Structure of Intratumor Heterogeneity: Is Cancer Hedging Its Bets?

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Development of resistance limits transferability of most anticancer therapies into curative treatment and understanding mechanisms beyond it remains a big challenge. Many high resolution experimental observations show enormous intratumor heterogeneity at molecular, genetic and cellular levels which is made responsible for emerging resistance to therapy. Therefore, researchers search techniques to influence development of intratumor heterogeneity, which requires understanding its role within the context of integrative, logically consistent, framework, such as evolutionary theory. Although it is agreed that intratumor heterogeneity increases probability of the emergence of therapy resistant clones, more instructive role of its structure in the process of cancer dynamics and metastasis is needed. In the paper, intratumor heterogeneity is viewed as a product of two, in general stochastic processes, evolutionary optimization and changing environment, respectively. In evolutionary theory, common risk-diversifying strategy displayed by isogenic populations in unpredictably changing environments is bet-hedging. We suggest, that the structure of intratumor heterogeneity is evolutionary trait evolving to maximize the clonal fitness in changing (or uncertain) environment and that its structure corresponds to bet-hedging strategy. We advocate our view by reviewing and combining important cancer relevant concepts.

BACKGROUND

Despite broad acceptance of evolutionary theory as a useful conceptual framework to understand fundamental features of cancer behavior, evolutionary aspect of cancer is often overlooked in development of novel therapeutic strategies. Although no disqualifying contradiction with evolutionary theory has been found, the dis-appreciation of therapeutic applicability of evolutionary theory might come from exaggerated expectations from too straightforward (or intuitive) applications of basic evolutionary concepts. More purpose exploitation of evolutionary nature of carcinogenesis in therapy necessitates deeper analysis of universal evolutionary concepts within the context of cancer data.

Many years since postulating the basic principles of evolution by Darwin, a lot of questions in evolution theory itself have remained open, such as selection unit, genotype-phenotype mapping, interactions of selection levels, the role of causation in evolutionary biology, etc. As biological organisms were, for a long time, the only 'experimental' system to study evolution, the principles of evolutionary theory merged with the implementation details. Developing evolutionary theory from the basic principles through the modern synthesis and genetic determinism towards the central dogma of molecular biology, the emphasis of the word 'gene' has, step by step, moved from its original meaning as 'the cause of an inheritable phenotype characteristic' to its present meaning as the physical structure. This conceptual change is a major source of present confusion in the question of causation in evolutionary biology and motivates researchers to concentrate on molecular aspects of cancer evolution. On the other hand, applications of evolutionary principles outside biology, in the fields such diverse as optimization, sociology, ecology, etc, the evolutionary principles must be implemented in the case-dependent way. Neglecting implementation details, evolutionary theory can potentially predict universal evolutionary dynamics across wide range of applications.

Many advanced tumors have poor clinical outcome due to development of resistance to therapy. As experimental studies have revealed enormous intratumor heterogeneity, it is intuitively agreed that intratumor heterogeneity increases the probability of harboring a therapy-resistant phenotypes. Consistently, while normal cells respond very similarly to drugs, mechanisms of resistance of cancer cells are extremely diverse. Deeper understanding of intratumor heterogeneity structure and dynamics therefore poses real challenge to cancer therapy. Cancer research concentrates mainly on recognizing molecular mechanisms beyond intratumor heterogeneity and their therapeutic exploitation. More systemic (or functional) role which intratumor heterogeneity plays in cancer initiation and progression remains less often studied and relatively poorly understood, limiting usually to its role in Darwinian evolution of tumors.

Presented genetic data shows that tumors contain complex combinations of low-frequency mutations thought to drive the cancer phenotypes. Previous studies showed that probably no prototypical cancer genotype exists and every tumor carries a unique set of mutations, indicating that multiple ge-
gene tic pathways may lead to invasive cancer as would be expected in a stochastic non-linear dynamical system \[13\]. It has been demonstrated that selection for in vitro drug resistance can result in a complex phenotype with more than one mechanism of resistance emerging concurrently or sequentially \[10\]. It was reported that genotoxic stress induces several cell death pathways, only part of which fall within the classical definition of apoptosis \[17\]. Accordingly to Witz and Levy-Nissenbaum \[18\], complexity of the signaling cascades in tumor microenvironment and the interactive cross-talk between these cascades generate the feeling that ‘anything that can happen will’ and they suggest to apply tools employed in hyper complex systems analysis \[18\]. Lewis generalizes that, in formally similar system of evolving bacteria, all of the theoretically logical possibilities of antibiotic resistance seem to have been realized in nature accomplishing the same task, which is to prevent the antibiotic from binding to its target \[15\].

It was proposed that cancer cells may possibly need only a modest number of phenotypic traits to deal with all the constraints and evolve into a tumor \[20\]. Facing huge genomic heterogeneity and microenvironmental uncertainty, the question is if these common "hallmarks of cancer" are present in all the cells all the time \[21\]. Focusing on a few phenotypic traits would crucially reduce dimensionality of the relevant search space of all the possible dynamics. Nevertheless, as any cancer cell can have myriad genetic causes, as natural selection selects for phenotype, not genotype, and population changes depend on local environmental selection forces \[22\], it leads, at genetic level, to an undetermined problem. Deeper insight into the problem requires correct interpretation of intratumor heterogeneity. If interpreted as a noise hiding a common pattern, the effort to generalize data from many samples of the relevant cancer type to see the pattern is justified. If, however, heterogeneity represents redundancy, i.e. no common pattern exists and each tumor has unique, nevertheless causative set of genes, to study cancer by reducing heterogeneity may be a flawed approach \[22\]. If this is the case, the optimization problem solved by cancer can be viewed as, in a sense, underdetermined, which means that there are enough degrees of freedom to find multiple reasonable fit solutions for many environments, i.e. multiple physically realizable solutions to the relevant mathematical problem. In \[24\], Krakauer and Plotkin applied the quasispecies model \[25\] and analyzed the evolutionary dynamics of redundancy and anti-redundancy in general case. They proposed conditions which favor evolution of redundancy or anti-redundancy, respectively, and list mechanisms responsible for creating redundancy and anti-redundancy at the cellular level \[24\].

It is intuitively clear that owing to its heterogeneity, evolving population of cancer cells can absorb and, in conjunction with natural selection, evolve many alternative solutions to many different environments, representing an efficient computational system. Produced by combination of many sources, population heterogeneity becomes extremely complex statistical quantity. The measures of heterogeneity may be defined in many different ways most of them chosen from the viewpoint of the statistical mechanics of non-extensive systems based on entropy \[26–28\]. Transforming heterogeneity into a tractable and computable property of cellular populations provides a rigorous starting point for determining which variation is random and which is meaningful \[29\].

The last decade has witnessed renewed interest of cancer research community in hierarchical model of cancer, well known as cancer stem cells (CSC) hypothesis \[30\] claiming that cancer cells populations are hierarchically structured with only a small subpopulation of the cells able to recapitulate tumor from which they were derived. CSC seems to possess self-renewal ability and reveal resistance against conventional therapies \[30, 32\]. As soon as the CSC hypothesis was proposed, CSC have become viewed as the target for therapeutic intervention \[33, 34\]. The strategies rely on the possibility of precise enough splitting of cancer cells population into CSC fraction and non-CSC fraction. The original cancer stem cell model suggested that CSC represent a subset of cancer cells population which is well distinguishable by a limited number of cell-surface markers. During the time, many controversial issues regarding their origin, proportions in cancer cells population, heterogeneity, flexibility of their state, etc. have emerged \[35–39\] and the existence and role of CSC in cancer initiation and progression remain a topic of intense debate \[40–44\]. Accordingly to Badve and Naksahatri \[46\], CSC should be viewed as representing an aggressive clone that has evolved during tumor progression, concluding that referring to these cells as CSC is, actually, a matter of semantics. Similarly Maenhaut et al. propose that the tumor propagating cells are multiple evolutionary selected cancer cells with the most competitive properties maintained by, at least partially, reversible mechanisms, quantitative rather than qualitative and resulting from a stochastic rather than deterministic process \[47\]. If, however, cancer cell stemness is defined by function \[41, 44, 46\] instead of specific molecular structure, approaches relying on the possibility of precise enough splitting of cancer cells population into CSC fraction and non-CSC fraction may lack efficiency.

There is growing evidence that many solid tumors may be composed of several distinct subtypes of tumors, which may have distinct CSC \[38, 42\]. Genetic heterogeneity of cancer stem cells \[30, 41, 43\] provides phenotypic and functional heterogeneity and tempts to designate the cancer stem cells to be the units of selection in the model of clonal evolution \[52\]. From an evolutionary perspective, limitation of
self-replicating capacity to a fraction of tumor cells means that the effective population size is restricted to this stem-like compartment, rather than encompassing a bulk of tumor cells \[53\].

**REVERSIBLE STATE TRANSITIONS**

A few papers proposed that some isogenic cancer cell populations consist of phenotypically different subpopulations and that cancer cells actually switch between different phenotypes (or cell types) in reversible way \[54–60\], putting the concept of stemness in question. In their influential paper, Gupta et al. \[61\] report observation that the only population of human breast cancer cells consists of three phenotypically different sub-populations (consisting of stem, basal and luminal cells, respectively). Studying dynamics of these cell-types fractions, they found that these stay, under stationary conditions, in equilibrium proportions. Moreover, if the cancer cells population is purified for any of the three cell types, the equilibrium is rapidly re-established \[61\]. The progression towards equilibrium proportions would require implausibly high proliferation rates, therefore they concluded that the progression towards equilibrium was not due to differential growth rates of cells in the respective states but rather to interconversions between cell types \[61\]. Summarizing their observations, Gupta et al. proposed a Markov model of cell-state dynamics \[61\], which assumes that cells within a population can exist in any one of \(M\) possible stable cell states (i.e., cell types) and under fixed genetic and environmental conditions, cells transition from one state to another with interconversion rates per unit time that are constant.

Identification of the cell-state dynamics with Markov process \[61\] enables to study physical and evolutionary aspects of cancer dynamics separately. Cancer research concentrates predominantly on the former, physical, aspects, trying to understand details of molecular mechanisms and genetic similarities beyond the cell types transitions. Assuming reported observation, that genetically identical cells under identical physical conditions differ in their response to a given chemotherapeutic to an extent that may impact on clinical response \[62\], probabilistic nature of Markov model of the cell-state dynamics may be, eventually, very appropriate. It follows common mathematical behavior of Markov processes, such as its convergence towards limiting distribution, which is fully determined by underlying it transition matrix. As each element of the transition matrix represents respective interconversion rate, i.e., the probability of a physical process, equilibrium cell types proportions in cells population (hence non-genetic heterogeneity) are fully determined by the transition probabilities and do not depend on instantaneous cell types proportions themselves. In this way, \(M\) equilibrium cell types proportions are determined by \(O(M^2)\) interconversion rates which leads, however, to underdetermined problem for \(M > 2\). Despite the fact that physical processes beyond the respective transitions are certainly not independent each other, huge number of degrees of freedom of the problem still may leave opportunity to get any equilibrium distribution of phenotypic proportions by multiple sets of interconversion rates (i.e., alternative combinations of the respective processes). Solving under-determined inverse problems is for evolution, in general, an easy task. Many forms and mechanisms of phenotypic switching have been theoretically studied \[63–69\] and observed at molecular, genetic and expression levels \[69, 70\].

**CANCER RELEVANT SELECTION UNIT, TIMESCALE AND CAUSATION**

Despite often referred observations that cancer cells actually switch between different cell types in reversible way \[54–60\], heterogeneity is, in cancer biology, traditionally attributed to genetic variance, implicitly implying one-to-one genotype-phenotype mapping. Many authors propose that genetic heterogeneity is unlikely to be the major contributor to phenotypic heterogeneity in general, but, underlying heritable differences, it fuels tumor evolution \[11\]. As non-genetic mechanisms, such as gene expression noise and multiplicity of stable states in gene networks, are responsible for phenotypic identities of normal cells, it was suggested that non-genetic heterogeneity, can contribute to somatic evolution of cancer cells, hence accelerating tumor progression and development of therapeutic resistance \[74, 75\]. Recently, the difference between two types of cancer cell instability, genetic and non-genetic, was accentuated and hierarchical link between the corresponding spaces, the fitness and epigenetic landscapes, was proposed \[77\]. Therein, each point in the fitness landscape (i.e., genome) provides epigenetic landscape of unique topology. Due to its mathematical complexity, each epigenetic landscape contains, stable areas (attractors) around stable cell-states \[77\], viewed as cell types or phenotypes (disputed below). Non-occupied attractors are not exposed to selection and, consequently, are not evolutionarily harmonized with the needs of the tissue \[77\] and stay pathological (or cancerous). If epigenetic landscape, due to genetic mutations or tumor microenvironment, changes, probability of the cell finding itself in cancerous attractors may increase \[77\]. Considering the timescale in which mutations spread in a cell population, non-genetic instability is made responsible for heterogeneity of cancer cell populations \[77\]. This is consistent with the observation that the population of isogenic cancer cells purified for one
of the stable cell-states re-establish equilibrium proportions of the cell types too fast to be explained by differential growth alone [61]. Consequently, the role of somatic evolution in cancer progression is put in doubt [77].

Keeping in mind Theodosius Dobzhansky's statement 'Nothing in biology makes sense except in the light of evolution', we propose the eventual role played by non-genetic heterogeneity in somatic evolution of cancer. To understand intratumor heterogeneity from evolutionary viewpoint, fundamental aspects of natural selection, namely selection unit, timescale and causation must be reconsidered. If somatic evolution is applied to explain cancer, cancerous features are implicitly attributed to a cell which is taken as cancer-relevant selection unit and its fitness is identified with its reproduction capability. Assuming one-to-one genotype-phenotype mapping, current cancer research focuses on differences in metabolic pathways responsible for cancer phenotype of the cell in order to distinguish cancer and normal cells as well as to predict proximate behavior of cancer cells. However, the focus on differences between the normal and cancer cells may be not adequate enough, as it was observed that (at least) some isogenic cancer cells populations consist of phenotypically different subpopulations [61], which implies cell’s multistability (or one-to-many genotype-phenotype mapping).

The term ‘phenotype’ is often used in very intuitive way, denoting usually observable traits of the cell, which are assumed to be static. If alternative sets of observable traits expressed by the same genome is observed, each of them is viewed as an alternative phenotype, and the change of phenotype without genetic cause is interpreted as ‘phenotypic switch’. Despite being appropriate in many biological contexts, if evolutionary theory is to be applied in cancer biology, the term ‘phenotype’ must be reconsidered. Evolution is based on the general premise that any population of entities which reveal variation, reproduction, and heritability may evolve [78]. The original biological meaning of a gene referred to the cause of an inheritable phenotypic characteristic [5]. It implies, that the genome, as a set of genes, refers to the cause of inheritable phenotype as a whole. The above evolutionary premise is formally fulfilled not only when the unique cell-type expressed by the genome is taken as phenotype, but, as well, if the epigenetic landscape, corresponding to the genome as its unique cause, is assumed to be the phenotype.

In the latter context, the term ‘phenotype’ encompasses all the relevant features of the epigenetic landscapes, such as the repertoire of attractors (i.e. cell types), the heights of barriers between them, etc. The fitness of the genome relates to the phenotype-relevant timescale. At proximate timescale, its phenotype (i.e. a cell-type) is the outcome of specific molecular mechanisms and its fitness is obvious. At longer timescale, after the genome produced a lineage of reasonable size, the size of its clone corresponds to the fitness of the genome (at this timescale). Obviously, evolutionary success (or failure) of the cell at short timescale does not necessarily correlate with its evolutionary success on long timescale. To quantify possible outcome of lineage (or clone) evolution in a more quantitative way, Palmer and Feldman introduced two metrics, \( k \)-fitness and \( k \)-survivability [79]. The former quantifies probability of increase of the size of the respective lineage after \( k \) generations, the latter relates to the likelihood that the species will avoid extinction after \( k \) generations. If \( k \) increases, \( k \)-fitness of the cell depends more and more on the eventual interaction of the cells in the clone. Regarding the timescale at which cancer has effects (cancer-relevant timescale), the clone seems to be more relevant structure to determine the genome fitness.

Leaving precise quantification of cancer-relevant timescale to further research, we ask how general evolutionary top-down causation [8] applies in cancer? At proximate timescale, the genomes’ fitnesses depend on their respective ‘built-in’ molecular machinery. On the other hand, molecular machinery has been selected accordingly to the evolutionary advantage they conferred to the ancestors of the respective genomes (Fig. 1) in the past. The above reconsideration of cancer-relevant selection unit, timescale and causation imply what is the selection force evolving epigenetic landscape and what is the optimum repertoire of stable cell-states (i.e. cell types) conferring to the cell the highest fitness at cancer-relevant timescale.

**BET-HEDGING**

Markov model of the cell-state dynamics by Gupta et al. [61] implies that non-genetic heterogeneity in isogenic cancer cells population (i.e. cancer clone) is actually determined by the probabilities of tran-
positions between different cell types, i.e. by the transition matrix of Markov process, and are not bound to genetic polymorphism. The cell types correspond to stable cell-states (or attractors) in the epigenetic landscape as conceptualized by Huang. In this way, genetically coded transition matrix prescribes the sizes of the cell types fractions (hence non-genetic heterogeneity) in any possible tumor microenvironment, and, from the evolutionary viewpoint, the fitness of the genome at the cancer relevant scale. As the cell types, in general, differ in their growth (and other) properties, the distribution of the cell states in the clone (non-genetic heterogeneity) becomes evolutionary important. To sum up, the cells evolve transition matrix producing the proportions of the cell types so that the clone increases at maximum rate. During their evolution the cells inevitably interact, which is traditionally viewed as 'cooperation' in the case of normal cells, while the cancer cells are often said to behave 'selfishly'. Revealing specific prototypical features. It was, however, proposed as well, that genetically distinct tumor cells cooperate as well to overcome certain host defenses by exchanging different diffusible products. Cooperation does not imply equality of the cells in the clone but rather a specialization of the cells to diverse roles, which increases clonal fitness. In the conceptual model of group selection, pure cooperator groups grow faster than pure defectors groups, whereas in any mixed group, defectors reproduce faster than cooperators, i.e. selection on the lower level (within groups) favors defectors, whereas selection on the higher level (between groups) favors groups consisting of cooperators.

Inspired by the above model of cooperation, we suggest that the cancer cell, its clone the evolutionary winner at the relevant timescale, produces the clone of well 'cooperating' cells, and propose what kind of cooperation between cancer cells can be expected. Instead of interpreting specific biochemical reactions as cooperative or selfish, cooperation is viewed in a more general way - as a coordinated action. This view enables its statistical interpretation as mutually correlated dispersal of the cell states in the state space. The observation by Gupta implies, that the cell types equilibrium (i.e. non-genetic heterogeneity) in isogenic clone of cells can be established by switching between a few possible states in a reversible way. Assuming that the cells in different states differ in their proliferation efficiency, intraclonal cooperation is determined by the cell-states heterogeneity when the cells in different states contribute to the clone growth by, in general, different amounts. Different environments confer different growth to the clones with different heterogeneity structure - in some environments clones consisting of the cells in the same state may provide faster clonal growth, while in the others more specialization (bound to specific structure of cell-states heterogeneity) leads to faster growth of the clone.

In this view, non-genetic heterogeneity becomes evolutionary trait and the fundamental question arises what heterogeneity structure gives to the cell the highest fitness (measured as the size of its clone).

From the viewpoint of evolutionary biology, phenotypic heterogeneity is an adaptation to environmental uncertainty and phenotypic diversification enables species to survive environmental adversity. Each phenotype (or, in here applied conceptualization, cell-type) proportion can be viewed as an investment of certain portion of population's reproductive effort. Two fundamental evolutionary strategies of population adapting to environmental uncertainty are well known. The former, generalist, produces constant phenotype which has been reasonably fit in any relevant environment. The latter, the bet-hedging strategy, generates non-genetic phenotypic diversity in the population producing phenotypes accordingly to probability distribution matching the distribution of the environments rewarding (in a sense of fitness) the respective phenotypes in the past. One can find instructive analogy with risk diversifying strategy in portfolio management. Facing uncertain future, investor divides his budget into a few (or many) assets instead of the only, whatever probable, asset to protect himself against fatal loss. Optimum investment strategy must somehow balance predictability of assets' prices with the cost of portfolio's restructuring. Optimal portfolio depends on the dynamics of the trends, and, at the same time, the investor's capability to restructure his 'portfolio'. It is of the utmost importance that the bet-hedging strategy is realized as alternative expressions of the only selection unit, not as a form of genetic polymorphism.

In laboratory studies of yeast and bacteria, the rate of phenotypic switching has appeared to adjust to match the frequency of environmental changes. Kussell et al. demonstrated that the optimal switching between normal cells and bacterial persister cells, characterized by slow growth and increased ability to survive antibiotic treatment, depends strongly on the frequency of environmental change and only weakly on the selective pressures of any given experiment. It is consistent with the finding that a critical feature of the process of tumor progression is selection of cells that can escape from resource limitations by achieving a relative microenvironmental independence.

To sum up, the bet-hedging strategy is a universal risk-diversification strategy evolved in the populations which face uncertain future and/or environment. Assuming formal similarity of evolving cancer cells population with the above evolutionary systems, we identify non-genetic heterogeneity corresponding to the observed equilibrium distribution of the cell types in isogenic cancer cells popula-
tion \[61\] with the bet-hedging strategy. This identification is motivated by the observation that a rapid progression towards equilibrium proportions would require implausibly high proliferation rates and cannot be explained by differential growth rates of the clones of cells in the respective states, but rather to interconversion between states \[61\]. Moreover, non-genetic heterogeneity develops at the timescale in which mutations barely spread in a cell populations \[77\].

To propose the role of non-genetic heterogeneity in clonal evolution of cancer, selection force which pushes the cell types proportions into the “optimum investment profile” in the respective environment, corresponding to the respective environmental frequencies, must be identified. Applying the above conceptualization by Huang \[77\], each the genome represents epigenetic landscape of unique topology, with the repertoire of attractors (i.e. cell types) as a fundamental mathematical feature. These attractors are separated by the barriers which heights determine probabilities of transition between them. In the conceptualization by Markov model \[61\], the repertoire of attractors corresponds to the limiting distribution of Markov model and the heights of the barriers to its transition probabilities.

On the other hand, environment predetermines optimum investment profile, which, if occupied by the respective (optimum) proportions of cancer cells, maximizes growth of the clone. In bet-hedging theory, the strength of selection towards the optimal bet-hedging strategy depends on how far the residents are from the optimal investment profile \[53\]. Straightforwardly, the fitness of the cancer cell is given by the deviations of the cell types proportions in its clone from the optimum bet-hedging proportions at cancer-relevant timescale. As the cell types proportions (hence the clone’s cell-state heterogeneity) are determined by the rates of interconversions between states \[61\], i.e. by the probabilities of specific physical processes, the genetically coded molecular mechanisms are under selection pressure preferring those which provide heights of the barriers between attractors leading to the optimum cell types fractions as required by the environment dynamics.

**EVOLUTIONARY OPTIMIZATION**

In 1932, Sewall Wright conceptualized evolution as a search process through the astronomically huge search space of all the possible combinations of genes \[91\]. Assigning a fitness value (a quality measure) to each genetic combination, the evolution was identified with a search for the highest peak (maximum fitness) in so-called fitness landscape. In this way, Wright linked evolution with the field of optimization as conceived in engineering and economy \[8\], initiating the new branch of stochastic optimization techniques, presently known as evolutionary algorithms (EA) \[92\]. Despite the fact that in evolutionary optimization one purposely applies evolutionary principles to evolve population of candidate solutions, while biological evolution is straightforward consequence of mere existence of the population of biological replicators (genomes), both the processes depend on universal aspects of fitness landscape. EA analyze the above features in implicitly abstract way, while in biological evolution they are bound to specific molecular machinery which complicates their analysis. Being applied in many different contexts, EA have significantly enriched evolutionary theory by sharpening the above “substrate-free” aspects of evolutionary dynamics.

In real-world optimization problems (including biological evolution) each fitness evaluation requires nonzero resources and must be attained within affordable time interval, which limits number of fitness evaluations. On the other hand, keeping in mind the fitness landscape uncertainty, rational strategy is exploring, with some probability, also not yet evaluated parts of the search space. Uncertainty of stationary fitness landscapes decreases proportionally to the number of fitness evaluations, therefore the typical strategy is to allocate, during the optimization, increasing number of trials to the observed best solution, and let explorative power of the algorithm vanish. But the question emerges how fast should the ratio between exploration and exploitation aspects decrease during optimization? The problem is known as the exploration vs. exploitation dilemma (or the optimal trial allocation) and optimization techniques differ in their way of solving it (Fig. 2).

Blind search (Fig. 2A) resigns exploitation preventing it from sticking in local optima but, at the same time, results in very low efficiency. Gradient search (Fig. 2B) always continues uphill, maximizing exploitation but it fails with high probability if the fitness landscape contains more local optima. Simulated annealing \[90\] (Fig. 2C) represents some compromise, starting as random search, decreasing continuously probability of acceptance of less fit points, converging, eventually, in global optimum. Obviously, efficiency of sampling strategies crucially depends on dimensionality, ruggedness, modality, stationarity, etc of the respective fitness landscape.

The problem of the optimal allocation of trials is especially challenging if the fitness landscape is changing, which is the crucial feature of biological fitness landscapes. Facing uncertain future, optimization procedure must maintain nonzero explorative ability. To be efficient in changing fitness landscape, an optimization procedure must i) detect change in the fitness landscape (exploration), and, ii) appropriately respond to it (exploitation). Due to nonzero detection and response time, tracing the optimum in dynamic optimization problem always expects nonzero
time correlation of fitness landscape which can be exploited. The maximum entropy principle states, that, if the probability distribution of random variable is not known, the probability distribution which best represents the current state of knowledge is the one with the largest information theoretical entropy. It implies preemptive distribution of the trials in evolutionary algorithms designed for dynamic environments. They keep fraction of candidate solutions, denoted as sentinels, unchanged [93]. The sentinels are population members that are statistically reasonably (in the above sense) distributed through the search space upon initialization, and kept in population to produce new population members through selection, but themselves are neither mutated nor replaced [93]. The sentinels themselves are, in average, neither more nor less fit than other solutions in the population. Their added value consists in preventing, in conjunction with the other sentinels, shrinking of the search space (Fig. 2D), i. e. maintaining the exploratory power of the algorithm.

**SUMMARY**

Clonal evolution model for tumor progression by Nowell [1] says that within a population of tumor cells natural selection, which favors cells that have acquired the most aggressive phenotype, occurs. To make this predication more applicable, better understanding what 'the most aggressive' means is necessary. As soon as the CSC hypothesis was proposed [30], the subpopulation of CSC was alleged to be the most competitive tumor fraction, and, consequently, tumor evolution was attributed to it. Despite its universality is still debated, the CSC hypothesis implies basic hierarchical structure of intratumor heterogeneity. Evolving cancer cells population reveals the structure of heterogeneity (CSC vs. non-CSC fractions) similar to the structure of population heterogeneity purposely applied in evolutionary optimization to make optimization procedure more efficient in changing fitness landscapes [93, 94]. This is not surprising, as the link between evolution and optimization was made long time ago [91]. To sum up, the intratumor heterogeneity structure of cancer clone (being the evolutionary winner) has balanced optimally exploration of the search space with its exploitation during cancer-relevant time period.

Cancer heterogeneity has been for a long time viewed as the consequence of genetic heterogeneity. Presently, an opinion is emerging that the stemness of cancer cells corresponds more to function state instead of distinguishable genetic (or epigenetic) pattern. This view is supported by reversible stochastic switching between cell states in cancer cells population [54–58, 61]. The results by Gupta et al [61] show, that the specific cancer genome encodes, at the same time, three alternative stable cell types, one of them being the 'stem' state, and switching between them creates, in stationary environment, equilibrium proportions of the cell types. In this way non-genetic heterogeneity is responsible for cancer and some papers present doubts about the role of somatic evolution in cancer.

In the paper, the equilibrium cell types proportions are viewed to be more refined structure of intratumor heterogeneity, beyond the basic division into...
CSC and non CSC fractions. The genome stays the main protagonist (i.e., selection unit) in the evolution of cancer cells, with non-genetic heterogeneity of its eventual clone being the crucial adaptive trait at cancer-relevant timescale. From the viewpoint of evolutionary biology, phenotypic heterogeneity is an adaptation to environmental uncertainty and phenotypic diversification enables species to survive environmental adversity. One of the observed and well studied strategies of population diversification in changing environment is the bet-hedging strategy, which divides reproductive investment in each environment to fit the respective environmental frequencies in the past. Straightforwardly, we suggest the hypothesis that non-genetic heterogeneity in cancer cells population evolves towards the universal bet-hedging diversification strategy. Affirmative answer to the hypothesis necessitates determination of the optimum bet-hedging profile in specific cancer case during some relevant past, which is the task far from trivial. Bound to the same evolving structure, the genome, the two components of phenotypic heterogeneity (genetic and non-genetic) interact in complex way, which dramatically complicates their more rigorous analysis.

How can be the affirmative answer to the question in the title helpful? Having attributed cancer dynamics to an appropriate universal dynamics (such as here proposed bet-hedging), one can, eventually, apply its general features to influence the process by modifying fitness landscapes in mathematically more purposeful way. It is known that diversification strategy adopted by evolving populations depends on the environment. Under some environments dynamics the generalist strategy are more successful than bet-hedging. Straightforwardly, purposeful manipulation with statistical features of environment (hence fitness landscape dynamics, may provide diversification strategy which is less fatal and/or better controllable.

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