Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Theoretical Biology 241 (2006) 158–171
Journal of Theoretical Biology
www.elsevier.com/locate/yjtbi

Mathematical modelling of radiotherapy strategies for early breast cancer

Heiko Enderling[a,*], Alexander R.A. Anderson[a], Mark A.J. Chaplain[a], Alastair J. Munro[b], Jayant S. Vaidya[b,**]

[a]Division of Mathematics, University of Dundee, Dundee DD1 4HN, Scotland, UK
[b]Department of Surgery and Molecular Oncology, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY, Scotland, UK

Received 18 May 2005; received in revised form 11 November 2005; accepted 11 November 2005
Available online 28 December 2005

Abstract

Targeted intraoperative radiotherapy (Targit) is a new concept of partial breast irradiation where single fraction radiotherapy is delivered directly to the tumour bed. Apart from logistic advantages, this strategy minimizes the risk of missing the tumour bed and avoids delay between surgery and radiotherapy. It is presently being compared with the standard fractionated external beam radiotherapy (EBRT) in randomized trials.

In this paper we present a mathematical model for the growth and invasion of a solid tumour into a domain of tissue (in this case breast tissue), and then a model for surgery and radiation treatment of this tumour. We use the established linear-quadratic (LQ) model to compute the survival probabilities for both tumour cells and irradiated breast tissue and then simulate the effects of conventional EBRT and Targit.

True local recurrence of the tumour could arise either from stray tumour cells, or the tumour bed that harbours morphologically normal cells having a predisposition to genetic changes, such as a loss of heterozygosity (LOH) in genes that are crucial for tumourigenesis, e.g. tumour suppressor genes (TSGs). Our mathematical model predicts that the single high dose of radiotherapy delivered by Targit would result in eliminating all these sources of recurrence, whereas the fractionated EBRT would eliminate stray tumour cells, but allow (by virtue of its very schedule) the cells with LOH in TSGs or cell-cycle checkpoint genes to pass on low-dose radiation-induced DNA damage and consequently mutations that may favour the development of a new tumour.

The mathematical model presented here is an initial attempt to model a biologically complex phenomenon that has until now received little attention in the literature and provides a 'proof of principle' that it is possible to produce clinically testable hypotheses on the effects of different approaches of radiotherapy for breast cancer.

Keywords: Mathematical modelling; Tumour invasion; Breast cancer; Radiotherapy; Targit; Intraoperative radiotherapy; Local recurrence

1. Introduction

In recent years the treatment of breast cancer has shifted away from the very radical to more conservative surgical operations followed by radiotherapy (Vaidya et al., 2001). Conventional external beam radiotherapy (EBRT) of about 50–55 Gy (1 Gy = 1 Gray = 1 Joule kg^{-1}) is given in fractions of 2 Gy over a 4–6 week period (Reitsamer et al., 2002). This external delivery may miss the target and estimates of such a 'geographical miss' range from 24% to 88% of cases (Vaidya et al., 2005). This could well contribute towards the persistent rate of local recurrence of about a third of the rate without radiotherapy (EBCTCG, 2000; Reitsamer et al., 2002).

Over the last few years studies have begun that focus on how to obtain local tumour control with radiotherapy directed at the tumour bed (Veronesi et al., 2001; Vaidya

*Corresponding author. Tel.: +44 1382 344470; fax: +44 1382 345516.
**Also to be corresponded to Tel.: +44 1382 496434; fax: +44 8701 307403.
E-mail addresses: heikman@maths.dundee.ac.uk (H. Enderling), anderson@maths.dundee.ac.uk (A.R.A. Anderson), chaplain@maths.dundee.ac.uk (M.A.J. Chaplain), a.j.munro@dundee.ac.uk (A.J. Munro), j.s.vaidya@dundee.ac.uk (J.S. Vaidya).

0022-5193/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtbi.2005.11.015

et al., 1999; Arthur, 2003). One reason for concentrating on the tissue adjacent to the tumour is that over 90% of early local recurrence occurs here (Vaidya et al., 1996). This region is defined by a 'shell' of tissue which may contain actual or potential tumour cells within a 10 mm margin (Ebert and Carruthers, 2003). The potential tumour cells are morphologically normal cells that may harbour genetic mutations similar to the alterations found in the tumour (Lakhani et al., 1999; Försti et al., 2001; Li et al., 2002; Steinarsdottir et al., 2004).

One genetic mutation that is common in the carcinogenesis cascade is the so-called loss of heterozygosity (LOH) (Dairkee, 1998; Tomlinson, 2001) in tumour suppressor genes (TSGs). These genes (i.e. their mutations) are important for the initiation or early progression of breast cancer development (Deng et al., 1996). The 'field effect' of the mutations in tissue adjacent to the tumour bed has been known for some time (Deng et al., 1996), but its aetiology is uncertain. It could be the result of clonal expansion (Tomlinson, 2001; Larson et al., 2002) of only a few stem cells that mutated before puberty (Lakhani et al., 1999) and thus spread to whole segments of the ducto-lobular tree. In an elegant study of patients treated with breast conservation surgery, LOH occurring in TSGs in tissues adjacent to the primary tumour was shown to increase the probability of recurrence 4–5-fold (Li et al., 2002).

Normal DNA-repair mechanisms at different check points during the cell cycle are able to detect and to repair most low-dose radiation-induced damage (Oldham, 2001) in a relatively short time (approximately 70.2 min, Brenner et al., 1998). DNA double-strand breaks, as induced by ionizing radiation, are repaired either by homologous recombination or non-homologous end-joining mechanisms (Sancar et al., 2004). The therapeutic index of radiotherapy relies on the fact that cancer cells lack such efficient DNA-repair mechanisms and therefore genetic damage is carried on. If the resulting mutations limit the cells' ability to survive, which is the case in about 1–2% of all radiaton-induced double-strand breaks (Turesson et al., 2003), a proportion dies with every small dose of radiation.

The traditional aim of radiotherapy has been to achieve a therapeutic index by inducing tumour cell death while limiting the damage to nearby healthy cells. Most radiobiological reaction-rate models lead to linear-quadratic (LQ) relations (Sachs et al., 1997). The biologically effective dose (BED) can be obtained from this relation, i.e.

$$\mathrm{BED} = n(\alpha d + \beta d^2), \tag{1}$$

where d is the physical dose delivered per fraction, n is the total number of fractions, α is the coefficient of single-hit double-strand breaks, and β is the coefficient of the combination of two sub-lethal single-strand breaks to form a lethal double-strand break. With the known BEDs for healthy cells and cancer cells one can calculate the surviving probability S (Guerrero and Allen Li, 2003) as

$$S = \exp\left[-nd\alpha\left(1 + \frac{d}{(\alpha/\beta)}\right)\right], \tag{2a}$$

which can then be used to predict the outcome of a treatment.

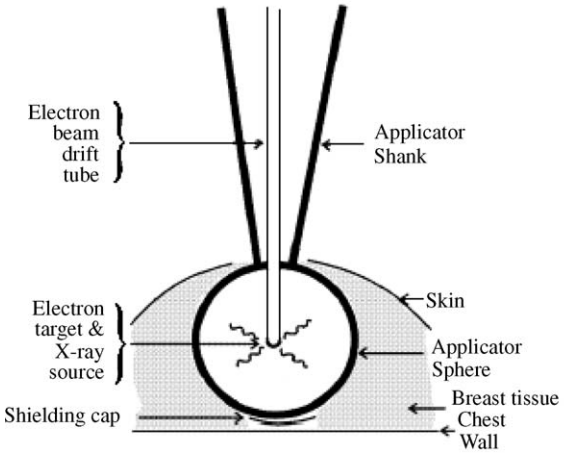
Although individual cell repair is not considered in the LQ-model, we use experimental results to estimate the proportion of cells that survive low-dose radiation damage after repair. From Eq. (2a) we obtain the proportion of cells that are not damaged by radiotherapy. However, 98% of the damage is likely to be repaired within the first few hours after radiation (Brenner et al., 1998; Guerrero and Allen Li, 2003) and hence the cells survive low-dose treatment. Turesson et al. (2003) have stated that each radiation dose of 1 Gy causes about 20–25 double-strand breaks per cell. If a dose of more than 5 Gy is delivered (and hence more than 100 double-strand breaks occur), it can be assumed that even after repairing of 98% of damage at least one crucial double-strand break is still left unrepaired which will force the cell to die. Therefore, we introduce the observed cell repair probability of 98% for doses $d \leqslant 5$ Gy and the new surviving portion S^* that will be applied to healthy cells arises from the proportion of cells that survive sub-lethal radiation damage (i.e. S) plus the proportion of the cells that were damaged and were repaired (i.e. $(1–S) \times 0.98$). Hence, we have

$$S^* = \begin{cases} S, & \text{if } d > 5, \\ S + [(1 - S) \times 0.98], & \text{if } d \leqslant 5. \end{cases} \tag{2b}$$

2. Targeted intraoperative radiotherapy (Targit) for early breast cancer

Intraoperative radiotherapy (IORT) delivers a large single dose to the tumour bed in the operating theatre while the patient is still anaesthetized. The applicator is placed in the tumour bed formed after wide local excision of the primary tumour (Vaidya et al., 2002). Since the applicator is placed directly in the tumour bed, a 'geographical miss' is avoided (Fig. 1). The tumour bed surrounds the radiation source, so that the breast tissue that was previously close to the tumour is now close to the spherical applicator and therefore receives the highest dose of radiotherapy (Vaidya et al., 2001).

An electron-beam driven X-ray source is used for Targit. This point source emits low energy (50 kV) X-rays isotropically and gives a uniform dose rate at the applicator surface. There is rapid attenuation of the dose within the tissues that absorb the radiation energy and thus there is only a small high-dose region and distant normal tissues are spared (Vaidya et al., 2001). Fig. 2 shows the experimentally measured loss of energy of soft X-rays over short distances (Vaidya et al., 2001) as well as an approximated function $f(x) = 5e^{1-x^{1.5}}$ of this exponential



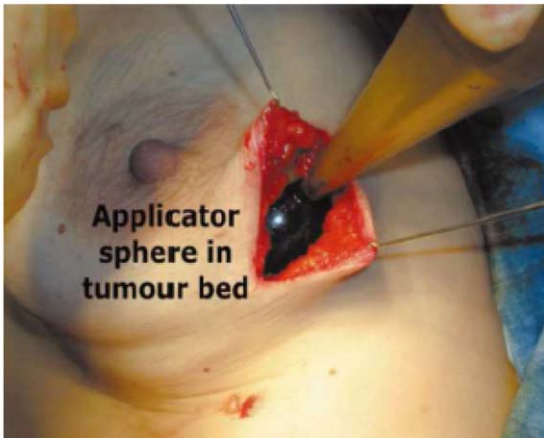


Fig. 1. Diagrams showing the Targit system. Left: schematic diagram of the Intrabeam system. X-rays are generated when electrons that are accelerated along the drift tube hit the tip. These X-rays are modulated by the spherical applicator delivering a uniform dose of radiation at the surface of the applicator sphere. Right: The applicator being placed in the tumour bed, immediately after the excision of the tumour. From Vaidya et al. (2001).

distance *cm*	dose *Gy*	$5e^{1-x^{1.5}}$ *Gy*
0.1	15	13.17
0.2	12.5	12.43
0.5	8.75	9.54
1.0	5.0	5.0
2.7	1.0	0.16

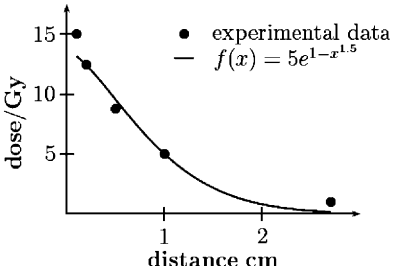


Fig. 2. Targit observed radiation dose over distance (Vaidya et al., 2001) and its approximation with a function $f(x)$ with exponential decay. The rapid attenuation reduces the treatment area to tissue close to the tumour bed.

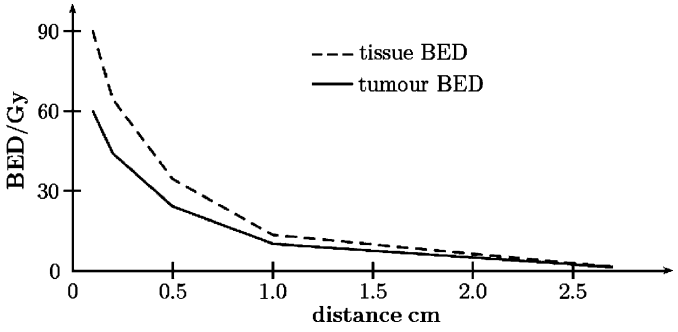


Fig. 3. Plot of the biologically effective dose (BED) in Targit against distance for both tissue and tumour cells. The biological ratios α/β are 5 Gy for tumour and 3 Gy for healthy breast tissue, respectively.

decay. The dose is 5 Gy at 1 cm distance from the applicator surface. The highest radiation dose of 20 Gy is located close to the source, whereas at a distance of 2.7 cm from the source only a dose of 1 Gy remains.

The approximated dose can be used to compute the BED and survival fractions for any area in the tissue. Guerrero and Allen Li (2003) have stated that the α/β ratio for breast tumour varies from 13 to 5 Gy, with a widely observed mean of 10 Gy. Throughout this paper we have assumed the biological ratio to be $(\alpha/\beta)_{tumour} = 10\,\text{Gy}$. The ratio for breast tissue is widely assumed to be $(\alpha/\beta)_{tissue} = 3\,\text{Gy}$ (Dale, 1996; Vaidya et al., 2001; Guerrero and Allen Li, 2003). If the ratio $(\alpha/\beta)_{tumour}$ turns out to be lower for single dose radiotherapy (as indicated in Vaidya et al., 2001; Yarnold et al., 2005), from Eq. 2(a) and 2(b) we obtain a much smaller survival probability and thus an even better therapeutic index. For Targit, by simply applying Eq. (1) we obtain the BED for both tumour and breast tissues, which are plotted against the distance from the radiation source in Fig. 3. Close to the applicator, the effective dose is higher for healthy tissue than for tumour cells, and in distant sites the radiation has almost the same low effect on either tissue. The high BED for healthy tissue is reduced by repair mechanisms.

By applying $\alpha_{tumour} = 0.3\,\text{Gy}^{-1}$ and $\alpha_{tissue} = 0.15\,\text{Gy}^{-1}$ for breast cancer (Guerrero and Allen Li, 2003) and breast tissue respectively (Booth, 2002) the survival rates S and S^* in Targit are calculated using Eq. (2a), (2b) and are as shown in Table 1 and plotted against distance from the radiation source in Fig. 4.

Targit is currently being compared in an international randomized trial (Vaidya et al., 1999; Vaidya et al., 2004) with the conventional 4–6 week course of postoperative radiotherapy. It is felt that Targit can virtually eliminate the risk of a 'geographical miss' of the tumour bed while sparing distant normal tissues (e.g. skin) and therefore can potentially better the cosmetic outcome. It avoids delays before radiotherapy treatment after surgery which could increase the risk of local recurrence (Wyatt et al., 2003), and has several logistic and financial advantages. Thus, if Targit is found as effective as conventional radiotherapy, it could become the new standard. Our mathematical model describes the effects of this new radiotherapy technique as well as that of the conventional radiotherapy. We provide

Table 1
Table shows the BEDs (in Gy) for tumour and breast tissue and the corresponding survival fractions S_{tumour} for tumour cells and S_{tissue} and S^*_{tissue} for breast tissue cells

Distance (cm)	Dose (Gy)	BED_{tumour}	S_{tumour}	BED_{tissue}	S_{tissue}	S^*_{tissue}
0.1	15	38	1.3×10^{-5}	90	1.4×10^{-6}	1.4×10^{-6}
0.2	12.5	28	2.2×10^{-4}	64	6.2×10^{-5}	6.2×10^{-5}
0.5	8.75	16	7.3×10^{-3}	34	5.9×10^{-3}	5.9×10^{-3}
1.0	5.0	8	0.1	13	0.14	0.983
1.3	4.0	5.6	0.19	9.3	0.25	0.985
2.0	2.0	2.4	0.48	3.3	0.6	0.992
2.7	1.0	1	0.7	1.3	0.82	0.996

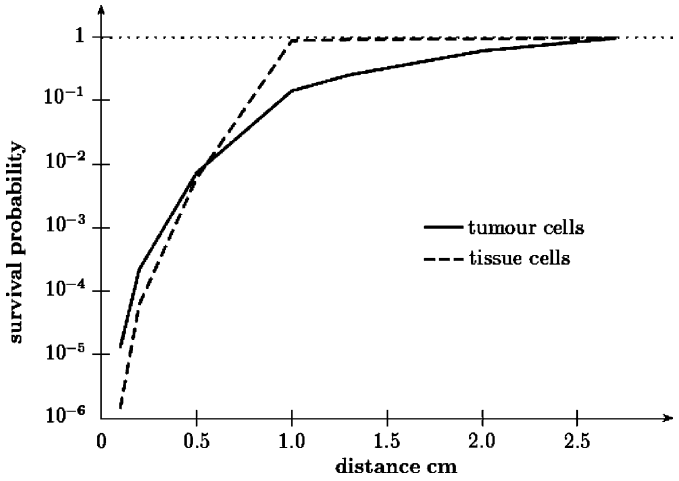


Fig. 4. Plot of survival probabilities for tumour and tissue cells in Targit as a function of distance as calculated in our model using equations (2a) and (2b).

an elegant demonstration of how mathematical modelling could help compare and contrast current treatments with new treatment strategies.

3. A mathematical model for solid tumour growth and invasion

The mathematical model is presented in three different phases of tumour development and treatment. In the first phase, we model a solid tumour invading the host breast tissue (cf. Anderson et al., 2000). After the tumour has reached a specific size we simulate the wide local excision of the tumour and then we introduce radiation therapy in the second phase of our modelling. In the final phase we simulate the development (or not) of local recurrence.

In the invasion model of Anderson et al. (2000), later developed and extended by Anderson (2005), the three variables considered in this first phase are tumour cells, extracellular matrix (ECM) and matrix-degrading enzymes (MDE). These variables are denoted as n, f and m, respectively. The random motion of tumour cells is modelled by simple diffusion (*random motility*). The directed movement of tumour cells up the gradients of fixed or bound chemicals is denoted by *haptotaxis*. The tumour cells are known to produce enzymes (modelled by *production* and *decay*) that *diffuse* throughout the surrounding matrix and degrade the tissue upon contact, which we model by a *degradation* term. In our model, we add a term to simulate the effect of *proliferation* of tumour cells. For simplicity we neglect cell death and only consider proliferation to be modulated by available space.

These assumptions lead to the following system of equations describing the interactions of the tumour cells, ECM and MDEs as detailed in the previous paragraph

$$\begin{aligned}
\frac{\partial n}{\partial t} &= \overbrace{\mu n(1-n/n_0-f/f_0)}^{proliferation} + \overbrace{D_n\nabla^2 n}^{radom\ motility} - \overbrace{\chi\nabla\cdot(n\nabla f)}^{haptotaxis},\\
\frac{\partial f}{\partial t} &= \overbrace{-\kappa m f}^{degradation},\\
\frac{\partial m}{\partial t} &= \overbrace{D_m\nabla^2 m}^{diffusion} + \overbrace{\zeta n(1-m/m_0)}^{production} - \overbrace{\omega m}^{decay}.
\end{aligned} \tag{3}$$

We assume that the tumour grows inside the breast without reaching the outer boundary—the skin. Hence we neglect the breast geometry and assume that the tumour grows in a radially symmetric manner inside a uniformly shaped domain of breast tissue. Therefore our system is considered to hold in a one-dimensional spatial domain Ω (a region of tissue) with appropriate initial conditions for each variable. This domain may be seen as an approximation to a radially symmetric tumour geometry within the breast. We assume that tumour cells, and consequently the MDEs, remain within the domain of tissue under consideration and therefore no-flux boundary conditions are imposed on $\delta\Omega$, the boundary of Ω.

In order to solve the system numerically, we first of all non-dimensionalize the equations in the standard way. We rescale distance with an appropriate length scale L (i.e. the size of the breast = 10 cm), time with $\tau = 1$ year, tumour cell density with n_0, ECM density with f_0 and MDE concentration with m_0 (where n_0, f_0, m_0 are appropriate reference density and concentration variables). Therefore, setting

$$\tilde{n} = \frac{n}{n_0},\ \tilde{f} = \frac{f}{f_0},\ \tilde{m} = \frac{m}{m_0},\ \tilde{x} = \frac{x}{L},\ \tilde{t} = \frac{t}{\tau},$$

in (3) and dropping the tildes for notational convenience, we obtain the scaled system of equations:

$$\begin{aligned}\frac{\partial n}{\partial t} &= \overbrace{\lambda n(1-n-f)}^{proliferation} + \overbrace{d_n\nabla^2 n}^{radom\ motility} - \overbrace{\gamma\nabla\cdot(n\nabla f)}^{haptotaxis},\\ \frac{\partial f}{\partial t} &= \overbrace{-\eta m f}^{degradation},\\ \frac{\partial m}{\partial t} &= \overbrace{d_m\nabla^2 m}^{diffusion} + \overbrace{\alpha n(1-m)}^{production} - \overbrace{\beta m}^{decay},\end{aligned} \tag{4}$$

where $\lambda=\tau\mu$, $d_n=\tau D_n/L^2$, $\gamma=\tau\chi f_0/L^2$, $\eta=\tau\kappa m_0$, $d_m=\tau D_m/L^2$, $\alpha=\tau\zeta n_0/m_0$, $\beta=\tau\omega$. We took a value for the cell random motility parameter D_n of $D_n\sim10^{-9}\,\mathrm{cm}^2\,\mathrm{s}^{-1}$ (cf. Bray, 1992). It is known that the MDEs secreted by cancer cells are quickly bound to the matrix and localized near the invading edge, i.e. the MDEs have a diffusion rate comparable to the cancer cell random motility. Hence, we took $D_m\sim10^{-9}\,\mathrm{cm}^2\,\mathrm{s}^{-1}$. The parameter f_0 was taken to be $f_0\sim10^{-11}\,\mathrm{M}$ estimated in line with Anderson et al. (2000). However, in contrast to Anderson et al. (2000) who modelled generic invasive tumour growth, we took the haptotactic parameter χ to be much smaller in order to reflect early stage breast tumour invasive behaviour which is characterized by more compact, localized spread. Estimates for the kinetic parameters κ, ζ, ω were not available since these are very difficult to obtain experimentally. The proliferation parameter μ was chosen to reflect breast cancer specific tumour growth with a mean tumour cell doubling time of 100 days (Guiot et al., 2003).

With the preceding estimates, in all the following simulations, the scaled parameter values used in Eq. (4) were as follows:

$$d_n=0.0001,\ d_m=0.0005,\ \gamma=0.00005,$$
$$\lambda=0.75,\ \eta=10,\ \alpha=0.1\ \text{and}\ \beta=0.$$

We now solve the above system of Eq. (4) numerically in order to simulate (i) breast cancer growth pre-treatment; (ii) breast cancer surgery, and finally (iii) postoperative treatment with radiotherapy (conventional and Targit).

Early breast cancer is defined by a solid mass of cancer cells which has a higher *density* than the surrounding tissue. We will interpret the term *density* in a slightly different way in this paper—it means the probability of tumour cells being present in a particular sample of tissue—so a low *density* here means a low probability of finding a tumour cell within the tissue and *not* 'the degree of compactness of a substance'. First of all we examine the effect of varying the proliferation rate on invasion.

The difference in the tumour *density* due to different proliferation rates is shown in Figs. 5a–c. Without proliferation ($\lambda=0$) diffusion is the dominant process for tumour cells. As the proliferation rate increases the tumour mass achieves a higher compactness and the border between tumour and healthy tissue becomes clearer. With a proliferation rate of $\lambda=0.75$ we can model a dense tumour lump that has a sharp boundary within surrounding breast tissue.

4. Modelling of surgery and radiotherapy

With increasing awareness about breast cancer and the widespread use of screening using mammography, more and more newly detected breast cancers are small in size. Indeed about 70% (Fritz et al., 2005) of all cases are of the early stages I and II. The usual treatment for a tumour of this size is breast conserving surgery and adjuvant radiotherapy.

Let us assume that the tumour develops as described in our numerical computations of the model (4) in the previous section and reaches a size of 2 cm. At this stage, we simulate breast conserving surgery at the beginning of the second phase in our model. In breast conserving surgery a solid tumour lump is removed along with a rim of the surrounding normal breast tissue. In our model, we simulate surgery by removing all areas of high tumour cell *density*. In our simulation these areas are defined by the concentration dominance of tumour cells over tissue cells. Therefore, the tumour cell concentration, the tissue concentration and the enzyme concentration in this area become zero after surgical removal of the lump.

To model conventional radiotherapy we apply the simple LQ model introduced in Eq. (1). This model has been modified by Dale (1985, 1989) and recently by Herskind et al. (2005) to include dynamic processes between two consecutive fractions, e.g. tumour repopulation. We, in contrast, consider every fraction of irradiation separately, and apply the model of solid tumour growth and invasion (4) between two fractions to enable tumour and tissue dynamics to influence the outcome during the course of conventional fractionated radiotherapy. Furthermore, to keep the model simple, we have not included hypoxia in the LQ-relation. This is because we are modelling the effect of radiotherapy on tissues left behind after surgery, and these tissues are unlikely to be hypoxic.

The dose delivered in conventional treatment is assumed to be uniform throughout the domain. Targit, in contrast, is characterized by a rapid dose fall off over distance. At about 3 cm from the applicator surface both tumour and healthy cells survive with a probability of 100%. The survival rates for cells closer to the radiation source are calculated using Eqs. (2a) and (2b) and the dose given in Fig. 2. The distance-dependent surviving probabilities are shown in Fig. 4. During the relatively short time when surgery and actual delivery of radiotherapy is performed, we can ignore all temporal processes described in the first phase of our model as the time-scales for surgery, Targit and each fraction of conventional treatment are in the range of minutes whereas potential breast cancer doubling time is in the range of months (e.g. a median of 100 days cf. Peer et al., 1993; Kopans et al., 2003; Michaelson et al., 2003).

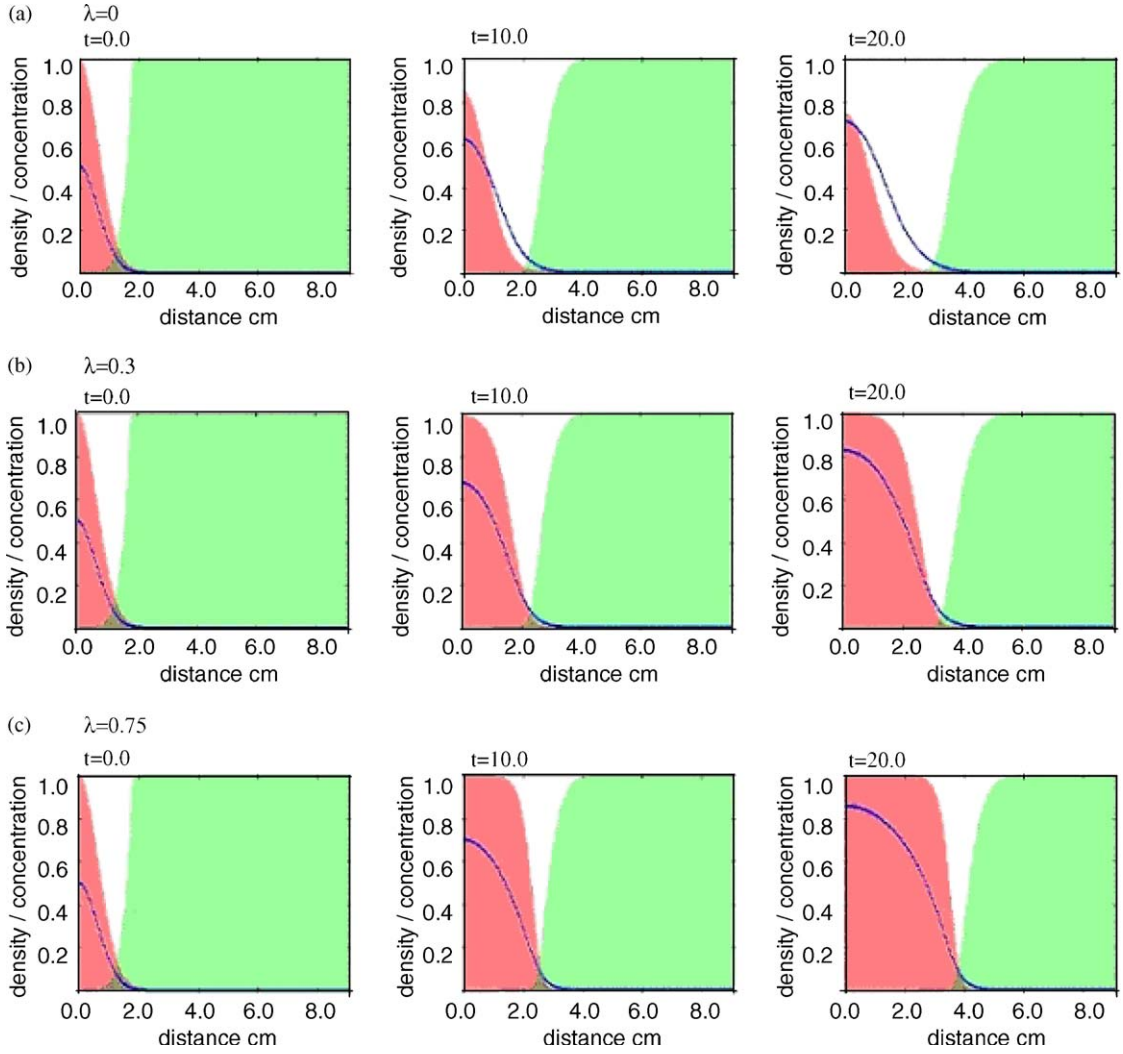


Fig. 5. Spatio-temporal evolution of growing and invading breast tumours with different proliferation rates $\lambda = 0$ (a), $\lambda = 0.3$ (b) and $\lambda = 0.75$ (c). Plots are shown at times $t = 0$, 10 and 20 in left, middle and right columns, respectively. Initially the tumour is located on the left-hand side of the domain (red), with tissue (green) elsewhere. The tumour releases matrix degrading enzymes (solid blue line) which degrade the tissue upon contact and thus create space for the tumour to grow into over time. With a higher proliferation rate λ the cancer cells proliferate more rapidly and form a solid dense tumour mass. Other parameter values as per text.

In the third phase of our framework, we apply the same mathematical model used in the first phase to simulate potential local recurrence. If the adjacent tissue still harbours tumour cells after surgery and radiotherapy, these cells may form a new tumour that invades the domain further. In the initial simulations we model the two radiotherapy strategies that include the effects of stray tumour cells in the tissue adjacent to the tumour bed.

In the final simulations, we model the presence of cells with LOH in TSGs in the tissues immediately surrounding the tumour and examine how this would change the clinical outcome with each of the two radiotherapy strategies. At this stage we have not modelled the actual emergence of recurrence from LOH because these cells are not yet transformed and modelling this transformation would need an individual-based cellular model rather than the continuum cell-population model that we have described. We have instead included this cell population as being a potential source of local 'recurrence' and assessed if a particular radiotherapy strategy can eliminate it.

5. Results

In the first phase of the mathematical model for solid tumour growth and invasion we simulate biologically observed breast tumour growth before diagnosis. In this specific situation, a solid and very dense tumour mass is produced and this degrades the surrounding tissue. Only a very small fraction of tumour cells escape into the tissue. The system of partial differential equations (4) was solved numerically using an explicit finite difference approximation scheme for the parameter values

$$d_n = 0.0001,\ \ d_m = 0.0005,\ \ \gamma = 0.00005,$$
$$\lambda = 0.75,\ \ \eta = 10,\ \ \alpha = 0.1 \ \text{ and } \ \beta = 0.$$

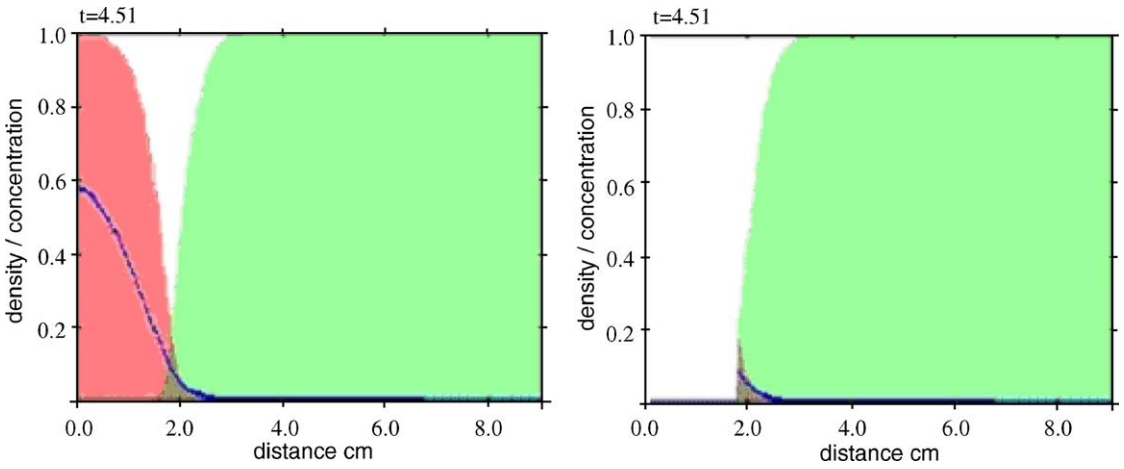


Fig. 6. Plots showing the simulation of surgery for early breast cancer. Tumour cell density is red, healthy tissue density is green and MDE concentration is the blue line. When the dense tumour diameter (dominance of tumour cells over tissue cells) reaches 2 cm (left), surgery is simulated and all cells and enzymes in the area of high tumour tissue density are removed (right). The presence of some tumour cells in the tissue after surgery (the small brown triangle at the bottom left of the green area near the x-axis) emphasizes the need of radiotherapy following breast conserving surgery.

The computational results were obtained using a Java implementation of the numerical scheme and all figures shown were generated in Java. [Simulation videos are available at URL http://www.maths.dundee.ac.uk/~heikman/targit; http://www.dundee.ac.uk/surgery/targit/model].

With the above parameter values, the undiagnosed and untreated tumour grows exactly as that shown in Fig. 5c. The distribution at $t = 0$ shows the initial conditions for our model with a cluster of tumour cells being located at the left-hand side of the domain (red), the presence of MDEs within this area (the solid blue line), and tissue cells (green) everywhere else. As time evolves the cluster of tumour cells grows and realeases MDEs, which degrade the ECM to make space for the tumour to invade the domain further. We found that the chosen values of the parameters for tumour cell random motility and haptotaxis, d_n and γ, respectively, simulate the very localized lump characteristic of early stage breast cancer. This is in contrast to the original simulations for more aggressive invading tumour cells with a higher random motility and haptotactic potential proposed by Anderson et al. (2000), where the initial cluster of tumour cells may be able to break into two separate clusters.

In the second phase of the model framework we model breast conserving surgery. Fig. 6 shows the effect of surgery. In our simulations we apply surgery as soon as the dense tumour reaches a 2 cm diameter, and all tissue on the left side of this boundary i.e., the tumour and some normal tissue is removed. The denser tumour is defined by a higher concentration of tumour cells than tissue cells. The tumour and tissue concentration, as well as the enzyme concentration in this area, is set to zero. In our simulations the tumour reaches a detectable size after about four and a half years. Guiot et al. (2003) state that with a mean tumour cell doubling time of 100 days and 30 cell doublings necessary to reach a 'diagnostic' stage the tumour had a developing time of roughly 8 years. In our simulations the tumour is 'detected' in about half of this time, which is in line with our initial conditions where the tumour is already at half the detectable size.

The presence of a few tumour cells (the small brown triangle at the bottom left of the green area near the x-axis in Fig. 6) within the healthy tissue after surgery now need to be treated with radiotherapy. We assumed that 99% of clonogenic cells are removed by surgery (Wyatt et al., 2003). Without radiotherapy, the tumour may recur even if only a few clonogenic cells are left in the breast tissue. If we assume that a small number of tumour cells remain (as seen in Fig. 6) and use this as the new initial conditions in our model (4) for the next phase, then as can be seen in Fig. 7 the tumour recurs. The computational simulations indicate a clinically detectable recurrence on a time-scale of 2–5 years which is in line with clinical data (Demicheli et al., 1999; Fisher et al., 2002). Now, we model the effect of different radiotherapy strategies on our tumour growth-and-surgery model.

6. Simulation of the effects of conventional fractionated radiotherapy

Fig. 6 shows the initial conditions used to model the effect of conventional EBRT. The dosage in EBRT is assumed to be uniform all over the domain (Vaidya et al., 2001). The healthy breast tissue gets the same dose as the tumour bed, but during the interval between fractions, normal cells are able to repair the radiation-induced DNA damage as discussed above. The model of phase I is applied between the fractions over the 5 week treatment period to consider the effect of regrowth and repopulation during the course of radiotherapy. Fig. 8 shows the simulation of EBRT treatment after breast conserving surgery with 25 fractions each of 2 Gy over a period of 5 weeks. The condition after surgery and the delivery of the first fraction (solid black line) is shown in the top row, and the results after first, second,

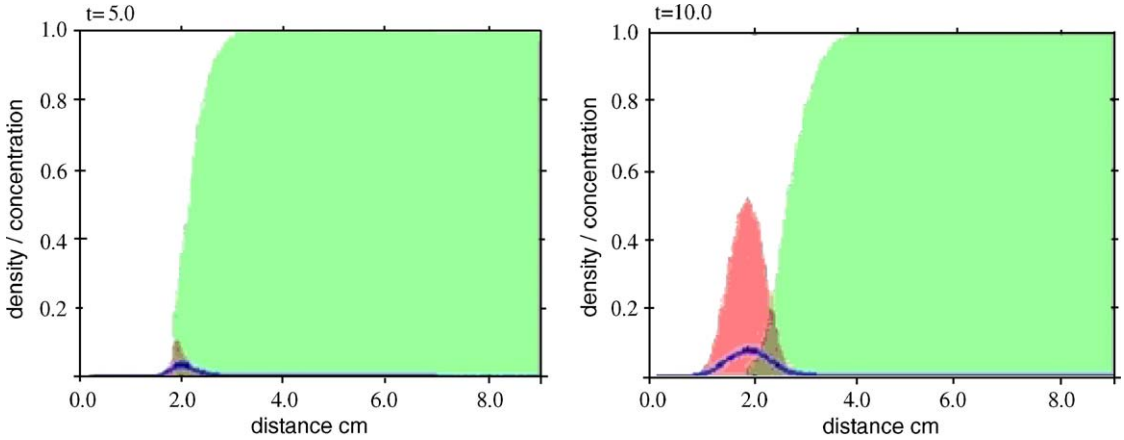


Fig. 7. Plots showing the spatio-temporal evolution of the recurrence of a breast tumour (red) when there are a few tumour cells left behind in the tissue (green) after the surgery as shown in Fig. 6. The matrix degrading enzyme concentration is plotted as the blue line. The plot at time $t = 5$ (left) shows the tumour density about 6 months after surgery (surgery was at time $t = 4.51$, see Fig. 6), and the plot at time $t = 10$ (right) represents about 5 years after surgery, by which time the tumour has already grown to a detectable size. Clinically, recurrence is typically detected between 2 and 4 years after surgery which is in line with our simulations results.

10th and the final 25th fraction is shown in the rows below. All tumour cells are eliminated, but over time (fractions 10–25) the healthy tissue throughout the domain also visibly suffers from irradiation. This damage to normal tissue is relatively small and distributed all over the breast.

7. Simulation of the effects of single fraction irradiation with Targit

Targit is given over a period of about 25 min (Vaidya et al., 2001). We apply the survival probability fractions $S\{n,f\}$ as shown in table 1 to the tumour and tissue densities. The results in Fig. 9 show a simulation that uses the parameters discussed earlier for 25 min of radiation with a 50 kV soft X-ray beam which affects normal tissue and tumour cells as seen in Fig. 4. The two images show the initial distribution and coverage of radiation (Fig. 9, left plot) and the tumour, tissue and MDE densities after irradiation (Fig. 9, right plot). As with EBRT, all tumour cells get eradicated. Additionally, with Targit the normal breast tissue close to the radiation source is also gradually destroyed.

Our simulations show that both treatment strategies eliminate stray tumour cells that are possibly left behind in the tumour bed after breast conserving surgery. So it appears that local recurrence due to tumour cells in the adjacent tissue would be completely eliminated by both the treatment strategies. However, we know that conventional radiotherapy only eliminates 2/3rds of recurrences (EBCTCG, 2000). Thus let us assume that some of the local recurrence arises from genetically unstable cells in the vicinity of the tumour.

8. Irradiation of cells harbouring LOH in tumour suppressor genes

LOH in TSGs is found at relatively high frequency in breast cancers (Berardo et al., 1998; Utada et al., 2000). Lacroix et al. (2004) argue that the origin of breast tumours could be breast cancer stem cells which have a long life and a large replicating potential. As discussed previously, recurrence of the tumour may occur due to genetically mutated cells in the adjacent tissue which are clones from the same stem cell as the primary tumour. It is plausible that the genetic mutations in TSGs in morphologically healthy cells in the tissue adjacent to the primary tumour increase their susceptibility to further mutations and eventually give rise to a new tumour. This hypothesis already has clinical support—the presence of LOH in certain TSGs is a strong predictor of local recurrence (Li et al., 2002). Additionally, Jamieson et al. (2003) has shown that LOH on a growth factor receptor involved in tumour suppression in head and neck cancer reduces the 5 year relapse-free survival significantly when patients are treated with radiotherapy alone. Since LOH in TSGs may disable the cells' ability to arrest the cell in a cell-cycle repair phase, these genetically unstable cells do not die after receiving mutative hits from irradiation and thus could accumulate and propagate low-dose radiation-induced genetic damage. This may be one reason why conventional radiotherapy fails to eliminate local recurrence completely and if these areas are treated it may eliminate the risk of local recurrence.

We now introduce an area of possible cells with LOH in TSGs into our breast tissue and discuss the clinical outcome after applying standard radiotherapy and Targit. We assume that an area of 2.5 cm radius around the original primary tumour may contain cells with LOH in TSGs similar to the primary tumour. After surgery, we assumed this shell to be about 1 cm deep. This is based on the following rationale. Gray's Anatomy (Bannister et al., 1995) describes 15–20 lobes and milk ducts in the female breast and we therefore assume an average of 18 main primordial ducts at the nipple. Thus there is approximately a 20° segment of breast tissue which would span a

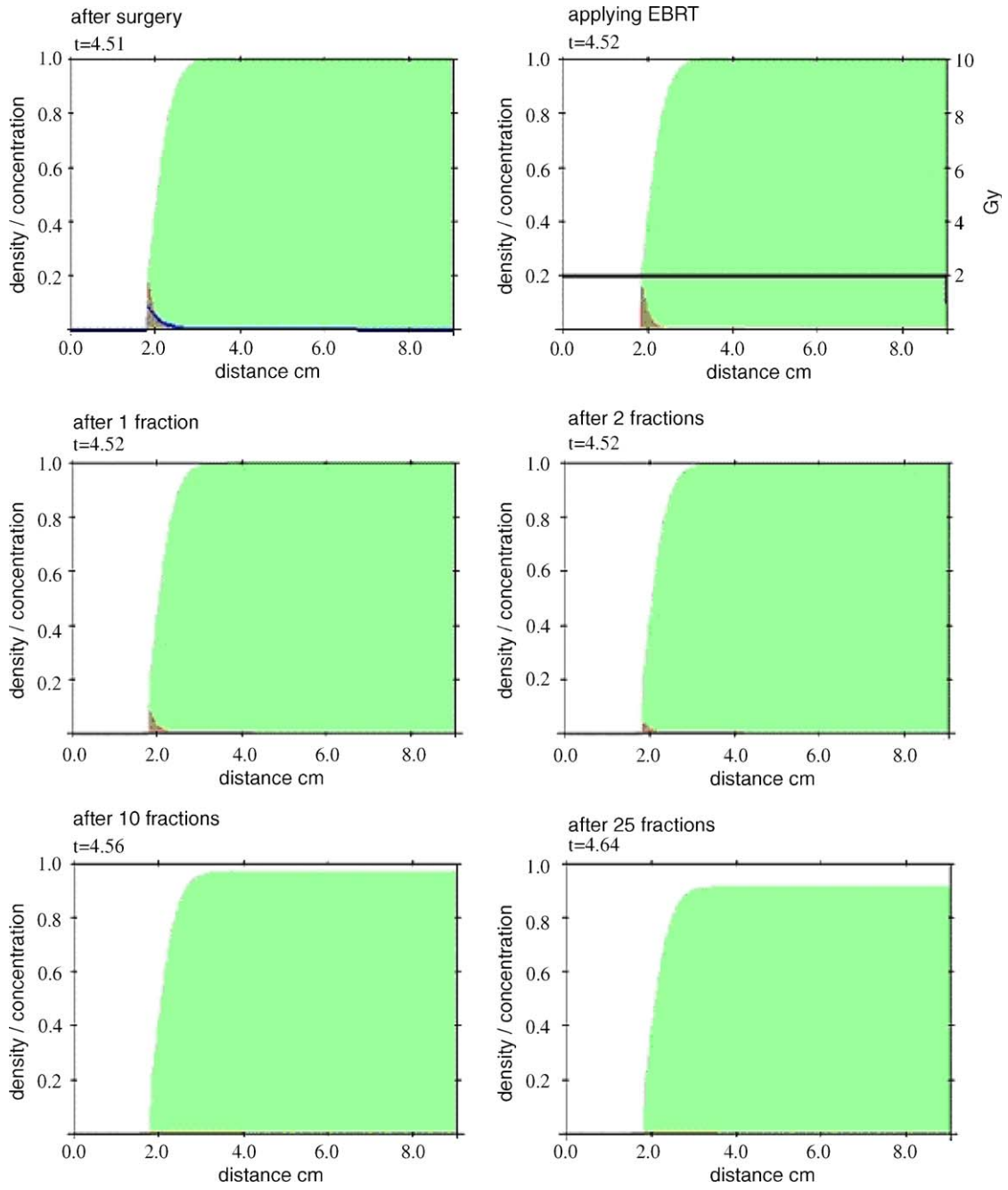


Fig. 8. Plots showing the simulation of conventional external beam radiotherapy treatment following breast conserving surgery (top left). Fractions with doses of 2 Gy each (solid black line in the top right picture) are delivered to the whole domain eradicating all tumour cells (brown) but also harming the healthy (green) breast tissue (bottom row).

maximum of about 1 cm at about halfway and about 3 cm at the perimeter of the breast (see Fig. 10). Identical to the simulation shown in Fig. 8, standard fractionated EBRT delivers multiple low doses of radiation and healthy cells have enough time to recover from irradiation during consecutive fractions. On the other hand, as discussed above, cells with LOH in TSGs (the yellow area, Fig. 11) suffer from radiation as well but do not die, consequently leaving behind an increased risk of local recurrence. Only massive genetic damages, such as those that could be caused by high-dose irradiation, may nullify the mutated cells' survival. In Fig. 12 we show the simulation of Targit, in which again a single lethal dose of radiation is delivered to a small region in the immediate vicinity of the tumour. This high-dose erradicates healthy cells, but also causes lethal damage in cells harbouring LOH in TSGs (the yellow area) which makes them unable to survive.

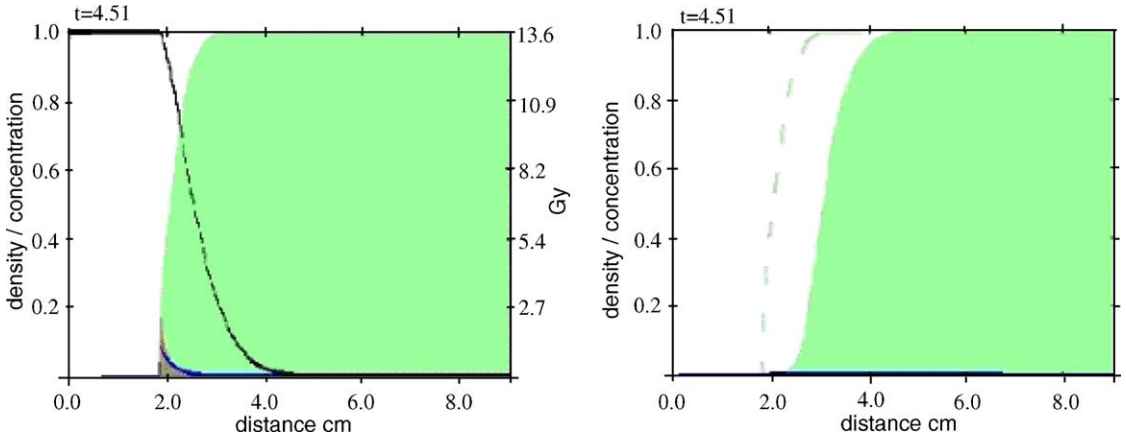


Fig. 9. Plots showing the results of simulating radiotherapy treatment post-surgery using the new Targit method. The plot on the left shows the initial distribution throughout the tissue of the radiation delivered by Targit (black line). The rapid attenuation of the radiation dose protects tissue at larger distances from the applicator (cf. Fig. 2). The plot on the right shows the domain after irradiation. We see that all the tumour cells are killed. However, note that the damage to the breast tissue due to irradiation is very localized. The dashed line represents the pre-treatment healthy tissue margin.

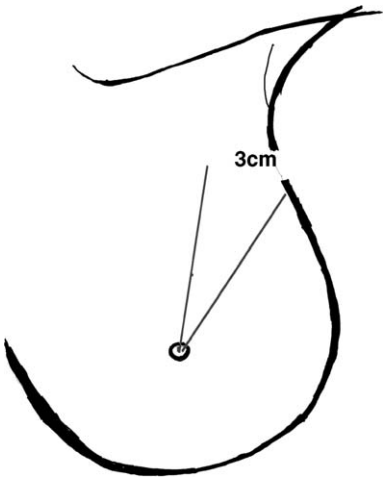


Fig. 10. Figure showing the geometry of the area of the breast tissue which potentially contains cells with LOH. Assuming 15–18 primordial ducts in the whole breast, a segment of breast tissue arising from one duct would span at most 3 cm at the periphery of the breast and about 1 cm nearer the nipple. Even if a 2 cm tumour develops at the very boundary of a segment, surgical excision of the tumour with a 1 cm margin would leave a 1 cm wide area which has clones from the same stem cell from which the primary tumour arose initially.

9. Discussion

Partial breast irradiation with single fraction radiation techniques such as Targit is a new approach to treat early stage breast cancer. In this paper, we have presented a model framework to predict the effect of this new treatment strategy and conventional radiotherapy treatment using the established LQ model. We can apply this simple model without any of the enhancements which have recently been discussed by Herskind et al. (2005), because we consider the effect of every irradiation fraction separately. The important dynamic processes during the course of the treatment, i.e. tumour regrowth and repopulation, are taken into account by our mathematical model of tumour growth and invasion which we apply in between single deliveries of fractionated therapy.

We have shown that both conventional EBRT and Targit following breast conserving surgery eliminate tumour cells that may have escaped into the surrounding healthy tissue. If we assume that local recurrence arises out of tumour cells left behind after surgery, then our model predicts that the new technique of targeted intraoperative radiotherapy (TARGIT) would have at least a similar curative effect as the conventional method. In fact, both methods should have eliminated local recurrence.

However, it is known that after breast conserving surgery, postoperative radiotherapy reduces local recurrence rates only by a factor of 2/3, from 27.2% to about 8.8% (EBCTCG, 2000). The origin of recurrence in these 8.8% of patients still remains unknown as does the reason why conventional radiotherapy is not able to prevent it.

Local recurrence arises in the area around the primary tumour irrespective of whether or not radiotherapy is given and whether or not stray tumour cells were found within a certain margin of the primary lump pre-treatment. It is plausible that the genetic mutations in TSGs in morphologically healthy cells, in the tissue adjacent to the primary tumour, increase their susceptibility to further mutations and eventually give rise to a new tumour. Li et al. (2002) has shown that the presence of LOH in certain TSGs is a strong predictor of local recurrence. These genetically unstable cells could accumulate and propagate low-dose radiation-induced genetic damage. It is this concept that we have modelled in the final stage of our model. The very design of the low-dose fractionated regime that spares normal tissues may be the flaw in conventional EBRT. The results of the simulation given in Fig. 11 shows that conventional radiotherapy would not only spare the

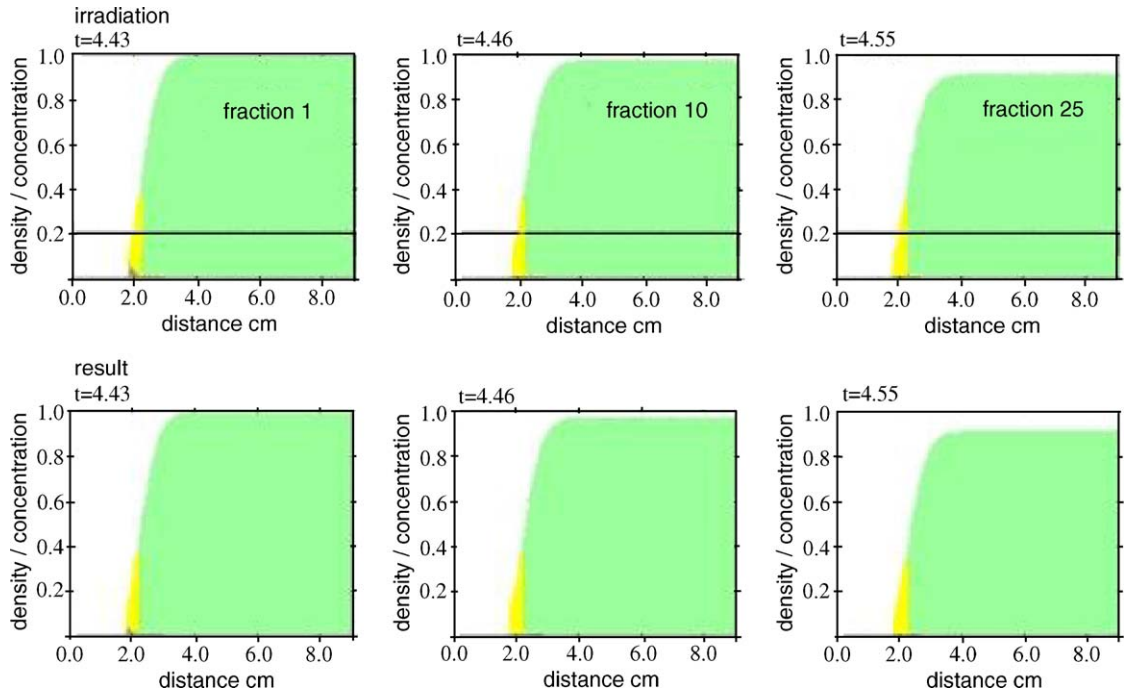


Fig. 11. Plots showing the simulation of standard fractionated radiotherapy following breast conserving surgery and its effect on cells with LOH. As can be seen from the plots, this standard treatment does indeed eradicate all tumour cells, but fails to eliminate cells with LOH on TSGs in the immediate vicinity of the tumour (yellow area), since these mutated cell-cycle check-point genes lack the detection of radiation-induced genetic damage.

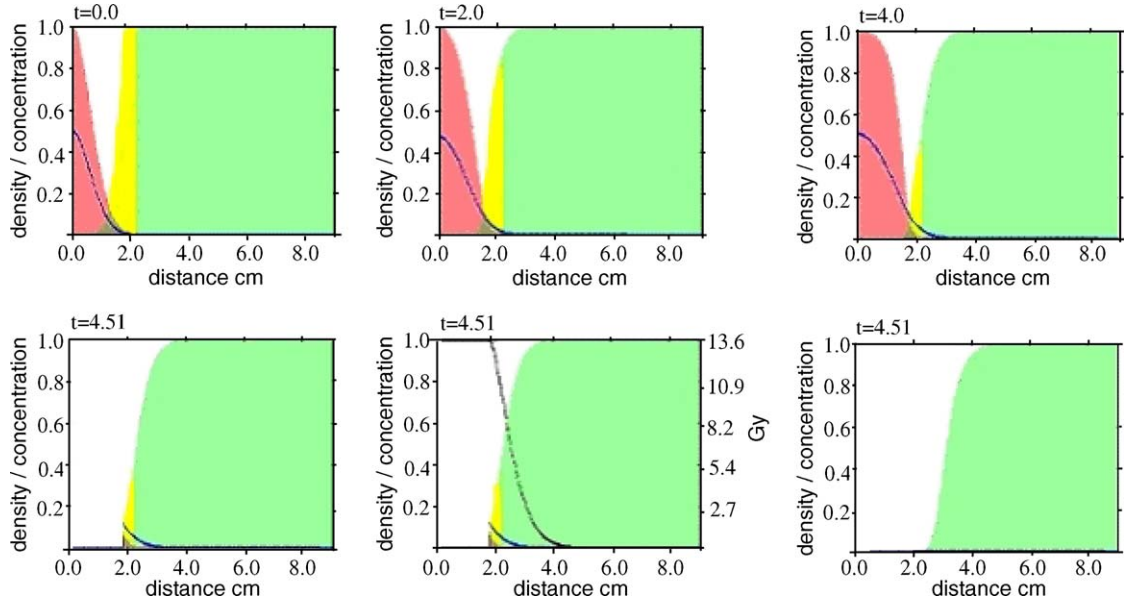


Fig. 12. Plots showing the simulation of a single high dose of Targit radiation (solid black line, middle picture bottom row) delivered to areas adjacent to the primary tumour and its effect on cells with LOH. As can be seen from the plots, Targit eliminates tissue cells that previously surrounded the (red) tumour and may have shared its clonal origin and may have harboured cells with LOH (yellow area). The (green) tissue is only affected near the tumour bed. Tissue at distant sites is spared damage. The top row shows the development and invasion of the primary tumour. The second row shows the simulation of surgery (left), the delivery of Targit radiation (middle) and the final effect of targeted intraoperative radiotherapy (right) killing not only all tumour cells but also all tissue containing cells with potential LOH.

healthy cells, but also the cells with crucial mutations in TSGs adjacent to the breast cancer. Only massive genetic damage, as could be caused by localized high-dose irradiation, would eradicate the mutated cells. In contrast to the conventional treatment, Targit delivers a high dose of radiation and destroys tissues close to the tumour and could therefore also eradicate genetically mutated cells in the tissue adjacent to the primary tumour. Therefore, Targit may be able to eliminate not only tumour cells that are left behind, but also the potentially malignant cells in

the immediate vicinity of the tumour that would usually be spared and possibly even promoted by the very nature of conventional fractionated radiotherapy.

We note that we have not modelled the indirect effects of radiation on completely normal cells in the immediate vicinity of the applicator. These cells are normally responsible for local paracrine secretions, such as growth factors, in response to trauma and higher-than-normal aromatase activity (O'Neill et al., 1988; Vaidya, 2002). These cells would be spared with conventional radiotherapy but not with Targit. This particular effect is difficult to include in our current partial differential equation model but would be expected to enhance the demonstrated effect. Finally, we note also that angiogenesis which is essential for the development of recurrence is not included in the model. However our simulations show that tumour cells left behind after surgery have the potential to reform a new tumour. Mathematical models of angiogenesis (e.g. Anderson and Chaplain, 1998) can be included in future developments of the present model in order to obtain more realistic simulations of recurrence. Arguably, the high local dose of radiotherapy may inhibit angiogenesis and this would again favour intraoperative radiotherapy (IORT). So Targit may yet prove to be a better treatment. Of course this conclusion can only be drawn once the results of the Targit trial are available.

The work presented here represents an initial attempt to model a biologically complex set of circumstances. We are well aware of its limitations, many of which are imposed by a lack of biological information. However, at the very least, our model draws attention to areas in which further basic research is needed.

We have used an α/β ratio of 3 Gy for the effect of radiation on normal breast tissue. This figure has been obtained from the literature and applies to the connective tissues of the breast (Guerrero and Allen Li, 2003). However, it may not apply to the critical cells of interest in this paper i.e. breast epithelial cells.

Furthermore, it is known that genomic instability arises in tumours both spontaneously and as a result of treatment with radiotherapy (Yang et al., 1989; Sarkaria et al., 1995; Durante et al., 1996; Colley-Durel et al., 2001; Lorimore et al., 2001; Trott, 2001; Goldberg, 2003; Roychoudhuri et al., 2004; Tozeren et al., 2005). As we have presented a partial differential equation continuum model, we cannot explicitly incorporate the fact that random genomic instabilities arise in single, individual cells. We are currently developing and extending the current model to an individual-based, single cell model in order to incorporate this fact theoretically at least (cf. Anderson, 2005). However, we do not know the relative effects of IORT and EBRT on genomic instability in non-irradiated tissues. Additionally, with IORT, low doses of radiation at the periphery of the irradiated volume may be dangerous, in terms of the clonal evolution of cancer. But one could also argue that the potential risk of a mutation occurring, due to the low dose given at a distance from the radiation source in IORT, is not higher than the risk of a mutation occurring in the same area with the very last low dose delivered in EBRT.

Despite these limitations our model does provide a 'proof of principle': by using a bottom-up approach through mathematical modelling it is possible to produce clinically testable hypotheses on the effects of different therapeutic approaches to the postoperative radiotherapy treatment of breast cancer. Our next task is to refine and expand the model, taking into account some of the issues described above and ultimately to provide a tool that clinicians and radiotherapists might use to explore and define optimal therapeutic strategies.

Acknowledgement

The work of HE was supported by a University of Dundee Nicholl-Lindsay Ph.D. Scholarship bursary. MAJC, ARAA and HE also gratefully acknowledge support from NIH Grant No. 1 P20 CA11300-01 (CFDA No. 93.397) 'Development of a Virtual Tumor'. ARAA also acknowledges the support of NCI Grant No. 1 P50 CA113007-01. The authors would also like to thank Prof. Lynn Matrisian for fruitful discussions.

References

Anderson, A.R.A., 2005. A hybrid mathematical model of solid tumour invasion: the importance of cell adhesion. Math. Med. Biol. 22, 163–186.

Anderson, A.R.A., Chaplain, M.A.J., 1998. Continuous and discrete mathematical models of tumour-induced angiogenesis. Bull. Math. Biol. 60, 857–899.

Anderson, A.R.A., Chaplain, M.A.J., Newman, E.L., Steele, R.J.C., Thompson, A.M., 2000. Mathematical modelling of tumour invasion and metastasis. J. Theor. Med. 2, 129–154.

Arthur, D., 2003. Accelerated partial breast irradiation: a change in treatment paradigm for early stage breast cancer. J. Surg. Oncol. 84, 185–191.

Bannister, L.H., Berry, M.M., Collins, P., Dyson, M., Dussek, J.E., 1995. Gray's anatomy, 38th ed. Churchill Livingstone, New York, USA, pp. 417–424.

Berardo, M.D., Allred, D.C., O'Connel, P., 1998. Breast cancer. In: Jameson, J.L. (Ed.), Principles of Molecular Medicine. Human Press, Totowa, NJ, pp. 625–632.

Booth, J.T., 2002. Modelling the impact of treatment uncertainties in radiotherapy. Ph.D. Thesis, University of Adelaide.

Bray, D., 1992. Cell Movements. Garland Publishing, New York.

Brenner, D.J., Armour, E., Corry, P., Hall, E., 1998. Sublethal damage repair times for a late-responding tissue relevant to brachytherapy (and external-beam radiotherapy): implications for new brachytherapy protocols. Int. J. Radiat. Oncol. Biol. Phys. 41 (1), 135–138.

Colley-Durel, S., Guitton, N., Nourgalieva, K., Leveque, J., Danic, B., Chenal, C., 2001. Genomic instability and breast cancer. Oncol. Rep. 8, 1001–1005.

Dairkee, S.H., 1998. Allelic loss in normal lobules adjacent to breast cancer. Cancer Detect. Prevent. 22 (1), 135A.

Dale, R.G., 1985. The application of the linear-quadratic dose–effect equation to fractionated and protracted radiotherapy. Br. J. Radiol. 58, 515–528.

Dale, R.G., 1989. Time-dependent tumour repopulation factors in linear-quadratic equations—implications for treatment strategies. Radiother. Oncol. 15, 371–381.

Dale, R.G., 1996. Dose-rate effects in targeted radiotherapy. Phys. Med. Biol. 41, 1871–1884.

Demicheli, R., Miceli, R., Brambilla, C., Ferrari, L., Moliterni, A., Zambetti, M., Valagussa, P., Bonadonna, G., 1999. Comparative analysis of breast cancer recurrence risk for patients receiving or not receiving adjuvant cyclophosphamide, methotrexate, fluorouracil (CMF). Data supporting the occurrence of 'cures'. Breast Cancer Res. Treat. 53 (3), 209–215.

Deng, G., Lu, Y., Zlotnikov, G., Thor, A.D., Smith, H.S., 1996. Loss of heterozygosity in normal tissue adjacent to breast carcinomas. Science 274, 2057–2059.

Durante, M., Grossi, G.F., Yang, T.C., 1996. Radiation-induced chromosomal instability in human mammary epithelial cells. Adv. Space Res. 18, 99–108.

Early Breast Cancer Trialists' Collaborative Group (EBCTCG), 2000. Favourable and unfavourable effects on long-term survival of radiotherapy for early breast cancer: an overview of the randomised trials. Lancet 355, 1757–1770.

Ebert, M.A., Carruthers, B., 2003. Dosimetric characteristics of a low-kV intra-operative X-ray source: Implications for use in a clinical trial for treatment of low-risk breast cancer. Med. Phys. 30 (9), 2424–2431.

Fisher, B., Anderson, S., Bryant, J., Margolese, R.G., Deutsch, M., Fisher, E.R., Jeong, J-H., Wolmark, N., 2002. Twenty-year follow-up of a randomized trial comparing total mastectomy, lumpectomy, and lumpectomy plus irradiation for the treatment of invasive breast cancer. N. Engl. J. Med. 347 (16), 1233–1241.

Försti, A., Louhelainen, J., Söderberg, M., Wijkström, H., Hemminki, K., 2001. Loss of heterozygosity in tumour-adjacent normal tissue of breast and bladder cancer. Eur. J. Cancer 37, 1372–1380.

Fritz, P., Cabrera, C.M., Dippon, J., Gerteis, A., Simon, W., Aulitzky, W.E., van der Kuip, H., 2005. c-erbB2 and topoisomerase IIalpha protein expression independently predict poor survival in primary human breast cancer: a retrospective study. Breast Cancer Res. 7 (3), 374–384.

Goldberg, Z., 2003. Clinical implications of radiation-induced genomic instability. Oncogene 22, 7011–7017.

Guerrero, M., Allen Li, X., 2003. Analysis of a large number of clinical studies for breast cancer radiotherapy: estimation of radiobiological parameters for treatment planning. Phys. Med. Biol. 48 (20), 3307–3326.

Guiot, C., Degiorgis, P.G., Delsanto, P.P., Gabriele, P., Deisboeck, T.S., 2003. Does tumor growth follow a "universal law"? J. Theor. Biol. 225 (2), 147–151.

Herskind, C., Steil, V., Kraus-Tiefenbacher, U., Wenz, F., 2005. Radiobiological aspects of intraoperative radiotherapy (IORT) with isotropic low-energy X-rays for early stage breast cancer. Radiat. Res. 163 (2), 208–215.

Jamieson, T.A., Brizel, D.M., Killian, .K., Oka, Y., Jang, H-S., Fu, X., Clough, R.W., Vollmer, R.T., Anscher, M.S., Jirtle, R.L., 2003. M6P/IGF2R loss of heterozygosity in head and neck cancer associated with poor patient prognosis. BMC Cancer 3 (4).

Kopans, D.B., Rafferty, E., Georgian-Smith, D., Yeh, E., D'Allesandro, H., Moore, R., Hughes, K., Halpern, E., 2003. A simple model of breast carcinoma growth may provide explanations for observations of apparently complex phenomena. Cancer 97 (12), 2951–2959.

Lacroix, M., Toillon, R-A., Leclercq, G., 2004. Stable 'portrait'of breast tumors during progression: data from biology, pathology and genetics. Endocr.-Relat. Cancer 11, 497–522.

Lakhani, S.R., Chaggar, R., Davies, S., Jones, C., Collins, N., Odel, C., Stratton, M.R., O'Hare, M.J., 1999. Genetic alterations in 'normal' luminal and myoepithelial cells of the breast. J. Pathol. 189, 496–503.

Larson, P.S., de las Morenas, A., Bennett, S.R., Adrienne Cupples, L., Rosenberg, C.L., 2002. Loss of heterozygosity or allele imbalance in histologically normal breast epithelium is distinct from loss of heterozygosity or allele imbalance in co-existing carcinomas. Am. J. Pathol. 161 (1), 283–290.

Li, Z., Moore, D.H., Meng, Z.H., Ljung, B.M., Gray, J.W., Dairkee, S.H., 2002. Increased risk of local recurrence is associated with allelic loss in normal lobules of breast cancer patients. Cancer Res. 62, 1000–1003.

Lorimore, S.A., Coates, P.J., Scobie, G.E., Milne, G., Wright, E.G., 2001. Inflammatory-type responses after exposure to ionizing radiation in vivo: a mechanism for radiation-induced bystander effects? Oncogene 20, 7085–7095.

Michaelson, J., Satija, S., Moore, R., Weber, G., Halpern, E., Garland, A., Kopans, D.B., 2003. Estimates of breast cancer growth rate and sojourn time from screening database information. J. Women's Imag. 5 (1), 11–19.

Oldham, M., 2001. Radiation physics and applications in therapeutic medicine. Phys. Educ. 36, 460–467.

O'Neill, J.S., Elton, R.A., Miller, W.R., 1988. Aromatase activity in adipose tissue from breast quadrants: a link with tumour site. Br. Med. J. (Clin. Res. Ed.) 296, 741–743.

Peer, P.G., van Dijck, J.A., Hendriks, J.H., Holland, R., Verbeek, A.L., 1993. Age-dependent growth rate of primary breast cancer. Cancer 71 (11), 3547–3551.

Reitsamer, R., Peintinger, F., Sedlmayer, F., Kopp, M., Menzel, C., Cimpoca, W., Glueck, S., Rahim, H., Kopp, P., Deutschmann, H., Merz, F., Brandis, M., Kogelnik, H., 2002. Intraoperative radiotherapy given as a boost after breast-conserving surgery in breast cancer patients. Eur. J. Cancer 38, 1607–1610.

Roychoudhuri, R., Evans, H., Robinson, D., Møller, H., 2004. Radiation-induced malignancies following radiotherapy for breast cancer. Br. J. Cancer 91, 868–872.

Sachs, R.K., Hahnfeld, P., Brenner, D.J., 1997. The link between low-let dose–response relations and the underlying kinetics of damage production/repair/misrepair. Int. J. Biol. 72 (4), 351–374.

Sancar, A., Lindsey-Boltz, L.A., Ünsal-Kaçmaz, K., Linn, S., 2004. Molecular Mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu. Rev. Biochem. 73, 39–85.

Sarkaria, J.N., Fowler, J.F., Lindstrom, M.J., Jordan, V.C., Mulcahy, R.T., 1995. The decreased influence of overall treatment time on the response of human breast tumor xenografts following prolongation of the potential doubling time (Tpot). Int. J. Radiat. Oncol. Biol. Phys. 31, 833–840.

Steinarsdottir, M., Jonasson, J.G., Vidarsson, H., Juliusdottir, H., Hauksdottir, H., Ogmundsdottir, H.M., 2004. Cytogenetic changes in nonmalignant breast tissue. Genes Chrom. Cancer 41 (1), 47–55.

Tomlinson, I.P.M., 2001. Mutations in normal breast tissue and breast tumours. Breast Cancer Res. 3, 299–303.

Tozeren, A., Coward, C.W., Petushi, S.P., 2005. Origins and evolution of cell phenotypes in breast tumors. J. Theor. Biol. 233, 43–54.

Trott, K.R., 2001. Non-targeted radiation effects in radiotherapy—roles of radiation-induced genomic instability and of the bystander effect in cancer cure by radiotherapy. Acta Oncol. 40, 976–980.

Turesson, I., Carlsson, J., Brahme, A., Glimelius, B., Zackrisson, B., Stenerlöw, B., 2003. Biological response to radiation therapy. Acta Oncol. 42 (2), 92–106.

Utada, Y., Emi, M., Yoshimoto, M., Kasumi, F., Akiyama, F., Sakamoto, G., Haga, S., Kajiwara, T., Nakamura, Y., 2000. Allelic loss at 1p34–36 predicts poor prognosis in node-negative breast cancer. Clin. Cancer Res. 6, 3193–3198.

Vaidya, J.S., 2002. A novel approach to local treatment of breast cancer. Ph.D. Thesis, University College London.

Vaidya, J.S., Baum, M., Tobias, J.S., Houghton, J., 1999. Randomised trials are not unethical (Letter). Lancet 535, 1714.

Vaidya, J.S., Vyas, J.J., Chinoy, R.F., Merchant, N., Sharma, O.P., Mittra, I., 1996. Multicentricity of breast cancer: whole-organ analysis and clinical implications. Br. J. Cancer 74, 820–824.

Vaidya, J.S., Baum, M., Tobias, J.S., D'Souza, D.P., Naidu, S.V., Morgan, S., Metaxas, M., Harte, K.J., Sliski, A.P., Thomson, E., 2001. Targeted intra-operative radiotherapy (Targit): an innovative method of treatment for early breast cancer. Ann. Oncol. 12, 1075–1080.

Vaidya, J.S., Baum, M., Tobias, J.S., Morgan, S., D'Souza, D.P., 2002. The novel technique of delivering targeted intraoperative radiotherapy (Targit) for early breast cancer. EJSO 28, 447–454.

Vaidya, J.S., Tobias, J.S., Baum, M., Keshtgar, M., Joseph, D., Wenz, F., Houghton, J., Saunders, C., Corica, T., D'Souza, D.P., Sainsbury, R., Massarut, S., Taylor, I., Hilaris, B., 2004. Intraoperative radiotherapy for breast cancer. Lancet Oncol. 5, 165–173.

Vaidya, J.S., Tobias, J.S., Baum, M., Wenz, F., Kraus-Tiefenbacher, U., D'Souza, D.P., Keshtgar, M., Massarut, S., Hilaris, B., Saunders, C., Joseph, D., 2005. TARGeted Intraoperative radioTherapy (TARGIT): an innovative approach to partial-breast irradiation. Semin. Radiat. Oncol. 15, 84–91.

Veronesi, U., Orecchia, R., Luini, A., Gatti, G., Intra, M., Zurrida, S., Ivaldi, G., Tosi, G., Ciocca, M., Tosoni, A., De Lucia, F., 2001. A preliminary report of intraoperative radiotherapy (IORT) in limited-stage breast cancers that are conservatively treated. Eur. J. Cancer 37, 2178–2183.

Wyatt, R.M., Beddoe, A.H., Dale, R.G., 2003. The effects of delays in radiotherapy treatment on tumour control. Phys. Med. Biol. 48, 139–155.

Yang, T.C., Stampfer, M.R., Tobias, C.A., 1989. Radiation studies on sensitivity and repair of human mammary epithelial cells. Int. J. Radiat. Biol. 56, 605–609.

Yarnold, J., Ashton, A., Bliss, J., Homewood, J., Harper, C., Hanson, J., Haviland, J., Bentzen, S., Owen, B., 2005. Fractionation sensitivity and dose response of late adverse effects in the breast after radiotherapy for early breast cancer: long-term results of a randomised trial. Radiother. Oncol. 75, 9–17.