Review

Cancer Research

Application of Evolutionary Principles to Cancer Therapy

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Abstract

The dynamic cancer ecosystem, with its rich temporal and spatial diversity in environmental conditions and heritable cell phenotypes, is remarkably robust to therapeutic perturbations. Even when response to therapy is clinically complete, adaptive tumor strategies almost inevitably emerge and the tumor returns. Although evolution of resistance remains the proximate cause of death in most cancer patients, a recent analysis found that evolutionary terms were included in less than 1% of articles on the cancer treatment outcomes, and this has not changed in 30 years. Here, we review treatment methods that attempt to understand and exploit intratumoral evolution to prolong response to therapy. In general, we find that treating metastatic (i.e., noncurable) cancers using the traditional strategy aimed at killing the maximum number of tumor cells is evolutionarily unsound because, by eliminating all treatment-sensitive cells, it enables rapid proliferation of resistant populations—a well-known evolutionary phenomenon termed "competitive release." Alternative strategies, such as adaptive therapy, "ersatzdroges," and double-bind treatments, shift focus from eliminating tumor cells to evolution-based methods that suppress growth of resistant populations to maintain long-term control. *Cancer Res; 75(22); 4675–80.* ©*2015 AACR.*

Introduction

Most disseminated cancers remain fatal despite the frequent availability of a large and growing number of potential treatments. While first-line therapy is often successful in reducing the tumor burden, it also applies intense Darwinian selection for resistant clones so that, even when complete response is achieved, tumor recurrence is almost inevitable by the phenomenon termed "competitive release" (1–3). Second-, third-, and fourth-line therapies may be available but are typically less effective as the cellular resistance strategies progressively broaden. Thus, evolution is the proximate cause of death in most cancer patients and will likely remain so in the absence of fundamental changes in the tumor treatment paradigm. Interestingly, Atkipis and colleagues (4) recently found that, despite this critical role of Darwinian dynamics, evolutionary principles were cited in less than 1% of cancer therapy publications, and this has not changed in the past three decades.

Most conceptual models of tumor evolution during chemotherapy emphasize resistance acquired in a stepwise fashion through some mutation following the start of therapy (5). If resistance arises stochastically and no resistant cells are present prior to treatment, then maximum dose-density therapy reduces the probability of resistance by minimizing the number of cells that can acquire the mutation. However, in most of the cases the resistant cells appear before chemotherapy starts (6).

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The assumption that a "resistance mutation" is necessary for tumor adaptation underestimates the vast information content, including xenobiotic pathways, that is available to cancer cells within the normal human genome (1). That is, many (probably most) resistance strategies simply require increased expression of one or more normal genes [P-glycoprotein (PgP; refs. 7–9), for example]. Thus, rather than an "all or none" phenomenon, resistance can be graded among cells in a population and, importantly, can change in the same cell over time as it acclimatizes to environmental stresses. For example, PgP is a HIF1α client (10) and its expression often increases in hypoxic and acidic environments even in the absence of cytotoxins. Furthermore, many other mechanisms of *de novo* therapy resistance have been identified. For example, in environmentally mediated drug resistance, components of the tumor mesenchyma protect cancer cells from otherwise lethal concentrations of cytotoxic drugs (11, 12). Tumor cells in regions of hypoxia may be protected due to increased expression of PgP, upregulation survival pathways, increased mutagenesis, and decreased drug delivery.

Evolutionary Dynamics of Cancer Therapy

Cancers can be described as open complex adaptive systems—"open" because they freely communicate with their surroundings, "complex" because they contain multiple components, and "adaptive" because each element can change over time and interact with other components in complicated, often nonlinear, ways. A critical attribute of such systems (based on their nonlinear dynamics) is that their dynamics can be nonintuitive and their response to a perturbation can yield unexpected and unintended consequences.

The traditional application of systemic therapy to cancer has largely rested on an intuitively appealing premise—maximum patient benefit is achieved by killing the maximum possible cancer cell (13, 14). This assumption that more is better is so

deeply ingrained that first phase in any cancer drug development seeks to find the maximum-tolerated dose. Traditionally, maximum cell death was obtained directly through maximum dose density of chemotherapy limited only by concern for fatal toxicity. An alternative approach is the "metronomic" strategy (15), which administers lower doses of therapy but more frequently. This has the benefit of reducing toxicity, permitting higher total drug administration, and increasing tumor cell death by inhibiting angiogenesis. However, the intent of modern therapy, whether administered through maximum or metronomic dosing remains inducing the greatest possible cell death.

When cancer therapy is viewed as an evolutionary process, significant flaws in the conventional assumptions emerge (1–3, 16–19). To be clear, when curative therapy is possible, then the treatment strategy must be designed to achieve that result. However, in a palliative clinical setting (e.g., most metastatic cancers) in which patients nearly always die of their disease, treatment for cure is futile and, in fact, evolutionarily unsound. By destroying the entire population of sensitive cells, maximum-dose therapy imposes intense selection for resistant phenotypes and, by eliminating all potential competitors, maximizes their proliferation—a well-known evolutionary phenomenon termed "competitive release" (2, 3).

Interestingly, insight into these dynamics can be found in an unlikely source—pest and weed management (20, 21). Application of high-dose pesticides was commonplace for decades but it became clear that this approach virtually never eradicated the pest and, in fact, promoted rapid emergence of uncontrollable, resistant strains. Since 1968, the policy of the U.S. Department of Agriculture toward pest management has been much more nuanced with greater emphasis on limited application of pesticides to minimize crop damage while also preventing emergence of resistant populations (20, 21). Incorporation of temporal data sampling and Darwinian dynamics into management of invasive species is now mandated by policy of the U.S. Agricultural Department. To assist agriculturalists in devising optimal treatment strategies, computational models to guide pest management, similar to those that we propose to, are widely available.

Application of Evolutionary Principles to Resistance in Cancer Therapy

Evolutionary therapy typically focuses on the competition for space and resources among cancer populations and, in particular, the Darwinian interactions between resistant and nonresistant populations. Any cancer therapy that results in cell death will impose strong selection forces for adaptive strategies, and the size and complexity of the human genome virtually assures the presence of multiple potential adaptive pathways to avoid cell death. Thus, while HIV with only nine protein-encoding genes can be controlled by targeting combinations of pathways, this strategy has not as yet proved successful in preventing emergence of resistance in human cells.

If resistance cannot be prevented, then tumor control requires therapy designed to slow or stop "proliferation" of resistant populations. Two general principles should be emphasized: (i) Growth of the resistant population is subject to evolutionary forces and, therefore, can be controlled by altering its fitness or that of competing populations. (ii) Evolving populations can only adapt to local and current environmental selection forces; they can "never" anticipate the future. Importantly, cancer therapists "can" anticipate the future and this knowledge of these temporal dynamics confer a key advantage by allowing treatment to change over time and, thus, use evolution to inhibit proliferation of resistant populations.

In evolutionary cancer treatment, a key component of the Darwinian dynamics is the cost of resistance. Cancer cells must alter their phenotype to become resistant typically through upregulation of established molecular defense mechanisms. Expression, maintenance, and utilization of these molecular pathways require resources, which, in an environment of limited substrate, must be diverted from proliferation and invasion. These dynamics are perhaps most clear in upregulation of xenobiotic pathways such as increased expression of P-glycoprotein (PGP), also known as multidrug resistance 1 (MDR1; refs. 7–9). PGP is a membrane transporter that effectively extrudes a large number of intracellular substrates, including chemotherapies and, consequently, reduces the effectiveness of these compounds. PGP and most other membrane pumps hydrolyze two ATP for every transported molecule. Indeed, in experimental studies this operation cost (as well as "capital cost" for synthesis and maintenance of the pumps) can approach 50% of the cell's energy budget (18). In cell-culture conditions with abundant resources, this may have little effect on cellular proliferation. However, when limited resources are available (*in vivo*, for example), cancer cells must trade-off resistance costs that permit survival with nonessential functions, including proliferation and invasion. This fitness cost can be inferred by the simple observation that the MDR phenotype is rare in pretherapy tumors and becomes common only following treatment (8). Furthermore, the drug-resistant phenotype is typically evolved through chronic exposure to a cytotoxic drug and quickly lost when the drug is removed (17).

Thus, in the presence of a cytotoxic drug, the fixed and operating costs of the molecular mechanisms of resistance are exceeded by the resulting survival benefit. However, in the absence of therapy, the cost of resistance is an evolutionary burden that can be exploited (22).

The evolutionary cost of resistance is most apparent in traditional chemotherapy in which the mechanisms of resistance and their associated "construction and operation" costs are fairly clear. This is less apparent in targeted therapies. However, Chmielecki and colleagues (23) have demonstrated an apparent cost of resistance to tyrosine kinase inhibitors (TKI) and proposed that alternative dosing strategies could be constructed based on exploiting this cost.

Exploiting the Cost of Resistance

Theodosius Dobzhansky famously stated "Nothing in biology makes sense except in the light of evolution" (24). Yet, the typical cancer therapy is administered in an evolutionarily static manner with drugs, doses, and schedules fixed according to protocol and changed only in the event of unacceptable toxicity or unequivocal evidence of cancer progression. In contrast, cancers are highly dynamic systems with enormous spatial and temporal heterogeneity that can change rapidly with perturbations such as applied therapy. Almost certainly, the tumor treated in the second cycle of chemotherapy is highly changed from that treated in the first cycle. Indeed any tumor that persists or recurs following therapy will likely be radically different from the tumor at diagnosis requiring new phenotype and genotype profiles.

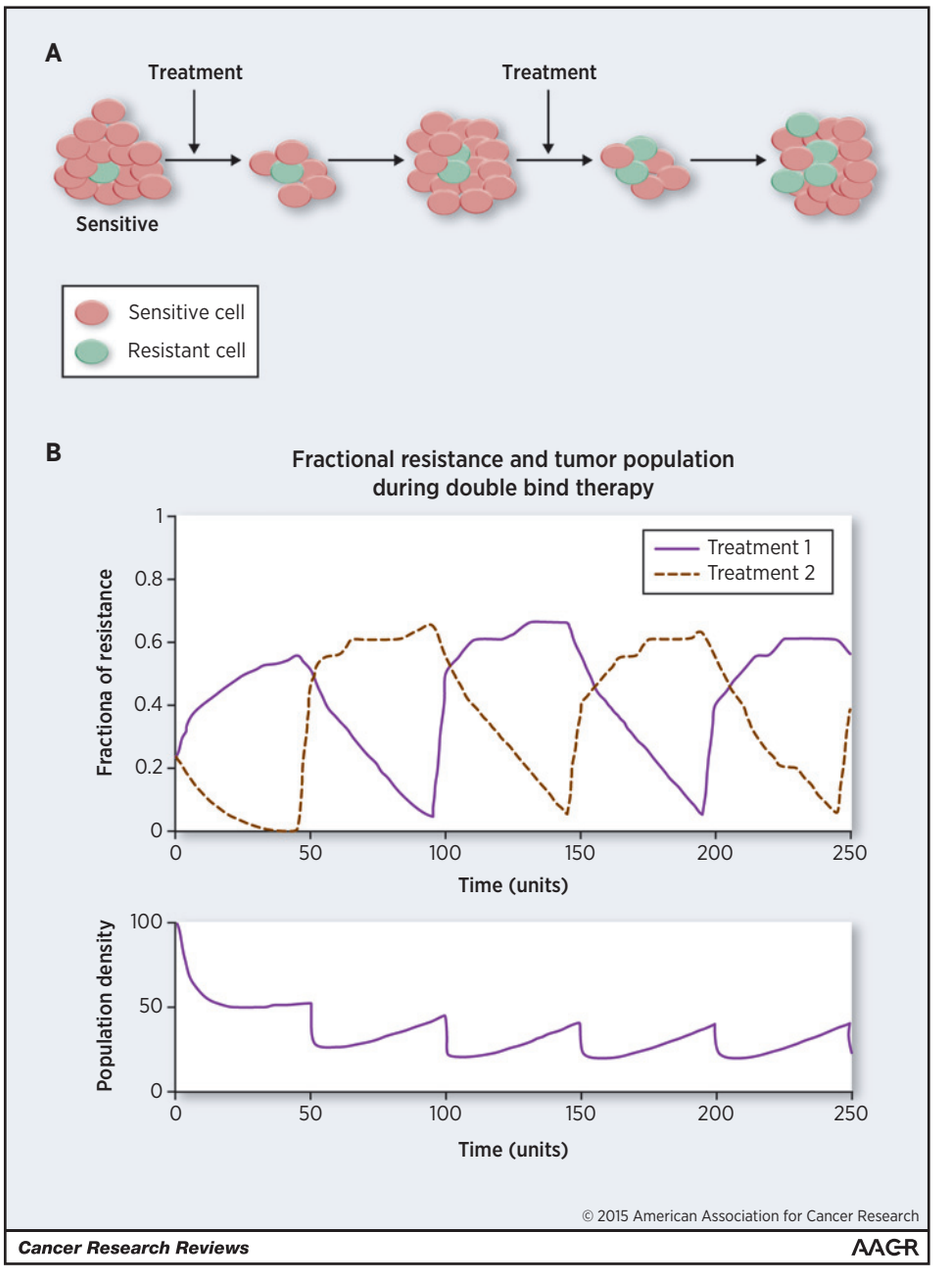

Figure 1.
Examples of evolutionary cancer treatment strategies. A, therapy for a mixed population of sensitive and resistant cells. Adaptive therapy reduces the tumor population but explicitly maintains a small population of treatment-sensitive cells. Once an initial tumor response is achieved, therapy is discontinued. In the absence of treatment, sensitive cells have a fitness advantage and will proliferate at the expense of the resistant cells. While the resistant cells will eventually dominate, the goal is to maintain tumor control with therapy for the longest possible time period. B, a double-bind approach in which the resistance mechanism to one therapy can be treated with the other therapy (see the text for example). Combining the two therapies will simply select for an alternative adaptive pathway and only slightly delays time to progression (not shown). However, by administering them in sequence, the evolutionary dynamics (termed "predator facilitation") forces the cancer cells to oscillate between phenotypes. This is an evolutionarily futile cycle that can permit long-term control of an invasive species.

In general, we propose that cancer therapy must become as dynamic as the tumor system that is treated. Ideally, treatment strategies should "stay ahead" of the intratumoral evolution through strategic application of different drugs and drug doses that move beyond the traditional goal of maximal cell death to one of optimal tumor control.

One such approach is adaptive therapy (1, 16). The premise of adaptive therapy (Fig. 1), similar to current pest management, is that the efficacy of extant anticancer drugs can be enhanced if their administration is guided by Darwinian principles. This approach has a number of features that differ from conventional chemotherapy strategies. First, the goal of adaptive therapy is maximizing progression-free survival and not reduction in cancer burden. Second, the amount of drug administered is not the maximum possible but the minimum necessary to maintain tumor stability and patient quality of life. Third, the drugs and dose schedules are not fixed but instead constantly adjusted to exploit evolutionary dynamics and maintain a stable tumor.

While there are a number of Darwinian dynamics that may be exploited during cancer therapy, the theory behind adaptive therapy typically focuses on the phenotypic cost of the molecular mechanism(s) of resistance. Proliferation of any phenotype in an adaptive landscape is dependent on the fitness of that phenotype compared with that of other extant populations. Importantly, the fitness of any phenotype is entirely contextual. A phenotype that, for example, expresses the PgP membrane pump is fitter than nonexpressing cells in the presence of chemotherapy. However, in the absence of therapy, the cost of the resistance mechanism

causes the fitness of the resistant cells to be lower than that of sensitive cells. To exploit this, adaptive therapy administers limited therapy with the explicit goal of maintaining a stable population of treatment-sensitive cells. Once the tumor size is stabilized, therapy is reduced or withheld. While this may permit some tumor cell proliferation, the fitness advantage of sensitive cells will allow their population to grow at the expense of the resistant cells. Repeated administrations of small doses of drugs are then used to reduce the tumor volume. The goal is always to administer not the maximum dose possible but the minimum dose necessary.

The adaptive therapy hypothesis was initially framed using catastrophe theory (25). It can be shown that, for any drug or drug combination, the probability of eradicating all of the tumor cells is maximum when their phenotypes and environments are homogeneous. In this mathematical model, intratumoral heterogeneity results in phase differences between therapy and tumor that permits survival. The biological interpretation of "phase difference" is that therapy fails not due to evolution of resistance during therapy but because of the pretreatment presence of resistant phenotypes or environments that are relatively sheltered from the toxic effects of therapy and permit rapid evolution of cellular resistant strategies.

To demonstrate the feasibility of this theoretical model, a preclinical model of ovarian cancer (OVCAR-3 cells) was developed and treated with carboplatin. Three groups were established: control, standard therapy (60 mg/kg q4 days $\times$ 3), and adaptive therapy. The adaptive therapy algorithm was based solely on tumor volume. Following the initial carboplatin dose of 50 mg/kg, tumor volumes were measured every 3 days and the dose of carboplatin adjusted to maintain a stable tumor volume. For example, if the tumor volume were to increase in size in two consecutive measurements, the administered dose of carboplatin would be increased and thus decreased if the tumor were to decrease in size. The result was prolonged tumor control and improved survival of adaptive therapy mice compared with control and standard therapy (16).

An unexpected observation in these experiments and in more recent studies using breast cancer cell lines, is a biphasic pattern in tumor response to adaptive therapy. That is, when treatment is initially applied, the tumors are typically growing exponentially. Forcing the growth curve to plateau required the full treatment dose. However, once the tumor volume was stabilized, the amount of drug necessary to maintain stability diminished rapidly. In the above experiment, for example, prolonged tumor control was often maintained using 5 mg/kg carboplatin. This phenomenon was not predicted by the computational models and remains under investigation but may be due to "normalization" of tumor vascularity during an enforced stability of tumor volume.

Increasing the Cost of Resistance—Ersatzdroges

As noted above, membrane extrusion pumps are a common mechanism of cancer cell resistance to chemotherapy. The metabolic capital and operation costs for maintenance of the pumps is significant. Broxterman and colleagues (26) demonstrated that the extrusion activity of PgP pumps activated by verapamil could consume 50% of the cell's ATP production. Prior clinical efforts have focused on either blocking PgP activity or administering other PgP substrate at the same time as chemotherapy to serve as competitive inhibitors and thus decrease extrusion of the cytotoxic drug. However, these approaches have generally not been successful due to combined toxicity and pharmacokinetics issues (27).

We have proposed a new strategy that, in the absence of therapy, seeks to "maximize" pump activity by administering nontoxic (or minimally toxic) substrate (17). That is, the chemotherapy and the "fake drug" are given in an alternative mode. Then, by forcing the cells to expend energy to extrude a "fake drug" (hence the name "ersatzdroges"), this strategy increases the phenotypic cost of the cells' resistance strategy. In a typical tumor microenvironment with limited substrate, the cells with an increased energetic demand for the extrusion of the ersatzdroges requires diverting resources from proliferation and invasion to support pump activity.

Currently available ersatzdroges are used to treat other diseases, including antibiotics and verapamil, a calcium channel blocker prescribed to treat arrhythmia. Thus, when the tumor cell detects the presence of these "fake" drugs, it uses its resistant mechanisms such as the MDR1 system to pump the drug from the cytosol.

In a preclinical model of breast cancer, exposure to verapamil dramatically altered the energy dynamics with cancer cells and reduced proliferation and invasion. *In vivo* and *in vitro*, administration of various ersatzdroges significantly increased glucose flux in tumors and reduced tumor growth (17).

Turning the Tables: Targeting the Adaptive Strategies

In general, application of evolutionary strategies to optimize tumor therapy requires "temporal" thinking. As demonstrated in adaptive therapy, the therapist must look beyond the immediate effects of treatment (i.e., tumor cell reduction) to anticipate longer term changes as new phenotypic properties are selected. Another example of this approach, termed "double bind" (28) or "sucker's gambit," (Fig. 1; ref. 29) uses a first-line treatment to induce a phenotypic adaptation that is then exploited in second-line therapy. Interestingly, this strategy has been successfully applied to antibiotic treatment of *Heliobacter pylori* (30). In cancer treatment, Antonia and colleagues examined the efficacy of a p53 vaccine in patients ($n = 29$) with small-cell lung cancer (31). While immune responses were elicited in most patients, only one partial response was observed. However, when the patients subsequently underwent chemotherapy, a response rate of 67% was observed (compared with the historic response rate of <5%) with increased efficacy in those patients that had the greatest immune activation (32). We interpret this as an example of an evolutionary double bind in which the tumor cells' adaptive strategy to the immunotherapy rendered them more vulnerable to cytotoxic drugs.

As demonstrated in Fig. 1, the ideal application of double-bind therapy is the creation of an evolutionarily futile cycle in which cyclical application of the treatments matches precisely the pattern evolution of resistance in the underlying cancer populations.

Exploiting the Properties of Complex Dynamic Systems for Cancer Control

All current cancer therapies act through some mechanism that kills cancer cells. This is certainly reasonable but also inevitably

produces Darwinian forces that promote resistance. An alternative approach targets environmental selection forces to alter the underlying evolutionary dynamics with the explicit goal of promoting a tumor phenotype, which is less proliferative or invasive. In general, cancers can be viewed as open complex dynamical systems: "complex" because it has many components, "dynamic" because the components interact and can change over time (often nonlinearly), and "open" because it freely interacts with the host. Traditional analysis of such systems (weather is probably the most familiar complex dynamic system) emphasizes the difficulty in predicting outcomes because of their nonlinear dynamics and sensitivity to initial conditions. For example, the famous "butterfly effect" posits an insect flapping its wings in Asia can cause a tornado in North America.

Importantly, however, it has also been noted that this tendency of complex systems to magnify some small perturbations can be exploited to steer the system along a desired course with minimal application of force (33). This, of course, requires sufficient understanding of the underlying intratumoral dynamics but does suggest there should be available strategies that can, if not dissipate the cancer system, at least nudge it along a less clinically aggressive path with selective application of small biologic force. For example, most tumors are net-producers of acid and intratumoral acidosis has been shown to select for phenotypes that are highly motile and invasive. However, small perturbations of the extracellular pH (an increase of approximately 0.2 pH units) can alter these Darwinian dynamics and select for less aggressive, more indolent phenotypes (34).

Clinical Applications

Mastering complex systems (as in weather forecasting; ref. 33) requires three components: (i) defining and mathematically framing first principles, (ii) necessary and sufficient data to parameterize mathematical models, and (iii) sophisticated computational methods.

We propose that evolutionary and ecological dynamics serve as first principles in cancer therapy and that computational models can be readily constructed (computer models to guide pest management are widely available, for example). The greatest impediment to clinical application of evolutionary principles to cancer therapy is the absence of usable data. While concerns for dealing with "big data" from molecular analysis are often expressed in oncology, these data lack spatial and temporal resolution and thus have limited value in evolutionary models. In fact, true dynamic data in oncology are largely limited to serum markers and repeated cross-sectional imaging. Thus, there is an urgent need to develop methods that can use sparse data for computational models and extract more information from available clinical data (e.g., radiomic analysis of CT and MRI images; ref. 35).

These limitations withstanding, some clinical trials that illustrate successful application of evolutionary dynamics have been and are being performed (36).

Summary and Future Directions

Cancers are complex, dynamic systems that will begin to evolve resistance strategies immediately upon application of any therapy. In contrast, current cancer treatment is typically applied in a static fashion so that the same drugs, doses, and schedules are administered until the protocol ends or tumor progresses. When a tumor responds to treatment, the typical strategy is to simply give more of the same. In contrast, evolution-based therapy seeks to become as adaptive and flexible as the tumor populations being treated. In adaptive therapy, for example, once tumor response to a specific treatment is observed, the best course might be to withdraw therapy or switch to a new strategy because further treatment with the successful drug will only result in greater selection for resistance.

In preclinical experiments (16–18), we have demonstrated that application of evolutionary principles to conventional chemotherapy agents can substantially prolong progression-free survival in both breast and ovarian cancer. Schweizer and colleagues (36) recently demonstrated that evolutionary principles could be used to prolong response to antiandrogen therapy in a cohort of men with castrate-resistant prostate cancer. A clinical trial using an adaptive-therapy algorithm for abiraterone therapy in men with castrate-resistant prostate cancer has recently opened.

However, there are a number of challenges in clinical application. These include the requirement to collect reliable data over time that allow the internal evolutionary dynamics of the cancer to be estimated. In fact, dynamic data that measure changes in tumors over time and space are typically quite sparse—largely limited to serum markers and clinical imaging. Thus, future directions must focus on converting clinical data into a dynamic understanding of the complex environmental and phenotypic changes that drives tumor evolution in response to therapy. Ultimately, this understanding will likely require sophisticated patient-specific computational models to provide treating physicians with decision support tools to optimize cancer therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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