VERSAL DEFORMATION AND STATIC BIFURCATION DIAGRAMS FOR THE CANCER CELL POPULATION MODEL

By

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Abstract. The paper studies the existence of rest-points and the static bifurcation diagrams of a given nonlinear differential system modeling the cancer cell population evolution from biology. To this aim, the nullclines, the equilibrium points, the transient set, the static bifurcation equation and the associated versal deformation are investigated. The results are further discussed in view of potential applications to cancer therapy.

1. Introduction. Recent research in cancer progression and treatment indicates that many forms of cancer arise from one abnormal cell or a small subpopulation of abnormal cells ([10]). These cells, which support cancer growth and spread are called *cancer stem cells* (CSCs). Targeting these CSCs is crucial because they display many of the same characteristics as healthy stem cells, and they have the capacity of initiating new tumors after long periods of remission ([3]). It is well known that cancer cell populations consist of a combination of *proliferating, quiescent (resting)* and *dead cells* that determine tumor growth or cancer spread ([6], [9]).

The understanding of the cancer mechanism has a significant impact on cancer treatment strategies which target diverse cell sub-populations at a specific stage of development. Although researchers have tried different treatments, of particular interest is the one based on the biological effect of ultrashort, high intensity electrical pulses nanosecond lasting pulsed electric fields (nsPEFs). These fields charge the membranes of intracellular organelles and allow the experimental researchers to handle the cellular functions ([11]). Moreover, recent studies showed that nsPEFs can induce *apoptosis* (i.e., programmed cell death) in cancer cells both *in vitro* and in tumors ([2]).

In this paper, we study a mathematical nondimensionalized model of a cancer cell population consisting of a 2-dimensional system of differential equations with 4 parameters, which was introduced in 2006 by Garner et al. ([7]). Section 1 provides a detailed presentation of the model, while in Section 2 we explicitly describe its nullclines and study its equilibrium points, with emphasis on the case of zero mortality rate of resting cells. In Section 3, after a brief introduction of the basic concepts and techniques used from the theory of versal deformations ([8]), are determined the normal form and the versal deformation for the equation which provides the abscissa of the equilibrium point. In Section 4 we explicitly obtain the transient set of the model and in Sections 5 and 6 we illustrate the theory by providing the bifurcation diagrams and characterizing the equilibrium points for certain given sets of parameters. Possible applications of the obtained results to therapy strategies relying on the chosen bifurcation parameter are discussed in the last section.

The model is based on the dynamical system proposed by Solyanik et al. ([12]), and estimates the behavior of the two types of cancer cell populations (proliferating and quiescent) based on approximately 10 measurements of the total cell population over one week [12]. Its main assumptions are as follows:

- the cancer cell population consists of proliferating and quiescent (resting) cells;
- the cells can lose their ability to divide under certain conditions and then transit from the proliferating to the resting state;
- resting cells can either return to the proliferating state, or die.

Figure 1 presents a block diagram of the system. We note that Solyanik's model utilizes two coupled, nonlinear differential equations with the final cell behavior dependent upon the initial total cell number and the ratio between proliferating and quiescent cells. The state variables are

- x - the number of proliferating cells, and
- y - the number of resting (quiescent) cells.

Their dynamics in time is described by the following differential equations introduced by Solyanik et al. [12]:

$$\begin{cases} x' = bx - Px + Qy, \\ y' = -dy + Px - Qy, \end{cases}$$

where the parameters involved have the following meaning:

- b is the rate of cell division of the proliferating cells;
- d is the rate of cell death of the resting cells (per day);
- Q and P describe the intensity of cell transition from the quiescent to proliferating cells and vice versa (per day).

Based on the experimental observation that cancer cells multiply in the presence of sufficient biological and physical factors, they assumed that P must depend on the number of cells present and, in the simplest form, can be written as $P = c(x + ay)$, where:

- a is a dimensionless constant that measures the relative nutrient uptake by resting vs. proliferating cancerous cells;
- c depends on the intensity of consumption by proliferating cells and gives the magnitude of the rate of cell transition from the proliferating stage to the resting stage in per cell per day.
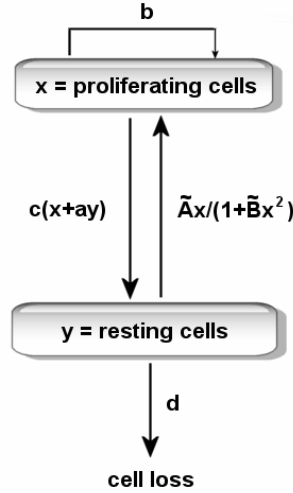
FIG. 1. Cancer cell population evolution

Solyanik et al. pointed out that the transition from the quiescent to the proliferating state was more complex. One can easily guess that increasing the number of proliferating cells would decrease the intensity of this transition; however, a decrease in the number of proliferating cells can also have the same effect ([14], [15]). Based on these remarks, one can assume that Q is nonmonotonic with respect to x (the number of proliferating cells) so that it increases with increasing x up to a certain point, and then decreases as x becomes very large. Accordingly, Q has the form $Q = \tilde{A}x/(1 + \tilde{B}x^2)$, where:

- $\tilde{A}$ is the initial rate of increase in Q at small x;
- $\tilde{A}/\tilde{B}$ represents the rate of decrease in Q when x becomes larger.

In their paper, Solyanik et al. studied the resulting steady states and stability, assuming that the transition rate Q is zero ([12]).

In order to simplify the notation and reduce the number of parameters, Garner et al. introduced in their paper [7] (2006) the following dimensionless parameters:

$$\bar{x} = xc/b, \quad \bar{y} = yca/b, \quad \bar{t} = bt.$$

After dropping bars for notational convenience, the nondimensional system can be rewritten as

$$\begin{cases} x' = x - x(x + y) + \dfrac{hxy}{1 + kx^2}, \\ y' = -ry + ax(x + y) - \dfrac{hxy}{1 + kx^2}, \end{cases} \tag{1.1}$$

where:

- $r = d/b$ is the ratio of the death rate of quiescent cells to the birth rate of proliferating cells;
- $h = \tilde{A} \cdot (ac)^{-1}$ represents a growth factor that preferentially shifts cells from the quiescent to proliferating state;

- $k = \tilde{B} \cdot (b/c)^2$ represents a slightly moderating effect.

Garner et al. analyzed the nondimensional model of a cancer cell population in order to estimate the long-term behavior of quiescent and proliferating cells. For simplicity, they set $k = 1$ and varied h to represent changes in the effects of these growth factors.

In the following we explicitly describe the nullclines of the model, study its equilibrium points, and further determine the normal form and the versal deformation for the equation which provides the abscissa of the equilibrium point. As well, we shall obtain the transient set, illustrate the theory for certain given sets of parameters, and discuss possible applications of the results to therapy strategies.

2. Nullclines and stationary points.
The nullclines of the system (1.1) are curves defined by $x' = 0$ and $y' = 0$, i.e.,

$$\begin{cases} x - x(x+y) + \dfrac{hxy}{1+kx^2} = 0, \\[2mm] -ry + ax(x+y) - \dfrac{hxy}{1+kx^2} = 0, \end{cases} \tag{2.1}$$

or equivalently

$$\begin{cases} x\left(y \cdot \dfrac{h-1-kx^2}{1+kx^2} - (x-1)\right) = 0, \\[2mm] y\left(ax - r - \dfrac{hx}{1+kx^2}\right) = -ax^2. \end{cases} \tag{2.2}$$

The equation $(2.2)_1$ provides either $x = 0$, i.e., trivial case (no proliferating cells), or a dependence of the domain of the nonnegative variables (x, y) in terms of the domain of the parameter h, as follows:

$D_h \subset [\,0, \infty) = \mathbb{R}_+$	$D_{(x,y)} \subset \mathbb{R}_+^2$
$[\,0, 1)$	$\{(x, f(x)) \mid x \in [\,0, 1]\}$
$[\,1, k+1)$	$\{(x, f(x)) \mid x \in (\sqrt{(h-1)/k}, 1]\}$
$\{k+1\}$	$\{(x, y) \mid x = 1, y \in \mathbb{R}_+\}$
$(k+1, \infty)$	$\{(x, f(x)) \mid x \in [\,1, \sqrt{(h-1)/k})\}$

where $f(x) = -\dfrac{(x-1)(1+kx^2)}{k\left(x^2 - (h-1)/k\right)}$. As well, $(2.2)_2$ leads to

$$y = g(x) \equiv \dfrac{-ax^2(1+kx^2)}{kax^3 - krx^2 + (a-h)x - r}.$$

The stationary points of (1.1) are located at the intersections of the nullclines. Let us denote by (x_0, y_0) an equilibrium point. From (2.2) it follows that x_0 and y_0 are related via

$$y_0 = -x_0 \cdot \dfrac{x_0\,(a-1) + 1}{x_0\,(a-1) - r}. \tag{2.3}$$

Replacing (2.3) in $(2.1)_1$ we get that $x = x_0 \in \mathbb{R}\backslash\{0, \frac{r}{a-1}\}$ satisfies the equation

$$P(x) \equiv x^3 + \dfrac{h(1-a) - kr}{k(a+r)}x^2 + \dfrac{a+r-h}{k(a+r)}x - \dfrac{r}{k(a+r)} = 0. \tag{2.4}$$

The nonnegative roots of the equation (2.4) are the abscissas of the stationary points of the model.

REMARK 2.1. In the following, we study the number and form of the roots of (2.4). These roots depend on the coefficients of (2.4), which we denote as

$$A = -\frac{kr + h(a - 1)}{k(a + r)}, \quad B = \frac{a + r - h}{k(a + r)}, \quad C = -\frac{r}{k(a + r)}.$$

Then (2.4) becomes

$$x_0^3 + Ax_0^2 + Bx_0 + C = 0.$$

Obviously $-C > 0$. Hence the roots of the equation (2.4) fit in one of the following cases:

a: three real positive roots;

b: three real roots from which two are negative and one is positive;

c: two complex conjugate roots and one real positive root.

From the biological point of view, we are only interested in the real positive roots. We further denote

$$p = B - \frac{A^2}{3}, q = \frac{2A^3}{27} - \frac{AB}{3} + C.$$

With this notation, equation (2.4) can be rewritten as

$$\left(x + \frac{A}{3}\right)^3 + p\left(x + \frac{A}{3}\right) + q = 0. \tag{2.5}$$

In $\mathbb{R}$, the equation (2.4) has a simple root in the following regions from the plane (p, q): $p > 0, q \in \mathbb{R}$; $p = 0, q \in \mathbb{R}^*$; $p < 0, |q| > \frac{2}{3}\sqrt{-\frac{p^3}{3}}$. For $p = q = 0$, the equation has a triple root $x_0 = -\frac{A}{3}$. For $p < 0$, when $|q| < \frac{2}{3}\sqrt{-\frac{p^3}{3}}$ we have three real distinct roots, and when $|q| = \frac{2}{3}\sqrt{-\frac{p^3}{3}}$ we have a double root and a simple root.

For the study of the static bifurcation our interest is focused only on the real roots of (2.5). For $p > 0, q \in \mathbb{R}$ and for $p < 0, |q| > \frac{2}{3}\sqrt{-\frac{p^3}{3}}$, we have:

$$x_1 = \sqrt[3]{-\frac{q}{2} + \sqrt{\Delta}} + \sqrt[3]{-\frac{q}{2} - \sqrt{\Delta}} - \frac{A}{3},$$

where $\Delta = \left(\frac{q}{2}\right)^2 + \left(\frac{p}{3}\right)^3$, and

$p = 0, q \in \mathbb{R}^*$	$x_1 = -\sqrt[3]{q} - \frac{A}{3}$
$p = q = 0$	$x_{1,2,3} = -\frac{A}{3}$
$p < 0, q = \frac{2}{3}\sqrt{-\frac{p^3}{3}}$	$\begin{cases} x_1 = -\frac{A}{3} - 2\sqrt{-\frac{p}{3}} \\ x_{2,3} = -\frac{A}{3} + \sqrt{-\frac{p}{3}} \end{cases}$
$p < 0, q = -\frac{2}{3}\sqrt{-\frac{p^3}{3}}$	$\begin{cases} x_1 = -\frac{A}{3} + 2\sqrt{-\frac{p}{3}} \\ x_{2,3} = -\frac{A}{3} - \sqrt{-\frac{p}{3}} \end{cases}$
$p < 0, |q| < \frac{2}{3}\sqrt{-\frac{p^3}{3}}$	$\begin{cases} x_1 = -\frac{A}{3} + 2\sqrt{-\frac{p}{3}}\cos(\frac{\varphi}{3}) \\ x_2 = -\frac{A}{3} + 2\sqrt{-\frac{p}{3}}\cos(\frac{\varphi+2\pi}{3}) \\ x_3 = -\frac{A}{3} + 2\sqrt{-\frac{p}{3}}\cos(\frac{\varphi+3\pi}{3}) \end{cases}$

with $\cos\varphi = -\frac{q}{2}\sqrt{-\frac{27}{p^3}}$. One can easily see that the real distinct roots can be found only in the case $p < 0, |q| < \frac{2}{3}\sqrt{-\frac{p^3}{3}}$ or, equivalently,

$$A^2 > 3B, \left|2A^3 - 9AB + 27C\right| < 2\left(A^2 - 3B\right)^{3/2}.$$

In order to find the relations fulfilled by the parameters a, r, k, h, for which the equation (2.4) admits three real positive roots, we apply the Hurwitz theorem for the polynomial

$$Q(x) = -P(-x) = x^3 + A_*x^2 + B_*x + C_*,$$

where $A_* = \dfrac{kr - h(1-a)}{k(a+r)}$, $B_* = \dfrac{a+r-h}{k(a+r)}$, $C_* = \dfrac{r}{k(a+r)}$. The positivity of the principal minors of the matrix $\begin{pmatrix} A_* & 1 & 0 \\ C_* & B_* & A_* \\ 0 & 0 & C_* \end{pmatrix}$ entails

$$\begin{cases} A_* > 0,\ C_* > 0 \\ A_*B_* - C_* > 0 \end{cases} \Leftrightarrow \begin{cases} a + r > h,\ ah + rk > h \\ a(a+r) + h > a(a+h) + r(k+1). \end{cases}$$

As well, all the roots should be real, i.e., $p = q = 0$, or $p \leq 0$ and $|q| < \frac{2}{3}\sqrt{-\frac{p^3}{3}}$. The first case can be rewritten in terms of the initial parameters $\{a, r, h, k\}$ as:

$$\begin{cases} 2k^3r^3 + (36r^2a + 3hr^2 + 18ra^2 + 9har + 6har^2 + 18r^3)k^2 \\ \quad + (-18ha^2r - 9h^2a + 18har + 9ha^2 + 6rh^2a^2 - 3h^2ar + 9hr^2 \\ \quad - 9ha^3 - 9har^2 - 3h^2r + 9h^2a^2)k + 2h^3a^3 - 6h^3a^2 + 6h^3a - 2h^3 = 0, \\ -k^2r^2 + (3r^2 + 3a^2 + 6ar - 2har - 3ha - hr)k + 2h^2a - h^2 - h^2a^2 = 0. \end{cases}$$

Moreover, the condition $y_0 > 0$ implies in view of (2.3) the following supplementary restrictions:

Case	$a < 1$	$a > 1$
Restriction	$x_0 < (1-a)^{-1}$	$x_0 < r \cdot (a-1)^{-1}$

and for $a = 1$ there exist no (biologically valid) stationary points with positive coordinates.

Regarding the case when $x_0 = 0$, this requires either the exceptional condition $r = 0$ (rate of resting-cells death being extremely small compared to the cell division of proliferating cells), or $y_0 = 0$.

We shall briefly comment on the case $r = d = 0$, when the SODE admits the whole axis Oy as a stationary subset. Then equation (2.4) becomes

$$x\left(x^2 + \frac{h(1-a)}{ka}x + \frac{a-h}{ka}\right) = 0. \tag{2.6}$$

The existence of other *real* roots (besides $x_0 = 0$) reverts to the condition

$$h^2(a-1)^2 - 4ka(a-h) \geq 0$$

and fits into the following cases:

a: $a \leq h$ (i.e., the relative advantage of the quiescent cells regarding the nutrient should be significantly small relative to h, which represents the growth factor that preferentially shifts cells from the quiescent to the proliferating state), or

b: $a > h$ but k (the moderating effect parameter) is bounded,
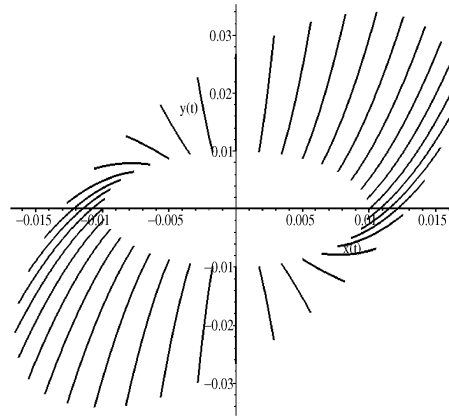
$$k \leq \frac{h^2(a-1)^2}{4a(a-h)}.$$

FIG. 2. Divergent paths around the origin (repeller), ($h = 1.236$, $k = .236$, $a = 1.998958904$, $r = 0.03$)

One can easily notice that for the subcase $a > h$ with $a < 1$, there exist no rest-points with $x_0 > 0$ (the roots of (2.6), if real, are both negative). As well, the positivity of the corresponding value y_0 from (2.3) entails the limitation $a < 1$ and

$$y_0 > 0 \Leftrightarrow x_0 < (1-a)^{-1} \Leftrightarrow k < \frac{a(a-h)}{1-a}. \tag{2.7}$$

Hence for $r = d = 0$ the existence of rest-points outside the y-axis is not achievable for $a \geq 1$.

Regarding the case when $x_0 = y_0 = 0$ and $r \neq 0$, the Jacobian matrix of the field at $(0,0)$ has positive eigenvalues, and hence the origin is a repeller rest-point (see Fig. 2).

From the biological point of view, this may be based on the following real fact: in the human body cancerous cells always appear, but healthy organisms are able to keep this process under control. Hence, the fact that the origin is a repeller rest-point exhibits the fact that in real life, the total amount of cancerous cells is never zero.

We conclude that the adjustment of parameters to obtain cases when the attractor is as close to the origin as possible might be a possible goal in obtaining "favorable" SODE field lines. In these cases the model exhibits stabilization of cancerous cells.

3. The versal deformation. In this section, we study topological properties of the static bifurcation diagram associated to our mathematical model coming from biology. For this purpose, we analyze the equation $F(r, x, \tilde{p}) = 0$, where x represents the abscissas of the equilibrium points of the system of ordinary differential equations. Here, r is the static bifurcation parameter and $\tilde{p}$ is the vector of the auxiliary parameters. By varying the components of the vector $\tilde{p}$, the static bifurcation diagram may change from a qualitative point of view, but it is well known that these changes can only occur in neighborhoods of the singular points of F.

In order to find the singularities of F, we need to solve the equations

$$F = \frac{\partial F}{\partial x} = ... = \frac{\partial^k F}{\partial x^k} = 0$$

in terms of $\tilde{p}$ and find a certain value $\tilde{p}_0$ of the vector $\tilde{p}$. In [8], Golubitsky and Schaeffer proved that in the parameter space, in a neighborhood of $\tilde{p}_0$, to the function F corresponds a polynomial form G, called *the normal form* of F. This normal form has a well-defined local behavior of F; i.e., the bifurcation problem $F(r, x, \tilde{p}) = 0$ is qualitatively equivalent to $G(r, x) = 0$ in a neighborhood of $\tilde{p}_0$. Also, this normal form of F helps us to build the *versal deformation* of F, which characterizes the qualitative changes of the static bifurcation diagram. In this way, one can determine the quasi-global properties of a model using purely local methods. Roughly speaking, a versal deformation is a class of small perturbations of G with the minimum number of parameters possible.

Those bifurcation diagrams in the versal deformation of G which remain unchanged (in the qualitative sense of equivalence) if subjected to an additional small perturbation are called persistent. Golubitsky and Schaeffer showed that there exist three sources of nonpersistence, namely, double limit points, hysteresis and bifurcation ([8]). Obviously, in each case any perturbation changes the number of solutions x as a function of r. In Section 5, we shall present sets of parameters for which the bifurcation diagrams are no longer persistent.

In the following we study the static bifurcation for the equation (2.4) of the abscissas of the equilibrium point (x_0, y_0). For biological reasons, we choose as the parameter of the static bifurcation r, which represents the ratio between the cell death of the quiescent cells and the cell division of the proliferating cells. This is motivated by experimental research proving that the parameter r can be significantly influenced (e.g., by the treatment with nsPEFs [7]).

The stationary point of the investigated model (1.1) has the $x-$component provided by equation (2.4), which can be rewritten as

$$F(r, x, \tilde{p}) \equiv k(a + r)x^3 - (kr + ha - h)x^2 + (a + r - h)x - r = 0, \qquad (3.1)$$

where r represents *the static bifurcation parameter*, $\tilde{p} = (a, h, k)$ is the vector of the other parameters of the system, and x is the variable of the function F. The vector parameter $\tilde{p}$ has the dimension equal to 3, so the static bifurcation codimension (the number of the parameters needed in the versal deformation) is less than or equal to 3.

The degree of the singularity cannot exceed 5. This means that we can have at most four vanishing subsequent partial derivatives of F w.r.t. x ([8]). In our case, we get

$$\begin{cases} F_x = 3k(a + r)x^2 - 2(kr + ha - h)x + a + r - h = 0, \\ F_{xx} = 6k(a + r)x - 2(kr + ha - h) = 0, \\ F_{xxx} = 6k(a + r) > 0, \end{cases} \qquad (3.2)$$

whence $\varepsilon = \text{sign}(F_{xxx}) = 1$. This implies that the family of possible normal forms $G(r, x)$ associated to our static bifurcation problem is restricted to the following cases ([8], pp. 201):

a) $x^3 + \delta r$, with $\delta = \text{sign}(F_r)$;

b) $x^3 + \delta rx$, with $F_r = 0$ and $\delta = \text{sign}(F_{xr})$;

c) $x^3 + \delta r^2$, with $F_r = F_{xr} = 0$ and $\delta = \text{sign}(F_{xr})$.

In order to evaluate the singularity, besides $(3.2)_1$-$(3.2)_2$ we need two more equations. According to the general theory ([8]), we impose $F_r = 0$, which leads to $x = 1$. Then
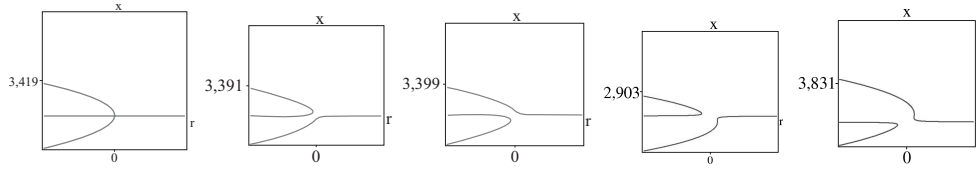
FIG. 3. The five standard admissible pitchforks of the model

from $(3.2)_2$ we get

$$kr + ha - h = 3k(a + r).$$

After computations, from (3.2) and $F_r = 0$, we get, as an intermediate step in obtaining the static bifurcation parameter $\tilde{p} = (a, h, k)$, the relations $x = 1$, $r = k(k+1)/(1 - 3k)$, and further

$$a = \frac{1 - k^2}{1 - 3k}, \quad h = k + 1, \quad k \in \left(0, \frac{1}{3}\right) \cup (1, \infty). \tag{3.3}$$

Then $x = 1$ implies $F_{xr} = k + 1 > 0$, whence $\delta = \mathrm{sign}\,(F_{rx}) = 1 \neq 0$.

Considering the set of obtained relations, the general algorithm for characterizing versal deformations ([8, Table 2.4, p. 201]; [4]) provides the normal form associated to (2.4), according to the thread:

$$F_{xx} \xrightarrow{=0} F_{xxx} \xrightarrow{\neq 0} F_r \xrightarrow{=0} F_{xr} \xrightarrow{\neq 0} \varepsilon x^3 + \delta rx,$$

where $\varepsilon = \mathrm{sign}\,(F_{xxx})$ and $\delta = \mathrm{sign}\,(F_{xr})$. Since in our case we have $F_r = 0$, $F_{rx} \neq 0$, $\delta = 1$ and $\varepsilon = 1$, the normal form associated to the static bifurcation problem is

$$G(r, x) = x^3 + rx. \tag{3.4}$$

This corresponds to the pitchfork of codimension 2, whose versal deformation is ([8]):

$$V(r, x, \alpha_1, \alpha_2) = x^3 + rx + \alpha_1 x^2 + \alpha_2,$$

where $\alpha_{1,2} \in \mathbb{R}$ are the deformation parameters. The qualitative types of bifurcation diagrams which can appear for this versal deformation were represented in Figures 3.1-3.5, by means of the software Maple 12. Figure 3.1 exhibits the case $\alpha_1 = \alpha_2 = 0$.

4. The transient set of the versal deformation. In the sequel, we are interested in the topologically nonequivalent changes of the static bifurcation diagram for G. In order to find them, we first define in the p-parameter space, four different sets, namely: D, *the double limit point set*; H, *the hysteresis set*; B, *the bifurcation set*, and Σ, *the transient set*, as follows:

$$\begin{aligned}
D = \quad & \{\tilde{p} = (a, h, k) \in \mathbb{R}_+^{*3} \mid (\exists)(r, x_1, x_2) \in \mathbb{R}_+^{*3}, \\
& x_1 \neq x_2 \text{ such that } F = F_x = 0 \\
& \text{at } (r, x_i, a, h, k), i = 1, 2\}, \\
H = \quad & \{\tilde{p} = (a, h, k) \in \mathbb{R}_+^{*3} \mid (\exists)(r, x) \in \mathbb{R}_+^{*2}, \\
& \text{such that } F = F_x = F_{xx} = 0 \text{ at } (r, x, a, h, k)\}, \\
B = \quad & \{\tilde{p} = (a, h, k) \in \mathbb{R}_+^{*3} \mid (\exists)(r, x) \in \mathbb{R}_+^{*2}, \\
& \text{such that } F = F_x = F_r = 0 \text{ at } (r, x, a, h, k)\}, \\
\Sigma = \quad & D \cup H \cup B.
\end{aligned}$$

In singularity theory, it is proved that in each region limited by the transient set Σ, the perturbations are persistent [8]. All the topologically nonequivalent changes occur only when crossing Σ from one region to another. For this reason, we construct below the transition set for $F(r, x, a, h, k) = 0$ defined by equation (3.1). We shall further subject the values of the parameters (a, h, k) to the biological admissible bounds (i.e., $(a, h, k) \in \mathbb{R}_+^{*\,3}$), eliminating thus irrelevant solutions, while determining the subsets D, H, B of the transient set.

4.1. *The double limit point set D.* To determine the double limit point set, we use the following result.

LEMMA ([8]). If $h(x)$ is a polynomial of degree 3 or less, such that $h = h_x = 0$ at two distinct points x_1 and x_2, then $h \equiv 0$.

As a consequence, since F is a nontrivial third-order polynomial in x, we infer $D = \emptyset$.

4.2. *The hysteresis set H.* In order to find the set H defined above, we have to solve the system $F = F_x = F_{xx} = 0$. We have already seen that $F_{xx} = 0$ leads to the triple equilibrium

$$x_0 = \frac{kr + ha - h}{3k(a + r)},$$

provided that

$$kr + ha - h > 0. \tag{4.1}$$

Replacing $x = x_0$ in equations $(3.2)_1$ and $(3.2)_2$, the system can be rewritten as

$$\begin{cases} \dfrac{(kr + ha - h)^2}{3k(a + r)} = a + r - h, \\ \dfrac{(kr + ha - h)^3}{27k^2(a + r)^2} = r, \end{cases} \tag{4.2}$$

entailing the condition

$$a + r - h > 0. \tag{4.3}$$

Further, dividing $(4.2)_1$ by $(4.2)_2$, one gets

$$8kr^2 + [(-a + 1 + k)h + 8ak]r + h(h - a)(a - 1) = 0, \tag{4.4}$$

and, using both $(4.2)_1$ and (4.4), we infer

$$\begin{aligned} r = [(8h^2 - 3h + hk - 24k)a^2 + (-13h^2 + 3h + 23hk - h^2k)a \\ + 5h^2 + h^2k]/[(3h + 24k + 8k^2 - 17hk)a - 10hk - 3h + k^2h], \end{aligned} \tag{4.5}$$

assuming a nonvanishing denominator. Then, plugging r into (4.4), one gets the necessary condition for $(a, h, k) \in H$:

$$\begin{aligned} (ak - ha + h)\{[-3k(9k + 5)h^2 + (27k - 1)h^3 - 48k^2h + 64k^3]a^3 \\ + [3(-19k + 1)h^3 - 48k^2(k - 1)h + 6k(13k + 5)h^2]a^2 \\ + [3(-1 + 11k + 8k^2)h^3 - 3k(5k^2 + 26k + 5)h^2]a - (k - 1)^3h^3\} = 0. \end{aligned}$$

In particular, for $k = h(a - 1)/a$, one gets from (4.5) that $r = -a \not> 0$. Hence, assuming that the conditions (4.3), (4.1) hold true, the denominator of (4.5) is nonzero and the corresponding ratio is positive, it follows that only the second factor from the equation above might lead to solutions $(a, h, k) \in H$.
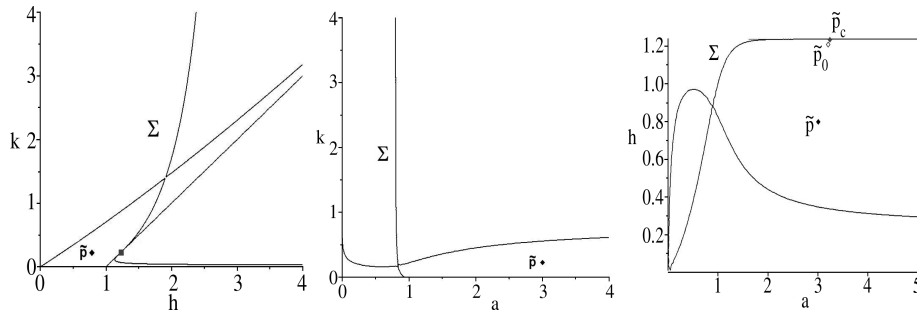
FIG. 4. The triple $\tilde{p} = (3; 0.8; 0.236)$ vs. the sections of the transition set Σ with the planes $\{a = 3\}$, $\{h = 0.8\}$ and $\{k = 0.236\}$, respectively

4.3. *The bifurcation set B.* The bifurcation set B is provided by the system: $F = 0$, $F_x = 0$, $F_r = 0$. The last equation has only the real solution $x_0 = 1$, which, when replaced in the first two equations, leads to

$$\begin{cases} a(k - h + 1) = 0, \\ r(k + 1) + 3ka - 2ha + h + a = 0. \end{cases} \tag{4.6}$$

The second equation of (4.6) admits real positive solutions for the bifurcation parameter r if only the following condition holds:

$$-3ka + 2ha - h - a > 0.$$

Using the first equation, it follows that $h = k + 1$ (since $a = 0$ leads to a contradiction). Then, for $k \in (0, 1)$ (i.e., within the only acceptable range), we infer $a > (1+k)(1-k)^{-1}$. It follows that the bifurcation set is

$$B = \left\{ (a, h, k) = (a, k + 1, k) \; \middle| \; a > \frac{1 + k}{1 - k}, \; k \in (0, 1) \right\}.$$

Assuming that the polynomial (3.1) maintains a constant degree, the general framework ([8], p. 207, fig. 6) provides a splitting given by $H \cup B$ of the parameter space $\mathbb{R}_+^{*\,3}$ into a basis in which the bifurcation diagrams are persistent.

There exist special cases (e.g., for $a = 3$, $h = 0.8$, $k = 0.236$) in which we note the nonconstancy of the degree, which drops for $k(a + r) = 0$. Then the line $\Delta : \; r = -a$ (emerging asymptotic line) splits the bifurcation diagram into two parts, deforming a branch on each of the two pitchforks located sidewise relative to Δ (see Figures 3.5-3.6). The absolute similarity between the existent and standard pitchforks ceases in this case, due to the fact that the main tool, the Taylor series equivalence, does not globally hold ([8], page ix).

As well, we observe a relatively large distance between the parameter triples $\tilde{p}$ and the transient set Σ (see the corresponding $a, h, k-$sections in Figures 4.1-4.3), and, moreover, we note a considerable distance (see D in Table 5.1) from $\tilde{p}$ towards the curve (5.1), which is in accordance with the considerable deviation from the standard pitchforks.

We note that for $\tilde{p} = (3; 0.8; 0.236)$ (Figure 5.5, case 5 in Table 5.1), the bifurcation diagram is topologically equivalent to the one of $\tilde{p}_o = (3.2; 1.21; 0.236)$, since $\tilde{p}$ and $\tilde{p}_o$

belong to the same region determined by the transient set Σ. On the other hand, $\tilde{p}_o$ is located close to $\tilde{p}_c = (3.233918; 1.236; 0.236)$, which belongs to (5.1) (see Figure 4.3), and hence its diagram is equivalent to the one depicted in Figure 3.2.

5. Bifurcation diagrams. From the relations (3.3) we can conclude that in a neighborhood of any vector

$$\tilde{p}_\alpha = (a_\alpha, h_\alpha, k_\alpha) = \left(\frac{1 - \alpha^2}{1 - 3\alpha}, 1 + \alpha, \alpha \right), \tag{5.1}$$

where $\alpha \in (0, \frac{1}{3}) \cup (1, +\infty)$, the function F admits the associated normal form G in (3.4).

The versal deformation of G plays an important role in understanding the behavior of the solution set of (3.1) for a, h and k close to $a_\alpha, h_\alpha, k_\alpha$. Namely, the bifurcation diagrams associated to a vector $\tilde{p}$ located in a "small" neighborhood of $\tilde{p}_\alpha$, are, from a qualitative point of view, equivalent with the standard admissible pitchforks from Figures 5.1-5.6 ([8]).

These diagrams correspond to the following sets of parameters:

TABLE 5.1. Sets of admissible parameters for the evolution system

#	a	h	k	r	T	B	D
1	3.234	1.236	0.236	.4	1	attract.	0
2	3.3	1.228	0.236	.0001	2	asympt.	0.01025
3	3.236	1.236	0.230	.0001	3	asympt.	0.006
4	2	1.246	0.256	.0001	4	asympt.	0.11174
5	3	0.8	0.236	3	N	attract.	0.4277887
6	0.6	1.228	0.236	0.075	N	attract.	0.4797606

where we have denoted:
- T = bifurcation type $(1, \ldots, 5)$,
- N = nonpersistent bifurcation,
- B = behavior w.r.t. the stationary point,
- *attract.* = existing attractor,
- *asympt.* = asymptotic behavior,
- D = distance between the triple (a, h, k) and the persistency curve (5.1),

where in case 1, we have chosen $\alpha_* = 0.236$, which leads to the auxiliary parameters vector $\tilde{p}_{\alpha_*} = (a_*, h_*, k_*) = (3.234, 1.236, 0.236)$.

The bifurcation diagrams which appear in the studied model for different positive values of the parameters a, h and k from Table 5.1 were provided below (the graphs 5.1-5.6) as Maple 12 plots.

REMARK 5.1. Although the employed methods related to the properties of the diagrams are local, it has been proven that the derived conclusions are globally valid ([1]).
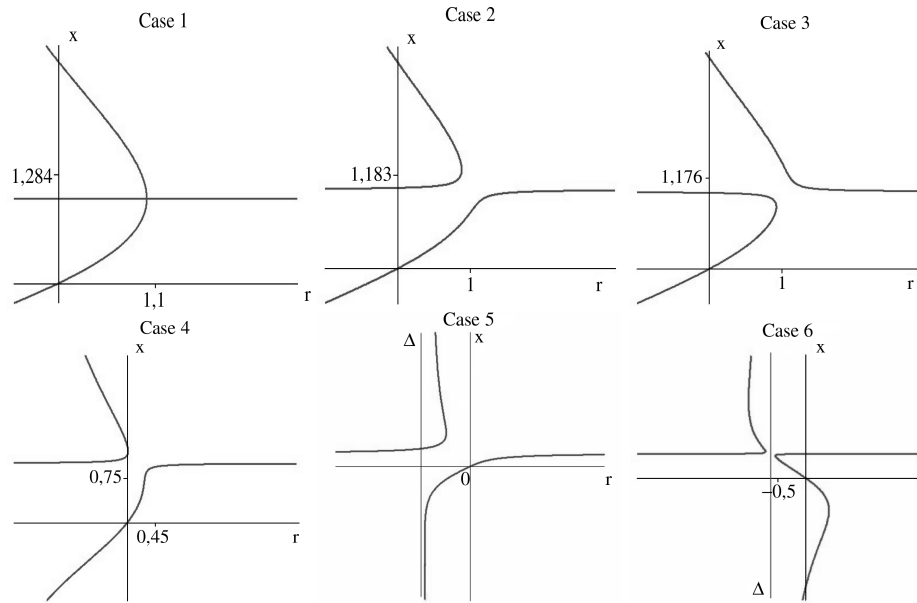
FIG. 5. The unperturbed pitchfork for the cancer cell population
model and the associated bifurcation diagrams

REMARK 5.2. From the biological point of view, the positive values of the parameters
are of particular interest. In particular, for a_*, h_* and k_* attended on the curve (5.1), one
gets the unperturbed pitchfork (Fig. 5.1), identical to the standard pitchfork from Fig.
3.1. In the latter images we have considered a, h, k in a neighborhood of a_*, h_* and k_*.
The first four bifurcation diagrams (Figs. 5.1-5.4) are very much similar to the standard
ones from a qualitative point of view, while the last two diagrams have the branches of
the fork asymptotically deformed due to the singularity at $a = -r$.

6. Attractors and repellers. For certain values of the parameters, the system ad-
mits stationary points. For instance, in case 1 (see Table 5.1) the evolution is a mixture
of asymptotic and limit-point behavior, while in case 6 the system has the admissible
stationary point $(x_0, y_0) = (.9944137450, 1.282883814)$, as plotted in Fig. 6.

This results in a stabilization of both the number of proliferating and resting cells, the
initial parameters corresponding to a cure strategy, which leads to a stabilization of the
malignant process.

The last cases 2-4 exhibit an asymptotic behavior, resulting in an explosive increase of
the resting cells and stabilization of the proliferating ones, corresponding to an evolution
which, though apparently stops the proliferation, dramatically increases the quiescent
cells, which always represent a potential danger of a malignant process burst. The
evolution of proliferating/resting cells has a similar pattern, as the one illustrated in Fig.
7.

The considered cases show that, for different sets of given system parameters, the
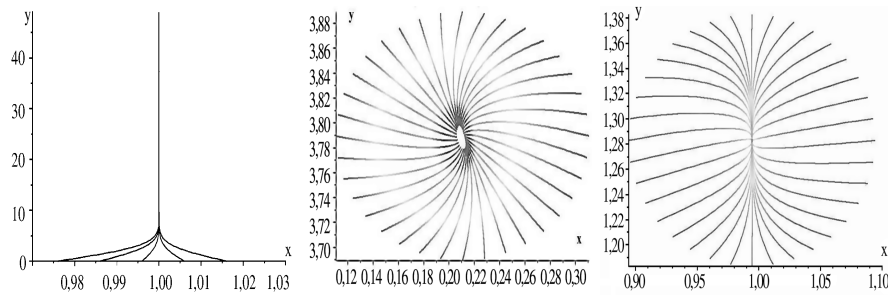evolution of the cancer-specific populations of cells dramatically changes. This supports

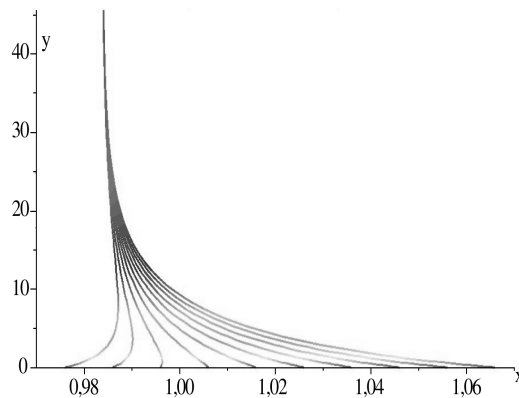Fig. 6. Admissible attractor (cases 1, 5 and 6)

Fig. 7. Asymptotic behavior of the field lines (case 2)

the idea that proper alteration of the parameters (induced, e.g., by nsPEFs) might lead to a favorable prognosis.

7. Biological interpretations. It is well known that, from a cancer-treatment perspective, the ability of predicting and minimizing the proliferating cell population is vital.

Based on experimental studies, it has been observed that one way of achieving this goal is to increase the growth rate of the resting cells compared to that of proliferating cells ([7, 14, 15]).

In this case, the quiescent cells will take more resources from the proliferating cells, causing their number to drop. Hence the treatments which are able to lower r by reducing the death rate of resting cells (and/or to increase a, the relative advantage of resting vs. proliferating cells in terms of nutrient consumption), ultimately lead to a significant drop of proliferating cells, reducing tumor growth and cancer spread.

We note that, though a small number of proliferating cells still exist, the rate Q of transition from a quiescent to a proliferating state is low (negligible, cf. Solyanik [12]), and therefore subsequent treatment methods might lead to the complete annihilation of cancerous cells.

It is known that for certain tubular neighborhoods of the curve (5.1) located in the parameter space (a, h, k), the persistent bifurcation diagrams are, from a qualitative point of view, equivalent with the standard admissible pitchforks. As shown in Section 2, the equation (3.1) which provides the abscissa of the stationary point always admits a positive solution x_0. This solution decreases to zero (while the parameters are located near the curve (5.1)) or approaches a positive constant, when r decreases to zero. Because x is the state variable which represents the number of the proliferating cells, we are interested in those x_0 as close to 0 as possible. Moreover, from a cancer treatment perspective, of particular interest are those stationary points which, besides the before-mentioned circumstances, satisfy the condition to be an attractor for the trajectories of the differential system which describes the cancer cell population. This leads to a stabilization of the amount of proliferating cells.

In conclusion, being able to minimize the ratio r/a is only part of the treatment strategy. Another part consists in the necessity to attract the trajectories towards the rest-point which is closest to the origin.

A related question might target supplementary conditions satisfied by the parameters allowing attractor-type stationary points which are located in the first quadrant and which are closest to the origin.

Another way to achieve the goal of dropping the amount of proliferating cells relies on the nsPEF treatment. This yields an increase of the length of the mitotic cycle and to an increased rate of resting cell death. Though this naturally leads to an increase of the parameter r, it also forces more cells to remain in the pre-mitotic division stage ([13]) and increases the parameter a. Then, according to Wallen's data ([14, 15]), in the case that r is significantly smaller than a, then the proliferating cell population can rapidly drop to its complete extinction. In this respect, the bifurcation diagrams 5.1-5.6 exhibit a drop of the x-component (representing to the amount of proliferating cells) of the equilibrium point (x, y), which in the attractor type of behavior, for low asymptotic values of x corresponds to an efficient therapy scenario.

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