Cancer is an evolutionary process that is driven by mutation and selection. Tumors are genetically unstable, and research has shown that this is the most efficient way for cancers to evolve. Genetic instability leads to genetic heterogeneity and dynamic change within a single individual’s tumor, in turn leading to therapeutic resistance. Cancer treatment has also evolved from an empirical science of killing dividing cells to the current era of ‘personalized medicine’, exquisitely targeting the molecular features of individual cancers. However, current personalized medicine regards a single individual’s cancer as largely uniform and static. Moreover, from a strategic perspective, current personalized medicine thinks primarily of the immediate therapy selection. Ongoing research suggests that new, nonstandard personalized treatment strategies that plan further ahead and consider intratumoral heterogeneity and the evolving nature of cancer (due to genetic instability) may lead to the next level of therapeutic benefit beyond current personalized medicine.

Keywords: evolution • intratumoral heterogeneity • personalized medicine • therapeutic resistance • treatment strategies

The future of oncology lies at least in part in personalized, targeted therapies [1–3]. The molecular characteristics of a patient’s tumor, including putative predictive biomarkers, are used to customize each patient’s therapy, selecting therapies that are hoped to be more efficacious for that particular patient. Prior to this personalized medicine paradigm, nonspecific cytotoxic approaches were the mainstay of medical therapy for cancer. Although this approach has produced significant effects in some patients, the lack of predictive biomarkers typically resulted in incremental benefits for unselected patient populations, requiring large clinical studies for their detection. This drove up the cost of oncology therapies, and the small-to-average benefits and high costs led to low cost–effectiveness. In addition, significant toxicities and low cure rates continued to limit the results of cancer treatment.

In the current age of targeted therapy, successes are still not universal, and predictive biomarkers must be carefully validated in the clinic [3]. Nevertheless, a number of important successes exist, including trastuzumab for HER2+ breast cancer [4,5], crizotinib for non-small-cell lung cancer with a translocation of the ALK gene [6], gefitinib and erlotinib for non-small-cell lung cancer with sensitizing mutations in the EGFR gene [7,8] and the BRAF inhibitor vemurafenib for BRAF V600E-mutant melanoma [9]. The case of vemurafenib illustrates the promise and pitfalls of the current personalized medicine paradigm. Approximately half of patients (48%) with the BRAF V600E mutation respond to vemurafenib and the survival benefit is highly significant relative to chemotherapy control, with a hazard ratio of 0.37 [9]. However, the median response duration is only 6.7 months [10]. By contrast, while therapy with the immune checkpoint inhibitor ipilimumab conferred a smaller survival benefit (hazard ratio: 0.68) and a lower response rate (10.9%), responses were
frequently maintained for 2 years or more [11]. The combination of two immune checkpoint inhibitors, ipilimumab and nivolumab, resulted in a higher response rate (53%) and, again, responses were highly durable [12].

Numerous resistance mechanisms to BRAF inhibition, both genetic and nongenetic, have been discovered [13]. In this Perspective, we focus on heritable (genetic and epigenetic) resistance and the implications for personalized medicine using targeted therapies.

Tumors are genetically unstable, and it has been shown that this is the most efficient way for them to evolve [14–16]. Carcinogenesis is a multistep process requiring the genetic alteration of oncogenic targets in order to confer the deadly characteristics of a cancer cell [17], generally numbering from two to 12 genetic alterations, as indicated by epidemiologic models [18]. Early acquisition of genetic instability was postulated as a mechanism for accelerating carcinogenesis and was termed the ‘mutator hypothesis’ [19]. Quantitative models demonstrated that early genetic instability is generally an integral part of the most efficient pathways of cancer evolution. This is true even when one takes into account confounders, including concurrent evolutionary selection of fitter partially transformed premalignant clones (which is also very important for efficient tumor evolution) and the need for an additional genetic instability mutation in order to confer genetic instability in the first place, as well as the possibly damaging accumulation of random deleterious mutations in genetically unstable subclones [14,15,20].

More efficient mechanisms of carcinogenesis are more likely to produce clinically observable tumors, leading to the prediction that genetic instability would be widespread in clinical tumors. These conclusions were subsequently confirmed independently using independent theoretical methods [21].

The predictions of these quantitative models were subsequently experimentally confirmed. Tumor sequencing efforts confirmed the high mutation burden and genetic variety of tumors [22,23], as well as the existence of genetic instability mutations, such as p53 mutations, as well as homologous recombination defects [24]. Moreover, the prediction that tumors that require less than three oncogenic mutations for transformation would not be genetically unstable was confirmed for retinoblastoma [25]. Finally, the prediction that convergent evolution would lead to multiple different ways to activate the same pathway within a given tumor was confirmed for renal cell carcinoma, where within a single lesion, different mutations affecting the mTOR pathway were seen in different biopsies [26].

Given this genetic instability, one would expect to see heterogeneity within a single individual’s malignancy, as well as the evolution of new genotypes. Indeed, genetic heterogeneity between the primary lesion and metastatic lesions has been documented for breast and pancreatic cancers [27–30]. For pediatric and adult acute lymphoblastic leukemia, subclonal structure analysis has made the deduction of evolutionary trees possible within single tumors [31–33].

These findings imply that, just as no two snowflakes are alike, each tumor cell within a single malignancy is unique. This genetic variability is potentially a rich source of pre-existing therapeutic resistance [34]. By contrast, the current personalized paradigm targets a static predictive signature that is generally derived from a consensus pattern on a bulk biopsy. Such a consensus pattern will be dominated by the largest subclone, but ignores minority subclones. In short, the current personalized medicine strategy addresses heterogeneity between tumors in different individuals (intertumoral heterogeneity), but not within a single tumor (intratumoral heterogeneity).

Moreover, a tumor is a constantly evolving, moving target, which is an additional source of acquired resistance [34]. However, currently molecular analyses are performed on tissues obtained earlier in the patient’s course (at diagnosis), although the tumor will have likely evolved since the acquisition of this analyzed sample. Finally, the current personalized medicine paradigm involves treating with the selected matching targeting therapy until the tumor either worsens or relapses, despite the fact that tumor evolution may necessitate an earlier change in therapy. The longer response durations observed with immunologic checkpoint inhibitors may be the result of the inherent adaptability of the immune system (i.e., its capacity to counterevolve).

Intratumoral heterogeneity and evolutionary dynamics are the key limitations to targeted therapy according to the current personalized medicine strategy. This article will explore nonstandard personalized medicine strategies that explicitly take subclonal structure and evolutionary dynamics into account. These nonstandard strategies have the potential to significantly improve the performance of targeted therapies throughout oncology [35]. The results from a minimal model are presented in order to evaluate the potential of nonstandard personalized medicine strategies across a very broad range of scenarios. This minimal model is utilized in order to permit computationally feasible and thorough exploration of all possible scenarios involving heritable variation and change, as well as evaluation of a large number of treatment strategies. The application of these strategies to individual cases...
Nonstandard personalized medicine strategies

What is a strategy?

We begin by defining the term ‘strategy’. A strategy is a data-driven method (or algorithm) for planning a sequence of therapies, such as when to use combinations, when to use monotherapy and when to switch therapies, among other considerations, in individual patients. A strategy is not a particular choice of therapy or a particular sequence of therapies. Current personalized medicine applies a constant strategy, individualizing the therapies employed. Nonstandard personalized medicine programs may individualize strategies as well as therapies. The same therapies may be applied in different sequences in similar patients due to the use of differing strategies.

The current personalized medicine strategy is a matching strategy. A consensus molecular pattern is identified on a bulk sample that is ideally current but is often from the past due to current technical limitations of repeat tissue sampling. A therapy or therapeutic combination is applied that matches this consensus pattern. This therapy is continued until the patient’s tumor worsens or, if the tumor has disappeared on therapy, until relapse. At that time, the process is repeated, subject to the availability of tissue, in order to obtain an updated consensus pattern.

The current personalized medicine strategy is not the only possible strategy. We will see below that the consideration of observed (and even predicted) subclonal structure and evolutionary dynamics in a knowledge-based way may lead to different strategies and, in turn, to occasionally counterintuitive choices of therapy and therapeutic sequences.

The question of whether to use multiple agents in simultaneous combination, which may address a greater variety of subclones, or to use sequential monotherapy, where it may be possible to achieve more effective doses (since dosages must often be reduced in combination due to toxicity), has been debated in the literature for decades [36–38]. When considering subclonal structure and evolutionary dynamics, this question becomes very complex and choices must be individualized.

A minimal model of intratumoral evolutionary dynamics

Consider the following model for the exploration of treatment strategies (Figure 1), which has been designed for the simplest possible case of a two-drug model: drug 1 and drug 2. In this case, ‘drug 1’ and ‘drug 2’ may actually be combinations: each refers to a single drug or combination that matches a particular genetic state and is optimal for its treatment. Indeed, ‘drug 1’ and ‘drug 2’ may represent short-term sequences of drugs that address short-term nongenetic dynamics. ‘Drugs’ may mean small-molecule drugs, biologics and/or radiation therapy. Extension of the model to immunotherapy and/or cell therapy would require additional features and would clearly be desirable in the future.

The model assumes four types of cells based on drug sensitivity and resistance phenotypes: S cells are sensitive to both drugs; R1 cells are resistant to drug 1, but sensitive to drug 2; R2 cells are resistant to drug 2, but sensitive to drug 1; and R1–2 cells are resistant to both drugs and are incurable in this model. Sensitivity is defined as the ability to slow the growth of the tumor by at least an amount corresponding to a 25% increase in survival, which is a common threshold for the approval of a new agent by health authorities.

Cell growth and death are affected by the drugs in a dose-dependent manner. Sensitivity means that...
high-dose monotherapy of the particular drug is capable of substantially reducing the net growth rate (growth rate minus death rate) and, ideally, could result in a negative growth rate. In combination, it is often necessary to reduce the dose of each drug because of additive toxicities. In this model, we make the simple assumptions that the total normalized dose of both drugs must equal a constant dose and that their effects are additive, broadly representing the most common scenario. In actual cases, the allowed doses would be substituted from Phase I safety data and would involve dose reduction, although not necessarily to a constant normalized sum. In some cases, it may be feasible to give full doses of both drugs or to benefit from the synergistic efficacy of these drugs, and in such cases, these facts can be specifically incorporated into the model.

Each cell type may be a cluster of many possible genetic states that gives the particular sensitivity phenotype. The model intentionally uses phenotypic states, which are fewer in number than the underlying genetic states, as this makes rapid computation of many strategies possible.

Each cell type has its own exponential growth rate in addition to its drug sensitivity phenotype. Reversible genetic and epigenetic transitions occur between these phenotypic states. Importantly, each patient may present with a mixture of these cell types that evolves over time.

The model does not assume that the different subclones compete for a limited ecological niche, as suggested by some authors [39]. Rather, each subclone grows independently, progressively colonizing the host, whose cells have lower fitness than all of the transformed cells, in order to create an expanding ecological niche.

Illustrative example of a nonstandard personalized medicine strategy

In this section, we compare the recommended treatment and results for the current personalized medicine strategy versus a nonstandard strategy in a single illustrative case. The case is computed using a quantitative model based on Figure 1.

In many cases, nonstandard and current personalized strategies may give similar recommendations. However, in some cases, nonstandard personalized medicine strategies may give counterintuitive recommendations. The case below illustrates a counterintuitive recommendation based on a general qualitative principle of nonstandard personalized cancer therapy. However, this is only one example, and other examples may reflect different underlying principles.

In this example (Figure 2), the patient presents with a single lesion of $10^6$ cells at the limit of visibility on a CT scan. The lesion is biopsied and assayed with a sensitive method that can detect one variant cell in $10^4$ cells. Thus, the assay method can detect a subclone of as small as $10^3$ cells in the lesion. The molecular test indicates that the lesion consists of pure S cells. In this example, S cells are more sensitive to drug 1 than to drug 2. In fact, they can be easily eradicated by drug 1, whereas drug 2 may shrink them slightly or may only slow their growth.

In Figure 2A, the current personalized medicine strategy and results are shown. The physician treats with drug 1, which is the best drug for the observed consensus state. This results in rapid reduction of the total tumor cell burden and the S cells. As the cell number falls below that which is observable on CT, the patient enjoys a complete response. However, unbeknownst to the physician or patient, a minor subclone of $10^5$ R cells exists at tenfold below the level of molecular detection on the original diagnostic assay. This subclone continues growing as it is unaffected by drug 1. In this example, R cells are also capable of rapidly acquiring resistance to drug 2, perhaps because they have a high degree of genetic instability or perhaps because there are many possible parallel mechanisms of drug 2 resistance acquisition. At approximately 13 months, the patient relapses with an apparently pure population of R cells. The patient receives drug 2, which is highly effective against R cells, and enjoys another complete response. However, while the R cells were permitted to survive, a fraction of them acquired resistance to drug 2. These doubly resistant $R_{1,2}$ cells grow out and cause an incurable relapse at month 28.

Figure 2. Example comparison of current and non-standard personalized medicine strategies. (See facing page). Illustrative case treated with (A) the current personalized medicine strategy and (B) a nonstandard personalized medicine strategy. The y-axes represent the log cell number and the x-axes represent time. The total cell population and the different subpopulations are represented by different colored lines, as shown in the keys (insets). Drug therapy is indicated by colored bars across the top of both graphs. A combination is indicated by both colored bars at half thickness. The limit of detection of disease on computed tomography and the smallest subclone that can be detected by molecular assay on biopsy are indicated by dashed horizontal lines. See the text for further details.

R: Cells resistant to drug 1 and sensitive to drug 2; $R_{1,2}$: Cells resistant to both drugs; $R_2$: Cells resistant to drug 2 and sensitive to drug 1; S: Sensitive cells.

Reproduced with permission from [35].
Nonstandard personalized medicine strategies for cancer may lead to improved patient outcomes

Perspective

A

Drug 1

Drug 2

Limit of radiologic detection

Limit of molecular detection on biopsy

B

Drug 1

Drug 2

Limit of radiologic detection

Limit of molecular detection on biopsy

A

Drug 1

Drug 2

Limit of radiologic detection

Limit of molecular detection on biopsy

B

Drug 1

Drug 2

Limit of radiologic detection

Limit of molecular detection on biopsy
In Figure 2B, the same situation is portrayed, but a nonstandard strategy is used. The strategy illustrates several broad principles of nonstandard personalized medicine. In this example, although the physician cannot directly observe R<sub>1</sub> cells, they consult bioinformatics knowledge sources, such as population studies, and find out that small R<sub>1</sub> subclones are relatively common in samples with apparent pure S samples and that these R<sub>1</sub> subclones have a tendency to rapidly acquire drug 2 resistance, leading to mortality. This illustrates the fact that nonstandard personalized medicine strategies must be highly knowledge based. It also illustrates the concept of working backwards from the states that are lethal, rather than forwards from diagnosis. The physician then performs a strategic analysis of the patient’s options, considering the risk of R<sub>1</sub> cells, even though they are not observable, and the future risk of R<sub>1-2</sub> cells. Considering the risk of unobservable or future states is another key attribute of nonstandard personalized medicine. Moreover, thinking several steps ahead to possible future states, similarly to an expert chess player, is also an attribute of nonstandard personalized medicine. Based on these considerations, the physician elects to treat with drug 2 – the inferior drug for S cells – for 4 months in order to eradicate a possible unobserved R<sub>1</sub> population, while watching the patient’s main tumor closely. The patient has stable disease overall, but the R<sub>1</sub> population is eradicated before any of its members can acquire double resistance. The doctor then prescribes an equal mixture of drugs 1 and 2 and cures the patient.

In this example, the optimal strategy was to give monotherapy of the less effective drug for the consensus molecular pattern of the tumor for 4 months, a highly counterintuitive approach. Note that initial combination therapy is an inferior strategy in this situation because the dose of drug 2 must be reduced, allowing the R<sub>1</sub> population to survive longer and so increasing the risk of it acquiring double resistance.

Of course, it is possible that there may be no unobserved R<sub>1</sub> population in some cases, or perhaps there may be an unobserved R<sub>2</sub> population. Whether initial combination therapy is superior or inferior overall, without knowing what unobserved subclones (if any) are present requires consideration of the outcome in all possible situations, given the measurements and the limits of their sensitivity and the probability of these different scenarios. Mathematically techniques such as game theory can address such questions.

A key principle of this example is illustrated in Figure 3. The dotted circle in Figure 3 encloses a region of the genetic subtypes that are treatable with available drugs. The large yellow circle represents the initial predominant state, which is the largest population on the diagnostic specimen. The circle is yellow because it merits concern. It is the major contributor to tumor bulk and may relate to important symptoms and survival risks. It is the state that is the focus of the current personalized medicine strategy.

However, the small red circle is the penultimate treatable state, which is the last treatable state before the tumor creates a cell outside the dotted circle of treatable states. Because it is genetically close to an untreatable ‘end state’ (black), it is a very-high-risk state and may merit greater attention than the initial predominant state. In short, the prevention of incurable, multiple-
resistant states may be a higher priority than the direct treatment of the bulk tumor in some instances.

**Generalizing the example**

The example above depicts a specific situation with particular growth rates, drug sensitivities, initial subpopulations and transition rates of genetic and epigenetic changes between the subpopulations. For this example to be meaningful, it must be generalizable across the many situations that are commonly encountered in oncology.

We sought to answer the following questions:

- How general are the benefits of the illustrative example?
- How great are the potential benefits of nonstandard personalized medicine?
- When is it important to focus on prevention of resistance as an even higher priority than treatment of the current tumor?

In order to investigate these questions, we performed a virtual clinical trial representing the two-drug system in Figure 1* in silico*. We examined 3 million different combinations of initial subpopulation structures and parameter values, including growth rates, transition rates and drug sensitivities. The ranges of these parameters were chosen in order to encompass the broadest ranges of possibilities as determined from the clinical and experimental literature [35]. For example, transition rates were varied over eight orders of magnitude, from very low genetic mutation rates at a single locus in cancer stem cells [40] to rates that might be associated with a mutator mutation in a somatic cell, with multiple parallel pathways to mutation. The overall trial was essentially a survey across possible conditions in oncology, a test of the generality of the usefulness of nonstandard personalized medicine. It should be noted that the simulation uses a minimal model incorporating only heritable variation and subclonal structures within tumors. A generalized survey of this nature incorporating all of the known complexities of cancer biology would not be computationally feasible.

The virtual trial assumed that information could be collected every 45 days regarding the cell numbers of all relevant subclones and that the growth rates and drug sensitivities of the subclones were known, as well as the transition rates between the subclones. This is not technically possible today, but may be approachable in the future.

No virtual patient began with incurable $R_{\text{1-2}}$ cells, since this would lead to mortality independent of strategy. All patients started with $10^9$ cells. Death was represented by $10^{13}$ total cells. Each of the 3 million sets of initial conditions and parameter values represented one ‘virtual patient’. Each virtual patient was treated with six different strategies:

- Strategy 0 was the current personalized medicine strategy. The patient was treated with the best drug or drug combination for their dominant subclone until experiencing total tumor growth or relapse and was then switched to the best therapy for the dominant subclone in the worsening or relapsed disease at that time;
- Other strategies employed the quantitative evolutionary model based on Figure 1 in order to predict the outcome at the next 45-day time point and involved the administration of therapy that minimized either the total cell burden or the risk of forming an incurable $R_{\text{1-2}}$ cell at the next 45-day time point:
  - Strategy 1 minimized the total cell population at the next 45-day time point;
  - Strategy 2.1 minimized the risk of forming an incurable cell if the total tumor burden was estimated to be less than $10^6$ cells (minimal residual disease) and otherwise minimized the total cell population;
  - Strategy 2.2 minimized the risk of forming an incurable cell if the total tumor burden was estimated to be less than $10^{11}$ cells (large disease burden) and otherwise minimized the total cell population;
  - Strategy 3 minimized the total cell population unless there was an immediate threat of forming an incurable cell, defined as an estimated non-zero incurable cell population at the next 45-day time point;
  - Strategy 4 estimated the most proximal threat by estimating the time to mortality from the increase in total cell numbers and the time to formation of an incurable cell and treated the most proximal threat.

The results are shown in Figure 4 and Table 1. The results from the current personalized medicine strategy are typical of end-stage metastatic cancer patients, validating the realism of the simulation. All of the nonstandard strategies performed similarly, and were markedly superior to the current personalized medicine strategy, doubling the mean and median survival and increasing the 5-year survival rate from less than 1% to 17–20%. Flattening of the Kaplan–Meier curves...
suggests that many of the patients surviving at 5 years will experience cure.

We note that some evolutionary theorists model subclones competing for a limited ecological niche, meaning that when one subclone is eradicated, the growth rate of the others is increased [39]. In those models, cure is not considered a possible outcome, and the strategies aspire to achieve stable disease by pitting one subclone against another. Because our model assumes all subclones are growing independently in an expanding ecological niche, cure is possible in our formulation.

Examining individual cases, we note that the average improvement is driven by a third of the virtual patients, approximately 1 million of the 3 million. For these patients, a dramatic benefit of nonstandard personalized medicine is realized. For the remaining two-thirds of these patients, current personalized medicine performs equivalently to nonstandard personalized medicine. Further investigation suggested that patients who benefited from nonstandard personalized medicine predominantly had either pre-existing heterogeneity within their tumors or some degree of genetic instability, or both. Other than these conditions, patient benefit was seen across the ranges of all of the other parameters, suggesting a general benefit across oncology. For example, significant relative benefit is seen whether the available drugs are marginally effective or highly effective. This suggests that improved strategies can be helpful in the current state of the art and can continue to be helpful as improved drugs are discovered.

Although the nonstandard personalized medicine strategies have similar average performance, they can be ranked based on the minority of patients for whom their performance differs. Table 1 lists the number of cases in which one strategy is significantly superior to all others. ‘Significantly’ is defined as at least a 2-month absolute improvement and 25% relative improvement in survival compared with the competing strategies. This definition of significant improvement mirrors the criteria for the approval of new therapies in demonstrating their superiority to existing therapies. It can be seen from Table 1 that in the majority of the cases where one strategy is significantly superior, that strategy is strategy 2.2. This strategy is similar to the strategy in the illustrative

Figure 4. Kaplan–Meier survival curves for the simulation of personalized medicine strategies over approximately 3 million virtual patients. The blue line represents the results for the current personalized medicine strategy. The other lines represent the results for five different nonstandard personalized medicine strategies, as shown in the key (inset). Reproduced with permission from [35].
example, in that the risk of developing incurable cells is minimized unless the patient has a large, immediately threatening total tumor burden, at which time attention is shifted treating the bulk tumor.

It is also notable that despite the similar average results of all of the nonstandard strategies, individual virtual patients differ in which strategy is optimal, and these differences can be significant (Table 1), indicating the need for individualization of strategies according to rules that are not yet fully understood. In the current work, we define the strategy piecewise in 45-day pieces. Ongoing work reveals that strategies that think further ahead require even more individualization on a per-patient basis.

Another interesting result is seen in Table 1. In 3 million virtual cases, encompassing initial conditions and parameter values spanning anything that is likely in oncology, not a single case was found in which the current personalized medicine strategy was significantly better. That is, if it were feasible to implement nonstandard personalized medicine, there would be no downside of using it instead of the current standard personalized medicine strategy.

### The role of combination therapy

A recent publication claims that combinations of at least three agents are required for the cure of cancer, based on theoretical estimates of the degree of genetic variability likely to be present in a malignancy [38]. The number of combination agents based on theoretical estimates of genetic variability can be reduced when the tumor burden is reduced [41]. These studies do not explicitly consider the likely need for dose reduction in combinations due to additive toxicities, which has provided arguments for sequential monotherapy in the past [37]. Targeted therapies are not free of toxicity, as many of them attack key pathways that are required for normal cells. While some targeted therapies may be given together at full doses, this is not the most common scenario.

The need for dose reduction in combination creates a strategic dilemma. We agree that at least three therapeutic agents will be needed in order to address the great plasticity of real tumors. Although our initial simulation has been conducted assuming the use of two drugs, we are simulating three drugs in order to address more complex subclonal structures in ongoing work. Furthermore, as we highlight below, ‘drug 1’ and ‘drug 2’ in our model may be combinations themselves, which are necessary in order to address nongenetic mechanisms of resistance within each genetically defined subclone. Within a single genetically defined subclone, it may be possible to take advantage of synergies between drugs in combination due to network biology. However, in some cases, the network dynamics may actually require sequential monotherapy in an ordered sequence rather than combination therapy [42]. If several agents are required for each subclone, one can imagine that a large menu of agents should optimally be available. However, whether these numerous targeted agents should always optimally be given in simultaneous combination is less clear.

We suspect that there is no single general answer for all cases, and the optimal use of combinations versus sequential monotherapy will depend on the initial conditions and parameter values governing

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### Table 1. Comparison of the treatment strategy results

<table>
<thead>
<tr>
<th>Result</th>
<th>Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Median survival(^\d) (weeks)</td>
<td>26</td>
</tr>
<tr>
<td>Mean survival(^\d) (weeks)</td>
<td>48.4</td>
</tr>
<tr>
<td>5-year survival (%)</td>
<td>0.7</td>
</tr>
<tr>
<td>Number of cases for which current strategy was numerically better than all others</td>
<td>1538</td>
</tr>
<tr>
<td>Number of cases for which current strategy was significantly(^\d) better than all others</td>
<td>0</td>
</tr>
<tr>
<td>Number of cases significantly(^\d) better than strategy 0</td>
<td>NA</td>
</tr>
<tr>
<td>Number of cases significantly(^\d) worse than strategy 0</td>
<td>NA</td>
</tr>
</tbody>
</table>

\(^1\) Performance in a virtual clinical trial of over 3 million evaluable cases. ‘Evaluable’ means that both drugs met minimal criteria for efficacy, providing strategic choices, and that the minimum survival of the worst strategy was ≤80% of the simulation length, allowing room for other strategies to demonstrate superiority.

\(^2\) Simulation truncated at 255 weeks, which is nearly 5 years and can be stored as 8 bits.

\(^3\) ‘Significantly better’ means at least 8 weeks’ absolute improvement and 25% relative improvement compared with the reference strategy, in analogy to the typical minimum improvement deemed clinically significant in randomized Phase III trials in cancer.

\(^4\) NA: Not applicable.

\(^5\) Reproduced with permission from [35].
the evolutionary dynamics. In some cases, complex sequences involving pulses of combination therapy interwoven with pulses of high-dose monotherapy will probably be optimal. In the illustrative example above, the optimal sequence was to treat with a pulse of monotherapy with ‘the wrong drug’ followed by combination therapy. Treating with the combination throughout was shown to be inferior in that instance.

Recreating the complexity of real tumor systems
The minimal model is intentionally simple in order to allow consideration of both a large number of scenarios and a large number of treatment sequences per scenario. In future applications, the model will be used in order to think multiple steps ahead, covering a large number of possibilities, especially when more than two drugs are involved. The core model is constructed so as to be easily customizable for certain details of specific cases, such as drug synergy and antagonism, as well as single-step acquisition of multistep drug resistance and sensitivity when these features are required by the case at hand.

Real malignancies feature a variety of additional complexities, including nongenetic mechanisms of resistance, cooperative and/or competitive interactions between tumor cells and between tumor cells and multiple subtypes within the stroma, different stromal microenvironments in different metastases that, in turn, differ from the primary, tumor cell kill by the immune system, tumor growth stimulation via inflammation and cancer stem cells and their relationship to metastasis and the epithelial–mesenchymal transition, among others. In real applications, the simple core model described herein will be fed complex information from multiple sources, thus representing the complexity via a network of interconnected models utilized in a modular fashion. These information sources will influence the parameter values, which will generally not be a single number, but rather a probability distribution. The system must be ‘knowledge based’ in that it makes use of such knowledge for real applications.

Representing the complexity with a parsimonious core model connected to other models in a modular fashion (rather than representing all of the known complexity in a single model) optimizes the likelihood of translating the approach into real clinical situations. First, many nongenetic resistance phenomena occur and reverse on shorter timescales and are not heritable. This means that they correspond to treatment decisions on a shorter timescale (within a single 45-day unit) and can naturally be optimized as a separate problem from longer-term issues of tumor evolution. To the extent that these phenomena are occurring on shorter timescales, they can contribute to probability distributions of parameters in the core model. Second, by having a modular structure of models, we can customize individual real applications and add knowledge only when it is available. For example, cooperative and competitive interactions between tumor cells and multiple cell types from the stroma are known to occur in some instances, but the details of this are typically unknown and unmeasurable in individual clinical cases. The addition of these complexities to the model when supporting experimental details are not available runs the danger of ‘overfitting’, in which a model has so many unknown and adjustable features that it can fit a dataset even when the model is false.

The drug sensitivity of a single genetic state may be influenced by the activity of nongenetic resistance mechanisms. Thus, when vemurafenib is used to treat colorectal cancer, BRAF inhibition leads to the upregulation of EGFR, resulting in upstream activation of RAS, as well as parallel activation of the PI3K pathway, both of which contribute to vemurafenib resistance. A similar upregulation of receptor tyrosine kinases occurs in response to the inhibition of AKT. Both resistance mechanisms are hard-wired and do not require genetic changes in order to be activated. Typically, nongenetic resistance mechanisms will occur – and also reverse – on a shorter timescale than the genetic and epigenetic changes that are considered in the model featured in this article. The current minimal model adjusts therapy every 45 days, but within each 45-day block, critical optimizations can be performed on a shorter timescale based on network biology, nongenetic resistance mechanisms and dormant nonproliferating states. When possible, ‘drug 1’ should be a combination or rapid sequence that is designed to address such resistance mechanisms if they are known. Thus, the effects of nongenetic resistance can be factored into the probability distribution of drug sensitivities for each given heritable state.

Another complexity affecting the results of therapy is uneven biodistribution of therapeutics, particularly therapeutic monoclonal antibodies, into tumor tissue. Tumor cells that are far enough away from the nearest blood vessel but within the diffusion range of oxygen and small-molecule nutrients may escape antibody therapy if the antibody does not distribute uniformly throughout the tumor tissue. In general, the exposure of different locations within the tumor to any type of therapy is heterogeneous, and this heterogeneity is further amplified by the heterogeneity of cell types within the tumor stroma. Again, given sufficient information, the sensitivity can be expressed as a probability distribution reflecting the possible different locations of the tumor cells.

The core model condenses greater complexity. For example, a limited number of phenotypic states of drug
Nonstandard personalized medicine strategies for cancer may lead to improved patient outcomes

Perspective

resistance and sensitivity each may correspond to many possible genetic and/or stable epigenetic states. Similarly, the transition rates are the sum of the rates of all possible genetic and epigenetic resistance mechanisms. For example, a number of mechanisms of resistance have been documented for crizotinib [52], and the total rate of resistance development would be the sum of the rates by all these different mechanisms.

In order to supply the information linking genetic and stable epigenetic states to phenotypic states of drug sensitivity and for estimating net growth and transition rates, a large number of possible sources can be used. These include direct measurements on tumor tissues ex vivo, the use of extensive cell line panels linking pharmacology to genetic and stable epigenetic states [53], the use of empirical databases of genetic and stable epigenetic characteristics of populations (ideally linked to clinical outcomes data) [54], the use of theoretical pathway knowledge in order to predict resistance mechanisms [55] and functional genomic screens [56]. Each of these information sources has challenges and limitations, which are beyond the scope of this article.

Required new technologies for the translation of nonstandard personalized medicine into the clinic

In this section, we will define some high-level needs of the nonstandard personalized medicine approach that create challenges for its immediate application and refer the reader to promising technologies that may enable these gaps to be filled in the future. This section is not meant to be comprehensive. We note that, in general, hematologic malignancies may be more technically amenable to this approach for initial proofs of concept.

Nonstandard personalized medicine places great demands on our ability to detect and isolate minority subclones. In this regard, the recent ability to reliably immortalize and culture patient samples is of great interest, although there could, in principle, be concerns that immortalization selects or favors certain subclones [57,58]. In addition, single-cell sequencing and single-cell PCR techniques are of interest [59]. Finally, a new technique called duplex DNA sequencing enables the accurate detection of mutations at targeted loci with as low a frequency as \(1/10^6\) in a mixture [60].

Repeated access to tumors will be essential in order to evaluate evolutionary dynamics optimally. Whereas this is clearly possible for hematologic tumors, it is more challenging for solid tumors. Circulating tumor cells (CTCs) have already been used for the early detection of known resistance mutations in non-small-cell lung cancer [61], and the ability to sequence single CTCs is approaching. The limited number of CTCs that are generally recovered, as well as the high cost of single-cell sequencing, both currently place limitations on the sensitivity of looking for rare subclones with this approach. CTCs may have the advantage of sampling from multiple lesions and therefore avoid the bias of sampling from only one lesion; however, the question concerning whether CTCs are representative of the actual tumor lesions themselves is still debated. Plasma DNA can be assayed for mutations that are likely to be present in the tumor [62]. The sensitivity for detecting rare mutations may be higher than that available for characterizing CTCs. However, in the current evolutionary dynamics model, the number of cells that simultaneously harbor multiple mutations is very important, and plasma DNA analysis cannot elucidate this. Imaging with specific molecular probes can noninvasively provide in situ molecular assessment [63]. Here, the limitations involve the limited sensitivity and spatial resolution for the detection of rare subclones, the cost and the limited ability to multiplex a large number of analytes.

Based on the above, it is clear that the optimization of therapy according to this paradigm will have to be attempted with incomplete, and sometimes noisy, experimental or clinical information. In many cases, as described above, probability distributions based on population data or other bioinformatics sources will have to be substituted for experimental or clinical measurements. The performance of nonstandard personalized medicine with incomplete information can be determined by simulations. Mathematical techniques, such as Markov Chain Monte Carlo [64] and machine learning algorithms [65], may improve performance with incomplete information. Game theory techniques may enable optimization of strategic therapy choices in the face of uncertainty [66].

Conclusion: key implications

There are four major differences between the current personalized medicine strategy for cancer and nonstandard personalized medicine strategies:

- Current personalized medicine focuses on the average molecular properties of a bulk sample. For nonstandard personalized medicine, minority subclones may be important;

- Current personalized medicine focuses primarily on the molecular state either in the present or at diagnosis. Nonstandard personalized medicine is particularly interested in the end states, the molecular states that may cause death and, in particular, in the ‘penultimate treatable states’, which are genetically close to the end states, but still treatable;
• Current personalized medicine attempts to optimize therapy one therapeutic maneuver at a time. Nonstandard personalized medicine tries to think several moves ahead, similarly to an expert chess player. This will be challenging given that we are still learning the thousands of pieces and moves involved in cancer treatment;

• Current personalized medicine applies mathematical optimization techniques to matching therapies to signatures. Nonstandard personalized medicine uses this information as basic input, but then attempts to achieve optimization over an entire treatment course.

The fundamental messages from this article are:

• The current strategy for the personalized therapy of cancer is not the only possible one;

• Genetic heterogeneity and evolutionary dynamics can greatly influence the optimal treatment strategy;

• Benefits can be highly significant across a broad range of tumor types and therapies;

• The systematic study of strategies for personalized medicine is an important new area for investigation.

Future perspective
Several changes to translational oncology may occur as a result of the adoption of non-standard personalized medicine strategies

Additional emphasis on the molecular analysis of rapid autopsies
Optimal strategy development requires thinking ahead all of the way to the end game, and sometimes working backwards from the end. Today, most of our molecular information is from diagnostic specimens. Instead, healthy people should be signed up for rapid autopsy in the event of cancer at the same time they are signed up for organ donation programs.

Mining ‘clinical pearls’ from simulations
‘Clinical pearls’ refers to valuable teaching lessons in medical education. The illustrative example presented herein teaches the oncologist to consider the prevention of resistance development as a greater priority at times than treating the bulk tumor. This illustrative example represents only one virtual patient in the strategy simulation. Approximately one million virtual patients benefited from non-standard strategies in the strategy simulation, and each virtual patient is different, corresponding to a different case. We suggest clustering these cases in order to find groups of analogous cases. Each cluster of similar cases and their optimal treatment sequences may represent its own unique clinical pearl.

The end of ‘lines of therapy’
During the American Civil War, many generals mounted massive frontal assaults against entrenched positions, resulting in massive casualties. However, other generals, such as Stonewall Jackson, applied more creative, fluid approaches in order to adapt to chaotic situations to their advantage, as demonstrated in his Shenandoah campaign of 1862. Prior to Jackson, Napoleon Bonaparte also benefited from flexible and adaptive strategies [67]. We see discrete lines of therapy in a similar light. Rather than a series of frontal assaults according to a fixed series of rules driven by simple matching to molecular patterns, therapy must be adaptive and proactive with a longer strategy horizon. Highly complex patterns of monotherapy and combination pulses of active agents will be individually designed for individual patients based not only on their malignancy’s static and predominant molecular pattern at diagnosis, but rather on the details of its subclonal structure and evolutionary dynamics. Lines of therapy may persist as the initial basis of regulatory approval (i.e. drug X is indicated for second-line non-small-cell lung cancer), but actual usage will be more complex.

A new field for study & optimization of therapeutic strategies
A new field will emerge involving the study of complex treatment strategies with available agents. These strategies will reach the level of sophistication that is currently devoted to complex games such as chess. Novel strategies will contribute as much to improved patient outcomes as novel agents, as well as illuminating priorities for novel targets based on mechanisms of resistance. In addition to the examples given herein, other creative proposed strategies have already emerged, including adaptive therapy for long-term stable disease maintenance [39], the ‘sucker’s gambit’ [68] and the ‘evolutionary double bind’ [69,70]. These strategies and others are based on differing assumptions from the model presented herein, and we project that different strategies will be optimal against different situations, providing further opportunities for strategy individualization.

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Nonstandard personalized medicine strategies for cancer may lead to improved patient outcomes

Executive summary

Background
- Tumors are genetically unstable because this is the most efficient way for them to evolve.
- Genetic instability leads to heterogeneity within a single individual’s tumor (a source of pre-existing therapeutic resistance) and evolutionary dynamics (a source of acquired resistance).

Nonstandard personalized medicine strategies
- A strategy is a data-driven method for planning a sequence of therapies. Strategies, similarly to therapies, should be individualized.
- The current personalized medicine approach is not the only possible strategy for planning targeted cancer treatment.
- Intratumoral heterogeneity and evolutionary dynamics greatly affect the optimal treatment plan.
- Nonstandard personalized treatment strategies of cancer, incorporating intratumoral heterogeneity and evolutionary dynamics, can lead to significantly increased survival and cure rates. The benefits are potentially very general across tumor and therapy types.

Future perspective
- The systematic study of strategies for planning cancer treatment as a function of subclonal structure and evolutionary dynamics is an important new field that could lead to improved patient outcomes. This article is not about the particular strategies presented, but rather about the beginning of a new field.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

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Complete study of the heterogeneity and branched evolution in a solid tumor, demonstrating heterogeneity within a single lesion, as well as convergent evolution.


Initial paper on nonstandard personalized medicine strategies for cancer.


Initial paper arguing for the importance of therapeutic combinations.


Initial paper arguing for the importance of serial high-dose monotherapy.


Nonstandard personalized medicine strategies for cancer may lead to improved patient outcomes

Perspective


** Example of using immortalized patient cells *ex vivo* to select optimal therapy.


** Presents a new sequencing method that can accurately detect one mutation among 10^6 wild-type DNA molecules.


** Demonstrates the use of circulating tumor cells for the real-time detection of genetically distinct tumor subclones.


