Toward Understanding of the Role of Reversibility of Phenotypic Switching in the Evolution of Resistance to Therapy.

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Abstract

Reversibility of state transitions is an intensively studied topic in many scientific disciplines over many years. In cell biology, it plays an important role in epigenetic variations of phenotypes, known as phenotypic plasticity. More interestingly, the cell state reversibility is probably crucial in the adaptation of population phenotypic heterogeneity to environmental fluctuations by evolving bet-hedging strategy, which might confer cancer cells resistance to therapy. In this article, we propose a formalization of the evolution of highly reversible states in environments of periodic variability. Two interrelated models of heterogeneous cell populations are proposed and their behavior is studied. The first model captures selection dynamics of the cell clones for the respective levels of phenotypic reversibility. The second model focuses on the interplay between reversibility and drug resistance in the particular case of cancer. Overall, our results show that multivariate threshold dependencies are emergent and common features of the investigated models that can have therapeutic relevance. Presented examples demonstrate importance of cell to cell heterogeneity within a system of clones with different reversibility potentials, quantified by appropriately chosen genetic and epigenetic entropy measures.

1 Introduction

Human diseases are typically caused by invading pathogenic microorganisms, such as viruses, bacteria, fungi, prions, neoplastic cells, etc. Despite immunity system is able to cope with the majority of pathogens, those escaping from innate immunity surveillance must be treated by therapy. The main obstacle to efficient therapy is the variability of the microorganisms within the population, such as strain or clone, conferring them resistance to therapy. Population variability flows from the evolutionary essence of population dynamics of microorganisms which equips evolving populations of pathogens with powerful adaptive capability. For example, evolutionary dynamics of carcinogenesis \cite{1,2,3} is considered the main reason why targeted therapy does not work \cite{4}.

As it is known for a long time, intratumor heterogeneity is not bound exclusively to the differences in DNA sequences of the respective cells (genetic heterogeneity), but to the epigenetic differences as well \cite{5}. It has been found that the role of epigenetic mechanisms, such as DNA methylation, histone modifications, chromatin remodeling, and small RNA molecules, in cancer initiation and progression is causative \cite{6,7}. In particular, variability in phenotypic characteristics of isogenic cells, known as phenotypic plasticity, is assumed to be an important cause of the therapeutic resilience of advanced cancers \cite{8}.

Recognizing tumor dynamics as evolutionary process, one can exploit known universal features of evolution to influence the evolution of the population in desirable direction in a mathematically more purposeful way. While the role of genetic intratumor heterogeneity in tumor evolution was accepted long time ago, the role of epigenetic heterogeneity is much less obvious. To implement evolutionary principles into the therapy design, the interplay between genetic diversity and epigenetic plasticity should be carefully studied at the model level within an evolutionary-based integrative conceptual framework. Recently, the concept of Waddington’s epigenetic landscape was proposed to formalize the relation between the cancer cell genome and epigenetic
mechanisms \[^10\] in mathematically more instructive way. Therein, each point in the fitness landscape (i.e., genome) provides epigenetic landscape of unique topology. Due to their mathematical complexity, the epigenetic landscapes contain many areas (attractors) around the stable cell-states corresponding to the respective stable phenotypes of a cell. Straightforwardly, the phenotypic switching corresponds to the transition between two such attractors.

Below we use the above formalization \[^10\] as an instructive backbone for our considerations. As different cell states in the epigenetic landscape differ in their fitness-related properties, the cell states composition (or non-genetic heterogeneity) becomes, from the viewpoint of the clone, evolutionary important at the clone’s respective timescale. It was observed that in the case of variable selective pressure, population of organisms evolve mechanisms to tune the phenotypic variability to the variability of the acting selective pressure \[^11\]. In bacteria, the well known risk-diversification strategy evolved in the populations when facing uncertain future and/or environment \[^12, 13, 14\] is the bet-hedging strategy \[^15, 16\]. Based on formal similarity of evolving cancer cells population with bacteria, viruses or yeast, it has been recently proposed that the structure of intratumor heterogeneity is evolutionary trait as well, evolving to maximize clonal fitness at a cancer-relevant timescale in changing (or uncertain) environment and that its structure corresponds to the bet-hedging strategy \[^17, 18, 19, 20, 21, 22\]. To sum up, the genome stays the main protagonist (i.e., selection unit) in the evolution of cancer cells, nevertheless with non-genetic heterogeneity of its eventual clone being the crucial adaptive trait at cancer-relevant, instead of proximate timescale.

Phenotypic plasticity confers to cellular tissues important properties, such as the ability of cancer cells to escape targeted therapy by switching to an alternative phenotype \[^23, 24, 25, 26, 27, 28\]. It motivates the effort to stimulate (or prevent) specific phenotype switching purposefully as a therapeutic strategy \[^29\], which requires deep understanding of the phenotype switching causation. Regarding the therapeutical perspectives, the one of significant, and, consequently, intensively investigated features is reversibility of the phenotype switching. As classical evolutionary theory derives phenotypic variation from random mutations that are independent of selective pressure, it seems probable that reversibility of phenotype switching can be underpinned exclusively by epigenetic modifications. Regarding the therapeutical perspectives, the difference between genetic and epigenetic changes is fundamental: while the genetic changes are, in principle, irreversible, the epigenetic modifications may be reversible at the therapy-relevant timescales \[^24, 30, 31\].

To sum up, there are well-founded reasons for studying phenotypic switching using quasispecies or population dynamics models, which address the specific characteristics of cancer. In Section \[^2\] we present the evolutionary based framework which assumes environmental dynamics shaping the evolution of reversible switching strategies. In the quasispecies framework (see Sections \[^38, 29, 30\]) the focus is placed on the phenotypic plasticity and reversibility. Studies of quasispecies models yielded important modelisation ideas about how to implement the immunotherapy aspects. Most of our efforts in this area have focused on the construction of the population-based cancer models where evolution of phenotypic reversibility is incorporated together with immunotherapy. The resulting models of cancer resistance are discussed in Section \[^4\].

2 Quasispecies model of phenotypical changes

In this section we introduce the qualitative time-continuous evolutionary model based on the system of ordinary differential equations. The model is applied to nonequilibrium scenarios, where constrained populations of irreversible phenotypes are evolutionary drawn towards an attractor populated by reversible phenotypes.

Mathematical formulation is based on the quasispecies model \[^32\] developed to clarify the role of mutations in the evolutionary process \[^33, 34, 35, 36\]. Owing to its versatility, the model have found applications in a broad range of scales - from molecular and virus scales \[^37\] up to the cellular systems, such as the populations of heterogeneous cancer cells \[^38, 39\]. Investigation of the population heterogeneity within the context of variable environments emerges as an interesting research area. The entropic variable constraints imposed on the heterogeneity were studied in \[^40\]. In \[^41\] the elimination of heterogeneity in the system of replicating entities was conceived as an inverse problem, with an eventual potential in therapeutic applications. Before going into details, we highlight three salient features of the model: (i) periodicity of the (micro)environmental variations; (ii) substantial genetic diversity underlying the switching rates between isogenic phenotypes; (iii) competition between the clones adopting either reversible or irreversible phenotype switching under the constraint of constant total cell population size.
Consider the effects of the evolutionary rate variation on the fractions \( c^{(z)}(k, t) \), \( k = 0, 1, \ldots, n_s - 1 \), of \( n_s \) clones (abstract ‘genotypes’), each of them allowed to occupy one of the two phenotypic states, indexed by \( z = 0, 1 \). We assume that the \( k \)-th genotype responds to the environmental variation in two alternative ways depending on its respective phenotypic state, \([k, z = 0]\) or \([k, z = 1]\). The time-variability of environment can be indirectly represented by the time-variability of the reproduction rates of the respective phenotypic states, \( r^{(z)}(t) \), \( z = 0, 1 \), which are supposed to be of harmonic periodic form

\[
r^{(z)}(t) = r_B + (-1)^z \Delta r \cos(2\pi t/T)
\]

defined by the period \( T \); \( \Delta r \) corresponds to the degree of diversification of the reproduction fitness and \( r_B \) to its basal level. It is worthy of noting that in [2] the oscillatory external (temperature) conditions are used to drive the evolution of the class of interacting information-carrying molecular replicators with the capability of reversible intermodal switches.

Presuming for a while that the replication dynamics is restricted to the concentration plane \( \phi(0)(k, t) \equiv c^{(1)}(k, t) \), the parameter \( r_B \equiv |r^{(0)}(t) + r^{(1)}(t)|/2 \) may be interpreted as static effective replication rate. Many natural cycles may imply changes in the replication rate, which can be, within the context of cancer research, exemplified by the cyclic hypoxia-reoxygenation exposure within solid tumors [3].

To study population dynamics with purposefully specified level of reversibility of the transitions between the phenotypes \( z = 0 \) and \( z = 1 \) in the respective clones, we define the parameter \( \varphi(k) \), below referred as the degree of reversibility of the \( k \)-th clone, in the form

\[
\varphi(k, n_s) = 1 + \frac{1 - k}{n_s - 2}, \quad (2)
\]

which enables to set to each clone, \( k = 1, \ldots, n_s - 1 \), different, uniformly discretized, levels of reversibility. The structure of \( \varphi(k) \) implies the symmetry \( \varphi(n_s - k, n_s) = 1 - \varphi(k, n_s) \). Let

\[
(1 - \varphi(k, n_s))c^{(1)}(k, t) \quad \text{and} \quad \varphi(k, n_s)c^{(0)}(k, t) \quad (3)
\]

specify the intensity of the transitions from the phenotype 0 to the phenotype 1, and vice versa, due to the phenotype switching. The terms Eq.3 are combined to describe the switching flow

\[
J_{sw}(k, n_s, t) = (1 - \varphi(k, n_s))c^{(1)}(k, t) - \varphi(k, n_s)c^{(0)}(k, t), \quad (4)
\]

which, when incorporated into dynamics, changes phenotypic fractions corresponding to \( z = 0 \) and \( z = 1 \) in each of the respective clones \( k = 1, 2, \ldots, n_s - 1 \). In further the direction of switching flow \((-1)^{m}J_{sw}\) is controlled by the prefactor \((-1)^z\), while the parameter \( m \) is used to control its amplitude. Both “boundary species values”\( \varphi(1, n_s) = 1 \) and \( \varphi(n_s - 1, n_s) = 0 \), belong to purely irreversible situations. The assumption of non-negativity of the fractions implies that “boundary” flows, \( J_{sw}(1, n_s, t) = -c^{(0)}(1, t) \leq 0 \) and \( J_{sw}(n_s - 1, n_s, t) = c^{(1)}(n_s - 1, t) \geq 0 \), are unidirectional, which sharply contrasts with the central species \( k_{central} = n_s/2 \), where \( \varphi(k_{central}, n_s) = 1/2 \) provides

\[
J_{sw}\left(\frac{n_s}{2}, t\right) = \frac{1}{2} \left( c^{(1)}\left(\frac{n_s}{2}, t\right) - c^{(0)}\left(\frac{n_s}{2}, t\right) \right), \quad (5)
\]

which reflects the fact that both directions of the flow (i.e. \( J_{sw} \geq 0, J_{sw} \leq 0 \)) are allowed for \( c^{(z)}(\frac{n_s}{2}, t) \geq 0 \). As \( J_{sw}(k, n_s, t) \) is constructed without considering explicit causal sensoric response [4], it can be viewed as a population-level consequence of evolved bet-hedging strategy within the context of quasispecies ODE (ordinary differential equations).

We postulate that the initial population is formed exclusively by the zero-th clone, which is gradually redistributed (by mutation mechanisms) among the concurrent clones, \( k = 1, 2, \ldots, n_s - 1 \). If the phenotype switching absents for \( k = 0 \), the dynamics of the population, \( c^{(z)}(0, t) \), follows

\[
\frac{dc^{(z)}(0, t)}{dt} = \left[ r^{(z)}(t) - \Phi(t) \right] - (n_s - 1)\mu c^{(z)}(0, t), \quad (6)
\]

and population fraction of \( k = 0 \) changes exclusively due to irreversible mutations. Their impact is modeled by \(-\mu c^{(0)}(0, t)\) terms proportional to the positive coefficient \( \mu \). The additional assumption is, that all mutants \( k = 1, 2, \ldots, n_s - 1 \) are produced with the same rate \( \mu c^{(1)}(0, t) \). The competition controls proliferation via the scalar \( \Phi(t) \) term. The Eq.6 is considered together with the ODE system

\[
\frac{dc^{(z)}(k, t)}{dt} = \left[ r^{(z)}(t) - \Phi(t) \right] c^{(z)}(k, t) - \mu(1)\mu c^{(z)}(0, t) + m(-1)^z J_{sw}(k, t), \quad (7)
\]

written for \( k = 1, 2, \ldots, n_s - 1 \). The additional notes should be made about the structure, description and interpretation of the switching process at the level of ODE: (i) As it follows from the Eq.7, the total rate of the clonal
fractions \( d(c^{(0)}(k, t) + c^{(1)}(k, t))/dt \) loses explicit dependence on \( J_{sw}(k, t) \); (ii) without mutation and replication terms the particular phenotypic equilibria can be formed when \([J_{sw}(k)]_{\text{equil}} = 0\), which leads to the ratio
\[
\frac{c^{(1)}(k)}{c^{(0)}(k)}_{\text{equil}} = \frac{\varphi(k, n_s)}{1 - \varphi(k, n_s)}
\] (8)

The competition among the clones is described conveniently using constraint of constant overall population density
\[
c_k^{(0)}(t) + c_k^{(1)}(t) = 1
\] (9)

with the particular terms
\[
c_k^{(z)}(t) = \sum_{k=0}^{n_s-1} c_k^{(z)}(k, t), \quad z = 0, 1.
\] (10)

If the sum of the left and right hand sides of Eq. 6 and Eq. 7 is carried out, and the result is compared with Eq. 9, the scalar correction to the reproduction rate may be constructed as
\[
\Phi(t) = \frac{\sum_{z=0,1} r^{(z)}(t) c_k^{(z)}(t)}{\sum_{z=0,1} c_k^{(z)}(t)} = \sum_{z=0,1} r^{(z)}(t) c_k^{(z)}(t).
\] (11)

This functional relationship is the source of non-linearity of the above system of equations.

In order to reduce the initial asymmetry, in our simulations (see Subsection 2.3 in below) of quasi-species model we have used the initial conditions
\[
c_k^{(0)}(0, t = 0) = c_k^{(1)}(0, t = 0) = \frac{1}{2}, \quad c_k^{(z)}(k, t = 0) = 0, \quad \text{for} \ k = 1, 2, \ldots, n_s - 1.
\] (12)

It means that, in the case of the initial demise of the \( k = 0 \) species, we expect the repopulation towards the species \( k = 1, 2, \ldots, n_s - 1 \). Since the mutations were designed without preference, contributing to the expansion of all remaining clones \( k \neq 0 \) with the same rate \( \mu c_k^{(z)}(0, t) \), the decisive contribution to the disparity of the fractions is expected solely from the switching mechanism where diversification is guaranteed by the use of \( \varphi(k, n_s) \).

2.1 Entropy measures of heterogeneity

To characterize selection strength and asymptotic behavior at the systemic level, we focus on the measures of heterogeneity of evolving population fractions. To quantify the dynamic heterogeneity we utilize two different measures. The first of them is Shannon entropy
\[
S_{EPIC}(t) = - \sum_{z=0}^{n_s-1} c_k(z, t) \ln c_k(z, t),
\] (13)

which reflects the epigenetic information. It is obvious, that this form is sensitive to the arrangement of phenotypic fractions. On the contrary, the introduction of measures based on the total concentration
\[
c_k(k, t) = c_k^{(0)}(k, t) + c_k^{(1)}(k, t)
\] (14)
or another meaningful algebraic combinations of \( c_k^{(0)}, c_k^{(1)} \) is a way how to easily remove phenotypic details (including subtle oscillations). Therefore, the genetic heterogeneity and variability can be better described by
\[
S_G(t) = - \sum_{k=0}^{n_s-1} c_k(k, t) \ln(c(k, t)).
\] (15)

Both \( S_{EPIC} \) and \( S_G \) measures can be constructed regarding often used analogy between probability and occupancy fraction [41].

2.2 Effective replication rate of the clone

In order to better understand the behavior of the system of equations [6] and [7] we proposed an alternative effective description. Within this, the effective dynamics of total fraction \( c_k(t) \) (see Eq. 14) can be expressed by simple formula
\[
\frac{dc_k(t)}{dt} = (r_{eff}(k, t) - \Phi(t)) c_k(t).
\] (16)

Here replication rate of \( k \)-th clone \( r_{eff} \) plays a key role for many results to follow. The phenomenology avoids the formal use of the mutations, switching or \( c_k^{(0)}(k, t), c_k^{(1)}(k, t) \). The summation of the equations Eq. 16 leads to the formula
\[
\Phi(t) = \sum_{k=0}^{n_s-1} r_{eff}(k, t),
\] (17)

which implies that \( \Phi \) can be interpreted as a total replication rate. Moreover, the consistence of Eq. 17 and Eq. 11 can be achieved for the ”mixed-type” solution
\[
r_{eff}(k, t) = r^{(0)}(t)c_k^{(0)}(k, t) + r^{(1)}(t)c_k^{(1)}(k, t).
\] (18)

2.3 Numerical investigation

Apart from few numerical exceptions, listed in the figure captions, the simulations have been performed for \( n_s = 6 \) species and parameters \( T = 10, r_B = 1, \Delta r \in \{0.1, 0.2, 0.4\}, m = 0.05, \mu = 0.01 \).
(unless other value specified in figure captions). In agreement with intuitively expected behavior of the model, Fig. 1 shows gradual qualitative progress in the redistribution of the species fractions. At the intermediate time scales (10 a.u. – 100 a.u.), there is a period of "hesitancy", where all the clones $k > 1$ nearly counterbalance their respective competitors. During this short initial phase, the variable environment causes that the putative equality among the clones $k = 1, 2, \ldots, 6$ gradually vanishes and the highest degree of reversibility $\varphi(k_{\text{max}}) = 1/2$ corresponding to the ratio $\varphi(k_{\text{max}})/(1 - \varphi(k_{\text{max}})) = 1$ [see Eq. 8] attributed to the clone $k_{\text{central}} = k_{\text{max}} = n_s/2 = 3$, leads at the long run ($\geq 1000$) to the largest $r_{\text{eff}}(k_{\text{max}}, t)$ [see the analytical approximation given by Eq. 28 in below].

Fig. 2 shows the non-trivial transient states and the initial increase common for $S_{\text{EPIG}}$ and $S_G$. The largest differences between $S_{\text{EPIG}}$ and $S_G$ are localized mainly in the long-time asymptotics. Here, the main question arises to what extent environmental variability affects the long-term heterogeneity of the populations. There are many obvious indicia that populations obtained for large and small $\Delta r$ significantly differ. Fig. 2 depicts both entropy measures as functions of $\Delta r$, which is the main determinant of environmental variability. The variability causes that $S_{\text{EPIG}}$ converges to the positive limit value, while trivial limit $S_G \rightarrow 0$ indicates dominance of the single species. For small $\Delta r$, there are only very low evolutionary benefits due to switching between phenotypic states, which leads to extremely slow evolution. This means that numerical approach becomes inappropriate to capture the differences between non-equilibrium decay and equilibrium long-time behavior. The numerical problems stimulated development of analytical approximation discussed in Subsection 2.3. Both approaches are consistent in indicating of the fact that convergence to the most reversible clone is faster as $\Delta r$ increases (see panel (C)). In addition, it appears that for given time horizon $t_h$ the increase in $\Delta r$ leads to the threshold-like behavior [see Fig. 3(B)]. According to Fig. 2(B), without sufficient environmental variability most of the population remains trapped from the beginning in the clone $k = 1$.

Fig. 3 summarizes the entropic responses induced by $\mu$, $\Delta r$, $T$ and $m$. Panels (C), (D) for example, show how the choice of $T$ affects the efficiency of the selection of the most reversible strategy. It turns out that large enough $T$ should support rapid selection. In agreement with the intuitive expectations, the process can be reinforced by super-threshold value of $m$.

It is clear that calculations of the characteristics for separate variables are not universal across parameteric values. The first step towards integrated view is manifested in Fig. 4 where the time averaged $S_{\text{EPIG}}(t)$ and max$_k r_{\text{eff}}(k, t)$ are plotted versus auxiliary variables $\xi$ and $\xi_c$ [see Eq. (25) and Eq. (26) in below] for different combinations of $m, T, \Delta r$ inputs. The explicit forms of $\xi^2(m, T, \Delta r)$ and $\xi_c(m, T, \Delta r)$ follow from the asymptotic results for the clone selection problem performed in the next subsection.

### 2.4 Long run asymptotics

Quasispecies nonlinear dynamic problem does not provide general symbolic solution for a system of many species. Nevertheless, numerical results from the previous section stimulate our interest in the asymptotic situation $t \gg 1/\mu$. We assume that at the late-time evolution the single clone with the index $n_s/2$ is fixed.

The case of highly localized (in $k$) species allows to consider more restrictive version of the constraint Eq. (9) in the form

$$c(n_s/2, t) = c^{(0)}(n_s/2, t) + c^{(1)}(n_s/2, t) = 1 \quad (19)$$

It is consistent with two settings

$$c^{(0)}_c(t) \simeq c^{(0)}(n_s/2, t) = \frac{1}{2} + \xi(t), \quad (20)$$

$$c^{(1)}_c(t) \simeq c^{(1)}(n_s/2, t) = \frac{1}{2} - \xi(t),$$

where the residual plasticity is transferred to the single auxiliary $-1/2 < \xi(t) < 1/2$. Consequently, using Eq. (9) we obtained $J_{\text{sw}}(n_s/2, t) = -\xi$ and the equation for $\xi(t)$ can be rewritten as

$$\frac{d\xi}{dt} = (r_0 - \Phi)\left(\frac{1}{2} + \xi\right) - m\xi. \quad (21)$$

Now, substituting $c^{(0)}(t)$, $c^{(1)}(t)$ from Eq. (20) into Eq. (18) we obtained

$$\Phi(t) \approx r_{\text{eff}}(t) = r_B + 2\Delta r\xi(t)\cos\left(\frac{2\pi t}{T}\right). \quad (22)$$

Its consequent substitution into Eq. (21) gives

$$\frac{d\xi}{dt} = 2\Delta r\left(\frac{1}{4} - \xi^2\right)\cos\left(\frac{2\pi t}{T}\right) - m\xi. \quad (23)$$

This nonlinear ODE problem can be solved using the single harmonic approximation (valid for $\xi^2 \ll 1/4$)

$$\xi(t) \simeq \xi_s \sin\left(\frac{2\pi t}{T}\right) + \xi_c \cos\left(\frac{2\pi t}{T}\right) \quad (24)$$

with the pair of amplitudes

$$\xi_s = \frac{\pi T \Delta r}{4\pi^2 + T^2 m^2}, \quad \xi_c = \frac{m T}{2\pi} \xi_s. \quad (25)$$
The formula clearly uncovers the relative effects of the processes operating at different time scales: $1/\Delta r$, $1/\mu$ and $T$. The dependence upon $1/\mu$ ab- sents due to assumption that clonal selection has basically vanished in the long run. For the solutions given by Eq. (24), the time averaging can be simply performed for the single period. The result is

$$\xi^2 \approx \frac{1}{2} (\xi_s^2 + \xi_e^2).$$

(26)

Similarly, for $T$-periodic $\xi(t)$ the mean effective replication rate can be defined by

$$\overline{r_{\text{eff}}} = \frac{1}{T} \int_0^T r_{\text{eff}}(t) dt .$$

(27)

Then, using Eq. (18), Eq. (20), Eq. (22), Eq. (24) and Eq. (27) we obtained

$$\overline{r_{\text{eff}}} = r_B + \Delta r \xi_c .$$

(28)

A posteriory confrontation with the condition $\xi^2 \ll 1/4$ provides bounding $\Delta r \ll (4\pi^2 + T^2m^2)/\pi T \max\{1, mT\}$. Based on the structure of Eq. (23), we have proposed the numerical analysis illustrated by examples in Fig. 3(C),(D) (see caption of this figure for more details). The combination with numerical tools in part justifies the use of asymptotic approximation presented in this subsection.

According to the assumption of only one final winner clone survival, we get a trivial limit $S_C(t \to \infty) \to 0$. However, the epigenetic alterations have non-trivial consequences. If Eq. (20) is substituted into Eq. (13), one obtains

$$S_{\text{EPIG}}(t) \simeq \sum_{j \in \{-1, 1\}} \left( \frac{1}{2} + j \xi(t) \right) \ln \left( \frac{1}{2} + j \xi(t) \right) .$$

(29)

The formula can be analyzed by calculating its time averages. For this aim, the Taylor series of the order $O(\xi^2)$ can be used. The entropy averaged over the period $S_{\text{EPIG}} \simeq \ln 2 - \xi^2$ used in combination with Eq. (23) and Eq. (26) provides ($S_{\text{EPIG}} - \ln 2) \sim (-\Delta r^2$) in a qualitative agreement with the simulation trend shown in Fig. 2(A),(D),(E),(F), Fig. 3(B) and also short sensitivity study reported in Fig. 4(A),(B).

3 Evolution of the tumor-immune dynamics

Based on our previous results, we formulate a complex growth model which represents specific application of our approach in tumor modeling. The objective of this section is to study evolutionary aspects of the resistance to immunotherapeutic drugs caused by nie phenotypic reversibility. In the below application we associate the quasispecies model with the exogenous oversimplified variant of the Kirschner-Panetta (KP) model of immunotherapy [15]. The original KP model consists of the three autonomous equations, for the effector cells production, tumor growth with tumor clearance, and, finally, equation for cytokine interleukin-2 (IL-2) production. Essential in the original KP model is the boosting of the IL-2 against tumors. There are many versions of the model that include simplified but biologically plausible approaches (see e.g. the review [46]). For example, in [47] the gene therapy characterized by populations of effector and cancer cells was described by means of two coupled autonomous ODE.

3.1 Simplified model of homogeneous tumor exposed to exogeneous effector cells

We continue with a simplified model of single cancer clone where a population of cancer cells varies accordingly to exogenous ODE

$$\frac{dC}{dt} = rC(1 - C) - \frac{e(t)C}{C + g} .$$

(30)

In agreement with the original KP model, the efficiency of therapeutic interventions is described by the clearance parameter $a$. The parameter $g$ plays the role analogous to that in the Michaelis-Menten term. Unlike the quasispecies model, the rate of replication $r$ does not change. The classical logistic term $rC(1 - C)$ is used for the nutrient-limited cancer growth of the rescaled tumor size $C$. Its use (loosely analogous to $\Phi$) causes that tumor size stabilizes at the carrying capacity equal to or less than 1 (in our present scaling). The role of dynamic environmental factor is mediated by the population of the effector cells. We assume that population size $e(t)$ varies according formula

$$e(t) = e_B + \Delta e \cos \left( \frac{2\pi t}{T} \right) .$$

(31)

This simplification is beneficial owing to a few aspects: (i) the exogenous formulation is parametri- cally less demanding than the original autonomous (endogenous) KP model; (ii) it can be easily linked to the quasispecies model variant proposed in this paper (see Eq. (1)); (iii) it avoids the stiffness problems typical of the original KP formulation; (iv) the harmonic character of Eq. (31) is qualitatively consistent with KP phase diagrams [16] belonging to the dynamical regimes with some limit cycle attractors.
Before studying more complex numerical examples, we continue with the parametrization of the elementary model based on Eqs. (30) and Eq. (31). According to the results, the system simulated with the initial condition \( C(0) = 0.1 \) for ten periods exhibits the largest susceptibility to the changes in \( g \) for the parameters

\[
a = 1, \quad T = 10, \quad r = 1, \quad \epsilon_H = 0.5, \quad \Delta e = 0.4, \quad g = 0.45.
\] (32)

The parameters are intended for further simulations of multiclonal populations of cancer cells.

### 3.2 Tumor growth and the evolution of immuno-therapeutic resistance

To illustrate the role of reversibility in the evolution of the therapy resistance, we construct the evolutionary model where reversibility is considered along with the heterogeneous (clone-dependent and state-specific) drug efficiency. It works with the population of seven rescaled species abundances, forming the set \( \text{set}_E \equiv \{C_P, C_{L0}, C_{L1}, C_{H0}, C_{H1}, C_H \} \), where \( C_P \) denotes the concentration of the primary cancer cells, and the abundances \( C_{L0}, C_{L1}, C_{H0}, C_{H1} \) are equipped with low level (L) of reversibility \( \varphi_L \in (0, 1) \). The effects of immunotherapy defined by Eq. (30) are diversified in a way that single scalar parameter \( \alpha \) is replaced by the species-dependent term \( \alpha_{jz} \), \( j \in \{0, 1\} \), \( z \in \{0, 1\} \) with the prefactor representing a modifier of the drug efficiency of the phenotype \( z \) of clone \( j \). The supplementary lower index 0 in \( C_{L0} \) (or, respectively, 1 in \( C_{L1} \)) indicates that immunotherapy is primarily directed on the phenotype \( z = 0 \) (or \( z = 1 \)) with the higher strength \( \alpha_H \), whereas \( z = 1 \) (\( z = 0 \)) is eliminated with lower efficiency \( \alpha_L \). The high reversibility (index \( H \)) is imposed on the clone characterized by the concentrations \( C_{H} \). (The overview of the theoretical structure of the model is schematically highlighted in Table I). In contrast to the quasispecies model, the sum

\[
C_{\Sigma} = \sum_{C_E \in \text{set}_E} C_E
\] (33)

is not normalized to unity but, instead, \( (1 - C_{\Sigma}) \) is used to construct the logistic form \( rC(1 - C_{\Sigma}) \) which assumes the carrying capacity equal to one and inequality \( C_{\Sigma} \leq 1 \) satisfied for proper initial conditions.

As we pay attention to the problem of reversibility within the context of heterogeneity, the switching flows within the clones \( L0, L1, \) and \( H \) are defined as it follows

\[
\begin{align*}
J_{swL0} &= (1 - \varphi_L)C_{L0} - \varphi_L C_{L0}, \\
J_{swL1} &= (1 - \varphi_L)C_{L1} - \varphi_L C_{L1}, \\
J_{swH} &= \frac{1}{2} \left( C_{H} - C_{\Sigma} \right).
\end{align*}
\] (34)

All population rates are defined by the universal formula consisting of the adaptions of Eq. (30) and former variant of the quasispecies model

\[
\mathcal{R}(\tilde{C}, \tilde{J}, \tilde{\alpha}, N_\mu) = r\tilde{C}(1 - C_{\Sigma})
\]
(35)

\[
+m\tilde{J} - \tilde{\alpha}_0 \frac{e(t)\tilde{C}}{C_{\Sigma} + g} + N_\mu \mu C_P,
\]
where \( \tilde{C}, \tilde{J}, \tilde{\alpha}, N_\mu \) are some auxiliary general variables which are later substituted by some appropriate specific variables and constants related to the clonal properties. In comparison with Eq. (30), we also included the mechanism of mutation (\( \sim N_\mu \mu C_P \)) and switching (\( \sim m\tilde{J} \)) already used in the quasispecies simulations. Accordingly to Eq. (35) the scalar drug sensitivity \( a \) is modified by the multiplicative modifier denoted as \( \tilde{\alpha} \). Four variants, \( \alpha_0, \alpha_1, \alpha_{1z}, \alpha_{1z} \), related to the particular phenotypes are used that express the heterogeneity of the species. The equality or inequality of the \( j, z \) indices of \( \alpha_{jz} \) determine the values

\[
\begin{pmatrix}
\alpha_0(0) & \alpha_0(1) \\
\alpha_1(0) & \alpha_1(1)
\end{pmatrix} = \begin{pmatrix}
\alpha_H & \alpha_L \\
\alpha_L & \alpha_H
\end{pmatrix}
\]
(36)

The definition involves only two marginal values (\( H \text{—} \text{high}, L \text{—} \text{low} \))

\[
\begin{align*}
\alpha_H(x_p) &= x_p, \\
\alpha_L(x_p) &= \frac{1}{x_p}
\end{align*}
\] (37)

parametrized by the single auxiliary parameter \( x_p \geq 1 \). The parametrization is designed to satisfy \( \alpha_0(0) \alpha_1(1) + \alpha_0(1) \alpha_1(0) = 1 \) and \( 0 < \alpha_L \leq 1 \leq \alpha_H \) for both phenotypic states \( z \in \{0, 1\} \). In the special case \( x_p = 1 \) which implies \( \alpha_L(1) = \alpha_H(1) = 1 \), there is no modification of \( a \). By combining Eq. (34) with Eq. (35), the population dynamics of (1 + 6) tumor species can be expressed as it follows

\[
\begin{align*}
\frac{dC_P}{dt} &= \mathcal{R}(C_P, 0, 1, -6), \\
\frac{dC_{L0}^{(z)}}{dt} &= \mathcal{R}(C_{L0}^{(z)}, (1 - z)^2 J_{swL0}, \alpha_0^{(z)}, +1), \\
\frac{dC_{L1}^{(z)}}{dt} &= \mathcal{R}(C_{L1}^{(z)}, (1 - z)^2 J_{swL1}, \alpha_1^{(z)}, +1), \\
\frac{dC_{H}^{(z)}}{dt} &= \mathcal{R}(C_{H}^{(z)}, (1 - z)^2 J_{swH}, \alpha_0^{(z)}, +1).
\end{align*}
\] (38)

The first formula of the list expresses that phenotype switching absents (zero at the second position
of $\mathcal{R}$) in the primary tumor clone. This choice is consistent with the quasispecies model. In addition, there is no modification of the drug sensitivity $a$ ($\alpha = 1$). The factor ($-6$) expresses that mutation process modifies the primary tumor growth by subtracting rate factor $\beta u C_P$. We assume that the "most reversible" and "symmetric" transitions between phenotypes corresponding to $C^{(0)}_H$ and $C^{(1)}_H$ are violated by the factor $\alpha_0^{(z)} \neq 1$.

As in the case of quasispecies model, now we propose measures that allow better interpretation of the results. Again, the appropriate transformations allow us to define proper measures of heterogeneity. For all $C_E \in \text{set}_{E}$ the rescaling $(C_E/C_{\Sigma})$ can be performed, that guarantees $\sum_{C_E \in \text{set}_{E}}(C_E/C_{\Sigma}) = 1$. In this case, the tumor can be monitored by epigenetic entropy-like measure

$$S_{\text{EPIG}}^C = -\sum_{C_E \in \text{set}_{E}} \frac{C_E}{C_{\Sigma}} \ln \frac{C_E}{C_{\Sigma}}.$$  \hspace{1cm} (39)

In analogy with Eq. (14), we define four-element auxiliary set $\text{set}_{G} = \{C_P, C^{(0)}_L, C^{(1)}_L, C^{(0)}_H, C^{(1)}_H\}$ which allows modification of the original definition of Eq. (15) to the form

$$S_{G}^C = -\sum_{C_G \in \text{set}_{G}} \frac{C_G}{C_{\Sigma}} \ln \frac{C_G}{C_{\Sigma}}.$$  \hspace{1cm} (40)

### 3.3 Simulation results - evolution of the therapeutic resistance

The results of numerical simulations are depicted in Fig. 5 and Fig. 6. Except for the initial condition $C_P(0) = 0.001, C^{(z)}_L(0) = C^{(z)}_H(0) = 0$ (for both cases $z \in \{0, 1\}$), $\varphi_L$ and $\varphi_L$, the calculation uses the parameters Eq. (22) from the subsection 3.1. The parametric settings $m = 0.05, \mu = 0.01$ were already used here within the model of quasispecies evolution. As is clear from the respective panels of Fig. 5, the different combinations of the drug efficiency ratio $x_P$ and reversibility levels $\varphi_L \in \{0.25, 0.37, 0.5\}$ trigger different competitive behaviors, which are manifested for different time horizons $t_h \in \{100, 500\}$. Again, as in the quasispecies model, the central emergent result of the simulation is the strength of the threshold effect in the therapeutic efficiency. One can see that threshold clearly separates dominating tumor growth regime from the regime of slow growth or decay dynamics. It also turns out, that the primary aspect shaping the evolutionary trajectory towards drug resistance is not necessarily maximal reversibility of the particular tumor phenotypes. In contrast, the results indicate that selection advantage conferred to a phenotype consists in the right combination of drug resistance and incomplete reversibility.

Interestingly, when examining time dependencies $S_{G}^C(t)$ and $S_{\text{EPIG}}^C(t)$, both, the quasispecies and cancer population models, become to exhibit many common and universal features such as overall single-peaked shape, direct proportions $S_{\text{EPIG}}^C \sim S_{G}^C$ as well as undulating $S_{\text{EPIG}}^C(t)$, which contrasts with the much smoother $S_{G}^C(t)$.

### 4 Discussion

The minimalist concept of nonequilibrium modeling of the evolution of phenotypic reversibility in time-varying environments is suggested. Special emphasis has been placed on the evolutionary changes and efficiency related to the bet-hedging strategy. The results demonstrate that the proposed ODE models can be helpful in understanding of the characteristic features of the evolutionary dynamics of genetic and epigenetic phenotypic states, including their interplay at the qualitative level. In particular, the quasispecies model confirmed that, under specific environmental conditions, the clones with extremal reversibility may outcompete their less reversible rivals, which is in agreement with recent experimental findings [48]. The modeling of environmental changes proposed in this work is limited to stylized periodic variations. Deeper insights would be obtained by exploiting stochastic dynamical modeling [49] [51].

The illustrative tumor population model demonstrates the model-specific therapeutic implications of evolved bet-hedging strategy and phenotypic plasticity of cancer cells. High and intermediate levels of immunotherapeutic efficiency that cause remarkable differences between genetic and epigenetic entropy measures become prevalent at late simulation times, where mutation changes become very rare.

Accordingly to both presented models, the high degree of reversibility represents an evolutionary advantage in the variable environments which disappears when the environments become static and vice versa. Less trivial properties that emerge from the both models are multivariate threshold dependencies of the entropy-like measures on the switching rate parameter. Regarding that the original quasispecies model naturally led to the concept of "error threshold" for selective competence (see e.g. [54]), the interesting question arises whether this idea is transferable to nonequilibrium problems, other contexts, and other parametric sets.

The most interesting implication of the illustrative population cancer model is the positive ther-
apeutic effect achieved with less aggressive therapy when moderately diversified immune responses are assumed. This model feature is interesting regarding increasing popularity of low-dose adaptive therapies [51].

Ability to manipulate the (micro)environment in the required way including passing through threshold, represents the principal challenge for therapeutic applications. In general, the period thresholds may serve as discriminative dynamic framework for separation of slow and fast time scales. The supplementary plan which may have important therapeutic implications could be to identify period thresholds for the slowdown of the somatic evolution.

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| $C_P$ | $C_{L0}$ | $C_{L1}$ | $C_H$ |
|-------|---------|---------|-------|
| → $6\mu$ | $\varphi_L \downarrow m$ | $\varphi_L \uparrow m$ | $1/2 \uparrow m$ |
| $\downarrow \mu$ | $\alpha_H$ | $\alpha_L$ | $1$ |
| $\uparrow \mu$ | $C_{L0}^{(1)}$ | $C_{L1}^{(1)}$ | $C_H^{(1)}$ |

Table 1: The scheme depicts the relations between abundances $C_P$, $C_{L0}^{(z)}$, $C_{L1}^{(z)}$, $C_H^{(z)}$, mutation ($\sim \mu$) and switching ($\sim m$) processes, reversibility measures ($\varphi_L$, $1/2$) and drug efficiencies $\alpha_L$, $\alpha_H$ within the model represented by Eq. (38).
Figure 1: Panel (A): Schematic diagram of the quasispecies model ($n_s = 6$) introduced in Section 2. Special emphasis is placed on the reversibility imposed by $\varphi$. The special emphasis on the reversible and irreversible mutations. Panel (B): The time dependence of $n_s = 6$ species fractions in the periodic environment ($T = 10$). The calculations have been performed for have been done for the parameters $\Delta r = 0.4$, $\mu = 0.01$, $m = 0.05$, $\varphi(3, n_s) = 1/2$ has been found. The fractions of the other species are suppressed in the long run asymptotics.
Figure 2: Panel (A) shows the time dependence of the entropy measure $S_{EPIG}$ (the inset includes comparison with $S_G$). The difference between the sub-threshold ($\Delta r = 0$, absence of the variation) and super-threshold fitness environmental variations (alternatives $\Delta r = 0.2, 0.4$). Calculated for the period $T = 10$. The remaining undetermined parameters are $\mu = 0.01$ and $m = 0.05$. In the panels (B) and (C) we plot the time dependencies of the effective index $k_{\text{max}}(t) + z_{\text{max}}(t) \times 0.3$. Here symbol max is related to the species of the maximal abundance and the auxiliary constant 0.3 is chosen for the visualization purposes only. For example in the case $z_{\text{max}} = 0$ the plot hits the mesh line [see $c^{(0)}(5), c^{(0)}(4), c^{(0)}(3), c^{(0)}(0)$ in the panel (C)] corresponding to the species of the maximal instant concentration showing the enhanced selection for the phenotype with highest reversibility, i.e. for $\varphi = 1/2$, $\varphi/(1 - \varphi) = 1$ (see Eq.(8)). In panel (B) the evolution in the static environment remains frozen at $k_{\text{max}} = 1$, $z_{\text{max}} = 1$, whereas the evolution obtained for $\Delta r = 0.2$ in panel (C) asymptotically supports the selection for the phenotype with highest degree of reversibility $k_{\text{max}} = 3$, $z_{\text{max}} = 0, 1$ and $\varphi(3) = 1/2$. (The results obtained for higher $\Delta r$ are qualitatively similar.) Panels (D), (E), (F) depict effects of the number of clones $n_s \in \{12, 24, 48\}$ and $\Delta r \in \{0.2, 0.4, 0.6\}$ on the $S_{EPIG}(t)$. 
Figure 3: The differences in the efficiency of selecting extreme reversibility in the frame of the nonequilibrium evolutionary dynamics. The panels show dependencies of the entropy measure $S_{EPIG}(t = t_h)$ calculated for various combinations of parameters and time horizons $t_h \in \{1000, 2000, 3000\}$. Panel (A) involves the threshold dependencies on the mutation rate $\mu$. We see that sufficiently large $\mu$ is needed to overcome the initial barrier of forming heterogeneous state from which selection can be realized. According to panel (B) the more intense environmental changes, the faster the selection for the most reversible clone is. Panels (C), (D) indicate non-monotonous dependence on $T$. In the case (D), obtained for $\Delta r \in \{0.1, 0.2, 0.4\}$, the efficiency of the selection reduces for the wide range of $T$ until global minima $T \sim (25 \sim 90)$ are attained. Obviously, as shown in panels (E), (F), selection mechanism can be easily accelerated by increasing the switching rate parameter $m$. 
\[ t_h = 1000 \quad \mu = 0.01 \]

\[ t_h = 3000 \quad \mu = 0.01 \]

\[ \ln 2 - \xi^2 \]

\[ \xi_c \]

Figure 4: Figure depicts results of the sensitivity analysis focused on the role of \( m, T, \Delta r \) parameters. The results were obtained for the time horizons \( t_h \in \{1000, 3000\} \) [see panels (A),(C) with \( t_h = 1000 \); panels (B),(D) with \( t_h = 3000 \)] and constant \( \mu = 0.01 \). Instead of particular dependencies on three parametric dimensions represented by \( m, T, \Delta r \) (see Fig. 3) (which lacks holistic perspective), we used \( \xi^2(m, T, \Delta r) \) (see Eq. (26)) as a single independent variable. We have studied sensitivity on \( \xi^2(m, T, \Delta r) \) generated by the \( (m, T, \Delta r) \) combinations with bounds \( m \in (0.01, 0.2) \), \( T \in (1, 20) \), \( \Delta r \in (0.1, 0.4) \). Since 8 evenly spaced values have been used for each particular variable, \( 8^3 = 512 \) samples were drawn from the cubic hyperlattice. Subsequently, each particular \( (m, T, \Delta r) \) vector was converted to the 2d projection consisting of the coordinates \( [\xi^2(m, T, \Delta r), S_{EPIG}(t_h - T, t_h)] \), where \( S_{EPIG}(t_h - T, t_h) \) is defined by Eq. (24) (see panels (A), (B)). Here the summation is performed for the period \( T \) preceding \( t_h \). In analogy with Fig. 3, the thresholds reappear. When the plots (A), (B) are compared with the approximate form \( \ln 2 - \xi^2 \) (see Eq. (29)), the particular agreement can be found for sufficiently high \( \xi^2 \) (low entropy) region where the selection process for the highest reversibility is more effective. The similar tendencies are exhibited by the mean replication measure \( \bar{r}_{eff, max}(t_h - T, t_h) \) defined by Eq. (26), where \( \xi_c(m, T, \Delta r) \) defined by Eq. (25) plays a role analogous to that of \( \xi^2 \); note that Eq. (18) is used to calculate \( r_{eff,k}(t_h) \). The long-time asymptotics \( r_B + \Delta r \xi_c \) is given by Eq. (28). In line with the expectations, the threshold is more pronounced and the relation look sharper at larger \( t_h \) [panels (B), (D)].
Figure 5: The numerical results obtained for the model incorporating the evolution of reversibility and resistance to immunotherapy. In addition, we used $\mu = 0.01$ and $m = 0.05$. The tumor is variable in size ($C_{\Sigma}$) and phenotypic structure. Panels (A), (B) show some differences in the course of the tumor progression ($x_p = 1.16$, “rapid progression”; $x_p = 1.14$, “slower” progression; $x_p = 1.11$, “shrinking”) for selected three values of $x_p$ characterizing qualitative differences in the therapeutic effects on the phenotypic states. Panel (B) shows specific phenotypic dynamics. It shows that despite the high reversibility, the component $C_{H}$ begins to fall at the late times as a result of the loss of the ability to compete. Panels (C), (D), (E), (F) reveal details of $x_p$ influence calculated for two time horizons $t_h \in \{100, 500\}$. The threshold between slow and rapid tumor progression can be identified easily. The transition due to $x_p$ becomes sharper as the $t_h$ increases. The sharpening is also noticeable for the panels (G), (H), where more detailed information on the population structure is available. Surprisingly, $C_{L1}^{(0)}$ dominates over a wide range of $x_p$ although the corresponding $\varphi_L = 0.25$ is quite far from the ideal reversibility measure $\frac{1}{2}$. The therapy causes remarkable shrinking of $C_{L0}$. 
Figure 6: The evolutionary dynamics of heterogeneity measures; the numerical results obtained for $S^C_G(t)$ and $S^C_{EPIG}(t)$. The scenarios corresponding to different $x_p$ are qualitatively similar: the initial increase associated with a large number of possibilities is replaced in the peak zones by the favorable growth of selected clones, i.e. clonal expansion. A weakened preference due to $x_p = 1$ in panel (A) leads to the highest entropy among the considered outcomes. Panels (B), (C) show to the transition stages. Panel (D) indicates that differences between epigenetic and genetic entropy measures are almost entirely neutralized if selective pressures due to immunotherapy are strong enough. For (B), (C), (D) panels the after peak behavior of $S^C_G(t)$ changes smoothly contrary to ”jagged” form of $S^C_{EPIG}(t)$. 
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