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ORIGINAL PAPER

Modelling the frequencies of chromosomal aberrations in peripheral lymphocytes of patients undergoing radiotherapy

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Abstract Recently, several attempts have been undertaken to correlate *in vitro* calibration curves with observed frequencies of chromosomal aberrations and micronuclei in lymphocytes of patients undergoing radiotherapy. The aim of such correlations is the search for a biological method to reconstruct the dose received during radiotherapy. While the *in vitro* dose-response curves are linear-quadratic, the observed *in vivo* relationship is usually linear and there is some controversy as to the nature of the observed linearity. We have, therefore, constructed a model to calculate the frequencies and distributions of chromosomal aberrations in lymphocytes of patients undergoing conventional radiotherapy. The model assumes that each fraction of radiation induces a certain number of Poisson-distributed aberrations in the irradiated blood volume. In addition, a simplified assumption is made that lymphocytes flow freely inside the body of the patient and no elimination of cells occurs. The model yields linear dose-response curves. The steepness of the curves increases with increasing size of irradiated block of tissue (referred to as irradiated volume) and increasing dose per fraction. The distributions of aberrations become increasingly overdispersed with increasing dose per fraction but are independent of the number of radiation fractions. The modelled dose-response curves agree well with the majority of published experimental results. Given the simple assumptions made, this indicates that cell elimination, which occurs during radiotherapy does not bias the results obtained experimentally. The linearity of the dose--response curve results from the fractionated irradiation. Hence, great care should be applied when attempting to use standard, linear-quadratic calibration curves to estimate the doses received by patients during radiotherapy.

Key words biological dosimetry • chromosomal aberration • peripheral lymphocytes • radiotherapy

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Introduction

Biological dosimetry based on the analysis of cytogenetic damage in peripheral lymphocytes of the exposed person is a well established technique [10]. The absorbed dose is estimated by comparing the scored number of aberrations with a calibration curve, obtained by *in vitro* irradiation of whole blood collected from control persons. Several authors have validated this procedure by testing experimentally that, after a uniform whole body exposure of animals, the frequency of aberrations induced by a given dose was essentially the same as that induced *in vitro* [2, 3, 18]. Also, [14] found good agreement between results of *in vitro* irradiation of blood and partial-body irradiation of rabbits, when the *in vivo* exposure time was long enough for the whole peripheral blood to flow through the irradiated field. The calibration curves and the dose--response curves following *in vivo* irradiation with gamma rays are best fitted by a linear-quadratic equation [10].

Following partial-body exposure of a short duration, the dose estimation becomes less precise because the cell population in a collected blood sample will be composed of irradiated and non-irradiated cells. Clearly, the uncertainty is inversely related to the volume of the exposed part of the body and the exposure time [14]. A situation where this problem is readily seen is the irradiation of patients during radiotherapy. The exposed part of the body is small

and the exposure time is in the range of a minute. In addition, the total tumour dose is applied in fractions, usually of 2 Gy per day, 5 days a week [20].

The frequencies of chromosomal aberrations and micronuclei in peripheral lymphocytes of patients undergoing radiotherapy have been analysed by several authors [1, 4, 7, 9, 11, 17, 23]. They all found a linear dose--dependency of aberrations with the tumour dose. More recently, several attempts have been undertaken to correlate the observed frequencies of micronuclei and aberrations with *in vitro* calibration curves [12, 13, 21, 22]. In order to bring the radiation, doses applied during radiotherapy to the same order of magnitude as those used to construct the calibration curves, equivalent whole-body doses were calculated by dividing the integral dose received by each patient by his body mass [21]. It was generally found that both curves overlap in the dose range of 0 to approximately 2 Gy (Fig. 1). Above that range, the *in vitro* calibration curves increase more steeply with dose than the *in vivo* dose-response curve. Interphase death and variation in the lymphocyte proliferation kinetics during the course of radiotherapy are made responsible for this [21, 22].

What is seemingly disregarded in the above correlations is the difference between the exposure conditions of *in vitro* irradiation of blood and *in vivo* irradiation of patients. During radiotherapy the total dose is applied in fractions and only a small part of the lymphocyte pool is irradiated during a single fraction. Since the radiation fractions are generally applied at intervals of 24 hours [20], it can be assumed that the lymphocytes in the circulating and extravascular pools will have reached equilibrium before the next fraction is applied [10]. Thus, each dose of radiation "hits" a certain number of previously non-exposed lymphocytes and a certain number of lymphocytes which have already been exposed. The latter increases with the number of radiation fractions. An interesting question is what are the expected aberration frequencies under such irradiation scenario.

A mathematical model describing the relationship between the yield of chromosomal aberrations in lymphocytes and the dose received during radiotherapy has been published previously [6]. However, the model dose

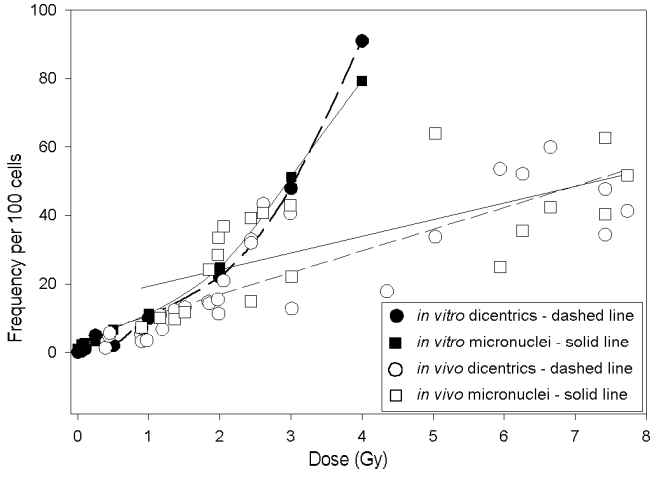

Fig. 1. Observed dose-response curves for dicentric chromosomes and micronuclei in lymphocytes irradiated *in vitro* (solid symbols) and *in vivo* (empty symbols) in the course of radiotherapy. The doses for lymphocytes irradiated *in vivo* are expressed as equivalent whole body doses (EWBD). Data from [22].

not take into account the relative volume of the irradiated blood and does not allow calculation of the aberration distribution. We have, therefore, constructed a mathematical model with the help of which the kinetics and the distribution of aberrations formed during radiotherapy can be calculated. The model assumes no elimination of lymphocytes from the irradiated pool and a complete mixing of cells before each fraction of radiation. This assumption is a simplification, because elimination of lymphocytes does occur during radiotherapy [21]. However, it is not clear how far this confounds the yield of aberrations. In fact, [15] observed no selective elimination of highly damaged lymphocytes under *in vitro* conditions. Hence, it was interesting to test how well the simplified model fits with the published results. The modelled dose-response curves and aberration distributions are in good agreement with the majority of published experimental results and indicate that cell elimination does not bias in any particular way the frequencies of chromosomal aberrations in peripheral lymphocytes. Furthermore, our model clearly shows that the observed linear dose-response curves result from the fractionated irradiation schemes of the patients and that attempts to apply standard, linear-quadratic calibration curves to estimate the doses received by patients during radiotherapy are not justified.

Description of the model

Before describing the mathematics of the model several assumptions must be made. First of all it is assumed that the cells in which chromosomal aberrations are scored flow freely and at random inside the body of the irradiated patient, meaning that the blood is fully mixed before each subsequent irradiation fraction is applied. The effect of each fraction is independent of the previous ones, i.e. is additive. Each single dose of the radiation acts on a limited number of cells which happened to be in the irradiation field. Since it would be difficult to operate with cell numbers, the term "irradiation volume" is introduced. This term was first used by [16] and was defined as the "size of the irradiated field multiplied by depth and expressed in gram assuming that 1 cm^3 of tissue was equivalent to 1 g". Thus, the irradiation volume is the block of tissue in which the total energy from the beam of radiation is absorbed. The size of the irradiated volume is expressed in cm^3.

Next, it is assumed that the frequency of aberrations (dicentric chromosomes) before irradiation = 0 and the distribution of aberrations induced by each fraction of radiation is Poisson. We think that both assumptions are permissible, since the base-line frequency of dicentric chromosomes in lymphocytes is known to be extremely low (in the order of 0.001 per cell) and the distribution of dicentrics following a whole-body exposure to low LET radiation is Poisson [10]. The values of dicentric frequencies induced in the irradiated volume by a single fraction of radiation were taken from [5] and are 0.06, 0.23, 0.49 and 1.35 per cell for 1, 2, 3 and 5 Gy, respectively (Co-60 irradiation). As mentioned in the introduction, no account is taken for cell elimination during the course of radiotherapy.

The model distribution of new aberrations is, as mentioned above, poissonian, i.e. is determined by the formula

$$P_\lambda(x) = \frac{e^{-\lambda}\lambda^x}{x!}, \tag{1}$$

where $x \in N_0 = \{0, 1, 2, \ldots\}$ and $\lambda > 0$ is the mean value of aberrations per cell which is a function (e.g. linear-quadratic or any other) of the radiation dose in Gy. The distribution is restricted to the population of irradiated cells, i.e. a fraction a of the entire body volume. The non-irradiated volume is described by the fraction $b = 1 - a$. Thus, a distribution of new aberrations in the whole population (the entire volume) is

$$Q_\lambda(x) = aP_\lambda(x) + bP_0(x), \tag{2}$$

where $P_0(x)$ denotes "no aberration" distribution, i.e. $P_0(0) = 1$ and 0 elsewhere.

Hence the distribution R_n of aberrations after the nth fraction of radiation has the form of a discrete convolution

$$R_n(x) = \left[\left(aP_\lambda + bP_0\right) \bullet R_{n-1}\right](x) \tag{3}$$

and obviously

$$R_n(x) = \left[\left(aP_\lambda + bP_0\right)^{\bullet n} \bullet R_0\right](x), \tag{4}$$

where R_0 is the primary distribution of aberrations before the sequence of fractionated irradiation. The convolution $\bullet$ of two functions on N_0 is defined as follows:

$$(f \bullet g)(x) = \sum_{z=0}^{x} f(z)g(x-z) \tag{5}$$

and $(...)^{\bullet n}$ denotes "nth power of convolutions", i.e. n successive convolutions.

The formula (4) may be rewritten in the form

$$R_n(x) = \sum_{i=0}^{n} \frac{n!}{(n-i)!} \cdot a^i \cdot b^{n-i} \cdot \left[P_\lambda^{\bullet i} \bullet P_0^{n-i} \bullet R_0\right](x). \tag{6}$$

Since the convolution of two Poisson distributions with parameters λ_1 and λ_2 is also a Poisson distribution with λ equal to the sum of λ_1 and λ_2 the formula (6) may be simplified:

$$R_n(x) = \sum_{i=0}^{n} B_{n,a}(i) \cdot \left[P_{i\lambda} \bullet R_0\right](x), \tag{7}$$

where $B_{n,a}$ is the Bernoulli distribution for n trials and probability of favourable event equal a. If one can assume no initial aberration (i.e. $R_0 = P_0$)

$$R_n(x) = \sum_{i=0}^{n} B_{n,a}(i) \cdot P_{i\lambda}(x). \tag{8}$$

The presence of Bernoulli distribution in the above formula is a manifestation of full mixing. The fractionated irradiation scheme for each cell may be treated as Bernoulli trials. The "favourable event" means that during a single exposure a cell finds itself in the irradiated volume. Obviously the probability of such event equals a and the fraction of cells exposed i times ($i = 0, 1, \ldots, n$) to radiation equals $B_{n,a}(i)$ provided that the mixing of blood is perfect.

It is easy to calculate (e.g. using the moment generating function method) for the distribution (4) the mean value of aberration per cell

$$s_n = n \cdot a \cdot \lambda + s_0 \tag{9}$$

and variance

$$v_n = n \cdot a \cdot \lambda \cdot (b \cdot \lambda + 1) + v_0, \tag{10}$$

where s_0 and v_0 are the initial mean value and variance, respectively.

Hence, the relative variance (also referred to as dispersion index in the literature) J_n of the distribution under consideration looks as follows:

$$J_n = \frac{v_n}{s_n} = 1 + \frac{b \cdot \lambda + \dfrac{v_0 - s_0}{n \cdot a \cdot \lambda}}{1 + \dfrac{s_0}{n \cdot a \cdot \lambda}}. \tag{11}$$

The index is a rough measure of how far a distribution deviates from that of Poisson. The above value indicates an overdispersed distribution of aberrations unless s_0 is greater enough than v_0 to make the numerator negative.

The assumption of no aberrations before the irradiation treatment, i.e. $R_0 = P_0$ and hence $v_0 = s_0 = 0$, implies that

$$s_n = n \cdot a \cdot \lambda, \tag{12}$$

$$w_n = n \cdot a \cdot \lambda \cdot (1 + b \cdot \lambda), \tag{13}$$

$$J_n = 1 + b \cdot \lambda. \tag{14}$$

Note that in this case the relative variance is independent of the number of fractions and its value indicates an overdispersion of the aberration distribution. It is evident that this property is subsequent to the partial irradiation of the body volume only.

Theoretically any two of the final parameters s_n, v_n and J_n may serve as fingerprints of fractional irradiation procedure provided that the initial values s_0 and w_0 are known and the fraction doses are equal. It means that the number of fractions n and the average number of aberrations per cell λ (connected with radiation dose) may be determined from the above equations. However, a problem of accuracy of n and λ arises if the final values s_n, v_n and the parameter a are determined empirically.

The assumption of ideal mixing seems to be realistic. Nevertheless, there is no obstacle to consider a more general case. The Bernoulli distribution $B_{n,a}(i)$ in the formula (8) may be substituted by a general "mixing function" $d(i)$ subject to the conditions

$$d(i) \geq 0, \tag{15}$$

$$\sum_{i=0}^{n} d(i) = 1. \tag{16}$$

Thus, the distribution

$$R_n(x) = \sum_{i=0}^{n} d(i) \cdot P_{i\lambda}(x). \tag{17}$$

In the case with no mixing at all (quite opposite to the ideal mixing) the function $d(i)$ looks as follows:

$$d(i) = \left\{ \begin{matrix} a, & i = n \\ b, & i = 0 \\ 0, & i \neq 0, n \end{matrix} \right\} \tag{18}$$

where a and b mean the same as above.

The values $d(i)$ of the "mixing function" may be treated as unknown parameters of the distribution (17) and estimated for a given sample if numbers of cells with i aberrations have been determined. The obtained values of estimators of $d(i)$ together with their confidence intervals may be compared to the values of Bernoulli distribution $B_{n,a}(i)$ to corroborate or not the assumption of the ideal mixing.

Application of the model and discussion

A computer program, in which the described model was implemented, was written in Pascal. With the help of the program the expected frequencies of dicentrics were calculated for a radiotherapy regime of 20 factions and a patient weight of 70 kg. First, the dependence of the size of the irradiated volume on the dicentric frequency was analysed. The results are plotted in Fig. 2. Linear dose--response curves are observed and the angle of the curves increase with increasing irradiation volume. This could be expected since the number of cells hit per fraction is proportional to the irradiation volume. This result is in agreement with the published results. For example, as shown by [1], the aberration yield for the same absorbed dose level in the treated volume is much greater after irradiation of ankylosing spondylitis patients, where the irradiated skin area is big, than after irradiation of mammary carcinoma. Also the frequency of micronuclei in peripheral lymphocytes of patients who underwent whole--body irradiation [8] is much higher than following partial body exposures [7, 12, 13, 21, 22]. As further expected, increasing the dose per fraction also results in steeper dose--response curves. The curves are linear at all tested doses (Fig. 3).

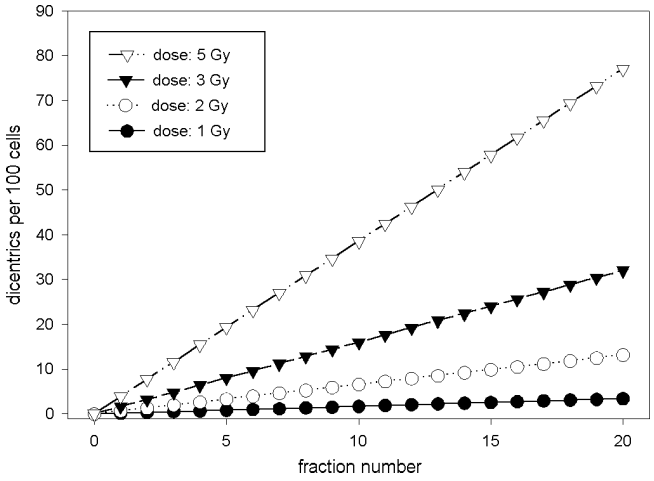

Fig. 3. Modelled frequencies of dicentric chromosomes in peripheral blood lymphocytes of a 70 kg patient receiving radiation fractions of 1, 2, 3 and 5 Gy to an irradiation volume of 2000 cm^3.

Next, we analysed the relation between the dose per fraction and the aberration distribution. The relative variance increases with increasing dose per fraction (Figs. 4 and 5), but, as assumed from equation (11), its value is independent of the number of fractions (Fig. 4). This is supported by the majority of experimental results, both for chromosomal aberrations [22] and micronuclei [7, 13, 21, 22]. Only [4] observed a slight increase in the relative variance of dicentrics with increasing number of fractions and similar results were reported by [12] for micronuclei.

Finally, the frequencies of aberrations were calculated for a single radiation exposure of increasing dose (Fig. 5). The dose-response curve was best fitted by a second order polynomial (Fig. 5) and its shape is similar to that obtained after *in vitro* irradiation of blood (see Fig. 1 for an example).

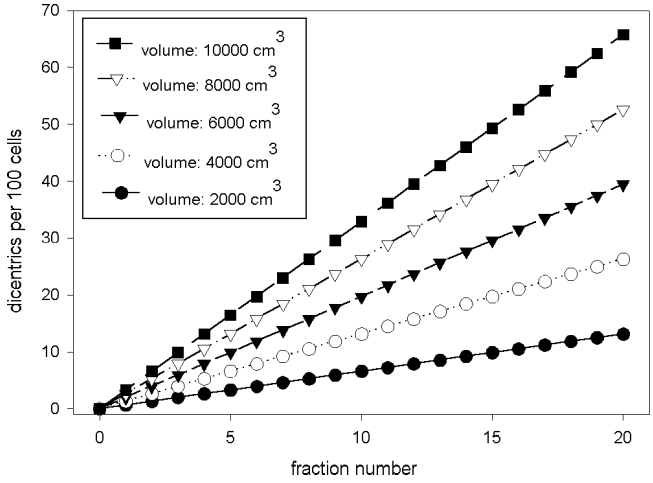

Fig. 2. Modelled frequencies of dicentric chromosomes in peripheral blood lymphocytes of a 70 kg patient radiation fractions of 2 Gy to irradiation volumes of 2000, 4000, 6000, 8000 and 10,000 cm^3.

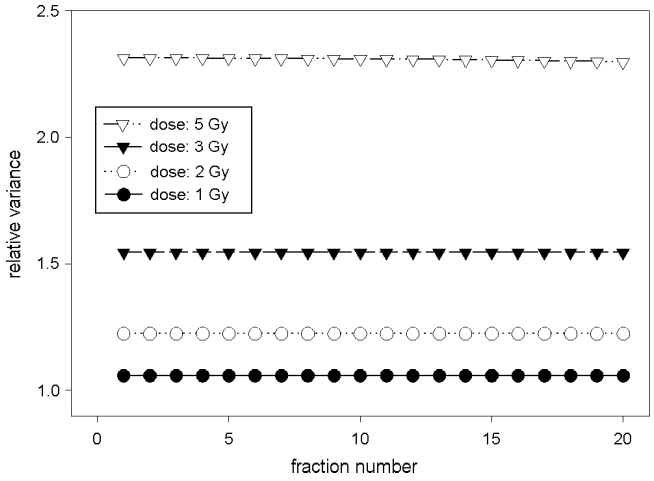

Fig. 4. Modelled distribution of dicentric chromosomes in peripheral blood lymphocytes of a 70 kg patient receiving radiation fractions of 1, 2, 3 and 5 Gy to an irradiation volume of 2000 cm^3.

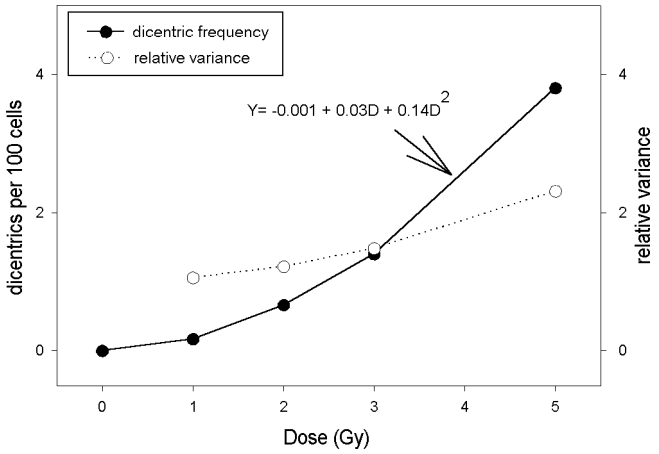

Fig. 5. Modelled frequencies of dicentric chromosomes and the values of relative variance in peripheral blood lymphocytes of a 70 kg patient receiving a single fraction of 1, 2, 3 and 5 Gy to an irradiation volume of 2000 cm^3. The dose-response curve for dicentrics was fitted by the least-square method.

This is readily expected but is difficult to verify experimentally *in vivo*, because we are not aware of any study performed on patients who received single, variable doses of radiation. Only [16] analysed the dicentric frequency in lymphocytes of patients who received two equal, bilaterally applied fractions, with a 50–60 s interval between the two doses. The dose-response curve was best fitted linearly, however, high interindividual variations in the dicentric frequency were observed.

Our model takes no account of either any perturbations of the proliferation of lymphocytes inside the body or of cell death during the treatment. There is, of course, no doubt that this is a simplification, because lymphocytes die during the course of irradiation [21]. However, it appears very difficult to model this effect [9, 24]. Cell elimination could be responsible for the increase of relative variance of dicentrics observed by [4], because in contrast to most other studies, the authors analysed the lymphocytes of a patient treated for Morbus Hodgkin, where the irradiation volume was large. Very interesting results were reported by [19] who studied aberrations in lymphocytes of patients who underwent a radiotherapy before or after a chemotherapy. They observed that chemotherapy given after a radiotherapy did not alter the frequency and distribution of dicentrics (which was significantly overdispersed) in peripheral lymphocytes. In contrast, when chemotherapy was given before radiotherapy, lower dicentric frequencies were scored and the distributions were not always overdispersed. The authors interpret this as an evidence for a complicated interplay of chemotherapy and radiotherapy with regard to cell elimination.

Given the complexity of the above, it is striking that the results of our simplified model agree so well with the majority of experimental results. In nearly all available reports linear dose-response curves were observed [4, 7, 9, 12, 13, 17, 21–23], with [1] being the sole exception. This strongly suggests that cell elimination, which could, among other factors, be responsible for the frequently observed high interindividual variability of the observed frequencies of dicentrics and micronuclei in patients undergoing radiotherapy, does not generally bias the results in any particular way. This conclusion fits well with the observation that heavily damaged lymphocytes are not selectively eliminated from the population of cells irradiated *in vitro* [15].

Our model shows that the linear dose-response curves observed in lymphocytes of patients undergoing radiotherapy are a consequence of the fractionated radiation schemes rather than of cell elimination. The linearity is a result of the fact that the entire dose D is not applied at once, but is a sum of n equal fractions acting subsequently. Let $\gamma(D)$ is a dose-response function obtained from "one dose" experiments. If the dose D is divided into n fractions D_0, i.e. $n = D/D_0$, then the cumulative effect of the treatment is given by $\gamma_C(D) = n \times \gamma(D_0) = (D/D_0) \times \gamma(D_0)$. Thus, for constant D_0 the cumulative dose-response function is linearly dependent on D no matter how the "basic" function $\gamma(D)$ (e.g. linear quadratic) looks like.

The effect of a fractionated irradiation scheme on the yield of aberrations in lymphocytes was considered in the model of [6]. Ideal mixing of lymphocytes was assumed and the obvious Bernoulli distribution (as a distribution of the number of singular irradiation doses received by a cell) was obtained but in a somewhat more complicated way then presented here. An averaging of linear-quadratic formula resulted in an average yield of aberrations, however, the authors did not consider the interesting question of aberration distribution. The linearity of dose-response relation occurred as a limit for a small irradiation volume.

In conclusion, it appears incorrect to use a linear--quadratic calibration curve, obtained from lymphocytes irradiated *in vitro* with single doses of radiation, as basis for estimating the cumulative dose received during radiotherapy. If such estimates are attempted, it is probably more safe to rely on dose-response curves obtained from analysing patient lymphocytes irradiated *in vivo* in the course of a radiotherapy.

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