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NFκB pathway modeling for Optimal Drug Combination Therapy on Multiple Myeloma

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***Abstract*—**NFκB activation plays a crucial role in anti-apoptotic responses in response to the apoptotic signaling during tumor necrosis factor (TNFα) stimulation in Multiple Myeloma (MM). Although several drugs have been found effective for the treatment of MM by mainly inhibiting NFκB pathway, there are no any quantitative or qualitative results of comparison assessment on inhibition effect between different single drugs or drug combinations. Computational modeling is becoming increasingly indispensable for applied biological research mainly because it can provide strong quantitative predicting power. In this study, a novel computational pathway modeling approach is employed to comparably assess the inhibition effects of specific single drugs and drug combinations on the NFκB pathway in MM, especially the prediction of synergistic drug combinations.

***Keywords*—**Computational modeling, Drug combination, Inhibition effect, Multiple Myeloma, NFkB pathway.

I. Introduction

Multiple myeloma (MM) is the second most common hematologic malignancy, with about 15,000 new cases per year in USA, and remains incurable with a median survival of 3 to 5 years [1]. It is a plasma cell malignancy characterized by complex heterogeneous cytogenetic abnormalities. The bone marrow microenvironment promotes MM cell growth and resistance to conventional therapies [2]. Failure of myeloma cells to undergo apoptosis plays an important role in the accumulation of myeloma cells within the bone marrow. Several anti-apoptotic proteins and anti-apoptotic signaling cascades have been identified that contribute to the anti-apoptotic phenotype of the myeloma cell [1]-[3]. Actually, adhesion of multiple myeloma cells to bone marrow stromal cells (BMSCs) triggers cytokine-mediated tumour cell growth, survival, drug resistance and migration. MM cell binding to BMSCs upregulates cytokine secretion from both BMSCs and tumour cells. These cytokines activate major signaling pathways: extracellular signal-regulated kinase (ERK); Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3); phosphatidylinositol 3-kinase (PI3K)/AKT; and nuclear factor κB (NFκB). These pathways not only promote growth, survival and migration of MM cells, but also confer resistance to conventional chemotherapy. Targeting these mechanisms or inhibiting these pathways offers a potential therapeutic strategy to induce the apoptosis of MM cells and overcome drug resistance.

It has preciously shown that canonical NFκB pathway in MM cells is mainly activated by TNFα [4][5]. Several drugs effective for the treatment of MM, including bortezomib (BZM), thalidomide, lenalidomide and arsenic trioxide (ATO), have been found to block NFκB activation [6]. Therefore, blockade of TNFα-induced NFκB signaling by different single drugs or different drug combinations represent a novel therapeutic strategy in MM. However, at least to the best of our knowledge, there are no any quantitative or qualitative results of comparison assessment on inhibition effect between these different single drugs or drug combinations. So we do not know how to choose drugs to inhibit the NFκB pathway, or we do not know which drug is the best one? What is the best dose for single drug? What is the best ratio and dose for some drug combination? How about the inhibition effect if a drug combination is chosen with fixed ratio and dose? If one want to answer these questions, he or she should predict and compare all the inhibition effects previously by using any kind of methodology, otherwise a mass of biological experiments should be designed and implemented for this purpose.

Computaional modeling is becoming increasingly indispensable for basic and applied biological research. Essentially, a mathematical model is a systematic representation of biological systems, whose analysis can confer quantitative predicting power. One of the common applications of mathematical modeling is to analyze cellular networks systematically and another use of mathematical modeling has been demonstrated in devising strategies to control cellular dynamics. Therefore, this kind of computational modeling is suitable for signal pathway analysis and drug combination response analysis in our study.

In this paper, we try to employ the methodology of computational modeling to assess or predict the specific drug responses on inhibition of NFκB pathway in MM. We firstly develop the computational model qualitatively, and then collect some specific experimental data to estimate the model parameters, and further design specific simulation protocols to predict the responses for single drugs or drug combinations. The rest of the paper is organized as follows. The details of

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methodology including the development of the pathway model and simulation protocols are present in section II. Section III provides the simulation results while section IV concludes this study.

II. Methodology

2.1 NFκB signal pathway description

In most cell types, NFκB resides in the cytoplasm and is inactivated by its association with IκB family inhibitors. In MM, the key cytokine TNFα binds to its receptor, leading to the recruitment of its adaptors and TRAFs, to form a complex which phosphorylates and activates IKKK, and the phosphorylated IKKK further activate IKK, leading to the phosphorylation and subsequent degradation of IκBα by 26s proteasome. The direct consequence is the translocation of NFκB from the cytoplasm into the nucleus, leading to transcription of target genes. NFκB also activates its own inhibitor, IκBα, giving rise to a negative feedback control [7]. The whole pathway can be divided by four modules, which are successively TNFα receptor system, IKK phosphorylation cascade system, the cytoplasmic and nuclear portion of IKK-IκB-NFκB system. Fig. 1 provides more details about this signal pathway with specific inhibition protocols in MM.

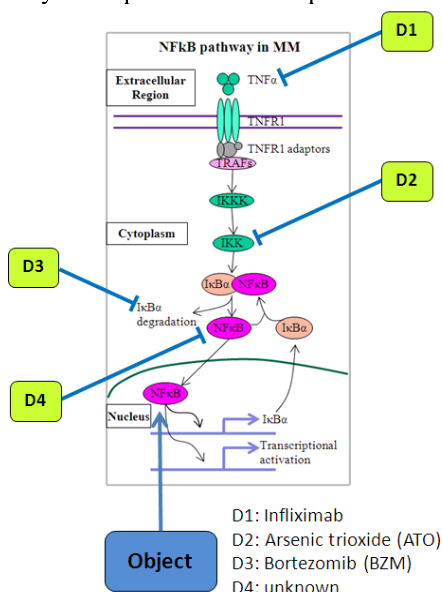

Fig. 1 Qualitative NFκB pathway with specific inhibition protocols in MM.

2.2 Development of a computational model

To understand the interactions of various molecular species in the NFκB activation module, we modeled this dynamical system using a set of ordinary differential equations (ODEs) by mass action kinetics and some systematic parameters on this model are fitted using some published data. Based on the qualitative network (Fig. 1), a graphical model was constructed (Fig. 2) and this model give us all the details about the considered NFκB pathway including all the reactions and all the molecules related to the pathway and all the symbols of parameters in the ODEs model. In fact, this model provides us a clear idea on how to build the whole ODE system for this model. Further, a detailed computational model with ODEs system was developed based on this graphical model.

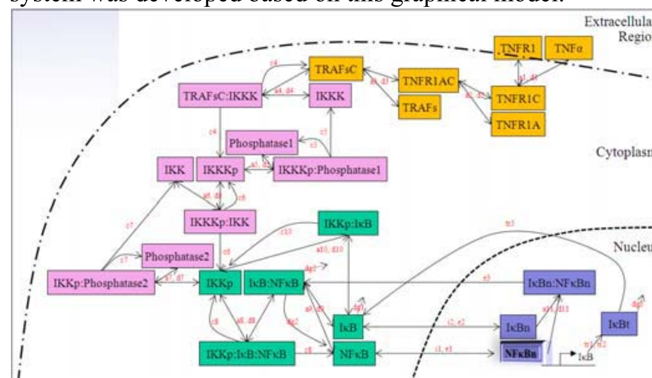

Fig. 2 The graphical model for the reactions of NFκB pathway in MM. Different functional components in the pathway are shown in different colors. Yellow denotes the TNFα receptor system; pink denotes the IKK phosphorylation cascade system; green and blue denote the cytoplasmic and nuclear portion for IKK-IκB-NFκB system, respectively. The parameter symbols are also shown at the side of the corresponding reaction arrows.

2.2.1 Basic assumptions

To facilitate the development of computational model for NFκB pathway in MM, the following basic assumptions were made.

(a) The cytoplasm can be considered as a uniform mixture in which all component molecules are uniform distributed and they can access to each other with equal probability. And this assumption reduces the complexity of biochemical reaction modeling by considering only temporal changes of molecules rather than their localization.

(b) The law of mass action is used for all the reactions in our model, and the reactions in the pathway include binding-dissociation reaction and the enzymatic reaction. Although the commonly used reaction model for enzymatic reaction is the Michaelis-Menten equation which is the famous simplification of the law of mass action, we only use the classic law of mass action for all the enzymatic reactions in the pathway modeling.

(c) In the pathway, IKKα and IKKβ are called the same name IKK and we don't explore their different functions no matter what in canonical or noncanonical NFκB activation pathway.

(d) We did not consider effects of inhibitor proteins IκBβ and IκBε because, under constitutive activity of IKK, NFκB does not directly induce re-synthesis of these proteins, so their presence becomes negligible in the steady state [8].

(e) We did not consider the reactions of the binding and dissociation between NFκB and the complex of IκBα and IKK which were also mentioned in [9].

(f) We did not specify the components about NFκB heterodimer isoforms and we just simply consider the single NFκB isoform p50/p65 in our model similarly as considered in other literatures.

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2.2.2 The ODE system of the model

From the description of Fig. 2, using the law of mass action we can build the whole ODE system for our considered NFκB model in MM. Generally, there are total 26 components in the model and, therefore, 26 ODEs, and the total number of the parameters in the model is 39. Due to the limitation of space, we have not list the ODE model in this manuscript, but readers can get the clear idea for the ODEs by referring to Fig. 2 in which all the reactions and systematic parameters are presented clearly. It is worth noting that this ODE model is motivated, but different, from various computational models of NFκB pathway in literatures [8]-[10]. By referring to these literatures, firstly we collect the parameter values and initial concentrations of the components. As expected that the simulation result from the ODE model with these parameter and initial value sets for IKKp (i.e. phosphorylated IKK), cytoplasmic IκB and nuclear NFκB presented an oscillation phenomenon.

2.2.3 Experimental data

Although there are a few computational models for the NFκB pathway and most of the model parameters have been identified [8]-[10], all these models did not focus on the specific MM cell lines. In this study, our specific purpose will be focused on NFκB pathway in MM. So it is necessary to validate and rectify the model obtained from the literatures using the real experimental data from human MM cell lines. For this purpose, we have collected some kinetic experimental data from literatures. Herein we obtained some time-course experimental data on protein expression for key components of NFκB pathway in MM, including western blot data for cytoplasmic IκB and electrophoretic mobility shift assay (EMSA) data for nuclear NFκB. The detail for the experimental data of MM can be seen from Fig. 3 and all of these data were focused on the human MM.1S cell line [4][5].

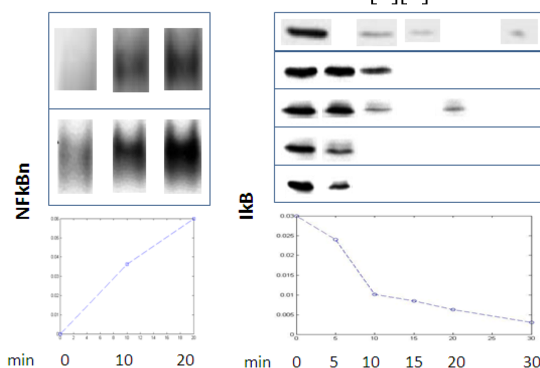


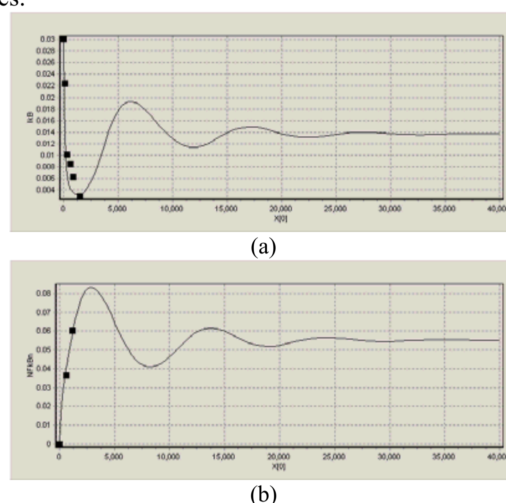
Fig. 3 Experimental data from literatures. The left subfigure presents the EMSA data for nuclear NFκB including two samples with three time-points, and the right subfigure shows the western blot data for cytoplasmic IκB including five samples with up to six time-points. The above subfigure shows the original experimental data and the corresponding quantitative data based on the mean value is shown in the below subfigure.

2.2.4 Parameter estimation

A direct attempt to use the existed model parameter set to describe our experimental data did not yield satisfactory results and the result has not been shown in this manuscript due to the limitation of space, which was not unexpected since different experimental models can yield different model parameters, and also determination of the model parameters of signaling pathways is subject to uncertainty and non-identifiability of kinetic parameters of the enzymes involved in signaling as mentioned in [11]. We therefore carried out parameter fitting of the model to the experimental data obtained on the human MM.1S cell line as described in the above experimental data subsection. The whole parameter estimation procedure in this study is referred to the method presented in [11] and the optimization procedure is implemented using DBsolve software with the version 7.48 [12][13] by minimizing the following objective function,

$$\Delta = \sum_{i=1}^{6}\left[Y_1^{(\mathrm{th})}(t_i) - Y_1^{(\mathrm{exp})}(t_i)\right]^2 + \sum_{i=1}^{3}\left[Y_2^{(\mathrm{th})}(t_i') - Y_2^{(\mathrm{exp})}(t_i')\right]^2, \quad (1)$$

where $Y_1^{(\mathrm{th})}(t_i)$ and $Y_1^{(\mathrm{exp})}(t_i)$ represent the theoretical and experimental data on the concentrations of IκB at time-points $t_i = 0$, 5, 10, 15, 20 and 30 minutes; similarly, $Y_2^{(\mathrm{th})}(t_i')$ and $Y_2^{(\mathrm{exp})}(t_i')$ represent the theoretical and experimental data on the concentrations of nuclear NFκB at time-points $t_i' = 0$, 10 and 20 minutes.



(a)

(b)

Fig. 4 Data fitting for cytoplasmic IκB (a) and nuclear NFκB (b). Black box and solid curve represent the experimental data point and simulated curve, respectively. In the coordinate system, X and Y axes present time and concentration, respectively.

In this procedure, the square error between the experimental and theoretical data is adopt for the cost function and then the Hook-Jeevse algorithm [14] is adopt to minimize the cost function in (1). It is worth noting that all the parameters for TNFα receptor system and IKK phosphorylation cascade system and all initial concentration values in the pathway are kept the same as those in the literatures, and we use the method to fit the parameters for IKK-IκB-NFκB sub-system to the experimental MM data, because the reactions in this module is specifically dependent on the cell line. Therefore, the total number of estimated parameters in this study is reduced to 21. The fitting curves on the model can be seen from the Fig. 4 which shows the fitting results for the cytoplasmic IκB and nuclear NFκB concentration. The summary for the estimated parameters is not listed here due to the limitation of space.

Although there are some differences between our fitted model and the model collected from literatures, the fitted model can fit the real experimental data well. Therefore, we will use this model for our further analysis in this study.

2.3 Simulation protocols

Once we have built the mathematical model for NFκB pathway, different drugs with different targets can be modeled into the constructed ODE model by specific mechanism, and then we can study the different inhibition profiles on single drugs or drug combinations by simulating the model, while this simulation protocol is also able to predict the optimal combination on these studied different drugs. In this study, we just focus on the following four kinds of drugs, i.e. Infliximab, Aresenic tricide (ATO), Bortezomib (ZBM) and the fourth drug with unknown name here and we just call them D1, D2, D3 and D4 for the purpose of simplification, and the targets are TNFα, IKKp, IκBα degradation and cytoplasm NFκB, respectively. Fig. 1 provides the rough idea for these inhibitors protocols.

2.3.1 Inhibition mechanisms of drugs and drug modeling

D1 (i.e. Infliximab) is a monoclonal antibody against TNFα and it has been approved by the FDA for the treatment of many diseases. It works by binding to TNFα, so it can inhibit the NFκB pathway. In this study, we assume that D1 competitively inhibit TNFα with binding kinetics which is the same as that of the natural reaction involving TNFα and TNFR1, that is, the binding rate is set as a1 and the dissociation rate is set as d1, as seen from Fig. 2. For D2 (i.e. ATO), it is an inorganic compound and a traditional Chinese medicine, and it also has been approved by the FDA for treatment of certain leukemias. Based on the information from DrugBank website, ATO inhibits the NFκB pathway by targeting the protein IKKp (phosphorylated IKK). So the effect of administering D2 is modeled in a similar manner as D1, i.e. D2 is assumed to competitively inhibit IKKp with the same binding kinetics as the reaction between IKKp and cytoplasmic complex IκB:NFκB. Based on the parameter symbol description in Fig. 2, the binding and dissociation rate between D2 and IKKp are set as a8 and d8, respectively. For D3 (i.e. BZM), it is the first therapeutic proteasome inhibitor to be tested in human and it has been approved in the US for treating relapsed MM. D3 works to inhibit the degradation of IκBα by blocking the activity of the proteasome. For simulating this drug's effect, we could not introduce an additional component to the system as D1 and D2 do because the degradation processes of IκBα is not explicit in the ODE model. By referring to [8], we can adjust the corresponding parameters in the terms for NFκB released after the degradation of IκBα, and the individual terms for IκBα and NFκB:IκBα molecules rescued from degradation. In order to describe the dose effect of D3 on the terms mentioned above, we introduce a Hill-type function to describe the inhibition rate for IκBα degradation by D3, which is defined as follows,

$$r = (D3)^{k0} \Big/ \left[K0 + (D3)^{k0} \right] \cdot$$

Where D3 denotes the concentration of drug D3 and k0 is set by 4 and K0 by 10e-10, and the corresponding curve can be seen from Fig. 5 in which the 50% inhibition concentration is about 0.0055 μM. Referred to Fig. 2, all the terms related to D3 are changed as follows, d10=d10+r*c10, c10=(1-r)*c10, d8=d8+r*c8 and c8=(1-r)*c8. At last, for D4 we just assume that there exists this kind of drug to inhibit the translocation of NFκB from cytoplasm to nucleus by binding mechanism [8], which is modeled similar as previously, i.e. it competitively inhibits cytoplasmic NFκB with the same binding kinetics as the reaction between NFκB and IκBα with rate constants a9 and d9.

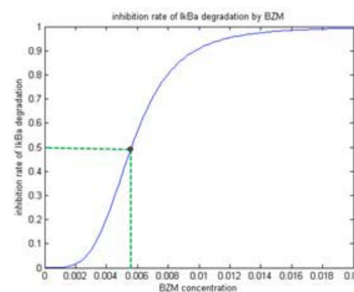


Fig. 5 Inhibition rate curve for IκBα degradation by BZM.

2.3.2 Inhibition percent curves and single-drug evaluations

Once we have modeled the drugs into our ODEs system, we can simulate the whole model by changing the single drug dose. Then we can predict the different steady output values for nuclear NFκB corresponding to the input with different single drug doses. By comparing these values with the control values (i.e. without drug input), the inhibition percent curves on single drugs can be calculated, meanwhile this kind of inhibition curve can be used as reference to asses the single drug effect.

2.3.3 Combination index and drug combination evaluations

It is well-known that, for drug combination, the action of two or more drugs working together maybe can produce an effect greater than the expected combined effect of the same agents used separately, and we call this case as synergy combination. Otherwise, we call the combination as additive (equivalent effect) or antagonism (less effect). In addition, different combinations for the same two drugs with different combination ratios sometimes can produce totally different effects, such as one combination is synergistic but another is antagonistic. Therefore, it is significant to predict the synergy combinations using computational model. Although a number of available mathematical combination indexes can be used to assess the effect of drug combination, in this study we prefer to select Bliss independence [15], because it is not only a famous synergy quantification method but also extremely convenient to calculate . We briefly introduce the Bliss independence idea as follows. Let f1, f2 and f12 denote the effects from single drug 1, single drug 2 and the drug combination drugs 1&2, then it is defined the combination as Bliss synergy if f12 > f1 + f2 – f1*f2, Bliss additive if f12 = f1 + f2 – f1*f2, and Bliss antagonism if f12 < f1 + f2 – f1*f2. In this study, following the above Bliss independence idea, we define a Bliss combination index as follows, CI_{Bliss} = (f1 + f2 – f1*f2)/f12. Given threshold_up and threshold_down, the effect of drug

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combination is defined as synergy if CI_{Bliss} < threshold_down, and antagonism if CI_{Bliss} > threshold_up, otherwise additive. In this study, the thresholds are fixed as threshold_down = 0.99 and threshold_up = 1.01, i.e. 1% perturbation by noise is tolerated. In the simulation procedure, the Bliss combination index will be used to assess the synergy of drug combinations.

III. Results

3.1 Nearly no effect for D1

It is shown from the single drug D1 simulation with the normal binding rate that the inhibition effect is negligible regardless of the huge and unreasonable dose as 1000 μM, as can be seen from the bottom curve in Fig. 6(a). It can be guessed spontaneously that this result may be due to the low rate of drug binding, so we reset the binding rate by 5, 10 and 100 times higher than normal one, then run the simulation again. The results in Fig. 6(a) show that the inhibition effects are still very low and just about 2%, 4%, 8% and 34% corresponding to different binding rates at fixed 500 μM dose. So the effect of binding rate is not enough significant to explain the ineffectiveness of D1. By another simulation, we seek the relationship between nuclear NFκB concentration and initial concentration of ligand TNFα. The predicted result shows that about 0.0003 μM, 0.001 μM and 0.0048 μM TNFα, i.e. about 0.15%, 0.5% and 2.4% of normal initial TNFα dose 0.2 μM, can sufficiently lead to 50%, 70% and 90% nuclear NFκB output comparing to normal case, as can be seen in Fig. 6(b). This result suggests that the stimulus TNFα with 0.2 μM concentration is largely redundant to stimulate the production of nuclear NFκB, which is consistency with the clinical result of high expression of TNFα on MM. Therefore, D1 is not effective to inhibit the NFκB pathway in MM due to the large redundancy of TNFα expression.

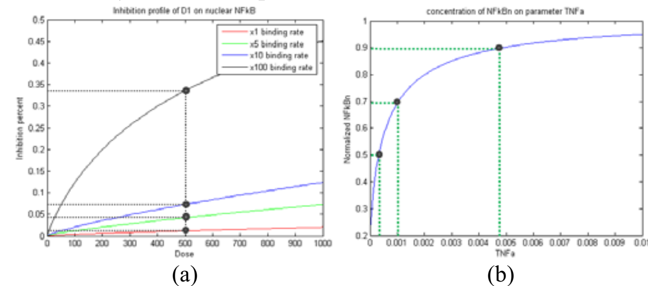

Fig. 6 (a) several inhibition profiles of D1 on nuclear NFκB corresponding to different binding rates; (b) normalized nuclear NFκB concentration curve on the initial concentration of TNFα.

3.2 Different inhibition profiles on D2, D3 and D4

It is shown from the inhibition profiles in Fig. 7 that there are different profiles for D2, D3 and D4. It can be concluded that D2 and D4 share the similar inhibition profile with hyperbolic type function, but D3 has the different inhibition profile with sigmoidal type function. Note that there are some extremely different properties between these two types of functions, as pointed out in Fig. 7 that tripling dose just increases the inhibition effect 20% and 30% for D2 and D4, but increases 15 fold of the effect for D3. From this property, to certain extent we can conclude that D3 is much better than D2 and D4 if we want to choose the single drug to inhibit the NFκB pathway. Of course, we omit some other factors, such as side-effect, economical consideration, and so on. It is worth noting that this drastic difference between these two types inhibition profiles underscores the difficulty to predict by inspection what would be the "additive effect" when two drugs are combined at a given ratio. It is also noting that the IC_{50} values, i.e. the drug concentration value with 50% inhibition effect, for each single drugs can be easy to predict from the simulated dose-effect curves as shown in Fig. 7, and these values of IC_{50} usually are applied in the drug combination valuation.

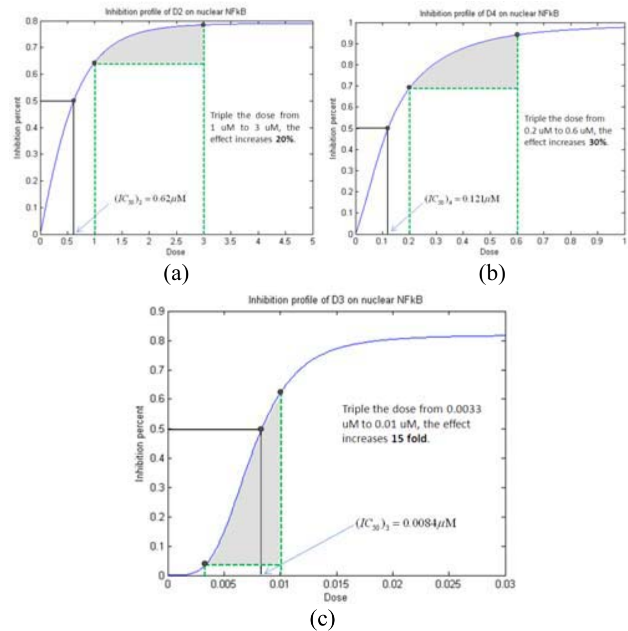

Fig. 7 Different inhibition profiles on nuclear NFκB production by different single drugs D2 (a), D4 (b) and D3 (c). Two types of profile functions are presented, in which D2 and D4 share the similar hyperbolic type function but sigmoidal type function for D3. Inhibition concentration IC_{50} values for each drug are also presented in each inhibition profile curve.

3.3 Synergy prediction for drug combination

Based on the prediction of inhibition profiles for D2, D3 and D4 shown in Fig. 7, we choose suitable ranges of dose for each drug to analysis the drug combinations, i.e. 0~4 μM for D2, 0~0.02 μM for D3 and 0~1 μM for D4. It is worth noting that the chosen dose ranges are consistent with biological consideration at least for D2 (ATO) and D3 (BZM). We evenly divide each range into 100 equal portions and then calculate the corresponding Bliss combination index defined previously for each combination. Note that the total number of dose combinations for each two-drug combination is equal to 10,000. The simulation results for heat-maps of Bliss combination index are shown in Fig. 8. Note that the threshold parameters, i.e. threshold_up and threshold_down previously defined in the Bliss evaluation are fixed at 1.01 and 0.99 respectively, i.e. 1% perturbation by noise is considered here, of course, other perturbations with more or less intensity are also considered for testing and the similar results also can be obtained. It can be

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easily found from Fig. 8 that all of three different combinations, i.e. D2&D3, D2&D4 and D3&D4, have different inhibition profiles. For D2&D3, most of combinations are detected as antagonistic effect, as most regions display in red color in the corresponding heat map in Fig. 8, and other small parts of combinations are detected as additive effect, and this result is also applicable if we just focus on the region within IC_{50} values. Note that this result is consistent with our previous study in [16][17] which suggests that although the synergy occurs on proliferation inhibition of human MM cells for D2&D3 drug combination treatment, this synergy effect is mainly reflected in JNK pathway rather than NFκB pathway. For D2&D4, synergistic effect is detected for most combinations fortunately, while no antagonistic effect is detected and all the remains are additive. Moreover, almost all of the combinations within IC_{50} region are shown as synergistic. For D3&D4, all of three types of combination effects are detected, but just additive and antagonistic effects are shown within IC_{50} region. From these combination profiles, it can be concluded that the D2&D4 combination is the best choice, D2&D3 is the worst one and D3&D4 is the mediacy, meanwhile the predicted synergistic regions in D2&D4 and D3&D4 combinations are potentially helpful to conduct the clinical drug combination experiment.

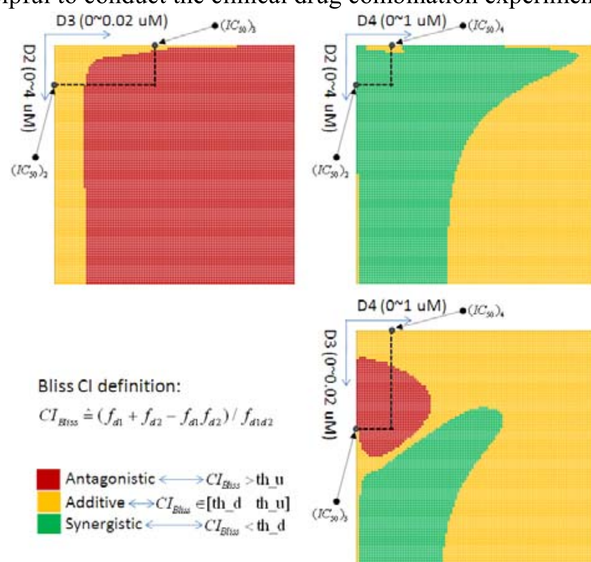

Fig. 8 Heat maps of different drug combinations, i.e. D2&D3, D2&D4 and D3&D4, based on Bliss combination index to predict the synergistic region for combination. Different types of combination effects are shown in different color in the heat maps, and the description of definitions for Bliss combination index and three types of combination effects are also shown in the bottom-left.

IV. Conclusion

This study focuses on the inhibition analysis of NFκB pathway with multiple inhibitors in MM using computational modeling. The specific experimental data related to the specific pathway for multiple myeloma is collected from literatures to construct the computational pathway model, and then the considered drugs are added into the model based on the corresponding inhibition mechanisms. Through the well-designed simulation protocols, the computational model can be employed to predict the inhibition profiles of single drugs and drug combinations, especially the synergistic effects of drug combinations. By the way, the further simulation study on this model will be focused on other quantification methods for assessment of drug combination synergy, such as Loewe synergy [18] and strong nonlinear blending [19], in order to test the consistency of the prediction results. In addition, the effect profile of pathway oscillation by the drugs will also be considered in the following study.

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