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A Mathematical Model Explains the Prognostic Influence of C1q Polymorphism on Rituximab Treatment of Nodular Lymphoma

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Abstract
A particular monoclonal antibody (mAB) binds to a particular antigen on the target cell, and thereby kills the target cell by direct killing, or by Antibody-dependent Cellular Cytotoxicity (ADCC), or by Complement-dependent Cytotoxicity (CDC), or by Antibody-dependent Cellular Phagocytosis (ADCP). Rituximab is one of the anti-CD20 mABs which has been found to be effective in killing the cancer cells in the patients of Nodular Lymphoma (NL). Three different treatment efficacies of Rituximab have been demonstrated in NL patients in the background of C1q polymorphism: in the background of null C1q Rituximab fails completely, in the background of low levels of C1q protein the patient response is high, and in the background of high levels of C1q protein the patient response is low. Assuming that the killing of the cancerous cells in the patients of NL by Rituximab treatment is CDC dominant, I propose a mathematical model which captures qualitatively the different treatment efficacies of Rituximab in NL patients in the background of C1q polymorphism. I also argue in the end that the killing of the cancerous cells in the patients of NL by Rituximab treatment is perhaps CDC dominant. The purpose of developing the mathematical model in this work has been to show the scientists the way to determine if CDC is the dominant mechanism of killing by Rituximab.

Keywords: Monoclonal antibodies; Antigen; Immune cells; Malignant; Complement unit.

1. Introduction

1.1 The biology

Around the years 1990s monoclonal antibodies mABs were found to be potent treatments/drugs against cancer, and the first such drug to be approved was Rituximab (Zahavi and Weiner 2020; Maloney et al. 1997). The antigen which Rituximab targets is CD20. CD20 is a protein found to be plentifully expressed on the cancerous B cells, otherwise normally expressed on mature B cells but not on immature B cells (Zahavi and Weiner 2020). Hence Rituximab is the drug of choice against Nodular Lymphoma (NL). NL is most popularly known as Follicular Lymphoma (FL). NL/FL is the uncontrolled malignant growth of the certain class of B cells known as centrocytes and centroblasts.

The simplified structure of an antibody is shown in Figure 1 (Chiu et al. 2019; Chailyan et al. 2011). The antibody is basically a multi-chain multi-protein unit also known as immunoglobulin. There are two light chains and two heavy chains, each possessing both the variable regions and the constant regions. The main active part of the antibody lies in the variable region, which binds to the target antigen. There are two such binding regions on a single antibody. That part of the variable region which binds to the target antigen is known as Complementarity Determining Region (CDR), and it comprises 3 loops each from the heavy chain and the light chain (Chailyan et al. 2011). The various fragments Fab, Fv, and Fc of the antibody are also shown in Figure 1. The antigen binding region is terminated by the free amine group (-NH2), hence also known as the N-terminal end of antibody. The Fc region of the antibody is terminated by a free carboxyl group (-COOH), hence also known as the C-terminal end of antibody.

The probable mechanisms involved in the treatment of cancer by mABs are direct killing (Zahavi and Weiner 2020), Antibody-dependent Cellular Cytotoxicity (ADCC) (Zahavi and

Weiner 2020; Wang et al. 2015), Complement-dependent Cytotoxicity (CDC) (Zahavi and Weiner 2020; Reis et al. 2018), and Antibody-dependent Cellular Phagocytosis (ADCP) (Zahavi and Weiner 2020; Gül and van Egmond 2015). In direct killing the mAB probably blocks the activation of the signaling pathways in control of the target antigen downstream of it; these signaling pathways are usually the ones that deregulate proliferation and apoptosis in cancer cells. In ADCC, upon binding of the Fab of mAB to target antigen the Fc (of mAB) binds to the Fc receptors (FcRs) expressed on effector immune cells. Thus an immune complex Antigen-Fab-Fc-FcR forms involving two cells- a cancer cell and a non-cancerous effector immune cell . Upon activation the FcR eventually is responsible for the death of the cancer cells. The probable immune cells causing death of the cancer cells by ADCC are Natural Killer cells (NK), monocytes, macrophages, neutrophils, eosinophils, and dendritic cells (Zahavi and Weiner 2020). But mostly NK controlled ADCC has been observed to deliver higher performance vis-à-vis other effector immune cells (Zahavi and Weiner 2020; Wang et al. 2015). In CDC the immune complex formed with mAB is one single cell- the cancer cell itself. Almost all the immune cells express the complement units (CUs) on their surfaces. Hence the CDC mediated death is observed mostly in cancers of the immune cells. The CU is a complicated network of several different types of molecules, at the apex of which is the molecule C1q (Reis et al. 2018). When the Antigen-Fab-Fc-C1q complex is formed on the same cell (a cancer cell in the context of this work), the CU is classically activated leading eventually to the death of the cell. In ADCP the macrophages internalize a cancer cell to which the IgG1 or IgG3 mABs have bound. The FcYRI expressed on the surfaces of macrophages binds to the Fc on the cancer cell bound IgG1 or IgG3 mABs.

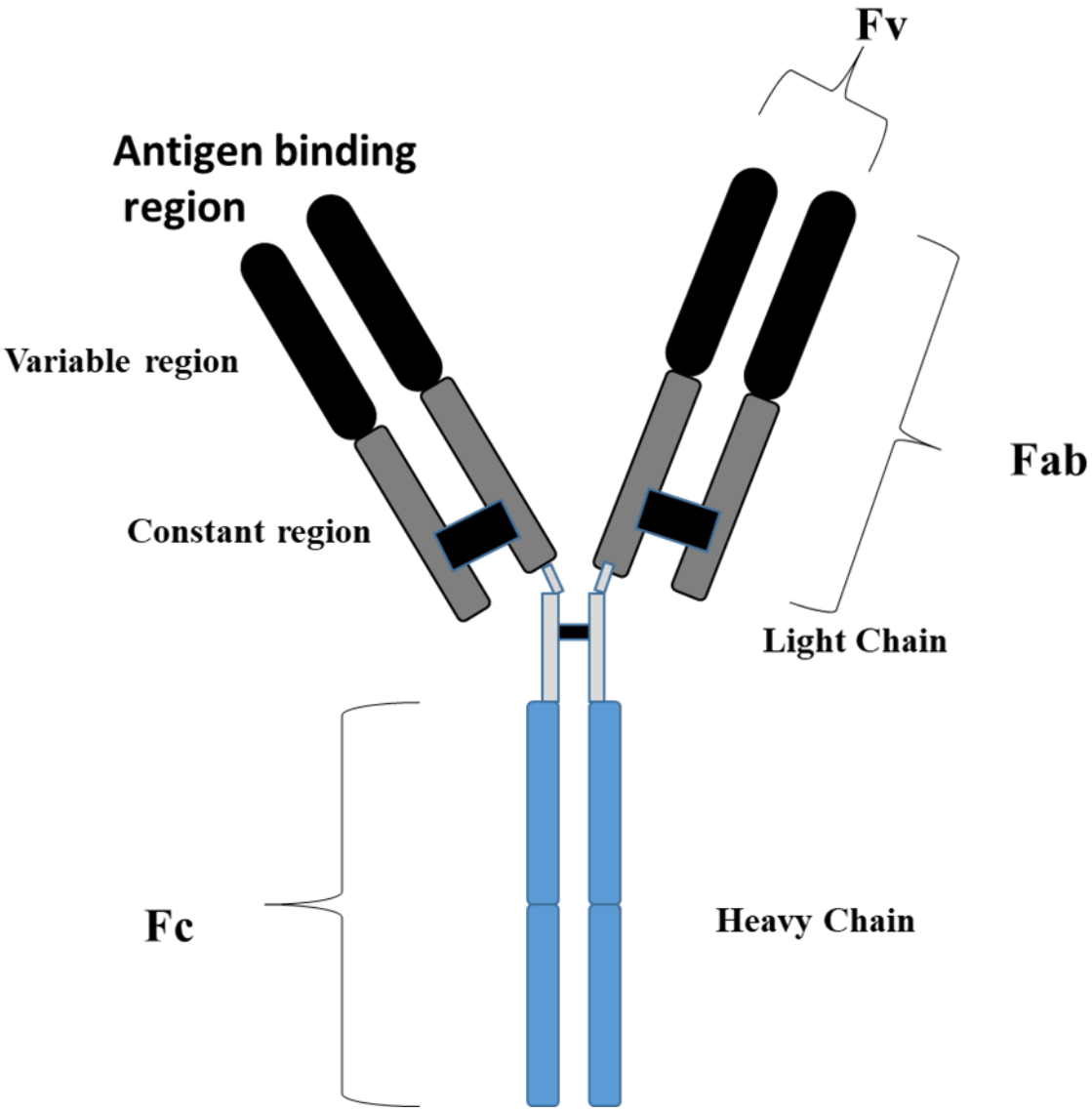

Fig. 1: The simplified structure of an antibody, the inter-chain bridges shown in black color are disulfide bonds.

1.2 The Mathematics

The three experimental/preclinical/clinical observations build up the mathematics for Rituximab treatment of FL. They are

(1) The $C1qa^{-/-}$ mouse with the intravenously injected EL4-$CD20^{+}$lymphoma cells failed completely to respond to the Rituximab treatment (Di Gaetano et al. 2003).

(2) In the sequence C1qA-$Gly70_{GGG}$ when the third guanine is replaced with adenine, we get a silent mutation with the sequence C1qA-$Gly70_{GGA}$ (Racila et al. 2003). This allele of the C1qA gene is called the A allele and the C1qA gene with the normal sequence is called the G allele (Racila et al.

2008). By a mechanism unknown the A allele results in the expression of low levels of C1q protein in serum. Amongst the FL patients who received Rituximab as first-line therapy, 53% of the patients homozygous for the A allele showed complete response [CR] (Racila et al. 2008).
(3) Amongst the FL patients who received Rituximab as first-line therapy, 14% of the patients homozygous for the G allele showed complete response [CR] (Racila et al. 2008).
Apart from the CR, the other measure for the anti-tumor effects of Rituximab is the median progression-free survival (mPFS) post CR. However, in my proposed mathematical model I do not include it. I also do not include in my mathematical model the clinical data for the patients heterozygous for the A and G allele of C1qA. Interpreted mathematically the CR percentage would mean the probability of the 100% of tumor cells undergoing apoptosis in a particular patient. I denote this probability P_{100}. The three observations listed above in this sub-section is plotted in Figure 2.

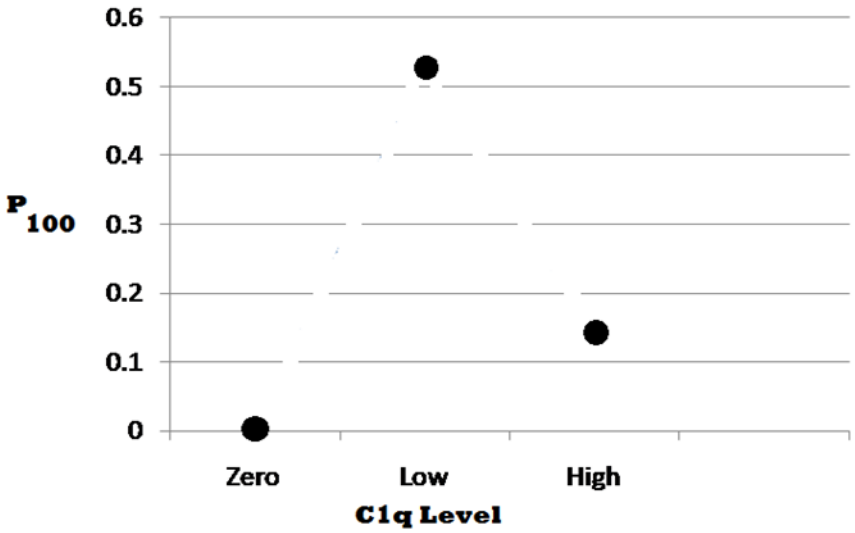

Fig. 2: The prognostic influence of Rituximab treatment of Nodular Lymphoma in the background of various levels of C1q protein in serum.

1.3 The purpose of this study

The purpose of this study is to ascertain if CDC is the dominant mechanism by which Rituximab kills the cancerous cells in patients of FL.

2. The Mathematical Model

2.1 Main Assumption

The main assumption in my proposed mathematical model is that the killing of the cancerous cells in the patients of FL by Rituximab treatment is CDC dominant. There are evidences that Rituximab or anti-CD20 mABs also kill the malignant B cells by direct killing (Byrd et al. 2002; Cardarelli et al. 2002; Pedersen et al. 2002; Shan et al. 1998), and ADCC (Cartron et al. 2002; Dall'Ozzo et al. 2004; Weng and Levy 2003). However, there are contradictory studies too: in a murine model the work (Di Gaetano et al. 2003) shows that Rituximab does not kill the cancer cells by ADCC, and the works (Golay et al. 2000; Manches et al. 2003) show that Rituximab does not exert anti-tumor effects by direct killing. The authors of the work (Zahavi and Weiner 2020) opine that CDC is a major mechanism by which Rituximab kills the cancer cells.
On first look the graph in Figure 2 would vote against CDC being the major mechanism of killing of the cancer cells by Rituximab. But my mathematical model supports qualitatively the result of graph in Figure 2 and hence proposes CDC to be one of the candidates of being a major player in the killing of the cancer cells by Rituximab.
The other major assumption is that there is just one C1q protein associated with one complement unit expressed on the surface of cell. Hence if there are n C1q proteins expressed by the cell, there would be n complement units on the surface of the cell.

2.2 The Rules that Make the Mathematical Model

The proposed rules that make the mathematical model are
(1) If the number of expressed C1q proteins in a cell are less than a particular threshold N_0, then the Rituximab treatment will fail completely even if the number of Rituximab molecules per cell is equal to or more than the number of C1q proteins on that cell. Also, if the number of Rituximab molecules per cell is less than this particular threshold N_0, then the Rituximab treatment will fail completely even if the number of C1q proteins per cell is equal to or more than N_0.
(2) If the percentage of C1q proteins on a cell associated with Rituximab molecules is below a particular threshold R_0, then the Rituximab treatment will fail completely.
(3) The Logical operation between Rule 1 and Rule 2 is "OR".
(4) If, however, the Rituximab treatment is voted (based on Rule 1 and Rule 2 above) not to fail, the higher percentage of C1q proteins associated with Rituximab molecules will yield higher P_{100} vis-à-vis the lower percentage of C1q proteins associated with Rituximab molecules.
(5) If, however, the Rituximab treatment is voted (based on Rule 1 and Rule 2 above) not to fail, the 100% of C1q proteins associated with Rituximab molecules will yield same P_{100} vis-à-vis the Rituximab molecules being greater than the number of expressed C1q proteins in cell.

3. Results

Figure 3 is constructed based on the proposed mathematical model. It shows, with a few mathematical calculations that are done below, that the results of Figure 2 are qualitatively

embedded in the mathematical model.

Assumption: $R^{//}$ (= $(N_0/N_A)*100$) > R_0
Calculations: $R^{/} = (N^{/}/N_A)*100$, which implies
$N^{/} = (R^{/} * N_A) / 100$ (1)
Let $R_G^{/}$ be the percentage of C1q proteins per cell associated with Rituximab in FL patients having the G allele of C1q gene if the number of Rituximab molecules per cell is $N^{/}$.
Then
$R_G^{/} = (R^{/}*N_A*100) / (100*N_G)$ (2)
where N_G→ Number of C1q proteins per cell for the G allele of C1q gene, and $N_G >> N_A$.
Hence
$R_G^{/} = (R^{/}*N_A) / N_G$ (3)

Obviously, $R_G^{/} << R^{/}$, and by the mathematical model the complete response percentage for the G allele patients will be much less than that for the A allele patients.

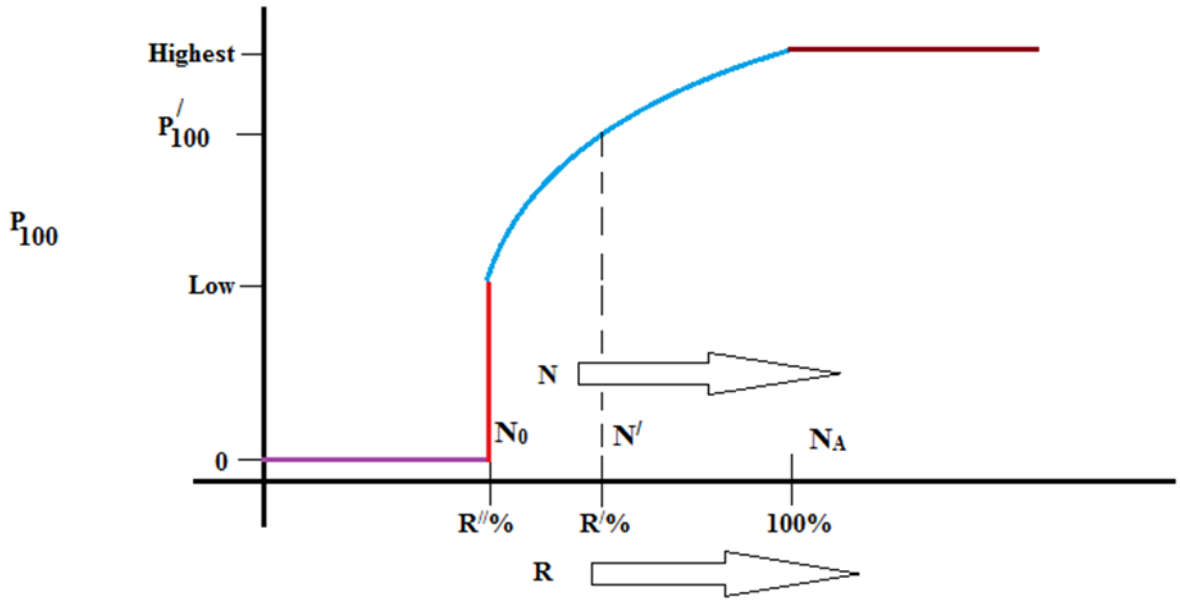

Fig. 3: Capturing qualitatively the prognostic influence of Rituximab treatment in Follicular Lymphoma patients having the A allele of C1q gene. N → Number of Rituximab molecules per cell. R → Percentage of C1q proteins per cell associated with Rituximab. N_A→ Number of C1q proteins per cell for the A allele of C1q gene. $N^{/}$→ Number of Rituximab molecules per cell in a generalized patient involved in the clinical study in work (Racila et al. 2008).

Let us now look at the mathematical model closely with some actual numbers.
Let $N_A = 1.5\ N_0$, and $N_A = 2.0\ N_0$
Let $N_G = 1.5\ N_A$, $N_G = 2.0\ N_A$, and $N_G = 3.0\ N_A$
Then for $N_A = 1.5\ N_0$, $N_G = 2.25\ N_0$, $N_G = 3.0\ N_0$, $N_G = 4.5\ N_0$,
And
for $N_A = 2.0\ N_0$, $N_G = 3.0\ N_0$, $N_G = 4.0\ N_0$, $N_G = 6.0\ N_0$.
Let $R^{/} = 100\%$, $R^{/} = 90\%$, $R^{/} = 80\%$, $R^{/} = 70\%$.
Calculations for $R_G^{/}$ for several cases:
Case1: $N_A = 1.5\ N_0$, $N_G = 2.25\ N_0$
Sub-case 1: $R^{/} = 100\%$
$R_G^{/} = (1.5\ N_0 * 100) / (2.25\ N_0) = 66.7\ \%$
Sub-case 2:$R^{/} = 90\%$
$R_G^{/} = 0.9*66.7 = 60\%$
Sub-case 3:$R^{/} = 80\%$
$R_G^{/} = 0.8*66.7 = 53\%$
Sub-case 4:$R^{/} = 70\%$
$R_G^{/} = 0.7*66.7 = 47\%$

Case2: $N_A = 1.5\ N_0$, $N_G = 3.0\ N_0$
Sub-case 1: $R^{/} = 100\%$
$R_G^{/} = (1.5\ N_0 * 100) / (3.0\ N_0) = 50\ \%$
Sub-case 2:$R^{/} = 90\%$
$R_G^{/} = 0.9*50 = 45\%$
Sub-case 3:$R^{/} = 80\%$
$R_G^{/} = 0.8*50 = 40\%$
Sub-case 4:$R^{/} = 70\%$
$R_G^{/} = 0.7*50 = 35\%$

Case3: $N_A = 1.5\ N_0$, $N_G = 4.5\ N_0$
Sub-case 1: $R^{/} = 100\%$
$R_G^{/} = (1.5\ N_0 * 100) / (4.5\ N_0) = 33\ \%$
Sub-case 2:$R^{/} = 90\%$
$R_G^{/} = 0.9*33 = 30\%$
Sub-case 3:$R^{/} = 80\%$
$R_G^{/} = 0.8*33 = 27\%$
Sub-case 4:$R^{/} = 70\%$
$R_G^{/} = 0.7*33 = 23\%$

We set three criteria for the Rituximab treatment in FL patients having the G allele of C1q gene showing non-zero CR. They are R_0>= 50%, R_0>= 40%, and R_0>= 30%
For R_0>= 50%, Case 1, CR will not be achieved for $R^/$ below 80%.
For R_0>= 50%, Case 2, CR will not be achieved for $R^/$ below 100%.
For R_0>= 50%, Case 3, CR will not be achieved at all.
For R_0>= 40%, Case 1, CR will not be achieved for $R^/$ below 70%.
For R_0>= 40%, Case 2, CR will not be achieved for $R^/$ below 80%.
For R_0>= 40%, Case 3, CR will not be achieved at all
For R_0>= 30%, Case 1, CR will not be achieved for $R^/$ below 70%.
For R_0>= 30%, Case 2, CR will not be achieved for $R^/$ below 70%.
For R_0>= 30%, Case 3, CR will not be achieved for $R^/$ below 90%.
This result is shown in Figure 4, and an important conclusion follows from the figure. Same result holds for $N_A = 2.0\ N_0$. From the figure it is clear that for all the cases combined together the G allele (of C1q gene) patients of FL will fail to show any CR for the percentage of C1q proteins per cell associated with Rituximab being below 70% in the FL patients having the A allele of C1q gene.

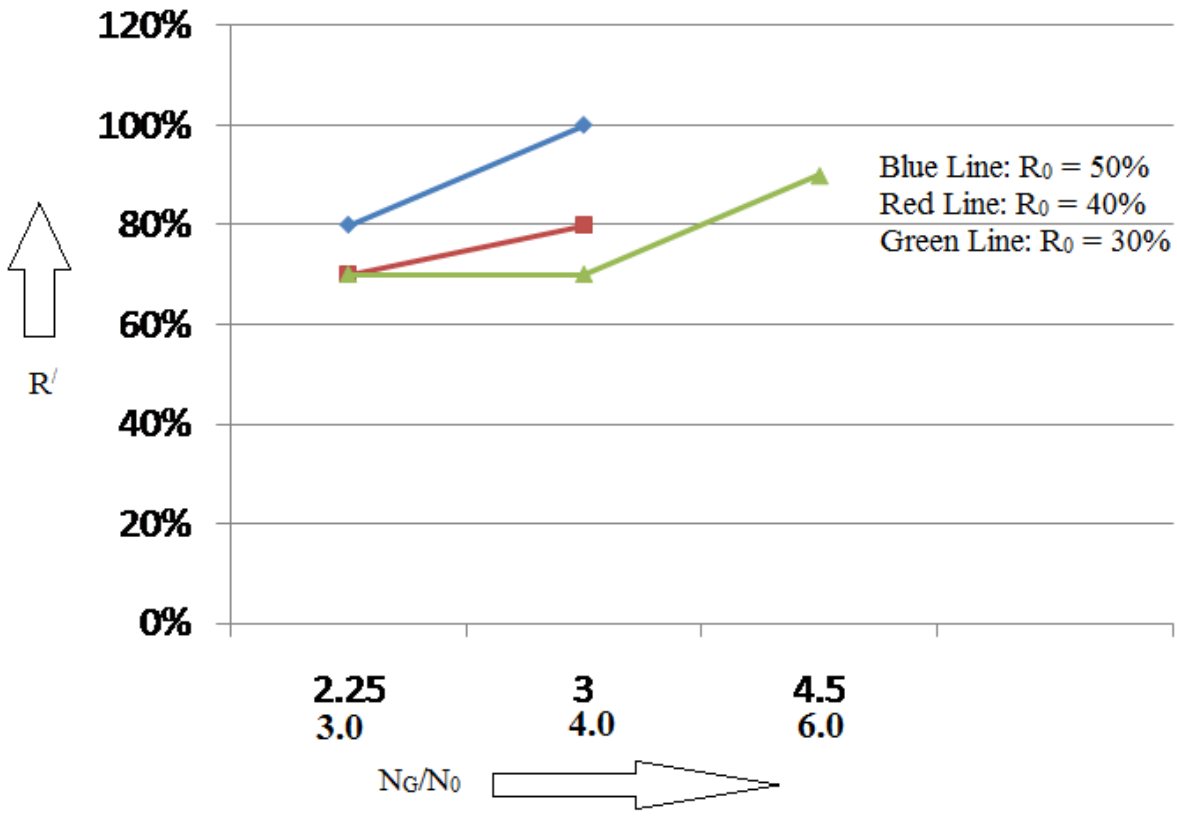

Fig. 4: Values of $R^/$ below which complete response will not be obtained in the Follicular Lymphoma patients having the G allele of C1q gene. $R^/$ is the percentage of C1q proteins per cell associated with Rituximab in the Follicular Lymphoma patients having the A allele of C1q gene.

4. Discussion

4.1 How to validate the mathematical model

If the dose of Rituximab is reduced below a particular value, the CR must not be achieved at all for both the A allele (of C1q gene) and the G allele (of C1q gene) patients of FL. From Figure 4 it follows that there must also be a certain dose of the Rituximab for which the CR will occur for the A allele (of C1q gene) patients of FL, but simultaneously the CR will not be achieved at all for the G allele (of C1q gene) patients of FL. If the dose of Rituximab is kept on increasing there will reach a stage when the CR occurring in the G allele (of C1q gene) patients of FL will match the maximum possible CR occurring in the A allele (of C1q gene) patients of FL; this stage is the one when $R_G^/ = 100\%$.
If all the above observations are made in a clinical setting, the mathematical model proposed in this paper is validated.

4.2 Autoimmunity and CDC

The validation of my mathematical model will prove that the killing of the cancerous cells in the patients of FL by Rituximab treatment is CDC dominant.
C1q is known to regulate immune tolerance by clearing up the apoptotic debris. If C1q protein levels in the serum is zero or low, apoptotic fragments are not cleared from the blood which then serve as antigens to activate the immune cells of adaptive immunity (Lu et al. 2008). 93% of the patients of autoimmune disorder systemic lupus erythematosus (SLE) are deficient in C1q protein (Macedo and Isaac 2016). The authors of the work (Racila et al. 2008) are of the opinion that because the A allele (of C1q gene) patients of FL have substantial amounts of apoptotic fragments (generated from the apoptosis of cancer cells) floating in blood they (the apoptotic fragments) activate the immune cells to attack the cancer cells. But I have a

question, “How do the apoptosis of cancer cells occur in first place?” The likely answer is that the CDC caused the apoptosis of cancer cells which eventually activated the immune cells of adaptive immunity to kill more cancer cells. Why could not ADCC or ADCP or direct killing be the dominant mechanism of generating apoptotic fragments from the cancer cells? The answer is that in the murine model of FL, deficiency of C1q caused the complete failure of Rituximab treatment (Di Gaetano et al. 2003). Hence the hypothesis that the CDC could be the dominant mechanism of killing cancer cells in FL patients post-Rituximab treatment is not a bad one. This hypothesis raises an important question, “What fraction of killed cancer cells are by CDC and what fraction are by adaptive immunity?” The author of this paper suggests that research be initiated in this direction.

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