatin concentration x time of exposure), which represents the memory effect of cisplatin exposure on the cellular growth (**Fig. 5A** and **Supplementary Text Section 2**).[24-26] *Sigmoid(AUC)* is the sigmoidal function (equation 7) that varies between 0 and 1, depending on the magnitude of AUC, which multiplied by the scale factor SCALE gives the cellular death rate.

AUC_5 and AUC_{95} represent the AUC values where 5% and 95% of the cisplatin death effect are achieved respectively. The SCALE, AUC_5 and AUC_{95} parameters are specific for the sensitive and the tolerant cell types.

$$\frac{dS}{dt} = -K_a S + K_b T + K_{Gs} S - SCALE_S \times sigmoid(AUC) \times S \quad (5)$$

$$\frac{dT}{dt} = -K_b T + K_a S + K_{Gt} T - SCALE_t \times sigmoid(AUC) \times T \quad (6)$$

$$sigmoid(AUC) = \left\{1 + exp\left[ln19\left(1 - 2\frac{AUC - AUC_5}{AUC_{95} - AUC_5}\right)\right]\right\}^{-1} \quad (7)$$

In total, the PSMSR includes 9 unknown parameters (16, when including cisplatin effect). We used roughly 2900 cell population data collected over several days of cellular growth under various conditions to fit (**Fig. 4B-C**) and analyze the model parameters. The fitting was performed using the global optimization method, Genetic Algorithm (GA) [27], as implemented in the package GA in R.[28] (also see **Supplementary text Section 2**). The suitability of the PSMSR model in describing the experimental observations was assessed by constructing the log likelihood profiles for each parameter as described in **Supplementary text Section 4 and Supplementary Figs. 8-9**.

PSMSR in monotypic and heterotypic cultures

Our experimental observations indicated that the sensitive and the tolerant cells behave quite differently when cultured alone, as opposed to being in presence of one another (**Supplementary Fig. 10A-C**). Therefore, by deconvoluting the growth trends using PSMSR, we examined whether a few or all parameters of the model are different between the monotypic and the heterotypic cultures. In general, several phenotypic parameters for the heterotypic cultures were different in magnitude compared to the values for the monotypic cultures (**Supplementary Fig. 10J**). This indicates an influence of the cellular phenotypes on each other. The parameters that showed a consistent difference between the mono- and heterotypic cultures include K_0 and K_b (the parameters for phenotypic switching), K_{Gt0} (growth rate for the tolerant phenotype) and K_s (rate of stress generation). One interesting observation is that the growth rate for the tolerant phenotype in monotypic and 3-week co-cultures is 7-10 times smaller than that of the sensitive phenotype (**Supplementary Fig. 10J**), reminiscent of the persister trait (please see “Discussion”).

By incorporating the cellular growth dynamics under different seeding ratios, The PSMSR model has the potential to provide insights into the mechanism of phenotypic switching in response to a changing microenvironment (**Fig. 4D-I**). Here, we have calculated the emergence of tolerant cell population from sensitive cells or vice versa in both monotypic (**Fig. 4D-E**, **Supplementary Fig. 11A, 11B, 11E** and **11F**) and heterotypic cultures (**Fig. 4G-H**, **Supplementary Fig. 12A, 12B, 12F** and **12G**). Of note, these cells are still experimentally detected as red or green, irrespective of their true phenotypes. Since in our model, stress is the driver for phenotypic switching, the switched phenotype cells only appear once stress builds up in the system over time (**Fig. 4I** and **Supplementary Fig. 12**). In both monotypic and heterotypic

391 cultures, the model predicts a rapid switch by the tolerant cells to the sensitive phenotype (up
392 to 97%, **Supplementary Fig. 11E, 12B, 12D** and **12G**), while maintaining a low but steady
393 tolerant population throughout the observations (**Fig. 4E**, **Supplementary Fig. 11B, 11F**). Thus,
394 we have seen how the cancer cells use phenotypic switching to maintain the overall fitness of
395 the community under different stress levels. To maintain steady growth, stress must be
396 mitigated, where switching to the tolerant phenotype pays off, since the tolerant cells are
397 capable of neutralizing stress. However, the fraction of tolerant phenotype in the population is
398 predicted to be low overall (10%, **Fig. 4G**, **Supplementary Fig. 12C** and **12H**). This may be due to
399 a balance between the necessity for stress removal and the energy or other costs required to
400 maintain the tolerant phenotype.

401 *Effects of phenotypic switching and stress give rise to diverse game-theoretical strategies in*
402 *mixed cell populations*

403 Cancer cell behavior is widely studied using game theory-based models where the inter-
404 species game strategies (competition and cooperation) are assumed to be constant throughout
405 the growth regime. A familiar example of such a model is the competitive Lotka-Volterra
406 equation, although more specialized models exist in the literature.[29] The phenotypic diversity
407 available to cancer cells suggest that their game strategic landscape will be considerably
408 complex, where the inter-species competition and cooperation are dynamically altered based
409 on changing scenarios.

410 Therefore, we have asked whether the PSMSR model can capture this complex strategic
411 landscape. Due to the complexity of the PSMSR equations, it is not possible to analytically
412 derive evolutionary payoffs (such as those given by the Lotka-Volterra equations[30]). Moreover,
413 the payoffs are likely to vary over time, unlike in the classical game theoretical models, where
414 they are assumed to be constant. Therefore, we followed a different approach, where we
415 numerically fitted the Lotka-Volterra equation to the growth rates given by PSMSR over moving
416 time windows (**Supplementary Fig. 13**). At small (12 h) and very large time-windows (6 days),
417 the fitting seemed to be worse, while at moderate time windows such as 4-5 days, the fitting
418 appeared reasonable between the two models (**Supplementary Fig. 13**). By fitting the growth
419 rates obtained from the PSMSR model to the competitive Lotka-Volterra equations, we
420 determined the inter-species competition parameters as function of time, for different sensitive
421 to tolerant seeding ratios (**Fig. 4J and K**). **Fig. 4J** shows that the tolerant cells are initially
422 competitive towards the sensitive cells, but they become cooperative after 2-3 days of growth.
423 This coincides with the accumulation of stress (**Fig. 4G**) indicating that the microenvironment
424 plays a major role in altering the game strategies of the cancer phenotypes. Notably, the effect
425 of the sensitive cells towards the tolerant cells (**Fig. 4K**) is significantly smaller in magnitude.
426 Within the tumor, where the microenvironment is significantly more complex than our
427 experimental setup, multiple agents such as the various cancer-associated macrophages and
428 immune cells can dynamically alter the game strategies adopted by the tumor cells and steer
429 resistance evolution [31]. In summary, the PSMSR model, combined with the payoff calculation
430 scheme described above, demonstrates the diverse strategic landscape explored by the
431 sensitive and tolerant cells under varying cell population and stress levels.

PSMSR model demonstrates the effectiveness of the intermittent cisplatin therapy

Using the PSMSR model, we simulated the continuous and intermittent therapy experiments, as explained before. We first calculated the model parameters by fitting to the experimental growth trends measured in presence of cisplatin. The best agreement was obtained when community cooperation such as phenotypic switching and stress removal were turned off (**Fig. 5B and C**). This implies that high cisplatin levels trigger the tolerant cells to focus on their own survival, similar to other social communities where imminent danger promotes self-survival. Also, at high cisplatin levels, the phenotypic switching to sensitive is detrimental to the survival of the community. Together, these observations are indicative of the adaptability of the cancer cells for survival in adverse environments. In **Fig. 5D**, the magnitudes of the SCALE parameter quantify the difference in cisplatin sensitivity between the sensitive and the tolerant phenotypes.

Using the PSMSR model, we simulated the cisplatin dose cycles as explained in **Supplementary Fig. 4**. Analogous to the experimental observations, the tolerant cell proportion increased with time in the continuous therapy, while it remained relatively small and increased at a slower rate during the intermittent cycles (**Fig. 5E-F**). Also, the intermittent 2 cycles of cisplatin dosage created more tolerant cell population than the single cycle, in agreement with the experiments (**Fig. 3D-F**). While the actual magnitudes of the tolerant cell population in the simulations are different than in the experiments, the qualitative behaviors agree. The quantitative difference between the predictions and the experiments could be partly attributed to the growth attenuation due to confluence that is not accounted for by the PSMSR model. Overall, these simulations show that the PSMSR model is able to qualitatively reproduce the drug-induced behavior of the cancer cell population.

Discussion

Several studies have applied evolutionary game theory to cancer[3-5,9,29,32-35] but as far as we are aware, the adaptive strategies NSCLC cancer cells adopt in response to environmental perturbations have not been investigated employing drug-naïve and drug-tolerant cells. We demonstrated that the growth dynamics of these cells can only be explained by invoking dynamic phenotypic switching upregulated by environmental stress. Consistent with this assumption, the present data with the epigenetic regulator together with our previous studies[16,36] and those from others[37,38], strongly support the possibility that the two cell types can stochastically switch their phenotypes via non-genetic mechanisms.

The present study also highlights the complex behavioral landscape of cancer cells, where the payoff strategies are dynamically evolving via phenotypic plasticity induced by environmental pressure (**Fig. 4J, K**). These inner level traits are cell frequency dependent and can affect the carrying capacity of the population. The proposed PSMSR model can provide important information about dynamic payoff strategies of multiple cellular phenotypes in a time and frequency-dependent manner. In contrast, traditional evolutionary models (e.g., the competitive LV) hold those payoffs to be fixed but are otherwise useful in understanding broad game strategies of the system, due to their mathematical simplicity. Combining the PSMSR with

473 a model analogous to LV can thus give additional insight into the complex group behavior of
474 multiple cellular phenotypes including the role of the microenvironment, as was demonstrated
475 in **Fig. 4J** and **K**.

476 The mathematical modeling combined with the experimental observations
477 demonstrated that accumulated stress (induced by cell growth and microenvironment),
478 promotes phenotypic switching of sensitive cells to tolerant phenotypes. Thus, cells that switch
479 phenotypes help to partly neutralize the stress and allow the fast-growing sensitive cells to
480 proliferate, thereby sustaining the carrying capacity of the system. Thus, the resulting carrying
481 capacity is a function of both stress and the level of tolerant phenotype in the system.
482 Interestingly, the behavior of the tolerant cells appeared to be beneficial to the overall
483 community. They helped the proliferation of the sensitive cells by removing stress, and they
484 themselves adopt to a slower proliferation rate (when co-cultured for 3 weeks with the
485 sensitive cells, the parameter K_{Gt0} in **table 1**), so as to not compete for limited resources
486 (altruism). This altruistic behavior is even more beneficial in the crowded environment of a
487 tumor, where nutrients and oxygen could run low. The tolerant cells elucidated the
488 evolutionary strategy of bet-hedging where they display low evolutionary fitness under normal
489 conditions, but high fitness under stressful conditions, such as in presence of cisplatin.[39] The
490 intermittent therapy simulations show that the PSMSR model reproduces this behavior of the
491 tolerant cell population (**Fig. 5E-F**). Comparing the growth data with and without cisplatin in
492 conjunction with the PSMSR model, we also find that the phenotypic switching (and the
493 consequent altruism) may be turned off at high stress, when cisplatin is administered.
494 Therefore, it appears that the altruistic stress removal benefit by the tolerant cells could be
495 effective under normal conditions in the tumor, where a small tolerant cell population benefits
496 the drug-sensitive cells to sustain proliferation. However, under high stress of chemotherapy,
497 such stress removal mechanisms may be insufficient to sustain the sensitive cell viability. It is
498 therefore prudent to turn off phenotypic switching under such situations and allow the
499 sensitive cells to become extinct and the tolerant cells to proliferate. While bet-hedging
500 strategies by drug-tolerant phenotypes are well discussed in the literature[39], altruism by such
501 phenotypes has hitherto been unexplored. There is overwhelming evidence that such tolerant
502 persister phenotypes exist in the tumor in small proportions, even in non-drug-resistant
503 disease. However, it is not clear whether they have a specific ecological role other than adding
504 to the tumor heterogeneity, although recent evidence indicate that persisters can facilitate
505 escape from drug induced toxicity by reversibly switching to slow cycling phenotypes [18].

506 Taken together, the behavior of the tolerant cells provides novel insights into the
507 phenotypic traits in cancer that emerge due to survival pressure as well as the cost-benefit
508 basis of such evolution. Of note, when co-cultured for 3 weeks prior to starting the experiment,
509 both the sensitive and tolerant cell types had equal opportunity to switch their strategy; either
510 they could have reduced or increased their proliferation rate to compete with each other, but
511 they followed an unexpected path where the sensitive cells remained unaffected and the
512 tolerant cells reduced their proliferation rate. This strategy could be helpful because most of
513 the genotoxic drugs used for chemotherapy target actively dividing cells. This behavior of
514 tolerant cells resembles phenotypic switching behavior reminiscent of persisters that are well
515 known in microbial systems[40] and more recently being recognized in cancer.[39,41-43]

From a translational perspective, the present study also suggests that intermittent rather than continuous chemotherapy may result in better outcomes in lung cancer. Although, it may not cure the patient of the disease, it could potentially result in stable disease that can be managed while sparing the patient of undesirable effects of excessive chemotherapy. The fact that intermittent therapy has shown promise in other solid tumors[7,9] should serve as a motivation to try it in lung cancer.

Materials and Methods

The details of the cell lines, antibodies and reagents used, the protocol used for live cell imaging, the ratios in which the heterotypic cultures were grown and observed in real time along with the details of the zebrafish microinjection, drug treatment, and animal handling, are provided in full detail in the Supplemental Section.

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Figure Legends

Figure 1. Schematic summarizing the experiment and the source of data used in developing the theoretical cell growth models. (**A**) The schematic representation of the different incubation duration, ratios, and treatments used for generating the data to develop the mathematical model. **(B)** schematic describing the principles based on which the mathematical model PSMSR was developed; **(C)** panel representing the functional form of PSMSR (please see the main text for further details).

Figure 2. Behavior of cisplatin-sensitive (S) and tolerant (T) NSCLC cells in 2D co-culture. (**A**) Schematic representation of the experimental design of co-culturing S and T cells in a ratio of 1:1 and collection of data points. Proliferation of sensitive (red) and tolerant (green) cells under different culture conditions in the absence or presence of cisplatin. Two-way ANOVA test (multiple comparison) showing statistical significance ****$p<0.0001$. (**B**) Sensitive and tolerant cells were plated in increasing T:S ratios and cultured for 3 weeks. Proliferation rate of sensitive cells (red) and tolerant cells (green) in heterotypic culture over the course of 144 hours. (**C**) Fold change in cell count of sensitive cells (red) and tolerant cells (green) in heterotypic culture was measured after 144 hours for ratios 1:1, 2:1, 4:1 and 8:1. Two-way ANOVA was used for calculating statistical significance ****$p<0.0001$, ns-not significant. (**D**) Fold change in cell count of sensitive cells (purple) and tolerant cells (blue) in heterotypic culture was measured after 144 hours in presence of cisplatin for ratios 1:1, 2:1, 4:1 and 8:1. Two-way ANOVA was used for calculating statistical significance ****$p<0.0001$, ns-not significant. (**E**) Change in tolerant/sensitive cells ratio with (orange) and without (black) 5 µM cisplatin over the course of 144 hours was measured. **(F)** Schematic representation of the conditioned medium experiment. (**G**) The left line graph representing the effect of tolerant cell conditioned medium on sensitive cells, and the right line graph representing the inhibitory effect of tolerant cell conditioned medium on sensitive cells growth. (**H**) Schematic representation of conditioned medium experiment to correlate the stoichiometry between cell number and inhibitory effect secreted by sensitive cells. (**I**) The bar graph representing the inhibitory effect of condition medium on different cell number of tolerant or sensitive cells. Statistical significance information can be found in **Supplementary Table 2 and 3**.

686 **Figure 3. Tolerant cells reversibly switch their phenotype to become sensitive with**
687 **intermittent therapy.** (**A-C**) Bar graph showing the ratio of tolerant versus sensitive cell
688 population over a period of 10 days. The cell ratio for the "Continuous" group wherein the cells
689 were continuously treated with cisplatin is shown in blue and the ratio for the "Intermittent"
690 group wherein the cells were treated with cisplatin for 2 days and released in fresh medium
691 (intermittent) is shown in black. (**D-F**) Media from "Intermittent – 2 cycles" group was removed
692 after 4 days of cisplatin treatment and replaced with fresh medium and the cells were allowed
693 to grow until confluent. These cells were monitored in real-time to determine the ratio of
694 tolerant vs sensitive over the course of 25 days. Similarly, the cells that only received cisplatin
695 once ("Intermittent – 1 cycle") throughout the experiment were also followed for 25 days. (**G**)
696 Sensitive (S, red fluorescence) and tolerant (T, green fluorescence) cells were mixed at S:T ratio
697 of 4:1 and microinjected into the perivitelline space of zebrafish larvae 48 h post fertilization
698 (hpf). Twenty-four hours after microinjection, larvae were randomly divided into 3 groups:
699 Group 1 received no drug treatment (Untreated), Group 2 received cisplatin 20 µM for 3 days
700 and released with no drug for 2 days (Intermittent), and Group 3 received cisplatin 20 µM
701 continuously for 5 days (Continuous). Ratio of tolerant versus sensitive cells was determined by
702 measuring fluorescence intensity. (**H**) Effect of suberoylanilide hydroxamic acid (SAHA) on
703 cisplatin-sensitive (H23), tolerant (H2009), and resistant (H1993) cells, demonstrating that
704 tolerant cells can reversibly switch their phenotype to become sensitive. Statistical significance
705 information can be found in **Supplementary Table 4**.
706
707 **Figure 4. Cooperativity and stress response as described by the PSMSR model.** (**A**) Schematic
708 describing the PSMSR model; initially, the sensitive and the tolerant cells proliferate
709 independently; as stress builds up, sensitive cells switch their phenotype to tolerant cells and
710 vice versa; tolerant cells remove stress and maintain a small population, while enabling the
711 sensitive cells to proliferate. (**B-C**) Fitting of the phenotype-switch model to the cellular growth
712 curves of sensitive and tolerant cell populations, where the cells were mixed at different
713 proportions and counting was started immediately; the colors represent the growth curves
714 from different initial seeding proportions, as indicated in the legend (sensitive to tolerant cell
715 seeding ratios); (**D-F**) predicted evolution of phenotypic switching and stress in monotypic
716 cultures; (D-E) populations of sensitive and (switched) tolerant phenotypes with time, when
717 seeded with sensitive cells only; (F) stress as function of time; (**G-I**) predicted evolution of
718 switched phenotypes and stress in heterotypic culture experiments, where cell growth was
719 monitored immediately after mixing; (G) fraction of sensitive cells that have switched to the
720 tolerant phenotype, as function of time; (H) fraction of tolerant cells that have switched to the
721 sensitive phenotype, as function of time; (I) stress with time; colors are according to the initial
722 seeding ratio of sensitive to tolerant cells as shown in the legend; the total cell population in
723 each case was close to 5000; (**J-K**) evolving game strategy landscape of cellular population due
724 to stress and phenotypic switching; the heatmaps of time varying payoff values representative
725 of inter-species competition/cooperation are shown as function of sensitive to tolerant seeding
726 ratio; payoff values are derived by fitting the PSMSR model to the competitive Lotka-Volterra
727 equations; orange areas in the maps represent competitive behavior, green areas represent
728 cooperative behavior; (J) α_{12} representing the effect of tolerant cells towards the sensitive cells;
729 (K) α_{21} representing the effect of sensitive cells towards the tolerant cells.

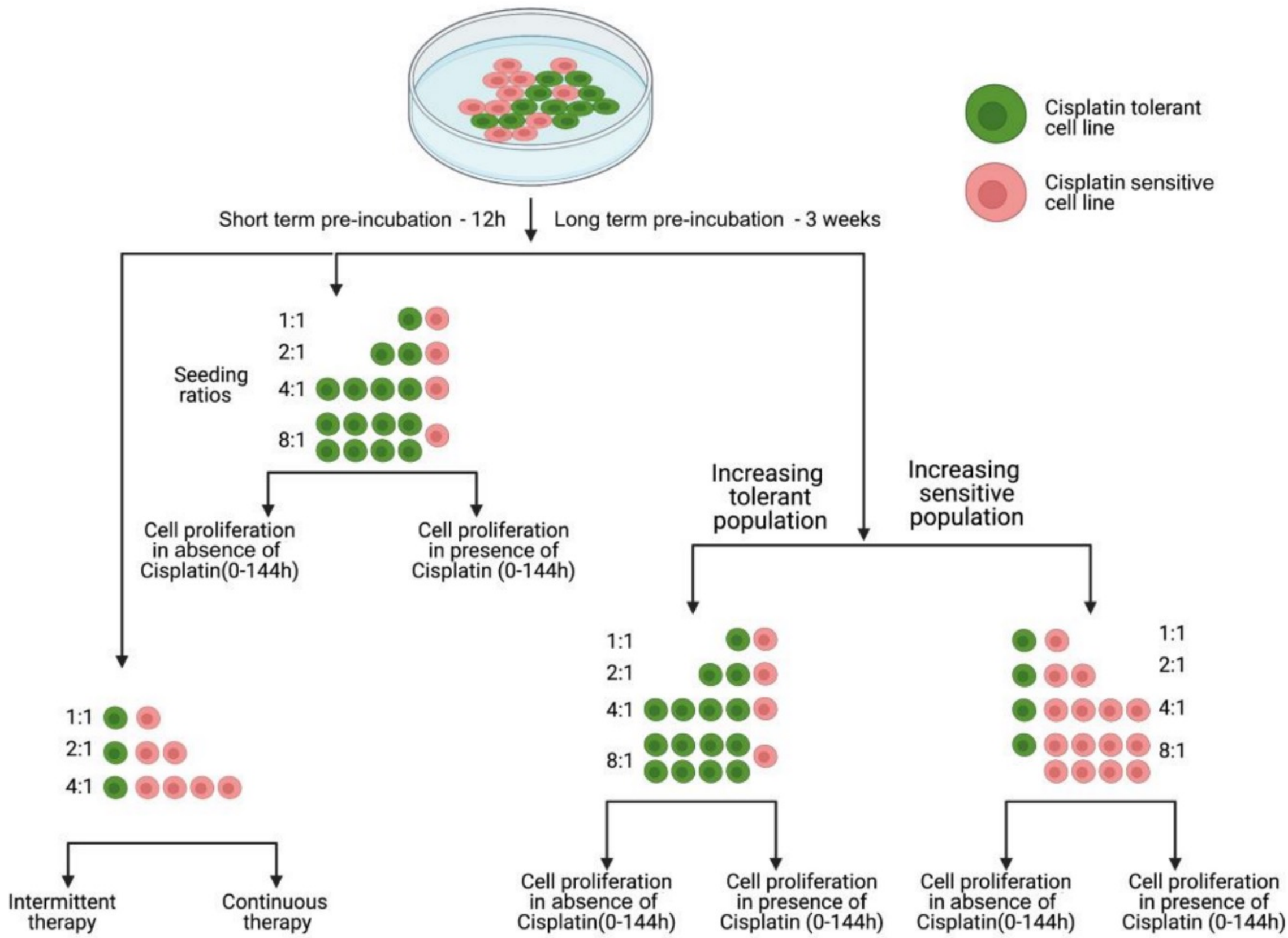
Figure 5. Mathematical model for cisplatin resistance. (**A**) Schematic demonstration of AUC and cellular death rate as function of AUC; (**B-C**) fitting of the experimental growth data where the cells were co-cultured for three weeks; B: sensitive cells; C: tolerant cells; circles and lines represent the experimental and fitted trends respectively; (**D**) SCALE parameter as measure of cisplatin sensitivity for the sensitive and the tolerant cells; the error bars represent 95% confidence limits (**E-F**) simulation of intermittent and continuous cisplatin treatment according to the protocols described in Fig. 3; the initial sensitive to tolerant cell ratio was set to 4:1 with a total cell population of 50,000. (**G**) An illustrative model depicting the presence (and absence) of group behavior among sensitive and tolerant cells under varying conditions of stress and effects of continuous versus intermittent therapy.

Table 1: Model parameters and parameter search ranges for PSMSR, including the 95% confidence limits.

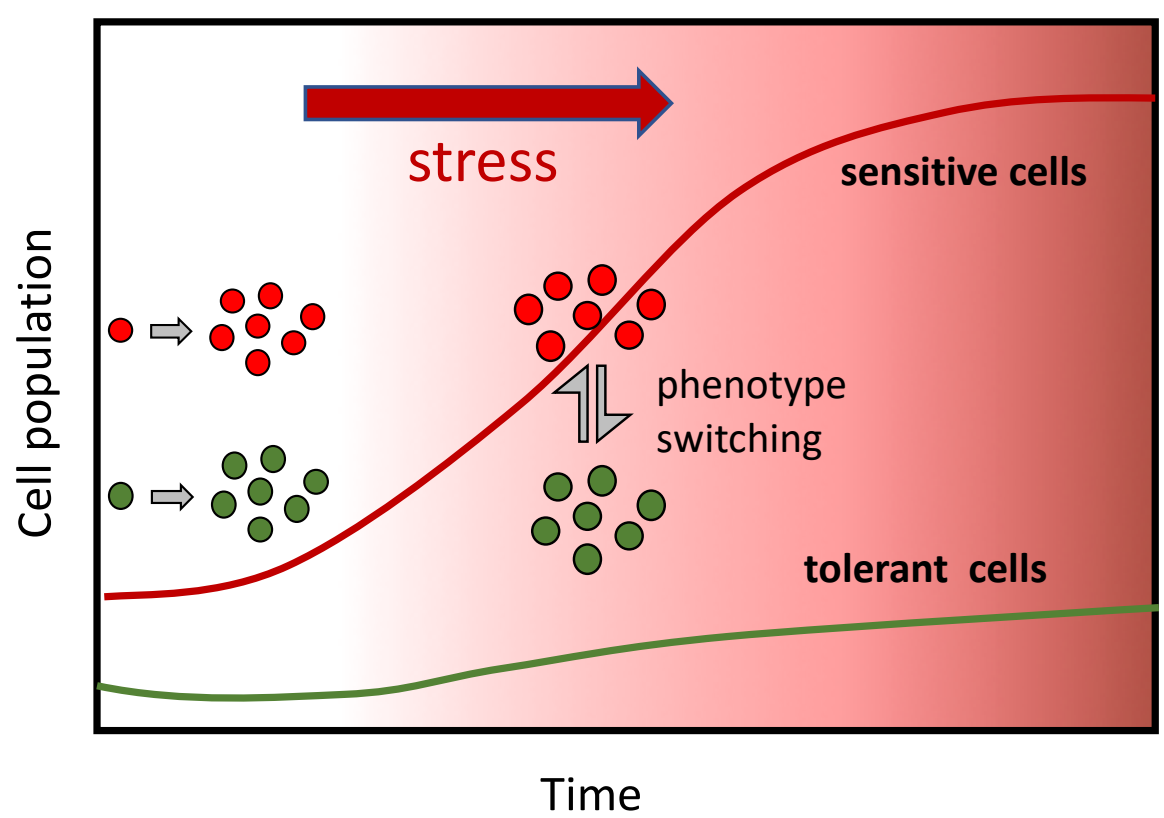
Condition	K_0	K_b	K_{Gs0}	K_{Gt0}	K_{str}	$K_{str,d}$	a	b	g	S_{drug}	AUC_s^5	AUC_s^{95}	$SCALE_s$	AUC_t^5	AUC_t^{95}	$SCALE_t$
Heterotypic	0.049 ±0.0003	3.57±0.02	0.713±0.001	0.687±0.004	7.14×10^{-4} $\pm3\times10^{-6}$	5.64×10^{-3} $\pm4\times10^{-5}$	0.046 ±0.0004	0.038 ±0.0002	0.018 ±0.0006							
Heterotypic, 3 weeks	0.052 ±0.0007	2.6±0.05	0.708±0.002	0.189±0.016	6.74×10^{-4} $\pm8\times10^{-6}$	5.52×10^{-3} $\pm1\times10^{-4}$	0.05 ±0.001	0.038 ±0.0006	0.02 ±0.0006							
Heterotypic, cisplatin 5μM	0.033 ±0.0017	0	1.033±0.046	0.976±0.054	5.9×10^{-4} $\pm2.8\times10^{-5}$	0	NA	0.04 ±0.003	0.034 ±0.003	108±14.5	239±12	1153±58	8.61±0.5	233±17	1201±49	5.79±0.6
Heterotypic, 3 weeks, cisplatin 5μM	0.033 ±0.0015	0	0.966±0.05	0.967±0.05	5.8×10^{-4} $\pm3.3\times10^{-5}$	0	NA	0.039 ±0.002	0.036 ±0.003	96.8±9.95	271±14	1098±44	8.91±0.5	250±14	1098±47	6.89±0.7
Parameter search range	0-0.1	0-5	0-2	0-2	0-0.001	0-0.02	0-0.1	0-0.1	0-0.1	1-500	1-500	10-1500	0.1-20	1-500	10-1500	0.1-20

Figure 1

A



B



C

Phenotype Switch Model with Stress Response (PSMSR)

sensitive

$$\frac{dS}{dt} = -K_a S + K_b T + K_{GS} S$$

tolerant

$$\frac{dT}{dt} = \underbrace{-K_b T + K_a S}_{\text{phenotype switching}} + K_{GT} T$$

net growth — phenotype switching — proliferation

stress

$$\frac{dC_{Str}}{dt} = K_{Str} S - K_{Str,d}\, T$$

generation — removal

Fig. 2

A

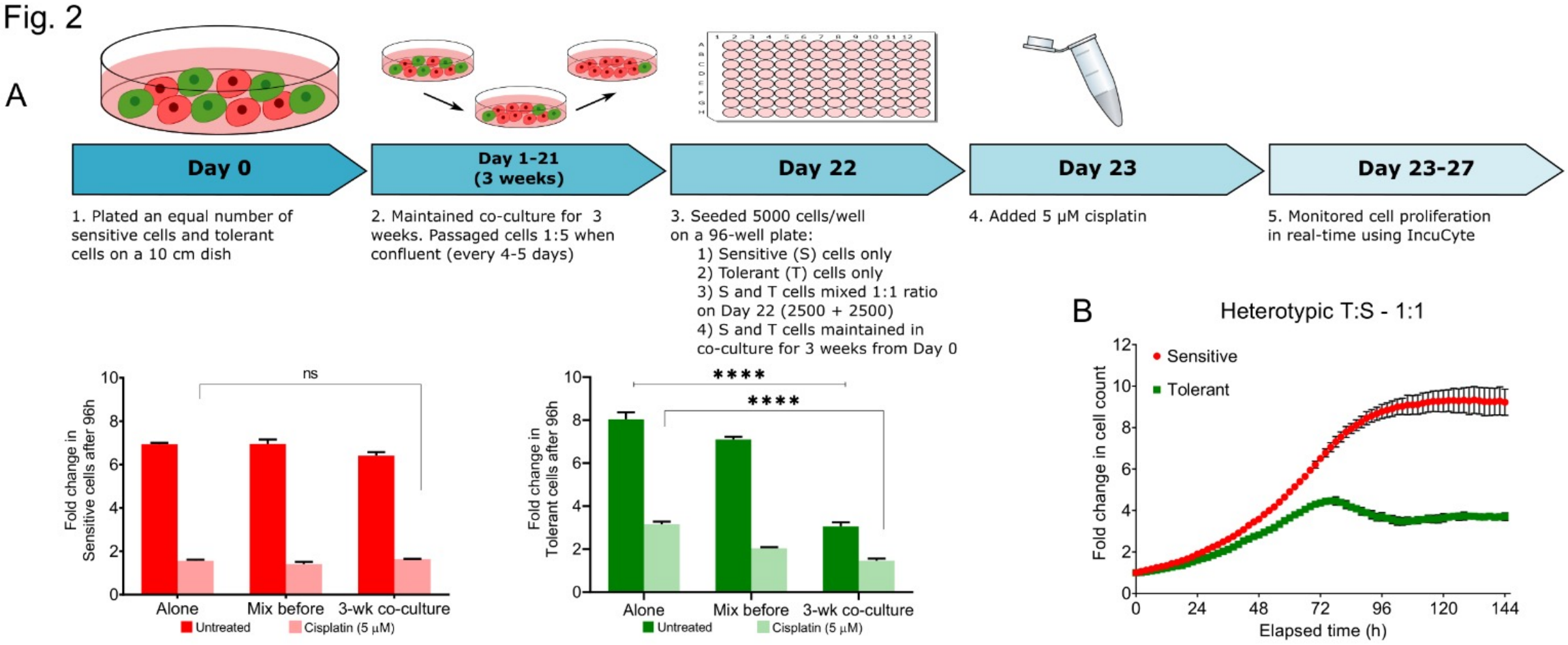

Day 0
Day 1-21 (3 weeks)
Day 22
Day 23
Day 23-27
1. Plated an equal number of sensitive cells and tolerant cells on a 10 cm dish
2. Maintained co-culture for 3 weeks. Passaged cells 1:5 when confluent (every 4-5 days)
3. Seeded 5000 cells/well on a 96-well plate:
1) Sensitive (S) cells only
2) Tolerant (T) cells only
3) S and T cells mixed 1:1 ratio on Day 22 (2500 + 2500)
4) S and T cells maintained in co-culture for 3 weeks from Day 0
4. Added 5 μM cisplatin
5. Monitored cell proliferation in real-time using IncuCyte
Fold change in Sensitive cells after 96h
ns
Alone
Mix before
3-wk co-culture
Untreated
Cisplatin (5 μM)
Fold change in Tolerant cells after 96h
****
****
Alone
Mix before
3-wk co-culture
Untreated
Cisplatin (5 μM)
B
Heterotypic T:S - 1:1
Sensitive
Tolerant
Fold change in cell count
Elapsed time (h)
C
Heterotypic 3-week culture - untreated
Fold change in cell count after 144 h
****
****
****
ns
1:1
2:1
4:1
8:1
Initial seeding ratio (tolerant:sensitive)
Untreated Sensitive
Untreated Tolerant
D
Heterotypic 3-week culture - treated for 144 h
Fold change in cell count after 144 h
ns
***
***
***
1:1
2:1
4:1
8:1
Initial seeding ratio (tolerant:sensitive)
Treated Sensitive
Treated Tolerant
E
Heterotypic 3-week culture
Ratio of T:S cells after 144 h
Tolerant/Sensitive cells ratio after 144 h
ns
*
****
****
1:1
2:1
4:1
8:1
Initial seeding ratio (tolerant:sensitive)
Untreated
Treated
F
Day 0
Day 1
Day 1-3
1. Plated 4 million cells on 10 cm dish to condition media
Seeded 200,000 cells/well on a 6-well plate
2. Collected conditioned media from 10 cm dish, centrifuged at 2500 RPM for 10 mins
Added sensitive-conditioned media to tolerant cells seeded on 6-well plate and vice versa (added centrifuged fresh media to cells as control)
3. Monitored cell proliferation in real-time using IncuCyte
G
Fresh
Tolerant conditioned
Fold change in Sensitive cell count
Time elapsed (h)
Fresh
Sensitive conditioned
Fold change in Tolerant cell count
Time elapsed (h)
H
Day 0
Day 1
Day 1-3
Post-experiment
1. Seeded an increasing number of sensitive cells and tolerant cells/well on a 96-well plate (2500, 5000, 7500, 10000, 20000)
Plated 4 million sensitive cells on 10 cm dish to condition media
2. Collected conditioned media from 10 cm dish, centrifuged at 2500 RPM for 10 mins
Added sensitive-conditioned media to cells on 96-well plate (added centrifuged fresh media to cells as control)
3. Monitored cell proliferation in real-time using IncuCyte
4. Measured fold change of cell count in fresh media and in sensitive-conditioned media
Plotted the difference
I
Tolerant cells fold change (Sensitive conditioned media)
Sensitive cells fold change (Sensitive conditioned media)
Fold change in inhibition
2500
5000
7500
10000
20000
Cell seeding number

Fig. 3

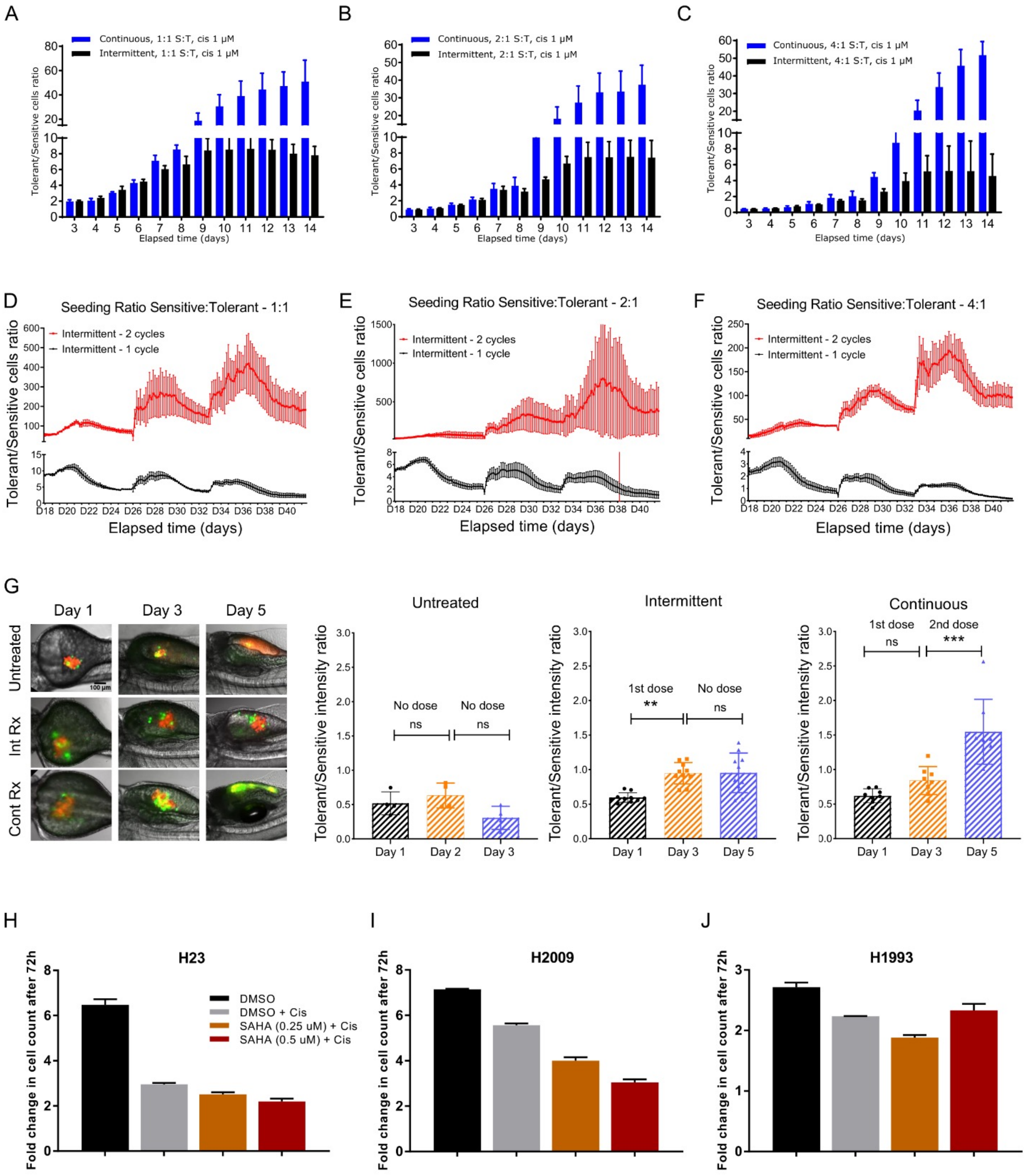

A
Continuous, 1:1 S:T, cis 1 μM
Intermittent, 1:1 S:T, cis 1 μM
Tolerant/Sensitive cells ratio
Elapsed time (days)
B
Continuous, 2:1 S:T, cis 1 μM
Intermittent, 2:1 S:T, cis 1 μM
Tolerant/Sensitive cells ratio
Elapsed time (days)
C
Continuous, 4:1 S:T, cis 1 μM
Intermittent, 4:1 S:T, cis 1 μM
Tolerant/Sensitive cells ratio
Elapsed time (days)
D
Seeding Ratio Sensitive:Tolerant - 1:1
Intermittent - 2 cycles
Intermittent - 1 cycle
Tolerant/Sensitive cells ratio
Elapsed time (days)
E
Seeding Ratio Sensitive:Tolerant - 2:1
Intermittent - 2 cycles
Intermittent - 1 cycle
Tolerant/Sensitive cells ratio
Elapsed time (days)
F
Seeding Ratio Sensitive:Tolerant - 4:1
Intermittent - 2 cycles
Intermittent - 1 cycle
Tolerant/Sensitive cells ratio
Elapsed time (days)
G
Day 1
Day 3
Day 5
Untreated
Int Rx
Cont Rx
100 μm
Untreated
Tolerant/Sensitive intensity ratio
No dose
ns
No dose
ns
Day 1
Day 2
Day 3
Intermittent
Tolerant/Sensitive intensity ratio
1st dose
**
No dose
ns
Day 1
Day 3
Day 5
Continuous
Tolerant/Sensitive intensity ratio
1st dose
ns
2nd dose
***
Day 1
Day 3
Day 5
H
H23
Fold change in cell count after 72h
DMSO
DMSO + Cis
SAHA (0.25 uM) + Cis
SAHA (0.5 uM) + Cis
I
H2009
Fold change in cell count after 72h
J
H1993
Fold change in cell count after 72h

Figure 4

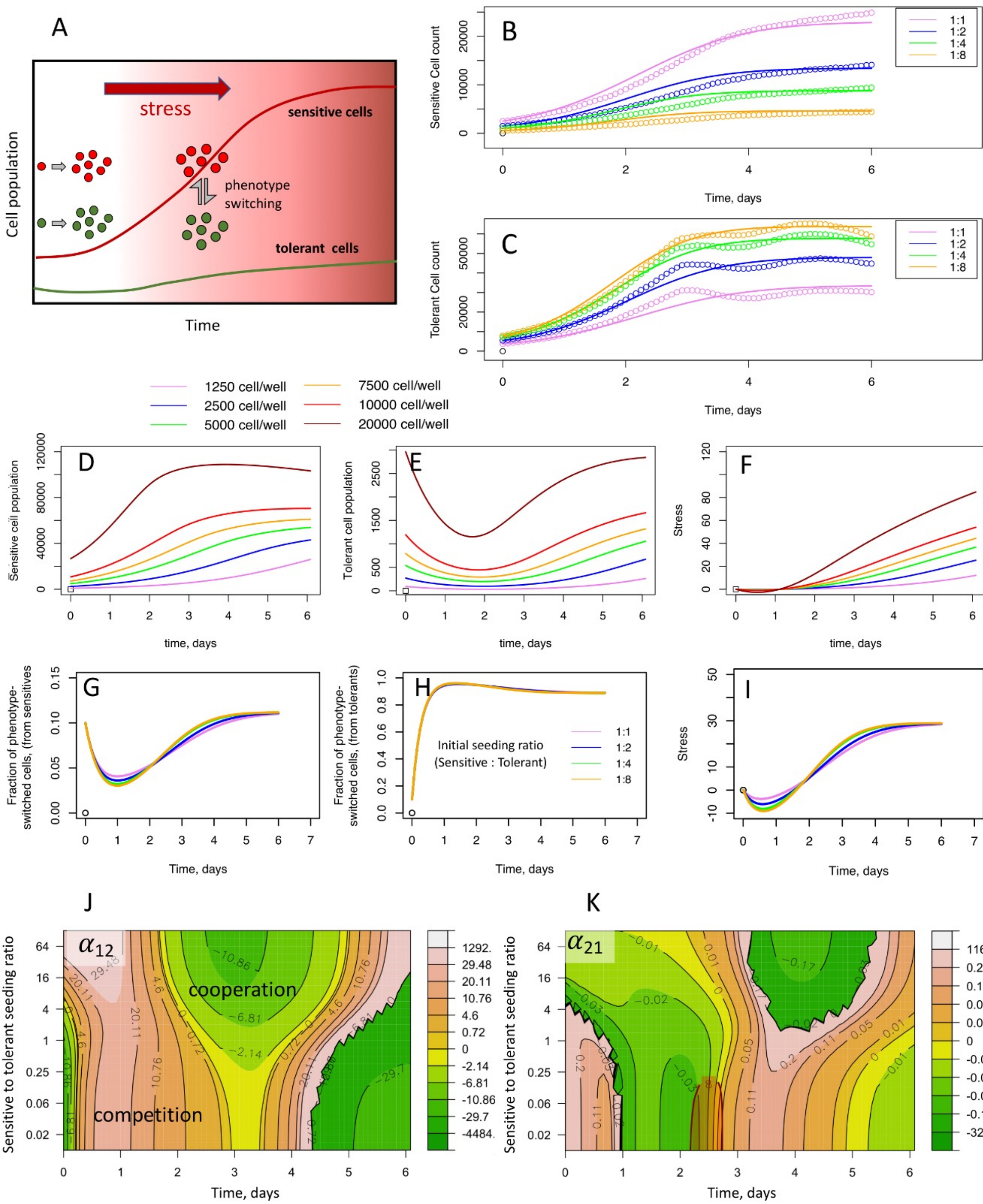
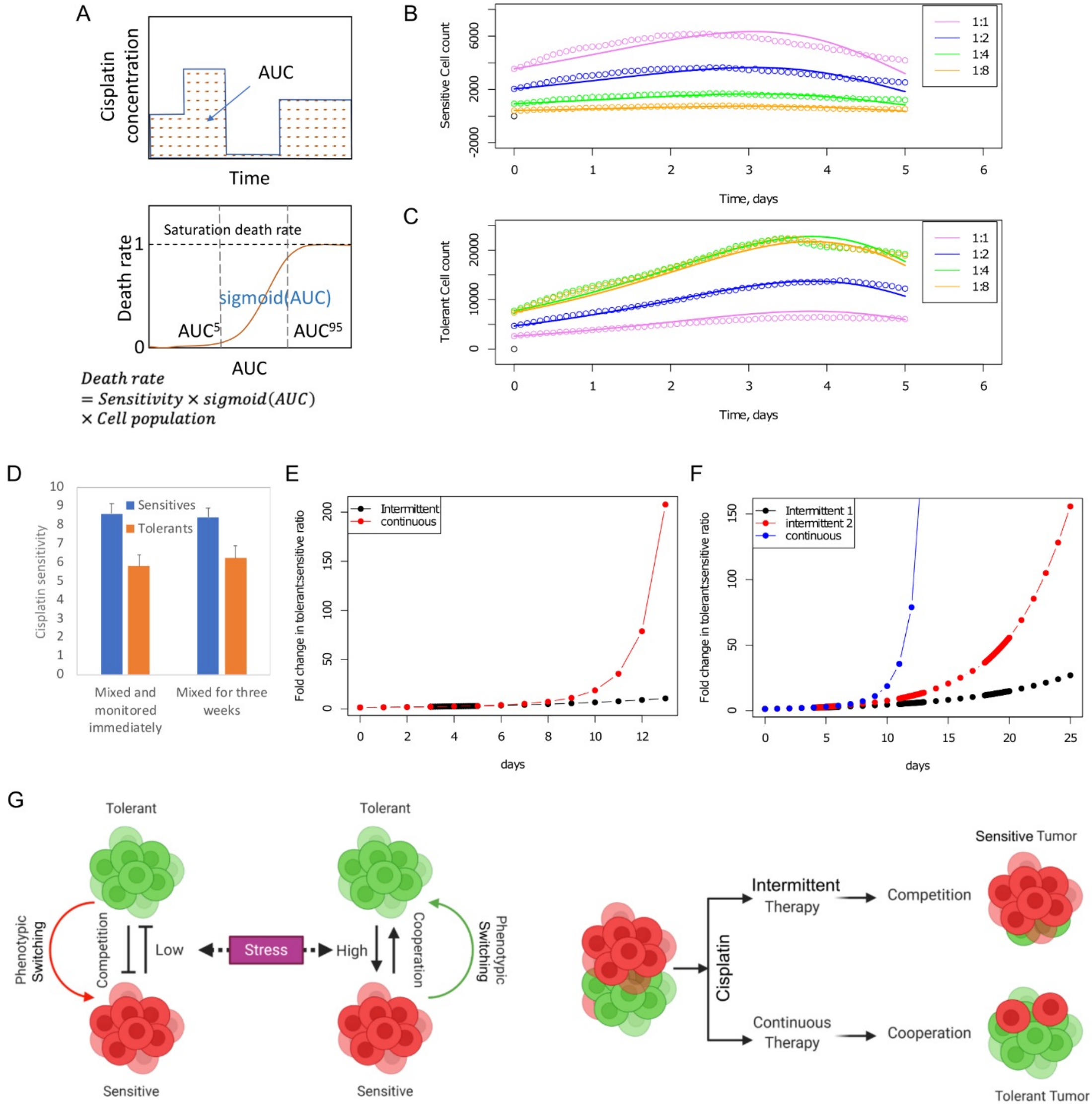

A
stress
sensitive cells
phenotype switching
tolerant cells
Cell population
Time
B
Sensitive Cell count
Time, days
1:1
1:2
1:4
1:8
C
Tolerant Cell count
Time, days
1250 cell/well
2500 cell/well
5000 cell/well
7500 cell/well
10000 cell/well
20000 cell/well
D
Sensitive cell population
time, days
E
Tolerant cell population
time, days
F
Stress
time, days
G
Fraction of phenotype-switched cells, (from sensitives
Time, days
H
Fraction of phenotype-switched cells, (from tolerants)
Initial seeding ratio
(Sensitive : Tolerant)
Time, days
I
Stress
Time, days
J
α_{12}
cooperation
competition
Sensitive to tolerant seeding ratio
Time, days
K
α_{21}
Sensitive to tolerant seeding ratio
Time, days

Figure 5


A
Cisplatin concentration
AUC
Time
Saturation death rate
Death rate
1
0
sigmoid(AUC)
AUC5
AUC95
AUC
Death rate
= Sensitivity × sigmoid(AUC)
× Cell population
B
Sensitive Cell count
1:1
1:2
1:4
1:8
Time, days
C
Tolerant Cell count
1:1
1:2
1:4
1:8
Time, days
D
Cisplatin sensitivity
Sensitives
Tolerants
Mixed and monitored immediately
Mixed for three weeks
E
Fold change in tolerant:sensitive ratio
Intermittent
continuous
days
F
Fold change in tolerant:sensitive ratio
Intermittent 1
intermittent 2
continuous
days
G
Tolerant
Tolerant
Phenotypic Switching
Competition
Low
Stress
High
Cooperation
Phenotypic Switching
Sensitive
Sensitive
Sensitive Tumor
Intermittent Therapy
Competition
Cisplatin
Continuous Therapy
Cooperation
Tolerant Tumor