Combination Therapy Design for Cancer: A Digital Systems Approach

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Abstract—Cancer encompasses various diseases associated with loss of cell-cycle control, leading to uncontrolled cell proliferation and/or reduced apoptosis. Cancer is usually caused by malfunction(s) in the cellular signaling pathways. Malfunctions occur in different ways and at different locations in a pathway. Consequently, therapy design should first identify the location and type of malfunction and then arrive at a suitable drug combination. We consider the growth factor (GF) signaling pathways, widely studied in the context of cancer. Interactions between different pathway components are modeled using Boolean logic gates. All possible single malfunctions in the resulting circuit are enumerated and responses of the different malfunctioning circuits to a ‘test’ input are used to group the malfunctions into classes. Effects of different drugs, targeting different parts of the Boolean circuit, are taken into account in deciding drug efficacy, thereby mapping each malfunction to an appropriate set of drugs.

I. INTRODUCTION

In eukaryotic multi-cellular organisms, life is sustained by a systematic coordination between different cells and all extra cellular signals. Each cell has its own functionality and its future is determined by various intrinsic and extrinsic biological signals. For instance, a cell’s proliferation, differentiation or induction of apoptosis are determined by a number of different signals. From the time of a cell’s birth (by division of its parent cell), the cell’s state is tightly controlled by different biological regulations. Cell signaling is a form of communication between different cells. These signals can be chemical or electrical impulses. Communication via electrical impulses is typically associated with nerve cells (neurons) which are attached to each other and the action potential transmits from neuron to neuron. For general somatic cells, proteins are usually the signaling molecules used for communication. The interactions between the different signaling molecules are multivariate in nature and hence difficult to study. As a result, historically biologists have focussed on studying the marginal interaction between the signaling molecules, leading to what is called pathway information. Although pathway knowledge cannot provide the complete multivariate picture of the overall cellular signal transduction, it is clear that one has to have a mechanism for incorporating this prior information into any signal transduction model that one develops. A procedure to do precisely that was recently developed in [1]. In that paper, tools from digital system design were used to generate Boolean networks consistent with given pathway information. Furthermore, it was shown using a specific biological example that a network designed using that approach could replicate relevant experimentally observed behavior from the published literature.

In this paper, our goal is to go a few steps further. Here, we are not content with just producing a Boolean network model from given pathway information. Instead our objective is to utilize such a model to (i) enumerate all the possible fault scenarios; (ii) use the response of the model to a test input to determine which fault or class of faults has occurred; and (iii) finally use this information to prescribe an appropriate therapeutic action. To keep the discussion biologically focussed, we will consider the specific case of growth factor signaling pathways.

The paper is organized as follows. In section II, pathways and networks are defined in a formal way. In section III, cancer is modeled as faults in the underlying signaling network. In section IV, drug therapies are modeled as interventions to alter aberrant network behavior emanating from a fault. Section V gives a biological example showing the power of our methodology. Specifically, fault classification and intervention results for our example are presented. Finally section VI contains some concluding remarks.

II. FROM PATHWAYS TO NETWORKS

From a systems viewpoint, the behavior of a living cell is analogous to that of a multi-input multi-output (MIMO) feedback system. Although the actual protein concentrations in the cell are continuous variables, there are at least three reasons why a discrete type of modeling would be preferred. First, although the continuous model may dictate the exact dynamics, using the current technology it is impossible to reliably measure the concentration of each protein inside the cell in real time. Second, many of the genes/proteins inside the cell exhibit ON/OFF switch-like behavior which is more readily accommodated using quantization within the digital domain. Third, the discrete-time systems are easier to analyze, model and control in real time in comparison to continuous-time systems. In the next subsection, we introduce Boolean Networks which constitute a popular framework for discrete-time discrete-space modeling of biological systems including genetic regulatory networks. In addition, we formally define signaling pathways.

A. Boolean Networks and Pathways

A Boolean Network (BN) ([2], [3], [4]), $B = (V, F)$, on $n$ genes/proteins is defined by a set of nodes (genes/proteins) $V = \{x_1, ..., x_n\}$, $x_i \in \{0, 1\}$, $i = 1, ..., n$, and a list $F = \{f_1, ..., f_n\}$, of Boolean functions, $f_i : \{0, 1\}^{n+m} \rightarrow \{0, 1\}$, $i = 1, ..., n, m \geq 0$ is the number of external inputs.
e.g., growth factors, stresses, metabolites etc. Each node $x_i$ represents the state/expression of the gene/protein $i$, where $x_i = 0$ means that gene/protein $i$ is OFF (unexpressed or inactive according to their biological significance) and $x_i = 1$ means that gene/protein $i$ is ON (expressed or active). The function $f_i$ is called the predictor function for gene/protein $i$. Updating the states of all genes/proteins in $B$ is done synchronously at every time step according to their predictor functions. If the predictor functions are known, the dynamics of the boolean network will solely depend on the set of input variables. The dynamic behavior of the boolean network is quite different in the presence of different external inputs. For $m$ different external binary inputs we can model the dynamical system as $2^m$ different closed BNs, each one of which we may define as a ‘context’. The switching between contexts here occurs in response to changes in the activity status of external input variables and is, therefore, deterministic. A stochastic view of context switching has been adopted in several earlier papers, for instance [5], [6].

In this paper, the starting point is the theoretical construction of context sensitive BNs or input-output BNs from the known biological knowledge of signal transduction pathways. Towards this end, define the term pathway segment $A \xrightarrow{t:a,b} B$ to mean that if gene/protein $A$ assumes the value $a \in \{0,1\}$ then gene/protein $B$ transitions to $b \in \{0,1\}$ in no more than $t$ subsequent time steps. A pathway is defined to be a sequence of pathway segments of the form $A \xrightarrow{t_1:a_1,b_1} B \xrightarrow{t_2:b_2,c} C$. A systematic general procedure for generating a family of boolean networks consistent with a set of given signaling pathways, or minor variations thereof, is presented in [1]. A network generated by such a procedure will form the starting point for the exposition of the results of this paper although, due to space limitations, the detailed steps involved will not be discussed here.

While on the topic of Boolean Network modeling, it is appropriate to point out that any Boolean Network can be of one of two types, either with feedback or without feedback. A non-feedback type boolean network can be considered to be a digital relay where the input signals are processed via digital circuitry to generate the relevant outputs. Although biological networks as a whole are of the feedback type, for the sake of simplicity of modeling we can decompose a large biological network (which is mostly unknown) into smaller modules (with and without feedback). This modular approach has yielded enormous benefits in designing very large digital networks for digital Integrated Circuit (IC) chips. It is possible that the adoption of a similar approach in systems biology could yield similar benefits. Although we believe that this modular approach to modeling in systems biology merits further study, we will not pursue it here since, as we will see, the biological example of this paper involving the Growth-Factor mediated signal transduction pathways can be adequately modeled using a non-feedback input-output digital circuit.

### III. Modeling Cancer as Faults in the Signaling Network

In molecular biology, the marginal behavior of the normal cell is described using signaling pathways. Boolean networks represent a paradigm that can be used to incorporate this information to model the overall dynamic behavior of the cell, consistent with the pathway knowledge. However, the translational motivation behind this type of dynamical modeling is to facilitate corrective intervention when the cell behaves abnormally. Cancer is actually a disease of several faults in the network. A ‘fault’ is defined by any structural error of the physical system, such that the dynamics become aberrant. For example, the accumulation of point mutations in the genomic DNA may cause the signaling pathways to behave erratically leading to proliferation. On the other hand, sometimes the fault may not be in the genetic code of a particular protein, rather in the protein synthesis factory ribosome, or in some control mechanism of alternative splicing. The fault could also be in the chromosomal spindle resulting in unequal splitting of the chromosomal DNA between the two daughter cells during cell division. Any of these different kinds of errors could cause structural changes in the regulatory network, thereby changing its dynamics and steady-state behavior. In this section, we try to model different types of biological errors within the Boolean network (digital electronics) framework. In a Boolean Network, the faults can be broadly divided into two types.

- **Stuck-at Fault**: A stuck-at fault means that a point in the network circuitry is stuck to a particular value. As a result, the incoming information is no longer communicated beyond the faulty point; instead, only the stuck-at value is passed on to the outgoing port. Clearly stuck-at faults can commonly be of two types: ‘stuck-at-1’ faults and ‘stuck-at-0’ faults with obvious interpretations. We next present an example to show that modeling via stuck-at faults makes biological sense.

In the Mitogen Activated Protein Kinase (MAPK) pathways, an important signaling protein kinase (molecule that adds a phosphate group) is the Ras protein. The Ras protein is activated when it forms a complex with guanosine triphosphate (GTP). The removal of one phosphate group from GTP produces guanosine diphosphate (GDP) and this renders the complex inactive. Ras is phosphorylated (given an additional phosphate group) by many upstream proteins (by Growth factor mediated pathways). Once activated, Ras activates downstream proteins which have transcriptional control on cyclin D1 and hence cell cycle progression. The inherent enzymatic GTPase (GTP degrading) activity of Ras hydrolyzes (breaks up with the addition of water) the active Ras-GTP complex into the inactive Ras-GDP complex, so that Ras activity ceases after some time delay. However, if due to some mutations in the Ras gene, the GTPase activity of the Ras protein is lost, the once activated Ras protein will be constitutively (always) active and will signal
the downstream transcription causing proliferation and cancer [7]. This constitutive activation of Ras can be modeled as a ‘stuck-at-1’ fault in the Ras node of the Boolean network model of the cell signal transduction.

- **Bridging Fault:** As the name suggests, a bridging fault refers to the disruption of old interconnections and incorporation of new interconnections in the network. Bridging faults also make biological sense. The molecular signal transduction relies on the sequences and 3 dimensional conformations of the molecules involved. So, any variation in the sequence and 3 dimensional conformation of a molecule (mainly protein) will alter its functionality. As a result, many pathways involving that molecule will become inactive while the altered molecule may open up new ones. Without any loss of generality, this kind of aberrant behavior could be modeled as a bridging fault in the boolean network.

Stuck-at faults and Bridging faults are illustrated in Fig. 1(a) & Fig. 1(b), where a fault free Boolean network is shown in Fig. 1a while the corresponding faulty network is shown in Fig. 1b.

Based on the preceding discussion, it is clear that cancer can be broadly modeled as multiple stuck-at and bridging faults in the boolean networks corresponding to the normal signaling pathways. In [8], extensive theoretical work on digital system testing and fault modeling is presented which engineers have been successfully using for digital circuit testing for quite some time now. One of the goals of this paper is to use a similar approach for the prediction of fault locations in cancerous networks and the design of intervention policies to compensate for the effect of these faults. For the sake of simplicity, we will focus only on single stuck-at faults. The more general case of cancer modeling involving multiple stuck-at and bridging faults will be taken up in future publications.

### A. Test Inputs and Fault Detectability

In this paper we will primarily focus on the non-feedback input-output modules of biological systems. Consider the BN of Fig. 1a which has 4 inputs and 2 outputs as shown. Now suppose that the only possible fault in this network is the stuck-at-1 fault shown in Fig. 1b. Following [8], for a combinatorial circuit (i.e, non-feedback BN) $N$, let $Z(x)$ denote the output vector for the input vector $x$. The presence of a fault $f$ transforms $N$ into $N_f$ with output function $Z_f(x)$ for the same input vector $x$. We say that a test vector $t$ detects the fault $f$ if $Z_f(t) \neq Z(t)$. Clearly, for the stuck-at-1 fault in Fig. 1b, the test input vector $ABCD = 0000$ can detect the fault because, $Z(0000) = 00$ while $Z_f(0000) = 10$. However, the test input vector $1111$ cannot detect the fault since $Z(1111) = Z_f(1111) = 11$. These ideas about fault detectability will be applied to a biological example in section V-B.

### IV. Modeling Drug Intervention

In a cancerous network, identification of the fault locations is only a part of the task. The major challenge lies in finding the best possible drug or drug combinations with which to intervene. From a theoretical perspective, we can consider the non-cancerous and cancerous (faulty) networks as two different boolean networks. In general, it will be impossible to make a cancerous network revert to the original non-cancerous one using any sort of drug intervention, because the mutations leading to cancer are usually irreversible. Instead, what the best drug combination could do is to nullify some of the lethal effects (like constitutive cell division) of the cancerous faulty system and try to kill the cell by inducing apoptosis (programmed cell suicide).

The following modeling of drug intervention is inspired by the biological effect of the drug on the pathways. A drug goes into the cell to bind a particular kinase to deactivate its phosphorylating capability. This means that the drug can cut the effect of that particular kinase on molecules further downstream. Hence, the drug can be modeled as an inverted input to an ‘AND’ gate at the target point of the boolean network. This schematic modeling of drug intervention is shown in Fig. 1(c).

In this paper, our goal is not to derive the mathematical expression for the optimal drug intervention policy, since most mathematically derived policies may be difficult, or impossible, to biochemically implement. Instead, our objective is to model known and well tested cancer drugs separately and then to find the best sub-optimal combination of drugs for a particular cancerous network. The method is described in detail in section V, where it is applied to a biological example.

### V. Biological Example: Growth Factors and Cellular Signal Transduction

Some of the most important cancer related signal transduction pathways are the growth factor activated pathways shown in Fig. 2. Here the inputs $EGF$, $HBEGF$, $IGF$ & $NRG1$ denote the mitogens or growth factors, and the outputs are $SP1$, $ELK1-SRF$, $ELK4-SRF$, $FOS-JUN$, $CCND1$, $BCL2$ and $BCL2L1$. Of these, $SP1$, $ELK1-SRF$, $ELK4-SRF$, $FOS-JUN$ are transcription factors of interest and $CCND1$, $BCL2$ and $BCL2L1$ are proteins that are indicative of ‘proliferation’ and ‘apoptosis’ respectively. In normal cells, growth and division are initiated only by proper signaling from different growth factors including
EGF, HBEGF, IGF, NRG1 etc. These pathways collectively work as a conduit of communication between the cell membrane and the genome to regulate the transcription of some genes important in cell cycle control. Mutations and alterations (faults) in these pathways actually lead to different types of cancer. In [9], a detailed description of the GF-activated pathways along with their role in cell cycle regulation and cancer development is presented. These important pathways can be modeled as a combinatorial boolean circuit which is shown in Fig. 3(a).

Fig. 2. A Schematic Diagram of the Growth Factor Signaling Pathways

A. Modeling Faults and Therapeutic Interventions Using the Boolean Circuit

Any mutation of any gene or post transcriptional modification of the corresponding protein can constitutively turn ‘ON’ or ‘OFF’ that particular protein. This fits in precisely within the stuck-at fault paradigm considered in section III. For the sake of simplicity, in our growth factor pathways case study, we will consider only single faults of the stuck-at type. In addition, we will only consider the stuck-at faults which can lead to cancer. For the Boolean circuit shown in Fig. 3(a) the possible locations for the different stuck-at faults, which can induce proliferation and stop apoptosis, are shown in Fig. 3(b). The numbers are color coded to distinguish between the ‘stuck at 1’ and ‘stuck at 0’ faults. Specifically, the black numerals refer to the stuck-at-one faults while the red numerals refer to the stuck-at-zero faults.

As discussed in section IV, a drug targets particular enzymes along the pathways and cuts off the connectivity of that enzyme to the downstream proteins. This connection cleavage can be achieved via various mechanisms. For instance, the drug may have the capability to bind a target protein and inhibit it from undergoing phosphorylation. For our case study, we consider six potent cancer drugs. Our objective here is not to study their detailed mechanisms of action. Instead, we are interested in using the knowledge from biologists to mark in their intervention locations and corresponding activities on the Boolean circuit of Fig. 3(a). This leads to the effects shown in Fig. 3(c). Such pictorial representation of the drug activity information is useful.

For instance, let us consider the drug ‘lapatinib’ which is known to work on EGFR, ERBB2, EGF or ERBB3 by inhibiting the signaling capabilities of these receptor tyrosine kinases. From Fig. 3(c), one can conclude that the drug ‘lapatinib’ will likely be more effective for the treatment of cancers caused by mutations in the receptor tyrosine kinases although it will probably be ineffective against cancers caused by mutations in the Ras protein, which lies further downstream. Two central objectives of this paper are: (i) to use the information contained in Fig. 3(b) to group the numbered faults into different classes; and (ii) to use the information in Fig. 3(c) to predict which set of drugs/drug combinations would be most effective against a particular fault. These objectives are pursued in the next two subsections.

B. Fault Analysis and Classification

From Fig. 3(b), we see that there are 24 possible fault locations. Alternatively, we could have arrived at the fault locations based on our biological understanding. As already indicated, in this paper we will be confining ourselves to the analysis of single faults only. So, for our purposes, the fault can be any one of the 24 faults in the figure. Carrying forward the discussion from section III, we use $f_i^j$ to denote the fault at the $i^{th}$ location. Then the sample space for the single fault modeling can be defined as
\( F^1 = \{ f_1^1, f_2^1, f_3^1, \cdots, f_{24}^1 \}. \) Here the superscript 1 refers to the fact that we are considering only single faults. Now if \( f_1^1 \in F^1 \) occurs, an input vector \( t \) detects the fault iff the output vector \( Z \) in the faultless system differs from the output vector \( Z_{f_1^1} \) in the faulty system. Mathematically \( Z(t) \neq Z_{f_1^1}(t) \). If we cannot find such an input \( t \), we say the fault is undetectable. In the circuit shown in Fig. 3(b), the only input vector which can detect any \( f_1^1 \in F^1 \) for this particular network is \( V = 00001 \) which is achieved with \( EGF = 0, HBEFG = 0, IGF = 0, NRG1 = 0 \) and \( PTEN = 1 \). This result is not at all surprising. Indeed, when there is no growth factor outside the cellular membrane and also the tumor suppressor protein \( PTEN \) is active, we expect to see all the proliferative transcription factors and anti-apoptotic factors deactivated or turned ‘OFF’. However, if there are faults (mutations) in the signal transduction pathways, we could see proliferation even in the absence of active input signals (mitogens).

1) Single Fault Simulation: In this subsection, computer analysis for the single fault model of the circuit in Fig. 3(a) is presented. The single fault model of the boolean circuit is shown in Fig. 3(b). The input vector is \( V = [EGF, HBEFG, IGF, NRG1, PTEN] \). Each input can take binary values. For this simulation we take \( V = 00001 \). The output vector is \( Z = [FOS-JUN, SP1, SRF-ELK1, SRF-ELK4, BCL2, BCL2L1, CCND1] \). For the fault free circuit we get the output \( Z(00001) = 0000000 \). Now for the 24 different faults which may induce cancer in the given circuit, the outputs are tabulated in Fig. 4(a).

![Fig. 4. Single Fault Simulation and the equivalent fault groups for input = 00001](image)

2) Fault Classification: From the outputs shown in Fig. 4(a), we can classify the faults into different groups of equivalent faults. Faults which generate the same output vectors for a particular test input vector are called ‘equivalent faults’ with respect to that input test vector. The information in Fig. 4(a) leads us to sets of equivalent faults for the test input vector \( V = 00001 \). The equivalent fault groups along with their corresponding outputs are shown in Fig. 4(b).

From Fig. 4(b), it is clear that any fault in the locations 13, 14, 15 cannot be detected from the output since the corresponding output is the same as that for the fault-free case. Hence, this class of faults is said to be ‘undetectable’. It is true that ‘undetectable faults’ cannot be compensated for based on observations of the output. However, this is not a major concern especially if we are only interested in the behavior of the outputs.

C. Simulation Results for Drug Intervention

Since we have only the 6 available drugs, we define a drug vector of length 6 as follows. If a particular drug is applied it is assigned the value 1, otherwise it is assigned the value 0. Consequently, the drug vector space has cardinality \( 2^6 = 64 \). The simulation is carried out for all of the possible faults, taken one at a time, and for each of the 64 different drug vectors, and the corresponding outputs are computed. The drug vector is defined by \( \{lapatinib, AG825, AG1024, U0126, LY294002, Temsirolimus\} \).

1) Continuous real mapping of the output vector: We take the same input vector (00001) that we have previously used for the fault analysis. In the no fault case, with the drug vector 000000 we get the output 000000 which is certainly non-proliferative. However, in the presence of faults, the outputs will be different. The objective of this simulation is to determine the best possible drug sequence which can nullify the effect of the fault, i.e, produce an output close to 000000 or away from the proliferative output 1111111. We note that although all the output vectors are represented as binary numbers, assigning the usual binary weights to the digits here does not make any biological sense. In other words, 1111111 here does not really mean 127 or 0001111 does not really mean 7. Consequently, we need to determine some transformation which will map these \( 128 = 2^7 \) output vectors to a continuous real number scale in a biologically meaningful way. One way to do this is to proceed as follows.

If we examine the components of the output vector, we see that out of the 7 components, 4 are transcription factors which express (turn ON) the important genes leading to proliferation. The remaining 3 components capture the activation status of some key proteins in the cytoplasm. So, these two groups of outputs have different biological significance and should be encoded separately. A possible mathematical transformation on the output vectors is described next. The output vector is \( OUTPUT = [FOS-JUN, SP1, SRF-ELK1, SRF-ELK4, BCL2, BCL2L1, CCND1] \).

Now suppose we take the number of active transcription factors as the first variable and the number of active remaining outputs as the second variable. The mathematical transformation makes use of these two variables as described in Eqn. 1 & Eqn. 2 below:

\[
\text{Output} = [a, b, c, d, e, f, g] \Rightarrow \\
\text{First} = a + b + c + d \Rightarrow \\
\text{Second} = e + f + g \tag{1}
\]
\[ P = \text{First} \times \text{Second} \]
\[ S = \text{First} + \text{Second} \]
\[ \psi(\text{Output}) = \alpha P + (1 - \alpha)S, \quad (2) \]

where \( \alpha \in (0, 1) \) is a design parameter. With \( \alpha \) chosen as 0.5, the function \( \psi \)'s values over the full sweep on the drug vectors and faults are shown in Fig. 5. Here the fault numbers and drug vectors are listed along the horizontal and vertical directions respectively. The results are color coded for easier visualization, and the color codes used are tabulated on the right side in Fig 5.

2) Interpretation of the Result: From the output tables and the color codes we see that the color green corresponds to non-proliferation while the color red corresponds to a high chance of proliferation even in the absence of mitogenic signals. So, the best drug vector will be the one which can drive the largest number of faulty circuits towards non-proliferative (green) outputs. For example, the drug vector 000110 drives all of the faults 1 – 6 to green and most of the remaining boxes along that row away from red. So, the drug combination of U0126 and LY294002 will likely be effective in producing a non-proliferative output. From Fig. 5, also note that neither of these drugs by themselves would have been able to achieve the same effect. Another point to note is that there can be faults (like fault 18 in Fig. 5) whose output cannot be altered using any drug sequence. This is not at all surprising and is consistent with the pathway information that we have. Indeed, the fault location 18 is at the ERK1/ERK2 protein and there is no available drug in our list downstream of that protein. Consequently, no drug in this particular case study would be able to block the effect of a mutated ERK1/ERK2 protein.

VI. CONCLUDING REMARKS

In this paper, we have presented a new approach for designing cancer therapies based on available pathway information and the manner in which drugs target specific pathway connections. Relevant pathway information is first used to produce Boolean networks whose state transitions are consistent with the given pathway information, or minor variations of it. The Boolean network is then realized as a digital circuit which is used to (i) enumerate all the possible fault scenarios; (ii) classify the faults into different classes based on their responses to a particular test input; and (iii) prescribe an appropriate course of therapeutic action, tailored to the fault or set of faults that has occurred. To keep the discussion focused on practical translational science, the entire presentation has been carried out specific to the growth factor signaling pathways. These pathways are widely studied in the context of cancer and also have a number of associated drugs known to target them at different points. Because the entire procedure is embedded in classical circuit theory, it can be implemented using slight variations of existing electrical engineering software.

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