Mechanistic modelling of radiotherapy-induced lung toxicity

^{1,2}E S RUTKOWSKA, PhD, ³I SYNDIKUS, MD, ¹C R BAKER, PhD and ¹A E NAHUM, PhD

¹Physics Department, Clatterbridge Cancer Centre, Bebington, UK, ²Department of Medical Physics and Clinical Engineering, Royal Liverpool University Hospital, Liverpool, UK, and ³Radiotherapy Department, Clatterbridge Cancer Centre, Bebington, UK

Objective: This work explores the biological basis of a mechanistic model of radiation-induced lung damage; uniquely, the model makes a connection between the cellular radiobiology involved in lung irradiation and the full three-dimensional distribution of radiation dose.

Methods: Local tissue damage and loss of global organ function, in terms of radiation pneumonitis (RP), were modelled as different levels of radiation injury. Parameters relating to the former could be derived from the local dose–response function, and the latter from the volume effect of the organ. The literature was consulted to derive information on a threshold dose and volume-effect mechanisms.

Results: Simulations of local tissue damage supported the alveolus as a functional subunit (FSU) which can be regenerated from a single surviving stem cell. A moderate interpatient variation in stem cell radiosensitivity (15%) resulted in a great variation in tissue response between 8 and 20 Gy. The threshold of FSU inactivation within a critical functioning volume leading to RP was found to be approximately 47% and the degree of health status variation (influencing the volume effect) in a population was estimated at 25%.

Conclusion: This work has shown that it is possible to make sense of the way the lung responds to radiation by modelling RP mechanistically, from cell death to tissue damage to loss of organ function.

Advances in knowledge: Simulations were able to provide parameter values, currently not available in the literature, related to the response of the lung to irradiation.

Received 6 July 2012
Revised 12 August 2012
Accepted 15 August 2012

DOI: 10.1259/bjr/28365782

© 2012 The British Institute of
Radiology

Normal-tissue complication probability (NTCP) modelling is the key to exploiting new planning and treatment technology in radiotherapy to optimal effect. The models help to select the best treatment plan, in terms of total dose, fraction size and beam configuration, for the individual patient's anatomy. Although the potential of current NTCP models for feeding clinical experience into treatment planning is becoming increasingly evident [1–5], they are largely empirically based, providing little or no insight into the underlying processes involved. Moreover, they ignore the spatial distribution of the dose deposited in the organ at risk. By contrast, this work explores the biological basis for a mechanistic model which makes a connection with the real cellular radiobiology involved in lung irradiation and takes into account the full three-dimensional (3D) dose distribution.

With conventional fractionation and prescribed radiation dose, lung tumour local control rates are of the order of 40% [6]; potential lung and oesophageal toxicity is generally put forward as the reason for such inadequate dosage. Therefore, it is imperative to understand how the lung responds to radiation, in order not to limit the dose

to the tumour unnecessarily. Irradiation of the lungs can lead to radiation pneumonitis (RP), which usually develops around 4–6 weeks after treatment [7]. Patients experience fever, dyspnoea and cough and are treated with oral steroids. Mild or moderate symptoms resolve. However, depending on respiratory reserve, radiotherapy treatment volume and radiation dose, patients may develop persistent symptoms. During RP many Type I pneumocytes, which line the alveolar surface, are lost, and the stem cells of the pulmonary epithelium, the Type II pneumocytes [8], show an increased rate of proliferation, migrate to denuded areas and some differentiate into Type I pneumocytes to replace the damaged cells. RP is also characterised by an inflammatory response [9, 10].

Considering the structure of the lung, two factors in particular should be important when estimating the risk of toxicity: the dose threshold for significant local tissue damage and the volume of damaged lung. An estimate of this threshold dose is given in a study by Gopal et al [11], in which the loss of diffusion capacity in the lung was related to the local dose. Here 8.4 Gy is the lowest total dose (normalised to 2-Gy fractions) for which a local tissue effect was detected.

The mechanisms behind radiation-induced normal-tissue injury are complex and not fully understood, but a mechanistic model can be used as a framework for summarising the current radiobiological and pathophysiological knowledge and for generating hypotheses

Address correspondence to: Dr Eva Rutkowska, Physics Department, Clatterbridge Cancer Centre, Clatterbridge Road, Bebington CH63 4JY, UK. E-mail: rutkowska.eva@gmail.com
This study was part of a PhD project funded by the School of Health Sciences, University of Liverpool, UK.

about radiation-induced normal-tissue effects [12]. In this work, information relevant to RP was collected from the literature and used to derive plausible parameter values for a recently published 3D model [13]. Following our earlier work, the model was extended to include interpatient variation in radiosensitivity and health status, and treatments were simulated for populations of patients while adjusting parameter values so that the functional subunit (FSU) and organ response, respectively, agreed with experimental and clinical data on lung toxicity.

Materials and methods

Model of normal-tissue effects

The 3D model used in this work to simulate normal-tissue effects has been described in detail [13]. Local tissue damage is represented by FSU inactivation, and the resulting loss of organ function depends on the distribution and severity of this damage, as well as on the architecture of the organ at risk.

An FSU is the tissue volume which can be regenerated from a single surviving stem cell. Consequently, an FSU is inactivated only if all its stem cells are killed. The cell survival fraction SF after fractionated radiotherapy is given by the linear quadratic (LQ) model [14, 15]:

$$SF = e^{\alpha d + \frac{\beta d^2}{2}} \quad (1)$$

assuming full repair of sublethal damage between treatment fractions and no repair during each fraction. These conditions are fulfilled by conventional external

radiotherapy using small fractions. Here n is the number of treatment fractions, d the dose per fraction, and α and β radiosensitivity parameters specific to each tissue type.

The organ is represented by an array of small volume elements (voxels), each containing a number of FSUs. Assuming the initial number of FSUs in a voxel is N_0^{FSU} , the surviving number of FSUs is given by Poisson statistics:

$$N_s^{\text{FSU}} = N_0^{\text{FSU}} (1 - e^{-N_{sc} \times SF}) \quad (2)$$

where N_{sc} is the number of stem cells per FSU before treatment. This distribution of N_s^{FSU} represents local tissue damage. The dose in each voxel (similar to treatment planning system dose grid sizes) is uniform; however, as generally is the case for normal tissues the dose varied greatly from voxel to voxel in our dose distributions. Figure 1 illustrates the array of voxels that each has a number of surviving FSUs after irradiating the tissue with a given dose distribution. The full array, excluding the gross tumour volume (GTV), was chosen to represent the total paired normal lung volume.

Loss of organ function follows depending on the integrated FSU inactivation over a characteristic volume, which varies between organs and end points. This volume is called the critical functioning volume (CFV). The simulated treatment results in a complication if the number of surviving FSUs falls below a threshold, $FSU_{\min}$, in any CFV. The CFV and $FSU_{\min}$ characterise the volume effect of an organ.

Patient populations were simulated by repeated model simulations using different dose distributions (depending on patient anatomy). Our method for generating dose distributions has been described previously [13].

Figure 1. Geometrical representation of a pair of lungs in the model. The blue volume is normal lung tissue, and the purple volume is the gross tumour volume, which is not included in the analysis of normal lung response. N_0^{FSU} , the number of pre-treatment functional subunits (FSUs) per voxel; N_s^{FSU} , the number of surviving FSUs per voxel.

Interpatient variation in stem cell radiosensitivity was modelled for each patient by randomly sampling α from a log-normal distribution, and thus a data set is characterised by the mean $\bar{\alpha}$ and its standard deviation σ_{α} . In the clinical setting patients will differ not only in radiosensitivity but also in health status and lung volume, and therefore in functional reserve. This is represented in the model by an interpatient variation in pre-treatment (uniform) FSU density (*i.e.* the number of pre-treatment FSUs per voxel; N_0^{FSU}).

Finding parameter values for a mechanistic model

In this work our model is used for summarising current knowledge of RP, and simulating treatments to compare with clinical data in order to find plausible values for parameters not readily available in the literature.

On a local level, the model represents tissue damage by inactivation of FSUs. Each FSU responds independently to irradiation, and thus only the local dose influences the survival probability of an individual FSU. The dose-response of a tissue therefore depends on the number of stem cells per FSU, N_{sc} , and the radiosensitivity of these stem cells (α). The radiosensitivity may vary between patients, and since generally only population-based data are available it is reasonable to consider the population mean, $\bar{\alpha}$. Together with the expression for the probability of FSU survival [Equations (1) and (2)] the threshold dose for FSU inactivation (8.4 Gy; see below) gives an isoeffect relationship between $\bar{\alpha}$ and N_{sc} . Although a plausible range for α was found in Stavrev et al [16], N_{sc} depends on the identity of the FSU, which has not yet been established. However, given the isoeffect relationship with $\bar{\alpha}$, N_{sc} could be determined by simulations of local tissue damage. A thousand “patients” were simulated at a range of local doses sampling the radiosensitivity for each patient from a log-normal distribution. All doses given in this article are in EQD2, *i.e.* they have been normalised to 2-Gy fractions using the LQ model with $\alpha/\beta=3$ Gy.

The literature was also searched for characteristics of the volume effect of the lung and how it can be represented in the model. Just as $\bar{\alpha}$ and N_{sc} can be determined from the local dose-effect of the tissue, the CFV and FSU_{min} depend on the functional reserve (redundancy of FSUs) of the organ. In both cases fixing one parameter gives the other, by comparing simulations with clinical data. Based on the size of the smallest damaged volume causing a complication in the mouse [16], the size of the CFV was approximated to be 75% of the total lung volume. Simulations with the model were then carried out for different values of FSU_{min} . 100 lung-type dose distributions were “delivered” at a prescription dose resulting in an average mean lung dose (MLD) of 29 Gy, which was expected to give 50% complication probability [17]. Simulations were repeated for different values of FSU_{min} until a 50% complication rate was achieved.

One source of information concerning the variability in health status among lung cancer patients is the degree of correlation observed between the MLD and complication probability. Simulations including this variability result in a less significant correlation than when all patients have the same health status; this variability acts as a

confounding factor in dose-volume analyses. Therefore, a suitable value for the population standard deviation in N_0^{FSU} , σ_{FSU} , was found by simulating data sets for a range of values and performing a univariate correlation analysis between MLD and the outcome given by the model. The results from three dose-volume studies on non-small cell lung cancer patients [18–20] were replicated; each study was simulated 50 times, selecting the appropriate number of patients, each with a value of N_0^{FSU} sampled from a log/log-normal distribution (since N_0^{FSU} is bounded by 0 and 1). Each set of simulations resulted in a different complication rate (depending on the randomly sampled parameters) and a different p -value in the correlation analysis. The value of σ_{FSU} resulting in a range of p -values matching the simulated studies was chosen.

Results

Modelling local tissue damage

The relationship between $\bar{\alpha}$ and N_{sc} of the local effect relevant to RP given by Equations (1) and (2) is shown in Figure 2, for the threshold dose estimated as 8.4 Gy. Importantly, this shows the range of possible numbers of stem cells per FSU, given the plausible range for α . Figure 3 shows the dose-response of the FSU for one combination of parameter values from Figure 2: $\alpha=0.24\text{ Gy}^{-1}$ and $N_{\text{sc}}=67$. The level of FSU survival is given by simulations of local tissue damage in 1000 patients with varying radiosensitivity for a range of local doses. Clues to the value of σ_{α} can be obtained from the variation in radiosensitivity between different mouse strains [16], which suggests $\sigma_{\alpha}=18\%$. To guarantee adequate FSU survival at doses tolerated for total lung irradiation (see below), a similar value of $\sigma_{\alpha}=15\%$ was selected.

Figure 2. Values of the radiosensitivity parameter α and the number of stem cells per functional subunit (FSU) before treatment (N_{sc}) resulting in 90% FSU survival for 8.4 Gy. The expected range of α , and the combinations of α and N_{sc} associated with the alveolus and acinus as FSUs, respectively, are indicated; see below regarding the FSUs associated with radiation pneumonitis.

Figure 3. Median functional subunit (FSU) survival with 5% and 95% quantiles for different local doses; simulations using the number of stem cells per FSU before treatment (N_{sc})=67, the radiosensitivity parameter $\alpha=0.24\text{ Gy}^{-1}$; and its standard deviation $\sigma_\alpha=15\%$. This represents the probability of local tissue damage with the uncertainty given by the population variation in radiosensitivity. The position and slope of the curve have been guided by the threshold dose for local loss of diffusion capacity, and the dose limit for total lung irradiation. EQD2, dose normalised to 2-Gy fractions.

Interestingly, these results indicate a large variation in dose–response of the FSU between patients, even for this moderate variation in radiosensitivity. Furthermore, this curve displays almost complete FSU inactivation for doses $>25\text{ Gy}$.

Modelling loss of global organ function

Using the parameter values for local tissue damage determined above, and $\text{CFV}=0.75$, simulations were done with dose distributions expected to give a 50% complication rate. The data set consisted of 100 “patients” and the simulations were repeated at different values of $\text{FSU}_{\min}$. It was found that a value of $\text{FSU}_{\min}=0.47$ gave a complication rate of approximately 50%, which matches the expected clinical complication rate for these dose distributions.

As discussed in the Materials and methods section there should be a relationship between σ_{FSU} (influencing the volume effect in the population) and the strength of correlation between MLD and outcome. As expected, the significance of the correlation decreased with increasing σ_{FSU} . Using the same value of σ_{FSU} for repeated simulations of three clinical studies resulted in a distribution of p -values for each study; at $\sigma_{\text{FSU}}=25\%$ the p -values observed in the real clinical studies fell within the range given by the repeated simulations (Figure 4).

Discussion

The functional subunit of radiation pneumonitis

Although the concept of the FSU is somewhat controversial (e.g. [21]) the current study makes no assump-

Figure 4. The distributions of the p -values of the correlation between mean lung dose (MLD) and outcome for 50 repeated simulations of 3 clinical studies using the population standard deviation in the number of pre-treatment functional subunits (FSUs) per voxel $\sigma_{\text{FSU}}=25\%$ (the logarithm of p is plotted for a better uniformity across the bins). The arrows indicate the p -values from the actual clinical studies. (a) The study of Yorke et al [20] included 49 patients, of whom 9 experienced grade ≥ 3 lung toxicity and this resulted in $p=0.04$ for the correlation between MLD and outcome. (b) Graham et al [18] found $p=0.10$ for the correlation between MLD and grade ≥ 2 radiation pneumonitis in 22 out of 99 patients. (c) Hernando et al [19] reported $p=0.005$ in a cohort of 201 patients, of whom 39 had grade ≥ 1 pulmonary toxicity.

tions about its physiological properties; it only assumes that local tissue damage depends on stem cell death and the volume of tissue one stem cell can regenerate. Its definition makes no assumption concerning its biological properties, *i.e.* the existence of an anatomically structural subunit of the organ, or how the FSU relates to such a structure. The model could also incorporate an inter-dependence of the FSU dose–response.

Stavrev et al [16] advocated the acinus as the FSU of the mouse lung, since the size of this structure matched the FSU size predicted by their model, and it seems anatomically possible for cells to migrate freely throughout the acinus. Timmerman et al [22], on the other hand, suggest that stem cells cannot migrate between alveoli, and conclude that the alveolus is the FSU of the lung. This is in accordance with the implications of our simulations, which showed that the radiosensitivity of the FSU requires the number of stem cells to be of the order of the alveolus (which contains 67 Type II pneumocytes on average [23]) in order for the dose–response threshold to match clinical data (Figure 2).

Local tissue response

A dose threshold for local tissue response was selected at 8.4 Gy from Gopal et al [11]. Although this was a study on only 26 patients, the 95% confidence interval for the threshold dose was small (7.0–9.8). However, this value should be considered approximate since the patients also received chemotherapy, and it is based on a model relating global lung function to the local dose rather than on measurements of the actual local effect. Their model was not a mechanistic one, but rather linked loss of local diffusion capacity to toxicity empirically. As shown in our earlier work [13, 24], however, information on dose–volume effects can be gained from fitting empirical

models to outcome data, and the type of model used is likely to be suitable for their data, which the small confidence interval of the dose threshold supports.

Other studies have indicated higher values for this dose threshold; in many studies on 3D conformal radiotherapy for lung tumours, the volume receiving at least 20 Gy (V_{20}) has been associated with RP [25], which suggests that the lower dose found in the study by Gopal et al could be the result of a sensitising effect of the chemotherapy. However, such findings are most likely to be treatment technique dependent [13, 26], and whether the lower dose threshold (V_5) indicated by recent studies, using data from intensity-modulated radiation therapy for mesothelioma [27] and 3D conformal radiotherapy for oesophageal cancer [28], is a result of the different radiotherapy treatment technique or the influence of chemotherapy/surgery is not clear. Two studies, one on mice [29] and one on rats [30], also indicate a higher dose threshold of approximately 12 Gy in a single fraction (36 Gy EQD2) for an increase in breathing rate.

Despite the uncertainty associated with the chosen value of the dose threshold it was preferred to other sources as there was no other study of the loss of diffusion capacity as a function of local dose, which seems to be the most relevant measure of the local effect of radiation for RP, since it measures gas exchange in the lungs [11].

However, doses as low as 8.4 Gy can only cause a low level of FSU inactivation, and therefore additional volumes irradiated at higher doses are required to trigger a complication [31]. Also, since 12 Gy is tolerated by patients during total body irradiation [32], the slope of the dose-response curve for FSU inactivation cannot be especially steep (Figure 3), or else these patients would die based exclusively on the dose received by the lungs. The slope of this curve is also in line with the local dose-response reported by Gopal et al [11], in which regions of <6.3, 6.3–13.4 and >13.4 Gy (EQD2) were associated with a 0%, 70% and 90% decrease in diffusion capacity respectively.

These results also indicate that, for the end point of RP, maximum local effect is reached at the relatively low dose of 25 Gy. However, the dose effect for other end points, such as fibrosis, may saturate at higher doses.

There are few data on organ-specific normal-tissue radiosensitivity in the literature. Two studies report values for the LQ parameters for lung, derived from radiobiological modelling [33, 34]. Very low values of α were reported (0.03–0.19 Gy⁻¹). However, these models did not include any volume effect but assumed that NTCP depended directly on FSU inactivation. When considering the large volume effect of the lung the radiosensitivity is expected to be greater, since a significant number of FSUs must be inactivated before symptoms are observed. As expected, modelling, including a volume effect [16] on mouse lung data, resulted in higher α values (between 0.26 and 0.37 Gy⁻¹).

Loss of global organ function

The importance of low doses indicated by Figure 3 is consistent with the classification of the lung as one of the most radiosensitive tissues [7]. To be able to explain why

much higher doses are frequently administered to the lung without causing clinical symptoms, the link between local tissue damage and the loss of global organ function must be considered. In our 3D model the loss of global organ function is determined by the CFV, as well as the distribution of surviving FSUs. A complication is triggered only if the number of FSUs falls below FSU_{min} in any one CFV. The ultimate function of the lung is gas exchange, which is dependent on a sufficient number of functioning alveoli [35]. Therefore, the CFV could be expected to encompass the total volume of the organ, and FSU_{min} might be given by the total lung volume minus the functional reserve. (FSU_{min} is the fraction of surviving FSUs each CFV needs in order to avoid a complication, whereas the functional reserve is the fraction of the organ volume which can be lost without causing a complication.) However, there is evidence of more complex volume-effect mechanisms in the lung.

Based on experimental findings with their mouse model, Stavrev et al [16] proposed that at low doses a portion of the FSUs were injured and recovered independently of each other, with the damaged FSUs randomly distributed within the irradiated volume. In contrast, above a threshold of tissue damage (*i.e.* local dose) all the FSUs in a surrounding subvolume of the lung were inactivated. This additional tissue damage throughout a contiguous region might be the result of an inflammatory process, triggered by the initial radiation-induced tissue damage. Thus, the radiation-induced tissue damage in itself might not have caused significant loss of organ function, but since it also triggered inflammation-induced tissue damage a complication occurred. In this case the CFV represents the large volume over which this inflammation-induced damage spreads, after the radiation-induced damage has caused the number of functioning FSUs in the volume to fall below FSU_{min} .

These volumes are illustrated in Figure 5, in which, for simplicity, partial uniform irradiation is assumed (only

Figure 5. Illustration of the application of the critical functioning volume (CFV) concept to the lung. A, irradiated volume; B, unirradiated but damaged volume; C, unirradiated and undamaged volume. If $CFV=A+B$ and if the minimum threshold for surviving functional subunits (FSU_{min})=B, at least A has to be damaged for a complication to occur.

A irradiated); A is the volume irradiated and damaged, B is the volume damaged indirectly (e.g. through inflammation) by the irradiation of volume A, and C is the (unirradiated and) undamaged volume. The CFV corresponds to the volume A+B, and the FSU_{min} to B. This illustration is for the critical case when a complication is just triggered.

The simulations carried out in this work yielded a value of FSU_{min}=0.47. The plausibility of this value is confirmed by the volume which must be spared from radiation damage, in order to avoid pulmonary complications, suggested by a correlation analysis for a threshold dose of 5 Gy [36]. The value of approximately 60% of the lung volume from this study corresponds to FSU_{min}=0.47. (Note that only an absolute lung volume was given, but a relative value was calculated assuming normal lung volumes. Also, when deriving FSU_{min} from the volume which must be spared, it is assumed that the volume outside the "critical" CFV receives doses below the threshold, and thus FSU_{min}=[volume spared-(1-CFV)]/CFV.) However, the patients in this study were treated with chemoradiotherapy, and a much lower value of around 0.11 is suggested by a large study by Seppenwoolde et al [37], who found that 33% of the total lung volume must be spared from doses >13 Gy. Thus, the clinical evidence for the value of FSU_{min} is conflicting, and of limited value for this analysis, since it is influenced by unknown data set characteristics.

The value of FSU_{min} was suggested to vary when different parts of the lung were irradiated by a study on mice [29]. The corresponding FSU_{min} varied from at least 76% when the base of the lung was irradiated to <24% for irradiation of the mid-region. An even lower value of approximately 9% was suggested by the study on rats [30], in which a critical volume model fitted to irradiation of the lungs (excluding the heart) resulted in a functional reserve of 32%. This great range of values does not help to increase the specificity of this parameter but suggests possible causes for the different values indicated by the two clinical studies mentioned above: both the location of the tumours in the cohorts and the inclusion of the heart in the radiation field are likely to influence the findings.

Future developments

The mechanistic model used in this study models both local tissue damage and overall organ function based on data in the literature. Currently any non-local effect of irradiation is treated as a contribution to the volume effect and thus handled by the CFV and FSU_{min} parameters. The volume effect can in fact be caused by several different mechanisms [24, 38, 39] and as those relevant to RP are identified and quantified they need to be modelled explicitly. The model could readily be modified to enable FSU inactivation to depend on the dose delivered to a volume at a distance.

Role of mechanistic modelling

Mechanistic models are useful as tools for generating hypotheses as well as for creating data sets for testing

data analysis methods; see our previous work [13]. We are currently using such data sets for testing the behaviour of empirical NTCP models in the presence of confounding factors.

Considering the difficulty of extracting systematic and reproducible data on radiation-induced normal-tissue damage from the literature, our mechanistic 3D model has proved an effective framework for summarising the radiobiological knowledge base of RP. Ideally, such a model should be used to predict the effect of a dose distribution during treatment planning for individual patients and would be an invaluable tool for the clinician. To make this possible more quantitative end point-specific data need to be collected [40–42]. Furthermore, until methods are developed to determine patient-specific values of radiosensitivity and health status, predictions will remain population based. Progress in modelling normal-tissue effects would enable individualised treatment planning without which modern planning and delivery techniques cannot reach their full potential.

Conclusions

The current work has shown that it is possible to make sense of the way the lung responds to radiation by modelling RP mechanistically, from cell death to tissue damage to loss of organ function. The radiosensitivity of lung tissue and the dose threshold for local tissue damage indicate that the size of the FSU equals that of the alveolus. Simulations also showed that the variation in health status, represented by FSU density, must be of the order of 25% to achieve clinical levels of correlation between the MLD and RP.

References

1. Fenwick JD, Lawrence GP, Malik Z, Nahum AE, Mayles WP. Early mucosal reactions during and after head-and-neck radiotherapy: dependence of treatment tolerance on radiation dose and schedule duration. *Int J Radiat Oncol Biol Phys* 2008;71:625–34.
2. Iori M, Cattaneo GM, Cagni E, Fiorino C, Borasi G, Riccardo C, et al. Dose-volume and biological-model based comparison between helical tomotherapy and (inverse-planned) IMAT for prostate tumours. *Radiother Oncol* 2008;88:34–45.
3. Sanchez-Nieto B, Nahum AE, Dearnaley DP. Individualization of dose prescription based on normal-tissue dose-volume and radiosensitivity data. *Int J Radiat Oncol Biol Phys* 2001;49:487–99.
4. Uzan J, Nahum AE. Radiobiologically guided optimisation of the prescription dose and fractionation scheme in radiotherapy using BioSuite. *Br J Radiol* 2012;85:1279–86.
5. Witte MG, van der Geer J, Schneider C, Lebesque JV, Alber M, van Herk M. IMRT optimization including random and systematic geometric errors based on the expectation of TCP and NTCP. *Med Phys* 2007;34:3544–55.
6. Willner J, Baier K, Caragiani E, Tschammler A, Flentje M. Dose, volume, and tumor control prediction in primary radiotherapy of non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys* 2002;52:382–9.
7. Dorr W. Pathogenesis of normal-tissue side-effects. In: Joiner M, van der Kogel AJ, eds. *Basic clinical radiobiology*. 4 edn. London, UK: Hodder Arnold; 2009. pp. 169–90.
8. Lumb AB, Nunn JF. *Nunn's applied respiratory physiology*. 6 edn. Philadelphia, PA.: Elsevier Butterworth Heinemann; 2005.

9. Phillips TL. Radiation fibrosis. In: Fishman AP, ed. *Pulmonary diseases and disorders*. 2nd edn. New York, NY: McGraw-Hill; 1988.
10. Tsoutsou PG, Koukourakis MI. Radiation pneumonitis and fibrosis: mechanisms underlying its pathogenesis and implications for future research. *Int J Radiat Oncol Biol Phys* 2006;66:1281–93.
11. Gopal R, Tucker SL, Komaki R, Liao Z, Forster KM, Stevens C, et al. The relationship between local dose and loss of function for irradiated lung. *Int J Radiat Oncol Biol Phys* 2003;56:106–13.
12. Joiner M. Quantifying cell kill and cell survival. In: Joiner M, van der Kogel A, eds. *Basic clinical radiobiology*. London, UK: Hodder Arnold; 2009. pp. 41–55.
13. Rutkowska E, Baker C, Nahum A. Mechanistic simulation of normal-tissue damage in radiotherapy: implications for dose-volume analyses. *Phys Med Biol* 2010;55:2121–36.
14. Steel G. Radiobiology of tumours. In: Mayles P, Nahum AE, Rosenwald J, eds. *Handbook of radiotherapy physics*. Boca Raton, FL: Taylor & Francis; 2007. pp. 127–48.
15. Steel G. Dose fractionation in radiotherapy. In: Mayles P, Nahum AE, Rosenwald J, eds. *Handbook of radiotherapy physics*. Boca Raton, FL: Taylor & Francis; 2007. pp. 163–78.
16. Stavrev P, Stavreva N, Sharplin J, Fallone BG, Franko A. Critical volume model analysis of lung complication data from different strains of mice. *Int J Radiat Biol* 2005;81:77–88.
17. De Jaeger K, Hoogeman MS, Engelsman M, Seppenwoolde Y, Damen EM, Mijnheer BJ, et al. Incorporating an improved dose-calculation algorithm in conformal radiotherapy of lung cancer: re-evaluation of dose in normal lung tissue. *Radiother Oncol* 2003;69:1–10.
18. Graham MV, Purdy JA, Emami B, Harms W, Bosch W, Lockett MA, et al. Clinical dose-volume histogram analysis for pneumonitis after 3D treatment for non-small cell lung cancer (NSCLC). *Int J Radiat Oncol Biol Phys* 1999;45:323–9.
19. Hernando ML, Marks LB, Bentel GC, Zhou SM, Hollis D, Das SK, et al. Radiation-induced pulmonary toxicity: a dose-volume histogram analysis in 201 patients with lung cancer. *Int J Radiat Oncol Biol Phys* 2001;51:650–9.
20. Yorke ED, Jackson A, Rosenzweig KE, Merrick SA, Gabrys D, Venkatraman ES, et al. Dose-volume factors contributing to the incidence of radiation pneumonitis in non-small-cell lung cancer patients treated with three-dimensional conformal radiation therapy. *Int J Radiat Oncol Biol Phys* 2002;54:329–39.
21. van Luijk P, Schippers JM. We need to bridge the gap between current practice in mathematical modeling and new insights obtained from radiobiology: comment on Zhou et al. [*Med. Phys.* 34, 2807–2815 (2007)]. *Med Phys* 2008;35:2558–9; author reply 60.
22. Timmerman R, Bastasch M, Saha D, Abdulrahman R, Hittson W, Story M. Optimizing dose and fractionation for stereotactic body radiation therapy. Normal tissue and tumor control effects with large dose per fraction. *Front Radiat Ther Oncol* 2007;40:352–65.
23. Stone KC, Mercer RR, Gehr P, Stockstill B, Crapo JD. Allometric relationships of cell numbers and size in the mammalian lung. *Am J Respir Cell Mol Biol* 1992;6:235–43.
24. Rutkowska E. Mechanistic simulation of normal-tissue damage in radiotherapy. Liverpool, UK: University of Liverpool; 2010.
25. Kong FM, Pan C, Eisbruch A, Ten Haken RK. Physical models and simpler dosimetric descriptors of radiation late toxicity. *Semin Radiat Oncol* 2007;17:108–20.
26. Marks LB, Ma J. Challenges in the clinical application of advanced technologies to reduce radiation-associated normal tissue injury. *Int J Radiat Oncol Biol Phys* 2007;69:4–12.
27. Allen AM, Czerminska M, Janne PA, Sugarbaker DJ, Bueno R, Harris JR, et al. Fatal pneumonitis associated with intensity-modulated radiation therapy for mesothelioma. *Int J Radiat Oncol Biol Phys* 2006;65:640–5.
28. Wang SL, Liao Z, Vaporciyan AA, Tucker SL, Liu H, Wei X, et al. Investigation of clinical and dosimetric factors associated with postoperative pulmonary complications in esophageal cancer patients treated with concurrent chemoradiotherapy followed by surgery. *Int J Radiat Oncol Biol Phys* 2006;64:692–9.
29. Travis EL, Liao ZX, Tucker SL. Spatial heterogeneity of the volume effect for radiation pneumonitis in mouse lung. *Int J Radiat Oncol Biol Phys* 1997;38:1045–54.
30. van Luijk P, Faber H, Meertens H, Schippers JM, Langendijk JA, Brandenburg S, et al. The impact of heart irradiation on dose-volume effects in the rat lung. *Int J Radiat Oncol Biol Phys* 2007;69:552–9.
31. Goitein M. How best to dispose of extra-tumoral dose: a cautionary note for intensity-modulated radiation therapy. *Int J Radiat Oncol Biol Phys* 2009;75:1–3.
32. Sampath S, Schultheiss TE, Wong J. Dose response and factors related to interstitial pneumonitis after bone marrow transplant. *Int J Radiat Oncol Biol Phys* 2005;63:876–84.
33. Kehwar TS. Analytical approach to estimate normal tissue complication probability using best fit of normal tissue tolerance doses into the NTCP equation of the linear quadratic model. *J Cancer Res Ther* 2005;1:168–79.
34. Thames HD, Hendry JH, Moore JV, Ang KK, Travis EL. The high steepness of dose-response curves for late-responding normal tissues. *Radiother Oncol* 1989;15:49–53.
35. Marks LB. The impact of organ structure on radiation response. *Int J Radiat Oncol Biol Phys* 1996;34:1165–71.
36. Tucker SL, Liu HH, Wang S, Wei X, Liao Z, Komaki R, et al. Dose-volume modeling of the risk of postoperative pulmonary complications among esophageal cancer patients treated with concurrent chemoradiotherapy followed by surgery. *Int J Radiat Oncol Biol Phys* 2006;66:754–61.
37. Seppenwoolde Y, Lebesque JV, de Jaeger K, Belderbos JS, Boersma LJ, Schilstra C, et al. Comparing different NTCP models that predict the incidence of radiation pneumonitis. Normal tissue complication probability. *Int J Radiat Oncol Biol Phys* 2003;55:724–35.
38. Hendry JH, Jeremic B, Zubizarreta EH. Normal tissue complications after radiation therapy. *Rev Panam Salud Publica* 2006;20:151–60.
39. Withers HR, Taylor JM, Maciejewski B. Treatment volume and tissue tolerance. *Int J Radiat Oncol Biol Phys* 1988;14:751–9.
40. Marks LB, Yorke ED, Jackson A, Ten Haken RK, Constone LS, Eisbruch A, et al. Use of normal tissue complication probability models in the clinic. *Int J Radiat Oncol Biol Phys* 2010;76(Suppl. 3):S10–19.
41. Schultheiss TE. The controversies and pitfalls in modeling normal tissue radiation injury/damage. *Semin Radiat Oncol* 2001;11:210–14.
42. Travis EL. Organizational response of normal tissues to irradiation. *Semin Radiat Oncol* 2001;11:184–96.