Cancer Systems Biology: Translational Mathematical Oncology

Ravi Salgia et al.

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CHAPTER

37 Group behaviour and drug resistance in cancer

Supriyo Bhattacharya, Atish Mohanty, Govindan Rangarajan, Ravi Salgia

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Abstract

Drug resistance is a major challenge in cancer. Contrary to prevailing wisdom, it is now evident that phenotypic plasticity mediated through non-genetic mechanisms plays an important role in evading drug effects and the emergence of a drug-tolerant state. These tolerant states eventually acquire mutations and lead to the development of permanent and irreversible drug-resistant states. Furthermore, a cancer cell resides with other cell types in the tumour microenvironment, and its ecological interaction with those cells and ensuing group behaviour also impact drug response. In this chapter, we briefly review game-theory-based models to understand the group behaviour of cancer cells and their contributions to developing drug tolerance. We then discuss the Phenotype Switch Model with Stress Response, a new mathematical approach to model real-time growth data of non-small-cell lung cancer (NSCLC) cells in the absence/presence of the drug. The model indicates that switching treatment from a continuous to an intermittent regimen can attenuate the emergence of tolerant, and hence the eventual resistant cells, suggesting that intermittent therapy may be a novel strategy to preclude or delay the onset of drug resistance in NSCLC. Given the generalizability of evolutionary principles, such therapeutic strategies can be applicable to other cancer types as well.

Keywords: cancer drug resistance, non-small-cell lung cancer (NSCLC), group behaviour, game theory, phenotypic switching, Phenotype Switch Model with Stress Response (PSMSR), non-genetic mechanisms, genetic/non-genetic duality

Subject: Clinical Medicine

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37.1. Introduction

Group behaviour refers to the collective property of a group or population that emerges due to interactions among individual members of the group [1], often benefiting the group as a whole. Cancer cells reside in a communal environment, in close proximity to one another as well as other cell types that constitute the tumour microenvironment (TME). These cells communicate and influence each other's behaviour through exchange of chemical messengers [2,3]. Such mutual interactions, coupled with the heterogeneity of the TME, give rise to complex ecological dependencies (e.g. competition and cooperation) within the tumour. Therefore, group behaviour is a major mechanism that is relevant to every aspect of cancer evolution, from initial progression to metastasis and therapy resistance.

One critical yet overlooked property of cancer cells is phenotypic plasticity that allows rapid adaptation to environmental changes without undergoing genetic mutations [4]. Mechanisms such as phenotypic plasticity and group behaviour facilitate the survival of cancer cells, especially during stressful events such as therapeutic intervention. These mechanisms are of considerable relevance to the emergence of drug resistance, alongside the more familiar mechanism through genetic mutations. Therefore, understanding the role of group behaviour, and the underlying non-genetic mechanisms, can lead to more efficacious treatment designs and minimize or delay the emergence of resistance.

For well over the past 150 years, cancer has been thought to be a predominantly genetic disease, where individual clones acquire driver mutations of increasing fitness through natural selection [5,6,7,8,9,10,11]. Furthermore, this thinking has also helped ingrain the idea that drug resistance in cancer, whether innate or acquired, is primarily driven by genetic mutations [12,13]. However, there is a growing appreciation that genetic evolution is unlikely to represent the only mechanism for acquiring drug resistance. Emerging evidence indicates that non-genetic mechanisms such as epigenetic modifications and rewiring of protein interaction networks also contribute to various aspects of cancer, including its origin, progression, and emergence of drug resistance [14,15,16].

A hallmark of cancer cells is their phenotypic plasticity, that is the ability to exhibit different phenotypes when exposed to variable environmental conditions without undergoing any genotypic changes [17]. The underlying mechanisms contributing to the development of phenotypic plasticity are non-genetic. Cancer cells can switch phenotypes reversibly which allows them to evade the toxic effects of a drug without acquiring any mutation or genetic alteration(s) while contributing to intra-tumour heterogeneity. Indeed, such heterogeneity induced through non-genetic mechanisms serves as an effective bet-hedging strategy that can help overcome the varying selection pressures faced by cancer cells [18,19]. Therefore, it is important to recognize the pervasive contribution of phenotypic plasticity and to develop strategies to effectively counteract this feature of cancer cells, in addition to the genomic-guided approach frequently used with targeted therapies. Of note, while genetic and non-genetic mechanisms of drug resistance are often recognized as separate entities to illustrate the concepts associated with them, most cancers appear to leverage both processes for therapeutic evasion that are not mutually exclusive evolutionary trajectories [14,20].

There is evidence that drug-resistant clones pre-exist within tumours prior to drug treatment, whereas the emergence of a drug-tolerant (i.e. weakly or moderately resistant) state is stochastic which could be exhibited by any cell in the tumour [21,22,23]. The cells exhibiting tolerance phenotypes are called persisters that are not very well characterized and usually present as a minor fraction of drug-sensitive cells [24]. Different processes, such as pathway rebound through the release of negative feedback loops, transcriptional rewiring mediated by chromatin remodelling, and autocrine/paracrine communication among tumour cells and between tumour cells and other cell types in the TME, are thought to contribute to the emergence of these cells [25]. Nonetheless, this begs the question how drug-sensitive and tolerant or resistant cells in a tumour influence each other's fitness (growth), and whether cooperation and competition (group behaviour) between the

sensitive and tolerant cells influence their response to drug treatment. Several studies in the literature have demonstrated the power of game-theory-based approaches to understand group behaviour and its contribution to ↳ drug resistance in different cancers. Furthermore, with advances in technology enabling live cell imaging and in the power of computing with big datasets, discerning group behaviour by monitoring interactions between drug-tolerant and drug-sensitive cells in real time in the absence or presence of the drug has emerged as a powerful tool to elucidate the role of group behaviour [26,27,28,29,30,31,32,33,34].

In this chapter, firstly, we briefly review game-theory-based models developed for understanding drug resistance, especially in non-small-cell lung cancer (NSCLC) [31,32,34], and draw attention to some of the challenges associated with applying classical game theory to cancer. We then discuss Phenotype Switch Model with Stress Response (PSMSR), a new mathematical approach with game theoretical underpinnings that we developed to model real-time growth data of NSCLC cells to discern patterns in response to treatment with cisplatin [1,35]. 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 behaviour strategies. Tolerant cells exhibit a 'persister-like' behaviour 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 NSCLC. We conclude by summarizing the enormous potential of mathematical modelling and quantitative cancer biology.

37.2. Introduction to game theory

Game theory is the mathematical framework for studying the strategic interactions between competing players in a communal environment. A strategy refers to a set of choices or actions adopted by a player at a certain time. The type of strategy that a player chooses will depend on their goals, the goals of the other players, and the rules of the game. Mathematically, each strategy is associated with a payoff matrix that lists the possible outcomes of the strategy and the costs and benefits incurred by different players under alternative scenarios. Classical game theory was developed to analyse the behaviours and strategies followed by human players or organizations, whose decisions are often expected to be rational and geared towards maximizing their payoffs [36]. This often leads to zero-sum games (one player's win is counterbalanced by the opponent's loss), equilibrium situations (e.g. Nash equilibrium, where a single-player strategy change does not lead to any gain, unless others change their strategies as well), or various forms of cooperations and collaborations [37].

The branch of game theory that studies evolutionary processes involving biological species is called evolutionary game theory. Unlike human players, the strategies adopted by biological entities (e.g. animal or plant species, microorganisms, and cancer cells) are not rational rather inherited through generations and evolved for the survival benefit of individuals or communities under a given environment. The dynamics of evolutionary games are driven by competition, cooperation, or other more complex strategies (e.g. bet-hedging and defection) among groups of individuals, where the outcomes (payoffs) of the strategies depend on the opponent strategies as well as relative group populations. In recent years, researchers have shown that phenotypic plasticity exhibited by certain microorganisms and cancer cells can lead to complex game landscapes [35,38], where strategies need not be fixed through inheritance rather switch depending on the environment or, in some cases, learned *de novo* from other players, as seen in the case of drug-sensitive lung cancer cells in the presence of drug-tolerant cells [35].

37.3. Game theory and drug resistance in cancer

Evolutionary game theory has been a valuable conceptual tool to understand the behaviour of cancer cells, the role of tumour heterogeneity, interaction with the microenvironment and immune system, and forecast disease prognosis and design effective therapy [2,27,28,30,31,33,34,35, 39,40,41,42,43,44]. The tumour ecosystem is comprised of multiple cell types, such as proliferative cancer cells, supportive stromal cells, immune cells, and fibroblasts (Figure 37.1A), each of which can be treated as players in an evolutionary game. Interaction among these cell types and with the microenvironment shapes the cellular phenotypes within the tumour. The group behaviours of individual clones and subclones that result from such interactions can be considered as heritable game strategies. For example, the cooperative subclones in the cancer milieu benefit each other by secreting diffusible factors [45]. However, non-cooperative subclones (cheaters) can compete with the cooperative cells to free ride on the diffusible factors for their own benefit.

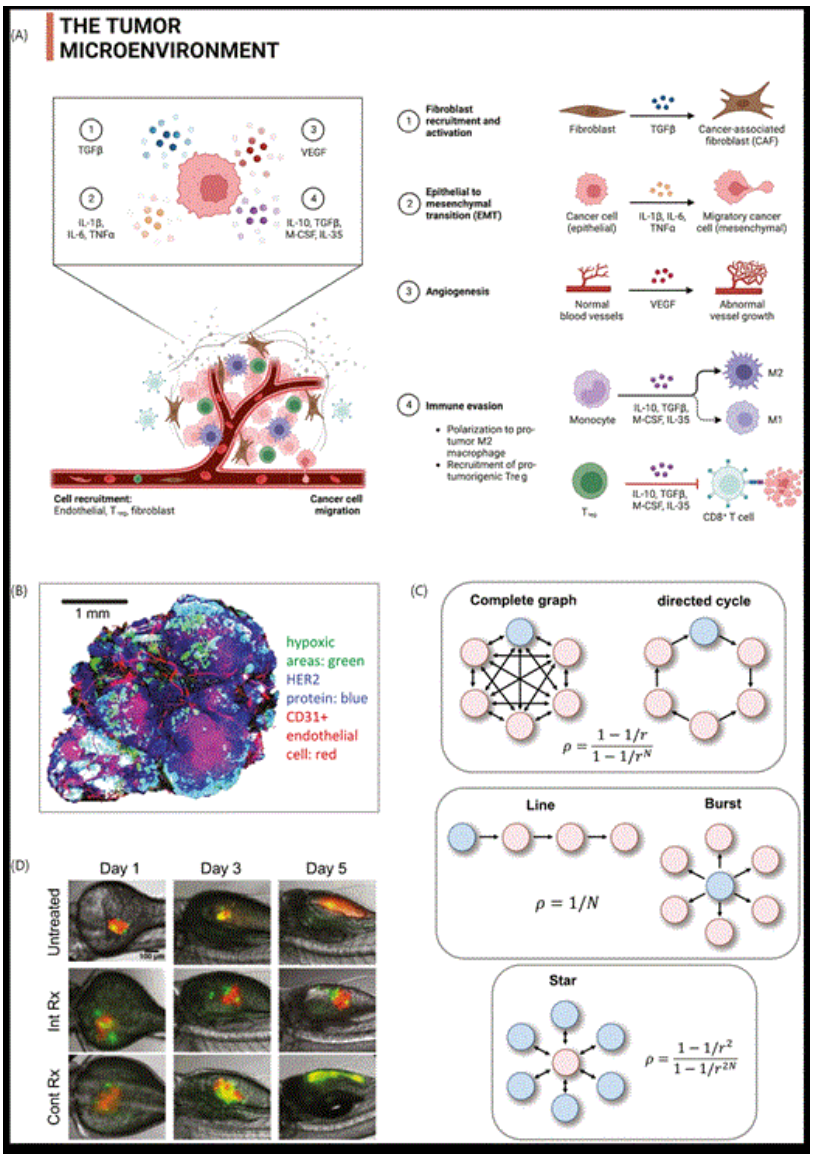

Figure 37.1. (A) Overview of the tumour microenvironment. Few of the common diffusible factors released by proliferating cancer cells are shown in the box. Role of each diffusible factor is depicted on the right. Cancer cells utilize the functional effects of such diffusible factors as part of their game strategies to survive in the host environment and compete/cooperate with other cells. *Source:* Created using Biorender (https//www.biorender.com). (B) High-resolution image of a growing tumour obtained using transparent tumour tomography, showing spatially heterogeneous regions expressing different biomarkers. *Source:* Obtained from https://www.flickr.com/photos/nihgov/27390448613 under license CC BY 2.0. Credit: Steve Seung-Young Lee, University of Chicago Comprehensive Cancer Center, National Cancer Institute, National Institutes of Health. (C) Description of evolutionary graph theory that studies evolutionary games in a spatially restricted environment. Parents and offsprings are organized as nodes in a graph. Mutants (blue nodes) can replace parental population only along the edges of the graph, in the direction specified by the arrows. Under such scenarios, fixation probability (taking over the entire population through successive generations) of a mutant, ρ, depends on the graph topology. Several topologies are shown along with their respective fixation probabilities. Here, r denotes the relative fitness of a mutant (compared to the parental population) and N is the population size. Topology names are according to Nowak et al. (D) Comparison of tumour growth in zebrafish over time, seeded with a mixture of fluorescence-tagged drug-sensitive (red) and drug-tolerant (green) cells under untreated, continuous, and intermittent therapy conditions. *Source:* Reproduced under Creative Common CC BY license. For more details, see Nam et al. 2021 [35].

These game strategies are subject to selection pressure from the microenvironment, and their payoffs (survival benefit) depend on the group populations. The more successful strategies that increase survival of the tumour

as a whole become dominant over time. The strategies dominant within a tumour determines the type of evolutionary game being played. Several studies have elegantly demonstrated that games, such as Prisoner's dilemma, Hawk-Dove, stag hunt, snowdrift, rock–paper–scissors, and Leader and Deadlock, are excellent models to explain many of the observations in clonal dynamics [31,39,41,42].

Therapeutic intervention represents a significant change in the tumour environment that reshapes the clonal composition by altering the fitness of existing clones and promoting newer ones. Drug treatment also leads to the emergence of new game strategies tailored towards evading drug toxicity. Conceptually, treatment and the emergence of drug resistance may be considered as an evolutionary predator–prey game between the cancer (prey) and the oncologist (predator). By analysing the nature of the game being played by the tumour (and carefully anticipating its future moves), the oncologist can design treatment strategies to effectively defeat the tumour [45]. Such strategies typically leverage the evolutionary costs associated with synthesis, maintenance, and operation of the molecular mechanisms necessary for evading and surviving drug treatment. Here, the benefit of resistance outweighs the costs. However, in the absence of treatment, particularly in the TME where resources are limited, the cost renders resistant cells less fit than their drug-sensitive counterparts. Thus, treatment withdrawal at regular intervals interspersed between treatment cycles (intermittent therapy) can encourage residual populations of drug-sensitive cells to exploit their fitness advantage at the expense of the
p. 381 less-fit resistant phenotypes. While withholding ↳

p. 382 ↳
treatment allows tumour regrowth, the resistant subpopulation remains small so that retreatment with the same drug(s) remains effective [44]. Thus, it follows that game theory-based studies have provided a novel framework for evolutionarily informed therapies wherein the physician aims to guide the eco-evolutionary dynamics of cancer towards better outcomes or outright cure [32,46,47]. Taken together, it is obvious that mathematical models provide valuable tools for formulating hypotheses and evaluating different scenarios pertaining to the interactions between cancer cell types and therapy [44].

Despite the apparent success, the complexity of cellular behaviour, such as soluble factors with non-linear effects on different cells as well as phenotypic plasticity, remains considerable challenges. For example, as discussed below, unlike in classical ecology models where players do not switch identities, cancer cells, drug-resistant, and drug-sensitive cells can switch their phenotypes because of their innate plasticity. Furthermore, since the behaviour of cancer cells is highly dependent on their unique microenvironment, it is often challenging to translate the findings from in vitro studies to *in vivo*. Secondly, the stochasticity involved at different levels of cellular behaviour, from intracellular biochemical networks to interactions among groups of cells, can result in multistability, and therefore, challenge claims of causal connection between quantitative phenotypic markers, such as the expression of cell surface receptors and their behavioural effects [48].

37.4. Tumour heterogeneity and significance of the spatial dimension

Cellular heterogeneity is a hallmark of cancer progression and reflected in the coexistence of multiple clones within the tumour and the diversity of the microenvironment. Much work has been carried out to explore the mutational landscape of developing tumours and its effect on treatment and resistance [49,50]. For instance, the positive correlation between tumour heterogeneity and worse clinical prognosis has been reported in multiple cancer types [51,52,53,54]. Recently, it was shown that linear (same clone successively acquiring multiple mutations) vs. branched (driver mutations distributed among multiple clones) evolution, as well as the sequence of acquiring mutations, makes a difference in clinical outcome in acute myeloid leukaemia [55]. Moreover, the frequency of certain mutations among subclones determines drug sensitivity. Such studies suggest that tumour heterogeneity evolves in response to selection pressure rather than as a by-product of cancer proliferation. In addition to mutations, non-genetic mechanisms play a major but underappreciated role in conferring phenotypic heterogeneity to the tumour ecosystem, which has been extensively discussed in several recent reviews by us and others [20,56,57]. Difficulty of measurement and the mutation-centric view of cancer are some of the reasons why non-genetic mechanisms are less appreciated. Besides clonal diversity, another major source of tumour heterogeneity is the TME (Figure 37.1A and B). As the disease progresses, crosstalk between the tumour and the TME shapes each other's heterogeneity and spatial organization, and this interaction is likely to be critical for the long-term survival of the disease. The benefits of the TME include providing proliferative and metabolic factors to the tumour and maintaining an immunosuppressive environment for the tumour to thrive. The ecological forces that shape tumour composition are challenging to study using current experimental techniques, leading to the development of theoretical and simulation frameworks as discussed next.

Solid tumours proliferate within a dense environment of host cells and extra-cellular matrix, where each cell mainly interacts with its nearest neighbours. This is in sharp contrast to scenarios, such as leukaemia, where cells are fully mobile and free to interact with any other cell in the environment. For example, in a spatially restricted environment, beneficial diffusible elements such as growth factors will primarily affect the near neighbourhood of the source cells since their levels will fall off with distance from the origin. Therefore, it has been proposed that spatial organization plays a vital role in tumour evolution, heterogeneity, and development of game strategies. To understand these effects from a theory standpoint, Nowak developed the evolutionary graph theory, where cells are organized as nodes in a graph [58]. Competition and cooperation are only allowed between neighbouring cells that share common edges in the graph network (Figure 37.1C). Nowak and co-workers have showed that evolutionary dynamics follows different trajectories in a spatially constrained environment [59], depending on the graph layout (i.e. fully connected, scale free or circular, etc.). Key properties, such as fixation probability (certain graph layouts can amplify or suppress natural selection) and payoffs, vary depending on the graph layouts, thus highlighting the importance of spatial dimension in tumour evolution. One interesting outcome of such theoretical analysis is the emergence of cooperation. In simulations of cooperator–defector dynamics on a spatial grid, it was shown that defectors invade the cooperator cells and outcompete them when the benefit to defection was above a certain threshold. However, the cooperators were never completely wiped out, and they survived by organizing themselves into tight clusters.

The above narrative carries a strong parallel to our observation of the interplay between drug-sensitive and drug-tolerant NSCLC cells in *in vivo* zebrafish model [35], where the drug-tolerant cells (cooperators) formed tight clusters, surrounded by the drug-sensitive cells (defectors) (Figure 37.1D). NSCLC can be classified as adenocarcinoma or squamous cell carcinoma or large cell carcinoma histologically. There are a number of oncogenes that can be abnormal in NSCLC, such as EGFR mutation, ALK fusions, ROS1 fusions, MET exon 14 splice variants, and others. There is large heterogeneity of lung cancer; however, the majority of NSCLC respond to platinum-based therapy. Independent *in vitro* studies, coupled with mathematical modelling,

confirmed the cooperative trait of the drug-tolerant cells in the form of diffusible factors. Works like the above that explore cancer progression from the ecological perspective are rare due to the challenges faced in monitoring the phenotypic behaviour of tumour components at the cellular level. One exciting development in this field is the microfluidic death galaxy developed by Austin and co-workers who can monitor the growth and spatial organization of multiple cell types under different ecological conditions, such as drug concentrations [43]. Combining experimental observations with game theory models, such constructs, can estimate hard to obtain parameters such as payoffs under varying selection pressures, which can be used for future prediction of prognosis under therapeutic intervention.

37.5. Group behaviour via non-genetic mechanisms facilitates therapy resistance

Historically, cancer research has focused on genetic alterations (e.g. mutations, copy number variations, and chromosomal instability) as the primary drivers of the disease. Emergence of resistance-conferring mutations in response to therapy is typically ascribed to the cause of tumour survival and disease relapse. Recently, however, the importance of non-genetic resistance mechanisms has come to light. Due to their genetic and epigenomic alterations, cancer cells are adept in rewiring their signalling networks to bypass the effect of anti-cancer drugs, as shown in the case of melanoma [60,61], or more recently through our own works on NSCLC [35,62]. These network rewiring acts are typically carried out at the transcriptional (e.g. regulation of transcription factors, DNA looping, and chromatin accessibility), post-transcriptional (e.g. alternative splicing and selective RNA degradation), translational (e.g. ribosomal regulation), or post-translational (e.g. protein phosphorylation and ubiquitination) levels and through various autocrine and paracrine processes. By network rewiring, cancer cells can temporarily switch to a different phenotype that enables them to survive under a rapidly changing environment, such as ambient drug concentrations. This behaviour is called phenotypic switching and has a strong presence in the fungal and bacterial world, where such mechanisms are used to combat sudden toxicity or invasion by competing microorganisms [63,64]. In the cancer world, one prime example of phenotypic switching is melanoma cells transitioning from a proliferative (drug sensitive) to an invasive phenotype (drug resistant) in response to mitogen-activated protein kinase (MAPK) pathway inhibitors, without undergoing genetic mutations [60].

An important indicator of non-genetic resistance mechanisms is drug-tolerant persister (DTP), a small subpopulation of drug-tolerant cancer cells that naturally exist within an otherwise sensitive population and survive drug treatment without undergoing genetic alterations. DTPs have been studied and referred to multiple times in the literature over the past decade, after they were first reported in 2010 [25,65]. Upon extended drug exposure, the DTPs were found to proliferate and reestablish *in vitro* colony of drug-tolerant cells, and these cells reverted to drug sensitivity upon drug withdrawal within a few cell divisions (~30). These reversible phenotypic transitions within a few generations indicate that heritable epigenetic modifications can stabilize drug-tolerant phenotypes and are supported by the involvement of histone demethylation, as in the case of melanoma cells.

Available evidence therefore indicates that in some cases non-genetic mechanisms, such as phenotypic switching and heritable epigenetic modifications, may be preferred over genetic mutations in developing therapy resistance. The question is why? From a game theory perspective where cells or groups of cells can be considered players, both non-genetic and genetic mechanisms are survival strategies that evolve under environmental pressures. If we analyse the benefits and costs associated with each type of strategy, it may be understandable why one would be preferable over the other. Non-genetic mechanisms are rapid and reversible and are therefore capable of addressing sudden changes in the environment. Genetic mechanisms lead to the fixation of heritable alterations in the genome that once acquired are not easily lost. However, evolution of such strategies through fitness conferring mutations require cellular proliferation and selection over multiple

generations (through stochastic trial-and-error attempts) and are typically slower in response to environmental changes. Moreover, genetic changes are permanent since reversing a mutation is not a spontaneous process. In comparison, non-genetic mechanisms can be reversible. While switching from a proliferative to an invasive phenotype, melanoma cells still retained their proliferative potential so that when the drug pressure was lifted, they were able to switch to the proliferative phenotype and reestablish their colony [60]. However, it is important to note that non-genetic mechanisms often come with a cost. For instance, they may require increased energy expenditure due to elevated transcription, protein synthesis, and kinase recruitment for phenotypic switching mechanisms.

The choice between non-genetic mechanisms and permanent genetic alterations as survival strategies depends on a careful assessment of the associated benefits and costs. In situations where the advantages of rapid response outweigh the costs, non-genetic mechanisms become the preferred option. It is hypothesized that the administration of anti-cancer drugs can induce an environmental shift that favours non-genetic mechanisms during the initial phase of adaptability as opposed to genetic alterations. Currently, the proposed hypothesis by our research team and other experts in the field suggests that non-genetic mechanisms can serve as a survival strategy for tumours facing environmental stress, such as exposure to cytotoxic drugs. These mechanisms provide a temporary solution until more permanent modifications, such as genetic mutations or epigenetic changes, evolve [1,20,66]. It could also mean that therapists can manipulate the environmental conditions that promote the dominance of non-genetic mechanisms within the tumour and delay the emergence of permanently resistant clones [35,66]. We will hold that thought and revisit it in a subsequent section (intermittent therapy).

37.6. Phenotypic switching, stress response, and intra-tumour cooperation

In the previous section, we have argued how drug-sensitive cancer cells can avert the effect of environmental stress by temporarily switching to a drug-tolerant phenotype. We recently addressed the question whether drug-tolerant phenotypes can also cooperate with drug-sensitive phenotypes and assist their survival under stress [35]. Such cooperation (and altruism) will benefit the tumour as a whole, being embodied in the theoretical framework of the Price equation, and was explored in the context of drug resistance evolution in bacteria [67]. To this end, we monitored the *in vitro* growth of drug-sensitive and tolerant NSCLC cells (shown to undergo phenotypic switching [62]) both in monotypic cultures and mixed at various ratios over a period of several weeks, in the presence or absence of the chemotherapeutic drug cisplatin. The growth rates of these cells when cultured together showed significant differences compared to their respective monotypic cultures. Additionally, the growth rates varied according to the proportion of sensitive to tolerant cells. Clearly, these cells altered their behaviour by sensing each other's presence in a frequency-dependent manner, underscoring the
p. 384 importance of group behaviour in ↳ tumour growth. We quickly realized that the complex growth dynamics of these cells could not be explained by invoking simple competition or cooperation, thus necessitating more intricate models. We also observed two interesting characteristics of these cells: (1) the sensitive cells secreted a diffusible factor that negatively affected cellular growth, including that of tolerant cells; (2) the tolerant cells could be reverted to cisplatin sensitivity by using a histone deacetylase inhibitor, indicating an epigenetic basis for their drug tolerance. These observations motivated us to develop a mathematical theory of drug resistance that incorporates the role of phenotypic switching, stress response, and cooperation of cancer cells in a community environment.

37.7. Phenotypic switching enables cancer cells to adapt to rapid environmental changes

Detailed formulation of PSMSR is already published elsewhere [35], so we briefly discuss it here. The chief hypothesis behind PSMSR is that cancer cell phenotype is not rigid but stochastically switch between drug-sensitive and drug-tolerant states. Further, this phenotypic switching can be influenced by environmental factors that adversely affect cellular growth, such as stress elements, lack of oxygen, or diffusible factors (collectively referred to as stress in this model) (Figure 37.2A). We also explored competition and cooperation through dissemination of 'public goods' and neutralization of stress by the drug-tolerant phenotype. As a result, evolutionary strategies dynamically altered with the level of stress in the environment (Figure 37.2B). The different parameters of PSMSR (e.g. cellular growth, phenotypic switching, and stress generation/neutralization rates) were derived by fitting real-time growth data of sensitive and tolerant cells in mixed cultures at different ratios (Figure 37.2C) [35]. In comparison to several other cellular growth models that account for group interactions, PSMSR fits the experimental data the best.

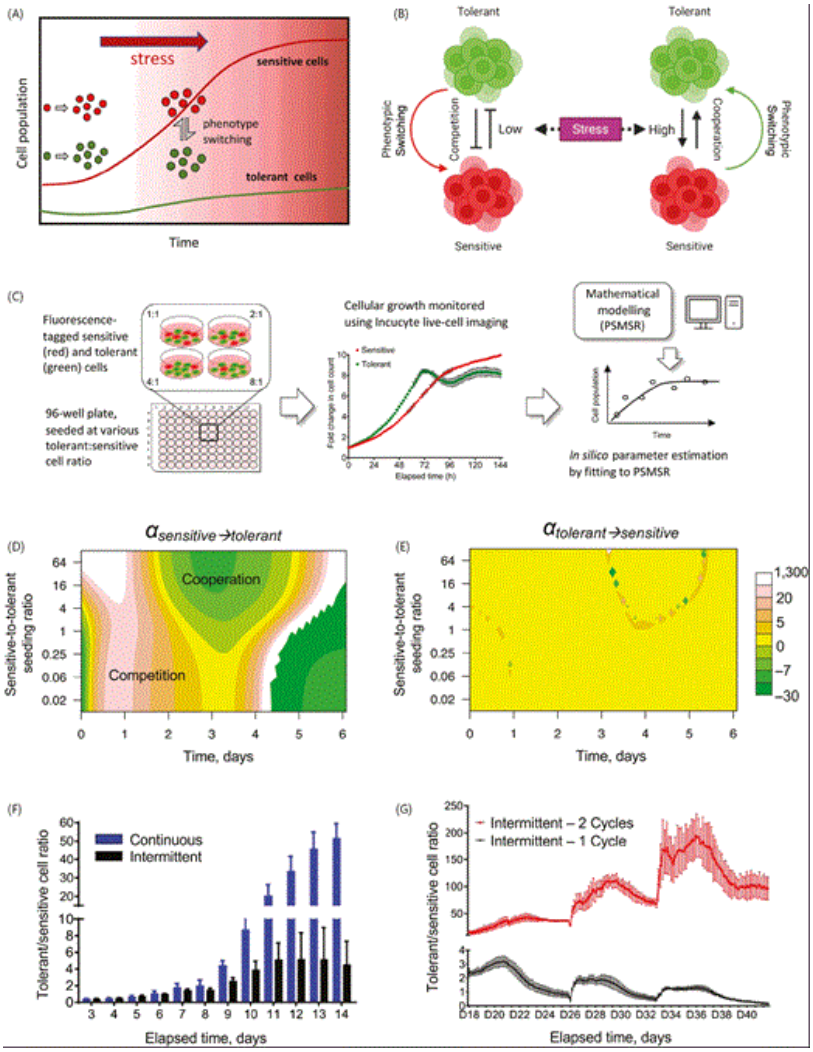

Figure 37.2. (A) Schematic describing the physical process (cellular growth and stress generation over time) that forms the basis of PSMSR. (B) Depiction of phenotypic switching between drug-sensitive and drug-tolerant lung cancer cells in response to environmental stress. (C) Schematic of cell growth monitoring process to determine group behaviour by fitting to PSMSR. (D, E) Dynamic game strategic landscapes of drug-sensitive and drug-tolerant phenotypes as a function of time, under various seeding conditions. Starting from initial populations of sensitive and tolerant cells mixed at different proportions (seeding ratios), cellular growth was simulated using PSMSR. Competition/cooperation was estimated by piecewise fitting of the competitive Lotka–Volterra equation to the PSMSR growth trends over a window of three days. Contour plots show the α parameters of the LV equation for different time and seeding ratios. Contours are coloured according to quantiles. Positive and negative values are indicative of competition and cooperation, respectively. For ease of comparison, the same scale is used in both (D) and (E). For more information, see Nam et al. Biomolecules, 2022. (F) Tolerant-to-sensitive cell ratio as a function of time under continuous and intermittent cisplatin therapy, monitored using the set-up depicted in (C). Sensitive and tolerant cells were cultured at an initial seeding ratio of 4:1. (G) Comparison of tolerant cell expansion under two different intermittent therapy regimens (see the text for more details). *Source:* Panels (F) and (G) are reproduced under Creative Common CC BY license. For more details, see Nam et al. 2021 [35].

PSMSR predicted that in addition to the cellular frequencies the level of growth-retarding diffusible factors released by the sensitive cells (termed stress in the model) determined the evolutionary strategy adopted by the tolerant cells. At low stress, passive competition existed between the two phenotypes, which quickly changed into cooperation by the tolerant cells (due to increased stress neutralization, thus benefiting the whole community) as stress built up (Figure 37.2D). The optimum fraction of tolerant phenotypes in the ecosystem (to ensure positive payoff for the whole community) was determined by the phenotypic switching rates, which

dynamically altered in response to stress and cellular frequencies. By combining PSMSR with competitive Lotka–Volterra model, we determined the magnitude of cooperative interaction between the sensitive and tolerant phenotypes as a function of time and seeding ratio, thus generating evolutionary strategy landscapes for both cell types (Figure 37.2D and E). We found that compared to the sensitive phenotype, the tolerant phenotype was more flexible in altering their game strategies depending on the environment. This suggests that the tolerant phenotype is more adaptive to increased stress, such as therapeutic pressure, and their cooperation with the sensitive cells could ensure the survival of the tumour as a whole. One caveat is that these inferences are drawn from *in vitro* studies and therefore cannot account for the effects of a real TME, which can alter the strategy landscape of the tumour and direct resistance evolution significantly.

37.8. Non-genetic resistance mechanism underscores the benefit of adaptive/intermittent therapy in delaying resistance

Traditionally, the first-line therapy in cancer involves continuous administration of targeted drugs or chemotherapeutic agents at the maximum tolerated dose. This inevitably creates a condition that favours the emergence of resistant disease. Recent awareness of the role of ecology in resistance development has led to innovations in therapy design, such as adaptive therapy, that aims to delay the onset of resistance [4,20,30,35,46]. The goal of adaptive therapy is to suppress the emergence of the drug-resistant phenotype and maintain drug sensitivity of the tumour by adjusting the dosage amounts and intervals. Adaptive therapy designs are usually based on assumptions, such as (1) pre-existence of drug-resistant clones in the original tumour and (2) low fitness of resistant cells in the absence of therapeutic pressure [40]. In a 2018 review article from our group, we argued that adaptive therapy through intermittent dosage can also be beneficial in cases where the resistance mechanism is non-genetic (as opposed to therapy-induced selection of pre-existing resistant clones) [20]. As was shown in multiple cases (and discussed in previous sections of this chapter), drug-tolerant phenotypes can emerge from drug-sensitive cellular population through non-genetic mechanisms, such as phenotypic switching and epigenetic alterations. Drug resistance in such cases do not need the pre-existence of resistant clones in the tumour. However, adaptive therapies can still be beneficial by suppressing the rate of transition to the tolerant phenotype or altering the group dynamics among the sensitive and tolerant phenotypes as well as the microenvironment.

We have shown that intermittent rather than continuous cisplatin treatment can suppress the growth of tolerant NSCLC cells and retain drug sensitivity in both *in vitro* and zebrafish models [35]. Starting with a mixture of fluorescence-tagged sensitive and tolerant cells at different seeding ratios, we cultured them for two weeks under continuous exposure to 1 μM cisplatin, as well as an initial exposure of three days, followed by growth in cisplatin-free media (intermittent dosage). The cellular growth was quantitatively measured in real time using Incucyte live cell analyser. As expected, we observed a massive expansion in tolerant cell population (60–80 times sensitive cell population) under continuous cisplatin treatment with the increasing trend continuing at the end of two weeks (Figure 37.2F). Conversely, under intermittent treatment, the tolerant-to-sensitive ratio was moderate [4,5,6] and was stabilized by day 10. Intermittent cisplatin treatment also suppressed the proliferation of tolerant cells *in vivo* in zebrafish, although the experiments had to be concluded within five days due to regulatory reasons (Figure 37.1D).

To see the long-term effect of intermittent cisplatin treatment, we continued the *in vitro* experiments for an extended period and noted that the tolerant-to-sensitive cell ratio was still low after 43 days (Figure 37.2G). Interestingly, a second cisplatin dose added on day 14 (intermittent: two cycles) after the first cisplatin treatment triggered the proliferation of tolerant cells (Figure 37.2G) suggesting that the exposure of the

 sensitive cell population to the first cisplatin ↳

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dose may have compromised their proliferative potential, thereby allowing tolerant cells to proliferate. We also used PSMSR to model the cellular growth under cisplatin treatment and showed that PSMSR captured the difference in growth dynamics between the continuous and intermittent conditions [35]. Further experiments are needed, potentially using mouse models of NSCLC to further establish the effectiveness of such therapeutic strategies under *in vivo* conditions. Mathematical and agent-based models that account for the group behaviour of mixed populations of sensitive and tolerant cells can be valuable tools to determine the optimal drug doses and intervals under intermittent treatment.

37.9. Conclusions and future directions

It is well established that, even within a given cancer type, there exist multiple mechanisms that regulate phenotypic switching and drug resistance. Furthermore, as discussed here, although intermittent therapy appears promising in some cases, several challenges still remain. Nonetheless, from the foregoing, it is obvious that insights from cancer systems biology and mathematical modelling can help identify new treatment strategies based on the principles of ecology and evolution. By incorporating these new concepts in clinical protocols, we could enhance the precision in which we deliver personalized medicine to all our patients, regardless of their economic status or their ability to access advanced medical centres. Furthermore, lowering the dose of the drug and its frequency as a result of intermittent rather than continuous therapy could not only lower the toxicity and undesirable side effects of the drugs but may also positively impact the financial burden carried by the patient and insurance providers [68].

References

1. Bhattacharya S, Mohanty A, Achuthan S, Kotnala S, Jolly MK, Kulkarni P, et al. Group behavior and emergence of cancer drug resistance. *Trends Cancer.* 2021;**7**(4):323–34.
Google Scholar WorldCat

2. Dominiak A, Chelstowska B, Olejarz W, Nowicka G. Communication in the cancer microenvironment as a target for therapeutic interventions. *Cancers (Basel).* 2020;**12**(5).
Google Scholar WorldCat

3. Brucher BL, Jamall IS. Cell-cell communication in the tumor microenvironment, carcinogenesis, and anticancer treatment. *Cell Physiol Biochem.* 2014;**34**(2):213–43.
Google Scholar WorldCat

4. Gupta PB, Pastushenko I, Skibinski A, Blanpain C, Kuperwasser C. Phenotypic plasticity: driver of cancer initiation, progression, and therapy resistance. *Cell Stem Cell.* 2019;**24**(1):65–78.
Google Scholar WorldCat

5. Hansemann DV. Ueber asymmetrische Zelltheilung in Epithelkrebsen und deren biologische Bedeutung. *Arch Pathol Anat Physiol Klin Med.* 1890;**119**:299–326.
Google Scholar WorldCat

6. Boveri T. *Zur Frage der Entstehung maligner Tumoren.* Jena: Gustav Fischer; 1914.
Google Scholar Google Preview WorldCat COPAC

7. Nowell PC. The clonal evolution of tumor cell populations. *Science.* 1976;**194**(4260):23–8.
Google Scholar WorldCat

8. Knudson AG. Two genetic hits (more or less) to cancer. *Nat Rev Cancer.* 2001;**1**(2):157–62.
Google Scholar WorldCat

9. Loeb LA, Harris CC. Advances in chemical carcinogenesis: a historical review and prospective. *Cancer Res.* 2008;**68**(17):6863–72.
Google Scholar WorldCat

10. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell.* 2000;**100**(1):57–70.
Google Scholar WorldCat

11. Martincorena I, Campbell PJ. Somatic mutation in cancer and normal cells. *Science.* 2015;**349**(6255):1483–9.
Google Scholar WorldCat

12. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: an evolving paradigm. *Nat Rev Cancer.* 2013;**13**(10):714–26.
Google Scholar WorldCat

13. Nussinov R, Tsai CJ, Jang H. Anticancer drug resistance: an update and perspective. *Drug Resist Updat.* 2021;**59**:100796.
Google Scholar WorldCat

14. Marine JC, Dawson SJ, Dawson MA. Non-genetic mechanisms of therapeutic resistance in cancer. *Nat Rev Cancer.* 2020;**20**(12):743–56.
Google Scholar WorldCat

15. Bell CC, Gilan O. Principles and mechanisms of non-genetic resistance in cancer. *Br J Cancer.* 2020;**122**(4):465–72.
Google Scholar WorldCat

16. Huang S. Reconciling non-genetic plasticity with somatic evolution in cancer. *Trends Cancer.* 2021;**7**(4):309–22.
Google Scholar WorldCat

17. Pigliucci M, Murren CJ, Schlichting CD. Phenotypic plasticity and evolution by genetic assimilation. *J Exp Biol.* 2006;**209**(Pt 12):2362–7.
Google Scholar WorldCat

18. Mathis RA, Sokol ES, Gupta PB. Cancer cells exhibit clonal diversity in phenotypic plasticity. *Open Biol.* 2017;**7**(2).
Google Scholar WorldCat

19. Jolly MK, Kulkarni P, Weninger K, Orban J, Levine H. Phenotypic plasticity, bet-hedging, and androgen independence in prostate cancer: role of non-genetic heterogeneity. *Front Oncol.* 2018;**8**:50.
Google Scholar WorldCat

20. Salgia R, Kulkarni P. The genetic/non-genetic duality of drug 'resistance' in cancer. *Trends Cancer.* 2018;**4**(2):110–8.
Google Scholar WorldCat

21. Hata AN, Niederst MJ, Archibald HL, Gomez-Caraballo M, Siddiqui FM, Mulvey HE, et al. Tumor cells can follow distinct evolutionary paths to become resistant to epidermal growth factor receptor inhibition. *Nat Med.* 2016;**22**(3):262–9.
Google Scholar WorldCat

22. Wang X, Zhang H, Chen X. Drug resistance and combating drug resistance in cancer. *Cancer Drug Resist.* 2019;**2**(2):141–60.
Google Scholar WorldCat

23. Mullard A. Stemming the tide of drug resistance in cancer. *Nat Rev Drug Discov.* 2020;**19**(4):221–3.
Google Scholar WorldCat

24. Venkatesan S, Swanton C, Taylor BS, Costello JF. Treatment-induced mutagenesis and selective pressures sculpt cancer evolution. *Cold Spring Harb Perspect Med.* 2017;**7**(8).
Google Scholar WorldCat

25. Swayden M, Chhouri H, Anouar Y, Grumolato L. Tolerant/persister cancer cells and the path to resistance to targeted therapy. *Cells.* 2020;**9**(12).
Google Scholar WorldCat

26. Tomlinson IP. Game-theory models of interactions between tumour cells. *Eur J Cancer.* 1997;**33**(9):1495–500.
Google Scholar WorldCat

27. Gatenby RA, Vincent TL. An evolutionary model of carcinogenesis. *Cancer Res.* 2003;**63**(19):6212–20.
Google Scholar WorldCat

28. Archetti M. Evolutionary game theory of growth factor production: implications for tumour heterogeneity and resistance to therapies. *Br J Cancer.* 2013;**109**(4):1056–62.
Google Scholar WorldCat

29. Hiltunen T, Virta M, Laine AL. Antibiotic resistance in the wild: an eco-evolutionary perspective. *Philos Trans R Soc Lond B Biol Sci.* 2017;**372**(1712).
Google Scholar WorldCat

30. Stanková K, Brown JS, Dalton WS, Gatenby RA. Optimizing cancer treatment using game theory: a review. *JAMA Oncol.* 2019;**5**(1):96–103.
Google Scholar WorldCat

31. Kaznatcheev A, Peacock J, Basanta D, Marusyk A, Scott JG. Fibroblasts and alectinib switch the evolutionary games played by non-small cell lung cancer. *Nat Ecol Evol.* 2019;**3**(3):450–6.

Google Scholar WorldCat

32. Staňková K. Resistance games. *Nat Ecol Evol.* 2019;**3**(3):336–7.
Google Scholar WorldCat

33. Grolmusz VK, Chen J, Emond R, Cosgrove PA, Pflieger L, Nath A, et al. Exploiting collateral sensitivity controls growth of mixed culture of sensitive and resistant cells and decreases selection for resistant cells in a cell line model. *Cancer Cell Int.* 2020;**20**:253. ↳
p. 387
Google Scholar WorldCat

34. Archetti M. Collapse of intra-tumor cooperation induced by engineered defector cells. *Cancers (Basel).* 2021;**13**(15).
Google Scholar WorldCat

35. Nam A, Mohanty A, Bhattacharya S, Kotnala S, Achuthan S, Hari K, et al. Dynamic phenotypic switching and group behavior help non-small cell lung cancer cells evade chemotherapy. *Biomolecules.* 2021;**12**(1).
Google Scholar WorldCat

36. von Neumann J, Morgenstern O, Rubinstein A. *Theory of games and economic behavior (60th Anniversary Commemorative Edition).* Princeton University Press; 1944.
Google Scholar Google Preview WorldCat COPAC

37. Osborne MJ, Rubinstein A. *A course in game theory.* Cambridge, MA: MIT Press; 1994.
Google Scholar Google Preview WorldCat COPAC

38. Borenstein E, Meilijson I, Ruppin E. The effect of phenotypic plasticity on evolution in multipeaked fitness landscapes. *J Evol Biol.* 2006;**19**(5):1555–70.
Google Scholar WorldCat

39. Bayer P, Gatenby RA, McDonald PH, Duckett DR, Staňková K, Brown JS. Coordination games in cancer. *PLoS ONE.* 2022;**17**(1):e0261578.
Google Scholar WorldCat

40. Gatenby RA, Silva AS, Gillies RJ, Frieden BR. Adaptive therapy. *Cancer Res.* 2009;**69**(11):4894–903.
Google Scholar WorldCat

41. McEvoy JW. Evolutionary game theory: lessons and limitations, a cancer perspective. *Br J Cancer.* 2009;**101**(12):2060–1; author reply 2–3.
Google Scholar WorldCat

42. Pacheco JM, Santos FC, Dingli D. The ecology of cancer from an evolutionary game theory perspective. *Interface Focus.* 2014;**4**(4):20140019.
Google Scholar WorldCat

43. Wu A, Liao D, Tlsty TD, Sturm JC, Austin RH. Game theory in the death galaxy: interaction of cancer and stromal cells in tumour microenvironment. *Interface Focus.* 2014;**4**(4):20140028.
Google Scholar WorldCat

44. Zhang J, Cunningham JJ, Brown JS, Gatenby RA. Integrating evolutionary dynamics into treatment of metastatic castrate-resistant prostate cancer. *Nat Commun.* 2017;**8**(1):1816.
Google Scholar WorldCat

45. Archetti M, Pienta KJ. Cooperation among cancer cells: applying game theory to cancer. *Nat Rev Cancer.* 2019;**19**(2):110–7.
Google Scholar WorldCat

46. Gatenby RA. A change of strategy in the war on cancer. *Nature.* 2009;**459**(7246):508–9.
Google Scholar WorldCat

47. Ibrahim-Hashim A, Robertson-Tessi M, Enriquez-Navas PM, Damaghi M, Balagurunathan Y, Wojtkowiak JW, et al. Defining cancer subpopulations by adaptive strategies rather than molecular properties provides novel insights into intratumoral evolution. *Cancer Res.* 2017;**77**(9):2242–54.
Google Scholar WorldCat

48. Pisco AO, Brock A, Zhou J, Moor A, Mojtahedi M, Jackson D, et al. Non-Darwinian dynamics in therapy-induced cancer drug resistance. *Nat Commun.* 2013;**4**:2467.
Google Scholar WorldCat

49. Ramón YCS, Sesé M, Capdevila C, Aasen T, De Mattos-Arruda L, Diaz-Cano SJ, et al. Clinical implications of intratumor heterogeneity: challenges and opportunities. *J Mol Med (Berl).* 2020;**98**(2):161–77.
Google Scholar WorldCat

50. Diaz-Cano SJ. Tumor heterogeneity: mechanisms and bases for a reliable application of molecular marker design. *Int J Mol Sci.* 2012;**13**(2):1951–2011.
Google Scholar WorldCat

51. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. *New Engl J Med.* 2016;**374**(23):2209–21.
Google Scholar WorldCat

52. Mroz EA, Rocco JW. MATH, a novel measure of intratumor genetic heterogeneity, is high in poor-outcome classes of head and neck squamous cell carcinoma. *Oral Oncol.* 2013;**49**(3):211–5.
Google Scholar WorldCat

53. Zhang J, Fujimoto J, Zhang J, Wedge DC, Song X, Zhang J, et al. Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. *Science.* 2014;**346**(6206):256–9.
Google Scholar WorldCat

54. Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science.* 2014;**344**(6190):1396–401.
Google Scholar WorldCat

55. Benard BA, Leak LB, Azizi A, Thomas D, Gentles AJ, Majeti R. Clonal architecture predicts clinical outcomes and drug sensitivity in acute myeloid leukemia. *Nat Commun.* 2021;**12**(1):7244.
Google Scholar WorldCat

56. Sharma A, Merritt E, Hu X, Cruz A, Jiang C, Sarkodie H, et al. Non-genetic intra-tumor heterogeneity is a major predictor of phenotypic heterogeneity and ongoing evolutionary dynamics in lung tumors. *Cell Rep.* 2019;**29**(8):2164–74.e5.
Google Scholar WorldCat

57. Black JRM, McGranahan N. Genetic and non-genetic clonal diversity in cancer evolution. *Nat Rev Cancer.* 2021;**21**(6):379–92.
Google Scholar WorldCat

58. Nowak MA. *Evolutionary dynamics exploring the equations of life.* Cambridge, MA: Harvard University Press; 2006.
Google Scholar Google Preview WorldCat COPAC

59. Noble R, Burri D, Le Sueur C, Lemant J, Viossat Y, Kather JN, et al. Spatial structure governs the mode of tumour evolution. *Nat Ecol Evol.* 2022;**6**(2):207–17.
Google Scholar WorldCat

60. Kemper K, de Goeje PL, Peeper DS, van Amerongen R. Phenotype switching: tumor cell plasticity as a resistance mechanism and target for therapy. *Cancer Res.* 2014;**74**(21):5937–41.
Google Scholar WorldCat

61. Zipser MC, Eichhoff OM, Widmer DS, Schlegel NC, Schoenewolf NL, Stuart D, et al. A proliferative melanoma cell phenotype is responsive to RAF/MEK inhibition independent of BRAF mutation status. *Pigment Cell Melanoma Res.* 2011;**24**(2):326–33.
Google Scholar WorldCat

62. Mohanty A, Nam A, Pozhitkov A, Yang L, Srivastava S, Nathan A, et al. A non-genetic mechanism involving the integrin beta4/Paxillin axis contributes to chemoresistance in lung cancer. *iScience.* 2020;**23**(9):101496.
Google Scholar WorldCat

63. Farquhar KS, Charlebois DA, Szenk M, Cohen J, Nevozhay D, Balázsi G. Role of network-mediated stochasticity in mammalian drug resistance. *Nat Commun.* 2019;**10**(1):2766.
Google Scholar WorldCat

64. Jain N, Hasan F, Fries BC. Phenotypic switching in fungi. *Curr Fungal Infect Rep.* 2008;**2**(3):180–8.
Google Scholar WorldCat

65. Sharma SV, Lee DY, Li B, Quinlan MP, Takahashi F, Maheswaran S, et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell.* 2010;**141**(1):69–80.
Google Scholar WorldCat

66. Gunnarsson EB, De S, Leder K, Foo J. Understanding the role of phenotypic switching in cancer drug resistance. *J Theor Biol.* 2020;**490**:110162.
Google Scholar WorldCat

67. Gjini E, Wood KB. Price equation captures the role of drug interactions and collateral effects in the evolution of multidrug resistance. *eLife.* 2021;**10**:e64851.
Google Scholar WorldCat

68. Mason NT, Burkett JM, Nelson RS, Pow-Sang JM, Gatenby RA, Kubal T, et al. Budget impact of adaptive abiraterone
p. 388 therapy for castration-resistant prostate cancer. *Am Health Drug Benefits.* 2021;**14**(1):15–20. ↳
Google Scholar WorldCat