Integrating Darwinian dynamics in prostate cancer treatment

Exploring the potential of evolution-based intermittent therapy for metastatic prostate cancer

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Abstract

Like most other metastatic cancers, metastatic prostate cancer (mPC) remains incurable, despite major advances in the oncology field. Development of drug-resistant tumor cells causes treatment to fail and ultimately leads to tumor progression. This thesis focuses on the potential benefits of incorporating evolutionary theory into treatment of mPC and castrate-resistant mPC variants (mCRPC). A new perspective is investigated, in which long-term tumor control is the central aim, ultimately turning cancer into a chronic disease. Mathematical models have demonstrated the potential of evolution-based intermittent therapy: the administration of appropriately timed on- and off-treatment periods, which takes into consideration evolutionary tumor dynamics. In the clinical field, this has been proven to have significant benefits compared to the current standard of care. These benefits include a delayed time to progression, reduced treatment toxicity and lowered medical expenses. However, as intratumoral dynamics are extremely complex and patient-specific, designing mathematical models and experimental trials remains challenging and further research is needed. Nonetheless, intermittent therapy appears to be a promising approach.

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Introduction

Prostate cancer is the most commonly diagnosed type of cancer and the second leading cause of cancer death in men in the United States (Siegel et al., 2019). Treatment options are effective, resulting in an average five-year survival rate of 98% for all stages of prostate cancer combined (Siegel et al., 2019). However, once it develops into metastatic prostate cancer (mPC), future prospects become poor. Despite the availability of over fifty approved drugs (Gatenby and Whelan, 2019), mPC usually remains incurable (Brady-Nicholls et al., 2020). The reason for this is that cancer cells have the exceptional capacity to acquire resistance to practically every treatment modality. Even when therapy initially seems to reduce tumor burden effectively, resistance inevitably evolves, resulting in treatment failure (Gatenby and Whelan, 2019).

The progression of cancer can be viewed as an evolutionary process. This does not only apply to the evolution of genes that affect cancer risk in a population; cells in particular can be considered as populations. Cells that undergo genetic or epigenetic mutations that increase their ability to replicate, evade the immune system, avoid cell death, invade tissues, or promote angiogenesis, will tend to predominate in later generations of cells within the tumor through the classical process of natural selection. A tumor is driven by somatic evolution of cells that have acquired an uncontrollable replication capacity (Crespi and Summer, 2005). Moreover, the evolution of genetic aberrations during therapy allows cancer cells to gain characteristics that make them insensitive to treatment, ultimately leading to tumor progression.

Although the use of evolutionary terms in cancer research has increased since the 1980s (Aktipis et al., 2011), integration of Darwinian dynamics into therapeutic trials remains limited. This is remarkable, given that the emergence of resistant tumor cells illustrates the relevance of evolutionary principles in cancer most clearly (Aktipis et al., 2011). Recently, integration of evolutionary principles in cancer therapy has successfully improved tumor control in pre-clinical and clinical trials with breast and ovarian cancers (Silva et al., 2012; Kam et al., 2014; Enriquez-Navas et al., 2015, Enriquez-Navas et al., 2016). This involved exploiting the evolutionary costs that a cell must pay to acquire and maintain resistance to therapy, a phenomenon that will be discussed extensively later in this thesis. As the same evolutionary principles apply to mPC, evolution-based therapies could have a similar potential for this specific type of cancer as the ones stated above. Therefore, the aim of this thesis is to investigate how Darwinian dynamics could be exploited in evolution-based therapies to prolong tumor control and ultimately improve mPC prognosis.

In order to answer this question, current standard therapies for metastatic prostate cancer and the emergence of resistance to cancer treatment will be discussed first, as well as common resistance mechanisms that lead to treatment failure. Cancer in an evolutionary context and the analogy between cellular and population evolution will be briefly explained. The major part of this thesis focuses on the integration of evolutionary principles into the development of metastatic prostate cancer and potential clinical applications. Evolution-based intermittent therapy, which is the administration of appropriately timed on- and off-treatment periods based on evolutionary intratumoral dynamics, will be reviewed for both mPC and the castrate-resistant variant mCRPC. Mathematical models, pre-clinical trials and clinical trials that explore these potential

therapies will be discussed. Finally, limitations and future perspectives of this evolutionary approach will be critically analyzed.

Treatment of metastatic prostate cancer and metastatic castration-resistant prostate cancer

Current therapies for metastatic prostate cancer

mPC treatment mainly consists of androgen deprivation therapy (ADT), as prostate cancer cells depend on androgens for survival and proliferation via the androgen receptor (AR) signaling pathway (Figure 1) (Vasaitis et al., 2010). In case of androgen deprivation, apoptosis is induced in prostate cells (Vasaitis et al., 2010). Both surgical and chemical castration are therapies that aim to substantially reduce the serum levels of androgens. Already in 1941, Huggins and Hodges demonstrated the favorable effects of ADT through surgical castration (Huggins et al., 1941) and this approach is still widely utilized. Additionally, chemical castration is obtained through gonadotropin-releasing hormone (GnRH) agonists and antagonists (Watson et al., 2015). A clinical response to hormone therapy is observed in almost all patients, but resistance typically evolves within one to three years after treatment initiation (Cunningham et al., 2018). Once resistance to these hormone therapies emerges, the disease progresses to a lethal stage and is referred to as metastatic castrate-resistant prostate cancer (mCRPC).

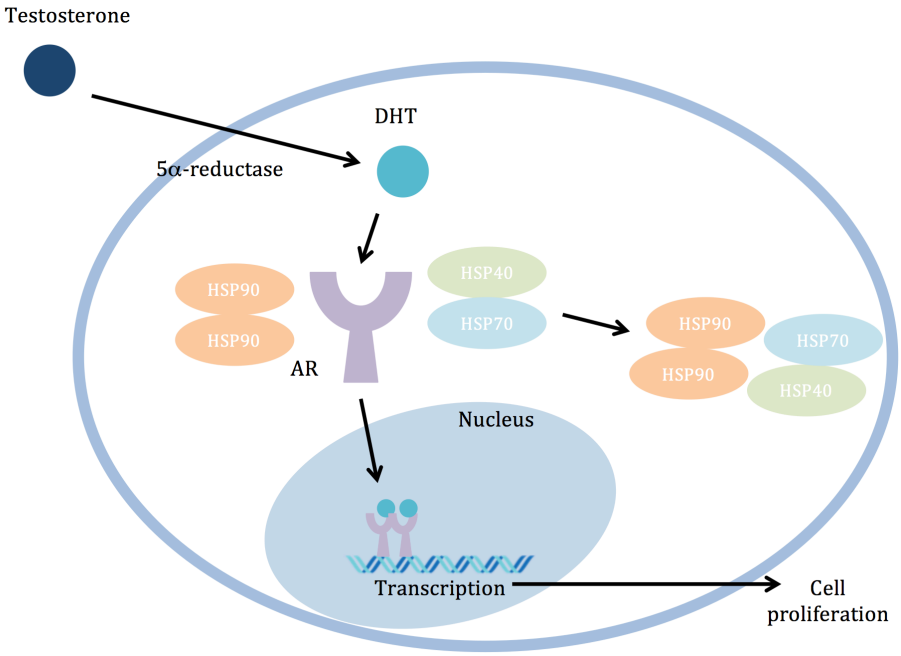

Figure 1
The androgen receptor signaling pathway. Testosterone enters the prostate cell, where 5α-reductase converts it to DHT. DHT binds to the AR, inducing conformational changes in the AR that lead to dimerization, phosphorylation and subsequent translocation to the nucleus. The modified AR then binds to specific sequences in the DNA, leading to transcription activation of target genes and ultimately cell proliferation (edited from Vasaitis et al., 2010).

Progression to metastatic castrate-resistant prostate cancer

Prostate cancer cells can acquire resistance to ADT through various mechanisms, of which two phenotypes are most commonly identified in mCRPC tumors (Cunningham et al., 2018). First, a fraction of the tumor cells remains androgen dependent, but becomes self-sufficient in its production through upregulation of CYP17α, an essential enzyme in androgen synthesis (Zhang et al., 2017; Cunningham et al., 2018). Increased expression of CYP17α generates enough testosterone synthesis to fully restore intratumoral testosterone levels, annihilating the effect of ADT (Zhang et al., 2017; Cunningham et al, 2018). Not only testosterone producing cells benefit from their newly acquired ability to synthesize testosterone themselves. Proliferation of nearby tumor cells sensitive to ADT can also be supported by this local source of testosterone (Cunningham et al., 2018).

Second, a fraction of the tumor cells proliferates completely independent of testosterone. These cells have evolved independency through variations in either the AR itself, or further downstream in the AR signaling pathway (Cunningham et al., 2018). For instance, in approximately 10% to 30% of all mCRPC patients, mutations in the AR have been found. The majority of these mutations (49%) are located within the ligand-binding domain (LBD). Mutations in this domain broaden binding specificity, making the AR sensitive for activation by multiple endogenous hormones, such as progesterone and estrogens. When mutations occur in the NTD and DBD, the receptor's affinity for coregulators could be changed, as well as nuclear localization (Livermore et al., 2016).

A drug that is commonly administered to men with mCRPC is abiraterone. This compound inhibits CYP17α, thus blocking autonomous androgen synthesis in prostate cancer cells (Zhang et al., 2017; West et al., 2019). Although this treatment yields a response in approximately 60% of mCRPC men (West et al., 2019), abiraterone-resistant tumor cells evolve over time as abiraterone strongly selects for resistant phenotypes. In the current standard of care, tumor progression occurs with a median time to radiographic progression of 16.5 months (Zhang et al., 2017).

Another drug that men with mCRPC initially respond to is docetaxel. This taxane drug induces microtubule stabilization, thereby disrupting mitosis, followed by apoptosis. Furthermore, a study by Darshan et al. in 2011 suggested that docetaxel inhibits ligand-induced nuclear translocation of AR and transcriptional activation of downstream AR target genes, including prostate-specific antigen (PSA), a serum biomarker of prostate cancer progression. This inhibition is caused by microtubule stabilization as well, as AR nuclear translocation relies on the cytoskeleton. However, evolution of resistance to docetaxel is inevitable as well and emphasizes the need for better therapy options (West et al., 2019).

Cancer in evolutionary perspective

Before we can discuss the possibility of exploiting evolutionary dynamics in order to optimize cancer treatment, we first need to establish a deeper understanding of the evolutionary roots of cancer. Molecular biologists have long recognized the analogy between evolutionary ecology and cancer origin, development and biology, and it is

suggested that cancer risk arose alongside the evolution of multicellular organisms (Crespi and Summers, 2005).

In multicellular organisms, cells coexist in an altruistic and cooperative manner and their growth is tightly controlled once the organism reaches its appropriate size. All cells have originally arisen from the same zygote, and are therefore virtually genetically identical. However, random somatic mutations occur in the genome and accumulate throughout an individual's life as changes in the genome are passed on to daughter cells during cell division (Crespi and Summers, 2005; West et al., 2016). These mutations can cause abnormalities that can ultimately lead to the development of cancer.

Analogous to evolution in systems biology, a tumor can be considered as a group of individuals (cells) that acquire genetic or epigenetic alterations and adaptively evolve over time because of interactions with their environment (selection) (Thomas et al., 2012). Thus, the progression of cancer is fundamentally an evolutionary process, in which a heterogeneous tumor cell population is subject to natural selection (Crespi and Summers, 2005; Somarelli et al., 2019). The evolutionary dynamics of cancer cells are analogous to the evolutionary dynamics of distinct organisms in evolutionary ecology, in which there are three fundamental concepts. First, variation in traits between different individuals is heritable and is therefore passed on to the next generation. Second, competition between individuals leads to differences in survival and reproductive success. This competition is, as a third rule, influenced by random inheritance of traits (West et al., 2019).

Integration of evolutionary principles in cancer treatment

Administration of the MTD may accelerate the emergence of drug-resistant cancer cells

Current cancer therapy typically administers the maximum tolerable dose (MTD) of cytotoxic drugs, aiming to kill as many tumor cells as possible until progression. The general assumption is that this will result in the best outcome for the patient (West et al., 2019). Indeed, a decrease in tumor burden is often observed after initial treatment, but this effect is only transient as resistance to therapy inevitably emerges over time (Figure 2) (Cunningham et al., 2018). To make matters worse, with the development of resistance to therapy, the tumor often transforms into a highly aggressive variant (Zou et al., 2019).

Although killing as many tumor cells as possible might appear to be an optimal strategy, administering continuous MTD therapy may be unwise as this approach does not take into consideration the intratumoral Darwinian dynamics, and as a result might actually accelerate the proliferation of resistant cells (Cunningham et al., 2018; West et al., 2019). MTD therapy creates a strong selection for resistant phenotypes as it eliminates all sensitive cells. In doing so, it eradicates the competition between sensitive and the remaining resistant cells, granting the latter exclusive access to the formerly shared resources: an evolutionary phenomenon known as competitive release (Cunningham et al., 2018).

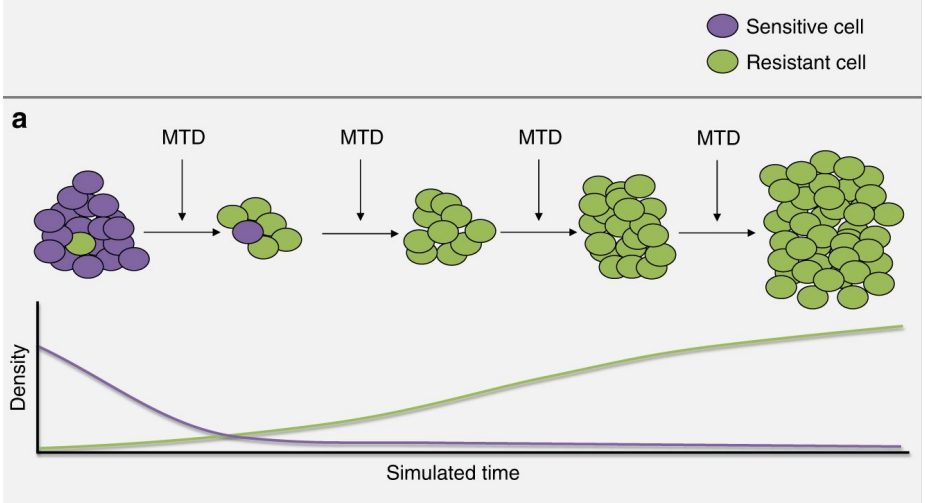

Figure 2
Evolution of resistance and tumor progression. Administering the maximum tolerable drug dose (MTD) may accelerate the development of resistance to therapy as it strongly selects for resistant phenotypes (Zhang et al., 2017).

Metronomic treatment is an alternative approach, in which lower doses of chemotherapeutic drugs are administered, but more frequently and periodically. Metronomic therapy has been shown to have positive effects in terms of reduced toxicity and therefore fewer side effects. However, despite being analogous to discontinuous treatment administration, metronomic therapy does not take into consideration any evolutionary principles. The fundamental aim is similar to that of continuous MTD therapy, namely to eliminate as many tumor cells as possible and reduce tumor blood flow, overlooking the consequences of this strategy on selection dynamics within the tumor (Gnoni et al., 2015; Gatenby and Brown, 2018).

Acceleration of the emergence of drug-resistant cell populations through competitive release occurs in virtually all cancers, mPC included. Therefore, it may be wise to take Darwinian dynamics into consideration for development of new cancer treatments. In the next section, three evolution-based strategies will be discussed.

Three evolution-based treatment strategies: targeting tumor heterogeneity, evolutionary herding and intermittent therapy

The problem in cancer treatment is not the lack of therapies, but therapies becoming ineffective through development of cells that are resistant to it. Changes in gene expression, leading to an altered metabolism, might cause this insensitivity, as well as genetic mutations that offer the cell an evolutionary escape route from the drug. Parallel to evolution in systems biology, such mutations can quickly spread through the population to give rise to a drug-resistant tumor. Given the many analogies between evolution of ecosystems and cancer evolution, several evolution-based strategies to target cancer cells have been developed, which will be discussed below.

Targeting tumor heterogeneity

The first example of an evolution-based cancer treatment is to target the tumor's heterogeneity and the mechanisms that contribute to the massive genetic diversity.

Tumors display a substantial level of heterogeneity, the presence of heritable variation among cancer cells within a tumor. This heterogeneity is not only existent between patients, but within a single patient as well (Dagogo-Jack and Shaw, 2018). Especially in metastatic disease, genetic and cellular differences among lesions are often found, as the tumor must acquire multiple changes to successfully establish a new lesion at a secondary site in the body (Lunt et al., 2009).

Intratumoral heterogeneity promotes the rapid adaptive evolution of tumors by increasing the amount of genetic "raw material" upon which natural selection can act. Mutations that contribute to tumor heterogeneity accumulate throughout the course of the disease. Moreover, tumors often exhibit additional mechanisms that increase the genetic variability even further, for instance defects in DNA damage repair and genomic instability, one of the hallmarks of cancer. These mechanisms can result in DNA mutations, chromosomal damage, epimutations and subsequently alterations in protein conformation, for instance (Thomas et al., 2012). The result is an immense competence to adapt to environmental changes. Therefore, when a cancer drug is introduced, it is not surprising that from this heterogeneous collection of tumor cells a portion invariably manages to find an escape route (Barzak et al., 2019).

Several research projects provide data on where these errors usually arise and which mutations are associated with an increased heterogeneity. Based on this, potential targets for evolution-based therapy can be identified. Unfortunately, tumors have already established a considerable level of heterogeneity at diagnoses in most cases. Even when initial heterogeneity is limited, evolution remains a complex process that is difficult to suppress, as tumor cells may adapt in ways that cannot be predicted. One way to overcome this limitation is to combine heterogeneity-suppressing therapy with another evolution-based therapy, such as evolutionary herding or intermittent therapy, which are discussed below (Barzak et al., 2019).

Evolutionary herding: exploiting collateral sensitivity

A second potential evolution-based strategy to target cancer is evolutionary herding, also known as an evolutionary double bind. This therapy focuses on exploiting a trade-off that tumor cells make. Trade-offs are not uncommon in biological adaptations. Adapting to one environment means lowering fitness in another. The same applies to drug resistance. In a phenomenon known as collateral sensitivity, it is occasionally found that mutations causing resistance to one drug create a vulnerability to another drug. Hall et al. reviewed this in 2009. For example, vincristine-resistant cell lines are hypersensitive to verapamil while cisplatin-resistant cell lines are sensitive to paclitaxel. To take advantage of this principle, evolutionary herding aims to predict effective drug combinations and their order of administration using mathematical models and experimental techniques. Unlike regular multidrug therapy, to which resistance eventually emerges and where toxicity and side effects are a major issue, the drugs are not administered at once but in a particular order. By repeated switching between two drugs exhibiting collateral sensitivity during cancer treatment, successful adaptation to either drug on the part of the tumor is prevented, since cells that have evolved resistance to the first drug are destroyed when the second drug is deployed, and vice versa. In summary, the aim of evolutionary herding is to make the tumor more

susceptible to one treatment through the development of resistance to another (Zhao et al., 2016).

Though in theory, evolutionary herding is a very potent treatment strategy, clinical application remains difficult. Collateral sensitivity is both rare and difficult to identify. Moreover, it often emerges unpredictably and the effects may be temporal, as the tumor keeps increasing its heterogeneity by the acquisition of new mutations. Thus, resistance to both drugs may still evolve eventually. Nonetheless, evolutionary herding provides a novel way to put existing drugs into practice, without the need for developing expensive new drugs, and extending the duration of their usefulness within an individual patient (Dhawan et al., 2017).

Intermittent therapy: take advantage of intratumoral competition

Intermittent therapy aims to restore intratumoral competition by giving drug-sensitive cells the chance to grow back between on-treatment periods. This keeps the drug-resistant population from taking over completely and causes the drug to remain effective (Gatenby, 2009).

During on-treatment periods, drug resistance provides a major fitness benefit to tumor cells, compared with drug sensitivity. Thus, treatment strongly selects for drug-resistant cells, which inevitably evolve over time, leading to rapid proliferation of an insensitive tumor population and ultimately, treatment failure. However, as drug resistance is costly for a cell – resources invested in resistance mechanisms cannot be devoted to growth and cell division – drug-sensitive cells have a competitive advantage in the absence of the drug (Enriquez-Navas et al., 2015). By alternating between appropriately timed on- and off-treatment periods, advantage can be taken of the competition between drug-resistant and drug-sensitive cells, potentially delaying or even preventing a tumor relapse (Gatenby, 2009).

The evolutionary strategies described above are applicable to virtually all cancers, but as intermittent therapy has been studied extensively in mPC, the remainder of this thesis will focus on this approach.

Application of intermittent therapy in metastatic prostate cancer

Intermittent androgen deprivation therapy: treating cancer like a chronic disease

Androgen deprivation therapy (ADT) has been the standard treatment for mPC. Most patients initially respond to ADT, but resistance usually evolves after 18 to 24 months on average, leading to progression to mCRPC (Kim and Kim, 2011).

Intermittent androgen deprivation therapy (IADT) is one example of an evolution-based therapy that aims to exploit intratumoral competition in order to delay of even prevent treatment failure and tumor relapse (Ideta et al., 2008; Enriquez-Navas et al., 2015). The goal of IADT is, unlike MTD, not to kill as many tumor cells as possible, but to monitor tumor size precisely and ultimately treat cancer like a chronic disease (Enriquez-Navas et al., 2015). Therapy is administered until the tumor size has decreased to a certain

extent, then proliferation of (mostly sensitive) tumor cells is permitted until the tumor reaches an upper limit and treatment is resumed. Treatment cycles are continued, resulting in stable tumor volume oscillations, until (hopefully delayed) tumor progression (Figure 3).

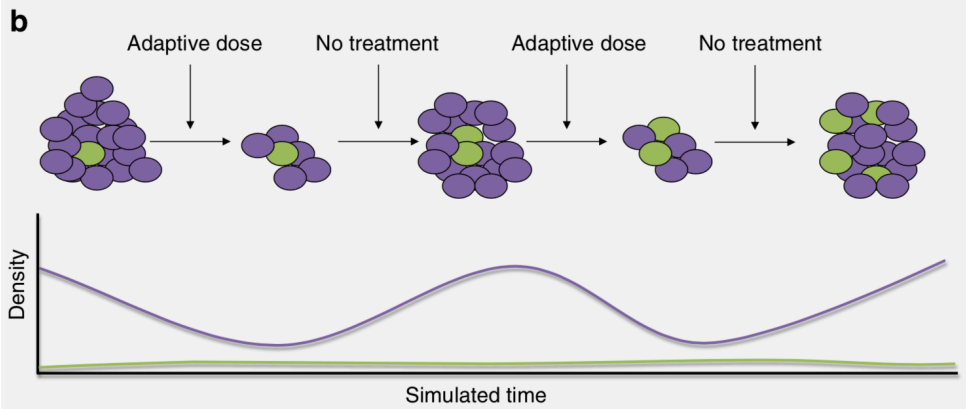

Figure 3
Administration of intermittent therapy. Appropriately timed on-treatment and off-treatment periods are used to control oscillations in tumor growth and regression, thus prolonging time to progression (Zhang et al., 2017).

Mathematical models provide a framework for complex intratumoral dynamics

Mathematical models are valuable tools to accurately simulate patient-specific tumor dynamics and can precisely predict a tumor's reaction to treatment (Zhang et al., 2017).

Ideta and colleagues proposed the first model that simulated a prostate tumor's response to intermittent androgen suppression (IAS) therapy, which is a form of ADT, in 2008. Although evidence from animal experiments and a number of phase II studies suggested the clinical potential of IAS therapy, the optimal timing of the intermittent treatment cycles remained elusive (Bhandari et al., 2005; Ideta et al., 2008). Therefore, Ideta and colleagues formulated a model that dynamically switched between on-treatment and off-treatment periods based on serum PSA level, as PSA is a reliable biomarker of prostate tumor progression. They aimed to propose a model for the effect of IAS therapy on prostate tumor growth in order to compare this to tumor progression under continuous androgen suppression (CAS) therapy. Another purpose of the study was to obtain insight into the optimal timing of treatment cycles (Figure 4).

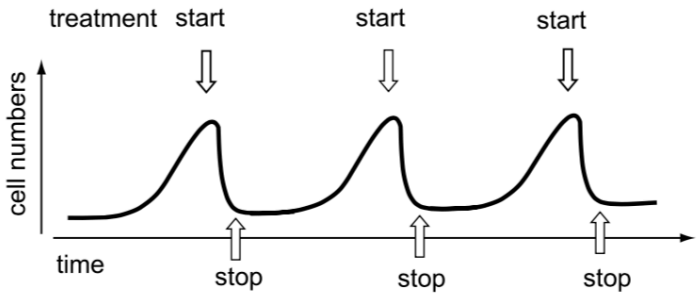

Figure 4
Schematic visualization of intermittent treatment administration. The upper and lower values of tumor cell number are based on serum PSA level (Ideta et al., 2008).

Ideta et al. based their model on a prior model by Jackson (2004a,b), which simulated the effect of CAS on a heterogeneous tumor cell population, consisting of both androgen dependent (AD) and androgen independent (AI) cells. Ideta and colleagues extended this model with the introduction of IAS therapy and application of the mutational effect, leading to AI relapse. They first proposed three hypothetical cases of the net growth rate of the AI cells:

(i) AI cells maintain a constant proliferation rate.
(ii) The total population of AI cells remains stable when androgen levels are normal.
(iii) The total population of AI cells decreases when androgen levels are normal.

Eight years later, Yang et al. obtained results similar to the Ideta model simulation of case (iii). Their model demonstrated cyclic growth and regression of the tumor without relapse under IAS therapy. The model was based on that of Ideta, with the addition of competitive interactions between AD and AI cells (Yang et al., 2016). Evolutionary game theory shows that natural selection favors those cells that optimize their fitness at the expense of their competitors (Cleveland et al., 2012). Therefore, it is hypothesized that tumor cells are capable of evolving strategies (i.e. heritable phenotypes) that benefit themselves while harming others (Tomlinson, 1997). Yang and colleagues aimed to improve relapse prevention by integrating this idea in a competitive model.

The results of both the Ideta and the Yang competition model are shown in Figure 5.

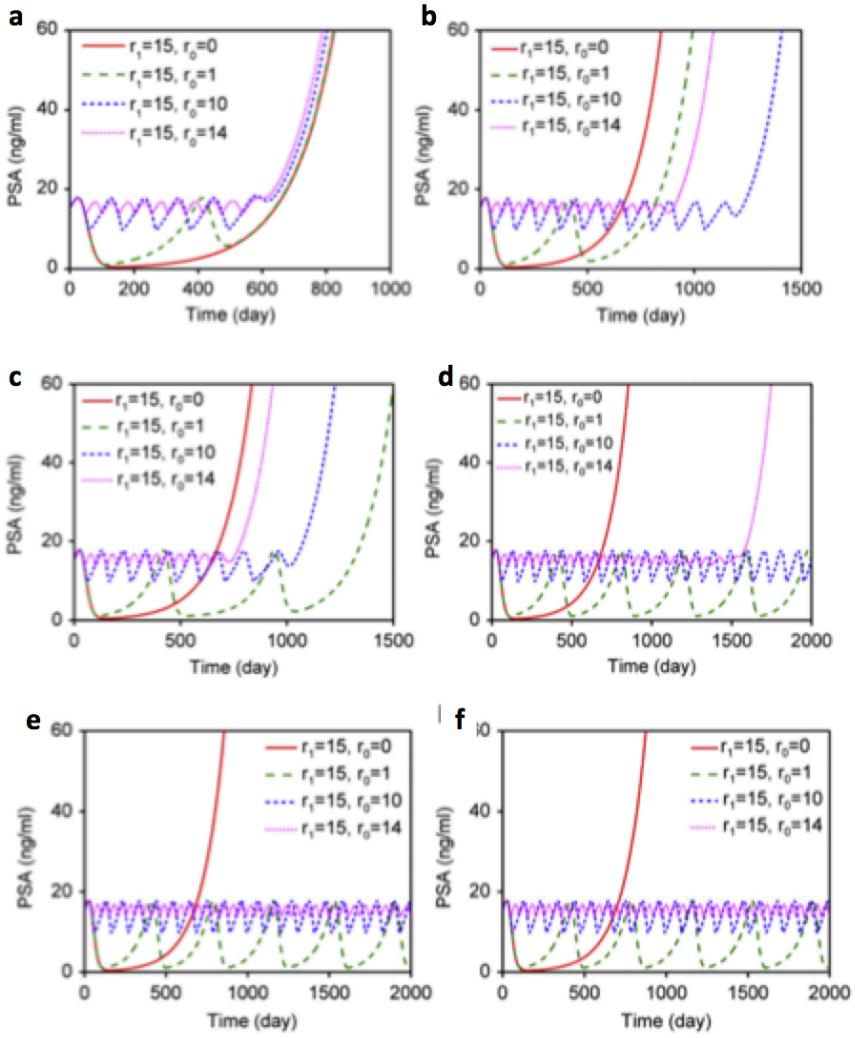

Figure 5
Results of the Ideta model (a, c, e) and Yang competition model (b, d, f). r_1 represents the upper value of serum PSA concentration, at which treatment should be restarted after an off-treatment period. r_0 represents the lower serum PSA concentration value, at which treatment should be suspended after an on-treatment period. The solid red line ($r_1 = 15$; $r_0 = 0$) shows PSA concentration under CAS therapy in all graphs. The other lines represent tumor progression under IAS therapy with different levels of r_0 (Ideta et al., 2008; Yang et al., 2016).

Case (i) The Ideta model (Figure 5a) indicates that CAS therapy is more effective in prolonging time to progression than IAS therapy. In contrast, the Yang competition model (Figure 5b) shows that for all r_0 values, IAS therapy is better than CAS therapy, though relapse cannot be prevented. Time to progression is dependent on the threshold values r_1 and r_0, which suggests that the efficiency IAS therapy depends on those values as well.
Case (ii) All IAS therapy trials prolong time to progression compared to CAS in the Ideta model (Figure 5c), though relapse still seems inevitable. Similar results are obtained with the model of Yang et al., as shown in Figure 5d. However, Yang et al. showed an even further prolonged relapse for the IAS therapy compared to CAS, and they demonstrated the possibility of establishing a stable IAS-control state.
Case (iii) Both the Ideta (Figure 5e) and Yang competition model (Figure 5f) indicate that IAS therapy results in a periodically oscillating PSA level without an exponential rise in tumor volume, regardless of the value of r_0, while CAS therapy still leads to an inevitable tumor relapse.

In summary, both the Ideta and the Yang competition models indicate that CAS therapy invariably leads to relapse in all three cases. The Ideta model showed the beneficial effect of IAS therapy cases (ii) and (iii), where relapse is prolonged or even prevented, respectively, compared to CAS therapy. Yang and colleagues obtained even better results with the addition of competitive interactions between AD and AI cells to the model. They showed that IAS therapy has a better treatment outcome than CAS therapy, regardless of the proliferation rate of AI cells, and relapse prevention is possible in cases (ii) and (iii). This implies that the competition effect between AD and AI cells could potentially enlarge the possibility of relapse prevention under IAS therapy.

In addition to this, Guo et al. analyzed optimal treatment schedules for IAS therapy and suggest that an optimal schedule for IAS therapy does exist (Guo et al., 2010), for which careful planning would be crucial. Several other studies have extended the Ideta model in order to analyze the effect of IAS therapy on tumor progression and optimal treatment timing (Hirata et al., 2010; 2014; 2015; 2018; Shimada and Aihara, 2008; Tanaka et al., 2010).

The studies mentioned above invariably demonstrated the potency of IAS therapy compared with CAS therapy in mathematical models, though the findings on the possibility of relapse prevention remain inconsistent.

At present, treatment schedules are based on information gained from preceding patients and past experience. Mathematical modeling can provide a more objective way for making decisions on optimal treatment timing, based on actual data. Several computational studies have demonstrated the potential of IAS therapy in comparison with CAS therapy, and although tumor relapse is not always preventable, it can be delayed by optimizing the timing of IAS therapy. This does not only prolong survival for patients with advanced prostate cancer, but also improves their quality of life. However,

no matter how precise, mathematical models cannot be taken as proof. Therefore, it is essential to evaluate these mathematical results through empirical research. In the next section, clinical trials with IAS therapy are investigated and the results are compared to those of mathematical models.

The potential of intermittent therapy in clinical oncology

Clinical studies on intermittent androgen deprivation therapy

Intermittent therapy has experimentally been shown to be clinically feasible and effective in several types of cancer (Gatenby et al., 2009; Enriquez-Navas et al., 2015; Enriquez-Navas et al., 2016). In this section, the application of this evolution-based approach in pre-clinical and clinical experiments will be discussed.

One Canadian Phase II study examined intermittent androgen suppression as a means to extend the hormone-dependent prostate tumor state, although it did not compare this to a historical control (Bruchovsky et al., 2007a). 103 patients were followed until development of androgen independence, which was defined as increased serum PSA level > 4.0 μg/L three consecutive times. Each cycle consisted of a total of 36 weeks of treatment, while serum PSA and testosterone were measured every 4 weeks. The number of weeks off treatment varied between patients, based on reduction in serum PSA level. In Figure 6 results from 4 consecutive treatment cycles are plotted. In all cycles, the graph flattens at approximately 95% serum PSA reduction after 40 weeks of treatment, although in cycle 1 the plateau stage is achieved earlier than in the other cycles.

Bruchovsky et al. found that the duration of the off-treatment interval under IAS therapy was inversely related to baseline and nadir (absolute lowest PSA level after treatment) serum PSA level. Their research suggested that nadir PSA level was a powerful predictor of the development of androgen independent cells (Bruchovsky et al., 2007a). In another article, they discussed the impact of IAS on side effects and quality of life and demonstrated that IAS therapy is a useful option for mPC treatment after radiation therapy. Quality of life improved both in terms of psychological and physiological function when treatment was paused (Bruchovsky et al., 2007b).

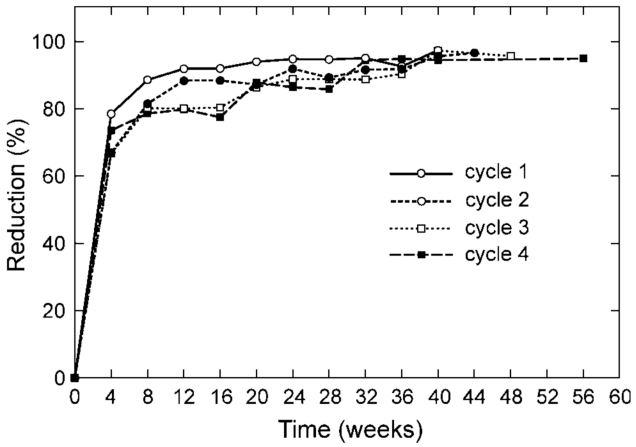

Figure 6
Rates of serum PSA reduction. The mean values of 4 consecutive treatment cycles are presented in this graph (Bruchovsky et al., 2007a).

Mottet et al. (2012) compared intermittent ADT with continuous ADT, but found no differences in time to tumor progression. However, they concluded that IADT is safe to apply to patients with respect to antitumor activity, as time to tumor progression was not reduced. Crook et al. (2012) and Hussain et al. (2013) confirmed these findings with similar studies. Furthermore, all three studies found that reduced medical costs are a benefit of IADT compared to continuous ADT, as fewer drugs are used with IADT. Another advantage of IADT is the potential decrease in adverse events such as hot flashes and headaches, two of the main side effects of androgen deprivation therapy.

Remarkably, the treatment protocols of these studies were initiated with an induction period of 6 to 8 months, in which continuous ADT was administered. This was later considered a major imperfection of these studies, as the induction period eliminated most drug-sensitive cells, thereby diminishing the potential beneficial effect of IADT (Zhang et al., 2017; Brady-Nicholls et al., 2020). Furthermore, the studies did not incorporate evolutionary intratumoral dynamics by predetermining the treatment schedule instead of administering tailor-made protocols to each individual patient, nor did they take into consideration evolution-based mathematical models. Zhang et al. (2017) developed a computational model of the treatment protocol used in the study of Hussain et al., which implied that the use of this protocol was comparable to metronomic treatment (also see Figure 7c).

This indicates the crucial role of incorporation of patient-specific tumor dynamics into the scheduling of treatment periods. For IADT to be superior to continuous treatment, evolutionary principles need to be fully taken into consideration, as was predicted by the mathematical models and is demonstrated in studies discussed hereafter.

Intermittent abiraterone therapy for metastatic castrate-resistant prostate cancer

Zhang et al. showed that intermittent therapy is clinically relevant in mCRPC (2017). They hypothesized that time to progression (TTP) could significantly be prolonged by intermittent administration of abiraterone, a CYP17α inhibitor, as compared to continuous MTD treatment.

To test this hypothesis, Zhang et al. first developed a mathematical model that simulated population dynamics of a heterogeneous tumor under various treatments (Figure 7). They defined three competing subpopulations within the tumor, based on clinical observations:

- T+ cells: cells that depend on exogenous androgen for proliferation.
- TP cells: cells that produce CYP17α, which enables them to autonomously produce testosterone.
- T- cells: cells that exhibit androgen-independent proliferation and are resistant to abiraterone.

Androgen deprivation therapy (ADT) does not affect the TP and T- subpopulations. Moreover, as the T+ population can use the testosterone produced by TP cells, T+ cells are minimally affected by ADT without contributing to the costly production of

androgens. When left untreated, the tumor, represented by the various subpopulations of cells to different extents, will progress to a fatal volume, as shown in Figure 7a.

Treatment with abiraterone, on the other hand, kills both the TP and the T+ population, by blocking testosterone synthesis through CYP17α inhibition. However, by continuous administration of abiraterone, conventional MTD treatment eliminates all TP and T+ cells, inducing competitive release of T- cells, as is explained in a previous section. This allows T- cells to rapidly proliferate to a lethal population size, as is visualized in Figure 7b.

Figure 7c demonstrates that not only the decreased drug dose, but also the evolution-based timing of treatment cycles is of importance to intermittent therapy. The simulation started with a considerable induction period, followed by predetermined intervals of treatment. This was based on a previous study performed by Hussain and colleagues (2013), who failed to demonstrate the benefits of intermittent treatment compared to continuous MTD treatment as explained in the previous section. The computer simulation displayed tumor progression similar to continuous MTD treatment.

Lastly, they simulated intermittent abiraterone therapy, in which treatment was discontinued when serum PSA was below 50% of its initial value (Figure 7d). Although a slow increase of T- cells during periods of low PSA concentrations, ultimately resulting in tumor relapse, could not be prevented, the simulation showed that intermittent abiraterone therapy was a superior treatment option to continuous MDT treatment.

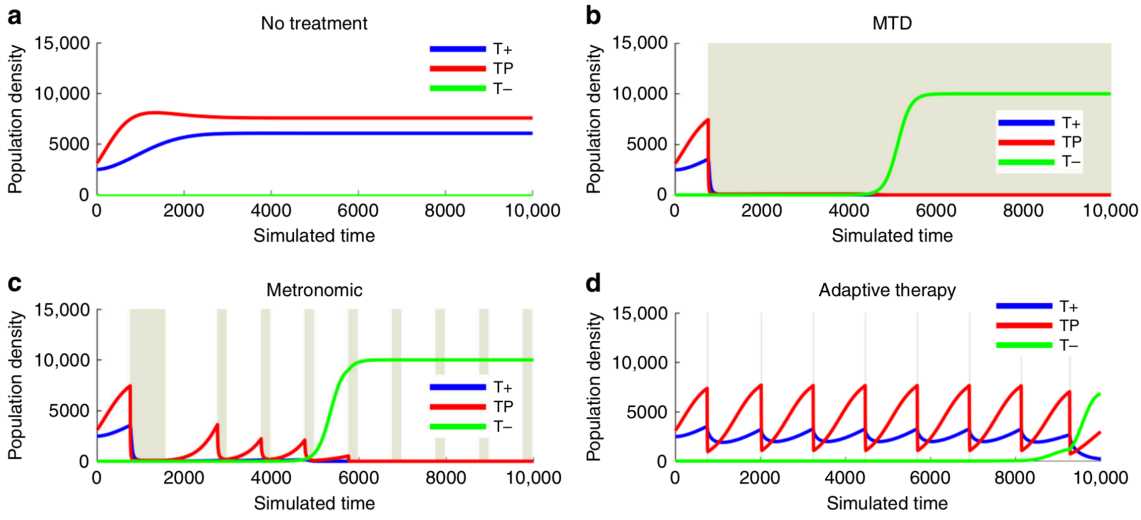

Figure 7
Computational models of tumor subpopulations under various treatment options. (a) no treatment, (b) standard of care: continuous maximum tolerable dose (MTD) of abiraterone, (c) metronomic treatment: a long induction period followed by predetermined treatment intervals, (d) intermittent abiraterone therapy (Zhang et al., 2017).

The competition between T+, TP and T- cells could be maintained by exploiting evolutionary dynamics in intermittent therapy. With appropriate timing of treatment cycles, the sensitive T+ and TP cells have the opportunity to grow back and competition is restored. The tumor composition is now almost equivalent to before treatment, preserving the effectiveness of retreatment with abiraterone and allowing tumor control

over several treatment cycles. However, a small increase of the T- populations during on-treatment periods cannot be prevented, which ultimately results in treatment failure. Still, time to progression is significantly longer than with continuous MTD treatment.

The mathematical simulations were followed by a clinical trial in which 11 mCRPC patients were assigned intermittent abiraterone therapy. Zhang and colleagues compared treatment outcomes to a contemporaneous cohort and historic controls of a phase III study by Ryan et al. (2013). Though their sample size was small, Zhang et al. demonstrated the superiority of intermittent abiraterone treatment for time to radiographic progression, compared to continuous MTD treatment in the cohort study (Figure 8). Intermittent abiraterone therapy significantly increased time to progression, while at the same time, cumulative drug dose was reduced to less than half that of continuous MTD treatment (Zhang et al., 2017).

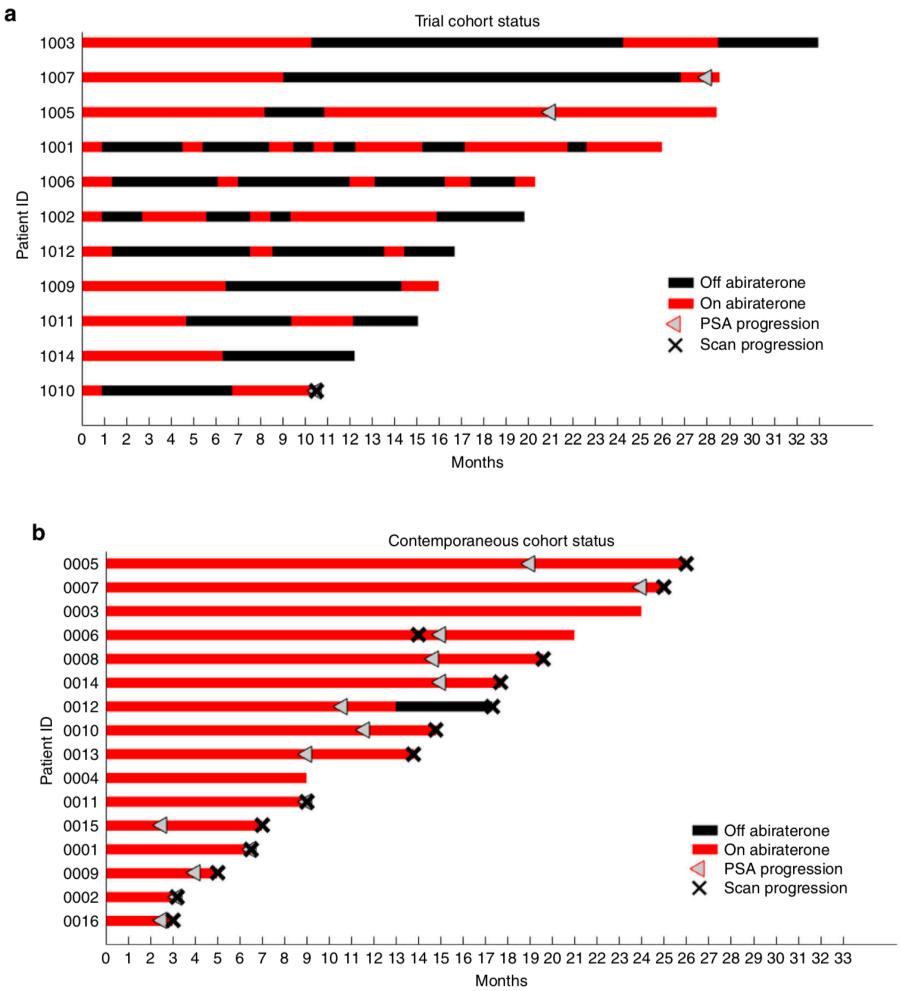

Figure 8
Comparison between the 11 patients of the pilot trial on intermittent abiraterone therapy (a), and 16 patients in the cohort study on continuous MTD abiraterone treatment (b). Tumor progression occurred 14 of 16 patients in the cohort study, in contrast to 1 of 11 in the intermittent therapy pilot trial (Zhang et al., 2017).

Intermittent therapy with abiraterone and docetaxel multidrug therapy

Most investigations of evolution-based cancer therapies have focused on monotherapies, as integrating evolutionary dynamics in multidrug therapy is even more

challenging. However, West et al. took the approach one step further by investigating intermittent therapy with both abiraterone and docetaxel (2019). Docetaxel is a taxane drug that reversibly binds to the β-subunit of tubulin. It disrupts microtubules during cell division, thereby inducing cell-cycle arrest and apoptosis (Ploussard et al., 2010). Prior studies have examined the simultaneous administration of abiraterone and docetaxel, but a patient-specific approach based on evolutionary dynamics was never realized (West et al., 2019). In this clinical trial, abiraterone administration depended on the patient's response. Both optimal treatment timing, which was calculated with evolution-based mathematical models, and the addition of docetaxel as secondary treatment were meant to reduce proliferation of drug-resistant cancer cells. West et al. developed a mathematical model that suggested the benefits of appropriately timed intermittent administration of both abiraterone and docetaxel compared to abiraterone alone (Figure 9).

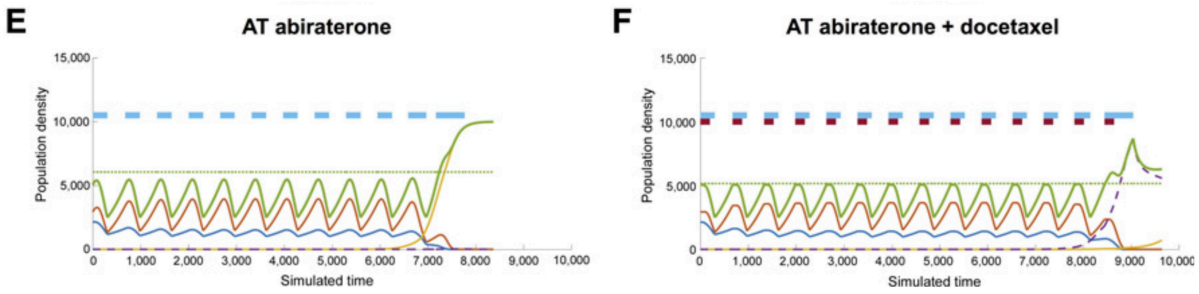

Figure 9
Mathematical models that compare the intermittent abiraterone therapy to the addition of appropriately timed docetaxel therapy. The model implies that multidrug treatment can extend the beneficial effect of intermittent therapy even further (West et al., 2019).

Data from two patients showed the number of days gained by administration of both abiraterone and docetaxel compared to abiraterone alone (Figure 10). West et al. demonstrated that inclusion of docetaxel delayed time to progression, and ongoing preclinical trials further investigate the potential of intermittent multidrug therapy.

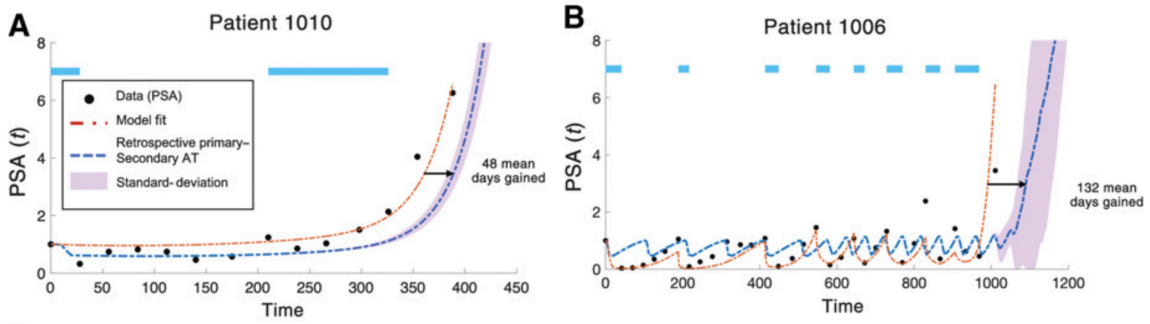

Figure 10
Docetaxel as a secondary therapy to intermittent abiraterone administration is superior to intermittent abiraterone therapy alone. Data from two patients who experienced tumor relapse indicate the number of days that are gained with inclusion of docetaxel therapy. Patient-specific model fitting is used to test the efficacy of secondary docetaxel therapy, optimally scheduled with each abiraterone cycle (West et al., 2019).

Limitations and future perspectives of evolution-based intermittent therapy

Evolution-based intermittent therapy has several limitations. The first, most recognizable restriction is its inability to completely prevent the emergence of drug resistance and tumor relapse in practice, although several mathematical modeling studies imply that relapse prevention is a possibility. This could be explained by the fact that mathematical models are only a simplified representation of reality, and ignorance of a certain important parameter always remains an option. Intermittent therapy does not aim to prevent the development of drug resistance, as it is much more advantageous to anticipate drug resistance and develop strategies to manage the resistant population. However, tumor relapse will remain inevitable in most cases, despite the prolonged survival times for patients with mPC and mCRPC. Continuous monitoring of evolving tumor populations with biopsies is needed to estimate the ratio between drug-resistant and drug-sensitive cells, and inclusion of a resistance management plan in clinical trials could be of assistance in maximally delaying time to progression (Stankova et al., 2019).

A second restraint is the complexity of the intratumoral dynamics, which makes mathematical modeling exceptionally challenging. Estimating patient-specific parameters is difficult, as tumors are extremely heterogeneous, a problem that is often encountered in mathematical medicine (Hirata et al., 2015). However, mathematical models are incredibly valuable for generating hypotheses and predicting optimal treatment timing.

Intermittent therapy can only have a beneficial effect over the current standard of care if both patient-specific tumor behaviour and evolutionary principles are incorporated into treatment design. This requires extensive monitoring of the patient's response to treatment, for which serum PSA is a good biomarker in prostate cancer. However, this might become a problem for other types of cancer as serum PSA cannot be used to monitor tumor progression. Moreover, treatment schedules cannot be determined in advance, making therapy laborious and raising ethical issues concerning the patient's well-being during treatment (Gatenby and Brown, 2018).

For evolution-based intermittent therapy to become incorporated in medical oncology, a paradigm shift is needed. Intermittent therapy is a counterintuitive concept, as the standard of care treatment is voluntarily interrupted in order for the tumor to grow back. In this approach, cancer is turned into a chronic disease instead of aiming to eliminate it completely. Chances are that both physicians and patients will not readily accept this counterintuitive approach. Therefore, the advantages of evolution-based therapy should be emphasized and intermittent therapy should gain public interest and become a recognized treatment approach in order to possibly become the new standard of care.

More research, both computational and experimental, is needed to further analyze the potential of intermittent cancer therapy and its complexity. The incorporation of evolutionary principles into cancer therapy represents an unrealized potential to postpone therapeutic resistance and thereby improve treatment outcome (Aktipis et al., 2011). Therefore, cancer treatment should change its goal to long-term tumor control, thereby turning cancer into a chronic disease (Cunningham et al., 2018).

Discussion and conclusion

Like most other metastatic cancers, metastatic prostate cancer (mPC) is incurable. Not because of lack of therapy, but because therapy loses its effectiveness over time due to the development of drug-resistant cancer cells. Current standard of care, continuous MTD therapy, aims to kill as many malignant cells as possible. Although this might intuitively seem in the best interest for the patient, the notion that this strategy may be unwise from an evolutionary point of view has recently gained attention. By eliminating all drug-sensitive cancer cells, continuous MTD therapy clears away the competition for the drug-resistant cancer cells, leading to maximized proliferation of the resistant population: a well-known phenomenon in evolutionary biology called competitive release.

In 1973, Theodosius Dobzhansky famously wrote, "Nothing in biology makes sense except in the light of evolution". However, despite the crucial role of Darwinian dynamics in treatment outcome, evolutionary principles are rarely applied to clinical oncology. This thesis has reviewed the potential of integrating evolutionary dynamics into mPC treatment. Several evolution-based strategies to treat cancer exist, of which this thesis has focused on intermittent therapy.

Tumors are extremely heterogeneous systems, and their genetic diversity contributes in large part to their unwavering capacity to develop drug-resistance. Gatenby and Brown stated in their 2018 review "the expression of a resistance mechanism does not by any means ensure that the resistant population will rapidly proliferate leading to tumor progression." In other words, if cancer therapy can control proliferation of resistant populations, which ultimately depends on evolutionary dynamics, emergence of resistance remains only a minor complication in cancer treatment. Therefore, intermittent cancer therapy, unlike conventional MTD treatment, focuses on long-term tumor control, regarding cancer as a chronic disease instead of trying to eliminate it. By appropriately scheduled cycling between on- and off-treatment periods, sensitive cancer cells can grow back and competition between the sensitive and resistant populations is restored. This is assumed to allow effective retreatment for multiple treatment cycles, thus prolonging time to tumor progression compared to conventional cancer therapy. By all means, if curative therapy is possible, treatment must be designed with that aim. However, since this is almost never the case, integration of evolutionary principles can significantly improve treatment outcome.

Both computational and experimental studies have confirmed the clinical potential and feasibility of intermittent administration of cancer therapy and demonstrated that intermittent therapy is superior to continuous MTD administration in three aspects. First, although the development of resistance is still virtually inevitable, several mathematical models predict that intermittent therapy significantly prolongs time to progression compared to conventional MTD treatment, and prevention of tumor relapse is possible. This is confirmed by several pilot clinical trials, although relapse prevention was not achieved. Second, intermittent treatment allows reduced drug administration. For example, in the study of Zhang and colleagues, the cumulative drug dose had dropped to only 47% of that of standard of care. Although abiraterone generally has few side effects, hormone therapy brings about many undesirable consequences, including weight gain, fatigue, loss of bone and muscle mass, metabolic and cardiac side effects

(Alva and Hussain, 2014). Decreased toxicity of cancer treatment reduces side effects and significantly improves the patient's quality of life. Third, a reduction of drug administration in intermittent therapy in comparison with continuous MTD treatment has a positive effect on medical expenses for mPC treatment.

Although numerous studies have found promising results, intermittent therapy has its limitations too. The largest obstacle for the clinical integration of evolution-based strategies to cancer therapy is the lack of usable data (Gatenby and Brown, 2018). Although mathematical models are a valuable source of information, they cannot be considered evidence for the benefits of intermittent therapy in itself. Moreover, there is always room for improvement, for instance by the incorporation of additional diagnostic tools such as DNA and circulating tumor cells (Zhang et al., 2017). More and larger clinical trials are needed to investigate prostate tumor development under intermittent therapy and the need for additional methods to assimilate both available computational and clinical data is pressing (Gatenby and Brown, 2018).

Another feature to take into account is the interactions between tumor cells and the microenvironment. Basanta and collegues developed a mathematical model that shows how prostate cancer progression is influenced by interactions with stromal cells (2012). They stressed the importance of understanding these interactions and their impact on potentially novel medical therapies. Sufficient spatial mixing of the tumor subpopulations should be taken into account as well, though this usually is not a problem with mCRPC (Zhang et al., 2017).

In conclusion, evolution-based intermittent cancer therapy appears to be very potent in treatment of mPC and mCRPC. As the development of drug resistance is still inevitable, intermittent therapy does not aim to eliminate the cancer, but rather aims for long-term tumor control, unlike the current standard of care. However, integrating this counterintuitive approach in current clinical oncology requires a paradigm shift in the cancer research field, in which the essential role of evolutionary principles is understood and appreciated. Moreover, for intermittent therapy to have a truly beneficial effect, patient-specific intratumoral dynamics need to be taken into consideration for optimal treatment timing, promoting the transition to precision medicine. Future clinical trials are needed to fully explore the benefits and weaknesses of intermittent therapy and other evolutionary approaches.

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