Chapter 4

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The classic approach to biological research is based on a ‘bottom-up’ methodology, where one attempts to identify and understand all pathways and signaling molecules involved in a biological process (i.e. to understand all constituents of a system and the dynamics between them). To function properly, biological pathways or signaling networks have adapted a carefully balanced tradeoff between robustness and sensitivity \(^1\), which is achieved by maximizing the information transfer between major signaling molecules and receptors of different signaling networks \(^2\). This need to maximize information exchange between pathways has resulted in biological processes which are highly complex and have an innate program which drives them towards maintaining normal cell function when perturbed by external influences. As a result of the inherent complexity of biological systems, mapping all of the individual components and their possible interactions can be an extremely complicated and arduous task. Additionally, cells in a diseased or impaired state are likely to act differently from healthy cells, as are cells in different stages of disease progression or cells which have been exposed to different therapies. It would therefore be a very laborious, if not impossible, task to attempt to understand or predict the response of a biological system to different treatments or external stimuli based on this approach.

A new method has begun to develop concerning the adaption of a ‘top-down’ methodology in biological research. The ‘top-down’ approach is frequently implemented in other fields of engineering and science, where predictions about how a system works are formed based on observation. These predictions can be supported or invalidated by experimental evidence. This type of approach no longer requires an in depth fundamental understanding of cellular mechanism, but only aims to predict and control overall cellular activity, greatly simplifying the work of the experimenter. It can be argued that this technique does not increase our overall understanding of the system; however, this is an approach intended to determine how to control cellular behavior and to develop viable treatment options for disease conditions as quickly as possible. Once the cellular responses to different environmental stimuli are determined and desired behaviors can be controlled, the mechanisms underlying these activities can always be retrospectively analyzed and studied.

The robustness of biological systems further increases the difficulty of treating complicated diseases, such as cancer, due to the fact that the therapy must overcome the innate nature of the biological systems to maintain normal (or diseased) function in the presence of external stimuli \(^3\). This is reflected in cancer therapy with the frequent development of drug resistance through up-regulation of different molecular pathways or mutation to overcome treatment. The field of cancer research is an excellent example in the field of biology where a ‘bottom-up’ approach has been vigorously implemented for years, but limited progress has been made due to the extremely complicated nature of the disease. Not
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only are the pathways involved in disease development and progression extremely complicated, but they vary between cancer types, patients and within a single tumor mass due to heterogeneous cell populations. Additionally, as cancer cells are believed to be genetically unstable, pathways activated by any one cell are likely to change over the course of disease progression or in response to therapy.

For the treatment of most cancer types, targeted agents exist which can slow or stop disease progression; however, this is frequently followed by the development of drug resistance and disease progression. Surmounting evidence from clinical trials has indicated the limitations of treating cancer with monotherapies, driving many researchers to therapeutic approaches involving combination therapies of multiple drugs and/or therapeutic modality. Additionally, there is still a lack of effective treatment options for many types of advanced or metastatic disease where average survival rates for patients remain very low.

Improvement in the field of targeted drug therapy for cancer may be achievable through the development of new, more selective, targeted agents, or in the form of combination therapies. The development of drug combinations with clinically-approved drugs can bypass the very expensive and timely process of developing new drugs, as it has been estimated to cost roughly $800 million and 12 years to develop a new drug. In addition, combination therapy strategies may provide unique advantages over single drug therapies. For example, the use of multiple targeted drugs inhibiting different pathways or cell populations within a tumor provides the potential for more effective and possibly synergistic therapies, which may carry a reduced probability for the development of drug resistance if the drugs combined have minimal cross-resistance (as has been seen for the treatment of HIV with drug cocktails). Heterogeneity in tumor cell populations may also play a key role in the frequent development of drug resistance (i.e. through the survival of cell populations that are not sensitive to a single drug treatment) and also supports the idea that enhanced tumor cell killing can be achieved with combination therapies. The combination of multiple drugs may allow for a reduction in individual drug doses, providing the potential for a concomitant reduction in drug-related toxicities, as well as the possibility to select compounds with different toxicity profiles in order to reduce the risk of additive or synergistic side effects. Additionally, some compounds may sensitize cells to other compounds, for example, by changing the cell-cycle stage or the growth of cells. Drug combinations possess a large potential to improve therapeutic outcomes in the field of cancer therapy if they are designed in such a way as to maximally exploit these possible advantages.

The efficacy of combination therapies utilizing up to as many as 5 different cytotoxic and/or specially targeted agents has been examined in a clinical setting for the treatment of various
cancer types, including various central nervous system tumors\(^\text{13}\), renal cell carcinoma (RCC)\(^\text{14}\), breast cancer\(^\text{15,16}\), pancreatic cancers\(^\text{17,18}\), etc. Although many of these drug combinations have shown treatment benefits over traditional therapeutic approaches, these benefits frequently come at the price of increased toxicities and side effects\(^\text{14}\). These toxicities are of particular importance in the treatment of metastatic disease, where quality of life is a very important factor in the selection of a treatment strategy and where combination therapies represent a large possibility for therapeutic improvement.

Many would argue that there is still great value in the potential of combination therapies for the treatment of cancer, and that its current limitations may, in large part, be due to the fact that we have yet to fully define the proper methods and considerations needed when designing combination therapy regimens. This can be seen from a brief glance at the historical context and current clinical approach to developing combination therapies.

The use of combination therapy approaches for the treatment of cancer has been evolving for more than half a century. In the 1940’s, the first forms of chemotherapy were being developed based on the observation of brief tumor remissions in patients exposed to nitrogen mustard, originally in the form of sulphur mustard gas during World War I and anti-folate drugs\(^\text{19}\). It would not be understood until years later that these drugs worked by targeting cancer cells versus normal cells based on their differing metabolic needs; however this coincidence introduced the idea that cancer cells could be selectively targeted by systemic drug administration and demonstrated that for many drugs, anti-cancer effects are frequently followed by drug resistance and tumor progression. The idea that drugs could be used in combination to reduce the probability of developing drug resistance came about as the result of a series of experiments\(^\text{20}\). The first showed that inheritable resistance in bacteria cells to bacterial viruses can arise as a result of mutation in virus exposed cells\(^\text{21}\). This was then shown to also be true for chemotherapy in bacteria\(^\text{22}\) and finally in the development of resistance in mice tumor models treated with anti-folates\(^\text{23}\). Given that there is a certain probability of developing drug resistance to any given agent, this probability is inherently reduced if two non-cross resistant drugs are administered together.

In 1964, Skipper et al. showed that a dose of chemotherapeutic drugs will kill the same proportion of cells regardless of the total tumor burden, which led to the idea that the best therapeutic effects could be achieved by administering drugs at their maximum tolerable dose (MTD)\(^\text{24}\). This led to the development of the 4-drug VAMP regimen in a study in 1964 by Freireich and Frei, where drugs were administered at MTDs\(^\text{25}\). This study showed improved treatment efficacy over other available therapies and initiated further study into combination chemotherapy regimens for many
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different forms of cancer. This initial success represented huge progress in the field of cancer therapy and marked the start of a new approach to cancer treatment. In many of these early studies, the designs of combination therapies was not approached in a systematic manner, but instead as a ‘shot in the dark’ where multiple effective drugs were administered together, at their MTD, in the hope to help treat patients with very poor prognoses. It is interesting to note that this idea still continues to dominate the approach to combination therapy today.

The success of a combination therapy is highly dependent upon the proper design of a therapeutic regimen. Many considerations must be taken into account for this design, from the selection of proper agents to be combined, to the selection of the method of drug administration, drug scheduling and drug doses. In designing the optimal combination therapy, the goal would be to maximize the therapeutic effects of treatment while minimizing side effects (therapeutic index) and drug resistance through manipulation of different therapeutic parameters.

Compounds included in a combination regimen are frequently selected based on single drug activity or due to their previous usage in the treatment of a selected cancer type, i.e. normally combination regimens are developed by attempting to identify compounds with single agent activity against the cancer type which will improve the activity of other drugs that are currently being used as the standard of care. Although it is logical to only consider agents with single drug activity for a specific cancer type, the interaction of different agents amongst each other can sometimes play a larger role in the overall effectiveness of the therapy and is frequently marginalized. For example, the combination of bevacizumab and erlotinib was clinically tested for the treatment of renal cell carcinoma and was well tolerated, showing promising efficacy with 25% of patients showing an objective response and 61% of patients having stable disease after 8 weeks of treatment. On the other hand, the combination of bevacizumab and sorafenib in the treatment of patients with advanced solid tumors showed both enhanced efficacy and enhanced toxicity. These results indicate how important and difficult it can be to select the proper compounds to be used in combination.

In addition to the selection of the individual compounds to be included, the relative dose of each agent must also be optimized. Varying the dose ratio between different compounds can change the overall effect of the drug combination, as well as its possible side effects. It is therefore very important to also optimize the doses of individual compounds in a mixture. This problem, however, is not as straight forward as one might think, as the number of possible drug combinations increases exponentially as more drugs are considered at multiple concentrations. For example, a combination of 4 drugs at 6 different concentrations represents $4^6$ possible drug
combinations. The optimization of a combination of multiple drugs at multiple concentrations can therefore be very time-consuming and expensive, if done in a systematic manner. Additionally, due to the highly complex and redundant nature of most biological signaling networks, which form robust pathways striving to maintain normal cell function in the presence of external stimuli, this task is nearly impossible to achieve if employing empirical intuition of drug actions and mechanisms alone. To further complicate an already complex problem, the time scheduling and method of drug administration in a treatment regimen may also have important effects on the outcome of therapy. For example, certain therapies may sensitize cancer cells to subsequent treatments or may have overlapping toxicities, making them only suitable for use when administered in succession. Designing a treatment schedule can be critical in developing a regimen of certain compounds, as can be seen in some of the studies presented in the following chapters, where the same drug combinations are tried with different schedules and methods of drug administration and result in drastically different therapeutic outcomes. The importance of drug doses and scheduling can be seen in the development of metronomic chemotherapy, where the administration of the same compound with a different schedule and dosage results in entirely different therapeutic effects. Even more interestingly, cancer cells that are resistant to high dose chemotherapy with a specific agent may still be sensitive to low-dose metronomic dosing of the same agent. This indicates that the dose and schedule of an agent can significantly change the mechanism by which it elicits anti-tumor effects and can critically affect its activity.

Therefore, three main challenges exist when optimizing multi-drug combinations: (i) the innately complex nature of a biological system, which makes it virtually impossible to predict optimal drug combinations based on empirical information, (ii) the large number of possible drug combination that exist when different drugs are considered at multiple concentrations, and (iii) determining optimal combinations of drugs while considering different drug doses, administration schedules and administration methods becomes practically impossible based on trial and error. Many of these challenges can be addressed through the implementation of a feedback system control (FSC) technique developed by the group of Prof. Chih-Ming Ho at the University of California, Los Angeles (UCLA), which provides a systematic, quantitative approach to determine optimal drug combinations and obtain a desired therapeutic outcome.

Many groups have investigated methods to optimize drug combinations, including techniques to model cancer progression and predict cell responses, systematic based searches, as well as various deterministic and stochastic search algorithms, including the Medicinal Algorithmic Combinatorial Screen driven by a sequential search based on a fitness function, Gur Game theory and similar stochastic algorithms, or sequential decoding algorithms. The limitation
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of modeling cell behavior lies in the complexity of cell signaling pathways. Although it is possible to make generalized observations about cell mechanisms and their interactions based on mathematical models, these models are inherently constrained by the information given to the system and the assumptions used in generating the models. Systematic based searches are also limited due to the number of possible existing drug combinations and therefore by the time, cost and effort required to test drug combinations. This effectively limits the size of the search space which can effectively be examined.

FSC.I is based on integrative system responses where the difference between desired and real system responses are used as an optimization criteria to be fed into a search algorithm, which can then iteratively drive the system to a desired systemic fate. An advantage of the feedback system control (or FSC.I) technique is that it is phenotypically driven and, as such, does not require any prior mechanistic information, such as complex cellular signaling information or target identification, in order to rapidly converge upon an optimal drug combination. For many biological applications, the FSC.I scheme is ideal, because it requires no knowledge of the mechanisms involved in determining the cellular response to a given drug stimulus or input. It only requires an output value that describes the overall cellular activity in response to a drug combination. This output is fed into the closed-loop feedback system, in order for the search algorithm to determine the next iteration of drug combinations to be tested on the cell system.

In the work described in this thesis, we used the FSC.I technique guided by the differential evolution (DE) search algorithm. Similar search algorithms have frequently been used in engineering research to optimize the design of electronic circuits and mechanical systems. Their use in biological applications is still very new, but has shown some promising initial successes. The DE algorithm implements parallel searches allowing for the robust identification of optimal drug combinations. FSC.I has already been successfully used in various complex biological systems, including the inhibition of viral infection, maintenance of human embryonic stem cells, and the differentiation of mesenchymal stem cells. In Chapter 6 we describe the use of the FSC.I technique to optimize an angiostatic drug combination in vitro. This optimization led to the identification of a three-drug combination which inhibited tumor growth in vivo in two preclinical tumor models with evidence of antiangiogenic mechanisms. FSC.I allows for the relatively rapid identification of optimal drug combinations, in most cases after only 10-15 iterative cycles of testing. This unexpected result is likely due to one of the defining characteristics of a complex system. Due to the robust nature of the complex system, the cellular response to different drug/drug combination inputs is relatively smooth. The smooth search space
makes it easier for the search algorithm to identify optimal combinations. The analysis of data from various drug optimizations led to the discovery that, in the cases analyzed, cell activity could be modeled using 2nd order regression analysis, as described in Chapter 5. This finding allowed for the development of a more rapid FSC.II technique.

The FSC.II is based on a statistical design of experiment approach called orthogonal array composite design (OACD). Using the FSC.II technique, designed experiments are used to ideally sample the response surface of the cell to drug combinations in order to generate accurate 2nd order linear regression models requiring minimal in vitro data. Regression analysis allows for the elimination of compounds that do not exhibit strong anti-cancer activity and subsequent testing of smaller subsets of drugs in order to rapidly identify highly effective combinations of 3 or 4 drugs. In Chapter 7, the FSC.II technique is applied to the optimization of a combination of targeted agents to inhibit the proliferation of a renal cell carcinoma cell line. This study demonstrates the potential of the FSC.II technology and shows how it allows for even more rapid identification of optimal drug combinations.

References

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