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ACQUIRED RESISTANCE IN TUMOR CELL POPULATION. SIMULATION AND PARAMETER ESTIMATION

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Acquired resistance to therapeutic treatments in a tumor cell population is considered with respect to one and two agents. A simulation model serves as a mathematical tool to the analysis of the effects of such a phenomenon in treatment planning and in curve fitting to available experimental data. Specialized cell compartments are considered, in order to divide the entire cell population, according to their reaction capabilities to therapeutic agents. Cell population data are computed and scaled on a direct proportionality basis to be compared with observed tumor volumes and measurement errors are taken into account in the model.

INTRODUCTION

Mathematical modelling in tumor growth and response to treatment may be examined under the point of view of tumor cell population and its division into specialized compartments. Such a model, with a minimum compartment configuration, is studied in [4], while other formulations and a general excursion on compartmental models is provided in [1]. [2] and [3] present other type of models dealing with similar problems, such as the random evolution of a tumor cell population resistant to therapeutic agents.

Problems of effectiveness of the theoretical scheme and of its adequacy to describe the actual clinical situations are ones of hardly satisfactory solutions: the attempt to examine biological mechanisms rather more closely than in most of the existing literature always implies the formulation of models of very disperse quality. On the other hand, the application of more studied modelling schemes with no other justification than their "age" or the nice setting of the solution very often turns up

to be misleading at such crucial points as therapeutic–agent–induced resistance or elimination of inactivated cells. The main problem arises when considering the existing relationship between tumor volume and tumor cell population: in vivo experiments only provide volume data, but reactions to treatment should be mainly conceived at microscopic level. Direct proportionality between volumes and the number of cells is then assumed and such assumption is a very limiting one; it is, nevertheless, the only valid alternative to the lack of reliable data on the argument.

In this paper a simulation model is presented to describe the modifications in a tumor cell population $X(t)$ induced by therapeutic treatments: two agents (A and B) are considered and cell population is divided into 5 compartments, according to its sensitivity to treatments. One–agent, two–agent and combined resistance acquisition is examined and inactivated cells are supposed to exit the system with a random delay ϑ. Untreated tumor cell population is supposed to grow according to the Gompertz distribution. As described in [5] and [6], this function well summarizes the various physical and biological effects (not examined in details in the present paper), which cause the reduction in tumor cell growth rate as the number of cells in the population increases.

Some of the hypotheses given in the next paragraph are very strong, while the weakest ones might drop when more experimental information will be available; nevertheless, all of them are introduced on strict biological basis. Measurement errors are also considered in the simulation program and a maximum likelihood estimation procedure is then established to verify the goodness of the fit of the simulation curves to experimental data.

1. BIOLOGICAL BASES OF THE MODE

The 5–compartment deterministic model here presented is a theoretical description of a 2–therapeutic agent–treatment scheme (agents A and B), where an initial average tumor cell population of $N(0)$ units grows and moves among compartments R_1, R_2, R_3, R_4 and R_5 according to the following hypotheses (see Fig.1):

i) if $N_i(t)$ is the average cell population of compartment R_i at time t, then

$$N(t) = \sum_{i=1}^{5} N_i(t) \qquad \text{for any } t$$

is the average total cell population at time t;

ii) At any time t, at which no treatment occurs, Gompertz growth law is assumed for the average total system population of active cells, summing up to

$N_a(t) = \sum_{i=1}^{4} N_i(t)$, i.e. the number of cells in compartment 1 through 4:

$$N_a(t) = N(0)\exp\left\{\frac{\beta}{\alpha}\left(1-e^{\alpha t}\right)\right\} = N(0)f(t) \tag{1}$$

iii) a matrix of cell transition rates p_{ij} from compartment *i* to compartment *j*, following a treatment, is assumed

$$\mathbf{P} = \begin{pmatrix} p_{11} & p_{12} & p_{13} & 0 & p_{15} \\ 0 & p_{22} & 0 & p_{24} & p_{25} \\ 0 & 0 & p_{33} & p_{34} & p_{35} \\ 0 & 0 & 0 & p_{44} & 0 \\ 0 & 0 & 0 & 0 & p_{55} \end{pmatrix} \tag{2}$$

where $\Sigma_j p_{ij} = 1$ for $i = 1...5$;

iv) constant doses of agents are administrated;

v) within each compartment, constant treatment effects on cell metabolism are supposed to occur instantly;

vi) no interaction is assumed between agents *A* and *B*, in case two different treatments should occur at the same time;

vii) cells reaching compartment R_5 at time *t* leave the system with a time delay due to a non immediate death and consequent elimination by lysis phenomena. Damaged cells are inactivated at time *t* and eliminated from the system at time $t + \vartheta$, where ϑ is an $N(\mu; \sigma)$ distributed random variable.

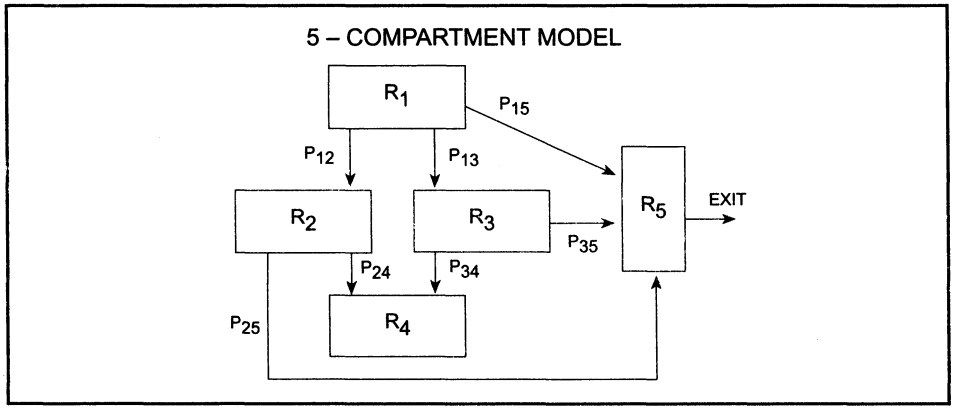

Fig. 1: Schematic representation of the 5–compartment model: R_1 is the compartment containing sensitive cells; R_2 and R_3 contain cells resistant to one agent only (A and B respectively); R_4 contains cells resistant to both agents and R_5 inactivated cells. Arrows and $p_{..}$ indicate possible transition of cells between compartments, following a treatment.

Observations

When a treatment occurs it can be deduced, from (2), that cells in compartments R_4 and R_5 will no longer alter their status and have, therefore, either acquired complete resistence to the therapeutic agent or been inactivated. On the other hand, some of the cells in compartments R_1, R_2 and R_3 will eventually acquire complete resistance "step by step", passing through resistence to agent *A* (compartment R_2) or to agent *B* (compartment R_3) and then adding further resistence to the other agent (compartment R_4). Remaining cell population will either exit the system or survive keeping their qualities unchanged.

2. MATHEMATICAL MODEL

Consider *n* completely ordered time units of observation $t_1 < \ldots < t_n$ at which treatment *F* (= F_A or F_B) occurs, then, for each compartment, average cell population at any *t* after *n*th treatment $N_i^n(t) = N_i(t_n + t)$ can be calculated.

Let us consider the following definitions:

$$g(t) = \frac{d}{dt} f(t) = \frac{d}{dt} \exp\left\{\frac{\alpha}{\beta}\left(1 - e^{-\alpha t}\right)\right\} \tag{3}$$

is the Gompertz rate of growth of cell population between two subsequent treatments;

$$p(\vartheta) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left\{-\frac{(\vartheta - \mu)^2}{2\sigma^2}\right\} \tag{4}$$

is the distribution of the random time ϑ between an arrival in compartment R_5 and exit from the system (time between cell inactivation and death);

$$\chi^j(F - F_k) = \begin{cases} 1 \text{ if } F = F_k \\ 0 \text{ o/w} \end{cases} \quad j = 1 \ldots n;\ k = A, B \tag{5}$$

is the indicator function of treatment with therapeutic agent *k;*

$$\delta_i(j) = \begin{cases} 1 \text{ if } N_i^{j-1}\left(t_j^-\right) = 0 \\ 0 \text{ o/w} \end{cases} \tag{6}$$

where

$$N_i^{j-1}\left(t_j^-\right) = \lim_{t \uparrow (t_j - t_{j-1})} N_i^{j-1}(t)$$

is the indicator function of a zero–population in compartment R_i before *j*th treatment;

t_j^*, solution to equation

$$N(0)f\left(t_j^*\right) = \sum_{i=1}^{5} N_i^j\left(t_j^+\right) \tag{7}$$

where

$$N_i^j\left(t_j^+\right) = \lim_{t\downarrow 0} N_i^j(t)$$

is the time which controls the rescheduling of the rate of growth after each treatment; in fact we suppose that, if $X(s) = X(r)$ at some time instants s and r, $X(.)$ being the total cell population, we must have

$$\frac{d}{dt}f(t)\bigg|_{t=s} = \frac{d}{dt}f(t)\bigg|_{t=r}$$

i.e. equal growth rates must correspond to equal cell populations.

Solution to (7) is explicitly provided by $t_j^* = -\frac{1}{\alpha}\ln\left[1-\frac{\alpha}{\beta}\ln\left(\frac{\sum_i N_i^j\left(t^+\right)}{N(0)}\right)\right]$. In the following limit cases we have:

i) extinction of the cell population:

$$N(0)f\left(t_j^*\right) = \sum_{i=1}^{5} N_i^j\left(t_j^+\right) = 0 \Rightarrow t_j^* = -\infty$$

ii) asymptotic maximum growth of cell population

$$N(0)f\left(t_j^*\right) = \sum_{i=1}^{5} N_i^j\left(t_j^+\right) = N(0)\exp\left\{\frac{\beta}{\alpha}\right\} \Rightarrow t_j^* = +\infty$$

In either cases, because of the asymptotic trend of the Gompertz function (see [5]), it means that the growth rate vanishes: in fact

$$\frac{d}{dt}f(t)\bigg|_{t=+\infty} = \beta\ \exp\left\{\frac{\beta}{\alpha}\left(1-e^{-\alpha t}\right)-\alpha t\right\}\bigg|_{t=+\infty} = 0$$

It should be noted that a negative value for t_j^* in case i) follows from the fact that the 0 value of the time variable is placed at the beginning of the hypothetical observation of the tumor cell population, which, in fact, starts when the total population amounts to $N(0)$. Any reduction in cell population, which brings its cardinality to less than $N(0)$ must then refer to the theoretical curve asymptotically originating at $-\infty$ with a 0 population.

Now, using (2)–(7), as defined above, we have the following average cell population for each compartment after the *n*th treatment:

$$
\begin{aligned}
N_1^n(t) = \; & \prod_{j=1}^{n}\left[(1-p_{12}-p_{15})\chi^j(F-F_A)+(1-p_{13}-p_{15})\chi^j(F-F_B)\right]x \\
& x\; N_1^0\left(t_1^-\right)+\sum_{k=0}^{n-2}\left(f\left(t_{k+1}^*-t_{k+2}-t_{k+1}\right)-f\left(t_{k+1}^*\right)\right)x \\
& x\; \prod_{j=k+1}^{n}\left[(1-p_{12}-p_{15})\chi^j(F-F_A)+(1-p_{13}-p_{15})x\right. \\
& x\; \left.\chi^j(F-F_B)\right]+\left(f\left(t_n^*+t\right)-f\left(t_n^*\right)\right)x \\
& x\; \left[(1-p_{12}-p_{15})\chi^n(F-F_A)+(1-p_{13}-p_{15})\chi^n(F-F_B)\right]
\end{aligned}
\tag{8}
$$

$$
\begin{aligned}
N_2^n(t) = \; & p_{12}N_1^{n-1}\left(t_n^-\right)\chi^n(F-F_A)+ \\
& +\prod_{j=1}^{n}\left[\chi^j(F-F_A)+(1-p_{24}-p_{25})\chi^j(F-F_B)\right]N_2^0\left(t_1^-\right)+ \\
& +\sum_{k=0}^{n-2}\left[\left(p_{12}N_1^k\left(t_{k+1}^-\right)\chi^{k+1}(F-F_A)+\left(p_{12}\chi^{k+1}(F-F_A)\right)+\right.\right. \\
& \left.+\delta_2(k+1)(1-p_{24}-p_{25})\chi^{k+1}(F-F_B)\right)x \\
& x\left(f\left(t_{k+1}^*-t_{k+2}-t_{k+1}\right)-f\left(t_{k+1}^*\right)\right)x \\
& x\prod_{j=k+2}^{n}\left(\chi^j(F-F_A)+(1-p_{24}-p_{25})\chi^j(F-F_B)\right)\Big]+ \\
& +\left[p_{12}\chi^n(F-F_A)+\delta_2(n)(1-p_{24}-p_{25})\chi^n(F-F_B)\right]x \\
& x\left(f\left(t_n^*+t\right)-f\left(t_n^*\right)\right)
\end{aligned}
\tag{9}
$$

$$
\begin{aligned}
N_3^n &= p_{13}N_1^{n-1}\left(t_n^-\right)\chi^n\left(F-F_B\right)+ \\
&+\prod_{j=1}^{n}\left[\chi^j\left(F-F_B\right)+\left(1-p_{34}-p_{35}\right)\chi^j\left(F-F_A\right)\right]N_3^0\left(t_1^-\right)+ \\
&+\sum_{k=0}^{n-2}\left[\left(p_{13}N_1^k\left(t_{k+1}^-\right)\chi^{k+1}\left(F-F_B\right)+\left(p_{13}\chi^{k+1}\left(F-F_B\right)+\right.\right.\right. \\
&+\delta_3(k+1)\left(1-p_{34}-p_{35}\right)\chi^{k+1}\left(F-F_A\right)\Big)x \\
&x\left(f\left(t_{k+1}^*-t_{k+2}-t_{k+1}\right)-f\left(t_{k+1}^*\right)\right)x \\
&x\prod_{j=k+2}^{n}\left(\chi^j\left(F-F_B\right)+\left(1-p_{34}-p_{35}\right)\chi^j\left(F-F_A\right)\right)\Big]+ \\
&\left[p_{13}\chi^n\left(F-F_B\right)+\delta_3(n)\left(1-p_{34}-p_{35}\right)\chi^n\left(F-F_A\right)\right]x \\
&x\left(f\left(t_n^*+t\right)-f\left(t_n^*\right)\right)
\end{aligned}
\tag{10}
$$

$$
\begin{aligned}
N_4^n(t) &= N_4^0\left(t_1^-\right)+\sum_{k=1}^{n}p_{24}N_2^{k-1}\left(t_k^-\right)\chi^k\left(F-F_B\right)+ \\
&+\sum_{k=1}^{n}p_{34}N_3^{k-1}\left(t_k^-\right)\chi^k\left(F-F_A\right)+ \\
&+\sum_{k=0}^{n-2}\left[p_{24}\delta_2(k+1)\chi^{k+1}\left(F-F_B\right)+p_{34}\delta_3(k+1)\chi^{k+1}\left(F-F_A\right)\right]x \\
&x\left(f\left(t_{k+1}^*-t_{k+2}-t_{k+1}\right)-f\left(t_{k+1}^*\right)\right)+ \\
&+\left[p_{24}\delta_2(n)\chi^n\left(F-F_B\right)+p_{34}\delta_3(n)\chi^n\left(F-F_A\right)\right]x \\
&x\left(t\left(t_n^*+t\right)-f\left(t_n^*\right)\right)
\end{aligned}
\tag{11}
$$

$$
\begin{aligned}
N_5^n(t) \quad &= N_5^0\left(t_1^-\right)\int_0^{t_n+t-t_1} p(\vartheta)d\vartheta + \\
&+p_{15}\left[N_1^{n-1}\left(t_n^-\right) - \sum_{k=1}^{n} N_1^{k-1}\left(t_k^-\right)\int_0^{t_n+t-t_k} p(\vartheta)d\vartheta\right] + \\
&+p_{25}\left[N_2^{n-1}\left(t_n^-\right)\chi^n(F-F_B) - \right. \\
&\left. -\sum_{k=1}^{n} N_2^{k-1}\left(t_k^-\right)\int_0^{t_n+t-t_k} p(\vartheta)d\vartheta\chi^k(F-F_B)\right] + \\
&+p_{35}\left[N_3^{n-1}\left(t_n^-\right)\chi^n(F-F_A) - \right. \\
&\left. -\sum_{k=1}^{n} N_3^{k-1}\left(t_k^-\right)\int_0^{t_n+t-t_k} p(\vartheta)d\vartheta\chi^k(F-F_A)\right]
\end{aligned}
\tag{12}
$$

In order to take measurement errors into account, total cell population *X(t)* at time *t* is thus sampled from a normal distribution with $N(t) = \Sigma_i N_i^n(t)$ mean and variance proportional to the square of the mean by some $\gamma(t)$ proportionality factor.

3. PARAMETER ESTIMATION

Estimation problems involve three different aspects:

i) estimation of parameters α and β, related to tumor free growth

ii) estimation of the mean–variance proportionality factor $\gamma(t)$ for the distribution of *X(t)*

iii) estimations of the cell transition rate matrix **P** and of the parameters μ and σ of the distribution of the random time ϑ.

ML estimates of parameters α and β of Gompertz function and of $\gamma(t)$ were evaluated using data $y_1 \ldots y_m$, $z_1(s) \ldots z_p(s)$ $s \in S$ from two different types of experiments: in the first one, values were provided from tumor volume measurements (considered proportional to its cell population in the model) and, in the second one, some cell lines were treated with radiations and a Ionidamine diet and their population was counted for a limited time period *S*. Using such estimates and a sample $\{\mathbf{x}(t); t \in T\}$ from the simulation computer program, log–likelihood function

is constructed:

$$L(\mathbf{P};\mu;\sigma|\mathbf{x}(t);t\in T)=-\sum_{t\in T}\left[\ln N(t)+\frac{\ln\gamma(t)}{2}+\frac{(x(t)-N(t))^2}{2(N(t))^2\gamma(t)}\right] \tag{13}$$

whose maximum provides values to transition rate matrix **P** and to parameters μ and σ of the distribution of ϑ.

Observations

Gompertz parameter estimations were computed from the sample $y_1...y_m$, using a standard Least Squares estimation computer program.

U.M.V.U. estimator for $\gamma(s)$:

$$\hat{\gamma}(s)=\sum_{i=1}^{p}\frac{(z_i(s)-N(s))^2}{pN^2(s)} \tag{14}$$

was then constructed, where $z_1(s)...z_p(s)$ $s \in S$ is the sample provided by the examined cell lines; the time series $\{\hat{\gamma}(s);s\in S\}$ was then interpolated to obtain $\{\gamma\}(t);\ t \in T\}$, used in (13).

Because of the large number of parameters involved in (13) (9 parameters, remembering that $\Sigma_j p_{ij}=1$ for i=1...5), its maximization was computed in two steps, using IMSL subroutines:

a) one–agent treatments were considered, providing estimates to p_{12},p_{13},p_{15},μ and σ;
b) two–agent treatments, using results from a), provide estimates to the remaining parameters p_{24},p_{25},p_{34},p_{35}.

This course of action is allowed by hypothesis vi) of 1., where no interaction between coincident treatments with two agents is stated.

4. RESULTS AND DISCUSSION

The theoretical model was tested using a computer program, written in FORTRAN and running on an MS–DOS personal computer. Two types of results are here analyzed: data fitting via the simulation program, when in vivo or in vitro experimental data are available, and the simulation of the effects of some hypothetical treatment, when its effects at microscopic level are known.

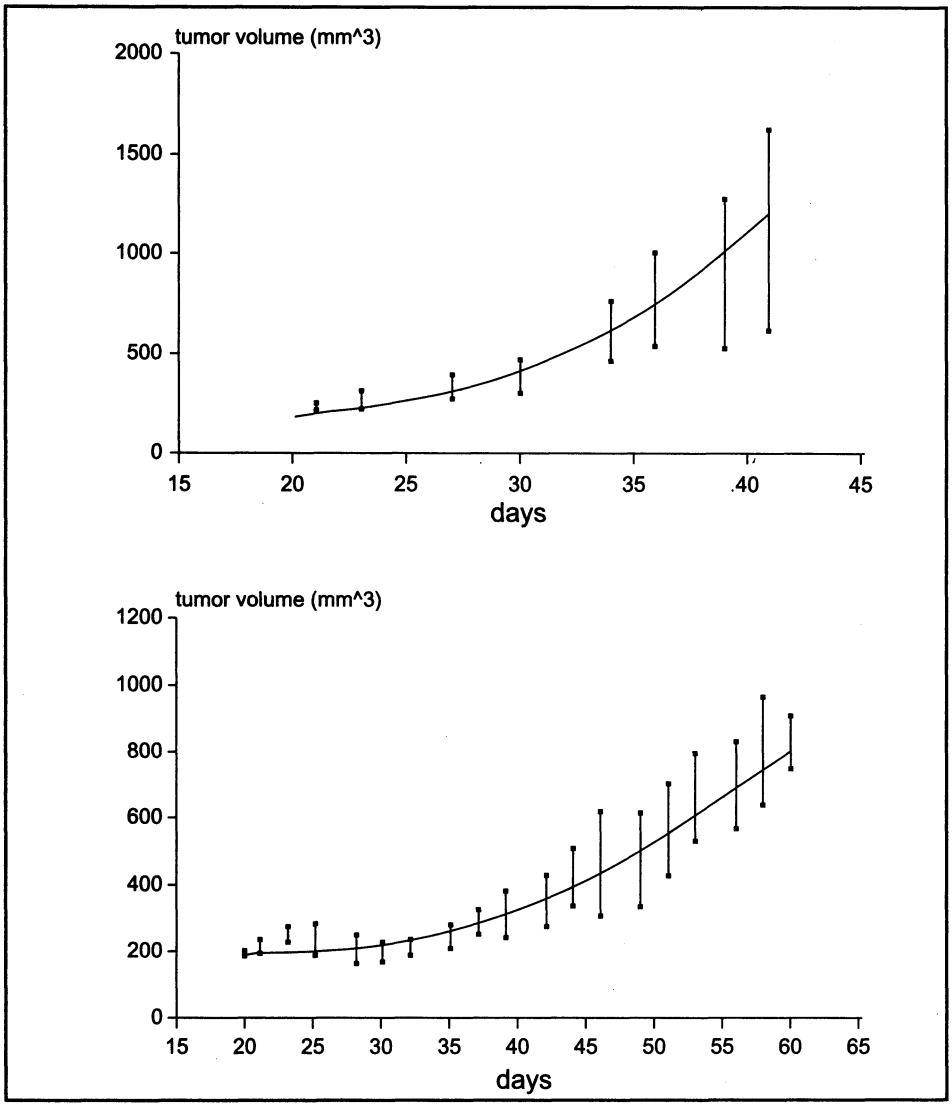

Fig. 2: Model fitting curve to experimental data. Vertical lines mark the range of data variability within a distance of one standard deviation from the mean value and the bold line is the model simulation of treatment effects.
A – one–agent treatment. B – two–agent treatment.

Fitting experimental data

In the first experiment (fig. 2–A) volume data refer to a one–agent treatment: a group of mice was treated with a lonidamine diet (lonidamine administered daily) starting on the 20th day after tumor inoculation (tumor volumes were not observable earlier).

In fig. 2–B a combination of two agents is considered: a lonidamine diet following the scheme as in the previous experiment, while radiation treatment occurring once only, on the 20^{th} day after tumor inoculation.

In both cases a direct proportionality is assumed between the number of cells and observable volumes; simulated data provided parameter ML estimates, as explained in 3. of the previous section.

The two graphs represent the model fitting curves to experimental data in the two possible cases considered by the theoretical model; the ML estimates of transition rates and inactivated cell elimination parameters, using function (13) with experimental data, and their expected values are here presented in table 1. Estimated values do not seem to differ significantly from the expected ones, although some discrepancy may be expected in the two–agent case, because of the possible interaction existing between the two types of agents used in the therapy. Data on the effects of interactions are, however, very misleading to analyze: statistical procedures only provide quantitative measurements of phenomena, without direct explenation on their origins and, therefore, effects that sometimes appear as ascribable to interaction often turn out to be a mere drop of some basic hypothesis on biological mechanisms controlling tumor growth and resistance acquisition. In view of such problems and of the general validity of the present model, any hypotheses on interaction between therapeutic agents was left aside.

Tab. I: Expected values and ML estimates for simulation model parameter (N(0)=150,000).

ONE AGENT		
Parameter	Expected values	ML Estimates
p_{11}	0.0090	0.0083890
p_{12}	0.0010	0.0009032
p_{15}	0.9900	0.9907000
μ	3.0000	3.0000000
σ	2.0000	2.0000000
TWO AGENTS		
Parameters	Expected values	ML estimates
p_{22}	0.090	0.07797
p_{24}	0.010	0.01453
p_{25}	0.900	0.90700
p_{33}	0.010	0.01592
p_{34}	0.090	0.08962
p_{35}	0.900	0.89400

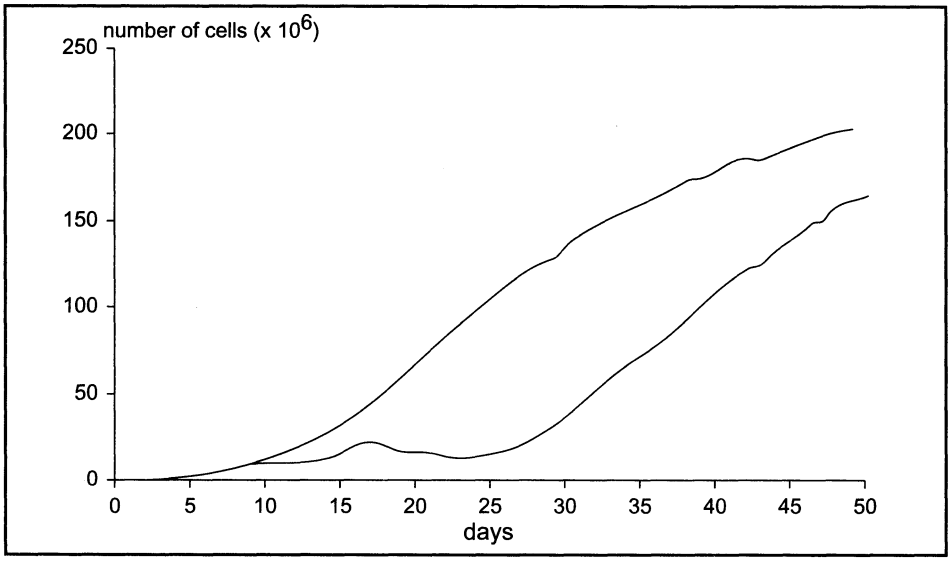

Fig. 3: Simulated control (upper line) and treatment (lower line) curves.

Data simulation

Simulated growth curves, referring to the same hypothetical type of tumor, were generated by the program, in order to test the simulation capability of the model: results for control and two– agent treatment groups are here plotted in fig.3. In this case agent A is supposed to induce resistance in 27% of sensitive cells and in 9% of cells already resistant to agent B; inactivating rates are 67.8% and 90%, respectively. Same values hold for agent B, while administrations occurr on day 10 (agent A), 18 (agent B) and 23 (agents A and B). Assumed transition rates, for both agents, are, therefore: p_{11}=0.052, p_{12}=0.27, p_{15}=0.678, p_{24}=0.09, p_{25}=0.9, p_{34}=0.09, p_{35}=0.9.

In both curves parameters μ and σ of the distribution of ϑ have values 3 and 2 respectively, while parameters α and β of the Gompertz distribution equal 0.0908 and 0.6625 respectively.

The importance of tumor cell resistance acquisition to therapeutic agents in designing an appropriate treatment planis particularly underlined by these simulated data:in spite of the high values of parameters p_{15}, p_{15}, and p_{15} treatment effects of cell population growth tend to vanish after second or third administration, as pointed out by treatment line in fig.3, which shows no downward trend correspinding to day 23 (combined treatment). This serious shortcoming should be thus taken into account when overfractioning doses of agents, since the reduction of organic damages may not completely make up for the loss of effects on tumor volume growth.

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RIASSUNTO

L'acquisizione di resistenza a trattamenti terapeutici da parte di una popolazione di cellule tumorali viene esaminata, con rispetto a trattamenti a uno o due agenti. Un modello di simulazione viene utilizzato come strumento matematico per l'analisi degli effetti di tali agenti, nella definizione dei piani di trattamento e nell'esame dell'accostamento delle curve teoriche ai dati sperimentali. Il modello di tipo compartimentale divide la popolazione cellulare in cinque compartimenti, in dipendenza della capacità di reazione delle cellule agli agenti terapeutici. La popolazione cellulare viene calcolata e comparata, su basi di proporzionalità diretta, con i volumi tumorali osservati e vengono fornite misure degli errori di misurazione.