The effect of lenvatinib and pembrolizumab on thyroid cancer refractory to iodine 131I simulated by mathematical modeling

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THE EFFECT OF LENVATINIB AND PEMBROLIZUMAB ON THYROID CANCER REFRACTORY TO IODINE ^{131}I SIMULATED BY MATHEMATICAL MODELING

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Immunotherapy and targeted therapy are alternative treatments to differentiated thyroid cancer (DTC), which is usually treated with surgery and radioactive iodine. However, in advanced thyroid carcinomas, molecular alterations can cause a progressive loss of iodine sensitivity, thereby making cancer resistant to radioactive iodine (RAIR). In the treatment of cancer, tyrosine kinase inhibitors are administered to prevent the growth of cancer cells. One such inhibitor, lenvatinib, forms a targeted therapy for RAIR-DTC, while the immunotherapeutic pembrolizumab, a humanized antibody, prevents the binding of programmed cell death ligand 1 (PD-L1) to the PD-1 receptor. As one of the first studies on treatments for thyroid cancer with mathematical model involving immunotherapy and targeted therapy, we developed an ordinary differential system and tested variables such as concentration of lenvatinib and pembrolizumab, total cancer cells, and number of immune cells (*i.e.*, T cells and natural killer cells (NK)). Analyzing local and global stability and the simulated action of drugs in patients with RAIR-DTC, revealed the combined effect of the targeted therapy with pembrolizumab. The scenarios obtained favor the combined therapy as the best treatment option, given its unrivaled ability to boost the immune system's rate of eliminating tumor cells.

Keywords: Thyroid tumor; Targeted therapy; Immunotherapy; Immune system; Mathematical model; Asymptotic stability; Lyapunov function.

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1. Introduction

Differentiated thyroid cancer (DTC) is usually treated with surgeries such as lobectomy, thyroidectomy, and, when necessary, radioactive iodine (RAI) therapy based on ^{131}I.[1] However, after malignant thyroid cells lose their ability to capture and concentrate iodine, other therapeutic options such as targeted therapies and immunotherapies should be applied.[2] According to Ref. 3, clinical and pathological evidence suggest that the increasingly poor differentiation of DTC until becoming anaplastic cancer is a natural process in the development of malignancy. In addition, papillary thyroid cancer (PTC) carrying mutations in the BRAF and RAS genes and RET–PTC rearrangements are more likely to progress through stages of increasingly poor differentiation. Those genetic changes can activate the mitogen-activated protein kinase (MAPK) signaling pathway, which is responsible for regulating various cellular activities. For that reason, those mutations have been explored as therapeutic targets in cancer treatment.[4,5]

An example of drug used to treat DTC due to a loss of sensitivity to iodine is lenvatinib[a]. According to Ref. 2, lenvatinib is a targeted therapeutic agent that acts as an inhibitor of multiple tyrosine kinases and primarily aims to reduce the proliferation of tumor cells by blocking vascular endothelial growth factors (VEGF).

In a randomized, double-blind, phase 3 study, lenvatinib was assessed by Ref. 6. The study had a total of 392 participants, 131 of whom received the placebo. The screening revealed several important results, including that responses rate to treatment of 64.8% and 1.5% using lenvatinib and placebo, respectively; the progression of cancer in 35.6% of patients receiving lenvatinib versus 83.2% in those taking placebo during the follow-up period, being approximately 17 months in both cases; and an average tumor progression-free survival of 18.3 months in the group using lenvatinib and 3.6 months in those using placebo. Some of the side effects observed in the study were hypertension, fatigue, and diarrhea.[6]

Considering the important role of the immune system in the development of cancer, Ref. 7 examined the infiltration of regulatory T cells (Tregs) and natural killer (NK) cells in thyroid gland of patients with PTC. Among the results, some effector T cells were strongly controlled by Tregs cells that inhibited their proliferation, as well as prevented attacks on the tumor by the immune system. The infiltration of Tregs was positively associated with advanced stages of tumor progression, whereas NK cells were negatively correlated with those stages. As for lenvatinib, Ref. 8 observed that in cases of murine melanoma and kidney cancer, the infiltration of NK cells into the tumor increased as a result of targeted therapy. Thus, the drug also caused immune modulation in tumors, thereby making it a promising complementary agent to be used with immunotherapies in order to increase tumor infiltration and NK cell activation.

The immune regulatory molecule known as programmed cell death ligand 1

[a]Medicine developed and marketed by Eisai Co. Ltd. under the label Lenvima.

(PD-L1), correlated with PTC involving a greater risk of metastasis or shorter disease-free survival, is indicated as a prognostic marker of cancer under treatment with immunotherapy.[9,10] PD-L1 blocks the immune response of activated T cells by binding to the PD-1 receptor, an immune checkpoint expressed in T cells, to modulate their activation or inhibition.[2,11] Pembrolizumab[b] is a monoclonal antibody of human immunoglobulin G4 able to prevent the binding of PD-1 to PD-L1 and thus restore the antitumor immune response of T cells.

The antitumor response observed by using targeted therapies in PTC that is refractory to RAI (RAIR) is usually limited, in the sense that the tumor always regrows at some point during or after treatment.[12] Several studies have addressed the combination of targeted therapies and immunotherapy as a treatment for RAIR-DTC. For example, ongoing clinical trials currently evaluate the combination of lenvatinib and pembrolizumab in patients with DTC (i.e., NCT02973997 and NCT02628067)[2,12–15] and other cancers.[16,17]

Mathematical modeling has been widely used to study tumor dynamics and cancer treatments. For instance, considering three coupled differential equations and variables at time t denoting the tumor cell population, the total level of NK cells, and the effectiveness of tumor-specific $CD8^+$ T cell, Ref. 18 modeled tumor-immune interactions to clarify the process of immune-mediated tumor rejection. In another study, Ref. 19, in addition to including tumor cells, host cells, and immune cells, the authors simulated drug interactions in order to identify the best protocols for chemotherapy. Immunotherapy has also been studied with mathematical models. Ref. 20 applied a system of ordinary differential equations (ODE) and the theory of optimal control to identify the best time of administration and dosage for dendritic cell vaccines designed to reduce or eliminate the tumor mass. In Ref. 21, the authors addressed the possibility of combining immunotherapy and radiotherapy based on a tumor growth model and examined the use of radiotherapy with inhibitors of the PD1–PDL1 axis and/or the CTLA4 pathway to describe how the antitumor immune response occurs. Ref. 22 present a review about mathematical models of thyroid cancer, one that discusses different models used and aspects investigated to date. In Ref. 23, a mathematical model with Allee effect is proposed to simulate the impact of different dosages of RAI in the treatment of PTC patients. The model used was an ODE system, and the Allee threshold was associated with doses that may sufficiently eliminate tumors.

A mathematical model with two-step development is proposed in this article. The first was designed to examine the effectiveness of lenvatinib in the treatment of RAIR-DTC, in which the drug's action occurs by reducing the tumor carrying capacity, inhibiting tumor growth and altering the anti-immune capacity by activating NK cells. In the second step, we add the immunotherapeutic effect of pembrolizumab to the aforementioned dynamics, which, by preventing the binding

[b]Drug developed and marketed by Merck & Co., Inc., under the label of Keytruda.

of PD-L1 to the PD-1 receptor, promotes the activation of antitumor T cells.

In what follows, Sections 2 and 3 introduce the model proposed, their parameters, and the analysis of the local and global stability of equilibria. Section 4 presents and discusses the results. Section 5 ends the article by articulating the major conclusions about the area of study.

2. Model with targeted therapy

We consider treatment with lenvatinib, with a concentration of the drug A, administered to patients with RAIR-DTC. Lenvatinib is administered orally in a daily dose of one to two capsules.[24] However, in our modeling we suppose that the concentration of the drug A is a continuous function in time. If the treatment cannot eradicate the tumor, it slows its growth to the maximum carrying capacity K_0, this delay is represented by the variable S, which is a function of the concentration of the drug A. Assuming that N and L, respectively represent the number of cancer cells and NK cells, the model considered is the following ODE

$$\begin{cases} \dfrac{dA}{dt} = D - \delta A, \\ \dfrac{dS}{dt} = -c(A)S, \\ \dfrac{dN}{dt} = rN\left(1 - \dfrac{N}{K(S)}\right) - \mu AN - \rho(A)LN, \\ \dfrac{dL}{dt} = \sigma(A) - nL, \end{cases} \tag{2.1}$$

where D, δ, r, μ, n are positive constants, the functions c, $K : \mathbb{R}^+ \longrightarrow \mathbb{R}^+$ are decreasing, and the functions ρ, $\sigma : \mathbb{R}^+ \longrightarrow \mathbb{R}^+$ are increasing. We set

$$c_0 = c(0), \quad K_0 = K(0), \quad \rho_0 = \rho(0) \quad \text{and} \quad \sigma_0 = \sigma(0).$$

For the numerical simulations, we choose the functions

$$c(A) = c_0 e^{-\bar{c}A}, \quad K(E) = K_0 e^{-\bar{K}S}, \quad \rho(A) = \rho_0 + \rho_1(1 - e^{-\bar{\rho}A})$$

and

$$\sigma(A) = \sigma_0 + \sigma_1(1 - e^{-\bar{\sigma}A}),$$

where c_0, $\bar{c}$, K_0, $\bar{K}$, ρ_0, ρ_1, $\bar{\rho}$, σ_0, σ_1 and $\bar{\sigma}$ are positive constants, described in Table 1.

The functions ρ and σ simulate the increase in antitumor immune capacity during the treatment with lenvatinib, as proposed by Ref. 8. In the absence of treatment, $D = 0$, $A \to 0$, the expressions used indicate a natural and limited response of the immune system, from the minimum values ρ_0 and σ_0. The function σ incorporates the expected increase in the number of NK cells due to treatment, represented by the expression $\sigma_1(1 - e^{-\bar{\sigma}A})$. The rate c simulates the velocity at which tumor cells reach the carrying capacity K_0 and, therefore, this rate should be decreasing as

a function of treatment with the antiangiogenic lenvatinib. The function K corresponds to the tumor growth towards carrying capacity, being directly affected by the antineoplastic action mentioned above through the control function S. Tumor cells can develop different mutations to resist to targeted therapy and continue to grow. This will be discussed later.

2.1. *Equilibria and their local asymptotic stability*

We consider the following parameter which is the equilibrium of the treatment A

$$d = \frac{D}{\delta}.$$

The trivial equilibrium, which always exists, is given by

$$E_0 := \left(d, 0, 0, \frac{1}{n}\sigma(d)\right).$$

A unique positive equilibrium is given by

$$E^* := (d, 0, N^*(d), L^*(d)),$$

with

$$L^*(d) = \frac{1}{n}\sigma(d) \quad \text{and} \quad N^*(d) = \frac{K_0}{r}\left(r - \mu d - \frac{1}{n}\rho(d)\sigma(d)\right).$$

A necessary and sufficient condition for the existence of the equilibrium E^* is

$$f(d) := \mu d + \frac{1}{n}\rho(d)\sigma(d) < r.$$

As the functions ρ and σ are positive and increasing, the function f is also positive and increasing with

$$f(0) = \frac{1}{n}\rho_0\sigma_0 \quad \text{and} \quad \lim_{d\to+\infty} f(d) = +\infty.$$

We have the following result.

- Suppose that $\frac{1}{n}\rho_0\sigma_0 \geq r$. Then, $f(d) \geq r$ for all $d \geq 0$. This means that only the trivial equilibrium $E_0 = (d, 0, 0, \sigma(d)/n)$ exists.
- Suppose that $\frac{1}{n}\rho_0\sigma_0 < r$. Then, there exists a unique $d_0 > 0$ such that $f(d_0) = r$. In this case, if $0 \leq d < d_0$, in addition to the trivial equilibrium E_0, there exists a unique non-trivial equilibrium $E^* := (d, 0, N^*(d), L^*(d))$. If $d \geq d_0$, only the trivial equilibrium E_0 exists.

Let $\bar{E} := (d, 0, \bar{N}(d), \sigma(d)/n)$ be any equilibrium of the system (2.1), with $\bar{N} = 0$, for $\bar{E} = E_0$, and $\bar{N} = N^*$, for $\bar{E} = E^*$. The graphic of $d \mapsto \bar{N}(d)$ seen as a function of d is shown in Figure 1. We can see that $\bar{N}$ decreases and suddenly drops to 0 when d approaches d_0. The four eigenvalues of the linearized system about the equilibrium point $\bar{E}$ are given by

$$-\delta, \quad -c(d), \quad r\left(1 - \frac{2\bar{N}(d)}{K_0}\right) - f(d) \quad \text{and} \quad -n.$$

The local asymptotic stability of $\bar{E}$ depends only on the sign of the real eigenvalue

$$\bar{\lambda}(d) = r\left(1 - \frac{2\bar{N}(d)}{K_0}\right) - f(d).$$

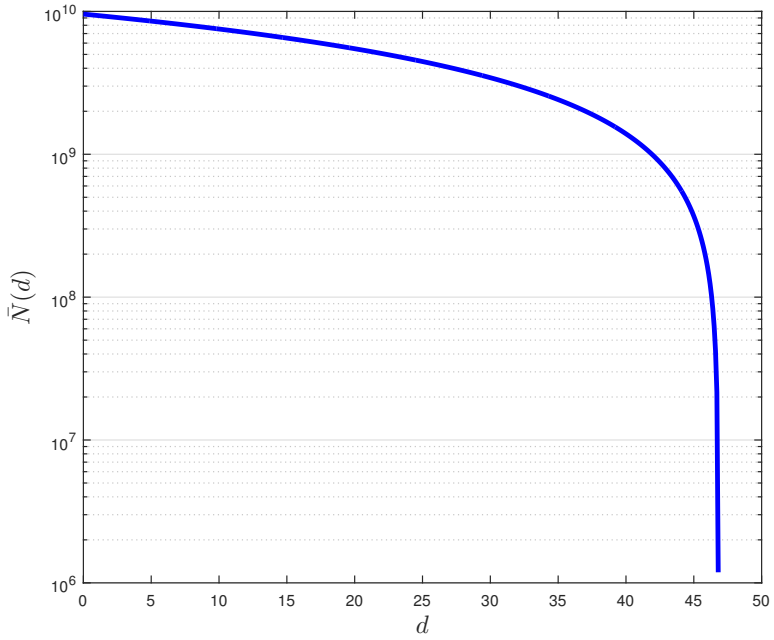

Fig. 1. Evolution of $\bar{N}$ as a function of d, the other parameters are chosen from Table 2.

- If $\frac{1}{n}\rho_0\sigma_0 > r$, then $\bar{N}(d) = 0$ and $\bar{\lambda} := \lambda_0(d) = r - f(d) < 0$ for all $d \geq 0$. In this situation, the trivial equilibrium E_0 is always locally asymptotically stable.
- Suppose that $\frac{1}{n}\rho_0\sigma_0 < r$ and consider the unique $d_0 > 0$ such that $f(d_0) = r$. If $d > d_0$, the trivial equilibrium E_0 is the unique steady state with $\lambda_0(d) = r - f(d) < 0$. Then, E_0 is locally asymptotically stable for $d > d_0$. Now suppose that $0 \leq d < d_0$. Then, the trivial equilibrium E_0 is unstable, because $\lambda_0(d) = r - f(d) > 0$. On the other hand, the positive equilibrium E^* exists and the eigenvalue $\bar{\lambda}(d) := \lambda^*(d)$ becomes

$$\lambda^*(d) = -f(d) + r - 2\left(r - \mu d - \frac{1}{n}\rho(d)\sigma(d)\right) = -r + f(d) < 0.$$

We conclude that E^* is locally asymptotically stable for $0 \leq d < d_0$.

Table 1. Parameters and their meanings.

	Description
D	Recommended daily dose of lenvatinib
δ	Elimination rate of lenvatinib drug
c_0	Minimum rate at which tumor cells reach the carrying capacity
$\bar{c}$	Coefficient of the antineoplastic effect of lenvatinib
r	Proliferation rate of tumor cells
K_0	Maximum tumor carrying capacity
$\bar{K}$	Coefficient of growth of tumor carrying capacity
μ	Rate of drug efficacy in eliminating tumor cells
ρ_0	Elimination rate of tumor cells due to immune system action
ρ_1	Elimination rate of tumor cells due to immune action with lenvatinib
$\bar{\rho}$	Coefficient of immune action due to lenvatinib
σ_0	Coefficient of the natural influx of immune cells to the tumor site
σ_1	Limit rate of activation of immune cells due to lenvatinib
$\bar{\sigma}$	Rate of activation of NK cells by lenvatinib
n	Natural mortality rate of immune cells

2.2. *Global asymptotic stability of the equilibria*

It is clear that the set $\Omega := [0, d] \times \mathbb{R}^+ \times \mathbb{R}^+ \times [0, \sigma(d)/n]$ is positively invariant under system (2.1). We make the change of variables

$$a = d - A \quad \text{and} \quad l = \frac{1}{n}\sigma(d) - L.$$

Then, the system (2.1) becomes

$$\begin{cases} \dfrac{da}{dt} = -\delta a, \\ \dfrac{dS}{dt} = -c(d-a)S, \\ \dfrac{dN}{dt} = rN\left(1 - \dfrac{N}{K(S)}\right) - \mu N - \rho(d-a)(\sigma(d)/n - l)N, \\ \dfrac{dl}{dt} = \sigma(d) - \sigma(d-a) - nl. \end{cases} \tag{2.16}$$

As σ is an increasing function and $a \in [0, d]$, we have

$$0 \leq \sigma(d) - \sigma(d-a) = \sigma_1 e^{-\bar{\sigma}d}[e^{\bar{\sigma}a} - 1] \leq \sigma_1 \bar{\sigma} a.$$

For $(a, S, N, l) \in \Omega$, we define the function

$$V_1(a, S, N, l) = \frac{1}{2}(a^2 + S^2).$$

The derivative of V_1 along the solution $(a, S, N, l) \in \Omega$ satisfies

$$\dot{V}_1(a, S, N, l) = -\delta a^2 - c(d-a)S^2.$$

We proved that V_1 is a Lyapunov function on Ω. Let us define the set

$$\Gamma_1 := \{(a, S, N, l) \in \Omega : \dot{V}_1(a, S, N, l) = 0\}.$$

Using LaSalle's invariance theorem, we confirm that the set Γ_1 is globally attractive in Ω. Furthermore, for $(a, S, N, l) \in \Gamma_1$, we have $a = S = 0$ and (N, l) satisfies the system

$$\begin{cases} \dfrac{dN}{dt} = rN\left(1 - \dfrac{N}{K_0}\right) - \mu dN - \rho(d)(\sigma(d)/n - l)N, \\ \dfrac{dl}{dt} = -nl. \end{cases} \tag{2.21}$$

Using the Lyapunov function $V_2(a, S, N, l) = \dfrac{1}{2}l^2$ and LaSalle's invariance theorem on the set Γ_1, we get the following globally attractive set of system (2.21)

$$\Gamma_2 = \{(a, S, N, l) \in \Gamma_1 : \dot{V}_2(a, S, N, l) = 0\}.$$

For $(a, S, N, l) \in \Gamma_2$, we have $a = S = l = 0$ and N satisfies the equation

$$\frac{dN}{dt} = rN\left(1 - \frac{N}{K_0}\right) - \mu dN - \frac{1}{n}\rho(d)\sigma(d)N.$$

In other words

$$\frac{dN}{dt} = \left(r - f(d) - \frac{rN}{K_0}\right)N. \tag{2.24}$$

To conclude, we consider the two cases:

- If $f(d) \geq r$, the trivial steady-state 0 of equation (2.24) is the only equilibrium and it is globally attractive for the solutions of (2.24). Then, the trivial equilibrium of system (2.1) is globally attractive on the set Ω.
- If $f(d) < r$, the positive equilibrium

  $$N^*(d) = \frac{K_0}{r}(r - f(d))$$

  of the logistic equation (2.24) is globally attractive. We conclude that the non-trivial equilibrium $E^* := (d, 0, N^*(d), L^*(d))$ of system (2.1) is globally attractive.

In fact, one can prove the global attractivity of the two equilibria on the set $(\mathbb{R}^+)^4$. The proof can be easily adapted from the above proof.

The antineoplastic action of lenvatinib as a retardant for the growth of the carrying capacity, expressed by the function c, is shown in Figure 2. The drug blocks factors as VEGF and its receptors, and causes the apoptosis of endothelial cells, among others.[25] As seen, the growth of the tumor carrying capacity is not interrupted despite of treatment, but it evolves slowly over time, indicating a limited but important therapeutic outcome.

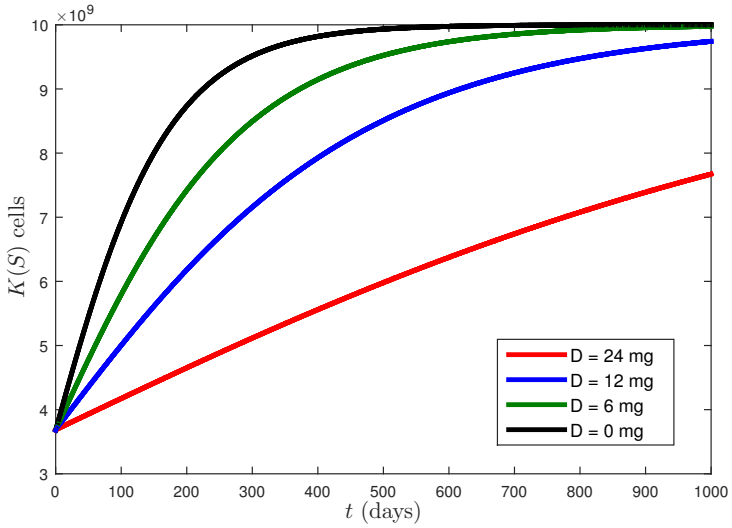

Fig. 2. Progression of tumor carrying capacity in RAIR-DTC patients treated or not with lenvatinib. Parameters used as shown in Table 2 and initial conditions $S(0) = 1$, $K_0 = 10^{10}$ cells and $D = 0,\ 6,\ 12$ and 24 mg.

The assigned values and units of each parameter are described in Table 2, which also presents the references used. A value was assigned to the parameter r based on the tumor doubling time observed in patients with metastatic PTC not responding to treatment with RAI ^{131}I, estimated in Ref. 26. For the parameters μ and ρ_1, which represent important effects of the treatment, different scenarios with degrees of antitumor activity and drug efficacy are considered. In addition to the values of 1.4×10^{-3} and 1×10^{-10}, the values 1.5×10^{-3} and 5×10^{-10} are also used in the simulations. The parameter ρ_0 represents the immune response against the tumor in the absence of treatment. The parameter $\bar{\rho}$, meanwhile, is associated with the increase in NK cells infiltrating the tumor, and thus an expansion of the antitumor immune response. The parameter σ_1 represents the constant activation rate of new NK cells due to the indirect action of lenvatinib.[8] The total number of cells activated in this manner represents an increase of approximately 1×10^7 over the expected number of cells responding naturally to the tumor, σ_0. Considering that the rate σ_1 is positively affected by treatment with targeted therapy and that the concentration of lenvatinib stabilizes at 40.4 mg over time,[24] a value of 10^{-1} is chosen for the parameter $\bar{\sigma}$ so that the equilibrium value for the population L is approximately 4×10^7 cells.

Table 2. Parameters with values, units and references.

Parameter	Value	Unity	Reference
D	24	mg $\times$ day^{-1}	Ref. 6
δ	0.5939	day^{-1}	Ref. 24
c_0	10^{-2}	day^{-1}	assumed
$\bar{c}$	5×10^{-2}	mg^{-1}	assumed
r	0.0707	day^{-1}	Ref. 26
K_0	10^{10}	cells	Ref. 27
$\bar{K}$	1	—	assumed
μ	1.4×10^{-3}	(mg $\times$ day)$^{-1}$	assumed
ρ_0	1×10^{-10}	(cells $\times$ day)$^{-1}$	assumed
ρ_1	1×10^{-10}	(cells $\times$ day)$^{-1}$	assumed
$\bar{\rho}$	10^{-2}	—	assumed
σ_0	3×10^5	cells $\times$ day^{-1}	Ref. 28
σ_1	1×10^5	cells $\times$ day^{-1}	assumed
$\bar{\sigma}$	10^{-1}	—	assumed
n	10^{-2}	day^{-1}	assumed

In the next section, we include in the model (2.1) a new variable, P, which corresponds to the concentration of the immunotherapeutic agent pembrolizumab. The introduction of this antibody aims to promote the activation of antitumor T lymphocytes by preventing the binding of the PD-L1 protein to its PD-1 receptor.

3. Model with targeted therapy and immunotherapy

We consider in this section, a combined treatment between targeted therapy and immunotherapy in patients with RAIR-DTC, with the new variable P denoting the concentration of Pembrolizumab. We have the following model

$$\begin{cases} \dfrac{dA}{dt} = D - \delta A, \\ \dfrac{dS}{dt} = -c(A)S, \\ \dfrac{dN}{dt} = rN\left(1 - \dfrac{N}{K(S)}\right) - \mu AN - \rho(A)LN, \\ \dfrac{dL}{dt} = \gamma(A,P) - nL, \\ \dfrac{dP}{dt} = Q - \varepsilon P, \end{cases} \tag{3.1}$$

where the new parameters Q and ε are positive, and instead of the function σ considered in the model (2), we introduce a new function $\gamma : \mathbb{R}^+ \times \mathbb{R}^+ \longrightarrow \mathbb{R}^+$, which is assumed to be an increasing function of each component state. We set

$\gamma_0 = \gamma(0,0)$ and for the numerical simulations, we take

$$\gamma(A,P) = \sigma(A) + \sigma_2(1 - e^{-\bar{\bar{\sigma}}P}) = \sigma_0 + \sigma_1(1 - e^{-\bar{\sigma}A}) + \sigma_2(1 - e^{-\bar{\bar{\sigma}}P}),$$

where the new parameters σ_2 and $\bar{\bar{\sigma}}$ are positive. The new parameters are described in Table 3.

Table 3. Meaning of the new parameters.

	Description
Q	Recommended daily therapeutic dose of pembrolizumab
ε	Elimination rate of pembrolizumab
σ_2	Limit rate of activation of T cells due to the action of pembrolizumab
$\bar{\bar{\sigma}}$	Coefficient of activation of T cells by pembrolizumab

3.1. *Local and global asymptotic stability of equilibria*

As for the previous model, we denote

$$q = \frac{Q}{\varepsilon}.$$

q is considered as a parameter for the second treatment. Similarly to the previously section, we obtain a trivial equilibrium $E_0 := (d, 0, 0, \gamma(d,q)/n, q)$ which always exists. A unique non-trivial equilibrium is given by

$$E^{**} = (d, 0, N^{**}(d,q), L^{**}(d,q), q),$$

where

$$L^{**}(d,q) = \frac{1}{n}\gamma(d,q) \quad \text{and} \quad N^{**}(d,q) = \frac{K_0}{r}\left(r - \mu d - \frac{1}{n}\rho(d)\gamma(d,q)\right).$$

A necessary and sufficient condition for the existence of E^{**} is given by

$$F(d,q) := \mu d + \frac{1}{n}\rho(d)\gamma(d,q) < r.$$

For any fixed $d \geq 0$, the function $q \mapsto F(d,q)$ is increasing and satisfies

$$F(d,0) = \mu d + \frac{1}{n}\rho(d)\sigma(d) \text{ and } F(d,+\infty) = \mu d + \frac{1}{n}\rho(d)(\sigma(d) + \sigma_2).$$

As a consequence, we obtain for a fixed $d \geq 0$, the following properties.

- Suppose $F(d,0) > r$. Then, for all $q \geq 0$, $F(d,q) > r$ and the trivial steady state E_0 is the only equilibrium.
- Suppose $F(d,+\infty) < r$. Then, for all $q \geq 0$, $F(d,q) < r$ and there exists a unique non-trivial equilibrium E^{**}.

- Suppose $F(d,0) < r < F(d,+\infty)$. There exists a unique $q_0(d) > 0$ such that $F(d,q_0(d)) = r$. In this case, if $0 \leq q < q_0(d)$, there exists a unique non-trivial equilibrium E^{**}. If $q \geq q_0(d)$, only the trivial equilibrium E_0 exists. Furthermore, $d \mapsto q_0(d)$ is a decreasing function.

As for the previous section, let $\hat{E}(d,q) = (d, 0, \hat{N}(d,q), \gamma(d,q)/n, q)$ be any equilibrium of the system (3.1). From the linearized system, the five eigenvalues obtained are given by

$$-\delta,\ -c(d),\ r\left(1 - \frac{2\hat{N}(d,q)}{K_0}\right) - F(d,q),\ -n \ \text{ and } \ -\varepsilon.$$

Accordingly, the local stability of $\hat{E}(d,q)$ depends only of the eigenvalue

$$\lambda(d,q) = r\left(1 - \frac{2\hat{N}(d,q)}{K_0}\right) - F(d,q).$$

Define the set of existence of the non-trivial equilibrium $E^{**}(d,q)$

$$R := \{(d,q) : F(d,q) < r\}.$$

It can be seen in Figure 3 that increasing the parameter d, which corresponds to treatment with lenvatinib, does not eradicate the tumor. Because, it is not possible to leave the region R of tumor stability. In particular, for a tumor that is not aggressive (r small), treatment with lenvatinib does not eliminate the tumor. On the other hand, the combination of the two treatments lenvatinib (d) and pembrolizumab (q) seems to be more effective, immunotherapy being the main responsible for the antitumor effect.

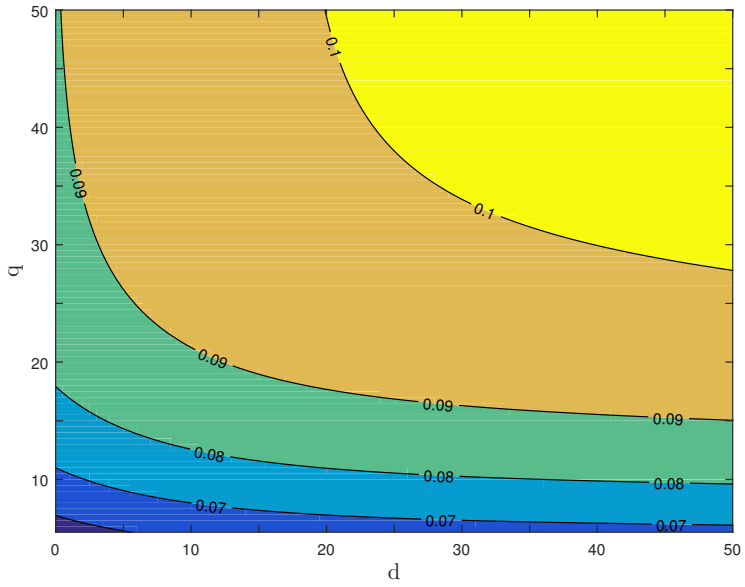

Fig. 3. Contour levels $F(d,q) = r$ for some values of r in the plane $d \times q$. Parameters used as shown in Table 2 with the exception of $\mu = 4 \times 10^{-10}$ and $\rho_0 = 1 \times 10^{-9}$.

Suppose that $F(d,q) > r$. Then, the only equilibrium is $\hat{E}(d,q) = E_0$, with $\hat{N}(d,q) = 0$. This implies that

$$\lambda(d,q) = r - F(d,q) < 0.$$

Hence, the trivial steady state E_0 is locally asymptotically stable. Now consider the case $(d,q) \in R$. Then, E_0 is unstable. On the other hand, the eigenvalue $\lambda(d,q)$ associated to $E^{**}(d,q)$ is given by

$$\lambda(d,q) = r - 2\left(r - \mu d - \frac{1}{n}\rho(d)\gamma(d,q)\right) - F(d,q) = -r + 2F(d,q) - F(d,q) < 0.$$

We conclude that $E^{**}(d,q)$ is locally asymptotically stable for $(d,q) \in R$.

As for system (2.1), using Lyapunov function and LaSalle's invariance theorem, one can prove the following result.

- If $(d,q) \notin R$, then the trivial steady state E_0 of system (3.1) is globally attractive.
- If $(d,q) \in R$, then the non-trivial equilibrium $E^{**}(d,q)$ of system (3.1) is globally attractive.

For the numerical simulations, the values of the parameters of the model (3.1) are presented in Table 4. The parameter ε is calculated from the estimated half-life

in Ref. 29 using the expression $\varepsilon = \frac{\ln(2)}{t_{1/2}}$. The parameters $\bar{\sigma}$ and $\bar{\bar{\sigma}}$ are assumed to increase the number of NK cells and T lymphocytes to a total value of approximately 7×10^5 cells. This means that the total number of immune cells activated by the combination of drugs is more than three times that obtained without treatment. The ODE systems were solved using the fourth-order Runge–Kutta method, with a time step $h = 0.01$.

Table 4. Parameters with values, units and references.

Parameter	Value	Unity	Reference
Q	15	mg $\times$ day^{-1}	Ref. 29
ε	0.0254	day^{-1}	Ref. 12
σ_2	6×10^5	cells $\times$ day^{-1}	assumed
$\bar{\bar{\sigma}}$	10^{-1}	—	assumed

In terms of modeling, only the situation with an attractive non-trivial equilibrium is confirmed by clinical data. The combination of treatments used does not usually eradicate the tumor. However, these treatments significantly slow the progression of the disease.[6,30]

4. Results and Discussion

The patients considered are RAIR-DTC, treated with lenvatinib A, or by combining lenvatinib A and pembrolizumab P. The effects of these therapeutic approaches include blocking the development of angiogenesis, represented by the function S and the carrying capacity K, the reduction in tumor growth rate, expressed as μAN, and the increase in the antitumor activity of the immune system with the activation of NK cells and T lymphocytes, as well as their infiltration into tumors, designated by the functions ρ, σ or γ.

The serum concentration limit for lenvatinib over time is approximately $D/\delta = 40.4$ mg. This drug is administered orally in a daily dose of one to two capsules.[24] Pembrolizumab is an anticancer drug that is administered every 2–3 weeks with an elimination half-life of approximately 27.3 days.[11,12,29] In this study, the administration of pembrolizumab is considered daily with a dose of 15 mg. This is an approximation of a treatment given every 2 weeks at a dose of 2 mg/kg, as indicated in Ref. 29.

The data reported in Ref. 12 represent a study of 22 patients with advanced thyroid cancer treated with pembrolizumab for 24 months or until whether tumor progression or intolerable toxicity is confirmed, then in the numerical simulations the treatment period considered is 1000 days.

The natural influx of NK immune cells or T lymphocytes to the site of interaction with the tumor is considered in the numerical simulations with 3×10^5 cells. The use

of lenvatinib generally activates NK cells, and the cellular increase corresponding to the use of this drug is shown in Figure 4, curve $L(A)$. The combination of this treatment with pembrolizumab results in blockade of PD-L1 and PD-1 and therefore reduced the inhibition of the immune system by the tumor.[12]

In the curves representing the increase in immune cells, $L(A)$ and $L(A, P)$, the initial number of immune cells, for $t = 0$, is assumed to be $L(0) = 10^6$ cells. Without any treatment, this cell population increases until a maximum of 3×10^7 cells. However, with the introduction of lenvatinib the maximum becomes approximately 4×10^7 cells. This slight increase is due to the fact that the expansion of the immune system observed in the experiments is a side effect not mentioned among the actions to be expected when using lenvatinib. The curve $L(A, P)$ represents the growth in the number of antitumor immune cells in terms of NK cells and T lymphocytes. With the addition of pembrolizumab, the maximum value becomes 10×10^7 cells, more than three times the number of immune cells in patients without treatment, curve L. The immunotherapeutic agent should cause a decrease in the tumor's ability to escape the immune response. However, in clinical practice, the approach has obtained only limited therapeutic effects. In Ref. 12, for example, among patients who showed partial response to treatment, the duration of the response ranged from 8 to 20 months.

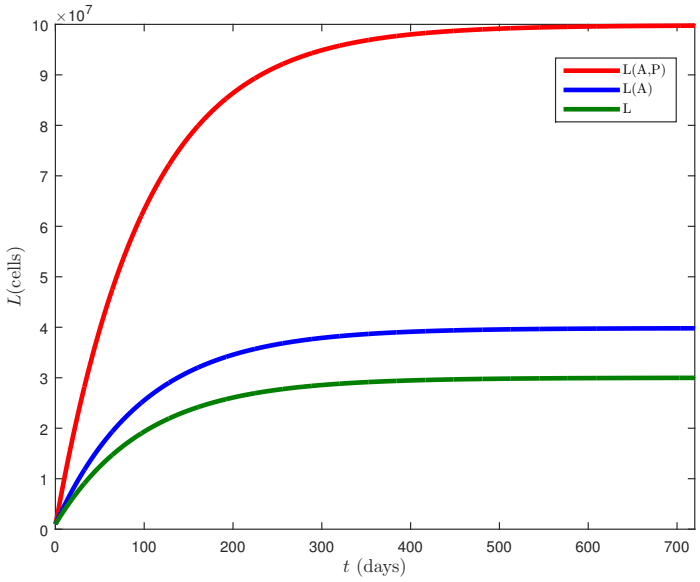

Fig. 4. Evolution of cell populations of NK and T lymphocytes without therapy, L, and during treatment with lenvatinib, $L(A)$, and the combination of lenvatinib with pembrolizumab, $L(A, P)$. Parameters are taken from Tables 2 and 4. Initial conditions are $S(0) = 1$, $A(0) = 24$ mg, $N(0) = 1.12 \times 10^9$ cells, $L(0) = 10^6$ cells and $P(0) = 15$ mg.

In general, the progression of iodine-refractory thyroid tumors under the treatments considered occurs in two ways: partial response, with a decrease in the number of cancer cells observed over a short period; and stable disease, in which tumor growth, despite an initial decrease, proceeds very slowly.[31]

Figure 5 illustrates the evolution of the tumor population with the two types of treatment: using only lenvatinib and using this drug in combination with pembrolizumab. Scenarios with different degrees of tumor elimination were obtained. In the simulations, although the initial decrease in the number of malignant cells is similar in both situations ($t < 50$ days), tumor evolution is different after the introduction of pembrolizumab, exhibiting greater capacity for removal of the tumor. Treatment with lenvatinib alone resulted in a partial tumor decrease within the first year, but the tumor started to grow again after this time. However, when considering the combination of treatments, the elimination of tumor cells is greater than that obtained with a single treatment, but the main result is that the tumor remains stable over time.

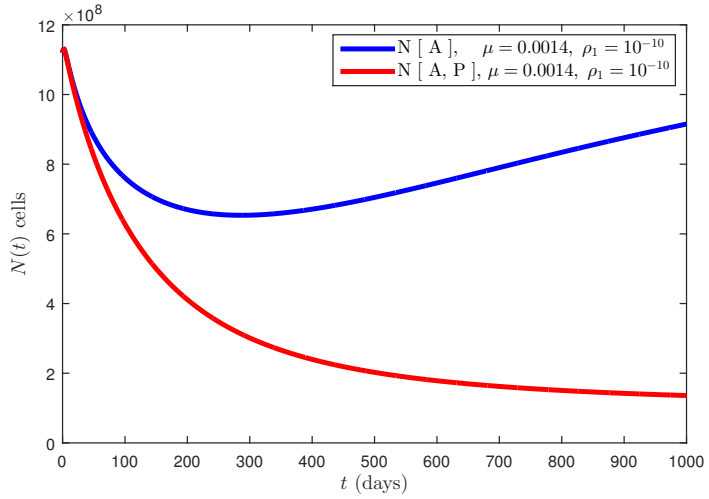

Fig. 5. Evolution of the population of malignant cells during treatments with lenvatinib alone, $N[A]$, and lenvatinib combined with pembrolizumab, $N[A, P]$. Parameters are taken from Tables 2 and 4. Initial conditions are $S(0) = 1$, $A(0) = 24$ mg, $N(0) = 1.12 \times 10^9$ cells, $L(0) = 10^6$ cells and $P(0) = 15$ mg.

The effect of the introduction of pembrolizumab in the treatment has a significant action on the tumor. In Figure 5, we consider some scenarios, by taking some values of the parameters μ and ρ_1, of the action of the treatments on the behavior of the tumor. The parameter ρ_1 could be linked to the effectiveness of the two treatments, while μ refers only to the action of lenvatinib in the elimination of tumor cells. Assuming a change in the rate of tumor cell elimination due to the im-

mune action, $\rho_1 = 5 \times 10^{-10}$, and without considering the immunotherapeutic drug pembrolizumab, Figure 6 shows a significant decrease in the number of malignant cells, but not enough to be similar to the combination of the two treatments. In the scenario assuming a greater efficacy of lenvatinib, $\mu = 1.5 \times 10^{-3}$, there is a decrease of N, however it is less than that observed with the use of the two therapies.

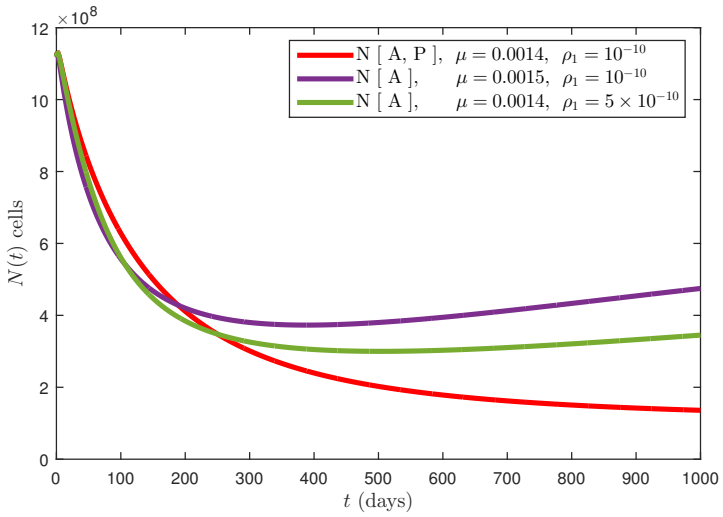

Fig. 6. Evolution of the malignant cell population during treatment with lenvatinib only, $N[A]$ and in combination with pembrolizumab, $N[A, P]$. The parameters are taken from Tables 2 and 4, with the exception of the parameters μ and ρ_1 to which we give the values 1.5×10^{-3} and 4×10^{-10}, respectively. The initial conditions are $S(0) = 1$, $A(0) = 24$ mg, $N(0) = 1.12 \times 10^9$ cells, $L(0) = 10^6$ cells, and $P(0) = 15$ mg.

Similar to lenvatinib, sorafenib is a multiple kinase inhibitor (MKI) used for different types of malignant tumors.[32] The observational study by Ref. 31 examined the efficacy of sorafenib in the treatment of metastatic thyroid carcinoma RAIR. In this study, 8 patients received 400 mg of the drug per day until disease progression or intolerable toxicity was observed. One patient showed partial response, with 35% tumor regression after 6 months of treatment, while 5 others showed stable disease. In the review conducted by Ref. 33, the authors found that the development of resistance after 1 or 2 years of treatment may occur in patients with a partial response or with stable disease. They then recommend using different MKIs. Various treatment options for RAIR-DTC are discussed in Ref. 15, which highlights the poor performance of some conventional treatments, including administration of chemotherapy via doxorubicin, a treatment recommended only when MKIs are not indicated. In discussing the use of lenvatinib to treat anaplastic thyroid cancer, Ref. 34 identified resistance mechanisms that could be activated by tumors to

survive treatment. RNA-seq analyses from this study revealed that prolonged treatment with lenvatinib caused changes in several cellular and oncogenic pathways. In another study of 94 patients regarding the efficacy and safety of lenvatinib as a treatment for RAIR-DTC, Ref. 35 observed partial responses and stable disease in only 36% and 41% of patients, respectively.

The antiangiogenic effect of lenvatinib, represented in our study by tumor carrying capacity, K, aims to mimic the action of the drug by blocking certain factors, for example VEGF or its receptor, and causes apoptosis of endothelial cells.[25] However, since the treatment has a limited effect, we propose that the tumor develops new molecular changes which re-expand its carrying capacity and reactivate its tumor growth process.[34] The action of lenvatinib as an inhibitor of fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF), involved in the processes of growth and cell division, is also represented by the term μAN.

For the numerical simulations, several values of the parameters representing the efficacy of lenvatinib against tumor growth μ and the rate of elimination of malignant cells via the immune action ρ_1, also enhanced by the second drug, were tested. We changed only these two parameters because they represent the direct action of drugs. Therefore, by considering doses similar to those taken in clinical studies, our model produces results that should expand current knowledge about targeted therapy and immunotherapies applied to RAIR-DTC.

5. Conclusions

A two-step development ODE model have been proposed to test therapies applied to patients with RAIR-DTC treated with lenvatinib alone or in combination with pembrolizumab. Numerical simulations have shown the effectiveness of the treatments in inducing a partial response or a stable disease. This response is more effective in the case of a combination of the two therapies. The parameters related to the action of the drugs were analysed and scenarios with different responses to the treatment were obtained. Our study provides new ideas for treatment discussions for patients with RAIR-DTC and highlighted the importance of combination therapies, including targeted therapies and immunotherapies.

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