Imaging dose affects *in vitro* survival following subsequent therapeutic irradiation

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**Abstract**

The aim of this study is to determine whether the additional dose from imaging procedures such as four-dimensional computed tomography (4DCT) and cone beam computed tomography (CBCT) affect cell survival when followed by therapeutic radiation. A clonogenic assay was used for one human normal (human umbilical vein endothelial cell) and two cancer (NCI-H460 and MM576) cell lines. Each cell line was exposed to a kilovoltage 4DCT or CBCT imaging dose prior to therapeutic doses of 0, 2 and 4 Gy. The time interval between imaging and therapy was 8 h (4DCT) and 6 min (CBCT). To increase the statistical power of the analysis, the results were combined and the effects of the imaging dose tested for significance. The survival fraction was compared with the predictions of the Lea–Catcheside linear-quadratic (LQ) model with repair. The observations show that a CBCT dose (0.6 cGy) with no therapeutic dose caused an increase in survival for NCI-H460, however this effect was marginally significant. The pooled data showed that a 4DCT dose (14 cGy) caused a significant decrease in survival (*p* = 0.0017) when followed by a 2 Gy therapeutic dose. This effect was larger than the theoretical prediction of the LQ model. Our results suggest two opposing low dose radiation effects: an increase in survival (hormesis) imparted by the smallest imaging doses and a decrease in survival (hypersensitivity) imparted by the larger imaging doses. The LQ model was a poor predictor of survival for small doses. The prime novelty of this work is the finding that 4DCT imaging can affect cell survival after a subsequent therapeutic dose. This study identifies the potential to increase the therapeutic ratio by using the imaging dose as a priming low dose partial fraction.

**Nomenclature**

| Abbreviation | Description |
|--------------|-------------|
| CBCT         | cone beam computed tomography |
| 4DCT         | four-dimensional computed tomography |
| IGRT         | image-guided radiotherapy |
| kV           | kilovoltage |
| MV           | megavoltage |
| LQ           | linear-quadratic model |

**1. Introduction**

Adaptive radiotherapy, gated treatments and image-guided radiotherapy (IGRT) offer advantages of accurate targeting of dose to the tumour volume and are becoming increasingly used. The additional imaging dose administered to the patient inherent in these techniques has been raised as a potential concern, because of an increase in deterministic effects to organs at risk and stochastic effects to the region subjected to the imaging dose (Stock *et al* 2012). More frequent image verification and use of IGRT in response to imperatives for better treatment...
localization may further increase the patient imaging dose (Dawson and Jaffray 2007). One such imperative is the need to obtain more information about tumour location and movement due, for example, to the effects of bladder and rectal filling (Fokdal et al 2004, McBain et al 2009, Lalondrelle et al 2011, Gwynne et al 2012, Bell et al 2014) or respiratory motion (Korremann et al 2008, Falk et al 2014, O’Brien et al 2014). A second imperative is the need for high accuracy in hypofractionated treatments to avoid delivering a high dose to a geographic miss (Nijkamp et al 2008, Bell et al 2014). This imperative is increasing in importance because of the developing trend towards fewer fractions of higher dose for example in prostate treatment (Valdagni et al 2005, Habermann et al 2013). The range of doses delivered by imaging procedures is discussed in the literature (Yang et al 2009, Hyer et al 2010, Tomic et al 2010, Ding and Munro 2013, Hyland et al 2014, Hu and McLean 2014, Létourneau et al 2015). Dose ranges are 0.04 cGy (Hyer et al 2010, Ding and Munro 2013) to 3.62 cGy (Létourneau et al 2015) for cone beam computed tomography (CBCT) and 11–17 Gy for four-dimensional computed tomography (4DCT) (Hoang et al 2015, Tomic et al 2015).

An imaging dose is a side effect of treatment localization, but may present an independent opportunity to increase the therapeutic ratio. One way in which this could occur is if the imaging dose were sufficient to induce low dose hyper-radiosensitivity (Joiner et al 2001). Short et al (1999, 2003, 2005) reported that for doses less than 50 cGy, low dose hyper-radiosensitivity results in a significantly greater reduction in cell survival than expected from the evidence on larger doses. It is conceivable that by giving a small dose, sufficient to cause hyper-radiosensitivity, a subsequent larger dose may have a greater than expected effect. This concept is supported by Lin and Wu (2005), who reported a study of eleven cell lines receiving different sequences of small and large partial fractions for a total 2 Gy dose. The smaller, or ‘priming’ dose was between 10 and 50 cGy, however, the majority were between 30 and 50 cGy and similar to those described by Short et al (1999, 2003, 2005). Lin and Wu found that the cell survival was lower when the small dose preceded the larger dose, than when the doses were delivered in the reverse order. Based on this evidence, it might be expected that the addition of a small imaging dose prior to the larger therapeutic dose would decrease the surviving number of tumour cells expected from therapeutic radiation alone.

Another way in which a small imaging dose could have an effect is through the induction of radiation hormesis, where an adaptive response is imparted to subsequent larger therapeutic doses that results in an increase in survival (Feinendegen 2005). Day et al (2006, 2007a, 2007b, 2007c) report an adaptive response induced by extremely low doses of radiation in the range 0.001–10 mGy. There was a reduction in chromosomal inversions induced by a subsequent 1 Gy dose in mouse prostate cells, pKZ1 (Day et al 2006, 2007b) and in the spleen and prostate of Atm knockout mice (Day et al 2007a). Suzuki et al (2001) report that a relatively larger x-ray dose of 2 and of 5 cGy, caused an increase in the cell number of both normal and lung cancer cell lines. They concluded that, for a limited range of low dose radiation, ERK1/2 kinases are activated, causing proliferation of both normal and lung cancer cell lines. The radiation hormesis reported for priming doses is in direct contrast to the hyper-radiosensitivity effects below 50 cGy discussed above. Since there are opposing effects reported for small and very small pre-treatment imaging doses, an appropriate design of experiments to disentangle them is needed. Further, such experiments should use clinically relevant imaging doses and dose rates to enable translation of the findings.

Repair in cells following radiation exposure was originally described using the Lea–Catcheside linear-quadratic (LQ) model (Lea and Catcheside 1942), in which a repair half-time parameter is used to progressively remove, as exposure time for a constant dose is protracted, the contribution of the quadratic term in the survival curve. Reported repair half-times range from minutes to tens of hours, so that repair from the imaging dose from CBCT, given only minutes before treatment, is unlikely to have taken place before the therapeutic dose is delivered. Conversely, 4DCT is usually administered a week or two before treatment begins, so that repair of damage induced by the imaging dose is likely to be complete by the time treatment commences.

The radiobiological effect of varying the temporal sequence of dose delivery of partial fractions for a given total dose has been reported in the literature in terms of its effect on repair (Altman et al 2006, Murphy et al 2007, Altman et al 2009, Bewes et al 2012). The temporal sequence may also influence the opportunity for bystander signals to express their effect. Bystander effects are responses communicated by signalling between exposed and unexposed cells and have been reported to also affect radiobiological outcomes. Bystander effects can be stimulated by a small priming dose, which could modify the outcome of the subsequent therapeutic dose (Prise and O’Sullivan 2009, Suchowerska et al 2010). Bystander effects therefore form part of the overall response and their contribution may also be subject to changes in the temporal sequence of dose delivery.

Hyland et al found that for DU-145 metastatic prostate cancer, NCI-H460 non-small-cell lung cancer and AGO-1522b normal tissue fibroblast cell lines, an imaging dose delivered prior to a therapeutic dose had no measurable effect on cell survival. The imaging exposure of Hyland et al was to a 6 MV photon beam to a dose of 5 cGy. In our study, both the CBCT and 4DCT use kilovoltage beams which are combined with a subsequent 6 MV therapeutic dose. We consider the effects of CBCT (0.6 cGy) and 4DCT (14 cGy), which
are lower and higher respectively than the 5 cGy dose for electronic portal imaging (EPI) considered by Hyland et al (2014). Our intention is to use the CBCT exposure as a test of possible low dose hormesis and the 4DCT exposure as a test of possible low dose hyper-radiosensitivity. The effects on cancer and normal cell lines need to be assessed since both will be exposed to the imaging doses as well as therapeutic doses.

2. Materials and methods

2.1. Theory

When a dose \( D_1 \) is given prior to another dose \( D_2 \) the total dose \( D \) is given by

\[
D = D_1 + D_2.
\]

(1)

The LQ formula has been used to predict survival \( S \), after the dose \( D \)

\[
S = e^{-\left(\alpha D + \beta GD^2\right)},
\]

(2)

where \( \alpha \) and \( \beta \) are the cell specific parameters that determine the response to radiation, to be determined experimentally. \( G \) is the Lea–Catcheside dose protraction factor, which for an exposure consisting of a short imaging dose, \( D_1 \) and a short therapeutic dose, \( D_2 \) separated by a time \( t \) is given by

\[
G = \frac{2}{D^2} \left[ \frac{1}{2} D_1^2 + \frac{1}{2} D_2^2 + D_1 D_2 e^{-\lambda t} \right].
\]

(3)

\( \lambda \) is the repair constant related to the repair half time \( R_{1/2} \)

\[
\lambda = \frac{\ln 2}{R_{1/2}}.
\]

(4)

In this work, the assumed value of the repair constant was that of Bewes et al (2012) where a repair half time, \( R_{1/2} \) of 5.9 min (354 s) was used for NCI-H460 cells. Values of \( \lambda \) have not been published for the other cell lines used in this study. The theoretical predictions of the Lea–Catcheside model are dependent on the accuracy of the values of \( \lambda \).

The change in survival, \( \Delta S \), caused by the imaging dose \( D_1 \), is given by differentiating equation (2) with respect to \( D_1 \),

\[
\Delta S = \frac{dS}{dD} D_1 = S(-\alpha - 2\beta GD)D_1.
\]

(5)

Defining the sensitivity of an experiment to a dose \( D_1 \) by the change in survival divided by \( D_1 \), from equation (5) and taking the absolute value, it can be seen that the sensitivity of the experiment is small when \( D \) is very small or very large. When \( D \) is very small, the sensitivity is \( S \alpha \) and when \( D \) is very large the sensitivity is zero, since \( S \) vanishes. A useful guide in designing an experiment to test the effect of a small dose, \( D_1 \) is to ensure that the total dose \( D \) should give approximately 50% survival, where the derivative \( \frac{dS}{dD} \) is large.

A second guide in designing an experiment that has the power to test the effect of a small dose is to ensure that the sample number, \( n \), is sufficient to yield statistical significance to the required level. For this reason, our experiment was designed to provide 8–12 samples for each of three cell lines and for each imaging modality. The data for all three cell lines was pooled to form a sample with \( n = 31 \) for each of the exposures to 4DCT and CBCT.

2.2. Experimental methods

2.2.1. Cell culture

A non-small cell lung cancer cell line (NCI-H460), a melanoma cell line (MM576) and a normal human umbilical vein endothelial cell line (HUVEC) were obtained from American Type Culture Collection (ATCC). Both cancer cell lines were maintained in RPMI-1640 medium (Gibco Life Technologies, Australia) supplemented with 10%v/v foetal bovine serum (FBS) (Gibco Life Technologies, Australia) as recommended by ATCC. Normal HUVEC cells were maintained in Medium M199 (Gibco Life Technologies, Australia) supplemented with 20%v/v FBS, 13.4 mM sodium bicarbonate, 20 mM HEPES, 1× non-essential amino acids, 1 mM sodium pyruvate, 1× Glutamax (all from Gibco Life Technologies, Australia) and 3 ul ml\(^{-1}\) of heparin (Sigma-Aldrich, MO, USA) and 0.048 g L\(^{-1}\) endothelial cell growth factor (ECGS) (In Vitro Technologies, Australia). Flasks for HUVEC cells were coated with 0.1% gelatin for a minimum of 15 min at room temperature prior to adding the cells and all experiments were carried out between passage 4–6. All cell cultures were kept in a humidified incubator with 5% CO\(_2\) at 37°C and were allowed to grow to 80% confluence. To minimize stress on the cells, no antibiotics or antifungal agents were used in these experiments (Potter et al 2011).

Once 80% confluent, the growth medium was discarded and the adherent cells were washed with phosphate buffered saline (Gibco Life Technologies, Australia) twice. Cells were then detached using 0.05% trypsin-EDTA solution for 4–6 min at 37°C as previously described (Franken et al 2006). The detached cells were centrifuged at 252 × g for 5 min at room temperature.

The cells were then plated in T25 cm\(^2\) flasks (Corning, MA, USA) at a density of 600 cells (cancer cell lines) or 1500 cells (normal cell line, on gelatin-coated flasks). Each flask was supplemented with 20 ml growth medium to create conditions of electronic equilibrium upon irradiation.

2.2.2. Selection of the time between imaging and treatment

For determining clinically relevant timing of imaging dose \( D_1 \) and therapeutic dose \( D_2 \), an analysis of the treatment history for 8 bladder patients was taken
using the Varian offline review programme (Varian Medical Systems, Palo Alto, CA). A total of 220 image sets were assessed. For the 8 patients considered, the time \( t \) between CBCT and treatment was between 2 min 29 s and 16 min 56 s. The average time of 6 min between imaging and treatment doses was used for these experiments.

In contrast, 4DCT images are typically taken at the planning stage of the treatment process, some 7–14 days prior to the start of treatment delivery. While it is recognized that cellular processes subsequent to irradiation may continue for longer than 8 h, it is not feasible to keep cells in culture for an extended period without intervention and passage. Consequently the therapeutic dose was delivered 8 h after the 4DCT dose, since it was considered that by this time, at least any repair as a consequence of the imaging dose would have been completed.

2.2.3. Irradiations

For the CBCT experiments, the plated cells were incubated overnight at 37 °C. A CBCT of the flasks was then performed on the Varian Novalis linear accelerator. After 6 min, the cells were irradiated with 6 MV photon beam on the same linear accelerator at a dose rate of 6 Gy min\(^{-1}\). To achieve full scatter conditions, the flasks containing the cells were placed in a custom built perspex phantom that accommodates T25 flasks (figure 1). The phantom was placed between slabs of solid water, to locate the cell layer at a depth of 50 mm when irradiated at gantry 180° as previously described (Claridge Mackonis et al 2012). The cells were irradiated to a uniform dose of 2 and 4 Gy. For all experiments, unexposed controls (0 Gy) were prepared as sham exposures.

For the 4DCT experiments, plated cells were incubated overnight at 37 °C, then exposed to a 4DCT on a Toshiba Aquilion wide bore CT, with the Varian RPM motion management system. The cells were then returned to the incubator for 8 h. The flasks were then irradiated on the Varian Novalis linear accelerator to doses of 2 and 4 Gy with controls as described above.

The dose to the cell layer in the flasks, positioned within the phantom from the imaging procedures was measured using a Roos parallel plate ionization chamber (PTW, Freiburg) which had been previously calibrated in an x-ray beam of similar kV and a known dose. The active volume of the ionization chamber was placed in the same position as the cell layer in a mock-up of the actual cell irradiation geometry (figure 1). The measured dose was independently confirmed using OSL dosimeters using a technique similar to that described by Létourneau et al (2015). The measured dose to the cell layer was 0.6 cGy from CBCT and 14 cGy from 4DCT.

Following irradiation, all flasks were incubated at 37 °C for 7–9 days (cancer cells) or 14 days (normal cells) until colonies of greater than 50 cells formed. Cancer colonies were fixed and stained with 0.3% methylene blue in 50% ethanol (Sigma-Aldrich, MO, USA). HUVEC cells were fixed and stained with crystal violet to increase the contrast of the disperse colonies. The number of colonies were counted using ColCount colony counter (Oxford Optronix, United Kingdom). All experiments were carried out in triplicate and on three separate occasions.

2.2.4. Validation of statistical analysis

The most powerful statistical test we can apply to the data, using all cell lines, is the one-tailed paired Student’s \( t \)-test. This was applied to test the hypothesis that an imaging dose caused a reduction in the survival of cells. The two-tailed \( t \)-test was also applied to test the balanced hypothesis that an imaging dose caused a difference in the survival of cells. The paired \( t \)-test is used in situations where there are uncontrolled variables. In this work these include cell type, number of passages and the date on which the experiment was carried out. Paired data are used that have the same values of these uncontrolled variables within each pair. The \( t \)-test is applicable provided that the distribution of the differences is approximately normal. For a

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**Figure 1.** (a) Cell irradiation set up. (b) Only the central two flasks on the axis contained cells, the others were filled with water only. (c) The dose to the cell layer was independently validated using a Roos ionization chamber positioned directly beneath each of the flasks of interest. The doses were also validated using OSL chips.
discussion of the applicability of the paired $t$-test in this way, the reader is referred to McDonald (2014). A test of the normality of the distribution of the differences within each pair in our case shows that the distribution is approximately normal with a mean difference in survival of 0.0971 and a standard deviation of 0.2309 using a bin size of 0.1 (figure 3, inset).

Where the one-tailed test is used, we are interested in the probability that a survival of less than the observed value will occur in random sampling. Where the two-tailed test is used, we are interested in the probability that the survival is either greater than or less than the observed value in random sampling.

### 3. Results

Figure 2 shows the experimental clonogenic survival fraction as a function of therapeutic doses of 0, 2 and 4 Gy of 6 MV photons. All survival fractions are normalized to the control without imaging or therapeutic dose. The black bars were for cells that received either a CBCT or 4DCT imaging dose prior to the therapeutic dose, white bars received therapeutic dose only. Mean survival for three cell lines (MM576, NCI-H460 and HUVEC) are shown. The error bars are $\pm$ standard error of the mean for 8–12 samples per group. No significant differences ($p < 0.05$) for any imaging dose for all cell lines were observed, although in two cases at 2 Gy therapeutic dose, $p$-values close to 0.05 were obtained (as shown).
$p = 0.0514$ for MM576 cells with a prior 4DCT imaging dose and $p = 0.06602$ for HUVEC cells where a CBCT was given prior to the 2 Gy dose.

A more powerful test of the hypothesis that an imaging dose causes a reduction in the cell survival can be obtained by pooling the data from all cell lines into single samples of larger number for each imaging modality at each therapeutic dose level (Figure 3, Table 3) and applying a paired $t$-test for the differences between samples that received the imaging dose and those that did not. In this pooled data set, effects that are present across all cell lines are emphasized relative to effects that are cell line dependent. Since our data set consists of a composite of different cell types, this test is statistically valid, provided that the pairwise differences are approximately normally distributed about their mean with a single variance. The distribution of differences about the mean is shown in Figure 3 (inset), confirming that it approximates a single normal distribution. In one case, for therapeutic dose of 2 Gy

| Table 1. Mean clonogenic survival for CBCT. |
|---------------------------------------------|
| 0 Gy −CBCT +CBCT 2 Gy −CBCT +CBCT 4 Gy −CBCT +CBCT |
| MM576 1.000 0.952 0.706 0.638 0.315 0.303 |
| NCI-H460 1.000 1.089 0.667 0.693 0.274 0.273 |
| HUVEC 1.000 1.001 0.901 0.787 0.673 0.596 |
| Pooled 1.000 1.011 0.771 0.712 0.441 0.403 |

| Table 2. Mean clonogenic survival for 4DCT. |
|---------------------------------------------|
| 0 Gy −4DCT +4DCT 2 Gy −4DCT +4DCT 4 Gy −4DCT +4DCT |
| MM576 1.000 0.959 0.622 0.493 0.223 0.283 |
| NCI-H460 1.000 0.998 0.835 0.747 0.300 0.364 |
| HUVEC 1.000 0.981 0.871 0.795 0.598 0.621 |
| Pooled 1.000 0.980 0.787 0.692 0.386 0.440 |

| Table 3. $p$-values from paired one- and two-tailed $t$-tests for the pooled cell line samples. |
|---------------------------------------------------------------|
| Dose (Gy) | − no CBCT—with CBCT +4DCT | − no 4DCT—with 4DCT +4DCT |
|---------------------------------------------------------------|
| 0 Gy | 0.3702 a | 0.40622 |
| 2 Gy | 0.05803 | 0.00165 b,a |
| 4 Gy | 0.06982 | 0.03653 c,b,a |

a Indicates that the one-tailed $t$-test was applied to test for an increase in survival rather than a decrease.
b Indicates significance.
c Indicates that a two-tailed $t$-test was applied.
with a prior 4DCT imaging dose of 14 cGy, there was a significant decrease in clonogenic survival ($p = 0.00165$) when tested using a one-tailed $t$-test. This remained significant when tested for a difference in survival using a two-tailed $t$-test ($p = 0.0033$). For CBCT the decrease in survival was close to 0.05 (table 3) indicating marginal significance. At a 4 Gy therapeutic dose, the 4DCT imaging dose increased survival significantly ($p = 0.0365$) using a one-tailed $t$-test, but when tested for a difference in survival using a two-tailed $t$-test there was no significance ($p = 0.073$).

Figure 4 shows the predicted clonogenic survival fractions for the three cell lines with and without CT. These predictions were made on the basis of a single repair time for all cell lines, the clinically relevant time delay between imaging and therapeutic doses and the $\alpha$ and $\beta$ cell line parameters from table 2.

The values of $\alpha$ and $\beta$ (table 4) were obtained by fitting the Lea–Catcheside equation (2) to the experimental survival curves (figure 5) in the absence of an imaging dose. The repair constant (4) was obtained from the literature (Bewes et al 2012). The predicted differences in survival with and without imaging doses were compared with those observed experimentally and tested for significance. The predicted mean difference in survival of all cell lines was 0.005, which is smaller than the experimentally observed mean difference of 0.095. In calculating the predicted survival we used the value of the repair constant for one of the cell lines (NCI-H460) (table 4). The prediction was significantly smaller than the observation ($p = 0.003$). However if repair is ignored in the prediction, yielding a mean difference in survival of 0.054, the prediction was not significantly different from the observation ($p = 0.089$).
The alpha and beta values for each cell line were calculated by fitting all available survival data as a function of dose shown in figure 3, where an imaging dose was not included. The fitted values are shown in table 4. For MM576 and HUVEC cell lines, the formula (2) was used to fit the experimental observations using α and β as free parameters. In the fitting process the dose protraction factor, G, was assumed to be 1, since the doses were administered in single short fractions. For NCI-H460 the fitting process led to a negative value of α (−0.0155 Gy−1). A negative value of α implies a very small radiation dose increases clonogenic survival, since for very small doses, the βD2 term in equation (2) can be neglected in comparison to the αD term. A negative value of α and an increase in clonogenic survival are consistent with a proliferative response to very small doses and therefore with low dose hormesis (Suzuki et al 2001). A negative value of α may be considered inconsistent with the traditional assumptions of the LQ model that associates cell death with the linear term. An alternative method was used to determine α and β for this cell line: a positive value of α was obtained by fitting the initial slope of the survival curve at 2 Gy, neglecting the term in βD2 and determining the value of β by fitting the survival at 4 Gy. This approach excludes the low dose information from influencing the value of α.

Table 4. α and β values obtained from clonogenic survival assays.

|                | MM576 (melanoma) | NCI-H460 (lung) | HUVEC (normal) |
|----------------|------------------|----------------|---------------|
| α              | 0.0799 Gy−1      | 0.0523 Gy−1    | 0.0084 Gy−1   |
| β              | 0.0615 Gy−2      | 0.0484 Gy−2    | 0.0261 Gy−2   |
| λ              | 0.001 97 s−1     | 0.001 97 s−1   | 0.001 97 s−1  |

* In the case of MM576 and HUVEC, the values were obtained by fitting the linear-quadratic formula without repair. The value of α for NCI-H460 was −0.0155 Gy−1 when all available data were fitted using the LQ formula. A positive value of α for NCI-H460 was obtained (listed in the table) by fitting the initial slope of the survival curve at 2 Gy and determining the value of β by fitting the survival at 4 Gy. Also shown is the repair constant λ assumed to be equal for all cell lines.

4. Discussion

We find that an additional imaging dose of 14 cGy, delivered by 4DCT prior to a therapeutic dose of 2 Gy, causes a reduction in the expected survival of cells. Note that our 4DCT imaging dose is much higher than our 0.6 cGy CBCT dose and higher than the 5 cGy MV portal imaging dose used by Hyland et al (2014). The 14 cGy dose is in the range where Short et al (1999) observed low dose hyper-radiosensitivity. Lin and Wu (2005) associate low dose hyper-radiosensitivity with the reduced survival they observe when a small dose of less than 50 cGy is followed by a larger dose giving a total dose of 2 Gy. Their data shows that a low dose has the effect of ‘priming’ the cells so that they are more sensitive to a subsequent larger dose. Our results for a 14 cGy 4DCT dose followed by a 2 Gy dose are highly significant and remain so when either a one- or two-tailed test is used and are in agreement with the findings of Lin and Wu (2005). In contrast, when followed by a 4 Gy therapeutic dose, we find the effect of the same 4DCT imaging dose was to increase survival. While the apparent increase in survival caused by the 4DCT imaging dose is significant (p = 0.0365), there is no significant difference in survival when a two-tailed t-test was applied (p = 0.073). Due to the small survival fraction at 4 Gy, we note that the sensitivity of our experiment is lower than the sensitivity at 2 Gy. The reason for the potentially different effects at 2 and 4 Gy therapeutic doses needs further investigation.

The effect we observe at 2 Gy following a 4DCT imaging dose is significantly larger than we predict on the basis of the LQ model with repair. This could be due to a ‘priming’ effect of the imaging dose that leads to dose hypersensitivity. However, a recalculation of predicted survival using the LQ model without repair gave a result significantly different from the measured survival (p < 0.05). Therefore, we surmise that at least part of the reason for the larger than predicted effect of an imaging dose could be attributed to a lack of knowledge of the true repair half-time. If the repair half-time of the cells was much longer than the assumed 5.9 min, then the predicted effect was consistent with the observation. The repair time we assumed in making the predictions is only approximate since it applies to only one cell line (NCI-H460) and was based on the difference in survival between a triangular and a ‘V’ shaped temporal dose pattern (Bewes et al 2012). Repair half-times of up to 154 min have been found by Amdur et al (1994) for the normal cell line AG1522 and 31 min with HTB-35 uterine-cervical cancer cell line. Schmitt et al (2012) quote a repair half time for normal lung tissue as long as 4 h and used repair half-times for the lung cancer cell line NCI-H460 of 15 and 90 min. Since we included both normal and cancer cell lines in our study, our repair constant of 5.9 min is likely to be an underestimate of the repair half-time.

Imaging doses when given alone, appear to cause proliferative responses, evidenced by a larger survival fraction than in the control group (figure 2, NCI-H460 cells, +/- CBCT). This response appears to be cell line and dose dependent. For NCI-H460 cells, our findings of a negative value of the parameter α in fitting the LQ model, suggest a single low dose causes a proliferative response in these cells. When exposed to a 0.6 cGy imaging dose followed by a 2 Gy therapeutic dose, we found a small increase in survival as a result of the imaging dose, however this was only marginally significant. An increase in survival is consistent with the findings of Yang et al (2009), who observed a significant increase in proliferation (12%) for NCI-H460 cells, for an imaging dose of 5 cGy followed by a 2 Gy therapeutic dose. However, Yang et al did not observe
the increase in proliferation for HUVEC or other cell lines and found that it disappeared for the NCI-H460 cells when the imaging dose was increased to 10 cGy. For the lower dose range of 2 and 5 cGy given without any subsequent therapeutic dose, for lung cancer and normal cells, Suzuki et al. (2001) report enhanced proliferation. The increase we observed in NCI-H460 survival for an imaging dose alone of 0.6 cGy, although marginally significant, would be consistent with the finding of Suzuki et al. We confirmed that the low dose proliferative effect of a 0.6 cGy imaging dose is cell line dependent, since it did not become significant when the data from all cell lines considered were pooled. Our findings, combined with those in the literature, point to the existence of competing low dose effects: one effect increasing survival and one effect decreasing survival.

The LQ model, when fitted to our survival data, yielded values of the parameter $\alpha$ very close to zero (HUVEC) or negative (NCI-H460). These values suggest a low dose can cause a proliferative response. Deficiencies of the LQ model have been discussed previously but only for large radiosurgery doses (Guerrero and Li 2004, Kirkpatrick et al. 2008, Joiner and van der Kogel 2009). Kirkpatrick et al. (2008) report that the LQ model overestimates clonogenic survival for large radiosurgery doses, whereas Guerrero and Li (2004) report that the LQ model underestimates it. There are refinements of the LQ model that, while not predicting proliferation, incorporate the effects of repopulation and resensitization. Brenner et al. (1995) and Yang and Xing (2005) have proposed an extension of the LQ model to include some of these effects, that may improve its predictive power for small doses.

The use of the one-tailed, paired $t$-test on the pooled data ($n = 30–32$) increases the statistical power of our study compared to that of Hyland et al. (2014) ($n = 9$). When we tested the effect of the imaging dose on the individual cell lines with sample sizes comparable to that of Hyland et al ($n = 8–12$), we also found no significance. In our pooled samples, the mean survival of cells exposed to an imaging dose differed by 12% from that of unexposed populations which could not have been detected by Hyland et al.

The effect of an imaging dose could be included in treatment planning by considering imaging and therapy as partial fractions, delivered with different energy beams and at different time points. If the interval of time between the imaging dose and the therapeutic dose is longer than the time needed for that cell to repair, the effect of the imaging dose may be
reduced. Note that the field size and distribution of dose in the patient for kilovoltage imaging are frequently different to those for therapeutic treatment. Clearly from the evidence presented here, any attempt to include the imaging dose in the overall treatment plan would need to give consideration to these differences. This study provides observational evidence that imaging dose can potentially affect the final therapeutic outcome and this finding should be tested in animal studies.

5. Conclusions

Our study, together with those in the literature, points to the presence of competing low dose effects. At very small doses there is proliferation or imparted radio-resistance for some cell lines. As the dose increases, this effect is lost. Although we found the proliferative effect of very small imaging doses given alone was marginally significant, the larger imaging doses caused a significant decrease in survival following a subsequent therapeutic dose.

The benefits of imaging in radiotherapy are clear. These include avoiding geographic miss and minimizing the dose to healthy tissue thereby improving the therapeutic ratio. These benefits motivate some centres to move towards increasing the frequency of imaging throughout treatment (Dawson and Jaffray 2007, Lalandrelle et al 2011, Huddart et al 2014). Changes in tumour position on a daily basis, or even within a treatment period drive the need for imaging before, during and following treatment.

Our work shows that imaging doses of the size given in 4DCT have the potential to affect cell survival to a subsequent therapeutic dose. Therefore the therapeutic gain could be optimized by including the effect of the imaging dose in treatment planning. We recommend that before modification of treatment plans to include the effects of the imaging dose, further studies of the repair time and of low radiation dose effects on the relevant cell lines, should be undertaken. Future animal studies will enable fractionation of the therapeutic dose and different imaging regimes to be explored.

Conflict of interest

The authors have no conflict of interest.

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