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The effect of delivering compensating doses on the survival of F10B16 melanoma and 4T1 breast adenocarcinoma treated with prolonged radiation delivery time

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► ***Original article***

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ABSTRACT

Background: Increasing the complexity in modern radiotherapy techniques have increased the delivery time lowering consequently the treatment efficacy. Through simulating the delivery time delay encountered in such techniques, its' effect on two cancer cell lines and the compensating doses given to prevent such effect was investigated. ***Materials and Methods:*** F10B16 and 4T1 cancer cell lines were exposed to simulated clinical fractionated radiotherapy procedures commonly used in complex techniques. The survival rate of the cells exposed to 2, 4, and 6 Gy of ionizing radiation with two equal subfractions given at various time intervals between the fractions (0.25-4 hours) were determined using the MTT assay. Then, relevant compensating doses were calculated and their efficacy in counterbalancing the time delay was assessed. ***Results:*** The cells' survival was increased with prolonged treatment times in the fractionated groups being more significant at the lower time intervals (up to 2 hours) and for the higher radiosensitive cells (4T1). Giving the compensated doses decreased the survival of the cells. ***Conclusion:*** Delivering appropriate compensating doses to the prolonged fractionated groups can counterbalance the effect of time delays encountered in complex radiotherapy techniques.

Keywords: *Survival fraction, ionizing radiation, radiobiological models, cancer cell lines, fractionation.*

INTRODUCTION

Dose fractionation in radiation therapy plays an important role in protecting normal tissues and tumor reoxygenation [(1)]. Many studies have investigated the outcome of the dose fractionation, prolonged treatment period, and patient absence from the sessions over the treatment period [(2-9)]. Results of those investigations have indicated considerable tumor recurrence that cannot be compensated if just the total number of sessions and doses is adhered by the end of the treatment. to compensate the effect of such procedures, researchers have focused on the basic radiobiological models and suggested some ways to calculate the extra compensating doses required to be delivered [(2-8)]. Some of these investigations have also showed that using shorter treatment time in more sessions and higher doses per fraction reduces the risk of tumor recurrence of some tumors compared to prolonged treatment periods [(3-5)].

Anyway, the usage of modern complex conformal modalities has opened new horizons on this issue being comprehensively considered before. Conformal dose distribution cannot be

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achieved when the shape of the target is complex or there are sensitive normal tissues surrounding the target [10]. At such situations, rather complicated modalities are used such as: intensity modulated radiation therapy (IMRT), image guided radiotherapy (IGRT) and so on. In such modalities, the overall treatment time for one fraction is often increased proportional to the complexity of the modalities [10-12]. Consequently, the radiobiological effectiveness of such modalities will be different from the conventional radiotherapy techniques due to the ongoing sublethal damage repair (SLDR) happening during the time intervals between the fractionated dose–delivery process [10, 13-18]. Previous studies [10, 13-18] have just investigated the effect of the total prolonged treaetment time on the outcome of complex radiotherapy modalities. They have investigated theoretically and experimentally such effects on some cell lines and claimed that increasing the radiation delivery times may decrease significantly the level of radiation cell killing and have a significant effect on the treatment results. However, those studies have neither embraced all the cell lines nor calculated and tested the compensating doses required to be given in such procedures.

Based on the recommendations proposed before[19-21], it seemed appropriate to implement a more complex form of the developed radiobiological linear quadratic (LQ) model enabling us to consider the effect of delays between subfractions encountered in modern radiotherapy modalities that have never been studied before [2-8].

Furthermore besides the models proposed for compensating the effect of the gaps between radiotherapy sessions (as commonly used in clinics), it also seemed more appropriate to investigate the efficacy of calculated doses delivered to compensate the effect of prolonged treatment times within one session encountered nowadays in complex radiotherapy techniques.

Hence, the effect of such prolonged radiation delivery time on the cell survival of two cancerous cells of interest (4T1 breast adenocarcinoma and F10B16 melanoma) was investigated to see whether these procedures have any significant effect on the treatment outcome, as previous studies [9, 18] had claimed that such prolonged procedures has no important effect on some types of cells. Thereafter the effect of compensating doses derived from the developed LQ model [19-21] and delivered to the above cells was analyzed.

MATERIALS AND METHODS

The cell lines

Monolayers of breast adenocarcinoma (4T1, ATCC CRL-2539) and melanoma (f10b16, ATCC CR-6475) cells were used in this study. The cells were cultured in RPMI-1640 medium containing 10% heat-inactivated FBS (Gibco Laboratories,Cergy Pontoise, France), 500 µg/ml geneticin (G418), 300 µg/ml glutamine, 0.25 µg/ml fungizone, 100 µg/ml streptomycin, and 100 units/ml penicillin G. The cells, were adherent and grew as monolayer at 37°C in a humidified 5% CO_2 incubator. The cells' concentration in the culture was adjusted to allow for exponential growth.

The radiobiological model used for calculating compensating doses

The basic LQ model is the main radiobiological model used to predict the cell survival following a given radiation dose [19-25] as described the following simple formula 1:

$$S = \exp(-\alpha D - \beta D^2) \qquad (1)$$

in which S is the cell survival for a single radiation dose, D is the dose delivered and α and β are two constants representing mathematically the "direct killing of the cells" and "impact of the cell killing due to double hits" respectively [22].

However, to consider the biological effect of the treatment regimes given in fractionated radiotherapy protocols composed of subfractions, the corrections due to incomplete repair of sublethal damages within a fraction must be taken into account. For this purpose, a developed form of the LQ model proposed [19-21] and used in recent studies [10, 13, 15, 18] was implemented. Based on this model the survival fraction is derived from equation 2:

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$$S = exp - (\alpha D + G\beta D^2) \qquad (2)$$

in which the G parameter is represented as a correction factor for the incomplete repair which assumes no recovery during actual irradiation but rather during the time between subfractions defined as equation 3 [10]:

$$G = \frac{2}{n'^2}\left[\frac{\theta}{1-\theta}\right]\left[n' - \frac{1-\theta^{n'}}{1-\theta}\right] + \frac{1}{n'} \qquad (3)$$

in which n' is the number of subfractions in one fraction and θ represents the exponential of sublethal damage derived from the equations 4 and 5:

$$\theta = \exp(-\frac{\delta T}{\tau}) \qquad (4)$$

$$\tau = \frac{T_{1/2}}{Ln2} \qquad (5)$$

in which δT, τ, and $T_{1/2}$ are "the time interval between different subfractions within one fraction", "the recovery time of sublethal damages" and "the half-time of sublethal damage" respectively [10].

Equation 2 was first used to calculate the G parameter. To achieve this, the relevant values of the cells' survivals, α and β values [30], and the level of the radiation doses to which the cells were exposed (D) were put in the equation. Thereafter, the calculated G value was used in equation 3 to calculate θ which was then put in equation 4 to calculated T. Finally, by using equation 5, $T_{1/2}$ was determined.

To determine the relevant compensating dose levels for the fractionated treatment groups by which the same rate of the survivals as that of the group exposed continuously to ionizing radiation, Equation 2 was used again. In this regard, the S value for all the fractionated groups was set equal to that of the continuous group and their G values were derived by putting their relevant known parameters in equations 3, 4, and 5.

Irradiation procedure

A Co-60 unit (Imatron, Canada) was used as the radiation source. The ionizing radiation was delivered in a 25×25 cm^2 field size. The source-half-depth distance was initially calculated to obtain a constant dose rate of 0.81 Gy/min. All irradiations were performed at a distance of 20 cm between the radiation sources and plate. A 4-cm polystyrene block was used under the plates during each irradiation to provide homogeneous backscattering γ-rays.

The MTT assay

The MTT assay was used to determine the survival curves and cell parameters as explained in other studies [26-30]. To determine the time constant for the repair of sublethal damages, an experiment was set up in which several groups of the cells were exposed to two subfractions with different time intervals between the sub-fractions (from 0.25 to 4 hours). The survival fraction was plotted against the time between fractions. Then, the $T_{1/2}$ and τ parameters were estimated as proposed before[10].

It was predicted that using the 2 Gy dose level (as commonly used in every clinical radiotherapy session) may lead to low differences among various treatment protocols designed and performed on the cell. Furthermore, the main objective of this study was to evaluate the ability of the developed LQ model in compensating the effects of delays and prolonged treatment times. Therefore a wider range of doses (2-6 Gy) was used firstly to find the suitable level of the dose leading to significant differences among various treatment protocols. Based on the result of this stage described later in the results the lowest dose level of 4 Gy was proved to be suitable. Then, the next stage of our study to calculate the relevant compensating doses was done only for the 4 Gy dose level to investigate whether the developed model can be used to calculate accurately the appropriate level of the dose to compensate the effect of prolonged treatment time and thereafter generalize it to other dose levels.

To investigate the effect of the total treatment time on the cells' survivals, several experiments were made on separate samples exposed to various radiation treatment regimes consisting of the continues doses of 0, 2, 4 and 6 Gy and three fractionated doses of 2, 4, and 6 Gy given in two equal fractions of 1, 2 and 3 Gy respectively with different time intervals (0.25, 0.5, 1,

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2, 3 and 4 hours) between them.
According to the results reported by others [15], increasing the number of subfractions and time variation among them in a fixed total treatment time of one fraction do not have any significant effect on the cell survival and just the total treatment time of subfractions within a fraction is an effective parameter. Accordingly, to simplify the calculations and comparisons between several experimental treatment groups, only two subfractions was used for all of them.
Although a total treatment time of more than 1 hour is not usual in clinical practices, due to the possible different responses of the cells to ionizing radiation, we followed the study up to a longer treatment time period of 4 hours to investigate the response of the cells in longer dose delivery times and also the ability of the developed LQ model used to compensate the effect of such extreme delays.

The second step was set up to investigate the ability of the developed LQ model in determine the relevant compensating dose levels for the fractionated treatment groups by which the same survival rates could be obtained as acquired for the groups exposed continuously. Therefore, Equation 2 was used in which the S value was set equal to that of the continuous irradiated groups.

Statistical analysis

Statistical analysis was performed using the SPSS software (version 16.0) based on the mean±SD values of the robustness of the samples. To assess the specific effect of the different irradiation protocols, the analysis of variance (ANOVA) was used. The differences between the groups were considered to be significant within a 95% confidence interval or a p-value ≤ 0.05.

RESULTS

The survival curves, α, β and $T_{1/2}$ parameters

The survival curves determined for the two cell lines (F10B16 and 4T1) are shown in figure 1. As seen, the 4T1 cell line exhibit a smaller shoulder and steeper linear compartment compared to F10B16 cell line.

The calculated values of the α, β and $T_{1/2}$ parameters for the F10B16 and 4T1cells are presented in table 1. All values shown in table 1 are significantly different for the two cell lines ($p<0.05$).

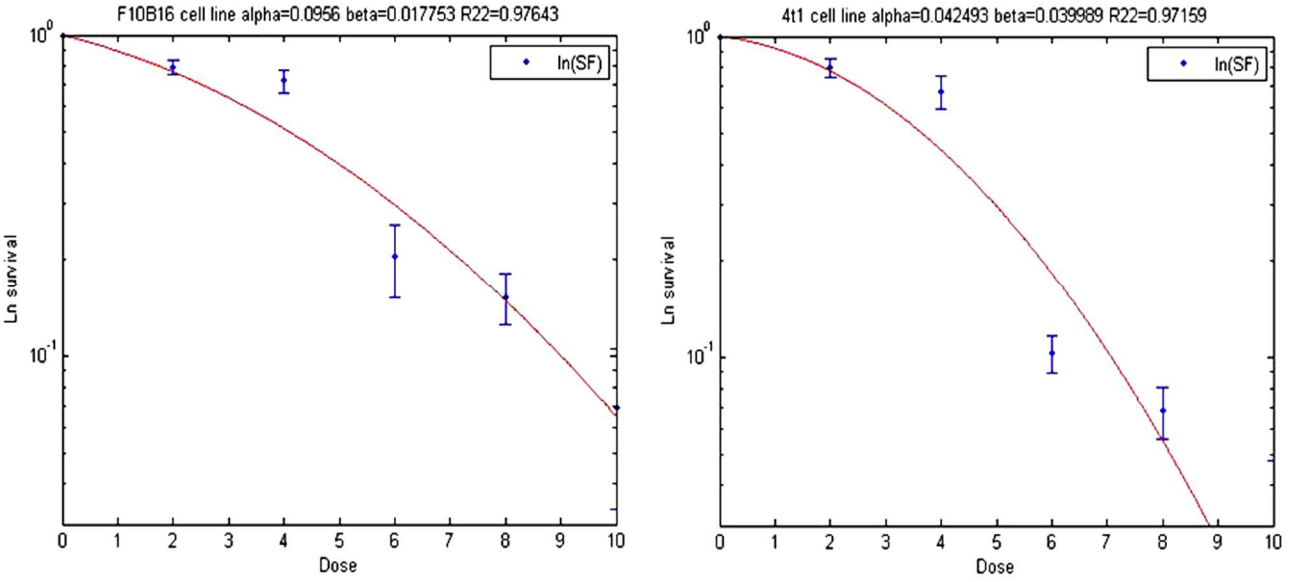

Figure 1. The survival curves of the melanoma F10B16 and breast adenocarcinoma 4T1 cell lines after exposure to various doses of gamma radiation.

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Table 1. The calculated values of α and β and T1/2 parameters determined for the F10B16 and 4T1 cells.

	F10b16	4T1
α(Gy^{-1})	0.0956(R^2=0.98)	0.0424(R^2=0.97)
β(Gy^{-2})	0.0177(R^2=0.98)	0.0399(R^2=0.97)
$T_{1/2}$(hour)	0.524±0.035	0.343±0.015

The effect of increasing the treatment time on survival fractions

The survival fractions of different groups exposed continuously to 2, 4 and 6 Gy of radiation and the groups exposed to the same level of radiation given in two equal subfractions and at various time intervals between the subfractions are shown in figure 2a and 2b for the F10B16 and 4T1 cells respectively.

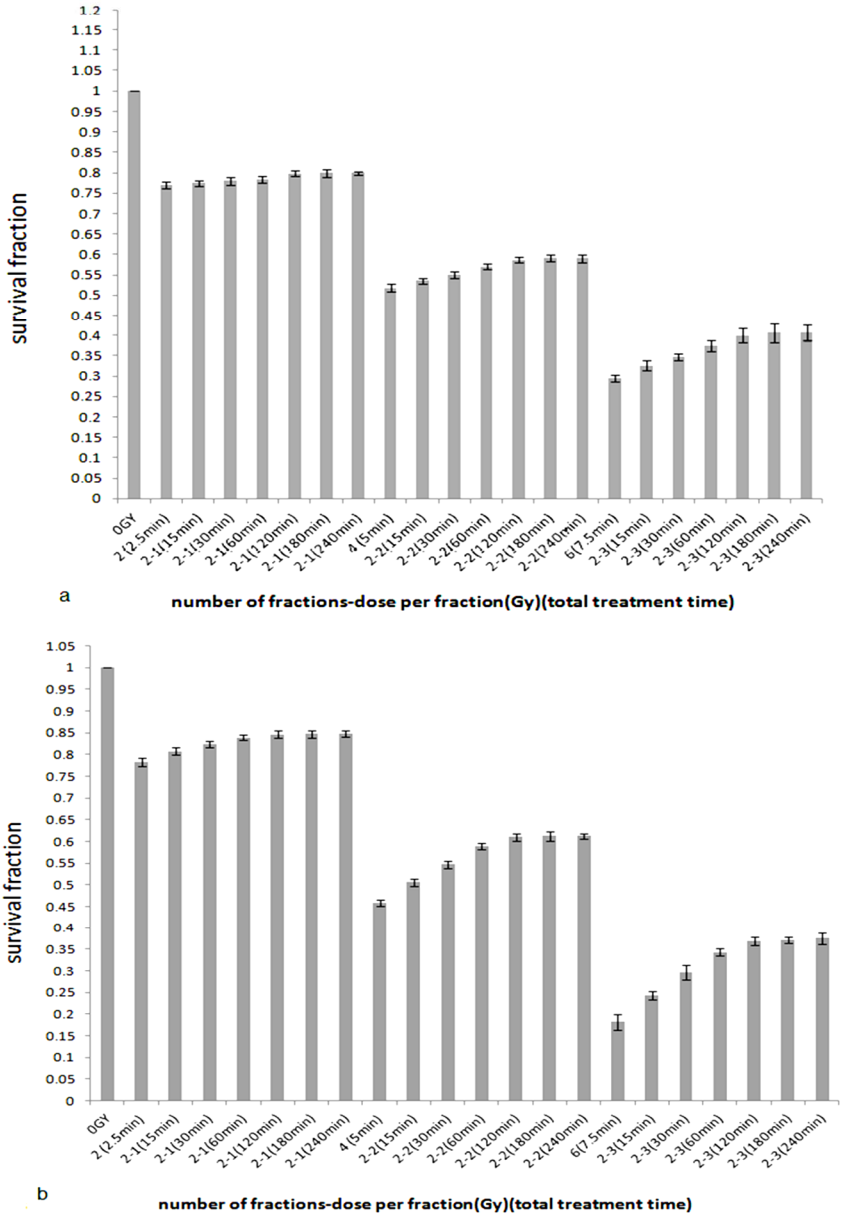

Figure 2. The effect of various fractionated radiation treatment protocols with various time intervals between the fractions on the survival fraction of the F10B16 (a) and 4T1 (b) cells.

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The results indicated that at 2 Gy, various fractionation regimes and increasing the treatment time makes no significant difference on the survival of the F10B16 cells. But, for the more radiosensitive cells of 4T1, increasing the treatment time increases the cell survival at all three levels of doses. However, at higher doses, various fractionated regimes given at the time intervals up to 2 hours caused an increase in the survival fraction of the cells both which was higher for the 4T1 having a lower α/β ratio and shorter $T_{1/2}$.

The effect of compensating doses on the survival fractions

The effect of giving the calculated compensating doses on the survival fractions of various fractionated groups with various time intervals are demonstrated in figure 3 in which 3a and 3b show the survival fractions against the usual doses while 3c and 3d show this parameter against the compensated doses given to the F10B16 and 4T1 cells respectively.

Figure 3 demonstrates the level of the differences happened after implementing the compensating doses for each group with various delay times between the fractions.

DISCUSSION

New fractionated radiotherapy techniques with rather complicated and more subfields prolong the treatment time. Consequently, it is claimed [10, 13-18] that these techniques are less effective, since their treatment prolongation lead to an increase of cancerous cell survival compared with conventional radiotherapy techniques delivering continuously the same level of dose. The reason of increasing the cell survival treated with prolonged treatment time is thought to be due to the SLDR process occurred during the time intervals between fractions/subfractions when there is enough time between fractions [10, 13-18].

Previous studies [2-9] have used various

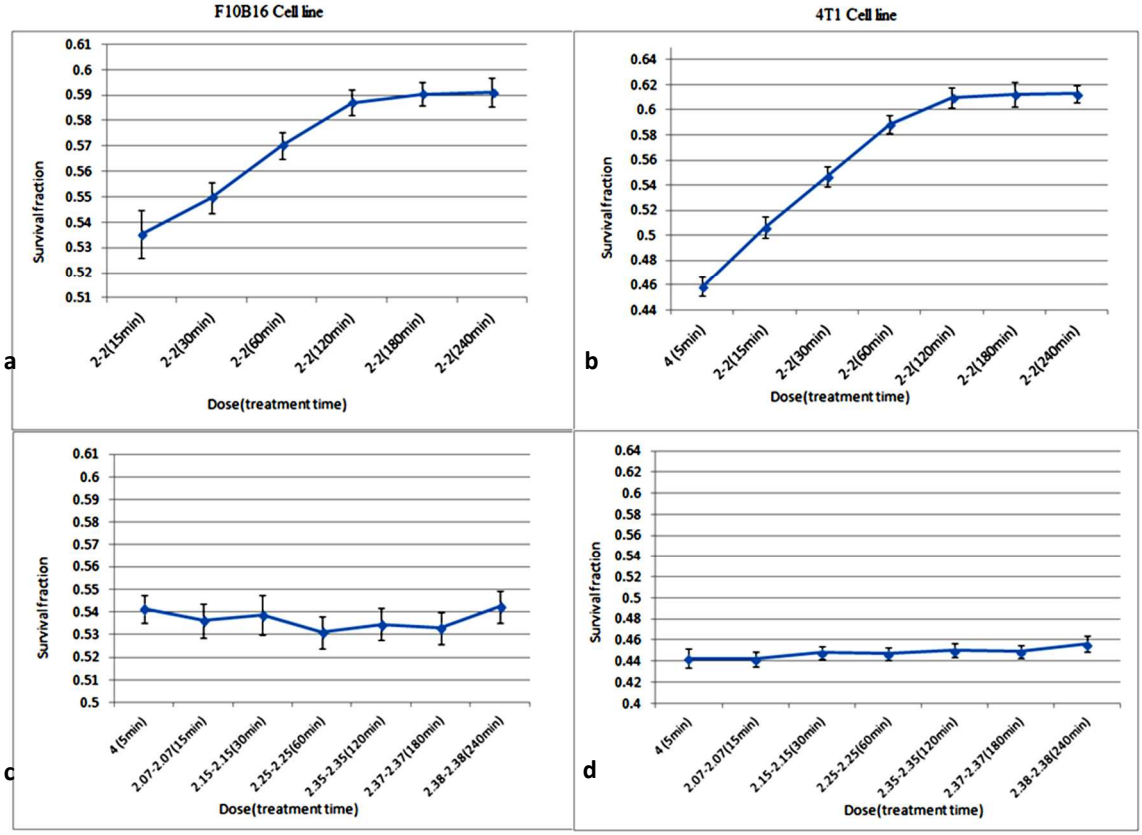

Figure 3. The survival fractions of the F10B16 (a and c) and 4T1 (b and d) cells with different time intervals.

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models just for compensating the effects of the delays due to either the gaps happened between radiotherapy sessions or the total prolongation of the radiotherapy procedure. But, in this research, based on the developed LQ model, the relevant doses were calculated and delivered to the cells to compensate the prolongation encountered due to the longer time spent for implementing subfractions within a radiotherapy fraction/session.

The formalism used in this study was the one proposed [10, 19] for the generalized incomplete repair model being based on the developed LQ model and limited to the constant exposure times and time intervals between the exposures. It is believed that the quadratic term in the developed LQ model reflects a cell capability to repair sublethal damages when fractionated delivery time is comparable or longer than the half-time for the repair process. The dose rate effects are included in this model by applying a so-called dose protraction factor, G, to its' quadratic term (βD^2) which depends on the characteristic repair rate of sublethal damages [10, 19-21].

In agreement with other studies [10, 13-18], as can be inferred from our results, the 4T1 cells having lower α/β and shorter $T_{1/2}$ characteristics has a large ability to undergo the SLDR process compared to the other cells (F10B16). Therefore, it could be concluded that the effect of prolongation of the treatment time in complicated radiotherapy modalities is expected to be significantly higher in the tumors with lower α/β ratio demanding more attention to be paid.

Although, the treatment time of more than 1 hour is not usual in clinical radiotherapy procedures we performed our experiments up to 4 hours. However, as indicated in Figure 3, for the higher time intervals (>2 hours), the differences between the survival fractions of the cells are negligible. But, for the time intervals less than 2 hours, which is common in clinical practices, increasing the time intervals between the subfractions increases the survival of both of the cells, but at a higher rate for the more radiosensitive cells (4T1).

A theoretical method used to increase the rate of the cell death for prolonged fractionated radiotherapy protocols is to increase the level of the radiation treatment dose [5] for each subfraction to higher levels known as the compensating dose and the developed LQ model can be used to estimate such doses. Therefore, as the results of this study indicated an increase in the cell survival fraction of the groups exposed to prolonged fractionated radiotherapy protocols, the compensating doses were estimated for these groups using the developed LQ model. The results confirmed that exposing these fractionated groups to the additional level of compensating doses decreases the percentage of cells' survival to the same level of the group exposed continuously to the common level of dose.

Therefore, by using the developed LQ model, the exact amount of the compensating dose to achieve the same survivals of conventional radiotherapy protocols could be calculated and used to increase the treatment efficiency of modern fractionated radiotherapy protocols.

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