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Mathematical modeling of drug resistance due to KRAS mutation in colorectal cancer

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HIGHLIGHTS

- KRAS mutated cells in a colorectal tumor is the sign of failure of moAb therapy.
- KRAS mutations in a tumor can also make the wild-type cells ineffective to therapy.
- KRAS sub-populations prevent the chemosensitization due to moAb drugs.
- Patient immune strength has no impact on therapeutic process of KRAS mutated tumors.
- Cetuximab cannot be recommended as a first-line therapy for KRAS mutated tumors.

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ABSTRACT

The most challenging task in colorectal cancer research nowadays is to understand the development of acquired resistance to anti-EGFR drugs. The key reason for this problem is the KRAS mutations appearance after the treatment with monoclonal antibodies (moAb). Here we present a mathematical model for the analysis of KRAS mutations behavior in colorectal cancer with respect to moAb treatments. To evaluate the drug performance we have developed equations for two types of tumors cells, KRAS mutated and KRAS wild-type. Both tumor cell populations were treated with a combination of moAb and chemotherapy drugs. It was observed that even the minimal initial concentration of KRAS mutation before the treatment has the ability to make the tumor refractory to the treatment. Minor population of KRAS mutations has strong influence on large number of wild-type cells as well rendering them resistant to chemotherapy. Patient's immune responses are specifically taken into considerations and it is found that, in case of KRAS mutations, the immune strength does not affect medication efficacy. Finally, cetuximab (moAb) and irinotecan (chemotherapy) drugs are analyzed as first-line treatment of colorectal cancer with few KRAS mutated cells. Results show that this combined treatment could be only effective for patients with high immune strengths and it should not be recommended as first-line therapy for patients with moderate immune strengths or weak immune systems because of a potential risk of relapse, with KRAS mutant cells acquired resistance involved with them.

1. Introduction

The World Health Organization (WHO) declared colorectal cancer (CRC) as the second most common cause of cancer mortality in Europe (http://www.euro.who.int/en/health-topics/non-communicable-diseases/cancer/news/news/2012/2/early-detection-of-common-cancers/colorectal-cancer). Monoclonal antibody (moAb) has been introduced as the most promising treatment to fight disease. The development of acquired resistance to the moAb drug, due to KRAS mutations, makes the problem very complex in terms of personalized treatment. We have developed a system of non-linear ordinary differential equations (ODEs) to model the impact of KRAS mutations on the moAb and chemotherapy combination treatment of colorectal cancer. We have studied the behavior of moAb and chemotherapy with respect to patient immune responses and we have explored one moAb drug as a potential candidate for first-line therapy of CRC, in combination with chemotherapeutic drug.

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1.1. Colorectal cancer therapy and KRAS mutations

Monoclonal antibodies are a major breakthrough in CRC therapeutic research because of their anti-EGFR activity (Deschoolmeester et al., 2010; Repetto et al., 2005). The Food and Drug Administration (FDA) approved moAb drugs for colorectal cancer including cetuximab and panitumumab (Gschwind et al., 2004). These drugs produce promising results when administered in combination with chemotherapeutic drugs (Van Cutsem et al., 2007; Martinelli et al., 2009). They kill tumor cells in three ways: by directly blocking the EGFR pathway, by enhancing the activity of chemotherapeutic drugs and by enabling antibody-dependent cellular cytotoxicity (ADCC) from natural killer cells.

The most relevant hypothesis concerning the CRC progression during the moAb therapy is the selection of the treatment of KRAS mutated cells. In particular, it is retained that there is a small fraction of KRAS mutated cells in to the majority of wild-type CRC cells, that will be selected by the moAb therapy while they will not be killed from the treatment and they will survive, given treatment acquired resistance (Tougeron et al., 2013). It has been frequently reported that patients having KRAS mutations show no significant response to moAb treatment (Parsons and Meng, 2009; Bando et al., 2011). KRAS mutations are found in approximately 35–45% of CRCs (Karapetis et al., 2008; Amado et al., 2008; Van Cutsem et al., 2009). For this reason KRAS mutational status is considered as predictive marker for determining the efficacy of anti-EGFR therapies, and KRAS screening tests are prescribed by physicians before the start of treatments (Fakih, 2010). Only patients having wild type KRAS are eligible for moAb therapy (De Roock et al., 2008). Interestingly, some patients who have initially only KRAS wild type cells before treatment, still remain irresponsive to the medication because of the emergence of KRAS mutations.

1.2. Previous models

Various colorectal cancer mathematical models have been developed for basic tumor cell populations, cell proliferation and for the more complex pharmacodynamic and pharmacokinetics in colorectal cancer treatment (Ballesta and Clairambault, 2014). These include models of colon crypts (van Leeuwen et al., 2006; Fletcher et al., 2012; Murray et al., 2011; Johnston et al., 2007) and models of chemotherapy for colorectal cancer (Monro and Gaffney, 2009; Boston and Gaffney, 2011). Recently, DePillis et al. proposed a model which includes both chemo and immunotherapy along with considerations of patient specific immunity parameters. This is a comprehensive model which includes tumor cell and immune cell populations, chemotherapy and monoclonal antibody treatment. Results show the effect of drugs on chemorefractory tumors (de Pillis et al., 2014).

The hypothesis of drug resistance of KRAS mutations in colorectal cancer is quite recent. Diaz et al. (2012) recently published a paper in which they proved that pre-existed small number of KRAS mutated cells are responsible for developing resistance to panitumumab, a monoclonal antibody drug. Another very recent paper by Stites (2014) describes a mathematical model which evaluates how different KRAS mutated polymorphisms show different sensitivity to the EGFR inhibitors.

This paper is the extended version of our previous study in which we explored the impact of KRAS mutations on the moAb treatment by a mathematical model (Sameen et al., 2015). In the current paper we have discussed our previous model in detail along with further experiments and explanations about the interplay between wild-type and mutated KRAS cells in the presence of monoclonal antibody drug and their impact on chemotherapeutic effectiveness.

2. Extending DePillis' model

The purpose of our model is to monitor tumor growth with respect to KRAS mutational status during and after the moAb therapy. Our model is an extension of the model developed by de Pillis et al. (2014). We extend DePillis' model by representing tumor cell populations using two equations, Eq. (1) for tumor cells with wild type KRAS and Eq. (2) for mutant KRAS tumor cells. All the other equations for natural killer (NK) cells, cytotoxic T lymphocytes (CTL), lymphocytes excluding NK cells and CTLs and medications are as in the original model by de Pillis et al. (2014). The model is implemented using the OCTAVE programming environment (http://www.gnu.org/software/octave/; Eaton et al., 2009). For detailed information and parameter values of the model see the paper by de Pillis et al. (2015). The model includes equations for:

1. wild type tumor cell (T_w) and mutant tumor cell (T_m) populations;
2. patient immune system including, natural killer cells (N), CD8+ T-cells (L), lymphocytes (C) and interleukins (I);
3. chemotherapy (M) and monoclonal antibody (A) treatment;
4. patient immune strength (D).

We illustrate these four groups of equations in Sections 2.1.1–2.4

2.1. Equations for tumor cells

2.1.1. Equation for KRAS wild-type tumor cells

Tumor cells with KRAS wild-type nature go through natural clonal expansion process to form a tumor mass. The only two factors that interrupt the logistic growth of tumor cells are immune system and therapy. This fact is modeled in the following equation:

$$\frac{dT_w}{dt} = aT_w(1-b(T_w+T_m)) - \left(c+\xi\frac{A}{h_1+A}\right)NT_w - DT_w - (K_t+K_{at}A)\frac{T_w}{\alpha T_m+T_w}(1-e^{-\delta TM})T_w - \psi AT_w \quad (1)$$

Logistic tumor growth is modeled by term $aT_w(1-b(T_w+T_m))$. The innate immune system of the body fights tumor cells with the help of natural killer cells (term $-cNTw$) and CD8+ T cells (term $-DT_w$). Two other ways by which tumor cells experience death are chemotherapy (term $Kt\frac{T_w}{\alpha T_m+T_w}(1-e^{-\delta TM})T_w$) and monoclonal antibody treatment. The triple action of monoclonal antibody, which is valid only for KRAS wild-type tumor cells, includes terms for:

- direct killing $(-\psi AT_w)$;
- killing by enhancement of chemotherapy $\left(K_{at}A\frac{Tw}{\alpha T_m+T_w}(1-e^{-\delta TM})T_w\right)$;
- killing by assisting natural killer cells $\left(-\xi\frac{A}{h_1+A}NT_w\right)$.

2.1.2. Equation for KRAS mutant tumor cells

KRAS mutant cells behave differently from the KRAS wild-types by disturbing the triple action behavior of monoclonal antibody treatment. The monoclonal antibody is not able to directly kill KRAS mutant tumor cells and also fails to create chemosensitization in KRAS mutants. This fact is modeled in the following equation:

$$\frac{dT_m}{dt} = aT_m(1-b(T_w+T_m)) - \left(c+\xi\frac{A}{h_1+A}\right)NT_m - DT_m - \left(K_t\frac{T_w}{\alpha T_m+T_w}\right)\left(1-e^{-\delta TM}\right)T_m \quad (2)$$

Thus Eq. (2) is obtained from Eq. (1) by removing the two terms for

moAb induced tumor death in KRAS wild-type tumor cell equation and moAb-induced tumor death by enhancing activity of chemotherapy.

Both of the equations for tumor contain the terms which describe the interaction of moAb therapy with natural killer cells or chemotherapy and their effect on tumor growth. The enhancement of natural killer cells activity induced by moAb therapy is the same for both mutated and wild-type cells. This is represented in both equations by the $-\xi\frac{A}{h_1+A}NT_w$ term. Chemotherapy has reduced effectiveness against tumor cells during monoclonal antibody treatment because of mutant cells. This is represented in the model by $-Kt\frac{T_w}{\alpha(T_m)+T_w}$. The chemotherapy effectiveness decreases with the increase of the number of mutated cells. This term is introduced in both the equations of wild-type and mutant tumour cells for controlling the rate of chemotherapy induced tumor death. K_t is the maximum rate of chemotherapy induced tumor death in the absence of KRAS mutant cells. The above term makes the effectiveness of the chemotherapy dependent on the ratio of wild-type and total tumor cells. This ratio is controlled by the parameter α in such a way that, by increasing α, the rate of chemotherapy induced death is decreased with respect to the increase in the mutant population. Similarly, by increasing the initial number of KRAS mutated cells or by decreasing the initial number of KRAS wild-type cells, the rate of chemotherapy induced tumor death becomes much lower. Hence, the function clearly models the phenomenon of chemotherapy ineffectiveness, in conjunction with monoclonal antibody treatment, in case of presence of KRAS mutant cells.

Determining accurate α is the key task for producing realistic results. α not only modulates the ratio of wild-type VS mutated cells but it also depicts the influence of mutated cells on wild-type cells in making them resistant to the chemotherapy as well. KRAS mutated cells has the ability to render the neighboring wild-type cells insensitive to the chemotherapy and the α determines the range of mutated cell microenvironment.

2.2. Equations for immune response

Natural killer cells, CD8+ T-cells, other lymphocytes, and interleukins all play a vital role in creating immediate immune response with the initiation of tumor. Thus, in order to analyze the effect of immune system response and strength on the tumor proliferation we introduce four equations.

2.2.1. Natural killer cells

Natural killer (NK) cells are a fundamental part of host first-line defense system. Their activity is modeled in the following equation:

$$\frac{dN}{dt}=eC-fN-\left(p+p_a\frac{A}{h_1+A}\right)N(T_w+T_m)+\frac{p_nNI}{g_n+I}-K_n(1-e^{-\delta NM})N \tag{3}$$

They are produced from circulating lymphocytes (term eC) and their activity is stimulated by interleukins (term $\frac{p_nNI}{g_n+I}$). NK turnover is modeled by term fN. In case of tumor cells NK cells exhibit a special killing mechanism known as "antibody-dependent cell-mediated cytotoxicity" (ADCC). In this process NK cells recognize tumor cells by special receptors that identify attached antibodies on the surface of tumor cells. After recognition, NK cells release some cytotoxic granules into the tumor cell which consequently cause death. The cytotoxic granules are actually tumor killing resources of NK cell; in case of exhaustion of these resources the NK cells die (term $\left(p+p_a\frac{A}{h_1+A}\right)N(T_w+T_m)$). In addition, NK cells may die due to chemotherapy toxicity (term $-K_n(1-e^{-\delta N_m})N$).

2.2.2. CD8+ T-cells

Cytotoxic lymphocytes are part of cell-mediated immunity. They kill target cells by releasing into them specialized granules that program them to undergo apoptosis. They are vital for killing tumor cells. Their activity is modeled in the following equation:

$$\frac{dL}{dt}=\frac{\theta mL}{\theta+I}+j\frac{T_w+T_m}{k+(T_w+T_m)}L-qL(T_w+T_m)+(r_1N+r_2C)(T_w+T_m)-\frac{uL^2CI}{\kappa+I}-K_l(1-e^{-\delta LM})L+\frac{p_iLI}{g_i+I} \tag{4}$$

CD8+ T cell turnover is modeled by term $\frac{\theta mL}{\theta+I}$ and the breakdown of their surplus in presence of of IL-2 is modeled by term $\frac{uL^2CI}{\kappa+I}$. CD8+ T cells activity is stimulated by dead tumor cells, lysed by themselves (term $j\frac{T_w+T_m}{k+(T_w+T_m)}L$), NK cells (term $r_1N(T_w+T_m)$) or the general lymphocyte population (term $r_2C(T_w+T_m)$). Interleukins also perform stimulating effect on CD8+ T cells (term $\frac{p_iLI}{g_i+I}$). CD8+ T cell may die because of exhaustion of these tumor killing resources (term $qL(T_w+T_m)$) or due to chemotherapy toxicity (term $K_l(1-e^{-\delta LM})L$).

2.2.3. Lymphocytes

Lymphocyte count is the most important parameter to be considered while modeling tumors undergoing chemotherapy. Chemotherapy kills normal cells along with the tumor cells; hence, patients are constantly checked for their lymphocyte count during treatment. Reduction in lymphocyte count means weakening of immune system, which makes the body more vulnerable. Lymphocyte activity is modeled in the following equation:

$$\frac{dC}{dt}=\alpha-\beta C-K_c(1-e^{-\delta CM})C \tag{5}$$

Lymphocytes are synthesized in the bone marrow (term α) and their turnover is modeled by term βC. In addition, lymphocytes may be killed by chemotherapeutic drugs (term $K_c(1-e^{-\delta CM})C$).

2.2.4. Interleukins

Interleukin-2 is a major regulatory factor of immune responses. It belongs to a immune signaling group of cytokines. Interleukin-2 works as an immune response system by increasing the activity of cytotoxic T-cells. Their activity is modeled in the following equation:

$$\frac{dI}{dt}=-\mu I+\phi C+\frac{\omega LI}{\varsigma+I} \tag{6}$$

Interleukin-2 is produced in response to activated CD8+ T-cells (term $\frac{\omega LI}{\varsigma+I}$) or by naive CD8+T cells and CD4+T cells in the body (ϕC). Its turnover is modeled by term $-\mu I$.

2.3. Equations for treatments

In order to monitor treatments, separate equations are defined for chemotherapy (irinotecan) and monoclonal antibody (cetuximab). Terms $V_{M(t)}$ and $V_{A(t)}$, in Eqs. (7) and (8), respectively, describe the amount of drug injected with respect to time.

2.3.1. Chemotherapy/irinotecan

The activity of chemotherapy depends on the concentration of drug present in body at a specific time. This can be understood by the rate of excretion of drug from body, which is modeled by term $-\gamma M$. Chemotherapy using irinotecan is modeled by the following equation:

$$\frac{dM}{dt}=-\gamma M+V_{M(t)} \tag{7}$$

2.3.2. Monoclonal antibody/cetuximab

Monoclonal antibodies bind to the epidermal growth factor receptors (EGFRs) present on the surface of tumor cells. As an average cell contains thousands of EGFRs, many molecules of moAb drug are consumed in a single tumor cell. The loss of moAb molecules due to their binding with the tumor (term $\lambda(T_w+T_m)\frac{A}{h_2+A}$) is an important factor to be considered while modeling moAb drug treatment to tumor. The rate of excretion of drug from body is modeled by term $-\eta A$:

$$\frac{dA}{dt} = -\eta A - \lambda(T_w+T_m)\frac{A}{h_2+A} + V_{A(t)} \tag{8}$$

2.4. Patient immune strength formula

Immune strength, i.e. the effectiveness of CD8+ T-cells, is calculated using Eq. (9). The formula uses the lymphocyte count L and total tumor mass T_w+T_m along with other parameters to compute immune strength.

$$D = d\frac{(L/(T_w+T_m))^l}{s+(L/(T_w+T_m))^l} \tag{9}$$

Immune strength D is calculated by considering the following parameters:

d = immune strength coefficient;
l = immune − system strength scaling coefficient;
s = value of ratio $(L/(T_w+T_m))^l$ necessary for half maximal CD8+ T − cell effectiveness against tumor.
(It tells how quickly CD8+ T − cells respond to the presence of tumor.)

In our simulation we varied the parameters to generate three types of immune strength values: strong, moderate and weak.

2.5. Initial conditions and drug dosages

The initial conditions for the model are taken from DePillis model except the number of KRAS mutated cells. The initial number of KRAS mutated cells, which can cause resistance to the treatment, is not available in the literature. Thus we assumed a small number for KRAS mutated cells, say 35, because even such a small number of mutated cells is able to cause resistance. The initial conditions for the model are as follows:

$Tw = 4.65928 \times 10^9$
$Tm = 35$
$N = 9 \times 10^7$
$L = 1.8 \times 10^5$
$C = 9 \times 10^8$
$M = 0$
$I = 1173$
$A = 0$

The parameter values in our model are also taken from DePilis except the rate of chemotherapy induced tumor death, which is reduced to the minimum level because of KRAS mutations. As DePillis, we assume that patients are already gone through first-line chemotherapy and are refractory to the treatment. Therefore, the initial tumor is assumed to have a very large number of cells: 4.65928×10^9. If tumor size becomes less than 2^7 cells during the treatment, it is assumed that the tumor is showing complete response to the therapy. Similarly, tumors which remain larger then 2^7 but do not continue to grow during the treatment are considered to have partial response.

Treatment comprised individual or combination of monoclonal antibody and chemotherapeutic drug, cetuximab and irniotecan, respectively. The drugs are administered according to standard FDA approved dosages and timings. For irinotecan, a 125 mg/m^2 dose is given over 90 min once a week, for 4 weeks. For cetuximab, a loading dose of 400 mg/m^2 is administered for 2 h, followed by a 250 mg/m^2 dose over 60 min given every week for one month.

3. Results

3.1. Monoclonal antibody effect on chemotherapy

As described before, the effectiveness of chemotherapy against tumor cells gradually reduce during monoclonal antibody therapy due to KRAS mutated cells. This gradual ineffectiveness with the increase of mutated cells is modulated by term α in the model. We have explored all of the possible α values ranging from 10 to 10^9. Initially we have started from very low range of α but our results contradict with the reported experimental data. The α value is then raised up to $\alpha = 10^6$ or $\alpha = 10^7$ to get the actual results (Figs. 1 and 2). The reason for this much high α is that a single KRAS mutated cell has the tendency to influence thousands of wild-type cells.

We have also analyzed our results by varying α values along with the varying initial number of mutated cells. Lower the α greater is the impact of chemotherapy but with the increase in number of mutated cell this effect is not much significant. But with lower α and small number of mutated cells the drug has profound effect, which is not an actual phenomenon. In reality, chemotherapy also tend to become ineffective with the passage of time.

In our simulations we used the value $\alpha = 10^7$ because this shows a gradual decrease in the efficiency of the chemotherapy as compared to a too rapid reduction experimented with the smaller value $\alpha = 10^6$.

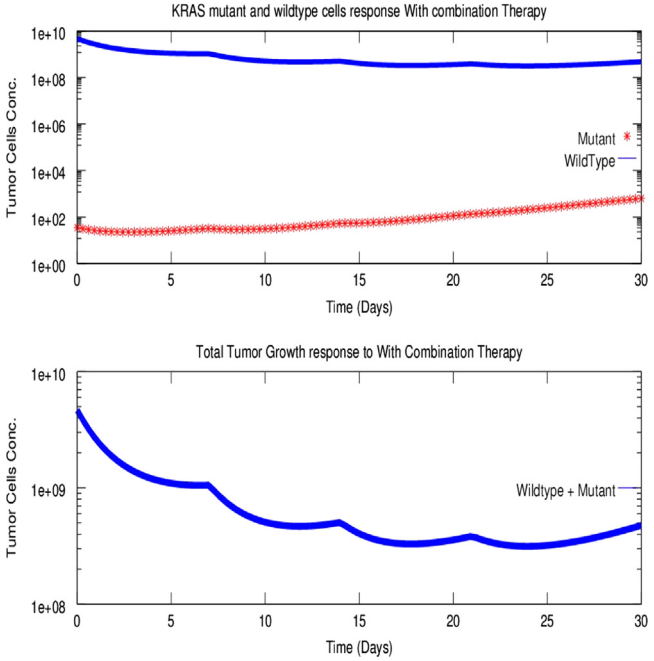

Fig. 1. α value: 10^6 shows rapid decrease in wildtype and increase in mutant KRAS cells (red: mutant and blue: wildtype). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

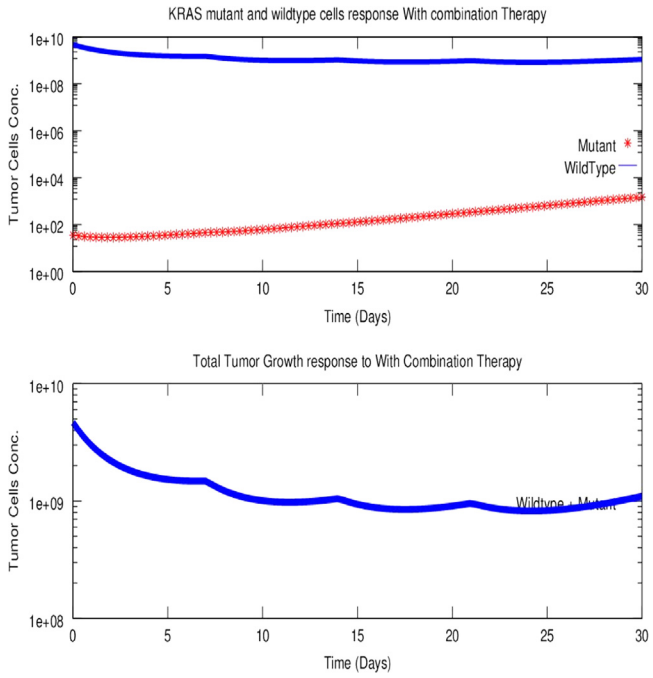

Fig. 2. α: 10^7 shows gradual decrease in wildtype and increase in mutant KRAS cells (red: mutant and blue: wildtype). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

3.2. Treatment trial simulations for KRAS mutated colorectal cancer tumors

Our model has been evaluated for standard treatments by chemotherapy and monoclonal antibodies for tumors with KRAS mutations. The KRAS mutated tumors are treated according to standard dosage of drugs and are evaluated for both monotherapy and combination therapy.

3.2.1. Cetuximab and irinotecan monotherapy

In accordance with the literature, in our model cetuximab monotherapy has no impact on colorectal tumors because of the number of elevated KRAS mutated tumor cells (Fig. 4). Similarly, irinotecan monotherapy has no impact on the tumor because of the chemorefractory status of tumor. Here, no increase in KRAS mutated cells is noticed (Fig. 3). Results show that, although both drugs fail as monotherapies, failure of cetuximab is specifically caused by an increase in the number of KRAS mutated cells.

3.2.2. Cetuximab and irinotecan combination therapy

For patients presenting metastatic colorectal cancer, cetuximab and irinotecan are recommended in combination. We used our model to test the combination of the two drugs. This allowed us to understand the impact of combined therapy on KRAS mutated tumor cells (Fig. 5). KRAS mutated cells grow with the passage of time and KRAS wild type cells start to reduce. However, as the initial number of KRAS mutated cells is very small, their increase is not clearly visible in the figure. Anyway, even this very low level of KRAS mutated cells is still able to gradually reduce the activity of drugs (Fig. 5). The combination therapy is only effective for KRAS wild-type tumours (Fig. 6).

3.3. Patient responses to the therapy

We simulated our model for patients with different immune strengths. Generally, it is believed that a strong immune system both helps the medication and facilitates quick recovery, while

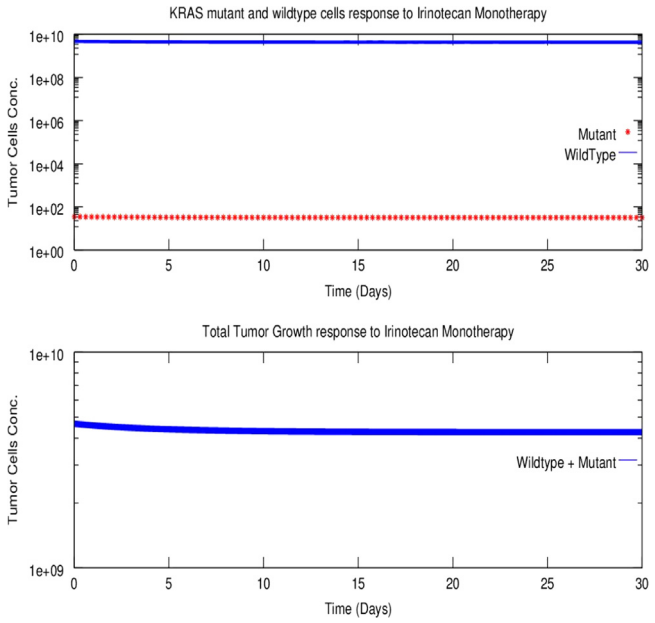

Fig. 3. Irinotecan monotherapy (red: mutant and blue: wildtype). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

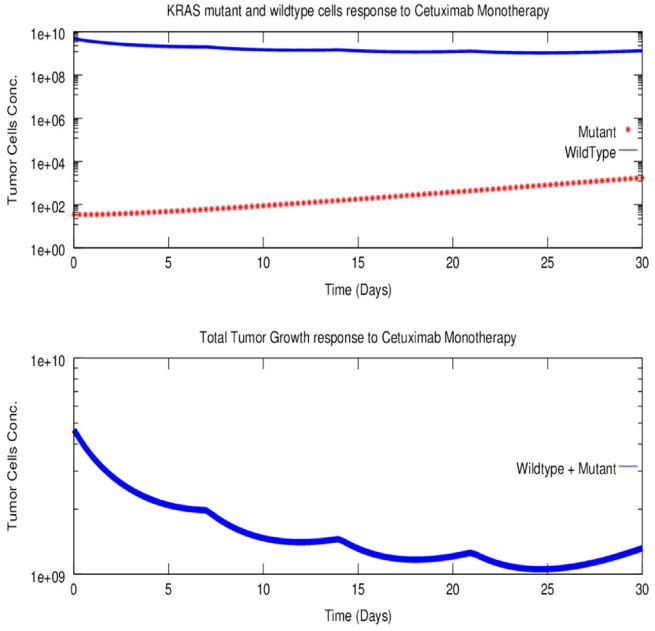

Fig. 4. Cetuximab monotherapy (red: mutant and blue: wildtype). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

patients with weak immunity do not respond well to the medicine. We analyzed the interaction between patient immune strength and treatment in case of mutation development during and after medication. The hypothetical immune strength values are calculated for generating weak, moderate and strong immune responses. These values are generated by the formula for immune strength (Eq. (9)) by changing the values of its parameters.

Our results are summarized in Table 1. Patients without KRAS mutations have complete response (CR), partial response (PR) and no response (NR) for strong, moderate and weak immunity, respectively. With KRAS mutations the immune strength has no significant impact on the treatment. KRAS mutated tumours

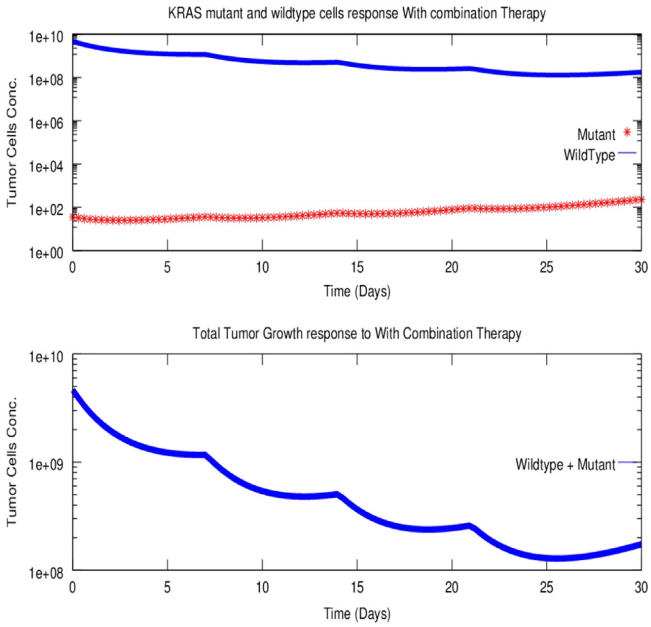

Fig. 5. Cetuximab and irinotecan as combination therapy with KRAS mutant (red: mutant and blue: wildtype). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

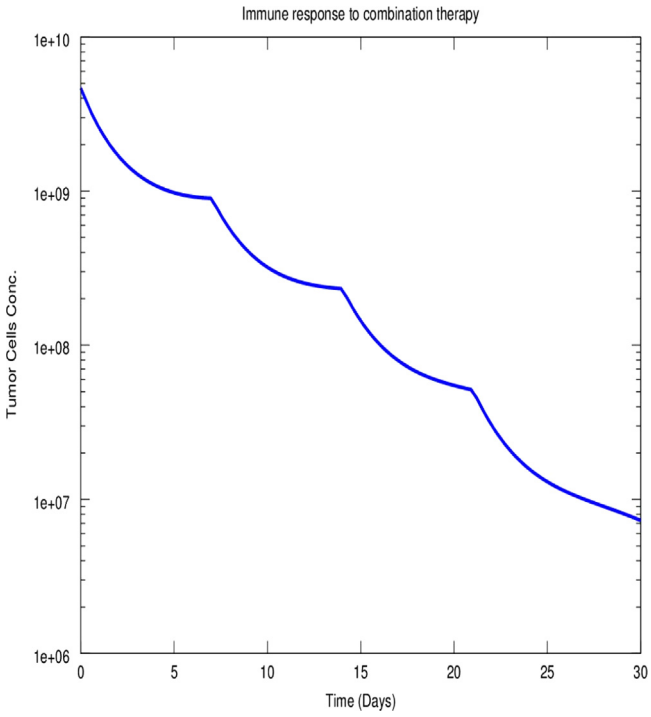

Fig. 6. Cetuximab and irinotecan as combination therapy without KRAS mutant (red: mutant and blue: wildtype). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

Table 1
Cetuximab and irinotecan combination therapy.

Immune strength	With KRAS mutation	Without KRAS mutation
Strong immunity	NR/PR (Fig. 7)	CR (Fig. 8)
Moderate immunity	NR (Fig. 9)	PR (Fig. 10)
Weak immunity	NR (Fig. 11)	NR (Fig. 12)

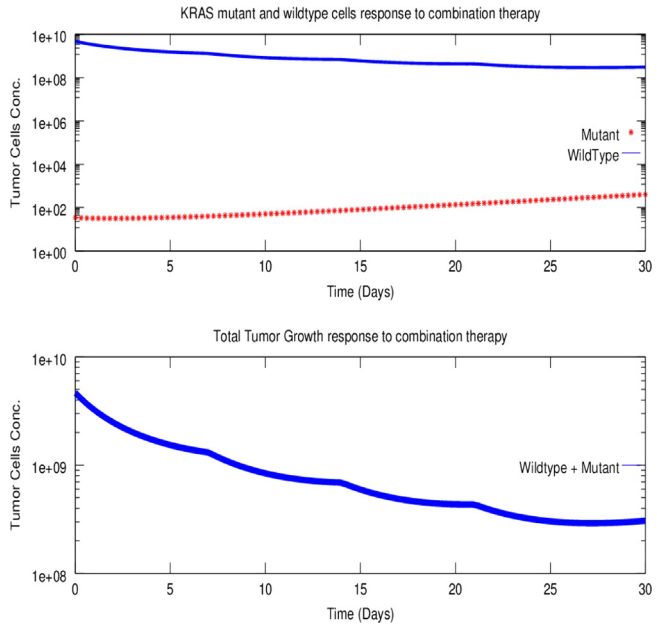

Fig. 7. Strong immunity response with KRAS mutation.

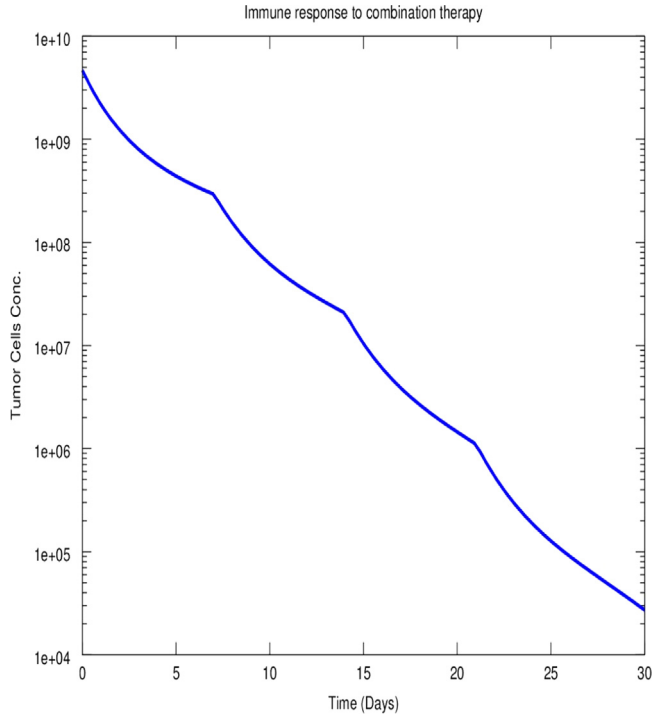

Fig. 8. Strong immunity response without KRAS mutation.

normally show no response to the treatment but sometimes there is a partial response in presence of a high immune strength. For moderate and weak immunity there is no response at all.

3.4. Cetuximab and irinotecan as first-line therapy

In this section we explore the possibility of using cetuximab and irinotecan as first-line therapy. Initial conditions are the same as shown in Section 2.5. Patients having weak immunity do not show any significant response to the cetuximab and irinotecan as first-line therapy (Fig. 13). Tumor size reduces significantly in patients with moderate immunity, but the number of KRAS mutated cells show a relevant increase (Fig. 14). The response to

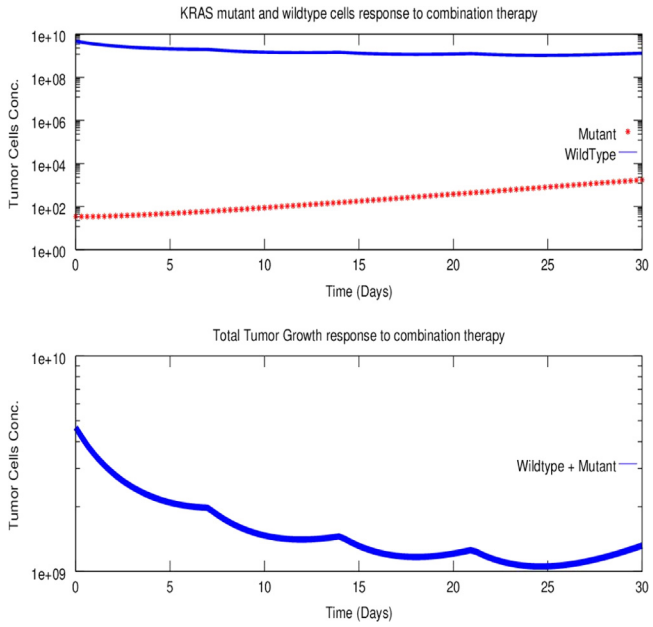

Fig. 9. Moderate immunity response to KRAS mutation.

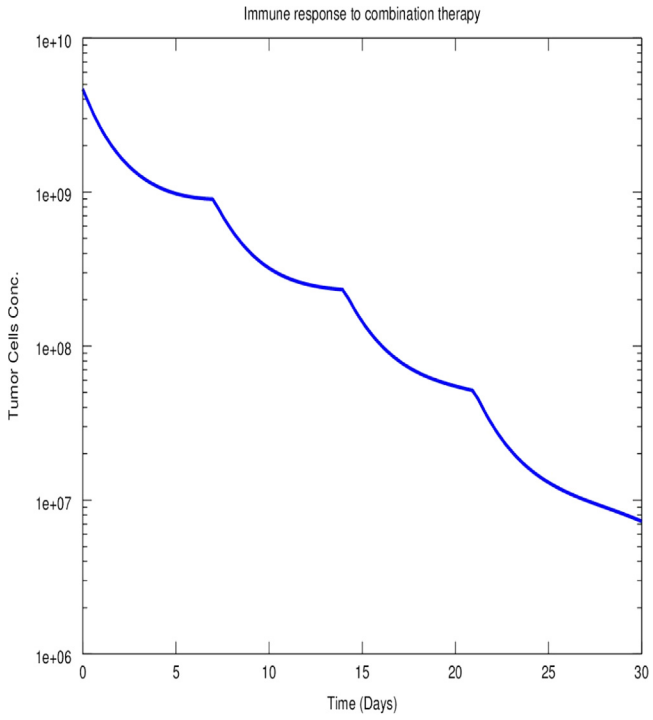

Fig. 10. Moderate immunity response without KRAS mutation.

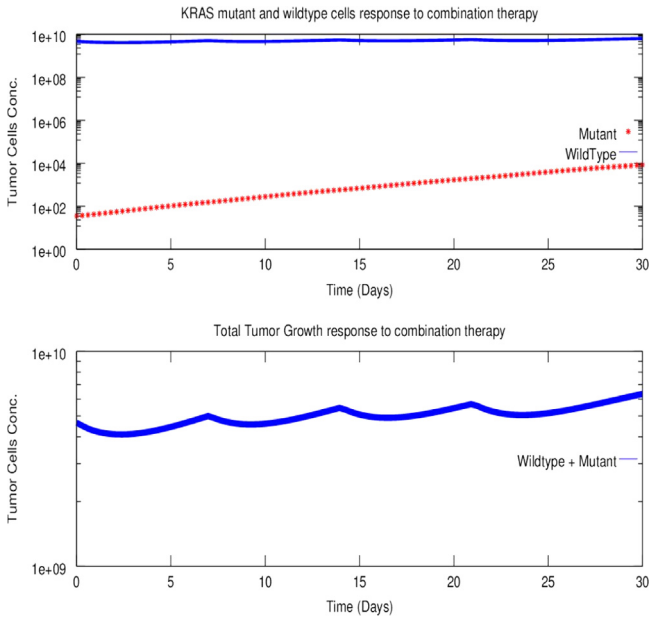

Fig. 11. Weak immunity response with KRAS mutation.

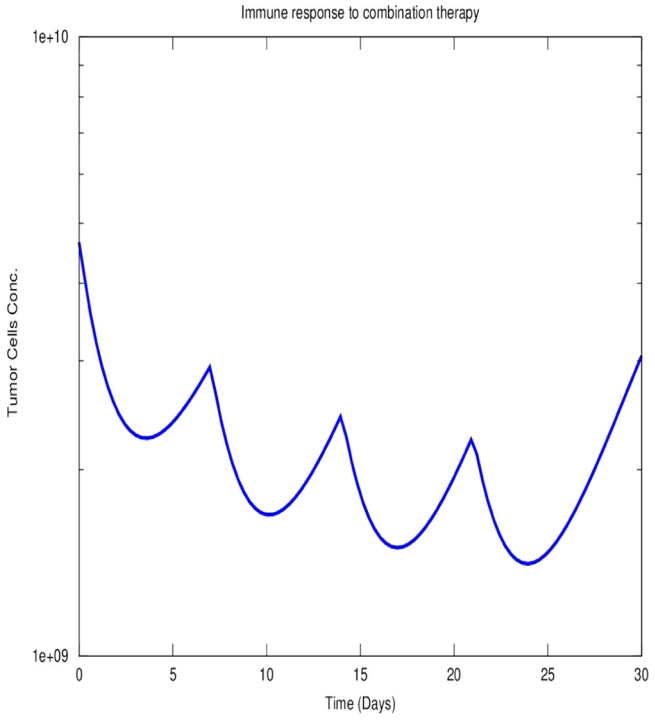

Fig. 12. Weak immunity response without KRAS mutation.

the therapy is only observed in patients with strong immunity and very low number of initial KRAS mutated cells (Fig. 15).

4. Discussion

Emergence of KRAS mutated status is an alarming situation for colorectal cancer patients being treated with anti-EGFRs. Presence of KRAS mutations in a tumor treated with monoclonal antibodies is a sign of becoming refractory to treatments. In order to understand the phenomenon of developing resistance to the anti-EGFRs we developed a mathematical model with separate equations for KRAS mutant and wild-type cells.

KRAS mutations are considered as driver of resistance to anti-EGFR therapy in colorectal cancer. Subset of KRAS wild-type cells in colorectal tumor initially responds very effectively to the anti-EGFR drugs but the presence of traces of KRAS mutations prior to treatment ultimately results in the development of acquired resistance to the drug. Hence, the treatment of colorectal tumor with anti-EGFRs is only recommended when the KRAS mutation status is zero. The anti-EGFR treatments to colorectal tumors containing minute quantity of KRAS mutated cells consequently develop resistance to the therapy. The pre-existing subclones of mutated cells multiply very rapidly during the treatment and on the other hand KRAS wild-type cells reduce significantly in their number due to efficient targeting procedure of monoclonal

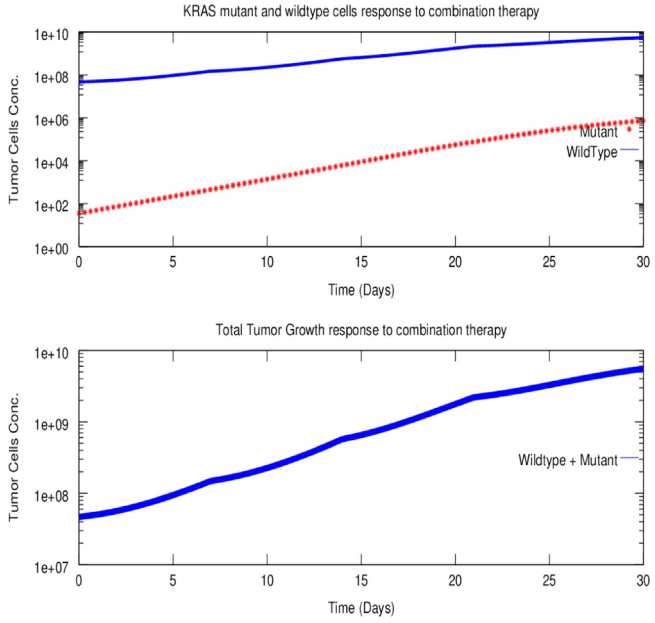

Fig. 13. Cetuximab and irinotecan as first-line therapy: weak immune response (red: mutant and blue: wildtype). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

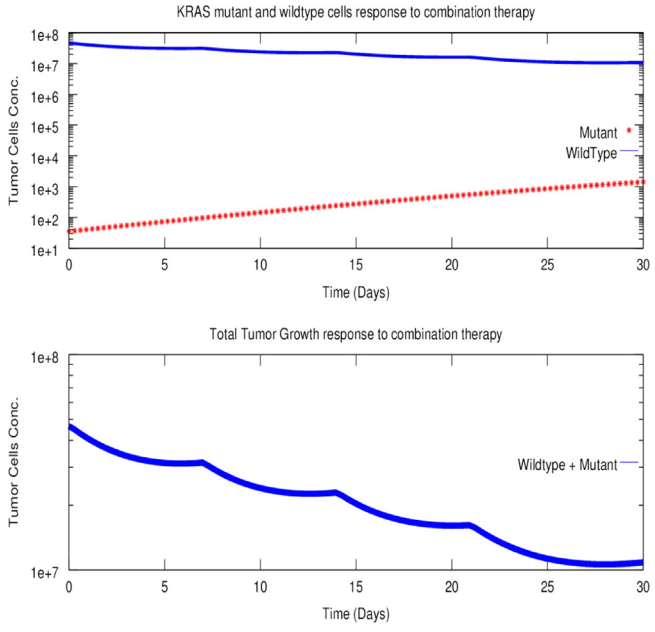

Fig. 14. Cetuximab and irinotecan as first-line therapy: moderate immune response (red: mutant and blue: wildtype). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

antibody drugs. Eventually, tumor mass repopulate with the mutated cells and makes tumor much more refractory to other treatments as well. So, the initial drop in the number of wild-type cells does not contribute much in shrinking the overall size of the tumor after the treatment. Diaz et al. was the first to discover the presence of small number of KRAS mutated cells in circulating tumor DNA at very early stage of drug treatment. This indicates that ostensibly looking KRAS wild-type tumors hide inside some fatal KRAS mutated cells as well. The mathematical model by Diaz et al. (2012) suggests that circulating DNA analysis for KRAS mutation detection can act as a marker for early detection of relapse of disease. Misale et al. (2012) also confirmed the Diaz et al. results by in vitro analysis and declared that the reason for

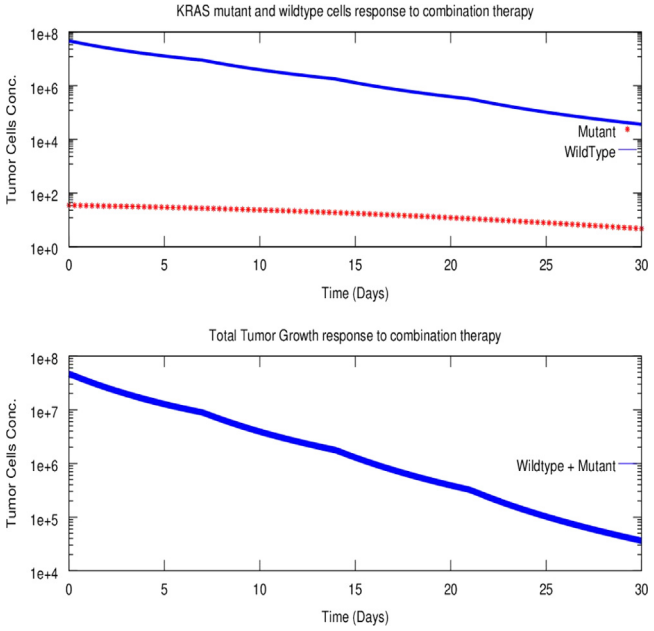

Fig. 15. Cetuximab and irinotecan as first-line therapy: strong immune response (red: mutant and blue: wildtype). (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this paper.)

development of resistance is the pre-existence of clones of KRAS mutated cells.

A major problem in colorectal cancer is to identify the behavior of monoclonal antibody therapy in presence of KRAS mutations and the impact of the mutations on other therapies. More specifically, exploring the sensitivity of monoclonal antibody drugs to the chemotherapy and natural killer cells activity in the presence of mutations is another key issue in understanding drug efficacy (Arnold and Seufferlein, 2010). We have speculated in our model that in case of natural killer cells, cetuximab has equal enhancing effect on both KRAS mutant and wild-type cells. In other words, KRAS mutational status has no significant impact on the antibody-dependent cellular cytotoxicity (ADCC) mediated by the drug (Wu et al., 2008).

The anti-EGFR drugs along with chemotherapy give promising results for colorectal tumors with wild-type KRAS. Monoclonal antibody is not only a perfect EGFR blocker but it also boosts the activity of chemotherapeutic drug molecules and results in enhancement of overall antitumor activity (Wong, 2005; Prewett et al., 2002, 2007; Jonker et al., 2007; Saltz et al., 2004; Adams and Louis, 2005). Hence, the monoclonal antibody therapy in combination with chemotherapy is proved to be effective in avoiding relapses and increasing the progression free survival period in colorectal cancer patients. The presence of KRAS mutation in colorectal tumor leaves this combination therapy with no profound effects on tumor size this means that the chemosensitization operation of moAb drugs does not imply on KRAS mutated tumor cells (Tol et al., 2009). The wild-type KRAS tumors have longer progression free survival as compared to mutated KRAS tumors when treated with combination therapy (Lievre et al., 2008; Bokemeyer et al., 2008; Van Cutsem et al., 2008). This reduced survival period due to mutated KRAS cells is also confirmed in our experiments. The ineffectiveness of both cetuximab and irinotecan drugs increases with increase in number of mutated cells as these drugs only influence the wild-type cells. Cetuximab has been frequently reported to increase chemotherapeutic activity upon combination with irinotecan drug in tumor cells (Jonker et al., 2007). Studies show that KRAS mutant cells do not allow cetuximab to produce such type of chemosensitization. Chemotherapy

is effective only at very early stage of treatment when KRAS mutated cells are significantly lower in numbers but when the number of mutated cells in the tumor start to increase the sensitivity of chemotherapy reduces gradually. In the initial phase of treatment with combination therapy the drugs seems to reduce tumor a little but soon it goes back to the much bigger and drug refractory state. Time tumor takes to go back in to maximum or more resistant state is the time between the relapse which is significantly lower in case of KRAS mutations.

Our results repeatedly confirmed that small number of mutated cells can have an influence on whole tumor for making it refractory to the therapies. The major point to ponder is that how a very small proportion of mutated cells make the drug insensitive even to the wild-type cells. The moAb drug effectiveness on the wild-type cells is well explained but understanding the change in behavior of wild-type cells because of growing number of mutated cells during treatment is a challenging task for the researchers. Parsons and Myers explained this myth of KRAS wild-type cells behavior by blaming KRAS mutant cells responsible for every unexplained resistance mechanism of the tumor. The mutated cells undergo negative selection process in poly clonal tumors. Parson and Myers suggested KRAS mutations as "transdriver mutations", these are the mutations which are able to speed up the tumor progression process even if they are in small proportion (Parsons and Myers, 2013b, 2013a).

The possible reasons for strong influence of small number of mutated cells on wild-type cells for producing phenomenal resistance against anti-EGFR drugs lies in tumor heterogeneity and in the theory of cancer stem cells. Tumor heterogeneity is the reason for failure of chemotherapy induced tumor cell death, not only for mutated cells but also for wild-type cells when treated along with the moAb drug (Vilar and Tabernero, 2012; Baldus et al., 2010; Hasovits et al., 2013). In order to model this phenomenon we have regulated the rate of chemotherapy induced tumor death. We assumed that the effect of chemotherapy decrease with the increase in KRAS mutated cells. Therefore, we cannot take any benefit from the chemosensitization activity of moAb drugs in case of KRAS mutations. The chemotherapy may work effectively only at the beginning of the treatment but then, with the increase of KRAS mutant population, it starts to loose its strength. Tumor heterogeneity explains the dispersal of mutated cells in the tumor mass. These distributed resistant cells have strong impact on their microenvironment (Junttila and de Sauvage, 2013). They perform like small radiators emitting some harmful radiations which affect a range of surrounding cells, leaving them resistant to the therapies irrespective of their original wild-type status. Hobor et al., reported that wild-type KRAS cells has the ability to grow during the cetuximab treatment when KRAS mutated cells are present in the tumor. Resistant mutated cells maintain micro-environment inside tumor by influencing their neighboring cells rendering drug sensitive wild-type cells to resistant cells. The KRAS mutated cells secrete increased amount of ligands that has the ability to protect wild-type cells from EGFR blockade by cetuximab drug. The secretion of TGF α and amphiregulin by moAb resistant KRAS mutated cells sustain the EGFR signaling in wild-type cells too (Hobor et al., 2014).

Tumor heterogeneity is also the major reason for the failure of initial KRAS mutation screening test. The incorrect assignment of wild-type status to the tumor is because of widely dispersed mutated cells inside the tumor. The test is only applied to a small chunk of tumor and there is a chance that the block selected for test may not contain KRAS mutation and hence give wrong result (Richman et al., 2011; Baldus et al., 2010).

Tumor heterogeneity role in therapeutic resistance is irrefutable but the root cause of heterogeneity development is the cancer stem cells (CSCs) (Marusyk et al., 2012; Shackleton et al., 2009). CSCs are best described as the cells having selective advantage of proliferation over the other cells in the tumor. They occur as minority in tumor and tend to have self renewing property that drive tumorigenesis (Clarke et al., 2006; Dean et al., 2005; Reya et al., 2001; Clevers, 2011). CSCs are rare cells with potential of being naturally resistant to chemotherapy. The area of CSC research is still underdeveloped that is why there is no concrete description about the process of chemotherapeutic drug resistance due to CSCs. If we consider KRAS mutations as CSC then failure of chemotherapy in our results is justified. KRAS mutated cells are recently been explored as potential CSCs in colorectal tumors. Evidences support the hypotheses of KRAS mutated cells as CSCs, which gives us enough explanation about the acquired resistance in colorectal tumor due to KRAS mutations (Fearon and Wicha, 2014; Moon et al., 2014).

Patient immune responses play a vital role in oncotherapeutic processes and this role varies from positive to negative with strong to weak immune strength respectively. The immune strength becomes unimportant for KRAS mutated patients because the initially strong immunity turns into a weak one due to the development of secondary KRAS mutations during the treatment (Smakman et al., 2005). Even with the highest immune strength, the response to the drugs is only partial (sometimes). In our simulations tumor size was set to its maximum and it is considered refractory to the chemotherapy given as first-line to the patients. The reason for adopting these criteria is because cetuximab is generally given as third- or fourth-line treatment to the patients as final rescue (Pfeiffer et al., 2008; Vincenzi et al., 2006). Hence it is proved that there is no correlation between immune strength and combination treatment for KRAS mutated patients.

The cetuximab and irinotecan combination therapy is proved to be very effective as first-line therapy for colorectal cancer but this is true only for KRAS wild-type patients (Folprecht et al., 2006; Van Cutsem et al., 2009). Although KRAS screening tests are always performed before starting monoclonal antibody treatments, there is a risk of minimal quantities of KRAS mutated cells that are not detected by common sequencing processes of laboratories. In this case critical questions arise about the patient's response to cetuximab and irinotecan as first-line therapy. Our results show complete response only in patients with strong immunity. High immune strength means little number of KRAS mutations, so there is a chance that the drug kills wild-type cells quickly and chemotherapy also gets the chance to kill mutant cells. The first-line therapy seems to work also for moderately immune persons but, at the same time, increases the KRAS mutation level, which is a sign of recurrence of disease. Patient responses are also dependent upon the initial KRAS mutant cell concentrations. If the initial mutant level is very low then a complete response can be obtained. However, in case of greater level of initial KRAS mutants, the response is only partial with decrease in tumor size and significant increase in KRAS mutant levels, which doubles the chances of relapse. The relapse after cetuximab as first-line therapy will be more lethal because of acquired resistance to the drugs due to increased KRAS mutant populations.

5. Conclusion and future work

In cetuximab and irinotecan combination therapy the rapid increase in levels of KRAS mutations and the partial or no response on the tumor size an indications of the development of resistance to the drugs. Using our model we could measure the level of KRAS mutations that can be tolerated to avoid resistance to anti-EGFRs. This could provide information to stop the anti-EGFR treatment before reaching the threshold value for KRAS mutant cells. The treatment could be switched from anti-EGFR to anti-KRAS drugs.

We do not know the clinical perspective about switching treatments, but this could provide a better way to solve the secondary KRAS mutation problem in colorectal cancers.

Patients with stronger immunity can be highly recommended cetuximab and irinotecan as first-line therapy but there is no instrument to accurately judge a person's immunity. Thus there is a potential risk associated with standard dosage cycles of drugs. The failure of the treatment will ultimately lead towards tumor progression with much higher rates. Moreover, the increased number of KRAS mutations makes the problem even more complex by creating resistance against the drugs. The co-occurrence of EGFR and KRAS mutations in a colorectal cancer patient is indeed the worst case scenario.

We want to further explore KRAS mutated cells fate as cancer stem cells and development of tumor heterogeneity. Tumor heterogeneity makes the problem of resistance against the drugs even worse as a small number of mutated cells is able to make drugs ineffective even for a large number of wild-type cells. We plan to further investigate this interplay between wild-type and mutant cells caused by tumor heterogeneity.

As future work, we also aim to develop a stochastic computational model for KRAS mutations and combine it with the current mathematical model in order to increase the accuracy of the model.

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