A Mathematical Model for Assessing KRAS Mutation Effect on Monoclonal Antibody Treatment of Colorectal Cancer

Sheema Sameen, Roberto Barbuti, Paolo Milazzo(✉), and Antonio Cerone

Dipartimento di Informatica, Università di Pisa, Pisa, Italy
{sameen,barbuti,milazzo,cerone}@di.unipi.it

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 produced after the treatment with monoclonal antibodies (mAb). KRAS screening tests done before the start of the treatment are not very sensitive to identify minute quantity of the mutated cells, which can produce resistance to the therapy after the beginning of the treatment. Here we present a mathematical model for the analysis of KRAS mutations behavior in colorectal cancer with respect to mAb treatments. To evaluate the drug performance we have developed equations for two types of tumors cells, i.e. KRAS mutated and KRAS wildtype. Both tumor cell populations were treated with a combination of mAb 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. 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 (mAb) and Irinotecan (chemotherapy) drugs are analyzed as first-line treatment of colorectal cancer with few KRAS mutated cells. Results show that this combined treatment is 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.

Keywords: Colorectal cancer · Mathematical model · Monoclonal antibody resistance · KRAS mutation

1 Introduction

The World Health Organization (WHO) declared colorectal cancer (CRC) as the second most common cause of cancer mortality in Europe [1]. Monoclonal antibody (mAb) has been introduced as the most promising treatment to fight disease. The development of acquired resistance to the mAb drug, due to KRAS mutations, makes the problem very complex in terms of personalized treatment.

C. Canal and A. Idani (Eds.): SEFM 2014 Workshops, LNCS 8938, pp. 243–258, 2015.
DOI: 10.1007/978-3-319-15201-1_16

We have developed a system of non-linear ordinary differential equations (ODEs) to model the impact of KRAS mutations on the mAb and chemotherapy combination treatment of colorectal cancer. We have studied the behavior of mAb and chemotherapy with respect to patient immune responses and we have explored one mAb drug as a potential candidate for first-line therapy of CRC, in combination with chemotherapeutic drug.

Colorectal Cancer Therapy and KRAS Mutations. Colorectal cancer is, in most cases, caused by the overexpression of epidermal growth factor receptor (EGFR). Monoclonal antibodies are a major breakthrough in CRC therapeutic research because of their anti-EGFR activity [2,3]. The Food and Drug Administration (FDA) approved mAb drugs for colorectal cancer including Cetuximab and Panitumumab [4]. These drugs produce promising results when administered in combination with chemotherapeutic drugs [5,6]. 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 emergence of KRAS mutations is the main obstacle to progresses in tumour treatments by monoclonal antibodies. It has been frequently reported that patients having KRAS mutations show no significant response to mAb treatment [7,8]. KRAS mutations are found in approximately 35 %–45 % of CRCs [9–11]. 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 [12]. Only patients having wild type KRAS are eligible for mAb therapy to avoid acquired resistance to drugs in case of mutant KRAS [13]. 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. There could be two possibilities for this phenomenon: either the mutations are produced by the drug, or there are initially subpopulations of KRAS mutants present in the body which are undetectable by conventional screening tests. Most scientists agree with the second hypothesis because minimal quantities of KRAS mutant cells cannot be detected by simple sequencing techniques, but can only be found by using the sensitive pyrosequencing method [14,15].

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 [16]. These include models of colon crypts [17–21] and models of chemotherapy for colorectal cancer [22,23]. 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 [26].

The hypothesis of drug resistance of KRAS mutations in colorectal cancer is quite recent. Diaz Jr. et al. 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 [24]. Another very recent paper by Stites describes a mathematical model which evaluates how different KRAS mutated polymorphisms show different sensitivity to the EGFR inhibitors [25].

The model presented here studies the impact of KRAS mutations on the mAb treatment.

2 Extending DePillis' Model

The purpose of our model is to monitor tumor growth with respect to KRAS mutational status during and after the mAb therapy. Our model is an extension of the model developed by DePillis et al. [26]. 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 cells (NK), cytotoxic T lymphocytes (CTL), lymphocytes excluding NK cells and CTLs and medications are as in the original model by DePillis et al. [26]. The model is implemented using the OCTAVE programming environment [27,28]. For detailed information and parameter values of the model see the DePillis paper [26]. The model includes equations for:

1. wild type tumor cell (Tw) and mutant tumor cell (Tm) 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 Sects. 2.1–2.4

2.1 Equations for Tumor Cells

Equation for KRAS Wild-Type Tumor Cells. Tumor cells with KRAS wildtype 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 Eq. (1).

$$\begin{aligned} \frac{dTw}{dt} &= aTw(1 - b(Tw + Tm)) - (c + \xi \frac{A}{h1 + A})NTw \\ -DTw &- (Kt + KatA)\frac{Tw}{\alpha Tm + Tw}(1 - e^{-\delta TM})Tw - \psi ATw \end{aligned} \tag{1}$$

Logistic tumor growth is modeled by term $aTw(1 - b(Tw + Tm))$. The innate immune system of the body fights tumor cells with the help of natural killer cells (term $-cNTw$) and CD8+ T cells (term $-DTw$). Two other ways by

which tumor cells experience death are chemotherapy (term $Kt\frac{Tw}{\alpha Tm+Tw}(1-e^{-\delta TM})Tw$) and monoclonal antibody treatment The triple action of monoclonal antibody, which is valid only for KRAS wildtype tumor cells, includes terms for:

- direct killing ($-\psi ATw$);
- killing by enhancement of chemotherapy ($KatA\frac{Tw}{\alpha Tm+Tw}(1-e^{-\delta TM})Tw$);
- killing by assisting natural killer cells ($-\xi\frac{A}{h1+A}NTw$).

Equation for KRAS Mutant Tumor Cells. KRAS mutant cells behave differently from the KRAS wildtypes 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 Eq. (2).

$$\begin{aligned}\frac{dTm}{dt} &= aTm(1-b(Tw+Tm)) - (c+\xi\frac{A}{h1+A})NTm \\ &\quad -DTm - (Kt\frac{Tw}{\alpha Tm+Tw})(1-e^{-\delta TM})Tm\end{aligned} \tag{2}$$

Thus Eq. (2) is obtained from Eq. (1) by removing the two terms for mAb induced tumor death in KRAS wildtype tumor cell equation and mAb-induced tumor death by enhancing activity of chemotherapy.

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.

Natural Killer Cells. Natural Killer (NK) cells are a fundamental part of host first-line defense system. Their activity is modeled in Eq. (3).

$$\begin{aligned}\frac{dN}{dt} &= eC - fN - (p+pa\frac{A}{h1+A})N(Tw+Tm) + \frac{pnNI}{gn+I} \\ &\quad -Kn(1-e^{-\delta NM})N\end{aligned} \tag{3}$$

They are produced from circulating lymphocytes (term eC) and their activity is stimulated by interleukins (term $\frac{pnNI}{gn+I}$). 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 $(p+pa\frac{A}{h1+A})N(Tw+Tm)$). In addition, NK cells may die due to chemotherapy toxicity (term $-Kn(1-e^{-\delta NM})N$).

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 Eq. (4).

$$\begin{aligned}\frac{dL}{dt} = \frac{\theta mL}{\theta + I} + j\frac{Tw + Tm}{k + T}L - qL(Tw + Tm) + (r1N + r2C) \\ (Tw + Tm) - \frac{uL^2CI}{\kappa + I} - Kl(1 - e^{-\delta LM})L + \frac{piLI}{gi + I}\end{aligned} \tag{4}$$

CD8+ T cell turnover is modeled by term $\frac{\theta mL}{\theta+I}$ and the breakdown of their surplus in presence 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 $j\frac{Tw+Tm}{k+T}L$), NK cells (term $r1N(Tw + Tm)$) or the general lymphocyte population (term $r2C(Tw + Tm)$). Interleukins also perform stimulating effect on CD8+ T cells (term $\frac{piLI}{gi+I}$). CD8+ T cell may die because of exhaustion of these tumor killing resources (term $qL(Tw + Tm)$) or due to chemotherapy toxicity (term $Kl(1 - e^{-\delta LM})L$).

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 Eq. (5).

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

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 $Kc(1 - e^{-\delta CM})C$).

Interleukins. Interleukin-2 is a major regulatory factor of immune responses. It belongs to a immune signaling group of cytokines. Interleukin-2 work as an immune response system by increasing the activity of cytotoxic T-cells. Their activity is modeled in Eq. (6).

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

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 $VM(t) and VA(t)$, in Eqs. (7) and (8), respectively, describe the amount of drug injected with respect to time.

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 Eq. (7)

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

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 mAb drug are consumed in a single tumor cell. The loss of mAb molecules due to their binding with the tumor (term $\lambda T \frac{A}{h2+A}$) is an important factor to be considered while modeling mAb drug treatment to tumor. The rate of excretion of drug from body is modeled by term $-\eta A$.

$$\frac{dA}{dt} = -\eta A - \lambda T \frac{A}{h2 + A} + VA(t) \tag{8}$$

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 tumor mass $'T' = (Tw + Tm)$ along with other parameters to compute immune strength.

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

Immune strength D is calculated by considering the following parameters:

d = immune strength coefficient;

l = immune-system strength scaling coefficient;

s = ratio of $(L/T)^l$ (It tells how quickly CD8+ T-cell 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.

$$\begin{aligned} Tw &= 4.65928 \times 10^9 \\ Tm &= 35 \\ N &= 9 \times 10^7 \\ L &= 1.8 \times 10^5 \\ C &= 9 \times 10^8 \\ M &= 0 \\ I &= 1173 \\ A &= 0 \end{aligned}$$

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 two hours, 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 and Natural Killer Cell Activity

The enhancement of natural killer cells activity induced by mAb therapy is the same for both mutated and wildtype cells. This is represented in both equations by the $-\xi \frac{A}{h1+A} NTw$ term. Chemotherapy has reduced effectiveness against tumor cells during monoclonal antibody treatment because of mutant cells. This is represented in the model by $-Kt \frac{Tw}{\alpha(Tm)+Tw}$. The chemotherapy effectiveness

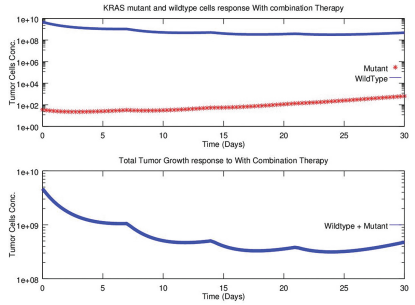

Fig. 1. α value: 10^6 shows rapid decrease in wildtype and increase in mutant KRAS cells (Red: mutant; Blue: wildtype) (Colour figure online)

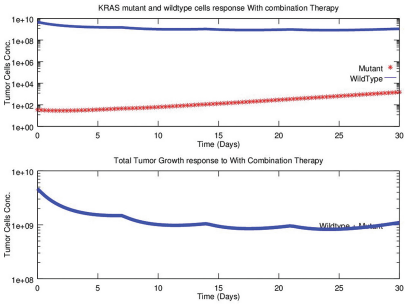

Fig. 2. α: 10^7 shows gradual decrease in wildtype and increase in mutant KRAS cells (Red: mutant; Blue: wildtype) (Colour figure online)

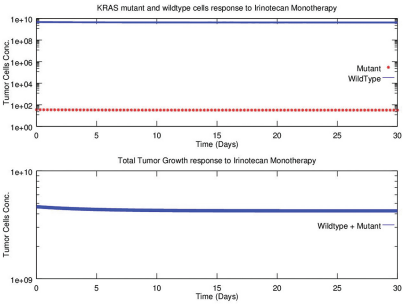

Fig. 3. Irinotecan monotherapy(Red: mutant; Blue: wildtype) (Colour figure online)

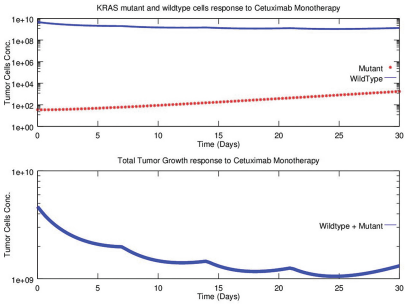

Fig. 4. Cetuximab monotherapy(Red: mutant; Blue: wildtype) (Colour figure online)

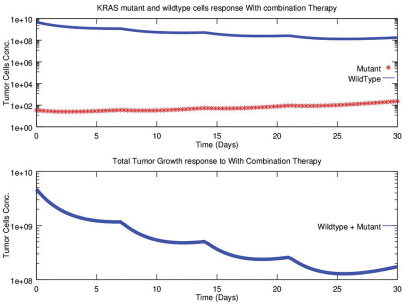

Fig. 5. Cetuximab and Irinotecan as combination therapy with KRAS mutant (Red: Mutant; Blue: Wildtype) (Colour figure online)

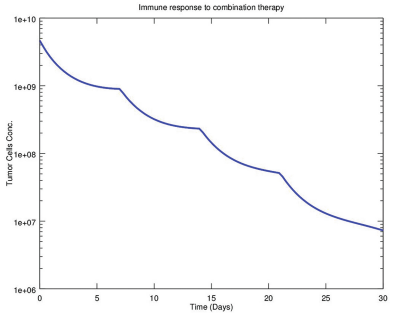

Fig. 6. Cetuximab and Irinotecan as combination therapy without KRAS mutant (Red: Mutant; Blue: Wildtype) (Colour figure online)

Table 1. Cetuximab and irinotecan combination therapy

	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)

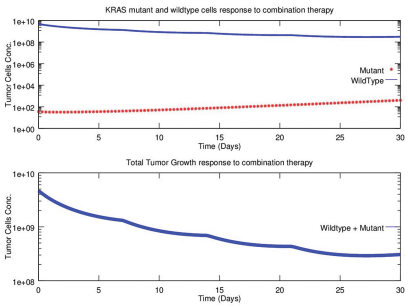

Fig. 7. Moderate Immunity response to KRAS mutation

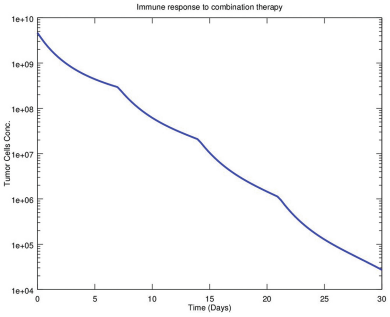

Fig. 8. Strong Immunity response without KRAS mutation

decreases with the increase of the number of mutated cells. This term is introduced in both the equations of wildtype and mutant tumour cells for controlling the rate of chemotherapy induced tumor death. Kt 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 wildtype 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 (Figs. 1 and 2). Similarly, by increasing the initial number of KRAS mutated cells or by decreasing the initial number of KRAS wildtype 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. It is hard to find more realistic values for α as we did not find any clue in the literature about the chemotherapy ineffectiveness rate due to increase in KRAS mutations. 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 $\alpha = 10^6$.

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

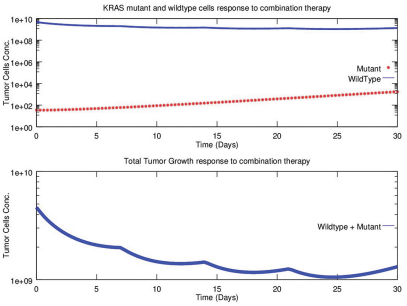

Fig. 9. Moderate Immunity response to KRAS mutation

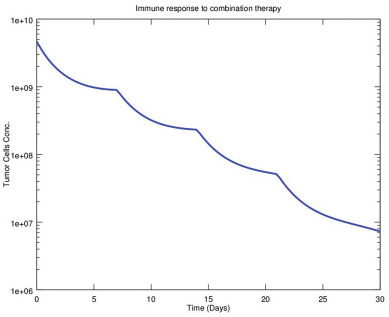

Fig. 10. Moderate Immunity response without KRAS mutation

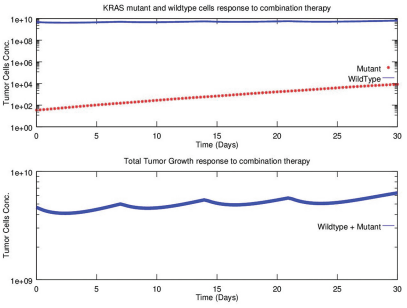

Fig. 11. Weak Immunity response with KRAS mutation

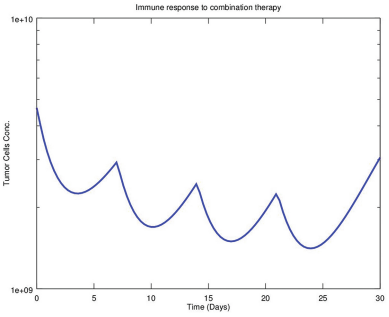

Fig. 12. Weak Immunity response without KRAS mutation

tumors are treated according to standard dosage of drugs and are evaluated for both monotherapy and combination therapy.

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.

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 wildtype 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 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 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 Sect. 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 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 wildtype 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

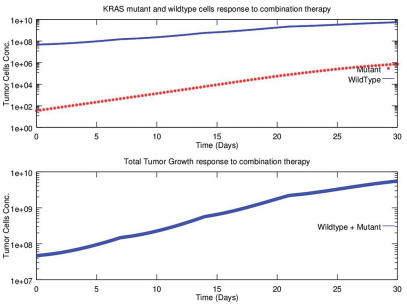

Fig. 13. Cetuximab and Irinotecan as first-line therapy: weak immune response (Red: Mutant; Blue: Wild-type) (Colour figure online)

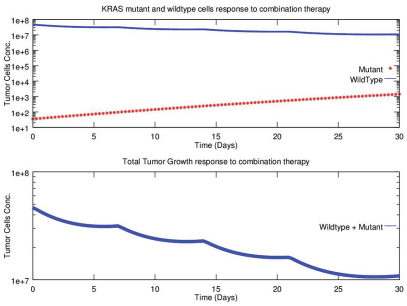

Fig. 14. Cetuximab and Irinotecan as first-line therapy: moderate immune response (Red: Mutant; blue: Wild-type) (Colour figure online)

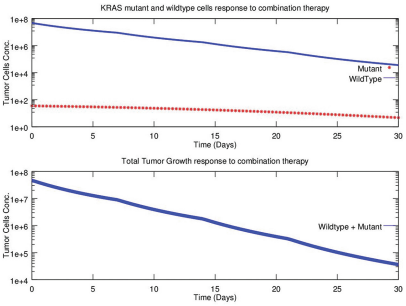

Fig. 15. Cetuximab and Irinotecan as first-line therapy: strong immune response (Red:Mutant; Blue:Wildtype) (Colour figure online)

in the presence of mutations is another key issue in understanding drug efficacy [29]. In case of natural killer cells, Cetuximab has equal enhancing effect on both KRAS mutant and wildtype cells. In other words, KRAS mutational status has no impact on the antibody-dependent cellular cytotoxicity (ADCC) mediated by the drug [32]. Cetuximab has been frequently reported to increase chemotherapeutic activity upon combination with Irinotecan drug in tumor cells [30,31]. Studies show that KRAS mutant cells do not allow Cetuximab to produce such type of chemosensitization [9,13]. In chemo-refractory colorectal cancer with mutated KRAS the chemotherapy failed to induce tumor cell death, not only for mutated cells but also for wildtype cells. The reason for this lies in the heterogeneity of KRAS mutations in colorectal tumors [33–35]. In order to model this phenomenon we have regulated the rate of chemotherapy induced tumor death. We assumed that the effect of chemotherapy decreases with the increase in KRAS mutated cells. Therefore, we cannot take any benefit from the chemosensitization activity of mAb 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, starts to loose its strength.

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 [36]. 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 [38,39]. 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 [11,37]. 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 for 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. The possibilities of Cetuximab and Irinotecan drugs as first-line therapy for treatment of KRAS mutated colorectal cancer can again be explored by varying dosages and timings of the drugs and also by applying other monoclonal antibodies, e.g. Panitumumab and Bevacizumab.

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.

References

1. WHO/Europe—Colorectal cancer. http://www.euro.who.int/en/health-topics/noncommunicable-diseases/cancer/news/news/2012/2/early-detection-of-common-cancers/colorectal-cancer
2. Deschoolmeester, V., Baay, M., Specenier, P., Lardon, F., Vermorken, J.B.: A review of the most promising biomarkers in colorectal cancer: one step closer to targeted therapy. Oncologist **15**, 699–731 (2010)
3. Repetto, L., Gianni, W., Aglianò, A.M., Gazzaniga, P.: Impact of EGFR expression on colorectal cancer patient prognosis and survival: a response. Ann. Oncol. **16**, 1557 (2005)
4. Gschwind, A., Fischer, O.M., Ullrich, A.: The discovery of receptor tyrosine kinases: targets for cancer therapy. Nat. Rev. Cancer. **4**, 361–370 (2004)
5. Van Cutsem, E., Peeters, M., Siena, S., Humblet, Y., Hendlisz, A., Neyns, B., Canon, J.L., Van Laethem, J.L., Maurel, J., Richardson, G., Wolf, M., Amado, R.G.: Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J. Clin. Oncol. **25**, 1658–1664 (2007)
6. Martinelli, E., De Palma, R., Orditura, M., De Vita, F., Ciardiello, F.: Anti-epidermal growth factor receptor monoclonal antibodies in cancer therapy. Clin. Exp. Immunol. **158**, 1–9 (2009)
7. Parsons, B.L., Meng, F.: K-RAS mutation in the screening, prognosis and treatment of cancer. Biomark Med. **3**, 757–769 (2009)
8. Bando, H., Yoshino, T., Tsuchihara, K., Ogasawara, N., Fuse, N., Kojima, T., Tahara, M., Kojima, M., Kaneko, K., Doi, T., Ochiai, A., Esumi, H., Ohtsu, A.: KRAS mutations detected by the amplification refractory mutation system-scorpion assays strongly correlate with therapeutic effect of cetuximab. Br. J. Cancer **105**, 403–406 (2011)
9. Karapetis, C.S., Khambata-Ford, S., Jonker, D.J., O'Callaghan, C.J., Tu, D., Tebbutt, N.C., Simes, R.J., Chalchal, H., Shapiro, J.D., Robitaille, S., Price, T.J., Shepherd, L., Au, H.J., Langer, C., Moore, M.J., Zalcberg, J.R.: K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N. Engl. J. Med. **359**, 1757–1765 (2008)

10. Amado, R.G., Wolf, M., Peeters, M., Van Cutsem, E., Siena, S., Freeman, D.J., Juan, T., Sikorski, R., Suggs, S., Radinsky, R., Patterson, S.D., Chang, D.D.: Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J. Clin. Oncol. **26**, 1626–1634 (2008)
11. Van Cutsem, E., Köhne, C.H., Hitre, E., Zaluski, J., Chang Chien, C.R., Makhson, A., D'Haens, G., Pintér, T., Lim, R., Bodoky, G., Roh, J.K., Folprecht, G., Ruff, P., Stroh, C., Tejpar, S., Schlichting, M., Nippgen, J., Rougier, P.: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N. Engl. J. Med. **360**, 1408–1417 (2009)
12. Fakih, M.M.: KRAS mutation screening in colorectal cancer: from paper to practice. Clin. Colorectal Cancer **9**, 22–30 (2010)
13. De Roock, W., Piessevaux, H., De Schutter, J., Janssens, M., De Hertogh, G., Personeni, N., Biesmans, B., Van Laethem, J.L., Peeters, M., Humblet, Y., Van Cutsem, E., Tejpar, S.: KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann. Oncol. **19**, 508–515 (2008)
14. Parsons, B.L., Myers, M.B.: KRAS mutant tumor subpopulations can subvert durable responses to personalized cancer treatments. Pers. Med. **10**, 191–199 (2013)
15. Tougeron, D., Lecomte, T., Pagés, J.C., Villalva, C., Collin, C., Ferru, A., Tourani, J.M., Silvain, C., Levillain, P., Karayan-Tapon, L.: Effect of low-frequency KRAS mutations on the response to anti-EGFR therapy in metastatic colorectal cancer. Ann. Oncol. **24**, 1267–1273 (2013)
16. Ballesta, A., Clairambault, J.: Physiologically based mathematical models to optimize therapies against metastatic colorectal cancer: a mini-review. Curr. Pharm. Des. **20**, 37–48 (2014)
17. Johnston, M.D., Edwards, C.M., Bodmer, W.F., Maini, P.K., Chapman, S.J.: Mathematical modeling of cell population dynamics in the colonic crypt and in colorectal cancer. Proc. Natl. Acad. Sci. U.S.A. **104**, 4008–4013 (2007)
18. van Leeuwen, I.M., Byrne, H.M., Jensen, O.E., King, J.R.: Crypt dynamics and colorectal cancer: advances in mathematical modelling. Cell Prolif. **39**, 157–181 (2006)
19. Fletcher, A.G., Breward, C.J.W., Chapman, S.J.: Mathematical modeling of monoclonal conversion in the colonic crypt. J. Theor. Biol. **300**, 118–133 (2012)
20. Murray, P.J., Walter, A., Fletcher, A.G., Edwards, C.M., Tindall, M.J., Maini, P.K.: Comparing a discrete and continuum model of the intestinal crypt. Phys. Biol. **8**, 1478–3975 (2011)
21. Johnston, M.D., Edwards, C.M., Bodmer, W.F., Maini, P.K., Chapman, S.J.: Mathematical modeling of cell population dynamics in the colonic crypt and in colorectal cancer. Proc. Natl. Acad. Sci. U.S.A. **104**(10), 4008–4013 (2007)
22. Monro, H.C., Gaffney, E.A.: Modelling chemotherapy resistance in palliation and failed cure. J. Theor. Biol. **257**, 292–302 (2009)
23. Boston, E.A.J., Gaffney, E.A.: The influence of toxicity constraints in models of chemotherapeutic protocol escalation. Math. Med. Biol. **28**, 357–384 (2011)
24. Diaz, L.A., Williams, R.T., Wu, J., Kinde, I., Hecht, J.R., Berlin, J., Allen, B., Bozic, I., Reiter, J.G., Nowak, M.A., Kinzler, K.W., Oliner, K.S., Vogelstein, B.: The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature **486**, 537–540 (2012)
25. Stites, E.C.: Differences in sensitivity to EGFR inhibitors could be explained by described biochemical differences between oncogenic Ras mutants. bioRxiv (2014). http://dx.doi.org/10.1101/005397

26. de Pillis, L.G., Savage, H., Radunskaya, A.E.: Mathematical model of colorectal cancer with monoclonal antibody treatments. Brit. J. of Med. and Medical Res. **4**(16), 3101–3131 (2014)
27. GNU Octave 3.8.1. http://www.gnu.org/software/octave/
28. Eaton, J.W., Bateman, D., Hauberg, S.: GNU Octave version 3.0.1 manual: a high-level interactive language for numerical computations, CreateSpace Independent Publishing Platform. ISBN: 1441413006 (2009). http://www.gnu.org/software/octave/doc/interpreter
29. Arnold, D., Seufferlein, T.: Targeted treatments in colorectal cancer: state of the art and future perspectives. Gut **59**, 838–858 (2010)
30. Prewett, M.C., Hooper, A.T., Bassi, R., Ellis, L.M., Waksal, H.W., Hicklin, D.J.: Enhanced antitumor activity of anti-epidermal growth factor receptor monoclonal antibody IMC-C225 in combination with irinotecan (CPT-11) against human colorectal tumor xenografts. Clin. Cancer Res. **8**, 994–1003 (2002)
31. Jonker, D.J., O'Callaghan, C.J., Karapetis, C.S., Zalcberg, J.R., Tu, D., Au, H.J., Berry, S.R., Krahn, M., Price, T., Simes, R.J., Tebbutt, N.C., van Hazel, G., Wierzbicki, R., Langer, C., Moore, M.J.: Cetuximab for the treatment of colorectal cancer. N. Engl. J. Med. **357**, 2040–2048 (2007)
32. Wu, L., Adams, M., Carter, T., Chen, R., Muller, G., Stirling, D., Schafer, P., Bartlett, J.B.: lenalidomide enhances natural killer cell and monocyte-mediated antibody-dependent cellular cytotoxicity of rituximab-treated CD20+ tumor cells. Clin. Cancer Res. **14**, 4650–4657 (2008)
33. Vilar, E., Tabernero, J.: Cancer: pinprick diagnostics. Nature **486**, 482–483 (2012)
34. Baldus, S.E., Schaefer, K.L., Engers, R., Hartleb, D., Stoecklein, N.H., Gabbert, H.E.: Prevalence and heterogeneity of KRAS, BRAF, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases. Clin. Cancer Res. **16**, 790–799 (2010)
35. Hasovits, C., Pavlakis, N., Howell, V., Gill, A., Clarke, S.: Resistance to EGFR targeted antibodies - expansion of clones present from the start of treatment. The more things change, the more they stay the same (Plus ca change, plus ca ne change pas!. Transl. Gastrointest. Cancer **2**, 44–46 (2013)
36. Smakman, N., Veenendaal, L.M., van Diest, P., Bos, R., Offringa, R., Borel Rinkes, I.H., Kranenburg, O.: Dual effect of Kras(D12) knockdown on tumorigenesis: increased immune-mediated tumor clearance and abrogation of tumor malignancy. Oncogene **24**, 8338–8342 (2005)
37. Folprecht, G., Lutz, M.P., Schöffski, P., Seufferlein, T., Nolting, A., Pollert, P., Köhne, C.H.: Cetuximab and irinotecan/5-fluorouracil/folinic acid is a safe combination for the first-line treatment of patients with epidermal growth factor receptor expressing metastatic colorectal carcinoma. Ann. Oncol. **17**, 450–456 (2006)
38. Pfeiffer, P., Nielsen, D., Bjerregaard, J., Qvortrup, C., Yilmaz, M., Jensen, B.: Biweekly cetuximab and irinotecan as third-line therapy in patients with advanced colorectal cancer after failure to irinotecan, oxaliplatin and 5-fluorouracil. Ann. Oncol. **19**, 1141–1145 (2008)
39. Vincenzi, B., Santini, D., Rabitti, C., Coppola, R., Beomonte Zobel, B., Trodella, L., Tonini, G.: Cetuximab and irinotecan as third-line therapy in advanced colorectal cancer patients: a single centre phase II trial. Br. J. Cancer. **94**, 792–797 (2006)