A MATHEMATICAL MODEL OF TUMOR-IMMUNE SYSTEM DYNAMICS COMBINED WITH RADIOTHERAPY ILLUSTRATING A POTENTIAL SYNERGISM

BY

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Abstract. Using nonlinear dynamics of immunogenic tumors, A. Kuznetsov proposed a quantitative model for the interaction between effector cells and cells in a growing tumor. Adding radiation therapy to Kuznetsov's original model, we obtain a system that takes into account both destruction, repair and repopulation of tumor cells as well as the destruction and inhibition of immune cells. The values for μ_1 and μ_2 coefficients derived from the literature and clinical trials, where μ_1 represents the radiotherapy action factor on effector immune cells and μ_2 is the effect of radiotherapy on tumoral cells. Using Matlab, we obtained therapeutic diagrams for different μ_1 and μ_2 parameter values and it

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is noted that for a well-defined range of these parameters, the tumors are reduced to the subclinical stage.

Keywords: Kuznetsov model; system dynamics; immune cells; Matlab; radiotherapy.

1. Introduction

Cancer is one of the major factors of death and a better understanding of it is necessary in order to increase the survival rate for the patients suffering from this condition. Tumors common features can offers the starting point to build mathematical models describing the neoplastic growth.

When the body is not infected, cells grow and divide in a very controlled way. Usually mutation in the DNA does occur but the reparation system in the body can obviate the damage. Sometimes the damage or change in the genetic material caused by these factors cannot be repaired though. In most cases the mutated cells do not cause cancer, but rarely can be occur. In these rare cases cells continue to multiply instead of dying. The mass of multiplying cells forms a neoplasia which can interfere with the whole human system.

Unlike regular cells, cancer cells can experience uncontrolled growth, and therefore, cause damage to the genes involved in the cell division. Cellular division is called proliferation and in the case of cancer it occurs in a rapid and excessive way.

Cell migration is a process which allows development and maintenance of multicellular organism, for instance when tissue is created and wound healing. On the other hand, cell invasion is the ability to actively invade tissue, this is in fact an extension of the cell migration. Invasion is the property of tumour cells. The invasive grade of the cell depends on the location of the tumour. Some tumours grow in a limited way and stay in one spot while others move to other locations in the body through the blood and lymph systems.

"**T-cell**, also called **T lymphocyte**, is a type of leukocyte (white blood cell) that is an essential part of the immune system. T-cells are one of two primary types of lymphocytes B cells being the second type-that determine the specificity of immune response to antigens (foreign substances) in the body.

T-cells originate in the bone marrow and mature in the thymus. In the thymus, T-cells multiply and differentiate into helper, regulatory, or cytotoxic T-cells or become memory T-cells. They are then sent to peripheral tissues or circulate in the blood or lymphatic system. Once stimulated by the appropriate antigen, helper T cells secrete chemical messengers called cytokines, which stimulate the differentiation of B cells into plasma cells (antibody-producing cells). Regulatory T-cells act to control immune reactions, hence their name. Cytotoxic T cells, which are activated by various cytokines, bind to and kill infected cells and cancer cells.

Without a doubt, introducing immune checkpoint inhibitors to the clinic was a major breakthrough in the war against cancer. For some patients tumor responses to anti-PD-1/PD-L1 or anti-CTLA4 therapies are spectacular and last long after the therapy is withdrawn. Interestingly, disease regression can occur even after an initial phase of tumor growth during the therapy. However, despite spectacular successes, therapies based on checkpoint inhibitors still suffer from relatively low response rates. There is an urgent need to establish reliable patient-specific biomarkers that would solve this problem. The current focus is on using, among others, measures of pretreatment T cell infiltration, PD-L1 expression profiles, inflammatory status, and mutational burden of the tumor. Finding a reliable correlation will allow for designing specific therapy scheduling or for incorporating appropriate adjuvant treatment" (https://computecancer.wordpress.com; https://www.britannica.com/science/T-cell; Brondz, 1987; Callewaert *et al*., 1988).

Combining radiotherapy with immune checkpoint blockade may offer considerable therapeutic impact if the immunosuppressive nature of the tumor microenvironment (TME) can be relieved. In this study, we adopt a mathematical model that was introduced by Kuznetsov and colleagues back in 1994 to investigate the effects of anti-PD-1/PD-L1 therapy depending on the pretreatment tumor characteristics, which may eventually illustrate a potential synergism between immune checkpoint inhibitors and radiotherapy. The way in which the model was formulated more than two decades ago allows for elegant and straightforward incorporation of such a treatment (Kuznetsov *et al*., 1994).

2. Short Remember of Kuznetsov Model

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In his paper Kuznetsov and his colleagues introduced the following relatively simple mathematical model describing tumor-immune system dynamics:

$$\dot{E}(t) = s + p\frac{E(t)N(t)}{g + N(t)} - d_0 E(t) - mE(t)N(t)$$

$$\dot{N}(t) = aN(t)\big(1 - bN(t)\big) - nE(t)N(t) \qquad (1)$$

where $E(t)$ and $N(t)$ are the numbers of immune effector cells and cancer cells, respectively. "Taking into account that anti-PD-1/PD-L1 treatment reduces the probability that cytotoxic T cell will be "turned off" during the interaction with cancer cell, we can assume that the treatment decreases the value of parameter m. To reduce the complexity of the following analysis, but without the loss of generality, we won't consider any pharmacokinetics and pharmacodynamics of

the drug, *i.e.* we assume simply that during the treatment parameter *m* has a lower value m_T " (Kuznetsov *et al.*, 1994).

First, we should check the predictive model dynamics for the nominal parameter values proposed by Kuznetsov and others. Let us simplify that task by performing non-dimensional procedure first. After defining $x = E/E_0$, $y = N/N_0$, and $\tau = nN_0 t$ with $E_0 = N_0 = 10^6$ we obtain the slightly simpler system

$$\dot{x} = \sigma + \rho \frac{xy}{\eta + y} - \delta x - \mu xy \qquad (2)$$

$$\dot{y} = \alpha y(1 - \beta y) - xy$$

where $\sigma = \dfrac{s}{nE_0 N_0}$, $\rho = \dfrac{p}{nN_0}$, $\eta = \dfrac{g}{N_0}$, $\mu = \dfrac{m}{n}$, $\delta = \dfrac{d_0}{nN_0}$, $\alpha = \dfrac{a}{nN_0}$ and $\beta = bN_0$. We can see that in the non-dimensional model μ is the parameter affected by treatment.

Using parameters proposed in Kuznetsov's original paper we obtain the following values of non-dimensional parameters:
$\sigma = 0.118, \rho = 1.131, \eta = 20.19, \mu = 0.00311, \delta = 0.374, \alpha = 1.635, \beta = 0.002$

"We can start our analysis with evaluating numerically the model behavior for the above set of parameters. In order to do that systemically we will plot the phase portrait in which behavior of the solutions in the whole phase space can be viewed. MATLAB is quite a convenient environment in which we can do that quite easily by using the built-in *quiver* function (a quiver plot displays velocity vectors as arrows with components (*u,v*) at the points (*x,y*): Program 1" (https://computecancer.wordpress.com).

After running the above script we should see an image similar to the one below.
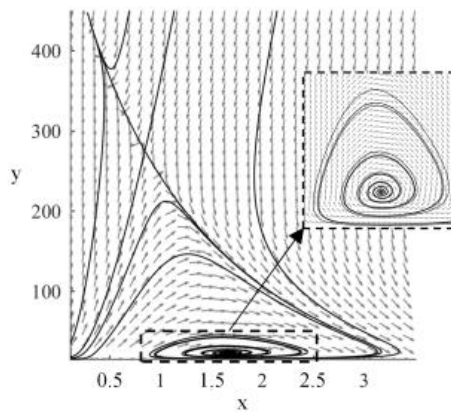
Fig. 1 – Phase portrait for the Kuznetsov & colleague's model
(https://computecancer.wordpress.com).

"We can see in the phase portrait that there are two steady states to which solutions tend to, one with substantial tumor burden, *i.e.* with large value of variable *y*, the second reflecting probably subclinical disease. Of course, those are the initial conditions that govern to which of them the solution will tend to. Let us now check analytically if the above behavior is not a numerical artifact.

First of all, the above system has a smooth vector field and thus, solutions exist and are unique. Moreover, it is easy to check that the solutions are bounded and non-negative. We are interested in calculating all of the possible steady states of the system and evaluating their stability. For any set of parameters we always have a semi-trivial steady state $\left(\dfrac{\sigma}{\delta}, 0 \right)$"

(https://computecancer.wordpress.com), *i.e.* the state without any tumor cells. By calculating null-clines and checking their intersections we obtain the following polynomial equation for other possible steady states.

$$W(y) = C_3 y^3 + C_2 y^2 + C_1 y + C_0 = 0 \qquad (3)$$

where

$$
\begin{aligned}
C_3 &= \mu\beta, \\
C_2 &= -\mu + \beta(\mu\eta + \delta - \rho), \\
C_1 &= \frac{\sigma}{\alpha} + \rho - \mu\eta - \delta + \delta\eta\beta, \\
C_0 &= \eta\left(\frac{\sigma}{\alpha} - \delta \right)
\end{aligned}
\qquad (4)
$$

We can see that, as we have cubic polynomial, there are up to three additional steady states. The exact number of solutions depends obviously on particular C_i values.

The local stability of the steady states can be evaluated by checking the eigenvalues of Jacobian matrix for the considered system:

$$
J(x, y) =
\begin{bmatrix}
\dfrac{\rho y}{\eta + y} - \mu y - \delta & ; & -\mu x + \dfrac{\rho\eta x}{(\eta + y)^2} \\
-y & & \alpha(1 - 2\beta y) - x
\end{bmatrix}
\qquad (5)
$$

"If all eigenvalues of *J*(*S*) have negative real parts then we know that the steady state *S* is locally asymptotically stable; if any of the eigenvalues have positive real part, then we know that the steady state is unstable.

An additional step that will be further required to draw definite conclusions is to show that there are no cycles in the system, *i.e.* all solutions

tend to one of the steady states. We can do that by using Dulac-Bendixon theorem with the function defined as $B(x,y)=1/(xy)$.

We are interested in behavior of the system for different values of parameter μ under the treatment. Thus, having the above equations we will plot a bifurcation diagram for the parameter $\mu = \mu_T$, *i.e.* we will plot existing steady states and their stability for different values of parameter μ_T. This can be achieved by using the following MATLAB script: Program 2. After running the code we should see a plot similar to the one below, but without the red dots that indicate the value of μ_T considered originally by Kuznetsov and colleagues" (Kuznetsov *et al*., 1994).
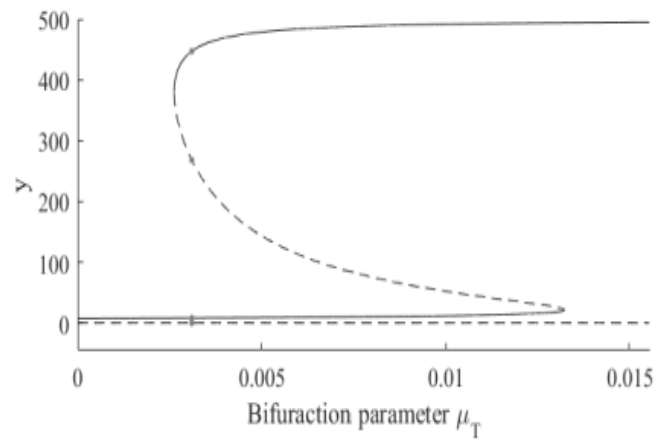

Fig. 2 – Bifurcation diagram (https://computecancer.wordpress.com).

"We can see in the bifurcation diagram that for nominal μ value we have two stable steady states, so the results of our initial numerical investigations are confirmed. Moreover, we see that:

– if nominal, *i.e.* without treatment, value of μ is too large then we can reduce the tumor size only temporary. In other words once we stop the treatment the tumor will grow back to the clinically detectable sizes.

– if nominal, *i.e.* without treatment, value of μ is smaller than a given threshold (≈ 0.013) then we can durably reduce the tumor to subclinical disease state, if during the treatment μ_T is sufficiently small (drug dose is sufficiently large) for sufficient amount of time. In other words, the tumor won't grow back once we stop the treatment.

This type of behavior is typically referred to as hysteresis. Most importantly results is consistent with clinical observation that in some patients long lasting responses may be achieved even after the drug withdrawal.

From a mathematical point of view everything boils down to the question if we can force the trajectory to intersect the curve separating basins of

attractions in the treatment free case. Once this is achieved we can withdraw the treatment and the patient's immune system will take care of the disease by itself. Thus, for each initial condition in the phase space we should look for the value of τ (scaled time) for which the intersection (if possible) occurs.

First, we need to approximate the curve separating the basins of attraction. This can be achieved by the MATLAB script: Program 3.

Then having the curve we can implement the numerical solver in a way that it stops integration once the separating curve is reached. This is done by the MALTAB script PROGRAM 4.

Using the above procedures we can look at how trajectories starting from the same initial condition (initial point) will behave for different μ_T values" (https://computecancer.wordpress.com).
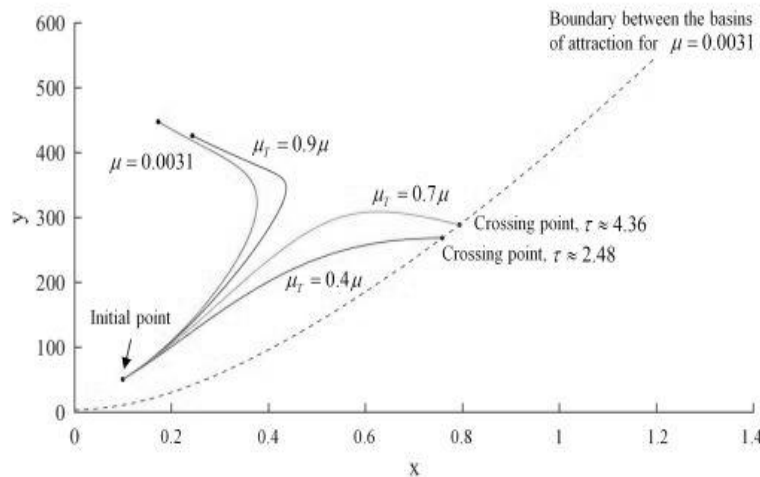

Fig. 3 – Separation curve between the basins of attraction and initial conditions.

"In the above plot we see that if the drug dose is not sufficient (μ reduction is not sufficient) then no matter how long we keep the patient on therapy, the tumor will grow to its maximal possible size (compare $\mu_T = 0.9\mu$) – therapy fails. However, if we increase the dosage, *i.e.* reduce μ even further, then we can move the tumor growth trajectory to the favorable outcome (compare $\mu_T = 0.7\mu$ and $\mu_T = 0.4\mu$). Interestingly, as observed in a clinic, the favorable outcome is achieved after initial phase of tumor growth under the therapy" (https://computecancer.wordpress.com).

3. Linear Quadratic Model and the "5 Rs" of Radiobiology

As known from radiobiology, the most important radiation damage is to the DNA in a cell, especially double-strand breaks (DSBs) of the DNA double helix. A dose of 1Gy results in thousands of ionizations in the cell's nucleus of

which a small part (about 40 in humans) induce DSBs. Most of these DSBs are repaired and some are miss-repaired. Many miss-repairs involve a reaction between two different DSBs. At a typical dose of several Grays, at least one miss-repair usually occurs, resulting in the cell's death.

The LQ-model incorporates these aspects and renders a survival fraction depending on the dose administered. This model is used to develop treatment schedules based on the best outcome of the Eq. (5).

The model describes cell killing by the following mechanisms: a single radiation track (*e.g.* γ-ray) produces various lethal lesions (DSBs) with a yield proportional to the dose, and miss-repair of pairs of DSBs produced from different radiation tracks produce lethal lesions with a yield proportional to the square of the dose. This results in the following equation for the yield of lethal lesions.

$$Y = \alpha D + \beta D^2 \quad \text{with } \alpha, \beta > 0 \tag{6}$$

D is the dose administered (Gy), αD lethal lesions produced per single radiation track and βD^2 are the lethal lesions produced from different radiation tracks (Brenner *et al.*, 1998; Cappuccio *et al.*, 2009; Sachs *et al.*, 2001; Thomson *et al.*, 1991). If more than one unrepaired break exists in a cell at the same time, a miss-joining can produce a lethal lesion. These lethal lesions follow a Poisson distribution from cell to cell. Therefore, the LQ-model reads

$$S = \frac{S^*}{S_0} = \exp(-Y) = e^{-\alpha D - \beta D^2} \tag{7}$$

where S is the survival fraction, S^* are the number of cells left after radiation and S_0 are the initial number of cells.
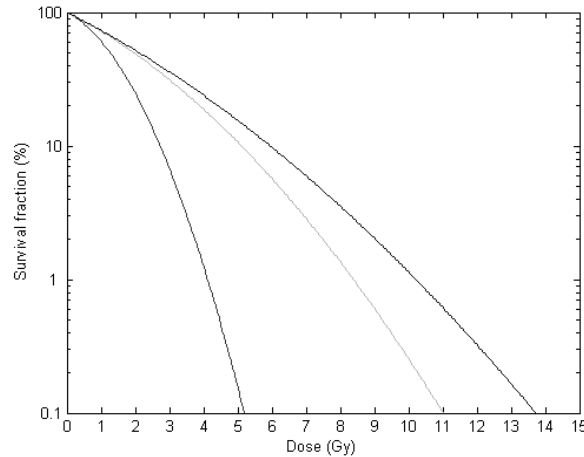

Fig. 4 – Cell survival curves after a single dose of radiation using Eq. (8), with red for $\alpha/\beta = 1.5$, green for $\alpha/\beta = 10$ and blue line for $\alpha/\beta = 20$.

Fig. 4 shows three survival curves using Eq. (6), each with a different value for the ratio α/β. The red curve depicts a survival curve for prostate cancer, the green curve for non-small cell cancers and the blue curve for advanced head and neck cancer. The ratios were taken from Rourke *et al.* (2009). From this plot one can see how different cancer types need different radiation doses to kill the same percentage of cells. Clearly, advanced head and neck cancer cells are more radio resistant and need a higher dose to die compared to prostate cancer cells.

The ratio α/β is a measure of a tissue's sensitivity. Prostate cancer is slow proliferating and the tissue responds late, thus having a higher repair capacity (since the cells have time to repair the damage before replicating) compared to advanced head and neck cancer, which is an early responding tissue with an aggressive cell proliferation rate and low repair capacity. Low α/β ratios are equivalent to a higher capacity for self-repair (*e.g.* normal tissue) and high ratios mean that the capacity for self-repair is low (*e.g.* tumours). Most normal tissue ratios are about 1-3Gy whereas most tumours have a ratio of about 10Gy.

The LQ-model in Eq. (6) only allows for a single dose of radiation. However, if the dose is not administered in one single session but in *n* fractions, each of dose *d*, the equation reads

$$S = e^{-\alpha n d - \beta n d^2} \tag{8}$$

So far this is a very simple model that describes cell killing depending on the dose of radiation. However, there are more factors that influence the capability of cells surviving radiation. **These are known as the "5 Rs" of radiobiology: repair, repopulation, re-distribution over the cell cycle, re-oxygenation and radio resistance**.

As we can easily figure out, the existing radiotherapy model (LQ-model) does not include the time course t of the treatment in its equation. This is a huge disadvantage as one cannot see the long term effect of radiation and should be included in future models.

4. Thomson Model for Human Blood Lymphocyte Population X-Ray Survival Curves. Single Hypothetical Model for the Shape of Survival Curve Population Prediction

Considering a theoretical population uniform in radio-sensitivity, then according to classical target theory the frequency distribution of hits per cell during irradiation would be Poisson in form. If the number of cells becoming dead by time t after irradiation were determined by the number of hits n received (*n* > 0 assumed lethal), the fraction of cells still alive (*i.e.* apparent plus real survivors) S at time t would be given by (Thomson *et al.*, 1991).

$$S = \frac{S_i}{S_0} = e^{-\alpha D}\left(1 + f_1(t)\alpha D + f_2(t)\frac{(\alpha D)^2}{2!} + \ldots f_n(t)\frac{(\alpha D)^n}{n!}\right) \qquad (9)$$

where α relates to the target size, αD is the average number of hits on-target, the measured survival S_i is expressed as a function of that (S_0) in the un-irradiated population and $f_n(t)$ is some function of t defining the fraction of cells still alive out of those receiving n hits. If it be assumed instead that the number dead by time t is independent of n, then

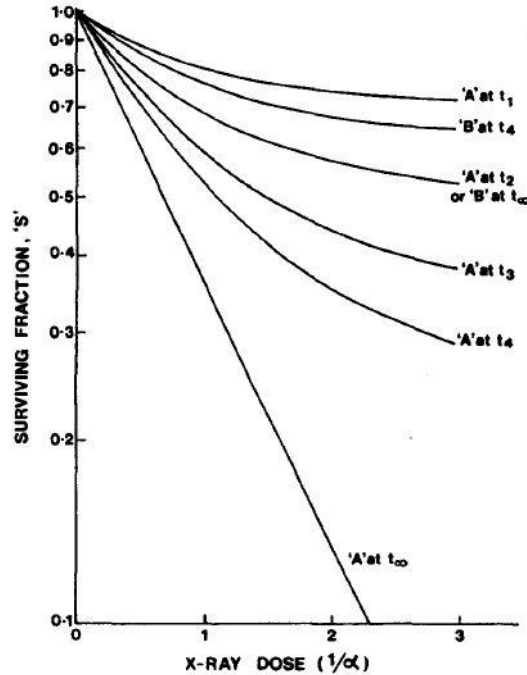$$S = \exp(-\alpha D) + f(t)[1 - \exp(-\alpha D)] \qquad (10)$$

Fig. 5 – Theoretical survival curves. 'A' is a single, uniformly radiosensitive cell population exhibiting the (log) survival-radiation dose relationship predicted by Eq. (10) for times t_1, t_2, t_3, t_4 and t_∞ after irradiation with $f(t)$ having the values 0.7, 0.5, 0.35, 0.25 and 0.00 respectively. The presence (where $t < t_\infty$) of lethally-hit cells still alive and indistinguishable from real survivors obscures the linear relationship for true (ultimate) survival by creating a continuous curve. The additional presence instead of totally radio resistant (unresponsive) cells has the same transforming effect; as illustrated here for population 'B' at t_∞ which comprises 50 per cent of 'A' at t_∞, and 50 per cent such radio resistant cells.

This expression defines the simple model. It apportions the surviving cell fraction into real survivors (the term exp (-αD)) and apparent survivors (the term $f(t)\{1 - \exp(-\alpha D)\}$. Fig. 5 shows how the shape of the survival curve afforded by this model for hypothetical population 'A' would differ at different times after irradiation corresponding to f(t) values of 0.7, 0.5, 0.35, 0.25, and 0.0, and in the dose range of 0 to $3/\alpha$; where $1/\alpha$ Gy is the dose (D 37) allowing 37 per cent real survival (that defines the radio-sensitivity), $2/\alpha$ is that allowing 13 per cent and $3/\alpha$ Gy is that allowing 5 per cent real survival.

The presence of apparent survivors (*i.e.* where $f(t)<1$ and > 0) alters the shape of the graphical relationship obtaining (where $f(t) = 0$) between the natural logarithm of true survival and radiation dose from a straight line to a smooth curve which flattens off as the number of real survivors approaches zero. Therefore, function $f(t)$ may be taken explicitly as $\exp(-\beta t)$, where β is the first-order rate constant.

$$S = \exp(-\alpha D) + \exp(-\beta t)[1 - \exp(-\alpha D)] \qquad (11)$$

A survival curve so-shaped if realized in experimental practice would be indistinguishable by eye from that representing a cell population heterogeneous in radio-sensitivity and/or additionally comprising cells totally resistant at the highest dose tested.

5. Adding Radiotherapy (Continuous Radiation) to the Tumor-Immune System

To derive a model that includes continuous radiation, we use the "5Rs" of radiobiology. We first add a term to the existing tumor-immune system that reduces the amount of tumor cells if radiation is administered:

$$\dot{N}(t) = aN(t)(1 - bN(t)) - nE(t)N(t) - \mu_2 DN(t) \text{ with } \mu_2 > 0 \qquad (12)$$

The right hand side of Eq. (12) is the same as the tumor growth model (Eq. (1)) except for the term $\mu_2 DN$ being subtracted from the growth term. D is the amount of radiation administered (Gy) and μ_2 related to how much the drug damages the tumoral cell per Gy. We assume continuous radiation and therefore set

$$D = d \quad \forall t \in (t_0, t_{max})$$

d is a constant amount of radiation given during a finite interval of time, from t_0 to t_{max}; after this time, one makes the measurements. If $d = 0$, *i.e.* no radiation is administered, the model is reduced to the growth model of Eq. (1).

Repair. Since we know that DSBs caused by radiation can be repaired, we want to include this in our model by adding a term, which increases the number of cells. The equation reads

$$\dot{N}(t) = aN(t)(1 - bN(t)) - nE(t)N(t) - \mu_2 DN(t) + \rho_2 \lambda_2 DN(t) \quad \text{with } \rho_2 > 0 \quad (13)$$

ρ_2 represents the factor of cells that are able to repair themselves after radiation and λ_2 is the repair rate, with $\lambda_2 = \ln 2/T_r$, T_r being the repair half-time.

Repopulation. In addition to repair, cells can also continue to proliferate, either between fractions of radiation or during continued low dose radiation. If we assume β_2 to be the amount of cells able to proliferate, then the model reads

$$\dot{N}(t) = aN(t)(1 - bN(t)) - nE(t)N(t) - \mu_2 DN(t) + \rho_2 \lambda_2 DN(t) + \beta_2 DaN(t)(1 - bN(t))$$
$$\text{with } \beta_2 > 0 \quad (14)$$

Also, we add a term to the existing model that reduces the amount of immune effector cells if radiation is administered, and get:

$$\dot{E}(t) = s + p \frac{E(t)N(t)}{g + N(t)} - d_0 E(t) - mE(t)N(t) - \mu_1 DE(t) \quad \text{with } \mu_1 > 0 \quad (15)$$

To be absolutely rigorous, we would have to employ the same "5 Rs" of radiobiology to act on the immune effector cells too, and introduce a repair and a repopulation term. Yet, this would have complicate too much the starting system (1) and we only wanted to test if adding radiation therapy to Kuznetsov's original model would change the behavior of the interaction between effector cells and cells in a growing tumor.

Consequently, introducing the effect of continuous radiation therapy Eqs. (14) and (15) by means of the terms μ_1, μ_2, ρ_2, λ_2, β_2, and $D=d$ the amount of continuous radiation administered (in Gy) and after some straightforward algebra manipulation, we get the same form for the new immunotherapy-radiotherapy system, as in Eq. (1)

$$\dot{E}(t) = s + p \frac{E(t)N(t)}{g + N(t)} - \Theta E(t) - mE(t)N(t)$$
$$\dot{N}(t) = AN(t)\left(1 - \frac{B}{A}N(t)\right) - nE(t)N(t) \quad (16)$$

where we made the notations

$$A = a + (a\beta_2 + \rho_2\lambda_2 - \mu_2)D;$$
$$B = ab(1 + \beta_2 D); \qquad\qquad\qquad (17)$$
$$\Theta = d_0 + \mu_1 D.$$

We can check easily, that if one takes $D = d = 0$ (*i.e.* no radiation is administered), the model (16) reduces to the growth model (1).

Finally, we get the same form for the new non-dimensional immunotherapy-radiotherapy system, as in Eq. (2)

$$\dot{x} = \sigma + \rho\frac{xy}{\eta + y} - \Delta x - \mu xy \qquad\qquad (18)$$
$$\dot{y} = \alpha' y(1 - \beta' y) - xy$$

with the following notations

$$\sigma = \frac{s}{nE_0 N_0}, \quad \rho = \frac{p}{nN_0}, \quad \eta = \frac{g}{N_0}, \quad \mu = \frac{m}{n}, \quad \Delta = \frac{\Theta}{nN_0}, \quad \alpha' = \frac{A}{nN_0} \quad \text{and}$$

$\beta' = \dfrac{B}{A}N_0$ with *A, B* and Θ given in Eq. (17).

We can see that in the new non-dimensional immunotherapy-radiotherapy we have μ, which is affected by the immune checkpoint blockade, and the parameters α', β' and Δ which are affected by the treatment with continuous radiation.

Using the unaffected parameters proposed in Kuznetsov's original paper and introducing the new parameters modified by the continuous radiation assumption, we obtain the following unified non-dimensional model's parameter values:

$$\rho_2 = 0.5; \quad \lambda_2 = \frac{\ln 2}{4}; \quad \beta_2 = 0.3; \quad \sigma = 0.118; \quad \rho = 1.131; \quad \eta = 20.19; \quad \mu = 0.00311$$

$$\Delta = 0.374 + 9.083 \cdot \mu_1 \cdot d; \quad \alpha' = 1.635 + \frac{(0.141 - \mu_2)d}{0.1101};$$
$$\beta' = \frac{0.00036(1 + 0.3 \cdot d)}{0.18 + (0.141 - \mu_2) \cdot d} \qquad\qquad (19)$$

After running the modified program 2 in Matlab, we analyze the bifurcation diagrams which can describe stationary states and their stability for different values of μ_1 and μ_2 parameters and we can see that they still behave in the same manner.
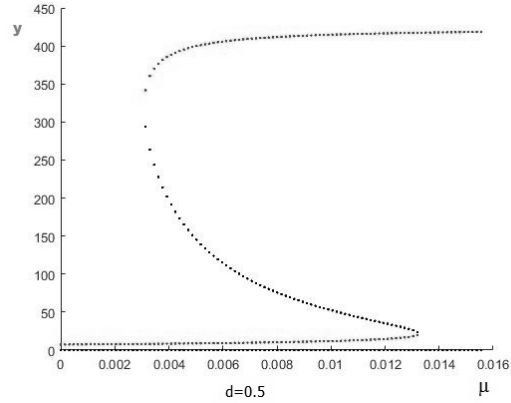
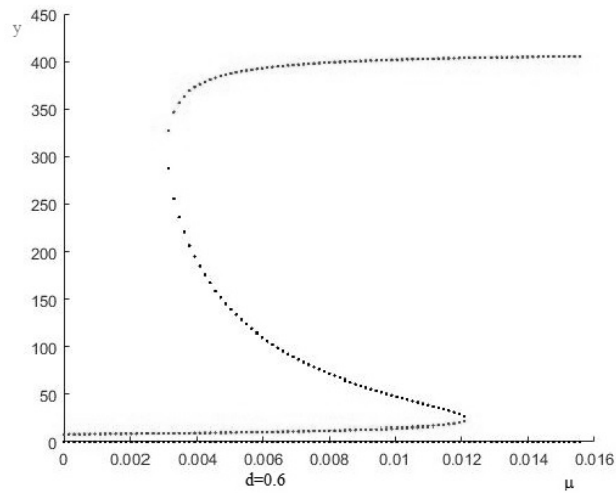Fig. 6 – Bifurcation diagram for $\mu_1 = 0.0005$, $\mu_2 = 0.15$ and $d = 0.5$.

Fig. 7 – Bifurcation diagram for $\mu_1 = 0.005$, $\mu_2 = 0.15$ and $d = 0.6$.

For a better understanding of the behavior predicted by Eqs. (18), we qualitatively characterized different regions of behavior according to the action coefficients of radiotherapy on tumor cells and on immune cells. These results are shown in Tables 1 and 4. The values for μ_1 and μ_2 coefficients are derived from the literature and clinical trials, where μ_1 represents the radiotherapy action factor on effector immune cells and μ_2 is the effect of radiotherapy on tumoral cells.

We want to find the behavior of the system for different values of the parameter in the treatment with radiation. This can be done using the MATLAB script: Program 4.

Table 1
*Therapeutic Diagram for $\mu_1 = 0.0005$; $\mu_2 = 0.15$ and
$d = (0\text{-}0.5)$ BS – Boundary of Separation*
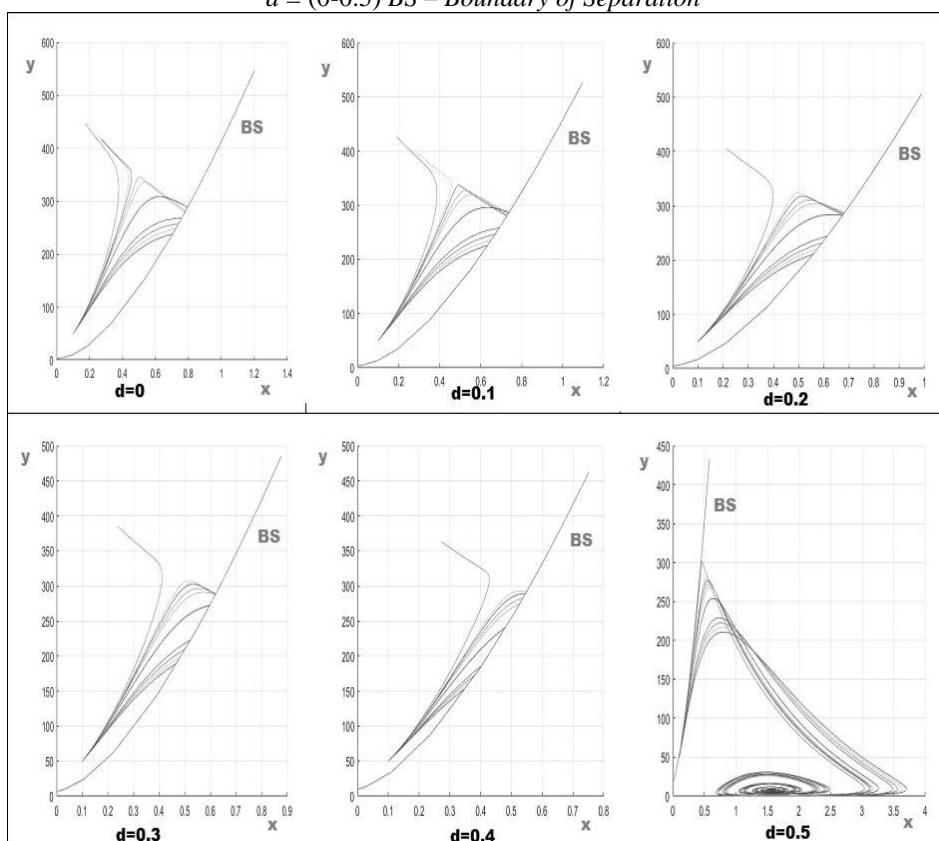
The variable x is the non-dimensional effector cell population and y the non-dimensional tumor cell population.

For the μ_1, μ_2 values estimated, it is noticed that for a value of dose $d \geq 0.5$, the state of the system becomes stable. This steady state is characterized by a relatively low TC (tumor cells) level and we refer to it as the "dormant tumor" steady state. Dormant states presumed that lethal tumor cells do not grow or they are growing at a slow rate during dormancy. This state appears after a radical treatment of a tumor. For the same initial conditions, the treatment curves running above the boundary of separation (BS) indicate that the tumor doesn't respond to radiation, and the treatment curves below BS, asymptotically approach the dormant tumor steady state. Thus the model is capable to explain both tumor dormancy and radio resistance.

The basin of attraction depends on the amount of immunotherapeutic medicine administered and the delivered dose of radiation.

In the next tables we delineate the parameter regimes in which these behaviors can be expected.

Table 2
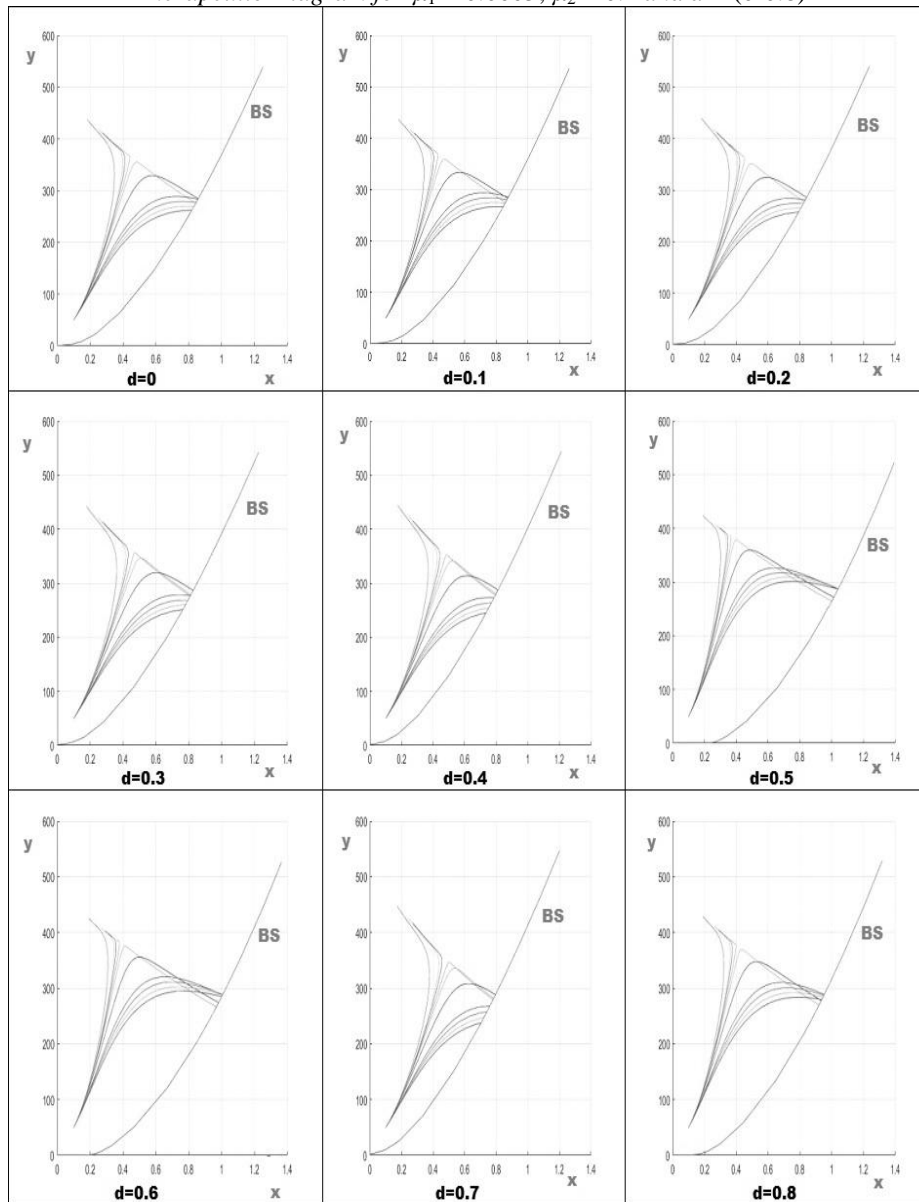*Therapeutic Diagram for $\mu_1 = 0.0005$; $\mu_2 = 0.1$ and $d = (0-0.8)$*
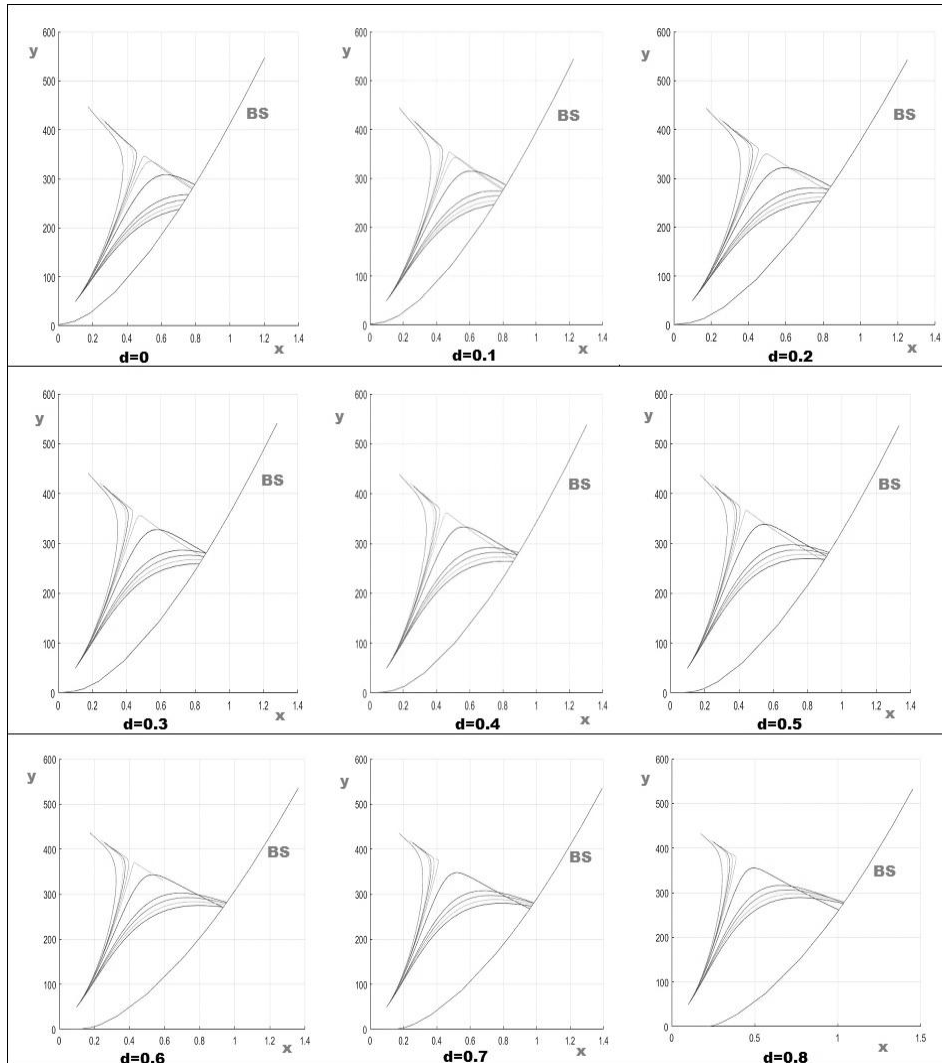
Table 3

Therapeutic Diagram for $\mu_1 = 0.005$; $\mu_2 = 0.1$ and $d = (0\text{-}0.8)$

In Tables 2 and 3, one can see for any radiation dose, the state of the system does not become stable.

Table 4
Therapeutic Diagram for $\mu_1 = 0.005$; $\mu_2 = 0.15$ and $d = (0$-$0.6)$
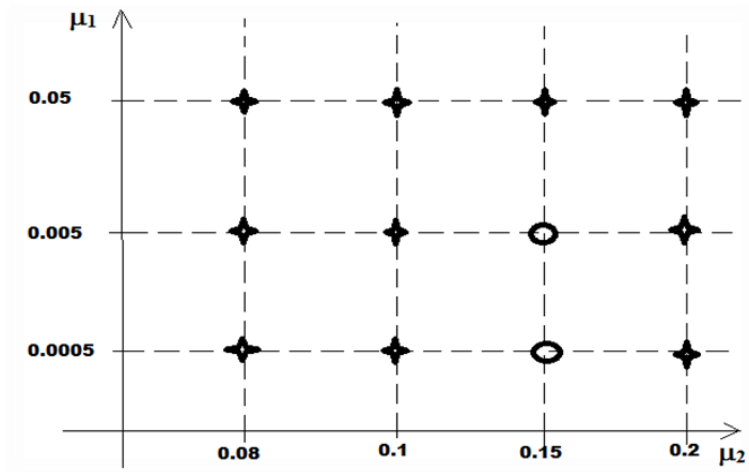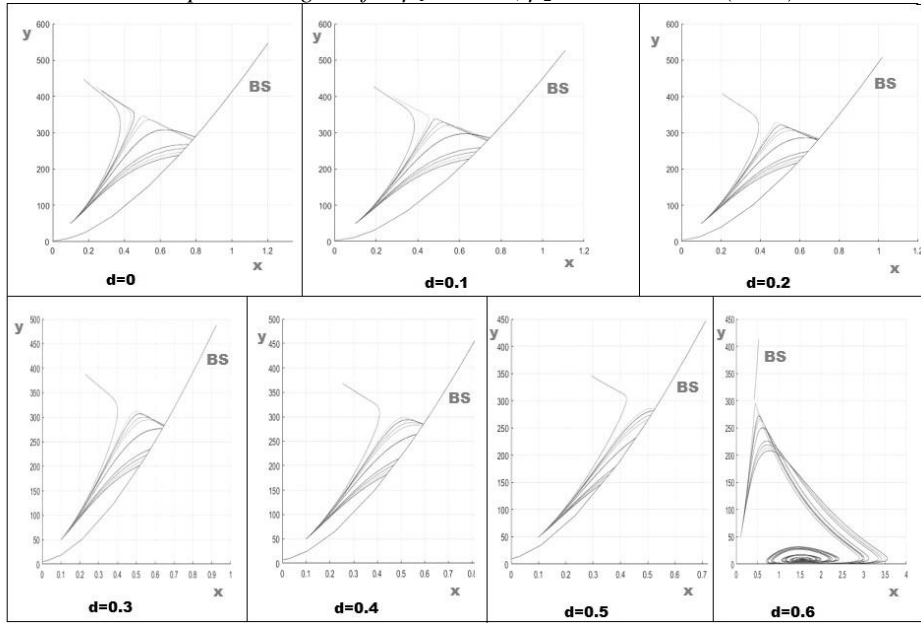

Fig. 5 – Diagram of results obtained from variations of parameters μ_1 and μ_2.

Depending on the values of the coefficients μ_1 and μ_2, we can reduce the tumor to the subclinical stage, *i.e.* the healing process is irreversible, or we can reduce the size of the tumor only temporarily.

5. Conclusions

We started from a non-linear quantitative dynamic tumor model for the interaction between effector cells and tumor cells proposed by Kuznetsov. The model investigates the tumor response to anti-PD-1/PD-L1 or anti-CTLA4 therapies.

We proposed the introduction of radiation therapy within this quantitative model through both-destruction, repair and repopulation functions of tumor cells as well as some terms with destructive and/or inhibitory action on immune cells.

The new immunotherapy-radiotherapy system, obtained by us with simple algebraic manipulation, has the same shape as the original Kuznetsov model, modifying the parameters a, b and d_0 (Eq. (17)).

From the literature, we used some programs written in MATLAB and modified them for the purpose of our analysis (see annexes). After running this programs we obtained the therapeutic diagrams (Tables 1-4).

Using realistic values of parameters (Fig. 5), there appears to be a synergism between the immune-stimulatory phenomena and the radio-sensitivity of the tumor growth. According to our findings there are two types of behavior, the inactive state ("dormancy") and the radio-resistant state. From the results of the concurrent action of immunotherapy and radiation, we can see that tumor reduction at the subclinical stage (irreversible healing process) is possible only for a well-defined parameter interval μ_1 and μ_2.

From Fig. 5, we conclude that for the estimated parameters μ_1 and μ_2 at the values (0.0005; 0.005) and respectively 0.15, we achieved a positive therapeutic response, acting concurrently with immunotherapeutic drug and continuous radiation. In both cases, the value of the radiation dose, d, after which the system passes the separation barrier and becomes stable is in the range (0.5 - 0.6).

APPENDIX - according to (https://computecancer.wordpress.com) with some modifications

"PROGRAM 1

```
%% DEFINING MODEL PARAMETERS
sigma = 0.118; rho = 1.131; eta = 20.19; mu = 0.00311;
delta = 0.374; alpha = 1.636; beta = 0.002;

%% DEFINING THE MODEL (INLINE FUNCTION)
rhs = @(t,x)([sigma+rho*x(1,:).*x(2,:)./(eta+x(2,:))-
mu*x(1,:).*x(2,:)-delta*x(1,:);...
alpha*x(2,:).*(1-beta*x(2,:))-x(1,:).*x(2,:)]);

%% FUNCTION RETURNING MODEL SOLUTION ON [0,100] FOR GIVEN
INITIAL CONDITION
```

```
solve = @(init)(ode45(rhs,[0 100],init));

%% EVALUATING THE VECTOR FIELD IN [0, 3.5]x[0, 450]
Npoints = 30;
x = linspace(0.,3.5,Npoints);
y = linspace(0,450,Npoints);
dx = x(2)-x(1);
dy = y(2)-y(1);
[X, Y] = meshgrid(x,y);
G = rhs([],[reshape(X,1,[]); reshape(Y,1,[])]);
U = reshape(G(1,:),Npoints,Npoints);
V = reshape(G(2,:),Npoints,Npoints)*dx/dy;
N = sqrt(U.^2+V.^2);
U = U./N; V = V./N;

%% EVALUATING MODEL SOLUTIONS FOR DIFFERENT INITIAL
CONDITIONS
initCond = [0, 2.58; 0, 1; 0, 0.1; 0, 20; 0.76, 267.8;...
0.755, 267.8; 1.055, 450; 0.6, 450; 2.1, 450];
sols = cell(1,size(initCond,1));
for i = 1:size(initCond,1)
sols{i} = solve(initCond(i,:));
end

%% PLOTTING THE RESULTS
figure(1)
clf
hold on
[X1, Y1] = meshgrid(0:Npoints-1,0:Npoints-1);
q = quiver(X1,Y1,U,V); %plotting vector field
q.Color = 'red';
q.AutoScaleFactor = 0.75;
for i = 1:length(sols) %plotting each solution
plot(sols{i}.y(1,:)/max(x)*(Npoints-
1),sols{i}.y(2,:)/max(y)*(Npoints-1),'k')
end
hold off "
```

"PROGRAM 2
```
%% DEFINING MODEL PARAMETERS
sigma = 0.118; rho = 1.131; eta = 20.19; mu = 0.00311;
delta = 0.374; alpha = 1.636; beta = 0.002;
%% DEFINING FUNCTION RETURNING JACOBIAN MATRIX FOR GIVEN
x,y AND mu
J = @(x,y,mu)([rho*y/(eta+y)-mu*y-delta, -
mu*x+rho*eta*x/(eta+y)^2 ; ...
-y, alpha*(1-2*beta*y)-x]);
```

```
%% DEFINING POLYNOMIAL COEFFICIENTS FOR DIFFERENT MU
C = @(mu)( [mu*beta; -mu+beta*(eta*mu+delta-rho); ...
sigma/alpha+rho-eta*mu-delta+beta*delta*eta; ...
eta*(sigma/alpha-delta)]);

%% PLOTTING BIFFURCATION DIAGRAM
muMesh = linspace(0,5*mu,100); %mesh for mu
figure(1)
clf
hold on
for mu = muMesh
StStatesY = roots(C(mu)); %solving W(y) = 0;
StStatesY(imag(StStatesY) ~=0) = []; %deleting complex
roots
StStatesY(StStatesY < 0) = []; %deleting negative roots
StStatesX = alpha*(1-beta*StStatesY); %calculating x
coordinate
indx = StStatesX < 0;
StStatesX(indx) = []; %deleting steady states with negative
x
StStatesY(indx) = []; %deleting steady states with negative
x
for i = 1:length(StStatesY) %evaluating stability and
plotting
Jeval = J(StStatesX(i), StStatesY(i), mu);
if all(real(eig(Jeval))< 0) %point is stable
plot(mu,StStatesY(i),'r.');
else
plot(mu,StStatesY(i),'k.');
end
end
plot(mu, 0,'k.'); %always unstable semi-trivial steady
state
end
hold off"
```

"PROGRAM 3

```
%% DEFINING MODEL PARAMETERS
sigma = 0.118; rho = 1.131; eta = 20.19; mu = 0.00311;
delta = 0.374; alpha = 1.636; beta = 0.002;

%% CALCULATING BIGGEST VALUE OF STEADY STATE FOR Y
Smax = max(roots([mu*beta; -mu+beta*(eta*mu+delta-rho); ...
sigma/alpha+rho-eta*mu-delta+beta*delta*eta; ...
eta*(sigma/alpha-delta)]));
```

```
%% DEFINING THE MODEL (INLINE FUNCTION)
rhs = @(t,x)([sigma+rho*x(1,:).*x(2,:)./(eta+x(2,:))-
mu*x(1,:).*x(2,:)-delta*x(1,:);...
alpha*x(2,:).*(1-beta*x(2,:))-x(1,:).*x(2,:)]);

%% FUNCTION RETURNING MODEL SOLUTION ON [0,100] FOR GIVEN
INITIAL CONDITION
solve = @(init)(ode45(rhs,[0 100],init));


%% CALCULATING LOWER PART OF THE SEPARATING CURVE
yInit = 50; %initial value
yTol = 1e-5; %tolerance
dy = 5; %initial step size

while dy > yTol
sol = solve([0 yInit]);
if abs(sol.y(2,end)-Smax) < 30 %reached maximal steady
state
yInitPrev = yInit;
yInit = yInit-dy;
else
dy = dy/2;
yInit = yInitPrev;
end
end

%% CALCULATING UPPER PART OF THE SEPARATING CURVE
xInit = 0.5; %initial value
xTol = 1e-5; %tolerance
dx = 0.5; %initial step size
while dx > xTol
sol = solve([xInit Smax+100]);
if abs(sol.y(2,end)-Smax) < 30
xInitPrev = xInit;
xInit = xInit+dx;
else
dx = dx/2;
xInit = xInitPrev;
end
end

%% CALCULATING THE FINAL CURVE
sol2 = solve([0 yInit]);
sol1 = solve([xInit Smax+100]);
sol1.y = sol1.y(:,1:find(diff(sol1.y(2,:))> 0,1,'first'));
sol2.y = sol2.y(:,1:find(diff(sol2.y(1,:))< 0,1,'first'));
```

```
curve.x = [sol2.y(1,:) sol1.y(1,end:-1:1) ];
curve.y = [sol2.y(2,:) sol1.y(2,end:-1:1) ];

%% PLOTTING
figure(1)
clf
plot(curve.x, curve.y)
function sol = solveKuznetsovNondimensionalBasins( par,
Tmax, init, curve )
init = init(:);
opts = odeset('RelTol',1e-8,'AbsTol',1e-8,'Events',@stops);
sol = ode45(@odesystem, [0 Tmax],init,opts);
function y = odesystem(~,x)
y = zeros(2,1);
y(1) = par.sigma+par.rho*x(1).*x(2)./(par.eta+x(2))-
par.mu*x(1).*x(2)-par.delta*x(1);
y(2) = par.alpha*x(2).*(1-par.beta*x(2))-x(1).*x(2);
end
function [value,isterminal,direction] = stops(~,y)
value = y(2)-int erp1(curve.x, curve.y, y(1));
isterminal = 1; % Stop the integration
direction = 0; % Any direction
end
end"
```

"PROGRAM 4
```
%% DEFINING MODEL PARAMETERS
sigma = 0.118; rho = 1.131; eta = 20.19; mu = 0.00311;
delta = 0.374; alpha = 1.636; beta = 0.002;

%% CALCULATING BIGGEST VALUE OF STEADY STATE FOR Y
Smax = max(roots([mu*beta; -mu+beta*(eta*mu+delta-rho); ...
sigma/alpha+rho-eta*mu-delta+beta*delta*eta; ...
eta*(sigma/alpha-delta)]));

%% DEFINING THE MODEL (INLINE FUNCTION)
rhs = @(t,x)([sigma+rho*x(1,:).*x(2,:)./(eta+x(2,:))-
mu*x(1,:).*x(2,:)-delta*x(1,:);...
alpha*x(2,:).*(1-beta*x(2,:))-x(1,:).*x(2,:)]);

%% FUNCTION RETURNING MODEL SOLUTION ON [0,100] FOR GIVEN
INITIAL CONDITION
solve = @(init)(ode45(rhs,[0 100],init));

%% CALCULATING LOWER PART OF THE SEPARATING CURVE
yInit = 50; %initial value
yTol = 1e-5; %tolerance
```

```
dy = 5; %initial step size
while dy > yTol
sol = solve([0 yInit]);
if abs(sol.y(2,end)-Smax)< 30 %reached maximal steady state
yInitPrev = yInit;
   yInit = yInit-dy;
else
   dy = dy/2;
   yInit = yInitPrev;
end
end

%% CALCULATING UPPER PART OF THE SEPARATING CURVE
xInit = 0.5; %initial value
xTol = 1e-5; %tolerance
dx = 0.5; %initial step size
while dx > xTol
sol = solve([xInit Smax+100]);
if abs(sol.y(2,end)-Smax) < 30
xInitPrev = xInit;
   xInit = xInit+dx;
else
    dx = dx/2;
    xInit = xInitPrev;
end
end

%% CALCULATING THE FINAL CURVE
sol2 = solve([0 yInit]);
sol1 = solve([xInit Smax+100]);
sol1.y = sol1.y(:,1:find(diff(sol1.y(2,:))>0,1,'first'));
sol2.y = sol2.y(:,1:find(diff(sol2.y(1,:))<0,1,'first'));
curve.x = [sol2.y(1,:) sol1.y(1,end:-1:1) ];
curve.y = [sol2.y(2,:) sol1.y(2,end:-1:1) ];

%% PLOTTING
par.sigma = 0.118; par.rho = 1.131; par.eta = 20.19; par.mu
= 0.00311;
par.delta = 0.374; par.alpha = 1.636; par.beta = 0.002;
figure(1)
clf
hold on
plot(curve.x, curve.y)
sol = solveKuznetsovNondimensionalBasins( par, 100, [0.1
50], curve );
plot(sol.y(1,:), sol.y(2,:))
par.mu = 0.00311*0.9;
```

```
sol = solveKuznetsovNondimensionalBasins( par, 100, [0.1
50], curve );
plot(sol.y(1,:), sol.y(2,:))
par.mu = 0.00311*0.7;
sol = solveKuznetsovNondimensionalBasins( par, 100, [0.1
50], curve );
plot(sol.y(1,:), sol.y(2,:))
disp(['Crossing point: ' num2str(sol.x(end))])
par.mu = 0.00311*0.4;
sol = solveKuznetsovNondimensionalBasins( par, 100, [0.1
50], curve );
plot(sol.y(1,:), sol.y(2,:))
disp(['Crossing point: ' num2str(sol.x(end))])
hold off ”
```

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https://www.britannica.com/science/T-cell
https://computecancer.wordpress.com

UN MODEL MATEMATIC CARE COMBINĂ DINAMICA
TUMORĂ-SISTEM IMUNITAR CU
RADIOTERAPIA ŞI ILUSTREAZĂ UN SINERGISM POTENŢIAL

(Rezumat)

Folosind dinamica neliniară a tumorilor imunogene, A. Kuznetsov a propus un model cantitativ pentru interacţiunea dintre celulele efectoare şi celulele tumorale. Adăugând radioterapia la modelul iniţial al lui Kuznetsov, obţinem un sistem care ia în considerare atât distrugerea, reparaţia şi repopularea celulelor tumorale, cât şi distrugerea şi inhibarea celulelor imune. Valorile pentru coeficienţii μ_1 şi μ_2 sunt derivate din literatura de specialitate şi din studiile clinice, unde μ_1 reprezintă factorul de acţiune al radioterapiei asupra celulelor imune efectoare şi μ_2 este efectul radioterapiei asupra celulelor tumorale. Folosind Matlab, am obţinut diagramele terapeutice pentru diferite valori ale parametrilor μ_1 şi μ_2 şi se observă că pentru o gamă bine definită a acestor parametri, tumorile sunt reduse la stadiul subclinic.