# Generalized Evolutionary Classifier for Evolutionary Guided Precision Medicine

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## ABSTRACT

**PURPOSE** Current precision medicine (CPM) matches patients to therapies using traditional biomarkers, but inevitably resistance develops. Dynamic precision medicine (DPM) is a new evolutionary guided precision medicine (EGPM) approach undergoing translational development. It tracks intratumoral genetic heterogeneity and evolutionary dynamics, adapts as frequently as every 6 weeks, plans proactively for future resistance development, and incorporates multiple therapeutic agents. Simulations indicated DPM can significantly improve long-term survival and cure rates in a cohort of 3 million virtual patients representing a variety of clinical scenarios. Given the cost and invasiveness of monitoring subclones frequently, we sought to determine the value of a short DPM window of only two 6-week adaptations (moves).

**METHODS** In a new simulation, nearly 3 million virtual patients, differing in DPM input parameters of initial subclone compositions, drug sensitivities, and growth and mutational kinetics, were simulated as previously described. Each virtual patient was treated with CPM, DPM, and DPM for two moves followed by CPM.

**RESULTS** The first two DPM moves provide similar average benefit to a 5-year, 40-move sequence in the full virtual population. If the first two moves are identical for DPM and CPM, patients will not benefit from DPM (65% negative predictive value). A patient subset (20%) in which 2-move DPM and 40-move DPM provide closely similar outcomes has extraordinary predicted benefit (hazard ratio of DPM/CPM 0.03).

**CONCLUSION** The first two DPM moves provide most of the clinical benefit of DPM, reducing the duration required for subclone monitoring. This also leads to an evolutionary classifier selecting patients who will benefit: those in whom DPM and CPM recommendations differ early. These advances bring DPM (and potentially other EGPM approaches) closer to potential clinical testing.

ACCOMPANYING CONTENT

Data Supplement

Visual Abstract

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## INTRODUCTION

In cancer, current precision medicine (CPM) matches therapies to static, consensus molecular patterns. Patients are treated with a therapy for as long as they are benefiting, and upon progression or relapse, the process is repeated with a different therapy. This approach has resulted in substantial patient benefits and is a major current direction in oncology. However, despite the benefits of this approach, duration of response is variable, and long-term remissions and cures remain elusive.

In this research paper, we report progress toward new approaches that, in the future, may significantly improve outcomes by preventing resistance development and moderate- to late-term relapse or progression. These methods rely on both intensive tracking of subclonal heterogeneity, and consider the rate of evolution to resistance and the likelihood of future states well in advance of their detection. We describe a novel biomarker classifier that enables us to select patients who can benefit most from this approach, as well as the minimum duration of costly and potentially invasive tracking required for benefit. Although our approach is currently undergoing laboratory testing, the work reported here will make future clinical studies feasible.

Cancers are constantly evolving subclonal heterogeneity. Ultradeep sequencing (20,000×) of colorectal cancers at diagnosis and a novel approach to modeling the evolution of very rare subclones indicate that any cancer containing enough cells to be detectable will have at least one cell with a resistance mutation to any single therapy. Furthermore, as

## CONTEXT

**Key Objective**

Current precision medicine (CPM) matches biomarker classifiers to therapies, but resistance inevitably limits long-term survival. We asked, using computer simulation, whether dynamic precision medicine (DPM), a highly adaptive and proactive evolutionary guided precision medicine (EGPM) paradigm under development, could benefit patients with only two 6-week treatment periods (moves), and still enhance survival by preventing late relapse or progression.

**Knowledge Generated**

Two DPM moves were highly effective, nearly as effective as 40 moves. Patients for whom DPM and CPM recommend the same treatment sequence for the first two moves will likely not benefit from DPM.

**Relevance**

A 2-move DPM paradigm is far more cost-effective and less invasive than a 40-move paradigm, and opens up the neoadjuvant period for future clinical studies. These findings establish a novel evolutionary classifier (EC) for selecting patients who will benefit from DPM. This approach to EC development may also work for other EGPM.

the cancer burden increases during a clinical course, cells may evolve that are simultaneously resistant to multiple elements of a therapeutic cocktail, at a rate substantially faster than previously anticipated.[1,2] Rare resistant cells may grow out relatively free of competition from other cancer cells (and thus more rapidly) in numerous micrometastases that are small enough to allow ready diffusion of oxygen and nutrients. Infiltration of major organs with metastatic disease, rather than growth of large primary lesions, generally leads to cancer mortality.[1,3]

Explicit consideration of subclonal heterogeneity and evolutionary dynamics may improve clinical results by preventing or slowing the evolution of resistance. Several strategies have been proposed for incorporating evolutionary dynamics in a personalized manner.[4-7] We term these evolutionary guided precision medicine (EGPM) strategies. A strategy is defined not as a particular therapy or regimen, but rather as a method for determining optimal therapy sequences on an individual basis.

Our work has been focused on dynamic precision medicine (DPM), a specialized EGPM that explicitly considers subclonal heterogeneity and dynamics, adapts very frequently, and proactively plans ahead on the basis of estimated risks of future events.[6] DPM, unlike earlier EGPMs, considers optimal sequencing of multiple non–cross-resistant therapies, rather than focusing on optimal scheduling of a single therapy. Many therapies are cross-resistant, and DPM requires that at least two of the therapies available be mutually non–cross-resistant. Extensive simulation has shown significant improvements in relapse prevention and a doubling of median survival.[6,8] Moreover, long-term survival and cure (all subclones eliminated) rates are significantly increased because of proactive planning for tumor evolution. Illustrative examples of cure are presented in detail.[6,8] Early resistance may be due to nongenetic plasticity including gene regulation or to preexisting mutations, whereas later relapses will increasingly be due to epigenetic and genetic subclonal evolution, the latter including not only mutations but also copy-number changes and other rearrangements. A more complex version of DPM integrating nongenetic plasticity with subclonal evolution has recently been developed[9]; however, this work uses the earlier, simpler version on the basis of genetic subclonal evolution.[6,8] Complex mathematical models may provide additional realism but may also provide greater challenges to both interpretation and clinical translation.[3] DPM is designed to allow introduction of additional complexity when possible, because DPM trajectories are calculated numerically and in short steps.[6] We have previously written about integration of DPM with other data sources when available, to account for complexities.[6,10]

DPM explicitly considers minority subclones and heritable genetic and epigenetic evolutionary dynamics, with the goal of maximizing survival by balancing treating current disease and preventing refractory disease relapse or progression, the latter accomplished by eliminating small singly resistant subclones before they can evolve sub-subclonal variants simultaneously resistant to multiple non–cross-resistant agents. DPM predicts that hypermutator subclones with a higher mutation rate will arise because of random mutations in proteins responsible for genome integrity, such as DNA replication and repair proteins.[6,11] These hypermutator subclones can rapidly evolve cells that are simultaneously resistant to all the agents in a therapeutic cocktail, and are a particular priority for early elimination. The DPM prediction that hypermutator subclones will be enriched in resistant samples has been verified using a fluorescent reporter assay for hypermutators as well as ultradeep DNA sequencing.[12] As a result, DPM will often recommend brief periods of prioritizing elimination of rare hypermutator subclones versus debulking the tumor.

DPM changes therapy very frequently and plans ahead for potential future evolution of rare hypermutator subclones and others using an adaptive evolutionary model to predict the optimal treatment regimen within a short adaptation window—for example, 6 weeks (equivalent to two typical 3-week chemotherapy cycles), although the length of this window can be adjusted. We term each therapy adaptation a move in analogy to chess. Frequent changes in therapy are a hallmark of DPM because this minimizes the constant, predictable selection pressure from treatment with a sustained single or combination therapy until progression or relapse. Instead, frequent adaptation complicates evolutionary pathways to multiple resistance.[13] DPM considers the probabilities of distant outcomes up to 5 years in the future when determining each move, and may be superior at prioritizing these longer timescales of interest versus other methods that focus on short-term outcomes such as tumor shrinkage.[8]

After each move, DPM assesses its input parameters (detectable subclones and their growth rates, genetic stability/instability, and drug sensitivities to drugs of interest) and determines the optimal next move in a personalized fashion, according to one of several DPM strategies, all of which balance immediate benefit (cytoreduction) and longer-term benefit (resistance prevention). For example, if there are two non–cross-resistant drugs (each drug being a single agent or a synergistic combination), DPM will choose one of three options at each move: full dose drug 1, full dose drug 2, or a simultaneous combination of drugs 1 and 2, possibly at a reduced dose as determined by clinical safety studies. DPM will not always choose simultaneous combinations, and we have shown that even when DPM is restricted to monotherapies alone, it is superior to simultaneous combinations employed using CPM, as in DPM, both drugs 1 and 2 can achieve early and consistent exposure because of frequent adaptation, for example, by interdigitation of short monotherapy courses.[8]

The most successful 40-move DPM strategy, also used in the current study of 2-move DPM, always chooses the option that minimizes the chance of evolving a cell that is simultaneously resistant to the two non–cross-resistant drugs through independent mutations, unless the total cancer burden exceeds a preset threshold, in which case the option that maximizes cytoreduction is prioritized. In the simulation, the cancer burden threshold was set at an optimal fixed value, but in practice, the oncologist would determine when additional cytoreduction needed to be urgently prioritized on the basis of the detailed clinical situation.[6]

In an extensive simulation of 3 million virtual patients, where each patient represented a unique starting state of prevalence of sensitive and resistant subclones, and a unique set of parameters including net growth rates, phenotypic transition rates of each subclone between sensitivity and resistance to two non–cross-resistant therapies, and sensitivity and resistance values, 40-move DPM doubled median survival compared with CPM-targeted therapy directed against the largest subclone, over a wide range of scenarios encompassing literature and clinical experience across oncology.[6]

DPM is designed to use approved drugs at approved dosages both singly and in simultaneous combination as determined to be safe by previous clinical studies. Therefore, it is not expected to increase acute toxicity. Because it does not treat with the same drug for long periods, it may lessen cumulative toxicities, but this can only be determined by careful future clinical studies.

We provide, in Table 1 and in the Data Supplement, a description of the DPM model: assumptions, robustness to measurement errors in input parameters, and emerging experimental evidence.

DPM in principle requires high-resolution data on subclonal composition at multiple time points, potentially leading to a high-cost paradigm, as well as one that is invasive, given that sensitivity for rare subclones in liquid biopsies might be limited. Hence, we asked whether two 6-week moves of DPM provided patient benefit, and how this might compare to the benefit from 40 moves over a 5-year period.

Moreover, the average benefit of 40-move DPM in the previous simulation was driven by 31% of the virtual patients who experienced significant benefit while the other 69% of patients had equivalent outcomes on CPM and DPM.[6] Hence, it is important to identify the subset of patients who will benefit from DPM to facilitate future clinical testing. This manuscript also concerns the development of an evolutionary classifier (EC) to select this important patient subset. In principle, this could be accomplished by direct calculation using the DPM equations over an entire clinical course, but that approach may not be as robust in clinical situations in which the available data may be incomplete compared with ideal DPM input data, particularly if data are available only at a small number of early time points.

DPM is one of many EGPM approaches under development by various investigators; a subset is discussed in the studies by Gatenby et al,[4] Chmielecki et al,[5] Beckman et al,[6] Leder et al,[7] and Yeang et al.[8] DPM is a parsimonious model, with simplifying assumptions (Table 1). No mathematical model available today can capture the full complexity of cancer. Increasingly complex models can provide additional realism, but often at the cost of decreased interpretability and translational feasibility, and at times decreased generalizability.[3] We note that CPM, although far simpler than DPM, has demonstrated clinical utility. DPM is designed to further extend that clinical utility by selectively adding additional features.

Our methods may be readily generalizable to other EGPM approaches that might require detailed molecular data at multiple time points and are cost-intensive and potentially

**TABLE 1.** Assumptions of the DPM Model

| Assumption | Comment | Impact |
|---|---|---|
| Considers only irreversible genetic alterations | A DPM model incorporating both genetic alterations and nongenetic resistance mechanisms is in review.[9] Genetic alterations include mutations, insertions, translocations, deletions, and amplifications | Having more mechanisms of resistance is a greater challenge, but the optimal treatment sequence in the presence of reversible genetic plasticity and irreversible genetic alterations (the DPM-J model) has a DPM backbone, and retains a substantial improvement over CPM.[9] To be published separately |
| Does not include TME | This knowledge may be incorporated if the TME's effect on input parameters is known and the patient's TME is characterized | Incorporation of TME effects is a pervasive challenge for CPM as well as for all EGPM approaches, including DPM. TME effects vary widely, even between microniches within individual patients. Impact could be assessed and DPM adjusted if and when the conditions described in the Comment column are fulfilled |
| Subclones grow independently of each other | Easily adaptable if influence of subclones on each other's growth is known. Cases are known where the subclones compete with each other, grow independently, have a host-parasite relationship, or benefit from mutual interactions. These dynamic interactions may also depend on the relative prevalence of subclones, lesion size, and drug therapy. Within radiologically visible lesions, cells often compete for nutrients. However, micrometastases, which are predominant causes of morbidity and mortality, are well nourished via diffusion[3] | DPM will be preferred to CPM if subclones cooperate with each other, since DPM reduces diversity, including minor subclones, more effectively than other methods, thereby limiting opportunities for cooperation. The case of independent growth has been extensively modeled, showing a robust advantage for DPM. If subclones compete with each other, adaptive therapy,[4] which exploits competition between subclones, may be preferred. However, it is likely that multiple interaction patterns may coexist within the same patient. Moreover, independent growth and subclonal cooperation are, in our opinion, more likely to present clinical problems, and addressing them may be more relevant[3] |
| At least two non–cross-resistant drugs exist | Although many drugs exhibit cross resistance, this is not always the case. For example, in double-positive breast cancer, we have shown that selection for resistance to anti-ER therapy does not lead to anti-HER2 resistance, and vice versa.[14] Moreover, several leading anti-HER2 therapies are monoclonal antibodies working at the cell surface, and not subject to small molecule drug efflux pumps that often cause cross-resistance | Drugs that exhibit a degree of cross-resistance may do so because they have overlapping mechanisms of action or because they are subject to removal from the cell by small molecule efflux pumps. Many chemotherapies show varying degrees of cross-resistance. DPM will not be effective if available drugs are fully cross-resistant to each other. Drugs less likely to be cross-resistant are those targeting distinct cellular processes and/or having nonoverlapping biodistribution mechanisms. This will encourage combining antibody therapies, antibody-drug conjugates, and biologic agents with conventional small molecule drugs. We have provided an example herein. Continued drug development will improve options |
| Drug is either a single agent or simultaneous combination of agents designed to eliminate a given class of subclones, and agents within a single drug may be cross-resistant | Oncologists must nominate at least two non–cross-resistant drugs for any desired application. DPM then gives a personalized recommendation for the optimal sequence of using these drugs, proactively playing out scenarios concerning future evolution | DPM benefits from the participation of drug developers to provide options and oncology experts to select drugs for specific applications. This assumption defines to clarify the process for achieving optimal results. It contrasts with CPM, where combinations are often directed at a single subclone |
| Only effective in patients who do not already possess cells with independent genetic resistance to both drugs | The more non–cross-resistant drugs can be identified, the lesser the chance that this will occur.[2] DPM has also been simulated with three non–cross-resistant drugs[8] | Previous work[2] suggests that cells simultaneously resistant to multiple non–cross-resistant drugs may exist in some patients but certainly not all. No current method is likely to be helpful if cells simultaneously resistant to all available therapies are preexisting. The likelihood of this problem occurring is discussed in the study by Loeb et al,[2] where we outline the risk of this problem as a function of several variables, including the number of non–cross-resistant therapies available. This challenge helps define priorities for future drug development, emphasizing the greater value of experimental drugs that are non–cross-resistant with preexisting ones |

(continued on following page)

**TABLE 1.** Assumptions of the DPM Model (continued)

| Assumption | Comment | Impact |
|---|---|---|
| Giving more than one non–cross-resistant drug simultaneously requires dose reduction due to toxicity | This is often but not always true. If full doses of all agents can be safely given simultaneously, DPM may recommend that. The recently developed joint DPM model, DPM-J[9] (not discussed in this work), accounting for both reversible nongenetic plasticity and genetic subclonal evolution would give more nuanced recommendations than the original DPM model[6] in the setting where simultaneous full dose therapy is feasible. In DPM-J, alternation of drugs 1 and drug 2 may in some instances be recommended to allow reversal of resistance to the drugs occurring due to nongenetic plasticity | Given that the degree of diversity encountered in cancers is far greater than previously anticipated,[2] it is unlikely that simultaneous dosing of all potentially useful drugs at full dose will be feasible. In rare cases where this possible, the recommendation of DPM will be to give the simultaneous combination, a recommendation likely corresponding to the standard of care. However, DPM-J[9] may still give novel recommendations, as outlined under the Comment section on the left |
| Allows for differences in mutation rates among subclones | A unique feature of DPM that is considered important and somewhat inevitable, given the large number of sites in the genome responsible for maintaining its stability. We have shown, both theoretically[11] and experimentally (unpublished), that hypermutator subclones are selected when we select for resistance | If a subclone is a hypermutator subclone and this is not detected, the priority of eliminating it could be underestimated. An ex vivo assay for detecting hypermutator subclones is under development (unpublished) |

NOTE. The DPM model has a variety of simplifying assumptions, but can readily be adapted to include additional complexity if the requisite knowledge and patient data are available. This is because each step is calculated numerically for short time intervals, and these numerical calculations can be done with any desired complex mathematics, if desired, subject to minor limitations. See text and the study by Beckman et al[3] for a discussion of the pros and cons of parsimonious versus complex mathematical models.
Abbreviations: CPM, current precision medicine; DPM, dynamic precision medicine; DPM-J, dynamic precision medicine, joint model; EGPM, evolutionary guided precision medicine; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; TME, tumor microenvironment.

invasive. Simulation methods will differ among EGPMs, and likely the minimum duration required for full benefit may vary. Nonetheless, our approach to deriving an EC, when adapted to other EGPMs, may facilitate clinical testing of that EGPM, by defining a simulation method for selecting patients who will benefit, and determining when to start and stop the EGPM. In short, an EGPM has delivered its benefit when a patient, predicted to benefit, has their cancer converted to a state where no further benefit is likely. This occurs when the therapeutic recommendation of the EGPM model no longer diverges from the CPM recommendation.

In this study, we have shown that 2-move DPM provides benefit nearly equivalent to 40-move DPM, thereby improving the cost-effectiveness and feasibility of DPM. We have also developed an EC that enriches for patients who will benefit from both 2-move and 40-move DPM.

## METHODS

### Clinical Evaluation of DPM

Clinical testing of EGPM faces several hurdles that may affect the ability to get complete input data sets.[6] Highly sensitive and specific noninvasive assays to detect rare subclones and predict their drug sensitivity and resistance properties and evolutionary dynamics are ideally required. However, these concerns are out of the scope of this manuscript, which instead focuses on selecting patients assuming the relevant input parameters are (to some degree) available, and thus enhancing the efficiency of clinical studies to evaluate the merit of EGPM. The EC developed in this work may be more robust in settings where data are available at early time points only, compared with direct computation using the DPM equations.

Clinical studies of highly adaptive EGPM algorithms that consider multiple therapies face a challenge. Highly adaptive EGPM presents a very large decision tree of therapy sequences, which will be assigned to patients individually on the basis of their initial state and evolutionary dynamic parameters,[8] and will, in real applications, need periodic reassignment if future states diverge from predictions. In DPM, which takes into account possible need for dose reduction in simultaneous combinations, if there are two partially non–cross-resistant therapies A and B, there are three options at each move: full dose A, full dose B, and a simultaneous combination of A and B at reduced dose. We note a therapy may itself be a combination of drugs. A therapy is defined as a drug or group of drugs targeted against a particular subclone. Because of frequent adaptation, both A and B can be delivered at high intensity early on, even if administered as monotherapy pulses. The number of possible paths if two therapies are considered over n moves is thus $3^n$. If we consider only two moves, or a 12-week window, there are nine sequences to consider.

### EC

In contrast to a conventional biomarker classifier, which informs a matching between patients and optimal therapies, we seek to develop an EC that will match patients to strategic

algorithms for computing optimal treatment sequences. Moreover, the classifier matches not just on the basis of static molecular properties but also on the basis of dynamic properties such as growth and mutation rates that may predict future states. To support the translation of DPM into the clinic, we note the EC must now perform two classifications, in itself unique for a biomarker classifier. EC1 must first identify patients most likely to benefit from DPM, who will then be the trial participants. EC2 must then sort patients selected by EC1 into optimal therapy sequences. Figure 1 shows how these two classifiers might function together in the context of a window pilot study in the neoadjuvant setting in estrogen receptor (ER)/human epidermal growth factor receptor 2 (HER2) double-positive breast cancer. Double-positive breast cancer is a strong potential use case for DPM: endocrine (anti-ER) and HER2-directed therapies are non–cross-resistant[14]; and these agents are safe and well tolerated when administered alone, in combination with each other, or even in a triple combination, with dose reduction in some patients, with other drugs such as CDK4/6 inhibitors.[15-17]

DPM simulations were performed on nearly 3 million virtual patients comprising different combinations of input parameters as described in the introduction.[6] A system of ordinary differential equations on the basis of the evolutionary model was integrated piecewise to simulate clinical time courses, and DPM and CPM recommendations for each move defined,[6] and used to determine EC1 and EC2.[6] The input parameters for DPM are the prevalence in the cancer of various subclones, their respective growth and mutation rates, and their respective drug sensitivities to the drugs of interest given individually or in combinations at approved dosages and schedules. The DPM differential equations compute expected total and subclonal cell numbers at the next observation point from available therapy options given at the current observation point, and heuristic strategies then decide which of these outcomes are most desirable, leading to a therapeutic choice. DPM chooses the therapy that maximizes prevention of the birth of multiply resistant cells at the next observation point unless the total cancer burden exceeds a predetermined threshold, whereupon overall cytoreduction is prioritized. In practice, determining when it is critical to prioritize overall cytoreduction would rely on the judgment of the treating physician. CPM mirrors current precision medicine by focusing on maximizing personalized cytoreduction, giving therapy directed at the most prevalent subclone, and maintaining this therapy until cancer relapse or progression. These strategies are described in detail in the Data Supplement. Death occurs in the simulation when cell numbers exceed a second predetermined threshold.[6]

Simulation time is 5 years in simulated time. Virtual patients predicted to have >4 years survival on CPM are excluded to permit observation of a 25% increase in survival if achieved. Virtual patients with <6 weeks' survival time on CPM are excluded, a slight difference from the simulation previously

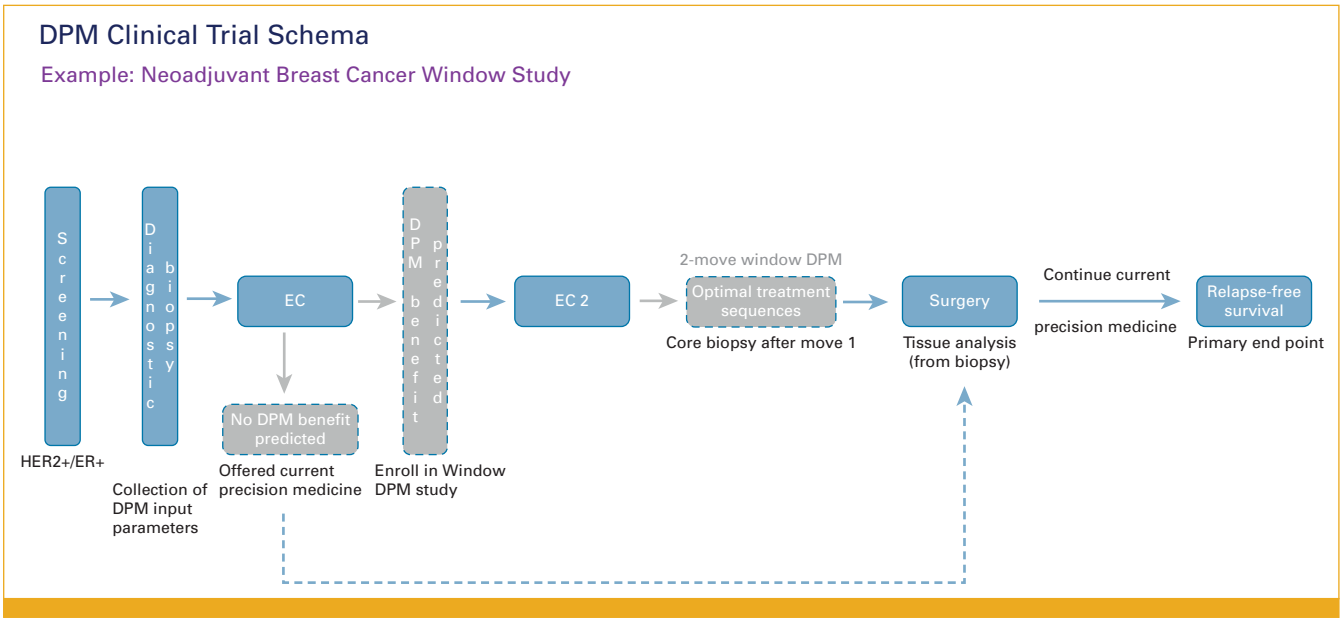
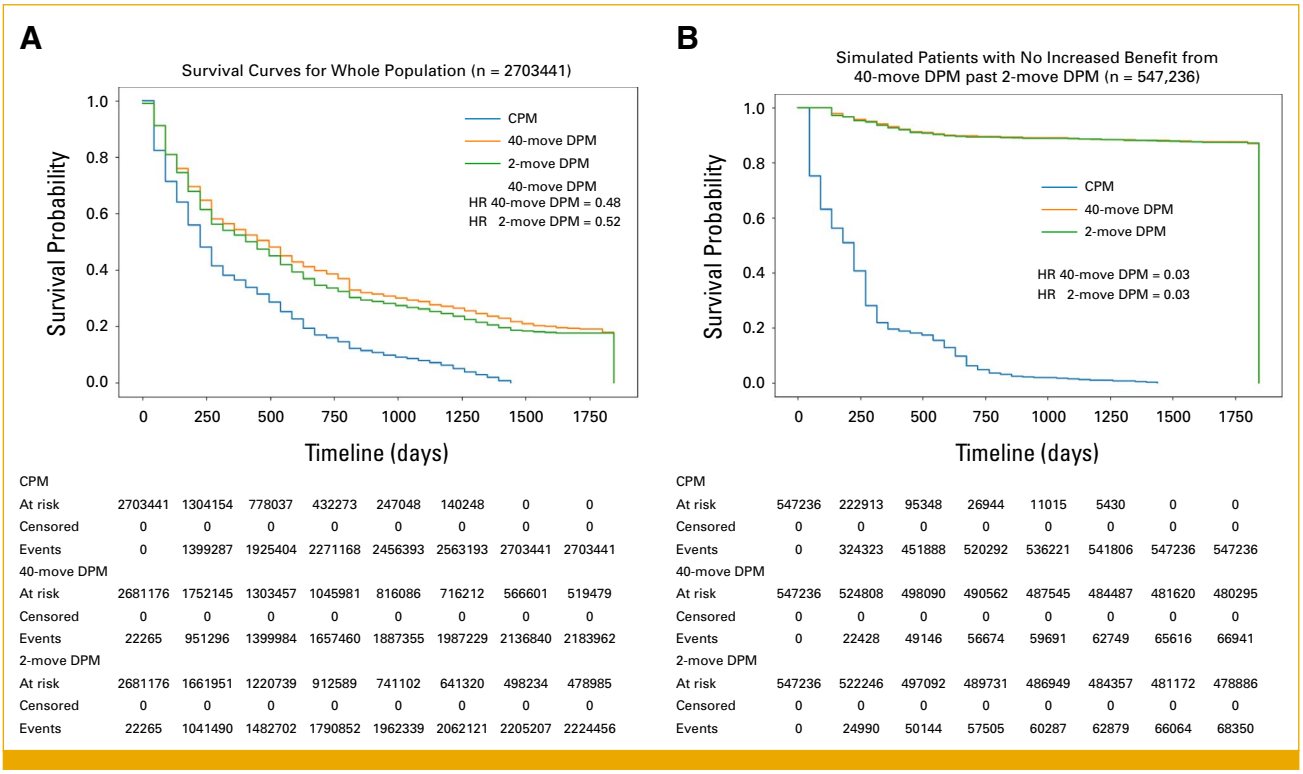



**FIG 1.** Vision of an evolutionary window pilot clinical trial using novel mathematical and computational tools of an EC. Within the neoadjuvant setting, patients with breast cancer are screened for double HER2- and ER-positive status. A diagnostic biopsy collects the individual DPM input parameters required for EC1 predictions. Patients expected to benefit from DPM are enrolled in a 2-move window trial. EC2 assigns patients to the evolutionary treatment sequence over the two moves predicted to be optimal. Core biopsy is performed at the end of move 1, and tissue is again collected for analysis at surgery. Patients continue on CPM after surgery. Patients who are not predicted to benefit from DPM will simply receive CPM before and after surgery. The primary end point will be relapse-free survival in a high-risk group. Subsequent clinical development steps (not shown) would contain randomization between DPM and CPM. CPM, current precision medicine; DPM, dynamic precision medicine; EC, evolutionary classifier; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2.




| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| CPM | | | | | | | | |
| At risk | 2703441 | 1304154 | 778037 | 432273 | 247048 | 140248 | 0 | 0 |
| Censored | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Events | 0 | 1399287 | 1925404 | 2271168 | 2456393 | 2563193 | 2703441 | 2703441 |
| 40-move DPM | | | | | | | | |
| At risk | 2681176 | 1752145 | 1303457 | 1045981 | 816086 | 716212 | 566601 | 519479 |
| Censored | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Events | 22265 | 951296 | 1399984 | 1657460 | 1887355 | 1987229 | 2136840 | 2183962 |
| 2-move DPM | | | | | | | | |
| At risk | 2681176 | 1661951 | 1220739 | 912589 | 741102 | 641320 | 498234 | 478985 |
| Censored | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Events | 22265 | 1041490 | 1482702 | 1790852 | 1962339 | 2062121 | 2205207 | 2224456 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| CPM | | | | | | | | |
| At risk | 547236 | 222913 | 95348 | 26944 | 11015 | 5430 | 0 | 0 |
| Censored | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Events | 0 | 324323 | 451888 | 520292 | 536221 | 541806 | 547236 | 547236 |
| 40-move DPM | | | | | | | | |
| At risk | 547236 | 524808 | 498090 | 490562 | 487545 | 484487 | 481620 | 480295 |
| Censored | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Events | 0 | 22428 | 49146 | 56674 | 59691 | 62749 | 65616 | 66941 |
| 2-move DPM | | | | | | | | |
| At risk | 547236 | 522246 | 497092 | 489731 | 486949 | 484357 | 481172 | 478886 |
| Censored | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Events | 0 | 24990 | 50144 | 57505 | 60287 | 62879 | 66064 | 68350 |

**FIG 2.** (A) Kaplan-Meier plot of simulated survival, which is increased over the standard therapeutic sequence (blue) when following the strategy defined by DPM for the entire clinical course (40-move DPM, orange) or the first two moves only (2-move DPM simulation, green). Benefits from 2-move DPM and 40-move DPM are similar, as shown by the curves and by the HRs. (B) Kaplan-Meier plot of those virtual patients predicted to have similar survival (within 25% relative and 60 days absolute) between 2-move DPM and 40-move DPM. This group of patients, 20% of those from the original population, experience substantial benefit from either 2-move DPM or 40-move DPM. Note *P* values in figures A and B (not shown) are highly significant, but with such large sample sizes, *P* values would be significant even with small differences. Similarly, 95% CIs would be extremely tight because of the large number of virtual patients, not truly reflecting the total uncertainty inherent in such an analysis. CPM, current precision medicine; DPM, dynamic precision medicine; HR, hazard ratio.

reported.[6] Drug 1 is by convention the more effective drug on sensitive cells, and the sensitivity is constrained such that full dose drug 1 and full dose drug 2 must inhibit growth of sensitive cells by at least 20%, which we define as the minimum threshold for effectiveness, as in the scenario for 40-move DPM previously reported.[6]

Kaplan-Meier analyses and calculation of EC1 statistical properties were performed using the survival analysis python library, lifelines.[18]

The simulation and analysis code can be found at GitHub[19] and the results can be downloaded at Zenodo.[20]

## RESULTS

### Nearly Equal Benefit From 40-Move DPM and 2-Move DPM

To consider a window study design in which DPM is given only within a 2-move window, we must determine the benefit conferred by only two moves of DPM. Figure 2A shows the surprising finding that the first two moves of DPM confer most of the benefit of 40-move DPM. In the entire simulation, 40 moves of DPM confers a hazard ratio (HR; DPM/CPM) of 0.48 and two moves of DPM confers a HR of 0.52.

### Extraordinary Benefit for Virtual Patients Whose Benefit From 40-Move DPM and 2-Move DPM Are Closely Equal

Figure 2B shows the benefit predicted for those virtual patients whose survival on 2-move DPM and 40-move DPM differs by <25% relative and <2 months absolute. These patients, representing 20% of the original population, experience an HR of 0.03.

### Construction of EC1 for 2-Move DPM and Its Performance

A patient was defined as benefitting from DPM in simulation if DPM provided at least 25% relative and 2-month absolute survival advantage compared with CPM. In the subpopulation that benefits from 2-move DPM (22.5%), nearly all the DPM recommendations diverged in the first treatment 6-week treatment period regardless of the same drug selection in the second window (94%). Therefore, we hypothesized that move 1 would have the greatest influence predicting

**TABLE 2.** Statistical Performance of an Initial Heuristic EC1 for Predicting Patients Who Will Benefit From 2-Move DPM on the Basis of Differences Between DPM's and CPM's Recommended Sequence of Two Drugs or a Combination for the First Move

| | EC | |
|---|---|---|
| Metric | No Benefit | Benefit |
| Simulation | | |
| No benefit | 1,146,289 | 949,830 |
| Benefit | 34,081 | 573,241 |

| | |
|---|---|
| Accuracy | 0.63 |
| Precision (PPV) | 0.38 |
| NPV | 0.97 |
| Sensitivity (positive recall) | 0.94 |
| Specificity (negative recall) | 0.55 |
| F1 | 0.54 |

NOTE. Benefit is defined by an overall increase in survival by at least 25%, and at least 60 days as a result of using DPM recommendations during the 2-move trial period compared with using the CPM treatment selections.

Abbreviations: CPM, current precision medicine; DPM, dynamic precision medicine; EC, evolutionary classifier; NPV, negative predictive value; PPV, positive predictive value.

those that will benefit from 2-move DPM. A patient was labeled by EC1 as potentially benefitting from the 2-move DPM if recommendations from DPM and CPM diverged in move 1, irrespective of the status of move 2. EC1 selects 56.3% of virtual patients as possibly benefiting from 2-move DPM, and 37.6% of this group benefits from 2-move DPM. Moreover, among the groups that are selected by EC1 and benefited, approximately 90% were in the extraordinary benefit group depicted in Figure 2. The odds ratio associated with EC1 is 20.3 (95% CI, 20.1 to 20.5), and the additional performance metrics are provided in Table 2.

### Predictive Classifier for 40-Move DPM

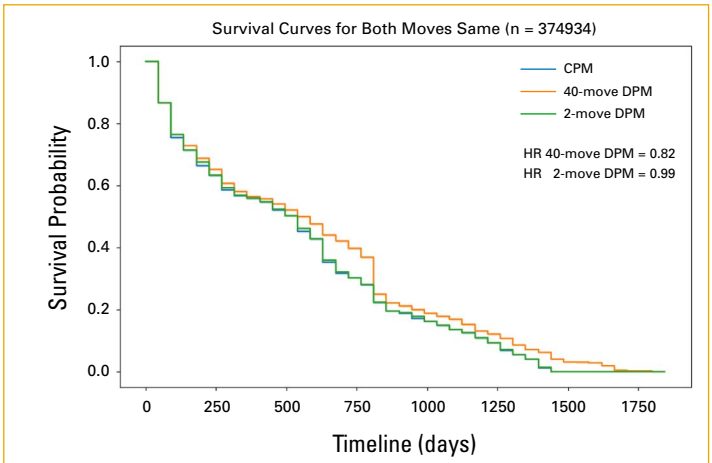
Among the 855,055 virtual patients (32% of total population) benefiting from 40-move DPM by simulation, 82% differ on recommended move 1 between DPM and CPM. We hypothesized that 40-move DPM would not be beneficial compared with CPM in patients with specific parameter configurations where the recommendations for both moves 1 and 2 were identical. We then considered this as a more precise criterion for predicting patients benefitting from the 40-move DPM. Figure 3 shows Kaplan-Meier curves of three treatment strategies applied to this subpopulation (14%)—CPM, 40-move DPM (applying the DPM algorithm over the full 40-move course), and 2-move DPM (applying DPM to the first two moves and reverting to CPM afterward)—no clinically significant advantage of DPM is seen under these conditions, and a classifier built on the basis of the first two moves had a negative predictive value of 92% for the entire population. The complementary population (86% of the full population), in which the recommendations differed between DPM and CPM in at least one of the first two moves, benefited from 40-move DPM (HR DPM:CPM 0.44). Note that *P* values are not shown in this research paper since the sample size in the virtual trial is extremely large, conferring statistically significant *P* values even in the face of clinically insignificant differences.

## DISCUSSION

A major goal of this work was to identify the subset of patients who are likely to benefit from DPM, so that future clinical trials may be conducted in an enriched population. Previously, biomarker classifiers have been used to categorize patients and match them to therapy according to CPM. These classifiers have consisted of a single biomarker such as a mutation, or a panel of biomarkers such as a gene expression profile, involving categorical or continuous variables, the latter converted to categorical by cutoffs.[21-25] We have developed a novel EC to determine if patients will benefit from 2-move DPM (EC1) and to match patients to individualized therapy sequences (EC2) of a predesignated set of drugs according to a method based on initial subclonal state variables (prevalence in the cancer, relevant drug sensitivities) and evolutionary dynamics parameters (subclonal growth rates and subclonal mutation rates).[6] The EC input values are thus unique, related in a complex way by a system of differential equations,[6] and deployed for unique purposes compared with previous biomarker classifiers.

Populations can be enriched for 2-move DPM benefit on the basis of comparison of therapy recommendations by DPM and CPM in the initial move. Moreover, 2-move DPM provides an average benefit similar to 40-move DPM, and patients for whom DPM does not differ in its recommendations in the first two moves from CPM have limited benefit from 40-move DPM. It is striking that this is true for DPM, which has the ability to plan each move against a future horizon far longer than the proposed window, that is, years.[8]

From the point of view of EGPM approaches, our results raise the question of how general this high-level approach to classifying patients can be. Can patients who will benefit from other EGPM approaches be selected on the basis of whether the recommended treatment differs from standard of care in an initial treatment window, and what in a given model determines the required length of this window? One might imagine, in patients who benefit equally from a small number of EGPM-directed moves and more extended EGPM, that the initial moves convert the patient's cancer to a state similar to the initial state of a patient who cannot benefit. Once these states precluding further benefit are identified, periodic checks can in principle be instituted to govern restarting of an EGPM as needed, again optimizing cost-effectiveness. Although details will differ for each EGPM, the high-level principle may be general.




| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| CPM | | | | | | | | |
| At risk | 374934 | 237719 | 189043 | 114015 | 61233 | 35493 | 0 | 0 |
| Censored | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Events | 0 | 137215 | 185891 | 260919 | 313701 | 339441 | 374934 | 374934 |
| 40-move DPM | | | | | | | | |
| At risk | 374934 | 244714 | 195905 | 149447 | 71318 | 45679 | 12461 | 1020 |
| Censored | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Events | 0 | 130220 | 179029 | 225487 | 303616 | 329255 | 362473 | 373914 |
| 2-move DPM | | | | | | | | |
| At risk | 374934 | 237978 | 189094 | 114131 | 61369 | 35688 | 115 | 115 |
| Censored | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Events | 0 | 136956 | 185840 | 260803 | 313565 | 339246 | 374819 | 374819 |

**FIG 3.** Kaplan-Meier Plot for a simulated trial of DPM, where three treatment strategies were compared. CPM (blue) recommendations are based on the therapy that provides the greatest response to the largest population of tumor cells, and the 40-move DPM (orange) recommendations minimize the emergence of multiple drug resistance at the expense of overall tumor shrinkage. The 2-move DPM (green) treatment strategy used the DPM recommendations for the first two therapeutic interventions before reverting to CPM. The resulting populations can be grouped on the basis of comparing the recommendations of DPM and CPM. Here we show that if DPM and CPM give identical recommendations for moves 1 and 2, there is little to no benefit of DPM. If only move 2 differs, 40-move DPM gives a clinically significant benefit (HR, 0.75), but 2-move DPM does not (HR, 0.84; data not shown). Note 95% CIs (not shown) are tight because of the large number of virtual patients, but this may not truly reflect the total uncertainty inherent in such an analysis. CPM, current precision medicine; DPM, dynamic precision medicine; HR, hazard ratio.

Future work is necessary to more deeply understand these findings. We will determine whether this process can in fact be used to stop and restart DPM. We will create clusters of the very large simulation population on the basis of recommended moves, initial state variables, and input parameters, and analyze representatives of each cluster to elucidate underlying evolutionary mechanisms for DPM benefit. There may be multiple mechanisms behind significant DPM benefit in the nearly 1 million virtual patients who demonstrated it. Furthermore, we are developing unique clinical study designs for optimal testing of DPM.

Another important goal of this work was to increase the feasibility of DPM in a clinical setting. DPM and potentially other EGPM approaches require repeated observations over time at deep subclonal resolution. Although single-cell methods are improving, they are expensive, and their sensitivity and accuracy may be insufficient to distinguish true rare cells from technical error. According to DPM simulations, one cell in 100,000 can alter the optimal strategy if it is an extreme hypermutator.[6] These challenges are magnified for liquid biopsies, which are nonetheless essential to minimize patient risk and inconvenience if prolonged monitoring is required. The finding that 2-move DPM provides similar benefits to 40-move DPM opens up the possibility of reduced patient risk and inconvenience, and reduced cost. The proposed 2-move, 12-week window may allow DPM to be deployed in high-risk neoadjuvant settings, where tissue availability at diagnosis and at surgery are routine and only one additional core biopsy between these time points would be required.

In this research paper, DPM illustrates a principle that could potentially be applicable to all EGPM. As cancers develop

drug resistance driven by subclonal heterogeneity and subsequent evolution, it is highly likely that one or more EGPM approaches will prove clinically useful. Our findings enable enrichment of future clinical studies with patients who are more likely to benefit, and reduce the cost and inconvenience associated with EGPM.

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**Final approval of manuscript:** All authors
**Accountable for all aspects of the work:** All authors

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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**Consulting or Advisory Role:** 1910 Genetics

**Robert A. Beckman**
This author is a member of the *JCO Precision Oncology* Editorial Board. Journal policy recused the author from having any role in the peer review of this manuscript.
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