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MEAN INACTIVATION DOSE (\bar{D})

A critical analysis of a neglected parameter in radiotherapy

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Abstract

By predicting treatment outcome to radiotherapy from *in vitro* radiobiological parameters, not only individual patient treatments can be tailored, but also new promising treatment protocols can be tried in patients in whom unfavorable outcome is predicted. In this respect, choosing the right parameter can be very important. Unlike D_0 and N which provide information of the distal part of the survival curve, mean inactivation dose (\bar{D}) estimates overall radiosensitivity. However, the parameters reflecting the response at the clinically relevant low-dose region are neglected in the literature. In a literature survey of 98 papers in which survival curves or D_0/N were used, only in 2 \bar{D} was used. In 21 papers the D_0/N values were important in drawing conclusions. By calculating \bar{D} in 3 of these 21 papers, we show that the conclusion drawn may be altered with the use of \bar{D} . The importance of 'low-dose-region-parameters' is reviewed.

Key words: Radiotherapy, radiobiological parameters, mean inactivation dose, literature review.

The work of Puck & Marcus (1) has led to an era in radiation therapy in which some clinical results could be explained by *in vitro* findings (2, 3). Initial *in vitro* work with human cell lines, using traditional parameters (D_0 and N), suggested that there were no differences in cell survival after irradiation, implying no inherent difference among cell lines in radiosensitivity to account for the observed clinical differences (4–10). However, several investigators have shown that the mean inactivation dose (\bar{D}) is a better parameter than N and/or D_0 (11–13). By using cell survival data described in the literature for human cell lines and calculating \bar{D} and recalculating surviving fraction at 2 Gy (SF_2), the *in vitro* data can be correlated to clinical and histopathological groups of tumors of different radiosensitivity/radiocurability (12–17). Differences in radiosensitivity between various tumors have also been shown in primary culture systems (i.e.,

from freshly excised/biopsied tumors) (16), and clinical failure can be correlated to enhanced repair capacity measured *in vitro* (18). The relative contributions of inherent radiosensitivity, potential lethal damage repair, hypoxia, etc. to clinical radiocurability are not known. To predict outcome of radiation therapy in individual human neoplasms, choosing the right parameters is very important. \bar{D} seems to be a useful parameter which, however, has been largely neglected in the literature.

By calculating \bar{D} from published survival data, we have analyzed whether the conclusions drawn with the use of conventional parameters (D_0 and N) have deviated from those derived from the use of \bar{D} .

Material and Methods

The mean inactivation dose, \bar{D} , was defined originally in 1966 (19, 20) and the ICRU described it in 1979 (21). We have reviewed that use of \bar{D} in radiobiological studies and concentrated this review to some reports published in 1981 and 1985. The journals *Acta Radiologica Oncology*, *International Journal of Radiation Oncology, Biology, Physics*, and *Radiation Research* were chosen since they are widely read by radiation oncologists/radiobiologists and related scientists.

All articles containing data on cell survival parameters were reviewed. We also studied the influence of the parameters D_0 and N values on the conclusions drawn in these articles. A few articles in which D_0 and/or N values were very important for the conclusions were selected for determining the \bar{D} and its correlation to the clinical findings (compared to that of D_0 and/or N).

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Table 1
Survey of number of articles using following parameters

Year/Journal	S.C. ⁺	N	\bar{D}_0	D_q	α/β	Use of N and/or D_0 *			
						\bar{D}	Impor- tant*	To some extent*	Not at all*
1981, 1985-1987, 1988 (FASC 1 and 2) Acta Radiologica Oncology	10	9	9	2	1	0	3	6	1
1981/Radiation Research	33	16	23	7	3	0	5	15	5
1985/Radiation Research	30	14	19	6	2	1	6	10	8
1981/International Journal of Radiation Oncology, Biology and Physics	7	5	6	2	1	1	1	3	0
1987/International Journal of Radiation Oncology, Biology and Physics	18**	7	10	4***	4	0	6	2	8

⁺ Number of articles in which survival curves/parameters are used.

^{*} Number of articles in which N and/or D_0 values are important in drawing conclusions.

^{**} In 2/18, SF₂ was used; in 1 of those 2, the SF₂ values were important in drawing conclusions.

^{***} In 1/4, D_q values were important in drawing conclusions.

The expressions of surviving curves and calculation of the mean inactivation dose \bar{D} . There are many expressions of dose-effect of cells in response to irradiation. Each form represents an assumed mechanism of radiation cell kill of an ensemble of cells. The simplest one is described by the single-target, single-hit survival expression:

$$S(D) = e^{-D/D_0} \quad (1)$$

where $S(D)$ is the surviving fraction after a single dose D , and D_0 is the slope of the curve on a semi-log plot. The dose required to reduce the surviving fraction $S(D)$ to 37% (i.e., 100/e%) is designed as D_{37} . In any event, the D_0 of a cohort of tumor cells is less than the inactivation dose of any one subtarget D_{0i} , and can be expressed as

$$\frac{1}{D_0} = \sum_i \frac{1}{D_{0i}} \quad (2)$$

where D_{0i} is the inactivation dose of subtargets of type i , each of which does not have a shoulder curve. The percentage cell kill per unit dose is a constant. There are other revised expressions of dose-response curves for cell systems of various subpopulations of mixture. The details can be found in the ICRU report (21). In practice a constant rate of increase of the slope of the surviving curve on a semi-log plot is adequate, i.e.

$$\frac{-d(I_n S[D])}{dD} = \alpha + 2\beta D \quad (3)$$

i.e., the first derivative of the semi-log curve is a straight line, or

$$S(D) = e^{-(\alpha D + \beta D^2)} \quad (4)$$

Where α is equal to $1/D_0$ in equation (1). The expression (4) contains a linear plus a quadratic term. It seems to fit many experimental situations and has been proposed as the most representative expression for analyzing the dose-response curve by Fertel et al. (15). We followed Fertel et al. (15) analysis of the linear quadratic equation and wrote a program to calculate the inactivation dose \bar{D} . (The program was written in Fortran language, and allows to calculate \bar{D} from survival data as well as from the linear quadratic parameters (α and β), using gaussian numerical integration. This is available upon request.)

Results and Discussion

Table 1 details the results of our survey of the literature. In 10 papers in the Acta Radiologica Oncology (1981, 1985-1988 volumes), in 33 and 30 articles in Radiation Research in years 1981 and 1985 respectively, and in 25 papers in International Journal of Radiation Oncology, Biology, Physics (1981 and 1987), use of cell survival curves and/or parameters describing cell survival was noted. The majority of the papers used one or more of the traditional parameters, viz. D_0 , N or D_q . Only a minority used the parameters α and β . Only in two of a total of 98 reports was \bar{D} utilized. By studying these papers, we found that in 57 of 98 papers, traditional parameters influenced the conclusion; in 21 of the 98 papers, these values were very important for the conclusions drawn.

We also determined \bar{D} for various survival curves in 3 of the 21 papers in which the values of the traditional parameters were important for the conclusions and ana-

Table 2
D₀ and D_q values reported by Okamoto et al. (22) versus \bar{D} values

Cell line	D ₀ (Gy)	D _q (Gy)	Cell line	\bar{D} (Gy)
I. In vitro irradiation** (Cell lines maintained in mice and irradiated in vitro)				
TMT-1	1.12 (1.03–1.24)	1.19	TMT-1 (4 days*)	2.02
			TMT-1 (12 days*)	1.76
TMT-2	1.12 (1.03–1.24)	1.19	TMT-2 (8 days)	2.28
			TMT-2 (9 days)	1.60
II. In vitro irradiation (Cells maintained in culture and irradiated in vitro)				
TMT-2	1.33	0.91	TMT-2	2.12
III. In vivo irradiation**				
TMT-1	3.13	0.55	TMT-1 (4 days*)	3.09
(4 and 6 days*)	(2.82–3.51)		TMT-1 (6 days*)	3.35
TMT-2	3.13	0.55	TMT-2 (4 days*)	3.85
(4 and 6 days*)	(2.82–3.51)		TMT-2 (6 days*)	4.10

* Irradiated in vitro at various times of growth in mice.

** D₀, N and D_q values of TMT-1 and TMT-2 cell lines are reported together by the authors.

Table 3
D₀ and D_q values reported by Rodriguez & Alpen (24) versus \bar{D} values

Radiation used	Test system	D ₀ (Gy)	D _q	\bar{D} (Gy)
I. 250 kVp x-rays	1. Cell in suspension	1.90	2.50	3.25
II. Carbon ions				
400 MeV/amu	1. Cell in suspension	1.80	2.80	2.84
Plateau region	2. Spheroids	2.60	3.40	3.60
III. Neon ions				
425 MeV/amu	1. Cell in suspension	1.65	2.60	3.98
Plateau region	2. Spheroids	2.20	2.75	5.17
IV. Carbon ions				
(Bragg peak)	1. Spheroids			
Proximal peak		2.40	0.70	3.08
Mid peak		2.10	0	2.36
Distal peak		1.90	0	1.78
V. Neon ions				
(Bragg peak)	1. Spheroids			
Proximal peak		1.90	0	2.11
Mid peak		1.60	0	1.80
Distal peak		2.45	0	2.34

lyzed whether the use of \bar{D} led to an alteration of the conclusions:

Okamoto et al. (22) determined values of traditional parameters in two types of mouse mammary tumors (TMT-1 and TMT-2 cell lines). Using colony-forming assay in soft agar, the in vitro response was compared with the in vivo response. Table 2 compares the values of D₀ reported by the authors with \bar{D} values calculated by us. The authors noted that the cells irradiated in vivo were more resistant than those irradiated in vitro. Calculation of \bar{D} values does not alter this conclusion. In general, D₀ and \bar{D} values correlate well in this study. However, one should note that the D_q values for in vivo irradiation are

lower than for in vitro irradiation (Table 2). As pointed out by Zeitz (23), traditional parameters 'do not necessarily define the shape of the curve for the best fit' and 'they may give a qualitatively incorrect impression'. This can be avoided by using \bar{D} .

Rodriguez & Alpen (24) compared survival characteristics of rat brain gliosarcoma (9L-21) cells irradiated as multicellular spheroids or in suspension with 225 kVp x-rays, 400 MeV/amu carbon ions and 425 MeV/amu neon ions. \bar{D} was calculated for the cell survival curves and the results are compared with D₀ and D_q-values. In each category the relative increases match (Table 3) However, the values of D₀ and \bar{D} between categories do not match.

Table 4
RBE calculated using \bar{D}

Radiation type	Test system	*RBE for survival of		**RBE calculated By D_0 /Test	†RBE using \bar{D} /test	
		50%	10%			
I. Carbon ions 400 MeV/amu Plateau region	1. Cell Suspension	0.94	1.0	1.06	1.14	
	2. Spheroids	0.97	0.97	1.0	1.20	
II. Neon ions 425 MeV/amu Plateau region	1. Cell Suspension	1.0	1.0	1.1	0.816	
	2. Spheroids	1.1	1.2	1.1	0.816	
III. Carbon ions (Bragg peak)	Spheroids	Proximal peak	2.1	1.5	1.08	1.41
		Mid peak	3.4	1.9	1.23	1.84
		Distal peak	3.7	2.1	1.36	2.4
IV. Neon ions (Bragg peak)	Spheroids	Proximal peak	4.0	2.1	1.36	2.06
		Mid peak	4.4	2.5	1.62	2.41
		Distal peak	2.9	1.6	1.06	1.85

* RBE values reported by Rodriguez & Alpen (24).

** D_0 for 225 kVp x-rays/ D_0 for test radiation.

† \bar{D} for 225 kVp x-rays/ \bar{D} for test radiation.

For example, the D_0 value of 1.90 Gy for cells in suspension treated with 250 kVp x-rays corresponds to a \bar{D} value of 3.25 Gy. For carbon (plateau), the D_0 value (1.80 Gy) deviates only slightly from that of x-rays (1.90 Gy); yet the \bar{D} (2.84 Gy) value deviates significantly from the D_0 values (3.25 Gy). D_q values for x-rays and carbon ions were fairly similar (Table 3). With the use of D_0 and D_q values, one may conclude that both the cells in suspension and spheroids have similar sensitivity to x-rays and carbon ions in the plateau region. However, the \bar{D} values indicate that the cell-kill is more pronounced with carbon ions. Using 50% survival, 10% survival and D_0 values, the RBE in the plateau regions of carbon and neon ions is about 1, both for cells in suspension and spheroids (Table 4). Using \bar{D} , RBE of carbon and neon ions differ. However, RBE is also equal for cell suspension and spheroids within each group (Table 4). RBE values calculated for proximal, mid and distal Bragg peaks for carbon and neon ions using \bar{D} are almost similar to the RBE values for 10% survival reported by the authors. The observation that the mid-peak of neon ions is more effective for cell killing than the distal-peak is also sustained using \bar{D} . The conclusion that there is an enhanced survival of cells irradiated in spheroids (both for x-rays and plateau region of heavy ions) is sustained with the use of \bar{D} (Tables 3 and 5). Even the degree of enhanced survival (1.3) is the same using D_0 or \bar{D} (Table 5). However, their conclusion of equal RBE (1.0) for both heavy ions (plateau) is not sustained with the use of \bar{D} (Table 4).

Rhee et al. (25) studied sublethal damage repair (SLDR)

Table 5
Radioresistance of cells in spheroids

Radiation used	D_0 spheroids/ D_0 cells* in suspension	\bar{D} spheroids/ \bar{D} cells in suspension
I. 225 kVp x-rays	1.36	1.33
II. Carbon ions 400 MeV/amu (Plateau region)	1.44	1.26
III. Neon ions 425 MeV/amu (Plateau region)	1.33	1.29

* D_0 values from Rodriguez & Alpen (24).

and dose-rate effect in hemopoietic cells from human acute premyelocytic leukemia and possible clinical implications for total body irradiation (TBI) in connection with bone marrow transplantation. In their split-dose experiments on measuring the SLDR, there was a 3-fold increase in survival when 8 Gy was delivered in two fractions (with cells maintained at 23°C between fractions) compared with a single dose of 8 Gy. However, the increase in survival was 8-fold when the cells were maintained at 37°C between fractions. This was postulated to be due to an additive effect of SLDR and progression through the cell cycle to a more resistant phase. To address this specific question, after a dose of 4 Gy, cells were maintained at 37°C for 3, 5, or 8 h after which

Table 6
*D₀ and \bar{D} values after split-dose radiation for HL-60 cells**

Time between 1st and 2nd doses (h)	D ₀ (Gy)	\bar{D} (Gy)
0	0.82	1.40
3	1.02	1.20
5	1.15	1.90
8	0.98	1.60

* D₀ values from Rhee et al. (25).

Table 7
D₀ and \bar{D} values for different dose rates for HL-60 cells

Dose rate Gy/min	D ₀ Gy	\bar{D} Gy
109.7	0.764	1.58
36.2	0.987	1.43
7.2	1.120	1.63
2.9	1.119	1.72

* D₀ values from Rhee et al. (25).

interval a second dose of radiation was administered. This resulted in reappearance of the shoulder of the survival curve as well as increased D₀ values. Table 6 shows D₀ values reported as well as \bar{D} values calculated by us. \bar{D} values after split-dose with 5-h interval confirm the increased survival suggested by D₀. However, after 3-h incubation, the \bar{D} value (1.2 Gy) is lower after a single dose (1.4 Gy). Similarly, the dramatic variation in D₀ values with different dose rates is not seen with \bar{D} values (Table 7). Based on D₀ values, Rhee et al. concluded the presence of a dose-rate effect for HL-60 cells. However, if one were to use \bar{D} values, a similar conclusion cannot be drawn.

In vitro cell survival curves are almost universally represented by a semi-logarithmic plot with dose on the abscissa (linear scale) and surviving fraction on the ordinate (logarithmic scale). This is convenient as it makes it easy to compare the exponential part of different curves and to calculate various traditional parameters (26). These parameters were originally based on theoretical models of radiation action that are no longer considered valid but are nevertheless still widely used in order to describe dose-effect relations (20, 21).

One factor responsible for the effect of radiation therapy is the postulated differences in radiosensitivity of different histological tumor types (27, 28). Can the differences in radioresponsiveness/radiocurability observed in clinical practice be measured in vitro?

Initial work using the parameters D₀ and N suggested

that there were no differences in in vitro survival (4–10), implying no inherent differences in radiosensitivity, among human cell lines. However, Fertil et al. using the parameters \bar{D} and SF₂, showed that the in vitro data can be correlated to clinical observations (12–15). Others have substantiated this observation (16, 17, 29–31). The usefulness of SF₂ and \bar{D} has been shown in non-tumor human cell lines also (32). These findings may not contradict the differences in potentially lethal damage repair (PLDR) noted among various human tumor cell lines in vitro by Weichselbaum et al. (33, 34). In fact, differences in \bar{D} and SF₂ may reflect differences in the extent/type of repair mechanisms among different tumors.

Fertil et al. (12, 13, 15), have established that in vitro cellular radiosensitivity can be correlated to clinical radioresponsiveness/curability. By recalculating SF₂, \bar{D} , etc. from published literature, (92 human cell lines), a correlation between 95% tumor control dose and mean SF₂, distinction of different histological cell lines, and separation of radiosensitivity of 6 different histological groups were possible. Their investigations, however, have the following short-comings (12, 13, 15): parameters were calculated retrospectively; data obtained from dissimilar protocols were compared; the source of the cells varied; test systems used were different; different LET radiations were used; not all histological types of tumors were represented; nothing was known about the radiosensitivity of original tumors; the radiosensitivity could have changed in established cell lines; the correlation was for *mean* values of \bar{D}/SF_2 . Individual histological groups had considerable range within them.

In addition, obtaining the dose and surviving fraction from figures published in the literature may not be accurate. Yet, Fertil et al. showed that the deviation between values obtained by them varied only by 4% and 10% from that reported by the original authors. The ideal solution for all the objections raised above would be calculation of \bar{D} by the original investigators prospectively.

Recently, there has been an interesting dialogue in the literature (14, 30, 31) regarding the usefulness of \bar{D} vis-à-vis SF₂. Both are model-free and reflect the response in the low-dose region. Are there any advantages in using one of them instead of the other?

By calculating \bar{D} for a wide range of theoretical curves, Tucker (31), showed that for the same SF₂, \bar{D} could vary significantly depending on the shape of the curve. The disparity between SF₂ and \bar{D} was negligible for SF₂ values of 0.2, 0.3, or 0.4; but could be quite pronounced for higher survival fractions at 2 Gy, e.g., 0.6, 0.8.

In addition, \bar{D} may not be model-free in practice due to lack of sufficient data on the SF for different dose groups. In that case, one will be forced to use a 'model to interpolate between data points'. How does \bar{D} compare with SF₂ if one were to use a model? \bar{D} was found to be more dependent on the model chosen; the average difference in \bar{D} values when calculated by LQ model versus the 2-com-

Table 8
Comparison of D_0 , N and \bar{D} for different cell lines

Parameter	Cell lines	Range	Arithmetic means	Coefficients of variation (CV)
I D_0	HeLa (10 lines)	0.76–2.08	1.36	33%
	Melanomas (12 lines)	0.67–1.72	1.15	25%
	Fibroblasts (10 lines)	0.89–1.59	1.26	19%
	AT fibroblasts	0.34–0.75	0.51	24%
II N	HeLa	1.4–14	5.6	69%
	Melanoma	2.4–35	12	86%
	Fibroblasts	0.48–3.4	1.9	51%
	AT fibroblasts	0.48–1.57	0.92	46%
III \bar{D}	HeLa	1.45–2.84	2.29	17%
	Melanoma	1.30–3.49	2.54	21%
	Fibroblasts	1.26–1.95	1.53	20%
	AT fibroblasts	0.40–0.62	0.51	16%

NOTE: 1) The CV for D_0 : N : \bar{D} = 19–33%: 46–69%: 16–21%; i.e., smaller for \bar{D} . 2) Derived from Table 1, Fertel et al. (15).

ponent cell survival model (TC) was 3.6%; it was 1.6% for the SF_2 . Variation dependent on the range of data available was 6.2% for \bar{D} and 1.1% for SF_2 .

Although SF_2 appears theoretically superior to \bar{D} , Tucker (31) notes that, in practice, there is a monotonous relationship between D and SF_2 . Of course, calculation of D is more cumbersome than calculation of SF_2 . Yet, Fertel & Malaise (14), pointed out that \bar{D} may be preferred for several reasons. In practice, 'mean curves' for 7 histological groups did not cross each other. They also pointed out that the discriminating SF need not be at 2 Gy. In their analysis, it was 1.5 Gy for categorizing tumors into 3 clinical groups; 2 Gy for discriminating 7 histopathological groups; whereas it was 3 Gy for separating 7 fibroblast cell line groups of different genetic origin. \bar{D} was able to discriminate the mentioned groups.

It can be concluded that, in general, \bar{D} or SF_2 can distinguish different radiosensitivity in the low-dose region. However, it is preferable to use both \bar{D} and SF_2 (14). More recently Rofstadt et al. (29) have compared in vitro radiosensitivity using Courtenay soft agar colony assays for cells directly isolated from human surgical specimens. Using D_0 , N , D_q and SF_2 , they showed that the values overlapped for 7 different histological groups. There was also significant range within individual histological groups. \bar{D} was not calculated by these authors.

The following observations by Fertel & Malaise are worth re-enumeration (Tables 8 and 9).

1) Analyzed survival curves exhibited different shapes: linear quadratic (LQ), pure exponential, or very strong quadratic. However, the majority exhibited a complex LQ dose dependence.

Table 9

Measurement of radiosensitivity in six histological groups of human tumor cell lines

Parameter	Coefficient for variation Range (CV)
α	37–117%
β	25–183%
N	38–265%
D_0	11–70%
SF_2	18–42%
SF_8	49–130%
D	21–38%

NOTE:

1) Derived from Table 1, Malaise et al. (13).

2) The narrowest range is for \bar{D} .

3) The widest range is for N .

4) The order of predicting radiosensitivity:

$\bar{D} \rightarrow SF_2 \rightarrow \alpha \rightarrow SF_8 \rightarrow \beta \rightarrow N$.

2) A wide spectrum of radiosensitivities were observed. For example, at 2 Gy, the surviving fraction (SF) varied from 0.15 to 0.90. Yet, within a given cell type, the variation was much narrower (e.g., SF for fibroblasts at 2 Gy = 0.17–0.38).

3) Since the HeLa cell line is very widely used, comparing the parameters of HeLa cell lines can be a test for the appropriateness of the conclusions drawn that inherent cellular radiosensitivity exist. They showed that the initial

part of the survival curves varied only slightly from author to author whereas the distal part was highly dependent on the technical factors. It is to be noted that the conventional parameters, D_0 and N , are obtained from the distal part of the survival curve. The D values have a coefficient of variation (CV) of only 17% compared with 33% and 69% for D_0 and N (Table 9) respectively.

4) Using SF_2 , cell types varied in radiosensitivity ($p < 2.5\%$). No such relationship was found with SF at 8 Gy.

5) D distinguished histologically different cells as distinct groups.

6) The range of radiosensitivity was of the order of the OER (oxygen enhancement ratio).

The above observations have been confirmed by others. Courdi et al. (16) assayed the response to 1.76 Gy of 14 breast and 9 melanoma tumor cell lines immediately after excision, using the colony method. The mean SF of the breast tumor cells (0.72) was statistically different ($p < 0.05$) from melanoma cells. This was unrelated to the number of cells plated or the plating efficiency.

Deacon et al. (17) have also found SF_2 to discriminate radioresistant and radiosensitive tumor types. These authors categorized the tumors into 5 groups, depending on their known clinical radiocurability (responsiveness). Cell lines from 15 different cancers from 14 different laboratories were used in their analysis. The mean values of SF_2 differed significantly between groups. The p -value between groups I/II (neuroblastoma, lymphoma, myeloma, medulloblastoma, and small cell lung cancer) and group V (melanoma, osteosarcoma, glioblastoma, and renal cell carcinoma) was < 0.001 ; between groups III/IV (breast, bladder, uterine cervix, pancreas, colorectal and squamous cell carcinoma) and group V, $p > 0.01$. D_0 and N failed to discriminate various groups. In addition, by adding the SF_2 for 30 fractions (the usual curative dose administered) (omitting recovery process, effect of hypoxia, etc.), the SF was compatible with cure for 'radiosensitive' tumors and not for 'radioresistant' tumors.

A 'unique survival curve for any given cell line is, like the holy grail, unattainable' (37). Yet, from a critical review of the literature, it can be concluded that the surviving fraction in the low-dose region (e.g., 2 Gy) or parameters reflecting the response of cells at low doses (e.g., D) are less variable than the conventional parameters reflecting the response of cells at high-dose (e.g., D_0 only). In addition, whereas the traditional parameters do not correlate with clinical observations of radioresponsiveness/sensitivity/curability, the 'low-dose-region-parameters' do. Still, even 20 years after its introduction, D is neglected. Our estimation of D from 3 papers in which conclusions were drawn from conventional parameters show that application of D may alter the conclusions.

D_0 and N provide information on the distal part of the survival curve only. On the other hand, D gives an overall estimation of the radiosensitivity. Interpretation of an in vitro cell survival curve in terms of 'sensitive or resistant'

based only on the parameters representing the distal portion of the cell survival curve can lead to erroneous conclusions.

Differences in the extent of potentially lethal damage repair (PLDR) exist between cells obtained from radiocurable and non-radiocurable tumors (18, 33, 34, 38). Differences in the value of D (and SF_2) exist between tumor cell lines of differing radiosensitivity. Further investigations to explore inter-relationships between PLDR and D are needed. This may lead to explanations of some of the radiobiological observations that are inexplicable by 'repair-models' at this time (39). These investigations might lead to prediction of radiocurability in individual patients (28). More recently, Elkind (40) points to the shortcomings of using D or SF_2 . Only a long-term clinical correlation of local tumor control with various radiobiological parameters (\bar{D} , N , D , SF_2 , PLDR) (40-42) can determine whether human tumor radiocurability can be predicted using in vitro radiobiological parameters.

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