 doi:10.20944/preprints202007.0547.v1

Transition therapy: tackling the ecology of tumor phenotypic plasticity

Guim Aguadé-Gorgorió,[1,2] Stuart Kauffman,[3] and Ricard Solé*[1,2,4]
[1]ICREA-Complex Systems Lab, Universitat Pompeu Fabra, 08003 Barcelona, Spain
[2]Institut de Biologia Evolutiva, CSIC-UPF, 08003 Barcelona, Spain
[3]Institute for Systems Biology, Seattle WA 98109, USA
[4]Santa Fe Institute, Santa Fe NM 87501, USA

Phenotypic switching in cancer cells has been found to be present across tumor types. Recent studies on Glioblastoma report a remarkably common architecture of four well-defined phenotypes coexisting within high levels of intra-tumour genetic heterogeneity. Tumors grown from any cell type recapitulate the original phenotypic composition. Similar dynamics have been shown to occur in breast cancer, melanoma and likely across further cancer types. Given the adaptive potential of phenotypic switching (PHS) strategies, understanding how it drives tumor evolution and how to break down these architectures is a major priority. Here we model the ecological dynamics behind PHS. The model is able to reproduce experimental results, and specific conditions for cancer progression and clearence reveal novel features of plastic tumors and its direct consequences on therapy resistance. Following our results we discuss *transition* therapy as a novel scheme to target not only combined cytotoxicity but also the rates of phenotypic switching.

Keywords: Cancer ecology, phenotypic switching, epigenetic plasticity, combination therapies, transition therapy.

I. INTRODUCTION

Phenotypic plasticity is a widespread phenomenon across the tree of life. From bacteria to multicellular development, epigenetic pathways generate a population of diverse phenotypes from homogeneous, stable genomes [1-4]. Phenotypic switching (PHS) is a stochastic phenomenon known to maintain population diversity in unicellular organisms as a means to survive in fluctuating environments [5,6]. This mechanism can also be found to boost non-genetic heterogeneity in a special multicellular context: cancer cell populations [7]. In this context, tumors can take advantage of already existing differentiation hierarchies to promote unlimited self-renewal or senescence and drug resistance with no need of selecting somatic mutations [8,9].

Phenotypic switching is a source for non-genetic heterogeneity in cancer [7,10,11]. The most recent example comes from Glioblastomas, where tumor cells are found to organize around four well-defined meta-modules resembling -though aberrant- healthy brain cell lines [12]. This arrangement is highly robust: tumors initiated by single cells from a biopsy evolve towards the previous phenotypic composition, regardless of the specific phenotype of the original cell, showing that stochastic transitions happen between all of the four phenotypes. Similar dynamics have been described in breast cancer [13], as well as in melanoma [14,15] and prostate cancer [16].

The existence of phenotypic plasticity in tumors has important consequences for therapy. Tumor relapse after therapy is usually acknowledged to be a consequence of pre-existing or acquired resistance mutations, present in a given subclone that survives and repopulates the tumor (see e.g [17]). This image is often correct, yet further mechanisms in many therapeutic settings, from stem cell senescence [18] to immunological editing [19] prove that a wider scope is key when trying to understand therapeutic failure. The stochastic nature of switching between rogue

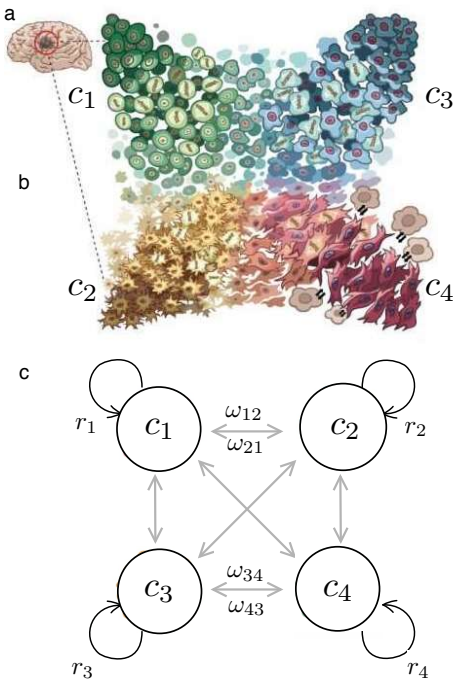

FIG. 1 **Phenotypic switching in cancer.** Genetic analysis reveals four transitioning phenotypes in Glioblastoma (a) and thus a set of cancer cell populations (b, after Neftell et al., 2019). Different transitions occur, linking phenotypes C_k by means of a matrix of transition rates, as sketched in (c).

*corresponding author

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 July 2020 doi:10.20944/preprints202007.0547.v1

cellular phenotypes allows robust and plastic tissue architectures, resulting in an adaptive mechanism that might be even harder to target [20]. How does this affect therapeutic strategies? Models of phenotypic switching have helped to explore cancer invasion [21-23] or the possible role of plasticity in maintaining a resistant phenotypes [24].

Here we present a toy model to study the characteristics of phenotypic plasticity in cancer by exploring the population dynamics of competing replicators exhibiting transitions among them (Fig. 1). The model allows in particular to analyze the rise of the switching populations and the equilibrium conditions for stable heterogeneity, as well as the requirements to tumor extinction with implications on novel therapeutic approaches.

II. PHENOTYPIC SWITCHING DYNAMICS

In this section we explore several features exhibited by different versions of a toy model of cancer cell populations exhibiting PHS. Our goal is to provide some basic bounds to the response of these systems to therapies acting on the switching dynamics and how they relate with the action of cytotoxic or targeted agents. Ecological models of heterogeneous cancer populations can be represented by means of a set of replicator equations [25]. Consider a set of N phenotypes, where $\mathbf{C} = (C_1, ..., C_N)$. The i-th cancer cell type population will change in time following:

$$\frac{dC_i}{dt} = \Gamma_i(\mathbf{C})C_i + \sum_{k\neq i}\omega_{ki}C_k - \sum_{k\neq i}\omega_{ik}C_i - C_i\phi(\mathbf{C}) \quad (1)$$

with $(i, k = 1, ..., N)$. Here $\Gamma_i(\mathbf{C})$ indicates the functional form of the replication rate associated with the $i-$th clone, which in general will be a nonlinear function of clone or tumor size [26]. The three last terms in the rhs correspond to (1) the phenotypic transitions from other phenotypes to phenotype C_i (i. e. $C_k \to C_i$) (2) the complementary transitions from C_i to the rest (i. e. $C_i \to C_k$) and (3) an outflow term that allows introducing competition effects and limited resources. Specifically, if we impose $\sum_k C_k = 1$, i. e. a constant population constraint (CPC), the explicit form of ϕ can be calculated. The previous set of equations can be re-written as follows:

$$\frac{dC_i}{dt} = \left(\Gamma_i(\mathbf{C}) - \sum_{k\neq i}\omega_{ik}\right)C_i + \sum_{k\neq i}\omega_{ki}C_k - C_i\phi(\mathbf{C}) \quad (2)$$

where we have aggregated those terms affecting C_i. In this way we can appreciate the fact that, because of phenotypic switching, the effective growth rate of C_i involves a trade-off between intrinsic replication and the likelihood that it shifts to a different cell type. Moreover a negative balance leading to a potentially decreasing growth of C_i can be counterbalanced by the net inflow from the rest of the phenotypes. Most models of cancer growth consider constant replication rates associated to each phenotype and as a first approximation we also start by considering this case (i. e. $\Gamma_i(\mathbf{C}) = r_i$).

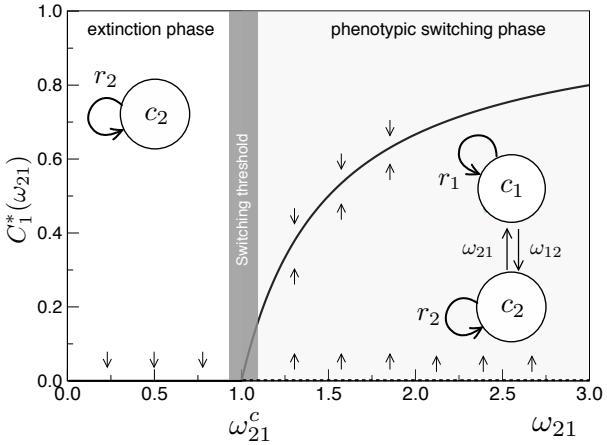

FIG. 2 Bifurcation diagram for the reduced $N = 2$ PHS model with two strains, as defined by equation (5) where the C_1 population is analyzed under CPC. This diagram represents the fixed points C_1^* against the transition rate ω_{21}. A critical switching threshold is defined here for a given ω_{21}^c separating a heterogeneous phase (gray) from a homogeneous one. Here $r_1 = 1, r_2 = 3/2$ and $\omega_{12} = 1/2$, which gives a critical value $\omega_{21} = 1.0$ (equation 8).

What is the impact of PHS on potential therapeutic approximations grounded on cytotoxic drugs? Are there novel attractors or alternative pathways to avoid targeted death? Relevant insight can be obtained by considering a minimal system, where a finite set of cancer clones replicate at rate r_i, defined as the effective difference $r_i = b_i - d_i$ between birth b_i and death d_i rates, and that can be negative when cytotoxic therapy is effective (increasing death beyond birth, see Fig. 3a). In this section we consider the simplest models of PHS in cancer populations with both limited and unlimited growth. While this first allows to show the presence of tipping points associated to switching rates, the later allows deriving basic relations concerning the cost of using transition therapy.

A. Any starting cell can recapitulate the original phenotypic composition

Experimental evidence in cancer populations exhibiting PHS shows that a secondary tumor evolves to the original phenotypic distribution of the primary malignancy, regardless of the initiating cell type [12,13]. This is an interesting outcome of PHS: the system has the potential to reliably restore population diversity in a predictable fashion. Instead of heterogeneity driven by somatic mutation, we have here a surrogate of developmental dynamics driven by epigenetic changes. The mathematical approach and its consequences are easily derived considering a population of two switchers ($N = 2$) under

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 July 2020 doi:10.20944/preprints202007.0547.v1

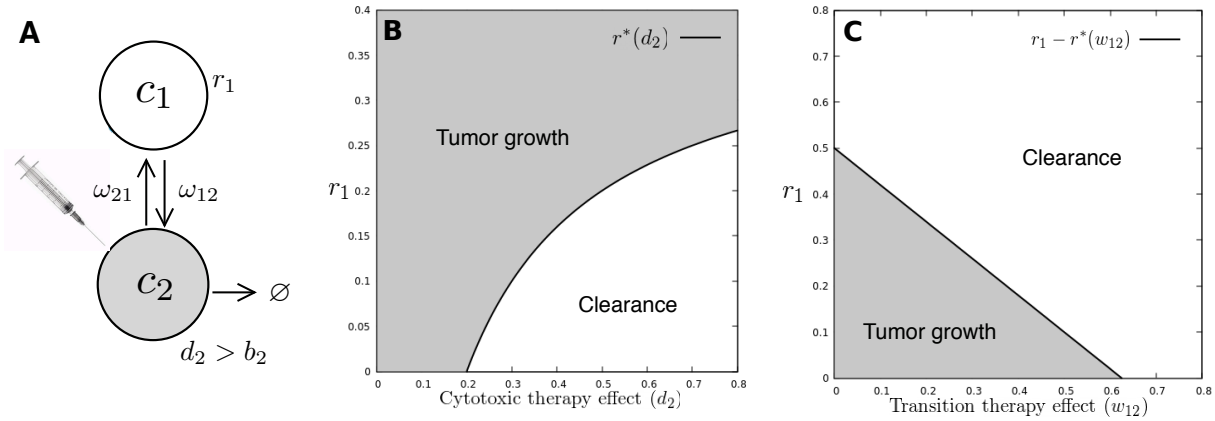

FIG. 3 **Transition therapy.** Targeting a single phenotype in a switching tumor (**A**). Cancer elimination hardly results from single targeted therapies if resistant populations and switching are at place (**B**). The addition of a therapy that increases the rate at which the resistant phenotype C_1 transitions to the targeted one C_2 can completely eliminate the tumor by draining type-1 cells (**C**).

CPC [6].

$$\frac{dC_1}{dt} = (r_1 - w_{12})C_1 + w_{21}C_2 - C_1\phi(\mathbf{C}) \tag{3}$$

$$\frac{dC_2}{dt} = w_{12}C_1 + (r_2 - w_{21})C_2 - C_1\phi(\mathbf{C}) \tag{4}$$

This equation reduces to a simple competition model when $\omega_{ij} = 0$. The winning population is decided by the highest r_i.

Assuming constant population, the competition term reads $\phi(\mathbf{C}) = r_1C_1 + r_2C_2$ and since C_1 are normalized, this is in fact the average replication rate, I. e. $\phi(\mathbf{C}) = \langle r \rangle$. Using this result, it is possible to reduce the system to a one-dimensional ordinary differential equation for one of the populations, say (C_1:

$$\frac{dC_1}{dt} = \gamma C_1(1 - C_1) - w_{12}C_1 \tag{5}$$

with $\gamma = (r_1 - r_2 - w_{21})$. This model displays two fixed points, namely $C_1^* = 0$ (extinction) and the heterogeneous point (where both populations persist) given by

$$C_1^* = 1 - \frac{w_{12}}{\gamma} \tag{6}$$

Interestingly, the presence of an heterogeneous attractor that is not dependent on initial phenotypic composition can be compared to experimental evidence of cell growth recapitulating original clonal distributions [12,13]. In particular, it can be seen that the attractor for population distributions, C_1^*/C_2^*, is consistent with the long-term stable distribution in the absence of intrinsic competition, $C_1(t)/C_2(t)$, because the CPC assumption is equivalent to formulating the model in terms of population concentrations (see SM). This result is consistent both analytically and through computer simulations, so that the minimal model is able to generate the basic *in vitro* properties of phenotypic switching.

The stability analysis of this system shows that this population will persist (i.e. $C_1^* > 0$) and any initial condition will recapitulate the whole attractor distribution provided that

$$\omega_{21} - \omega_{12} > r_2 - r_1. \tag{7}$$

This inequality has an interesting, intuitive interpretation: C_1 will be positive, even if $r_2 > r_1$, provided that the difference between transition rates is larger than the difference between growth rates, highlighting the ability of PHS to maintain tumor heterogeneity (Fig. 2). This allows defining a threshold value: heterogeneity will be observed when

$$\omega_{21}^c = \omega_{12} + (r_2 - r_1) \tag{8}$$

which determines the threshold condition for the switching rate ω_{21} required to sustain C_1, being other parameters fixed. The basic bifurcation diagram associated to this model is shown on figure 2. Two phases are indicated. The first is associated to the diverse switching phenotypes (for $\omega_{21} > \omega_{21}^c$, gray area). Here a single attractor exists, which can be reached from any initial condition. Another, homogeneous phase occurs for $\omega_{21} < \omega_{21}^c$ where only the fastest replicating population persists.

The transition defines a tipping point that is determined (with other parameters fixed) by the rate of recovery provided by the PHS mechanism. The diagram is obtained under unfavorable replication: we use $r_1 < r_2$ which, in the absence of PHS, would inevitably lead to the extinction of C_1. The presence of a heterogeneous phase indicates that one population can rescue the second from decay and this will have relevant consequences for therapy thresholds based on treatments affecting switching rates.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 July 2020 doi:10.20944/preprints202007.0547.v1

B. Cytotoxic therapy failure: Transition thresholds for $N = 2$ tumor growth

Consider again the simplest scenario of two clones away from their carrying capacity (assuming no limited resources, i. e. early tumor growth). In order to formulate this model, we just remove the competition term $C_i\phi(\mathbf{C})$ in the previous equations (3-4). This is a more interesting approach for two reasons: one one hand, the CPC constraint is lifted and secondly a more realistic scenario (a growing tumor with no stable states) is taken into account.

Neglecting nonlinear competition dynamics, we study the following linear system

$$\frac{dC_1}{dt} = (r_1 - w_{12})C_1 + w_{21}C_2 \tag{9}$$

$$\frac{dC_2}{dt} = w_{12}C_1 + (r_2 - w_{21})C_2 \tag{10}$$

As a linear system, it does not admit a single-population solution: the tumor either gets extinct or $C_1(t)$ and $C_2(t)$ undergo exponential growth. Interestingly, long-term phenotypic composition C_1/C_2 is still maintained and independent from initial conditions (see SM), as observed in experimental setups [12,13]. Nevertheless, the model makes possible to show how single-target therapies are eminently inefficient in targeting switching tumor types. We know that the $(0, 0)$ attractor is stable if both *effective* growth rates are negative. Since $r_i = b_i - d_i$, this can be true if death rates for both cell types are increased beyond their birth rates by means of two different drugs. What happens if only one of the two phenotypes can be targeted?

Assume that cell type C_1 has a positive replication rate $r_1 > 0$. The death rate of cell type C_2 can be increased by means of a cytotoxic therapy, so that $r_2 = b_2 - d_2$ could shift from be positive to negative (Fig. 3a). The stability analysis shows that there is a threshold replication rate for C_1,

$$r_1^* = \frac{w_{12}}{1 - \left(\frac{w_{21}}{r_2}\right)} \tag{11}$$

If C_1 replicates faster than this threshold level, it will repopulate the tumor and maintain the targeted population C_2 (Fig. 3b). This is consistent with recent analytical results from [26] for the progression of a tumor in the presence of a drug-tolerant phenotype.

The potential therapeutic implications of this result are straightforward: A single-target therapy might fail if the other population is able to seed the tumor. For very strong cytotoxicity, the limit sets at $r_1^* = w_{12}$: if the resistant population can grow beyond switching it will maintain tumor growth and diversity (Fig. 3b). This might be a clue on why single-target therapies fail at complete eradication of phenotypic switching tumors. However, the same result opens a novel therapeutic possibility. If r_1 cannot be targeted, we could increase w_{12}, the rate at which C_1 switches to C_2, to drain the replicative phenotype into the one we can kill by cytotoxic therapy (Fig. 3c). Can transition rates w_{ij} be targeted as novel a therapeutic strategy?

C. Sequential therapy: Multiple phenotypes ($N > 2$)

We have used the $N = 2$ case to illustrate the concept of cancer growth with switching and how different growth-transition tradeoffs can influence the final outcome. But tumor architectures include more than two coexisting phenotypes [12,13]. Given a larger system with N phenotypes that switch stochastically, what are the effects of sequential therapies? The analytical approach for $N > 2$ independent phenotypes becomes harder as we add dimensions, and results depend on N^2 parameters. However, we can predict the average effect of sequential targeting by assuming that all phenotypes behave and respond, on average, in similar ways.

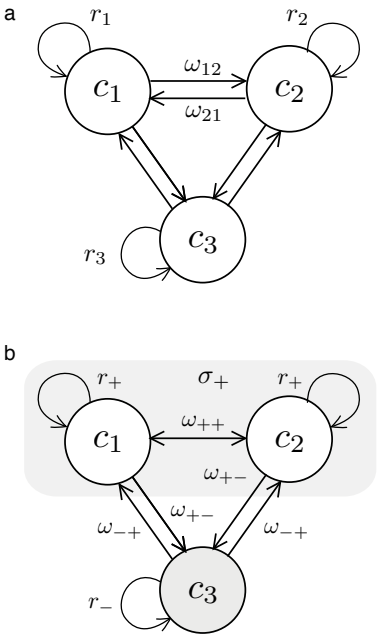

FIG. 4 **Transitions for N=3 phenotypes**. For a $N = 3$ case study, the flow diagram (a) indicates all the transition and replication rates. In order to determine the requirements for successful therapy when a cytotoxic drug is used against C_3, a homogeneous model (b) is used.

The problem can be tackled as follows. Let us first consider the $N = 3$ case, as indicated in figure 3a. In order to reduce the complexity of our calculations, we consider a coarse-graining assumption: all replicating and dying cells do so at equal rates, r_+ and r_- respectively, and transition rates between replicating and dying cells are also homogeneous. This is summarized in figure 3b.

In this scenario, suppose a system with two phenotypes that replicate at $r_+ > 0$ and hold a dying phenotype

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 23 July 2020 doi:10.20944/preprints202007.0547.v1

$r_- < 0$:

$$\frac{dC_1}{dt} = (r_+ - w_{++} - w_{+-})C_1 + w_{++}C_2 + w_{-+}C_3 \quad (12)$$

$$\frac{dC_2}{dt} = (r_+ - w_{++} - w_{+-})C_2 + w_{++}C_1 + w_{-+}C_3 \quad (13)$$

$$\frac{dC_3}{dt} = (r_- - 2w_{-+})C_3 + w_{+-}(C_1 + C_2) \quad (14)$$

Let us now indicate by σ_+ the total population of replicating cells, I. e. $\sigma_+ = C_1 + C_2$ (figure 3b). In this case, the system reduces to

$$\frac{d\sigma_+}{dt} = \sigma_+ r_+ - \sigma_+ w_{+-} + 2w_{-+}C_3 \quad (15)$$

$$\frac{dC_3}{dt} = C_3 r_- - 2C_3 w_{-+} + w_{+-}\sigma_+ \quad (16)$$

For this two-compartment system, it can be shown that the minimal threshold for the positive population replication rate is:

$$r_+^* = \frac{w_{+-}}{\left(1 + 2\frac{w_{-+}}{|r_-|}\right)}. \quad (17)$$

This calculation, under our homogeneity assumptions, can be done in a systematic way for a switching population with of N cell types (see SM). Specifically, we can consider n_+ replicators with a positive effective growth rate r_+ and n_- cell types targeted by therapy, so that their death rate increases beyond birth and $b_- - d_- = r_- < 0$.

By aggregating the two different populations in σ_+ and σ_- compartments, the problem of a tumor with N switching phenotypes can be studied (see SM). It can be shown that the minimal growth rate for the positive replicators to sustain the tumor is

$$r_+^*(n_+, n_-) = n_- \frac{w_{+-}}{\left(1 + n_+ \frac{w_{-+}}{|b_- - d_-|}\right)}. \quad (18)$$

This result provides a lower bound to the requirements for transition therapy for can be translated into glioblastoma therapy design. Complete cancer eradication can happen if all phenotypes are targeted. Targeting less than four phenotypes can prove useless if the other cell types maintain diversity by replicating faster than (3). Through sequential targeted therapy, we can increase n_- by one and decrease n_+ accordingly. This results in a nonlinear increase in the pressure to maintain diversity and growth (Fig. 4). This is a specially relevant result here, since it provides a rough estimate of the potential obstacles to cytotoxic therapy posed by the presence of switching.

III. DISCUSSION

Several considerations on therapy design arise directly from the previous results (and our simplifying assumptions). A well-adapted population can maintain non-adapted cell types, provided replication and transition rates are tuned accordingly. Evidence for skewness in experimental transition rate values [13] could indicate their evolution towards enhancing well-adapted phenotypes. PHS offers an alternative pathway to cancer heterogeneity and consequent drug resistance [20,24,27]. Targeting a PHS-based malignancy need to take into account this result. Single-phenotype strategies are likely to fail, steering tumor evolution towards other phenotypes instead of providing a cure.

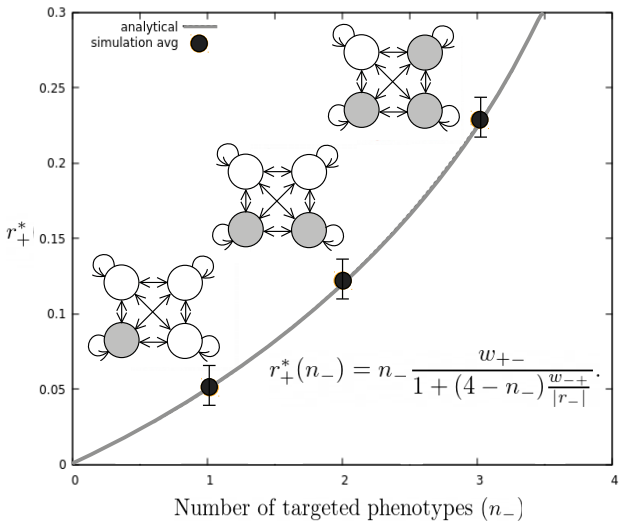

FIG. 5 **Sequential therapy efficiency.** Replicating phenotypes (empty circles) maintain drug-targeted phenotypes (gray) through stochastic switching. Therapy targeting sequentially a four-phenotypes architecture increases nonlinearly the cost of maintaining diversity by resistant cells (continuous line, equation displayed in the inset). Stochastic Gillespie simulations result in a certain degree of deviation, where extinction can eventually happen for values of $r > r_+$. Filled dots indicate the value for which 95% of the computational experiments result in population extinction, with errors bars indicating 5% deviations from this value (see SM for computational details).

Instead, what is to be tackled is diversity itself: if only one phenotype can be targeted, the model indicates that others can be drained by increasing the rates at which they transition to the dying one. Therapeutic strategies that target differentiation pathways are already in place [28], and much is known about dedifferentiation and reprogramming across cell types [29,30]. Clinical and experimental evidence points to differentiation-regulating genes as potential targets of *transition therapy*. An example would be TBX3 affecting inter-phenotype switching in breast cancer cell lines [13]. Epigenetic drugs targeting DNA methylation are nowadays a therapeutic opportunity [31,32], and combinatorial antibody libraries as regulators of cell fate [33] or stem cell transdifferentation [34] might provide further options to induce phenotypic transdifferentation as a therapeutic strategy.

When more than two phenotypes coexist it is likely that several cell types have evolved oncogenic advantage. Our approach indicates that sequential targeting of phenotypes increases non-linearly the pressure for tumor survival. Drug combination targeting several cell types together with transition rates to drain non-targeted phenotypes could result in increased benefits for patient survival.

Sequential therapy schemes are known to drive tumor evolution by inducing pressures that drive clonal selection [35]. Even in tumors where phenotypes show self-renewal capacity after cytotoxic therapy, our modeling approach is a predictive tool for the resulting phenotypic trajectories. Since we can compute the stable phenotypic composition for any combination of parameters, knowing how they change after therapy results in a quantitative prediction of the new tumor state.

This can prove helpful to understand tumor evolution after each drug [36]. It has been studied for clonal evolution tumor schemes [37], but accumulated knowledge indicates that epigenetic plasticity introduces novel conditions for eradication of resistant cell types [27]. The ability to push the system towards equilibria predicted by our model puts forward the opportunity of directing evolution to pre-sensitize the tumor to a second drug [38]. Following the notion of cancer attractors and combination therapy [39], acting on transition rates offers new ways of thinking in how to tackle cancer heterogeneity under PHS under a more "developmental" view. Future extensions might need to be considered, including gene network regulation, spatially explicit structure, niche architecture and tissue hierarchy. Each extra layer will undoubtedly modify our basic bounds, but we conjecture that the ways PHS influence tumor responses will be basically the same.

Acknowledgments

G. Aguadé-Gorgorió thanks the SFI 2019 crew and all the CSL lab members. S. Kauffman thanks Sui Huang for inspiring conversations on the cancer attractors and the open possibilities of transdifferentation. Special thanks to J.C. Scott for his inspiring ideas. This work was supported by the Botín Foundation by Banco Santander through its Santander Universities Global Division, the Spanish Ministry of Economy and Competitiveness, grant FIS2016-77447-R MINECO/AEI/FEDER, an AGAUR FI 2018 grant, and the Santa Fe Institute where most of this work was done.

Bibliography

1. Sultan SE. 2000 Phenotypic plasticity for plant development, function and life history. Trends Plant Sci. 5, 537–542. (doi: 10.1016/S1360-1385(00)01797-0)
2. Piggliuci M. 2001 Phenotypic plasticity: beyond nature and nurture. JHU Press, 328pp.
3. Margueron R, Reinberg D. 2010. Chromatin structure and the inheritance of epigenetic information. Nat. Rev. Genet. 11, 285–296.
4. Balalszi G, Oudenaarden AV, Collins JJ. 2011 Cellular Decision Making and Biological Noise: From Microbes to Mammals Cell 144, 910–925. (doi: 10.1016/j.cell.2011.01.030)
5. Kussell E, Leibler S. 2005 Phenotypic Diversity, Population Growth, and Information in Fluctuating Environments. Science 309, 2075–2078. (doi:10.1126/science.1114383)
6. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. 2004 Bacterial persistence as a phenotypic switch. Science 305, 1622–1625.
7. Flavahan WA, Gaskell E, Bernstein BE. 2017 Epigenetic plasticity and the hallmarks of cancer Science 357, eaal2380. (doi:10.1126/science.aal2380)
8. Dean M, Fojo T, Bates S. 2005 Tumour stem cells and drug resistance. Nat. Rev. Can. 5, 275–284.
9. Shackleton M, Quintana E, Fearon ER, Morrison SJ. 2009 Heterogeneity in cancer: cancer stem cells versus clonal evolution. Cell 138, 822–829.
10. Marusyk A, Almendro V, Polyak K. 2012 Intra-tumor heterogeneity: a looking glass for cancer? Nat. Rev. Can. 12, 323–334.
11. Brock A, Chang H, Huang S. 2009 Non-genetic heterogeneity–a mutation-independent driving force for the somatic evolution of tumours. Nature Reviews Genetics 10, 336–342.
12. Neftel C, *et al.* 2019 An integrative model of cellular states, plasticity, and genetics for glioblastoma. Cell 178, 835–849.
13. Gupta PB, Fillmore CM, Jiang G, Shapira SD, Tao K, Kuperwasser C, Lander ES. 2011 Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. Cell 146, 633–644.
14. Quintana E, *et al.* 2008 Efficient tumour formation by single human melanoma cells. Nature 456,593–598.
15. O'connell MP, Weeraratna AT. 2013 Change is in the air: the hypoxic induction of phenotype switching in melanoma. Jour. Inves. Dermatol. 133, 2316–2317.
16. Jolly MK, Kulkarni P, Weninger K, Orban J, Levine H. 2018 Phenotypic plasticity, bet-hedging, and androgen independence in prostate cancer: Role of non-genetic heterogeneity. Front. Oncol. 8, 50.
17. Diaz Jr LA, *et al.* 2012 The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 486, 537–540.
18. Jordan CT, Guzman ML, Noble M. 2006 Cancer stem cells. New Engl. Jour. Med. 355, 1253–1261.
19. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. 2017 Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell 168, 707–723.
20. Sharma SV, *et al.* 2010 A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 141,69–80
21. Gerlee P, Nelander S. 2012 The impact of phenotypic switching on glioblastoma growth and invasion. PLoS computational biology, 8(6).

22. Jolly MK, Ware KE, Gilja S, Somarelli JA, Levine H. 2017 EMT and MET: necessary or permissive for metastasis?. Molecular oncology, 11, 755–769.
23. Mathis RA, Sokol ES, Gupta PB. 2017 Cancer cells exhibit clonal diversity in phenotypic plasticity. Open biology, 7(2), 160283.
24. Gunnarsson E, De S, Leder K, Foo J. 2020 Understanding the role of phenotypic switching in cancer drug resistance. Journal of Theoretical Biology, 110162.
25. Nowak MA. 2006 Evolutionary dynamics: exploring the equations of life. Harvard University Press, 363pp.
26. Roose T, Chapman SJ, Maini PK. 2007 Mathematical models of avascular tumor growth. SIAM review 49, 179–208.
27. Easwaran H, Tsai HC, Baylin SB. 2014 Cancer epigenetics: tumor heterogeneity, plasticity of stem-like states, and drug resistance. Mol. cell 54, 716–727.
28. de Thé, H. 2018 Differentiation therapy revisited. Nat. Rev. Can. 18, 117.
29. Jopling C, Boue S, Belmonte JCI. 2011 Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. Nature reviews Mol. Cell Biol. 12, 79–89.
30. Huang S. 2009 Reprogramming cell fates: reconciling rarity with robustness. Bioessays 31, 546–560. item Gore SD, Baylin S, Sugar E, Carraway H, Miller CB, Carducci M, *et al.* 2006 Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. Cancer research, 66(12), 6361–6369.
31. Juergens RA, Wrangle J, Vendetti FP, Murphy SC, Zhao M, Coleman B, *et al.* 2011 Combination epigenetic therapy has efficacy in patients with refractory advanced non–small cell lung cancer. Cancer discovery, 1(7), 598–607.
32. Lerner RA, Grover RK, Zhang H, Xie J, Han KH, Peng Y, Yea K. 2015. Antibodies from combinatorial libraries use functional receptor pleiotropism to regulate cell fates. Quarterly reviews of biophysics, 48(4), 389-394.
33. Xie J, Zhang H, Yea K, Lerner RA. 2013. Autocrine signaling based selection of combinatorial antibodies that transdifferentiate human stem cells. Proceedings of the National Academy of Sciences, 110(20), 8099-8104.
34. Gatenby R, Brown J. 2018 The evolution and ecology of resistance in cancer therapy. Cold Spring Harb. Perspect. Med. 8, a033415.
35. Amirouchene-Angelozzi N, Swanton C, Bardelli A. 2017 Tumor evolution as a therapeutic target. Cancer discov. 7, 805–817.
36. Bozic I *et al.* 2013. Evolutionary dynamics of cancer in response to targeted combination therapy. eLife 2,e00747. (doi:10.7554/eLife.00747)
37. Basanta D, Gatenby RA, Anderson AR. 2012 Exploiting evolution to treat drug resistance: combination therapy and the double bind. Molecular pharmaceutics, 9(4), 914–921.
38. Huang S, Ernberg I, Kauffman S. 2009 Cancer attractors: a systems view of tumors from a gene network dynamics and developmental perspective. Sem. cell develop. biol. 20, 869–876.
39. Huang S, Kauffman S. 2013 How to escape the cancer attractor: rationale and limitations of multi-target drugs. Sem. Can. Biol. 23, 270–278.